CLINICAL STUDY REPORT

Clinical Trial:	Bendamustine Combined with Alemtuzumab in Pretreated Chronic Lymphocytic Leukemia (CLL) – A Phase I/II Trial with Concomitant Evaluation of Safety and Efficacy
Clinical Phase:	I/II
Protocol Number:	AGMT CLL-6
EudraCT:	2008-004987-37
Coordinating Investigator:	Prim. Univ.- Prof. Dr. Richard Greil Universitätsklinik für Innere Medizin III Universitätsklinikum der PMU Salzburger Landeskliniken Müllner Hauptstraße 48, 5020 Salzburg Tel.: +43 662/4482-2879 Fax: +43 662/4482-3400 e-mail: r.greil@salk.at
Sponsor:	AGMT gemeinnützige GmbH Prim. Univ.- Prof. Dr. Richard Greil Plenergasse 5/31, 1180 Wien Zweigniederlassung und Zustelladresse Wolfsgartenweg 31, 5020 Salzburg
Sponsor Contact:	Dr. Daniela Wolkersdorfer Tel.: +43 664 1422504 e-mail: d.wolkersdorfer@agmt.at
Study initiation date (FPI)	07-Apr-2009
Study completion date (LPO)	22-Aug-2012

Clinical Trials Lead Manager, AGMT:
Dr. Daniela Wolkersdorfer

18.09.2013 .....
date, signature

Clinical Project Manager, AGMT:
Mag. Alexandra Keuschnig

19.09.2013 .....
date, signature

Protocol Contact Person:
Univ.- Doz. Dr. Alexander Egle

11.09.2013 .....
date, signature

Coordinating Investigator:
Univ.- Prof. Dr. Richard Greil

24. Sep. 2013 .....
date, signature

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1 ETHICS

The study was conducted in accordance with GCP and all applicable local laws and the Declaration of Helsinki, including archiving of study documents.

The protocol was approved by local ethics committees and informed consent was obtained from all patients.

2 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Coordinating Investigator: Prim. Univ.- Prof. Dr. Richard Greil

List of investigators and participating sites is attached (Appendix 1).

3 RATIONALE FOR PERFORMING THE STUDY

We have previously investigated a combination of fludarabine and alemtuzumab in pretreated CLL (Flusalem Study) and found very rapid onset of responses and deep remissions as judged by MRD analysis. However, the combined T cell toxic effects of fludarabine combination were substantial and we experienced a high rate of (mostly subclinical) CMV reactivation.

A large proportion of CLL patients in Austria (and specifically in the AGMT cohorts) receive fludarabine-containing regimens as part of their first line therapy. This raises a need for the use of non cross-reactive substances in consecutive treatment regimens. One treatment option that is currently available is bendamustine, which seems to exhibit a therapeutic potential at least equal to fludarabine, while at the same time having a favourable toxicity profile. In addition bendamustine has a non crossreactive mechanism to fludarabine and cyclophosphamide (the most highly used backbone for first-line therapies).

Alemtuzumab (although applied for registration for 1st line usage) is well established as a routine therapeutic option in relapsed and refractory disease. The main shortcoming of the treatment is the fact that alemtuzumab monotherapy has a clear weakness with regard to the control of nodal disease (specifically in the situation of bulky adenopathy). A combination with a chemotherapy agent may therefore present a relevant advantage (see also our previous Flusalem Study).

Other reported protocols combining fludarabine with alemtuzumab have used much lower cummulative doses of alemtuzumab in the past (as low as 6 total weeks of therapy), while our Flusalem study evaluated the combination with 16 weeks of alemtuzumab therapy. This design derived the rationale from alemtuzumab data suggesting the importance of a relevant cumulative dose for the outcome of the treatment. In our previous study we found that continuous dosing of alemtuzumab throughout the complete chemotherapy cycle was feasible in the majority of cycles, with weekly patient visits allowing for dose reductions (pause of alemtuzumab) according to cytopenias. Taking into account the pretreated state of the cohort, the rate of G3/4 hematotoxicities and infections was relatively low [1, 2], most likely due to the extremely rapid clearance of the bone marrow CLL infiltration.

From the relevant toxicity profiles a combination of bendamustine and alemtuzumab seems feasible and may be superior to our previously investigated regimen. Furthermore given the pre-treatment constellation, an even better response than for the fludarabine combination may be foreseen, due to the lack of cross-resistance that can be expected. The aim of this study will therefore be to evaluate

the combination of bendamustine and alemtuzumab with regard to toxicity and efficacy in comparison with relevant historical controls from the previous Flusalem cohort.

4 INVESTIGATIONAL PLAN

4.1 STUDY OBJECTIVES

Primary objective

- The primary objective of this study is to determine the percentage of patients achieving a response, defined as the percentage of patients achieving complete response, partial response and stable disease/ no change upon treatment with the combination therapy according to NCI response criteria (also established according to IWCLL guidelines) upon treatment with a combination of bendamustine and alemtuzumab.

Secondary Objectives

- To determine the safety profile of bendamustine/ alemtuzumab combination therapy in terms of observed toxicities according to NCI CTCAE v3
- To evaluate the efficacy of a bendamustine/ alemtuzumab combination therapy in terms of complete response rates
- To evaluate the achievable cumulative doses of bendamustine and alemtuzumab in terms of maximum tolerated doses while on treatment
- To determine response rates in all phases by 4-colour flow cytometric MRD analysis
- To identify and characterize potential risk factors via FISH cytogenetics, CD38/ Zap-70 expression and mutational status
- To define clonal evolution by use of longitudinal FISH cytogenetics
- To define T cell subsets including prognostic EM T cells and Treg cells
- To document change upon quality of life by use of a standardized QoL questionnaire

4.2 INCLUSION CRITERIA

- Male or female patients with CD23+, CD5+, CD19+ light chain monoclonal B-CLL with treatment indication according to IWCLL criteria
- 1st or greater relapse after fludarabine or any other primary treatment regimen OR Refractory to any previous treatment and simultaneous indication for treatment according to IWCLL criteria
- Age 18 years and older
- ECOG status 0 – 2
- Life expectancy > 6 months
- Written informed consent given by the patient
- Patient using a reliable means of contraception (e.g. physical barrier, contraceptive pill or patch, spermicide and barrier, or IUD) for the duration of the study. Male patients have to use an adequate contraception method for the duration of study treatment and for 6 months following completion of study treatment. Women of childbearing potential have to use an effective method of contraception for the duration of study participation.

4.3 EXCLUSION CRITERIA

- HIV positive or positive for Hepatitis B or C
- Active uncontrolled infection

- Pregnant or lactating women
- Hypersensitivity with anaphylactic reaction to humanised monoclonal antibodies or to the excipients of any of the applied drugs (e.g. bendamustine hydrochloride or mannitol)
- Previous treatment with bendamustine
- Treatment with an experimental drug within the previous 2 months
- Patients with a history of other malignancies within 2 years prior to study entry, except for adequately treated carcinoma in situ of the cervix; basal or squamous cell skin cancer; low grade, early stage localized prostate cancer treated surgically with curative intent; good prognosis DCIS of the breast treated with lumpectomy alone with curative intent.
- Transformation to aggressive B-cell malignancy (e.g. large B-cell lymphoma, Richter's syndrome, or prolymphocytic leukemia (PLL)
- Decreased kidney function with creatinine clearance < 30 ml/min
- Patients with severe co-morbidities or major organ dysfunctions (e.g. known severe liver damage, jaundice)
- Patients with a history of severe cardiac disease; e.g. NYHA Functional Class III or IV heart failure, myocardial infarction within 6 months, ventricular tachyarrhythmias requiring ongoing treatment, or unstable angina
- Any co-existing medical or psychological condition that would preclude participation in the study or compromise ability to give informed consent, or patients unable to comply with requirements of study protocol.

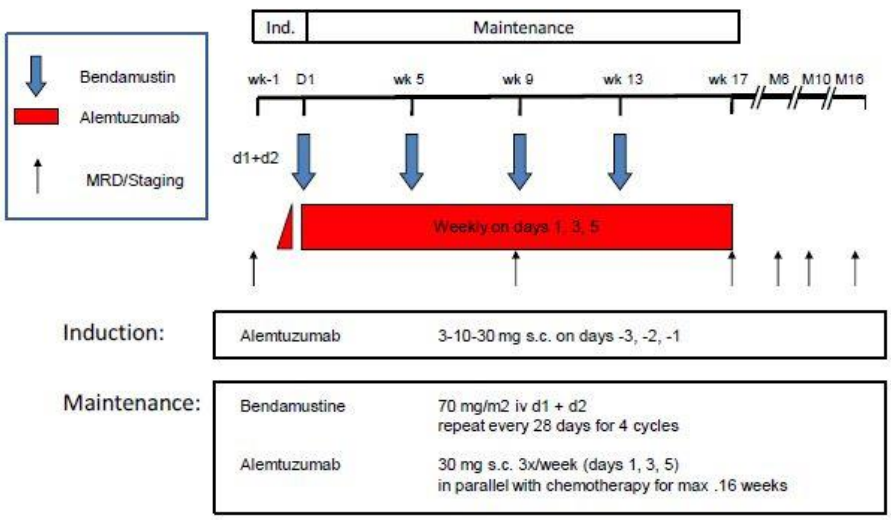
4.4 NUMBER OF PATIENTS

20 patients with pretreated B-CLL has been recruited following a Gehan design with an interim analysis for safety and efficacy after the first 7 patients. List of patients is attached (Appendix 2).

5 STUDY DESIGN

5.1 OVERALL STUDY DESIGN

Eligible patients received bendamustine as 4 courses of 70 mg/m2 on days 1 and 2 every 28 days and 30 mg alemtuzumab s.c. continuously on days 1, 3 and 5 of every week, for a maximum of 16 weeks. Safety assessments were conducted weekly; efficacy assessments including imaging were performed at months 2, 4, 6, 10 and 16. Bone marrow biopsies were performed upon CR or fixed at 6 and 16 months.



5.2 DURATION

This study started in Q2 2009. Overall recruitment was completed in Q4 2011. Overall study end (LPLV) was in Q3 2012.

6 STATISTICS

This phase I/II study followed a two stage design according to Gehan. In a first stage with 7 patients a non-response hypothesis was tested to be able to quickly refute the therapy in case of complete lack of efficacy. In addition this interim analysis was used to evaluate the therapy for safety using grade III/IV toxicities (specifically infections with special emphasis on CMV) and SAE definitions as endpoints. In addition, the observed response rate was used to identify the minimal number of additional patients that was needed to be recruited into the second phase of the trial in order for the trial to be informative regarding the initial hypothesis.

7 EFFICACY EVALUATION

7.1 INTERIM ANALYSIS

The results of the planned interim analyses were published.

In the first 7 patients response assessment at the end of treatment (4 cycles) showed 3 complete remissions (including 1 unconfirmed CR, without bone marrow sampling), 2 partial remissions and 1 stable disease. One treatment failure with early progressive disease occurred in a patient with a very complex karyotype. This patient had received 7 prior treatment lines and had been refractory to the last two lines (FCR and ofatumumab). The overall response rate was 71% and slightly lower than that reported in the previous Flusalem trial. [1].

In summary 5 out of 7 patients responded to the study treatment and according to study protocol further 13 patients were enrolled to achieve the minimum planned sample size of 20 patients.

7.2 RESPONSE EVALUATION

Results including efficacy evaluation of all enrolled patients and including the follow up phase will be published.

8 SAFETY EVALUATION

8.1 INTERIM ANALYSIS

Safety analysis was done after 7 patients, the results were published.

Regarding toxicities, almost exclusively hematological and infectious complications were observed. Grade 3 and 4 leukopenia was expected in this combination and occurred in all patients, necessitating G-CSF treatment in 6/7 patients. Two patients had grade 3 or 4 thrombocytopenia and 3 patients received transfusions. No tumor lysis syndrome was detected. The median cumulative dose of alemtuzumab was 1453 mg (maximal scheduled dose: 1483 mg), reflecting the feasibility of the regimen. Bendamustine dose had to be reduced by greater than 25% in 3/7 patients due to hematologic toxicity. CD4 T cell depletion was profound and rapid. After 2 cycles of treatment the median CD4 count was 55/µl. This was not relevantly different from the previous experience with the

combination of fludarabine and alemtuzumab. In the current study 3 of 7 patients had asymptomatic CMV reactivation, but no symptomatic infection was observed. Five of 7 patients had serious adverse events due to infections, including one fatal outcome due to pneumonia 2 months after the end of treatment. [1]

8.2 GRADE 3/4 ADVERSE EVENTS

All reported adverse events grade 3/4 are listed in Table 1. Toxicity grading was done using the CTCAE version 3.0.

Patient number	AE-term	CTC Grade	Severity scale	SAE	Reasonable related to study medication
101	NEUTROPENIA	3		no	yes
101	NEUTROPENIA	3		no	yes
101	NEUTROPENIA	4		no	yes
101	ZYTOPENIA	4		no	yes
103	LEUCOPENIA	4		no	yes
103	LEUCOPENIA	3		no	yes
103	LEUCOPENIA	4		no	yes
103	NEUTROPENIC INFECTION		severe	yes	yes
104	LEUCOPENIA	3		no	yes
104	HYPERKALEMIA	3		no	no
104	LEUCOPENIA	3		no	yes
104	PNEUMONIA		life-threatening	yes	no
104	NEUTROPENIA	3		no	yes
105	LEUCOPENIA III°	3		no	yes
105	LEUCOPENIA IV°	4		yes	yes
105	LEUCOPENIA III°	3		no	yes
105	NEUTROPENIA III°	3		no	yes
106	NEUTROPENIA	4		no	yes
107	LEUCOPENIA	4		no	yes
108	NEUTROPENIA IV	4		no	yes
109	NEUTROPENIC FEVER		severe	no	yes
109	NEUTROPENIA	3		no	yes
109	NEUTROPENIA	4		yes	yes
109	NEUTROPENIA	4		no	yes
201	PNEUMONIA	3		yes	yes
201	PNEUMONIA LEFT	3		yes	yes
201	PNEUMONIA LEFT UPPER LOBE	3		yes	yes
301	LEUKOPENIA	3		no	yes
301	LEUKOPENIA	4		no	yes
301	NEUTROPENIA	3		no	yes
302	ATYPICAL PNEUMONIA		severe	yes	no
302	THROMBOPENIA	4		no	yes
302	FEBRILE NEUTROPENIA	3		yes	no
302	REDUCED GENERAL CONDITION		severe	yes	no
302	NEUTROPENIA	3		no	no
302	EDEMA IN BOTH LEGS	3		no	no
303	LEUCOPENIA	4		no	yes
306	RASH	3		yes	no
306	LEUCOPENIA III	3		no	yes

306	LEUCOPENIA IV	4		no	yes
306	LEUCOPENIA	3		no	yes
306	LEUCOPENIA III	3		no	yes
306	LEUCOPENIA	4		no	yes
401	ANEMIA	3		no	yes
401	ANEMIA	3		no	yes
401	ANEMIA	3		no	yes
401	ANEMIA	3		no	yes
401	PANCYTOPENIA		severe	yes	yes
501	NEUTROPENIA	3		no	no
501	NEUTROPENIA	4		no	no
501	NEUTROPENIA	4		no	no
501	PNP	3		no	no
501	NEUTROPENIA	4		no	no
501	NEUTROPENIA	3		no	no
502	ANEMIA	4		yes	no
502	THROMBOPENIA	4		no	yes
502	NEUTROPENIA	4		no	yes
601	DG OF MELANOMA			no	no
601	DG OF MELANOMA			yes	no

Table 1: List of AEs grade ≥ 3, severe or life-threatening respectively

8.3 SERIOUS ADVERSE EVENTS

30 SAEs (26 cases) were reported. 22 of these were considered related to study treatment and 1 of them was classified as SUSAR. The list of SAEs including a precise description is attached (Table 2).

SAE Nr	Pat	AE-term	Start date	CTC Grade	Severity scale	SAE	SUSAR	Reasonable related	Outcome	Date resolved	Date of death
A100085	103	NEUTROPENIC INFECTION	19/10/2009		severe	yes	no	yes	completely recovered	28/10/2009	
A100112	104	PNEUMONIA	30/11/2009		life-threatening	yes	no	yes	patient died		15/12/2009
A100419	105	LEUCOPENIA IV°	31/08/2009	4		yes	no	yes	completely recovered	03/09/2009	
A100103	105	STATUS FEBRILIS (38.5 °C)	07/11/2009	2		yes	no	yes	completely recovered	09/11/2009	
A100116	105	FEBRILE INFECTION	02/12/2009		moderate	yes	no	yes	completely recovered	11/12/2009	
A100420	105	DIARRHOEA	17/12/2009		moderate	yes	no	yes	completely recovered	21/12/2009	
A100268	108	COUGH (INFECT OF NON SPECIFIC GENESIS)	07/02/2011		mild	yes	no	yes	completely recovered	10/02/2011	
A100356	109	NEUTROPENIA	12/07/2011	4		yes	no	yes	completely recovered	13/082011	
A100286	201	PNEUMONIA	04/04/2011	3		yes	no	yes	completely recovered	11/04/2011	
A100292	201	FEBRILE REACTION	18/04/2011	2		yes	no	no	completely recovered	19/04/2011	
A100299	201	PNEUMONIA LEFT	16/05/2011	3		yes	no	yes	completely recovered	24/05/2011	
A100302	201	PNEUMONIA LEFT UPPER LOBE	26/05/2011	3		yes	no	yes	completely recovered	07/06/2011	
A100051	302	ATYPICAL PNEUMONIA	05/06/2009		severe	yes	no	yes	completely recovered	26/06/2009	
A100059 a	302	FEBRILE NEUTROPENIA	14/07/2009	3		yes	no	yes	patient died		17/08/2009
A100059 b	302	REDUCED GENERAL CONDITION	14/07/2009		severe	yes	no	yes	patient died		17/08/2009

A100081	303	EXANTHEMA	26/09/2009	2		yes	no	no	completely recovered	29/09/2009	
A100134	304	FATIGUE DUE TO ANEMIA	22/02/2010		moderate	yes	no	no	completely recovered	26/02/2010	
A100216	304	PNEUMONIA, SEPSIS, EBV ASSOCIATED LYMPHOID DISSORDER	27/09/2010		moderate	yes	yes	yes	patient died		23/10/2010
A100191	304	PNEUMONIA ATYP.	05/07/2010			yes	no	yes	completely recovered	14/07/2010	
A100203	304	FATIQUE	06/09/2010			yes	no	yes	completely recovered	15/09/2010	
A100208	306	RASH	26/09/2010	3		yes	no	no	completely recovered	04/10/2010	
A100167	401	SUSPICION OF TVT	18/05/2010		mild	yes	no	no	recovered with sequelae	21/05/2010	
A100181a	401	NEUTROPENIC FEVER	15/06/2010		mild	yes	no	yes	completely recovered	16/06/2010	
A100181b	401	PANCYTOPENIA	15/06/2010		severe	yes	no	yes	completely recovered	25/06/2010	
A100190a	401	FATIGUE	01/07/2010		moderate	yes	no	yes	completely recovered	15/07/2010	
A100190b	401	DIARRHOE	01/07/2010		moderate	yes	no	yes	completely recovered	15/07/2010	
A100190c	401	NEUTROPENIA	01/07/2010		moderate	yes	no	yes	completely recovered	09/09/2010	
A100217	502	ANEMIA	25/06/2010	4		yes	no	no	completely recovered	07/07/2010	
A100218	502	FATIGUE	16/07/2010			yes	no	no	completely recovered	24/07/2010	
A100379	601	DG OF MELANOMA	09/12/2011			yes	no	no	ongoing	29/12/2011	

Table 2: List of serious adverse events

9 LITERATURE

[1] Egle et al., 2006 Planned first safety and efficacy analysis of oral fludarabine combined with subcutaneous alemtuzumab in 2(nd) line therapy of B-chronic lymphocytic leukaemia (B-CLL): The FLUSALEM study. Blood (ASH Annual Meeting Abstracts). 2006; 108: Abstract 4990.

[2] Egle et al., 2009 FINAL RESULTS OF ORAL FLUDARABINE WITH CONCOMITANT SUBCUTANEOUS ALEMTUZUMAB IN RELAPSED/REFRACTORY B-CHRONIC LYMPHOCYTIC LEUKAEMIA (B-CLL). THE FLUSALEM STUDY. Haematologica – the hematology journal (14th Annual Meeting of the European-Hematology-Association). 2009; 94: Abstract 0353

[3] Egle et al., 2010 The BENDALEM CLL 6 AGMT Study – Bendamustine Combined with Alemtuzumab in Relapsed/Refractory Chronic Lymphocytic Leukemia (CLL): Results of a Planned Interim Analysis. Blood (ASH Annual Meeting Abstracts). 2010; 116: Abstract 4633.

APPENDIX 1: List of Participating Sites

Site#	Site	Department	Principal Investigator	Country
01	Landeskrankenhaus Salzburg Universitätsklinikum der Paracelsus Medizinischen Privatuniversität	Universitätsklinik für Innere Medizin III	Prim. Univ.- Prof. Dr. Richard Greil	Austria
02	Klinikum Wels-Grieskirchen GmbH	Abteilung für Innere Medizin IV	Prim. Univ.- Prof. Dr. Josef Thaler	Austria
03	Landeskrankenhaus-Universitätskliniken Innsbruck	Univ.-Klinik für Innere Medizin V, Hämatologie und Onkologie	Univ.- Prof. Dr. Michael Steurer	Austria
04	AKh Linz	Interne 3 - Zentrum für Hämatologie und Med. Onkologie	Prim. Univ.- Doz. Dr. Michael Fridrik	Austria
05	LKH Leoben-Eisenerz	Department für Hämato-Onkologie	Univ.- Prof. Dr. Felix Keil	Austria
06	A.ö. Krankenhaus der Elisabethinen Linz	1. Interne Abteilung: Hämatologie mit Stammzellentransplantation, Hämostaseologie und medizinischer Onkologie	OA Dr. Otto Krieger	Austria
07	Landeskrankenhaus Feldkirch	Interne E (Hämatologie und Onkologie)	OA Dr. Alois Lang	Austria

APPENDIX 2: List of Patients

Patient	YOB	Inclusion	Status	Last Visit
101	1930	07.04.2009	Completed	23.08.2010
102	1928	29.04.2009	Failure	NA
103	1929	05.05.2009	Completed	11.10.2010
104	1955	08.05.2009	Completed	15.12.2009
301	1937	26.05.2009	Completed	22.09.2010
302	1942	28.05.2009	Withdrawal	17.08.2009
105	1941	26.06.2009	Completed	14.12.2010
303	1936	25.08.2009	Completed	13.08.2010
106	1941	07.09.2009	Completed	16.12.2010
304	1950	04.02.2010	Completed	23.10.2010
501	1939	22.02.2010	Completed	10.05.2011
401	1936	29.04.2010	Withdrawal	02.06.2010
502	1948	22.06.2010	Completed	05.11.2011
305	1942	28.06.2010	Completed	31.12.2010
306	1938	20.09.2010	Completed	12.03.2012
107	1948	05.11.2010	Completed	05.03.2012
108	1950	28.01.2011	Completed	11.04.2012
201	1945	15.03.2011	Withdrawal	01.06.2012
109	1941	08.07.2011	Withdrawal	22.08.2012
601	1965	18.10.2011	Withdrawal	20.04.2012