22 February 2018
EMA/155284/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Mylotarg

International non-proprietary name: gemtuzumab ozogamicin

Procedure No. EMEA/H/C/004204/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

Term	*Definition*
AAS	Amino Acid Substitution
AcBut	(4-(4'acetylphenoxy)butanoic acid)
ADA	anti-drug antibody
ADC	antibody-drug conjugate
ADE	AraC/DNR/etoposide
ADR	adverse drug reaction
AE	adverse event
ALFA	Acute Leukemia French Association
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
APL	acute promyelocytic leukaemia
AraC	cytarabine
AST	aspartate aminotransferase
AT	As Treated
AUC	area under the plasma concentration-time curve
BCRP	breast cancer resistance protein
BMA	bone marrow aspirate
BSA	bovine serum albumin
CD	Cluster of Differentiation
CDR	Complementarity determining region
CFU	Colony Forming Units
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
CL	Clearance
CLL1	C-type lectin-like molecule-1
Cmax	Maximum Serum Concentration
CNS	central nervous system
COG	Children's Oncology Group
COSY	correlation spectroscopy
CQA	critical quality attribute
CR	complete remission
CR1	first CR
CRF	case report form
CRp	complete remission with incomplete platelet recovery
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
CYP	Cytochrome P450
DClo	daunorubicin and clofarabine
DL	dose level
DMH	dimethylhydrazide
DNA	deoxyribonucleic acid
DNR	daunorubicin
DS	drug substance
EC	European Community
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ED50, ED90, ED95	dose that produces 50%/90%/95% of maximum response
EFS	event-free survival
ELISA	enzyme-linked immunosorbent assay
ELN	European Leukemia Network
EMA	European Medicines Agency
Emax	maximum achievable response
EOP	end of production

EU	European Union
EU	Endotoxin Units
FDA	Food and Drug Administration
FLAG-Ida	fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin
FLT3	FMS-like tyrosine kinase 3 gene
G-CSF	granulocyte colony-stimulating factor
GMP	Good Manufacturing Practice
GO	gemtuzumab ozogamicin (Mylotarg)
GOELAMS	Groupe Ouest Est d'Etude des Leucémies aiguës et Autres Maladies du Sang
HC	Heavy chain
HiDAC	high-dose AraC
HL-60	CD33-positive human leukaemia cell line
HMBC	heteronuclear multiple-bond correlation spectroscopy
HPLC	High-Performance Liquid Chromatography
HR	hazard ratio
HSCT	haematopoietic stem cell transplant
ICD	Informed Consent Document
ICH	International Conference on Harmonisation
Ida	idarubicin
IIR	investigator-initiated research
IPD	individual patient data
IR	infrared radiation
ISS	Integrated Summary of Safety
IV	intravenous
IWG	International Working Group
K	Lysine
kdes,	decay coefficient of the time-dependent clearance
LC	Light chain
LDAC	low dose AraC
LSC	leukaemic stem cell
MAA	marketing authorisation application
mAb	monoclonal antibody
MAH	marketing authorisation holder
MDS	myelodysplastic syndromes
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
MRC	Medical Research Council
MRD	minimal residual disease
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NAc	N-acetyl
MCB	Master Cell Bank
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NCRI	National Cancer Research Institute
NDA	New Drug Application
NE	not estimable
NEC	not elsewhere classified
NMR	nuclear magnetic resonance spectroscopy
No GO	chemotherapy alone, without GO
NOPHO	Nordic Society of Paediatric Hematology and Oncology
NPM	nucleophosmin gene
NS	not significant
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
OR	odds ratio
ORR	overall response rate
OS	overall survival
PBS	phosphate-buffered saline
PD	pharmacodynamics
PDX	patient-derived xenograft

P-gp	P-glycoprotein
PIP	Paediatric Investigation Plan
Ph. Eur.	European Pharmacopoeia
PK	pharmacokinetic(s)
PMAR	Population Modelling Analysis Reports
PP	per protocol
PS	performance status
PSUR	Periodic Safety Update Report
PT	Preferred Term
PXRD	Powder X-ray diffraction
QoL	Quality of Life
R	Arginine
RBC	red blood cell
RFS	relapse-free survival
RP-HPLC	Reverse Phase High-Performance Liquid Chromatography
RSI	Reference Safety Information
SAE	serious adverse event
SCE	Summary of Clinical Efficacy
SCS	Summary of Clinical Safety
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	size exclusion chromatography
SMQ	Standardised MedDRA Query
SOC	System Organ Class
SOS	sinusoidal obstruction syndrome
SWOG	Southwest Oncology Group
t½	terminal phase half-life
TEAE	treatment-emergent AE
TLS	tumour lysis syndrome
UGT	UDP-glucuronyl transferase
USA	United States of America
ULN	upper limit of normal
UV	ultraviolet
V1	volume of distribution in central compartment
V2	volume of distribution in peripheral compartment
VOD	veno-occlusive disease
Vd	volume of distribution
WBC	white blood cell
WCB	Working Cell Bank
WHO	World Health Organization
WT1	Wilms' tumour suppressor gene

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Pfizer Limited submitted on 1 December 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Mylotarg, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 March 2015.

Mylotarg was designated as an orphan medicinal product EU/3/00/005 on 18 October 2000 in the following condition: treatment of acute myeloid leukaemia.

The applicant applied for the following indication: for combination therapy with daunorubicin (DNR) and cytarabine (AraC) for the treatment of adult patients with previously untreated, de novo acute myeloid leukaemia (AML).

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Mylotarg as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/Find medicine/Human medicines/European public assessment reports.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that gemtuzumab ozogamicin was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicant's own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0078/2016 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0078/2016 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

New active Substance status

The applicant requested the active substance gemtuzumab ozogamicin contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Scientific Advice

The applicant received from the CHMP on 28 April 2016. The Scientific Advice pertained to quality aspects of the dossier.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Nithyanandan Nagercoil Co-Rapporteur: Sinan B. Sarac

- The application was received by the EMA on 1 December 2016.
- The procedure started on 23 December 2016.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 10 March 2017. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 16 March 2017. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 24 March 2017.
- During the meeting on 21 April 2017, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 12 October 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 20 November 2017.
- During the PRAC meeting on 30 November 2017, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP.
- During the CHMP meeting on 14 December 2017, the CHMP agreed on a list of outstanding issues to be sent to the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 22 January 2018.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 7 February 2018.
- The CHMP adopted a report on similarity of Mylotarg with Vidaza (azacitidine), Dacogen, (decitabine), Ceplene, (histamine dihydrochloride) and Rydapt (midostaurin) on 22 February 2018 (Appendix 1).
- During the meeting of 19-22 February 2018, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Mylotarg on 22 February 2018.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Mylotarg is intended for the treatment of adolescents aged 15 to 17 years and adult patients with previously untreated, de novo CD33-positive acute myeloid leukaemia (AML), except acute promyelocytic leukaemia (APL), in combination therapy with daunorubicin (DNR) and cytarabine (AraC).

2.1.2. Epidemiology and risk factors, screening tools / prevention

In Europe, the annual incidence of AML in adults is 5 to 8 cases per 100.000 individuals with a mortality rate of 4 to 6 cases per 100.000. (1) The median age at diagnosis is 67 years, but the incidence increases by age with a projected incidence of 15 to 25 cases per 100.000 in patients who are 70 years of age or older. (2) (3)

2.1.3. Biologic features

Acute myeloid leukaemia is a form of leukaemia, characterised by infiltration of proliferative, clonal, abnormally differentiated, and occasionally poorly differentiated haematopoietic cells of myeloid lineage in the bone marrow, blood, and other tissues.

2.1.4. Clinical presentation, diagnosis and stage / prognosis

AML is a heterogeneous disease; the classification is based on morphologic, cytogenetic, molecular, and immunophenotypic features, which, along with baseline patient characteristics such as age and performance status (PS), influence outcome and treatment recommendations (4). Among these, baseline cytogenetic risk constitutes one of the most significant prognostic markers of disease outcome (5). Age is the most prominent patient-specific risk factor, and cytogenetics the most disease-specific risk factor.

In AML, leukaemic blasts replace normal blood cells in bone marrow and peripheral blood, which leads to anaemia, neutropenia, and thrombocytopenia. This is associated with symptoms of fatigue, shortness of breath, disturbed wound healing, infections and bleedings. If left untreated, AML results in death within a few weeks to months.

Long-term survival in adult patients with AML is only 35% to 40% for patients ≤60 years of age, and drops to 5% to 15% in patients who are >60 years of age. (6) The majority of patients with AML will have relapsed disease within 3 years. (7)

2.1.5. Management

The general therapeutic strategy in patients with AML has not changed substantially in more than 30 years. The standard regimen ‘‘3+7’’, established in 1973, consisted of 3 consecutive daily infusions of DNR and 7 days of continuous infusion of AraC.

The standard treatment has for many years consisted of an induction treatment in order to achieve complete remission (CR). When CR has been obtained, 2 courses of consolidation therapy usually are performed, in order to eliminate undetected residual disease. Patient who do not achieve CR after the induction have a poor prognosis. Although some patients older than 60 years, with ECOG PS 0 to 2 and minimal comorbidity may benefit from standard 3+7, the therapeutic options for patients with poor functional status or comorbidities often include low intensity therapy such as subcutaneous AraC, azacitidine, or decitabine. The majority of patients with AML whether in CR after consolidation therapy or not, will have relapsed disease within 3 years. Achievements in the treatment of AML have mainly focused on younger patients. The introduction of allogeneic stem cell transplantation has improved the outcome in selected patient groups, especially those with intermediate and high risk disease with 3 years LFS of 60%. However patients who are not in remission at the time of transplantation have a dismal prognosis. It is of clinical benefit, that patients achieve CR are risk stratified in order to select those patients that might benefit from an HSCT.

In the EU (European Union), recently approved agents include decitabine (Dacogen) which is authorised for the treatment of adult patients with newly diagnosed de novo or secondary AML, according to the World Health Organisation (WHO) classification, who are not candidates for standard induction chemotherapy. Azacitidine (Vidaza) is also authorised for the treatment of adult patients Vidaza is indicated for the treatment of adult patients who are not eligible for haematopoietic stem cell transplantation (HSCT) with AML with 20-30 % blasts and multi-lineage dysplasia, according to WHO classification and AML with >30% marrow blasts according to the WHO classification. In addition, histamine dihydrochloride (Ceplene) is authorised for adult patients with AML in first remission concomitantly treated with interleukin-2 (IL-2). Finally, midostaurin (Rydapt) is authorised in combination with standard daunorubicin and cytarabine induction and high dose cytarabine consolidation chemotherapy followed by midostaurin single agent maintenance therapy for adult patients with newly diagnosed AML who are FLT3 mutation positive.

Still new therapeutic options that could improve survival of patients, and prevent or delay relapse of the disease remain an important unmet medical need for patients with previously untreated de novo AML.

About the product

Gemtuzumab ozogamicin is a CD33-directed ADC. Gemtuzumab is a humanised immunoglobulin class G subtype 4 (IgG4) antibody which specifically recognises human CD33. The antibody portion binds specifically to the CD33 antigen, a sialic acid-dependent adhesion protein found on the surface of myeloid leukaemic blasts and immature normal cells of myelomonocytic lineage, but not on normal haematopoietic stem cells. The small molecule, N acetyl gamma calicheamicin, is a cytotoxic semisynthetic natural product. N acetyl gamma calicheamicin is covalently attached to the antibody via an AcBut (4-(4-acetylphenoxy) butanoic acid) linker. Non-clinical data suggest that the anticancer activity of gemtuzumab ozogamicin is due to the binding of the ADC to CD33-expressing tumour cells, followed by internalisation of the ADC CD33 complex, and the intracellular release of N acetyl gamma calicheamicin dimethyl hydrazide via hydrolytic cleavage of the linker. Activation of N acetyl gamma calicheamicin dimethyl hydrazide induces double stranded deoxyribonucleic acid (DNA) breaks, subsequently inducing cell cycle arrest and apoptotic cell death. (SmPC, section 5.1).

The applicant requested the approval for the following indication:

Mylotarg is a CD33-directed antibody-drug conjugate indicated for combination therapy with daunorubicin (DNR) and cytarabine (AraC) for the treatment of adult patients with previously untreated, de novo acute myeloid leukaemia (AML).

The final indications following CHMP review of this application is:

MYLOTARG is indicated for combination therapy with daunorubicin (DNR) and cytarabine (AraC) for the treatment of patients age 15 years and above with previously untreated, de novo CD33-positive acute myeloid leukaemia (AML), except acute promyelocytic leukaemia (APL) (SmPC, section 4.1).

For the induction phase, the recommended dose of Mylotarg is 3 mg/m^2/dose (up to a maximum of one 5 mg vial) infused over a 2 hour period on Days 1, 4, and 7 in combination with DNR 60 mg/m2/day infused over 30 minutes on Day 1 to Day 3, and AraC 200 mg/m2/day by continuous infusion on Day 1 to Day 7. Mylotarg should not be administered during second induction therapy (SmPC, section 4.2).

For the consolidation phase, for patients experiencing a complete remission (CR) following induction, defined as fewer than 5% blasts in a normocellular marrow and an absolute neutrophil count (ANC) of more than 1.0 × 10^9 cells/L with a platelet count of 100 × 10^9/L or more in the peripheral blood in the absence of transfusion, up to 2 consolidation courses of intravenous DNR (60 mg/m^2 for 1 day [first course] or 2 days [second course]) in combination with intravenous AraC (1,000 mg/m^2 per 12 hours, infused over 2 hours on Day 1 to Day 4) with intravenous Mylotarg (3 mg/m^2/dose infused over 2 hours up to a maximum dose of one 5 mg vial on Day 1) are recommended (SmPC, section 4.2).

Type of Application and aspects on development

The applicant had CHMP Scientific Advice in February 2016 (EMEA/H/SA/3285/1/2016/PA/I) on the proposed data package to demonstrate acceptable product quality in relation to the amino acid substitution (AAS) in the antibody portion of the drug substance.. The applicant had no CHMP Scientific Advice regarding the clinical development.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as powder for concentrate for solution for infusion containing 5 mg of gemtuzumab ozogamicin as active substance. After reconstitution, the solution contains 1 mg gemtuzumab ozogamicin per mL. Other ingredients are: dextran 40, sucrose, sodium chloride, sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate anhydrous. The product is available in an amber Type 1 glass vial, with butyl rubber stopper and crimp seal with flip-off cap.

Although this dossier is not considered a Quality by Design application, certain elements of an enhanced approached were applied as described in the relevant sections below.

Mylotarg (gemtuzumab ozogamicin) has been in commercial production and on the market in the US from 2000-2010 and again in 2017, and in Japan since 2005. The current application is a re-submission. In 2007, the CHMP considered that the Quality package for Mylotarg was generally acceptable. During development, a significant level of amino acid substitution (AAS) was discovered at multiple sites within the antibody part of the active substance. The root cause of the AAS was identified. Primarily based on the AAS observed, several major deficiencies, two joint with clinical, were identified in the initial quality package submitted in support of the marketing authorisation application (MAA). None of the clinical studies supporting the current MAA used exclusively elevated AAS gemtuzumab batches, and as such do not fully represent the proposed commercial product. During the review, the applicant has provided additional data confirming the comparability of the clinical material with the proposed commercial

material in regards to the amino acid substitution in the antibody portion of the drug substance. The control strategy has also been reviewed to ensure a consistent product.

2.2.2. Active Substance

Gemtuzumab ozogamicin (Mylotarg) is an antibody drug conjugate (ADC) of humanized CD33-directed monoclonal IgG4 antibody covalently bonded to the activated calicheamicin derivative, a semi-synthetic derivative of gamma calicheamicin.

Gemtuzumab was selected to target CD33 expressed on the majority of leukemic cells from patients with acute myeloid leukemia (AML); it has an average theoretical molecular mass of 148 KDaltons.

Calicheamicin is a potent cytotoxin that, once conjugated to the anti-CD33 monoclonal antibody, allows target directed therapy. The calicheamicin is bound to the antibody via a linker which forms an amide bond with the antibody and forms a disulfide bond with the calicheamicin. The linker also contains an internal hydrazone bond, which is acid-labile.

The structure of the active substance is provided in Figure 1.

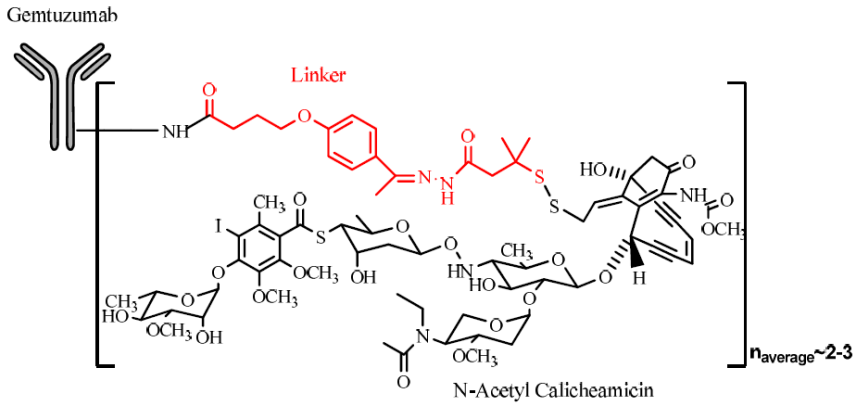


Figure 1 Structure of Gemtuzumab ozogamicin

Manufacture, process controls and characterisation

Wyeth Pharmaceuticals Division of Wyeth Holdings Corporation, a subsidiary of Pfizer Inc., Pearl River, NY, USA, is responsible for the manufacture, release and stability testing, and storage of the active substance gemtuzumab ozogamicin and of the finished product, Mylotarg. The marketing authorisation holder (MAH) is Pfizer Limited, Sandwich UK.

In the manufacture of Mylotarg two separate intermediate materials are used: the **activated calicheamicin derivative** and the monoclonal antibody **gemtuzumab**.

The manufacture, characterisation, control and stability of the two intermediates (the **activated calicheamicin derivative** and **gemtuzumab**) and the active substance **gemtuzumab ozogamicin** are described below in three separate sections.

Intermediate activated calicheamicin

General Information (Intermediate activated calicheamicin)

The chemical structure of activated calicheamicin was elucidated by a combination of infrared radiation (IR), ultraviolet (UV), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR). The results demonstrate that the activated calicheamicin derivative has the expected structure.

The activated calicheamicin derivative is a white to off white powder, amorphous solid, exhibits no crystalline properties, is hygroscopic and is not soluble in water or aqueous solutions.

Description of manufacturing process and process controls *(Intermediate activated calicheamicin)*

Starting from a working cell bank, fermentation of M. echinospora strain produces γ-calicheamicin. The γ-calicheamicin solution is used to produce N-acetyl calicheamicin. Lastly, the activated linker is added to N-acetyl calicheamicin to produce activated calicheamicin derivative.

The activated calicheamicin intermediate is transferred into glass bottles prior to drying and subsequent storage at the recommended storage temperature.

Control of Materials *(Intermediate activated calicheamicin)*

Sufficient information on raw materials used in the intermediate activated calicheamicin manufacturing process has been submitted. Sufficient information is provided regarding the cell bank system Master Cell Bank (MCB) and Working Cell Bank (WCB) and its testing. The banks are monitored according to a pre-approved stability protocol.

Control of critical steps and intermediates *(Intermediate activated calicheamicin)*

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the manufacturing process of the activated calicheamicin derivative, including its purification and isolation, is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests.

Process validation *(Intermediate activated calicheamicin)*

The intermediate activated calicheamicin manufacturing process has been validated adequately. Consistency in production has been shown. All acceptance criteria for the critical operational parameters and likewise acceptance criteria for the in-process tests are fulfilled demonstrating that the purification process consistently produces intermediate activated calicheamicin of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

Characterisation *(Intermediate activated calicheamicin)*

The structure and characteristics of the activated calicheamicin derivative have been determined using several physico-chemical analyses. Full characterisation by IR, UV, MS, NMR has been carried out. The results demonstrate that the activated calicheamicin derivative has the expected structure.

Specification *(Intermediate activated calicheamicin)*

Specifications tests and limits are provided and are overall considered acceptable.

Analytical methods *(Intermediate activated calicheamicin)*

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines. The specifications make adequate reference to the internal analytical method identifiers.

Batch analysis *(Intermediate activated calicheamicin)*

Batch analysis data have been provided, including production batches manufactured with the current manufacturing process. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials *(Intermediate activated calicheamicin)*

A reference standard has been established for activated calicheamicin derivative.

Stability *(Intermediate activated calicheamicin)*

The stability results under long term and accelerated conditions have been provided indicating that the intermediate activated calicheamicin is sufficiently stable and justify the proposed retest period in the proposed container.

Intermediate gemtuzumab

General Information *(Intermediate gemtuzumab)*

Gemtuzumab (KD1, hP67.6 antibody) is an engineered monoclonal antibody (mAb) and consists of a human IgG4 kappa framework with putative complementarity determining region (CDR) grafted mouse sequences, which form the antigen-binding site. The theoretical molecular masses (average) for the predominant N-linked glycoforms, assuming C-terminal G residues in both H chains, and full disulfide bond connectivity, is 148 KDaltons. The sites of AAS are located in both light and heavy chains including the CDR.

Description of manufacturing process and process controls *(Intermediate gemtuzumab)*

The gemtuzumab intermediate manufacturing process has been adequately described. Main steps are fermentation, recovery and purification.

The manufacturing process for gemtuzumab uses a recombinant NS0 mouse myeloma cell line that contains the DNA encoding the sequence for gemtuzumab. Cells from the WCB are thawed, and the culture is progressively expanded into a production bioreactor. The production bioreactor culture is harvested removing cells and debris, concentrated and filtered. Following harvest, the product is processed by chromatographic and viral inactivation steps into containers and stored under appropriate conditions. The container closure system components comply with the EC directive 2002/72/EC and EC 10/2011 as amended.

Control of materials *(Intermediate gemtuzumab)*

Sufficient information on raw materials used in the intermediate gemtuzumab manufacturing process, including the composition of the cell culture media, has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. The history and details of cell line establishment are given. The applicant has provided a thorough risk assessment and conducts viral testing in support of both the BSE/TSE and the viral safety of gemtuzumab, which is found acceptable.

A full genotypic characterisation of the MCB, WCB and EOP cells, under current manufacturing process conditions at commercial scale, has been provided. All results are deemed satisfactory. Testing and control of starting/raw materials as well as introducing new in process controls have been included in the control strategy, which is now satisfactory.

Control of critical steps and intermediates *(Intermediate gemtuzumab)*

A comprehensive overview of the control strategy performed throughout the gemtuzumab intermediate manufacturing process is given. Acceptable information has been provided on the control system. The intermediate gemtuzumab manufacturing process is considered acceptable.

Process validation *(Intermediate gemtuzumab)*

The intermediate gemtuzumab manufacturing process is now considered as validated adequately. Validation data from fermentation and purification batches were presented. Data from these studies were in compliance.

It is concluded that the process consistently produces intermediate gemtuzumab of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

Characterisation *(Intermediate gemtuzumab)*

In the original submission, characterisation data, primarily from one single reference batch of gemtuzumab reference materials from the commercial process, was provided. However, since this batch represented only the post-AAS batches, it was not considered as representative of all the intermediate gemtuzumab used for the clinical material; a major objection was raised. As a result substantial additional characterisation data have been provided. No new sites of calicheamicin were found to have been introduced in the shifted batches. Glycosylation was also demonstrated comparable in pre and post-shit batches. Effector functions are not part of the mechanism of action. The applicant has demonstrated that gemtuzumab does not have Antibody-Dependent Cell-Mediated Cytotoxicity, and Complement Dependent Cytotoxicity activity.

Comparable cytotoxicity was initially demonstrated for two lots that spanned the AAS levels. During the procedure, data on binding to the CD33 target and FcRn were provided supporting the conclusion that the AAS has not had any effect on the affinity of gemtuzumab to CD33 or FcRn. The applicant has been recommended to conduct full characterisation of the AAS for an agreed number of gemtuzumab batches manufactured. Should an update of the test for Amino Acid Substitution be needed, this should be applied for in a variation application. In any case (if no need for update of the specification is identified), the data from full characterisation of the AAS sites should be provided to the Agency and Rapporteur.

Specification *(Intermediate gemtuzumab)*

The specification which are based on clinical and comparability data, are accepted.

Analytical methods *(Intermediate gemtuzumab)*

Validation reports and transfer reports for the gemtuzumab analytical procedures have been submitted and are satisfactory.

Batch analysis *(Intermediate gemtuzumab)*

Batch analysis data used for non-clinical, clinical, stability, process validations, and filling reference material have been provided. All batches met the specifications at the time of release.

Reference materials *(Intermediate gemtuzumab)*

For reference standards used throughout gemtuzumab development, the data from extended characterisation tests have been provided. The data shown confirm the suitability of the reference materials.

Stability *(Intermediate gemtuzumab)*

The stability results indicate that the intermediate gemtuzumab is sufficiently stable and justify the proposed shelf life in the proposed container.

Manufacture, process controls and characterisation *(Gemtuzumab ozogamicin)*

Description of manufacturing process and process controls

The gemtuzumab ozogamicin active substance manufacturing process has been adequately described. The main steps are conjugation of two active substance intermediates, activated calicheamicin derivative and gemtuzumab antibody, and purification. A solution of activated calicheamicin derivative is combined with a solution of gemtuzumab in a reactor. The conjugated material is purified. The purified conjugate is combined with excipients, filtered, and stored. The process parameters and the controls are described for each step. The active substance manufacturing process is considered acceptable. The unique batch number allows traceability of all materials associated with the batch.

The gemtuzumab ozogamicin active substance is filled in sterile bags, compliant with European Pharmacopoeia (Ph. Eur.) and food contact legislation (EC Regulation No 1183/2012 replacing No 10/2011). Extractable and leachables evaluation is satisfactory. The active substance is stored at appropriate storage conditions.

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. A statement confirms that the active substance is in compliance with the “Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products” (EMA/410/01 rev.3).

Control of critical steps and intermediates

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the gemtuzumab ozogamicin active substance manufacturing process is now given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests. The control strategy is consistent with the principles outlined in ICH Q9, Q10 and Q11. The description of the control strategy in place for the active substance is generally acceptable.

Process validation

The gemtuzumab ozogamicin active substance manufacturing process is considered as validated adequately.

Characterisation

Gemtuzumab ozogamicin has been sufficiently characterised by physicochemical and biological state-of-the-art methods revealing that the active substance has the expected structure of an antibody drug conjugate molecule presenting both the antibody (gemtuzumab) and the ozogamicin components. Furthermore, post-translational modifications, charge and size heterogeneity, conjugation sites, extent of calicheamicin derivative conjugation, higher order structure, and biological activity have been adequately characterised, confirming the primary structure and expected cytotoxicity. The analytical results are consistent with the proposed structure. In summary, the characterization is considered appropriate for this type of molecule.

A discussion of the impact of AAS on the active substance characterisation has been provided during the review. The AAS in gemtuzumab does not overly affect the characterization of gemtuzumab ozogamicin. Comparability in the gemtuzumab intermediate, active substance and finished product was demonstrated. In addition, side-by-side characterization using an array of analytical methods demonstrates comparability in primary structure, post-translational modifications, and higher-order structure among gemtuzumab ozogamicin batches that vary in the level of amino acid substitutions. Furthermore, side-by-side data to evaluate the sites of conjugation and the higher order structure of these entities of gemtuzumab and gemtuzumab ozogamicin refrigerated active substance and reconstituted finished product were found to be comparable.

The active substance impurities have been described. The absence of novel conjugation sites with the AAS is implied by an absence of changes in binding or cytotoxicity, and to the secondary/tertiary structure of the molecule, which were all found to be comparable between pre- and post-shift batches. Furthermore, additional analyses of batches containing high AAS confirmed the absence of novel conjugation sites. Unconjugated gemtuzumab and unconjugated calicheamicin are controlled.

Specification (Gemtuzumab ozogamicin)

The specification for the Gemtuzumab ozogamicin active substance has been provided and includes tests for characteristics, identity, purity, biological activity, product related impurities and safety. Limits reflect the clinical experience and are considered acceptable.

Acceptance criteria for several gemtuzumab specifications were reviewed and tightened during the procedure.

At submission, the definition of CQAs was considered insufficient, which lead to a major objection. During the procedure, CQAs were redefined and additional attributes were included in the intermediate gemtuzumab specification.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch analysis data were provided. The results are within the specifications at the time of release and confirm consistency of the manufacturing process, for the parameters tested.

Reference materials

The reference material of the finished producthas been established.

Stability (Gemtuzumab ozogamicin)

The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container.

Real time, real condition stability data according to the ICH guidelines were provided.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

The finished product, Mylotarg powder for concentrate for solution for infusion, contains 5 mg lyophilised gemtuzumab ozogamicin with no ovefill. Other components are dextran 40 (bulking agent), sucrose (cryoprotectant), sodium chloride (tonicity adjusting agent); sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate anhydrous (buffering agents). All the excipients are well known pharmaceutical ingredients. Monobasic sodium phosphate, monohydrate, is not described in the Ph. Eur.; hence, compliance with USP is accepted. The quality of all the other excipients is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The primary packaging is a Type I borosilicate amber glass vial. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

When reconstituted to a 1 mg/mL concentration as directed, the extractable content of the vial is 4.5 mg (4.5 mL). The extractable volume is supported by data. Following reconstitution, the solution is further diluted with sterile saline and administered to patients by intravenous infusion. During dilution, reconstitution and administration Mylotarg needs to be protected from light, and a low protein binding filter must be used for the infusion.

The batches of gemtuzumab ozogamicin that have been commercially manufactured demonstrated adequate quality and stability of the commercial formulation.

Manufacture of the product and process controls

The finished product manufacturing process consists of aseptic filtration and filling of the formulated active substance into vials, lyophilisation, and final stoppering/capping. The process is adequately described.

The applicant has summarised the approach to the control. The applicant has submitted process validation data and is acceptable. The applicant is using mainly compendial methods for the analytical assays. Validation reports were provided where applicable.

Product specification

The specifications for Mylotarg at release include tests for the following quality attributes: characteristics, identity, purity, biological activity (by target binding and cytotoxicity), product related impurities and safety.

CQAs for the finished products and related test parameters were updated during the procedure. Specifications have been tightened for certain attributes and the applicant has been recommended to

review and adjust the finished product specifications for other attributes. The information provided is acceptable.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch analysis data of the finished product, at commercial scale, were provided. The results are within the specifications set at the time of release.

Reference materials

A reference material of the finished product has been established.

Stability of the product

Stability studies in line with ICH guidelines were presented. Finished product stability lots were manufactured with the commercial active substance. All test results at the recommended storage conditions met the commercial specifications. Based on available stability data, the proposed shelf-life is 5 years, at the recommended storage conditions (store in a refrigerator (2°C-8°C). Do not freeze. Store the vial in the original carton to protect from light).

Adventitious agents

The applicant has given an overview of the approach to adventitious agent control and has conducted viral clearance studies to support the ability of the mAb purification process ability to remove adventitious viruses. The information provided for validation of adventitious virus removal is found sufficient. The applicant has also provided a thorough risk assessment in support of the viral testing performed to establish viral safety of gemtuzumab, which is found acceptable.

TSE

Certificates of suitability have been provided where applicable for materials utilised in the current manufacturing process. A risk assessment for TSE was provided as outlined in the 'Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 current revision).

Comparability exercise for Finished Medicinal Finished product

See above under Manufacture of the Finished Product.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Several major deficiencies were identified in the initial quality package submitted in support of the Mylotarg MAA. All issues regarding the quality for Mylotarg were satisfactorily addressed and are now resolved.

Primarily based on the AAS observed, several major deficiencies were identified in the initial quality package submitted in support of the Mylotarg MAA. None of the clinical studies supporting the current

Mylotarg MAA used exclusively elevated AAS gemtuzumab batches, and as such do not fully represent the proposed commercial product. The fraction of gemtuzumab ozogamicin batches used in the clinical studies displaying elevated levels of AAS increased according to the accrual period of the studies. In the original submission, it was stated that it is not possible to perform heightened characterisation of the original product using state-of-the art orthogonal methods, due to unavailability of sufficient material, so comparability studies were limited in scope. This was considered a major deficiency.

The root cause of the AAS was identified. Since the evidence to fully support this was not sufficient, a major objection was raised to fully investigate the impact of several parameters on AAS levels. Data provided in the dossier suggest that changes to the production process could be implemented to eradicate the AAS and provide an antibody moiety with a consistent primary sequence; since the applicant has stated that this change would take time, it has committed to implement a new, improved process for gemtuzumab, designed to reduce AAS and improve consistency post-approval.

A major objection (comprising part of a joint clinical MO) was raised regarding the potential impact of the AAS on the functional activity of Mylotarg. CD33 and FcRn binding between pre- and post-AAS shift batches was shown to be comparable on the whole. Additional data provided by the applicant also provide further evidence that no new sites for calicheamicin conjugation are introduced as a result of the AAS.

Another major objection (comprising part of a second joint clinical MO) was raised regarding the use of batches with basal levels of AAS in the pivotal clinical trial. The applicant has now demonstrated convincingly that, except for the AAS, there is overall comparability at the quality level between pre- and post-AAS shift batches, and this point is considered resolved.

A major objection regarding comparability of gemtuzumab throughout development is also considered resolved. Additional data provided by the applicant, which comprises a mixture of new data and a more thorough presentation of comparative data allows for a more comprehensive assessment of the relative quality attributes of pre- and post-AAS shift batches. No significant differences are found in binding to the CD33 target. Glycosylation, aggregation, and stability of the antibody are considered comparable. No new sites of conjugation were found, and the low conjugated and unconjugated fractions were found comparable. The provided data support the notion that the AAS has not had any significant effect on the critical quality attributes of Mylotarg and its functionality, as far as can be ascertained from analyses of quality attributes. A further major objection was raised regarding process robustness. Data provided in the initial submission suggested that the process may not be sufficiently robust to withstand any necessary future changes to the manufacturing process which might therefore lead to further variation in AAS or other quality attributes. The applicant has provided a substantial amount of process performance data. Important changes have been made to the control process, including increased testing and control of starting/raw materials as well as introducing new in process controlsand a general tightening of process controls and acceptance criteria for release.

In summary, several major objections and a number of other concerns were raised. All issues regarding the quality for Mylotarg were satisfactorily addressed and are now resolved.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends several points for investigation.

2.3. Non-clinical aspects

2.3.1. Introduction

All definitive toxicology studies (repeat dose toxicity, reproductive and developmental, genetic toxicity), and tissue cross-reactivity studies were conducted in accordance with US FDA GLP regulations, unless otherwise noted. Safety pharmacology studies were not GLP compliant.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro pharmacodynamics

Binding affinity to soluble CD33

The binding of gemtuzumab ozogamicin and hP67.6 antibody to soluble CD33 was evaluated using Biacore surface plasmon resonance (SPR) (RPT-56053). hP67.6 antibody exhibited a high affinity for CD33, with an average KD of 0.073 nM. This high affinity was not compromised by conjugation of N-Ac-γ-calicheamicin DMH via the AcBut linker to hP67.6 antibody where the KD of gemtuzumab ozogamicin was 0.082 nM.

Binding to a CD33-positive leukaemia cell line

The relative binding affinity of gemtuzumab ozogamicin to a CD33-positive tumour cell line was determined in a competitive radioimmunoassay using HEL 92.1.7 erythroleukemia cells (MIRACL-26754). Gemtuzumab ozogamicin had an EC50 value of 11.6 nM (1.69 µg/mL) which was comparable to 11.9 nM (1.73 µg/mL) for the hP67.6 Ab which confirmed that conjugation of the linker-payload to the hP67.6 antibody does not alter its binding affinity to the cells (Table 1).

Binding to normal peripheral blood and bone marrow cells

Binding characteristics of hP67.6 antibody and gemtuzumab ozogamicin were evaluated in peripheral blood and normal bone marrow cells from 5 allogeneic transplant donors as compared by flow cytometry (MIRACL-27251). While this study examined a very small sample size, overall, there does not appear to be a substantial difference in the binding characteristics with regard to cellular specificity for hP67.6 antibody compared to gemtuzumab ozogamicin (Table 1).

Table 1. Summary of Key Pharmacologic Properties of Gemtuzumab Ozogamicin

BIAcore Analysis of Binding to soluble CD33		
	hP67.6[a]	Gemtuzumab Ozogamicin
K_D (nM)	0.073	0.082

Binding to HEL92.1.7 Cell Line		
	hP67.6	Gemtuzumab Ozogamicin
EC50[b] (nM)	11.9	11.6

Binding to Normal Peripheral Blood and Bone Marrow										
	Blood[c] (% positive staining)				Marrow[c] (% positive staining)					
	Mono	SD	Gran	SD	Mono	SD	Gran	SD	Myelo	SD
hP67.6	96.0	1.9	83.4	10.8	76.6	18.3	81.2	6.6	63.6	5.0
GO	93.4	2.9	86.6	8.3	73.2	14.0	73.0	8.3	59.6	4.5

Source: RPT-56053, MIRACL-26754, and MIRACL-27251.
a. Average of 2 batches;
b. In this competitive radioimmunoassay, EC50 is the concentration of test conjugate required to obtain 50% inhibition of binding of the labeled mAb.
c. Average of 5 donors
GO = Gemtuzumab ozogamicin; Mono = Monocytes; Gran = Granulocytes;
Myelo = Myeloblasts; SD = Standard deviation.

Cross-species binding data

A tissue cross-reactivity study was conducted to determine whether hP67.6 cross reacts with cynomolgus monkey and Sprague-Dawley rat tissues (MIRACL-26808). Specific staining by hP67.6 was absent in all tissues of both animal species. In addition, the binding of hP67.6 antibody to Chinese hamster ovary (CHO) cells transfected with human and cynomolgus CD33 was evaluated. hP67.6 bound strongly to human CD33-transfected CHO cells but did not bind to the monkey CD33-transfected CHO cells (study 084032).

Internalisation of CD33 antibody

Binding and internalization analysis of two radio iodinated anti-human CD33 antibodies, hP67.6 and M195 benchmark antibody (8) was conducted with the CD33-expressing HL-60 cell line (GTR-36629). A published study of 315 leukemic bone marrow samples showed the highest concentration of CD33 in AML samples, with an average of 10,000 and a range of 700 to 55,000 copies per cell (9). These results were confirmed in HL-60 cell where the CD33 expression was estimated to be 22,000 molecules per cell (GTR-36629). The results indicated that 45 to 55% of the initially-bound hP67.6 was internalized over 22 hours at 37°C. A separate electron microscopy study (GTR-36630) confirmed that trafficking of mP67.11 antibody (an antibody with no known differences to mP67.6) was mediated by endocytosis through the endosome/lysosome pathway in HL-60 cells.

Intracellular elease of NAc-gamma calicheamicin DMH from gemtuzumab ozogamicin

Once gemtuzumab ozogamicin has trafficked to the lysosomes, the calicheamicin cytotoxin is released by hydrolysis in the acidic environment. The rate of hydrolysis of N-Ac-γ- calicheamicin DMH was studied across a pH range (4.5, 6.0, and 7.4) (MIRACL-26756). The gemtuzumab ozogamicin sample did not show significant decomposition at pH 7.4. However, the ADC was relatively unstable at a pH value of 4.5,

which approximates the pH of a lysosome, and approximately half of the observed release of N-Ac-γ calicheamicin DMH occurred in approximately 6 hours. Payload release at a pH value of 4.5, but not 7.4, is consistent with the therapeutic rationale.

Activation of N-Ac-γ-calicheamicin DMH by glutathione

Following hydrolysis of the hydrazone moiety in the AcBut linker, the released N-Ac-γ-calicheamicin DMH must be activated in order to damage DNA. Prior studies have shown that the DNA cleavage reaction requires a reducing agent, and in its absence the calicheamicin binds to DNA with no destructive effect (10). GSH, an abundant constituent of the cytoplasm, has been shown to react readily with γ-calicheamicin to form an active DNA-damaging agent at concentrations that would be found in the cell (Myers et al, 1994). N-Ac-γ-calicheamicin DMH was designed to improve the stability of the disulfide bond.

The activated, DNA-damaging product, the diradical form of N-Ac-ε-calicheamicin, is formed following exposure of N-Ac-γ-calicheamicin DMH to GSH. Results of a study showed that the formation of N-Ac-ε-calicheamicin appeared to be directly proportional to the concentration of GSH in the reaction mixture (RPT-50556). The concentration of glutathione outside of cells in general circulation has been found to be in the low micromolar range (11);while, for example, the concentration of GSH in rat hepatocytes is 5 mM in the cytosol and as high as 20 mM in the nucleus. (12) The results of this study suggest that N-Ac-ε-calicheamicin can be produced without any enzymatic facilitation. In addition, the reaction of N-Ac-γ-calicheamicin DMH with glutathione was low at slightly acidic pHs but robust at a physiological pH values of 7.4.

In vitro cytotoxicity of gemtuzumab ozogamicin

The cytotoxic activity of gemtuzumab ozogamicin was compared to N-Ac-γ calicheamicin DMH and N-Ac-γ calicheamicin DMH AcBut in CD33-positive HL-60 and CD33-negative Raji cell lines (MIRACL-26753). Potent cytotoxic activity of gemtuzumab ozogamicin was observed for CD33-positive cells (IC50 of 0.3 pM calicheamicin payload, or 0.46 pg/mL calicheamicin equivalents) and a selectivity index of 77,609 for CD33-positive versus CD33-negative cells. Gemtuzumab ozogamicin had greater potency when compared to non-specific cytotoxicity of N-Ac-γ calicheamicin DMH and N-Ac-γ calicheamicin DMH AcBut (IC50 values of 6.4 and 13.0 nM calicheamicin payload, or 9.2 and 18.6 ng/mL calicheamicin equivalents, respectively) for CD33-positive cells. Cytotoxicity of gemtuzumab ozogamicin was also compared to a non-binding murine plasmacytoma mAb control antibody conjugated to calicheamicin, MOPC-21, in HL-60 and Raji cells (MIRACL-26749 and MIRACL-26900). Gemtuzumab ozogamicin was 10,500 times more cytotoxic to HL-60 compared to the MOPC-21 antibody-calicheamicin conjugate. The cytotoxic effects of gemtuzumab ozogamicin were also investigated comparing cells with high levels of CD33 (HL60, NOMO-1, NB4, and NKM-1), low CD33- positive (K562), and no CD33 (Daudi), as well as P-gp-expressing cell lines, MONO-1/ADR (adriamycin-resistant subline) and NB4/MDR (mdr1 DNA-transfected (GTR-37661). Concentration-dependent, CD33-directed cytotoxicity was observed (IC50 approximately 0.084 nM calicheamicin payload) in the cell lines tested. Gemtuzumab ozogamicin had cytotoxic activity against MONO-1/ADR and NB4/ MDR only in the presence of MDR inhibitors, MS209 and PSC833 (IC50 ranged from 0.175 to 17.5 nM).

Gemtuzumab ozogamicin activity in primary human leukaemic bone marrow samples

In order to assess the ability of gemtuzumab ozogamicin to inhibit cell growth from progenitor cells in patients, colony forming cell growth was assessed in diagnostic blood or bone marrow specimens from patients with AML and normal healthy donors (MIRACL-26757). Inhibition of colony growth was observed in all gemtuzumab ozogamicin treated samples. In the 27 samples incubated with 7.0 nM gemtuzumab ozogamicin, 15 had >25% inhibition of which 12 had >60% inhibition, while in samples incubated with

1.4 nM gemtuzumab ozogamicin 10 had >25% inhibition of which 4 with >60% inhibition. Normal bone marrow samples (n=3) were exposed to gemtuzumab ozogamicin of 7 to 28 nM calicheamicin payload and showed no inhibition of colony growth. Non-specific inhibition of colony formation was observed after AML patient samples were treated with 70 nM calicheamicin payload of a non-targeting hCTM01control but not at lower doses.

In Vivo Pharmacodynamics

Antitumor Effects of Single Dose Gemtuzumab Ozogamicin in the HL-60 Xenograft Model

The *in vivo* antitumor effects of a single dose of gemtuzumab ozogamicin were studied in a CD33-positive HL-60 xenograft model (MIRACL-26749 and MIRACL-26900). Athymic mice (n = 5 per test group; n = 10 for the saline control group) were dosed IP with 30, 60, 90, and 120 mg/m^2 of gemtuzumab ozogamicin 8 days after subcutaneous implantation of tumour cells. A single dose of 120 mg/m^2 of gemtuzumab ozogamicin resulted in 20% survival. The 30 mg/m^2 dose resulted in 100% survival with 40% of the mice tumour-free, while at 60 and 90 mg/m^2 gemtuzumab ozogamicin, survival was 80%, with 60% of the mice tumour free. Single dose gemtuzumab ozogamicin showed antitumor efficacy but had a poor survival rate at higher doses in mice at the doses tested; therefore, a fractionated dosing approach was explored as a possible improvement to the dosing regimen. Athymic mice implanted with HL-60 tumours allowed to grow to approximately 150 mg (n= 5 per test group, n = 10 in the saline control group) were given 3 IP doses once every four days (Q4Dx3) of gemtuzumab ozogamicin on days 7, 11, and 15 after tumour implantation for total doses of 9, 18, 54, and 108 mg/m^2 (MIRACL-26749 and MIRACL-26900). In the gemtuzumab ozogamicin-treated mice, 100% survival was observed following administration of 9, 18, and 54 mg/m^2 doses. After doses of 9 and 18 mg/m^2, 40% of the animals were tumour free while 60% were tumour free in the 54 mg/m^2 dose group at study termination. After administration of 108 mg/m^2 of gemtuzumab ozogamicin, 60% of the animals survived and were also tumour free at Day 37. In the saline-treated control group, there was no antitumor activity by Day 37. A separate experiment with the same Q4Dx3 dosing regimen of gemtuzumab ozogamicin in mice bearing larger 200 mg HL-60 tumours resulted in complete tumour regression and 100% survival at 18, 54, and 108 mg/m^2 total doses of gemtuzumab ozogamicin administered Q4Dx3.

A study comparing gemtuzumab ozogamicin and a control ADC (N-Ac-γ calicheamicin DMH AcBut conjugated to a non-binding murine plasmacytoma control antibody, MOPC-21) was conducted with Q4Dx3 IP administration for total doses of 9, 18, 54, and 108 mg/m^2 in the HL60 xenograft model (n= 5 per group, 150 mg tumours) in athymic mice. Tumour growth inhibition of >80% was observed across all 4 gemtuzumab ozogamicin doses. All mice survived at 9, 18, and 54 mg/m^2, while 2 deaths were observed at the 108 mg/m^2 dose. In contrast, treatment with the MOPC-21 conjugate resulted in <20% tumour growth inhibition across all of the 4 doses, and 100% lethality was observed at the 108 mg/m^2 dose. Therefore, the efficacy of gemtuzumab ozogamicin in this tumour model was dependent on the CD33-directing properties of gemtuzumab ozogamicin.

Antitumor Effects of Low Doses of Gemtuzumab Ozogamicin Administered IV in the HL-60 Xenograft Tumor Model

More recent studies evaluated efficacy over a lower dose range and NOD/SCID mice were followed for a much longer period, 124 days (030406). Total doses of 1.2, 3.6, and 12 mg/m^2 gemtuzumab ozogamicin were administered in a subcutaneous HL60 xenograft model in immune-compromised NOD/SCID mice. Mice (n = 10) with staged tumours (average volume 225 mm^3) received 4 IV doses every 4 days (Q4Dx4) of either a non-binding calicheamicin conjugated antibody control or gemtuzumab ozogamicin. Treatment with 12 mg/m^2 gemtuzumab ozogamicin caused sustained tumour regressions, with only 2 animals presenting tumor relapse around Day 45 and Day 80. Six (6) out of 10 animals remaining on study at Day

100 were tumour free. Treatment with a non-binding control ADC at 12 mg/m^2 caused only transient reduction in tumour volume, and tumours relapsed at Day 32. The 3.6 mg/m^2 dose of gemtuzumab ozogamicin delayed tumour growth significantly, but the tumours eventually relapsed and these animals were taken off study by Day 37. In contrast, the tumors of all animals in the non-binding ADC control group at 3.6 mg/m^2 progressed, and all animals had to be taken off the study by Day 16 due to tumour burden. There was no observed efficacy in the group dosed with 1.2 mg/m^2 of gemtuzumab ozogamicin.

Antitumor Effects of Gemtuzumab Ozogamicin in Combination with Daunorubicin/ Cytarabine (DA) Chemotherapy in AML Xenograft Models with Leukemic Stem Cell Outgrowth

The combination of gemtuzumab ozogamicin with conventional chemotherapy has showed significant improvement in the overall survival of AML patients (13) (14). However, when DA therapy was administered alone, despite early anti-leukemic responses, a high percentage of disease recurrence was observed. This has been attributed to the outgrowth of chemo-resistant LSCs characterized by their inherent ability for self-renewal (15) BM0407 and BM2407 PDX and human AML cell line (MV4-11-Luc) disseminated disease models in NOD/SCID mice were used to compare the antileukemic activity of DA chemotherapy, gemtuzumab ozogamicin monotherapy, and the combination of both therapies (study 082753).

High dose DA chemotherapy (cytarabine at 15 mg/kg subcutaneously daily for 5 days and daunorubicin at 1.5 mg/kg on Days 1, 3, and 5) resulted in elimination of human AML CD33+CD45+ blasts in the peripheral blood, but residual disease remained in the bone marrow. Combining DA chemotherapy with GO compared to either therapy alone resulted in nearly complete elimination of CD33+/CD45+ human tumor cells from both the peripheral blood and bone marrow. In the MV4-11-Luc disseminated model, the GO/DA combination also showed a statistically-significant increase in survival compared to DA or GO monotherapies.

The combinatorial effect of DA and gemtuzumab ozogamicin was further tested in the AML BM0407 PDX disseminated model where DA (cytarabine at 10 mg/kg SC on Days 1-5 and 22-26 and daunorobucin at 1 mg/kg IV on Days 1, 3, 5, 22, 24, and 26) or gemtuzumab ozogamicin monotherapy (at a dose of 0.06 mg/kg per dose on Day 1 and 22, or 0.36 mg/m^2 total dose, which is lower than the dose required for complete elimination of CD33+CD45+ blasts from the bone marrow) caused partial inhibition of leukemic growth, while the combination of DA with gemtuzumab ozogamicin completely depleted the CD33+/CD45+ AMLs from the bone marrow.

AML PDX models, BM2407 and BM0407, were established in immunocompromised mice, and following DA chemotherapy, the residual disease, as measured by the percentage of CD33+/CD45+ blasts in the bone marrow, were analysed for expression of several cell surface markers associated with LSCs. In the BM2407 PDX model, CD34+ cells were enriched in the residual disease and characterized as a potential LSC marker, while in the BM0407 model, a CLL1+/CL117-population was enriched and identified as a potential LSC phenotype. The residual AML blasts in the bone marrow of BM0407 and BM2407 models treated with DA were sorted into subpopulations using potential LSC markers and retransplanted into a separate cohort of naïve mice to monitor for tumour engraftment. In the BM2407 model, CD33+/CD45+ were further sorted into CD34+ and CD34- cells. Implanting 2 x 105 or 6 x 105 CD33+/CD34+ cells resulted in all mice (5/5) developing bone marrow leukaemia burden. In contrast, CD33+/CD34- cells failed to engraft in any of the mice regardless of the cell concentration. Similarly, in the BM0407 model, when 1 x 105 of the sorted CLL1+/CD117- cells were injected into mice, all of the mice (5/5) developed bone marrow leukemic CD33+/CD45+ blast. On the contrary, the CLL1+/CD117+ cells failed to engraft in mice.

Secondary pharmacodynamic studies

A summary of the results of a secondary pharmacology study (GTR-37604) is presented in Table 2.

Table 2. Tabulated summary of secondary pharmacology study

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report Number
The Effects of Gemtuzumab Ozogamicin on Normal Human Megakaryocyte Differentiation	CD34-positive Cells Isolated From Normal Human Bone Marrow Cells	Ex Vivo	Final concentrations from 17.2 to 345 ng/mL calicheamicin equivalents (0.5 to 10 µg/mL protein equivalents).	N/A	CD34-positive, progenitor cells isolated from healthy donors were highly variable in their sensitivity to gemtuzumab ozogamicin as measured by megakaryocyte and total colony formation. With low CD33 expression, the earliest progenitor/ stem cells are the most likely to be resistant to gemtuzumab ozogamicin.	No	GTR-37604

CD = Cluster of differentiation; GLP = Good Laboratory Practices;.N/A = Not applicable.

Safety pharmacology programme

An overview of gemtuzumab ozogamicin safety pharmacology studies is presented in Table 3.

Table 3 Overview of Gemtuzumab Ozogamicin Safety Pharmacology Studies

Study	Study Number (Sponsor Reference Number)	Concentration or Dose[a,b,c]	Tabulated Summary	GLP Compliance
In Vitro Screening Study				
Effect of N-Ac-γ-Calicheamicin DMH on the hERG Potassium Current Stably Expressed in HEK293 Cells	N-acetyl-gamma-calicheamicin-DMHHERG	1, 3, 6.77 µM	2.6.3.4	Non-GLP
In Vitro Autonomic Nervous System and Smooth Muscle				
Effect of Gemtuzumab Ozogamicin on Spontaneous Motility and Contractile Response	LJT3075 (GTR-37315)	0.75 µg/mL calicheamicin equivalents	2.6.3.4	Non-GLP
General Safety Pharmacology – Mouse, Rat				
Single-Dose IV Safety Pharmacology Study of Gemtuzumab Ozogamicin in Male Mice (Central Nervous System, General Activity and Behavior) and Male Rats (Body Temperature, Digestive System, Hepatic Function, Kidney Function, Vehicle Effects)	LJT3075 (GTR-37315)	Mouse: 0, 3, 9, 30, and/or 90 mg/m^2 Rat: 0, 2.1, 7, 21 mg/m^2	2.6.3.4	Non-GLP
Single-Dose IV Safety Pharmacology Study of the Effect of Gemtuzumab Ozogamicin on Urine Volume and Urinary Concentrations of Electrolytes in Male Mice	LJT3175 (GTR-37316)	0, 3, 9, 30 mg/m^2	2.6.3.4	Non-GLP
Cardiovascular – Conscious Dog				
Single-Dose IV Cardiovascular Safety Pharmacology Study of Gemtuzumab Ozogamicin in Male and Female Dogs	MIRACL-26834	0, 4, 13, 40 mg/m^2	2.6.3.4	Non-GLP

DMH = Dimethylhydrazide; GLP = Good Laboratory Practice; HEK = Human embryonic kidney; hERG = Human ether-à-go-go-related gene; IV = Intravenous; N-Ac = N-Acetyl.
a. Doses of gemtuzumab ozogamicin in mice expressed as calicheamicin equivalents on the basis of µg/kg of body weight were 0, 25, 75, 250, or 750 µg/kg, and when expressed as dose equivalents of the hP67.6 antibody on the basis

of mg/kg of body weight were 0, 1, 3, 10, or 30 mg/kg. hP67.6 antibody doses in mg/kg of body weight were converted to mg/m^2 of body surface area using a conversion factor of 3 for mouse.
b. Doses of gemtuzumab ozogamicin in rats expressed as calicheamicin equivalents on the basis of µg/kg of body weight were 0, 7.5, 25, or 75µg/kg, and when expressed as dose equivalents of the hP67.6 antibody on the basis of mg/kg of body weight were 0, 0.3, 1, or 3 mg/kg. hP67.6 antibody doses in mg/kg of body weight were converted to mg/m2 of body surface area using a conversion factor of 7 for rat.
c. Doses of gemtuzumab ozogamicin in dogs expressed as calicheamicin equivalents on the basis of µg/kg of body weight were 0, 4.8, 16, or 48 µg/kg, and when expressed as dose equivalents of the hP67.6 antibody on the basis of mg/kg of body weight were 0, 0.19, 0.63, or 1.9 mg/kg. h67.6 antibody doses in mg/kg of body weight were converted to mg/m2 of body surface area using a conversion factor of 21 for dog.

To evaluate potential effects on cardiac repolarization, N-Ac-γ-calicheamicin DMH was tested for its effect on the hERG potassium channel stably expressed in HEK293 cells (N-acetyl-gamma-calicheamicin-DMHhERG). At concentrations up to 6.77 µM, <1% inhibition of the hERG current amplitude was observed. The IC50 value for hERG current inhibition was not determined because concentrations greater than 6.77 µM (10,000 ng/mL) could not be tested.

In vitro, gemtuzumab ozogamicin at a concentration of 0.75 µg/mL calicheamicin equivalents caused no noticeable changes in spontaneous motility or contractile responses to acetylcholine, histamine, serotonin, or barium chloride in isolated guinea pig ileum (GTR-37315).

Gemtuzumab ozogamicin was evaluated in mice, rats, and dogs after a single IV administration. Potential effects of gemtuzumab ozogamicin on the CNS (mice, rats), digestive system (mice), kidney function (mice), and hepatic function (rats) were evaluated. Potential cardiovascular effects were evaluated in dogs. Gemtuzumab ozogamicin caused no noticeable changes in gross activity or behaviour of mice, and no effects on spontaneous locomotor activity, potentiation of convulsions, on pain threshold, gastrointestinal transit, or changes in urinary volume or urinary electrolytes at doses of ≤30 mg/m^2. Gemtuzumab ozogamicin administered to rats at ≤21 mg/m^2 had no effect on body temperature compared with the control group, and caused no noticeable changes in the excretion of sodium bromosulphalein indicating no effect on hepatic function (GTR-37315).

Gemtuzumab ozogamicin was administered IV to male mice (20/group) at doses of 3, 9, or 30 mg protein/m^2 and pooled urine samples from each cage (5 animals/cage, 4 cages/group) was collected over 5 hours. Gemtuzumab ozogamicin caused no noticeable changes in urine volume or urinary excretion of electrolytes (GTR-37316).

Gemtuzumab ozogamicin was administered to conscious Beagle dogs at doses of 4 (30-minute infusion), 13 (30-minute infusion), or 40 (bolus) mg/m^2 (MIRACL-26834). Following bolus injection at 40 mg/m^2, there was a reduction in cardiac output at 5 to 10 minutes postdose that gradually returned to baseline by 40 minutes post dose. A decrease in mean arterial blood pressure was also observed at 5 to 20 minutes post dose, and was likely secondary to the reduction in cardiac output. Heart rate increased following gemtuzumab ozogamicin administration which appeared to be a reflexive response to maintain cardiac output. Heart rate was also increased at 13 mg/m^2. There were no effects of gemtuzumab ozogamicin on hemodynamic or cardiac function at 4 mg/m^2 nor on electrocardiogram (ECG) parameters at any dose. In addition, there were no effects on ECG parameters (monkeys) or cardiac histopathology findings (rats and monkeys) in the 6-week repeat-dose studies.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction study with gemtuzumab ozogamicin has been conducted (see discussion on non-clinical).

2.3.3. Pharmacokinetics

Enzyme-linked immunosorbent assays (ELISAs) were used for the quantitation of total hP67.6 antibody, total calicheamicin, and unconjugated calicheamicin in rat, dog, and/or monkey plasma. To quantify conjugated and unconjugated calcheamicin in monkey plasma from the 12 week study, LC-MS/MS was used.

To support the 6-week toxicity studies in rats and monkeys, bioanalytical methods were developed and characterized. Non-validated gel permeation chromatography with radiochemical detection or ELISA methods were used for the qualitative assessment of ADA in rat and monkey plasma. To detect ADA in monkey plasma in the 12 week study, a validated ECL method was used.Qualified and/or validated assays used to support the TK and ADA evaluations in repeat-dose toxicity studies conducted under Good Laboratory Practices (GLP).

The single-dose PK/TK of gemtuzumab ozogamicin was characterized after IV administration in Sprague-Dawley rats and cynomolgus monkeys. The mean PK Parameters after a single iv dose of [3H]gemtuzumab ozogamicin are displayed in Table 4.

Table 4. Mean PK Parameters after a Single IV Dose of [3H]Gemtuzumab Ozogamicin

Species	Dose	Analyte	Sex/ n	CL (mL/min/kg)	V_{ss} (L/kg)	$t_{½}$ (h)	C_{max} [a] (ng/mL)	AUC_{inf} [a] (ng•h/mL)
Rat (MIRACL-26888)	9.6 mg/m^2 [b] 1.6 mg/kg [c] 49 µg/kg [d]	Total radioactivity [e]	M/4	0.037 [f]	0.206 [f]	95 [f]	1000	22900 [f]
		Total calicheamicin	M/4	0.042 [f]	0.165 [f]	66 [f]	1280	20100 [f]
Rat (GTR-30503)	7.8 mg/m^2 [b] 1.3 mg/kg [c] 29.5 µg/kg [d]	Total radioactivity [e]	M/3 [g]	0.03	0.162	81	430 [h]	18800
Monkey (MIRACL-26887)	15.6 mg/m^2 [b] 1.3 mg/kg [c] 45 µg/kg [d]	Total radioactivity [e]	M/5	0.016	0.088	119	1620	51200
		Total calicheamicin	M/5	0.023	0.196	162	1540	35700

Notes: Gemtuzumab ozogamicin = PF-05208747, CMA-676, or CL 555,201.
Total calicheamicin = Unconjugated calicheamicin + conjugated calicheamicin.
Abbreviations: AcBut = 4-(4'-acetylphenoxy) butanoic acid; AUCinf = Area under the concentration-time curve from time 0 to infinity; CD33 = Cluster of differentiation 33; CL = Systemic plasma clearance; Cmax = Maximum observed concentration; DMH = Dimethylhydrazide; eq = Equivalents; IV = Intravenous; M = Male; min = Minute; n = Number of animals; N-Ac = N-acetyl; PK = Pharmacokinetic; t½ = Apparent elimination half-life; Vss = Apparent steady-state volume of distribution.
a. Concentrations reported as ng calicheamicin/mL.
b. Protein (hP67.6 antibody) dose equivalent; mg protein/kg was converted to mg protein/m^2 using a conversion factor (km) of 6 for rat and 12 for monkey.
c. Protein (hP67.6 antibody) dose equivalent; mg protein/kg determined using the loading of calicheamicin onto the CD33 antibody.
d. N-Ac-γ-calicheamicin DMH AcBut dose equivalent.
e. Concentrations determined from total radioactivity are expressed as ng eq/mL with AUCinf expressed as ng eq•h/mL.
f. n = 3; 1 rat was excluded.
g. 3 animals per time point; total number of animals = 27.
h. Concentration at 5 minutes postdose.

After single IV administration of N-Ac-γ-calicheamicin DMH, N-Ac-γ-calicheamicin DMH AcBut, or N-Ac-ε-calicheamicin to male and female Sprague-Dawley rats (MIRACL-26628; MIRACL-26709; MIRACL-25706) and/or male and female Beagle dogs (MIRACL-26629; MIRACL-25707), the plasma concentrations of calicheamicin equivalents were evaluated using an ELISA that measured multiple forms of calicheamicin (including the administered calicheamicin derivatives and/or its metabolites). After single IV administration of N-Ac-γ-calicheamicin DMH to rats or dogs, the PK of calicheamicin equivalents

was characterized by moderate systemic CL and high Vss. After IV administration of N-Ac-γ-calicheamicin DMH AcBut to rats, the PK of the calicheamicin equivalents was characterized by low CL and a low to moderate Vss. PK parameters could not be determined in rats or dogs administered N-Ac-ε-calicheamicin, as systemic concentrations of calicheamicin equivalents were either BLQ (<2.5 ng/mL) or only quantifiable at the early time points.

The TK of hP67.6 antibody, total calicheamicin, and/or unconjugated calicheamicin and the presence of ADA were assessed for up to 6 weeks (1 dose/week) in male and female Sprague-Dawley rats (MIRACL-26813) or male and/or female cynomolgus monkeys (MIRACL-26812; GTR-27677) after IV administration of gemtuzumab ozogamicin or hP67.6 antibody alone. Mean systemic hP67.6 antibody and/or total calicheamicin AUC exposure increased in a dose-proportional manner in rats and monkeys after single- and/or repeat-dose administration of gemtuzumab ozogamicin. Plasma concentrations of unconjugated calicheamicin were typically below the limit of quantitation (BLQ) and were only observed in the high dose group from each species. After repeat-dose administration of gemtuzumab ozogamicin, the total incidence of ADA-positive animals was 95% in rats and 8.3% to 33% in monkeys. Due to the high incidence of ADA in the 6-week repeat-dose rat toxicity study, time-dependent changes in exposure could not be assessed in these species. In the 6-week repeat-dose comparative toxicity study in monkeys, accumulation of hP67.6 antibody was observed after repeated administration of gemtuzumab ozogamicin or hP67.6 antibody, with AUC168 accumulation ratios ranging from 2.3 to 2.7. Mean, dose-normalized systemic exposure to hP67.6 antibody was similar in monkeys dosed with gemtuzumab ozogamicin prepared with hP67.6 antibody produced from either the initial or commercial cell lines.

Plasma protein binding of N-Ac- γ -calicheamicin DMH was high in mouse, rat, rabbit, monkey, and human plasma (≥ 97%). In rats, distribution of [3H]gemtuzumab ozogamicin radioequivalents into red blood cells and tissues was limited, with tissue-to-plasma AUC ratios ≤ 0.54 for all tissues evaluated. The highest tissue-to-plasma ratios were observed in the blood (0.54), liver (0.36), kidney (0.26), spleen (0.25), and lung (0.22). Radioequivalents in brain were < 2% of plasma.

In vivo metabolism of gemtuzumab ozogamicin was not evaluated in nonclinical species; however*, in vivo* metabolism was evaluated in rats after IV administration of [3H]inotuzumab ozogamicin, which shares the same linker-payload and mAb IgG subclass as gemtuzumab ozogamicin and, like gemtuzumab ozogamicin, does not bind to its target antigen in nonclinical species. In rats, [3H]N-Ac-γ-calicheamicin DMH was extensively metabolized following release from [3H]inotuzumab ozogamicin. The primary metabolic pathway for [3H]N-Ac-γ-calicheamicin DMH in rats was reduction of the disulphide moiety, leading to formation of N-Ac-ε-calicheamicin and deglycosylated tetrasaccharide metabolites. Hydrolysis (of the hydrazide moiety), oxidation, and adduction (with pyruvic acid) were minor metabolic pathways. Inotuzumab ozogamicin accounted for 66% of total drug-related material (AUC) in circulation of rats, with 2 unidentified, higher molecular weight (MW) species accounting for the remainder of circulating radioactivity. *In vivo* metabolism of gemtuzumab ozogamicin in humans was assessed by analysis of urine samples collected from patients administered unlabeled gemtuzumab ozogamicin. All urinary metabolites detected in patient samples were also observed in rats administered [3H]inotuzumab ozogamicin.

The effect of pH on the hydrolytic stability of the hydrazone linker of gemtuzumab ozogamicin was evaluated in buffer at various pH. Gemtuzumab ozogamicin was stable to hydrolytic cleavage at pH 7.4, but at pH 4.5, approximately 50% of the total N-Ac-γ-calicheamicin DMH was hydrolytically cleaved from gemtuzumab ozogamicin in 6 hours.

Minimal deconjugation of calicheamicin (<10%) occurred following incubation of gemtuzumab ozogamicin or [3H]inotuzumab ozogamicin in rat, monkey, or human plasma for up to 96 hours. N-Ac-γ-calicheamicin DMH was stable in the presence of glutathione (GSH) at concentrations similar to

those found in plasma but was readily reduced at intracellular concentrations. The contribution of glutathione-S-transferase (GST) was negligible compared to the effect of GSH alone.

Following incubation of [3H]N-Ac-γ-calicheamicin DMH in liver S9 (supplemented with a full complement of cofactors including glutathione) and plasma, metabolic profiles were similar across species as [3H]N-Ac-γ-calicheamicin DMH underwent metabolism primarily by reduction. In addition, incubation of [3H]N-Ac-γ-calicheamicin DMH in buffer controls were similar to those including liver S9 or plasma, suggesting that a majority of N-Ac-γ-calicheamicin DMH biotransformation occurs non-enzymatically. Metabolic profiles of [3H]N-Ac-γ-calicheamicin DMH observed in vitro were qualitatively similar to those observed in in vivo samples collected from rats dosed with [3H]inotuzumab ozogamicin. Collectively, these data indicate that the hydrolytic release of N-Ac-γ-calicheamicin DMH from gemtuzumab ozogamicin or inotuzumab ozogamicin in circulation is limited and the metabolism of N-Ac-γ-calicheamicin DMH occurs primarily via non-enzymatic reduction. Therefore, coadministration of gemtuzumab ozogamicin with inhibitors or inducers of CYP or UGT drug metabolizing enzymes are unlikely to alter exposure to N-Ac-γ-calicheamicin DMH.

After IV administration of [3H]gemtuzumab ozogamicin to rats, the majority of the radioactive dose was recovered in the faeces (59%), with urinary excretion as a minor route of elimination (13%). Radioactivity was eliminated slowly over time and was still being excreted in the faeces and urine of rats at the end of the study (14 days), accounting for the incomplete mass balance observed.

Assessment of drug-drug interaction (DDI) potential with gemtuzumab ozogamicin and N-Ac-γ-calicheamicin DMH for reversible or time-dependent CYP inhibition was based on the 50% inhibitory concentration (IC50) and/or inactivation kinetics determined from *in vitro* studies and the mean steady-state total Cmax of 3280 ng/mL (0.0221 µM) for hP67.6 antibody and 5.81 ng/mL (0.00393 µM) for unconjugated calicheamicin achieved in humans after multiple dose administration of 9 mg/m^2 gemtuzumab ozogamicin.

Gemtuzumab ozogamicin did not cause induction of CYP3A4 in the transfected HepG2 cells at up to 0.0424 µM of gemtuzumab ozogamicin (highest concentration evaluated). In humans, the mean steady-state total hP67.6 antibody Cmax after multiple dose administration of 9 mg/m2 of gemtuzumab ozogamicin was 3280 ng/mL (0.0221 µM). Gemtuzumab ozogamicin did not induce CYP3A4 at concentrations up to 0.0424 µM, which was approximately 2x the Cmax. N-Ac-γ-calicheamicin DMH did not cause induction of CYP1A2, CYP2B6, or CYP3A4 mRNA expression and/or enzyme activity in any of the 3 hepatocyte lots evaluated to 0.3 µM. This was >50x the Cmax of unconjugated calicheamicin. Finally, N-Ac-ϵ-calicheamicin did not cause induction of CYP3A4 in the transfected HepG2 cells at up to 0.120 µM, which was >50x the Cmax of unconjugated calicheamicin.

N-Ac-γ-calicheamicin DMH demonstrated little or no reversible inhibition of UGT1A4, UGT1A6, UGT1A9, and UGT2B7 catalysed activities; however, N-Ac-γ-calicheamicin DMH inhibited UGT1A1 activity. N-Ac-γ-calicheamicin DMH showed little or no inhibition of the bidirectional transport of digoxin (P-gp substrate) or pitavastatin (BCRP substrate). The efflux ratio of digoxin in the absence and presence of N-Ac-γ-calicheamicin DMH (0.3 µM) was 39.1 and 48.2, respectively. N-Ac-γ-calicheamicin DMH inhibited the OATP1B1- and OATP1B3-mediated transport of pravastatin and rosuvastatin by 30% and 16%, respectively, in a concentration-dependent manner. N-Ac-γ-calicheamicin DMH, over the concentrations evaluated, did not inhibit OAT1, OAT3, or OCT2. The IC50 for inhibition was estimated to be >0.5 µM (Ki >0.25 µM) for OAT1 and >0.1 µM (Ki >0.05 µM) for OAT3 and OCT2.

2.3.4. Toxicology

Single dose toxicity

A summary of results of single dose toxicity studies with gemtuzumab ozogamicin is displayed in Table 5.

Table 5 Summary of results of single dose toxicity studies with gemtuzumab ozogamicin

Species/ Strain	Method of Administration (Vehicle/ Formulation) Test Article Lot Number	Doses[a] Protein (hP67.6 Antibody) Dose Equivalent (mg/m^2)	Doses[a] Protein (hP67.6 Antibody) Dose Equivalent (mg/kg)	Doses[a] N-Ac-Gamma Calicheamicin DMH AcBut Dose Equiv. (µg/kg)	Number/ Sex/ Group	Observed Maximum Nonlethal Dose (mg/m^2)	Approx. Lethal Dose (mg/m^2)	Noteworthy Findings	Study Number/ GLP Compliance
Mouse/ CD-1	Intravenous, 0.1 mL (dextran-40, sucrose, sodium chloride, sodium phosphate monobasic and dibasic, sterile water for injection /solution) Lot: 1325608A[b]	0 15	0 5	0 150	5M, 5F	15	>15	None	RPT-77183/ Yes
Rat/ Sprague-Dawley	Intravenous, 0.4 - 12 mL/kg (100 mM sucrose, 100 mM sodium chloride, 5 mM phosphate buffer, sterile water for injection/solution) Lot: 4489A24-102593-R1592-24[c]	0 2.4 24 72	0 0.4 4 12	0 10 100 300	5M, 5F	2.4	>2.4	**≥2.4 mg/m²:** Few or no feces **24 mg/m²:** Four (4) males and 2 females were euthanized in moribund condition on Days 3 or 6, ↓ body weight, ↓ body weight gain, ↓ food consumption, liver (mottled), spleen (white, rough) **≥24 mg/m²:** Pale, jaundice, hunched, hypoactive, inactive appearance, orange or orange-brown urine, wet/stained perianal area, chromodacryorrhea, lacrimation, chromorhinorrhea, dyspnea, tachypnea, hypothermia, ptosis, proteinuria, glucosuria, hematuria, bilirubinuria, urobilinogen in urine, liver (rough surface, ↑ reticular pattern), yellow perianal area; unkempt, jaundiced, and pale appearance; orange-tinged urine; ptosis; ↑ total bilirubin; proteinuria, hematuria, urobilinogen in urine; yellow discoloration (internal organs, skin); liver (rough surface, red, hepatocellular necrosis, periportal mixed cell infiltrate, sinusoidal mixed cell infiltrate, congestion); kidney (tan, tubular casts); stomach mucosa (discolored foci) **18 mg/m2:** Three (3) males and 3 females were found dead on Day 3, 2 males and 2 females were euthanized in moribund condition on Day 3, ↓ hemoglobin, ↓ hematocrit, ↓ reticulocyte, bilirubinuria discoloration (internal organs, skin), red areas (stomach, heart, lungs, thymus, testes) **72 mg/m²:** Four (4) males and 4 females were found dead on Days 2 or 3, 1 male and 1 female were euthanized in moribund condition on Days 2 or 3	MIRACL-26631/ Yes

Rat/ Sprague-Dawley	Intravenous, 0.8-3 mL/kg (Sterile water for injection/Solution) Lot: 4489A24-102593-R1592-24[c]	4.8 8.4 12 18	0.8 1.4 2 3	20 35 50 75	5M, 5F	8.4	>8.4	**≥4.8 mg/m²:** Kidney (tubular vacuolation, tubular dilation) **≤8.4 mg/m²:** ↓ Body weight, ↓ body weight-gain **≥8.4 mg/m²:** Hypoactivity, ↓ platelet; ↑ AST, ↑ ALT, liver (hepatocellular karyocytomegaly) **8.4 and 12 mg/m²:** Liver (hepatocyte vacuolation, hemosiderin in Kupffer cells, single cell necrosis, oval/cell bile duct proliferation) **12 mg/m²:** Two (2) males and 3 females were found dead on Days 3, 4, or 6; body weight loss; kidney (karyocytomegaly, ↑ tubular basophilia) **≥12 mg/m²:** Hypoactivity/ inactivity; few feces; wet/stained	MIRACL-26711/ No
Monkey/ Cynomolgus	Intravenous, 3-6 mL/kg (100 mM sucrose, 100 mM sodium chloride, 5 mM phosphate buffer, sterile water for injection/solution) Lot: 4489A24-102593-R1592-24[c]	0 36 54 72	0 3 4.5 6	0 75 112.5 150	4M	36	>36	**36 mg/m²:** None **54 mg/m²:** Two (2) males were found dead on Day 17, injected sclera, rales, dyspnea, chromorhinorrhea **≥54 mg/m²:** Emesis, soft/liquid feces, pale gums, hypoactivity, hunched appearance, ↓ appetite (in early deaths), liver (rough surface, mottling, reticular pattern), gall bladder (red), abdominal cavity (red-tinged fluid), and red areas or red foci in or on the retroperitoneum, heart, lung (also red foamy contents), diaphragm, stomach, ileum (dark areas only), colon/cecum, mesentery, kidney capsule, urinary bladder, and/or seminal vesicles **72 mg/m²:** Two (2) males were euthanized in moribund condition on Days 7 or 8, inactivity, hypothermia, eyes unresponsive to touch and stimuli, atonia, ↓ body weight, body weight loss	MIRACL-26710/ Yes
Chimpanzee	Intravenous 2-hour infusion, 0.3 mL/kg (100 mM sucrose, 100 mM sodium chloride, 5 mM phosphate buffer, sterile water for injection/solution) Lot: 4489A24-102593-R1592-24[c]	0.5	0.015	0.39	2M	0.5	>0.5	None	MIRACL-26817/ No

Toxicokinetic Parameters (M) - Study MIRACL-26817[a]:
Total Calicheamicin Derivatives

Dose (µg/kg)	0	0.39
Plasma Concentration (ng/mL) (0 HPD)	NA[e]	4.74[f]
Plasma Concentration (ng/mL) (2 HPD)	NA[e]	3.60[f]
Plasma Concentration (ng/mL) (24 HPD)	NA[e]	2.00[g,h]
Plasma Concentration (ng/mL) (240 HPD)	NA[e]	1.85[g,h]

ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ELISA = Enzyme-linked immunosorbent assay; F = Female; GLP = Good Laboratory Practice; HPD = Hours postdose; IV = Intravenous; M = Male; N = Number of animals; NA = Not applicable.
a. Doses of PF-05208747 are expressed as calicheamicin equivalents on the basis of µg/kg of body weight, and as dose equivalents of the hP67.6 antibody on the basis of mg/kg of body weight and on the basis of mg/m² of body surface area. Doses in mg/kg were converted to mg/m² using a conversion factor of 3 for mouse, 6 for rat, and 12 for monkey.
b. Lot/batch of PF-05208747 was manufactured with antibody produced from the commercial cell line.
c. Lot/batch of PF-05208747 was manufactured with antibody produced from the initial cell line.
d. Bioanalysis was conducted using a non-validated ELISA method.
e. A toxicokinetic control group was not included.
f. N = 2.
g. Total calicheamicin concentrations were below quantifiable limits in 1 of 2 animals.
h. N = 1.

Repeat dose toxicity

- *6-Week Toxicity Study in Rats* (*study MIRACL-26813)*

In study MIRACL-26813 the potential toxicity of gemtuzumab ozogamicin and associated systemic exposure and anti-drug antibody (ADA) response after 6 weeks of once-weekly dosing and the reversibility of any effects after a 4-week non-dosing period was assessed in Sprague-Dawley rats. Gemtuzumab ozogamicin was administered IV (bolus) to male and female rats (15/sex/group for the toxicity animals; 12/sex/group for the toxicokinetic animals) at doses of 0 (PBS control), 7.2 (hP67.6 antibody control), 0.6, 2.4, or 7.2 mg/m^2/week. There was no mortality and no ocular findings during the study. Gemtuzumab ozogamicin-related clinical signs of toxicity occurred at ≥0.6 mg/m^2/week and included a dose-related increased incidence and frequency of few or no faeces and a wet/stained perianal area at 7.2 mg/m^2/week. Gemtuzumab ozogamicin caused slightly lower mean body weight at ≥2.4 mg/m^2/week in males (22% to 34%) and at 7.2 mg/m^2/week in females (19%). Gemtuzumab ozogamicin-related haematologic effects occurred on red blood cell (RBC) mass (RBC, haemoglobin, and haematocrit), and WBC counts. There were no clinical pathology changes observed after administration of hP67.6 antibody alone. The primary gemtuzumab ozogamicin-related microscopic findings were observed in the, kidney, liver, spleen, bone marrow, testes/epididymides, and male mammary gland. Slight to marked tubular dilation, casts, and basophilia were observed in the kidney of males and females at ≥ 2.4 mg/m^2/week. The microscopic findings in the kidney correlated with pale kidneys observed in males macroscopically at 7.2 mg/m^2/week, slight increases in kidney weight (12% to 25%) at ≥ 2.4 mg/m^2/week, and proteinuria and decreased serum albumin. Vacuolation (minimal to slight, males only), hepatocellular karyocytomegaly (minimal to marked), atrophy of hepatocytes and dilation of sinusoids (minimal to moderate), oval cell/bile duct proliferation (minimal to slight), and haematopoiesis (minimal) were observed in the liver of both sexes at 7.2 mg/m^2/week.

After a 4-week non-dosing period, overall body weight gain (Days 41 to 69) was comparable in animals given gemtuzumab ozogamicin and control animals. Clinical pathology values in animals administered gemtuzumab ozogamicin were similar to control animals at the end of the 4-week non-dosing period except for WBC, ALT, and globulin, and proteinuria. On Day 65, mean WBC count was still lower in males at ≥0.6 mg/m^2/week. The ALT values in males were still moderately higher, and globulin was slightly higher in females at 7.2 mg/m^2/week. Proteinuria was still present (2+ to 4+) at ≥0.6 mg/m^2/week on Day 66. Gemtuzumab ozogamicin-related microscopic changes were observed in kidney, liver, testes, and male mammary gland at the end of the 4-week non-dosing period. There were no organ weight, macroscopic, or microscopic changes related to administration of the hP67.6 antibody alone at the end of the dosing or recovery phases of study.

The NOAEL was considered to be 0.6 mg/m^2/week based on adverse effects on male reproductive organs, kidney, liver, bone marrow, and spleen at ≥ 2.4 mg/m^2/week. The mean Cmax exposure value for total hP67.6 antibody after the first dose was 1840 ng/mL and the mean AUC168 exposure value for total hP67.6 antibody after the first dose was 90,600 ng•h/mL at 0.6 mg/m^2/week.

- *6-Week Toxicity Study in Monkeys* (study *MIRACL-26812)*

This study assessed the potential toxicity of gemtuzumab ozogamicin and associated systemic exposure and ADA response following 6 weeks of once-weekly dosing in cynomolgus monkeys and the reversibility of any effects after a 4-week non-dosing period. Gemtuzumab ozogamicin was administered IV (bolus) to male and female monkeys (5/sex/group) at doses of 0 (PBS control), 22 (hP67.6 antibody control), 2.4, 7.2, or 22 mg/m2/week.

There were no unscheduled deaths or hP67.6 antibody- or gemtuzumab ozogamicin-related clinical signs, ECG, or ophthalmoscopic observations noted during the study. Slightly lower body weight (3% to 19%;

Day 41) was observed at ≥7.2 mg/m2/week; however, there were no changes in qualitative food consumption. Gemtuzumab ozogamicin-related haematologic effects occurred on Days 9 and 37 at ≥.2 mg/m^2/week and consisted of slightly to moderately lower RBC mass (11% to 42%), WBCs (5% to 68%), polymorphonuclear cells (33% to 78%), and lymphocytes (41% to 63%). Gemtuzumab ozogamicin-related clinical chemistry effects occurred at 2.4 mg/m2/week on Days 9 and/or 37 and consisted of higher AST, total protein, globulin, and sodium, and lower albumin and albumin/globulin ratio (A/G). Gemtuzumab ozogamicin-related microscopic findings were noted in the kidney, liver, thymus, spleen, lymphatic tissues, and bone marrow. Minimal to moderate dilation, basophilia, casts, vacuolation of glomerular tufts, and/or accumulation of red granules were observed in the kidney at ≥7.2 mg/m^2/week. Slight to marked dilation of liver sinusoids with hepatocyte atrophy and minimal to slight amounts of brown pigment (hemosiderin) was observed in the liver at ≥7.2 mg/m^2/week. Minimal to moderate hepatocellular single cell necrosis was observed at 22 mg/m^2/week. Microscopic changes in the liver correlated with macroscopic findings of red foci/discoloured at ≥2.4 mg/m2/week. Changes in the lymphohaematopoietic system included slight to marked depletion of lymphocytes in the thymus; atrophy of germinal centers and depletion of lymphocytes in the spleen, cervical and mesenteric lymph nodes, and tonsils; and/or hypocellularity of the bone marrow at ≥7.2 mg/m2/week.

Most gemtuzumab ozogamicin-related clinical pathology changes were fully reversible by the end of the 4-week non-dosing period. RBC and lymphocyte counts were still lower at ≥7.2 mg/m^2/week or 22 mg/m^2/week, respectively, but less than that observed at the end of the dosing period. Changes in the kidney and liver were partially to fully reversible after the non-dosing period. Changes observed in the lymphohaematopoietic system were not reversible. Slight to marked depletion of lymphocytes was observed in the thymus, spleen, and lymph nodes, and slight to marked atrophy of germinal centers was observed in the spleen, mesenteric lymph nodes, and tonsils at ≥7.2 mg/m^2/week.

The NOAEL was considered to be 2.4 mg/m^2/week based on target organ toxicity in the kidney, liver, and lymphohaematopoietic organs at ≥7.2 mg/m^2/week. Mean Cmax and AUC168 exposure values (males and females combined) for gemtuzumab ozogamicin (as measured by total hP67.6 antibody) at Week 1 (data for Week 6 was not collected) were 3500 ng/mL and 258,000 ng· h/mL, respectively.

- *6-Week Toxicity and Comparability Study in Monkeys (study GTR-27677)*

The purpose of this study was to evaluate the comparability, toxicity, and associated systemic exposure and ADA response of gemtuzumab ozogamicin manufactured with hP67.6 antibody produced from the initial and commercial cell lines following 6 weeks of once-weekly dosing in cynomolgus monkeys. Gemtuzumab ozogamicin manufactured with antibody produced from the commercial cell line was administered IV (bolus) to male monkeys (3/group) at doses of 0 (saline control), 18 (hP67.6 antibody control), 2.4, 6, or 18 mg/m^2/week. There were no unscheduled deaths and no gemtuzumab ozogamicin-related ophthalmologic findings. Gemtuzumab ozogamicin-related clinical signs were limited to a rash in 2 monkeys (7.2 mg/m^2/week [initial cell line] and at 18 mg/m^2/week).

Mild decreases in RBC mass (RBC, haemoglobin, haematocrit) were observed at 18 mg/m^2/week. Decreases in WBCs and lymphocytes, and increases in neutrophils were also observed at 18 mg/m^2/week. Clinical pathology values were of similar magnitude for monkeys given 7.2 mg/m^2/week (initial cell line) or 6 mg/m^2/week, suggesting a similar biological effect of these two cell lines.

Gemtuzumab ozogamicin-related findings were noted in haematolymphopoietic tissues (lymph nodes, tonsils, thymus, and spleen), testes and epididymides, bone marrow, kidney, and liver. Microscopic findings in the kidney and liver were similar to those reported in a previous 6-week study in monkeys with gemtuzumab ozogamicin (MIRACL-26812).

When compared with monkeys given gemtuzumab ozogamicin at 7.2 mg/m^2/week (initial cell line) in this study, changes in monkeys given 7.2 mg/m^2/week (initial cell line) in the previous 6-week study (MIRACL-26812) included slightly more severe and widespread lymphoid depletion/atrophy. Additionally, liver cell atrophy and renal tubular alterations were also noted in the previous study. In contrast, bone marrow hypocellularity was slightly more prevalent in monkeys in the current study. The testicular and epididymal degenerative changes observed at 18 mg/m^2/week in the current study were not reported in the previous study with the initial cell line.

- *12-Week Toxicity Study in Monkeys (study 16GR242)*

A GLP-compliant general toxicity study in monkeys was also completed in which 12 doses of gemtuzumab ozogamicin were given once weekly by intravenous bolus injection to groups of 3 male and 3 female cynomolgus monkeys at doses of 0 (sterile water), 2.2, 6.6, or 11 mg/m^2/week. It was formulated at 0.98 mg/ml.

Doses of ≥11 mg/m^2/weekk caused severe toxicity and monkeys were euthanized early, despite initiation of a dose-free period. All monkeys in the higher two dose groups were euthanize early for this reason, between days 33 and 53 (between 4 and 8 doses). Monkeys showed body weight loss with clinical decline attributed to primarily to drug-induced liver toxicity with evidence of decreased red cell mass due, at least in part, to test article-related bone marrow injury. In addition, at ≥2.2 mg/m^2/week, there was body weight loss, decreases in red cell mass and microscopic findings in liver, bone marrow, lymphoid organs, kidney, male and female reproductive organs, and/or eyes which meant that in this study, a no observable adverse effect level (NOAEL) was not identified. Monkeys administered 2.2 mg/m2/kg were euthanized after 12 doses, 1 week earlier than initially intended. Monkeys in all doses groups, including the lowest, showed body weight loss with a reduction in food intake. There were multiple abnormalities in clinical chemistry testing. These included increases in liver enzymes (aspartate aminotransferase (AST), gamma glutamyltransferase and total bilirubin with decreased albumin and A:G ratio. On microscopic examinations, the livers showed sinusoidal dilatation and/or hepatocyte atrophy in monkeys given 6.6 or 11 mg/m^2/week. At post-mortem, organ weight data were only collected from monkeys given 2.2 mg/m^2/week. There were findings of lower weight of testes and epididymes, correlated with microscopic findings of degeneration of the seminiferous tubules in the testis and increased luminal debris, epithelial degeneration, and oligospermia. Liver weights were lower in males. Thymus weights in females given 2.2 mg/m^2/weekwere reduced (non-significantly so) but thymuses showed decreased lymphocytes. There were also increases in the weight of kidneys and spleen. There were changes seen on microscopic examination in liver, bone marrow (sternum), kidney, lymphoid organs (thymus, spleen, and mesenteric lymph node), eyes, testis, epididymis, seminal vesicle, ovary, oviduct, uterus and cervix. Immunostaining for VEGFR2 in controls resulted in staining of the endothelial cells uniformly throughout the liver; in monkeys given gemtuzumab ozogamicin, there was minimally to moderately decreased staining consistent with alteration and/or loss of sinusoidal endothelial cells in affected regions. In the bone marrow of the sternum, there was an increase in myeloid / erythroid ratio (resulting from decreased population of erythroid precursors), considered adverse at ≥6.6 mg/m^2/week and which were associated with decreases in red blood cell mass. In the kidneys, again at ≥ 6.6 mg/m^2/week, there were changes of minimal to moderate tubular epithelium degeneration / regeneration, minimal or slight karyocytomegaly of tubular epithelium, minimal to slight glomerulopathy and moderate pigment in tubular epithelial cells. Lymphoid organ findings were observed at ≥2.2 mg/m^2/weekand consisted of decreased lymphocyte cellularity in splenic follicles, in the thymus (cortex and medulla), and in the mesenteric lymph node (characterized by decreased follicle size or absence of follicles). The male reproductive tract was adversely affected, consistent with the organ weight changes described above and in the reproductive tract of females, there were also adverse changes of ovarian atrophy and disruption or loss of a normal menstrual cycle would be expected. There were a multitude of other changes noted

that were attributed to secondary consequences of the primary effects just described. There was a finding of myocardial degeneration / necrosis in a male at 2.2 mg/m^2/week, but its acute nature suggested to the applicant that it had occurred near the time of euthanasia and was not due to gemtuzumab ozogamicin.

Therefore, in this study, a NOAEL was not identified; the lowest observable adverse effect level (LOAEL) for the study was 2.2 mg/m^2/week. Mean overall exposure values (male and female combined) at the LOAEL for total antibody (hP67.6) were 7,420 ng/mL and 541,000 ng•h/mL for C_{max} and AUC_{168}, respectively, at Week 1 and 13,900 ng/mL and 1,470,000 ng•h/mL for C_{max} and AUC_{168}, respectively, at Week 6 (exposures at Week 12 were similar to Week 6).

- *13-Day Tolerability Study in Rabbits (study GTR-33261)*

The purpose of this study was to evaluate the tolerability of gemtuzumab ozogamicin after 13 days of once-daily dosing in female New Zealand White rabbits followed by a 15-day non-dosing period. Gemtuzumab ozogamicin was administered IV (bolus) via a marginal ear vein to female rabbits (4/group) at doses of 0 (vehicle control), 1.2, 3.6, or 7.2 mg/m^2/day. All animals survived the dosing period; however, 2 animals given 7.2 mg/m^2/day were electively euthanized during the post-dose period (Days 16 or 23) as a result of adverse clinical observations (lacrimation, white discharge around eyes, lethargy, ptosis, lack of food consumption in 1 animal, and body weight loss for both animals [15% to 25%]). Cultures of samples from the eyes and paranasal sinus were negative for pathogenic bacteria, and the cause of ocular discharge could not be determined. The remaining observations were attributed to administration of gemtuzumab ozogamicin. Dose-related mean decreases in food consumption and body weight occurred in all groups administered gemtuzumab ozogamicin, and these effects occurred generally during and after the second week of dosing. In the 1.2 mg/m^2/day group, 1 of 4 females was affected, whereas all animals were affected in the 3.6 and 7.2 mg/m2/day groups. During the second week of dosing at $\geq$ 1.2 mg/m^2/day, mean body weight loss ranged from 112 to 263 grams, and mean food consumption decreased from 33% to 57% during the same period. Body weights continued to decrease during the non-dosing period (Days 14 to 28) at 7.2 mg/m^2/day, but partial recovery from body weight losses occurred at $\leq$ 3.6 mg/m^2/day. Macroscopic findings at 7.2 mg/m^2/day in animals at the scheduled necropsy included discoloration of the anterior lobes of the lung (bilateral) in 1 animal, and multifocal tan discoloration of the liver in another animal.

The MTD was considered to be 1.2 mg/m^2/day based on the absence of gemtuzumab ozogamicin-related changes in 3 of 4 animals in this group, mortality at 7.2 mg/m^2/day, and dose-related decreases in body weight and food consumption at ≥3.6 mg/m2/day.

Genotoxicity

Gemtuzumab ozogamicin was evaluated for potential genotoxicity in an *in vivo* mouse micronucleus study. In addition, *in vitro* studies were conducted with the unconjugated calicheamicin derivative N-Ac-γ-calicheamicin DMH. All studies were conducted in compliance with GLP regulations.

In vitro

Table 6 Genotoxicity studies with N-Ac-gamma-calicheamicin DMH

Type of test / study ID / GLP	**Test system**	**Concentrations / Concentration range / Metabolising system**	**Results Positive / negative / equivocal**
Gene mutations in bacteria (WIL-655125)	*Salmonella* strains (TA98, TA100, TA1535, TA1537) Escherichia coli strain (WP2 *uvr*A pKM101)	+/- S9 0.0121, 0.0241, 0.0482, 0.0964, 0.193, 0.386, 0.771, 1.54, 3.09, 6.17, 12.3, 24.7, 49.4, 98.8, 198, and 395 µg/plate	N-Ac-γ-Calicheamicin DMH (Lot no. 00706183-0156) was +ve for mutagenic activity in the E. coli strain WP2 *uvrA* pKM101, with and without metabolic activation, and -ve for mutagenic activity in the *Salmonella* strains TA1537, TA98, TA100, and TA1535 with and without metabolic activation
Chromosomal aberrations in vivo (WIL-655124)	Micronucleus in TK6 cells	+/- S9 vehicle, positive control, 0.0556 ng/mL (7%), 0.0989 ng/mL (19%), and 0.198 ng/mL (37%) for the 4-hour treatment without metabolic activation; 0.00418 ng/mL (0%), 0.0417 ng/mL (21%), and 0.0989 ng/mL (49%) for the 27-hour treatment without metabolic activation; and 0.0989 ng/mL (4%), 0.395 ng/mL (24%), and 0.618 ng/mL (36%) for the 4-hour treatment with metabolic activation	N-Ac-gamma-calicheamicin-DMH (Lot no. 00706183-0156) was considered positive for inducing micronuclei in TK6 cells with and without metabolic activation

In vivo

Table 7. Genotoxicity studies with gemtuzumab ozogamicin

Type of test / study ID / GLP	**Test system**	**Concentrations / Concentration range / Metabolising system**	**Results Positive / negative / equivocal**
Chromosomal aberrations in vivo (17418-0-455CO)	Micronuclei in bone marrow	(vehicle), positive control, 225, 450 and 900 µg/kg (batch no. 4489A-35-301195-R1592-163)	Toxic/cytotoxic effect: Severe toxicity at all doses. Reduced PCE/NCE ratio at all doses. Genotoxic effect: Significant increase in micronuclei of bone marrow PCEs at all doses. The positive response in the assay

			was expected and is consistent with the induction of DNA breaks by the calicheamicins and other enediyne antitumor antibiotics.

Carcinogenicity

No carcinogenicity studies have been conducted with gemtuzumab ozogamicin (see discussion on non-clinical aspects).

Reproduction Toxicity

A summary of the results of the non-pivotal reproductive and developmental toxicity studies is displayed in Table 8.

Table 8. Reproductive and Developmental Toxicity: Non- pivotal Studies

Species/Strain	Method of Administration (Vehicle/Formulation)/ Test Article Lot Number[a]	Dosing Period	Dose (mg/m^2/day)	Number/ Sex/ Group	Noteworthy Findings	Study Number/ GLP Compliance
Rat/ Sprague-Dawley	Intravenous, QD, 1 mL/kg (1% rat serum albumin, phosphate buffered saline, 1.55% sucrose, water for injection/solution) Lots 111196-R2024-2 and 050597PSG2024-14	28 Days (pre-mating)[c]	0 (vehicle)[b] 0.36 0.72 1.08 1.44	5M	**≥0.36 mg/m^2:** Testes (↓ spermatogonium, ↓ spermatocytes) **≥0.72 mg/m^2:** ↓ Body weight gain, ↓ red blood cell, ↓ prothrombin time, ↑ ALT, testes (↓ absolute weight), spleen (atrophy of the marginal zone) **≥1.08 mg/m^2:** ↓ Food consumption, ↓ hemoglobin, ↓ hematocrit, ↑ reticulocyte, ↓ sperm count, testes (↓ relative [to body weight] weight, small), ↓ spermatids (round and elongate), vacuolation of nucleus in spermatids (round), presence of giant cells, epididymis (↓ sperm), kidney (proteinaceous casts) **1.44 mg/m^2:** ↓ white blood cell, ↓ lymphocyte, ↑ neutrophil, ↑ platelet, ↓ sperm motility, ↑ sperm anomalies (1 animal), thymus (↓ absolute weight)	LJT3256R/ No
Rat/ Sprague-Dawley	Intravenous, QD, 1 mL/kg (1% rat serum albumin, phosphate buffered saline, 1.55% sucrose, water for injection/solution) Lot 050597-PSG2024-14	14 Days (pre-mating)[d]	0 (vehicle)[e] 0.12 0.36 0.72 1.08	5 or 10F	**0.36 mg/m^2:** ovary (↓ weight [absolute and relative]) **≥0.36 mg/m^2:** ↓ Body weight gain, ↓ food consumption, ↓ number of live fetuses, ↓ fetal viability, ↑ number of dead fetuses **1.08 mg/m^2:** ↓ corpora lutea, ovary (↓ weight [absolute and relative])	LJT3176R/ No
Rat/ Sprague-Dawley	Intravenous, QD, 1 mL/kg (0.06% sodium phosphate dibasic, 0.01% sodium phosphate monobasic, 1.55% sucrose, 0.91% dextran-40, 0.58% sodium chloride, sterile water for injection /solution) Lot 040497-PSG2024-13	GD 6-17	0 (vehicle)[e] 0.15 0.45 1.2	8F, 12F[g], 3 or 5[h]	**≥0 mg/m^2:** Vehicle (dextran-40)-related swelling of front and hind feet and nose **≥0.15 mg/m^2:** ↓ Maternal body weight, ↓ maternal body weight gain, ↓ maternal food consumption, ↓ gravid uterus weight, ↓ fetal weight **0.45 mg/m^2:** Fetal digital malformations (one or both hind feet), ↑ postimplantation loss (early and late resoprtions), ↓ number of live fetuses/litter, complete resorption of litter (3/8 litters) **1.2 mg/m^2:** Thin appearance (1 animal), complete resorption of litter (8/8 litters)	GTR-33248/ Yes

AcBut = 4-(4'-Acetylphenoxy)butanoic acid, a bifunctional linker; DMH = Dimethylhydrazide; F = Female; GD = Gestation Day; GLP = Good Laboratory Practice; NA = Not applicable; QD = Once daily.
a. Lot/batch of PF-05208747 was manufactured with antibody produced from the commercial cell line.
b. Doses of PF-05208747 are expressed as dose equivalents of the hP67.6 antibody on the basis of mg/m2 of body surface area. In the study report, doses of PF-05208747 are expressed as dose equivalents of the hP67.6 antibody on the basis of mg/kg of body weight (0, 0.06, 0.12, 0.18, and 0.24 mg/kg, respectively).
Doses were converted to mg/m2 using a conversion factor of 6 for rat.
c. On Day 27 (final day of dosing) and Day 28 all treated males were mated with untreated females.

d. After 14 daily doses, 5 of 10 females in the control group and all treated females (5/group) in the PF-05208747 groups were mated with untreated males. The other 5 control females were not mated.
e. Doses of PF-05208747 are expressed as dose equivalents of the hP67.6 antibody on the basis of mg/m2 of body surface area. In the study report, doses of PF-05208747 are expressed as dose equivalents of the hP67.6 antibody on the basis of mg/kg of body weight (0, 0.02, 0.06, 0.12, and 0.18 mg/kg, respectively). Doses were converted to mg/m2 using a conversion factor of 6 for rat.
f. Doses of PF-05208747 are expressed as dose equivalents of the hP67.6 antibody on the basis of mg/m2 of body surface area. In the study report, doses of PF-05208747 are expressed as dose equivalents of the hP67.6 antibody on the basis of mg/kg of body weight (0, 0.025, 0.075, and 0.2 mg/kg, respectively).
Doses were converted to mg/m2 using a conversion factor of 6 for rat.
g. An additional 12 females/group at doses of 0.15, 0.45, or 1.2 mg/m2 were included as satellite animals for determination of toxicokinetic parameters and antibody response.
h. An additional 3 (timed-mated) or 5 (non-gravid) females/group were dosed daily for 3 days by IV injection without dextran-40 or with the same and lesser amounts of dextran-40, respectively, to determine if the swelling observed in all groups in the developmental toxicity dose-ranging aspect of the study were attributable to the presence of dextran-40 in the dosing formulations. In these groups, tissue swelling did not occur in the rats given formulation diluent without dextran-40 or with lesser amounts (40, 14%) of dextran while similar tissue swelling occurred in the rats given dextran-40 (0.91%) in phosphate buffered saline. These results indicated that the tissue swelling in rats observed in the developmental toxicity dose-ranging aspect of the study was due to the dextran-40 component of the vehicle.

In the 6 week toxicity study with gemtuzumab ozogamicin (MIRACL-26813) effects on male reproductive organs (testes, epididymides, and mammary gland) were observed at ≥ 2.4 mg/m^2/week (approximately 3.7 or 18 times the human clinical exposure after the second human dose of 9 mg/m^2, or after the third human dose of 3 mg/m2, respectively, based on AUC). Effects on male rat reproductive organs were partially reversible or not reversible following a 4-week non-dosing period. These findings did not resolve following a 9-week recovery period. Gemtuzumab ozogamicin adversely affected male fertility in rats (RPT-40369), effects included lower spermatogonia and spermatocytes, decreases in testicular spermatids and epididymal sperm, vacuolation of the nucleus in spermatids, and/or appearance of giant cells at ≥ 0.12 mg/m^2/day. Additional findings included effects on the testes and epididymides; both organs were macroscopically small and decreased in weight as well as fertility (1.08 mg/m^2/day). When male rats were mated again after a 9-week non-dosing period, effects on sperm and fertility were worse but there was partial recovery of the lower spermatogonia and spermatocytes in the testes.

The potential for gemtuzumab ozogamicin to have effects on fertility and early embryonic development was evaluated in female Sprague-Dawley rats (RPT-40230). Female rats (32/group) were administered gemtuzumab ozogamicin IV once daily at doses of 0 (vehicle), 0.12, 0.36, or 1.08 mg/m2/day for 14 days. There were no gemtuzumab ozogamicin-related effects on copulation and fertility index in any females that mated at the end of the dosing or non-dosing periods; however, in females at the end of the dosing period there was a decrease in corpora lutea at 1.08 mg/m^2/day indicating an effect on ovulation. This decrease in corpora lutea resulted in lower numbers of implants at 1.08 mg/m^2/day. At the end of the dosing phase, the average number of live embryos per litter was 14.33, 13.07, 11.86, and 7.13, and the dead embryo indices were 6.52%, 7.98%, 15.74%, and 47.95% at 0 (vehicle control), 0.12, 0.36, or 1.08 mg/m^2/day, respectively; this increase in embryo lethality at 0.36 and 1.08 mg/m2/day was considered gemtuzumab ozogamicin related. The lower numbers of corpora lutea resulted in lower numbers of implants in the 1.08 mg/m^2/day group at the end of the dosing phase. In dams that mated at the end of the non-dosing period, no effects of gemtuzumab ozogamicin were observed in the numbers of corpora lutea, implants, or embryos. The NOAEL for general and female reproductive toxicity was 0.12 mg/m^2/day based on effects on maternal body weight, maternal food consumption, female fertility (lower numbers of corpora lutea), and early embryonic development (embryo lethality) at ≥0.36 mg/m^2/day in females that were mated immediately after the gemtuzumab ozogamicin-dosing phase. No gemtuzumab ozogamicin-related effects on early embryonic development were observed in dams that mated at the end of the 6-week non-dosing period.

Dose-dependent reductions in maternal food consumption, body weight gain, uterine weights and fetal body weight was observed after daily dosing with gemtuzumab ozogamicin (0.06; 0.15 and 0.36 mg/m^2/day) to pregnant rats (GTR-33829). The highest dose (approximately 0.04 times the recommended human single dose on a mg/m^2 basis) produced increased embryo-foetal mortality, and

gross external, visceral, and skeletal malformations. Gemtuzumab ozogamicin-related fetal morphological anomalies were observed at ≥ 0.15 mg/m^2/day and included digital malformations, absence of the aortic arch, anomalies in the long bones in the forelimbs, misshapen scapula, absence of a vertebral centrum, and fused sternebrae at 0.36 mg/m^2/day. The maternal and developmental NOAEL was 0.06 mg/m^2/day, although decreased skeletal ossification was observed in one foetus of the low-dose group (not different from vehicle). The lowest dose with embryo-foetal effects correlated with 9.7 times the human clinical exposure after the third human dose of 3 mg/m^2, based on AUC.

Toxicokinetic data

The threshold plasma concentrations of gemtuzumab ozogamicin associated with toxicologically important findings from *in vivo* nonclinical safety studies are shown in Table 9. Safety margins are presented based on a comparison of gemtuzumab ozogamicin AUC values associated with NOAELs for toxicology findings to human clinical exposure (AUC) at human doses for both indications (AML in first relapse and untreated de novo AML).

Table 9. Threshold Concentrations of Gemtuzumab Ozogamicin Associated with Toxicologically Important Findings

Toxicologically Important Finding(s)[a,b]	Dose[c] (mg/m^2)	AUC[d] (ng•h/mL)	C_{max}[e] (ng/mL)	Exposure Margin[f] (AML in First Relapse[g])	Exposure Margin[h] (Untreated De Novo AML[i])
Repeat-Dose IV Toxicity Studies[j]					
6-Week Toxicity Study in Rats with 4-Week Recovery[k]					
Clinical pathology: ↑ AST, ALT ↓ WBC, PMN, LYM, MONO	0.6 (NOAEL)	90600	1840	0.8	4.1
Clinical pathology: ↑ CHOL, ALP, GLOB ↓ RBC, HGB, HCT, ALB Target organs: Male mammary gland, kidney, liver, spleen	2.4	403000	7890	3.7	18
Target organs: Male reproductive, bone marrow	7.2	1180000	31700	11	54
6-Week Toxicity Study in Monkeys with 4-Week Recovery[k]					
Clinical pathology: ↑ AST Target organs: Liver	2.4 (NOAEL)	258000	3500	2.4	12
Clinical pathology: ↓ RBC, HGB, HCT, WBC, PMN, LYM Target organs: Lymphoid organs/tissues, kidney, bone marrow	7.2	795000	11100	7.4	36
Same as lower doses	22	2530000	35700	23	115
6-Week Toxicity and Comparability Study in Male Monkeys[l]					
Clinical pathology: ↑ AST, ALT, TP, GLOB, TRIG ↓ ALP, A/G Target organs: Lymphoid organs/tissues, bone marrow	2.4	201000	3460	1.9	9.1
Same as 2.4 mg/m^2	6	670000	10800	6.2	30
Clinical pathology: ↑ NEUT ↓ RBC, HGB, HCT, WBC, LYM Target organs: Male reproductive, kidney, liver	18	1790000	30500	17	81
Reproductive and Developmental IV Toxicity Studies[m]					
Embryo-fetal Development Dose-Ranging Study in Female Rats[l]					
↓ Maternal body weight, food consumption, gravid uterus weight, fetal weight	0.15 (LOAEL)	30600	1640	2.0	9.7
Fetal digital malformations ↑ Postimplantation loss ↓ Number of live fetuses/litter, complete resorption of litter	0.45	124000	5840	8.0	39
Complete resorption of litters	1.2	346000	17500	22	110

A/G = Albumin/globulin ratio; ALB = Albumin; ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AML = Acute myeloid leukemia; AST = Aspartate aminotransferase; AUC = Area under concentration-time curve; C2h = Concentration at 2 hours postdose; C2min = Concentration at 2 minutes postdose;
Cmax = Maximum observed concentration; CHOL = Cholesterol; GD = Gestational day; GLOB = Globulin;
HCT = Hematocrit; HGB = Hemoglobin; LOAEL = Lowest observed adverse effect level;
LYM = Lymphocyte; MONO = Monocyte; NEUT = Neutrophil; NOAEL = No observed adverse effect level;
OECD = Organization for Economic Co-Operation and Development; PMN = polymorphonuclear cell;
RBC = Red blood cell; TP = Total protein; TRIG = Triglyceride; WBC = White blood cell.

a. Toxicologically important findings were observed at successively higher doses. Findings were not presented at doses where they were not considered toxicologically important. Only studies with toxicokinetic data are presented in this table.

b. All studies were conducted under Good Laboratory Practice regulations in an OECD mutual acceptance of data (MAD) compliant member state.

c. Doses of gemtuzumab ozogamicin are expressed as dose equivalents of the hP67.6 antibody on the basis of mg/m^2 of body surface area. In study reports, doses may also be expressed as dose equivalents of the hP67.6 antibody on the basis of mg/kg of body weight and/or as calicheamicin equivalents on the basis of µg/kg of body weight. Doses in mg/kg were converted to mg/m^2 using a conversion factor of 6 for rat and 12 for monkey.

d. Total hP67.6 antibody AUC168 values after the first dose for the repeat-dose toxicity studies and AUC24 values at GD 17 for the dose-ranging embryo-fetal development study. Total hP67.6 antibody AUC values represent mean combined male and female plasma concentrations, unless otherwise indicated. Reported values were obtained near termination, or as specified.

e. Total hP67.6 antibody Cmax values after the first dose for the repeat-dose toxicity studies (C2min for the second monkey toxicity study) and C2h values at GD 17 for the dose-ranging embryo-fetal development developmental study. Total hP67.6 antibody concentration values represent mean combined male and female plasma concentrations, unless otherwise indicated. Reported values were obtained near termination, or as specified.
f. Exposure margins (ie, safety margins) were calculated by dividing AUC168 values in animal toxicity studies by the predicted human AUC168 value of 108,000 ng•h/mL at 9 mg/m2 following the Day 15 dose. Note that for the dose-ranging embryofoetal evelopment study (once-daily dosing), the animal AUC24 values were multiplied by 7 to calculate AUC168 values.
g.gemtuzumab ozogamicin administered at 9 mg/m2 with up to 2 doses administered 14 to 28 days apart, for ML in first relapse.
h. Exposure margins (ie, safety margins) were calculated by dividing AUC168 values in animal toxicity studies y the predicted human AUC168 value of 22,000 ng•h/mL at 3 mg/m^2 following the Day 7 dose. Note that for the dose-ranging embryo-fetal development study (once-daily dosing), the animal AUC24 values were multiplied by 7 to calculate AUC168 values.
i. Gemtuzumab ozogamicin administered at 3 mg/m2 on Days 1, 4 and 7 in combination with cytarabine and daunorubicin, for untreated de novo AML.
j. 1 dose/week.
k. Gemtuzumab ozogamicin manufactured with antibody produced from the initial cell line.
l. Gemtuzumab ozogamicin manufactured with antibody produced from the commercial cell line.
m. Daily dosing.

Local Tolerance

Local tolerance was evaluated in single- and repeat-dose toxicity studies in rats and/or monkeys after IV administration; there were no gemtuzumab ozogamicin-associated effects (macroscopic and/or microscopic pathology) observed at the injection sites.

Other toxicity studies

Table 10. Overview of other toxicity studies (*in vitro* and *in vivo*)

Type of Test	Test Cells/ Tissues	Test Article Lot Number	Test Concentrations	Number of Donors/ Tissue	Noteworthy Findings	Study Number/ GLP Compliance
In Vitro						
Studies with Gemtuzumab Ozogamicin						
In Vitro Blood Compatibility	Human Whole Blood	4489A-24-102593-R1592-24	12 µg protein/mL[a] (0.3 µg N-Ac-γ-calicheamicin DMH AcBut/mL)	NA	No RBC hemolysis in human whole blood or protein flocculation in human plasma.	MIRACL-26816/ Yes
Studies with hP67.6 Antibody and/or Gemtuzumab Ozogamicin						
Tissue Cross-Reactivity	Sprague-Dawley Rat, Monkey/Cynomolgus Tissue	10008374/2a	7.2, 72 µg/mL hP67.6 antibody	1 or 2	No tissue reactivity with hP67.6 was seen for any tissue from either species.	IM116/ Yes
Tissue Cross-Reactivity	Monkey/Cynomolgus Tissue	16-000559	2, 10 µg/mL biotinylated gemtuzumab ozogamicin	2	No staining was observed with biotinylated gemtuzumab ozogamicin in any cynomolgus monkey tissue examined.	20112882/ Yes
Tissue Cross-Reactivity	Human Tissue	6709-3-3	10 µg/mL hP67.6 antibody	1	Binding of hP67.6 with 44 different types of human tissue occurred only to histiocytes/lymphocytes and blood vessels. Staining appeared to be antigen-specific and was attributed to cytoplasmic antigen.	94-CDP771-222/00/ Yes
Tissue Cross-Reactivity	Human Tissue	6709-3-3	10 µg/mL hP67.6 antibody	1 to 3	Tissue reactivity of hP67.6 with 47 different types of human tissue did not show any unexpected or potentially adverse staining pattern. Specific staining was associated with macrophages/monocytes or histiocytes. Nonspecific staining was associated with neutrophils or, occasionally, with collagen and pigments.	94-CDP771-222A/ Yes
Tissue Cross-Reactivity	Human Tissue	4489A-24-102593-R1592-24	10 µg/mL PF-05208747	1 to 3	Tissue reactivity of PF-05208747 with 50 different types of human tissue did not show any unexpected or potentially adverse staining pattern. Specific staining was associated with macrophages/monocytes or histiocytes. Nonspecific staining was associated with neutrophils or, occasionally, with collagen and pigments.	94-CDP771-233/ Yes
Tissue Cross-Reactivity	Human Tissue	hP67.6: 26372QC PF-05208747: 4489A-35-301195-R1592-163	10 µg/mL hP67.6 antibody or PF-05208747	1 to 3	Tissue reactivity of hP67.6 or PF-05208747 with 34 different types of human tissue did not show any unexpected or potentially adverse staining pattern. Specific staining did not occur with hP67.6. Specific staining with PF-05208747 in 3 tissues (colon, lung, spleen) was associated with polymorphonuclear cells. Nonspecific staining observed in several tissues was associated with polymorphonuclear neutrophils or, occasionally, with mast cells and mucin.	95-CDP771-280/ Yes

In Vitro						
Type of Test	**Test Cells/ Tissues**	**Test Article Lot Number**	**Test Concentrations**	**Number of Donors/ Tissue**	**Noteworthy Findings**	**Study Number/ GLP Compliance**
Tissue Cross-Reactivity	Human Tissue	16-000559	2, 10 µg/mL biotinylated gemtuzumab ozogamicin	3	Membrane and cytoplasmic staining was observed with biotinylated gemtuzumab ozogamicin in mononuclear cells in most tissues (including monocytes in blood smears), granulocytes in blood smears, and hematopoietic precursor cells in bone marrow. No unexpected tissue staining was observed with biotinylated gemtuzumab ozogamicin.	20100387/ Yes
Studies with N-Ac-γ-Calicheamicin DMH						
Phototoxicity	BALB/c 3T3 Mouse Fibroblasts	00706190-0223	0.17, 0.3, 0.53, 0.95, 1.69, 3.00, 5.34, 9.5 µg/mL	NA	No phototoxic potential demonstrated.	20090641/ Yes
Mouse/CD-1	IV, 10 mL/kg (Lot 14664B-137)	1 Day (with ~ 6-Week Observation)	0, 50, 100, 300, 400, 500	10M	**≥300 µg/kg:** Liver (↑ weight, hepatocellular pleomorphism, nodular regeneration) **≥400 µg/kg:** Liver (rough surface) **500 µg/kg:** ↓ Body weight, ↓ food consumption, ↓ fecal output The NOAEL in this study was 100 µg/kg.	MIRACL-24627/ No
Rat/Sprague-Dawley	IV, 10 mL/kg (Lot PC 1249)	1 Day (with ~ 6-Week Observation)	0, 10, 100, 300, 1000	5	**100 µg/kg:** ↓ RBC, ↓ HCT, ↑ MCV, ↑ MCH, ↑ MCHC, ↓ WBC, ↓ lymphocyte **≥100 µg/kg:** Soft/liquid feces, few/no feces, ↓ body weight, ↓ body-weight gain, ↓ food consumption, liver (hepatocellular karyocytomegaly), kidney (karyocytomegaly in renal cortical tubular epithelium) **≥300 µg/kg:** Mortality in 10/10 animals, hypoactive, prostrate, unkempt appearance, chromodacryorrhea, wet peri-anal area, spleen (small, ↓ extramedullary hematopoiesis, lymphoid depletion), thymus (small, lymphoid depletion), seminal vesicle (small), adrenal gland (enlarged), stomach (ulceration, inflammation, distended), bone marrow (hypocellularity), acanthosis **1000 µg/kg:** Liver (pale, hepatocellular single cell necrosis), kidney (tubular epithelium necrosis) The NOAEL in this study was 100 µg/kg.	MIRACL-26628/ Yes
Dog/Beagle	IV, 2.5 mL/kg (Lot PC 1249)	1 Day (with ~ 6-Week Observation)	0, 2.5, 25, 75, 250	2	**25 µg/kg:** Kidney (renal tubular degeneration or necrosis/regeneration) **≥25 µg/kg:** Soft/loose feces, hematest-positive feces, emesis; kidney (renal tubular degeneration/ regeneration) **75 µg/kg:** Mortality in 1/4 animals, ↑ APTT **≥75 µg/kg:** Inactive or thin appearance, ↓ body weight, ↓ body weight gain, ↓ food consumption, spleen (small, lymphoid depletion), thymus (small, lymphoid depletion), multiple pinpoint-to-diffuse reddened areas of the intestinal tract mucosa, lymph nodes (lymphoid depletion), bone marrow	MIRACL-26629/ Yes

In Vivo						
Species/Strain	Method of Administration (Test Article Lot Number)	Duration of Dosing	Doses (µg/kg)	Number/ Sex/ Group	Noteworthy Findings	Study Number/ GLP Compliance
					(hypocellularity), small/large intestine (karyocytomegaly with necrosis, congestion, or hemorrhage)	
Rat/Sprague-Dawley	IV, 1 mL/kg (Lot PC 1249)	6 Weeks[b]	0, 1, 10, 30, 100	10	**250 µg/kg:** Mortality in 4/4 animals, hypothermia, excessive salivation The NOAEL in this study was 2.5 µg/kg. **≥10 µg/kg:** ↓ Body weight, ↓ body weight gain, ↓ food consumption, ↑ reticulocyte, liver (hepatocytic karyocytomegaly) **≥30 µg/kg:** Red crust around mouth and nose, few feces, wet/stained perianal area, unkempt appearance, ↓ RBC, ↓ HGB, ↓ HCT, ↑ MCV, ↑ MCH, ↑ MCHC, ↓ WBC, ↓ lymphocyte, ↑ blood urea nitrogen, liver (↑ weight, karyocytomegaly), kidney (tubular degeneration/ regeneration, tubular and/or collection duct epithelial karyocytomegaly), spleen (↑ weight, ↑ hematopoietic activity, atrophy of marginal zones), thymus (lymphoid depletion) **100 µg/kg:** Moribundity (1 male after 6th dose), ↑ calcium, ↑ phosphorous, ↑ cholesterol, ↑ ALT, ↓ sodium, ↓ chloride, ↓ potassium, spleen (small, ↑ weight), thymus (small), bone marrow (pale, hypocellularity), large/small intestine (mucosal changes of crypt epithelial hyperplasia), male mammary gland (atrophy, feminization) The NOAEL in this study was 10 µg/kg.	MIRACL-26630/ Yes
Dog/Beagle	IV, 1 mL/kg (Lot PC 1249)	6 Weeks[b]	0, 1, 5, 25, 50	4	**≥5 µg/kg:** Emesis, ↓ RBC, ↓ HGB, ↓ HCT **≥25 µg/kg:** Reduced/no feces, ↓ food consumption, ↓ reticulocyte, ↓ WBC, ↑ platelet, ↓ total protein, ↓ albumin, ↓ globulin, ↑ LDH, red discoloration of lymph nodes and colon, cervical lymph node (lymphoid depletion), mesenteric lymph node (lymphoid depletion, hemorrhage), thymus (↓ weight, lymphoid depletion), testes (↓ weight, germinal epithelium	MIRACL-26707/ Yes

In Vivo						
Species/Strain	Method of Administration (Test Article Lot Number)	Duration of Dosing	Doses (µg/kg)	Number/ Sex/ Group	Noteworthy Findings	Study Number/ GLP Compliance
					atrophy), epididymis (aspermia), prostate gland (atrophy), pancreas (exocrine atrophy, acinar necrosis, islet cell hyperplasia) **50 µg/kg:** Thin appearance, ↓ body weight, ↓ body weight gain, ↓ calcium, ↓ alkaline phosphatase, lymphoid depletion (tonsils, ileum, spleen), bone marrow (hypocellularity), ovary (arrested follicular development), vagina (atrophy of epithelium), fundic gastric glands (necrotic debris), thyroid (morphologic activation) The NOAEL in this study was 5 µg/kg.	
Studies with N-Ac-γ-Calicheamicin DMH AcBut						
Rat/Sprague-Dawley	IV, 10 mL/kg (Lot R-668)	1 Day (with ~ 6-Week Observation)	0, 10, 100, 300, 1000	5	**≥10 µg/kg:** ↓ Body weight **100 µg/kg:** Testes (atrophy) **≥100 µg/kg:** Kidney (tubular dilation, karyocytomegaly, tubular degeneration, ↑ tubular basophilia), liver (karyocytomegaly) **300 µg/kg:** Mortality in 6/10 animals, ↑ BUN, ↑ creatinine, bone marrow (dark red, hypocellularity) **≥300 µg/kg:** ↓ Motor activity (hypoactive, inactive, and/or prostrate), fecal (no or few feces; soft, liquid, and/or mucoid feces), wet perianal area, unkempt appearance, spleen (small, lymphoid depletion), thymus (small, lymphoid depletion), liver (pale foci), nonglandular stomach (intraepithelial abscesses, discolored foci) **1000 µg/kg:** Mortality in 10/10 animals, kidney (tubular casts), liver (necrosis), seminiferous tubules (atrophy), seminal vesicles (small) The NOAEL in this study was 10 µg/kg.	MIRACL-26709/ Yes
Studies with N-Ac-ε-Calicheamicin						
Rat/Sprague-Dawley	IV, 10 mL/kg (Lot PC 1251)	1 Day (with ~ 6-Week Observation)	0, 10, 100, 300, 1000	5	**≥10 µg/kg:** Heart (↓ weight, F), **≥300 µg/kg:** Thymus (↑ weight, M) **1000 µg/kg:** Thymus (↑ weight, F) The NOAEL in this study was 1000 µg/kg.	MIRACL-25706/ Yes
Dog/Beagle	IV, 2.5 mL/kg (Lot PC 1251)	1 Day (with ~ 6-Week Observation)	0, 2.5, 25, 75, 250	2	None. The NOAEL in this study was 250 µg/kg.	MIRACL-25707/ Yes
Other Studies with an ADC Containing Calicheamicin						
Monkey/Cynomolgus	IV, 1 mL/kg PF-06647259[c] (Lot PF-06647259-00-0005)	9 Weeks[d]	0, 0.5 mg/kg	M (7 or 8)	**0.5 mg/kg:** ↓ RBC, ↓ HGB, ↓ HCT, ↑ reticulocyte, ↑ RDW, ↓ platelet (complete recovery after first dose, incomplete recovery after subsequent doses), ↑ APTT, ↑ fibrinogen, ↓ WBC, ↓ lymphocyte, ↑ AST, ↑ ALT, ↑ total protein, ↓ albumin, ↑ globulin, ↓ calcium, ↓ thrombopoietin, ↑ hyaluronic acid, spleen (Days 3 and 63 necropsy: ↑ cellularity in sinusoids; Day 63 necropsy: ↓ lymphoid cellularity), liver (Day 3 necropsy: loss of sinusoidal endothelial cells with platelet sequestration; Day 63 necropsy: multifocal sinusoidal dilatation and/or hepatocyte atrophy, recovery of sinusoidal endothelial cells with CD34 overexpression)	14GR346/ No

2.3.5. Ecotoxicity / environmental risk assessment

Phase I: Estimation of Exposure

The cytotoxic agent or active ingredient in gemtuzumab ozogamicin is calicheamicin, an antitumor antibiotic derived from the bacterium Micromononspora echinospora found in chalky soil. The active ingredient or payload consists of conjugated and unconjugated calicheamicin. Total calicheamicin, including the payload and the linker components 4-(4-acetylphenoxy) butanoic acid (AcBut) and 3-mercapto-3-methyl butanoic acid, hydrazide (DMH), was determined to be the environmentally relevant component of gemtuzumab ozogamicin used in this assessment. No experimental value for the the log Kow for the active ingredient has yet been determined. The log D values estimated by calculation are provided below.

Screening for Persistence, Bioaccumulation and Toxicity (PBT)

Payload				
Component	Tautomer	Log D[4]		
		pH 5.0	pH 7.4	pH 9.0
unconjugated calicheamicin	Major	2.79	3.83	3.93
conjugated calicheamicin	Minor	1.65	2.32	1.87
Linkers				
Component		Log D[4]		
		pH 5.0	pH 7.0	pH 9.0
4-(4-acetylphenoxy)butanoic acid		1.15	-0.69	-1.95
3-mercapto-3-methyl butanoic acid, hydrazide		-0.27	-0.26	-0.27

Calculation of the Predicted Environmental Concentration (PEC) in Surface Water

Fpen Refinement

As per the original public summary of opinion on orphan designation of gemtuzumab ozogamicin for the treatment of AML2, as well as subsequent Rev 1 (June 2013)3, the prevalence of the condition in the European Union is 0.66 in 10,000 persons 0.66 patient / 10,000 population = 0.000066 EU population

Fpenrefined = 0.000066

Dosing Regimen

For patients with previously untreated de novo CD33-positive AML, the recommended dose of gemtuzumab ozogamicin during induction is 3 mg/m^2 (body surface area) up to a maximum dose of 5 mg, on Days 1, 4, and 7. Therefore, over a 7 day period, patients will receive a maximum dose of 15 mg total drug. Since gemtuzumab ozogamicin is not dosed on a daily basis, but over a 7 day period, the maximum daily dose, based on total calicheamicin (payload and linker), defined as Nacetyl gamma calicheamicin DMH Ac-But acid, is derived as follows:

$$\text{Maximum daily dose} = [(35\ \mu g/mg \times 15\ mg)] / 1000$$

7 days Maximum daily dose = 0.075 mg/day

Where: total calicheamicin (35 micrograms/mg protein, max acceptance criteria) maximum dose gemtuzumab ozogamicin administered (15 mg/7 days) conversion factor (1000)

Predicted Environmental Concentration (PEC) in Surface Water

$$PECsw\ [mg\ L] = \frac{DOSEai \times Fpen}{WASTEWinhab \times Dilution}$$

Where:

PEC_{SW}	Predicted environmental concentration in surface water	-- mg/L
DOSEai	Maximum daily dose consumed per inhabitant	0.075 mg/(inh·d)
Fpen	Market penetration	0.000066
WASTEWinhab	Amount of wastewater per inhabitant per day	200 L/(inh·d) [Default]
DILUTION	Dilution factor	10 [Default]

$$PECsw = \frac{0.075\ mg/(inh \cdot d) \times 0.000066}{200\ L/(inh \cdot d) \times 10}$$

$$PEC_{sw} = 2.5 \times 10^{-9}\ mg/L = 2.5 \times 10^{-6}\ \mu g/L$$

$PECsw = 2.5 \times 10^{-6}\ \mu g/L$ < Action limit of 0.01 µg/L

Outcome of Phase I

The PECsurfacewater value is less than the 0.01 µg/L action limit. Based on the PEC value, a Phase II environmental fate and effects analysis is not required.

Table 11. Summary of main study results

Substance (INN / Invented Name):			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107 or …	Inconclusive – experimental value not available	Potential PBT (Y/N)
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	Inconclusive – experimental value not available	B/not B
	BCF		B/not B
Persistence	DT50 or ready biodegradability		P/not P
Toxicity	NOEC or CMR		T/not T
PBT-statement :	The compound is not considered as PBT nor vPvB The compound is considered as vPvB The compound is considered as PBT		

Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC surfacewater , default or refined (e.g. prevalence, literature)	2.5 x 10-6	µg/L	> 0.01 threshold (N)
Other concerns (e.g. chemical class)			(N)

2.3.6. Discussion on non-clinical aspects

The hP67.6 antibody exhibited a high affinity for CD33, with an average KD of 0.073 nM. Gemtuzumab ozogamicin had an EC50 value of 11.6 nM (1.69 µg/mL) which was comparable to hP67.6 antibody, indicating that conjugation of the linker-payload to the hP67.6 antibody does not alter its binding affinity to the cells. Binding and internalization analysis of radioiodinated hP67.6 antibody demonstrated efficient delivery of the cytotoxic payload of the product into CD33-expressing HL-60 cells and payload release was determined at a pH value of 4.5, which is consistent with the pH in the acidic lysosomal vesicular compartment of the cell.

Gemtuzumab ozogamicin exhibited *in vitro* cytotoxicity in CD33+ cells, and inhibition of colony growth in blood or bone marrow specimens from AML patients, but not in normal samples.

In HL60 AML subcutaneous xenografts in immune-compromised mice, gemtuzumab ozogamicin reduced tumour growth and enhanced survival at doses of 1 mg/kg/day on a q4dx4 schedule.

Gemtuzumab ozogamicin safety pharmacology was evaluated in mice, rats, and dogs after a single IV administration. In an *in vitro* hERG current inhibition study with N-Ac-γ-calicheamicin DMH, no significant hERG current inhibition was observed up to 6.77 µM.

Nonclinical studies assessing drug-drug interaction potential of gemtuzumab ozogamicin and/or N-Ac-γ-calicheamicin DMH were presented and indicated a low potential to inhibit activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 at clinically relevant concentrations. N-Ac-γ-calicheamicin DMH demonstrated little or no reversible inhibition of UGT1A4, UGT1A6, UGT1A9, and UGT2B7 catalysed activities; however, N-Ac-γ-calicheamicin DMH inhibited UGT1A1 activity. N-Ac-γ-calicheamicin DMH showed little or no inhibition of the bidirectional transport of digoxin (P-gp substrate) or pitavastatin (BCRP substrate).N-Ac-γ-calicheamicin DMH inhibited the OATP1B1- and OATP1B3-mediated transport of pravastatin and rosuvastatin by 30% and 16%, respectively, in a concentration-dependent manner. A low potential for an interaction between N-Ac-γ-calicheamicin DMH and these two transporters at clinically relevant concentrations exists. N-Ac-γ-calicheamicin DMH, over the concentrations evaluated, did not inhibit OAT1, OAT3, or OCT2.

Overall, N Ac γ-calicheamicin DMH showed a low potential to inhibit P gp, BCRP, BSEP, MRP2, MATE1, MATE2K, OATP1B1, OATP1B3, OCT1, OAT1, OAT3, and OCT2 at clinically relevant concentrations.

The main toxicities occurred in the liver, bone marrow and lymphoid organs, haematology parameters (decreased RBC mass and WBC counts, mainly lymphocytes), kidney, eye and male and female reproductive organs. Effects on liver, kidney and male reproductive organs in rats, and on lymphoid tissues in monkeys (approximately 18 times for rats, and 36 times for monkeys, the human clinical

exposure after the third human dose of 3 mg/m^2 based on AUC_{168}) were not reversible. Effects on female reproductive organs and the eye in monkeys were adverse in the 12-week study (approximately 193 and 322 times, respectively, the human clinical exposure after the third human dose of 3 mg/m^2 based on AUC_{168}). The relevance of the irreversible animal findings to humans is uncertain (SmPC, section 5.3).

Clinical chemistry changes in rats and monkeys included dose-related changes in AST, ALT, and/or ALP after single and/or repeat dosing with GO. Mylotarg has the potential to cause elevations in liver-related laboratory tests and hepatotoxicity. Severe (Grade $\geq$ 3) and/or serious hepatotoxicity including all VOD/SOS has been classified as an identified risk in the Risk Management Plan (see discussion on clinical safety).

In rats and monkeys, haematology changes consisted of decreases in RBC mass, WBC counts (mainly lymphocytes), and platelets (rats only at high doses associated with hepatocellular necrosis) in single and/or repeat-dose studies up to 6 weeks in duration with GO. Myelosuppression (Severe and/or serious infection and haemorrhage has been classified as an identified risk in the Risk Management Plan (see discussion on clinical safety).

Nervous system effects have not been observed in rats or monkeys after single or repeat dosing with GO. Nervous system alterations were identified after repeat doses in rats with other antibody-calicheamicin conjugates and are considered to be a class effect (SmPC, section 5.3). Neurotoxicity has been classified as a potential risk in the Risk Management Plan (see discussion on clinical safety).

Renal tubular degeneration associated with tubular casts, dilation, karyocytomegaly, and basophilia, accompanied by proteinuria and decreased serum albumin was observed in rats after in single- or repeat-dose studies up to 6 weeks in duration with GO. Renal toxicity has been classified as a potential risk in the Risk Management Plan (see discussion on clinical safety).

Gemtuzumab ozogamicin was found to be clastogenic. This is consistent with the known induction of DNA breaks by calicheamicin and other enediyne antitumour antibiotics. N acetyl gamma calicheamicin DMH (the released cytotoxin) was found to be mutagenic and clastogenic (SmPC, section 5.3). Second primary malignancy has been classified as a potential risk in the Risk Management Plan.

Formal carcinogenicity studies have not been conducted with gemtuzumab ozogamicin. In toxicity studies, rats developed preneoplastic lesions (minimal to slight oval cell hyperplasia) in the liver approximately 54 times, the human clinical exposure after the third human dose of 3 mg/m^2 based on AUC_{168}). There were no preneoplastic or neoplastic lesions observed in monkeys up to approximately 115 times the human clinical exposure after the third human dose of 3 mg/m^2 based on AUC_{168}). The relevance of these animal findings to humans is uncertain (SmPC, section 5.3).

In a female rat fertility study slightly lower numbers of corpora lutea and increased embryolethality were observed in the presence of maternal toxicity (approximately 9.7 times, the human clinical exposure after the third human dose of 3 mg/m^2 based on AUC_{168}). Effects on the reproductive tract of female monkeys were observed in the 12-week study (atrophy of the ovary, oviduct, uterus, and cervix; approximately 193 times the human clinical exposure after the third dose of 3 mg/m^2) (SmPC, section 5.3).

In a male fertility study, effects on male reproduction included lower spermatogonia and spermatocytes, decreases in testicular spermatids and epididymal sperm, vacuolation of the nucleus in spermatids, and/or appearance of giant cells. Additional findings included effects on the testes, epididymides and mammary gland as well as fertility. When male rats were mated again after a 9 week non-dosing period, effects on sperm and fertility were worse but there was partial recovery of the lower spermatogonia and spermatocytes in the testes. Effects on male rat reproductive organs were partially reversible or not reversible. Male reproductive effects (testes, epididymides, seminal vesicles) in monkeys were observed

at approximately 66 times the human clinical exposure after the third dose of 3 mg/m^2) (SmPC, section 5.3).

In an embryo-foetal toxicity study lower foetal body weight, higher incidence of foetal wavy ribs, and lower incidence of foetal skeletal ossification were observed. Increased embryolethality and foetal morphological anomalies included digital malformations, absence of the aortic arch, anomalies in the long bones in the forelimbs, misshapen scapula, absence of a vertebral centrum, and fused sternebrae. Increased embryolethality was also observed in the presence of maternal toxicity. The lowest dose with embryo-foetal effects correlated with 9.7 times the human clinical exposure after the third human dose of 3 mg/m^2, based on AUC_{168} (SmPC, section 5.3).

A tissue cross-reactivity study using rat or cynomolgus monkey cryosections determined that specific staining by hP67.6 was absent in all tissues of both animal species. In view of the absence of cross-reactivity of gemtuzumab ozogamicin with CD33 from laboratory species, studies of kinetics in animals can only be of limited value, as there will be no binding, specific intracellular uptake, and metabolism of the conjugate that underlies its therapeutic action in humans. Further PK data were derived from the 13-week toxicology study in cynomolgus monkeys. The mechanism proposed for liver toxicity is suggested to be related to tissue vascularisation and hence exposure to the product ie the liver, as a highly perfused organ, is exposed to more gemtuzumab ozogamicin than poorly vascularised tissues. Toxicity is suspected to arise from uptake by liver sinusoidal cells and breakdown of gemtuzumab ozogamicin leading to local cellular toxicity where this occurs. Therefore this does not correlate with tissue binding results.

The phototoxic potential of gemtuzumab ozogamicin is low, based on the type of product and a low distribution to eye and skin in rats.

The introduction of Mylotarg would not be expected to result in an environmental risk based on the data generated. However, this conclusion is based on an estimated value for log Kow; the applicant is recommended to provide an experimentally derived log Kow as a post authorisation measure.

2.3.7. Conclusion on the non-clinical aspects

Overall, the non-clinical documentation submitted was considered adequate. The relevant information has been included in the SmPC (sections 4.4, 4.6, 5.1, 5.3).

The applicant is recommended to provide an experimentally derived log Kow as a post authorisation measure.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 12 Overview of Clinical Studies with gemtuzumab ozogamicin

Study Name	Study Design	Study Population	Efficacy Population (Safety Population) [GO/No GO]	Induction Treatment
Studies with GO in Combination with Chemotherapy				
Pivotal Phase 3 Study				
Study ALFA-0701 (MyloFrance 3, WS936568)	Phase 3, open-label, randomised 1:1 study to assess benefit and toxicity of adding fractionated GO to standard induction chemotherapy.	Adults 50-70 years of age with previously untreated de novo AML	271 [135/136] (268 [131/137])	GO: 3 mg/m² (maximum dose 5 mg) D1, 4, 7. Chemotherapy: DNR 60 mg/m²/d D1-3; AraC 200 mg/m²/d D1-7 vs Chemotherapy without GO
Supportive Individual Patient Data (IPD) Meta-Analysis				
IPD Meta-Analysis	Five (5) randomised studies of GO added to chemotherapy during induction	Patients ≥15 years of age with newly diagnosed AML and MDS	3331 [1663/1668] (3331 [1663/1668])	Intensive induction chemotherapy, designed to induce CR in patients. The meta-analysis included trials that allowed an unconfounded comparison of GO in Course 1 of induction chemotherapy (ie, chemotherapy plus GO versus chemotherapy without GO), excluding trials where GO was used in place of part of a chemotherapy regimen, before chemotherapy, or only in consolidation. Trials involving less intensive regimens such as low-dose AraC (LDAC) were excluded from the IPD meta-analysis.
Individual Phase 3 Studies Included in the Supportive IPD Meta-Analysis [a]				
Study SWOG S0106 (WS936510)	Phase 3, open-label, randomised study to assess the benefit of adding GO to standard induction therapy, followed by a post-consolidation randomisation to receive either 3 additional doses of GO or no additional therapy.	Adults 18-60 years of age with previously untreated de novo non-M3 AML.	595 [295/300] (595 [295/300])	GO: 6 mg/m² D4 Chemotherapy: DNR 45 mg/m²/d D1, 2, 3; AraC 100 mg/m²/d Course I D1-7; vs Chemotherapy without GO
Study MRC AML15 (WS1974568)	Phase 3, open-label, randomised study to assess the benefit of adding GO to induction and/or consolidation chemotherapy.	Adults <60 years of age with previously untreated de novo AML or secondary AML, APL[b]	1099 [548/551] (1099 [548/551])	GO: 3 mg/m² D1 of Course 1 plus Chemotherapy: 1) ADE 2) DNR 50 mg/m² D1, 3, 5 AraC 100 mg/m²/q12h, D1-10 3) FLAG-Ida vs Chemotherapy without GO
Study NCRI AML16 (WS936667)	Phase 2/3, open label, randomised study to assess the benefit of adding GO to standard induction chemotherapy.	Adults >60 years of age with previously untreated de novo or secondary AML or high-risk MDS	1115 [559/556] (1115 [559/556])	GO: 3 mg/m² D1 Chemotherapy: 1) DNR 50 mg/m²/d D1, 3, 5 AraC 100 mg/m²/q12h, D1-10 2) DNR 50 mg/m²/d D1, 3, 5 clofarabine 20 mg/m²/d D1-5; +GO 3 mg/m² D1 vs Chemotherapy without GO
Study GOELAMS AML2006IR (WS936554)	Phase 3, open label, randomised study to assess the benefit of adding GO to standard induction and consolidation chemotherapy.	Patients ≤60 years of age with newly diagnosed intermediate-risk cytogenetics CD33-positive AML	251 [126/125] (251 [126/125])	GO: 6 mg/m² D4 Chemotherapy: DNR 60 mg/m² D1-3 AraC 200 mg/m²/d D1-7 vs Chemotherapy without GO

Study Name	Study Design	Study Population	Efficacy Population (Safety Population) [GO/No GO]	Induction Treatment
Other Supportive Studies of GO in Combination with Chemotherapy				
0903B1-205-US/EU/AU (Study 205)	Phase 1/2, open label, single-arm, multicentre study to assess the safety and efficacy of GO given in combination with AraC.	Phase 1: Adults ≥18 years of age with relapsed or refractory AML or patients ≥60 years of age with previously untreated de novo CD33-positive AML	Phase 1: N/A[c] (21)	Phase 1: GO: 1) 6 mg/m² D1, 15 1a) 6 mg/m² D1 and 4 mg/m² D8 2a) 6 mg/m² D1 and 4 mg/m² D8 3a) 9 mg/m² D1 and 6 mg/m² D8 Chemotherapy: (1, 2a and 3a): AraC 100 mg/m² D1-7
		Phase 2: Adults ≥60 years of age with previously untreated de novo CD33-positive AML	Phase 2: 21 (21) with 17 patients from Phase 2 and 4 patients from Step 2a of Phase 1	Phase 2: GO dose schedule as in Step 2a from Phase 1 plus Chemotherapy
0903B1-206-US/EU/AU (Study 206)	Phase 1/2, open-label, single-arm, multicentre study to assess safety and efficacy of GO given in combination with AraC and DNR.	Phase 1: Adults ≥18 and <60 years of age with de novo AML or adults ≥60 years of age with relapsed or refractory AML	Phase 1: N/A[c] (22)	Phase 1: GO: 6 or 9 mg/m² D4 Chemotherapy: DNR 45 mg/m² D1-3 AraC 100 or 200 mg/m²/d D1-7
		Phase 2: Adults ≥18 and <60 years of age with de novo AML	Phase 2: 53 (53) with 49 from Phase 2 and 4 patients from regimen a of Phase 1	Phase 2: GO: 6 mg/m² D4 Chemotherapy: DNR 45 mg/m² D1-3 AraC 100 mg/m²/d D1-7
MyloFrance 2 (WS936540)	Phase 1/2, open label study to determine optimal doses of DNR and AraC to be combined with fractionated doses of GO.	Adult 50-70 years of age with AML in first relapse	20 (20)	GO: 3 mg/m² D1, 4, 7 Chemotherapy: 3+7 DNR+AraC at 45/100 or 60/100 or 60/200 mg/m²
Supportive Studies with Single-Agent GO in Relapsed AML				
0903B1-201-US/CA (Study 201)	Phase 2, open-label, single-arm, 3-part, multidose, multicentre study, to examine the effects of GO in patients with CD33-positive AML in first relapse.	Adults with CD33-positive AML in first relapse	84 (84)	GO: 9 mg/m² for 2 or 3 doses 14 days apart
0903B1-202-EU (Study 202)	Phase 2, open-label, single-arm, 3-part, multidose, multicentre study, to examine the effects of GO in patients with CD33-positive AML in first relapse.	Adults with CD33-positive AML in first relapse	95 (95)	GO: 9 mg/m² for 2 or 3 doses 14 days apart
0903B1-203-US/EU (Study 203)	Phase 2, open-label, single-arm, 3-part, multidose, multicentre study, to examine the effects of GO in patients with CD33-positive AML in first relapse.	Adults ≥60 yrs with CD33-positive AML in first relapse	98 (98)	GO: 9 mg/m² for 2 or 3 doses 14 days apart
0903A1-101-US (Study 101)	Phase 1, single-arm, dose escalation study to examine the safety and PK of GO.	Adults with relapsed or refractory CD33-positive AML	40 (40)	GO: 0.25, 0.5, 1, 2, 4, 5, 6, and 9 mg/m² (>14 days apart); maximum of 3 doses.
0903A1-102-US (Study 102)	Phase 1, 2 part, single-arm, open-label dose-escalation study to assess safety, efficacy, and PK of GO in paediatric patients	Children (≤17 yrs) with refractory or relapsed CD33-positive AML	29 (29)	GO: 6, 7.5, and 9 mg/m² for up to 2 doses. For patients <3 years of age, per kg dosing was used.

Study Name	Study Design	Study Population	Efficacy Population (Safety Population) [GO/No GO]	Induction Treatment
Supportive Studies with Single-Agent GO in AML (continued)				
0903A1-103-JP (Study 103)	Phase 1/2, single-arm, open-label study in Japanese patients to study safety, efficacy, PK, and to confirm the tolerance of GO at 9 mg/m²	Japanese adults 18 to 70 yrs with relapsed or refractory CD33-positive AML in first relapse	Phase 1: 20 (20) Phase 2: 20 (20)	Phase 1: GO: 6, 7.5, and 9 mg/m² for up to 2 doses. Phase 2: GO: 9 mg/m² for up to 2 doses.
0903X-100374 (Study 100374)	Phase 4, single-arm, dose-finding study to assess safety of GO as single-agent treatment of patients with relapsed AML who have received prior HSCT	Patients with relapsed AML after autologous or allogeneic HSCT	37: Allogeneic HSCT: 27, Autologous HSCT: 10 (37)	Phase 1: 2, 4, and 6 mg/m² GO; up to 2 doses. Phase 2: GO: up to an additional 4 doses
0903X-100863 (Study 100863)	Phase 4, single-arm multicentre study to assess efficacy of corticosteroids prophylaxis on frequency and severity of GO infusion-related AEs and to evaluate the effect of corticosteroids on GO efficacy after 1 month of treatment	Adults ≥18 years with CD33-positive AML, resistant or relapsed	23 (23)	GO: 2 to 9 mg/m²×2 doses (Day 1 and Day 15).
0903X-100847 (Study 100847)	Phase 4, single arm, prospective observational study to primarily estimate the rate of VOD and to identify risk factors for VOD and to collect safety data in routine clinical practice	Patients with CD33-positive AML in first relapse	512 enrolled[e] (482)	GO, IV, as per the treating physician or clinical study.
Supportive Studies with Single-Agent GO in AML (continued)				
MyloFrance 1[d]	Phase 2, single-arm, prospective, multicentre study to assess the safety and efficacy of fractionated doses of GO	Adult ≥18 years of age with CD33-positive AML in first previously untreated relapse with a duration of CR1 3-18 months	57 (57)	GO: 3 mg/m² IV Days 1, 4, 7
Supportive Study with Single-Agent GO in Patients With MDS				
0903B1-207-US/EU (Study 207)	Phase 2, single arm, multidose study to assess total survival and QoL after GO treatment	Adults >18 years of age with intermediate or high-risk MDS	26[f] (26)	GO: 9 mg/m² Arm A: 1 dose; Arm B: 2 doses.

AE=adverse event; ADE=AraC/DNR/etoposide; ALFA=Acute Leukemia French association; AML=acute myeloid leukaemia; AraC=cytarabine; APL=acute promyelocytic leukaemia; AU=Australia; CA=Canada; CD=Cluster of Differentiation; CR=complete remission; CO=Clinical Overview; CR1=first CR; D=day; DNR=daunorubicin; EU=European Union; FLAG-Ida=fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin; GO=gemtuzumab ozogamicin (Mylotarg); GOELAMS=Groupe Ouest Est d'Etude des Leucémies aiguës et Autres Maladies du Sang; IPD=individual patient data; HSCT=haematopoietic stem cell transplantation; IV=intravenous; LDAC=low dose AraC; MDS=myelodysplastic syndrome; MRC=Medical Research Council; N/A=not applicable; NCRI=National Cancer Research Institute; No GO=chemotherapy alone; PK=pharmacokinetic; q=every; QoL=Quality of Life; SCE=Summary of Clinical Efficacy; SWOG=Southwest Oncology Group; US=United States; VOD=veno-occlusive disease.

a. Study ALFA-0701 presented in previous row was also included in the meta-analysis.
b. APL patients were not included in the meta-analysis.
c. In the Phase 1 part of Studies 205 and 206, efficacy data were reported only for the 4 patients in each study with de novo AML treated at the Phase 2 dose; these patients were analysed with the Phase 2 patients.
d. Study MyloFrance 1 was a dose-finding study to evaluate the safety and efficacy of the fractionated dosing regimen of GO in patients with AML in first relapse.
e. No efficacy data were reported.
f. No efficacy data are presented in the SCE or the CO for Study 207, given that it was conducted in patients with MDS.

2.4.2. Pharmacokinetics

Pharmacokinetic data were obtained from 8 Phase 1 and Phase 2 clinical trials with GO (study A1-101-US, study B1-201-US/CA, study B1-202-EU, study B1-205-AU/EU/US, study B1-206-AU/EU/US), including one paediatric Phase 1 study (study A1-102-US), one Phase 2 trial in elderly patients age ≥ 60 years only (study B1 203 US/EU), and one Phase 1/2 trial conducted in Japanese patients (study A1-103-JA).

Population PK model

The dataset for the pooled analysis of hP67.6 comprised 5643 concentrations obtained from 407 patients, including 505 (9%) concentrations < LLOQ. The dataset for the pooled analysis of unconjugated calicheamicin comprised 4281 concentrations obtained from 338 patients, including 730 (17%) concentrations < LLOQ.

Absorption

Gemtuzumab ozogamicin is administered intravenously, therefore absorption is not applicable.

Distribution

In vitro, the binding N-acetyl gamma calicheamicin dimethyl hydrazide to human plasma proteins is approximately 97%. In vitro, N-acetyl gamma calicheamicin dimethyl hydrazide is a substrate of P-glycoprotein (P-gp). In patients, the total volume of distribution of hP67.6 antibody (sum of V1 [10 L] and V2 [15 L]) was found to be approximately 25 L (SmPC, section 5.2).

Elimination

The primary metabolic pathway of gemtuzumab ozogamicin is anticipated to be hydrolytic release of N acetyl gamma calicheamicin dimethyl hydrazide. In vitro studies demonstrated that N acetyl gamma calicheamicin dimethyl hydrazide is extensively metabolised, primarily via nonenzymatic reduction of the disulphide moiety. The activity (cytotoxicity) of the resultant metabolites is expected to be significantly attenuated. In patients, unconjugated calicheamicin plasma levels were typically low, with a predicted mean Cmax of 1.5 ng/mL following the third dose (SmPC, section 5.2).

Based on Population PK analyses, the predicted clearance (CL) value of hP67.6 from plasma was 3 L/h immediately after the first dose and then 0.3 L/h. The terminal plasma half-life (t½) for hP67.6 was predicted to be approximately 160 hours for a typical patient at the recommended dose level (3 mg/m2) of Mylotarg (SmPC, section 5.2).

Dose proportionality and time dependencies

Table 13 displays the summary of dose normalized pharmacokinetic parameters for hP67.6 in study 101 by dose level and dose event (Dose Periods 1 and 2).

Table 13 Summary of Dose-normalized Pharmacokinetic Parameters for hP67.6 in Study 0903A1-101-US by Dose Level (Dose Periods 1 and 2)

GO Dose Level (mg/m^2)	n	C_{max} / Dose (ng/mL/mg)		AUC_{inf} / Dose (ng·h/mL/mg)	
		Period 1	Period 2	Period 1	Period 2
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
0.25	4	33±23	30	180±201	266
0.5	3	31±15	50±39	593±598	1655±2010
1	4	26±24	51±38	485±380	861±743
2	2	129±102	117±107	3554±3630	3726±3928
4	6	83±48	107±55	1459±1566	2529±2733
5	6	133±40	173±66	2788±2467	4471±1640
6	7	205±102	324±201	6292±6574	12010±13140
9	7	193±62	270±91	5202±5130	13480±9432

All values are mean±SD unless n<2.
Note: All dose periods are included in the summary statistic. Summary statistics are weighted for the number of dose periods to reflect individual patient variability.
AUCinf=area under the concentration-time curve from time zero extrapolated to infinity; Cmax=peak plasma drug concentration.

For most of the studies where 2 doses of GO were administered, exposure increased approximately 2-fold in Dose Period 2. In Studies 201, 202, and 203, the mean increase in C_{max} for Dose Period 2 was approximately 19%, compared to Dose Period 1. However, AUC_{inf} increased by 92%, from 137400 ng·h/mL in Dose Period 1 to 263700 ng·h/mL in Dose Period 2. Unconjugated calicheamicin exposure also increased in the second dose period by 15% for C_{max} and by 25% for AUC_{inf}, compared to the first dose period. A similar trend was observed in Studies 101 and 103 in adult AML patients, as well in as paediatric AML patients in Study 102, where mean AUC_{inf} increased by 53% and 44%, for the 6 mg/m^2 and 9 mg/m^2 groups, respectively.

Special populations

Age

The adult population modelling showed that age did not significantly affect the PK of hP67.6. Similarly, age was not a significant covariate in the exposure-response modelling for efficacy and safety endpoints within the adult population.

Table 14 PK studies in elderly population

PK Trials	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
0903A1-101-US 0903A1-103-JA 0903B1-201-US/CA 0903B1-202-EU 0903B1-203-US/EU 0903B1-205-US/EU/ AU 0903B1-206-US/EU/ AU	105/407	24/407	1/407

The adult population PK analysis required incorporation of a fixed effect of BWT (allometric scaling) on V1 and CL1 for hP67.6. Similarly, for unconjugated calicheamicin, a fixed effect of BWT (allometric scaling) was included for CL/F and V1/F.

For the paediatric population modelling, BWT was incorporated as a fixed effect on hP67.6 CL1, central volume of distribution (V1), and decay coefficient of the time-dependent clearance (kdes) using the theoretical allometric values of 0.75 and 1 for clearance and volume, respectively, and estimating the effect of body weight on kdes.

Renal impairment

The clearance of hP67.6 in patients with mild renal impairment (N=149) and with moderate renal impairment (N=47) was similar to the clearance of hP67.6 in patients with normal renal function (N=209). Severe renal impairment could not be assessed due to lack of information (N=1). For unconjugated calicheamicin, the same measure of renal impairment was tested as a covariate in the population model and was not found to significantly affect the PK.

The median estimated total CL values were 0.113, 0.112, 0.0889, and 0.0670 L/hour for patients in the normal (A), mild (B1), mild (B2), and moderate (C) groups, respectively; there were no patients in the severe (D) group.

Hepatic impairment

The results showed the clearance of hP67.6 in patients with mild hepatic impairment (B1, N=58), mild hepatic impairment (B2, N=19), or moderate hepatic impairment (C, N=6) was similar to that in patients with normal hepatic function (A, N=322). In the 6 patients with moderate hepatic impairment (C), the median hP67.6 clearance appeared to be lower; however, the mean hP67.6 clearance was not lower. For unconjugated calicheamicin, the same measures of hepatic impairment were tested as covariates in the population model and none were found to significantly affect the PK.

Gender

The volume of distribution in the central compartment (V1) was decreased by 16.2% in females patients. Gender was not a significant covariate for the PK parameters of unconjugated calicheamicin. The exposure-response model showed that gender did not significantly affect the efficacy or safety outcomes examined.

Race

The adult population modelling showed that race did not significantly affect the PK of hP67.6 or unconjugated calicheamicin. Population PK modelling showed that race, in particular Asian vs non-Asian (White 89%, Black 2%, Other 2%, Asian 7%), was not a significant covariate on the pharmacokinetics of gemtuzumab ozogamicin.

Pharmacokinetic interaction studies

No clinical drug interaction studies have been conducted with gemtuzumab ozogamicin.

Potential Inhibition of CYP Enzymes

The potential for gemtuzumab ozogamicin to reversibly inhibit the catalytic activity of 7 CYP enzymes (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) was investigated in human liver microsomes (HLM). Assessment of DDI potential with gemtuzumab ozogamicin for reversible CYP inhibition, based on the IC50 values of >15 µM determined from *in vitro* studies and the mean steady-state total hP67.6 Cmax of 3280 ng/mL (0.0221 µM) after multiple dose administration of 9 mg/m^2 of gemtuzumab ozogamicin to humans, indicated a low potential for gemtuzumab ozogamicin to inhibit the activities of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 (R1 value of <1.1, and Cmax/Ki <0.02).

The potential for N-Ac- γ -calicheamicin DMH to reversibly inhibit the catalytic activity of 7 CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) was investigated in HLM. N-Ac- γ -calicheamicin DMH showed little or no reversible inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6 with IC50 values >10 µM (highest concentration tested) and an estimated Ki of >5 µM. *In vitro*, N acetyl gamma calicheamicin dimethyl hydrazide and gemtuzumab ozogamicin had a low potential to induce the activities of CYP3A4 at clinically relevant concentrations with IC50 values of 5.5, 0.42, and 0.40 µM for testosterone 6β-hydroxylation, midazolam 1 γ -hydroxylation, and nifedipine oxidation, respectively.

N-Ac- γ -calicheamicin DMH showed time-dependent inhibitory activity of CYP2C8 and CYP3A4/5 activity (as measured by testosterone 6β-hydroxylation and midazolam 1'-hydroxylation). N-Ac- γ -calicheamicin DMH showed very weak time-dependent inhibition of CYP2C8, with the concentration at half the maximal rate of enzyme inactivation (KI) of >30 μM; thus, definitive in vitro inactivation kinetic parameters could not be estimated; the KI and maximal rate of enzyme inactivation (kinact) parameters were 0.077 μM and 0.00368 min-1 for midazolam and 0.237 μM and 0.00434 min-1 for testosterone, respectively.

Assessment of DDI potential with N-Ac- γ -calicheamicin DMH for time-dependent CYP3A4/5 inhibition, based on the inactivation parameters determined *in vitro*, a CYP3A4/5 kdeg value of 0.00032 min-1 ((16) (17)), and the mean steady-state unconjugated calicheamicin total Cmax of 5.81 ng/mL (0.00393 μM), indicates the potential for N-Ac- γ -calicheamicin DMH to inhibit CYP3A4/5 in a time-dependent manner at clinically relevant concentrations (R2 values of 1.22-1.56). However, assessment of DDI potential using the mean steady-state unbound Cmax for unconjugated calichemaicin, 0.000110 μM, indicated a low potential for N-Ac- γ -calicheamicin DMH to inhibit CYP3A4/5 in a time-dependent manner at clinically relevant concentrations (R values of 1.01-1.02). Moreover, mechanistic static modeling of the effect of N-Ac- γ -calicheamicin DMH on the CYP3A4/5 substrate midazolam indicated a low potential for N-Ac- γ -calicheamicin DMH to inhibit CYP3A4/5 in a time-dependent manner at clinically relevant concentrations, with an estimated midazolam AUCR value of 1.02.

Potential Induction of CYP Enzymes

Gemtuzumab ozogamicin did not induce CYP1A2, CYP2B6, and CYP3A4 in 3 lots of cryopreserved human hepatocytes at concentrations .50x the Cmaxof total hP67.6 antibody in patients after multiple dose administration of 3 mg/m2 gemtuzumab ozogamicin (0.00426 µM). These results indicate that the potential for gemtuzumab ozogamicin to induce CYP1A2, CYP2B6, or CYP3A4 is low at clinically relevant concentrations.

N-Ac- γ -calicheamicin DMH did not cause induction of CYP3A4 in the transfected HepG2 cells up to 0.090 µM (highest concentration evaluated). N-Ac- γ -calicheamicin DMH had a low potential to inhibit the CYP1A2, CYP2B6, or CYP3A4 mRNA expression and/or enzyme activity in any of the 3 hepatocyte lots evaluated at up to 0.3 µM of N-Ac- γ -calicheamicin DMH (highest concentration evaluated). In humans, the mean steady-state unbound Cmax of unconjugated calicheamicin multiple dose administration of 9 mg/m^2 gemtuzumab ozogamicin was 0.000110 µM.

Potential Inhibition of UGT Enzymes

In the presence or absence of 2% Bovine serum albumin (BSA), N-Ac- γ -calicheamicin DMH showed little or no reversible inhibition of UGT1A4, UGT1A6, UGT1A9, and UGT2B7 catalyzed activities (IC50 >10 µM). However, N-Ac- γ -calicheamicin DMH inhibited UGT1A1 activity with IC50 values of 0.61 and 1.4 µM, in the absence or presence of 2% BSA, respectively. The unbound IC50 value could not be determined due to instability of N-Ac- γ –calicheamicin DMH in the 5 hour *in vitro* HLM-BSA binding assay.

Assessments based on the comparison of the IC50 (total) and unconjugated calicheamicin Cmax values (0.00393 µM and 0.000110 µM for total and unbound, respectively after multiple dose administration of

9 mg/m^2 of gemtuzumab ozogamicin to humans indicated a low likelihood of DDI involving N-Ac- γ –calicheamicin DMH and the evaluated UGT enzymes (R1 value of <1.1, and total or unbound Cmax to Ki values <0.02).

Effect on drug transporter substrates

In vitro, N acetyl gamma calicheamicin dimethyl hydrazide had a low potential to inhibit the activities of P gp, breast cancer resistance protein (BCRP), bile salt export pump (BSEP), multidrug resistance associated protein (MRP) 2, multidrug and toxin extrusion protein (MATE)1 and MATE2K, organic anion transporter (OAT)1 and OAT3, organic cation transporter (OCT)1 and OCT2, and organic anion transporting polypeptide (OATP)1B1 and OATP1B3 at clinically relevant concentrations.

Efflux Transporters

The potential interaction of N-Ac- γ -calicheamicin DMH (over a concentration range of 0.002 to 0.3 µM) with efflux transporters P-gp and BCRP was investigated in vitro in MDCKII-MDR1 cells and MDCKII low efflux cells expressing the BCRP transporter (MDCKII-LE-BCRP) using the prototypical substrates digoxin (10 µM) and pitavastatin (0.5 µM) for the respective efflux transporters.

Results indicate a low potential for an interaction between N-Ac- γ -calicheamicin DMH and P-gp and BCRP at clinically relevant concentrations (mean steady-state total and unbound Cmax values of unconjugated calicheamicin of 0.00393 µM and 0.000110 µM, respectively. The ratio of total Cmax/Ki was <0.1, and 50x the unbound Cmax did not exceed the estimated Ki value of >0.15 µM.

Hepatic Uptake Transporters

N-Ac- γ -calicheamicin DMH, at a concentration range of 0.00005 to 0.1 µM, was evaluated for its potential to inhibit the human hepatic uptake transporters OATP1B1 and OATP1B3 expressed in human embryonic kidney (HEK) 293 cells using pravastatin (10 µM) and rosuvastatin (5 µM), respectively, as the prototypical probe substrates.

N-Ac- γ -calicheamicin DMH inhibited the OATP1B1- and OATP1B3-mediated transport of pravastatin and rosuvastatin, respectively, in a concentration-dependent manner. Inhibition of OATP1B1 and OATP1B3 at the highest concentration of N-Ac- γ -calicheamicin DMH evaluated (0.1 µM) was 30% and 16%, respectively, while rifamycin SV (100 µM, positive control inhibitor) inhibited the transport of pravastatin and rosuvastatin by 98% and 57%, respectively. The IC50 of N-Ac- γ -calicheamicin DMH-mediated inhibition of OATP1B1 and OATP1B3 was estimated to be >0.1 µM, and the Ki was estimated to be >0.05 µM.

Results indicate a low potential for an interaction between N-Ac- γ -calicheamicin DMH and these two transporters at clinically relevant concentrations (mean steady-state total and unbound Cmax values of unconjugated calicheamicin of 0.00393 µM and 0.000110 µM, respectively. The ratio of total Cmax/IC50 was <0.1, and 50x the unbound Cmax did not exceed the estimated Ki value of >0.05 µM.

Renal Uptake Transporters

The inhibitory potency of N-Ac- γ -calicheamicin DMH for the human renal transporters, OAT1, OAT3, and OCT2, was assessed using HEK293 cells over-expressing each transporter with [3H]p-aminohippurate (2 µM), [3H]estrone-3-sulfate (0.2 µM), and [14C]metformin (10 µM) as the respective probe substrates. Inhibition was evaluated at an N-Ac- γ -calicheamicin DMH concentration range of 0.002 to 0.5 µM for OAT1 and at a concentration range of 0.0004 to 0.1 µM for OAT3 and OCT2.

N-Ac- γ -calicheamicin DMH, over the concentrations evaluated, did not inhibit OAT1, OAT3, or OCT2, while the positive control inhibitors (probenecid for OAT1 and OAT3 and verapamil for OCT2) inhibited the

activity for these transporters by >95%. The IC50 of N-Ac- γ -calicheamicin DMH-mediated transporter inhibition was estimated to be >0.5 µM (Ki >0.25 µM) for OAT1 and >0.1 µM (Ki >0.05 µM) for OAT3 and OCT2.

A low potential for an interaction between N-Ac- γ -calicheamicin DMH and OAT1, OAT3, OCT2 transporters at clinically relevant concentrations (mean steady-state total and unbound Cmax values of unconjugated calicheamicin of 0.00393 µM and 0.000110 µM, respectively) has been observed. The ratio of unbound Cmax/IC50 was <0.1, and 50x the unbound Cmax did not exceed the estimated Ki value of >0.05 to >0.25 µM.

No formal clinical DDI studies have been conducted, or are planned, to evaluate the PK of GO in patients in combination with other drugs.

Pharmacokinetics using human biomaterials

N/A

Immunogenicity

In clinical studies of Mylotarg in patients with relapsed or refractory AML, the immunogenicity of MYLOTARG was evaluated using 2 enzyme-linked immunosorbent assays (ELISAs). Patients in the Phase 2 trials did not develop antidrug antibodies (ADAs) and 2 patients in a Phase 1 trial developed antibodies against the calicheamicin-linker complex, 1 of whom had reduced hP67.6 plasma concentrations. Overall, the incidence rate of ADA development after Mylotarg treatment was < 1% across the 4 clinical studies (Study 101, 201, 202, 203) with ADA data (SmPC section 4.8).

2.4.3. Pharmacodynamics

No clinical pharmacodynamic studies were submitted.

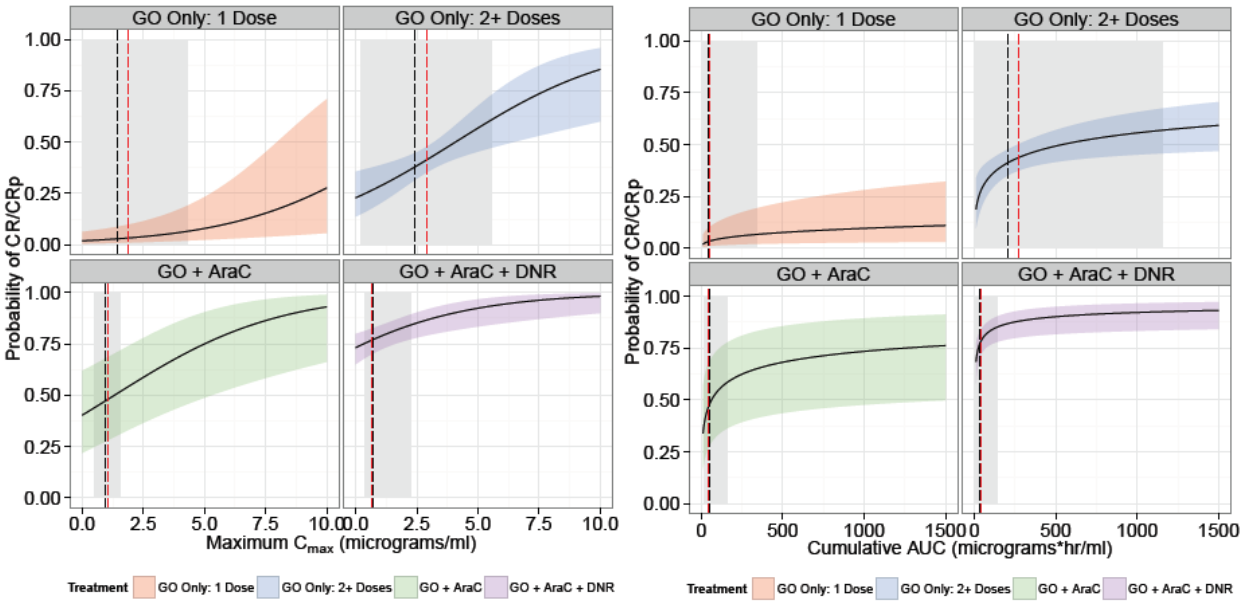
Primary and Secondary pharmacology

Pharmacodynamic data supportive to the suggested mechanism of action have been submitted.

Several single-agent studies measured target CD33 saturation post-GO dose in patients with relapsed and refractory AML, including: Study 101, the initial dose-escalation study, wherein GO was administered by IV at doses ranging from 0.25 mg/m² to 9 mg/m²; Study 102, a dose escalation study in paediatric patients with AML, wherein GO doses ranged from 6 to 9 mg/m^2; Study 103, a dose escalation study in Japanese patients with AML, where GO doses ranged from 6 to 9 mg/m^2; and Phase 2 Studies 201, 202, and 203, where GO was administered at 9 mg/m^2. Across all studies, near maximal peripheral CD33 saturation was observed post-GO dose at all dose levels of 2 mg/m² and above. Furthermore, by fitting a simple Emax to the CD33 saturation data from dose escalation Study 101, which included GO doses ranging from 0.25 mg/m² to 9 mg/m^2 the estimated ED50 value was approximately 0.2 mg/m^2 corresponding to an ED90 of 1.6 mg/m^2 and an ED95 of 3.3 mg/m^2.

An exposure-response analysis was conducted (PMAR-EQDD-B176asNDA-491) using PK, PD, and efficacy data from 8 previous Wyeth studies, and also data from study ALFA-0701, where the PK data for the fractionated dosing regimen were simulated using the population PK model.

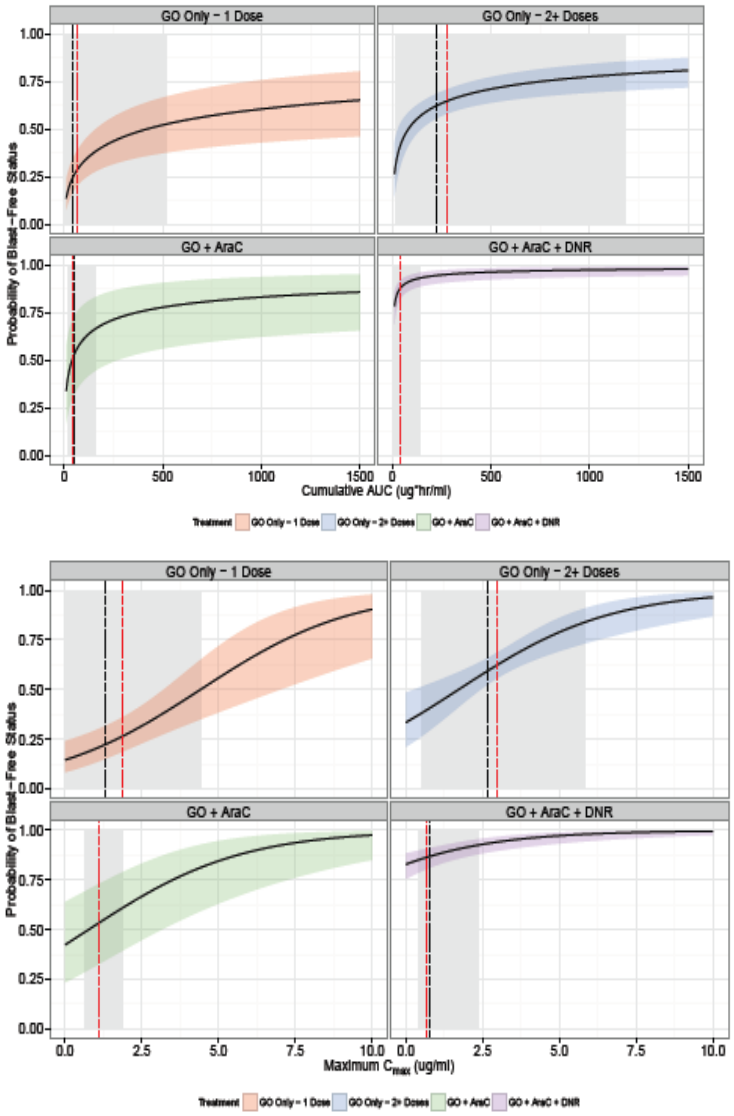
Figure 2 shows the prediction probabilities for the final models for CR/CRp using cumulative AUC and overall Cmax, respectively.



The gray shaded area represents the 2.5% and 97.5% percentiles of the simulated cumulative AUCs from patients who received the specified dosing regimen. The cumulative AUCs for patients whom post-hoc PK estimates were not available had their PK imputed using the population estimate. The black and red dashed lines correspond to the geometric mean and median of the exposure parameter represented. AraC=cytarabine; AUC=area under the hP67.7 concentration-time curve; CR=complete remission; CRp=complete remission without platelet recovery; DNR=daunorubicin; GO=gemtuzumab ozogamicin; PK=pharmacokinetics.

Figure 2 Prediction Probabilities for the Final Model for CR/CRp Using Overall AUC (L) and Cmax (R)

The impact of cumulative AUC and the overall Cmax on the probability of achieving blast-free status can be seen in Figure 3.



The grey shaded area represents the 2.5% and 97.5% percentiles of the simulated cumulative AUCs from patients who received the specified dosing regimen. The cumulative AUCs for patients whom post-hoc PK estimates were not available had their PK imputed using the population estimate. The black and red dashed lines correspond to the geometric mean and median of the exposure parameter represented. AraC=cytarabine; AUC=area under the hP67.7 concentration-time curve; DNR=daunorubicin; GO=gemtuzumab ozogamicin; PK=pharmacokinetics.

Figure 3 Prediction Probabilities for Final Model for Blast-Free Status Using Cumulative AUC (top) and Cmax (bottom)

An exposure-response analysis was conducted using PK and safety data from 8 Wyeth studies, and also data from Study ALFA-0701, where the PK data for the fractionated dosing regimen were simulated using the population PK model (PMAR-EQDDB176a-sNDA-491). On the basis of the incidence and severity of treatment-related adverse events (AEs) following treatment with GO, 7 safety terms were selected as the safety endpoints of interest for exposure-response analysis: neutropenia, thrombocytopenia, elevated AST, elevated bilirubin, hypoalbuminemia, elevated alkaline phosphatase, and hepatic VOD.

A significant exposure-response relationship was found between the Cmax of hP67.6 antibody occurring after the first dose of GO and elevated bilirubin, hypoalbuminemia, and elevated AST. However, no

relationship was found between either overall Cmax or overall AUC and VOD occurring within 28 days after any GO dose (PMAR-EQDD-B176asNDA-491). The stepwise logistic regression analysis, which included VOD observed at any time (ie, not restricted to within 28 days after any dose of GO), did not show a relationship between VOD and the dose of GO; however, that analysis did not include PK results, which were quite variable, with 62% geometric %CV for Cmax and 136% geometric %CV for AUCinf at the 9 mg/m^2 dose level. The combination of GO with AraC and DNR did not have a significant effect on the incidence of VOD.

Most patients that reached remission or blast-free status experienced Grade 4 neutropenia, Grade 4 thrombocytopenia, and Grade 4 leukopenia. PK/PD modeling was conducted to characterize the relationship between plasma concentrations of hP67.6 and the time course of neutropenia and thrombocytopenia (PMAR-EQDD-B176asNDA-491).

2.4.4. Discussion on clinical pharmacology

Based on a population PK analysis, age, race, and gender did not significantly affect gemtuzumab ozogamicin disposition (SmPC section 5.2). No dose adjustment is required in elderly patients (≥ 65 years (SmPC section 4.2).

The results of the population modelling showed that the PK behaviour of gemtuzumab ozogamicin (hP67.6 antibody and unconjugated calicheamicin) is similar between adult and paediatric AML patients following the 9 mg/m^2 dosing regimen (SmPC section 5.2).

The safety and efficacy of Mylotarg in patients less than 15 years of age has not been established. No recommendation on a posology can be made (SmPC section 4.2).

Pharmacokinetic data using the proposed posology supporting the use in adult patients is solely based on PK modelling. The population PK model for gemtuzumab ozogamicin and unconjugated calicheamicin in adult AML patients showed that allometric exponentials are appropriate and therefore a BSA-based dosing approach is justified. In addition, a population PK model for paediatric AML subjects (down to 2 years old of age) showed that no factors other than body weight appeared to significantly impact the PK of total antibody or unconjugated calicheamicin. Allomeric scaling on CL and volume of distribution accounted for the wide range of age and body size in the in the overall paediatric population (2-17 years). Similar to adults, BSA-based dosing would hence also be recommended in adolescents age 15-17 years of age. Overall, there is no scientific rational to assume that adolescents between 15-17 years of age experience different exposure levels, nor is there any rationale to assume that, based on similar exposure, this would result in a clinically meaningful response difference in patients with CD33-positive de novo AML treated in line with the adult 3+7 induction chemotherapy backbone in combination with Mylotarg.

No formal PK studies of gemtuzumab ozogamicin have been conducted in patients with hepatic impairment. Based on a population PK analysis, the clearance of gemtuzumab ozogamicin (hP67.6 antibody and unconjugated calicheamicin) is not expected to be affected by mild hepatic impairment status, as defined by National Cancer Institute Organ Dysfunction Working Group (NCI ODWG). The analysis included 406 patients in the following NCI ODWG impairment status categories: mild (B1, n=58 and B2, n=19), moderate (C, n=6), and normal hepatic function (n=322) (SmPC section 5.2). No adjustment of the starting dose is required in patients with hepatic impairment defined by total bilirubin ≤ 2 × upper limit of normal (ULN) and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤ 2.5 × ULN. Mylotarg should be postponed until recovery of total bilirubin to ≤ 2 × ULN and AST and ALT to ≤ 2.5 × ULN prior to each dose (SmPC section 4.2).

No formal PK studies of gemtuzumab ozogamicin have been conducted in patients with renal impairment. Based on a population PK analysis in 406 patients, the clearance of gemtuzumab ozogamicin in patients with mild renal impairment (creatinine clearance [CLcr] 60-89 mL/min; n=149) or moderate renal impairment (CLcr 30 59 mL/min; n=47), was similar to patients with normal renal function (CLcr $\geqslant$ 90 mL/min; n=209). The PK of gemtuzumab ozogamicin has not been studied in patients with severe renal impairment (SmPC section 5.2). No dose adjustment is required in patients with mild to moderate renal impairment. Mylotarg has not been studied in patients with severe renal impairment. Mylotarg does not undergo renal clearance, the pharmacokinetics in patients with severe renal impairment is unknown (SmPC section 4.2).

No formal drug-interaction studies have been performed with GO (SmPC section 4.5). *In vitro*, N-acetyl gamma calicheamicin dimethyl hydrazide is primarily metabolised via nonenzymatic reduction. Therefore, coadministration of gemtuzumab ozogamicin with inhibitors or inducers of cytochrome P450 (CYP) or uridine diphosphate glucuronosyltransferase (UGT) drug metabolising enzymes are unlikely to alter exposure to N-acetyl gamma calicheamicin dimethyl hydrazide (SmPC section 5.2).

Based on population PK analyses, the combination of gemtuzumab ozogamicin with hydroxyurea, DNR, and AraC is not predicted to cause clinically meaningful changes in the PK of hP67.6 or unconjugated calicheamicin(SmPC section 5.2).

N-acetyl gamma calicheamicin dimethyl hydrazide and gemtuzumab ozogamicin had a low potential to inhibit the activities of CYP1A2, CYP2A6 (tested only using gemtuzumab ozogamicin), CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 at clinically relevant concentrations. In vitro, N-acetyl gamma calicheamicin dimethyl hydrazide and gemtuzumab ozogamicin had a low potential to induce the activities of CYP1A2, CYP2B6, and CYP3A4 at clinically relevant concentrations (SmPC section 5.2).

In vitro, N-acetyl gamma calicheamicin dimethyl hydrazide had a low potential to inhibit the activities of UGT1A1, UGT1A4, UGT1A6, UGT1A9, and UGT2B7 at clinically relevant concentrations (SmPC section 5.2).

In vitro, N-acetyl gamma calicheamicin dimethyl hydrazide had a low potential to inhibit the activities of P-gp, breast cancer resistance protein (BCRP), bile salt export pump (BSEP), multidrug resistance associated protein (MRP) 2, multidrug and toxin extrusion protein (MATE)1 and MATE2K, organic anion transporter (OAT)1 and OAT3, organic cation transporter (OCT)1 and OCT2, and organic anion transporting polypeptide (OATP)1B1 and OATP1B3 at clinically relevant concentrations (SmPC section 5.2).

Based on population pharmacokinetic (PK) analyses, the combination of gemtuzumab ozogamicin with DNR and AraC is not predicted to cause clinically meaningful changes in the PK of these agents (SmPC section 5.2).

Saturation of a high percentage of CD33 antigenic sites is presumed to be required for maximum delivery of calicheamicin to leukaemic blast cells. Several single agent studies measured target (CD33) saturation post-MYLOTARG dose in patients with relapsed and refractory AML. Across all studies, near maximal peripheral CD33 saturation was observed post-MYLOTARG dose at all dose levels of 2 mg/m² and above, suggesting that a low dose of gemtuzumab ozogamicin is sufficient to bind all available CD33 sites (SmPC, section 5.1).

As with all therapeutic proteins, there is potential for immunogenicity. Definitive conclusions cannot be drawn between the presence of antibodies and potential impact on efficacy and safety due to the limited number of patients with positive ADAs. The detection of ADAs is highly dependent on the sensitivity and specificity of the assay. The incidence of antibody positivity in an assay may be influenced by several

factors, including assay methodology, circulating gemtuzumab ozogamicin concentrations, sample handling, timing of sample collection, concomitant treatments and underlying disease. For these reasons, comparison of incidence of antibodies to gemtuzumab ozogamicin with the incidence of antibodies to other products may be misleading (SmPC section 4.8). Mylorarg will be administered to patients who are going to be immunosuppressed over the course of the treatment, with little meaningful clinical impact expected; taking into consideration that Mylotarg seems not to be very immunogenic (<1%).The CHMP recommended the applicant to evaluate immunogenicity of the batches intended for licensing as part of post-marketing commitment. Immunogenicityhas been classified as a potential risk in the Risk Management Plan (see discussion on clinical safety).

While the PK of gemtuzumab ozogamicin has been well-characterised over a wide range of doses, there is no confirmation of the proposed dose and it's PK in the target population. The pharmacokinetics of gemtuzumab ozogamicin and unconjugated calicheamicin in patients with AML has been described using a population PK model, with the pharmacokinetics following the proposed gemtuzumab ozogamicin dose simulated in the target population. The CHMP recommended the applicant to evaluate gemtuzumab ozogamicin metabolites as part of the planned additional PK study mentioned below.

The lack of secondary PD data, particularly on QT interval prolongation is reflected as missing information in the RMP, however and the CHMP recommended the applicant to evaluate the effect of GO on QTc as described in the planned clinical study with the fractionated regimen (see Risk Management Plan).

The lack of comprehensive data related to AML genetics such as hierarchy of the leukaemic population, drug efflux intensity in relation to pharmacodynamics and clinical response is unfortunate. The applicant provided additional literature to substantiate this lack of information, based on which the applicant agreed to add the requirement for CD33-positive AML to the indication (see discussion on clinical efficacy).

Based on the above, the CHMP recommended the applicant to conduct a study in patients with relapsed/ refractory CD33-positive AML, using single agent Mylotarg to allow for the collection of additional PK, exposure-response, QTc and anti-drug antibody (ADA) data using the current, batches of Mylotarg.

2.4.5. Conclusions on clinical pharmacology

The PK of gemtuzumab ozogamicin has been reasonably well investigated.

2.5. Clinical efficacy

2.5.1. Dose response studies

Mylotarg was initially developed as monotherapy for patients with AML in first relapse. The initial single agent dosing recommendation was 9 mg/m², infused over a 2-hour period, with a total of 2 doses with a 14 days treatment free interval. This dosing schedule had been chosen based on the results from the dose escalation phase 1 study 101, during which the dose was not escalated beyond 9 mg/m^2 because of myelosuppression, even though no prospectively defined dose-limiting toxicity (DLT) had been encountered.

Study 205 then evaluated the MTD for Mylotarg in combination with AraC to be 6 and 4 mg/m² on Days 1 and 8, respectively, plus AraC 100 mg/m² × 7 days. Study 206 confirmed the MTD for Mylotarg in combination with 3+7 induction chemotherapy to be 6 mg/m² plus AraC 100 mg/m²/day and DNR 45 mg/m². Both studies were conducted including patients with de novo AML.

MyloFrance 1 study further evaluated the fractionated Mylotarg dosing regimen in combination. MyloFrance 1 was a multicentre phase 2 uncontrolled trial to assess the safety of fractionated doses of GO, given at a dose of 3 mg/m^2 on days 1, 4 and 7 (max of 5mg per dose) in adult patients age 18 and above with CD33-positive AML in first relapse. There were no signs of prolonged myelosuppression. During the treatment period, grade 3 TEAEs that occurred in >1% patients included sepsis (31.5%), fever (15.8%), rash (10.5%), pneumonia (7%), bleeding (7%), mucositis (3.5%), diarrhea (1.75%), headaches (1.75%), tachycardia (1.75%) and oedema (1.75%). No grade 4 toxicity was observed. No infectious deaths occurred. No grade 3 or 4 liver toxicity was observed. No episodes of VOD occurred. The efficacy results were comparable to Phase 1 data using the 9 mg/m^2 dose.

MyloFrance 2 study (18) was a multicentre Phase 1/2 dose escalation study aimed to determine the optimal doses of daunorubicin and cytarabine in combination with fractionated doses of Mylotarg (3 mg/m^2 on day 1, 4, and 7) in patients age 50–70years with CD33-positive AML in first relapse and adequate PS (ECOG 0-1) and organ function. The primary endpoint was to determine the MTD for DNR and AraC doses in combination with 3 mg/m2 GO on days 1, 4, and 7 (max of 5mg per dose), among three different dose schedules, namely (45, 100), (60, 100), and (60, 200) mg/m^2. A total of 20 patients were included; no DLT was observed in neither of the three dose levels even after expanding the third dose level to an additional four patients. One grade 4 liver toxicity was observed in the second dose cohort and one grade 3 liver toxicity in the third, but all considered not related to the chemotherapy, but to events of sepsis. The highest tested dose level of DNR 60 mg/m^2 x 3 days and AraC 200 mg/m2 for 7 days was considered tolerable in combination with the low dose fractioned Mylotarg. CR was obtained in 11/20 patients, CRp in two patients and PR in one patient. A total of 4 patients experienced disease progression. There were 2 early deaths, one from infection and one from disease progression.

Furthermore, analyses of the different GO dosing regimens (3 mg/m^2 single dose, 3×3 mg/m^2 fractionated and 6 mg/m2 single dose) during induction for the following safety endpoint (30-day and 60-day mortality, haemorrhage, infections, hepatotoxicity and haematotoxicity) showed that the single dose of 6 mg/m² resulted in a significant increase early mortality, however, the 3x3 mg/m² fractionated GO regimen showed similar 30-day and 60-day mortality rates than the single 3 mg/m² GO regimen. The risk of Grade 3/4 Haemorrhage, Grade 3/4 infections and hepatotoxicity seems not increased with the 3x3 mg/m² fractionated Mylotarg regimen compared to single 3 mg/m² or 6 mg/m². The risk of persistent neutropenia was not increased by the addition of GO to the intensive chemotherapy, but the risk of persistent thrombocytopenia increased with the 3x3 mg/m² fractionated regimen.

Regarding the single dose of Mylotarg administered during the 2 courses of consolidation, this didn't result in cumulative toxicity; certainly not for those who tolerate GO during induction.

Overall this confirmed the 3x3 mg/m2 fractionated GO dosing regimen in combination with DNR 60 mg/m^2 x 3 days and AraC 200 mg/m^2 for 7 days to be tolerable with comparable efficacy results to Phase 1 data using the 9 mg/m^2 dose.

2.6. Main study – ALFA0701

Methods

This was a multicenter, randomized,comparative phase 3 study of fractionated doses of the monoclonal antibody gemtuzumab ozogamicin (Mylotarg) in addition to daunorubicin + cytarabine versus daunorubicin + cytarabine alone for induction and consolidation therapy in patients with acute myeloid leukemia aged 50 to 70 years.

Study Participants

Main inclusion criteria

Eligible patients were expected to meet the following criteria:

- Previously untreated morphologically documented AML.
- Age ≥ 50 years and ≤ 70 years.
- ECOG PS 0 to 3.
- Cardiac function within normal limits as explored by scintigraphy or echocardiography.
- Signed the ICD.
- Had blood and bone marrow specimens taken for molecular biology assessment.

Main exclusion criteria:

Patients were ineligible to participate in this study if any of the following criteria were met:

• AML3 (APL).

• AML arising from myeloproliferative syndrome.

• AML arising from known myelodysplastic syndromes, documented by myelogram and diagnosed more than 6 months earlier.

• AML secondary to previous chemotherapy and/or radiotherapy for another neoplastic disease.

• Central nervous system (CNS) involvement.

• Uncontrolled infection.

• Other active malignant disease.

• Seropositivity to HIV, hepatitis B, or hepatitis C (except post vaccination).

- Creatinine ≥ 2.5 × ULN; ALT or AST ≥ 2.5 × ULN; total bilirubin ≥ 2 × ULN.

• Prior antileukemia treatment, except with hydroxyurea in case of hyperleukocytic leukemia.

• Positive pregnancy test in women of childbearing age.

Treatments

The treatment algorithm is shown in Figure 6.

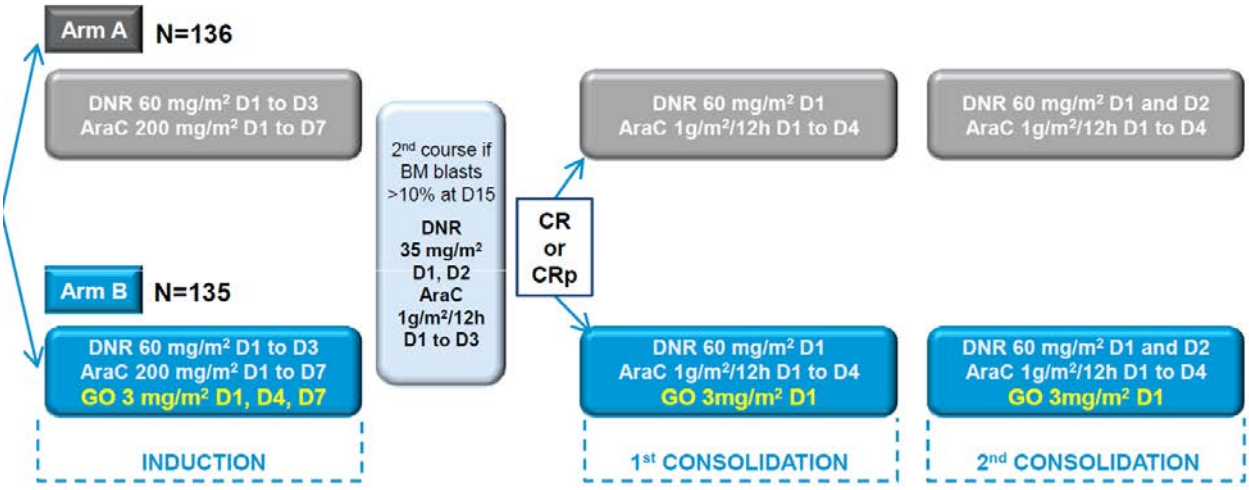


Figure 4 Study scheme (ALFA0701)

Premedication, including Polaramine (dexchlorpheniramine) ± Solumedrol (methylprednisolone) 1 mg/kg, was required 1 hour before the GO infusion and could be repeated if needed. Mylotarg infusion was performed under close clinical monitoring, including pulse, blood pressure, and temperature, and was to be interrupted if patients experienced dyspnoea or clinically significant hypotension.

In case of hyperleukocytic AML (leukocyte count >30,000/mm^3), rasburicase or allopurinol was administered for prevention of tumour lysis syndrome. It was also recommended to postpone Mylotarg administration and to perform leukoreduction with oral hydroxyurea first, to administer only AraC for 2 days before administering DNR and GO according to the usual regimen.

Salvage therapy:

Patients who did not receive the second course of induction therapy and did not achieve a CR after the first course of induction could receive a salvage course comprised of Idarubicin + AraC as long as the patient had an ECOG PS <3 and a creatinine clearance >30 mL/min. Salvage therapy consisted of:

- Idarubicin 12 mg/m^2 on Day 1 and Day 2
- AraC 1 g/m^2 × 2/day on Days 1 to 4
- G-CSF starting on Day 6

Patients who did not respond to induction therapy (including salvage course) discontinued the study.

Allogeneic transplant

Patients who experienced CR could be considered for allogeneic transplant. Patients with favourable and intermediate I cytogenetic and molecular risk categories were not sent for transplant in first CR. Whereas patients with intermediate II or unfavourable cytogenetic and molecular risk categories with a CR were considered for transplant if qualified. An interval of 2 months between the last dose of Mylotarg and transplant was recommended. The type of pre-transplant conditioning chemotherapy was left to the discretion of the treating centre.

A protocol amendment in December 2009 added that GO should not be used during consolidation in patients with a platelet count <100,000/mm^3 by Day 45 after the initiation of chemotherapy.

Patients who experienced CR could be considered for allogeneic transplant. Patients with favourable and intermediate I cytogenetic and molecular risk categories were not sent for transplant in first CR. An interval of 2 months between the last dose of Mylotarg and transplant was recommended. In that respect is noted that publications recommend an even longer interval between last Mylotarg dose and HSCT, ie 3 month (18). Nonetheless, the chosen interval was based on investigators' recommendation. The type of pre-transplant conditioning chemotherapy was left to the discretion of the treating centre.

Evaluation schedule/ Follow-up

Clinical and haematological responses were assessed after induction, before each course of consolidation and at one to two months after hematologic recovery from the last cycle of consolidation therapy. Full blood count (FBC) was assessed 1) at the pre-treatment evaluation, 2) three times per week during induction and consolidation, and 3) at the end of treatment visit. The BMA was done 1) on day 15 after start of treatment, 2) after haematological recovery from each course of therapy (at least 7 days after end of G-CSF treatment) or just before each cycle of consolidation therapy, and 3) at the end of treatment visit.

Patients were evaluated after hematologic recovery every 1 to 2 months from the last cycle of consolidation therapy while still in CR/CRp for 2 years, after which the evaluation visits were extended to every 6 months. At the time of relapse, both FBC and BMA results were collected. All patients were followed until death.

Objectives

Primary objective was to compare the efficacy of DNR + AraC + Mylotarg (GO arm) versus DNR + AraC (control arm), as measured by event-free survival (EFS).

Secondary objectives included comparison of the following between the GO arm and control arm:

- CR and CRp rates. Definition of CR:
  - o Absence of leukemic blast cells in blood and disappearance of all tumours.
  - o Percentage of marrow blast cells < 5% after morphological study of bone marrow aspiration.
  - o Haemoglobin > 9 g/dL, platelets > 100,000/mm, Neutrophils > 1000/mm and no need for transfusion.

A patient will be considered in CRp if all the above criteria are satisfied with the exception of platelet count > 100,000/mm^3.

- Relapse rate
- Duration of remission, as measured by relapse-free survival (RFS)
- Overall survival (OS)
- Safety profile of Mylotarg in addition to chemotherapy in comparison with the control arm.
- Predictors of response: multi drug resistance (MDR) proteins, cytogenetic risk categories, mutation or overexpression of FLT3, MLL, CEBPa, NPM genes.
- Relationship between residual disease levels measured by WT1 or NPM1 transcript levels (in NPM+ patients) at M1, M3 and M6 and length of remission.

Outcomes / endpoints

Primary endpoint

The primary endpoint was EFS defined as the time from date of randomisation to date of induction failure, relapse, or death due to any cause, whichever came first.

The date of induction failure was the date of evaluation of bone marrow response after the last induction cycle if a CR (by investigator assessment) had not been achieved.

The definition of CR was:

- Absence of leukemic blast cells in peripheral blood and no investigator report of extramedullary, molecular, or cytogenetic disease
- Blast cells <5% in BMA with no Auer rods
- Neutrophils >1000/mm^3, platelets >100,000/mm^3, and transfusion independent

A patient was considered in CRp if all the above criteria were satisfied with the exception of platelet count >100,000/mm^3; however, the eCRF did not distinguish between CR or CRp, thus, all responder patients were assessed as CR/CRp. For patients who experienced a post-induction CR, the IRC reviewers determined whether (and if so, when) relapse had occurred, as indicated by any of the following:

- Presence of ≥ 5% blasts in BMA or presence of Auer rods
- Blasts in the FBC not attributable to BM recovery following chemotherapy or G-CSF
- Investigator report of extramedullary disease, molecular or cytogenetic disease

Study investigators assessed and classified disease progression in accordance with the International Working Group criteria (19).

Secondary endpoints

- Overall Survival defined as the time from date of randomisation to date of death due to any cause.
- Relapse-Free Survival defined for patients experiencing CR or CRp as the time from the date of remission to the date of relapse or death from any cause, whichever came first. For the primary analysis, the date of relapse determined by individual investigators was used to define the RFS.
- Hematologic Response. Responses were determined based on investigator's assessment. A patient was considered in complete remission (CR) by the investigator if the following conditions were all met:
  - Leukemic blasts absent from the peripheral blood and no clinical tumours;
  - Percentage of blasts in the bone marrow is <5%, as measured by morphologic studies (aspirates);
  - Haemoglobin >9 g/dL, platelets >100,000/mm^3, ANC > 1000/mm^3;
  - The patient is red cell and platelet transfusion independent.
- Minimal Residual Disease (MRD) as measured by WT1 expression for post baseline samples was defined as negative if the WT1/ABLx100 (%)is <ULN (ULN is 0.5% for peripheral blood samples and 2.5% for bone marrow samples). Peripheral blood samples were classified as positive if the

ratio (%) was ≥0.5. Bone marrow samples were classified as positive if the ratio (%) was ≥2.5. MRD status as measured by WT1 expression for post baseline samples was based on peripheral blood samples. Minimal Residual Disease as assessed by detection of mutated NPM1 transcripts by RT-qPCR was classified as positive if NPM1mut/Abl transcripts x 100 (%) was ≥0.1% (0.1% was the quantitative detection limit of the assay) for peripheral blood and bone marrow samples. MRD status as assessed by detection of mutated NPM1 transcripts for post baseline samples was based on bone marrow samples.

Sample size

Event-free survival at 3 years was assumed to be 25% in the control arm and 40% (HR=0.66 based on an underlying exponential distribution) in the experimental arm. Assuming a type I error of 5% (2-sided) and a type II error of 20% for 80% power, a sample size of 140 patients was required in each treatment arm (total of 280 patients), in order to obtain a total of 184 events required for EFS analysis.

Randomisation

Patients were randomly assigned in a 1:1 ratio to receive either standard induction therapy with DNR + AraC without (control arm) or with Mylotarg (GO arm).

Randomization was centralized, and stratified by center.

Blinding (masking)

The study treatment was not blinded to patients or treating investigators. The IRC reviewers were blinded to the treatment arms.

Statistical methods

The modified intent-to-treat (mITT) population was the primary population for evaluating efficacy endpoints and patient characteristics. The mITT population included all patients who were randomized, unless consent was withdrawn prior to the start of treatment.

The as-treated (AT) population defined as all patients who received at least 1 dose of study medication and who were analysed according to the actual treatment received.

Time-to-event endpoints were summarized using the Kaplan-Meier method and displayed graphically when appropriate. Two-sided 95% confidence intervals (CIs) for median time-to-event were estimated using the Brookmeyer-Crowley method with log-log transformation. Time-to-event endpoints were compared between the 2 treatment arms with 2-sided log-rank tests. The log-rank test was used for the primary analysis, and the stratified log-rank test was used for sensitivity analyses. HRs and the associated 2-sided 95% CIs were estimated using the Cox proportional hazards model. For sensitivity analyses, HRs were estimated using the Cox proportional hazards model stratified by key prognostic factors and adjusted by other baseline characteristics.

For the primary EFS analyses, the following method to determine the event dates was used to define the EFS.

Primary (Method A)

The date of the first documentation of induction failure based on investigator's assessment, and the date of relapse based on investigator's assessment, and death date, was used to define the EFS. The date of induction failure was considered as the date of evaluation of bone marrow response after the last induction cycle if complete remission per the investigator has not been achieved by that time.

For sensitivity analyses, the following methods to determine the event dates were used to define the EFS.

• Method A refers to the primary analysis, by investigator assessment and date of post induction assessment used as date of induction failure events.

• Method B refers to independent review.

• Method D refers to investigator assessment but uses the date of randomization as date of induction failure events.

• 1 refers to the reference date of 01 August 2011.

• 2 refers to the reference date of 30 April 2013.

• 3 refers to the reference date of 01 August 2011, with censoring at the last assessment before the HSCT.

The primary study endpoint was derived from investigator assessment. The retrospective efficacy data was reviewed by a blinded independent review committee (BIRC) in order to reduce possible bias introduced by the local investigators.

Results

Participant flow

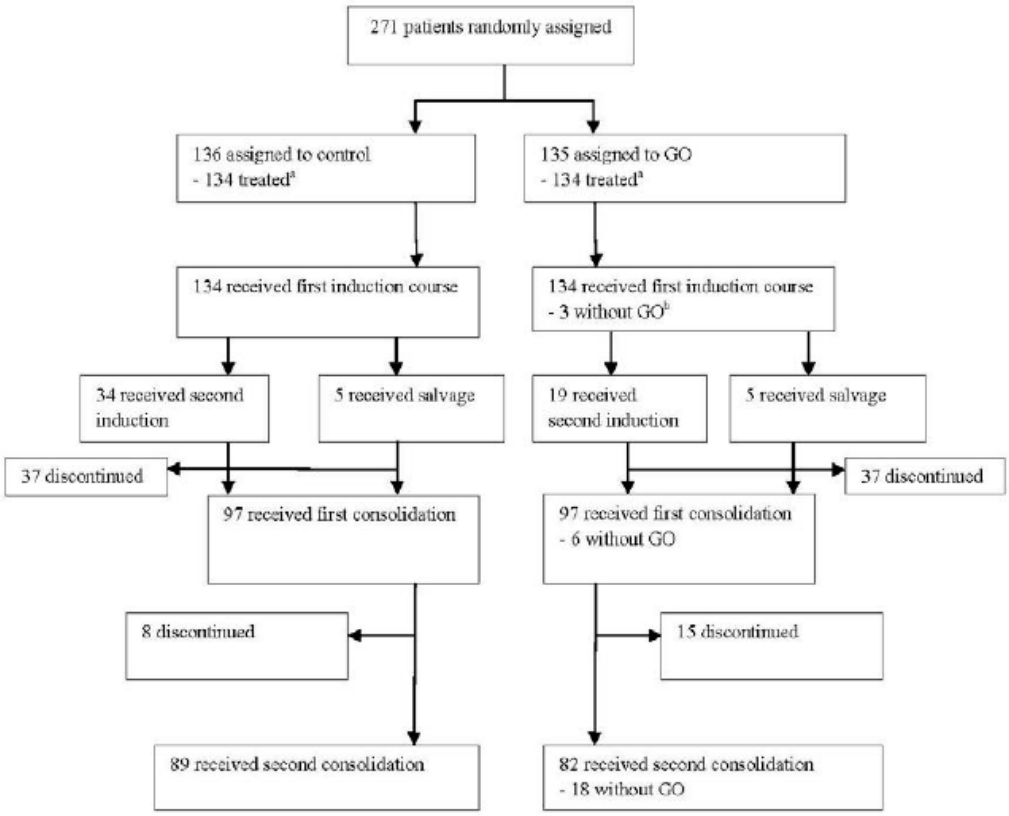


Figure 5 Participant flow (ALFA0701 study)

Table 15. Patient Disposition - Discontinuations from treatment (As-Treated Population) (ALFA0701 study)

Number (%) of Patients	GO + Daunorubicin + Cytarabine (N=131)		Daunorubicin + Cytarabine (N=137)	
	Daunorubicin + Cytarabine Treatment	GO Treatment	Daunorubicin + Cytarabine Treatment	GO Treatment
Completed treatment	82 (62.6)	64 (48.9)	89 (65.0)	NA
Reason for treatment permanent discontinuation[a]	49 (37.4)	67 (51.1)	48 (35.0)	NA
Adverse event	19 (14.5)	35 (26.7)	5 (3.6)	NA
Related to study treatment[b]	18 (13.7)	33 (25.2)	3 (2.2)	NA
Not related to study treatment	1 (0.8)	2 (1.5)	2 (1.5)	NA
Resistant disease[c]	17 (13.0)	16 (12.2)	26 (19.0)	NA
Patient relapse	1 (0.8)	1 (0.8)	4 (2.9)	NA
Symptomatic deterioration (not meeting criteria of progressive disease)	1 (0.8)	1 (0.8)	0	NA
Patient started new treatment for disease under study[d]	3 (2.3)	3 (2.3)	3 (2.2)	NA
Patient died	6 (4.6)	6 (4.6)	5 (3.6)	NA
Protocol violation	0	0	1 (0.7)	NA
Lost to follow-up	0	0	0	NA
Patient no longer willing to continue treatment for reason other than AE	0	0	0	NA
Noncompliance with study treatment	0	0	0	NA
Other	2 (1.5)	5 (3.8)	4 (2.9)	NA

Recruitment

The study was conducted in 27 centres in France (out of which one did not randomised a patient) and sponsored by the Centre Hospitalier de Versailles. The first patient first visit was on 8 January 2008 and the last patient last visit was on 30 April 2013 (data cut-off for overall survival data) and 01 November 2013 (data cut-off date of retrospective data collection).

Conduct of the study

Table 16. Protocol amendment history (ALFA0701 study)

Amendment Number	Date of Amendment	Brief Description of the Changes	Primary Reason for the Amendment
1	22 Oct 2007	Addition of recommendation around consolidation course (complete laboratory tests to be performed before each consolidation course and GO should not be administered in case of liver abnormalities). Addition of recommendations around GO infusion in case of hyperleukocytic leukemia.	Changes resulting from discussions with the French regulatory agency.
2	18 Feb 2008	Addition of eligibility criteria (biological specimen for molecular biology/AML arising from known myelodysplastic syndromes documented by myelogram and diagnosed more than 6 months earlier). Additional clarity on Day 15 BMA. Addition of biological sampling for residual disease assessment. Conditions for re-induction course were modified (trigger for re-induction course was revised from Day 15 BMA blasts >10% to Day 15 BMA blasts >5%, and DNR dosing was modified from 60 to 35 mg/m^2/day). Addition of guidelines for allogeneic transplant	Changes resulting from discussions with study investigators.
3	25 May 2009	Addition of the salvage course. Additional clarity on bone marrow transplant with respect to last dose of GO.	Changes resulting from discussions with study investigators.
4	21 Dec 2009	Addition of recommendations around management of persistent Thrombocytopenia: GO to be discontinued ig platelets did not recover to at least 100,000/µL 14 days at the latest after the planned date of next treatment course.	Changes resulting from safety signal observed during the course of the study.

Source: Section 16.1.1
Abbreviations: AML=acute myeloid leukemia; BMA=bone marrow aspirate; DNR=daunorubicin; GO=gemtuzumab ozogamicin.

Protocol deviations

Only major protocol deviations pertaining to eligibility criteria and dosing or randomisation errors were collected; identified during the retrospective data collection, monitoring visits or programmatically derived from the data collected.

Major protocol deviations were reported in 139 patients; 8 patients had a treatment allocation deviation, 30 patients had a GO dosing error, 68 patients had a chemotherapy dosing error, 2 patients had source data missing during retrospective data collection, and 31 patients had eligibility criteria deviations (copy of signed consent from not available at site (9 patients), cardiac function not within normal limits at the time of study entry (7 patients), not adequate hepatic and renal function (6 patients), other active malignant disease at the time of study entry (2 patients), serology positive for HIV, HBV, or HCV at the time of data entry (5 patients), uncontrolled infection at the major time of data entry (1 patient), randomisation mistake (1 patient). 19 patients were reported to have more than 1 major protocol deviation. The percentage of major protocol deviations per site varied from 0% (site 16; 2 patients recruited) to 100% (ie site 21; 1 patient recruited).

Baseline data

The demographics and baseline characteristics are displayed in Table 17.

Table 17. Demographic and Baseline Characteristics (mITT Population- ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=135)	Daunorubicin + Cytarabine (N=136)	Total (N=271)
Gender, n (%)			
Male	74 (54.8)	60 (44.1)	134 (49.4)
Female	61 (45.2)	76 (55.9)	137 (50.6)
Age (years), n (%)			
<60	38 (28.1)	52 (38.2)	90 (33.2)
≥60	97 (71.9)	84 (61.8)	181 (66.8)
Age (years)			
Mean (Std Dev)	62.1 (5.02)	60.8 (5.39)	61.5 (5.24)
Median (Range)	62.0 (50-70)	61.0 (50-70)	62.0 (50-70)
Body surface area (m^2)[a], N	135	135	270
Mean (Std Dev)	1.83 (0.182)	1.83 (0.214)	1.83 (0.198)
Median (Range)	1.83 (1.5-2.3)	1.85 (1.3-2.4)	1.83 (1.3-2.4)
ECOG PS, n (%)			
0-1	121 (89.6)	117 (86.0)	238 (87.8)
≥2	14 (10.4)	18 (13.2)	32 (11.8)
Missing	0	1 (0.7)	1 (0.4)
Risk (ELN)			
Poor/Adverse	37 (27.4)	36 (26.5)	73 (26.9)
Favorable/Intermediate	86 (63.7)	91 (66.9)	177 (65.3)
Unknown	12 (8.9)	9 (6.6)	21 (7.7)
Risk (NCCN)			
Poor/Adverse	43 (31.9)	46 (33.8)	89 (32.8)
Favorable/Intermediate	80 (59.3)	80 (58.8)	160 (59.0)
Unknown	12 (8.9)	10 (7.4)	22 (8.1)
Cytogenetics[b]			
Favorable	3 (2.2)	6 (4.4)	9 (3.3)
Intermediate	91 (67.4)	89 (65.4)	180 (66.4)
Unfavorable	27 (20.0)	30 (22.1)	57 (21.0)
Unknown	14 (10.4)	11 (8.1)	25 (9.2)
Genotype[b]			
Favorable risk	27 (20.0)	24 (17.6)	51 (18.8)
Unfavorable risk	44 (32.6)	40 (29.4)	84 (31.0)
Unknown	2 (1.5)	11 (8.1)	13 (4.8)
Missing	62 (45.9)	61 (44.9)	123 (45.4)
WBC count categories (10^9/L)			
<30	108 (80.0)	114 (83.8)	222 (81.9)
≥30	26 (19.3)	21 (15.4)	47 (17.3)
CD33 expression (positivity)			
N	100	94	194
<30%	17 (12.6)	20 (14.7)	37 (13.7)
≥30%	83 (61.5)	74 (54.4)	157 (57.9)
<70%	37 (27.4)	31 (22.8)	68 (25.1)
≥70%	63 (46.7)	63 (46.3)	126 (46.5)

Abbreviations: CD=cluster of definition; CHV=Centre Hospitalier de Versailles; ECOG=Eastern Cooperative Oncology Group; ELN=European
LeukemiaNet; GO=gemtuzumab ozogamicin; mITT=modified intent-to-treat; n=number of patients; N=number of patients; NCCN=National Comprehensive Cancer Network; PS=performance status; Std Dev=standard deviation; WBC=white blood cell.
a. BSA is defined as weight [kg]0.425 × height [cm]0.725×71.84/104. b. As classified by CHV.

Numbers analysed

The summary of the dataset analysed is summarised in Table 18.

Table 18. Analysis populations (ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=135) n (%)	Daunorubicin + Cytarabine (N=136) n (%)	Total (N=271) n (%)
Randomized to study treatment	135	136	271
Treated (as randomized to treatment)	134 (99.3)	134 (98.5)	268 (98.9)
Analysis populations			
Modified ITT	135 (100)	136 (100)	271 (100)
As-Treated (actual treatment received)	131 (97.0)	137[a] (100.7)	268 (98.9)

Source: Table 14.1.1.1
The mITT population included all patients who were randomized, unless withdrawal of consent prior to start of treatment and were analyzed according to initial randomization arm. The AT population included all patients who received at least 1 dose of study medication and were analyzed according to actual treatment received.
Abbreviations: AT=as-treated; GO=gemtuzumab ozogamicin; mITT=modified intent-to-treat; n=number of patients; N=number of patients.
a. This includes the 134 patients randomized to the control arm and who actually received daunorubicin + cytarabine (2 patients randomized to this treatment arm were not treated) and 3 patients who were randomized to the GO arm but only received daunorubicin + cytarabine; hence, the total number of patients receiving daunorubicin + cytarabine was greater than the number of patients randomized to the control arm, resulting in percentage population greater than 100.

Outcomes and estimation

Primary endpoint EFS

The results of the primary efficacy analysis (cut-off date August 2011) are displayed in Figure 6 and in Table 19.

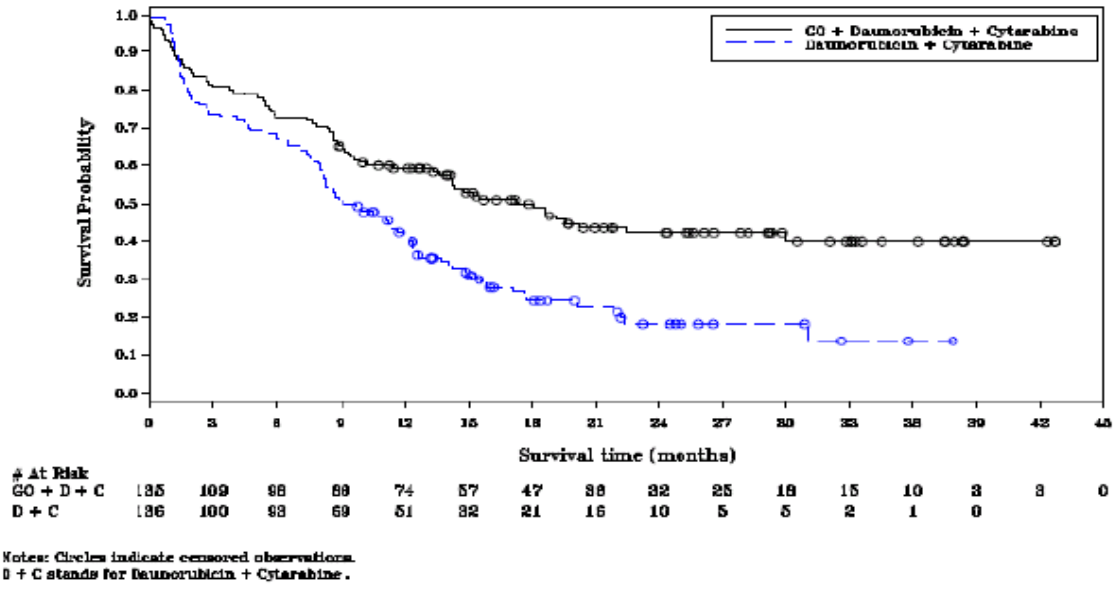


Figure 6. Kaplan-Meier plot of Event Free Survival by Investigator Assessment, mITT Population- (ALFA0701 study, cut-off date Aug 2011)

Table 19 Event Free Survival Primary Endpoint by Investigator Assessment, mITT Population- (ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=135)	Daunorubicin + Cytarabine (N=136)
Number of events, n (%)	73 (54.1)	102 (75.0)
Induction failure	17 (12.6)	29 (21.3)
Relapse	44 (32.6)	58 (42.6)
Death	12 (8.9)	15 (11.0)
Number of censored patients, n (%)	62 (45.9)	34 (25.0)
Reason for censoring, n (%)		
Event-free at reference date	62 (45.9)	34 (25.0)
Event-free at last assessment prior to reference date	0	0
No on-study disease assessment	0	0
Patient withdrew consent	0	0
Lost to follow-up	0	0
KM estimate of median time to event (months) [95% CI][a]	17.3 [13.4, 30.0]	9.5 [8.1, 12.0]
Probability of being event-free at 2 years [95% CI][b,c]	42.1 [32.9, 51.0]	18.2 [11.1, 26.7]
Probability of being event-free at 3 years [95% CI][b,c]	39.8 [30.2, 49.3]	13.6 [5.8, 24.8]
Versus daunorubicin + cytarabine – unstratified		
Hazard ratio[d] [95% CI]	0.562 [0.415, 0.762]	
p-Value[e]	0.0002	
Versus daunorubicin + cytarabine – stratified by risk (NCCN guideline)		
Hazard ratio[d] [95% CI]	0.575 [0.418, 0.790]	
p-Value[e]	0.0006	
Versus daunorubicin + cytarabine – stratified by risk (ELN guideline)		
Hazard ratio[d] [95% CI]	0.559 [0.408, 0.767]	
p-Value[e]	0.0003	

Source: Table 14.2.1.1

Based on the primary definition of EFS: event dates (induction failure, relapse, or death) determined by investigator assessment and censoring date being the reference date of 01 Aug 2011 or the last disease assessment date before the reference date where patient was event-free (no induction failure, relapse, or death). If data existed after reference date confirming the patient was event-free, patient was considered event-free at reference date; otherwise, patient was event-free at the last assessment prior to reference date.

Abbreviations: CI=confidence interval; EFS=event-free survival; ELN=European LeukemiaNet; GO=gemtuzumab ozogamicin; KM=Kaplan-Meier; mITT=modified intent-to-treat; n=number of patients; N=number of patients; NCCN=National Comprehensive Cancer Network.

a. Based on the Brookmeyer and Crowley Method with log-log transformation.

b. Estimated from the KM curve.

c. Calculated from the product-limit method/Calculated from the log[-log(x-<year,month> survival probability)] using a normal approximation and back transformation.

d. Based on the Cox Proportional Hazards Model.

e. 2-sided p-value from the log-rank test.

EFS Sensitivity Analysis

Table 20 summarizes the results of sensitivity analyses of EFS derived by using alternative assessors and event, and alternative reference and censoring dates.

Table 20. Event-Free Survival – Sensitivity Analyses (mITT Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine Versus Daunorubicin + Cytarabine – Unstratified		
	Hazard Ratio[a]	95% CI of Hazard Ratio	p-Value[b]
Method A1 (investigator, 01 Aug 2011)	0.562	[0.415, 0.762]	0.0002
Method A2 (investigator, 30 Apr 2013)	0.639	[0.484, 0.843]	0.0014
Method A3 (investigator, 01 Aug 2011, HSCT censored)	0.591	[0.431, 0.811]	0.0010
Method B1 (IR, 01 Aug 2011)	0.661	[0.491, 0.891]	0.0059
Method B2 (IR, 30 Apr 2013)	0.705	[0.536, 0.928]	0.0121
Method B3 (IR, 01 Aug 2011, HSCT censored)	0.707	[0.520, 0.961]	0.0261
Method D1 (investigator, 01 Aug 2011, IF date=randomization)	0.556	[0.410, 0.753]	0.0001
Method D2 (investigator, 30 Apr 2013, IF date=randomization)	0.631	[0.478, 0.832]	0.0010
Method D3 (investigator, 01 Aug 2011, IF date=randomization; HSCT censored)	0.584	[0.426, 0.801]	0.0007
Alternative Method (salvage therapy classified as IF and alternative relapse date)	0.599	[0.445, 0.807]	0.0007
ALFA definition (events of relapse and death only)	0.597	[0.439, 0.812]	0.0009

Abbreviations: ALFA=Acute Leukemia French Association; CI=confidence interval; GO=gemtuzumab ozogamicin; HSCT=hematopoietic stem cell transplant; IF=induction failure; IR=independent review; mITT=modified intent-to-treat; n=number of patients.
a. Based on the Cox Proportional Hazards Model.b. 2-sided p-value from the log-rank test.

EFS subgroup analysis

A summary of EFS derived from investigator assessment at the reference date of 1 August 2011 (Method A1), by AML risk classification is summarized in Table 21 and displayed in Figure 7 and Figure 8.

Table 21 Event-Free Survival by AML Risk Classifications – by Investigator Assessment at the Reference Date of 01 August 2011 (Method A1) (mITT Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine	Daunorubicin + Cytarabine
Cytogenetics (Favorable/Intermediate), N	94	95
Number of events, n (%)	44 (46.8)	68 (71.6)
Induction failure, n (%)	6 (6.4)	14 (14.7)
Relapse, n (%)	33 (35.1)	45 (47.4)
Death, n (%)	5 (5.3)	9 (9.5)
KM estimate of median time to event (months) [95% CI][a]	22.5 [15.5, NE]	11.6 [8.3, 13.7]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.460 [0.313-0.676]	
p-Value	<0.0001	
Cytogenetics (Unfavorable), N	27	30
Number of events, n (%)	23 (85.2)	26 (86.7)
Induction failure, n (%)	9 (33.3)	13 (43.3)
Relapse, n (%)	9 (33.3)	9 (30.0)
Death, n (%)	5 (18.5)	4 (13.3)
KM estimate of median time to event (months) [95% CI][a]	4.5 [1.1, 7.4]	2.8 [1.6, 8.7]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	1.111 [0.633-1.949]	
p-Value	0.7151	
NCCN (Favorable/Intermediate), n	80	80
Number of events, n (%)	37 (46.3)	55 (68.8)
Induction failure, n (%)	6 (7.5)	13 (16.3)
Relapse, n (%)	27 (33.8)	35 (43.8)
Death, n (%)	4 (5.0)	7 (8.8)
KM estimate of median time to event (months) [95% CI][a]	20.3 [15.5, NE]	12.4 [8.5, 15.6]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.480 [0.314, 0.732]	
p-Value	0.0005	
NCCN (Poor/Adverse), n	43	46
Number of events, n (%)	30 (69.8)	39 (84.8)
Induction failure, n (%)	9 (20.9)	14 (30.4)
Relapse, n (%)	15 (34.9)	20 (43.5)
Death, n (%)	6 (14.0)	5 (10.9)
KM estimate of median time to event (months) [95% CI][a]	8.6 [4.5, 14.2]	7.0 [2.7, 8.8]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.738 [0.457, 1.190]	
p-Value	0.2111	
ELN (Favorable/Intermediate), n	86	91
Number of events, n (%)	40 (46.5)	63 (69.2)
Induction failure, n (%)	6 (7.0)	13 (14.3)
Relapse, n (%)	29 (33.7)	43 (47.3)
Death, n (%)	5 (5.8)	7 (7.7)
KM estimate of median time to event (months) [95% CI][a]	22.5 [15.5, NE]	12.2 [8.5, 14.3]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.485 [0.325, 0.724]	
p-Value	0.0003	
ELN (Poor/Adverse), n	37	36
Number of events, n (%)	27 (73.0)	32 (88.9)
Induction failure, n (%)	9 (24.3)	14 (38.9)
Relapse, n (%)	13 (35.1)	12 (33.3)
Death, n (%)	5 (13.5)	6 (16.7)
KM estimate of median time to event (months) [95% CI][a]	7.4 [3.7, 14.3]	4.0 [1.7, 8.6]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.720 [0.430, 1.205]	
p-Value	0.2091	

Method (A1): Event date determined by investigator assessment; censoring date was reference date (01 Aug 2011) or the last disease assessment date before the reference date.
Abbreviations: AML=acute myeloid leukemia; CI=confidence interval; ELN=European LeukemiaNet; GO=gemtuzumab ozogamicin; KM=Kaplan-Meier; mITT=modified intent-to-treat; n=number of patients;

N=number of patients; NCCN=National Comprehensive Cancer Network; NE=not estimable.
a. Based on the Brookmeyer and Crowley Method with log-log transformation.
b. Based on the Cox Proportional Hazards Model.
c. 2-sided p-value from the log-rank test.

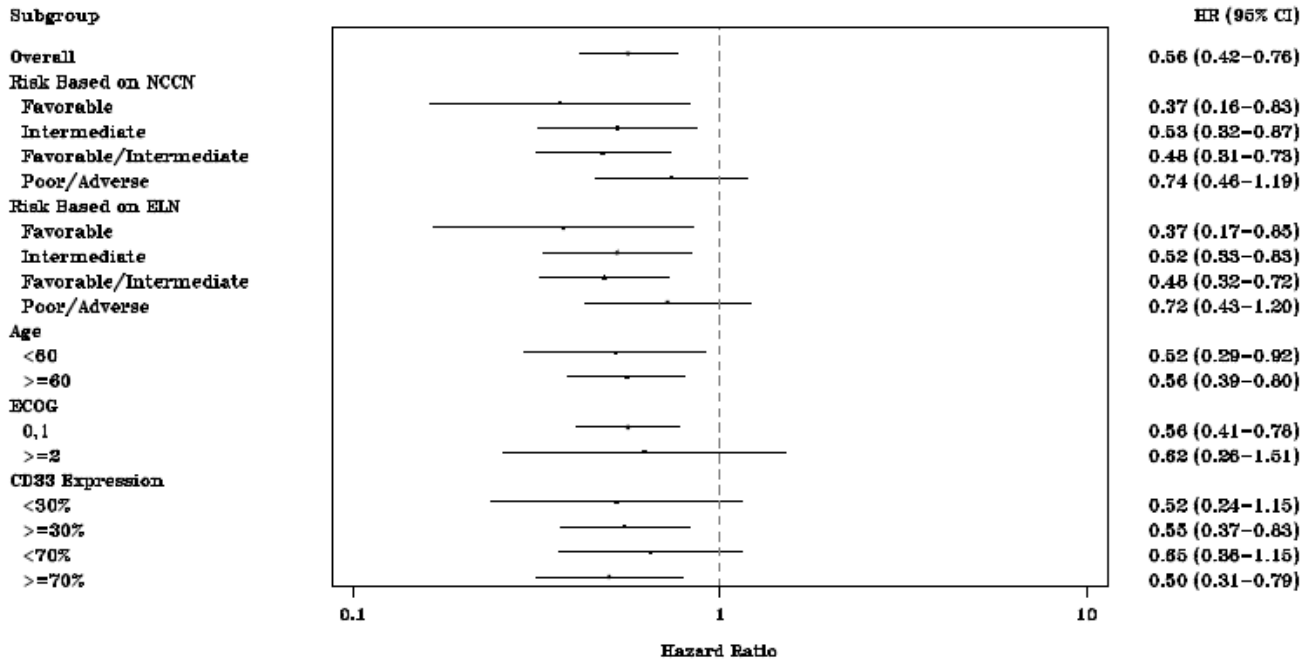


Figure 7 Forest Plot for Event-Free Survival - by Investigator Assessment at the Reference Date of 1 August 2011 (Method A1) (mITT Population) (ALFA0701 study)

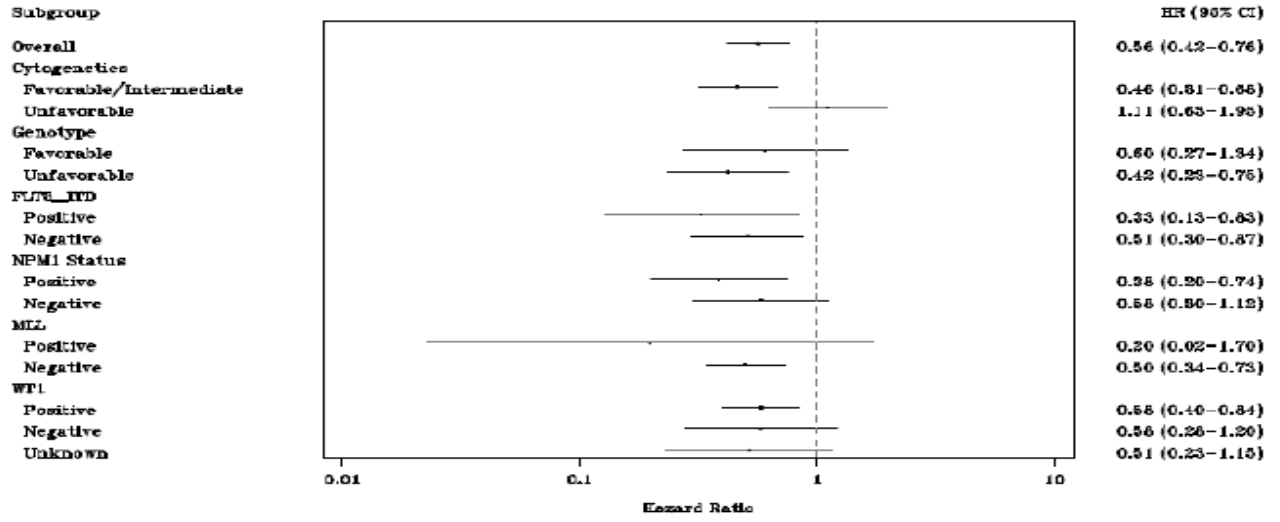


Figure 8 Forest Plot for Event-Free Survival - by Investigator Assessment at the Reference Date of 01 August 2011 (Method A1) (mITT Population) (ALFA0701 study)

A summary of EFS based on independent review with reference date of 1 August 2011 (Method B1), by AML risk classifications is summarized in Table 22 and Figure 9 and Figure 10.

Table 22 Event-Free Survival – by NCCN and ELN Risk Classifications – by Independent Review at the Reference Date of 1 August 2011 (Method B1) (mITT Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine	Daunorubicin + Cytarabine
NCCN (Favorable/Intermediate), n	80	80
Number of events, n (%)	42 (52.5)	54 (67.5)
Induction failure, n (%)	13 (16.3)	17 (21.3)
Relapse, n (%)	26 (32.5)	31 (38.8)
Death, n (%)	3 (3.8)	6 (7.5)
KM estimate of median time to event (months) [95% CI][a]	18.0 [9.4, NE]	12.2 [8.1, 15.6]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.650 [0.433, 0.976]	
p-Value	0.0352	
NCCN (Poor/Adverse), n	43	46
Number of events, n (%)	30 (69.8)	38 (82.6)
Induction failure, n (%)	9 (20.9)	14 (30.4)
Relapse, n (%)	15 (34.9)	17 (37.0)
Death, n (%)	6 (14.0)	7 (15.2)
KM estimate of median time to event (months) [95% CI][a]	7.7 [4.0, 14.3]	6.1 [2.7, 9.1]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.735 [0.455, 1.187]	
p-Value	0.2145	
ELN (Favorable/Intermediate), n	86	91
Number of events, n (%)	45 (52.3)	63 (69.2)
Induction failure, n (%)	11 (12.8)	14 (15.4)
Relapse, n (%)	29 (33.7)	39 (42.9)
Death, n (%)	5 (5.8)	10 (11.0)
KM estimate of median time to event (months) [95% CI][a]	18.6 [9.5, NE]	11.3 [8.1, 15.2]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.607 [0.413, 0.893]	
p-Value	0.0099	
ELN (Poor/Adverse), n	37	36
Number of events, n (%)	27 (73.0)	30 (83.3)
Induction failure, n (%)	11 (29.7)	17 (47.2)
Relapse, n (%)	12 (32.4)	9 (25.0)
Death, n (%)	4 (10.8)	4 (11.1)
KM estimate of median time to event (months) [95% CI][a]	5.4 [0.1, 9.9]	3.0 [0.0-9.1]
Versus daunorubicin + cytarabine		
Hazard ratio[b] [95% CI]	0.781 [0.464, 1.315]	
p-Value	0.4258	

Method (B1): Event date determined by independent review; censoring date was reference date (1 Aug 2011) or the last disease.
Abbreviations: AML=acute myeloid leukemia; CI=confidence interval; ELN=European LeukemiaNet; GO=gemtuzumab ozogamicin; KM=Kaplan-Meier; mITT=modified intent-to-treat; n=number of patients; NCCN=National Comprehensive Cancer Network.
a. Based on the Brookmeyer and Crowley Method with log-log transformation.
b. Based on the Cox Proportional Hazards Model.
c. 2-sided p-value from the log-rank test.

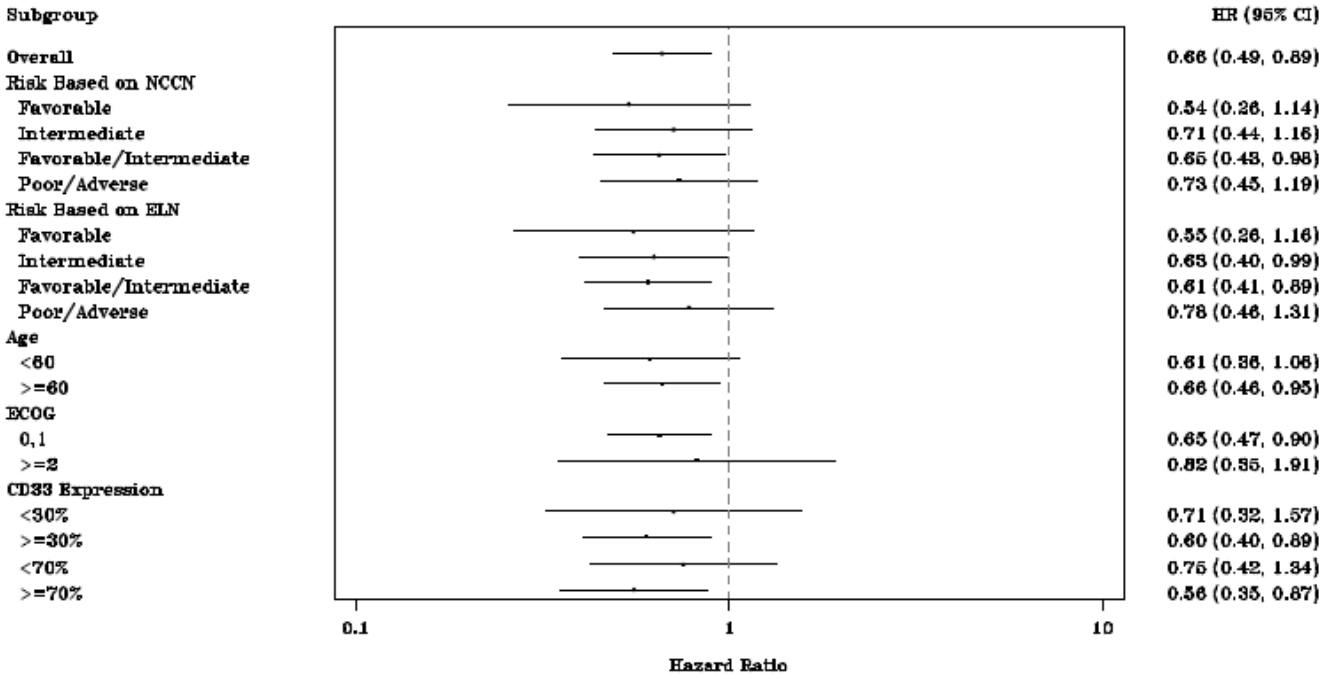


Figure 9 Forest Plot for Event-Free Survival - by Independent Review at the Reference Date of 01 August 2011 (Method B1) (mITT Population) (ALFA0701 study)

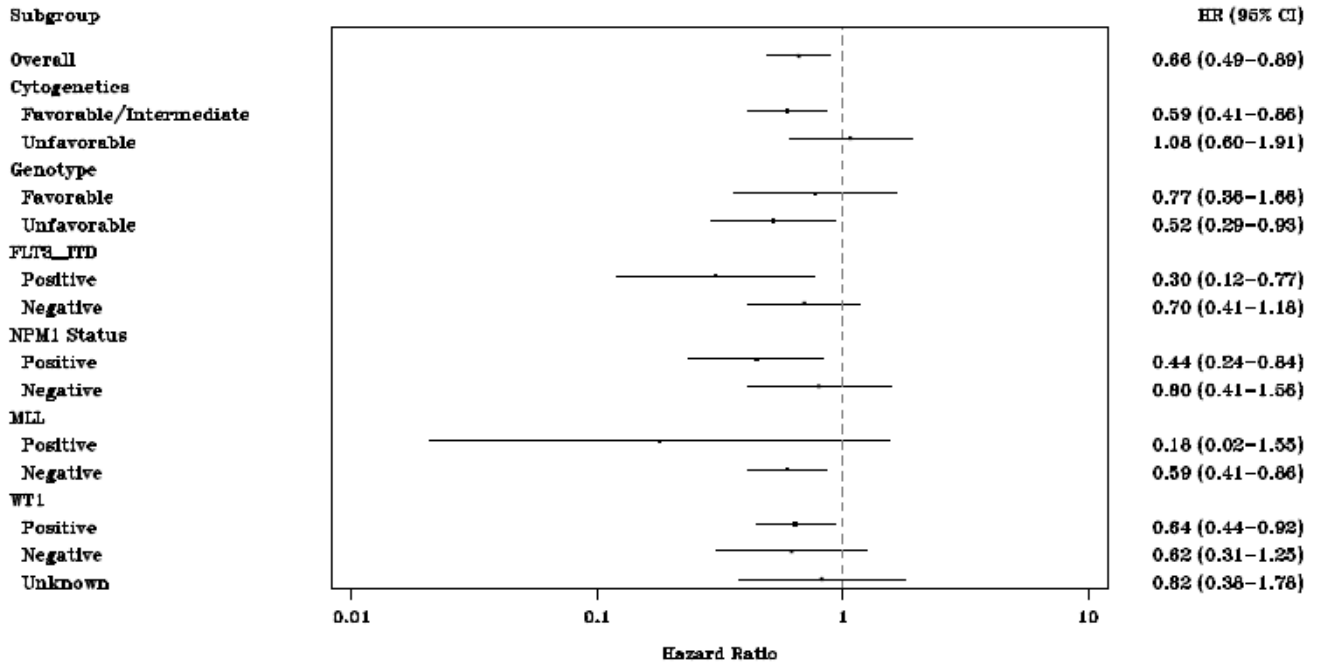


Figure 10 Forest Plot for Event-Free Survival - by Independent Review at the Reference Date of 1 August 2011 (Method B1) (mITT Population) (ALFA0701 study)

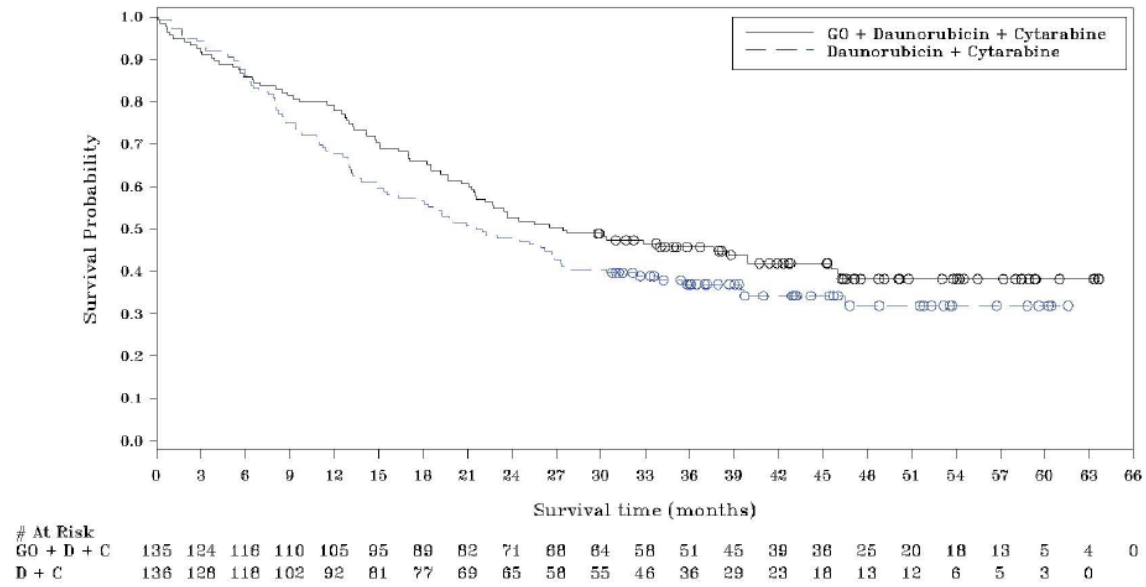
Secondary endpoint-Overall survival

Table 23 and Figure 11 summarize the OS analysis. A total of 80 (59.3%) patients in the GO arm and 88 (64.7%) patients in the control arm died prior to the reference date of 30 April 2013.

Table 23 Overall Survival Analysis (mITT Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=135)	Daunorubicin + Cytarabine (N=136)
Number of deaths, n (%)	80 (59.3)	88 (64.7)
Number of censored patients, n (%)	55 (40.7)	48 (35.3)
Reason for censoring, n (%)		
Alive at reference date	55 (40.7)	48 (35.3)
Patient withdrew consent	0	0
Lost to follow-up	0	0
Alive at last assessment or contact date prior to reference date	0	0
KM estimate of median time to event (months) [95% CI][a]	27.5 [21.4, 45.6]	21.8 [15.5, 27.4]
Survival probability at 2 years (95% CI) [b,c]	52.6 [43.8, 60.6]	47.8 [39.2, 55.9]
Survival probability at 3 years (95% CI) [b,c]	45.7 [37.2, 53.9]	37.0 [28.8, 45.1]
Versus daunorubicin + cytarabine - unstratified		
Hazard ratio[d] [95% CI]	0.807 [0.596, 1.093]	
p-value[e]	0.1646	
Versus daunorubicin + cytarabine – stratified by risk (NCCN guideline)		
Hazard ratio[d] [95% CI]	0.887 [0.647, 1.216]	
p-Value[e]	0.4547	
Versus daunorubicin + cytarabine – stratified by risk (ELN guideline)		
Hazard ratio[d] [95% CI]	0.869 [0.635, 1.190]	
p-Value[e]	0.3824	

Abbreviations: CI=confidence interval; ELN=European LeukemiaNet; GO=gemtuzumab ozogamicin; KM=Kaplan-Meier; mITT=modified intent-to-treat; n=number of patients; N=number of patients; NCCN=National Comprehensive Cancer Network.
a. Based on the Brookmeyer and Crowley Method with log-log transformation.
b. Estimated from the KM curve.
c. Calculated from the product-limit method/Calculated from the log[-log(x-<year,month> survival probability)] using a normal approximation and back transformation.
d. Based on the Cox Proportional Hazards Model.
e. 2-sided p-value from the log-rank test.



Notes: Circles indicate censored observations.
D + C stands for Daunorubicin + Cytarabine .

Figure 11 Kaplan-Meier Plot of Overall Survival- Modified ITT Population (ALFA0701 study)

Secondary endpoint-Response rate

A summary of response rate by investigator assessment and independent review is provided in Table 24.

Table 24 Response Rate - by Investigator Assessment and Independent Review(mITT Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=135)	Daunorubicin + Cytarabine (N=136)	Total (N=271)
Investigator assessment			
Overall response[a], n (%)	110 (81.5)	100 (73.5)	210 (77.5)
95% CI[b]	[73.89, 87.64]	[65.28, 80.72]	[72.05, 82.32]
Risk difference [95% CI][c]			7.95 [-3.79, 19.85]
Odds ratio [95% CI][d]			1.58 [0.86, 2.96]
p-Value[e]			0.1457
CR	95 (70.4)	95 (69.9)	190 (70.1)
CRp	15 (11.1)	5 (3.7)	20 (7.4)
Independent review[f]			
Overall response[a], n (%)	100 (74.1)	96 (70.6)	196 (72.3)
95% CI[b]	[65.83, 81.23]	[62.17, 78.09]	[66.59, 77.57]
Risk difference [95% CI][c]			3.49 [-8.20, 15.50]
Odds ratio [95% CI][d]			1.19 [0.67, 2.10]
p-Value[e]			0.5875

Table 25 Response status- Per Investigator- By phase, Modified ITT population (ALFA0701 study)

Treatment Phase	Status (Post-Treatment Phase)	GO + Daunorubicin + Cytarabine (N=135)	Daunorubicin + Cytarabine (N=136)	Total (N=271)
Induction	CR	94 (69.6)	94 (69.1)	188 (69.4)
	CRp	15 (11.1)	5 (3.7)	20 (7.4)
	Alive But Treatment Failure	18 (13.3)	30 (22.1)	48 (17.7)
	Early Death Before Day 15	0	0	0
	Death in Non-blastic Aplasia	4 (3.0)	0	4 (1.5)
	Death After Day 15 of indeterminate cause	0	2 (1.5)	2 (0.7)
Salvage	CR	2 (1.5)	4 (2.9)	6 (2.2)
	CRp	0	1 (0.7)	1 (0.4)
	Alive But Treatment Failure	4 (3.0)	0	4 (1.5)
	Early Death Before Day 15	0	0	0
	Death in Non-blastic Aplasia	0	0	0
	Death After Day 15 of indeterminate cause	0	0	0
Consolidation 1	CR	61 (45.2)	90 (66.2)	151 (55.7)
	CRp	37 (27.4)	7 (5.1)	44 (16.2)
	Relapse	1 (0.7)	1 (0.7)	2 (0.7)
	Died	0	0	0
	Dropped Out of the Study	0	1 (0.7)	1 (0.4)
	Lost to Follow-up	0	0	0
	Not Assessed	0	0	0

Notes: Status as collected on Part 1: CHV CRF after each treatment phase (re-induction was assessed by the investigator as part of induction with no separate CRF page). CR= Complete Remission; CRp= Complete Remission with incomplete platelet recovery.
Death in non-blastic aplasia was assessed by investigator as: Deaths occurring >= 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia.
Death after Day 15 from indeterminate cause was assessed by investigator as: Deaths occurring >= 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available.

Secondary endpoint-Relapse-free survival

A summary of RFS derived from investigator assessment with the reference date of 1 August 2011 is provided in Table 26.

Table 26. Relapse-Free Survival (mITT Population)-(ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=110)	Daunorubicin + Cytarabine (N=100)
Number of events, n (%)	49 (44.5)	66 (66.0)
Relapse, n (%)	44 (40.0)	58 (58.0)
Death, n (%)	5 (4.5)	8 (8.0)
Number of censored patients, n (%)	61 (55.5)	34 (34.0)
Reason for censoring, n (%)		
Event-free at reference date	61 (55.5)	34 (34.0)
Event-free at last assessment prior to reference date	0	0
No on-study disease assessment	0	0
Patient withdrew consent	0	0
Lost to follow-up	0	0
KM estimate of median time to event (months) [95% CI][a]	28.0 [16.3, NE]	11.4 [10.0, 14.4]
Versus daunorubicin + cytarabine - unstratified		
Hazard ratio[b] [95% CI]	0.526 [0.362, 0.764]	
p-Value[c]	0.0006	

Summary of main study

The following tables summarise the efficacy results from the main study supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 27 Summary of efficacy for trial ALFA0701

Title: Multicenter, randomized, phase 3 study of fractionated doses of the monoclonal antibody [drug conjugate] gemtuzumab ozogamicin (GO) in addition to daunorubicin + cytarabine for induction and consolidation therapy in patients with acute myeloid leukemia (AML) Aged 50 to 70 Years		
Study identifier	ALFA0701	
Design	Multicenter, randomized, open label, two arm study	
	Duration of main phase:	FPFV: 8 January 2008 Primary completion: 01 August 2011
Hypothesis	Superiority	
Treatments groups	Daunorubicin + Cytarabine	Induction chemotherapy: DNR 60 mg/m^2/day as a 30-minute IV infusion on Days 1 to 3 and AraC 200 mg/m^2/day as a continuous infusion on Days 1 to 7. Second induction course: DNR + AraC (DNR 35 mg/m^2/day as a 30-minute IV infusion + AraC 1 g/m^2/12 hours as a 12-hour infusion from Days 1 to 3) - If the creatinine clearance was <30 mL/min verified before initiation, without AraC. Salvage therapy : Idarubicin + AraC (12 mg/m^2 on Day 1 and Day 2 and 1 g/m^2 × 2/day on Days 1 to 4) if PS<3 and clearance >30ml/min. Consolidation therapy: 2 courses of treatment including DNR + AraC (DNR 60 mg/m^2 as a 30-minute IV infusion on Day 1 and AraC 1 g/m2/12 hours as a 2-hour infusion from Days 1 to 4). The second consolidation course consisted of an additional second dose of DNR 60 mg/m^2 as a 30-minute IV infusion on Day 1 and 2. 136 patients assigned, 89 completed treatment (consolidation 2)

	GO + Daunorubicin + Cytarabine		Induction chemotherapy: Same DNR and AraC dosing and schedule as the control am and Mylotarg 3 mg/m^2/day as a 2-hour IV infusion on Days 1, 4, and 7. Second induction course: Same as control group – GO may have been given if ≥5% blasts in D15 BMA (or 10% depending on the protocol amendment). Salvage therapy: Same as control group. Consolidation therapy: Same as control plus GO 3 mg/m^2/day as a 2-hour IV infusion on Day 1 according to initial randomisation. 135 patients assigned, 82 completed chemotherapy treatment, 64 completed GO (GO received in consolidation 2)
Endpoints and definitions	Primary endpoint	Event free survival (EFS) by investigator	Time from date of randomization to date of induction failure, relapse, or death due to any cause, whichever came first, determined by each investigator individually.
	Secondary endpoint	Overall survival (OS)	Time from date of randomization to date of death due to any cause.
	Secondary endpoint	Relapse-free survival (RFS)	Time from the date of remission to the date of relapse or death from any cause, whichever came first (for patients experiencing CR or CRp)
	Secondary endpoint	Hematologic Response	A patient was considered in complete remission (CR) by the investigator if the following conditions were all met: Leukemic blasts absent from the peripheral blood and no clinical tumors; Percentage of blasts in the bone marrow is <5%, as measured by morphologic studies (aspirates); Hemoglobin >9 g/dL, platelets >100,000/mm3, ANC > 1000/mm3; The patient is red cell and platelet transfusion independent.

	Secondary endpoint	Minimal Residual Disease (MRD)	Negative (if based on WT1): WT1/100xABL ratio is <ULN (0.5% for peripheral blood samples and 2.5% for bone marrow samples). Peripheral blood samples were classified as positive if the ratio (%) was ≥0.5. Bone marrow samples were classified as positive if the ratio (%) was ≥2.5. MRD monitoring was based on peripheral blood. Negative (if based on NPM-1mut): NPMmut/100xABL ratio is <ULN (0.1% for peripheral blood samples and bone marrow samples). Peripheral blood and bone marrow samples were classified as positive if the ratio (%) was ⩾0.1. MRD monitoring was based on bone marrow.
Database lock	LPLV (cut-off for OS): 30 April 2013 Data cut-off for retrospective data collection: 01 November 2013		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Modified Intent to treat (mITT) – excluding 9 randomized patients due to missing ICF (4 control arm/ 5 GO arm) For all analyses: censoring date being the reference date of 01 Aug 2011 or the last disease assessment date before the reference date.		
Descriptive statistics and estimate variability	Treatment group	Daunorubicin+ Cytarabine	GO + Daunorubicin + Cytarabine
	Number of subjects	136	135
	EFS (median; months) 95% CI	9.5 8.1 – 12	17.3 13.4 – 30
	OS (median; months) 95% CI	21.8 15.5-27.4	27.5 21.4-45.6
	RR (%) 95% CI	73.5 73.89-87.64	81.5 65.28-80.72
	RFS (median; months) 95% CI	11.4 10.0-14.4	28 16.3, NE
Effect estimate per comparison	Primary endpoint EFS by investigator assessment	Comparison groups	Control vs GO
		HR (unstratified)	0.562
		95% CI	0.415 – 0.762
		P-value (two sided by log rank)	0.0002
	Secondary endpoint OS	Comparison groups	Control vs GO
		HR	0.807
		95% CI	0.596 – 1.093
		P-value (two sided by log rank)	0.1646
	Secondary endpoint Hematologic Response	Comparison groups	Control vs GO
		Odds ratio	1.58
		95% CI	0.86- 2
		P-value (Fishers exact test)	0.1457
	Secondary endpoint RFS	Comparison groups	Control vs GO
		HR	0.526

		95% CI	0.362-0.764
		P-value (two sided by log rank)	0.0006
Notes	Primary and secondary endpoints were confirmed by sensitivity analyses, using different censoring dates, the data determined by the independent review and censoring patients prior HSCT. But significance levels became less compelling.		

Analysis performed across trials (pooled analyses and meta-analysis)

Individual patient data (IPD) meta-analysis

The IPD meta-analysis consisted of 5 randomised investigator-initiated research (IIR) studies, including the pivotal Study ALFA-0701 as well as 4 other studies (Medical Research Council [MRC] AML15, National Cancer Research Institute [NCRI] AML16, Southwest Oncology Group [SWOG] S0106, and Groupe Ouest Est d'Etude des Leucémies aiguës et Autres Maladies du Sang [GOELAMS] AML2006IR).

The characteristics of the 5 selected trials are summarized in Table 28.

Table 28 Trials Included in the Individual Patient Data Meta-Analysis

Study Name Study Type	Endpoints: Primary (Secondary)	Study Population	Induction Therapy		Enrollment	No. Patients Randomized (GO/No GO)		Median Follow-up in Current Analysis [a] (months)
			Chemotherapy	GO		Publication	Current Analysis	
MRC AML15 Phase 3, 6-arm, open-label, randomized	OS and CR, CRi, (RD, RFS, CIR, CIDCR, toxicity)	AML, de novo or secondary, APL[b]	DA (3+10, 3+8) or ADE (3+10+5, 3+8+5) or FLAG-Ida	3 mg/m² Day 1	2002-2006	1113 (556/557)[c]	1099 (548/551)	110.45
NCRI AML16 Phase 3, 4-arm, open-label, randomized	OS (CR, CRi, RFS, RR, DCR1, toxicity)	AML, de novo, secondary, or high risk MDS	DA (3+10, 3+8) or DClo (D 1, 3, 5/clofarabine D 1-5)	3 mg/m² Day 1	2006-2010	1115 (559/556)[c]	1115 (559/556)	69.06
ALFA-0701 Phase 3, 2-arm, open-label randomized	EFS (CR, CRp, RFS, OS, toxicity)	de novo AML	DA (3+7)	3 mg/m² D 1,4,7 (≤5 mg/dose)	2008-2010	280 (140/140)	271 (135/136)	45.44
GOELAMS AML2006IR Phase 3, 2-arm, open-label, randomized	EFS at 3 years (OS, CIR, and CIDND at 3 years, CR, toxicity)	de novo AML, intermediate cytogenetics	DA (3+7)	6 mg /m² Day 4	2007-2010	254[d]	251 (126/125)	66.2
SWOG S0106 Phase 3, 2-arm, open-label, randomized	DFS, CR (CRi, PR, RD, OS, RFS, toxicity)	de novo AML	DA (3+7)+growth factor	6 mg/m² Day 4	2004-2009	637 (317/320)[e]	595 (295/300)	66.23

Results

A total of 3331 patients were included in the meta-analysis. Of these, 1663 patients (49.9%) were randomized to GO while 1668 patients (50.1%) were randomized to No GO. There were no post-randomization exclusions from the meta-analysis. The number of elderly patients age 60-69 represented the majority (33.1%), but ranging from 7.7% of patients age 15-29 up to 10.7% of patients age 70 and older; with a total of 22 patients under the age of 18 years included. Sensitivity analysis indicated no difference due to age. Slightly more male patients were included (55.4%) and 93% of patients with a PS of 0 or 1; 88.1% were treated for de novo AML with 75.5% having had cytarabine, daunorubicin, and etoposide based chemotherapy; the majority were in the favourable/intermediate cytogenetic or ELN risk group (MRC cytogenetics 62.2% ; ELN 44.9% [62.2% imputed ELN], with negative FLT3 or NPM1 status; the minority (12.9%) had known CD33 expression <30%. However, the high percentage of data not known needs to be appreciated as well (ie ELN risk group 37.6%, imputed ELN 20.3%, FLT3 46.2%, NMP1 50.8%, CD33 positivity 47.5%).

Primary efficacy endpoint OS

The OR for GO versus No GO was 0.91 (95% CI: 0.84-0.99, 2-sided stratified log-rank p=0.02), in favour of the GO arm (Figure 12 and Figure 13).

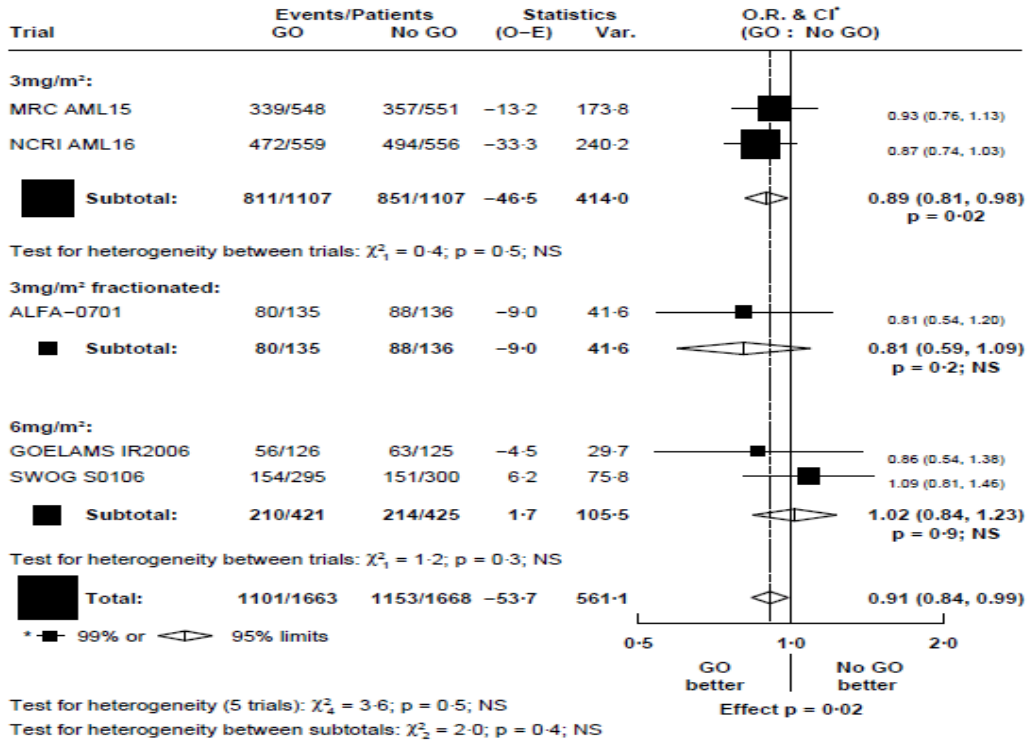


Figure 12 Overall Survival by GO Dose Group and Trial: Unstratified

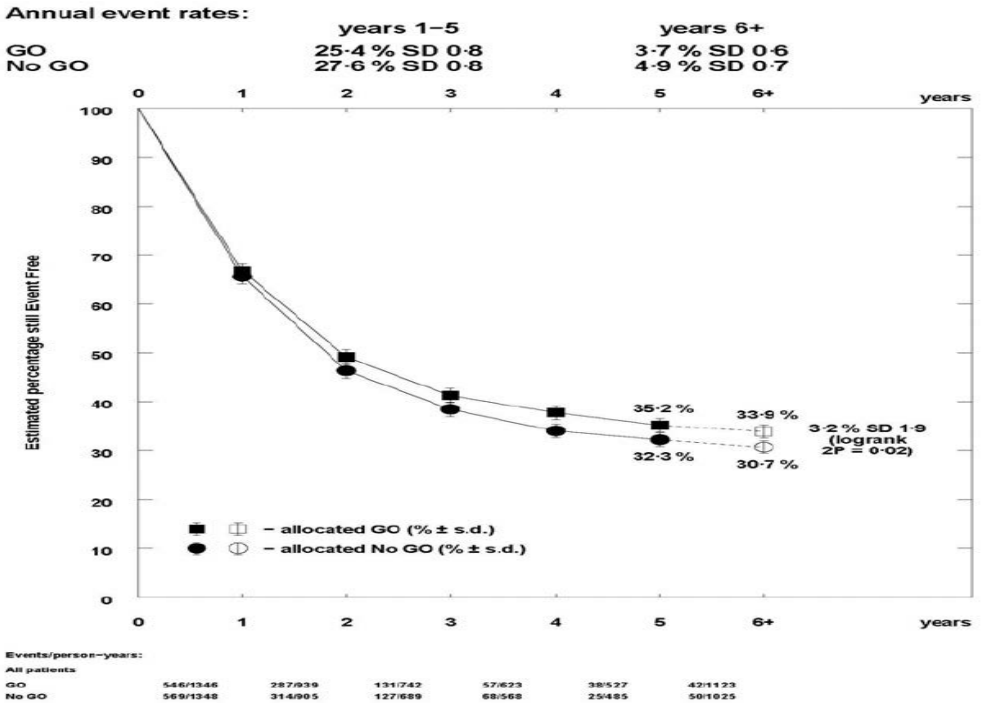


Figure 13 Overall Survival for GO versus No GO

- *OS by risk categories*

Figure 14 shows varying GO effects according to cytogenetic risk group (heterogeneity p=0.01 and trend test p=0.009).

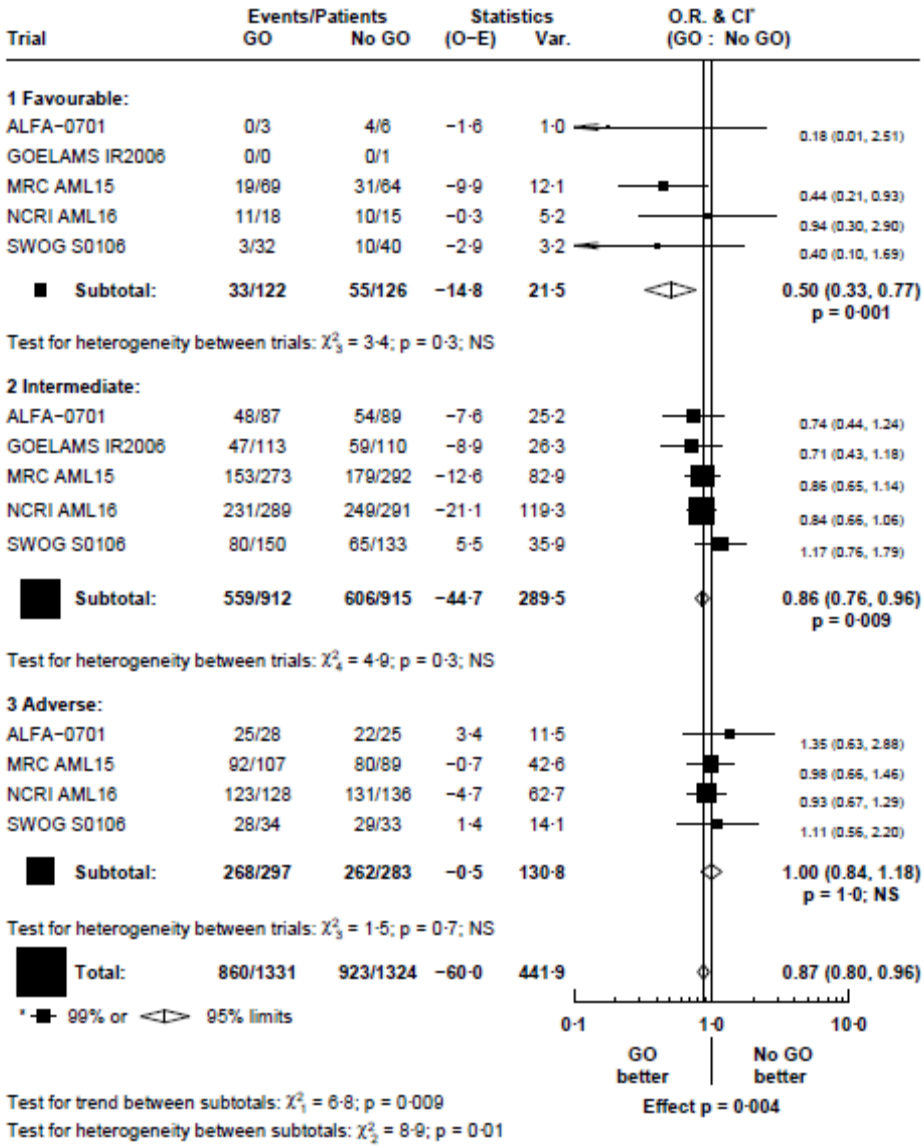


Figure 14 Overall Survival by MRC Cytogenetics: Favourable, Intermediate, and Adverse Risk Groups

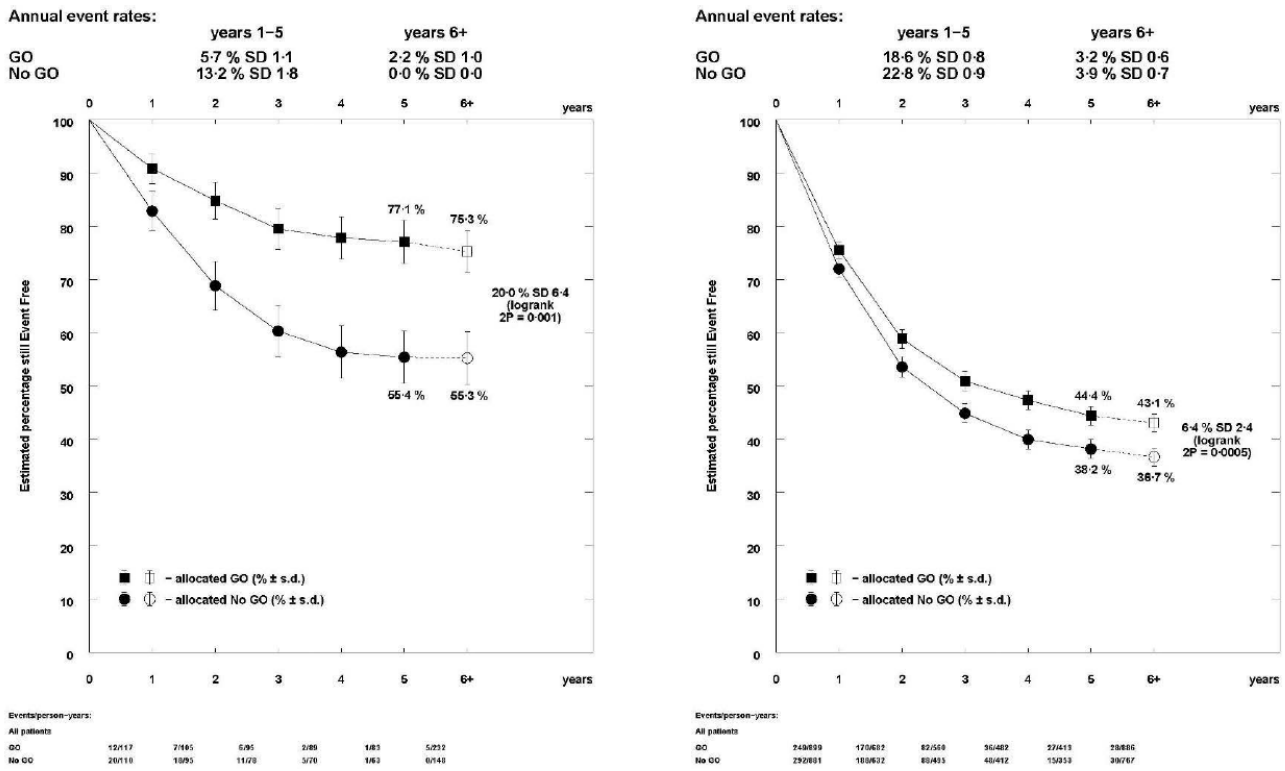


Figure 15 Overall survival curve GO versus no GO in favourable (left figure) and in favourable or intermediate MRC cytogenetic risk Group (right)

- *ELN risk group*

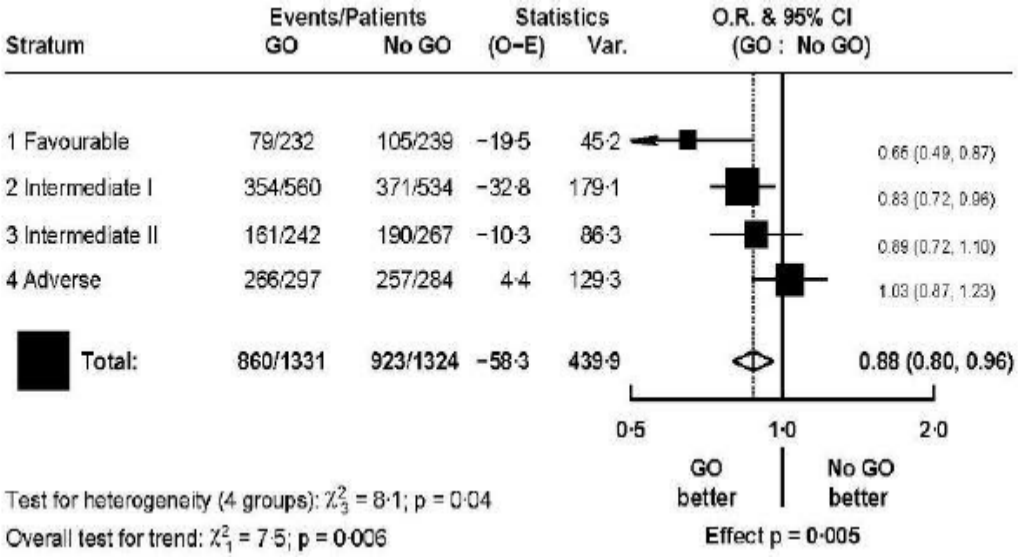


Figure 16 Overall Survival, by Imputed ELN Risk Group

- *OS Subgroups by Age, PS and CD33 positivity*

The OS Subgroups results by Age, PS and CD33 positivity are displayed in Figure 17, Figure 18 and Figure 19.

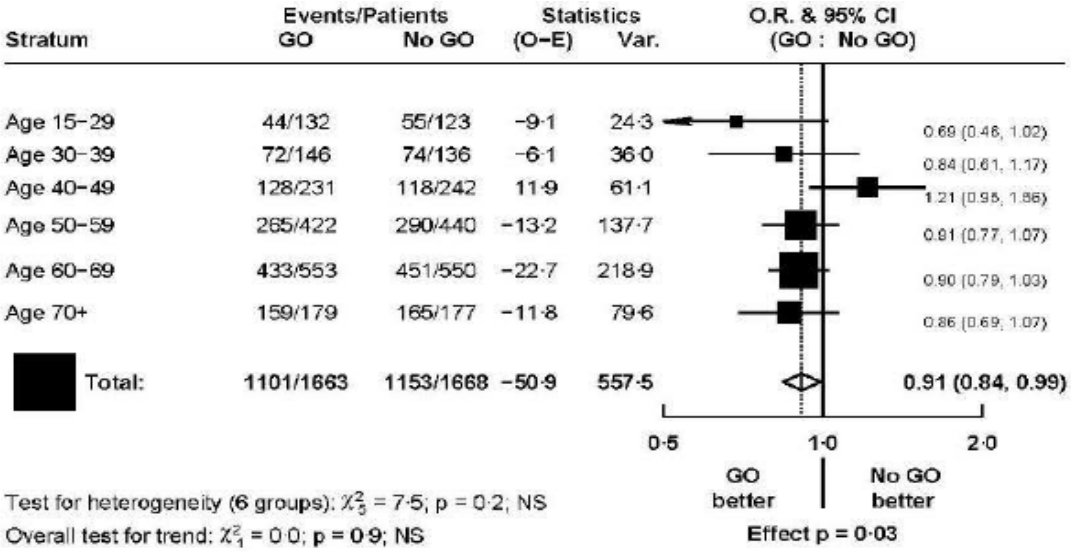


Figure 17 Overall Survival by Age

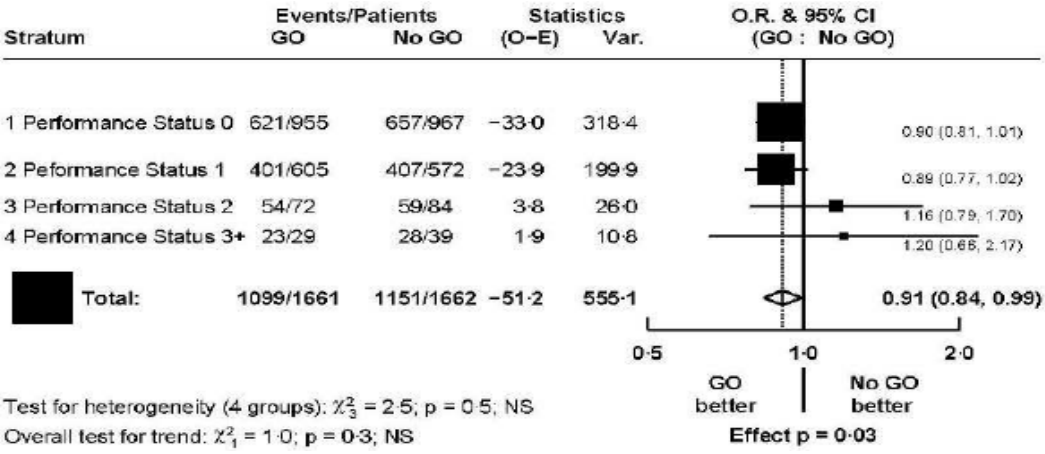


Figure 18 Overall Survival, by Performance Status

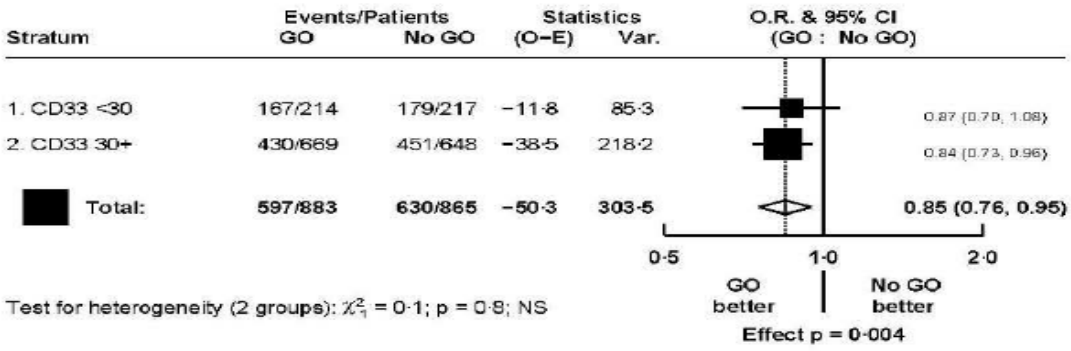


Figure 19 Overall Survival, by CD33-positivity

Secondary efficacy endpoints

The secondary endpoint of EFS was prolonged in the GO arm compared to No GO (OR 0.85, 95% CI: 0.78-0.93, 2-sided stratified log-rank p=0.0002), corresponding to a 15% reduction in events, with no heterogeneity by dose or trial within dose group.

In the GO arm, 1308 patients (78.7%) achieved an overall response, compared to 1285 (77.1%) in the No GO arm. The odds of achieving an Overall Response were not significantly increased in the GO arm (OR 0.91, 95% CI: 0.77-1.08, p=0.3), or in any of the dose groups.

In the GO arm, 1189 patients (71.5%) achieved a CR compared to 1166 patients (70.0%) in the No GO arm. GO treatment did not significantly increase the odds of achieving CR (OR 0.93, 95% CI: 0.80-1.08, p=0.3).

Of the 1307 GO patients and 1284 No GO patients who achieved a response, 868 patients in the GO arm and 916 patients in the No GO arm relapsed or died during follow-up. Overall GO treatment was found to significantly prolong RFS compared to No GO (OR 0.84, 95% CI: 0.77-0.93, p=0.0004).

Clinical impact of change in AAS levels – efficacy

In a review of drug supply records the AAS status was identified for all GO lots used in the studies considered relevant for the Mylotarg development and submitted as either pivotal (ALFA0701) or within the IPD meta-analyses (Table 29).

Table 29 Distribution of GO lots with elevated AAS by study

	MRC AML15	SWOG S0106	NCRI AML16	GOELAMS AML 2006IR	ALFA-0701
Study accrual (Start date – End date)	Jul 2002-Jun 2006	Aug 2004-Aug 2009	Dec 2006-Jul 2010	2007-2010	Jan 2008-Nov 2010
Number of lots with base AAS	4	6	7	5	3
Number of lots with elevated AAS	0	1	4	4	3
Percent of lots with elevated AAS	0%	14%	36%	44%	50%

Abbreviations: AAS=Amino acid substitution; ALFA=Acute Leukemia French Association; GO=Gemtuzumab ozogamicin; GOELAMS= Groupe Ouest Est d'Etude des Leucémies aiguës et Autres Maladies du Sang; MRC= Medical Research Council; NCRI=National Cancer Research Institute; SWOG= Southwest Oncology Group.

In the current product, approximately 32% of the gemtuzumab molecules contain AAS. A retrospective analysis was undertaken to characterise the extent of exposure to GO with elevated AAS across the clinical development program, evaluating the potential impact of AAS on safety and efficacy of GO in the studies included in the meta-analysis. Data on the GO lot administered to individual patients is only

available for the pivotal trial, with the AAS status either not known or unknown for at least one dose (AAS status unknown due to missing GO lot number) for 25% of the patients.

The report analysed the following groups:

- Only elevated AAS GO group: all GO administrations came from lots with elevated AAS, and there were no infusions with an unknown GO lot,
- Only base AAS GO group: all GO administrations came from lots without elevated (ie, base) AAS, and there were no infusions with an unknown GO lot,
- Any elevated AAS GO group: at least 1 GO administration came from a lot with elevated AAS. Patients could have had infusions with an unknown GO lot. All patients in the only elevated AAS GO group are also included in this group.

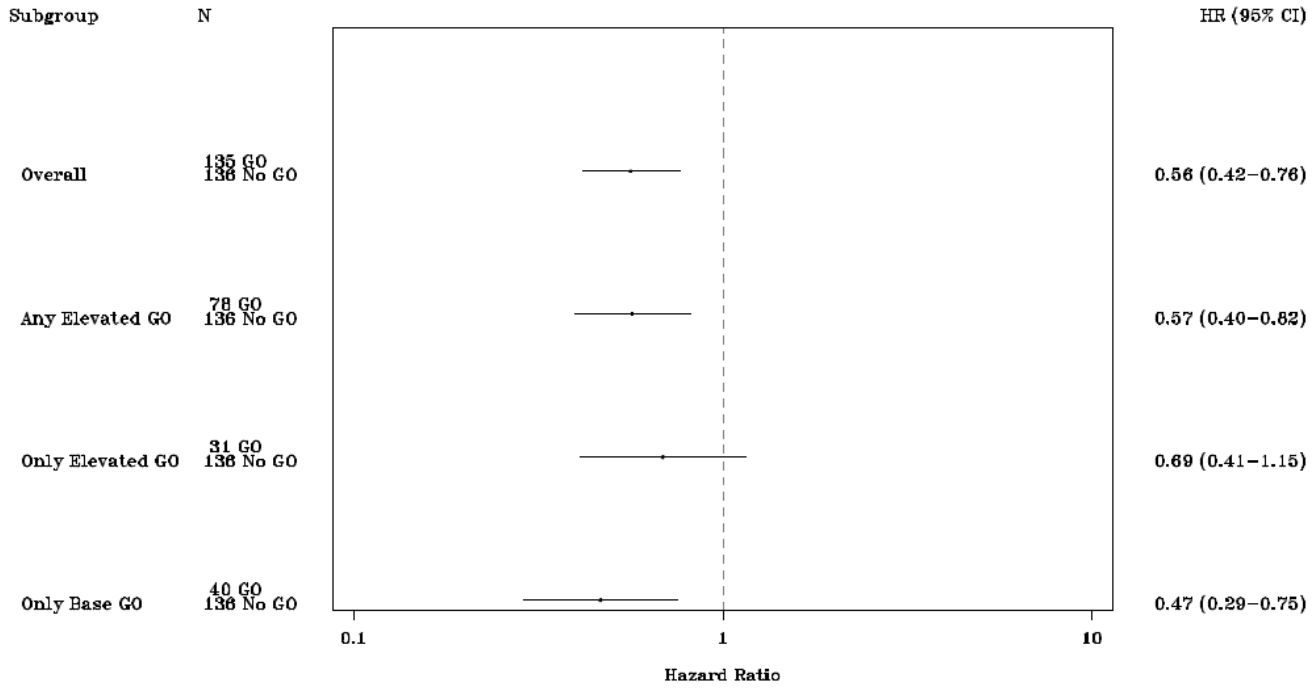
Results

Event-Free Survival

Table 30 Event-Free Survival Events in the ALFA-0701 Study – mITT Population

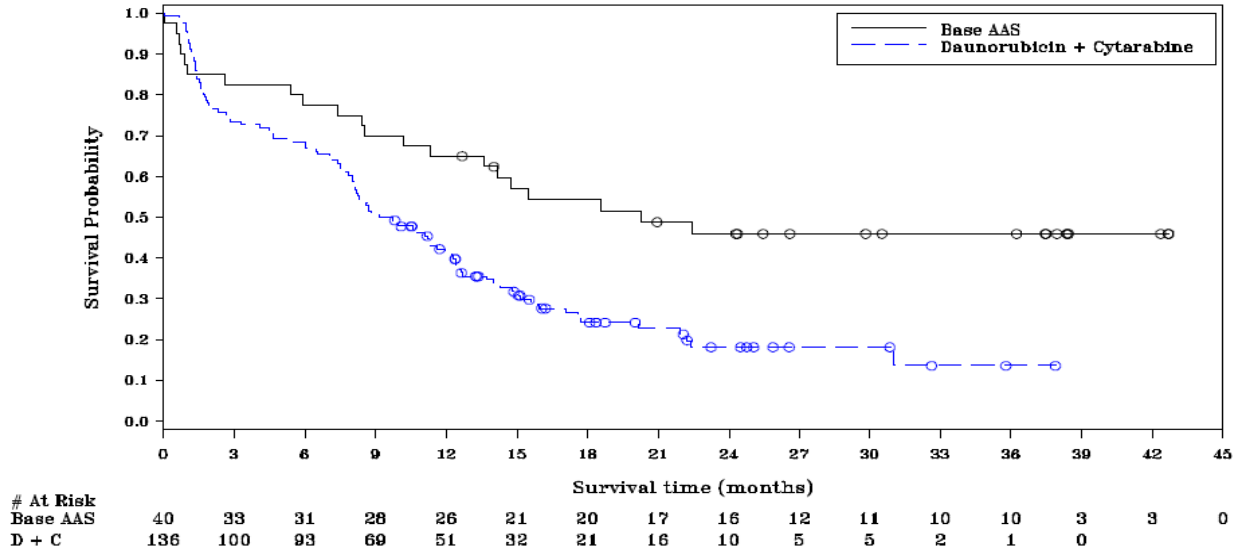
	Number of patients	Events (relapse, death, induction failure)	
		n	%
Only Elevated AAS GO	31	17	54.8
Any Elevated AAS GO	78	41	52.6
Only Base AAS GO	40	21	52.5
Overall GO arm	135	73	54.1
Control Arm	136	102	75.0

Abbreviations: AAS=Amino acid substitution; ALFA=Acute Leukemia French Association; GO=Gemtuzumab ozogamicin; mITT=Modified Intent to Treat; n=number affected.



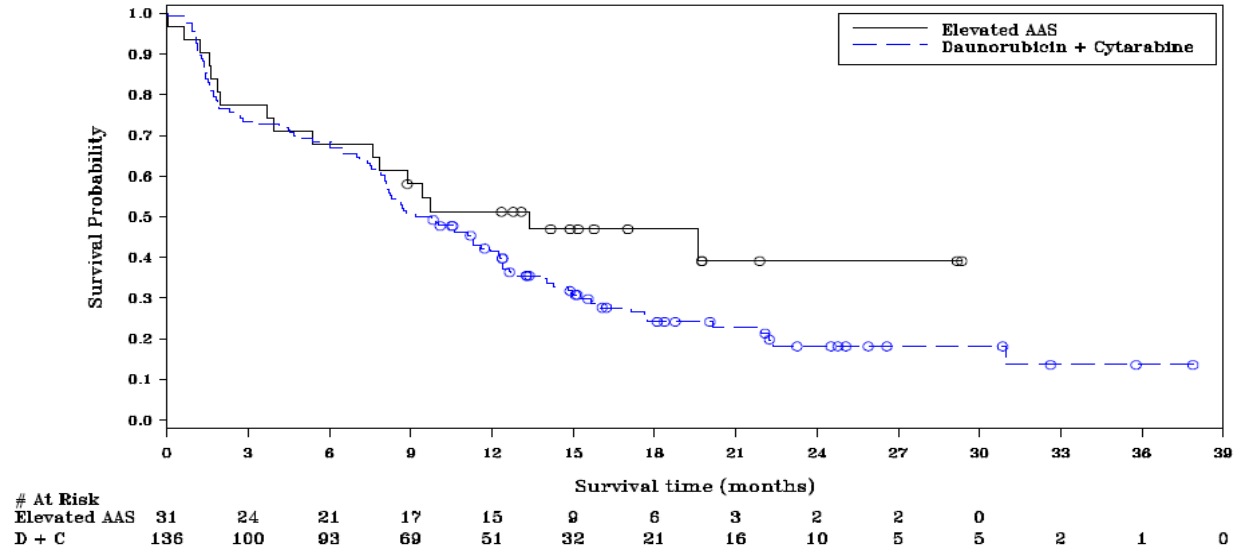
Notes: Based on the Cox Proportional Hazards Model. Hazard ratio >1 favors Daunorubicin + Cytarabine group.

Figure 20 Forest Plot of Event-free Survival for AAS Subgroups -Method Al: Event Date Determined by Investigators Assessment, Censoring - Date is Date of Last Disease Assessment Before the Reference Date (August 1, 2011) - Modified ITT Population



Notes: Circles indicate censored observations.

Figure 21 Event Free Survival- Kaplan-Meier Plot for Patients Who Received Only Doses of Baseline AAS Subgroup- Method A1: Event Date - Determined by Investigators Assessment, Censoring Date is Date of Last Disease Assessment Before the Reference Date (August 1, 2011) - Modified ITT Population



Notes: Circles indicate censored observations.
D + C stands for Daunorubicin + Cytarabine .

Figure 22 Event Free Survival- Kaplan-Meier Plot for Patients Who Received Only Doses of Elevated AAS Subgroup- Method A1: Event Date - Determined by Investigators Assessment, Censoring Date is Date of Last Disease Assessment Before the Reference Date (August 1, 2011) - Modified ITT Population

Relapse-Free Survival

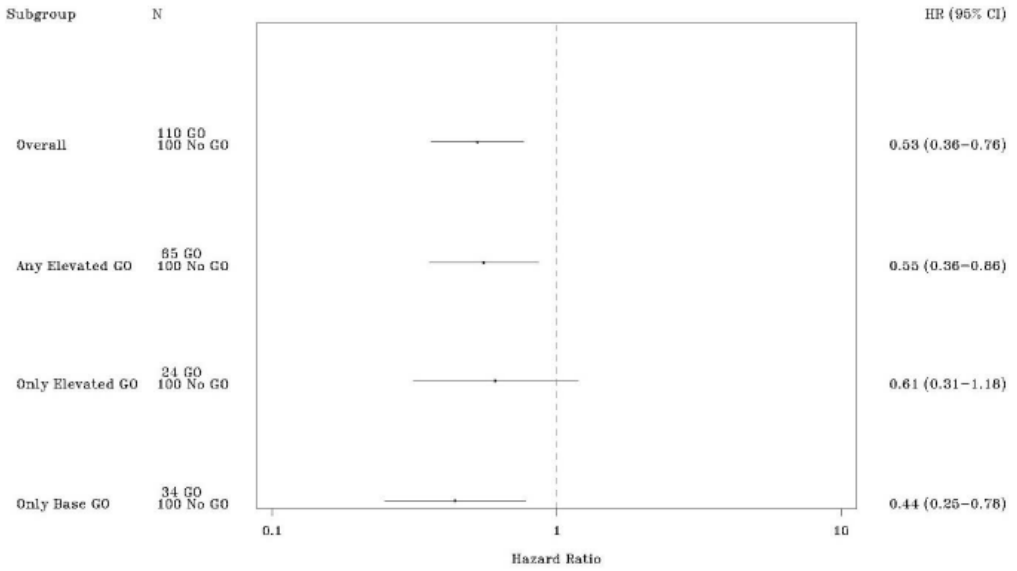


Figure 23 Forest Plot of Relapse-Free Survival: GO AAS Groups Compared to the Control Arm in ALFA-0701 – Modified Intent-to-Treat Population

Overall Survival

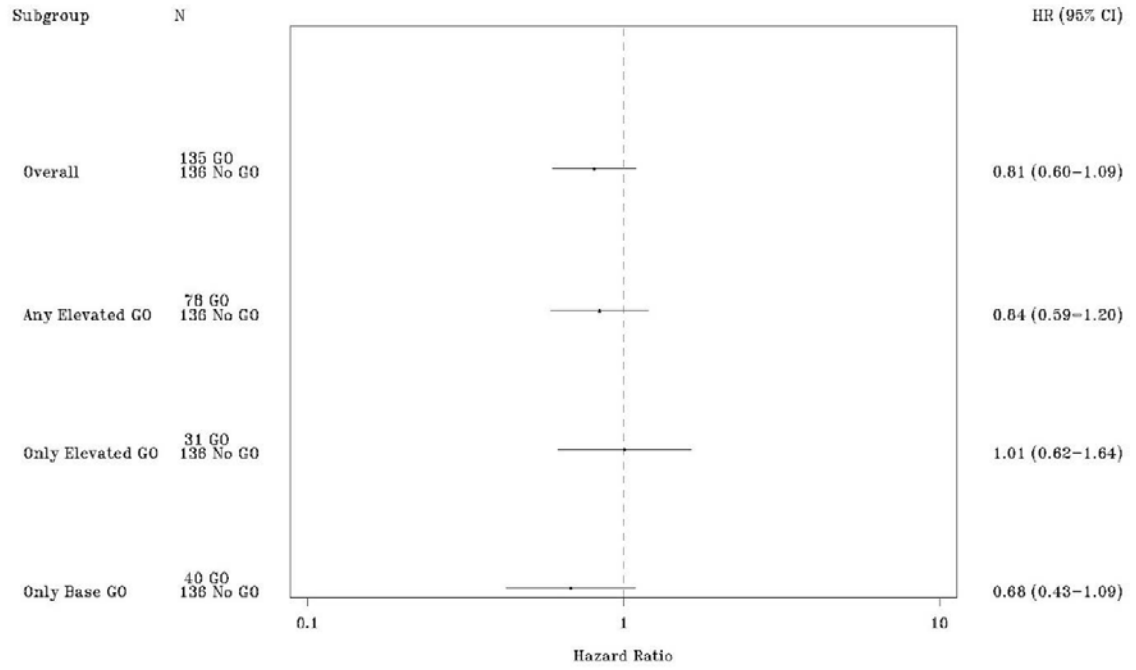


Figure 24 Forest Plot of Overall Survival: GO AAS Groups Compared to the Control Arm in ALFA-0701 – Modified Intent-to-Treat Population

Clinical studies in special populations

Table 31 Number of patients per age included in clinical studies

	Teenager and Young Adults, including paediatrics (age 15-29)	Age 60-69 (Older subjects number /total number)	Age ≥70 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
Controlled Trials (see also meta-analysis below)	ALFA 0701: 0 MRC AML15: 150 NCRI AML16: 0 GOELAMS AML2006IR: 25 SWOGS0106: 80 Total of 7.7% [255/3331]	ALFA 0701: 169 MRC AML15: 151 NCRI AML16: 755 GOELAMS AML2006IR: 9 SWOGS0106: 19 Total of 33.1% [1103/3331]	ALFA 0701: 12 MRC AML15: 3 NCRI AML16: 341 GOELAMS AML2006IR: 0 SWOGS0106: 0 Total of 10.7% [356/3331]	No information available
Non Controlled trials		MyloFrance1: included patients up to 80 years MyloFrance2: included patients up to 70 years Study 201: included patients up to 82 years Study 202: included patients up to 79 years Study 203: included patients up to 87 years Study 205: included patients up to 84 years		

Supportive studies

Out of the 19 clinical trials submitted, the 5 studies included in the meta-analysis are considered supportive of the intended indication of treatment of patients with de novo AML, though only the ALFA of the proposed posology.

The overall results of these 4 trials were that remission rates were not improved, although relapse was reduced in 4 of 5 trials with a significant survival benefit observed in NCRI trial AML16 comparing a single dose of GO (3 mg/m²) to induction chemotherapy, consisting of either DA 3+10 in Course 1 and 3+8 in Course 2 or DClo (DNR plus clofarabine) (data not shown).

Paediatric Data

In a randomised study (COG AAML0531) that evaluated standard chemotherapy alone or combined with Mylotarg in 1,022 newly diagnosed children (94.3% of patients < 18 years of age), and young adults (5.7% of patients); median age was 9.7 years (range: 0.003 29.8 years), patients with de novo AML were randomly assigned to either standard 5 course chemotherapy alone or to the same chemotherapy with 2 doses of Mylotarg (3 mg/m^2/dose) administered once in induction Course 1 and once in intensification Course 2. The study demonstrated that addition of Mylotarg to intensive chemotherapy improved EFS (3 years: 53.1% versus 46.9%; HR 0.83; 95% CI: 0.70 0.99; p=0.04) in de novo AML owing to a reduced relapse risk, with a trend towards longer OS in the Mylotarg arm which was not statistically significant (3 years: 69.4% versus 65.4%; HR 0.91; 95% CI: 0.74 1.13; p=0.39). However, it was also found that increased toxicity (post remission toxic mortality) was observed in patients with low risk AML which was

attributed to the prolonged neutropenia that occurred after receiving gemtuzumab ozogamicin during intensification Course 2. Thus, the optimal dose of gemtuzumab ozogamicin for paediatric patients was not established (SmPC, section 5.1).

Literature Review

A systematic literature review was conducted with the aim to identify paediatric clinical trials involving GO as a single agent or in combination with chemotherapy in order to obtain additional safety data.

Published papers selected for review from the 376 articles fulfilled all of the following criteria: "Paediatric" or "children" in the title, written in English, GO administered in patients with AML, and each study enrolled and treated at least 10 patients. Review articles, meeting abstracts, nonclinical studies, meta-analyses, case reports, and papers focusing on APL only were excluded from the literature review. Fifteen studies were identified that matched these criteria and they are listed below in Table 32.

Table 32 Paediatric Clinical Trials of GO

Sponsor/Study ID	AML Population	Age (years) Range and Median	N	Dose / Regimen	Reference
PHASE 1					
Wyeth/0903A1-102	Relapsed or refractory	1-16 median 12	29	Dose escalation x2 monotherapy	(20)
COG/ AAML00P2	Refractory	0.8-19.8 median 11.5	45	3mg/m^2 + AraC +mitoxantrone 3mg/m^2 or 2mg/m^2 + AraC+ L-asparaginase	(21)
New York Presbyterian Children's Hospital	Relapsed or refractory	1-17 Median 3	12	Dose escalation x 1 + busulfan and cyclophosphamide as conditioning	*(22)
COG / Columbia University	AML in CR1 or CR2	1-21 Median 13.5	14	Dose escalation x2 monotherapy after RIC HSCT	* (23) * (24)
PHASE II					
COG/ AAML03P1	Previously untreated, de novo	0-21 median 9.5	340	3 mg/m^2 +ADE induction 1 and 3mg/m^2 + MA in consolidation 2	(25)
International BFM/ Rel AML 2001/02	Relapsed or refractory	1.4-16.6 median 9.4	30	7.5 mg/m^2 x 2 14 days apart monotherapy	(26)
PHASE III					
COG/ AAML0531	Previously untreated, de novo	0-29 median 9.7	1022	3 mg/m^2 + ADE induction and 3 mg/m^2 + MA consolidation	(27)
NOPHO/ NOPHO-AML 2004	Previously untreated, de novo	0-18	120	5 mg/m^2/dose x 2 monotherapy post-consolidation	(28)
SJCRH/ AML02	Previously untreated	0.0-21.4 Median 9.1	232	3 mg/m^2 x 1 + ADE for induction 2 or 6 mg/m^2 x 1 monotherapy for induction 3	*(29) (30)

Sponsor/Study ID	AML Population	Age (years) Range and Median	N	Dose / Regimen	Reference
COMPASSIONATE USE					
Hôpital Saint-Louis	Relapsed or refractory	0.9-22.3 Median 10.8	17	GOCYT: 3 mg/m^2 x3 +AraC	(31)
Hôpital Saint-Louis	Relapsed or refractory	1.0-17.2 Median 5.5	12	3-9mg/m^2 1-5x monotherapy	*(32)
Children's Hospital, University of Münster	Relapsed or refractory	0.2-16.5 1. Median 2.1	12	1.8-9.0 mg/m^2 2x monotherapy	*(33)
AML-BFM, DCLSG, NOPHO	Relapsed or refractory	0.7-17.3 Median 8.9	15	2-9mg/m^2 x 2 monotherapy	*(34)
Queen Mary Hospital, Prince of Wales Hospital, Hong Kong	Relapsed or refractory	2.2-18.4 Median 10.9	15	2-9mg/m^2 Or 3- 3 mg/m^2 x 3 Combination: GO + FLAG, GO + MA or DA	
Bristol Royal Hospital for Children, Bristol UK	Relapsed or Refractory	1-13 Median 8	12	9 mg/m^2 monotherapy	*(35)

* Newly identified studies that match search criteria that do not appear in the literature search provided for the approved Paediatric Investigation Plan (PIP)
ADE = cytarabine+daunorubicin+etoposide; AML=Acute myeloid leukaemia; BFM = Berlin-Frankfurt-Münster; COG = Children's Oncology Group; DCLSG = Den Haag, The Netherlands; HSCT = haematopoetic stem cell transplant; iBFM=International Berlin-Frankfurt-Münster ; MA = mitoxantrone and cytarabine; N/A=Not applicable; NOPHO = Nordic Society of Paediatric Haematology and Oncology Children's Research Hospital Consortium; RIC = reduced intensity conditioning; R/R-relapse/refractory

The 8 studies using monotherapy dosing regimens for GO ranged between GO 1.8 and GO 9 mg/m^2 with different intervals for repeat dosing, and included 3 compassionate use trials with a median age of children in these studies at 2.1, 5.5 and 8.9 years. Five studies focused on children receiving a combination of GO with chemotherapy; 3 were in the previously untreated setting, one in relapsed patients and another in the setting of haematopoietic stem cell transplant. The most frequent AEs reported were represented by myelosuppression, infection, constitutional symptoms and liver enzyme alteration.

2.6.1. Discussion on clinical efficacy

Design and conduct of clinical studies

ALFA trial

Inclusion and exclusion criteria are generally acceptable. The fact that only patients age 50-70 were included is not considered an issue in view of the proposed target population, as it reflects a representative majority of patients with de novo AML eligible to undergo intensive chemotherapy with curative intent. The issue which consequently needed to be addressed in this context is what the acceptable lower age cut-off is for which extrapolation of efficacy is considered acceptable, assuming similar exposure levels and disease similarity (see discussion below). Patients were not required to be CD33-positive, but expression of CD33 will be a requirement for the intended indication considering the mechanism of action of Mylotarg. The chosen treatment schedule of induction/ consolidation therapy as

add on to Mylotarg is considered standard of care for patients with de novo AML. The issue around the response assessment being performed by the unblinded investigators involved in the treatment of the patients has been addressed by the introduction of a BIRC, though defined in retrospectively.

The sample size is acceptable. The methods of analyses are also acceptable. However, for the primary endpoint the sensitivity analysis using the BIRC and most mature data set (ie April 2013) is the most appropriate and will therefore be the main focus.

Major protocol deviations were recorded for almost 50% of all patients (139/280). A total of 22 patients (8%) had a major protocol deviation around lack of eligibility, slightly more in the control arm (7 patients GO arm; 15 control arm). Overall, this posed the question to whether trial documentation was according to protocol and raises concerns around the robustness of the primary data. However, the additional sensitivity analyses confirmed the robustness of the results observed.

Efficacy data and additional analyses

Demographics and baseline characteristics were as expected for a de novo AML population age 50-70. There were no major discrepancies between the two groups apart from slightly more male (54.8% vs 44.1%) and patients older than 60, respectively 65 years of age in the GO arm (71.9% vs 61.8%; 37% vs 29.4%). This can be considered to be in its favour, appreciating that elderly patients usually perform worse. The majority had a PS of 0-1 (87.8%) and favourable/intermediate risk AML as per NCCN/ELN criteria as well as according to cytogenetics. Genotyping revealed more patients with unfavourable genetics, but with almost 50% of data missing or unknown, which limits its usefulness. Patient numbers were equally balanced during induction. More patients in the control arm needed a second course of induction (34 vs 19). This could be a sign of early efficacy or due to a higher number of patients with resistant disease in the control arm (discontinuation due to resistant disease in the control arm 26 vs 17 patients in GO). Patients receiving one, respectively two courses of consolidation were also equally balanced across arms. The different follow-up interventions used for patients (with at least 1 follow-up), particularly consolidation regimens for those in CR/CRp were equally distributed between the control and investigational arm either (all GO 27.3% vs control 28%; consolidation GO 19.1% vs control 18%), with no impact expected on EFS.

The primary efficacy analysis, investigators review and data cut of August 2011 showed an EFS difference of 7.8 months (HR 0.562; 95% CI: 0.415-0.762; 2-sided p=0.0002), corresponding to a 44% reduction in the risk of an event for patients in the GO arm. These results were consistent when stratified by NCCN or ELN classification. Additional sensitivity analyses, derived by using BIRC data, different censoring and more mature data cut off dates were consistent with the primary analysis, but less compelling. Using the most conservative analysis performed, (BIRC and most mature data set - April 2013), the HR was 0.705 (95% CI [0.536-0.928]; p= 0.0161), when stratified according to ELN. Additional sensitivity analyses confirmed the robustness of the primary endpoint, including analysis using data from the BIRC, with latest data cut off, salvage therapy classified as induction failure and the date of randomisation as date of induction failure event (according to Guideline EMA/CHMP/205/95 Rev.5).

Regarding the secondary endpoints, RFS confirmed a statistical significant difference in favour of the GO arm (HR 0.656, 95% CI [0.466, 0.922], p= 0.02480) stratified for ELN risk category. Response rate, including early response (CR/CRp at Day 15 as part of a post hoc analyses), and most importantly OS did not show any statistically significant difference. Survival data are available up to April 20130 and can be considered mature.

Overall, the efficacy results from the pivotal trial supported by the meta-analysis showed that Mylotarg added to induction chemotherapy improved EFS through prolongation of remission following initial

chemotherapy, rather than increasing the number of patients who achieve complete remission, as confirmed by the absence of a statistically significant difference in the ORR. Subgroup analyses of EFS indicated a more encouraging treatment effect with the Mylotarg combination in patients with favourable/intermediate risk cytogenetics (HR 0.591; 95% CI (0.407, 0.857), p= 0.0047; vs unfavourable HR 1.08). Reflecting on the differences observed for the different risk groups, it can be hypothesised that patients with adverse cytogenetics who receive fractioned low dose of Mylotarg seem to exhibit less deep responses, translating into shorter, not statistically significant periods of remission. It can be argued that, based on distinct biological characteristics, with the pathophysiological route causes yet to be fully elucidated, the hard-to-treat poor cytogenetic patient group is less susceptible to Mylotarg based induction chemotherapy. Adequate wording has been added in section 4.4 of the SmPC to reflect on the need to individually consider the benefit/risk profile in patients, particularly with adverse cytogenetics, once results become available.

The results of the IPD meta-analysis showed a statistically significant difference in terms of the secondary endpoint of EFS, for the subgroup of adverse risk cytogenetics. This is in addition to RFS, not statistically significant, but showing a supportive trend of benefit in patients with adverse cytogenetics. While it is agreed that the total numbers of the meta-analysis which include all risk groups, are supportive of a survival benefit, the differences in the sub-groups are evident in the meta-analysis and broadly consistent with the pivotal trial, showing no benefit of Mylotarg treatment in patients with adverse risk cytogenetics (OR of 1), as opposed to the favourable/ intermediate risk group.

Additionally, it must be emphasized that APL is not included in the pivotal trial and this has been reflected in the indication.

AML is a heterogeneous disease, stratified into different disease risk groups. There are also additional established independent risk factors, such as age, which is a poor risk factor in adults, associated with higher rates of poor risk cytogenetics. Efficacy for the intended indication for patients less than 50 years of age is based on full extrapolation, as the pivotal ALFA trial only recruited patients age 50-70 years. It is agreed that Mylotarg is considered to have a positive benefit/risk in all patients with newly diagnosed CD33-positive AML age 18 and above. This is based on disease similarity, acknowledging that any associated (known or unknown) biological differences due to age do not alter the assumed clinically meaningful benefits for this patient group.

No clinical impact of the shift in the AAS could be observed based on the comparability exercise. No clear efficacy differences were observed in the data submitted (see also 2.2.4 Discussion on chemical, pharmaceutical and biological aspects).

Assessment of paediatric data on clinical efficacy

During the initial evaluation, the CHMP raised a concern about the indication needing to be further discussed, with reference to the lower age cut-off for which extrapolation of efficacy is considered acceptable based on disease similarity. It is now difficult to acknowledge to why one would consider a treatment benefit in a patient with AML treated with Mylotarg in combination with 3+7 induction chemotherapy at the age of 18 years established, but not at the age of 17 years. The CHMP acknowledged that there are differences in the frequency of AML subtypes and common molecular aberrations between adults and children in general. In a large survey evaluating the age effect on AML biology and response to therapy among paediatric patients, differences were seen in infants, but a distinct biology in TYA patients could not be identified (36). In addition, prospective studies that included both paediatric and adult patients did not report any outcome differences for the group of TYA patients ((37) (38)). This is reflected in clinical practice, as indeed TYA patients with de novo AML may be treated using adult protocols, such

as 3+7 induction chemotherapy (39). Overall, based on disease similarity, it can be concluded that TYA patients age 15-17 years with CD33-positive AML derive the same benefits than adults from the adult 3+7 induction chemotherapy in combination with Mylotarg. Furthermore, the PK modelling results confirmed the PK similarity between adults and adolescents, supporting the assumption of similar exposure between adults and adolescents using BSA based dosing (see discussion on clinical pharmacology). This is further supported by the efficacy data from the meta-analyses, which is used as supportive evidence to bridge efficacy assumptions to patients less than 50 years of age. The subgroup of TYA patients (15-29 years of age, n=132), showed efficacy trends similar to the overall population, if not slightly better. Regarding safety it is noted that the 30 and 60-day mortality for TYA patient (15-29 years of age) in the Mylotarg arm was none. Despite the limited number, all of this is reassuring, as it confirms what is already known, younger TYA patients tend to tolerate intensive chemotherapy better than older patients.

2.6.2. Conclusions on the clinical efficacy

Overall the robustness of the EFS endpoint and its clinical benefit for patients age 15 and above with de novo CD33-positive AML, excluding APL, is considered established. This was supported by improvements on relevant secondary endpoints.

2.7. Clinical safety

The summary of clinical safety comprised data from a total of 19 studies conducted in patients with AML; 11 studies of GO monotherapy conducted by Wyeth Pharmaceuticals Inc, a subsidiary of the applicant and 9 studies that support the use of Mylotarg in combination with DNR and AraC, of which the 5 IPD meta-analysis studies were conducted in patients with de novo AML.

Patient exposure

A total of 2,747 patients received GO either as monotherapy or in combination chemotherapy:

1) GO as monotherapy as part of the legacy Wyeth-sponsored studies: 953 patients with AML (Studies 201, 202, 203, 101, 102, 103, 100374, 100847, 100863 and 205 [monotherapy part])

2) GO in combination as part of the legacy Wyeth-sponsored studies:

- 38 patients received GO in combination with AraC (Study 205)
- 71 patients received GO in combination with DNR and AraC (Study 206)

3) GO in combination with chemotherapy in patients with de novo AML as part of academia sponsored studies: total of 1,659 patients with

- 131 patients received GO in combination with DNR and AraC in Study ALFA-0701 age 50-70 years
- 1,528 patients were randomized to GO in combination with chemotherapy in induction in other IIT studies (Studies GOELAMS AML2006IR, MRC AML15, NCRI AML16, SWOG S0106), ranging from age 15 to ≥70.

Study ALFA0701

The duration of study treatment is summarized for each treatment arm in Table 33.

Table 33 Duration of Study Treatment (As-Treated Population-ALFA0701 study)

Duration (weeks)		GO + Daunorubicin + Cytarabine (N=131)	Daunorubicin + Cytarabine (N=137)
All patients			
Overall duration[a]	N	131	137
	Median (range)	12.14 (0.6, 22.1)	11.71 (0.3, 19.0)
Induction duration[b]	N	131	137
	Median (range)	6.00 (0.6, 13.9)	5.86 (0.3, 15.1)
Consolidation duration[c]	N	97	97
	Median (range)	6.57 (0.6, 13.6)	6.43 (0.6, 10.1)
CR/CRp patients (per investigator)			
Overall duration[a]	N	108	101
	Median (range)	12.71 (1.0, 22.1)	12.43 (0.7, 19.0)
Induction duration[b]	N	108	101
	Median (range)	6.29 (1.0, 13.9)	6.14 (0.7, 15.1)
Consolidation duration[c]	N	97	94
	Median (range)	6.57 (0.6, 13.6)	6.50 (0.7, 10.1)
NonCR/CRp patients (per investigator)			
Induction duration[b]	N	23	36
	Median (range)	1.14 (0.6, 6.6)	2.57 (0.3, 9.7)

Abbreviations: CR=complete remission; CRp=complete remission with incomplete platelet recovery; GO=gemtuzumab ozogamicin; HSCT=hematopoietic stem cell transplant; N=number of patients.
a. Overall duration defined as duration from first dose to last dose of any study treatment (excluding HSCT).
b. Induction duration defined as duration from first dose of induction to the start of consolidation or to last dose of induction +1 if there was no consolidation treatment, including any re-induction or salvage treatment.
c. Consolidation duration defined as duration from first dose of consolidation 1 to the last dose of consolidation 2.

Table 34 Dose Administration of Study Treatments - GO (As-Treated Population-ALFA0701 study)

Phase		GO + Daunorubicin + Cytarabine (N=131) n (%)
Induction	Patients receiving all doses	123 (93.9)
	Patients with any dose interruptions (any dose day)	7 (5.3)
	Patients receiving Day 1 dose	129 (98.5)
	Patients with dose interruption Day 1	2 (1.5)
	Administered Day 1 dose >5 mg	6 (4.6)
	Patients receiving Day 4 dose	127 (96.9)
	Patients with dose interruption Day 4	5 (3.8)
	Administered Day 4 dose >5 mg	6 (4.6)
	Patients receiving Day 7 dose	125 (95.4)
	Patients with dose interruption Day 7	3 (2.3)
	Administered Day 7 dose >5 mg	6 (4.6)
Consolidation 1	Patients receiving dose	91 (69.5)
	Administered dose >5 mg	1 (0.8)
Consolidation 2	Patients receiving dose	64 (48.9)
	Administered dose >5 mg	1 (0.8)

Table 35 Dose Administration of Chemotherapy – Daunorubicin + Cytarabine (As-Treated Population-ALFA0701 study)

Phase		GO + Daunorubicin + Cytarabine (N=131) n (%)		Daunorubicin + Cytarabine (N=137) n (%)	
		Daunorubicin	Cytarabine	Daunorubicin	Cytarabine
Induction	Received treatment	131 (100)	131 (100)	135 (98.5)	137 (100)
	Received expected prescribed dose[a]	125 (95.4)	126 (96.2)	125 (92.6)	126 (92.0)
Consolidation 1	Received treatment	96 (73.3)	97 (74.0)	97 (70.8)	97 (70.8)
	Received expected prescribed dose[a]	92 (95.8)	84 (86.6)	95 (97.9)	88 (90.7)
Consolidation 2	Received treatment	82 (62.6)	82 (62.6)	89 (65.0)	89 (65.0)
	Received expected prescribed dose[a]	79 (96.3)	72 (87.8)	85 (95.5)	77 (86.5)

Adverse events

Table 36 Treatment Emergent Adverse Events- as Treated Population (ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=131)		Daunorubicin + Cytarabine (N=137)		Total (N=268)	
	ALL CAUSALITY ADVERSE EVENTS	RELATED ADVERSE EVENTS	ALL CAUSALITY ADVERSE EVENTS	RELATED ADVERSE EVENTS	ALL CAUSALITY ADVERSE EVENTS	RELATED ADVERSE EVENTS
Number (%) of Subjects						
Subjects with adverse events	129 (98.5)	129 (98.5)	129 (94.2)	126 (92.0)	258 (96.3)	255 (95.1)
Subjects with serious adverse events	70 (53.4)	62 (47.3)	55 (40.1)	35 (25.5)	125 (46.6)	97 (36.2)
Subjects with grade 3 or 4 or severe infection adverse events	114 (87.0)	114 (87.0)	113 (82.5)	104 (75.9)	227 (84.7)	218 (81.3)
Subjects fatal events [1]	8 (6.1)	7 (5.3)	7 (5.1)	3 (2.2)	15 (5.6)	10 (3.7)
Subjects who permanently discontinued study treatment due to AE	41 (31.3)	38 (29.0)	10 (7.3)	4 (2.9)	51 (19.0)	42 (15.7)

Notes: Percentages are based on the number of subjects in the As Treated Population.

[1] Subjects with fatal events included subjects with grade 5 adverse events and fatal infections. Multiple occurrences of the same adverse event in a subject at the Preferred Term level or System Organ Class level are counted as one AE per treatment in each row.

Per the additional data capture, significant infections, haemorrhage, veno-occlusive disease, and other AEs which led to permanent discontinuation of study drugs were collected and reported. As such, only SAE reported on the eCRF meeting these criteria are tabulated in this summary.

Treatment Related was defined as a reasonable possibility the AE is related to any of the study treatments received.

MedDRA v18.0 coding dictionary applied.

Table 37 Predefined Treatment-Emergent Adverse Events (All Causalities) by Maximum CTCAE Grade (As-Treated Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=131) n (%)	Daunorubicin + Cytarabine (N=137) n (%)	Total (N=268) n (%)
CHV CRF[a] Predefined CRF Term			
Skin toxicity, total	14 (10.7)	23 (16.8)	37 (13.8)
Grade 3	14 (10.7)	23 (16.8)	37 (13.8)
Grade 4	0	0	0
Muscosal toxicity, total	21 (16.0)	9 (6.6)	30 (11.2)
Grade 3	16 (12.2)	8 (5.8)	24 (9.0)
Grade 4	5 (3.8)	1 (0.7)	6 (2.2)
Pain, total	19 (14.5)	5 (3.6)	24 (9.0)
Grade 3	16 (12.2)	5 (3.6)	21 (7.8)
Grade 4	3 (2.3)	0	3 (1.1)
Nausea, vomiting, diarrhea, total	22 (16.8)	14 (10.2)	36 (13.4)
Grade 3	18 (13.7)	14 (10.2)	32 (11.9)
Grade 4	4 (3.1)	0	4 (1.5)
Constipation, total	1 (0.8)	1 (0.7)	2 (0.7)
Grade 3	0	1 (0.7)	1 (0.4)
Grade 4	1 (0.8)	0	1 (0.4)
Pulmonary toxicity, total	17 (13.0)	19 (13.9)	36 (13.4)
Grade 3	10 (7.6)	14 (10.2)	24 (9.0)
Grade 4	7 (5.3)	5 (3.6)	12 (4.5)
Cardiac rhythm disorder, total	5 (3.8)	4 (2.9)	9 (3.4)
Grade 3	4 (3.1)	4 (2.9)	8 (3.0)
Grade 4	1 (0.8)	0	1 (0.4)
Other cardiac toxicity, total	6 (4.6)	5 (3.6)	11 (4.1)
Grade 3	4 (3.1)	4 (2.9)	8 (3.0)
Grade 4	2 (1.5)	1 (0.7)	3 (1.1)
Central neurological toxicity, total	8 (6.1)	4 (2.9)	12 (4.5)
Grade 3	7 (5.3)	2 (1.5)	9 (3.4)
Grade 4	1 (0.8)	2 (1.5)	3 (1.1)
Peripheral neurological toxicity, total	4 (3.1)	2 (1.5)	6 (2.2)
Grade 3	2 (1.5)	2 (1.5)	4 (1.5)
Grade 4	2 (1.5)	0	2 (0.7)
Retrospective Data Collection[b]			
Infections and infestations Severe (≥Grade 3)	102 (77.9)	106 (77.4)	208 (77.6)
HAEMORRHAGE All Grades (≥Grade 1) Cluster TEAEs , Total [c]	118 (90.1)	107 (78.1)	225 (84.0)
Grade 3	23 (17.6)	12 (8.8)	35 (13.1)
Grade 4	4 (3.1)	0	4 (1.5)
Grade 5	3 (2.3)	1 (0.7)	4 (1.5)
VOD All Grades (≥Grade 1) Cluster TEAEs , Total [c,d]	6 (4.6)	2 (1.5)	8 (3.0)
Grade 3	2 (1.5)	1 (0.7)	3 (1.1)
Grade 4	1 (0.8)	1 (0.7)	2 (0.7)
Grade 5	2 (1.5)	0	2 (0.7)

Source: Table 14.3.1.2.1.2, Table 14.3.1.2.4.2, Table 14.3.1.2.5.5, Table 14.3.1.3.1, Listing 16.2.7.1.1.1
Abbreviations: AE=adverse event; CHV=Centre Hospitalier de Versailles; CRF=case report form; GO=gemtuzumab ozogamicin; MedDRA=Medical Dictionary for Regulatory Activities; N=number of patients; n=number of patients; NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse

Table 38 Summary of GO Treatment-Emergent Adverse Events by Phase (ALFA0701 study)

	Induction N=131	2nd Induction Salvage* N=24	Consol 1 N=97	Consol 2 N=82	Follow-up N=131
	n (%)	n (%)	n (%)	n (%)	n (%)
Infections	61 (46.6)	12 (50.0)	52 (53.6)	40 (48.8)	0
Haemorrhage	109 (83.2)	15 (62.5)	58 (59.8)	51 (62.2)	3 (2.3)
VOD	4 (3.1)	0	0	0	3 (2.3)
* 2nd Induction / Salvage treatment did not contain GO as described in the ALFA-0701 protocol					

TEAEs for Patients in CR/CRp

Table 39 displays the TEAEs for all patients and responder patients (patients in CR/CRp) in the ALFA-0701 trial.

Table 39 Predefined Treatment-Emergent Adverse Events (All Causalities) by Maximum CTCAE Grade (As-Treated Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine N n (%)	Daunorubicin + Cytarabine N n (%)
Retrospective Data Collection		
All Patients	131	137
Infections and infestations Severe (Grade ≥3)	102 (77.9)	106 (77.4)
Grade 3/4	100 (76.3)	102 (74.4)
Grade 5	2 (1.5)	4 (2.9)
HAEMORRHAGE All Grades (Grade ≥1) Cluster TEAEs, Total	118 (90.1)	107 (78.1)
Grade 3	23 (17.6)	12 (8.8)
Grade 4	4 (3.1)	0
Grade 5	3 (2.3)	1 (0.7)
VOD All Grades (Grade ≥1) Cluster TEAEs, Total	6 (4.6)	2 (1.5)
Grade 3	2 (1.5)	1 (0.7)
Grade 4	1 (0.8)	1 (0.7)
Grade 5	2 (1.5)	0
Responder Patients (Patients in CR/CRp)	108	101
Infections and infestations Severe (Grade ≥3)	88 (81.5)	85 (84.2)
Grade 3/4	88 (81.5)	83 (82.2)
Grade 5	0	2 (2)
HAEMORRHAGE All Grades (Grade ≥1) Cluster TEAEs, Total	98 (90.7)	82 (81.2)
Grade 3	21 (19.4)	10 (9.9)
Grade 4	4 (3.7)	0
Grade 5	1 (0.9)	0
VOD All Grades (Grade ≥1) Cluster TEAEs, Total	5 (4.6)	2 (2)
Grade 3	2 (1.8)	1 (1)
Grade 4	1 (0.9)	1 (1)
Grade 5	1 (0.9)	0
Responder patients refers to Patients in CR/CRp		

TEAEs by ELN Subgroups

A summary of predefined TEAEs for the Overall AT population and by ELN risk categories is presented in Table 40.

Table 40 Predefined Treatment-Emergent Adverse Events (All Causalities) by Maximum CTCAE Grade (As-Treated Population) (ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N) n (%)	Daunorubicin + Cytarabine (N) n (%)
Retrospective Data Collection		
Overall Population	(N = 131)	(N = 137)
Infections and infestations Severe (Grade ≥3)	102 (77.9)	106 (77.4)
Grade 3/4	100 (76.3)	102 (74.4)
Grade 5	2 (1.5)	4 (2.9)
HAEMORRHAGE All Grades (Grade ≥1) Cluster TEAEs, Total	118 (90.1)	107 (78.1)
Grade 3	23 (17.6)	12 (8.8)
Grade 4	4 (3.1)	0
Grade 5	3 (2.3)	1 (0.7)
VOD All Grades (Grade ≥1) Cluster TEAEs, Total	6 (4.6)	2 (1.5)
Grade 3	2 (1.5)	1 (0.7)
Grade 4	1 (0.8)	1 (0.7)
Grade 5	2 (1.5)	0
ELN Risk: Favourable/Intermediate	(N = 84)	(N = 92)
Infections and infestations Severe (Grade ≥3)	68 (80.9)	71 (77.2)
Grade 3/4	67 (79.8)	69 (75.0)
Grade 5	1 (1.2)	2 (2.2)
HAEMORRHAGE All Grades (Grade ≥1) Cluster TEAEs, Total	79 (94.0)	75 (81.5)
Grade 3	18 (21.4)	7 (7.6)
Grade 4	4 (4.8)	0
Grade 3/4	22 (26.2)	7 (7.6)
Grade 5	0	0
VOD All Grades (Grade ≥1) Cluster TEAEs, Total	3 (3.6)	1 (1.1)
Grade 3	2 (2.4)	1 (1.1)
Grade 4	0	0
Grade 3/4	2 (2.4)	1 (1.1)
Grade 5	0	0
ELN Risk: Poor/Adverse	(N = 35)	(N = 36)
Infections and infestations Severe (Grade ≥3)	26 (74.3)	28 (77.8)
Grade 3/4	25 (71.4)	26 (72.2)
Grade 5	1 (2.9)	2 (5.6)
HAEMORRHAGE All Grades (Grade ≥1) Cluster TEAEs, Total	30 (85.7)	24 (66.7)
Grade 3	3 (8.6)	2 (5.6)
Grade 4	0	0
Grade 3/4	3 (8.6)	2 (5.6)
Grade 5	1 (2.9)	1 (2.8)

	GO + Daunorubicin + Cytarabine (N) n (%)	Daunorubicin + Cytarabine (N) n (%)
VOD All Grades (Grade ≥1) Cluster TEAEs, Total	3 (8.6)	1 (2.8)
Grade 3	0	0
Grade 4	1 (2.9)	1 (2.8)
Grade 3/4	1 (2.9)	1 (2.8)
Grade 5	2 (5.7)	0

TEAEs are defined as AEs that commence on or after the first dose date but within 28 days of last dose.
Patients are counted only once per treatment in each row. Maximum CTCAE grades are displayed.
The System Organ Class (SOC) displayed is the SOC associated with the Adverse Reaction.
MedDRA (v18.0) coding dictionary is applied. Adverse events graded according to the NCI CTCAE, version 3.0.
Adverse reactions that are upper case are grouped preferred terms.
Per the additional data capture data entry guidelines, fatal significant infections were marked as permanently withdrawn from the study. Only significant infections were collected and all were considered severe (≥Grade 3).
CTCAE = Common Terminology Criteria for Adverse Events; GO=gemtuzumab ozogamicin; ; MedDRA=Medical Dictionary for Regulatory Activities; N=number of patients; n=number of patients; NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events; SOC=System Organ Class; TEAE=treatment emergent adverse event; v=version; VOD=Veno-occlusive disease

IPD meta-analysis – AEs Grade 3/4

Table 41 displays the proportion of patients who experienced at least 1 case of Grade 3-4 adverse event of special interest in each safety period.

Table 41 Patients with at Least One Grade 3-4 Adverse Event of Special Interest in Each Safety Period from the Prelisted and Other Safety Sources

	GO Patients		No GO Patients		Odds Ratio (95% CI)	p-Value
	N	%	N	%		
Prelisted and Other Safety Sources						
All Safety Period	1663	1251 (75.2)	1668	1199 (71.9)	1.21 (1.03, 1.42)	0.02
Tt Safety Period	1663	1235 (74.3)	1668	1177 (70.6)	1.23 (1.05, 1.45)	0.01
Induct Safety Period	1663	1092 (65.7)	1668	1026 (61.5)	1.22 (1.05, 1.41)	0.009

Abbreviations: AE=adverse event, CI=Confidence interval, N=Number, n=Number affected, No GO=Treatment arm(s) without GO.
All Safety Period: All toxicities reported at any time.
Tt Safety Period: All toxicities reported at any time, excluding the extra reporting period for ALFA-0701 and OELAMS AML2006IR trials.
Induct Safety Period: Toxicities reported during the induction courses of treatment including second induction or salvage treatments.

Treatment related AEs (ADRs)

Table 42 presents the ADRs in the primary pooled GO monotherapy studies (Studies 201/202/203).

Table 42 Summary of ADRs by MedDRA System Organ Class and Maximum CTCAE Grade by Descending Order of Frequency (All Causalities, All Cycles, Grade 3 or higher) – Studies 201/202/203

System Organ Class	ADR Terms	Frequency Category	All Grades n (%)	Grades 3-4 n (%)
Blood And Lymphatic System Disorders	Thrombocytopenia[a]	Very Common	134(48.38)	133(48.01)
	Neutropenia[b]	Very Common	84(30.32)	81(29.24)
	Anaemia[c]	Very Common	75(27.08)	67(24.19)
	Leukopenia[d]	Very Common	74(26.71)	74(26.71)

System Organ Class	ADR Terms	Frequency Category	All Grades n (%)	Grades 3-4 n (%)
	Febrile neutropenia	Very Common	53(19.13)	32(11.55)
	Pancytopenia[e]	Common	14(5.05)	12(4.33)
	Lymphopenia[f]	Common	10(3.61)	9(3.25)
Cardiac Disorders	Tachycardia[g]	Very Common	36(13)	12(4.33)
Gastrointestinal Disorders	Nausea	Very Common	197(71.12)	109(39.35)
	Vomiting	Very Common	168(60.65)	93(33.57)
	Stomatitis[h]	Very Common	100(36.1)	34(12.27)
	Diarrhoea	Very Common	94(33.94)	41(14.8)
	Abdominal pain[i]	Very Common	92(33.21)	20(7.22)
	Constipation	Very Common	70(25.27)	14(5.05)
	Dyspepsia	Common	24(8.66)	3(1.08)
	Ascites	Common	8(2.89)	1(0.36)
	Oesophagitis	Common	5(1.81)	2(0.72)
General Disorders And Administration Site Condition	Pyrexia[j]	Very Common	229(82.67)	145(52.35)
	Chills	Very Common	188(67.87)	48(17.33)
	Fatigue[k]	Very Common	114(41.16)	31(11.19)
	Oedema[l]	Very Common	59(21.3)	9(3.25)
	Multi-organ failure	Common	6(2.17)	5(1.81)
Hepatobiliary Disorders	Transaminases increased[m]	Very Common	68(24.55)	52(18.77)
	Hyperbilirubinaemia[n]	Very Common	36(13)	29(10.47)
	Venoocclusive liver disease[o]	Common	8(2.89)	6(2.17)
	Hepatomegaly	Common	7(2.53)	2(0.72)
	Hepatic function abnormal[p]	Common	7(2.53)	4(1.44)
	Jaundice	Common	6(2.17)	3(1.08)
	Gamma-glutamyltransferase increased	Common	5(1.81)	2(0.72)
	Budd-Chiari syndrome	Uncommon	1(0.36)	1(0.36)
	Hepatic failure	Uncommon	1(0.36)	1(0.36)
Immune System Disorder	Infusion related reaction[q]	Common	21(7.58)	10(3.61)
Infections And Infestations	Infection[r]	Very Common	189(68.23)	112(40.43)
Investigations	Blood lactate dehydrogenase increased	Very Common	46(16.61)	20(7.22)
	Blood alkaline phosphatase increased	Common	24(8.66)	17(6.14)
Metabolism And Nutrition	Decreased appetite	Very Common	75(27.08)	17(6.14)
	Hyperglycaemia[s]	Very Common	31(11.19)	19(6.86)
	Tumour lysis syndrome	Common	7(2.53)	5(1.81)
Nervous System	Headache	Very Common	106(38.27)	34(12.27)
Respiratory, Thoracic And Mediastinal Disorder	Dyspnoea[t]	Very Common	76(27.44)	35(12.64)
Skin And Subcutaneous Tissue Disorders	Rash[u]	Very Common	55(19.86)	16(5.78)
	Erythema[v]	Common	26(9.39)	6(2.17)
	Pruritus	Common	15(5.42)	1(0.36)
Vascular	Haemorrhage[w]	Very Common	186(67.15)	76(27.44)
	Hypotension[x]	Very Common	56(20.22)	41(14.8)
	Hypertension[y]	Very Common	48(17.33)	29(10.47)

Abbreviation: ADR=adverse drug reaction; CTCAE=Common Terminology Criteria for Adverse Events; SOC=system organ class
TEAEs are defined as AEs that commence on or after the first dose date but within 28 days of last dose. Patients are counted only once per treatment in each row. Maximum CTCAE grades are displayed. The SOC displayed is the SOC associated with the Adverse Reaction.
MedDRA (v18.0) coding dictionary is applied. Maximum CTCAE grades are displayed. Adverse events are graded according to NCI CTCAE v1.CTCAE v1 includes Grades 1-4.
a: Thrombocytopenia includes the following reported PTs: Platelet count decreased and Thrombocytopenia
b: Neutropenia includes the following reported PTs: Neutropenia, Granulocytopenia, and Neutrophil count decreased.
c: Anaemia includes the following reported PTs: Anaemia and Haemoglobin decreased.
d: Leukopenia includes the following reported PTs: Leukopenia and White blood cell count decreased.
e: Pancytopenia includes the following reported PTs: Pancytopenia and Bone marrow failure.
f: Lymphopenia includes the following reported PTs: Lymphopenia and Lymphocyte count decreased.
g: Tachycardia includes the following reported PTs: Tachycardia, Sinus tachycardia, Heart rate increased, and Supraventricular tachycardia
h: Stomatitis includes the following reported PTs: Mucosal inflammation, Oropharyngeal pain, Stomatitis, Mouth ulceration, Oral pain, Oral mucosal blistering, Aphthous stomatitis, Tongue ulceration, Glossodynia, Oral mucosal erythema, Glossitis, and Oropharyngeal blistering
i: Abdominal pain includes the following reported PTs: Abdominal pain, Abdominal pain lower, Abdominal pain upper, Abdominal discomfort, and

System Organ Class	ADR Terms	Frequency Category	All Grades n (%)	Grades 3-4 n (%)
Abdominal tenderness j: Pyrexia includes the following reported PTs: Pyrexia, Body temperature increased, and Hyperthermia k: Fatigue includes the following reported PTs: Fatigue, Asthenia, Lethargy, and Malaise. l: Odema includes the following reported PTs: Oedema peripheral, Oedema, Face oedema, Generalised oedema, Swelling face, and Periorbital oedema m: Transaminases increased includes the following reported PTs: Transaminases increased, Hepatocellular injury, Alanine aminotransferase increased, Aspartate aminotransferase increased, and Hepatic enzyme increased n: Hyperbilirubinaemia includes the following reported PTs: Blood bilirubin increased and Hyperbilirubinaemia o: Venoocclusive liver disease includes the following reported PTs: Venoocclusive disease and Venoocclusive liver disease p: Hepatic function abnormal includes the following reported PTs: Liver function test abnormal and Hepatic function abnormal q: Infusion related reaction includes the following reported PTs: Infusion related reaction, Urticaria, Hypersensitivity, Bronchospasm, Drug hypersensitivity, and Injection site urticaria r: Infection includes the following reported PTs: Oral herpes, Pneumonia, Sepsis, Device related infection, Bacteraemia, Oral candidiasis, Cellulitis, Herpes simplex, Sinusitis, Herpes virus infection, Septic shock, Staphylococcal sepsis, Folliculitis, Catheter site infection, Conjunctivitis, Infection, Tooth abscess, Bronchopulmonary aspergillosis, Candida infection, Enterococcal sepsis, Gingivitis, Staphylococcal bacteraemia, Abscess limb, Bacterial sepsis, Catheter site cellulitis, Escherichia bacteraemia, Oral fungal infection, Pneumonia fungal, Streptococcal sepsis, Urinary tract infection, Anal abscess, Bacterial infection, Cellulitis orbital, Clostridium difficile colitis, Enterococcal bacteraemia, Escherichia sepsis, Fungal infection, Furuncle, Genital herpes, Lung infection, Neutropenic sepsis, Streptococcal bacteraemia, Subcutaneous abscess, Upper respiratory tract infection, Abdominal abscess, Bronchopneumonia, Candida sepsis, Clostridium difficile infection, Corynebacterium infection, Corynebacterium sepsis, Cytomegalovirus enteritis, Cytomegalovirus viraemia, Diverticulitis, Enterobacter sepsis, Enterococcal infection, Epiglottitis, Escherichia infection, Eye infection bacterial, Febrile infection, Genital infection bacterial, Hepatitis B, Herpes dermatitis, Herpes zoster, Herpes zoster disseminated, Hordeolum, Klebsiella sepsis, Lobar pneumonia, Localised infection, Lower respiratory tract infection, Neutropenic infection, Orchitis, Oropharyngeal candidiasis, Osteomyelitis, Parotitis, Periodontitis, Perirectal abscess, Pharyngitis, Phlebitis infective, Pneumonia klebsiella, Pneumonia pneumococcal, Proctitis herpes, Pseudomonal sepsis, Pseudomonas infection, Respiratory moniliasis, Respiratory syncytial virus infection, Sepsis syndrome, Sinusitis fungal, Skin bacterial infection, Soft tissue infection, Staphylococcal infection, Streptococcal infection, Systemic candida, Tinea barbae, Tooth infection, Toxic shock syndrome, Urinary tract infection staphylococcal s: Hyperglycemia includes the following reported PTs: Hyperglycaemia and Blood glucose increased t: Dyspnoea includes the following reported PTs: Dyspnoea and Dyspnoea exertional. u: Rash includes the following reported PTs: Rash, Dermatitis, Dermatitis allergic, Dermatitis bullous, Dermatitis contact, Dermatitis exfoliative, Drug eruption, Pruritus allergic, Rash erythematous, Rash macular, Rash maculo papular, Rash papular, Rash Pruritic, and Rash Vesicular v: Erythema includes the following reported PTs: Catheter site erythema, erythema and infusion site erythema. w: Haemorrhage includes the following reported PTs: Epistaxis, Petechiae, Gingival bleeding, Haematuria, Mouth haemorrhage, Ecchymosis, Vaginal haemorrhage, Haemoptysis, Haematemesis, Melaena, Haematoma, Contusion, Catheter site haemorrhage, Occult blood positive, Cerebral haemorrhage, Disseminated intravascular coagulat, Purpura, Rectal haemorrhage, Gastrointestinal haemorrhage, Post procedural haemorrhage, Blood urine present, Haematochezia, Haemorrhage, Haemorrhoidal haemorrhage, Diarrhoea haemorrhagic, Eye haemorrhage, Menorrhagia, Retinal haemorrhage, Tongue haemorrhage, Catheter site haematoma, Conjunctival haemorrhage, Metrorrhagia, Scleral haemorrhage, Subdural haematoma, Anal haemorrhage, Ear haemorrhage, Eyelid haematoma, Increased tendency to bruise, Post procedural haematoma, Pulmonary alveolar haemorrhage, Traumatic haematoma, Blood blister, Central nervous system haemorrhage, Gastric haemorrhage, Haemarthrosis, Haemorrhage intracranial, Lip haemorrhage, Mallory-Weiss syndrome, Procedural haemorrhage, Puncture site haemorrhage, Retroperitoneal haematoma, Subarachnoid haemorrhage, Thrombocytopenic purpura, Tooth socket haemorrhage, Ulcer haemorrhage, Upper gastrointestinal haemorrhage, and Vessel puncture site bruise x: Hypotension includes the following reported PTs: Hypotension and Blood pressure decreased y: Hypertension includes the following reported PTs: Hypertension and Blood pressure increased				

Table 43 presents the ADRs in Study ALFA-0701.

Table 43 Summary of Selected ADRs by MedDRA System Organ Class and Maximum CTCAE Grade by Descending Order of Frequency (All Causalities, All Cycles, Grade 3 or higher) – (ALFA0701 study)

System organ class *Frequency* Preferred term	MYLOTARG + daunorubicin + cytarabine (N=131)		daunorubicin + cytarabine (N=137)	
	All grades %	Grade 3 / 4 %	All grades %	Grade 3 / 4 %
Infections and infestations				
Very common				
Infection*[a]	77.9	76.3	77.4	74.4
Vascular disorders				
Very common				
Haemorrhage*[b]	90.1	20.6	78.1	8.8
Hepatobiliary disorders				
Common				
Venoocclusive liver disease*[c]	4.6	2.3	1.5	1.5
Investigations * **				
Very common				
Haemoglobin decreased	100	86.2	100	89.7
Platelets decreased	100	100	100	100
White blood cells decreased	100	100	99.3	99.3
Lymphocytes (absolute) decreased	98.5	90.7	97.8	89.6
Neutrophils decreased	97.7	96.1	98.5	97.0
Hyperglycaemia	92.0	19.2	91.1	17.8
Aspartate aminotransferase (AST) increased	89.2	14.0	73.9	9.0
Prothrombin time increased	84.8	3.3	89.1	0
Activated partial thromboplastin time prolonged	80.0	6.4	57.5	5.5
Alkaline phosphatase increased	79.7	13.3	68.9	5.3
Alanine aminotransferase (ALT) increased	78.3	10.9	81.3	15.7
Blood bilirubin increased	51.6	7.1	50.8	3.8
Hyperuricaemia	32.5	2.6	28.5	0

- Abbreviations: N=number of patients; PT=preferred term.
- *Including fatal outcome.
- **Only selected safety data were collected in this study of newly diagnosed AML.
- ***Frequency is based on laboratory values **(**Grade per NCI CTCAE v4.03).
- [a.] Infection includes Sepsis and Bacteraemia (53.4%), Fungal infection (15.3%), Lower respiratory tract infection (5.3%), Bacterial infection (9.2%), Gastrointestinal infection (8.4%), Skin infection (2.3%), and Other infections (28.4%).
- [b.] Haemorrhage includes Central nervous system haemorrhage (3.1%), Upper gastrointestinal haemorrhage (33.6%), Lower gastrointestinal haemorrhage (17.6%), Subcutaneous haemorrhage (60.3%), Other haemorrhage (64.9%), and Epistaxis (62.6%).
- [c.] Venoocclusive liver disease includes the following reported PTs: Venoocclusive disease and Venoocclusive liver disease*.

Description of selected adverse reactions

Neutropenia

During the induction phase, 121 (92.4%) patients in the GO arm and 125 (91.2%) patients in the control arm had a documented neutrophil recovery to ANC of 500/mm^3, and 118 (90.1%) patients in the GO arm and 120 (87.6%) patients in the control arm had a neutrophil recovery to ANC of 1000/mm^3. Overall the majority of patients experienced severe myelosuppression with similar rates in both treatment arms and by treatment period. This is supported by the similar median number of RBC transfusions required per patient (14.0 in each arm). There was slightly increasing proportion of patients who recovered in the subsequent treatment phases post induction (all patients, threshold 1000/mm^3: consolidation 1 90.1%, consolidation 2 93.8%).

The IPD meta-analysis confirmed that the odds to experience severe neutropenia tends not to be different between GO and no GO treatment.

Thrombocytopenia

The median times to recovery of platelets at either threshold (50.00/mm^3 or 100.000/mm^3) were longer for patients in the GO arm than in the control arm (days 34 vs 29, respectively 35 vs 30 days). This is supported by the higher median number of platelet transfusions required in the GO arm compared to the control (median 23 vs 12). More patients tended to recover to the respective threshold following subsequent cycles (100.000/mm^3 GO arm: consolidation 1 73.2%, 85.4% consolidation 2), which could be explained by the less intensive Mylotarg schedule (ie only one dose per consolidation cycle). Data from the pooled monotherapy studies 201, 202, 203 showed that patients younger than 60 years had a higher probability to recover to the above platelet thresholds. Similar figures presented for the AFLA trial showed no such correlation.

Although the IPD meta-analysis could not show a statistically significant difference to experience an increase odds for severe thrombocytopenia in the GO arm, significant heterogeneity was observed between the single and fractioned 3 mg/m2 dosing schedule (p=0.00007), with persistent thrombocytopenia occurring in 16.3% of patients randomized to GO 3 × 3 mg/m² fractionated, compared to 1.5% of patients in the No GO arm (OR 6.23, 95% CI: 2.70-14.39, p=0.00002). This in general emphasises a potential risk increase due to the fractioned dosing schedule.

Haemorrhage

TEAEs of Haemorrhage of any grade occurred in the ALFA trial in the majority of patients in both treatment arms, with a higher rate in the GO arm: 118 (90.1%) patients in the GO arm and 107 (78.1%) patients in the control arm. This included Grade ≥ 3: 22.9% GO arm vs 9.5% control; serious events 8.4% vs 1.5% in control; unresolved 13.7% vs 7.3% and death GO 2.3% vs 0.7%.

Treatment with GO resulted in a delay in recovery of thrombocytopenia as well as higher percentages of persistent thrombocytopenia and an increased incidence of bleeding. The IPD meta-analysis showed that the odds to experience grade 3/4 haemorrhage was higher during induction (prelisted safety source: induction OR 2.16 [95% CI (1.46-3.20); p=0.0001] vs treatment period OR 1.87 [95% CI (1.32-2.65); p=0.0004].

Infections

In the combination therapy study, in patients with de novo AML treated with fractionated doses of gemtuzumab ozogamicin in combination with chemotherapy (N=131), 102 (77.9%) patients experienced all causality severe (Grade ≥ 3) infections. Treatment-related death due to septic shock was reported in

2 (1.5%) patients. Fatal severe infection was reported in 2 (1.53%) patients in the MYLOTARG arm and 4 (2.92%) patients in the control arm (SmPC, section 4.8).

The incidence of severe infections (Grade 3/4) tended to be lower in the monotherapy studies in the relapsed AML setting (studies 201, 202, 203) compared to the setting of add-on Mylotarg to chemotherapy in the de novo population (monotherapy: 40.5% of patients with Grade ≥ 3 vs 78% in ALFA trial and 81.4% in IPD meta-analysis). There is also no difference in frequency of events between GO and no GO in the ALFA trial (GO: 102 patients [77.9%] vs control 106 patients [77.4%]; serious 40.5% GO vs 36.5% in control; not resolved 3.8% GO vs 3.8% control; death 1.5% GO vs 2.9% control) and no difference in time with severe infections between the arms (median weeks 2.7 GO vs 2.4 control), though a broader time range can be seen in the GO arm (0-70.4 weeks GO vs 0-56.6 control).

The meta-analysis showed no statistically significant increase in odds to experience a Grade 3/4 infection. There was a trend for a slight increase in the OR when comparing the odds during all treatment periods and the induction phase (All treatment period OR 0.85 (95%CI [0.6-1.21]) vs induction treatment period OR 1.08 (95%CI [0.8-1.46]).

MRC cytogenetic risk was found to suggest heterogeneity in OR (p=0.07); patients in the GO arm with favourable risk disease had lower odds of infection than those in the No GO arm (OR 0.41, 95% CI: 0.19-0.90, p=0.03).

Hepatotoxicity, including hepatic VOD/SOS

In the combination therapy study, VOD and hepatic laboratory abnormalities were collected. Additional characterisation of hepatotoxicity adverse reactions is provided from the monotherapy studies.

In the combination therapy study (N=131), VOD was reported in 6 (4.6%) patients during or following treatment, 2 (1.5%) of these reactions were fatal. Five (3.8%) of these VOD reactions occurred within 28 days of any dose of gemtuzumab ozogamicin. One VOD event occurred more than 28 days of last dose of gemtuzumab ozogamicin; with 1 of these events occurring a few days after having started an HSCT conditioning regimen. The median time from the last gemtuzumab ozogamicin dose to onset of VOD was 9 days (range: 2-298 days). VOD was also reported in 2 patients who received Mylotarg as a follow-up therapy following relapse of AML after chemotherapy treatment in the control arm of the combination therapy study. Both of these patients experienced VOD more than 28 days after the last dose of gemtuzumab ozogamicin. One of these patients experienced VOD 25 days after the subsequent HSCT (SmPC, section 4.8).

The potential association of key variables with the risk of developing VOD (both VOD observed at any time following exposure to GO and VOD within 28 days of any dose of GO) was assessed by logistic regression. The analysis included adult patients treated with GO monotherapy from all relapsed/refractory AML studies except dose-finding Study 103, and had data for all key variables. The covariates evaluated in this analysis were age, sex, starting dose of GO (mg/m2), total dose of GO (in mg), number of GO doses, baseline ALT, baseline AST, baseline bilirubin, hepatic impairment baseline (categorised into 2 categories moderate/severe and none/mild) and indicator of HSCT (prior and follow-up).

Based on this analysis of potential risk factors, adult patients who received Mylotarg as monotherapy, patients who had received an HSCT prior to gemtuzumab ozogamicin exposure were 2.6 times more likely (95% CI: 1.448-4.769) to develop VOD compared to patients without HSCT prior to treatment with gemtuzumab ozogamicin; patients who had received an HSCT following treatment with gemtuzumab ozogamicin were 2.9 times more likely (95% CI: 1.502-5.636) to develop VOD compared to patients without HSCT following treatment with gemtuzumab ozogamicin; and patients who had moderate/severe

hepatic impairment at baseline were 8.7 times more likely (95% CI: 1.879-39.862) to develop VOD compared to patients without moderate/severe hepatic impairment at baseline (SmPC, section 4.8).

Myelosuppression

In the combination therapy study in patients with previously untreated *de novo* AML treated with fractionated doses of gemtuzumab ozogamicin in combination with chemotherapy, Grade 3/4 decreases in leukocytes, neutrophils, and platelets were observed in 131 (100%), 124 (96.1%), and 131 (100%) patients, respectively (SmPC, section 4.8).

During the induction phase, 109 (83.2%) and 99 (75.6%) patients had platelet recovery to counts of 50,000/mm^3 and 100,000/mm^3, respectively. The median times to platelet recovery to counts of 50,000/mm^3 and 100,000/mm^3 were 34 and 35 days, respectively. During the consolidation 1 phase, 92 (94.8%) and 71 (73.2%) patients had a platelet recovery to counts of 50,000/mm^3 and 100,000/mm^3, respectively. The median times to platelet recovery to counts of 50,000/mm^3 and 100,000/mm^3 were 32 and 35 days, respectively. During the consolidation 2 phase, 80 (97.6%) and 70 (85.4%) patients had a platelet recovery to counts of 50,000/mm^3 and 100,000/mm^3, respectively. The median times to platelet recovery to counts of 50,000/mm^3 and 100,000/mm^3 were 36.5 and 43 days, respectively (SmPC, section 4.8).

Thrombocytopenia with platelet counts < 50,000/mm^3 persisting 45 days after the start of therapy for responding patients (CR and incomplete platelet recovery [CRp]) occurred in 22 (20.4%) of patients. The number of patients with persistent thrombocytopenia remained similar across treatment courses (8 [7.4%] patients at the induction phase and 8 [8.5%] patients at the consolidation 1 phase and 10 [13.2%] patients at the consolidation 2 phase) (SmPC, section 4.8).

During the induction phase, 121 (92.4%) and 118 (90.1%) patients had a documented neutrophil recovery to ANC of 500/mm^3 and 1,000/mm^3, respectively. The median time to neutrophil recovery to ANC of 500/mm^3 and 1,000/mm^3 was 25 days. In the consolidation 1 phase of therapy, 94 (96.9%) patients had neutrophil recovery to counts of 500/mm^3, and 91 (94%) patients recovered to counts of 1,000/mm^3. The median times to neutrophil recovery to ANC of 500/mm^3 and 1,000/mm^3 were 21 and 25 days, respectively. In the consolidation 2 phase of therapy, 80 (97.6%) patients had neutrophil recovery to counts of 500/mm^3, and 79 (96.3%) patients recovered to counts of 1,000/mm^3. The median times to neutrophil recovery to ANC of 500/mm^3 and 1,000/mm^3 were 22 and 27 days, respectively (SmPC, section 4.8).

In the combination therapy study (N=131), all grades and Grade 3/4 bleeding/haemorrhagic reactions were reported in 118 (90.1%) and 27 (20.6%) patients, respectively. The most frequent Grade 3 bleeding/haemorrhagic reactions were epistaxis (1.5%), haemoptysis (3.1%), and haematuria (2.3%). Grade 4 bleeding/haemorrhagic reactions were reported in 4 (3.1%) patients (gastrointestinal haemorrhage, haemorrhage, and pulmonary alveolar haemorrhage [2 patients]). Fatal bleeding/haemorrhagic reactions were reported in 3 (2.3%) patients (cerebral haematoma, intracranial haematoma, and subdural haematoma) (SmPC, section 4.8).

Serious adverse events and deaths

Serious adverse events

Serious adverse events (SAEs) were collected following the standard definition as any manifestation that suggested the occurrence of an important risk for the patient and/or the existence of a contraindication to continuation of treatment, from the time of signing the ICD until withdrawal from the study or 30 days after the end of study treatment.

In the ALFA trial more patients experienced a related SAE in the GO compared to the control (61.1% vs 51.5%). The most common treatment-related SAEs by SOC were: Infections and infestations: 50 (38.2%) patients in the GO arm and 46 (33.6%) patients in the control arm, blood and lymphatic system disorders: 45 (34.4%) patients in the GO arm and 15 (10.9%) patients in the control arm and hepatobiliary disorders: 16 (12.2%) patients in the GO arm and 5 (3.6%) patients in the control arm. Treatment-related SAEs experienced by >5% of patients in either treatment arm, by MedDRA Preferred Terms, were: thrombocytopenia: 32 (24.4%) patients in the GO arm and 5 (3.6%) patients in the control arm, bronchopulmonary aspergillosis: 13 (9.9%) patients in the GO arm and 10 (7.3%) patients in the control arm, febrile bone marrow aplasia: 12 (9.2% patients) in the GO arm and 7 (5.1%) patients in the control arm and septic shock: 9 (6.9%) patients in the GO arm and 7 (5.1%) patients in the control arm.

Information on the occurrence of SAEs in the IPD Meta-Analysis was collected for each trial and the number of patients in whom one or more SAEs occurred (including SAEs with fatal outcome) was compared by treatment arm is displayed in Figure 25.

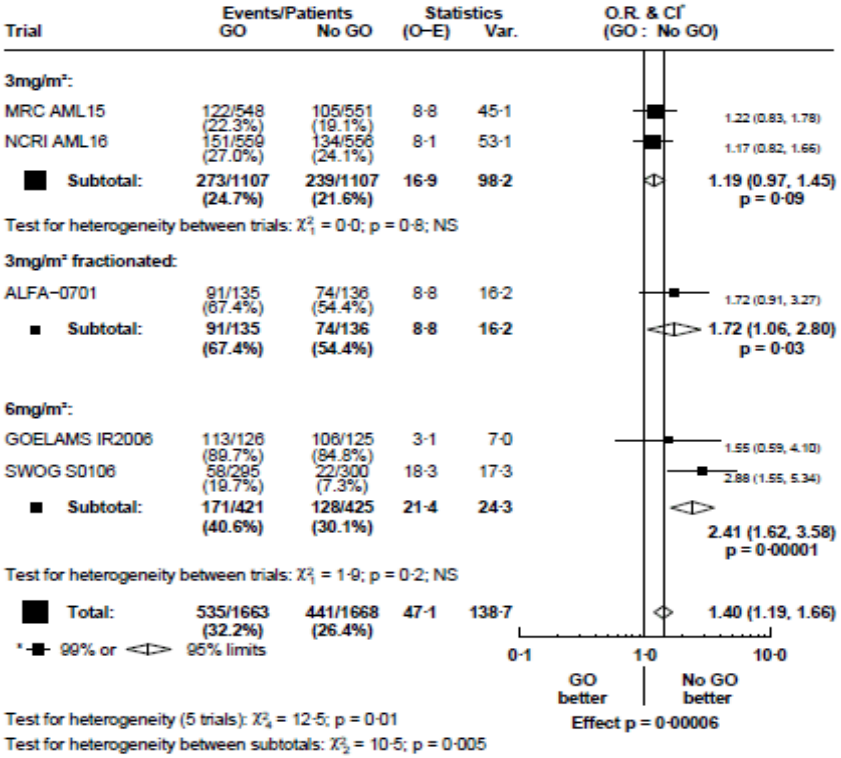


Figure 25 Patients with a Serious Adverse Event by Dose Group and Trial – IPD Meta-Analysis

Serious Adverse Events by ELN Risk Category

Overall, 164 (61.2%) patients experienced an SAE (all-causality): 88 (67.2%) patients in the GO arm and 76 (55.5%) patients in the control arm. In the Favourable ELN risk category 32 (60.4%) patients experienced an SAE (all causality): 15 (57.7%) patients in the GO arm and 17 (63.0%) patients in the control arm. In the Intermediate ELN risk category, 79 (64.2%) patients experienced an SAE (all causality): 44 (76.0%) patients in the GO arm and 35 (53.8%) patients in the control arm. In the Poor/Adverse ELN risk category, 42 (59.2%) patients experienced an SAE (all causality): 21 (60.0%) patients in the GO arm and 21 (58.3%) patients in the control arm.

The most common all-causality SAEs by SOC were:

- Infections and infestations: 54 (41.2%) patients in the GO arm and 52 (38.0%) patients in the control arm. Considering by ELN risk category, this SOC was reported in 10 (38.5%), 28 (48.3%) and 13 (37.1%) patients in the GO arm and in 11 (40.7%), 23 (35.4%) and 17 (47.2%) patients in the control arm for the Favourable, Intermediate and Poor/Adverse risk subgroups, respectively.
- Blood and lymphatic system disorders: 49 (37.4%) patients in the GO arm and 19 (13.9%) patients in the control arm. Considering by ELN risk category, this SOC was reported in 5 (19.2%), 25 (43.1%) and 16 (45.7%) patients in the GO arm and in 3 (11.1%), 8 (12.3%) and 7 (19.4%) patients in the control arm for the Favourable, Intermediate and Poor/Adverse risk subgroups, respectively.
- Hepatobiliary disorders: 17 (13.0%) patients in the GO arm and 8 (5.8%) patients in the control arm. Considering by ELN risk category, this SOC was reported in 2 (7.7%), 8 (13.8%) and 6 (17.1%) patients in the GO arm and in 2 (7.4%), 1 (1.5%) and 5 (13.9%) patients in the control arm for the Favourable, Intermediate and Poor/Adverse risk subgroups, respectively.

All-causality SAEs experienced by >5% of patients in either treatment arm, by MedDRA Preferred Term, were:

- Thrombocytopenia: 34 (26.0%) patients in the GO arm and 6 (4.4%) patients in the control arm. Considering by ELN risk category, thrombocytopenia was reported in 3 (11.5%), 17 (29.3%) and 13 (37.1%) patients in the GO arm and in 1 (3.7%), 2 (3.1%) and 2 (5.6%) patients in the control arm for the Favourable, Intermediate and Poor/Adverse risk subgroups, respectively.
- Bronchopulmonary aspergillosis: 14 (10.7%) patients in the GO arm and 10 (7.3%) patients in the control arm. Considering by ELN risk category, Bronchopulmonary aspergillosis was reported in 2 (7.7%), 9 (15.5%) and 3 (8.6%) patients in the GO arm and in 1 (3.7%), 6 (9.2%) and 2 (5.6%) patients in the control arm for the Favourable, Intermediate and Poor/Adverse risk subgroups, respectively.
- Septic shock: 12 (9.2%) patients in the GO arm and 9 (6.6%) patients in the control arm. Considering by ELN risk category, Septic shock was reported in 2 (7.7%), 5 (8.6%) and 4 (11.4%) patients in the GO arm and in 0, 5 (7.7%) and 4 (11.1%) patients in the control arm for the Favourable, Intermediate and Poor/Adverse risk subgroups, respectively.
- Febrile bone marrow aplasia: 12 (9.2%) patients in the GO arm and 8 (5.8%) patients in the control arm. Considering by ELN risk category, Febrile bone marrow aplasia was reported in 1 (3.8%), 6 (10.3%), and 3 (8.6%) patients in the GO arm and in 2 (7.4%), 4 (6.2%), and 2 (5.6%) patients in the control arm for the Favourable, Intermediate, and Poor/Adverse risk subgroups, respectively.

Deaths

As of the OS analysis reference date of 30 April 2013, a total of 168/271 (62.0%) patients died, including 80 (59.3%) patients in the GO arm and 88 (64.7%) patients in the control arm.

Table 44 Summary of Overall Deaths - Through 30 April 2013 (mITT Population-ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=135) n (%)	Daunorubicin + Cytarabine (N=136) n (%)	Total (N=271) n (%)
Overall Number of Deaths	**80 (59.3)**	**88 (64.7)**	**168 (62.0)**
Mechanism(s) of death			
Disease progression or relapse	50 (37.0)	59 (43.4)	109 (40.2)
Septic shock	19 (14.1)	13 (9.6)	32 (11.8)
Infection	15 (11.1)	17 (12.5)	32 (11.8)
Graft versus host disease	4 (3.0)	7 (5.1)	11 (4.1)
Liver toxicity	5 (3.7)	1 (0.7)	6 (2.2)
Haemorrhage	11 (8.1)	6 (4.4)	17 (6.3)
Other[a]	22 (16.3)	33 (24.3)	55 (20.3)
Death In CR/CRp[b]	4 (3.0)	4 (2.9)	8 (3.0)

Abbreviations: CR=complete remission; CRF=case report form; CRp=complete remission with incomplete platelet recovery; GO=gemtuzumab ozogamicin; mITT=modified intent-to-treat; N=number of patients; n=number of patients.
a. Includes all other mechanisms not included in the predefined categories, such as Cardiac failure, Respiratory distress, and Multi-organ failure
b. Deaths in CR/CRp are defined as patients who experienced CR/CRp by investigator assessment and died without relapse by investigator assessment and did not have a transplant.

Treatment-related deaths included all patients who died because of events, reported at any time during the study either in the clinical or safety databases, and assessed as related to the study drug. A summary of treatment-related deaths is displayed in Table 45.

Table 45 Summary of Treatment-Related Deaths (As-Treated Population- ALFA0701 study)

	GO + Daunorubicin + Cytarabine (N=131) n (%)	Daunorubicin + Cytarabine (N=137) n (%)	Total (N=268) n (%)
Treatment-related deaths	7 (5.3)	5 (3.6)	12 (4.5)
Cause of death			
Disease under study	1 (0.8)	3 (2.2)	4 (1.5)
Study treatment toxicity	5 (3.8)	3 (2.2)	8 (3.0)
Unknown	1 (0.8)	0	1 (0.4)
Other	0	0	0
Mechanism of death			
Disease progression or relapse	1 (0.8)	1 (0.7)	2 (0.7)
Septic shock	1 (0.8)	3 (2.2)	4 (1.5)
Infection	1 (0.8)	2 (1.5)	3 (1.1)
GVHD	0	0	0
Liver toxicity	2 (1.5)	0	2 (0.7)
Haemorrhage	4 (3.1)	0	4 (1.5)
Other	3 (2.3)	3 (2.2)	6 (2.2)
Treatment related deaths during CR/CRp[a]	2 (1.5)	2 (1.5)	4 (1.5)

Table 46 summarizes the number of patients who died within 30 or 60 days from the first dose of study treatment regardless of the post induction status of the patient (ie, responder or induction failure).

Table 46 Induction Deaths - From Date of First Study Treatment (As-Treated Population)

	GO + Daunorubicin + Cytarabine n (%)	Daunorubicin + Cytarabine n (%)	Total n (%)
Overall[a]	131	137	268
Within 30 days	5 (3.8)	3 (2.2)	8 (3.0)
Within 60 days	7 (5.3)	7 (5.1)	14 (5.2)
ELN Risk[b]			
Favorable, N	26	27	53
Within 30 days	1 (3.8)	0	1 (1.9)
Within 60 days	1 (3.8)	0	1 (1.9)
Intermediate, N	58	65	123
Within 30 days	0	1 (1.5)	1 (0.8)
Within 60 days	1 (1.7)	3 (4.6)	4 (3.3)
Poor/Adverse	35	36	71
Within 30 days	2 (5.7)	2 (5.6)	4 (5.6)
Within 60 days	3 (8.6)	4 (11.1)	7 (9.9)
Cytogenetics[c]			
Favorable, N	2	7	9
Within 30 days	0	0	0
Within 60 days	0	0	0
Intermediate, N	89	88	177
Within 30 days	1 (1.1)	1 (1.1)	2 (1.1)
Within 60 days	2 (2.2)	3 (3.4)	5 (2.8)
Unfavorable, N	26	31	57
Within 30 days	2 (7.7)	2 (6.5)	4 (7.0)
Within 60 days	3 (11.5)	4 (12.9)	7 (12.3)

Time to death during induction was calculated from date of first dose regardless of remission status.
Abbreviations: ELN=European LeukemiaNet; GO=gemtuzumab ozogamicin; n=number of patients.
a. Denominator included all patients within the as-treated population.
b. Denominator included all patients within the as-treated population within the respective ELN risk group.
c. Denominator included all patients within the as-treated population within the respective cytogenetic risk group.

IPD meta-analysis

In 1663 patients randomized to GO (6.6%), 109 deaths occurred within 30 days and 85 deaths occurred in 1668 patients randomized to the no GO arm (5.1%). The risk was not significantly increased (OR 1.29, 95% CI: 0.97-1.71, 2-sided stratified log-rank p=0.08). In 1663 patients in the GO arm, within 60 days after randomization 167 deaths (10.0%) were observed and 141 deaths (8.5%) were observed in 1668 patients in the no GO group. The risk of death within 60 days was not significantly increased (OR 1.19, 95% CI: 0.95-1.49, p=0.1).

Laboratory findings

Table 47 Summary of Haematology Laboratory Results by Maximum CTCAE Grade (All Phases) (As-Treated Population) – (ALFA0701 study)

Parameter	N	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Total n (%)
GO Arm						
Hemoglobin (L)	130	0	18 (13.8)	112 (86.2)	0	130 (100.0)
Leukocytes (H)	131	0	0	1 (0.8)	0	1 (0.8)
Leukocytes (L)	131	0	0	1 (0.8)	130 (99.2)	131 (100.0)
Lymphocytes (H)	129	0	9 (7.0)	1 (0.8)	0	10 (7.8)
Lymphocytes (L)	129	2 (1.6)	8 (6.2)	37 (28.7)	80 (62.0)	127 (98.4)
Neutrophils (L)	129	0	2 (1.6)	7 (5.4)	117 (90.7)	126 (97.7)
Platelets (L)	131	0	0	1 (0.8)	130 (99.2)	131 (100.0)
Control Arm						
Hemoglobin (L)	136	1 (0.7)	13 (9.6)	122 (89.7)	0	136 (100.0)
Leukocytes (H)	136	0	0	3 (2.2)	0	3 (2.2)
Leukocytes (L)	136	0	0	1 (0.7)	134 (98.5)	135 (99.3)
Lymphocytes (H)	135	0	7 (5.2)	0	0	7 (5.2)
Lymphocytes (L)	135	3 (2.2)	8 (5.9)	31 (23.0)	90 (66.7)	132 (97.8)
Neutrophils (L)	135	1 (0.7)	1 (0.7)	11 (8.1)	120 (88.9)	133 (98.5)
Platelets (L)	136	0	0	2 (1.5)	134 (98.5)	136 (100.0)

Abbreviations: CTCAE=Common Terminology Criteria for Adverse Events; GO=gemtuzumab ozogamicin; H=high; L=low; N=number of patients; n=number of patients; NCI=National Cancer Institute; v=version.

ECG

Clinically, ECG tests were performed in 11 GO studies and post-baseline ECG tests were obtained in 8 studies. In 3 studies, namely, ALFA-0701, 100374 and 100863, ECG tests were performed only at screening or baseline. Since the clinical studies predated the current QT interval prolongation guidance, these trials did not collect sufficient data to enable a formal concentration-QTc assessment; primarily qualitative ECG assessments were reported (data not shown).

Safety in special populations

The treatment-emergent adverse events (>5% of patients) by MedDRA system organ class, preferred term (all causalities) by subgroup age (as-treated population) are displayed in Table 48.

Table 48. Treatment-Emergent Adverse Events (>5% of Patients) by MedDRA System Organ Class, Preferred Term (All Causalities) - by Subgroup Age (As-Treated Population) – (ALFA0701 study)

System Organ Class Preferred Term	GO+Daunorubicin+Cytarabine (N=131)			Daunorubicin+Cytarabine (N=137)		
	≥50 and <55 years (N=10) n (%)	≥55 and <65 years (N=72) n (%)	≥65 years (N=49) n (%)	≥50 and <55 years (N=23) n (%)	≥55 and <65 years (N=73) n (%)	≥65 years (N=41) n (%)
Any TEAE	10 (100.0)	70 (97.2)	49 (100.0)	21 (91.3)	71 (97.3)	37 (90.2)
Blood and lymphatic system disorders	0	10 (13.9)	11 (22.4)	0	0	1 (2.4)
Eye disorders	2 (20.0)	12 (16.7)	3 (6.1)	0	2 (2.7)	2 (4.9)
Gastrointestinal disorders	5 (50.0)	31 (43.1)	27 (55.1)	7 (30.4)	25 (34.2)	10 (24.4)
General disorders and administration site conditions	3 (30.0)	21 (29.2)	19 (38.8)	7 (30.4)	24 (32.9)	7 (17.1)
Hepatobiliary disorders	0	10 (13.9)	1 (2.0)	1 (4.3)	1 (1.4)	0
Venoocclusive liver disease	0	6 (8.3)	0	0	0	0

System Organ Class Preferred Term	GO+Daunorubicin+Cytarabine (N=131)			Daunorubicin+Cytarabine (N=137)		
	≥50 and <55 years (N=10) n (%)	≥55 and <65 years (N=72) n (%)	≥65 years (N=49) n (%)	≥50 and <55 years (N=23) n (%)	≥55 and <65 years (N=73) n (%)	≥65 years (N=41) n (%)
Infections and infestations	8 (80.0)	56 (77.8)	38 (77.6)	18 (78.3)	62 (84.9)	26 (63.4)
Injury, poisoning and procedural complications	1 (10.0)	9 (12.5)	10 (20.4)	1 (4.3)	9 (12.3)	5 (12.2)
Investigations	1 (10.0)	2 (2.8)	2 (4.1)	1 (4.3)	2 (2.7)	0
Renal and urinary disorders	1 (10.0)	13 (18.1)	12 (24.5)	4 (17.4)	7 (9.6)	3 (7.3)
Reproductive system and breast disorders	0	5 (6.9)	1 (2.0)	2 (8.7)	3 (4.1)	2 (4.9)
Respiratory, thoracic and mediastinal disorders	8 (80.0)	47 (65.3)	33 (67.3)	10 (43.5)	33 (45.2)	17 (41.5)
Skin and subcutaneous tissue disorders	6 (60.0)	37 (51.4)	25 (51.0)	7 (30.4)	24 (32.9)	10 (24.4)
Vascular disorders	4 (40.0)	17 (23.6)	15 (30.6)	2 (8.7)	18 (24.7)	11 (26.8)

Abbreviations: GO=gemtuzumab ozogamicin; N/n=number of patients; TEAE=treatment-emergent adverse event

Safety related to drug-drug interactions and other interactions

No formal drug-interaction studies have been conducted with GO.

Discontinuation due to AES

A total of 51 (19.0%) patients discontinued study drug due to TEAEs (Table 49).

Table 49 Number (%) of Patients Experiencing TEAEs Leading to Permanent Discontinuation of Study Drug (As-Treated Population) (ALFA0701 study)

System Organ Class Preferred Term	GO + Daunorubicin + Cytarabine (N=131) n (%)	Daunorubicin+ Cytarabine (N=137) n (%)	Total (N=268) n (%)
Permanent drug discontinuation in patients with AEs	**41 (31.3)**	**10 (7.3)**	**51 (19.0)**
Blood and lymphatic system disorders	20 (15.3)	0	20 (7.5)
Thrombocytopenia	20 (15.3)	0	20 (7.5)
Cardiac disorders	2 (1.5)	1 (0.7)	3 (1.1)
Acute coronary syndrome	1 (0.8)	0	1 (0.4)
Left ventricular failure	0	1 (0.7)	1 (0.4)
Ventricular hypokinesia	1 (0.8)	0	1 (0.4)
Gastrointestinal disorders	0	1 (0.7)	1 (0.4)
Gastrointestinal haemorrhage	0	1 (0.7)	1 (0.4)
General disorders and administration site conditions	1 (0.8)	0	1 (0.4)
Death	1 (0.8)	0	1 (0.4)
Hepatobiliary disorders	8 (6.1)	1 (0.7)	9 (3.4)
Hepatic cirrhosis	1 (0.8)	0	1 (0.4)
Hepatitis cholestatic	1 (0.8)	0	1 (0.4)
Hepatocellular injury	1 (0.8)	1 (0.7)	2 (0.7)
Hepatotoxicity	1 (0.8)	0	1 (0.4)
Veno-occlusive liver disease	4 (3.1)	0	4 (1.5)
Infections and infestations	3 (2.3)	2 (1.5)	5 (1.9)
Septic shock	3 (2.3)	2 (1.5)	5 (1.9)
Injury, poisoning and procedural complications	1 (0.8)	0	1 (0.4)
Subdural haematoma	1 (0.8)	0	1 (0.4)
Investigations	2 (1.5)	2 (1.5)	4 (1.5)
Ejection fraction	1 (0.8)	0	1 (0.4)
Ejection fraction decreased	0	1 (0.7)	1 (0.4)
Liver function test abnormal	1 (0.8)	0	1 (0.4)
Oxygen saturation decreased	0	1 (0.7)	1 (0.4)
Nervous system disorders	3 (2.3)	2 (1.5)	5 (1.9)
Cerebral haematoma	1 (0.8)	0	1 (0.4)
Cerebral haemorrhage	0	1 (0.7)	1 (0.4)
Cerebrovascular accident	0	1 (0.7)	1 (0.4)
Intracranial haematoma	1 (0.8)	0	1 (0.4)
Neuropathy peripheral	1 (0.8)	0	1 (0.4)
Renal and urinary disorders	1 (0.8)	0	1 (0.4)
Acute kidney injury	1 (0.8)	0	1 (0.4)
Respiratory, thoracic and mediastinal disorders	0	1 (0.7)	1 (0.4)
Acute respiratory distress syndrome	0	1 (0.7)	1 (0.4)

Abbreviations: AE=adverse event; GO=gemtuzumab ozogamicin; MedDRA=Medical Dictionary for Regulatory Activities; N=number of patients; n=number of patients; TEAE=treatment-emergent adverse event; v=version.

Post marketing experience

On September 1, 2017, FDA approved Mylotarg for the treatment of newly-diagnosed CD33-positive AML in adults and for treatment of relapsed or refractory CD33-positive AML in adults and in paediatric patients 2 years and older. Mylotarg may be combined with Ara-C and daunorubicin (as in the EU) for adults with newly-diagnosed AML, or as a stand-alone treatment for certain adult and pediatric patients (in USA only). Mylotarg is marketed in Japan (since July 2005) for the treatment of patients with relapsed or recurrent CD33-positive AML.

Safety assessments performed as part of global pharmacovigilance monitoring have been reported in PSURs over the past 15 years.

In the first 2 PSURs for the time period 17 May 2000 to 16 May 2001, changes were made to the Reference Safety Information (RSI) provided in the Investigator's Brochure regarding hepatotoxicity/VOD and hypersensitivity reactions, and the corresponding changes were subsequently included in a warning for the US market (prior withdrawal). Renal failure secondary to tumour lysis syndrome was also added to the tumour lysis syndrome section under the warnings and precautions. Since 2001, no new safety findings were identified in the post-marketing setting. Additionally, assessments were done during routine pharmacovigilance monitoring including all known safety

information, spontaneous reports, literature, non-interventional studies, and clinical trial reports, with no new safety concerns identified and a consistent safety profile as known for Mylotarg.

A Drug Use Investigation (DUI) also conducted in Japan, under approval conditions as an all-patient survey from September 2005 to December 2009. Of the 753 patients included in this exercise, 367 patients were aged 65 years or older (48.7%), 366 patients aged 15 to less than 65 years (48.6%), and 20 children aged less than 15 years (2.7%). There were 1,444 serious ADRs in 508 patients. Major ADRs were as follows: 197 ADRs of platelet count decreased, febrile neutropenia, 169 ADRs of neutrophil count decreased, 134 ADRs of white blood cell count decreased, 118 ADRs of sepsis, 98 ADRs of febrile neutropenia, 66 ADRs of anaemia, 47 ADRs of pneumonia, 42 ADRs of neutrophil count decreased, 42 ADRs of venoocclusive liver disease, 31 ADRs of disseminated intravascular coagulation, 28 ADRs of thrombocytopenia, 21 ADRs of pyrexia. With regard to the important identified risk of VOD, the following has been described: the incidence rate of adverse reactions of VOD was 5.58% (42 AEs of VOD in 42 patients) and 4.38%, (33 AEs of Grade >3 venoocclusive liver disease in 33 patients) respectively.

Clinical impact of change in AAS levels – safety

A comprehensive review of Mylotarg cases meeting PSUR criteria, and reporting preferred terms (PTs) relevant to the specified search strategies for each of the important risks of GO in the Pfizer global safety database during the exclusively pre-AAS (base AAS) shift time period (cumulatively through 31 December 2006; 6 years) versus cases reported during the exclusively post-AAS shift (elevated AAS) time period (01 January 2012 through 31 May 2017; 5.5 years) has been submitted. This included clinical trial cases through this period.

2.7.1. Discussion on clinical safety

Safety data collected in a prospective open-ended manner, in line with regulatory requirements, describing all Grades of severity (Grade 1 and 2 missing) and accurately reflecting the respective frequencies are not available from the ALFA trial. But the extensive experience with Mylotarg in clinical trials and the fact that it is still licensed in Japan allows a reassuring qualitative description of the safety profile. A total of 2.747 patients received GO either as monotherapy or in combination chemotherapy.

The most common adverse reactions (> 30%) in the combination therapy study were haemorrhage and infection. In monotherapy studies the most common adverse reactions (> 30%) included pyrexia, nausea, infection, chills, haemorrhage, vomiting, thrombocytopenia, fatigue, headache, stomatitis, diarrhoea, abdominal pain, and neutropenia (SmPC section 4.8).

Most AEs associated with GO use could be considered expected in the patient population, ie meylosuppression, infections or GI events. But there are AE SOCs (predefined) observed with increased incidence, such as grade 3/4 mucosal toxicity (16.0% vs 6.6%), pain (14.5% vs 3.6%), and the composite of nausea, vomiting, and diarrhoea (16.8% vs 10.2%). Others considered more directly related to Mylotarg and it's on and off target effects, are prolonged thrombocytopenia and consequential increased incidence of serious events of haemorrhage (GO 8.4% vs 1.5%), or hepatotoxicity (8.6% vs 2.2%), including VOD (GO 4.6% vs 1.5%). The increased incidence of toxicity is supported by the meta-analysis showing a significantly higher odd to experience a Grade 3 or 4 toxicity with Mylotarg added to chemotherapy (Odds 1.21 [95% CI 1.03, 1.42], p=0.02), which tends to be even slightly higher for the induction period (Odds 1.22 [95% CI 1.05, 1.41], p=0.009).

The same holds true for treatment related SAEs, with SOCs of infections and infestations (GO 38.2% vs 33.6%), blood and lymphatic system disorders (GO 34.4% vs 10.9%), and hepatobiliary (GO 12.2% vs

3.6%) observed most. The higher risk to experience a SAE is also supported by the IPD meta-analysis (OR 1.40, [95% CI: 1.19-1.66], p=0.00006).

In the combination therapy study ALFA-0701, clinically relevant serious adverse reactions were hepatotoxicity, including VOD/SOS (3.8%), haemorrhage (9.9%), severe infection (41.2%), and tumour lysis syndrome (1.5%). In monotherapy studies, clinically relevant serious adverse reactions also included infusion related reactions (2.5%), thrombocytopenia (21.7%), and neutropenia (34.3%)(SmPC section 4.8).

The most frequent (≥ 1%) adverse reactions that led to permanent discontinuation in the combination therapy study were thrombocytopenia, VOD, haemorrhage and infection. The most frequent (≥ 1%) adverse reactions that led to permanent discontinuation in monotherapy studies were infection, haemorrhage, multi organ failure, and VOD (SmPC section 4.8). In addition, permanent drug discontinuation due to TEAEs tends also to be high in the GO arm (GO discontinuation 49.2% [33/67]; chemotherapy discontinuation 36.7% [18/49]). When comparing to the control arm this trend is confirmed by less TEAEs reported leading to discontinuation of chemotherapy (13.7% GO arm vs 2.2% control arm). This means the likelihood of permanently discontinuing Mylotarg due to AEs was around 25%, and discontinuation of chemotherapy due to any study treatment related AE was almost 6 times higher in the GO arm. Initially, this raised concerns around drug tolerability and would need to be outweighed by a clinically meaningful improvement in EFS. This also showed the importance to only consider patients for treatment with Mylotarg induction combination who are considered fit enough by their treating physician. Based on this, the CHMP considered that Mylotarg should be used only in patients eligible to receive intensive induction chemotherapy (SmPC section 4.2).

The rates of selected TEAEs (Infections/ infestations; Haemorrhage and VOD) were similar between the all treated and responder population. The clear difference between the GO arm and the control arm in terms of all grade haemorrhage (90.7% in the GO arm versus 81.2% in the control arm and 90.1% in the GO arm versus 78.1% in the control arm in responder patients and the overall AT population, respectively) and all grade VOD (4.6% in the GO arm versus 2% in the control arm and 4.6% in the GO arm versus 1.5% in the control arm in responder patients and the overall AT population, respectively) remains. It is overall agreed that Mylotarg leads to an increase of certain TEAEs, but that there was no worsening of the imbalance between patients who responded and all patients (AT). As mentioned, in the overall AT population and responder patients, the two most frequent reasons for permanent Mylotarg discontinuation were identified to be thrombocytopenia and hepatotoxicity. In addition to this, Mylotarg treatment either before or after HSCT and baseline moderate/ severe hepatic impairment have been identified as risk factors for an increased risk for developing VOD. Both are now reflected in the proposed SmPC, including adequate monitoring recommended as follows: Due to the risk of VOD/SOS, signs and symptoms of VOD/SOS should be closely monitored; these may include elevations in ALT, AST, total bilirubin, and alkaline phosphatase, which should be monitored prior to each dose of Mylotarg, hepatomegaly (which may be painful), rapid weight gain, and ascites. Monitoring only total bilirubin may not identify all patients at risk of VOD/SOS. For patients who develop abnormal liver tests, more frequent monitoring of liver tests and clinical signs and symptoms of hepatotoxicity is recommended. For patients who proceed to HSCT, close monitoring of liver tests is recommended during the post-HSCT period, as appropriate. No definitive relationship was found between VOD and time of HSCT relative to higher Mylotarg monotherapy doses, however, the ALFA-0701 study recommended an interval of 2 months between the last dose of Mylotarg and HSCT (SmPC, section 4.4).

Management of signs or symptoms of hepatic toxicity may require a dose interruption, or discontinuation of Mylotarg. In patients who experience VOD/SOS, Mylotarg should be discontinued and patients treated according to standard medical practice (SmPC, sections 4.2 and 4.4).

In clinical studies, neutropenia, thrombocytopenia, anaemia, leukopenia, febrile neutropenia, lymphopenia, and pancytopenia, some of which were life-threatening or fatal, were reported. Complications associated with neutropenia and thrombocytopenia may include infections and bleeding/haemorrhagic reactions respectively. Infections and bleeding/haemorrhagic reactions were reported, some of which were life-threatening or fatal.

Complete blood counts should be monitored prior to each dose of Mylotarg. During treatment, patients should be monitored for signs and symptoms of infection, bleeding/haemorrhage, or other effects of myelosuppression. Routine clinical and laboratory surveillance testing during and after treatment is indicated. Management of patients with severe infection, bleeding/haemorrhage, or other effects of myelosuppression, including severe neutropenia or persistent thrombocytopenia, may require a dose delay or permanent discontinuation of Mylotarg (SmPC sections 4.2, 4.4 and 4.8). Myelosuppression (severe [Grade ≥ 3] and/or serious infection and haemorrhage) been classified as an identified risk in the Risk Management Plan.

Regarding the different toxicities in relation to the different risk subgroups, it is agreed that similar rates of all grade pre-defined TEAEs in both arms when classified by ELN risk groups has been shown. This is certainly the case for infections and infestations (GO arm All Patients 77.9%; 80.9% favourable/ intermediate risk; 74.3% adverse risk group), as well as haemorrhage (GO arm all patients 90.1%; 94% Favourable/ intermediate risk; 85.7% adverse risk group). It is however evident that all grades VOD appeared with a higher incidence in patients with adverse risk AML (8.6%), compared to the favourable/ intermediate group (3.6%) and to the all patient population (4.6%). This is in addition to the two fatal cases of VOD occurring in the adverse risk group. This is consistent with all-causality SAEs by the SOC 'Hepatobiliary disorders', which was reported with increasing incidence of 7.7%, 13.8% and 17.1% in patients in the GO arm for the favourable, intermediate and poor/adverse risk subgroups, respectively, compared to an overall incidence of 13.0% of all patients in the GO arm. Similar increase can be observed for the all-causality SAEs experienced by >5% of patients for septic shock, reported in 2 (7.7%), 5 (8.6%) and 4 (11.4%) patients in the GO arm as well as thrombocytopenia reported in 3 (11.5%), 17 (29.3%) and 13 (37.1%) patients in the GO arm for the favourable, intermediate and poor/adverse risk subgroups. The remaining retrospectively collected data showed similar rates of all-grade predefined SAEs in both treatment arms when patients were classified by ELN criteria. Consistent positive benefit/risk across all cytogenetic risk groups can hence not be concluded (see discussion on clinical efficacy). This has been adequately reflected in section 4.4 of the SmPC.

The 30-day mortality rate was numerically higher in the Mylotarg arm (3.8% vs 2.2%), but similar across arm at 60-days (GO 5.3% vs control 5.1%). More induction deaths at day 60 were observed in the Poor/Adverse risk groups (GO 8.6% [3/35] vs control 11.1% [4/36]) compared with the Favourable/ Intermediate risk groups (3.5% [3/84] GO arm vs 4.3% [4/92] control arm). This is consistent for the cytogenetic subgroups, showing more induction deaths in the unfavourable group (D30 GO 11.1% [3] vs control 3.3% [1]; D60: 14.8% [4] in GO arm vs 10% [3] in control arm) compared to the intermediate group (2.2% [2] in GO arm), none with favourable cytogenetics. The trend in mortality difference is supported by the 30 and 60-day mortality results from the meta-analysis with heterogeneity in OR observed between cytogenetics risk groups. No overall increase in risk of early mortality between arms is observed.

No clinical impact of the shift in the AAS could be observed based on the comparability exercise. No clear safety differences were observed in the comprehensive analysis submitted (see also 2.2.4 Discussion on chemical, pharmaceutical and biological aspects).

In clinical studies infusion related reactions, including anaphylaxis were reported (see section 4.8). There have been reports of fatal infusion reactions in the post marketing setting. Signs and symptoms of

infusion related reactions may include fever and chills, and less frequently hypotension, tachycardia, and respiratory symptoms that may occur during the first 24 hours after administration. Infusion of MYLOTARG should be performed under close clinical monitoring, including pulse, blood pressure, and temperature. Premedication with a corticosteroid, antihistamine and acetaminophen (or paracetamol) is recommended 1 hour prior to MYLOTARG dosing (see section 4.2). Infusion should be interrupted immediately for patients who develop evidence of severe reactions, especially dyspnoea, bronchospasm, or clinically significant hypotension. Patients should be monitored until signs and symptoms completely resolve. Discontinuation of treatment should be strongly considered for patients who develop signs or symptoms of anaphylaxis, including severe respiratory symptoms or clinically significant hypotension (SmPC section 4.4). Infusion-related reactions (including anaphylaxis) from start of infusion to within 24 hours of end of infusion have been classified as an identified risk in the Risk Management Plan.

In clinical studies, TLS was reported. Fatal reports of TLS complicated by acute renal failure have been reported in the post marketing setting. In patients with hyperleukocytic AML, leukoreduction should be considered with hydroxyurea or leukapheresis to reduce the peripheral WBC count to below 30,000/mm^3 prior to administration of Mylotarg to reduce the risk of inducing TLS. Patients should be monitored for signs and symptoms of TLS and treated according to standard medical practice. Appropriate measures to help prevent the development of tumour lysis-related hyperuricaemia, such as hydration, administration of antihyperuricemics (e.g., allopurinol) or other agents for treatment of hyperuricaemia (e.g., rasburicase) must be taken (SmPC sections 4.2 and 4.4). Tumour lysis syndrome has been classified as an identified risk in the Risk Management Plan.

No effect of the low levels of unconjugated calicheamicin observed after a 3 mg/m^2 or 9 mg/m^2 dose of GO on ECG parameters have been identified in non-clinical studies (see discussion on non-clinical aspects). No safety signal has been identified in clinical trials, but data are insufficient to make a firm conclusion. In conclusion, as no cardiac safety signal has been identified from the clinical trial data of >6000 patients, the CHMP recommended the applicant to evaluate the effect of GO on QTc as described in the planned clinical study with the fractionated regimen. Furthermore cardiac function is considered missing information and this is reflected in the Risk Management Plan.

Overall, the incidence rate of ADA development after MYLOTARG treatment was < 1% across the 4 clinical studies with ADA data. Definitive conclusions cannot be drawn between the presence of antibodies and potential impact on efficacy and safety due to the limited number of patients with positive ADAs. Mylorarg will be administered to patients who are going to be immunosuppressed over the course of the treatment, with little meaningful clinical impact expected; taking into consideration that Mylotarg seems not to be very immunogenic (<1%). The CHMP recommended the applicant to evaluate immunogenicity of the batches intended for licensing as part of post-marketing commitment. Immunogenicity has been classified as a potential risk in the Risk Management Plan.

Management of patients with severe infection, bleeding/haemorrhage, or other effects of myelosuppression, including severe neutropenia or persistent thrombocytopenia, may require a dose delay or permanent discontinuation of Mylotarg (SmPC sections 4.2 and 4.4).

There are no or limited amount of data from the use of gemtuzumab ozogamicin in pregnant women. Mylotarg must not be used during pregnancy unless the potential benefit to the mother outweighs the potential risks to the foetus. Pregnant women, or patients becoming pregnant whilst receiving gemtuzumab ozogamicin, or treated male patients as partners of pregnant women, must be apprised of the potential hazard to the foetus (SmPC, section 4.6).

There is no information on fertility in patients. Based on non-clinical findings, male and female fertility may be compromised by treatment with gemtuzumab ozogamicin. Both men and women should seek advice on fertility preservation before treatment (SmPC, section 4.6).

Women of childbearing potential should be advised to avoid becoming pregnant while receiving Mylotarg. Women of childbearing potential, or partners of females of childbearing potential should be advised to use 2 methods of effective contraception during treatment with MYLOTARG for at least 7 months (females) or 4 months (males) after the last dose (SmPC, section 4.6).

There is no information regarding the presence of gemtuzumab ozogamicin or its metabolites in human milk, the effects on the breast-fed child, or the effects on milk production. Because of the potential for adverse reactions in breast-fed children, women should not breast-feed during treatment with Mylotarg and for at least 1 month after the final dose (SmPC, section 4.6).

Mylotarg has moderate influence on the ability to drive and use machines. Patients should be advised they may experience fatigue, dizziness and headache during treatment with Mylotarg. Therefore, caution should be exercised when driving or operating machines (SmPC section 4.7).

No cases of overdose with Mylotarg were reported in clinical experience. Single doses higher than 9 mg/m^2 in adults were not tested. Treatment of Mylotarg overdose should consist of general supportive measures (SmPC section 4.9).

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

2.7.2. Conclusions on the clinical safety

Overall, the safety profile of gemtuzumab ozogamicin is considered acceptable, with haemorrhage and infection being the most common adverse reactions (> 30%) in the combination therapy.

2.8. Risk Management Plan

Safety concerns

Table 50 Summary of safety concerns

Summary of safety concerns	
Important identified risks	Severe (Grade ≥3) and/or serious hepatotoxicity including all VOD/SOS Myelosuppression • Severe (Grade ≥3) and/or serious infection • Haemorrhage Tumour lysis syndrome Infusion-related reactions (including anaphylaxis) from start of infusion to within 24 hours of end of infusion
Important potential risks	Renal toxicity Reproductive and developmental toxicity (post exposure during pregnancy, including breastfeeding) Neurotoxicity Second primary malignancy

Summary of safety concerns	
	Immunogenicity Off label use in paediatric patients
Missing information	Use in patients with severe hepatic impairment Use in patients with severe renal impairment Effect on cardiac conduction

Pharmacovigilance plan

There are no additional pharmacovigilance activities proposed to assess the effectiveness of risk minimisation measures, as there are no additional risk minimisation measures proposed. The PRAC Rapporteur considers that no additional measures are required at this point.

Risk minimisation measures

Table 51 Summary Table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Severe (Grade ≥3) and/or serious hepatotoxicity (including all VOD/SOS)	**Routine risk minimisation measures:** SmPC Sections 4.2; 4.4; 4.8. PIL Section 2; 4	Routine
Myelosuppression	**Routine risk minimisation measures:** SmPC Sections 4.2; 4.4; 4.8. PIL Section 2; 4	Routine
Tumour Lysis Syndrome	**Routine risk minimisation measures:** SmPC Sections 4.2; 4.4; 4.8. PIL Section 2; 4	Routine
Infusion-Related Reactions (including Anaphylaxis) from start of infusion to within 24 hours of end of infusion	**Routine risk minimisation measures:** SmPC Sections 4.2; 4.4; 4.8. PIL Section 2; 4	Routine
Renal Toxicity	**Routine risk minimisation measures:** SmPC Section 5.3	Routine
Reproductive and Developmental Toxicity (post exposure during pregnancy, including breastfeeding)	**Routine risk minimisation measures:** SmPC Section 4.6; 5.3. PIL Section 2.	Routine
Second Primary Malignancy	**Routine risk minimisation measures:** SmPC Section 5.3.	Routine

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Neurotoxicity	**Routine risk minimisation measures:** SmPC Section 5.3.	Routine
Immunogenicity	**Routine risk minimisation measures:** SmPC Section 4.8.	Routine
Off Label Use in Paediatric Patients	**Routine risk minimisation measures:** SmPC Section 4.2; 4.8; 5.1; 5.2. PIL Section 2.	Routine
Use in Patients with Severe Hepatic Impairment	**Routine risk minimisation measures:** SmPC Section 4.2; 4.4; 4.8; 5.2. PL Section 2.	Routine
Use in Patients with Severe Renal Impairment	**Routine risk minimisation measures:** SmPC Section 4.2; 5.2.	Routine
Effect on Cardiac Conduction	None	Routine

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.2, dated January 2018, is acceptable.

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 17th May 2000. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant compared the structure of gemtuzumab ozogamicin with active substances contained in authorised medicinal products in the European Union and declared that it is not a salt, ester, ether, isomer, mixture of isomers, complex or derivative of any of them.

The CHMP, based on the available data, considers gemtuzumab ozogamicin to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Mylotarg (gemtuzumab ozogamicin) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Mylotarg (gemtuzumab ozogamicin) is proposed for treatment in combination with daunorubicin (DNR) and cytarabine (AraC) in adult patients with previously untreated de novo CD33-positive AML.

3.1.2. Available therapies and unmet medical need

The current standard of care for the treatment of de novo AML is based on intensive 3+7 (DNR/ AraC) induction chemotherapy; in case of remission this is followed by usually two courses of consolidation therapy or transplant in patients eligible based on the individual risk category.

3.1.3. Main clinical studies

The main clinical study was study ALFA070, a multicenter, randomized, comparative phase 3 study of fractionated doses of gemtuzumab ozogamicin in addition to daunorubicin + cytarabine versus daunorubicin + cytarabine alone for induction and consolidation therapy in patients with AML aged 50 to 70 years.

3.2. Favourable effects

Study ALFA070 has provided convincing evidence of clinical efficacy of gemtuzumab ozogamicin in combination with daunorubicin + cytarabine compared to daunorubicin + cytarabine alone in terms of the primary endpoint EFS, for induction and consolidation therapy in patients with AML. The primary efficacy

analysis (investigators review - data cut of August 2011), showed an EFS difference of 7.8 months (HR 0.562; 95% CI: 0.415-0.762; 2-sided p=0.0002), consistent when stratified by NCCN or ELN classification. The most conservative sensitivity analysis performed, (BIRC; data set April 2013), confirmed the primary analysis (HR 0.705; 95% CI: 0.536-0.928, p=0.0161), when stratified according to ELN. The robustness of the EFS was confirmed by appropriate additional sensitivity analyses.

Regarding the secondary endpoints, RFS confirmed a statistical significant difference in favour of the GO arm (HR 0.656, 95% CI: 0.466, 0.922, p= 0.02480) stratified for ELN risk category. In terms of response rate and OS there was a numerical advantage in favour of GO arm although this was not statistically significant.

Efficacy is supported by the primary endpoint OS of the IPD meta-analysis showing it was significantly improved in patients randomized to GO than in No GO patients. The OR for GO versus No GO was 0.91 (95% CI: 0.84-0.99, p=0.02), in favour of the GO arm. Overall pooled median OS was 23.62 months (95% CI: 21.22-27.33) in the GO arm and 21.49 months (95% CI: 19.42-23.20) in the No GO arm.

3.3. Uncertainties and limitations about favourable effects

The uncertainties that were identified during the assessment, including the initially proposed indication, the age cut-off and the efficacy in patients with adverse cytogenetic risk disease were satisfactorily addressed (see discussion on clinical efficacy).

3.4. Unfavourable effects

The most common adverse reactions (> 30%, all grades) in the combination therapy study were haemorrhage (90.1% vs 20.6%) and infection (77.9% vs 77.4%). In the combination therapy study (N=131), VOD was reported in 6 (4.6%) patients during or following treatment, 2 (1.5%) of these reactions were fatal. Five (3.8%) of these VOD reactions occurred within 28 days of any dose of gemtuzumab ozogamicin.

Thrombocytopenia with platelet counts < 50,000/mm3 persisting 45 days after the start of therapy for responding patients (CR and incomplete platelet recovery [CRp]) occurred in 22 (20.4%) of patients. The number of patients with persistent thrombocytopenia remained similar across treatment courses (8 [7.4%] patients at the induction phase and 8 [8.5%] patients at the consolidation 1 phase and 10 [13.2%] patients at the consolidation 2 phase).

The 30-day mortality rate was numerically higher in the GO arm (3.8% vs 2.2%), but similar across arms at 60-days (GO 5.3% vs control 5.1%). The increased risk for treatment related mortality in the GO arm was driven by increased deaths due to haemorrhage (3.1% vs 0%) and liver toxicity (VOD) (1.5% vs 0%).

The most frequent (≥ 1%) adverse reactions that led to permanent discontinuation in the combination therapy study were thrombocytopenia, VOD, haemorrhage and infection.

3.5. Uncertainties and limitations about unfavourable effects

No cardiac safety signal has been identified in clinical trials, but data are insufficient to make a firm conclusion. In conclusion, as no cardiac safety signal has been identified from the clinical trial data of >6000 patients, the CHMP recommended the applicant to evaluate the effect of GO on QTc as described

in the planned clinical study with the fractionated regimen. Furthermore cardiac function is considered missing information and this is reflected in the Risk Management Plan.

The incidence rate of ADA development after gemtuzumab ozogamicin treatment was < 1% across the 4 clinical studies with ADA data. Definitive conclusions cannot be drawn between the presence of antibodies and potential impact on efficacy and safety due to the limited number of patients with positive ADAs. Mylotarg will be administered to patients who are going to be immunosuppressed over the course of the treatment, with little meaningful clinical impact expected.The CHMP recommended the applicant to evaluate immunogenicity of the batches intended for licensing as part of post-marketing commitment. Immunogenicity has been classified as a potential risk in the Risk Management Plan.

3.6. Effects Table

Table 52 Effects Table for Mylotarg use in combination with chemotherapy – ALFA trial (data cut-off: 1 November 2013; cut-off date of retrospective data collection)

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence
Favourable Effects (mITT population)					
Event-free Survival (EFS)	Time from randomization to induction failure, relapse, or death due to any cause (local evaluation)	Months	17.3 (13.4-30.0)	9.5 (8.1-12.0)	• HR 0.562 (0.415-0.762), 2-sided p=0.0002
Overall survival (OS)	Time form randomization to death regardless of cause	Months	27.5 (21.4-45.6)	21.8 (15.5-27.4)	• HR 0.807 (0.596-1.093), 2-sided p=0.1646 • Pooled median OS in meta-analysis was 24 v. 21 months for GO v. non-GO group, respectively
Unfavourable Effects (AT population)					
Haemorrhage	Grade 3-4	%	20.6	8.8	
Venoocclusive liver disease	Grade 3-4	%	2.3	1.5	
Infection	Grade 3-4	%	76.3	74.4	
Thrombocytopenia	Grade 3-4	%	24.4	3.6	

Abbreviations: AEs: adverse events, CR/CRp: complete remission / complete remission with incomplete platelet recovery, EFS: Event free survival, mITT: modified intent-to-treat, OS: Overall survival, HR: hazard ratio, RFS: relapse-free survival

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The proposed indication concerns all patients with untreated de novo AML, except APL. Few improvements have been achieved in the treatment of this disease in the last decades and survival

expectance remains poor, highlighting the unmet medical need. Any treatment benefit in the de novo setting would be reflected in an increase of patients achieving sustainable first remission rates, allowing more patients proceeding to transplant in case eligible as per individual risk profile, ultimately translating into improvement in OS. However, any prolongation of remission could be considered of clinical benefit. Particularly when looking at first line treatment and the benefit patients might gain by having a prolonged time off further therapy in a disease in which relapse usually occurs early. The primary endpoint of EFS, evaluated in the ALFA trial is relevant in this clinical context and it is agreed that EFS is a clinically meaningful endpoint. An improvement in EFS of around 6 months and a risk reduction to experience an event of around 30% is of clear clinical relevance. The effect on overall survival was less clear although, also based on supportive evidence from a meta-analysis a small favourable effect seems likely. In any case, a detrimental effect in terms of OS in the whole population can be ruled out.

The efficacy results from the pivotal trial supported by the meta-analysis showed that Mylotarg added to induction chemotherapy improved EFS through prolongation of remission following initial chemotherapy, rather than increasing the number of patients who achieve complete remission, as confirmed by the absence of a statistically significant difference in the overall response rate. The toxicity associated with GO, although expected based on the mechanism of action, was also important, including treatment discontinuation due to AEs that occurred more frequently in the GO arm (13.7%) vs. control arm (2.2 %) and were mainly due to thrombocytopenia and VOD.

3.7.2. Balance of benefits and risks

In view of the effect in terms of EFS and the observed toxicity, and that any remaining uncertainties have been addressed, the benefit-risk balance in the proposed indication is considered positive.

3.7.3. Additional considerations on the benefit-risk balance

Efficacy for the intended indication for patients less than 50 years of age is based on full extrapolation, as the pivotal ALFA trial only recruited patients age 50-70 years. It is agreed that Mylotarg is considered to have a positive benefit/risk in all patients with newly diagnosed CD33-positive AML age 18 and above. This is based on disease similarity, acknowledging that any associated (known or unknown) biological differences due to age do not alter the assumed clinically meaningful benefits for this patient group. However it is difficult to acknowledge to why one would consider a treatment benefit in a patient with AML treated with Mylotarg in combination with 3+7 induction chemotherapy at the age of 18 years established, but not at the age of 17 years. The CHMP acknowledged that there are differences in the frequency of AML subtypes and common molecular aberrations between adults and children in general. However literature data and efficacy data from the meta-analyses were considered as supportive evidence to bridge efficacy assumptions to patients less than 50 years of age. The subgroup of TYA patients (15-29 years of age, n=132), showed efficacy trends similar to the overall population, if not slightly better. Regarding safety it is noted that the 30 and 60-day mortality for TYA patient (15-29 years of age) in the Mylotarg arm was none. Despite the limited number, all of this is reassuring, as it confirms what is already known, younger TYA patients tend to tolerate intensive chemotherapy better than older patients.

The term "de novo" is used in the indication to exclude secondary leukemia, i.e., AML evolving from previous myelodysplasia and forms of acute leukaemia developing after exposure to environmental or therapeutic toxins or radiation (therapy related), as reflected in the pivotal trial (see SmPC sections 4.1 and 5.1).

Additionally, it must be emphasized that APL is not included in the pivotal trial and this has been reflected in the indication.

Subgroup analyses of EFS indicated a more encouraging treatment effect with the Mylotarg combination in patients with favourable/intermediate risk cytogenetics. Reflecting on the differences observed for the different risk groups, it can be hypothesised that patients with adverse cytogenetics who receive fractioned low dose of Mylotarg seem to exhibit less deep responses, translating into shorter, not statistically significant periods of remission. It can be argued that, based on distinct biological characteristics, with the pathophysiological route causes yet to be fully elucidated, the hard-to-treat poor cytogenetic patient group is less susceptible to Mylotarg based induction chemotherapy. Adequate wording has been added in section 4.4 of the SmPC to reflect on the need to individually consider the benefit/risk profile in patients, particularly with adverse cytogenetics, once results become available.

3.8. Conclusions

The overall B/R of Mylotarg is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus decision is of the opinion that Mylotarg is not similar to Vidaza, Dacogen, Ceplene and Rydapt within the meaning of Article 3 of Commission Regulation (EC) No. 847/200.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Mylotarg is favourable in the following indication:

MYLOTARG is indicated for combination therapy with daunorubicin (DNR) and cytarabine (AraC) for the treatment of patients age 15 years and above with previously untreated, de novo CD33-positive acute myeloid leukaemia (AML), except acute promyelocytic leukaemia (APL).

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC

and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that gemtuzumab ozogamicin is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

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