Aus der Medizinischen Klinik und Poliklinik III (Hämatologie und Onkologie),
Klinikum Großhadern der
Ludwig-Maximilians-Universität München

Direktor: Prof. Dr. Wolfgang Hiddemann

EARLY AND RISK ADAPTED THERAPY WITH FLUDARABINE IN HIGH RISK BINET STAGE A CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Jensen Imawan

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Berichterstatter	: Prof. Dr. med. M. Hallek
Mitberichterstatter	: Prof. Dr. Wolfram Dempke
Mitbetreuung durch die promovierte Mitarbeiterin	: Fr. Dr. med. Manuela Bergmann
Dekan	: Prof. Dr. med. Dr. h.c. M. Reiser, FACR, FRCR
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GLOSSARY OF ABBREVIATION

AE	Adverse Events
ANC	Absolute Neutrophil Count
CLL	Chronic Lymphocytic Leukemia
CR	Complete Remission
CT	Computer Tomography
CTC	Common Toxicity Criteria
ECOG	Eastern Cooperative Oncology Group
EFS	Event Free Survival
EORTC	European Organization for Research and Treatment of Cancer
FACS	Fluorescence Activated Cell Sorting
FCGCLL	French Cooperative Group on CLL
GCLLSG	German CLL Study Group
HR-F	High Risk-Fludarabine
HR-WW	High Risk-Watch and Wait
ITT	Intention To Treat
LDH	Lactate Dehydrogenase
LDT	Lymphocyte Doubling Time
LR	Low Risk
NCI	National Cancer Institute
nCR	Nodular Complete Remission
NHL	Non Hodgkin Lymphoma
OS	Overall Survival
PR	Partial Response
PD	Progressive Disease
PFS	Progression Free Survival
QOL	Quality of Life
ULN	Upper Level of Normal Value
SAE	Severe Adverse Events
S-$ß_2$M	Serum-$ß_2$-Microglobulin
SD	Stable Disease
S-TK	Serum Thymidine Kinase
SWOG	Southwest Oncology Group
W&W	Watch and Wait
WHC	White Blood Count
WHO	World Health Organization

1. INTRODUCTION

1.1. Chronic Lymphocytic Leukemia

1.1.1. Definition and Epidemiology

CLL is the most common type of adult leukemia in the western world, accounting for 24% of all leukemia's. The disease occurs at a median age of 65 to 70 years. Over the last years the number of younger patients under 60 years has increased, now representing about 20% of all patients (Diehl et al. 1999, Dighiero et al. 1996). It is also a disease with a frequent association in families (10 to 20% of patients have at least one first-degree relative with CLL) as well as other cancers (Catovsky 2004).

CLL is renowned for its variable natural history with respect to time to progression and response to standard cytotoxic therapies. About one third of the patients never require treatment and have a long survival; in another third, an initial indolent phase is followed by progression of the disease; the remaining third of the patients seem to have an aggressive disease at the onset and need early treatment (Dighiero et al. 2000).

The development of the Rai (Rai et al. 1975) and the Binet (Binet et al. 1981) staging systems for CLL has allowed the separation of three prognostic groups: good, intermediate and poor prognosis (Binet stages A, B, C). Both systems clearly identify patients with poor prognosis who need immediate therapy, but do not predict the risk of early disease progression in the good prognosis group. The Binet stage A comprises approximately two thirds of patients, who have a median age of 64 years and an expected median survival of >10 years, which is close to the life expectancy of a normal population matched for sex and age (Dighiero et al. 1998)

The median survival of patients with CLL ranges from 5 to 10 years, but a trend to a longer survival has become evident in the last years, partially due to earlier diagnosis (Brugiatelli et al. 2006)

1.1.2. Pathogenesis

CLL is characterized by the progressive accumulation of monoclonal peripheral (mature, but immune incompetent) B-Cells in peripheral blood, lymphoid tissues, bone marrow, and spleen. The monoclonal population of B-Cells in CLL expresses CD19, CD5, and CD23 on the surface (Chiorazzi et al. 2005).
Chromosomal aberrations occurs in 82% of CLL-cases, the most common is the deletion at 13q with 55% and it is associated with a long interval between diagnosis and the need for treatment (the treatment-free interval). The deletion at chromosome 11q is at second place with 18 %, trisomy 12q follows with 16 %, whereas the deletion at 17p (7%) and 6q (6%) are seldom.
10-17 % of the CLL-patients have a mutation at the p-53 gen, which regulates DNA repairing and apoptosis. These patients have a highly increased risk of progression, as the p-53 mutation, mostly to be found in Binet C Stadium, is the strongest negative prognosis factor for the survival (Döhner et al. 2000). Moreover, the anti-apoptotic BCL2 gene is reported to be over expressed in 65 to 70% of B-CLLs (Korz et al. 2002).

1.1.3. Clinical Symptoms

CLL is a disease which progresses slowly and it is until now not fully curable. The cardinal symptoms are: Lymph nodes swelling, hepatomegaly, splenomegaly and leucocytosis. The bone marrow suppression through the CLL-cells causes in later stages anemia and thrombocytopenia. Many patients suffer from unspecific symptoms such as fatigue and infections caused by the hematopoietic insufficiency. B-symptoms like fever, night sweats, and weight loss are in 20% of the cases at the time of diagnosis present.
Principal causes of death in CLL cases are infections (pneumonia and sepsis) and second malignancies.

1.1.4. Disease-associated Complications

Further complications in the course of the disease are AIHA (autoimmune hemolytic anemia) in 15-20% of the cases, autoimmune thrombocytopenia, and the „pure red cell"anemia (Mauro et al 2000).
A transformation into a high malignant Non-Hodgkin-Lymphoma (the so-called Richter-syndrome) or into an acute lymphoblastic leukemia occurs seldom.
CLL patients show furthermore an increased tendency to produce second malignancies such as bronchial carcinoma, malignant melanoma, and malignant tumors from the gastro-intestinal-tract (Travis et al 1992, Dighiero et al 1998).

1.1.5. Diagnostic Procedures

Following criteria, based on the ESMO (European Society for Medical Oncology) clinical recommendations (Eichhorst et al. 2008) are newer than the ones based on the Guidelines published in 1996 by NCI-WG (National Cancer Institute-Sponsored Working Group) and are important for the clinical diagnosis of CLL :

- *Peripheral Blood*
  1. Sustained increase of peripheral blood lymphocytes $\geq 5x10^9$ cells/l not explained by other clinical disorders.
  2. Predominance of small, morphologically mature lymphocytes in the blood smear.

- *Bone Marrow Examination*

A bone marrow aspirate and biopsy are generally not required to come to the diagnosis of CLL. But it is not to forget that basically CLL is a disease of the bone marrow, and it is therefore appropriate and recommended to evaluate the involvement of the bone marrow. The aspirate smear must show $\geq$30% of all nucleated cells to be lymphoid. A bone marrow examination also provides useful prognostic information by determining whether there is a diffuse or non diffuse involvement (Rozman et al. 1984), and permits an assessment of the erythroid precursors and megakaryocytes.

- *Immunphenotype*

The composite immunphenotype CD5+, CD19+, CD20+(low), CD23+, sIG low, CD79b low, FMC7- allows the distinction of most cases of B-cell type CLL from other CD5+ B-cell lymphoma.

1.1.6. Staging Systems

The fact that some patients with CLL could survive for many years without therapy and eventually succumb to unrelated diseases, whereas others have a rapidly fatal disease despite aggressive therapy led Rai and colleagues (Rai et al. 1975, 1987) and Binet and colleagues (Binet et al. 1981) to develop devised staging systems for use in assessing the extent of disease in an individual patient. Both are based on the extent of lymphadenopathy, splenomegaly, and hepatomegaly on physical examination and on the degree of anemia and thrombocytopenia in peripheral cell counts. These simple examinations are inexpensive and can be applied to every patient without special or complicated technical equipment.

These systems remain the cornerstones on which decisions regarding medical follow-up and treatment are built, but they have some limitations, the most important of which is their inability to distinguish between patients with early disease who will remain stable for many years and require no therapy and those who will develop progressive disease and require treatment, in other words, they fail to predict the course of the disease in patients in whom CLL is diagnosed in early stages (Molica 1991).

Below are described both the established staging systems of CLL according to Rai (Rai et al. 1975) and Binet (Binet et al. 1981)

Table 1: Rai staging of CLL (Rai et al. 1975)

Stage	Definition	Survival rate
Low risk		> 10 years
0	Absolute lymphocytosis >15.000/µl Bone marrow infiltration >40 %	
Intermediate risk		7 years
I	Absolute lymphocytosis with lymphadenopathy	
II	Absolute lymphocytosis with either hepatomegaly or splenomegaly (with or without lymphadenopathy)	
High risk		2,3 – 5 years
III	Absolute lymphocytosis and anemia (Hb <11 g/dl) (with or without lymphadenopathy, hepatomegaly, or splenomegaly)	
IV	Absolute lymphocytosis and thrombocytopenia (<100.000/mm^3) with or without lymphadenopathy, hepatomegaly, splenomegaly, or anemia	

Table 2: Binet staging of CLL (Binet et al. 1981)

Stage	Definition	Survival rate
Low risk		> 10 years
A	Hb ≥10 g/dl Platelet count normal Less than three areas of lymphoid enlargement*	
Intermediate risk		7 years
B	Hb ≥10 g/dl Platelet count normal Three or more areas of lymphoid enlargement*	
High risk		2,3 – 5 years
C	Hb <10 g/dl and/or Platelet count <100.000/mm^3 regardless of the number of areas of lymphoid enlargement*	

*Lymphoid areas include cervical, axillary, inguinal (≥1cm) unilateral or bilateral, liver, and spleen.

Both Rai's stage 0 and Binet's stage A consist of patients with a long survival, the median survival time exceeding 10 years. Binet's stage A comprises 66% of CLL patients (all Rai's stage 0, about $^2/_3$ Rai's stage I, $^1/_3$ Rai's stage II, and $^1/_5$ Rai's stage III) with a 78% 5-year survival rate, while Rai's stage 0 comprises 31% of CLL patients with an 82.5% 5-year survival rate (FCGCLL 1988).

An exception does exist, however, with the so called "smouldering CLL", which includes around one-third of all CLL patients in Binet stage A. Based on the fact that progression strongly affects the overall survival of the patients, also considering the importance of adapting therapy to the severity of the disease and not to forget the potential harmful effect of chemotherapeutic agents for the patients, it is understandable that it is important to define criteria to identify a subgroup of patients with Binet A (or Rai 0) with low probability of disease progression and death. In 1998, Montserrat et al first proposed the "smouldering CLL" with the criteria needed to identify it (see table below).
"Smouldering CLL" is seen as the mild variant of the CLL Binet stage A with a survival rate equivalent to an age- and sex-matched normal population; this is of clinical relevance because patients with it require therefore no treatment unless progression takes place (Montserrat et al 1988, Cheson 1996, FCLLG 1990).

Table 3: smouldering CLL; criteria

Criteria for Smouldering CLL	
1.	CLL Binet stage A
2.	Non-diffuse bone marrow histology
3.	Lymphocyte doubling time (LDT) > 12 months
4.	Hemoglobin level > 13 g/dl
5.	Absolute lymphocyte count < 30.000/ µl

1.1.7. Prognostic factors

Little is known regarding the initiation and progression of CLL (Bullrich et al. 2001). Nevertheless, several factors that can predict the clinical course have been identified (Rassenti et al. 2004, Crespo et al. 2003, Orchard et al. 2004, Damle et al. 1999, Hamblin et al. 1999). Cases in which the leukemic cells have few or no mutations in the immunoglobulin heavy-chain variable-region (IgV_H) gene or a high level of expression of the 70-kD zeta-associated protein (ZAP-70) show an aggressive course, whereas cases involving mutated CLL clones or few ZAP-70 cells show an indolent course (Chiorazzi et al. 2005). Genomic aberrations in CLL are also independent predictors of disease progression and survival (Döhner et al. 2000). However, the molecular basis of these associations is largely unknown.

Neither the Rai nor the Binet staging system can predict which patients among the early stage prognosis group will shift into progressive disease. Lymphocyte doubling time, serum levels of ß2-microglobulin, thymidine kinase (Hallek et al. 1996), soluble CD23 (Sarfati et al. 1996), as well as CD38 expression on malignant cells (Damle et al. 1999) can help predict disease activity and rapid progression, but the presence of cytogenetic abnormalities like 11q or 17p deletions in the leukemic B-cells (Döhner et al. 2000) or somatic mutations in the immunoglobulin heavy chain genes (Damle et al. 1999, Hamblin et al. 1999) are better predictors of survival. A retrospective study from the French Cooperative Group on 146 patients for whom the Ig sequence could be obtained and with a long follow-up stressed the importance of the mutational profile of Ig genes in predicting the progression in Stage A patients. Stage A patients expressing mutated Ig genes have a 75% 12-year survival and a progression free survival of 156 months, as compared to a median overall survival of 97 months and a progression free survival of 42 months in those without them.

These results suggest that a high percentage of stage A patients requiring early treatment are included within this group. However, this study also showed that a small percentage of mutated cases (about 10%) may also require early treatment and may die from disease related causes (Vanconcellos et al. 2003). These data confirm the results of a monocentric German study which represents the greatest Binet stage A population so far with 189 patients. The estimated median overall survival time of the group with IgVH homology of 98% or greater was 79 months whereas the median OS was 152 months for the group with IgVH homology less than 98% (Kröber et al. 2002).

A recent preliminary analysis performed on a common data base of the German and French cooperative study groups identified short LDT and high sTK as the strongest predictors of rapid progression. Nonetheless, a model testing the four following parameters (sTK, LDT, mutational status and unfavorable cytogenetic) was accurate to segregate low and high risk of progression when high risk was defined as two or more factors of bad prognosis.

In order to give an easier and clearer overview, below are once again listed in the table some established additional parameters which serve to help predict the prognosis of CLL patients in common.

Table 4: CLL Prognostic Parameters (Hallek 2006)

CLL Prognostic Parameters		
1.	Aberrations in chromosomes 13(13q-), 11(11q-) and 17(17p-)	Döhner 2000, Cheson et al. 1996
2.	Cytoplasmic ZAP70 in CLL cells	Moreton et al. 2005, Wendtner et al. 2004
3.	Expression of CD38 on CLL cells	Bosch et al. 2002, Anaissie et al. 1998
4.	LDT (Lymphocyte Doubling Time)	Montserrat et al. 1986, Plunkett et al. 1993
5.	Serum $ß_2$-microglobulin concentration	Rai et al. 2000
6.	Serum levels of soluble CD23	Johnson et al. 1996, Leporrier et al. 2001
7.	Serum thymidine kinase activity	Hallek et al. 2004
8.	Somatic hypermutations of the immunoglobulin V_H-gene region	Bosch et al. 2002, O"Brien et al. 2001

1.1.8. Treatment Options

A therapy is recommended to be commenced in CLL patients with Binet stage C or Rai stage III/IV and also in patients having lower CLL stage (Binet A & B, Rai II) with active disease (for example B-symptoms, rapid lymphocyte doubling time, anemia or thrombocytopenia). Active disease should be clearly documented for protocol therapy and the following criteria must be met (Cheson 1996):

1. A minimum of any one of the following disease-related symptoms must be present:
   (a) Weight loss $\geq$10% within the previous 6 months
   (b) Extreme fatigue (i.e. ECOG PS 2 or worse; cannot work or unable to perform usual activities)
   (c) Fevers of greater than 100.5°F for $\geq$2 weeks without evidence of infection
   (d) Night sweats without evidence of infection
2. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia
3. Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroid therapy
4. Massive (i.e. >6 cm below the left costal margin) or progressive splenomegaly
5. Massive nodes or clusters (i.e. >10 cm in longest diameter) or progressive lymphadenopathy
6. Progressive lymphocytosis with an increase of >50% over a 2-month period, or an anticipated doubling time of less than 6 months
7. Marked hypogammaglobulinemia or the development of a monoclonal protein in the absence of any of the above criteria for active disease is not sufficient for protocol therapy

Patients with smouldering CLL should not (as already stated above) be offered therapy until they exhibit clear evidence of disease progression.

Chlorambucil

Chlorambucil, an aromatic derivative of nitrogen mustards taken orally, was first introduced for the treatment of CLL in 1952 and has since then established itself as the most common treatment for CLL for around 30 years (Foon et al. 1987), although its beneficial role has never been proved. A large randomized clinical trial (FCGCLL 1990) compared the usage of daily Chlorambucil with the "watch and wait" strategy. It could be demonstrated that Chlorambucil has a beneficial effect in terms of 9-month remission and of preventing disease progression to stage B. However, no benefit was observed in terms of overall survival, and a harmful effect can be evoked since Chlorambucil seems to favor the occurrence of epithelial cancers and increase resistance to further treatment among those patients with disease progression. It is also clear that Chlorambucil is able to prevent disease progression to stage B but it does not appear to affect progression to stage C.

The appearance of purine analogs has opened a new page in the treatment of CLL. Two large randomized studies (Rai et al. 2000 and Eichhorst et al. 2003) and a metaanalysis of randomized studies (Zhu et al. 2004), proved that Fludarabine, used as single agent and compared with Chlorambucil at standard doses, induced higher complete response rates and improved patients' quality of life, irrespectively of age. Even the combination of Cyclophosphamide, Adriamycin, and Prednison (CAP) is still inferior in terms of the number of complete remissions and the duration of remission in first- and second-line treatment of CLL (FCGCLL 1996, Leporrier et al. 2001). However, none of these studies could demonstrate a benefit on median overall survival.

One study initiated by the German CLL study group (CLL-5-study) intended to evaluate the effect of Fludarabine versus Chlorambucil in first line therapy of elderly patients above the age of 65 years with advanced CLL, because it was not clear if elderly or physically non-fit patients would benefit from more intense first line strategies as well (this was the reason why Chlorambucil is still widely used in first line therapy of elderly CLL patients). The result showed that elderly patients have no significant clinical benefit from first line therapy with Fludarabine in comparison to Chlorambucil. Despite being able to induce higher CRR and ORR Fludarabine failed to show any benefit in terms of PFS and OS. Chlorambucil and Fludarabine therefore represent themselves as similar potent first-line treatment options for elderly CLL patients (Eichhorst et al. 2009).

Fludarabine

Fludarabine (Fludara®) is distributed in Germany by the company Bayer Schering Pharma AG. It is the best studied purine analogue used for the treatment of CLL. Fludarabine is available in injection vials containing 50 mg dry substance. A fluorinated adenine (active ingredient: Fludarabine-dihydrogen phosphate) and thus by definition an antimetabolite, Fludarabine acts by inhibiting DNA synthesis. The recommended adult dose of Fludarabine is 25 mg/m^2 administered intravenously over a period of approximately 30 minutes daily for five consecutive days. Each 5 day course of treatment should commence every 28 days. Dosage may be decreased or delayed based on evidence of hematologic or non-hematologic toxicity. Physicians should consider delaying or discontinuing the drug if neurotoxicity occurs. Dose reduction (20%) is needed for adult patients with moderate impairment of renal function (creatinine clearance 30-70 ml/min/1.73m^2). Severely impaired renal function (creatinine clearance less than 30 ml/min/1.73m^2) prohibits the use of Fludarabine.

The major side effects are myelosuppression with neutropenia and immunosuppression and reduction of the T-helper cells, at higher doses CNS toxicity. Mucositis/stomatitis, diarrhea, anorexia, nausea, vomiting, and elevation of transaminases are also possible (Adkins et al. 1997, Pott et al. 1997).

Myelosuppression may be severe, cumulative, and may affect multiple cell lines. Bone marrow fibrosis occurred in one CLL patient, several instances of trilineage bone marrow hypoplasias or aplasias resulting in pancytopenia, sometimes resulting in death, were also reported. Life threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia / thrombocytopenic purpura (ITP), Evan's syndrome, and acquired hemophilia were also reported. Steroids may or may not be effective in controlling these hemolytic episodes. The majority of patients re-challenged with Fludarabine developed a recurrence in the hemolytic process. The mechanism(s) which predispose patients to the development of this complication has not been identified. Discontinuation of therapy with Fludarabine is therefore recommended in case of hemolysis.

Serious and sometimes fatal infections, including opportunistic infections and reactivations of latent viral infections such as VZV (herpes zoster), Epstein-Barr virus, JC virus (progressive multifocal leukoencephalopathy) have been reported in patients treated with Fludarabine.

When used at high doses in dose-ranging studies in patients with acute leukemia, Fludarabin was associated with severe neurologic effects, including blindness, coma, and death. This severe CNS toxicity occurred in 36% of patients treated with doses approximately four times greater (96 mg/m^2/day for 5-7 days) than the recommended dose. Similar severe CNS toxicity, including coma, seizures, visual disturbances, optic neuropathy, optic neuritis, blindness, objective weakness, agitation and confusion have been reported in patients treated at doses in the range of the dose recommended for CLL.

Skin toxicity, consisting primarily of skin rashes has been reported. Erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, and pemphigus have also been reported, with fatal outcomes in some cases. Worsening or flaring up of pre-existing skin cancer lesions, as well as new onset of skin cancer was also noted.

Based on its mechanism of action, Fludarabine phosphate can cause fetal harm when administered to a pregnant woman. Fludarabine phosphate was embryolethal and teratogenic in both rats and rabbits. If it is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Woman of childbearing potential should be advised to avoid becoming pregnant. Women of childbearing potential and fertile males must take contraceptive measures during and at least for six months after cessation of treatment with Fludarabine.

Retrospective cohort studies showed that Fludarabine did not increase the rate of secondary cancers, as compared to the rate in untreated patients. However, Fludarabine has some drawbacks, such as the increased risk of opportunistic infections, the occurrence of Fludarabine-associated autoimmune hemolytic anemia (AIHA), and decreased stem cell mobilization although data's supporting this remain controversial.

Actual recommendations suggest that patients without co-morbidity should receive Fludarabine plus Cyclophosphamide, whereas elderly and unfit patients with co-morbidity (in particular renal insufficiency) Chlorambucil or alternatively a dose-reduced Fludarabine monotherapy, which appear to be less myelotoxic than the FC combination. Younger patients with unfavorable biological risk factors should be considered for high-dose chemotherapy and autologous or allogeneic stem cell transplantation within approved clinical trials. Patients who are refractory or relapsed after first-line Chlorambucil should receive Fludarabine-containing regimens. Patients either relapsing soon (within one year) after or not responding to Fludarabine-based chemotherapy should be considered for

schedules including non-cross-reactive agents, such as Alemtuzumab, possibly followed by high-dose chemotherapy and autologous transplantation in the context of a clinical trial or by allogeneic stem cell transplantation (Brugiatelli et al. 2006).

Considering the possible, not to be underestimated adverse events due to Fludarabine administration, patients undergoing therapy should be closely observed for signs of hematologic and non hematologic toxicity. Periodic assessment of peripheral blood counts is recommended to detect the development of anemia, neutropenia and thrombocytopenia.
In patients with impaired state of health, Fludarabine should be given with caution and after careful risk/benefit consideration. This applies especially for patients with severe impairment of bone marrow function (thrombocytopenia, anemia, and/or granulocytopenia), immunodeficiency or with a history of opportunistic infection. Prophylactic treatment should be considered in patients with increased risk of developing opportunistic infections.

In a clinical investigation using Fludarabine in combination with Pentostatin (deoxycoformycin) for the treatment of refractory chronic lymphocytic leukemia, there was an unacceptably high incidence of fatal pulmonary toxicity. The use of Fludarabine in combination with Pentostatin is therefore not recommended.

Response rates were further improved by Fludarabine-containing combination regimens, especially by the Fludarabine plus Cyclophosphamide combination, which was shown to have a higher efficacy with a higher rate of overall response rate and more complete remissions than Fludarabine alone also in subgroups at higher risk, such as patients with del(17) or p53 mutation. The same study also showed that the FC (Fludarabine and Cyclophosphamide) has an edge against Fludarabine alone regarding the progression-free survival time and the treatment-free survival time. Thus far, no difference in median overall survival has been observed, suggesting that a longer median observation time (in this study 22 months) is needed to determine a survival benefit for any first-line treatment (Eichhorst et al. 2006). 2 other publications in 2007 by Catovsky et al. (Fludarabine / Chlorambucil vs. Fludarabine + Cyclophosphamide) and Flinn et al. (Fludarabine vs. Fludarabine + Cyclophosphamide) confirmed the data of the trial conducted by Eichhorst et al. FC was since then the standard first-line therapy in CLL patients who are physically fit until the era of RFC (Rituximab, Fludarabine, and Cyclophosphamide), which began 2008 and was marked by the emergence of Rituximab as a powerful option.

Rituximab (Mabthera®), an anti-CD20 monoclonal antibody was a surprise newcomer in the so far established therapy regime. Somewhat surprisingly, combinations of Rituximab with other chemotherapy agents have proven to be very efficacious, thus, could possibly prevail to be the new standard in the CLL therapy in the future. There is preclinical evidence for synergy between Rituximab and Fludarabine (Di Gaetano et al. 2001). A large phase II trial conducted at the MD Anderson Cancer Center showed that the combination of FCR (Fludarabine, Cyclophosphamide, and Rituximab) showed superior response rates and complete remission rate than the FC alternative (Keating et al. 2005). This result, together with some other studies suggests that Rituximab plus Fludarabine-based therapies, in particular the FCR combination represent a significant advance in the therapy for CLL.

CLL8-trial initiated by the German CLL study group confirms those previous trials and proved the efficacy of the highly anticipated FCR by comparing it with FC in totally 817 patients within a randomized trial. The trial found that treatment with FCR chemoimmunotherapy improves response rates and progression free survival when compared to the FC chemotherapy. The result is decisive, as it sets now a new standard in CLL therapy for physically fit patients, which is FCR (Fludarabine, Cyclophosphamide, and Rituximab) (Hallek et al. 2008)

1.2. Rationale and study purpose

The fact that today CLL is increasingly diagnosed within young patients (11% are younger than 55 years) and in early stages shows a very inhomogeneous patient group regarding its illness course and progression risk. It has also been agreed, that patients with smouldering-CLL will not be treated because of their almost normal survival rate. It is possible to identify using new parameters a risk-group under the remaining stage A patients, which is very likely to suffer progression within 12 months (Hallek et al. 1997).

Based on the hypothesis that qualitatively good complete remission leads to prolonged free of illness interval and overall survival, this study aims to verify whether a therapy with Fludarabine, today the backbone substance for CLL therapy, would prevent or at least delay the progression of the high risk patients in early stages of CLL. The CLL-1 protocol (a multicentre, risk stratified and randomized Phase III study) was therefore designed. The reason for the risk stratification is first of all to prevent needless therapy for patients with indolent CLL and secondly to make sure that only patients who anyway within 2 years should receive treatment, would be treated. (Hallek et al. 1997)

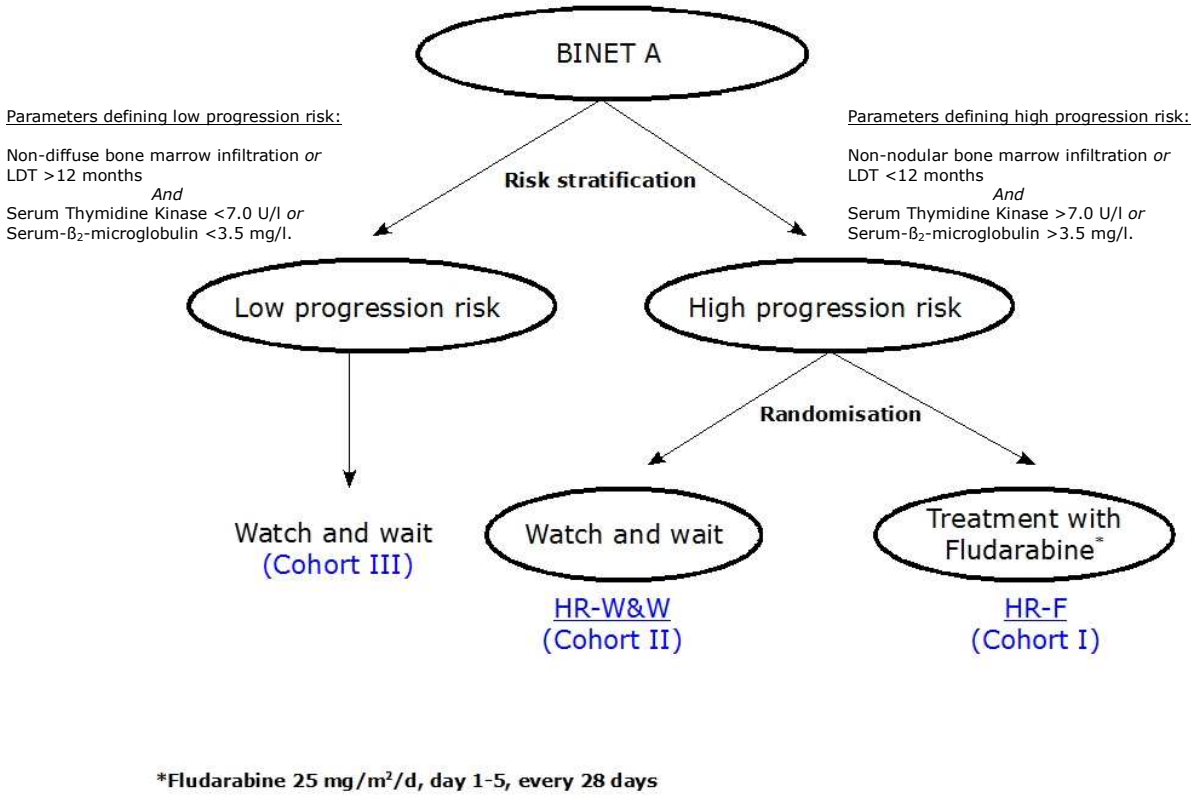
Patients who are stratified within the HR (High risk) group are in a second step randomized to "upfront" Fludarabine or "Watch and Wait" strategy until progressive disease occurs. The quality of the remission, the progression free survival, the overall survival will all then be evaluated.

2. MATERIALS AND METHOD

2.1. CLL-1-Protocol: Study Design

Below is the simplified design of the CLL-1 study described, the complete flow sheet of the study is in appendix 2 (see chapter 9.2).

Fig.1. Simplified study design



This is a multicentre, risk stratified trial evaluating the therapy and prognosis factors of CLL in Binet stage A patients. It consists of one group which includes all patients with low progression risk, and another group for all patients with high progression risk. The group consisting of high risk patients would then be divided into 2 cohorts after a phase III randomization (1:1) in order to compare the Fludarabine therapy with the standard option, which is observation without therapy (the so called "Watch and Wait" strategy).

Following questions should be answered:

1) Is it possible to extend the progression free survival and the overall survival of CLL patients in Binet A through an early, risk adapted Fludarabine therapy?

2) How significant is the role of the new prognosis factors for CLL patients in Binet A?

2.1.1. Protocol for risk-adapted therapy of CLL in the Binet stage A

The Binet A patients are assessed in terms of their progression risk with bone marrow histology (infiltration type), lymphocyte doubling time (LDT), serum thymidine kinase, and the serum-$ß_2$-microglobulin. High progression risk is defined as:

> Not-nodular bone marrow infiltration *or* LDT < 12 months
>
> *and*
>
> Serum Thymidine Kinase > 7.0 U/l *or* Serum-$ß_2$-microglobulin > 3.5 mg/l

Patients with high progression risk would then after randomization either receive treatment with Fludarabine in a controlled trial (cohort I) or only be observed (cohort II), whereas the patients without high progression risk according to the definition above (cohort III) would not be treated but observed.

Within this analysis only patients with high risk for progression are assessed.

2.1.1.1. Objectives

- Primary Objective
  Progression free survival

- Secondary Objectives
  - Overall survival
  - Therapy efficacy (CR, PR, SD, Progression, remission duration)
  - Adverse events related to treatment (treatment safety)
  - Occurrence of infections
  - Quality of life

2.1.2 Assessment of the significance of new prognosis factors for CLL patients in Binet stage A

Following parameter assessments are needed at inclusion time to the study in order to analyze the prognosis factors:

- Age
- Gender
- Binet-stage
- Rai-stage
- Peripheral blood lymphocyte
- Hemoglobin
- Blood platelets
- Bone marrow histology and cytology
- Lymph node histology, when lymph node enlargement with at least 2 cm diameter exists and a punction (with small risk) is possible (including assessment of the histological subtype of B-CLL and B-CLL with plasmacellular differentiation) (Harris et al 1994)
- Lymphocyte doubling time
- Serum immunoglobulin level (IgA, IgM, IgG)

- Serum-ß$_2$-microglobulin
- Serum thymidine kinase
- Serum LDH
- Serum albumin
- Number of lymph node enlargements through clinical examination plus chest X-ray plus abdominal ultrasound
- Hepatomegaly
- Splenomegaly
- Molecular cytogenetic with FISH method (e.g. p53-mutation, 11q-deletion)
- B-symptoms
- ECOG performance status

2.1.2.1. Objectives

- Primary objective
  Progression free survival

- Secondary objective
  Overall survival

2.2. Study Population

2.2.1. Inclusion Criteria (Cheson et al. 1996)

1. Established diagnosis of B-CLL in Stage Binet A.
   The diagnosis criteria for B-CLL are:
   - Permanent (>3 months) absolute lymphocytosis >5000/µl in the peripheral blood.
   - >30% mature lymphocytes in bone marrow.
   - Immunphenotype confirmation of the diagnosis in the form of low expression of surface immunoglobulin, CD5+, CD19+, CD20+, CD23+, inclusive CD5/CD19 (Matutes et al 1994, Rozman et al 1995).
2. First diagnosis within 3 years before inclusion in study.
3. No previous CLL therapy.
4. Age between 18 and 75 years old.
5. ECOG performance status 0-2.
6. No insufficiency of important organ functions.
7. Written informed consent of patient for participation in the study.
8. Presence of all necessary parameters for risk stratification.
9. Willingness to accept contraception if randomized to cohort I during the therapy.

2.2.2. Exclusion Criteria (Cheson et al. 1996)

1. Age under 18 years old and over 75 years old.
2. ECOG performance status >2.
3. Clinically apparent immune hemolysis.
4. Positive Coombs test.
5. Clinically apparent immune thrombocytopenia.
6. Active secondary malignancy.
7. Chemotherapy/radiotherapy for any neoplastic disease other than B-CLL prior to the study.
8. HIV-infection.
9. Pregnancy and/or nursing.
10. Participation in another trial before and during the study.

11. Concurrent severe diseases, as listed following:
    - Clinical apparent heart insufficiency
    - Cardiomyopathy
    - Myocardial infarction within the past 6 months prior to the study
    - Severe chronic obstructive lung disease with hypoxemia
    - Severe diabetes mellitus
    - Hypertension difficult to control
    - Infection hard to control
    - Impaired liver function with serum bilirubin >2mg/dl and/or Transaminase over 3-times of ULN
    - Impaired renal function with creatinine >3 mg/dl
    - Clinically apparent cerebral dysfunction
    - Severe neurological or psychiatric diseases which hinder a good cooperation

2.3. Protocol Schedule

2.3.1. Procedures of risk stratification and randomization

The first step following after the registration is the assessment of lymphocyte doubling time (LDT) by the study center; at least 4 blood lymphocyte counts are therefore needed. Bone marrow histology is also important to evaluate the infiltration. These two parameters, together with serum thymidine kinase and serum-$ß_2$-microglobulin, would then be used to perform risk stratification in order to determine whether the patients high progression risk have or not. Patients stratified to the high risk group will furthermore be randomized between the treatment arm (cohort I) and the watch and wait arm (cohort II).

2.3.2. Treatment Schedule

2.3.2.1. Remission Criteria

The remission status is evaluated according to a modified version of the NCI sponsored Working Group guidelines (Cheson 1996, 1998), therapy results are compared with initial status at study inclusion, not at the beginning of therapy.

Complete Remission (CR)

Following criteria must for at least 2 months be met:

- Lymph nodes <1 cm (previous lymph nodes enlargement no more detectable), evaluated by clinical examination, chest x-ray, abdomen ultrasound and in case of doubt CT-scan.
- Normal liver and spleen sizes.
- Absence of disease related symptoms.
- Peripheral blood lymphocytes <4.000/µl.
- Peripheral blood neutrophils $\geq$1.500/µl.
- Platelets $\geq$100.000/µl.
- Hemoglobin >11g/dl (untransfused).
- Bone marrow lymphocytes <30%.

Patients with CR, who however still has bone marrow nodules (histological), are given the remission status nCR (nodular complete remission).

Partial Remission (PR)
The definition of a partial remission requires **all** of the following features (if abnormal prior to therapy) for at least 2 months:

- ≥50% decrease in peripheral blood lymphocyte count from the pretherapeutic staging.
- ≥50% reduction in lymphadenopathy.
- ≥50% reduction of the size of the enlargement of liver and/or spleen.

As well as **one or more** of the remaining features:

- ANC ≥1.500/µl or 50% improvement over baseline.
- Platelets >100.000/µl or 50% improvement over baseline.
- Hemoglobin >11 g/dl or 50% improvement over baseline without transfusions.

Progression (PD) or relapse
Progression occurs when at least one of the following criteria is met:

- Progression to symptomatic stage Binet B or stage Binet C.
- Progressive lymphocytosis with an increase of ≥100% (doubling) of the absolute lymphocyte count.
- Transformation into a highly aggressive NHL (Richter-syndrome) or into Prolymphocytoid leukemia (>55% Prolymphocytes).
- 25% size enlargement of liver and spleen or new evidence of previously not detected hepato/splenomegaly (confirmed by ultrasound examination).
- Evidence of new lymph node enlargement (at least 1 cm diameter) in 2 consecutive examinations with at least 2 weeks interval between them.
- Clear lymph node enlargement, at least to the double (100%) of the previous size in 2 consecutive examinations with at least 2 weeks time in between. One of the lymph nodes must have at least 2 cm diameter.

Stable Disease (SD)
Stable Disease (SD) is defined as the remission status if the criteria for Complete Remission (CR), Partial Remission (PR), and Progression (PD) are not met.

2.3.2.2. Guideline for concomitant supportive therapy
Patients should receive full supportive care including transfusions of blood and blood products, antibiotics, anti-emetic drugs etc when they are reasonably needed. Concomitant antiemetic treatment with Metoclopramide (e.g. Paspertine) 2 x 1 vial i.v. daily during the Fludarabine therapy is highly recommended. Common, adequate hygiene measures are sufficient for infection prophylaxis. Prophylactic administration of antibiotics and G-CSF (e.g. Neupogen®) or other hematopoietic growth factors are not required.

2.3.2.3. Dose Modification
Following adverse effects indicates for dose reduction of Fludarabine during the next cycles:

- Occurrence of severe common infections during the neutropenia phase after the application of Fludarabine: 25 % dose reduction
- Occurrence of severe neutropenia (if it didn't occur before therapy):
  Nadir of neutrophile < 500/µl: 50 % dose reduction
  Nadir of neutrophile < 1000/µl: 25 % dose reduction
- Occurrence of severe thrombocytopenia (if it didn't occur before therapy):
  Nadir of platelet count < 20.000/µl: 50 % dose reduction
  Thrombocytopenia and simultaneous bleeding complications: 50 % dose reduction

2.3.2.4. Criteria for therapy termination

Treatment with Fludarabine is stopped precociously in case of:

- Lack of response or disease progression after 2 cycles.
- Occurrence of severe adverse effects, especially life threatening complications.

No further chemotherapy is scheduled after the discontinuation until reasonable indications show up.

2.3.3. Procedures for patients having the "watch and wait" strategy (cohort II & III)

Patients stratified to the group with low progression risk (cohort III) and therefore counted among the control arm or patients randomized to the control arm of the study despite having high progression risk (cohort II) receive no chemotherapy, but would only be evaluated according to the „watch and wait" strategy until the occurrence of therapy indications.

2.3.4. Treatment guidelines for progressive/relapsed patients

Indications for treatment due to progression/relapse are defined as following (Hellriegel 1997):

- Progression to stage Binet C or symptomatic stage Binet B.
- Progression with continuous increase of absolute lymphocyte count $\geq$100% and/or increase of leukocyte count >300.000/µl.
- Development of severe B-symptoms.

Patients included in the „watch and wait"arm (cohort II and III) would, in case of therapy indications, be included into the CLL4-Study (Fludarabine vs. combination of Fludarabine-Cyclophosphamide) if they are under 65 years old. Patients over 65 years old would be included into CLL5-Study (Fludarabine vs. Chlorambucil).

On the other hand, cohort I patients with progression or relapse after Fludarabine therapy should receive further treatment according to the CLL6-Protocol (therapy combination consisting Fludarabine, Cyclophosphamide, and Mitoxantrone).

In case the patients reject the therapy above, it is allowed to treat them with Fludarabine, unless they have history of no-response under Fludarabine therapy.

2.3.5. Interim staging, final staging, and follow-up evaluation in cohort I

Interim staging is scheduled after 2 and 4 cycles of therapy (month 2 and 4), whereas the final staging is scheduled after 6 cycles, more precisely it is scheduled 2 months after the last Fludarabine cycle (month 8). Follow-up evaluations after the final staging are performed at months 9, 12, 18, and 24 (end of study) and annually thereafter.

2.3.6. Follow-up evaluation in cohort II and III

For patients belonging to cohort II and III the follow-up evaluations are scheduled at months 3, 6, 9, 12, 18, 24 and annually thereafter.

2.4. Statistic Analysis

2.4.1. Progression Free Survival (PFS) and Overall Survival (OS)

The analysis of the progression free survival and overall survival will be estimated using the Kaplan-Meier product-limit estimator. Log-rank test would also be used to analyze the survival curves. The level of significance (one sided test) is at 5 %.

2.4.2. Efficacy of the Fludarabine therapy

The efficacy of Fludarabine will be evaluated on the patients who did receive Fludarabine. The analysis of the response rate (Occurrence of CR, PR, PD, and Stable Disease) is descriptive.

2.4.3. Adverse Events (AE) related to treatment

The analysis of the occurrence and severity of adverse events related to the treatment is also descriptive and any dose adjustment is always to be considered. All patients who have received at least one infusion of Fludarabine should be evaluated.

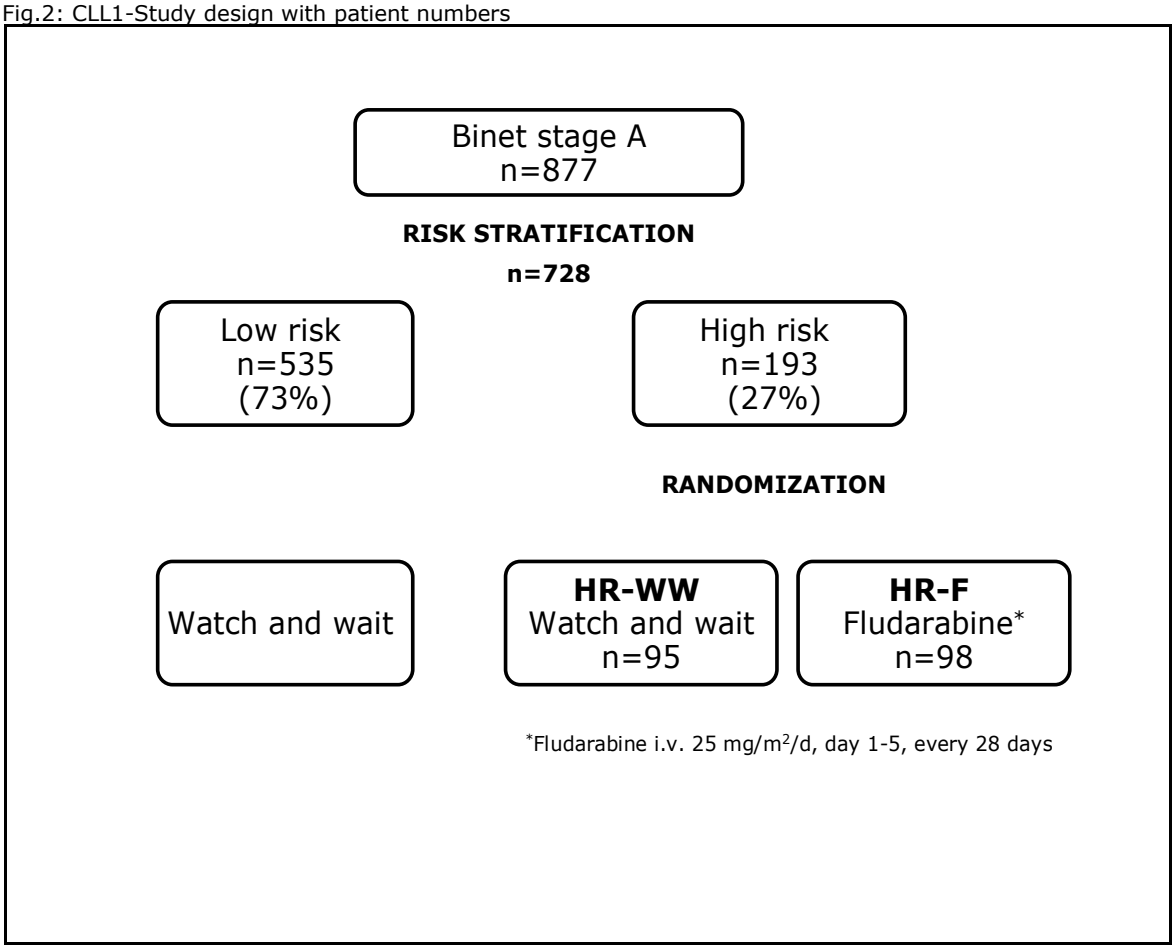
3. RESULTS

3.1. Patients Characteristics

A total of 877 patients were registered into the study between 1997 and 2004, 728 of them fitted to the inclusion and exclusion criteria, thus then underwent risk stratification, the youngest patient at the beginning of the trial was 32 years old, the oldest 81 years old, the average age was 59,7 years old. 60.4% were male patients. 535 Patients (73%) were classified into the low-risk group (cohort 3), the other 193 (27%) into the high-risk group (cohort I, 98 patients and cohort II, 95 patients)

Below is once again the CLL-1 study design placed, this time with the numbers of patients from each cohorts.

Fig.2: CLL1-Study design with patient numbers



The following table gives an overview of the characteristics from the high risk patients (n=193) included in this analysis.

Table 5: Patients characteristics on trial begin

Characteristics	HR-F	HR-WW	p-value
Age (y; median)	60	62	n.s.
Sex (male)	61.2 %	72.6 %	n.s.
ECOG (PS)* 0 ≥1	85.2% 14.8%	92.3% 7.7%	n.s.
B-symptoms	9.3%	10%	n.s.
Comorbidities	39.8%	45.2%	n.s.
LN cervical	33%	28.4%	n.s.
LN axillar	22.1%	14.1%	n.s.
LN inguinal	15.9%	6.7%	n.s.
Splenomegaly	11.8%	10.3%	n.s.
Hepatomegaly	3.7%	4.1%	n.s.
WBC count, $x10^9$/L (range)	36.2 (6.4-249.4)	26.8 (4.6-184)	n.s.
ALC, $x10^9$/L (range)	30.5 (3.2-244.4)	21.8 (0-162.8)	n.s.
HGB level, g/dL (range)	14.1 (10.5-18.1)	14.2 (10.7-17.3)	n.s.
PLT count, $x10^9$/L (range)	171 (102-352)	195 (111-418)	n.s.
BM Lymph, % (range)	67% (5-100)	70% (0-100)	n.s.

* Also see: Appendix

Comparison of patients in both arms indicated no significant difference regarding the main clinical features and the risk categories.

3.2. Dose of study medication and duration of therapy

Patients randomized into the treatment arm (cohort I) will receive following medication:

Fludarabine 25 mg/m^2/day as a 30-minutes i.v. infusion from day 1-5 Cycle repeat on day 28

Study medication will be given for at least 2 cycles, but not more than 6 cycles. Interim stagings are scheduled after 2 and 4 cycles of therapy (month 2 and 4). The number of treatment cycles is based on the following guideline:

- Complete Remission (CR) after 2 cycles: Administration of another 2 cycles (totally 4) Fludarabine
- Complete Remission (CR) first after 4 cycles: Administration of another 2 cycles (totally 6) Fludarabine
- Partial Remission (PR) after 4 cycles : Administration of another 2 cycles (totally 6) Fludarabine
- Progression after 2 or more cycles: Therapy stop
- No change after 2 cycles: Administration of another 2 cycles Fludarabine
- No change after 4 cycles: Therapy stop

Each treatment cycle takes one month; the maximum duration of therapy for cohort I patients is thus 6 months long.

3.3. Response to treatment with Fludarabine (also see chapter 2.3.2.1)

Table 6: Response rate to Fludarabine

Response to Fludarabine (n=74)				
ORR	*CR*	*PR*	*SD*	*PD*
85%	32% 24 pts	53% 39 pts	7% 5 pts	8% 6 pts

The evaluation of the response according to the NCI-WG guidelines of 1996 to Fludarabine was possible for totally 74 patients who really received the therapy. An overall response rate of 85 % from these patients was reached, with higher partial remission rate (PR 53%) than the complete remission rate (CR 32%).

3.4. Side Effects of Fludarabine

Being used to help evaluate the side effects of Fludarabine was the CTC (Common Toxicity Criteria), a standardized classification of side effects used in assessing drugs for cancer therapy. Most US and UK drug trials base their observations on this system which has a range of grades from 1-5. Specific conditions and symptoms may have values or descriptive comment for each level, but the general guideline is as following:

1^0-mild
2^0-moderate
3^0-severe
4^0-life threatening
5^0-lethal

The evaluation was done after each cycle of Fludarabine administration. Below are listed some of the severe side effects of CTC grade 3 or 4.

Table 7: Review of the side effects of Fludarabine

Main Toxicities (CTC° 3 and 4)			
CTC⁰	***3⁰***	***4⁰***	***Total***
WBC (White Blood Count)	7	0	7
Infections	3	0	3
HGB (Hemoglobin level)	1	1	2
PLT (Platelet level)	1	1	2
Thrombosis	2	0	2
Granulocytes	1	0	1
Systemic panepidermic lesion	1	0	1
Arrhythmia	1	0	1

Hypokaliaemia	1	0	1
Lymphocytes	1	0	1
Bilirubine	1	0	1
Appetite loss	1	0	1
Urinary retention	1	0	1

Categorically seen there are totally 4 main aspects of side effects, which are described in the table below.

Table 8: Fludarabine side effects by category (CTC^0 1-4)

Side Effect	Hematological	Infections	Gastrointestinal	Neurologic
Number	68	29	29	12
Percentage	37%	16%	16%	7%

Hematological side effects were the most among all the side effects, ranging from anemia, leucopenia, neutropenia and thrombopenia, followed by infections and gastrointestinal side effects.

3.4.1. Hematological side effects

One of the most common side effects of Fludarabine is myelosupression (Bergmann 1993, Wijermans 1993). Furthermore possible side effect is an increase of the occurrence of Fludarabine-associated autoimmune hemolytic anemia (AIHA), from 4 till 21 % of the patients. Autoimmune thrombopenias (AITP) are also possible (Mauro et al 2000, Hamblin et al 1986, De Rossi et al 1988, Byrd et al 1994, Di Raimondo et al 1993, Myint et al 1995).

Anemia was the least among the hematological side effects with 9%. Leucopenia had a higher rate with 56%, while thrombopenia had 35%.

3.4.2 Gastrointestinal side effects

Gastrointestinal side effects, for example nausea, vomitus, diarrhea, stomatitis remain as common side effects of the most antineoplastic drugs. The gastrointestinal side effects were mainly mild. There were no gastrointestinal symptoms of CTC grade 3 or 4; all gastrointestinal side effects were only CTC grade 1 or 2, thus representing themselves as mild till moderate side effects.

Table 9: Gastrointestinal side effects

Gastrointestinal side effects				
CTC^0	*1^0*	*2^0*	*3^0*	*4^0*
Nausea	5	7	0	0
Vomitus	1	8	0	0
Diarrhea	1	3	0	0
Stomatitis	3	0	0	0

3.4.3. Neurological side effects

Occurrence of neurotoxicity through Fludarabine therapy in CLL patients were described in some cases (Zabernigg 1994, Johnson 1994).
Listed in the CTC-criteria among the neurologic side effects are for example sensory, sleep disorders, motorics, consciousness, coordination, affect, headache, vertigo, behavior disorder, hearing and sight.

Table 10: Neurological side effects

Neurological side effects				
CTC^0	*1^0*	*2^0*	*3^0*	*4^0*
Sensory	1	0	0	0
Motorics	1	0	0	0
Affect	1	0	0	0
Headache	4	2	0	0
Vertigo	4	0	0	0
Sight	1	0	0	0

Most of the neurological side effects were in the category CTC grade 1, which represent mild symptoms; none were in the higher CTC grade (3^0 or 4^0). Headaches and vertigo were the most among them.

3.4.4. Other side effects

Apart from the common side effects mentioned above, there are also other uncommon/seldom side effects which occurred in a small number of the patients, such as coughing, skin lesions, elevation of liver enzymes, arrhythmia, thrombosis, dyspnoea, loss of appetite, urinary retention and allergic reaction.

Another main aspect of side effects, the infections, took the second place together with the gastrointestinal side effects among the side effects after the group of hematological side effects.
Infections during a therapy with Fludarabine are common especially because of the vast T-cell depressions (Bergmann et al 1993, Wijermans et al 1993). Included in the group of infections were the following, based on the CTC-criteria, symptoms: fever, infections, and chilling.

Table 11: Infection rate

	HR-F	**HR-WW**
Infections	23.5%	9.6%

Table 12: Etiology of the infections

Etiology of the infections								
Etiology	bacterial		viral		mycotic		other	
Percentage	42.4%		51.5%		2.2%		3.9%	
	HR-F	*HR-WW*	*HR-F*	*HR-WW*	*HR-F*	*HR-WW*	*HR-F*	*HR-WW*
	45.3%	44.0%	43.4%	52%	1.9%	0%	9.4%	4%

As seen in the table above, there were more infections registered in the cohort I (treatment arm) in comparison with cohort II (watch and wait arm) with 23.5% vs. 9.6%. Viral infections were the most, followed by bacterial infections, but none of them reached the CTC^0 4, while 3 of them reached the CTC^0 3.

3.4.5. SAE (Severe Adverse Events)

AE (Adverse Events) which match the criteria defined below are recorded as serious/severe adverse events (SAE):

- Fatal (results in death; NOTE: death is an outcome, not an event!)
- Life threatening (NOTE: the term refers to an event in which the patient was at immediate risk of death at the time of the event, it does not refer to an event which could hypothetically have caused death had it been more severe)
- Disabling
- Requires hospitalization or prolongation of existing hospitalization
- Congenital anomaly (birth defect)
- Medically significant event or event requiring intervention to prevent the outcomes listed above.

Occurrence of a SAE does not directly indicate Fludarabine (when administered) as the cause behind the SAE, although it is possible. Other probable or common reasons such as ischemic stroke or myocardial infarction should always be considered. Possible involvement of Fudarabine in the occurrences of SAE should always be assessed.

There were 7 SAE cases reported during the CLL-1 study, which involved 6 males and only 1 female. Death occurred in 3 events; the other 4 patients survived the SAE (see also the table below).

Table 13: Severe Adverse Events (SAE)

Severe Adverse Events (SAE)		
Outcome : Death	*Outcome : Survival*	*Total*
3 pts	4 pts	7 pts

SAE resulting in death

One multi morbid patient died despite having intensive treatment because of acute renal failure, cardiac decompensation, together with septic shock due to obstructed kidney with ureteral calculus.

Another one died after suddenly falling down in the garden of his house, probably due to heart attack, attempts to reanimate him were unsuccessful.

One patient was brought to the hospital due to an unclear, therapy refractory fever and reduction of his general condition. The patient received treatment for 3 months, including intravenous antibiotics, antifungal agents, blood culture and several other diagnostic procedures to find the reason of the fever, unfortunately without satisfying results. Substitutions of erythrocytes and platelets were then needed to keep him stable. Considering all the clinical findings and the development of more abdominal lymphomas in course of the treatment, it was assumed that the reason behind the refractory, unclear elevation of the body temperature was the combination of B-symptoms due to CLL and potentially viral superinfection within the scope of reduced immune system. The patient passed away after 3 months of treatment because of multi organ failure.

SAE survived by the patients

One patient had syncope and was then brought to hospital in order to clarify the origin of her syncope, which existed anamnestic since 20 years. The event was fortunately without any severe or life threatening consequences, another one had epileptic seizure during an ischemic stroke of the brain areas supplied by the left middle cerebral artery, the patient eventually then recovered from the event.

One patient suffered from dysesthesia of the legs, which later progressed into beginning paralysis of the legs, and unspecific sweating tendency.

One life threatening event occurred in one patient two weeks after receiving four months long Fludarabine administration, the patient was brought to the hospital by emergency physician due to fever of 40° C. Listeriosis with sepsis and meningitis was later diagnosed and with antibiotics medicated.

3.5. Progression Free Survival (PFS) under Fludarabine Therapy

The progression free survival (PFS) was evaluated using the Kaplan-Meier method and the survival chart using log ranks test (Kaplan & Meier 1958). 96 patients (intent-to-treat analysis) from the therapy arm and 94 patients from the "watch and wait" arm were included in the PFS evaluation and had follow-up observations. The median observation time was for HR-F 44.6 months (95% CI, range: 0-109 months) and for HR-WW 40.0 months (95% CI, range: 6.9-92 months).
Below is described the comparison between both cohort I and II in terms of progression free survival. A longer time span of PFS (progression free survival) had as expected the patients from therapy arm (cohort I) with 27.9 months, whereas the patients from watch and wait arm (cohort II) had 15.3 months of PFS time span (p-value = 0.002). This shows a benefit of 12 months for the patients receiving Fludarabine.

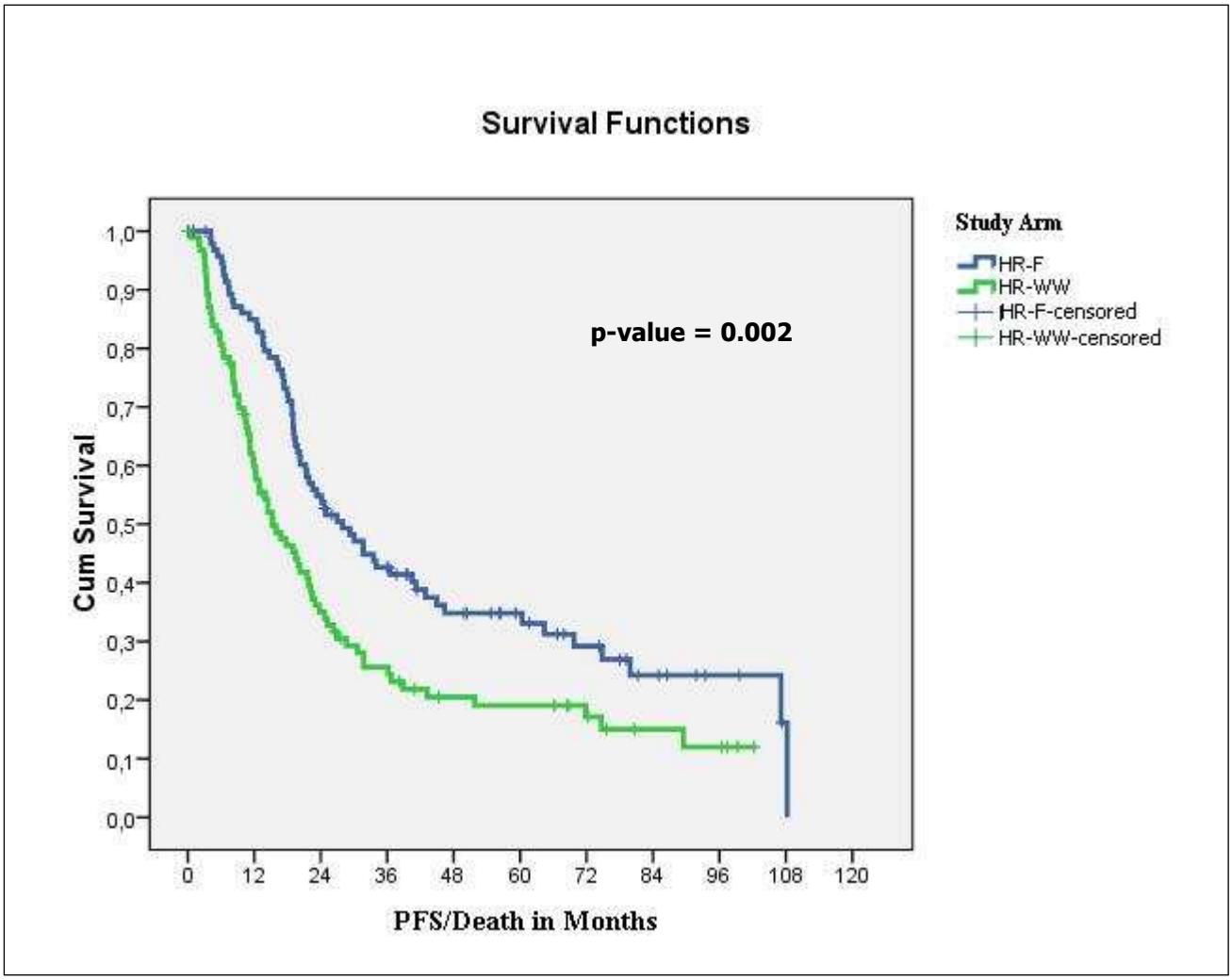
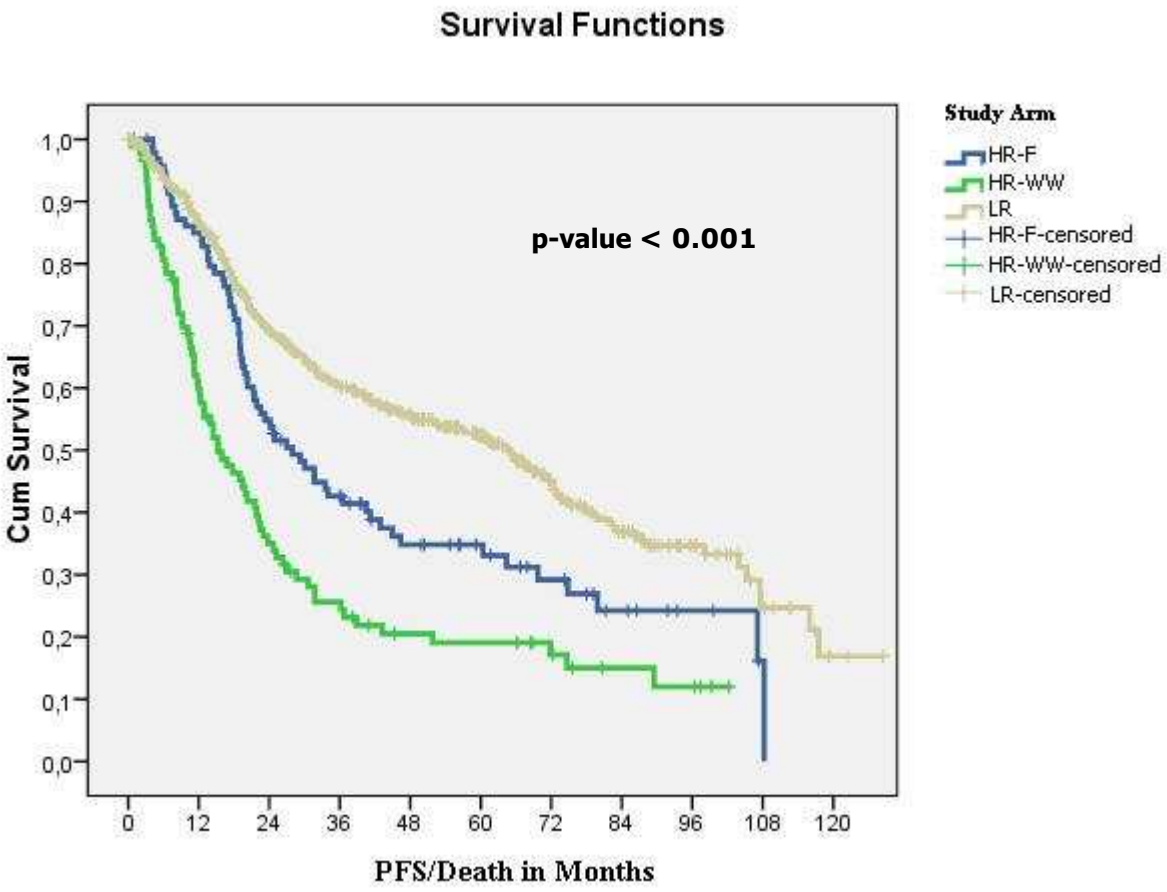


Diagram 1: PFS HR-F vs. HR-WW

PFS:
HR-F vs. HR-WW
27.9 months vs. 15.3 months

Below is once again the diagram of the PFS (progression free survival), this time with all three cohorts included together. Patients from the low-risk arm (cohort III), *per definition* also with less risk and tendency of progressing from Binet stage A to Binet stage B or C, had as expected among the three cohorts the best PFS time span (p-value < 0.001)

Diagram 2: PFS HR-F vs. HR-WW vs. LR

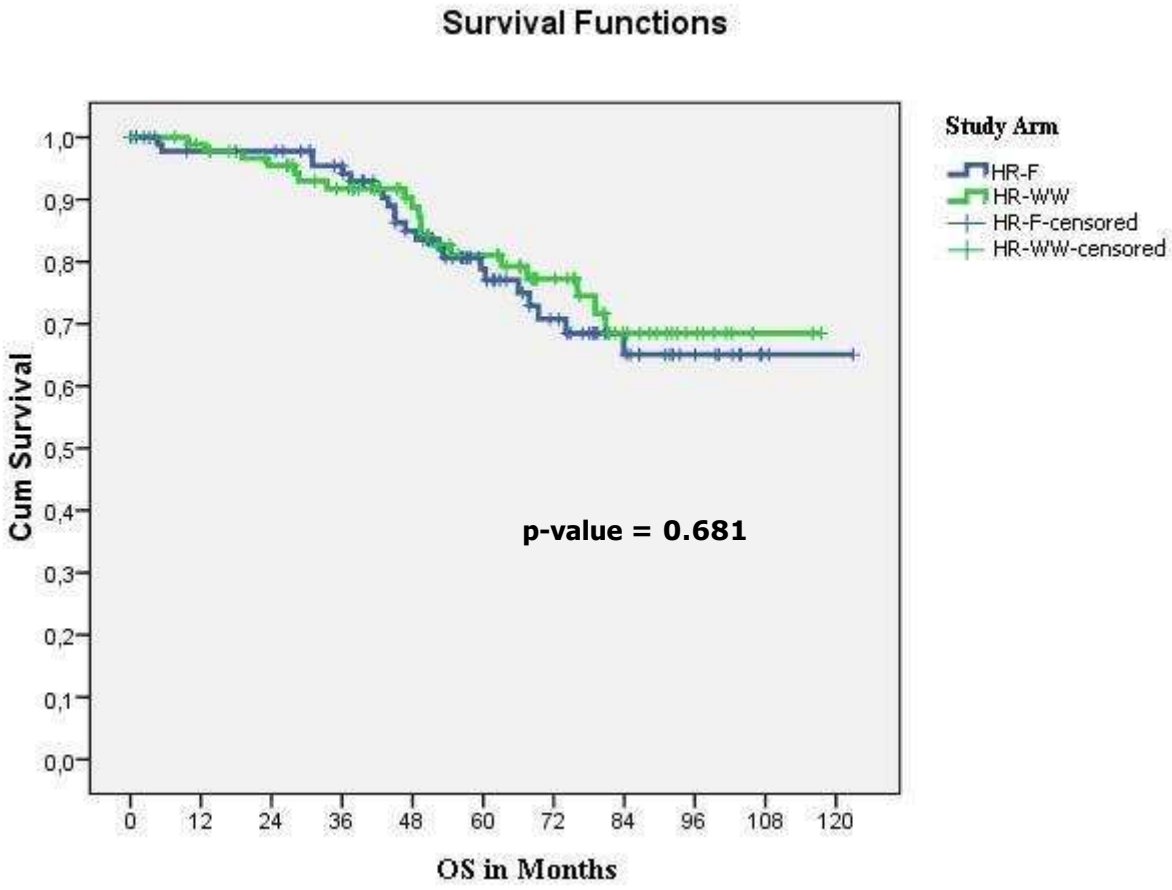


PFS:
HR-F vs. HR-WW vs. LR
27.9 months vs. 15.3 months vs. 64.8 months

3.6. OS (Overall Survival) under Fludarabine therapy

The comparison between the therapy arm and the "watch & wait" arm regarding OS (overall survival) was evaluated also using the Kaplan-Meier method and the survival chart using log ranks test (Kaplan & Meier 1958).

Diagram 3: Overall Survival HR-F vs. HR-WW

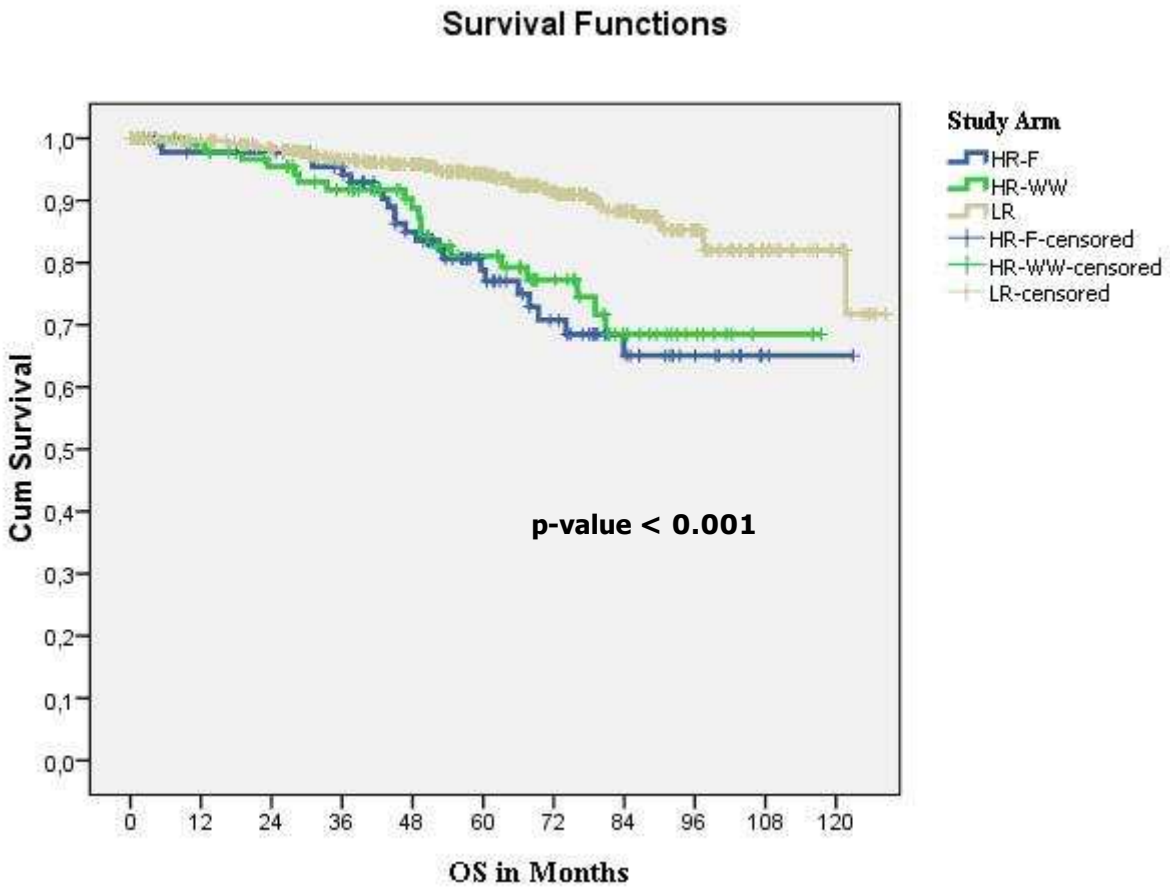


OS :
HR-F vs. HR-WW
Median not yet reached

As one could see from the diagram above, in terms of the overall survival no prolongation through Fludarabine administration is seen in comparison with cohort II. Fludarabine prolongs the progression free survival but not the overall survival.

To complete the comparisons, it is once again described below the comparison between all 3 cohorts regarding their overall survival rate.

Diagram 4: Overall Survival HR-F vs. HR-WW vs. LR



OS:
HR-F vs. HR-WW vs. LR
Median not yet reached

Patients with low progression risk showed the best survival rate among the three cohorts (p-value < 0.001).

4. DISCUSSIONS

Fludarabine's effectiveness as a powerful agent for untreated patients with advanced CLL was demonstrated in some studies (Keating et al 1993, Leporrier et al 2001). In the year 2000 Rai et al. published the results of their study, which involved 509 patients between 1990 and 1994 in order to compare the efficacy of Fludarabine with that of Chlorambucil and a combination of Fludarabine with Chlorambucil in the primary treatment of CLL (Rai et al. 2000), their findings showed that Fludarabine is superior to Chlorambucil in the initial treatment of CLL. The rate of complete remission and the overall rate of response (complete remission or partial remission), as well as the duration of the response and of progression-free survival were significantly better among them treated with Fludarabine than among them with Chlorambucil (20 vs. 14 months), thus being in resonance with the results from previous studies. Treatment with Fludarabine plus Chlorambucil produced response rates similar to those with Fludarabine alone, but with greater toxicity, thus cannot be recommended.

The long period of Chlorambucil era –more than 3 decades with lack of progress and persistently low rates of objectively measured responses- came to an end as Fludarabine emerged to set the new standard in CLL treatment and challenged physicians to find other effective agents that, when combined with Fludarabine, will lead to more incremental advances and, ultimately, to increased survival among patients with CLL.

Further hope of increased survival was then brought by the monoclonal antibody Rituximab (anti CD-20), as its combination with Fludarabine and Cyclophosphamide (known as "FCR"), investigated in studies including one initiated by the German CLL Study Group (CLL-8 study), was able to improve response rates and survival rates, therefore setting since 2008 a new era in CLL treatment (Hallek et al. 2008).

Patients in early stage of CLL showed, however, till now only unsurely clinical advantage regarding an early start of chemotherapy (Dighiero et al 1997, Brugiatelli et al 1995, FGCLLG 1990). The question, whether administrations of Fludarabine for patients with CLL stage Binet A who have a high risk of progressing to stage Binet B or C would bring clinical benefits for them has not found an answer through recent studies and presents itself as the base of our CLL-1 study.

Previous studies showed that patients with advanced CLL and first line Fludarabine therapy without any previous therapy could reach a progression free survival time span between 25 to 30 months (Rai et al 2000, Spriano et al 2000, FGCLLG 1999). The evaluation of CLL-1 study showed a progression free survival time span of 27.9 months for patients with first line Fludarabine therapy in early CLL (Binet stage A), thus being in accordance to those previous studies.

4.1. Side Effects & Infections

On the top of the adverse events list stood as expected hematological side effects such as leucopenia, thrombopenia or anemia like in previous studies.
Infection rate was higher in cohort I with 23.5% in comparison with 9.6% from cohort II, likely also due to the myelosupression through Fludarabine, thus resulting in somewhat a

weaker immune system of those Fludarabine-treated patients. Fortunately though, none of these infections reached the CTC grade 4, while only 4 times was CTC grade 3 reached. They were largely mild or moderate infections, thus do not display a hurdle against the application of Fludarabine in CLL stage A patients with high progression risk.

In order to win an overview of the extent of possible side effects caused by Fludarabine, we compared the CLL-1 study with another study also initiated by the GCLLSG (CLL-4 study) which has some design similarities with CLL-1 study, as it evaluated the usage of Fludarabine vs. the combination of Fludarabine and Cyclophosphamide in indolent CLL Binet stage A patients, and with some other previous studies and also the official Fludarabine product information in terms of the side effects of Fludarabine. The Fludarabine product information inserted 2 single-arm open-label clinical studies involving 133 patients conducted by M.D. Anderson Cancer Center (MDAH) and by the Southwest Oncology Group (SWOG) which evaluated Fludarabine administration in adult patients with CLL refractory to at least one prior standard alkylating-agent containing regimen.

4.1.1. Hematological side effects.

Myelosuppression is renowned as a major side effect of therapy with Fludarabine. Hematological side effects made the most of all side effects in this study with 37%. 7% (of all cases) reached even the CTC grade 3 or 4. Almost 70% of the patients had hematological side effects, thus confirming the general assumption. A lower rate of 39.4% was reported by Eichhorst et al. in the CLL-4 study (from a total of 173 patients), this can be explained by recognizing that Eichhorst et al published only the ones reaching CTC grade 3 or 4, while CLL-1 study recorded those with grade 1 or 2 as well.

Biggest impact of Fludarabine therapy is felt concerning the leucopenia, weighing 56% of the hematological AE. 13% of the hematological AE were severe leucopenias (severe means that the AE reach CTC grade 3 or 4), CLL-4 study reported that 26% of their patients had severe leucopenias reaching CTC grade 3 or 4. This fairly big difference (CLL-1 study recorded only 9.2%) is likely due to the patients collective in the CLL-4 study which were Binet B or C patients or at least Binet A patients with B-symptoms, meaning the patients had more "advanced" CLL than our patients with only Binet A patients. Fludarabine product information stated in the study done by MDAH and SWOG that 59% of the patients endured neutropenia, which indirectly also reflects a part of leucopenia.

Thrombopenia presents itself as the second most common hematological AE under Fludarabine therapy. CLL-1 study showed that thrombopenia took 35% fraction of the hematological AE, and around 24% of the patients suffered it, severe thrombopenia had however only 2% of the patients, thus playing only a minor role. CLL-4 study recorded 12.7% thrombocytopenias among their patients.

Anemias due to Fludarabine therapy in this study were with only 9% the most seldom among the hematological AE and made only 6% of the patients. 11.6% of the patients in the CLL-4 study were reported having anemia.

4.1.2. Infections

8.7% of the patients in the CLL4-study had infection (again only those with CTC grade 3 or 4 were reported), and showed no difference against the FC (Fludarabine + Cyclophosphamide) arm, in a sense maybe because both arms obtained Fludarabine. In CLL-1 study the rate of infections in the Fludarabine arm was higher than in the "watch and wait" arm (23.5% vs. 9.6%). The impact of the infections in CLL-1 study was under control, as none of them reached the CTC^0 4, while only 3 of them reached the CTC^0 3, bolstering the suggestion that Fludarabine is feasible for CLL patients.

The studies described in the Fludarabine product information showed infection rate of 33% and 44% in the MDAH (N=101) and, respectively, in the SWOG (N=32) study, higher than in CLL-1 study. It is however to remember that the patients collective in the MDAH and SWOG studies were those with CLL refractory to at least one prior standard alkylating-agent containing regimen, while in CLL-1 study the patients were all without any previous CLL therapy.

4.1.3. Gastrointestinal side effects

Adverse events concerning the digestive tract, particularly nausea and vomiting are on the one hand in fact common and occur frequently during chemotherapy. On the other hand, they are in most cases objectively mild, though subjectively were without any doubt very uncomfortable for the patients.
Gastrointestinal side effects were the least among the adverse events in the CLL-4 study, making just 1.7% of the patients, however, those AE reached CTC grade 3 or 4, while the 29% recorded in the CLL 1 study represented only CTC grade 1 or 2, thus were all mild or moderate. 46% was noted in the Fludarabine product information based on the MDAH study with nausea/vomiting once again playing the biggest part.

4.1.4. Neurological side effects

CLL-1 study evaluated that neurological side effects as a group were among the least in CLL-1 study, and most of them were only grade 1 (CTC) with headache and vertigo playing the biggest part among them. This fact suggests too that Fludarabine is in terms of neurotoxicity very feasible. CLL-4 study supports this idea, as no neurological side effects were published in the paper from Eichhorst et al., but this does not mean that there were no neurological side effects in CLL-4 study at all, it is explainable because only AE that reached CTC grade 3 or 4 were published in the paper. 21% of the patients in the study done by MDAH (stated in Fludarabine product information) had neurological adverse events with weakness being the most among them.

While Fludarabine was connected with the appearance of severe neurologic effects including blindness, coma, and death, one shall notice that they appeared in patients treated with high doses of Fludarabine in dose-ranging studies in patients with acute leukemia (the dose was approximately four times greater (96 mg/m2/day for 5 days) than the recommended dose of 25 mg/m2/day for 5 days). 13 of 36 patients treated with the higher dose regime developed this severe neurotoxicity, according to the study noted in the product information. Used at the normal, recommended dose, like in CLL-4 or CLL-1 study, no such severe neurologic effects as stated above were noticed, suggesting that the dose used in both study is a feasible Fludarabine dosage for treating chronic lymphocytic leukemia.

4.2. SAE (Severe Adverse Events)

Interesting is also the theme severe adverse events (SAE) during CLL-1 study, and the question whether Fludarabine usage a direct correlation with those events has or not, or even turns out to be the main causal reason behind those seven events.

In the 3 SAE eventually leading to death, direct correlation between them and Fludarabine usage was evaluated as "unlikely", as one died because of urosepsis due to ureter calculus which then resulted in many organ complications, another one died most likely because of cardiac problems with history of former myocardial infarction.

The last patient died due to a therapy refractory fever, later interpreted as refractory B-symptoms due to progressive CLL with obvious growth of his abdominal lymphomas. 3 times Fludarabine administration were then given, unfortunately without positive response.

Here too is a causal connection with Fludarabine as "unlikely" interpreted, the fever existed already before the Fludarabine administration and was actually together with the abdominal lymphomas the reason behind the Fludarabine usage.

In 2 patients, who survived their SAE, one with epileptic seizure due to ischemic stroke and the other with syncope which existed supposedly since 20 years, again no "likely" direct correlation between Fludarabine usage and their SAE was found.

A "possible" correlation between Fludarabine usage and one life threatening SAE was interpreted in 1 patient, who, 2 weeks after receiving the third cycle of Fludarabine (i.v. 45 mg), started to develop persisting dysesthesia of the legs with unspecific sweating tendency without fever, which after another 10 days progressed into beginning paralysis of the legs thus requiring thorough treatment in the intensive care unit.
Another possible correlation was found in one other patient, the correlation this time actually as "likely" interpreted. The patient was brought to the clinic by emergency physician due to fever of 40° C 2 weeks after the last Fludarabine (50 mg i.v.) treatment. Sepsis and meningitis were diagnosed with Listeriosis as the main reason behind them. Antibiotics were required to bring the life threatening SAE under control.

4.3. Response rates

Fludarabine, given at 25 mg/m^2/day i.v. for 5 days long with cycle repeat on day 28 is now not only the most effective mono therapy for treating CLL, it has now established itself as the base agent in developing the right chemotherapy combinations for CLL, such as in the CLL-4 trial which evaluates the combination of Fludarabine plus Cyclophosphamide and CLL-8 study which evaluates the "FCR" combination (Fludarabine plus Cyclophosphamide plus Rituximab).

Response rates achieved by Fludarabine mono therapy surpassed those achieved by other agents also used as mono therapy. Chlorambucil, as the old standard therapy for CLL, was evaluated by Dighiero et al in terms of response rates, and reported an ORR of 76% (Dighiero et al. 1998). The result from a study done by FCGCLL in 1990 reported a similar ORR (68%). Several studies evaluating Fludarabine given as first line CLL therapy showed an overall response rate between 63% and 80%, and complete remission rate between 20-46% (Leporrier et al 2001, Spriano et al 2000, Rai et al 2000, O`Brien et al 1998, FCGCLL 1996), higher than the rate achieved by Chlorambucil.

CLL-1 study registered an overall response rate of 85%, complete remission rate was 32%, partial remission rate was 53%, thus being in consistent accordance with the other published studies. 15% of the patients were non-responders (7% had stable disease and 8% had progression).
CLL-4 study, initiated by Eichhorst et al., noted a similar ORR with 82.9%, however with an obvious lower CR rate of 18.3%. One possible explanation for this particular difference is that patients with more advanced Binet stage C disease were included in the study, while all the patients in CLL-1 study had Binet stage A disease.

4.4. Progression Free Survival (PFS)

CLL-1 study showed that Fludarabine, given in patients with Binet stage A CLL, were able to induce a prolongation of the progression free survival in comparison with the "watch and wait" arm, with 27.9 months vs. 15.3 months (p-value = 0.002), respectively, for both arms.

Other publications showed that patients, with advanced CLL though, who were treated with Fludarabine gained a progression free survival time span between 25 and 30 months, thus once again standing in harmony with the result from CLL-1 study (Rai et al 2000, Spriano et al 2000). CLL-4 study showed a median PFS of 20 months for the Fludarabine arm.

One can say that a positive effect of Fludarabine in terms of PFS does exist not only in patients with advanced CLL, but also in patients with Binet stage A CLL as seen in CLL-1 study.

4.5. Overall Survival (OS)

No significant difference or advantage is seen between cohort I and cohort II in terms of the overall survival. This could indicate that maybe the median observation time were not long enough to foresee the advantages Fludarabine could bring forth, or could also suggest that Fludarabine, despite able to give patients significant advantages in terms of their Progression Free Survival (PFS) time span, does not prolong the Overall Survival (OS) time span.

Summing-up things, there is a positive impact of a first line Fludarabine therapy in CLL stage A patients with high risk of disease progression unto stage Binet B, first of all concerning the aspect of progression free survival, which were prolonged in comparison with patients without any therapy. There is, however, a setback, because no advantage in terms of overall survival (OS) could be seen, as it is not prolonged through Fludarabine.

The findings and comparisons of the AE in several different studies are, as one can see, consistent and not contradictive; they support each other's findings. Looking further into the aspect of the impact made by those adverse events, they remain so far under control and relatively manageable, with no major handicap for Fludarabine in comparison with "watch and wait" strategy, one could say then that the use of Fludarabine is safe and feasible in Binet stage A of chronic lymphocytic leukemia.

5. CONCLUSIONS

The much longed cure for chronic lymphocytic leukemia has, despite decent improvements of chemotherapy regimes recently, until today unfortunately not yet been discovered. Fludarabine, an alkylator from the purine analogue type, showed itself in previous studies as a powerful option, whether used as a monotherapy or as part of a therapy combination with other antineoplastic drugs, such as Cyclophosphamide, or the much promising newcomer "Rituximab" from the group monoclonal antibody (anti CD-20), which recently has set a new dawn of hope for CLL patients, as it brought the new therapy standard since 2008 (in combination with Fludarabine and Cyclophosphamide, also known as the "FCR" chemotherapy) for physically fit CLL patients.

Particular information, however, concerning the efficacy of Fludarabine in CLL stage Binet A patients with high risk of progressing into Binet stage B still unfortunately lack. CLL-1 study would then be one of the first providers of information concerning this aspect.

CLL-1 study intended therefore to answer the question, whether administrations of Fludarabine as a monotherapy in CLL stage A patients with high risk of progression would bring an advantage for the patients in comparison with the until now standard option, which is the "watch and wait" strategy.

The evaluation was based on the response to Fludarabine (including complete response, partial response, or no response, meaning a stable disease or progression), AE (adverse events), SAE (severe adverse events), PFU (progression free survival), and also not to forget the overall survival rate. From the total of 193 patients stratified as CLL stage A patients with high progression risk, 98 patients were randomized into the therapy arm as cohort I, while the remaining 93 patients into the "watch and wait" arm as cohort II. Cohort III included Binet stage A patients with low risk of progressing into Binet stage B; these patients, too, receive no treatment.

Patients who received Fludarabine (25 mg/m^2/day for 5 days, maximum 6 cycles) had quite a high overall response rate with 85% (complete remission rate was 32%, partial remission rate was 53%), only 8% had progression, 7% stable disease.
They also showed after a median observation time from 44.6 months a prolonged progression free survival time span of 27.9 months, compared with patients from "watch & wait" arm with progression free survival of 15.3 months after a median observation time from 40 months (p-value: 0.002).

Looking at the comparison concerning the overall survival between both arms, no advantage of prolongation for the therapy arm could be noticed though; suggesting that Fludarabine is unable to induce a prolongation of the overall survival time span, or maybe that a longer median observation time is needed to determine a survival benefit for any first-line regimen.

Infection rate was higher in the therapy arm than in the "watch and wait" arm (23.5% vs. 9.6%); most of them were mild though. Other adverse events rates were in accordance with the findings of previous trials and remain tolerable.

Summary:

Fludarabine is feasible, prolongs the progression free survival but not the overall survival in CLL Binet A patients with high risk of progressing into Binet stage B or C. The "watch and wait" strategy cannot therefore be replaced by Fludarabine administration and remains as the standard therapy option for CLL Binet stage A patients with or without high risk of progressing into Binet stage B or C.

Further improvement may be possible with the use of the chemoimmunotherapy combination "FCR" (Fludarabine + Cyclophosphamide + Rituximab), which was the subject of the CLL-7 study and the CLL-8 study initiated by the German CLL Study Group and is now the new standard therapy for physically fit and advanced CLL patients. The results showed that the FCR combination has the best ORR (overall response rate), even better than the former standard therapy, which was FC (Fludarabine and Cyclophosphamide). Whether FCR could improve the progression free survival and the overall survival in CLL Binet stage A patients with high risk of progressing, shall be cleared in further upcoming trials.

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8. NOTES

9. Appendix

Appendix 1: ECOG Performance Status

Table 14: ECOG performance status scale

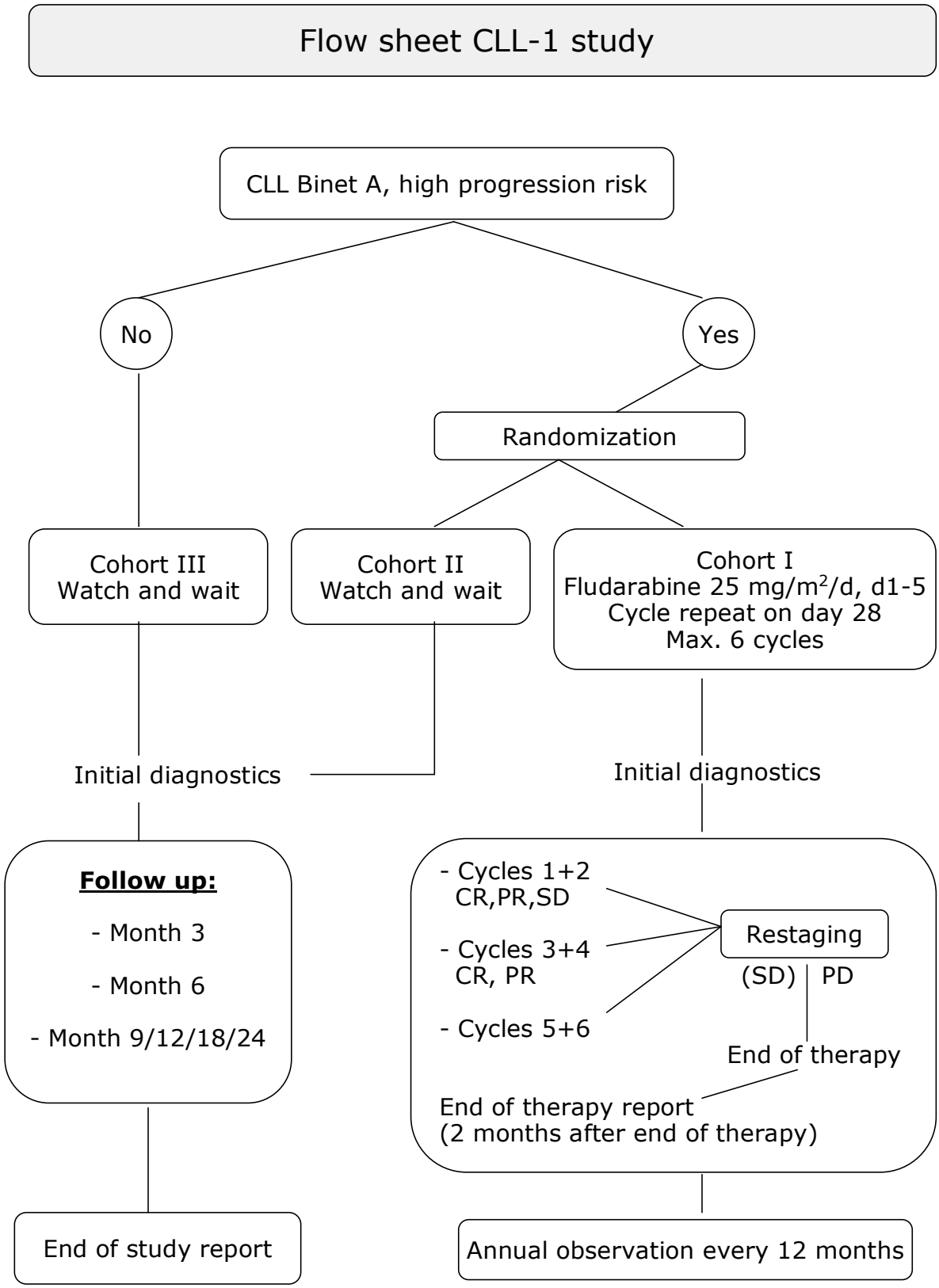
ECOG Performance Status	
Score	*Description*
0	Asymptomatic (Fully active, able to carry on all pre-disease activities without any restriction)
1	Symptomatic but completely ambulatory (Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work)
2	Symptomatic, <50% in bed during the day (Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours)
3	Symptomatic, >50% in bed, but not bedbound (Capable of only limited self-care, confined to bed or chair 50% or more of waking hours)
4	Bedbound (Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair)
5	Death

“ECOG (PS)”, stated above, represents the “performance status” of the patients according the criteria used by ECOG (Eastern Cooperative Oncology Group) (Oken et al 1992). This ECOG score (also called the WHO or Zubrod score) runs from 0 to 5, with 0 denoting perfect health and 5 death.

The performance statuses were analyzed ahead of the therapy in every patient. Patients with an ECOG-status of more than 2 would not be able to meet the inclusion criteria and therefore would then be excluded from the study.

Appendix 2: Flow sheet of the CLL-1 study

Fig. 3: CLL-1 study flow sheet



Curriculum Vitae

Personal information

Name:	Jensen Imawan
Status:	Single
Nationality:	Indonesian
Date of birth:	10 August 1983
Place of birth:	Jakarta, Indonesia

Education

1989-1995	Elementary school Regina Pacis Jakarta, Indonesia
1995-1998	Junior high school Regina Pacis Jakarta, Indonesia
1998-2001	Senior high school Regina Pacis Jakarta, Indonesia
2002-2003	Studienkolleg Munich, Germany
2003-2009	Medical college; Ludwig-Maximilians-University Munich, Germany

Medical activities

2009-	Assistant doctor in Klinikum Bad Hersfeld; Division of Gastroenterology

Lebenslauf

Angaben zur Person

Name:	Jensen Imawan
Familienstand:	Ledig
Staatsangehörigkeit:	Indonesisch
Geburtsdatum:	10. August 1983
Geburtsort:	Jakarta, Indonesien

Ausbildung

1989-1995	Grundschule Regina Pacis Jakarta, Indonesien
1995-1998	Mittelschule Regina Pacis Jakarta, Indonesien
1998-2001	Oberschule Regina Pacis Jakarta, Indonesien
Abschluss :	Allgemeine Hochschulreife
2002-2003	Studienkolleg München
2003-2009	Studium der Humanmedizin an der Ludwig-Maximilians-Universität München

Medizinische Tätigkeiten

2009-	Assistenzarzt in der Klinik für Gastroenterologie; Klinikum Bad Hersfeld