Clinical Study Protocol

Protocol Title:	**Prospective, phase II/III, randomized clinical study to compare BEGEDINA® versus "conventional treatment" for treating steroid resistant acute graft-versus-host disease**
Protocol Number:	**ADN011**
Date of Protocol:	**Version 5.0: 13 March 2017** **Version 4.0: 14 October 2016** **Version 3.0: 25 June 2015** **Version 2.0: 12 May 2015** **Version 1.0: 09 April 2015**
Product:	**BEGEDINA® (begelomab)**
IND No.:	**117373**
EudraCT No.:	**2015-001360-19**
Study Phase:	**Phase II/III**
Sponsor:	**ADIENNE SA** **Via Zurigo, 46** **6900 – Lugano, Switzerland** **Telephone number: +41.912.104.726** **Fax number: +41.919.211.978**

Approved on behalf of Sponsor:

Giovanni Amabile, PhD — **Date** 13-Mar-2017
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Professor Andrea Bacigalupo, MD — **Date** 13/03/2017

Synopsis

Name of Sponsor/Company:	ADIENNE SA
Name of Finished Product:	BEGEDINA®
Name of Active Ingredient:	Murine monoclonal antibody against CD26, BT 5/9 antibody
Title of Study:	Prospective, phase II/III, randomized clinical study to compare BEGEDINA® versus "conventional treatment" for treating steroid resistant acute graft-versus-host disease
Protocol No:	ADN011
Study centers:	Approximately 30 Bone Marrow Transplantation Units in Europe, United States and Canada
Study duration: for an individual subject: 6 months, including screening and the 180-day follow-up.	**Phase:** phase II/III

Objectives:

Primary objective:

To determine the efficacy of BEGEDINA® versus conventional therapy in steroid-resistant acute graft-versus-host disease (GvHD) in terms of overall response (OR) at 28 days and transplant-related mortality (TRM) up to 180 days.

Key Secondary objective:

- To compare BEGEDINA and conventional therapy with respect to overall survival (OS) up to 180 days.

Other secondary objectives:

Efficacy: To compare BEGEDINA and conventional therapy with respect to:

- Change from baseline in stages of GvHD by target organ
- Incidence of chronic GvHD up to 180 days
- Cumulative steroid dose
- Incidence of Relapse and Relapse-Related Mortality
- Change from baseline in Karnofksy Performance Status

Pharmacokinetic (PK)

- The PK objective for this study is to characterize the pharmacokinetics of BEGEDINA in subjects with Grades II-IV acute GvHD, who have failed to respond to steroid treatment.

Safety:

- To compare the safety and tolerability of BEGEDINA and conventional therapy.
- To gather additional information on the safety of BEGEDINA in subjects with Grades II-IV acute GvHD who have failed to respond to steroid treatment.
- To evaluate the immunogenicity of BEGEDINA.
- To evaluate the effect of BEGEDINA on glucose metabolism.
- To compare the incidence of second malignancies at the end of the follow-up between BEGEDINA and conventional therapy.

Exploratory objectives:

- To evaluate the change in quality of life (QoL) from baseline
- To evaluate duration of response

• To evaluate OR by standard-risk and high-risk GvHD at onset.	
Methodology: Prospective, multicenter, randomized, open-label, phase II/III clinical study.Randomization will be stratified by the baseline GvHD severity grade.	
Planned number of subjects:	184 subjects
Study period	Estimated date for first subject enrollment: Q1 - 2016 Estimated date for last subject completed: Q1 - 2018
Diagnosis and main criteria for inclusion:	***Inclusion criteria:*** 1. Age ≥18 and ≤65 years of age. 2. Recipient of an allogeneic hematopoietic stem cell transplantation (HSCT). Note: Subjects with GvHD following donor lymphocyte infusion post-HSCT are also eligible 3. Steroid-resistant acute GvHD, Grade II-IV, defined as: progressive disease (deterioration of at least 1 stage in 1 organ) after 3 days of primary treatment with methylprednisolone 2 mg/kg, or equivalent. or lack of at least a partial response (PR) after 7 days of primary treatment with methylprednisolone 2 mg/kg or equivalent. or lack of a complete response (CR) after 14 days of primary treatment with methylprednisolone 2 mg/kg or equivalent. Note: Subjects who may have received an increase in their steroid dose treatment prior to randomization will be eligible for enrollment. An increase in steroid dose will not be considered as second-line therapy. 4. Evidence of previous myeloid engraftment (absolute neutrophil count $\geq 0.5 \times 10^9$/L). 5. Karnofsky Performance Status Scale ≥50%. 6. Adequate renal function as defined by serum creatinine ≤2 × upper limit of normal or calculated creatinine clearance (CrCl) of ≥30 mL/min using the Cockroft-Gault equation: Calculated CrCl= ([140-age in years] x [ideal body mass {IBM} in kg])/72 x (serum creatinine value in mg/dL), where IBM = IBM (kg) = ([height in cm–154] × 0.9) + (50 if male, 45.5 if female). 7. Subject must be willing and able to comply with study requirements, remain at the clinic, and return to the clinic for the follow-up evaluation, as specified in this protocol during the study period. 8. Able and willing to provide signed informed consent.
	Exclusion criteria: 1. Prior second-line systemic treatment for GvHD. 2. Received agents other than steroids for primary treatment of acute GvHD. 3. Stage 1-2 skin acute GvHD alone (with no other organ involvement). 4. Acute steroid resistant GvHD beyond 28 days from first-line therapy (primary treatment).

	5. Evidence of severe hepatic veno-occlusive disease or sinusoidal obstruction. 6. Evidence of encephalopathy. 7. Life expectancy <3 weeks. 8. Presence of chronic GvHD 9. Second or subsequent allogeneic transplant. 10. Previous solid organ transplant (with the exception of a corneal transplant >3 months prior to screening). 11. Relapsed disease after last transplant. 12. Human immunodeficiency virus positive. 13. Evidence of lung disease that is likely to require more than 2 liter per minute of O2 via face mask or an estimated FiO2 of 28% via other delivery methods in order to sustain an O2 saturation of 92% within the next 3 days. 14. Any underlying or current medical or psychiatric condition that, in the opinion of the investigator, would interfere with the evaluation of the subject including uncontrolled infection, heart failure, pulmonary hypertension. Any other serious medical condition, as judged by the investigator, which places the subject at an unacceptable risk if he or she were to participate in the study or confounds the ability to interpret data from the study. 15. Administration of any other investigational agents (not approved by the United States Food and Drug Agency [FDA] or European Medicines Agency [EMA] for any indication) within 30 days of randomization. Participated in any interventional clinical trial for an acute GvHD therapeutic agent or for an immunomodulatory drug, within the past 30 days or within 5 half-lives of the study treatment, whichever is the greater. Participated or is currently participating in any bone marrow derived autologous and allogeneic stem cell or gene therapy study. 16. Known hypersensitivity to murine proteins. 17. Women who are pregnant, breastfeeding or at risk to become pregnant during study participation; female subjects of childbearing potential who have not been started on an anti-ovulatory regimen prior to initiation of chemo-inductive regimen must test negative for pregnancy (serum) at the time of enrollment. 18. Male and female subjects who do not agree to take adequate measures to avoid pregnancy prior to study entry and for the duration of participation in the study (or for at least 3 months following the last dose of study drug, whichever is longer) (acceptable methods of birth control are described in protocol Section 6.2.1.6).
Test product, dose and mode of administration:	BEGEDINA (murine monoclonal antibody against CD26, BT 5/9 antibody) 2.7 mg/m^2/day for 5 consecutive days from Study Day 1 through to Study Day 5, and on Study Days 10, 14, 17, 21, 24, and 28.
Reference therapy, dose, and mode of administration:	Subjects in the conventional treatment arm will receive a single second-line treatment, according to the standard practice at the study center (physician's best choice).

Salvage treatment:	There are currently no recommended salvage (third-line) therapies for steroid-resistant acute GvHD; recommended treatment at this point could follow the European Group for Blood and Marrow Transplantation and the European LeukemiaNet or American Society of Blood and Marrow Transplantation (ASBMT) guidelines (per investigator discretion).

Sample size considerations: This study will have two primary efficacy endpoints as health authorities (EMA/FDA) have requested different primary endpoints: OR at Study Day 28 is the single primary endpoint for FDA, TRM up to Day 180 is the single primary endpoint for EMA.

For OR at Study Day 28, it is assumed that the OR rate for conventional treatment would be 50% and that the OR rate for BEGEDINA would be 75%. In order to have 80% power for a one-sided test at alpha=0.025, 58 patients per treatment are required.

For the other primary endpoint, TRM up to Study Day 180, the cumulative incidence for the conventional treatment is assumed to be 50% and the one for BEGEDINA is assumed to be 30% (relative reduction by 40%). 80% power for a one-sided log-rank test with significance level 0.025 requires a total of 103 transplant-related deaths. These can be expected with 92 subjects per treatment group, provided there are no early dropouts and no diagnoses of relapse. If drop-outs or diagnoses of relapse are observed during the recruitment period, the sample size may be slightly increased, e.g., to 100 subjects per treatment group.

Since the required sample size for TRM is larger than the one for OR, a total sample size of 184 (up to 200, respectively) will be used. The planned sample size will also provide adequate power to reject the null hypothesis of equal OS up to Study Day 180 (key secondary efficacy endpoint) assuming 20 to 25 percent differences in survival probabilities in favor of BEGEDINA.

Statistical methods:

Analysis of Primary Efficacy Endpoints

- The null hypothesis of equal OR probabilities for BEGEDINA and conventional treatment will be tested using a Cochran-Mantel-Haenszel test stratified for baseline GvHD severity grade (as used in the randomization) at the one-sided α =0.025 level. The treatment effect will be expressed as odds ratio with a two-sided 95% confidence interval (CI).
- The null hypothesis of same transplant-related cumulative incidences (up to Study Day 180) for both study treatments will be tested by a one-sided test stratified for the baseline GvHD severity grade (as used in the randomization) taken into account the competing risk of relapse diagnosis. The one-sided significance level is set to α=0.025. Regression analysis of cause-specific hazards stratified for the baseline GvHD severity will be performed as supportive analysis in order to quantify the treatment effect in terms of hazard ratios and two-sided 95% CI.

Analyses of Secondary Efficacy Endpoints

Key secondary efficacy endpoint:

The key secondary efficacy endpoint is OS up to 180 days. The null hypothesis of same OS curves (up to Study Day 180) for both study treatments will be tested by a one-sided log-rank test stratified for the baseline GvHD severity grade (as used in the randomization). The one-sided significance level is set to α=0.025. A proportional hazards regression analysis stratified for the baseline GvHD severity will be performed as supportive analysis in order to quantify the treatment effect in terms of a hazard ratio and a two-sided 95% CI. OS will be graphically displayed by Kaplan-Meier plots.

Other secondary efficacy endpoints:

All other secondary and supportive analyses will be tested at a one-sided alpha=0.025 significance level (where applicable), with no adjustments for multiplicity. Any further exploratory efficacy endpoints will be evaluated by appropriate descriptive statistics.

PK Analyses The population PK parameters will be determined using Nonlinear Mixed Effects Modeling.
Safety All adverse events (AEs) will be analyzed in terms of descriptive statistics and qualitative analysis. Adverse events will be listed for each subject and summarized by system organ class and preferred term by using the Medical Dictionary for Regulatory Activities. Safety laboratory data, vital signs measurements and ECG data will be listed for each subject and presented descriptively by treatment group, visit and assessment time.
Data and Safety Monitoring: A Data and Safety Monitoring Board (DSMB) will be set up for the trial reviewing and evaluating unblinded safety data, and propose appropriate actions as necessary. An initial assessment of safety will be performed after the first approximately 12 subjects in the BEGEDINA arm have stopped study treatment. **Interim Analysis:** In addition to the regular monitoring of safety, one interim analysis including certain efficacy data is planned when Day 28 response data is available for approximately 50% of the subjects. This analysis will be conducted by an independent, unblinded statistician and statistical programmer and results will be reviewed by the DSMB. Two objectives are associated with this interim analysis: • a non-binding futility analysis that may lead to stop of the study if chances for a successful study outcome are too low • a submission of interim results to Health Authorities for conditional approval if interim results for overall response at Study Day 28 would be very convincing (i.e., with a one-sided p-value less than 0.001). Even in this case, the study will be continued as planned to determine the final results for response at Study Day 28, response and in particular the results for TRM, overall survival, other secondary efficacy endpoints and safety (all relevant for the assessment of unconditional approval).
Date of protocol: 13 March 2017

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1.0 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADR	Adverse drug reaction(s)
AE	Adverse event(s)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASBMT	American Society of Blood and Marrow Transplantation
AST	Aspartate aminotransferase
BL	Baseline
BMI	Body mass index
BSA	Body surface area
CD26	T-cell antigen
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CL	Total body clearance
C_{max}	Peak serum concentration
CMV	Cytomegalovirus
CR	Complete response
CrCl	Creatinine clearance
CRO	Contract Research Organization
CSR	Clinical study report
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CV%	Coefficient of variation in percentage
DNA	Deoxyribonucleic acid
DPP-IV	Dipeptidyl peptidase IV
DSMB	Data and Safety Monitoring Board
EBV	Epstein Barr Virus
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic Data Capture
ELISA	Enzyme-linked immunoassay
EMA	European Medicines Agency
EudraCT	European Clinical Trials Database

FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GvHD	Graft-versus-host disease
HAMA	Human anti-mouse antibodies
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
HCV Ab	Hepatitis C virus antibodies
HIV	Human immunodeficiency virus
HOMA	Homeostasis Model Assessment
HSCT	Hematopoietic stem cell transplant
IBM	Ideal body mass
IBMTR	International Bone Marrow Transplant Registry
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IFN	Interferon
IgG	Immunoglobulin G
IMP	Investigational medicinal product
IRB	Institutional Review Board
IUD	Intrauterine device
IUS	Intrauterine system
IV	Intravenous
IVRS	Interactive Voice Activated Response System
k_{12} and k_{21}	Distribution rate constants
MedDRA	Medical Dictionary for Regulatory Activities
NCI	National Cancer Institute
NONMEM	Nonlinear Mixed Effects Modeling
NSG	NOD/SCID gamma [strain of mouse]
OR	Overall response
PBMCs	Peripheral blood mononuclear cells
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)

PR	Partial response
PS	Performance status
QoL	Quality of life
R_{acc}	Accumulation ratio
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCT	Stem cell transplant
SD	Stable disease
SF36	Short Form 36
SOC	System organ class
SOP	Standard operating procedures
STD	Standard deviation
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{½}$	Elimination half-life
TK	Toxicokinetic
TNF	Tumor necrosis factor
TRM	Transplant-related mortality
ULN	Upper limit of normal
US	United States
Vd	Volume of distribution
WHO	World Health Organization

2.0 INTRODUCTION

2.1 Background Information

2.1.1 Graft-Versus Host Disease

Recipients of an allogeneic hematopoietic stem cell transplant (HSCT) receive graft-versus-host disease (GvHD) prophylactic pharmacological treatment before the procedure. Nevertheless, despite this and improvements in post-HSCT immunosuppression, acute GvHD still develops in up to 30% of transplant recipients (Qian et al., 2013). It is a complex immunological disease and remains a major post-HSCT life-threatening complication. It is also a predictor of morbidity and mortality (Bacigalupo and Palandri, 2004; Van Lint et al., 2006; Goker et al., 2001).

Three conditions are generally necessary to develop GvHD: (1) the graft has to contain immunocompetent cells; (2) the recipient has to express tissue antigens which are not present in the donor; and (3) the recipient has to be unable of mounting an effective response to destroy the transplanted cells (Ferrara et al., 2009). It has been proposed that acute GvHD develops in 3 separate steps: 1) tissue damage in the host caused by radiotherapy and chemotherapy, with release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma; 2) an activation phase during which alloreactive donor T cells are activated by host antigens, presented by host antigen presenting cells; 3) cell proliferation, additional cytokines secretion and emerging of both cytotoxic and effectors T cells. This set of events is known as the Ferrara model (Ferrara et al., 2009), and highlights the complexity of the disease, and consequently the difficulty for satisfactory treatment.

Historically, GvHD was categorized as 'acute' or ‘chronic’ based on time of presentation relative to the HSCT: GvHD before Day 100 post-HSCT was known as 'acute,' and after Day 100 it was known as 'chronic’. This temporal distinction is arbitrary. For example, a recent National Institutes of Health classification includes late-onset acute GvHD (after Day 100) and an overlap syndrome with features of both acute and chronic GvHD (Filipovich et al., 2005).

Typically, acute GvHD affects the skin, liver and gastrointestinal (GI) tissues; infrequently, the eyes and oral cavity may also be targeted. It is now considered preferable to arrive at a diagnosis of acute GvHD through a variety of clinicopathological analyses, including biopsy of an affected organ (Qian et al., 2013). However, it is also important to eliminate other non-GvHD complications of the affected tissues, such as cytomegalovirus enteritis or drug eruption from medications (Carpenter & Macmillan, 2010). Chronic GvHD is now similarly

recognized without reference to time after HSCT by the presence of diagnostic or distinct GvHD manifestations which resemble autoimmune disease (Carpenter and Macmillan, 2010).

2.1.1.1 Acute Graft-Versus Host Disease

2.1.1.1.1 First-line Treatment in Acute GvHD

Acute GvHD is a life-threatening condition and long-term survival in patients is directly related to the severity of skin, liver and gut involvement (Qian et al., 2013). In a comprehensive review, Martin et al found that at the onset of acute GvHD, 81% of patients had skin involvement, 54% had GI involvement, and 50% had liver involvement (Martin et al., 1990). Patients with acute GvHD typically present with maculopapular rash (± bullous formation and desquamation), diarrhea, abdominal cramps and/or elevated serum bilirubin levels that can dramatically worsen in case of severe acute GvHD.

First-line treatment for patients with acute GvHD (Grade II or higher) is 6-methylprednisolone 2 mg/kg/day (Bacigalupo and Palandri, 2004; Van Lint et al., 2006; Goker et al., 2001; Ruutu et al., 2014). The standard pattern of treatment is 6-methylprednisolone at a dose of 2 mg/kg/day (as two divided doses) for 7 days starting within 48 hours from an acute GvHD diagnosis. Treatment can be changed in case of clear progression after a few days, but there is no evidence that change in treatment will affect the outcome (Ruutu et al., 2104). No reduction of the dose is done during the first 7 days. Tapering of the dose is done slowly and depending on the response. No marked dose reductions are done in the early phase. 6-methylprednisolone is not discontinued before all signs of GvHD have disappeared. However, although this is effective in over 50% of patients (Martin et al., 1990; Weisdorf et al., 1990), it produces durable responses in only a third of patients (Deeg et al., 2007; Hings et al., 1994; MacMillan et al., 2002).

Moreover, both the acute GvHD and the treatment increase the risk of severe infection because of the influence on the immune system.

2.1.1.1.2 Second-line Treatment in Acute GvHD

Acute GvHD is considered steroid-resistant when it progresses within 3 days or is not improved after 5–7 days of initial treatment with methylprednisolone 2 mg/kg (Deeg, 2007). Failure of treatment (corticosteroid resistance) is defined as no response after 7 days of treatment or clear progression after 5 days (Ruutu et al., 2104). Steroid-resistant acute GvHD is associated with a high rate of morbidity and mortality, primarily from infections and/or multi-organ failure.

Although non-responders are offered second-line therapy, there are no authorized treatments for this clinical situation, and current options are based on combinations of immunosuppressive agents not registered in this indication. Many therapeutic strategies have been studied for treating steroid-resistant acute GvHD, including: anti-thymocyte globulin (results with high dose anti-thymocyte globulin have been unsatisfactory [Van Lint et al., 2006; Bacigalupo, 2005; Remberger et al., 2001; McCaul et al., 2000; MacMillan, 2002]); monoclonal antibodies, such as anti-CD 147 (Van Lint et al., 2006); basiliximab (Massenkeil et al., 2002), daclizumab, alemtuzumab and visulizumab; mycophenolate mofetil; anti-TNF-alpha; pentostatin; dinileukin diftìtox; N-acetyl-cysteine (Colombo et al., 1999); and sirolimus (Benito et al, 2001).

None of these agents have shown a significant better efficacy and all have been correlated with a high incidence of life-threatening infectious complications (fungal, bacterial and viral) and multi-organ failure (Remberger et al., 2001; Wolff et al., 2005; Benito et al, 2001).

Treatments tested in recent years have not shown benefit in terms of improving patient survival (Bacigalupo, 2005; Qian et al. 2013). Despite many molecules having been studied in the last years, the second-line therapy is largely unsatisfactory with 1-year survival at 30% in most large trials (Bacigalupo, 2011). For these reasons, a standard second-line therapy has not been identified yet and steroid-resistant acute GvHD represents an important unmet clinical need. Currently, no drugs are registered for the treatment of steroid-resistant GvHD.

2.1.2 BEGEDINA®

The investigational medicinal product (IMP), BEGEDINA, is a murine immunoglobulin G (IgG) 2b monoclonal antibody against T-cell antigen CD26 that has been isolated by immunizing BALB/c mice with human-activated T lymphocytes. It is produced by a P3X63-Ag8.654 hybridoma and has a molecular weight of approximately 150 KDa (Moretta et al., 1982). BEGEDINA binds the CD26 antigens (dipeptidyl peptidase IV [DPP-IV]) expressed on about 10-60% of CD4+ T lymphocytes (De Meester et al., 1993), down-regulating the CD26 signal. This pathway induces an inhibition and a reduction in the number of helper T cells and, finally, an inhibition of immune response.

CD26 is a multifunctional glycoprotein with DPP-IV enzymatic activity, expressed both as a soluble form and on the cell surface of activated T lymphocytes and of various tissues (e.g., epithelial cells of the liver, kidney and gut). The DPP-IV enzymatic activity belongs to a subgroup of prolyl oligopeptidases and it is able to cleave N-terminal dipeptides from many biologically active polypeptides, hormones, cytokines and chemokines (De Meester et al., 1993). Dipeptidyl peptidase IV plays a major role in glucose metabolism. It is responsible for

the degradation of incretins such as glucagon-like peptide-1 (GLP-1) (Barnett, 2006; Dicker 2011). CD26 is also involved in adenosine deaminase binding in humans and it is able to transmit an activating signal to the T cells and regulate immune responses in vitro (Cordero et al., 2007).

Donor T-cell activation, differentiation and migration represent a crucial phase for the development and the evolution of acute GvHD, and neutralization of CD26 activity clearly results in reduced immune response (Mattern et al., 1991). Donor activated lymphocytes are thus the target of anti-CD26 antibody and their partial depletion may lead to a clinically relevant modulation of steroid-resistant acute GvHD.

Based on this mechanism of action, it is hypothesized that BEGEDINA could be beneficial in the treatment of steroid-resistant acute GvHD.

2.1.2.1 Non-Clinical Pharmacology

The non-clinical development program followed the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline: S6 (R1) – Preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA, 2011). *In vivo* pharmacodynamics (PD) studies are presented below; further non-clinical study data is available in the Investigator's Brochure (IB).

2.1.2.1.1 In vivo PD studies

Investigating the therapeutic effects of BEGEDINA in a xenograft GvHD disease mouse model

The aim of this study was to verify that BEGEDINA was capable of inhibiting the development of GvHD in a xenograft GvHD model based on the transfer of human peripheral blood mononuclear cells (PBMCs) in immunocompromised mice. Additionally, the study screened for immunological biomarkers potentially putatively associated with efficacy and/or mechanism of action of BEGEDINA.

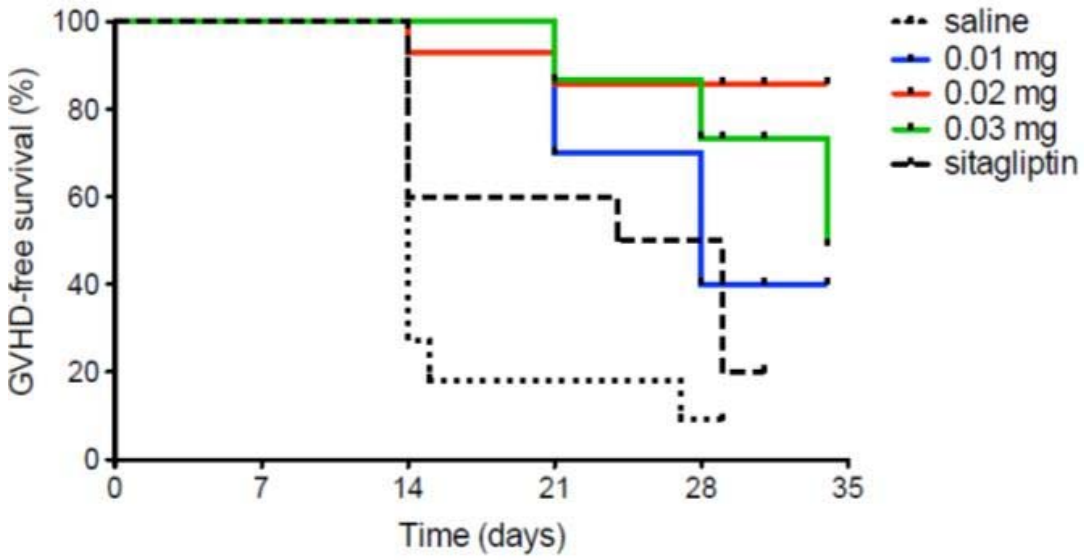
The model used in this study has been already used for testing novel therapeutic approaches for GvHD (Bondanza et al., 2006). This model is based on the transfer of PBMCs from human healthy donors into gamma-irradiated immunodeficient mice. More recently, this model was adapted to the NOD/SCID gamma (NSG) strain, which is characterized by a higher degree of immunodeficiency. GvHD across xenogeneic barriers has the unique advantage that the targets expressed by effector cells, including CD26, are of full human origin, and therefore completely suitable for BEGEDINA. Furthermore, the fact that the

BEGEDINA target, CD26 is highly expressed on T cells of human origins makes this model particularly suitable for testing antibody-based therapeutics.

After PBMCs transfer into irradiated immunocompromised mice, human T cells promptly recognize xenoantigens, proliferate and differentiate into effectors capable of attacking classical GvHD target organs, including skin, gut and liver (ibidem). Importantly, the model is highly reproducible and GvHD incidence and severity are easy to follow, based on circulating human CD3+ T cells and weight loss.

A load-dosing schedule was set up, in which, by analogy with its clinical use (see Section 2.1.2.2.2), BEGEDINA was administered daily (starting from Day 0) for 5 consecutive days and mice were observed for at least 4 weeks. As a control, sitagliptin was administered twice daily (starting from Day 0) for 2 consecutive days. Results showed that even at the lowest dose of BEGEDINA (0.01 mg/day), treated mice were significantly protected from GvHD ($P=0.0026$ compared with saline). When compared with control mice, protection from GvHD was also evident, but only at the intermediate (0.02 mg/day; $P<0.0019$) or at the highest dose (0.03 mg/day; $P<0.0092$). The results indicated that the therapeutic effects of BEGEDINA in xenograft GvHD were dose-dependent (Figure 1).

Figure 1 GvHD-free Survival



Abbreviation: GvHD

By studying the kinetics of CD26 expression of human circulating T cells in NSG mice, it was found that its up-regulation preceded the clinical manifestations of GvHD. Although generalized, this phenomenon was more evident on CD4+ T cells than on other CD3+ T cells subpopulations. The results suggested that, regardless of its mechanism of action, the therapeutic effects of BEGEDINA were preceded, and therefore may be predicted, by a failure of CD4+ T cells to up-regulate CD26 *in vivo*.

The study results supported the conclusion that BEGEDINA is effective at inhibiting the early development of GvHD in a clinically relevant xenograft murine GvHD model.

Since CD26 is a T-cell activation antigen that contains DPP-IV, the well-known chemical inhibitor sitagliptin was used in the study to investigate the involvement of CD26 enzymatic activity in BEGEDINA mechanism of action. Interestingly, when immunocompromised mice were transplanted with human PBMCs and treated with sitagliptin, it was found that the chemical molecule had no effect on the levels of human circulating CD3+ T cells, suggesting that DPP-IV inhibition does not interfere with the immunobiology of xenograft GvHD.

Pharmacokinetic and Pharmacodynamic study in marmosets

In a second study, *in vivo* PD was also evaluated in marmosets which proved to be a relevant model for further toxicology studies since BEGEDINA showed a binding to a subset of T lymphocytes (activated T lymphocytes) similar to that expected in humans.

Pharmacodynamic profile of BEGEDINA was evaluated after single intravenous (IV) administration in marmosets by measuring its binding to CD3+, CD4+ and CD8+ lymphocytes at different dose levels and different post-treatment times. The percentage of BEGEDINA bound to lymphocyte subsets in treated animals was compared to the percentage of BEGEDINA bound after adding the drug to *in vitro* lymphocytes culture control.

The IV route has been selected as it was the intended route of administration of BEGEDINA in the phase III study. The dose levels were selected as 4.4 x and 10 x of the anticipated maximum human dose of 4.5 mg/m^2. Applying the conversion factor to Marmoset equivalent dose based on body surface area (BSA), human doses of 20 mg/m^2 and 45 mg/m^2 correspond to 3.3 and 7.5 mg/kg. Two animal groups were used; each animal group included 2 male and 1 female marmosets.

BEGEDINA binding to activated T lymphocytes by flow cytometry results showed an increase in the % of CD3+ and CD3+CD8+ cells that were bound to BEGEDINA (CD26+ cells) in both groups of animals at all the timepoints. Percentage of CD3+ and CD3+CD8+ cells that were bound to BEGEDINA (CD26+ cells) in samples spiked with 10 µg/mL BEGEDINA were used as reference for the maximal binding of antibodies to CD26 (100% binding). The % of CD3+ and CD3+CD8+ cells versus pre-dose spiked samples showed a high binding of BEGEDINA at all the timepoints in both groups.

In summary, BEGEDINA binding to its target on activated T lymphocytes was confirmed with a binding >70% versus spiked samples in the cell populations analyzed for both treatment groups at both timepoints. Moreover, analysis of samples collected soon after dosing showed a dose response increase of BEGEDINA binding, reaching a high binding at 3.3 mg/kg and a plateau phase at 7.5 mg/kg suggesting a 100% of target-binding at the highest concentration. Lymphocyte subset profile by flow cytometry (CD4, CD8, CD45RA and CD45RO surface markers) did not show any clear alteration following BEGEDINA treatment at any dose tested.

BEGEDINA preliminary toxicity study in marmosets

Additionally, a PD evaluation was included as part of the repeated-dose toxicity studies. Results of the preliminary toxicity study confirmed the specific BEGEDINA binding to its target on T lymphocyte subpopulations since a high percentage of binding was reported in all treated animals at all timepoints tested.

2.1.2.2 Clinical Studies

BEGEDINA has been investigated in 2 completed clinical studies: a pilot study (European Clinical Trials Database [EudraCT] No. 2007-005809-21) and a dose-finding study (EudraCT No. 2012-001353-19).

2.1.2.2.1 Pilot Study (EudraCT No. 2007-005809-21)

Study Design

The objectives of this open-label, uncontrolled pilot study were to investigate the feasibility, efficacy and safety of BEGEDINA in subjects with steroid-resistant acute GvHD; the primary objective was the percentage of responders at Day 10.

Subjects with acute GvHD not responsive to a first-line treatment with steroids lasting 5 days received a 2 mg/day fixed dose of BEGEDINA for 5 consecutive days. This dose was chosen based on the findings of previous studies conducted at the same investigational site (Bacigalupo et al., 1985). Assessments were performed at Day 10 and Day 30 (where Day 1 corresponded to the first day of BEGEDINA administration) and at 1 year.

Blood samples for the analysis of the pharmacokinetic (PK) profile of BEGEDINA were obtained before and immediately (15 minutes) after a 1-hour infusion at Days 1, 2, 3, 4 and 5, and then at 1 week, 2 weeks, and 1, 2 and 3 months after treatment.

A sensitive enzyme-linked immunoassay (ELISA) was developed and validated to measure BEGEDINA serum levels after IV administration. This immunoassay was validated and the results obtained were checked according to the European Medicines Agency (EMA) guidelines. The relevant parameters were: linearity of the standard calibration curve within the concentration range; limit of quantification; assessment of monoclonal antibody stability; validation (precision and accuracy); and application of the analytical method to the clinical samples.

For each subject, the infusions were considered as a 1-treatment course and analyzed as 1 group in the PK study. The PK parameters were determined with a 2-compartment open model, with first-order distribution rates between compartments. The following PK parameters were considered: total body clearance (CL), volume of distribution (Vd), distribution rate constants (k_{12} and k_{21}) and elimination half-life ($t_{1/2}$).

Study Population

Fourteen subjects were enrolled at a single investigational site in Italy between December 2010 and February 2012. Thirteen subjects received treatment with BEGEDINA and are

included in safety (SAF) evaluations. One of the 13 treated subjects was determined to have been enrolled with chronic, not acute GvHD, and was alive and evaluable at 1 year but is excluded from the efficacy Full Analysis Set (FAS). Of the 12 subjects in the FAS, all were evaluable for GvHD through Day +30; 7 subjects were alive and evaluable through Day +180 and 4 subjects were alive and evaluable at Day +365.

Table 1 Subject Disposition (Pilot Study, EudraCT No. 2007-005809-21)

	Subject Disposition
Enrolled, n	14
Treated, n (%)	13 (92.9%)
Completed 1-year follow-up, n (%)	
No	9 (64.3%)
Yes	5 (35.7%)
Reason for discontinuation, n (%)	
Death	8 (57.1%)
New pathology	1 (7.1%)

Abbreviations: n, number of patients.

Source: Clinical study report, EudraCT No. 2007-005809-21, Table 4.

Efficacy Results

In the 12 FAS subjects, a response to BEGEDINA treatment was seen in 7 (58.3%) subjects at Day 10 and in 10 subjects (83.3%) at Day 30.

At Day +180, 7/12 (58.3%) subjects were alive and at Day +365, 4/12 (33.3 %) were alive.

Eight subjects (66.7%) died during the study; none of the deaths were considered related to BEGEDINA treatment by the investigator. The mean (STD) OS in the 8 subjects who died was 169.3 days (median 145.0 days; range 104-284 days (Table 2). The cause of death was transplant-related mortality (TRM) in 4 subjects (2 due to GvHD; 2 due to an infection) and relapse of the original disease in 4 subjects.

Table 2 Overall Survival Full Analysis Set (Pilot Study, EudraCT No. 2007-005809-21)

	Statistic	Full Analysis Set (N=12)
Death		
No	n (%)	4 (33.3%)
Yes	n (%)	8 (66.7%)
Overall survival (days)		
	n	8
	Mean (STD)	169.3 (69.1)
	Median	145.0
	Min, max	104, 284

Abbreviations: N, total number of patients; n,number of patients; STD, standard deviation.

Source: Clinical study report Addendum1, EudraCT No. 2007-005809-21, Table 14.2.4

Pharmacokinetic Results

Pharmacokinetic data were available from 11 subjects, of whom 3 received a second cycle of treatment.

A 2-compartment, open-model seemed to adequately describe the trend of serum concentrations of BEGEDINA during multiple dosing (2 mg once a day for 5 consecutive days) and during follow-up.

BEGEDINA binds to its target expressed on different cells and tissues, including T lymphocytes, melanocytes, epithelia of the renal tubule of colonic mucosa and endothelial cells. It is important to underline that for macromolecular protein drugs such as BEGEDINA, it is possible and perhaps likely that a significant fraction of drug elimination occurs from tissue sites that are not in rapid equilibrium with serum.

The PK data are summarized in Table 3. After administration of a single dose of 2 mg/day of BEGEDINA, the steady-state distribution volume was 91.6±58.4 L and the CL was 1.47±0.90 L/h. The mean $t_{1/2}$ of BEGEDINA was 2.29±1.65 days, which corresponds to the average values for the $t_{1/2}$ of murine antibodies, which are lower than $t_{1/2}$ of human IgG (approximately 21 days).

There was a direct correlation between administered dose and serum concentration of BEGEDINA. The results of serum concentration of all subjects and their accumulation ratio showed that the concentrations of BEGEDINA after the fifth dose were about 2 times the values at the corresponding times after a single (first) dose:

BEGEDINA peak serum concentration, quantified 15 minutes after the last administration (peak serum concentration [C_{max}], fifth dose), corresponded to a median (range) value of 33.0 ng/mL (range 15.7-158.7 ng/mL). If these concentrations are compared to the corresponding

values after the first dose (C_{max}, first dose = 18.00 ng/mL [range 6.2-77.0 ng/mL]) the ratio C_{max}, fifth dose/C_{max}, first dose corresponds to 1.94 (range: 0.33-2.9), and is an index of the extent of accumulation (R_{acc}).

Table 3 Summary of BEGEDINA Pharmacokinetic Parameters (Pilot Study, EudraCT No. 2007-005809-21)

	CL (L/day)	CL (L/h)	Vd (L)	k_{12} (Day 1)	k_{21} (Day 1)	$t_{½}$ (days)	BEGEDINA Serum Levels (ng/mL)		R_{acc}
							Post-dose 1	Post-dose 5	
Mean	35.25	1.47	91.6	0.595	0.052	2.29	32.38	45.12	1.71
Median	30.03	1.25	69.2	0.642	0.044	1.65	18.00	33.00	1.94
Min, max	5.53, 79.80	0.23, 3.33	28.6, 219.5	0.334, 0.750	0.028, 0.102	0.52, 6.02	6.2, 77.00	15.7, 158.7	0.33, 2.90
STD	21.66	0.90	58.4	0.119	0.022	1.65	25.78	39.55	0.79
CV%	61.4	61.4	63.7	19.9	42.1	72.1	79.62	87.67	46.30

Abbreviations: CL, total body clearance; CV%, coefficient of variation in percentage; k_{12} and k_{21}, distribution rate constants; R_{acc}, accumulation ratio; STD, standard deviation; $t_{1/2}$, elimination half-life; Vd, volume of distribution.

Safety Results

Exposure

Thirteen patients received 5 planned consecutive daily doses of BEGEDINA 2 mg/kg.

Three subjects received a second cycle of five days treatment and one patient received three additional days of treatment.

Summary of adverse events

An overview of the adverse events (AEs) reported during the study is presented in Table 4.

All 13 subjects (100.0%) reported at least one AE. Death occurred by Day +365 in 8 subjects (61.5%) and SAEs were reported in 9 subjects (69.2%). All 13 subjects (100.0%) had at least one Grade ≥3 AE. Treatment-related AEs were reported in 5 subjects (38.5%). No subject discontinued the study due to AEs.

Table 4 Summary of Adverse Events (Pilot Study, EudraCT No. 2007-005809-21)

	Safety Population (N=13)
Number of deaths, *N (%), E*	8 (61.5%), 12
AEs, *N (%), E*	13 (100.0%), 488
SAEs, *N (%), E*	9 (69.2%), 27
Grade ≥ 3 AEs, *N (%), E*	13 (100.0%), 70
Treatment-related AEs, *N (%), E*	5 (38.5%), 8
AEs leading to treatment discontinuation, *N (%), E*	0 (0.0%), 0

Abbreviations: AE, adverse events; E, number of events; N, number of subjects; SAE, serious adverse event.

Source: Clinical study report, EudraCT No. 2007-005809-21, Section 14, Table 14.3.1.1

The safety profile of BEGEDINA was good and no allergic AEs were reported for any subject.

The most commonly presented AEs by preferred term (in at least 25% of subjects overall) are presented in Table 5.

Table 5 Most Commonly Reported Adverse Events (in at least 25% Subjects), All Causality (Safety Population)

System Organ Class Preferred Term	Safety Population (N=13) *N (%), E*
Blood and Lymphatic System Disorders	**10 (76.9%) 24**
Leukopenia	4 (30.8%) 8
Pancytopenia	4 (30.8%) 5
Gastrointestinal Disorders	**12 (92.3%) 73**
Abdominal pain	6 (46.2%) 13
Diarrhoea	11 (84.6%) 24
Nausea	4 (30.8%) 11
Vomiting	5 (38.5%) 9
General Disorders and Administration Site Conditions	**11 (84.6%) 20**
Asthenia	4 (30.8%) 5
Pyrexia	6 (46.2%) 9
Immune System Disease	**10 (76.9%) 80**
GvHD in intestine	8 (61.5%) 25
GvHD in liver	7 (53.8%) 22
GvHD in skin	7 (53.8%) 31
Infections and Infestations	**12 (92.3%) 55**
Cytomegalovirus infection	8 (61.5%) 24
Investigations	**13 (100.0%) 57**
Blood bilirubin increased	4 (30.8%) 8
Gamma-glutamyl transferase increased	5 (38.5%) 12
Metabolism and Nutrition Disorders	**8 (61.5%) 14**
Decreased appetite	4 (30.8%) 5
Musculoskeletal and Connective Tissue Disorders	**8 (61.5%) 23**
Sjorgen's syndrome	4 (30.8%)
Skin and Subcutaneous Tissue Disorders	**11 (84.6%) 46**
Rash	7 (53.8%) 16
Skin exfoliation	4 (30.8%) 6
Skin hyperpigmentation	6 (46.2%) 9

Abbreviations: E, number of events; N, number of subjects.

Source: Clinical study report, EudraCT No. 2007-005809-21, Table 14.2.4

Treatment-related adverse events

The incidence of treatment-related AEs was low. Eight AEs out of 488 (1.6%) in 5 of 13 subjects (38.5%) were considered related to BEGEDINA by the investigator (Table 6). Of these 8 treatment-related AEs, 4 (muscle spasms, malaise, abdominal pain and hepatic steatosis) were reported in 1 subject; all other treatment-related AEs occurred in 1 subject each (dyslipidemia anemia, abnormal neurological examination, exfoliative dermatitis).

Table 6 Treatment-Related Adverse Events (Safety Population)

System Organ Class Preferred Term	Safety Population (N=13) *N (%), E*
Blood and Lymphatic System Disorders	**1 (7.7%) 1**
Anaemia	1 (7.7%) 1
Gastrointestinal Disorders	**1 (7.7%) 1**
Abdominal pain	1 (7.7%) 1
General Disorders and Administration Site Conditions	**1 (7.7%) 1**
Malaise	1 (7.7%) 1
Hepatobiliary Disorders	**1 (7.7%) 1**
Hepatic stenosis	1 (7.7%) 1
Investigations	**1 (7.7%) 1**
Neurological examination abnormal	1 (7.7%) 1
Metabolism and Nutrition Disorders	**1 (7.7%) 1**
Dyslipidaemia	1 (7.7%) 1
Musculoskeletal and Nutrition Disorders	**1 (7.7%) 1**
Muscle spasms	1 (7.7%) 1
Skin and Subcutaneous Tissue Disorders	**1 (7.7%) 1**
Dermatitis exfoliative	1 (7.7%) 1

Abbreviations: E, number of events; N, number of subjects.

Source: Clinical study report, EudraCT No. 2007-005809-21, Table 14.3.1.4

Adverse events by severity

All 13 patients (100.0%) had at least one Grade ≥3 AEs. The Grade ≥3 AEs by preferred term reported in ≥10% of subjects are presented in Table 7.

Table 7 Incidence of Grade ≥3 Adverse Events Occurring in ≥10% of Subjects (Safety Population)

System Organ Class Preferred Term	Safety Population (N=13) *N (%), E*
Immune System Disorders	**4 (30.8%) 12**
GvHD in intestine	2 (15.4%) 3
GvHD in liver	3 (23.1%) 7
Infections and Infestations	**7 (53.8%) 10**
Bronchopneumonia	3 (23.1%) 3
Cytomegalovirus infection	2 (15.4%) 2
Neoplasms benign, Malignant and Unspecified (Incl. Cysts and Polyps)	**3 (23.1%)**
Leukaemia recurrent	3 (23.1%) 3
Respiratory, Thoracic and Mediastinal Disorders	**3 (23.1%) 9**
Dyspnea	3 (23.1%)
Lung disorder	2 (15.4%) 2
Skin and Subcutaneous Tissue Disorders	**1 (7.7%) 1**
Rash	2 (15.4%) 2

Abbreviations: E, number of events; N, number of subjects.

Source: Clinical study report, EudraCT No. 2007-005809-21, Table 14.3.1.5.

Deaths

Death occurred in 5 subjects (38.5%) by Day 180, and in 8 subjects (61.5%) by Day +365 (Table 4). The causes of death (Day +365) were:

- Relapsed leukemia
- Leukemia
- Acute respiratory failure complicating pneumonia
- GvHD (primary cause) and gastric-enterorrhage (secondary cause)
- Disease progression and pneumonia with respiratory failure
- Acute GvHD
- Bronchopneumonia and acute respiratory failure
- Leukemia relapse (primary cause) and infection (secondary cause)

Serious adverse events

A total of 27 serious adverse events (SAEs) were reported in 9 subjects (69.2%). The most commonly reported SAEs by preferred term were bronchopneumonia (3 subjects, 23.1%) and dyspnea (2 subjects, 15.4%). None of the other SAEs by preferred term were reported in more than one subject each. None of the SAEs was considered as treatment-related.

Clinical laboratory findings

Individual Clinically Significant Abnormalities

Clinical laboratory parameter values that were considered as clinically significant and reported as AEs are summarized in Table 8. Only one case of anemia among the clinically significant abnormalities of laboratory parameters was considered as treatment-related.

Table 8 Incidence of Clinically Significant Laboratory Abnormalities Reported as Adverse Events (Safety Population)

System Organ Class Preferred Term	Safety Population (N=13) *N (%)*
Blood and Lymphatic System Disorders	
Anaemia	2 (15.4%) 2
Leukocytosis	1 (7.7%) 1
Leukopenia	4 (30.8%) 8
Pancytopenia	4 (30.8%) 5
Thrombocytopenia	3 (23.1%) 4
Investigations	
Alanine aminotransferase increased	1 (7.7%) 1
Blood alkaline phosphatase increased	1 (7.7%) 1
Blood bilirubin increased	4 (30.8%)
Blood creatinine increased	3 (23.1%) 6
Blood glucose increased	1 (7.7%) 1
Gamma-glutamyltransferase increased	5 (38.5%) 12
Haemoglobin decreased	2 (15.4%) 2
Hepatic enzyme increased	2 (15.4%) 2
Platelet count decreased	2 (15.4%) 2
Transaminases increased	3 (23.1%) 11
Metabolism and Nutrition Disorders	
Hyperglycaemia	1 (7.7%) 1
Hyperkalaemia	2 (15.4%) 2
Hypermagnesaemia	1 (7.7%) 1

Abbreviations: E, number of events; N, number of subjects.

Source: Clinical study report, EudraCT No. 2007-005809-21, Table 14.3.1.2.

Vital signs

There were no treatment-related abnormalities of vital signs parameters in any subject.

Human anti-mouse antibodies (HAMA)

Subjects were assessed for the development of HAMA. Most of the concentration levels of HAMA were not quantifiable based on the ELISA method, i.e. they were below the limit of

quantitation (3 ng/mL). Five subjects (38.5%) developed HAMA. There was no evidence of any correlation between the concentrations of BEGEDINA and HAMA formation and therefore an influence of antibody formation on the clearance of BEGEDINA can be excluded.

2.1.2.2.2 Dose-finding Study (EudraCT No. 2012-001353-19)

Some subjects analyzed in the pilot study showed only a fraction of circulating CD3+CD26+ cells bound to BEGEDINA, suggesting that there was not enough monoclonal antibody to interact with most of the circulating CD3+CD26+ cells (Section 2.1.2.2.1). The low binding between BEGEDINA and the circulating CD3+CD26+ cells and the good safety profile of the treatment led to the proposal for a dose-finding study in 9 subjects to identify the minimum effective dose of BEGEDINA in the treatment of steroid-resistant acute GvHD treatment.

Study Design

The primary objective of the dose-finding, single-center, open-label, phase II, uncontrolled study was to determine the minimum effective dose of BEGEDINA in subjects with steroid-resistant acute GvHD through a PD/PK assessment of counting BEGEDINA-positive cells staining with anti-mouse IgG within Day 5 of BEGEDINA treatment. The study planned to evaluate the following 3 dosages of BEGEDINA, in different cohorts of subjects, enrolling 3 subjects per dose level:

- Cohort DL1: First dose level: 2 mg/m^2/day for 5 consecutive days.
- Cohort DL2: Second dose level: 3 mg/m^2/day for 5 consecutive days.
- Cohort DL3: Third dose level: 4.5 mg/m^2/day for 5 consecutive days.

Because it is known that subjects have GvHD flares following treatment of acute GvHD (e.g., 41% subjects treated with basiliximab; Funke et al., 2006), and following advice from the United States (US) Food and Drug Administration (FDA) and the EMA, it was decided to add 7 more subjects to the study, each to be treated at a dose level of 3 mg/m^2/day of BEGEDINA for 5 consecutive days, plus 6 additional 3 mg/m^2/day doses at Days 10, 14, 17, 21, 24 and 28 (Cohort DL2bis) in order, possibly, to further improve the clinical efficacy in the most critical phases of the disease. For these 7 subjects, the analysis presented in the clinical study report (CSR) was limited to the first 28 days.

As a secondary endpoint of the study, PD data were studied together with the PK data in order to correlate the serum concentration of BEGEDINA before and after drug administration.

Study Population

Overall, 16 subjects were enrolled in the study, 3 in the DL1 cohort, 3 in the DL2 cohort, 7 in the DL2bis cohort, and 3 in the DL3 cohort (Table 9). All subjects completed treatment and entered the 180-day follow-up phase, except 1 subject in the DL2bis cohort (who died during the treatment phase). The follow-up was completed by 2 subjects in the DL2 cohort, 5 of the subjects in the DL2bis cohort, and by all 3 subjects in DL3 cohort. None of subjects in the DL1 cohort completed the follow-up phase.

Table 9 Subject Disposition (Dose-Finding Study, EudraCT No. 2012-001353-19)

	Treatment Group			
	DL1 (N=3)	**DL2 (N=3)**	**DL2bis (N=7)**	**DL3 (N=3)**
Enrolled & Treated	3	3	7	3
Completed follow-up, N (%)				
No	3 (100%)	1 (33.3%)	2 (100%)	0
Yes	0	2 (66.7%)	5	3 (100)
Reason for Discontinuation, n (%)				
Death	3 (100)	1 (100)	2 (100)	0

Source: Clinical study report Addendum1, Dose-finding study, EudraCT No. 2012-001353-19, Table 14.1.1.

Efficacy Results

The results of TRM, OS and OR rate, as well as of the other efficacy endpoints demonstrate a similar efficacy profile for BEGEDINA at the different dose levels.

Primary endpoint

None of the subjects treated with BEGEDINA in any dose cohort showed a maximum %BEGEDINA-positive cells on Day 5 of treatment of ≥80%, suggesting the possibility that no saturation effect was reached and therefore the dose escalation to the next dose level was performed for each 3 subjects.

Secondary endpoints

Transplant-related Mortality

In the 6-month follow-up period, TRM occurred in 4 subjects: 1 subject (33.3%) in the DL1 cohort (death due to acute GvHD); 1 (33.3%) subject in the DL2 cohort (death due to acute

respiratory failure following pulmonary edema); 2 (28.6%) subjects in the DL2bis cohort (death due to multiple organ dysfunction caused by sepsis and death due to acute GvHD); and no subjects in the DL3 cohort.

Of these, TRM occurred within Day 28 in 2 subjects: 1 subject (33.3%) in the DL2 cohort (acute respiratory failure following pulmonary edema, OS 24 days); and 1 subject (14.3%) in the DL2bis cohort (acute GvHD, OS 17 days).

Frequency of Overall Response

In the overall population, at Day 28, a response (complete response [CR] or partial response [PR]) was observed in 11 out of 16 subjects (68.8%). By cohort, a response at Day 28 was observed in 2 out of 3 subjects (66.7%) in each of the DL1, DL2 and DL3 cohorts, and in 5 out of 7 subjects (71.4%) in the DL2bis cohort (Table 10).

Table 10 Frequency of Responders at Day +28 (Dose-Finding Study, EudraCT No. 2012-001353-19)

	Treatment Group				Total
	DL1 (N=3)	DL2 (N=3)	DL2bis (N=7)	DL3 (N=3)	(N=16)
Responders at +Day 28, N (%)					
No [a]	1 (33.3%)	1 (33.3%)	2 (28.6%)	1 (33.3%)	5 (31.2%)
Yes	2 (66.7%)	2 (66.7%)	5 (71.4%)	2 (66.7%)	11 (68.8%)

[a] Subjects who died before or at Day +28 were considered as not responders.

Source: Clinical study report Addendum1, Dose-finding study, EudraCT No. 2012-001353-19, Table 14.2.1.1.

Overall Survival at Day 180

Overall survival in the FAS is presented in Table 11. At Day +180, death had occurred in 6 out of 16 subjects (37.5%). By cohort, the deaths had occurred in all 3 subjects (100%) in the DL1 cohort, in 1 subject (33.3%) in the DL2 cohort, in 2 subjects (28.6%) in the DL2bis cohort, and in none of the subjects in the DL3 cohort.

The one-year overall survival was 56.2% (9/16 subjects).

Table 11 Overall Survival Day 180: Full Analysis Set (Dose-Finding Study, EudraCT No. 2012-001353-19)

	Treatment Group				Total
	DL1 (N=3)	DL2 (N=3)	DL2bis (N=7)	DL3 (N=3)	(N=16)
Death, n (%) [a]					
No	0 (0.0%)	2 (66.7%)	5 (71.4%)	3 (100.0%)	10 (62.5%)
Yes	3 (100.0%)	1 (33.3%)	2 (28.6%)	0 (0.0%)	6 (37.5%)
Overall survival (days) [b]					
n	3	1	2 [c]	0	6
Mean (standard deviation)	54.0 (31.5%)	24.0	66.5 (70.0%)	0	NC
Median	45.0	24.0	66.5	-	NC
Min, Max	28, 89	24, 24	17, 116	-	17, 116

[a] Percentages are based on the number of subjects with data available in the FAS within each group

[b] Overall survival was calculated on death

Source: Clinical study report Addendum1, Dose-finding study, EudraCT No. 2012-001353-19, Table 14.2.5.1.

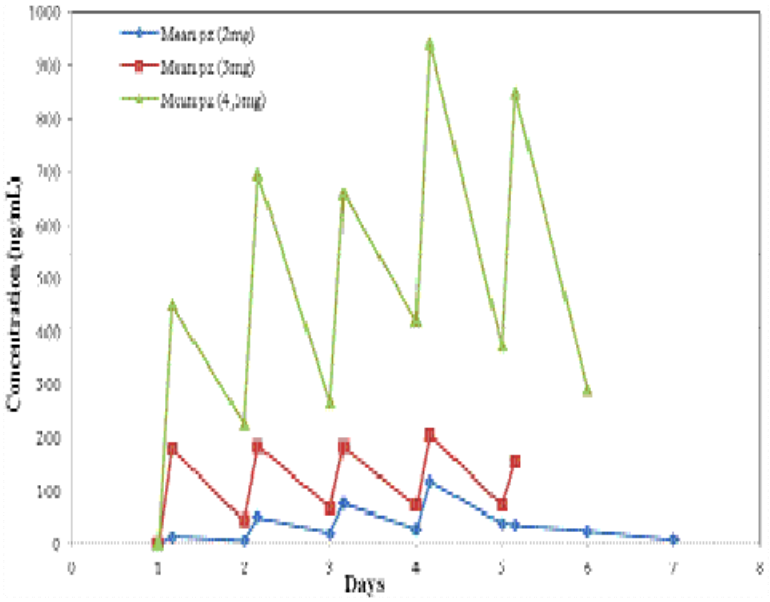
Pharmacokinetic Results

DL1, DL2 and DL3 cohorts

Figure 2 shows the mean profile of BEGEDINA concentration in the 3 subjects in the DL1 cohort (dose 2 mg/m^2), of the 3 subjects in the DL2 cohort (dose 3 mg/m^2) and in the 3 subjects in the DL3 cohort (dose of 4.5 mg/m^2), within 5 days of administration.

The PK results showed linearity in dose-concentration: highest mean BEGEDINA concentration was observed in subjects who received 4.5 mg/m^2 of drug (DL3 cohort), which was greater than the mean concentration in subjects who received 3 mg/m^2 of drug (DL2 cohort), which in turn was greater than mean concentration in those treated with the 2 mg/m^2 dose (DL1 cohort).

Figure 2 BEGEDINA Concentration Profile at Dose Levels 2 mg, 3 mg, and 4.5 mg/kg/day for 5 Days (Dose-Finding Study, EudraCT No. 2012-001353-19)



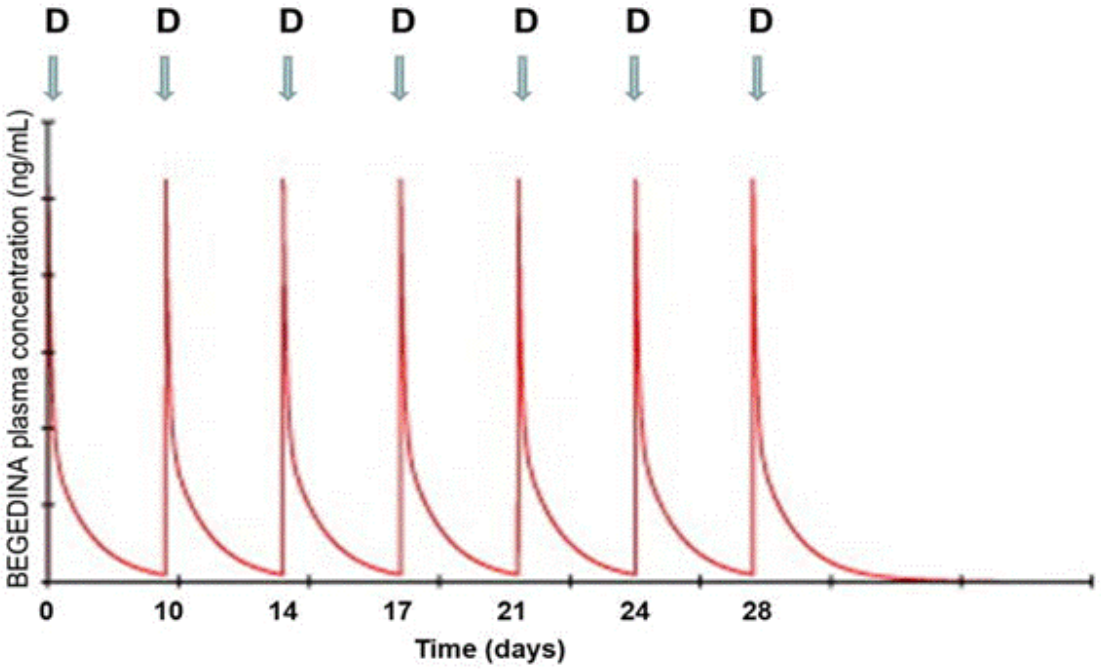
The relationships between the values of the concentrations pre- and post-administration in subjects in the DL1, DL2 and DL3 cohorts showed that the average ratio of $D_{4.5mg}/D_{2mg}$ was about 4 times higher than the average ratio of D_{3mg}/D_{2mg}. The mean accumulation ratios were 1.88, 0.87 and 3.7, respectively in the DL1, DL2 and DL3 cohorts.

DL2bis cohort

PK assessments were performed in 7 subjects in the DL2bis cohort (treated with 3 mg/m^2/day BEGEDINA for 5 consecutive days, plus 6 additional 3 mg/m^2/day doses at Days 10, 14, 17, 21, 24 and 28).

Figure 3 shows the mean profile of BEGEDINA concentration in the 7 subjects in the DL2bis cohort (multiple dosing). The results were indicative of a PK profile comparable to that predicted on the basis of data obtained after a single dose.

Figure 3 BEGEDINA Concentration Profiles in Subjects in the DL2bis Cohort (Dose-Finding Study, EudraCT No. 2012-001353-19)



As the mean half-life of BEGEDINA has been estimated equal to 1.8 days, a dosing interval ranging from 3 to 5 days should ensure BEGEDINA plasma levels just before the next dose of around 10-30% of C_{max} levels.

The clearance of BEGEDINA after a dose of 3 mg/m^2 in cohort DL2bis was about 25% of the corresponding value observed after the administration of the 2 mg/day dose. These data are in accordance with what was observed during the part of this dose-finding study which analyzed the relationship between serum concentration and dose of BEGEDINA in a dosage range of between 2 mg/m^2 and 4.5 mg/m^2 (see Figure 2).

At a dose of 3 mg/m^2, the apparent volume of distribution was about 27% compared to the corresponding value observed after the administration of 2 mg/day. As a consequence of variations in the volume of distribution and clearance, the elimination half-life appeared unchanged.

BEGEDINA demonstrated dose-dependent elimination, which was consistent with target-mediated elimination, where clearance decreased as a function of dose.

Safety Results

The incidence of AEs, deaths, SAEs, treatment-related AEs and Grade ≥3 AEs, as well as of clinically significant laboratory parameters and vital signs abnormalities, did not substantially differ between treatment dose cohorts, and therefore BEGEDINA could be escalated to the highest dose level and regimen with no increased risk of toxicities.

Adverse events

All subjects in all dose cohorts reported at least one AE. None of the subjects discontinued the study due to AEs.

The incidence of subjects with AEs, fatal AEs, SAEs, treatment-related AEs and Grade ≥3 AEs did not substantially differ between treatment dose cohorts, and therefore the incidence of AEs, fatal AEs, SAEs, treatment-related AEs and Grade ≥3 AEs was not dose-related.

Table 12 Summary of Adverse Events, by Cohort (Safety Population; Dose-Finding Study, EudraCT No. 2012-001353-19)

	Treatment Group			
	DL1 (N=3)	**DL2 (N=3)**	**DL2bis (N=7)**	**DL3 (N=3)**
Number of deaths, *N (%)*	3 (100.0%)	1 (33.3%)	2 (28.6%)	0
AEs, *N (%)*	3 (100.0%), 70	3 (100.0%), 55	7 (100.0%), 146	3 (100.0%), 108
Deaths, *N (%)*	3 (100.0%)	1 (33.3%)	2 (28.6%)	0
SAEs, *N (%)*	1 (33.3%), 2	2 (66.7%), 3	2 (28.6%), 3	2 (66.7%), 4
Grade ≥3 AEs, *N (%)*	3 (100.0%), 22	3 (100.0%), 9	7 (100.0%), 22	3 (100.0%), 33
Treatment-related AEs, *N (%)*	0	0	1 (14.3%), 6	0
AEs leading to treatment discontinuation, *N (%)*	0	0	0	0

Abbreviations: AE, adverse events; E, number of events; N, number of subjects; SAE, serious adverse event.

Source: Clinical study report Addendum1, Dose-finding study, EudraCT No. 2012 001353-19, Table 14.3.1.1

All adverse events

The most commonly involved SOCs (i.e. in at least 50% of subjects overall) are displayed in Table 13 and the most commonly reported AEs by preferred term (i.e. in at least 25% of subjects overall) are displayed in Table 14.

Table 13 Most Commonly Reported Adverse Events by System Organ Class and Cohort (Safety Population; Dose-Finding Study, EudraCT No. 2012-001353-19)

System Organ Class	Treatment Group			
	DL1 (N=3)	DL2 (N=3)	DL2bis (N=7)	DL3 (N=3)
	N (%) E	N (%) E	N (%) E	N (%) E
Blood and lymphatic system disorders	0	2 (66.7%) 2	5 (71.4%) 28	1 (33.3%) 1
Gastrointestinal disorders	2 (66.7%) 10	2 (66.7%) 4	6 (85.7%) 20	3 (100%) 13
General disorders and administration site conditions	3 (100%) 8	2 (66.7%) 5	6 (85.7%) 15	2 (66.7%) 8
Infections and infestations	3 (100%) 11	2 (66.7%) 19	7 (100.0%) 17	3 (100%) 25
Investigations	3 (100%) 6	1 (33.3%) 2	4 (57.1%) 15	2 (66.7%) 14
Musculoskeletal of connective tissue disorders	2 (66.7%) 2	1 (33.3%) 1	3 (42.9%) 7	2 (66.7%) 12
Nervous system disorders	2 (66.7%) 4	1 (33.3%) 1	4 (57.1%) 4	1 (33.3%) 1
Skin and subcutaneous tissue disorders	3 (100%) 16	1 (33.3%) 1	5 (71.4%) 14	2 (66.7%) 18

Abbreviations: AE, adverse events; E, number of events; N, number of subjects; SAE, serious adverse event.

Source: Clinical study report Addendum1, Dose-finding study, EudraCT No. 2012 001353-19, Table 14.3.1.2.

Table 14 Most Commonly Reported Adverse Events by Preferred Term and Cohort (Safety Population; Dose-Finding Study, EudraCT No. 2012-001353-19)

System Organ Class Preferred Term	Treatment Group DL1 (N=3) N (%) E	 DL2 (N=3) N (%) E	 DL2bis (N=7) N (%) E	 DL3 (N=3) N (%) E
Blood and lymphatic system disorders	**0 (0.0%)**	**2 (66.7%) 2**	**5 (71.4%) 28**	**1 (33.3%) 1**
Anaemia	0 (0.0%)	0 (0.0%)	4 (57.1%) 9	0 (0.0%)
Gastrointestinal Disorders	**2 (66.7%) 10**	**2 (66.7%) 4**	**6 (85.7%) 20**	**3 (100.0%) 13**
Diarrhoea	2 (66.7%) 5	2 (66.7%) 2	5 (71.4%) 9	2 (66.7%) 7
Nausea	1 (33.3%) 2	1 (33.3%) 1	2 (28.6%) 2	1 (33.3%) 1
General Disorders and Administration Site Conditions	**3 (100%) 8**	**2 (66.7%) 5**	**6 (85.7%) 15**	**2 (66.7%) 8**
Oedema peripheral	0 (0.0%)	1 (33.3%) 1	3 (42.9%) 4	0 (0.0%)
Pyrexia	1 (33.3%) 2	2 (66.7%) 2	2 (28.6%) 3	1 (33.3%) 2
Infections and infestations	**3 (100%) 11**	**2 (66.7%) 19**	**7 (100.0%) 17**	**3 (100%) 25**
Bacterial infection	2 (66.7%)	1 (33.3%)	0 (0.0%)	1 (33.3%)
Cystitis	0 (0.0%)	0 (0.0%)	2 (28.6%) 2	2 (66.7%) 2
Cytomegalovirus infection	1 (33.3%) 1	2 (66.7%) 7	3 (42.9%) 6	1 (33.3%) 4
Investigations	**3 (100%) 6**	**1 (33.3%) 2**	**4 (57.1%) 15**	**2 (66.7%) 14**
Hepatic enzyme increased	0 (0.0%)	1 (33.3%) 2	1 (14.3%) 1	2 (66.7%) 5
Musculoskeletal of connective tissue disorders	**2 (66.7%) 2**	**1 (33.3%) 1**	**3 (42.9%) 7**	**2 (66.7%) 12**
Arthralgia	1 (33.3%) 1	0 (0.0%)	3 (42.9%) 4	1 (33.3%) 1
Renal and Urinary Disorders	**1 (33.3%) 3**	**2 (66.7%) 2**	**2 (28.6%) 5**	**2 (66.7%) 4**
Cystitis hemorrhagic	1 (33.3%) 1	0 (0.0%)	2 (28.6%) 4	1 (33.3%) 1

Skin and subcutaneous tissue disorders	**3 (100%) 16**	**1 (33.3%) 1**	**5 (71.4%) 14**	**2 (66.7%) 18**
Rash	2 (66.7%) 5	1 (33.3%) 1	4 (57.1%) 7	2 (66.7%) 9
Skin exfoliation	1 (33.3%) 2	0 (0.0%)	2 (28.6%) 2	2 (66.7%) 2
Skin hyperpigmentation	2 (66.7%) 3	0 (0.0%)	2 (28.6%) 2	2 (66.7%) 6
Vascular disorders	**1 (33.3%) 1**	**2 (66.7%) 3**	**2 (28.6%) 2**	**0 (0.0%)**
hypertension	1 (33.3%) 1	2 (66.7%) 2	2 (28.6%) 2	0 (0.0%)

Abbreviations: AE, adverse events; E, number of events; N, number of subjects; SAE, serious adverse event.

Source: Clinical study report Addendum1, Dose-finding study, EudraCT No. 2012 001353-19, Table 14.3.1.2.

Treatment-related adverse events

Six treatment-related AEs were reported in 1 subject (14.3%) in the DL2bis cohort (leukopenia, neutropenia, edema peripheral, heart rate increased, total protein decreased and weight increased).

Severity of adverse events

All subjects, except one in the DL 2bis cohort, had at least one Grade ≥3 AEs.

The Grade ≥3 AEs by preferred term reported in more than one subject in any group were:

- Pain: 2 subjects (66.7%) in the DL1 cohort
- Thrombocytopenia: 3 subjects (42.9%) in the DL2bis cohort
- Fungal infection: 2 subjects (66.7%) in the DL3 cohort

All the other Grade ≥ 3 AEs were reported in no more than one patient in any group.

Deaths

Six subjects (37.5%) died: all 3 subjects (100%) in the DL1 cohort, 1 subject (33.3%) in the DL2 cohort, and 2 subjects (28.6%) in the DL2bis cohort. No deaths occurred in the subjects treated at the highest dose in the DL3 cohort (Table 12). The causes of death are summarized in Table 15.

Table 15 Summary of Deaths (Safety Population; Dose-Finding Study, EudraCT No. 2012-001353-19)

Subject No.	Treatment Arm	Date [a]		Cause of Death	Transplant-Related Death	Other SAEs
		Transplant	Death			
101	DL1	18-Apr-12	18-Jul-12	Acute GvHD	Yes	Bacterial sepsis Convulsion
102	DL1	20-Sep-11	04-Jul-12	Leukaemia relapse	No	None reported
103	DL1	01-Jun-12	11-Sep-12	Leukaemia	No	None reported
104	DL2	30-May-12	14-Jul-12	Acute respiratory failure following pulmonary edema in patient with GvHD	Yes	None reported
111	DL2bis	15-Nov-13	10-Apr-14	Multiple organ dysfunction due to sepsis (multi-drug resistant *Strenotrophomonas*)	Yes	None reported
113	DL2bis	14-Jan-14	06-Mar-14	Acute GvHD	Yes	None reported

Abbreviation: GvHD, graft-versus host disease; SAE, serious adverse event.

[a] Date: Day-Month-Year format

Source: Clinical study report, Dose-finding study, EudraCT No. 2012 001353-19, Listing 16.3.2

Serious adverse events

Six subjects experienced SAEs; they are summarized in Table 16.

Table 16 Summary of Serious Adverse Events (Safety Population; Dose-Finding Study, EudraCT No. 2012-001353-19)

Subject No.	Treatment Arm	Date [a]		Preferred Term	Treatment-related / Severity	Outcome
		Start	stop			
101	DL1	06-Jul-12		Bacterial sepsis	Not related / Severe	Unresolved
		15-Jul-12		Convulsion	Not related / Severe	Unresolved
104	DL2	05-Jul-12		Acute respiratory failure	Not related / Life-threatening	Fatal
		05-Jul-12		Pulmonary oedema	Not related / Life-threatening	Fatal
105	DL2	13-Nov-12	28-Nov-12	Diverticulitis	Not related / Severe	Resolved
108	DL3	08-Nov-12	09-Dec-2013	GvHD	Not related / Severe	Resolved
109	DL3	08-May-13	18-Mar-13	Renal failure	Not related / Severe	Resolved
		06-Jun-13	12-Jun-13	Escherichia infection	Not related / Severe	Resolved
		12-Jun-13	20-Jun-13	Renal failure	Not related / Severe	Resolved
111	DL2bis	10-Apr-14	10-Apr-14	Sepsis	Not related / Life-threatening	Fatal
		10-Apr-14	10-Apr-14	Multi-organ failure	Not related / Life-threatening	Fatal
112	DL2bis	06-Mar-14	21-Mar-14	Influenza A virus test positive	Not related / Severe	Resolved

Abbreviation: GvHD, graft-versus host disease; SAE, serious adverse event.

[a] Date: Day-Month-Year format

Source: Clinical study report, Dose-finding study Addendum1, EudraCT No. 2012 001353-19, Table 16.3.1.3.

Clinical laboratory findings

The number of subjects was too small for a reliable assessment of mean changes from baseline of hematology and biochemistry parameters. Clinically significant abnormalities of laboratory parameters reported as AEs are summarized in Table 17 by cohort. Two of these AEs, neutropenia and total protein decreased, in a subject in the DL2bis cohort, were considered related to the study treatment by the investigator.

Table 17 Incidence of Clinically Significant Laboratory Abnormalities Reported as Adverse Events (Safety Population; Dose-Finding Study, EudraCT No. 2012-001353-19)

System Organ Class Preferred Term	Treatment Group DL1 (N=3) N (%) E	 DL2 (N=3) N (%) E	 DL2bis (N=7) N (%) E	 DL3 (N=3) N (%) E
Blood and Lymphatic System Disorders	**o**	**2 (66.7%) 2**	**5 (71.4%) 17**	**1 (33.3%) 1**
Anaemia	0	0	4 (57.1%) 9	0
Neutropenia	0	0	1 (14.3%) 3	0
Pancytopenia	0	1 (33.3%) 1	0	0
Thrombocytopenia	0	0	3 (42.9%) 5	0
Investigations	**3 (100%) 4**	**1 (33.3%) 2**	**4 (57.1%) 8**	**2 (66.7%) 13**
Blood albumin decreased	0	0	0	1 (33.3%) 1
Blood bilirubin decreased	1 (33.3%) 1	0	0	1 (33.3%) 1
Blood creatinine increased	0	0	1 (14.3%) 1	0
Protein total decreased	0	0	1 (14.3%) 1	0
Metabolism and Nutrition Disorders	1 (33.3%) 1	1 (33.3%) 1	1 (14.3%) 1	2 (66.7%) 3
Hyperglycaemia	0	0	1 (14.3%) 1	0

Abbreviations: E, number of events; N, number of subjects.

Source: Clinical study report, Dose-finding study, EudraCT No. 2012 001353-19, Table 14.3.1.2.

Vital signs

The number of subjects was too small for a reliable assessment of mean changes from baseline of vital signs parameters.

HAMA assessments

Most of the concentration levels of HAMA were not quantifiable based on the ELISA method, i.e. were below the limit of quantitation (3 ng/mL). Two subjects in the DL1 cohort, 3 in the DL2bis cohort and 3 in the DL3 cohort developed HAMA; no subject in the DL2 cohort developed HAMA. There was no evidence of correlation between serum concentrations of BEGEDINA and the possibility of developing HAMA.

There was no evidence of any correlation between the administered dose and concentrations of BEGEDINA with HAMA formation, and therefore, an influence of the antibody formation on the clearance on BEGEDINA can be excluded.

Pharmacodynamic assessments

The level of circulating lymphocytes CD3+/ CD26+ bound to BEGEDINA is the PD surrogate endpoint recommended by the Committee for Medicinal Products for Human Use (CHMP).

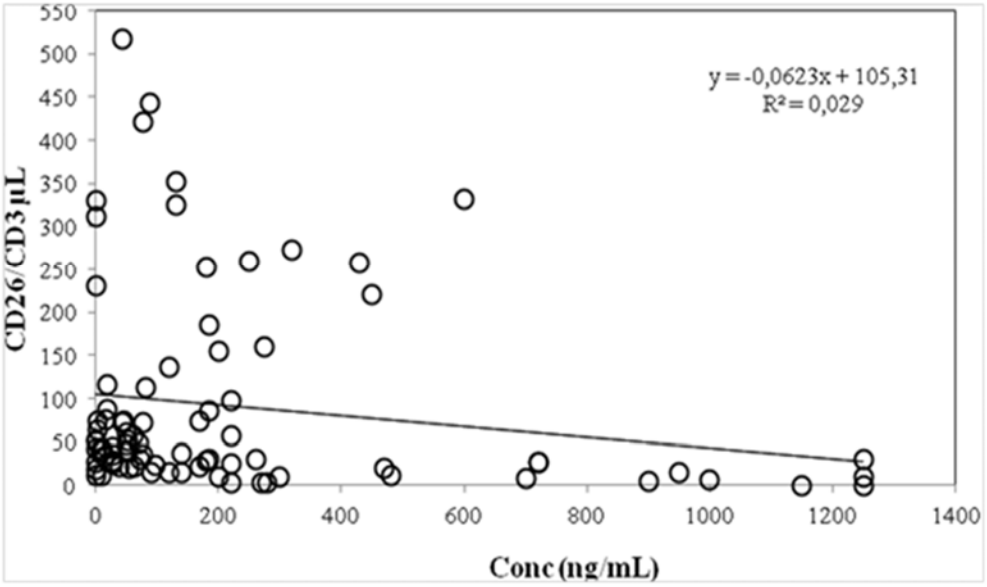
BEGEDINA-positive cell count (for counting lymphocytes CD26+ and CD3+) was performed using flow cytometry and staining with anti-mouse IgG within Day +5 of BEGEDINA treatment.

Dose change was scheduled as follows:

- In case of a maximum % BEGEDINA-positive cells on Day +5 of treatment ≥80% at the first dose level (2 mg/m^2), 3 additional subjects were to be treated on the same dose;
- In case of a maximum % BEGEDINA-positive cells <80% at the first dose level → Second dose level: 3 subjects had to receive 3 mg/m^2 for 5 consecutive days;
- In case of a maximum % BEGEDINA-positive cells ≥80% at the second dose, 3 additional subjects were to be treated on the same dose;
- In case of a maximum % BEGEDINA-positive cells <80% at the second dose level → Third dose level: 3 subjects had to receive 4.5 mg/m^2 for 5 consecutive days;
- In case of 6 subjects treated at the same dose level:
    a. In case of a maximum % BEGEDINA-positive cells <80%, subjects had to be escalated to superior dose level
    b. In case of a maximum % BEGEDINA-positive cells ≥80%, the dose level represented the MED level.

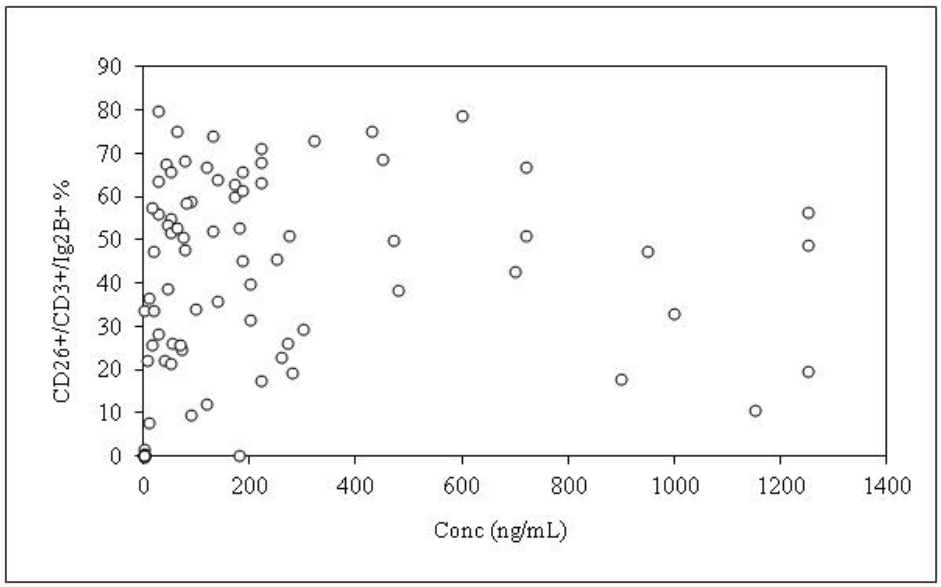
The concentration of BEGEDINA vs the respective levels of CD26/CD3/µL is presented in Figure 4 and concentration of BEGEDINA vs the respective values of % binding to target CD26 (CD26 + / CD3 + / + Ig2B%) is shown in Figure 5. There was a wide inter-individual variability.

Figure 4 BEGEDINA Concentration vs Respective Levels of CD26/CD3/µL



(Dose-Finding Study, EudraCT No. 2012 001353-19)

Figure 5 BEGEDINA Concentration vs % Binding to Target CD26 (CD26 + / CD3 + / + Ig2B%) (Dose-Finding Study, EudraCT No. 2012 001353-19)



None of the patients treated with BEGEDINA in any dose cohort study showed a maximum % BEGEDINA-positive cells on Day +5 of treatment of ≥80% at each dose level, and therefore the dose escalation to the next dose level was performed all 3 subjects.

Furthermore, the highest dose was not associated with the highest % of CD3-CD26 binding due to a high inter-individual variability

Data from subjects treated with BEGEDINA for 5 consecutive days are presented in Table 18. From the cohort with dosage of 4.5 mg/m^2/day administered for 5 consecutive days, the results showed that binding is at least as much as the 3 mg/m^2/day cohort and far in excess with respect of the 2 mg/m^2/day cohort. However, no correlation was observed between the 2 mg/m^2/day and 3 mg/m^2/day cohorts; on the basis of the currently available data, a poor correlation between dosage and percentage of binding of circulating CD3+CD26+ cells has been demonstrated.

Table 18 Binding Results from Subjects Treated with BEGEDINA for 5 Consecutive Days (Dose-Finding Study, EudraCT No. 2012 001353-19)

Subject Number	BEGEDINA Dose	Median % CD3+CD26 Binding
01	2 mg/m^2/day	46.8%
02		20^
03		Not available
04	3 mg/m^2/day	47%
05		63%
06		63%
07	4.5 mg/m^2/day	76%
08		45%
09		20%

In conclusion, a significant correlation could not be identified between the administered dose of BEGEDINA dose and the proportion of circulating cells stained with BEGEDINA. The number of subjects was too small to test for a correlation between CD26+ circulating cells and response.

2.1.3 Conventional Treatments for Graft-versus Host Disease

There are no approved second-line treatments for this group of subjects, although many have been investigated, as discussed in Section 2.1.1.1.2. However, guidelines for treatment have been published by the American Society of Blood and Marrow Transplantation (ASBMT) (Martin et al., 2012) and by the Working Group of the European Group for Blood and Marrow Transplantation and the European LeukemiaNet (EBMT-ELN) (Ruutu et al., 2014).

2.2 Rationale

Currently, there are no approved regimens for second-line therapy in subjects with acute GvHD who develop steroid-resistant disease. Survival in this group of subjects is poor and new treatment options are urgently needed.

Any clinically relevant result in this population would be of significant benefit as it would offer a clinically relevant advantage for steroid-resistant acute GvHD subjects.

BEGEDINA binding to CD26 antigen on T cells is able to inhibit activation, expansion and migration of T-cell population that plays a major role in GvHD development.

The rationale for the choice of endpoints is discussed in Section 4.3.3.

2.3 Risk Assessment

Steroid-resistant acute GvHD is associated with a high rate of morbidity and mortality, primarily from infections and/or multi-organ failure. Currently, there are no approved second-line regimens for treating subjects who develop steroid-resistant acute GvHD and those second-line therapies that are administered are largely unsatisfactory in terms of efficacy. For 30% of subjects, survival is no more than 1 year (Qian et al., 2013).

Any clinically relevant result in this population would be of significant benefit as it would offer a clinically relevant advantage for steroid-resistant acute GvHD patients.

The dose-finding study confirmed that BEGEDINA binds to circulating CD26+ T lymphocytes in patients with steroid-resistant acute GvHD. The efficacy endpoint was overall response (OR), which included patients with CR or PR to the BEGEDINA treatment (resolution of all signs of GvHD or improvement in grading of GvHD). Furthermore, the transplant mortality rate represents another efficacy endpoint to confirm the survival benefit.

The response rate on Study Day 28 for adults given BEGEDINA at the recommended schedule (3 mg/m^2 on Days +1, +2, +3, +4, and +5 and then on Days +10, +14, +17, +21, +24, and +28, considering Day +1 as the first day of treatment with BEGEDINA) was 85.7% and TRM at Day 180 was 28.6%.

Thus, if the efficacy of BEGEDINA can be confirmed in this study, it may provide benefit to this group of subjects in whom no satisfactory treatment options are available.

In clinical studies conducted to date, BEGEDINA has shown a good safety and tolerability profile; there was no evidence that there was an increased risk of toxicity at the higher doses.

This study will be performed in compliance with the protocol, ICH Good Clinical Practice (GCP) and applicable regulatory requirements, including the 2001/20/CE European Directive and the US FDA directive 21 CFR 314.126 - Adequate and well-controlled studies.

3.0 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of the study is to determine the efficacy of BEGEDINA versus conventional therapy in the treatment of subjects with steroid-resistant acute GvHD subjects in terms of OR at 28 days and TRM up to 180 days.

3.1.1 Secondary Objectives: Efficacy

The key secondary objective is to compare BEGEDINA and conventional therapy with respect to OS up to 180 days.

The other secondary efficacy objectives are to compare BEGEDINA and conventional therapy with respect to

- Change from baseline in stages of GvHD by target organ
- Incidence of chronic GvHD up to 180 days
- Cumulative steroid dose
- Incidence of Relapse and Relapse-Related Mortality
- Change from baseline in Karnofksy Performance Status

3.1.2 Secondary Objectives: Pharmacokinetic (PK)

The PK objective for this study is to characterize the PK of BEGEDINA in subjects with Grades II-IV acute GvHD who have failed to respond to steroid treatment.

3.1.3 Secondary Objectives: Safety

The secondary safety objectives are:

- To compare the safety and tolerability of BEGEDINA and conventional therapy.
- To gather additional information on the safety of BEGEDINA in subjects with Grades II-IV acute GvHD, who have failed to respond to steroid treatment.
- To evaluate the immunogenicity of BEGEDINA.
- To evaluate the effect of BEGEDINA on glucose metabolism.
- To compare the incidence of second malignancies at the end of the follow-up between BEGEDINA and conventional therapy.

3.2 Exploratory Objectives

The exploratory objectives for this study are:

- To evaluate the change in quality of life (QoL) from baseline.
- To evaluate duration of response.
- To evaluate OR by standard-risk and high-risk GvHD at onset (MacMillan et al., 2015).

4.0 INVESTIGATIONAL PLAN

4.1 Summary of Study Design

This will be a prospective, multicenter, randomized, open-label, phase II/III clinical study in which subjects will be randomly assigned in a 1:1 ratio to receive BEGEDINA treatment or the best conventional treatment. Randomization will be stratified by the baseline GvHD severity grade to either Grade B GvHD or Grade C/D GvHD.

The study will be conducted at approximately 30 bone marrow transplantation units in Europe, US and Canada. It is planned to enroll 184 subjects with steroid-resistant acute GvHD. The subjects will be randomized (1:1) to one of two treatment arms:

- BEGEDINA IV at a dose of 2.7 mg/m^2/day for 5 consecutive days from Study Day 1 through to Study Day 5, and on Study Days 10, 14, 17, 21, 24, and 28.*
- Conventional second-line treatment: a single treatment (agent), chosen at the discretion of the investigator (physician's best choice) and based on the clinical conditions of the individual subject. The treatment will be given as per prescribing instructions (and/or standard practice of the center).

Patients in both arms are to continue (or initiate) baseline therapy of methylprednisolone (or other steroid equivalent) and calcineurin inhibitor (see Section 5.7.2.4.1).

*Please note: the term "Study Day" as used throughout the protocol is used in relation to the day that randomized study treatment started and not in relation to the day of transplant.

All subjects will undergo a screening visit to assess for eligibility and for screening assessments; this visit must take place within 2 days of Baseline. After screening, subjects will undergo randomization and the baseline visit, which is also the first day of study treatment (Study Day 1) (i.e., subjects must commence study treatment within two days of meeting eligibility). Study visits will be conducted at various timepoints (Table 19) through Study Day 180.

The experimental phase of the study will end for each subject on Study Day 180. An extended, long-term, observational follow-up is also planned in order to capture survival, response/relapse, glucose metabolism, health outcomes and safety data out to Study Day 180. Thus, the expected duration for an individual subject who completes the study according to the protocol will be approximately 6 months, including screeningand 180-day follow-up.

Table 19 Schedule of Events

Assessments	Screening [a]	Experimental Phase																		Early Withdrawal
		BEGEDINA Treatment Period																		
Study Day	**-2 to -1**	BL[a]/1	2	3	4	5	6	7	8	9	10	14	17	21	24	28	56	90	180[b]	
Visit Windows											**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±2**	**±7**	**±7**	
Informed Consent	X																			
Inclusion/exclusion criteria	X	X																		
Demographics	X																			
Complete medical/surgical history	X [c]																			
Transplant history	X [d]																			
Transplant medications	X [e]																			
Prior and concomitant medications [f]	X	X – To be assessed at each visit and recorded through Study Day 58 or up to 30 days after the last dose of study treatment, whichever is later [f]																		
Complete physical examination [g, h]	X	X				X					X	X		X		X	X	X	X	
Height and weight [g, i]	X	X				X					X	X		X		X	X	X		
Oxygen saturation [g, j]	X	X	X	X	X	X					X	X		X		X	X	X		

Assessments	Screening [a]	Experimental Phase																	Early Withdrawal
		BEGEDINA Treatment Period																	
Study Day	**-2 to -1**	BL[a]/1	2	3	4	5	6	7	8	9	10	14	17	21	24	28	56	90	180[b]	
Visit Windows											**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±2**	**±7**	**±7**	
Vital signs [g, j]	X	X				X					X	X		X		X	X	X		
12-lead ECG [k]	X	X				X						X				X				
Laboratory safety [g, l]	X	X	X	X	X	X					X	X		X		X	X	X	X[x]	
Pregnancy test [m]	X																			
Glucose metabolism [n]		X														X			X	X
Infection markers [o]	X															X[o]	X			
Karnosfky PS [g, p]	X	X				X					X	X		X		X	X	X	X	X
Acute GvHD assessment, organ staging [q]	X	X	X	X	X	X					X	X	X	X	X	X	X	X		
Adverse events [w]	X	X – All AEs to be assessed at each visit and recorded through Study Day 58 or up to 30 days after the last dose of study treatment, whichever is later [w]																		
QoL evaluation [r]	X															X		X	X[x]	
Randomization		X																		
Administration of BEGEDINA		X	X	X	X	X					X	X	X	X	X	X				

Assessments	Screening [a]	Experimental Phase																		Early Withdrawal
		BEGEDINA Treatment Period																		
Study Day	**-2 to -1**	BL[a]/1	2	3	4	5	6	7	8	9	10	14	17	21	24	28	56	90	180[b]	
Visit Windows											**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±2**	**±7**	**±7**	
Administration of conventional treatment		Per Prescribing information for selected conventional treatment																		
Overall survival		To be assessed/recorded at each visit through Day 180 (excluding Days 6 through 9)																		
TRM		To be assessed/recorded at each visit through Day 180 (excluding Days 6 through 9)																		
PK blood sample [s]		X				X					X	X	X	X	X	X				
HAMA blood sample [t]		X				X					X	X	X	X	X	X	X	X		
Steroid use (route, dose duration)		To be assessed/recorded at each visit through Day 90																		
Response to treatment																X[u]	X	X		
Relapse		To be assessed/recorded at each visit through Day 180 (excluding Days 6 through 9)																		
Second malignancy		To be assessed/recorded at each visit through Day 180 (excluding Days 6 through 9)																		
Chronic GvHD assessment [v]		X	X	X	X	X					X	X	X	X	X	X	X	X	X	X

Abbreviations: BL, baseline; CT, computed tomography; ECG, electrocardiogram; GvHD, graft-versus-host disease; HAMA, human anti-mouse antibody; PK, pharmacokinetic(s); PS, performance status; QoL, quality of life; TRM, transplant-related mortality.

[a] Screening, randomization and the first administration of study treatment may occur on the same day if the study treatment is maintained at the study center. If baseline occurs on the same day as screening, then there is no need to repeat any evaluations that have already been done as screening assessments. However, before administering the drug, vital signs should be measured and oxygen saturations collected.

[b] Every effort should be made to arrange follow-up visits at the study site at Study Days 180; in the event, however, that a subject is remote from the site and unable to return within this window, a telephone call should be made to his/her local treating physician for as much information as possible to complete these visits.

[c] Complete medical and surgical history, including history of GvHD diagnosis (with stages by organ), hepatic, renal or cardiovascular disease, history of chronic diseases, history of diabetes, alcoholism or drug abuse, and non-disease relevant clinical history.

[d] Date of transplant, donor relation, gender and date of birth, human leukocyte antigen matching, stem cell source, diagnosis, phase of the disease at the time of transplant, assessment of engraftment, date of neutrophil and platelet engraftment, date of donor chimerism assessment and details of donor lymphocyte infusion.

[e] Record administration of medication (date, dose, variations) associated with transplant/post-transplant: conditioning regimen, GvHD prophylaxis and all drugs administered to the subject.

[f] Record other prior and concomitant medications, including history of adverse reactions to medications at each visit through Day 58 or up to 30 days after the last dose of study treatment, whichever is later.

[g] The assessments should be conducted on the same day as the BEGEDINA infusion or conventional treatment, when applicable.

[h] A complete physical examination will include, at a minimum, assessment of the following systems: skin, head, ears, eyes, nose, and throat, respiratory system, cardiovascular system, gastrointestinal system, neurological condition, blood and lymphatic systems, and the musculoskeletal system.

[i] Height only measured at Screening.

[j] Vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature) and oxygen saturation to be performed prior to BEGEDINA or conventional treatment administration, 30 and 60 minutes from the start of the first administration and prior to and at 60 minutes from the start of subsequent administrations. Patients in the BEGEDINA arm will continue to be monitored (under hospital observation) through 2 hours after the start of infusion.

[k] ECG is to be performed at on Study Day 1 (prior to start of BEGEDINA or conventional therapy), and on Study Days 5, 14, and 28. A window of 7 days for screening ECG results collected per standard of care prior to Screening visit is allowed. For patients in the BEGEDINA arm, the ECG on Study Days 5, 14, and 28 is to be performed after completion of the infusion (and at the time of the post-infusion PK draw, when applicable). See section 6.2.3.2 for details.

[l] To be coordinated with local routine/standard collections, as is possible, with window ±2 days.

Hematology: Basophils, eosinophils, hematocrit, hemoglobin, lymphocytes, monocytes, neutrophils, platelets, red blood cells, total white blood cells, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin. Optional (if routinely available): ean platelet volume, red blood cell distribution width, prothrombin time, prothrombin time-international normalization ratio. Serum chemistry: Albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, direct bilirubin, glucose, inorganic phosphorus, lactate dehydrogenase, lipase, potassium, sodium, total bilirubin, total protein, gamma-glutamyl, creatine phosphokinase. Lipid panel: Total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides. Urinalysis: Blood, glucose, ketones, microscopic exam, pH, protein, specific gravity, bilirubin. To be coordinated with local routine/standard collections, as is possible, with window ±2 days.

[m] Serum pregnancy test (beta-human chorionic gonadotropin) at screening only for women of childbearing potential only who have not been started on an anti-ovulatory regimen prior to initiation of chemo-inductive regimen.

[n] Glucose metabolism will be assessed through the evaluation of fasting glucose, insulin, c-peptide, glycosylated hemoglobin and Homeostasis Model Assessment (HOMA) calculation and through glucose in urine. Blood and urine samples for these assessments will be collected at baseline and on Study Days 28 and 180 (see Section 6.2.2.4 for details).

[o] Infection markers (most recently available results, as collected per standard of care, with window up to 90 days pre-study screening): HIV-1 Ab, HIV-2 Ab, HBsAg, HBc Ab,cytomegalovirus (CMV) DNA, HCV Ab, EBV DNA, erythrocyte sedimentation rate, C-reactive protein. CMV and EBV to be taken for analysis on Study Day 28 and Day 56 (window +/-2).

[p] A copy of the Karnofsky Performance Status scale is provided in Appendix 2 (see Section 12.2)

[q] GvHD assessment and staging using the modified International Bone Marrow Transplant Registry (IBMTR) index and according to common criteria of Glucksberg to be performed by a physician (see Section 6.1.1.1.1).

[r] Quality of Life (QoL) will be assessed using the Short Form 36 test. A copy of this questionnaire is provided in Appendix 3 (see Section 12.3).
[s] For subjects in the BEGEDINA arm only: Blood sample for PK measurements will be collected prior to and 15 minutes post-infusion, on Study Days 1, 5, 10, 14, 17, 21, 24, and 28 (see Section 6.3 for details). A window (+/1 1 day) is permitted from Day to Day 28, however, all efforts should be made to plan these doses with at least 48 hours between doses.
[t] For subjects in the BEGEDINA arm only: Blood sample for HAMA measurements will be taken before the first infusion and after the fifth infusion and on Study Days 10, 14, 17, 21, 24, 28, 56, and 90 (see Section **Errore. L'origine riferimento non è stata trovata.** for details). On study days in which BEGEDINA infusion is planned, the sample will be drawn to infusion (i.e., earlier that day or immediately prior to the infusion)..
[u] Note that during the administration of BEGEDINA/conventional treatment, the response to treatment is to be done at Study Day 28.
[v] Subjects are to be assessed at all visits for the appearance of chronic GvHD.
[w] Record all AEs observed in both arms during the study, from the time that informed consent is signed through Study Day 58 or up to 30 days after the last dose of study treatment, whichever is later. Any ongoing adverse drug reactions (ADRs) and/or SAEs will be followed until resolution (for a maximum to Study Day 180). Second malignancies and deaths will be recorded as SAEs through Study Day 180.
[x] Laboratory safety parameters at Day 180: Glucose to be collected; other laboratory safety parameters are optional.

4.2 Study Overview

4.2.1 Screening (Days –2 to 1)

Note: Screening, randomization and the first administration of study treatment may occur on the same day.

At the screening visit, potential subjects who are thought to meet the eligibility criteria will be informed of the requirements of the study and will give their written informed consent to participate. This consent must be provided before any study-specific procedures are carried out. Subjects will only be randomized when they meet all inclusion / exclusion criteria.

Subjects will complete the schedule of assessments as presented in Table 19.

In some cases, examinations already available before screening, as per standard practice, can be used at the screening visit (e.g. infection markers).

Subjects who are screened for eligibility into the trial but do not meet the inclusion/exclusion criteria for trial-entry are considered screen failures. Subjects who provide consent but discontinue the trial (by their own decision or by the decision of the investigator) prior to being randomized are also considered screen failures. The following information must be recorded on the respective electronic case report forms (eCRFs) for all screen failures who have signed informed consent:

- Demography
- Inclusion/exclusion criteria
- AEs
- End of screening disposition.

4.2.2 Baseline (Randomization and Study Day 1)

Subjects will participate in a baseline visit on Study Day 1 within 2 days of screening. All screening baseline assessments are performed prior to randomization. However, if baseline and screening occur on the same day, assessments planned for baseline but which have been previously performed at screening do not need to be repeated with the exception of vital signs and oxygen saturation assessments, which should be repeated before administration of study treatment.

4.2.3 Study Day 1 through to Study Day 180

Time windows are:

No visit windows are permitted from Study Day 1 and up to and including Study Day 5; other windows are provided in Table 19.

Assessments will be performed as shown in Table 19.

The end of study assessments will be performed Study Day 180 (±7 days) or at the time of withdrawal from study. The end of study is defined as any one of the following, whichever occurs first:

- The date of the last scheduled visit for the last subject
- The date of death of the last subject
- The date of withdrawal of the last subject.

Individual subjects will be deemed to have completed study when they have completed all required protocol procedures and assessments. For subjects with ongoing AEs at the last scheduled visit, the end of study date will be deemed to be the last scheduled visit date. The study site staff will perform the appropriate closeout/last visit assessments.

4.2.4 Hospitalization of Subjects During the Study

To be eligible for the study, subjects have to be nonresponsive to corticosteroids for GvHD following HSCT. In these situations many patients will already be hospitalized at screening. They are recommended to remain in the hospital for initial BEGEDINA treatment from Study Days +1 to +5. Subjects enrolled in both arms may, at the discretion of the investigator, be treated in day-hospital facilities, but must be monitored as per the Schedule of Events (Table 19), with special attention to risk of infusion related reactions (5.1.1.1, 6.2.1.5). Subjects receiving BEGEDINA must be under clinical observation for at least 2 hours after the start of the infusion.

The length of hospitalization will therefore vary between subjects and per the investigator's discretion.

4.3 Discussion of Study Design

4.3.1 Rationale for BEGEDINA Dose

The dose to be tested in this study (BEGEDINA 2.7 mg/m^2/day for 5 consecutive days, plus 6 additional 2.7 mg/m^2/day doses at Days 10, 14, 17, 21, 24 and 28) was selected based on the results from the phase II dose-finding study (EudraCT No. 2012-001353-19; see Section 2.1.2.2.2). In the dose-finding study, this regimen was administered to the subjects in

the DL2bis cohort (multiple dosing) and the results were indicative of a PK profile comparable to that predicted on the basis of data obtained after a single-dose in the phase I/II pilot study (EudraCT No. 2007-005809-21; see Section 2.1.2.2.1). The PK data in the DL2bis cohort confirmed that the treatment schedule extended to 6 additional administration at Days +10, +14, +17, +21, +24 and + 28 ensured high BEGEDINA plasma levels to possibly further improve the clinical efficacy in the most critical phases of the disease.

Thus, among the dosing regiments tested, the DL2bis regimen (BEGEDINA 3 mg/m^2/day for 5 consecutive days, plus 6 additional 3 mg/m^2/day doses at Days 10, 14, 17, 21, 24 and 28) was found to provide the best outcomes of the 4 doses evaluated (see Section 2.1.2.2.2). It was selected based on the following reasons:

- It was associated with the most satisfactory efficacy profile.
- It did not increase the risk of toxicity compared with the other lower dosages.
- The use of a dose-interval ranging from 3 to 5 days is fully appropriate taking into account that the mean half-life of BEGEDINA has been estimated equal to 1.8 days, and therefore this proposed dose regimen should ensure the maintenance of adequate plasma levels for the entire treatment period.

The BEGEDINA concentration of the product for this study was recalculated using the empirically established molecular extinction coefficient and resulted to be 0.9 mg/ml. The 3 mg/m^2 dose utilized in the preliminary dose-finding study when recalculated, corresponds to 2.7 mg/m2/day, the dose which is utilized for this study.

4.3.2 Rationale for the Control Arm (Conventional Treatment)

There are currently no approved regimens for the treatment of steroid-refractory acute GvHD in adults. Therefore, in this study the choice of conventional treatment is at the discretion of the investigator (physician's best choice).

Because the administration schedule could vary between the different conventional treatments chosen and also between the conventional treatments and BEGEDINA, the study will be conducted unblinded.

4.3.3 Rationale for Choice of Outcome Variables

Very few prospective studies have evaluated the efficacy and safety of second-line therapy for acute GvHD, and interpretation of these studies is made difficult due to the lack of standardization (Qian et al., 2013). Response rates and OS have frequently been used as the endpoints in these trials (Martin et al., 2012). For example, in a study investigating the

efficacy of the monoclonal antibody daclizumab as treatment for acute GvHD, response at Study Day 43 was the primary endpoint of the study. Survival was a secondary endpoint and was scored on Study Day 120 (Przepiorka et al., 2000).

However, in this current study, the selection of Study Day 28 post-treatment as the timepoint for assessing short-term response to treatment is consistent with other trials that have investigated the endpoints for acute GvHD treatment (Inamoto et al., 2014; Levine et al., 2010; MacMillan et al., 2010; Saliba et al., 2012).

Overall response to treatment is based on a change from baseline in the severity of the disease. The most commonly used grading system for severity of acute GvHD is the Glucksberg system (Grades I to IV), originally published in 1974. Although the system is still widely used and has recognized prognostic value, it is complex and relies on a combination of objective assessment of organ function (or organ-disease involvement) and subjective assessment of performance status (Glucksberg et al., 1974, Przepiorka et al., 1995). An alternative to this system is the International Bone Marrow Transplant Registry (IBMTR) index, which uses the same organ staging system, but is independent of performance status, which is sometimes difficult to document (Cahn et al., 2005; Rowlings et al., 1997). This system has been validated and is the grading system (Grades A, B, C and D) used in the current study for the assessment of this primary endpoint.

Grading is important in terms of assessing the response to prophylaxis or treatment, impact upon survival, and association with graft-versus-leukemia effect. Subjects with moderate to severe GvHD have a significantly higher mortality rate compared with those with mild disease. As an example, estimated 5-year survival rates of subjects with Grade C and Grade D acute GvHD are 25 and 5 percent, respectively (Cahn et al., 2005). However, caution must be used when applying these estimated survival rates to current subject population given changes in post-HSCT care. Current preventive regimens may alter overall outcomes and expressions of the disease.

Importantly, subjects with the same grade but different patterns of skin, gut or liver involvement often have significantly different outcomes. On occasion, subjects will present with Grade IV GvHD of a single organ; although most clinicians would consider such a manifestation severe disease, the particular organ involved has clear prognostic implications. As an example, a subject with stage IV cutaneous GvHD alone would be expected to have a much more favorable outcome than a subject with stage IV GI GvHD alone, although both have overall Grade IV GvHD (see Table 22 and Table 23). As a result, attempts have been made to create a new staging system that provides better prognostic information and permits subject comparison (Leisenring et al., 2006). Although no new staging system has yet found

universal acceptance, a refined risk score which has been recently proposed for prediction of response based on identified high and low risk GvHD at onset (MacMillan et al., 2015) will be utilized for an exploratory analysis with the data collected from this study.

BEGEDINA binds to the CD26 antigen (DPP-IV) thereby down-regulating its expression (Section 2.1.2). A possible consequence of the block of the CD26 activity is the inactivation of GLP-1, an incretin hormone that is excreted from the intestinal L cells. This inactivation in turn leads to an increase of blood concentrations of GLP-1 (Drucker and Nauck, 2006). GLP-1 plays a major role in the regulation of insulin secretion and glucose homeostasis. Dipeptidyl peptidase IV inhibitors are already available and approved for the treatment of type 2 diabetes (e.g., sitagliptin). Therefore, this study will explore if BEGEDINA could have a similar effect in the regulation of insulin secretion and glucose homeostasis, via an evaluation of its effect on glucose metabolism.

4.3.4 Other Comments on Study Design

An upper age limit of 65 years was selected for the study population. An age between 55 and 65 years is no longer considered a barrier to HSCT. However, subjects with higher age and/or comorbidities, such as organ impairment or ongoing infection are less often selected to receive HSCT because they will have a higher mortality rate and are expected to be less responsive to therapy (Brunner et al., 2013). The aforementioned will affect assessment of study results.

4.3.5 Benefits and Risks

The benefits and risks of the study are discussed in Section 2.3.

4.4 Selection of Study Population

4.4.1 Inclusion Criteria

Male or female subjects may be entered in the study only if they meet all of the following criteria:

1. Age ≥18 and ≤65 years of age.
2. Recipient of an allogeneic HSCT.

   Note: Subjects with steroid-resistant GvHD following donor lymphocyte infusion post-HSCT are also eligible
3. Steroid-resistant acute GvHD, Grade II-IV, defined as:

- progressive disease (deterioration of at least 1 stage in 1 organ) after 3 days of primary treatment with methylprednisolone 2 mg/kg, or equivalent.

or

- lack of at least a PR after 7 days of primary treatment with methylprednisolone 2 mg/kg or equivalent.

or

- lack of a CR after 14 days of primary treatment with methylprednisolone 2 mg/kg or equivalent.

Note: Subjects who may have received an increase in their steroid dose treatment prior to randomization will be eligible for enrollment. An increase in steroid dose will not be considered as second-line therapy.

4. Evidence of previous myeloid engraftment (absolute neutrophil count ≥0.5 x 10^9/L).
5. Karnofsky Performance Status score ≥50%.
6. Adequate renal function as defined by serum creatinine ≤2 × upper limit of normal (ULN) or calculated creatinine clearance (CrCl) of ≥30 mL/min using the Cockroft-Gault equation: Calculated CrCl= ([140-age in years] x [ideal body mass {IBM} in kg])/72 x (serum creatinine value in mg/dL), where IBM = IBM (kg) = ([height in cm–154] × 0.9) + (50 if male, 45.5 if female).
7. Subject must be willing and able to comply with study requirements, remain at the clinic, and return to the clinic for the follow-up evaluation, as specified in this protocol during the study period.
8. Able and willing to provide signed informed consent.

4.4.2 Exclusion Criteria

Subjects will not be entered in the study for any of the following reasons:

1. Prior second-line systemic treatment for GvHD.
2. Received agents other than steroids for primary treatment of acute GvHD.
3. Stage 1-2 skin acute GvHD alone (with no other organ involvement).
4. Acute steroid-resistant acute GvHD beyond 28 days from first-line therapy (primary treatment).
5. Evidence of severe hepatic veno-occlusive disease or sinusoidal obstruction.

6. Evidence of encephalopathy.
7. Life expectancy <3 weeks.
8. Presence of chronic GvHD (Dignan et al., 2012).
9. Second or subsequent allogeneic transplant.
10. Received a solid organ transplant (with the exception of a corneal transplant >3 months prior to screening).
11. Relapsed disease after last transplant.
12. Human immunodeficiency virus (HIV) positive.
13. Evidence of lung disease that is likely to require more than 2 liter per minute of O_2 via face mask or an estimated FiO_2 of 28% via other delivery methods in order to sustain an O_2 saturation of 92% within the next 3 days.
14. Any underlying or current medical or psychiatric condition that, in the opinion of the investigator, would interfere with the evaluation of the subject including uncontrolled infection, heart failure, pulmonary hypertension. Any other serious medical condition, as judged by the investigator, which places the subject at an unacceptable risk if he or she were to participate in the study or confounds the ability to interpret data from the study.
15. Administration of any other investigational agents (not approved by the FDA or EMA for any indication) within 30 days of randomization. Participated in any interventional clinical trial for an immunomodulatory drug, within the past 30 days or within 5 half-lives of the study treatment, whichever is the greater. Participated in any interventional clinical trial for an acute GvHD therapeutic agent or for an immunomodulatory drug, within the past 30 days or within 5 half-lives of the study treatment, whichever is the greater. Participated or is currently participating in any bone marrow derived autologous and allogeneic stem cell or gene therapy study.
16. Known hypersensitivity to murine proteins.
17. Women who are pregnant, breastfeeding or at risk to become pregnant during study participation. Female subjects of childbearing potential who have not been started on an anti-ovulatory regimen prior to initiation of chemo-inductive regimen must test negative for pregnancy (serum) at the time of enrollment.
18. Male and female subjects who do not agree to take adequate measures to avoid pregnancy prior to study entry and for the duration of participation in the study (or for at least 3

months following the last dose of study drug, whichever is longer) (acceptable methods of birth control are described in protocol Section 6.2.1.6).

4.4.3 Subject Restrictions

The following restrictions may affect subject participation in this study:

- Availability to attend follow-up visits according to the protocol.

4.4.4 Subject Withdrawal

The duration of the study is defined for each subject as the date signed written informed consent is provided through to the last follow-up visit.

4.4.4.1 Reason for Withdrawal/Discontinuation

All subjects are free to withdraw from participation in the study at any time, for any reason, specified or unspecified, and without prejudice to further treatment. The criteria for enrollment are to be followed explicitly.

If a subject who does not meet enrollment criteria is inadvertently enrolled, the Sponsor or Sponsor designee (Contract Research Organization [CRO]) must be contacted to evaluate if the subject is to continue treatment. In these rare cases, the investigator must obtain documented approval to allow the subject to continue study treatment.

In addition, subjects will be discontinued from study drug (but not withdrawn from the study and subsequent assessments) in the following circumstances:

- The investigator decides that the subject's treatment should be discontinued. If this decision is made because of an intolerable AE or a clinically significant laboratory value, which compromises the safety, health or welfare of the subject, the study drug is to be discontinued and appropriate measures are to be taken. The Sponsor or Sponsor designee is to be notified immediately.
- Lack of compliance with protocol.

If a subject discontinues from study drug but continues in the study for follow up, a last PK sample should be collected on the next scheduled visit after study drug discontinuation. No further samples will be required. HAMA and other scheduled assessments are to be followed as per protocol.

Subjects will be discontinued from study drug and withdrawn from the study protocol in the following circumstances:

- The subject is unwilling to continue in the study and consent is withdrawn by the subject or their legal guardian. A subject may choose to discontinue treatment but agree to be followed for study endpoints and safety, as per 4.4.4.2 below.
- Lost to follow-up.
- Death.

Subjects who are withdrawn from the study early will have early termination procedures performed as shown in the Schedule of Events (Table 19). Subjects who are withdrawn from the study will not be replaced (see Section 4.4.4.3).

4.4.4.2 Handling of Withdrawals

Subjects who discontinue active participation and/or withdraw informed consent in the study will no longer receive study treatment. If a subject discontinues treatment for any reason, the subject will be asked to continue in the study for endpoints for the duration of the study (in this case, they are not considered to have withdrawn from the study, but only from study treatment).

When a subject withdraws from the study, where available, the reason(s) for withdrawal shall be recorded by the investigator on the relevant page of the eCRF. In addition, when possible, the Sponsor should be notified of the withdrawal of a subject from the study. Whenever possible, for all subjects who withdraw from the study prematurely for any reason, all early termination procedures should be performed on the day of withdrawal (or the day after withdrawal) as shown in the Schedule of Events (Table 19). Subjects who fail to return for final assessments will be contacted by the study center in an attempt to have them comply with the protocol.

If the subject discontinues study treatment and has an ongoing AE at the time of discontinuation, the subject must receive follow-up until the AE is resolved or is stable. If a clinical visit(s) is (are) necessary, the procedures performed during the follow-up visit(s) will be recorded on unscheduled visit pages of the eCRF.

4.4.4.3 Replacements

Any subject with a severe AE or clinical condition emerging after the enrolment of the subject and before randomization, which is considered by the investigator as compromising the continuation of the study and being life-threatening, may be replaced. No other replacements will be allowed.

5.0 STUDY TREATMENTS

5.1 Treatments Administered

At the baseline visit, subjects will be randomly assigned in a 1:1 ratio to receive BEGEDINA or conventional second-line treatment. The study treatments will be administered at the study center by appropriately trained health care professionals.

Subjects in both treatment arms will receive baseline therapy with methylprednisolone (or other steroid equivalent) and a calcineurin inhibitor (see Section 5.7.2.4) along with their assigned study treatment.

5.1.1 BEGEDINA Treatment Arm

Subjects in the BEGEDINA treatment arm will receive BEGIDINA 2.7 mg/m^2/day IV for 5 consecutive days (Study Days 1, 2, 3, 4, 5) and then on Study Days 10, 14, 17, 21, 24 and 28, for a total of 11 doses. As per Table 19, a window (+/- 1 day) is permitted for doses from Day 10 to Day 28, however, all efforts should be made to plan these doses with at least 48 hours between doses.

The total volume of BEDEGINA to be administered should be further diluted in 100 mL of sodium chloride 9 mg/mL (0.9%) solution for injection prior to administration. The infusion lasts for 60 minutes. For instructions on dilution prior to administration, see Section 5.2.1.

BEGEDINA is a murine antibody and the possibility of an allergic reaction occurring in a subject administered this drug cannot be excluded. Therefore, although allergic reactions have not been observed in Phase I-II studies, premedication is recommended (see 5.7.2.3).

Subjects are to be monitored under hospital observation through 2 hours after the start of the infusion (see Section 6.2.3.1 Vital signs). No other medications should be given during the BEGEDINA infusion unless determined medically necessary by the investigator.

There are no dosing restrictions regarding food and drink.

5.1.1.1 Special Guidance for Investigators

Allergic reactions during the administration of a BEGEDINA infusion are to be handled per the institutional guidelines and distinguished from infusion related reactions.

During administration of BEGEDINA, the infusion must be stopped:

1. If the subject has symptoms or signs of respiratory compromise such as tachypnea, cyanosis, complains of shortness of breath, etc., regardless of the oximetry reading.

   Or

2. If SaO_2/SAT decreases to <85% over a continuous period of 3 to 5 minutes, whether or not the subject has symptoms of respiratory distress, the dose should not be restarted and the Medical Monitor should be contacted prior to administering further doses. If the SaO_2/SAT is <85% in the absence of symptoms or signs, the accuracy of the pulse oximeter reading should be confirmed by using another machine, relocating the sensor or testing the device on a normal subject.

In addition, the infusion of BEGEDINA may be stopped at the discretion of the investigator if there is another AE that the investigator believes is related to BEGEDINA, there is an issue with BEGEDINA infusion or the subject withdraws consent. If the toxicity evaluation during an infusion results in a worsening of a subject's respiratory functioning by one or more grades (per the Common Terminology Criteria for Adverse Events (CTCAE) assessment criteria) in comparison to the pre-infusion status, and the respiratory status does not return to baseline levels after symptomatic treatment, the investigator should consider to interrupt therapy and contact the Medical Monitor in order to discuss each specific case and determine whether the subject will be precluded from additional BEGEDINA infusions.

5.1.2 Conventional Second-line Treatment Arm

Subjects in the conventional treatment arm will receive a second-line treatment (single agent): each center will choose the best conventional second-line treatment, according to the standard practice at the study center (physician's best choice).

Assessments are to be performed as per Table 19.

5.2 Identity of Investigational Product

BEGEDINA is supplied as a 5.4 mg concentrate for solution for infusion (Table 20).

Table 20 Identity of Investigational Product

Investigational product (generic name)	Active ingredient	Dosage form and strength and route of administration	Manufacturer
BEGEDINA (begelomab)	Murine monoclonal antibody against CD26, antibody BT 5/9	0.9 mg/mL concentrate in vials of 6 mL (5.4 mg of active substance) for reconstitution for intravenous infusion via parenteral route	ADIENNE S.r.l. S.U., Via Galileo Galilei, 19, Caponago (MB) – 20867 Italy

ADIENNE SA or delegate will distribute the supplies of BEGEDINA to the study centers.

The conventional treatments will be obtained by the study centers from commercial suppliers.

5.2.1 Preparation of BEGEDINA

BEGEDINA should be visually inspected for particulate matter prior to administration.

The solution should be allowed to warm to room temperature (25°C) for 10-15 minutes prior to administration. The transfer procedures require strict adherence to aseptic techniques.

The required amount of BEGEDINA (calculated according to posology) should be drawn from the vial(s) using a sterile syringe and transferred into a 100 mL 0.9% saline solution infusion bag.

The infusion bag containing the diluted solution should be gently agitated to ensure thorough mixing of the product and diluent. Any unused BEGEDINA left in the vial should be discarded, as the product contains no preservatives. Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

Detailed instructions regarding investigational agent preparation, handling and administration procedures are included in the Directions for Use and the Study Procedures Manual.

5.3 Management of Clinical Supplies

5.3.1 Packaging and Labelling

BEGEDINA will be packaged and supplied according to local legal requirements.

A label (in the local language) will be attached to the outside of each cases as well as to the immediate container (vial). The text will be compliant with local regulatory requirements and may include some of the following information:

- Protocol number
- Subject number/study center number
- Name, formulation, administration route and contents
- Lot number
- Investigator name or code
- Storage instructions

- Caution statement (for clinical study use only)
- Expiry date
- Sponsor name and address.

Each case includes Directions for Use with detailed instructions regarding investigational agent preparation, handling and administration procedures.

All study treatment supplies must be stored in accordance with the manufacturing instructions and according to local regulations.

5.3.2 Study Treatment Accountability

The investigator, a member of the investigational staff or a hospital pharmacist must maintain accurate records of receipt of all test articles, including dates of receipt. In addition, accurate records will be kept regarding when and how much study treatment is dispensed and used by each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. This information must be recorded using the Drug Accountability Form, which must be available for inspection at any time.

All study medication supplies should be accounted for at the termination of the study and a written explanation provided for discrepancies. All unused study medication supplies and packaging materials are to be inventoried and returned to the CRO by the investigator. The investigator is not permitted to return or destroy unused clinical drug supplies or packaging materials unless authorized by the CRO.

5.4 Method of Assigning Subjects to Treatment Group

Once the subject meets all of the inclusion criteria and none of the exclusion criteria and has provided an informed consent, the study center will request the study medication assignment using the Interactive Voice Activated Response System (IVRS).

The subject will be randomized to either the active treatment group (BEGEDINA) or the conventional treatment arm (randomization ratio 1:1). Randomization will be stratified by the baseline GvHD severity grade to either Grade B GvHD or Grade C/D GvHD.

The randomization will be managed centrally.

Subjects must start study treatment the day of randomization. Specific randomization instructions will be provided in the study procedures manual.

5.5 Selection and Timing of Dose for Each Subject

All subjects randomized to the BEGEDINA treatment arm will receive 2.7 mg/m^2/day IV for 5 consecutive days (Study Days 1, 2, 3, 4, 5) and on Study Days 10, 14, 17, 21, 24 and 28.

All subjects randomized to the conventional treatment arm will receive the treatment according to the relevant prescribing instructions (and/or standard practice of the center).

The rationale for the BEGEDINA dosing regimen and the choice of conventional treatment are discussed in Sections 4.3.1 and 4.3.2, respectively.

5.6 Blinding

Because the administration schedule could vary between the different conventional treatments chosen and also between the conventional treatments and BEGEDINA, the study will be conducted as an open-label study. The study team of the Sponsor and CRO (except the on-site monitors, pharmacovigilance and personnel as specified in the study-specific blinding plan), however, will be blinded regarding the study treatment up to database lock and general unblinding. The same holds for the independent hematologist who will adjudicate response.

The DSMB (see Section 6.2.6) members will receive unblinded outputs generated by a dedicated unblinded statistician and statistical programmer (both separated from the blinded study team members).

5.7 Prior and Concomitant Treatments

Prior medication refers to medication started within 30 days prior to screening and stopped prior to the first administration of the study treatment. Concomitant medication refers to medication started prior to and continued after the first administration of study treatment or taken any time after the first administration of the study treatment up to the follow-up visit.

Use of all concomitant medications will be recorded in the subject's eCRF, regardless of whether they are permitted or prohibited medications, up to Study Day 58 or up to 30 days after the last dose of study treatment, whichever is later.

The minimum requirement is that the drug name, dose, indication and the dates of administration are to be recorded. This will include all prescription drugs, herbal products, vitamins, minerals and over the counter medications. Any changes in concomitant medications will also be recorded in the subject's eCRF.

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the investigator. However, it is the responsibility of the investigator to ensure that details regarding the medication are recorded in full in the eCRF.

5.7.1 Excluded Medications

The following medications are prohibited during study (their use is a protocol violation and should be documented as such) and concomitant use during screening will result in subject exclusion:

- Agents for treatment of steroid-resistant acute GvHD other than BEGEDINA in the BEGEDINA arm or more than 1 single agent for treatment of steroid-resistant acute GvHD in the conventional treatment arm. Administration of any additional agent(s) for treatment of steroid-resistant acute GvHD within 28 days of randomization, either concomitantly or in succession, will be considered third-line therapy for treatment failure (i.e. salvage treatment; see Section 5.8).
- Any other investigational agents (not approved by the FDA or EMA for any indication) concurrently during study participation.

5.7.2 Allowed Medications and Therapies

- Blood products, including irradiated granulocytes.
- Steroid therapy and calcineurin inhibitors (or other ongoing GvHD prophylaxis, see Section 5.7.2.2) is allowed as concomitant baseline therapy per protocol (5.7.2.4.1). Subjects may continue any prior therapy used for prophylaxis at a stable dose from baseline as long as it has not been discontinued and restarted after initiating steroid treatment for acute GvHD (see Section 5.7.2.2).
- Subjects with stable disease (SD) or progressive disease (Section 6.1.1.1) at Study Day 28 or subjects who cannot tolerate the randomized study treatment may receive a third-line treatment for steroid-resistant acuteGvHD at the investigator's discretion (Section 5.8).
- Other routine supportive care as per standard practice.

5.7.2.1 Conditioning Regimen for Hematopoietic Stem Cell Transplantation

There are no restrictions on the conditioning regimen administered before HSCT; it will be per local practice, the original disease, and the subject's clinical status and age. However, data on the conditioning regimen will be collected and might be used in exploratory analyses.

5.7.2.2 Prophylaxis for GvHD

There are no restrictions on GvHD prophylaxis (each study center will use appropriate prophylactic treatments according to local standards); subjects may continue any prior therapy used for prophylaxis at a stable dose from baseline as long as it has not been discontinued and restarted after initiating steroid treatment for acute GvHD. Patients who have developed steroid-resistant acute GvHD while on multiple ongoing GvHD prophylaxis may be enrolled, however, life expectancy must be carefully evaluated prior to their enrolment to fulfill with Exclusion Criteria no. 7.

After study start, baseline therapy with calcineurin inhibitors and steroids are to be administered as per Section 5.7.2.4.1.

5.7.2.3 Premedication for Subjects Receiving BEGEDINA

Although allergic reactions have not been observed in Phase I-II studies, premedication, consisting of prednisolone 40 mg and diphenhydramine (e.g., 25-50 mg) (or another steroid equivalent and/or antihistamine drug), are recommended to be administered parenterally at least 30 minutes prior to the BEGEDINA infusion.

Note: for those subjects already on high doses of steroids, premedication with additional steroid may not be necessary (may be omitted at the discretion of the investigator).

5.7.2.4 Standard of Care for Subjects with Acute GvHD

All enrolled subjects will receive institutionally defined standard of care as described in Section 5.7.2.4.1. In addition, investigators should provide standard of care for prevention and treatment of viral infections, particularly if there is evidence of viral reactivation as discussed in Section 5.7.2.4.2 (unless qualifies as excluded medications, see section 5.7.1).

5.7.2.4.1 Concomitant Treatment

Alongside the study treatments, the following drugs will be administered to subjects in both treatment arms as baseline therapy

- Methylprednisolone IV at the dose of 2 mg/kg/day (or other steroid and dose equivalent)

- A calcineurin inhibitor (or other alternate, continuing GvHD prophylaxis) (see also Section 6.2.4.2), adjusted to therapeutic drug levels per study center standard practice.

They will be administered per the Principal Investigator's discretion and according to standard practice and institutional/international guidelines. Methylprednisolone or steroid equivalent dose may be escalated for worsening of acute GvHD as per Section 5.10. Tapering of steroids can start only after response (at least 1 stage in 1 organ) is observed and after at least 5 days of BEGEDINA or conventional treatment. A steroid taper rate of at least 10% of the dose per week, but not exceeding 25% of the dose per week, is recommended as summarized in Table 25 (see Appendix 1 in Section 12.1), with the goal of discontinuation of steroids by 10 weeks after initiating taper. Tapering may be commenced earlier or rate increased for documented strong safety indications (e.g., uncontrolled infection).

5.7.2.4.2 Supportive Care for Subjects with GvHD

Supportive care of subjects with GvHD will be administered according to local regulations and standards of care and institutional and international guidelines. The care could include, but is not limited to, the following:

- Oral decontamination and oral hygiene.
- Antibacterial, antiviral, antifungal prophylaxis; notably for: cytomegalovirus (CMV), gram positive (encapsulated) bacteria, *Pneumocystis carinii* and fungal infections per institutional practice.
- Monitoring and treatment of CMV, Epstein-Barr virus (EBV), human herpesvirus 6 and adenovirus viremia (for "matched unrelated donor" follow specific Epstein-Barr virus monitoring). CMV and EBV are to be recorded in the eCRF at screening and Day 28 and Day 56.
- Transfusions of erythrocyte and platelet concentrates; all blood products must be leukocyte depleted and irradiated.
- Antiemetic prophylaxis and pain therapy.

A publication by the EBMT-ELN proposes guidelines that could be used in transplantation centers (Ruutu et al., 2014).

5.8 Salvage Treatment

There are currently no recommended salvage (third-line) therapies for steroid-resistant acute GvHD; recommended treatment at this point could follow the EBMT-ELN guidelines (Ruutu et al., 2014) or ASBMT (Martin et al., 2012), per investigator discretion.

In case of salvage treatment, subjects randomized to BEGEDINA, may be continued BEGEDINA according to the scheduled dose (up to Day 28), along with third-line therapy. For subjects randomized to conventional treatment, the initial conventional treatment may be administered with a third-line therapy. BEGEDINA is not permitted as salvage (third-line) treatment in the conventional treatment arm.

5.9 Management of a GvHD Flare

An acute GvHD flare is defined as worsening of acute GvHD in any organ by at least one stage beyond Study Day +28, subsequent to achieving PR or CR to Initial Therapy.

Subjects who have been randomized to the BEGEDINA arm and who have a GvHD flare after Study Day 28 and before Study Day 90 may receive additional BEGEDINA treatment as follows:

- Subjects must have had documented CR or PR at Day+28.
- Treatment consists of the Initial Therapy Plan (i.e. 5 consecutive days plus days 10, 14, 17, 21, 24 and 28 from the start of the flare) as in the Schedule of Events (Table 19).
- Subjects are recommended to remain in the hospital for treatment of flare, however, at the discretion of the investigator, may be treated in day-hospital facilities; subjects must be monitored (under hospital observation through 2 hours after the start of the infusion) and as per the Schedule of Events (Table 19), with special attention to risk of infusion related reactions (5.1.1.1, 6.2.1.5).'
- If a subject has begun other steroid-resistant acute GvHD therapy within the active study treatment period (up to and including Day 28), they would not be eligible to receive further BEGEDINA therapy.

Subjects in the conventional treatment arm may continue or restart therapy as per investigator judgment.

5.10 Worsening of Acute GvHD

Protocol guidelines for increasing the steroid dose for worsening of acute GvHD disease include:

1. Worsening of symptoms for at least 3 days despite second line treatment.

   or

2. Grades III-IV acute GvHD persisting for at least 1 week despite second line treatment.

   or

3. Grade II acute GvHD persisting for at least 2 weeks despite second line treatment.

Escalation of steroid therapy prior to Day 28 does not constitute withdrawal of a subject from the study. If escalation of therapy occurs, the subject is to continue on study and receive all treatments and assessments per protocol.

If worsening of GvHD requires a third-line agent, please follow instructions as on Section 5.8 for salvage therapy.

5.11 Dose Modification for Toxicity

No specific toxicities have been observed to date with BEGEDINA.

Important identified risks include the development of HAMA and infusion-related reactions (described in Section 5.1.1).

Therefore, dose modification for toxicity is left to the discretion of the Principal Investigator. However, treatment should be delayed/withdrawn in case of severe (Grade 3 or above) toxicity considered related to the study treatment or that, in the opinion of the investigator, might be life-threatening if treatment is continued.

5.12 Medical Care of Subjects After End of Study

In both treatment arms, after subjects leave the study they will continue to receive institutionally defined standard of care.

5.13 Treatment Compliance

The prescribed dosage, timing and mode of administration may not be changed. Any departures from the intended regimen must be recorded in the eCRFs.

Subjects in the BEGEDINA arm will receive their assigned study treatment via IV infusion under the supervision of a healthcare professional. Their compliance will be documented by drug accountability as described in Section 5.3.2.

Subjects in the conventional arm will be administered their assigned treatment by a healthcare professional. Details of this drug therapy will be recorded in the eCRF.

Noncompliance is defined as taking less than 80% of study medication during any evaluation period (visit to visit).

6.0 EFFICACY, SAFETY, AND PHARMACOKINETIC ASSESSMENTS

During assessment visits in which subjects are administered BEGEDINA the assessments must be performed according to Table 19. GvHD assessments will be used to determine whether the subject's GvHD status has progressed from baseline according to the definition of progression of disease provided in Section 6.1.1.1.

6.1 Efficacy

6.1.1 Primary Efficacy

There are two primary endpoints.

1. The OR (CR or PR) at Study Day 28.

2. TRM (any deaths without prior diagnosis of relapse) up to Study Day 180

These endpoints are both considered primary as health authorities (EMA/FDA) have requested different primary endpoints.

6.1.1.1 Overall Response to Treatment at Study Day 28

The change in grade from baseline to Study Day 28 (-1/+2 day) will be used to determine the response of GvHD to treatment using the following classifications:

- *Complete response (CR)*: complete resolution of all signs of GvHD.
- *Partial response (PR)*: improvement of 1 overall grade (i.e., change from baseline in IBMTR to a less severe grading).
- *Stable disease (SD)*: No change in GvHD grading (i.e., no change from baseline in IBMTR grade).
- *Disease progression (PD)*: Deterioration in one overall grade in GvHD (i.e., worsening in IBMTR by at least 1 grade compared to baseline).
- Death (up to Study Day 28).

Overall response at Study Day 28 (i.e. change in GvHD grade compared to baseline) is defined as

- Complete response or PR at Study Day 28 response assessment
- and no third-line therapy has been introduced prior to the Study Day 28 response assessment

Non-response at Study Day 28 is defined as

- Stable disease or PD or missing response assessment at Study Day 28 (including death up to Study Day 28) or
- Complete response or PR at Study Day 28 response assessment and third-line therapy has been introduced prior to the Study Day 28 response assessment

Response at Study Day 28 will be adjudicated by an independent blinded hematologist; this adjudicated response will be used for the primary analysis of OR at Study Day 28.

6.1.1.1.1 Grading the Severity of Acute GvHD

At the visits presented in Table 19, the severity of the subject's GvHD will be graded using the IBMTR system (see Table 21) and the Glucksberg criteria (see Table 23).

GvHD will be graded and documented by stage of each organ, with date of onset/change in stage; gut GvHD will be documented as upper GI tract (limited to anorexia, nausea, vomiting) and/or lower GI tract. This will permit also an exploratory analysis of refined response by standard-risk or high-risk GvHD at onset, in which standard risk is defined by single organ involvement (stage 1 to 3 skin or stage 1 to 2 GI) or 2 organ involvement (stage 1 to 3 skin plus stage 1 GI or stage 1 to 3 skin plus stage 1 to 4 liver), and high-risk are those which are not standard risk (MacMillan et al., 2015).

The IBMTR

For the analysis of the primary endpoint, severity of acute GvHD will be graded using the IBMTR system. In this system, severity is determined by a clinical assessment of the degree of involvement of the skin, liver and GI tract (Table 21). Grade A GvHD is characterized as mild disease, Grade B GvHD as moderate, Grade C as severe and Grade D life-threatening (Cahn et al., 2005; Przepiorka et al., 1995; Rowlings et al., 1997).

Table 21 Grading of Graft-versus-Host Disease According to the IBMTR System

Grade	Category	Skin	Intestine	Liver
A	Mild	1	0	0
B	Moderate	2	0	0
		0-2	1	0-1
		0-2	0-1	1
		0-2	2	0-2
		0-2	0-2	2
C	Severe	3	1	0-1
		3	0-1	1
		3	0	0
		0-3	0-3	2-3
		3	0-2	0-3
D	Life-threatening	0-3	0-3	4
		0-3	4	0-4
		4	0-4	0-4

The maximum possible grading should be selected. The highest organ stage determines the grade.
Reference: Cahn et al., 2005; Rowlings et al., 1997.

Glucksberg Criteria

An overall grade for acute GvHD is obtained by first assigning a stage for each of the 3 target organs (skin, liver gut). Staging of each organ, a clinical assessment, is summarized in Table 22.

With the Glucksberg approach to grading the severity of acute GvHD, this organ staging is then combined with a functional performance status assessment, using the Eastern

Cooperative Oncology Group performance status measure, to assign an overall grade for acute GvHD, according to Glucksberg and as presented in Table 23.

Table 22 Staging of Graft-versus-Host Disease (Glucksberg Criteria)

Stage	Skin	Liver	Gastro-enteric Tract
1	Rash <25% body surface[a]	Bilirubin 2-3 mg/dL[b]	>500 mL diarrhea/day (300 mL/m^2/day) or anorexia, nausea, vomiting
2	Rash 25-50% body surface[a]	Bilirubin 3-6 mg/dL[b]	1000 mL diarrhea/day (>600 mL/m^2/day)
3	Rash >50% body surface[a] (generalized erythroderma)	Bilirubin 6-15 mg/dL[b]	>1500 mL diarrhea/day (>1000 mL/m^2/day)
4	Generalized erythroderma with bullae and desquamation	Bilirubin >15 mg/dL[b]	Severe abdominal pain with/without ileus

[a] To simplify the calculation of body surface the rule of nine could be considered.

[b] Cut-off value for µmol/liter.

Source: Glucksberg et al., 1974.

Table 23 Overall Grade of Graft-versus-Host Disease (Glucksberg Criteria)

	Staging of GvHD Disease in Target Organ				ECOG PS
Grade	**Skin**	**Liver**		**Gut**	
I	1-2	0		0	0
II	1-3	1	*and/or*	1	0-1
III	2-3	2-3	*and/or*	2-4	2-3
IV	2-4	2-4	*and/or*	2-4	3-4

Abbreviations: ECOG, Eastern Cooperative Oncology Group; GvHD, graft-versus-host disease; PS, performance status.
Source: Glucksberg et al., 1974, Przepiorka et al, 1995.
For ECOG Performance Scale, see Appendix Section 12.4.

6.1.1.2 Transplant-Related Mortality up to 180 Days

A transplant-related death is defined as any death without prior diagnosis of relapse. Time to transplant-related death up to 180 days is calculated as date of transplant-related death minus date of randomization plus 1. Competing risk is the diagnosis of relapse up to 180 days with

time to diagnosis of relapse calculated as date of diagnosis of relapse minus date of randomization plus 1.

For a subject without a transplant-related death or diagnosis of relapse up to 180 days, the time will be censored at the earliest of the days below:

- at Study Day 180 if the subject is known to be alive at Study Day 180
- at day of last contact if subject gets lost to follow-up prior to Study Day 180.

6.1.2 Secondary Efficacy

6.1.2.1 Overall Survival up to 180 Days

The key secondary efficacy endpoint is OS up to 180 days.

OS up to 180 days will be calculated as date of death (from any cause) minus date of randomization plus 1. It will be censored

- at Study Day 180 if the subject is known to be alive at Study Day 180
- at day of last contact if subject gets lost to follow-up prior to Study Day 180.

6.1.2.2 Change from Baseline in Stages of GvHD by Target Organ

Stages of GvHD, by target organ, will be evaluated at the visits presented in Table 19. These stages will be used to determine the change from baseline in stages of GvHD by target organ at Study Day 28.

6.1.2.3 Chronic Graft-versus Host Disease up to 180 Days

The presence of GvHD is to be assessed at each visit (Table 19). The date of occurrence of chronic GvHD in an individual subject is to be recorded.

Chronic GvHD not requiring systemic treatment is defined as:

1. Mild abnormalities involving a single site, with platelet count >100,000 and no steroid treatment at the onset of chronic GvHD, which could include:

    - Oral abnormalities consistent with chronic GvHD, a positive skin or lip biopsy, and no other manifestations of chronic GvHD.
    - Mild liver test abnormalities (alkaline phosphatase [ALP] ≤2 × ULN, aspartate aminotransferase [AST] or alanine aminotransferase [ALT] ≤3 × ULN and total bilirubin ≤1.6) with positive skin or lip biopsy, and no other manifestations of chronic GvHD.

- Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of BSA, dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy and no other manifestations of chronic GvHD.
- Ocular sicca (Schirmer's test ≤5 mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of chronic GvHD.
- Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of chronic GvHD.

2. Platelet count <100,000 or steroid treatment at the onset of chronic GvHD:

- Involvement of two or more organs with symptoms or signs of chronic GvHD, with biopsy.
- Documentation of chronic GvHD in any organ.
- ≥15% baseline body weight loss not due to other causes, with biopsy documentation of chronic GvHD in any organ.
- Skin involvement more extensive than defined for clinical limited chronic GvHD, confirmed by biopsy.
- Scleroderma or morphea.
- Onycholysis or onychodystrophy thought to represent chronic GvHD, with documentation of chronic GvHD in any organ.
- Decreased range of motion in wrist or ankle extension due to fasciitis caused by chronic GvHD.
- Contractures thought to represent chronic GvHD.
- Oral involvement with functional impairment, refractory to topical treatment.
- Vaginal involvement with functional impairment, refractory to topical treatment.
- Bronchiolitis obliterans not due to other causes.
- Positive liver biopsy or abnormal liver function tests not due to other causes with ALP >2 × ULN, AST or ALT >3 × ULN, or total bilirubin >1.6, and documentation of chronic GvHD in any organ.
- Positive upper or lower GI biopsy.
- Fasciitis or serositis thought to represent chronic GvHD and not due to other causes.

Time to diagnosis of chronic GvHD up to 180 days is calculated as date of diagnosis of chronic GvHD minus date of randomization plus 1. Competing risk is death (if prior to Study

Day 180 and prior to diagnosis of chronic GvHD) with time to death calculated as date of death minus date of randomization plus 1.

For a subject without a diagnosis of chronic GvHD or death up to 180 days, the time will be censored at the earliest of the days below:

- at Study Day 180 if the subject is known to be alive without diagnosis of chronic GvHD at Study Day 180
- at day of last contact if subject gets lost to follow-up prior to Study Day 180.

6.1.2.4 Cumulative Steroid Dose

The dose and route of steroids used will be reported at the visits presented in Table 19.

6.1.2.5 Relapse and Relapse-Related Mortality

Clinical relapse of the underlying malignancy will be recorded, as identified by standard procedures. This could include, but is not limited to, physical examination, assessment of symptoms, complete blood count, imaging assessments, and bone marrow biopsy/aspirate.

The identification of minimal residual disease should not be considered as a clinical relapse.

Time to relapse up to 180 days is calculated as date of relapse diagnosis minus date of randomization plus 1. Competing risk is death prior to diagnosis of relapse (if prior to Study Day 180) with time to death calculated as date of death minus date of randomization plus 1.

For a subject without a relapse diagnosis or death up to 180 days, the time will be censored at the earliest of the days below:

- at Study Day 180 if the subject is known to be alive without a relapse diagnosis at Study Day 180
- at day of last contact if subject gets lost to follow-up prior to Study Day 180.

A relapse-related death is defined as any death with prior diagnosis of relapse. Time to relapse-related death up to 180 days is calculated as date of relapse-related death minus date of randomization plus 1. Competing risk is death without prior diagnosis of relapse (if prior to Study Day 180) with time to death without prior diagnosis of relapse calculated as date of death minus date of randomization plus 1.

For subjects without a death up to 180 days it will be censored at the earliest of the days below:

- at Study Day 180 if the subject is known to be alive at Study Day 180
- at day of last contact if subject gets lost to follow-up prior to Study Day 180.

6.1.2.6 Change from Baseline in Karnofsky Performance Status

The Karnofsky Performance Status scale allows subjects to be classified according to their functional impairment.

The change from baseline in the Karnofsky Performance Status scale will be derived for the timepoints noted in Table 19.

A copy of the Karnofsky Performance Status scale is provided in Appendix 2 (Section 12.2).

6.1.3 Exploratory Efficacy

Exploratory efficacy include:

- Quality of life (QoL) based on the SF36 assessed at the visits noted in Table 19.
- Duration of response up to Study Day 90 (calculated from the date at which PR or CR is documented).
- Overall response by standard-risk and high-risk GvHD (MacMillan et al., 2015).

6.2 Safety

6.2.1 Adverse Events

The investigator is responsible for recording all AEs observed in during the study, from the time that informed consent is signed through Study Day 58 or up to 30 days after the last dose of study treatment, whichever is later; at that time, any ongoing adverse drug reaction (ADR) and/or SAE event are to be followed until resolution (up to Study Day 180). AEs will be recorded in the subject's eCRF.

Second malignancies and deaths will be recorded as SAEs through Study Day 180.

Information about all AEs, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the AE page of the eCRF and followed as appropriate.

Diagnostic and therapeutic non-invasive and invasive (i.e. surgical) procedures will not be reported as AEs. However, the medical condition for which the procedure was performed must be reported if it meets the definition of an AE, unless it is a pre-existing (prior to stem cell transplantation (SCT)/chemotherapy) condition.

Underlying disease being studied (GvHD) will not be reported as an AE, unless is deemed as more severe than expected.

Medical conditions/diseases present before starting study drug are only considered AEs if they worsen after starting study treatment.

6.2.1.1 Definitions of AEs, Severity, Relationship (Causality), Action Taken and Outcome

Adverse events will be classified and their severity will be graded using the National Cancer Institute (NCI) CTCAE version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf).

Each AE is to be evaluated by the investigator for duration, severity, seriousness and causal relationship to the investigational drug. The action taken and the outcome must also be recorded.

NCI CTCAE Grade 3-4 laboratory abnormalities are to be recorded as AEs independent of associated signs or symptoms. The associated signs and symptoms should be considered as additional event terms and graded according to their own criteria.

Definitions of AEs

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this 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 product, medical treatment or procedure whether or not considered related.

An SAE, experience or reaction, is any untoward medical occurrence or effect (whether or not considered to be related to study drug) that at any dose meets one or more of the Seriousness (or Gravity) criteria listed below:

- Results in death.
- Is life-threatening (the subject is at a risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization: Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.
- Results in persistent or significant disability/incapacity.

- Is a congenital abnormality/birth defect.
- Other: Medically significant events, which do not meet any of the criteria above, but may be considered serious when, based on medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the other serious outcomes listed in the definition above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias (e.g. neutropenia or anemia requiring blood transfusion, etc.) or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

An ADR is defined as all untoward and unintended responses to an IMP related to any dose administered.

An Unexpected ADR is defined as any adverse reaction, whose nature, severity or frequency is not consistent with the applicable product information specified in the IB. Adverse Drug Reactions which are unexpected and satisfy one of the seriousness criteria specified above, meet the definition of Suspected Unexpected Serious Adverse Reactions (SUSARs). In addition, the following events are expected following stem cell transplant and / or diagnosis of acute GvHD (Copelan, 2006) and as such should not be considered as ADRs, unless in the investigator's opinion there is at least a possible relationship with study drug:

febrile neutropenia, mucositis (nausea, vomiting, abdominal pain, diarrhea), veno-occlusive disease (also known as sinusoidal obstructive syndrome, with resulting hepatomegaly, fluid retention, ascites, renal or respiratory failure), transplantation related lung injury (with pneumonitis/pulmonary infiltrates, respiratory failure), transplantation related infections, recurrence of acute GvHD (with rash, jaundice, abdominal pain, diarrhea, bloody stool), chronic GvHD (with resulting bronchiolitis, malabsorption, cholestasis, hematocytopenia), relapse of underlying disease and secondary malignancies (including but not limited to the skin, oral mucosa, brain, thyroid and bone).

Severity

Adverse events will be classified and their severity will be graded using the NCI CTCAE version 4.0.

Relationship

The causal relationship between the study medication and the AE will be determined by the investigator according to the following criteria:

- Not related: The event or laboratory test abnormality is most likely produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs, and does not follow a known response pattern to the study drug, or the temporal relationship of the event to study drug administration makes a causal relationship unlikely.
- Possibly related: The event or laboratory test abnormality follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug, but could have been produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs. Information on drug withdrawal may be lacking or unclear.
- Probably related: The event or laboratory test abnormality follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug, and unlikely to be explained by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs. There is a positive response to drug withdrawal. Rechallenge is not required.
- Certainly related: The event or laboratory test abnormality follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug, and cannot be reasonably explained by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs. There is a positive response to drug withdrawal. Rechallenge is positive.

Adverse Events with a causality of “Possibly”, “Probably” or “Certainly” related meet criteria of ADRs.

All efforts should be made to classify the AE according to the above categories.

Action Taken

Action taken with study treatment due to the event which bases on the following categories:

- None (when no action is undertaken with the investigational product due to the event)
- Drug temporarily withdrawn

- Drug permanently withdrawn

Outcome of AE

Adverse events may result in one of the following outcomes:

- Death (only if the AE term reported is considered to be the cause of Death. Please note: for consistency, if outcome is 'Death' also seriousness must be 'Death')
- Recovered/resolved
- Recovered with sequelae
- Recovering/resolving
- Not Recovered/resolved: the event does not resolve

6.2.1.2 Reporting of Adverse Events

All AEs occurred during the study, from the time that informed consent is signed through Study Day 58 or up to 30 days after the last dose of study treatment (whichever is later), and regardless of severity, are to be recorded on the appropriate AE pages (either 'serious' or 'non-serious') in the eCRF.

The investigator should complete all the details requested including dates of onset, severity, action taken, outcome, relationship to study drug. Each event should be recorded separately.

Further instructions are available in the training materials (i.e. eCRF and SAE Completion Guidelines).

6.2.1.3 Reporting of Serious Adverse Events and Unexpected Adverse Drug Reactions to the Regulatory Authorities and Investigators

All SAEs have to be reported, whether or not considered causally related to the IMP, or to the study procedure(s). All SAEs will be recorded in the eCRF.

If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate ADIENNE representatives immediately, or **no later than 24 hours** of when he or she becomes aware of it.

The designated ADIENNE representative works with the investigator to ensure that all the necessary information is provided to the ADIENNE Patient Safety data entry site **within one calendar day** of initial receipt for fatal and life-threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform ADIENNE representatives of any follow-up information on a previously reported SAE immediately, or **no later than 24 hours** of when he or she becomes aware of it.

The reference document for definition of expectedness/listedness is the IB for begelomab.

Investigators should follow local IRB/EC guidelines regarding reporting of AEs to their IRB/EC.

The Sponsor will be responsible for safety reporting to Competent Authorities, Investigators and IRBs/ECs as per requirements specified in the European Directive 2001/20/EC and in the Code of Federal Regulations 312.32 and associated guidances. In particular the Sponsor will be responsible for reporting:

- SUSARs that are fatal or life-threatening to the Competent Authorities and to the ECs no later than 7 days after knowledge of such cases, thus providing relevant follow-up information within an additional 8 days.
- All other SUSARs (i.e., non-fatal or life-threatening) to the Competent Authorities and to the ECs no later than 15 days after first knowledge of such cases.
- Inform all Investigators of any SUSARs.
- Once a year throughout the clinical trial, provide a listing of all Suspected Serious Adverse Reactions which have occurred during this period and a report of the subject's safety to the Member States in whose territory the clinical trial is being conducted and the ECs.

Hospitalization itself is a seriousness criterion by definition and is not considered as SAE. An event which led to hospitalization or prolongation to hospitalization should be reported as SAE.

6.2.1.4 Follow-Up of Adverse Events

Any AEs observed from screening/randomization up to Study Day 58 or up to 30 days after the last dose of study treatment, whichever is later, will be followed up to resolution (up to Study Day 180).

Resolution means that the subject has returned to a baseline state of health or the investigator does not expect any further improvement or worsening of the AE.

6.2.1.5 Adverse Events of Special Interest

A theoretical risk of decreased respiratory function during the infusion exists, though to date, no infusional toxicities have been reported. Infusional toxicity will be evaluated by monitoring the subject's vital signs and SaO_2/SAT via pulse oximetry prior to BEGEDINA (or conventional treatment) administration, 30 and 60 minutes from the start of the first administration and prior to and at 60 minutes from the start of subsequent administrations. Patients in the BEGEDINA arm will continue to be monitored (under hospital observation) through 2 hours after the start of infusion.

Hypoxia, Decreased in O_2 Sat, respiratory insufficiency, pulmonary failure, bronchospasm, infusion related reactions, anaphylactic reactions, infusion site reactions (phlebitis) will be recorded as AEs of special interest (AESI).

6.2.1.6 Pregnancy

Due to the nature and clinical conditions of the subjects receiving the investigational product in this study, pregnancy is highly unlikely to occur. Pregnant women are excluded from the study. In addition, male and female subjects enrolled in the study should take adequate measures in order to avoid any pregnancies of their sexual partners during the course of the study (or for at least 3 months following the last dose of study drug, whichever is longer). Acceptable methods of birth control include oral, injected or implanted hormonal methods of contraception, placement of an intrauterine device (IUD) or intrauterine system(IUS), barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. Abstinence is only considered an acceptable form of contraception when it is the usual life style of an individual (see Exclusion Criteria, Section 4.4.2).

At screening, a serum pregnancy test will be performed by the site personnel for all female subjects of childbearing potential who have not been started on an anti-ovulatory regimen prior to initiation of chemo-inductive regimen.

6.2.1.6.1 Reporting of Pregnancy

In the case that pregnancy should occur, the investigator must report any pregnancy to the ADIENNE Drug Safety Unit and the Medical Monitor as soon as possible and no later than 24 hours after becoming aware of it. The subject must be immediately discontinued from further treatment with study treatment. All pregnancies will be followed through to birth.

Pregnancies will be captured if they occur in female subjects from the time the subject is first exposed to the investigational product until 30 days after last exposure to the investigational product. Pregnancies will be captured if they occur in the sexual partners of male subjects from the time the subject is first exposed to the investigational product until 90 days after last exposure to the investigational product.

Any congenital abnormalities in the offspring of a subject who received investigational drug will be reported as an SAE. The outcome of any pregnancy and the presence or absence of any congenital abnormality will be recorded in the source documentation and reported to the Medical Monitor and Sponsor.

The investigator should regard the pregnancy or congenital abnormalities in the offspring as SAEs for the purposes of assessment, recording and reporting the data.

6.2.2 Clinical Laboratory Evaluations

Information on collection, processing and storage/shipping of sample material will be provided to all study centers in the study laboratory manual.

Clinical laboratory tests will be reviewed for results of potential clinical significance at all timepoints throughout the study. The investigator will evaluate any change in laboratory values. If the investigator determines a laboratory abnormality to be clinically significant, independent of CTCAE grading, this will have to be considered a laboratory AE; however, if the abnormal laboratory value is consistent with a current diagnosis, it may be documented accordingly.

All subjects should be fasted for 8 to10 hours prior to blood draws when indicated by local procedures (e.g., lipids, glucose).

If necessary, laboratory assessments may be performed by a laboratory other than the designated laboratory. In this case, normal ranges of test values for this laboratory should be provided to the Sponsor.

6.2.2.1 Laboratory Safety

Routine safety laboratory evaluations will be performed at the visits as outlined in Table 19. The laboratory safety parameters detailed in Table 24 will be analyzed at a local laboratory, using standard, validated laboratory methods. Serum beta-human chorionic gonadotropin pregnancy tests at screening will be performed by an appropriate clinical laboratory local to the study center, using standard, validated laboratory methods for female subjects of child-bearing potential.

Table 24 Laboratory Safety Assessments

Hematology	Basophils, eosinophils, hematocrit, hemoglobin, lymphocytes, monocytes, neutrophils, platelets, red blood cells, total white blood cells, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin. Optional (if routinely available): mean platelet volume, red cell distribution width.
Coagulation	Partial thromboplastin time, prothrombin time and international normalized ratio.
Serum chemistry	Albumin, ALP, ALT, AST, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, direct bilirubin, glucose, inorganic phosphorus, lactate dehydrogenase, lipase, potassium, sodium, total bilirubin, total protein, gamma-glutamyl transferase, creatine phosphokinase.
Lipid panel	Total cholesterol, HDL, LDL, triglycerides.
Urinalysis	Blood, glucose, ketones, microscopic exam, pH, protein, specific gravity. Optional (if routinely available): bilirubin.

6.2.2.2 Infection Markers

The infection markers to be collected will include: HIV-1 antibody, HIV-2 antibody, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, CMV DNA, Epstein-Barr virus DNA, hepatitis C antibody, erythrocyte sedimentation rate, C-reactive protein.

Blood samples for these assessments will be collected at the timepoints shown in Table 19.

Further details will be provided in the study laboratory manual.

6.2.2.3 Immunogenicity

In patients randomized to receive BEGEDINA, immunogenicity will be assessed based on serum anti-BEGEDINA antibodies (HAMA).

A blood sample for HAMA assays will be taken on Study Days 1, 5, 10, 14, 17, 21, 24, 28, 56, 90 to evaluate exposure-response relationship. HAMA serum concentrations will be quantified by means of an ELISA method. On study days in which BEGEDINA infusion is

planned, the sample will be drawn before infusion (i.e. earlier that day or immediately prior to the infusion). Further details will be provided in the study laboratory manual.

If a patient agrees to participate in the optional exploratory biomarkers research part of this study, leftover biological samples (serum) that remain after the planned immunogenicity samples may be analyzed for further investigations, e.g., exploratory biomarkers to assess correlations with disease activity, effects of study drug, and clinical outcomes. The results of this research will be reported separately from the results of this study and will not be included in the CSR.

6.2.2.4 Glucose Metabolism

Blood samples for the analysis of fasting glucose, insulin, c-peptide and glycosylated hemoglobin will be collected at the timepoints shown in Table 19. Urine samples for the analysis of glucose will be collected at the timepoints shown in Table 19.

Insulin resistance and beta-cell function will be analyzed using the corresponding Homeostasis Model Assessment (HOMA) indices (HOMA Calculator © The University of Oxford 2013).

Further details will be provided in the study laboratory manual.

6.2.3 Vital Signs, Physical Findings and Other Safety Assessments

6.2.3.1 Vital Signs

Vital signs will be assessed at the timepoints shown in Table 19. The measurements will include systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, oxygen saturation (peripheral capillary oxygen saturation [SpO_2], pulse oximetry) and body temperature. Vital signs will be collected prior to BEGEDINA (or conventional treatment) administration, 30 and 60 minutes from the start of the first administration and prior to and at 60 minutes from the start of subsequent administrations. Patients in the BEGEDINA arm will continue to be monitored (under hospital observation) through 2 hours after the start of infusion.

Vital signs will be measured after approximately 5 minutes of quiet rest with the subject in a sitting position.

Any clinically significant findings in vital sign measurements compared with baseline measurements should be recorded as an AE.

6.2.3.2 12-Lead Electrocardiogram

A 12-lead electrocardiogram (ECG) will be performed at the timepoints shown in Table 19, to assess any changes in cardiac physiology.

Subjects should be in a supine or semi-supine position for at least 5 minutes prior to the recording. The same recording position (supine or semi-supine) and the same equipment should be used for each subject throughout the study. All ECG printouts must be (1) reviewed by a medically qualified member of the study team, (2) annotated to indicate any clinical finding, (3) signed and dated by the medically qualified member, and (4) filed with the notes for the subjects. ECG parameters will be entered into the eCRF, including heart rate, PR, QRS and QTc and any ECG abnormality associated with an AE must be entered in the AE eCRF.

For the present study QTc will be calculated according to the formulas proposed by Fridericia (Fridericia, 1920) and by Bazett (Bazett, 1920):

- Fridericia's formula: $QTc = QT/RR^{0.33}$
- Bazett's formula: $QTc = QT/RR^{0.5}$

6.2.3.3 Physical Examination

A complete physical examination, including but not limited to a targeted examination for evidence of AEs, will be performed at the timepoints shown in Table 19.

A complete physical examination will include, at a minimum, assessment of the following systems: skin, head, ears, eyes, nose, and throat, respiratory system, cardiovascular system, GI system, neurological condition, blood and lymphatic systems, and the musculoskeletal system.

Information about physical examinations must be available in the source documentation at the study site. Significant findings that are present prior to the start of the study treatment must be included in the relevant eCRF on medical history/current medical conditions. Significant new findings made after the start of the study treatment that meet the definition of an AE or SAE must be recorded on the Adverse Event eCRF.

At the screening visit, height and weight should be measured; weight will also be measured at various other timepoints as shown in Table 19. The weight measurement at screening will be used to determine the dose of study treatment to be used throughout the subject's

treatment period. Measurement of height from a standing position should be performed with the subject's shoes removed, the knees straightened and head held upright. Measurement of weight should be performed without shoes or extra layers of clothing (e.g., sweater or jacket) during the measurement. Subjects should be weighed on the same scales at all visits.

6.2.4 Prior, Background, Concomitant Medications and Other Therapies

6.2.4.1 Medications for Prophylaxis, First-, Second- and Third-line Treatment

All agents to treat GvHD will be coded using the World Health Organization (WHO) Drug Dictionary (latest available version) and presented by preferred term for GvHD. If an agent used for prophylaxis of acute GvHD is discontinued and restarted prior to initiation of corticosteroids to treat acute GvHD, it is considered a prophylactic agent. If the agent is restarted after initiation of corticosteroids to treat acute GvHD, it would be considered a second-line agent.

6.2.4.2 Concomitant Medication and Supportive Therapy

Information on all concomitant medications will be collected for this study, including information on all concomitant therapy to treat acute GvHD. All pharmacologic and nonpharmacologic treatment used by study subjects to treat acute GvHD prior to screening should be recorded in the corresponding eCRF. This will include the treatment for HSCT procedure.

Subjects will continue to be treated with a stable dose of systemic steroid therapy until able to be tapered following response (at least 1 stage in 1 organ) to treatment (see section 5.7.2.4.1). Subjects may also continue any prior therapy used for prophylaxis at a stable dose from baseline if the therapy agent was not discontinued and restarted after initiating first-line steroid treatment for acute GvHD. No other GvHD medications are to be introduced to subjects during the initial 28 days post-BEGEDINA administration unless disease has progressed. Addition of other agents prior to Study Day 28 would constitute failure to respond (endpoint OR at Study Day 28), however the subject will remain on study for follow-up.

6.2.4.3 Standard of Care for Acute GvHD

All enrolled subjects will receive institutionally defined standard of care (i.e., maintenance of steroid treatment and other prophylactic treatment for acute GvHD). In addition, investigators should provide standard of care for prevention and treatment of viral infections,

particularly if there is evidence of viral reactivation. See Sections 5.7.2.4.1 and 5.7.2.4.2 for further discussion.

6.2.5 Acute Reaction to BEGEDINA Infusion

The possibility of an allergic reaction to the BEGEDINA infusion is discussed in Sections 5.1.1.1 and 5.7.2.3, and 6.2.1.5. Acute drug reactions associated with BEGEDINA infusion (i.e., those occurring within 1-2 hours of infusion) will be closely monitored and managed according to institutional guidelines.

No specific SAEs have been reported for BEGEDINA, and therefore the Sponsor will provide all study centers with a "global" safety monitoring plan according to usual complications that arise during acute GvHD.

6.2.6 Safety Monitoring

A Data and Safety Monitoring Board (DSMB) will be set up for the trial reviewing and evaluating unblinded data, and propose appropriate actions as necessary. The members of the DSMB will comprise physicians and a biostatistician with expertise in oncology, stem cell transplantation and conduct of clinical trials.

The composition of the DSMB and its specific working procedures will be described in a separate charter. This charter will clearly specify attendees, rules and responsibilities, procedures, review meeting schedule and the data to be analyzed. The DSMB will follow the DSMB Charter and data analyses will be done according to the DSMB Statistical Analysis Plan (SAP).

An initial assessment of safety will be performed after the first approximately 12 subjects in the BEGEDINA arm have stopped study treatment.

In addition to the regular monitoring of safety, one interim analysis including certain efficacy data is planned (see Interim Analysis, Section 8.6).

The DSMB may also meet in *ad hoc* meetings at its discretion as needed in response to events occurring in the trial.

6.3 Pharmacokinetic Assessments

Blood samples for PK analysis (including evaluation of circulating CD26) will be taken at the timepoints shown in Table 19 from subjects in the BEGEDINA arm. Analysis will be performed at the central laboratory. Further details will be provided in the study laboratory manual.

If a subject refuses blood collection for PK analysis, this will not be considered a protocol violation. If a subject discontinues from study drug but continues in the study for follow up, a last PK sample should be collected on the next scheduled visit after study drug discontinuation. No further samples will be required. HAMA and other scheduled assessments are to be followed as per protocol.

The following information will be captured for blood sample collection in each subject's eCRF:

1. Time and date of dose administration.
2. Time and date of each blood sample collected for PK analysis.

6.4 Health Outcomes

The subject's QoL will be assessed at the timepoints shown in Table 19 using the Short Form 36 (SF36) test. It is completed by the subject.

The SF36 is a 36-item scale constructed to survey health status and QoL (Ware and Sherbourne, 1992). The SF36 assesses 8 health concepts: limitations in QoL physical activities because of health problems; limitations in social activities because of physical or emotional problems; limitations in usual role activities because of physical health problems; bodily pain; general mental health (psychological distress and well-being); limitations in usual role activities because of emotional problems; vitality (energy and fatigue); and general health perceptions.

The standard form of the instruments asks for participants to reply to questions according to how they have felt over the previous week. The items use Likert-type scales, some with 5 or 6 points and others with 2 or 3 points. Sample items include "How much bodily pain have you had during the past 4 weeks" and "How much of the time during the past 4 weeks have you felt so down in the dumps nothing could cheer you up?"

A copy of the SF36 test is provided in Appendix 3 (see Section 12.3).

6.5 Appropriateness of Measurements

The appropriateness of measurements is discussed in Section 4.3.3.

7.0 QUALITY CONTROL AND QUALITY ASSURANCE

According to the Guidelines of GCP (CPMP/ICH/135/95), the Sponsor is responsible for implementing and maintaining Quality Assurance and quality control systems with written Standard Operating Procedures (SOPs).

Quality control will be applied to each stage of data handling.

The following steps will be taken to ensure the accuracy, consistency, completeness, and reliability of the data:

- Investigator meeting(s).
- Central laboratories for PK and immunogenicity testing.
- Center Initiation visit.
- Early center visits post-enrollment.
- Routine center monitoring.
- Ongoing center communication and training.
- Data management quality control checks.
- Continuous data acquisition and cleaning.
- Internal review of data.
- Quality control check of the final CSR.

In addition, Sponsor and/or authorized representative's Clinical Quality Assurance Department may conduct periodic audits of the study processes, including, but not limited to study center, center visits, central laboratories, vendors, clinical database and final CSR. When audits are conducted, access must be authorized for all study related documents including medical history and concomitant medication documentation to authorized Sponsor's representatives and Regulatory Authorities.

7.1.1 Monitoring

The Sponsor has engaged the services of a CRO, to perform all monitoring functions within this clinical study. The CRO monitors will work in accordance with the CRO's SOP and have the same rights and responsibilities as monitors from the Sponsor organization.

Monitors will establish and maintain regular contact between the Investigator and the Sponsor.

Monitors will evaluate the competence of each study center, informing the Sponsor about any problems relating to facilities, technical equipment or medical staff. During the study, monitors will check that written informed consent has been obtained from all subjects correctly and that data are recorded correctly and completely. Monitors are also entitled to compare entries in eCRFs with corresponding source data and to inform the investigator of any errors or omissions. Monitors will also control adherence to the protocol at the study center. They will arrange for the supply of investigational product and ensure appropriate storage conditions are maintained.

Monitoring visits will be conducted according to all applicable regulatory requirements and standards. Regular monitoring visits will be made to each center while subjects are enrolled in the study. The monitor will make written reports to the Sponsor on each occasion contact with the investigator is made, regardless of whether it is by phone or in person.

7.1.2 Data Management/Coding

Data generated within this clinical study will be handled according to the relevant SOPs of the Data Management and Biostatistics departments of the CRO.

Electronic Data Capture (EDC) will be used for this study, meaning that all eCRF data will be entered in electronic forms at the study center. Data collection will be completed by authorized study center staff designated by the investigator. Appropriate training and security measures will be completed with the investigator and all authorized study center staff prior to the study being initiated and any data being entered into the system for any study subjects.

All data must be entered in English. The eCRFs should always reflect the latest observations on the subjects participating in the study. Therefore, the eCRFs are to be completed as soon as possible during or after the subject's visit. To avoid inter-observer variability, every effort should be made to ensure that the same individual who made the initial baseline determinations completes all efficacy and safety evaluations. The investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available or not applicable or unknown, the investigator should indicate this in the eCRF. The investigator will be required to electronically sign off on the clinical data.

The monitor will review the eCRFs and evaluate them for completeness and consistency. The eCRF will be compared with the source documents to ensure that there are no discrepancies

between critical data. All entries, corrections and alterations are to be made by the responsible investigator or his/her designee. The monitor cannot enter data in the eCRFs. Once clinical data of the eCRF have been submitted to the central server, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who performed the change, together with time and date will be logged. Roles and rights of the site staff responsible for entering the clinical data into the eCRF will be determined in advance. If additional corrections are needed, the responsible monitor or Data Manager will raise a query in the EDC application. The appropriate study center staff will answer queries sent to the investigator. This will be audit trailed by the EDC application meaning that the name of investigational staff, time and date stamp are captured.

The eCRF is essentially considered a data entry form and should not constitute the original (or source) medical records unless otherwise specified. Source documents are all documents used by the investigator or hospital that relate to the subject's medical history, that verify the existence of the subject, the inclusion and exclusion criteria and all records covering the subject's participation in the study. They include laboratory notes, ECG results, memoranda, pharmacy dispensing records, subject files, etc.

The investigator is responsible for maintaining source documents. These will be made available for inspection by the study monitor at each monitoring visit. The investigator must submit a completed eCRF for each subject who receives study medication, regardless of duration. All supportive documentation submitted with the eCRF, such as laboratory or hospital records, should be clearly identified with the study and subject number. Any personal information, including subject name, should be removed or rendered illegible to preserve individual confidentiality.

The eCRF records will be automatically appended with the identification of the creator, by means of their unique User ID. Specified records will be electronically signed by the investigator to document his/her review of the data and acknowledgement that the data are accurate. This will be facilitated by means of the investigator's unique User ID and password; date and time stamps will be added automatically at time of electronic signature. If an entry on an eCRF requires change, the correction should be made in accordance with the relevant software procedures. All changes will be fully recorded in a protected audit trail, and a reason for the change will be required.

Adverse events will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). Concomitant medications will be coded using the latest version of the World Health Organization Drug Dictionary. Concomitant diseases/medical history will be coded using the latest version of MedDRA.

7.1.3 Quality Assurance Audit

Study centers, the study database and study documentation may be subject to Quality Assurance audit during the course of the study by the Sponsor or the CRO on behalf of the Sponsor. In addition, inspections may be conducted by regulatory bodies at their discretion.

8.0 STATISTICS

The SAP will be finalized prior to randomization of the first 30% of the planned number of subjects. Further statistical details can be found by referring to the SAP. The basis for the analysis plan can be found in this section.

8.1 Determination of Sample Size

This study will have two primary efficacy endpoints (see Section 6.1.1).

For the primary efficacy endpoint OR at Study Day 28, it is assumed that the conventional treatment would have a response rate of 50%, and further assumed that the response rate for the BEGEDINA treatment arm would be 75%. In order to have 80% power for a one-sided test at alpha=0.025, 58 patients per treatment group would be required.

For the other primary endpoint, TRM up to Study Day 180, the cumulative incidence for the conventional treatment is assumed to be 50% and the one for BEGEDINA is assumed to be 30% (relative reduction by 40%). 80% power for a one-sided log-rank test with significance level 0.025 requires a total of 103 deaths without prior diagnosis of relapse. These can be expected with 92 subjects per treatment group, provided there are no early dropouts and no diagnoses of relapse. If drop-outs or diagnoses of relapse are observed during the recruitment period, the sample size may be slightly increased, e.g., to 100 subjects per treatment group. Since the required sample size for TRM is larger than the one for overall response, a total sample size of 184 (up to 200, respectively) will be used.

Overall survival up to 180 days is the key secondary endpoint and the planned sample size provides adequate power to reject the null hypothesis of equal OS, assuming 20 to 25 percent differences in survival probabilities in favor of BEGEDINA.

8.2 Analysis Sets

Full Analysis Set

The FAS comprises all subjects to whom study treatment has been randomized. Statistical analyses will be based on study treatment groups as per randomization (and strata used within randomization), irrespective of the study treatment actually received. The FAS will be used for demographics, baseline disease characteristics and all efficacy analyses.

Safety Analysis Set

The Safety Analysis Set (SAF) includes all subjects who received at least one dose of study treatment. Subjects will be analyzed according to the study treatment (regimen) they actually received: if a subject has received any dose of BEGEDINA, the subject is assigned to the

BEGEDINA group within the Safety Analysis Set; otherwise the subject is assigned to the conventional treatment group. The Safety Analysis Set will be used for all safety analyses.

Pharmacokinetic analysis set

The pharmacokinetic analysis set consists of all subjects randomized to BEGEDINA with at least one study drug concentration value.

8.3 Statistical Methods

Descriptive statistics [e.g., number of subjects, mean and standard deviation, median, minimum, and maximum] will be provided for continuous variables and frequency counts and percentages will be displayed for categorical variables. All outputs will be generated by study treatment group.

All statistical tests will be conducted at a one-sided significance level of 2.5% unless otherwise specified. Treatment effect estimates will be reported with two-sided 95% confidence interval (CI).

8.3.1 Analysis of Demographics and Baseline Disease Characteristics

Demographics and baseline disease characteristics will be summarized by descriptive statistics.

Demographic data will include: gender, age, race and ethnicity, smoking status (never smoked, current smoker, previous smoker), weight, height, BMI. Baseline disease characteristics will include: medical history and medications related to GvHD, general medical history, physical examinations, vital signs, donor compatibility, HSCT source, grade of acute GvHD at onset, and grade of acute GvHD at baseline.

8.3.2 Analysis for Extent of Exposure

Extent of exposure to study treatment will be summarized by visit as well as for duration of study medication (date of last dose minus date of first dose +1).

8.3.3 Analysis of Concomitant Medication for Treatment of aGvHD

Treatments of acute GvHD will be tabulated, separately prior to randomization and after randomization.

8.3.4 Analysis of Primary Efficacy Endpoints

There are two primary efficacy endpoints as health authorities (EMA/FDA) have requested different primary endpoints: OR at Study Day 28 (see Section 6.1.1.1) is the single primary

endpoint for FDA, TRM up to Day 180 (see Section 6.1.1.2) is the single primary endpoint for EMA. The analysis methods are:

- the null hypothesis of equal OR probabilities for BEGEDINA and conventional treatment will be tested using a Cochran-Mantel-Haenszel test stratified for baseline GvHD severity grade (as used in the randomization) at the one-sided α =0.025 level. The treatment effect will be expressed as odds ratio with a two-sided 95% CI.
- TRM up to Study Day 180 will be graphically displayed by non-parametric cumulative incidence curve estimates. The null hypothesis of same TRM (defined as death without prior diagnosis of relapse) cumulative incidences for both study treatments will be tested by a one-sided test stratified for the baseline GvHD severity grade (as used in the randomization) taken into account the competing risk of relapse diagnosis. The one-sided significance level is set to α=0.025. Regression analysis of cause-specific hazards stratified for the baseline GvHD severity will be performed as supportive analysis in order to quantify the treatment effect in terms of hazard ratios and two-sided 95% CI.

8.3.5 Analyses of Secondary Efficacy Endpoints

8.3.5.1 Analysis of Key Secondary Efficacy Endpoint

The null hypothesis of same OS curves (up to Study Day 180) for both study treatments will be tested by a one-sided log-rank test stratified for the baseline GvHD severity grade (as used in the randomization). The one-sided significance level is set to α=0.025. A proportional hazards regression analysis stratified for the baseline GvHD severity will be performed as supportive analysis in order to quantify the treatment effect in terms of a hazard ratio and a two-sided 95% CI. OS will be graphically displayed by Kaplan-Meier plots.

The following two sequential testing procedures will be applied to control the one-sided type-I-error probability by 0.025:

A. For the primary endpoint preferred by the FDA:

1. Test of “OR at Day 28” by a stratified Cochran-Mantel-Haenszel test with one-sided significance level 0.025
2. Only if significant, then test “OS up to Study Day 180” by a stratified log-rank test with one-sided significance level 0.025
3. Only if significant, then test “TRM up to Study Day 180” by a stratified log-rank test with one-sided significance level 0.025.

Whenever a test does not lead to rejection of a null hypothesis, the test procedure A will be stopped.

B. For the primary endpoint preferred by the EMA:

1. "TRM up to Study Day 180" by a stratified log-rank test with one-sided significance level 0.025.
2. Only if significant, then test "OR at Study Day 28" by a stratified Cochran-Mantel-Haenszel test with one-sided significance level 0.025.
3. Only if significant, then test "OS up to Study Day 180" by a stratified log-rank test with one-sided significance level 0.025

Whenever a test does not lead to rejection of a null hypothesis, the test procedure B will be stopped.

8.3.5.2 Analysis of Other Secondary Efficacy Endpoints

All other secondary and supportive analyses will be tested at a one-sided α=0.025 significance level (where applicable), with no adjustments for multiplicity. These endpoints and their statistical analysis method are:

- Change from baseline in stages of GvHD by target organ at Study Day 28: shift tables displaying the frequencies for each possible change from baseline to Study Day 28.
- Cumulative incidence of chronic GvHD up to Study Day 180 will be graphically displayed by non-parametric cumulative incidence curve estimates. The null hypothesis of same cumulative incidences (up to Study Day 180) for both study treatments will be tested by a one-sided test stratified for the baseline GvHD severity grade, taken into account the competing risk of death. Stratified regression analysis of cause-specific hazards will be performed as supportive analysis in order to quantify the treatment effect in terms of hazard ratios and two-sided 95% CI. The cumulative steroid dose on Study Days 28, 56 and 90: descriptive statistics and non-parametric Wilcoxon-Mann-Whitney test stratified by for the baseline GvHD severity grade.
- Cumulative incidence of relapse up to Study Day 180 will be graphically displayed by non-parametric cumulative incidence curve estimates. The null hypothesis of same cumulative incidences (up to Study Day 180) for both study treatments will be tested by a one-sided test stratified for the baseline GvHD severity grade, taken into account the competing risk of death prior to diagnosis of relapse. Stratified regression analysis of cause-specific hazards will be performed as supportive analysis in order to quantify the treatment effect in terms of hazard ratios and two-sided 95% CI.
- Cumulative incidence of relapse-related mortality up to Study Day 180 will be graphically displayed by non-parametric cumulative incidence curve estimates. The null hypothesis of

same cumulative incidences (up to Study Day 180) for both study treatments will be tested by a one-sided test stratified for the baseline GvHD severity grade, taken into account the competing risk of transplant-related death (defined in Section 6.1.1.2). Stratified regression analysis of cause-specific hazards will be performed as supportive analysis in order to quantify the treatment effect in terms of hazard ratios and two-sided 95% CI.

- Change from baseline in Karnofsky Performance Status scale: shift tables displaying the frequencies for each possible change from baseline.

8.3.6 Analyses of Exploratory Efficacy Endpoints

Any further exploratory efficacy endpoints will be summarized by descriptive statistics. These endpoints include:

- The change in QoL from baseline based on the SF36.
- Duration of response.
- Overall response by standard-risk and high-risk GvHD (MacMillan et al., 2015).

8.3.7 Safety Analyses

For each study treatment, numbers of AEs and incidence rates will be tabulated by MedDRA system organ class and preferred term. An event that occurred one or more times on the date of, or subsequent to, randomization will contribute one observation to the numerator of the incidence rate. The denominator of the rate will comprise all randomized subjects exposed to the study treatment. If the intensity or seriousness of the AE changes, then the overall intensity or seriousness will be the maximum intensity or seriousness of the multiple occurrences.

Serious adverse events, AEs leading to death and AEs leading to withdrawal of subjects will be tabulated for each treatment group. Commonly occurring AEs, i.e. those which occur in 5% or more of the subjects in either treatment group, will be summarized using descriptive statistics.

Descriptive statistics will be used to present physical examination results, weight, BMI, vital signs measurements; clinical laboratory test results; and ECG results – for both raw values and change from baseline. Shift tables will be presented for selected tests.

8.3.8 Missing Data

Appropriate missing data techniques will be implemented for relevant efficacy endpoints. Multiple imputation will be used as the primary method for accounting for missing data. Details will be described in the SAP.

8.4 Pharmacokinetic Analyses

BEGEDINA concentrations will be evaluated using Nonlinear Mixed Effects Modeling (NONMEM®) to construct a Population Pharmacokinetic (PK) model. The model will be used to assess the significance of various covariates on PK of BEGEDINA, e.g., patient age, gender, body weight, renal function and relevant laboratory parameters.

8.5 Health Outcomes Analyses

The subject's QoL data will be summarized using descriptive statistics.

8.6 Interim Analyses

One unblinded interim analysis is planned when Day 28 response data is available for approximately 50% of the subjects. This analysis will be conducted by an independent, unblinded statistician and statistical programmer and results will be reviewed by the DSMB (see Section 6.2.6).

Two objectives are associated with this interim analysis:

- A non-binding futility analysis for which the conditional power will be calculated given the unblinded interim results with future projection under the observed trend as well as other more optimistic or skeptical assumptions for the future data. If the resulting conditional power is deemed to be too low, the study can be considered to be stopped. Since there is no possibility to stop for superiority, there will be no adjustment to the final significance level.If interim results for response at Study Day 28 is very convincing (i.e., with a one-sided p-value less than 0.001), the DSMB may recommend

  that an independent unblinded team (excluding the sponsor/CRO study specific team) submit the interim results to Health Authorities for conditional approval. The study will be continued as planned in order to determine the final results for response at Study Day 28, the results for TRM, overall survival, other secondary efficacy endpoints and safety. Since there is no possibility to stop for superiority, there will be no adjustment to the final significance level for response at Study Day 28 (as only the final results will be relevant for unconditional approval).

9.0 ETHICS

9.1 Institutional Review Board (IRB) or Independent Ethics Committee (IEC)

An Ethics Committee should approve the final protocol, including the final version of the Informed Consent Form (ICF) and any other written information and/or materials to be provided to the subjects. The investigator will provide the Sponsor or the CRO with documentation of IRB/IEC approval of the protocol and informed consent before the study may begin at the study center(s). The investigator should submit the written approval to the Sponsor or representative before enrolment of any subject into the study.

The Sponsor or representative should approve any modifications to the ICF that are needed to meet local requirements.

The investigator will supply documentation to the CRO of required IRB/IEC's annual renewal of the protocol, and any approvals of revisions to the informed consent document or amendments to the protocol.

The Sponsor or representative will report promptly to the IRB/IEC, any new information that may adversely affect the safety of subjects or the conduct of the study. Similarly, the Sponsor or representative will submit written summaries of the study status to the IRB/IEC annually, or more frequently if requested by the IRB/IEC. Upon completion of the study, the Sponsor (or delegated CRO) will provide the Ethics Committee with a brief report of the outcome of the study, if required.

The Sponsor or representative will handle the distribution of any of these documents to the national Regulatory Authorities.

The Sponsor or representative will provide Regulatory Authorities, Ethics Committees and Investigators with safety updates/reports according to local requirements, including Suspected Unexpected Serious Adverse Reactions, where relevant.

Each investigator is responsible for providing the IRB/IEC with reports of any serious and unexpected ADRs from any other study conducted with the study drug. The Sponsor or representative will provide this information to the investigator so that he/she can meet these reporting requirements.

9.2 Ethical Conduct of the Study

This study will be conducted and the informed consent will be obtained according to the ethical principles stated in the Declaration of Helsinki (52nd General Assembly, Edinburgh, Scotland, October 2000), the applicable guidelines for GCP (CPMP/ICH/135/95), or the applicable drug and data protection laws and regulations of the countries where the study will be conducted.

GCP is an international ethical and scientific quality standard for designing, conducting, recording and reporting studies that involve the participation of human subjects. The study will be conducted in compliance with GCP and the applicable national regulations so as to assure that the rights, safety and well-being of the participating study subjects are protected consistent with the ethical principles that have their origin in the Declaration of Helsinki.

9.3 Subject Information and Informed Consent

The ICF will be used to explain the risks and benefits of study participation to the subject in simple terms before the subject will be entered into the study. The ICF contains a statement that the consent is freely given, that the subject is aware of the risks and benefits of entering the study, and that the subject is free to withdraw from the study at any time. Written consent must be given by the subject and/or legal representative, after the receipt of detailed information on the study.

The investigator is responsible for ensuring that informed consent is obtained from each subject or legal representative and for obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study medication. The investigator will provide each subject with a copy of the signed and dated consent form.

10.0 STUDY ADMINISTRATION

10.1 Data Handling and Record Keeping

It is the investigator's responsibility to maintain essential study documents (protocol and protocol amendments, completed eCRFs, signed ICFs, relevant correspondence, and all other supporting documentation). The study site should plan on retaining such documents for approximately 15 years after study completion. The study site should retain such documents until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years after the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by the applicable regulatory requirements or the hospital, institution, or private practice in which the study is being conducted. Subject identification codes (subject names and corresponding study numbers) will be retained for this same period of time. These documents may be transferred to another responsible party, acceptable to Sponsor, who agrees to abide by the retention policies. Written notification of transfer must be submitted to Sponsor. The investigator must contact the Sponsor prior to disposing of any study records.

The US FDA regulations (21 CFR §312.62[c]) and the ICH Guideline for GCP (see Section 4.9 of the guideline) require that records and documents pertaining to the conduct of this study and the distribution of study drug, including eCRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 15 years after the last marketing application approval in an ICH region or after at least 15 years have elapsed since formal discontinuation of clinical development of the investigational. The Sponsor will notify the Principal Investigator of these events.

No records should be disposed of without the written approval of the Sponsor.

For studies conducted outside the United States under a U.S. IND, the Principal Investigator must comply with U.S. FDA IND regulations and with those of the relevant national and local health authorities.

10.2 Direct Access to Source Data/Documents

The investigator will prepare and maintain adequate and accurate source documents to record all observations and other pertinent data for each subject randomized into the study.

The investigator will allow the Sponsor, the delegated CRO, and authorized Regulatory Authorities to have direct access to all documents pertaining to the study, including individual subject medical records, as appropriate.

10.3 Investigator Information

10.3.1 Investigator Obligations

This study will be conducted in accordance with the ICH Harmonized Tripartite Guideline for GCP (GCP, 1997), the US CFR Title 21 parts 50, 56, and 312; and European Legislation; and the ethical principles that have their origin in the Declaration of Helsinki.

A summary of Investigator Obligations is provided in Appendix 5 (see Section 12.5).

The investigator agrees to conduct the clinical study in compliance with this protocol after the approval of the protocol by the IEC/IRB in compliance with local regulatory requirements. The Investigator and the Sponsor will sign the protocol to confirm this agreement.

10.3.2 Protocol Signatures

After reading the protocol, each investigator will sign the protocol signature page and send a copy of the signed page to the Sponsor or representative (Appendix 6, see Section 12.6). By signing the protocol, the investigator confirms in writing that he/she has read, understands and will strictly adhere to the study protocol and will conduct the study in accordance with ICH Tripartite Guidelines for GCP and applicable regulatory requirements. The study will not be able to start at any center where the investigator has not signed the protocol.

10.3.3 Publication Policy

The data generated by this study are confidential information of the Sponsor. The Sponsor will make the results of the study publicly available. The publication policy with respect to the investigator and study center will be set forth in the Clinical Trial Agreement.

10.4 Financing and Insurance

Sponsor will provide insurance in accordance with local guidelines and requirements as a minimum for the subjects participating in this study. The terms of the insurance will be kept in the study files.

11.0 REFERENCES

Bacigalupo A., Corte G, Ramarli D et al. Intravenous monoclonal antibody (BT 5/9) for the treatment of acute GVHD. *Acta Haematologica 1985, 73:185.*

Bacigalupo A. Antilymphocyte/thymocyie globulin for GVHD prophylaxis: efficacy and side effects. *Bone Marrow Transplant*. 2005;**35**:225–31.

Bacigalupo A. Acute graft-versus-host disease. *Immunotherapy*. 2011;**3**(12):1419–22.

Bacigalupo A, Palandri F. Management of acute graft versus host disease (GvHD). *Hematol J*. 2004;**5**(3):189-96.

Barnett A. DPP-4 inhibitors and their potential role in the management of type 2 diabetes. *Int J Clin Pract*. 2006;**60**(11):1454–70.

Bazett HC. An analysis of the time-relations of electrocardiograms. Heart. 1920;7:353-70

Benito AI, Furlong T, Martin PJ, et al. Sirolimus (rapamycin) for the treatment of steroid refractory acute GVHD. *Transplantation.* 2001;**72**(12):1924–9.

Bondanza A, Valtolina V, Magnani Z, et al. Suicide gene therapy of graft-versus-host disease induced by central memory human T lymphocytes. *Blood.* 2006;**107**(5):1828–36.

Brunner AM, Kim HT, Coughlin E, et al. Outcomes in patients age 70 or older undergoing allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Biol Blood Marrow Transplant*. 2013;**19**(9):1374–80.

Cahn JY, Klein JP, Lee SJ, et al. Prospective evaluation of 2 acute graft-versus-host (GVHD) grading systems: a joint Société Française de Greffe de Moëlle et Thérapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMTR) prospective study. *Blood*. 2005;**106**(4):1495–500.

Carpenter PA, MacMillan ML. Management of acute graft-versus-host disease in children. *Pediatr Clin North Am*. 2010;**57**(1):273–95.

Colombo AA, Alessandrino EP, Bernasconi P, et al. N-acetylcysteine in the treatment of steroid-resistant acute graft-versus-host disease: preliminary results. Gruppo Italiano Trapianto di Midollo (GITMO). *Transplantation* 1999;**68**(9):1414–6.

Copelan, EA. Hematopoietic stem-cell transplantation. *N Engl J Med*. 2006; **354**: 1813–1826

Cordero OJ, Yang CP, Bell EB. On the role of CD26 in CD4 memory T cells. *Immunobiology*. 2007;**212**(2):85–94.

Deeg HJ. How I treat refractory acute GVHD. *Blood*. 2007;**109**(10):4119–26.

De Meester I, Scharpé S, Vanham G, et al. Antibody binding profile of purified and cell bound CD 26. Designation of BT 5/9 and TA5.9 to the CD26 cluster. *Immunobiology*. 1993;**188**(1-2):145–58.

Dicker D. DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors. *Diabetes Care*. 2011;**34** Suppl 2:S276-8.

Dignan FL, Amrolia P, Clark A, et al.; Haemato-oncology Task Force of British Committee for Standards in Haematology; British Society for Blood and Marrow Transplantation. Diagnosis and management of chronic graft-versus-host disease. *Br J Haematol*. 2012;**158**(1):46-61.

Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*. 2006;**368**(9548):1696–705.

European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Guideline on preclinical safety evaluation of biotechnology-derived pharmaceuticals. London, June 2011. EMA/CHMP/ICH/731268/1998. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002828.pdf

Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;**373**(9674):1550–61.

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. *Biol Blood Marrow Transplant*. 2005;**11**(12):945–56.

Fridericia, LS. Die systolendauer im elektrokardiogramm bei normalem menschen und bei herzkranken. Acta Med Scand 1920; 53:489–494

Funke VA, de Medeiros CR, Setúbal DC, et al. Therapy for severe refractory acute graft-versus-host disease with basiliximab, a selective interleukin-2 receptor antagonist. *Bone Marrow Transplant*. 2006;**37**(10):961-5.

Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA matched sibling donors. *Transplantation*. 1974;**18**(4):295–304.

Goker H, Haznedaroglu CI, Chao NK. Acute GVHD: Pathobiology and management. *Exp Hematol*. 2001;**29**(3): 239–77.

Hings IM, Severson R, Filipovich AH, et al. Treatment of moderate and severe acute GVHD after allogeneic bone marrow transplantation. *Transplantation*. 1994;**58**(4):437–42.

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Inamoto Y, Martin PJ, Storer BE, Mielcarek M, Storb RF, Carpenter PA. Response endpoints and failure-free survival after initial treatment for acute graft-versus-host disease. *Haematologica*. 2014;**99**(2):385–91.

Leisenring WM, Martin PJ, Petersdorf EW, et al. An acute graft-versus-host disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens. *Blood*. 2006;**108**(2):749–55.

Levine JE, Logan B, Wu J, et al; Blood and Marrow Transplant Clinical Trials Network. Graft-versus-host disease treatment: predictors of survival. *Biol Blood Marrow Transplant*. 2010;**16**(12):1693–9.

MacMillan ML, Weisdorf DJ, Davies SM, et al. Early antithymocyte globulin therapy improves survival in patients with steroid-resistant acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2002;**8**(1):40–6.

MacMillan ML, DeFor TE, Weisdorf DJ. The best endpoint for acute GVHD treatment trials. *Blood*. 2010;**115**(26):5412–7.

MacMillan ML, Robin M, Harris AC, et al. A Refined Risk Score for Acute Graft-versus-Host Disease that Predicts Response to Initial Therapy, Survival, and Transplant-Related Mortality. *Biol Blood Marrow Transplant.* 2015;21(4):761-7.

Moretta A, Corte G, Mingari MC, Moretta L. Human T cell subpopulations defined by a monoclonal antibody. II. Evidence for cell cooperation in the response to alloantigens and generation of cytolytic cells. *J Immunol*. 1982;**128**(1):20–3.

Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. *Blood*. 1990;**76**(8):1464–72.

Martin PJ, Rizzo JD, Wingard JR, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2012;**18**(8):1150–63.

Massenkeil G, Rackwitz S, Genvresse I, Rosen O, Dörken B, Arnold R. Basiliximab is well tolerated and effective in the treatment of steroid-refractory acute graft-versus-host-disease after allogenic stem cell transplantation. *Bone Marrow Transplant*. 2002;**30**(12):899–903.

Mattern T, Scholz W, Feller AC, Flad HD, Ulmer AJ. Expression of CD26 (dipeptidyl peptidase IV) on resting and activated human T-lymphocytes. *Scand J Immunol*. 1991;**33**(6):737–48.

McCaul KG, Nevill TJ, Barnett MJ, et al. Treatment of steroid-resistant acute graft-versus-host disease with rabbit antithymocyte globulin. *J Hematother Stem Cell Res*. 2000;**9**(3):367–74.

Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;**15**(6):825–8.

Przepiorka D, Kernan NA, Ippoliti C, et al. Daclizumab, a humanized anti-interleukin-2 receptor alpha chain antibody, for treatment of acute graft-versus-host disease. *Blood*. 2000;**95**(1): 83–9.

Qian L, Wu Z, Shen J. Advances in the treatment of acute graft-versus-host disease. *J Cell Mol Med*. 2013;**17**(8):966–75.

Remberger M, Aschan J, Barkholt L, Tollemar J, Ringdén O. Treatment of severe acute graft-versus-host disease with anti-thymocyte globulin. *Clin Transplant*. 2001;**13**(3):147–53.

Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR severity index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. *Br J Haematol*. 1997;**97**(4):855–64.

Ruutu T, Gratwohl A, de Witte T et al. Prophylaxis and treatment of GvHD: EBMT-ELN working group recommendations for a standardized practice. *Bone Marrow Transplantion*. 2014; **14**:168-173.

Saliba RM, Couriel DR, Giralt S, et al. Prognostic value of response after upfront therapy for acute GVHD. *Bone Marrow Transplant*. 2012;**47**(1):125–31.

Van Lint MT, Milone G, Leotta S, et al. Treatment of acute graft-versus-host disease with prednisolone: significant survival advantage for day +5 responders and no advantage for nonresponders receiving anti-thymocyte globulin. *Blood*. 2006;**107**(10):4177–81.

Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Med Care*. 1992;**30**(6);473–83.

Weisdorf D, Haake R, Blazar B, et al. Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. *Blood*. 1990;**75**(4):1024–30.

12.0 APPENDICES

12.1 Appendix 1: Recommendations for Steroid Taper

Table 25 Recommendations for a Steroid Taper (Intravenous Methylprednisolone)

Dose of intravenous methylprednisolone	Day of taper
2 mg/kg/day divided into 2-3 doses	Days 0-6
2 mg/kg/day, once daily	Days 7-13
1.5 mg/kg/day	Days 14-21
1.0 mg/kg/day	Days 21-28
0.5 mg/kg/day	Days 29-35
0.4 mg/kg/day	Days 36-42
0.3 mg/kg/day	Days 43-49
0.2 mg/kg/day	Days 50-56
0.1 mg/kg/day	Days 57-63
0.1 mg/kg/day every other day	Days 63-69
Discontinue	Day 70

12.2 Appendix 2: Karnofsky Performance Status

	Karnofsky Performance Status scale ≥ 16 yrs.
100%	Normal, no complaints, no evidence of disease
90%	Able to carry on normal activity, minor signs or symptoms of disease
80%	Normal activity with effort, some signs or symptoms of disease
70%	Cares for self, unable to carry on normal activity or to do active work
60%	Requires occasional assistance from others but able to care for most needs
50%	Requires considerable assistance from others and frequent medical care
40%	Disabled, requires special care and assistance
30%	Severely disabled, hospitalization indicated, death not imminent
20%	Very sick, hospitalization necessary, active support, treatment necessary
10%	Moribund, fatal process progressing rapidly

12.3 Appendix 3: Short Form 36

Short Form (36) Health Survey

Hays, RD (1994). The *Medical Outcomes Study (MOS) Measures of Patient Adherence.* Retrieved April 19, 2004, from the RAND Corporation web site: http://www.rand.org/health/surveys/MOS.adherence.measures.pdf.

Short Form (36) Health Survey

1. In general, would you say your health is:	Excellent	Very Good	Good	Fair	Poor
	[1]	[2]	[3]	[4]	[5]

2. **Compared to one year ago**, how would you rate your health in general **now**?	Much better now than one year ago	Somewhat better now than one year ago	About the same	Somewhat worse now than one year ago	Much worse now than one year ago
	[1]	[2]	[3]	[4]	[5]

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

(Circle One Number on Each Line)	Yes, Limited a Lot	Yes, Limited a Little	No, Not limited at All
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	[1]	[2]	[3]
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	[1]	[2]	[3]
5. Lifting or carrying groceries	[1]	[2]	[3]
6. Climbing **several** flights of stairs	[1]	[2]	[3]
7. Climbing **one** flight of stairs	[1]	[2]	[3]
8. Bending, kneeling, or stooping	[1]	[2]	[3]
9. Walking **more than a mile**	[1]	[2]	[3]
10. Walking **several blocks**	[1]	[2]	[3]
11. Walking **one block**	[1]	[2]	[3]

12. Bathing or dressing yourself	[1]	[2]	[3]

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

(Circle One Number on Each Line)	Yes	No
13. Cut down the amount of time you spent on work or other activities	[1]	[2]
14. **Accomplished less** than you would like	[1]	[2]
15. Were limited in the **kind** of work or other activities	[1]	[2]
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	[1]	[2]

During the **past 4 weeks,** have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

(Circle One Number on Each Line)	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	[1]	[2]
18. **Accomplished less** than you would like	[1]	[2]
19. Didn't do work or other activities as **carefully** as usual	[1]	[2]

20. During the **past 4 weeks,** to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?	Not at all	Slightly	Moderately	Quite a bit	Extremely
	[1]	[2]	[3]	[4]	[5]

21. How much **bodily** pain have you had during the **past 4 weeks**?	None	Very mild	Mild	Moderate	Severe	Very Severe
	[1]	[2]	[3]	[4]	[5]	[6]

22. During the **past 4 weeks,** how much did **pain** interfere with your normal work (including both work outside the home and housework)?	Not at all	Slightly	Moderately	Quite a bit	Extremely
	[1]	[2]	[3]	[4]	[5]

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

(Circle One Number on Each Line)	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
23. Did you feel full of pep?	[1]	[2]	[3]	[4]	[5]	[6]
24. Have you been a very nervous person?	[1]	[2]	[3]	[4]	[5]	[6]
25. Have you felt so down in the dumps that nothing could cheer you up?	[1]	[2]	[3]	[4]	[5]	[6]
26. Have you felt calm and peaceful?	[1]	[2]	[3]	[4]	[5]	[6]
27. Did you have a lot of energy?	[1]	[2]	[3]	[4]	[5]	[6]
28. Have you felt downhearted and blue?	[1]	[2]	[3]	[4]	[5]	[6]
29. Did you feel worn out?	[1]	[2]	[3]	[4]	[5]	[6]
30. Have you been a happy person?	[1]	[2]	[3]	[4]	[5]	[6]
31. Did you feel tired?	[1]	[2]	[3]	[4]	[5]	[6]

32. During the **past 4 weeks,** how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)? **(Circle One Number)**	All of the time [1]	Most of the time [2]	Some of the time [3]	A little of the time [4]	None of the time [5]

How TRUE or FALSE is <u>each</u> of the following statements for you.

(Circle One Number on Each Line)	Definitely True	Mostly True	Don't not Know	Mostly False	Definitely False
33. I seem to get sick a little easier than other people	[1]	[2]	[3]	[4]	[5]
34. I am as healthy as anybody I know	[1]	[2]	[3]	[4]	[5]
35. I expect my health to get worse	[1]	[2]	[3]	[4]	[5]
36. My health is excellent	[1]	[2]	[3]	[4]	[5]

12.4 Appendix 4: ECOG Performance Scale

Developed by the Eastern Cooperative Oncology Group, Robert L. Comis, MD, Group Chair.*

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

*Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-655.

12.5 Appendix 5: Summary of Investigator Obligations

The responsibilities of the investigator include to:

- Be thoroughly familiar with the properties of the IMP as described in the IB.
- Ensure that he/she has sufficient time to conduct and complete the study, has adequate staff and appropriate facilities and that other studies do not divert essential subjects or facilities away from the study in hand.
- Provide, upon request, retrospective data on numbers of subjects who satisfied the proposed entry criteria during preceding time periods, in order to assure an adequate recruitment rate for the study.
- Submit an up-to-date curriculum vitae and other credentials to the Sponsor/CRO and where required to relevant authorities.
- Agree and sign the Clinical Trial Protocol (CTP) with the Sponsor confirming that he/she will work according to the CTP and GCPs and accepting the role of the Monitor and the need for control procedures as stated in this protocol.
- Nominate (if appropriate) a local Study Co-ordinator or Co-investigator(s) to assist in the management of the study.
- Follow the submission of notification/application to relevant bodies including local hospital management and to Ethics Committee jointly with the Sponsor/CRO, where appropriate.
- Provide information to all staff members involved with the study.
- Fully inform study subjects about the clinical study and obtain their Informed Consent.
- Certify that all IMP has been correctly delivered, stored, and safely handled, and that reconciliation of stock can be justified. Account must be given of any discrepancies; certificates of delivery and returns must be signed following the trial randomization code.
- Collect, record, and report data properly.

- Notify the Sponsor/CRO immediately in the case of an SAE and take appropriate measures to safeguard subjects.
- Promptly report to Ethics Committee and Sponsor changes increasing the risk to subjects and/or affecting significantly the conduct of the study and new information that may affect adversely the safety of the subjects or the conduct of the study.
- Agree with and sign the Final Report of the study, if requested.
- Ensure that the confidentiality of all information about subjects is respected by all persons involved as well as the information supplied by the Sponsor.
- Make all data available for direct access to the Sponsor/CRO personnel (e.g., the Monitor, Auditor), Ethics Committee C or Competent Authorities for validation/audit/review/inspection purposes.
- Ensure that medical records are clearly marked to show that the subject is participating in a clinical study.
- Inform the family doctor, with subject's consent, about subject's participation in the study.
- Provide a list of appropriately qualified persons (Study Personnel Form or equivalent) to whom the investigator has delegated some duties relevant to the conduct of the study, together with their signatures and initials.
- Submit, during the study, on a regular basis, written summaries of the study status to the Ethics Committee, when requested.

12.6 Appendix 6: Signature of Investigator

PROTOCOL TITLE: Prospective, phase II/III, randomized clinical study to compare BEGEDINA® versus "conventional treatment" for treating steroid resistant acute graft-versus-host disease

PROTOCOL NO: *ADN011*

This protocol is a confidential communication of the Sponsor (ADIENNE SA). I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices (GCP) and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from ADIENNE SA.

I have read this protocol in its entirety and agree to conduct the study accordingly:

Signature of Investigator: _____ Date: _____

Printed Name: _____

Investigator Title: _____

Name/Address of Center: _____

_____

_____