Analysis of prognostic factors in chronic lymphocytic leukemia

Doctoral (Ph.D.) theses summary

Gábor Kovács MD

University of Pécs, Clinical Center,

1st Department of Internal Medicine

Interdisciplinary Medicine Doctoral School

Hematologic Program

Head of Doctoral School: Prof. Balázs Sümegi, MD, DSc

Head of Doctoral Program: Hussain Alizadeh, MD, DSc

Mentor: Prof. Hajna Losonczy, MD, DSc

University of Pécs

Medical School

2017

1. Introduction

Chronic lymphocytic leukemia (CLL) is an indolent hematological malignancy, characterized by slow progression and long-term survival, however, in some cases the progression is fast, the expected survival time is short. The **clinical staging systems** of CLL (Binet, Rai) defined an early (Rai 0, Binet A), intermediate (Rai I-II, Binet B) as well as an advanced (Rai III-IV, Binet C) stages of the disease together with their expected survival times. These staging systems (especially in case of nowadays often verified early stage diseases) could not predict the possibility of a fast progression. There was a special need to defined factors that could predict the estimated progression of the disease.

Biological factors, such as **age**, **gender**, **the pattern of bone marrow (BM) and lymph node infiltration** were identified to correlate with the prognosis of CLL.

Biochemical factors, such as **serum beta-2-microglobuline** (sB2M), **lactate dehydrogenase** (LDH), **thymidine kinase** (sTK), and **soluble CD23** (sCD23) were identified to indicate a correlation with the tumor mass and the expected survival time.

Cytochemical parameters, such as negative prognostic value of **CD38 positivity** (with using a cut-off with 20%) was published by Ibrahim, and was confirmed (with using a cut-off with 30%) by Damle. The correlation between the increased expression of **zeta-associated protein-70** (ZAP-70) and fast disease progression was verified in 2003 by Crespo.

Metaphase cytogenetic studies detected genetic aberrations in 40-50% of patients. With the availability of interphase cytogenetic assays (fluorescents in situ hybridization [FISH]) genetic abnormalities were identified in 80% of all cases: deletion on long arm of chromosome 13 (**del(13q)**) 55% and chromosome 11 (**del(11q)**) 18%, trisomy of chromosome 12 (**+12**) 16%, deletion on short arm of chromosome 17 (**del (17p)**) 7% and long arm of chromosome 6 (**del (6q)**) 6%. The normal karyotype and the sole del(13q) were found to be good, while the appearance of del(11q), del(17p) proved to be a poor prognostic sign. The prognostic role of +12 and del(6q) remained controversial.

Direct sequencing of the immunoglobulin heavy chain gene (***IgHV***) after polymerase chain reaction (PCR) verified a ≥98% sequence identity with the germline heavy chain gene (unmutated [**UM**] *IgHV* gene status) in 45.2% of cases and > 2% somatic mutation (mutated [**M**] *IgHV* gene status) in 54.8 % of cases. The overall survival time was found to be 95 and 293 months, respectively. Further studies were performed to determinate of the type of heavy chain gene

family. It was proved that the presence of the **$V_{3\text{-}21}$** sequence has a negative effect on the prognosis despite of M *IgHV* status.

During this period, there was a dynamic evolution of the treatment of CLL. Chemotherapies in combination with monoclonal antibody treatment resulted in significantly longer overall survival (OS). In addition, significantly longer progression free survival (PFS) was demonstrated with several new targeted therapies (kinase inhibitors, bcl-2 inhibitors, etc.). The early measurement of the effectivity of these new treatment options and the further estimation of prognosis after the therapy was necessary. In the light of this, supported by the American National Cancer Institute (NCI), the CLL Working Group established the criteria for **clinical responses** in guidelines in 1988 and 1996 (complete [CR] and partial remission [PR], non-responding and progressive disease [PD]). Since therapeutic response after treatment was well correlated with prognosis and survival, it was used to estimate the effectivity of different treatment modality.

The possibility of a more accurate assessment of the depth of the therapeutic response was provided by peripheral blood flow cytometry to detect CLL cells. By standardizing the method, minimal residual disease (**MRD**) and MRD negativity were defined (with a criterion of <10-4 CLL phenotypic B lymphocytes / total leukocytes). The International CLL Working Group (International Workshop on CLL [IWCLL]) provided a new guideline on CLL in 2008 that included the MRD measurement from peripheral blood for CR patients, further refining the clinical response to MRD-positive and negative CR. In case of MRD negativity, better prognosis, longer PFS and OS were detectable. Since MRD measurement gives an opportunity to estimate the prognosis and survival of disease and the effectivity of the treatment, the US Food and Drug Administration (FDA) as the European Medicines Agency (EMA) was considering using MRD as a clinical trial endpoint. According to these guidelines, MRD was initially used in CR patients to further evaluate the depth of remission. However, with the using of MRD measurement in further trials, not only CR patients but also with PR were observed to reach MRD negativity in bone marrow as well as peripheral blood. The relationship between clinical response and MRD in predicting the prognosis and survival after treatment remained an open question.

2. Objectives

The aim of our research was to investigate these prognostic factors and their effects on the clinical outcome, and to investigate further factors involved in the prognosis of CLL. Our studies were performed on two patient populations: on 241 CLL patients of the University of Pécs, Clinical Center, 1st Department of Internal Medicine and on 554 patients of two phase III trials of the German CLL Study Group (GCLLSG).

On the first group of patients we were looking for answers to the following questions:

1. Can the role of prognostic factors in the literature be demonstrated on our own patient population?
2. How do factors with unclear prognostic significance correlate with the course of the disease in our own patient population?
3. Which prognostic factors show an independent correlation with the course of the disease?
4. Are prognostic factors changing during the course of the disease?
5. Is there any new factor involved in the prognosis in addition to the known markers?

On the second patients population we were looking for answers to the following questions:

1. The clinical therapeutic response or the MRD analysis from the peripheral blood has a better correlation with the further prognosis of the disease?
2. Patients with MRD-negative PR or MRD-positive CR have better prognosis?
3. Can we verify the better prognostic role of bone marrow MRD measurement compared to the peripheral blood MRD test?
4. Is the prognosis of patients with residual lymph node enlargement, hepatosplenomegaly, or residual bone marrow involvement differed in the group of patients with MRD-negative PR? Which compartment involvement affects a worse prognosis?

3. Patients and methods

3.1. Pre-therapeutic prognostic factors

Between May 2003 and May 2012, the different prognostic factors and their relationship to the clinical course of the disease were investigated on 241 CLL patients of our clinic. We recorded the **gender** and **age** of patients, the **Rai stage** of their disease, the **morphology of the bone marrow infiltration** and **sB2M** level in our database.

Flow cytometric studies were performed on peripheral blood samples (anticoagulated with ethylenediaminetetraacetic acid [EDTA]) by using FACSscan and FACSCalibur (Becton Dickinson) flow cytometers to determinate **CD38** and **ZAP-70** expression, in the Pathology and Laboratory Medicine Institute (University of Pécs). Two cut-off values (≥20% and ≥ 30%) were used to define the CD38 and ZAP70 positivity in our studies, and for both cut-off values their relationship to the clinical course was analyzed.

Peripheral blood samples with heparin anticoagulation were stimulated 3-days with tetradecanoyl-O-phorbol-13-acetate (TPA) in vitro to perform **metaphase cytogenetic analysis** with G-banding. After the isolation of CLL cells from these samples, with using locus-specific probes (Vysis Inc., Downers Grove, USA), FISH analysis were performed for detection of **del(13q14)** (D13S319), **del(11q22)** (LSI ATM), **del(17p13)** (LSI p53), and **+12** (CEP12).

After the isolation of white blood cells from these blood samples, complete deoxyribonucleic acid (DNA) isolation and with using of FR1 and JH consensus primers, polymerase chain reaction (PCR) and direct sequencing was performed to determine the **mutational status of the *IgHV* gene**.

In addition to the outcome of different prognostic factors, the time from diagnosis to the first treatment (time-to-first-treatment [TTFT]) and the survival time ([OS] = time from diagnosis to death of any cause)) of each patients were recorded during our observation period.

3.2. Post-therapeutic prognostic factors

Based on data of two phase III studies of the GCLLSG (CLL8: fludarabine [F] and cyclophosphamide [C] in combination with rituximab [FCR] versus [vs.] FC; CLL10: FCR vs. bendamustin [B] and R [BR]), we further analyzed the prognostic value of the pre-treatment

factors, the minimal residual disease, as well as the clinical response observed after treatment on 554 patients.

Pre-treatment factors possibly influence the prognosis in this patients population were: age, gender, performance status of the patients (scored according to the recommendation of the Eastern Cooperative Oncology Group [ECOG]), comorbidity (based on Cumulative Illness Rating Scale [CIRS]), Binet stage of the disease, sB2M and sTK, del(13q), del(11q), del(17p), +12 and *IgHV* status. Measurements of serum parameters and FISH analysis were performed at the University Clinic of Cologne, and *IgHV* gene rearrangement was analyzed at University Clinic of Ulm.

Due to the potentially influencing effect on the prognosis, the **type of treatment** (FC, FCR and BR) and the **clinical trial population** (CLL8 and CLL10) were also taken into consideration in the analysis of the patient population.

As post-treatment prognostic marker, **MRD** measurements were performed on peripheral blood samples – as well as in some cases on bone marrow aspirate - with flow cytometric analysis, 3 months after the beginning of the last treatment cycle (final restaging [RE]) in the GCLLSG MRD Laboratory (Kiel) as previously described.

The classification of the post-treatment **clinical response** was based on the report of the investigating physician and verified by an independent medical review according to the IWCLL 2008 criteria system at the end of treatment (EOT). The therapeutic response of patients with complete remission, but incomplete bone marrow recovery (CRi) was considered as CR.

PFS (time from randomization until progression or death of any cause) and OS (time from randomization until death of any cause) was recorded for each patient.

3.3. Statistical analysis

Survival curves were calculated with using Kaplan-Meier analysis and compared with two-sided, non-stratified log-rank test. Univariate analysis was performed with Cox regression model to verify the effect of each prognostic factor on TTFT, PFS and OS. With using factors showing significant effect in univariate analyzes, multivariate Cox regression analysis was performed with stepwise selections to identify independent prognostic factors. The correlation was considered significant for $p < 0.05$.

4. Results

4.1. Pre-therapeutic prognostic factors

The median age in our patient population (n=241) was 64.6 years (range 33-89 years), with 56% male (n=136) patients. The majority of patients were diagnosed with an early stage disease (Rai 0: n=112, 46.5%, Rai I: n= 78, 32.4%). Bone marrow biopsy was performed in 106 patients (diffuse infiltration was verified in 51 cases [48%]). sB2M level was measured at the time of diagnosis in 212 cases (>3.5 mg/l, 41 cases [19%]). CD38 measurements were performed in 222 cases (≥20% 84 and ≥30% in 67 cases). ZAP70 positivity was detected with a very high frequency with using a cut-off of 20% and 30% as well (≥20% 85.4% and ≥30% 78.9% of all cases, respectively). Chromosome analyses were performed in 150 patients, that was not informative in 2 cases. The method was able to detect genetic aberrations in 71 cases (47.3%). The most frequent aberrations were: del(13q) (n=27, 18%), del(11q) (n=16, 10.7%), +12 (n=15, 10%), del(17p) (n=6, 4%) and del(6q) (n=7, 4.7%). In 34 cases, further rare, sporadic aberrations were observed. Complete FISH analysis for the detection of all frequent aberrations (del(13q), del(11q), del(17p) and +12) were performed in 101 patients, in 81 cases (80.2%) were a genetic aberration detectable. As summary of the metaphase and interphase genetic analysis the most common genetic aberrations were observed with the following frequency: del(13q) 52.4%, del(11q) 17.2%, +12 11.0%, del(17p) 8.8%, del(6q) 4.7%. With using a cut-off with ≥98% by the sequence analysis of *IgHV*, in 58.2% of all cases (n=50) were an UM status detectable. With the analysis the presence of 41 different *IgHV* gene families were verified. Most common sequence was $V_{1\text{-}69}$ and $V_{3\text{-}21}$ (17/86, 19.8% and 5/86, 5.8%, respectively). The median observation time of the patient population was 5.7 years (range 0-27.7 years). During the observation period 33.2% (n=80) of patients died. Treatment was required in 49.8% of all cases (n=120).

4.1.1. Analysis of prognostic factors

Comparing the survival curves calculated with **Kaplan-Meier analysis**, significantly shorter **TTFT** and **OS** were observed for male patients, in cases with advanced Rai stage, elevated sB2M, diffuse bone marrow infiltration, CD38 positivity (even with a cut-off with 20% and 30%), not-sole del(13q) abnormality and UM *IgHV* status. According to the hierarchical model, significant differences in TTFT and OS were observed between the different patients groups.

ZAP70 positivity did not show significant correlation with either TTFT or OS neither with using a cut-off with 20% nor with 30%. Therefore we left out ZAP70 expression in our further analyzes.

To further analyze the effect of the presence of each factor on **TTFT**, **univariate analysis** with Cox regression model was performed, in which we have added the age of patients as a continuous variable. The groups of the hierarchical model were compared to a group of patients with sole del(13q) abnormality, observed as a good prognostic marker in the previous analysis. Gender (male) (HR: 1.71, *p*=0.005), elevated sB2M (HR: 4.65, *p*=0.000), diffuse bone marrow infiltration (HR: 2.24, *p*=0.000), intermediate and advanced Rai stages (HR: 4.05, *p*=0.000 and Rai III-IV: HR: 12.02, *p*=0.000), CD38 positivity with a cut-off with 20% and 30% (HR: 2.57, *p*=0.000 and HR: 2.90, *p*=0.000), del(17p) (HR: 3.00, *p*=0.002), del(11q) (HR 4.76, *p*=0.000), +12 (HR: 2.80, *p*=0.011), and UM *IgHV* status (HR: 4.20, *p*=0.000) indicated a significant negative effect on TTFT. Considering the fact that other genetic variables can be associated in patients within +12 group, according to the hierarchical model, the presence of +12 as sole abnormality was further analyzed. Despite of the small number of cases (n=9) in this group, significantly shorter TTFT was detected (HR: 3.31, *p*=0.009).

Similarly, we further investigated the effect of the presence of the different factors on the **OS**. Significantly reduced OS was observed in men (HR: 1.64, *p*=0.035), advanced age (HR: 1.04, *p*=0.001), elevated sB2M (HR: 4.18, *p*=0.000), intermediate and advanced Rai stages (Rai I-II: HR: 2.97, *p*=0.000 and Rai III-IV: HR: 11.80, *p*=0.000), diffuse bone marrow infiltration (HR: 2.41, *p*=0.002), CD38 positivity with using both cut-offs (20%: HR: 1.79, *p*=0.015 and 30%: HR: 1.97, *p*=0.006), del(17p) (HR: 4.01, *p*=0.000), del(11q) (HR: 3.38, *p*=0.001) and UM *IgHV* status (HR: 5.15, *p*=0.000). Neither the +12 groups of the hierarchical model nor the +12 as sole abnormality showed a significantly shorter OS compared to the reference group (HR: 1.90, *p*=0.192 and HR: 2.10, *p*=0.206).

To analyze the independent effect of the different prognostic factors on the course of the disease, **multivariate analysis** was performed. As in the univariate analysis a closer relationship with TTFT and OS was detected by using 30% cut-off for CD38 positivity, we used this cut-off in our multivariate analysis. Since bone marrow biopsies are not part of the disease diagnosis, therefore, it was performed only in 135 cases (56%). As it would lead to a remarkable reduction in the number of cases in our multivariate model, we had to omit the investigation of this factor. The analysis was performed with the other factors involved with significant effect on TTFT in the univariate analysis. On **TTFT** the gender (male) (HR: 2.57, *p* = 0.005), intermediate and advanced

Rai stage (Rai I-II: HR: 2.25, *p*=0.022 and Rai III-IV: HR: 7.46, *p*=0.000) and UM *IgHV* status (HR: 4.22, *p*=0.000) had an independent, negative prognostic value.

Similarly to this analysis, the factors having an independent negative effect on **OS** were: age (HR: 1.06, *p*=0.002), intermediate and advanced Rai stage (Rai I-II: HR: 2.66, *p*=0.028 and Rai III-IV: HR: 9.69, *p*=0.000), del(17p) (HR: 4.46, *p*=0.000) and UM *IgHV* status (HR: 4.18, *p*=0.000).

4.1.2. Repeated analyzes

In rare cases, especially in case of clinical progression, we also had the opportunity to repeat prognostic analyzes. Repeated **bone marrow biopsy** was performed in 5 cases prior to start of treatment. In each case, an earlier nodular infiltration was observed, and due to the progression, the diffuse bone marrow infiltration of the disease occurred. **SB2M** was regularly controlled during the course of the disease. However, data collection and further statistical analysis were not performed, there was an elevated level detectable in case of progression of the disease and enlarge of the tumor mass, while a decrease was observed after therapy. **CD38** was repeated in 105 patients. With using a cut-off with 30%, in 9 cases (18.1%) a switch to CD38 positivity was detected together with the progression. Repeated **chromosomal analysis** was performed in 12 patients during the observation. There were new aberrations detectable in 4 cases: in 1 case t(13;18) with new del(11q), and in the other 3 cases, rare complex abnormalities were observed. Repeated **FISH** analysis was performed due to the progression or after the treatment of the disease in 33 cases. Aberrations were observed in 5 patients (15%). The appearance of del(11q) and del(13q) was observed in 2-2 patients. In one case the disappearance of del(13q), del(11q) was observed after completed high dose chlorambucil (CLB) therapy and with the further progression, del(11q) was detectable repeatedly. Repeated ***IgHV*** analyzes were not performed due to financial reasons and due to published data about its stability during the course of the disease.

4.1.3. Chromosome analysis vs. fluorescents in situ hybridization

71 of 150 (47.3%) patients had genetic aberration on chromosomal analysis. Compared to the FISH result: 45 cases of del(13q), 5-5 cases of del(11q) and del(17p), and 2 cases of +12 were not detected by chromosome analysis. In rare cases, despite of the negative FISH results, the genetic aberration was observed by chromosome analysis: del(11q) in 2 cases, del(13q), del(17p) and +12 in 1-1 cases. Rare abnormalities were mainly part of complex abnormalities in 34 patients

with chromosomal analysis (see earlier). Due to their sporadic incidence, no statistical analysis was possible. Most commonly, the presence of del(6q) (7 cases - 4.7%) was observed, however, due to this low number and the associated other genetic aberrations, its influencing effect on progression could not be analyzed.

To investigate the possible role of complex karyotype abnormalities in the prognosis, we analyzed the group of patients with ≥3 different genetic aberrations. There were 23 patients in the group, of whom 21 (91%) had the complex abnormalities confirmed only by chromosome analysis. A short TTFT and OS were detectable (median 6 and 66 months, respectively) (**Figure 1/a-b.**). Compared with the group with sole del(13q) abnormality, patients with complex aberrations had significantly shorter TTFT and OS (HR: 3.12, 95% p=0.001 and HR: 3.40, p=0.001, respectively). Due to the low patient number, further multivariate analysis were not performed.

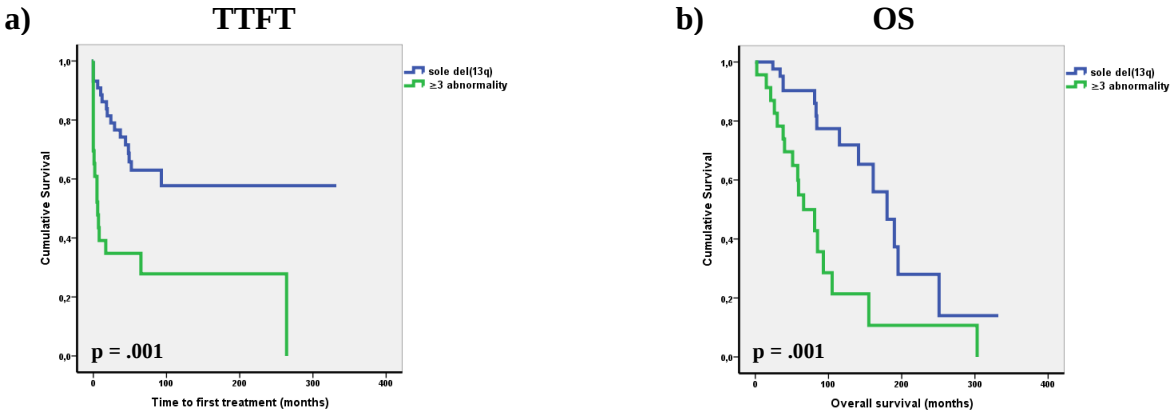

Figure 1/a-b.: *Effect of the presence of ≥3 genetic abnormalities (vs. sole del(13q) abnormality) on a) TTFT and b) OS*

4.1.4. IgHV sequence analysis

IgHV sequence analysis identified 41 different *IgHV* gene families in our patient population. $V_{1\text{-}69}$ (17/86, 19.8%) gene family was verified in 17 (19.8%) patients. In all cases UM status was detectable. The second most common *IgHV* gene family was the $V_{3\text{-}21}$ sequence in 5 (5.8%) patients. Of those, 4 cases had UM status.

In case of both gene families, short TTFT ($V_{1\text{-}69}$ and $V_{3\text{-}21,}$ median 26 and 17 months, respectively) and OS ($V_{1\text{-}69}$ and $V_{3\text{-}21,}$ median 85 and 29 months, respectively) were verifiable. In univariate analysis in comparison with the group of sole del(13q) abnormality, the appearance of

both gene families had statistically negative effect on TTFT ($V_{1\text{-}69}$ and $V_{3\text{-}21}$, HR: 4.27, p=0.000 and HR: 3.88, p=0.035, respectively) and OS ($V_{1\text{-}69}$ and $V_{3\text{-}21}$, HR: 3.86, p=0.003 and HR: 14.26, p=0.000, respectively). Due to the low number of cases we could not perform a further multivariate analysis.

4.2. Post-therapeutic prognostic factors

On the patients population of 2 phase III trials (CLL8 and CLL10) of the German CLL Study Group we examined the prognostic value of the MRD measurement from peripheral blood and the clinical response observed after the first line treatment and analyzed their relationship in the prediction of survival. The mean age of patients (n=554) was 61 years (range 33-81). Majority of patients (n=427 [77%]) were male. The median of the ECOG status of cases was 0 (range 0-2), and the median CIRS score was 2 (range 0-7). The Binet stage of the disease at the start of treatment in the population was as follows: Binet A 76 (13.7%), Binet B 284 (51.4%) and Binet C 193 (34.9%). The frequency of the genetic abnormalities were: del(13q) 206 cases (38.4%), del(11q) 133 cases (24.8%), +12 58 cases (10.8%), del(17p) 8 cases (1.5%), while normal karyotype was observed in 131 cases (24.4%). 326 of all cases (62.2%) had UM *IgHV* status. 388 patients (74.0%) had an increased sTK (>10 U/l), while 177 (33.8%) a higher sB2M (>3.5 g/l) level. CR, PR and MRD negativity was observed in 40.6% (n=225), 59.4% (n=329) and 62.7% (n=347) of all cases after first line therapy. The median PFS of the patient population was 51 months, while median OS was not reached during the observation time.

4.2.1. Analysis of prognostic factors

In the **univariate analysis**, the sole del(13q) (HR: 1.41, p=0.009), del(17p) (HR: 11.75, p<0.001), del(11q) (HR: 1.81, p <0.001), the UM *IgHV* status (HR: 2.45, p<0.001), the type of treatment (FC vs. FCR: HR: 1.43, p=0.012 and BR vs. FCR: HR: 1.62, p=0.002), the clinical response (HR: 2.06, p<0.001) and MRD (HR: 3.30, p<0.001) had significant correlation with **PFS**. Regarding to the **OS**: the age of patients (HR: 1.66, p=0.022), co-morbidities according to CIRS (HR: 1.21, p=0.002), del(17p) (HR: 7.31, p<0.001), *IgHV* mutational status (HR: 3.41, p<0.001), clinical response (HR: 1.63, p=0.030), and MRD (HR: 2.20, p<0.001) indicated significant prognostic value.

In our **multivariate analysis**, del(17p) (HR: 9.67, $p<0.001$), del(11q) (HR: 1.32, $p=0.049$), *IgHV* mutational status (HR: 2.40, $p<0.001$), type of treatment (BR vs. FCR) (HR: 1.63, $p=0.003$), clinical response (HR: 1.48, $p=0.007$) and MRD (HR: 3.55, $p<0.001$) had independent effect on the **PFS**. The strongest impact was observed for del(17p), MRD and *IgHV* status. Regarding to **OS**: beside age (HR: 1.65, $p=0.038$) and CIRS score of patients (HR: 1.21, $p=0.010$), del(17p) (HR: 5.02, $p<0.001$), *IgHV* status (HR: 3.35, $p<0.001$) and MRD (HR: 2.34, $p<0.001$) had the strongest independent effect.

4.2.2. MRD and clinical response

After grouping our patients according to their MRD status measured from peripheral blood and clinical response, MRD-negative CR, PR, and MRD-positive CR, PR were observed in 186 (33.6%), 161 (29.1%), 39 (7.0%) and 168 (30.3%) cases, respectively. For these patient groups median PFS was 61, 54, 35 and 21 months, respectively. Median OS was not reached in the first 3 groups, while 72 months was detected for patients with MRD-positive PR.

In our univariate analysis, in comparison to MRD-negative CR, significantly shorter **PFS** was observed for patients with MRD-positive CR (HR: 1.99, $p=0.004$) and MRD-positive PR (HR: 4.27, $p<0.001$). In contrast to this, there was no significant difference between patients with MRD-negative CR and PR (HR: 1.24, $p=0.228$). In addition, in the comparison of patients with MRD-negative PR and MRD-positive CR, a significantly shorter PFS was detectable for patients with CR, but MRD positivity (HR: 0.63, $p=0.048$).

Regarding to the **OS**: in comparison to the MRD-negative CR group, significantly shorter OS was only confirmed for patients with MRD-positive PR (HR: 2.38, $p=0.001$). As compared with the same reference group, there was no significant difference detectable in survival for patients with MRD-negative PR and MRD-positive CR (HR: 0.85, $p=0.612$ and HR: 0.92, $p=0.853$, respectively) (**Figure 2/a-b**.).

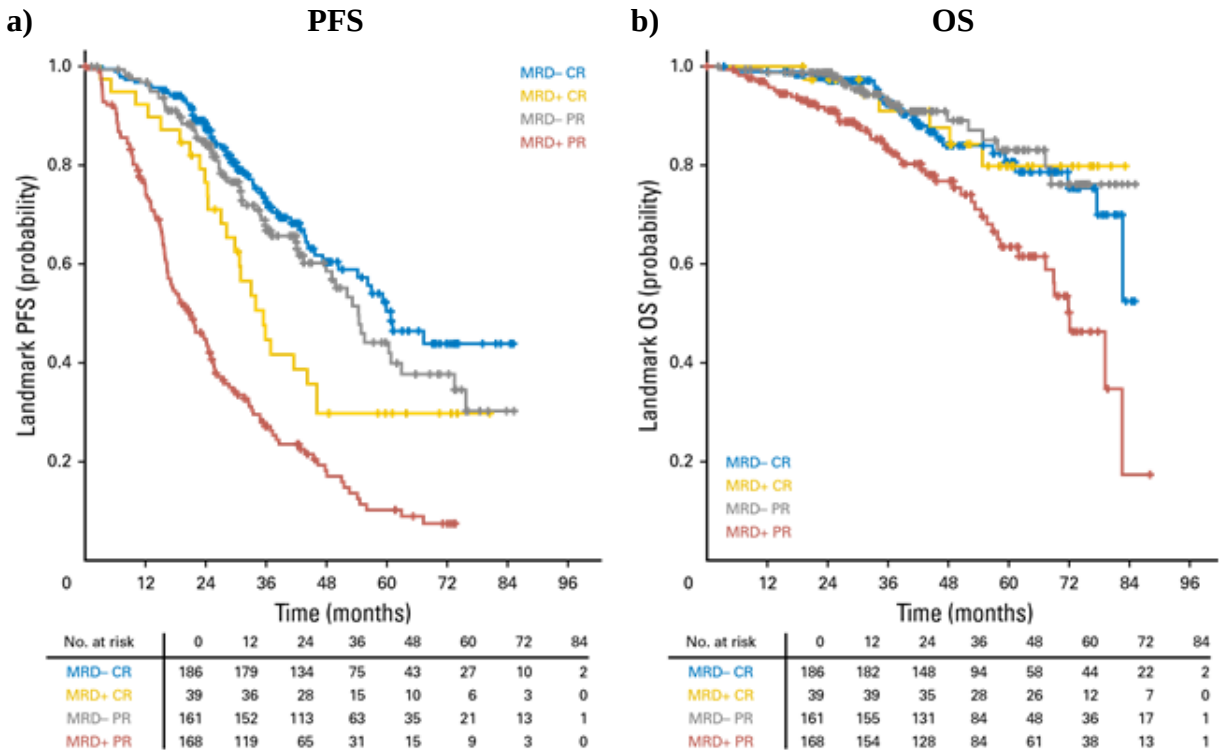

Figure 2/a-b .: *Landmark analysis on (a) the PFS and (b) the OS for patients grouped by clinical response and peripheral blood MRD results*

4.2.3. MRD from peripheral blood and bone marrow

In order to compare the prognostic value of MRD response from bone marrow and peripheral blood, we further investigated the group of patients having both measurement done (n=351).

Significantly longer **PFS** was demonstrated for patients (n=143 [40.7%]) showing MRD negativity in bone marrow and in peripheral blood compared to patients with MRD negativity in peripheral blood, but positivity in bone marrow (median NR vs. 43 months, HR: 3.13, p=0.005). For patients with MRD positivity in both compartments, median PFS was only 23 months.

After the integration of MRD measurement in our **multivariate model** for PFS, both bone marrow and peripheral blood MRD positivity had an independent prognostic significance (HR: 3.08, p<0.001 and HR: 2.13, p<0.001, respectively).

After grouping our patients according to their **bone marrow MRD measurement and clinical response**, median PFS were not reached for cases with MRD-negative CR and PR, and it was 36 months for MRD-positive CR, while 26 months for MRD-positive PR. Similarly to our

results based on MRD measurement from peripheral blood, there was no significant difference between patients with MRD negativity in bone marrow and having a CR or PR (HR: 1.19, p=0.638). While for MRD-positive CRs and PRs the PFS was significantly shorter as compared with MRD-negative CRs (HR: 3.15, p<0.001 and HR: 5.81, p<0.001, respectively). In addition, similarly to the peripheral blood MRD results, PFS was significantly longer for patients with bone marrow MRD negativity and PR, than patients having a CR, but MRD positivity in bone marrow (HR: 0.38, p=0.003).

4.2.4. The prognostic effect of residual disease compartments

In our further analysis, we investigated the possible effect of the residual disease compartments (spleen, lymph node, bone marrow) on the prognosis of the disease in MRD-negative patients. Of the 161 patients 78 (48.4%) had a single splenomegaly, 25 (15.5%) single lymphadenomegaly, 18 (11.2%) only bone marrow infiltration, and in 40 cases (24.8%) more than 1 compartments were involved. Median PFS of these patients was 63, 31, 49, and 52 months, respectively. Median OS was 68 months for patients with bone marrow involvement, while not reached for the other groups.

In our univariate analysis, PFS was significantly shorter only for **patients with lymphadenomegaly** as compared to MRD-negative CR (HR: 2.60, p<0.001). There was no significant difference detectable between the MRD-negative CRs and the other 3 groups (in case of splenomegaly: HR: 0.79, p=0.354; in case of bone marrow involvement: HR: 1.39, p=0.412, in case of more than 1 compartment involved: HR: 1.42, p=0.217).

In addition, we compared the MRD-negative PR groups with the MRD-positive CRs. In this case, PFS was again significantly longer for **patients with single splenomegaly** but associated with MRD negativity (HR 0.40, p=0061).

With using the bone marrow MRD result instead of peripheral blood, the difference was maintained. To exclude the potential bias due to the definition of spleen enlargement, we further analyzed our results with using different definition (cut-off with 12, 14 and 16 cm) for splenomegaly. Our results remained unchanged regardless of these cut-offs.

5. Discussion

5.1. Pre-therapeutic prognostic factors

On our patient population with 241 CLL cases, we confirmed the negative prognostic effect on TTFT and OS of gender (***male***). The negative prognostic effect for male patents could only be demonstrated for TTFT in multivariate analysis. The lack of its independent effect on OS, could be explained by the appearance of other negative prognostic factors, such as *IgHV* UM status, del(17p) and del(11q), that was verified in this patient group.

In accordance with other authors, the ***advanced age*** of patients has also proved to be a negative prognostic factor in our analysis. This was confirmed by our univariate and multivariate analysis regarding to OS.

The ***clinical stage*** (according to Rai and Binet) of disease also showed close correlation with the prognosis of disease in our patient population as confirmed by other authors. This was proved by multivariate analysis for both TTFT and OS.

We could confirm the negative effect of the increase of s***B2M*** (>3.5 mg/l), although the independent prognostic role of the marker was not demonstrated for TTFT and OS. This may be explained by the fact that the increase of sB2M correlates with the growth of whole tumor mass, thus correlating with the stage of disease described above.

We could confirm that diffuse ***bone marrow infiltration*** has a negative prognostic effect on both TTFT and OS.

We could confirm a closer correlation with the clinical course of the disease with using a cut-off with 30%, than 20% for ***CD38*** positivity. However, in our further analysis, the independent prognostic role of the marker was not verified either for TTFT or OS. This is consistent with the results of Hamblin et al., which states that the CD38 assay closely correlates especially with *IgHV* status.

The prognostic role of ***ZAP70*** was not confirmed, which could be explained by the difficulties in reproducibility of the ZAP70 measurement by using flow cytometry. We had no possibility for further immunohistochemical, Western blots analysis for this marker.

It has to be noted, that with the analysis of genetic aberrations according to the hierarchical model, in our univariate analysis we could confirm not only the positive effect of ***del(13q)*** and

normal karyotype, and the negative prognostic role of ***del(11q)*** and ***del(17p)***, but also negative effect of ***+12***. We also confirmed this negative prognostic role of +12 as sole abnormality. Regarding to OS we also confirmed the positive effect of del(13q) and normal karyotype, as the negative effect of del(11q) and del(17p). The presence of del(17p) was also confirmed as an independent marker for OS in our patient population. Due to the low number of cases, the prognostic role of ***del(6q)*** was not statistically evaluable in our analysis, however in line with the published data, the clinical course of these cases were not represented a clearly negative effect.

In our analysis we verified, that patients with ***multiplex*** (≥3) ***genetic aberrations*** have significantly shorter TTFT and OS, so that multiplex genetic abnormalities, regardless of their type, have a negative prognostic effect. Combined aberrations were confirmed only by chromosomal analysis in 91% of all cases, therefore the analysis - despite of their technical difficulties - is recommended for young patient with curative therapies.

In rare cases, we had the possibility for repeated tests. There were new abnormalities detectable at the time of clinical progression in 33% and in 15% by chromosome analysis and by FISH.

In the analysis of ***IgHV gene mutational status***, with using a cut-off with ≥98%, in contrast to the results observed by Hamblin et al., and in accordance with data published by Tobin et al., we demonstrated a higher number of UM status (n=50, 58.2%) in our patient population. We confirmed the excellent prognostic role of this marker, as according to the published data, a strong, independent effect was verified in our multivariate analysis for both TTFT and OS.

Analyzing the type of specific ***immunoglobulin heavy chain gene family***, we could confirm the known negative prognostic role of the appearance of $V_{1\text{-}69}$ and $V_{3\text{-}21}$ sequences on both TTFT and OS.

5.2. Post-therapeutic prognostic factors

With the analysis of two prospective phase III trials of the German CLL Study Group, we could confirm the prognostic role of peripheral blood MRD and the clinical response in the univariate analysis regarding to PFS and OS. Our multivariate analysis confirmed the prognostic effect of the MRD and clarified the value of the clinical response regarding to the PFS. The strongest correlation with PFS and OS was detectable for del(17p), MRD and *IgHV* status.

Regarding to OS, in accordance with other authors, the role of the clinical response were not verifiable in our analysis.

Looking at the relationship between ***the MRD and the clinical response***, we proved that despite the high number of patients, there is no significant difference in the PFS for patients with MRD-negative CR and PR. In contrast, similarly to previous observations, there was a significant difference in PFS between patients with MRD-negative and MRD-positive CR. In addition, we have confirmed that patients with MRD-negative PR can expect better prognosis than patients with CR, but with MRD positivity. With our results, we confirmed that - as opposed to the recommendation of the EMA - the MRD measurement not only for patients with CR, but also with PR is suggested.

Analyzing the prognostic value of ***MRD measurement from peripheral blood and bone marrow***, significantly longer PFS was demonstrated for patients with MRD negativity in both compartments vs. patients with peripheral blood negativity, but with bone marrow positivity. In our multivariate model, complemented by bone marrow MRD results, confirmed the independent prognostic role of the MRD measured in both compartments. The bone marrow result showed a closer correlation with PFS. Our findings confirm the better prognostic value of MRD measurement compared to clinical response and bone marrow compared to peripheral blood MRD.

In our further analysis, we investigated the ***prognostic effect of different residual disease compartments*** in patients with MRD-negative PR. We demonstrated that ***splenomegaly*** alone does not shorten PFS compared to patients achieving MRD-negative CR. We have also verified that these patients have significantly longer PFS than patients with CR, but MRD positivity. It could be further verified that this observation is independent of the cut-off value used for the definition of splenomegaly. Based on this observation, it can be assumed that residual spleen enlargement alone after chemoimmunotherapy does not refer to the presence of CLL. The biological activity of the disease can be better assessed by MRD in case of residual splenomegaly. We have confirmed that the sole ***residual lymph node enlargement*** in PR patients significantly shorten the PFS as compared to MRD-negative CR. This difference in prognosis can be explained by the recently published key role of lymph nodes in tumor proliferation and disease progression. It can be assumed that the lymph node microenvironment can support a faster division of residual CLL cells and a faster relapse of the disease.

6. Summary

Our analysis confirmed that the age, gender of patinets, Rai stage of the disease, morphology of bone marrow infiltration, sB2M, CD38, normal karyotype, del(13q), del(11q), del(17p), mutation status of *IgHV*, $V_{3\text{-}21}$, $V_{1\text{-}69}$ sequences are factors influencing the disease prognosis. We demonstrated that the CD38 positivity with a cut-off with 30% has a closer correlation with the prognosis, than 20%.

We have proved that the presence of +12, even in the case of a single occurrence, is clearly a negative prognostic factor for TTFT. For this reason, the analysis of the marker may be recommended in the clinical practice as a frequent control of these patients is necessary.

With our genetic analyzes (especially with chromosome analysis) **we proved that multiple genetic aberrations (≥3), irrespective of their type, also have a negative effect on prognosis regarding to TTFT and OS**. For this reason, chromosomal analysis may be recommended for young patients with curative therapy.

We have confirmed the appearance of new, negative prognostic factors with disease progression. For this reason - considering the therapeutic consequence of del(11q), del(17p) -, the repeated analysis of these markers is required in clinical practice before initiation of treatment.

We confirmed that the mutation status of *IgHV* has a very strong correlation with the course of the disease. For this reason, and due to the fact, that it can be applicable for the estimation of expected efficacy of the B-cell signaling pathway inhibitor new drugs (such as ibrutinib, idelalisib), its analysis can be recommended in the clinical practice.

With our further analysis we confirmed that compared to the therapeutic response, the MRD from the peripheral blood and compared to the MRD from peripheral blood, the bone marrow MRD have a closer correlation to the expected course of the disease.

For the first time, we have demonstrated that patients with MRD-negative PR have a significantly better prognosis compared to cases with a CR, but with MRD positivity. With this – in contrast to the current recommendation -, we have verified that **MRD measurement is recommended not only in patients with CR, but also with PR**.

In the case of patients with MRD-negative PR, we **first proved that splenomegaly alone does not impair the prognosis of the disease**, while **in case of lymph node enlargement an earlier progression can be expected.**

7. Acknowledgment

Our clinical research was granted by the application of "Multi-clinical prospective analysis on chronic lymphoid leukemia (CLL)", supported by the Ministry of Healthcare.

I would like to express my thanks to all colleagues of the University of Pécs, Clinical Center, 1st Department of Internal Medicine and the Department of Hematology, especially to Prof. Dr. Hajna Losonczy, who introduced me to the clinical hematology, helped me insistently in all of my work and Prof. Dr. Kálmán Tóth, Dr. Hussain Alizadeh, who supported me during my work in Hungary and in Germany.

I would like to thank the support of my previous and current members of my Hungarian working group: Dr. Lenke Molnár, Dr. Marrianna Dávid, Dr. Ágnes Nagy, Dr. Árpád Szomor, Dr. Tímea Vidra, Dr. Szabolcs Kosztolányi, Dr. Renáta Csalódi, Dr. Orsolya Tóth, Dr. Tamás Szendrei, Dr. Judit Pammer and Dr. Erna Gulyás.

I would like to thank for carrying out the flow cytometric analysis to Prof. Dr. Tamás Magyarlaki and Dr. Pál Jáksó, the genetic analyses to Prof. Dr. László Pajor, Prof. Dr. Gábor Méhes, Dr. Béla Kajtár, Dr. László Kereskai, Dr. Donát Alpár, Veronika Kalász, Ágnes Lacza, Roberta Kiss and Linda Deák, the statistical analysis to Dr. Kornélia Farkas and Dr. László Pótó.

GCLLSG trials were supported by Zentrum Klinische Studien (ZKS), Cologne, and Kompetenznetz Maligne Lymphome (KML), Cologne. The CLL8 and CLL10 trials were supported by Roche and Mundipharma companies and the German Federal Ministry of Education and Research.

I would like to thank for the help of all members of the German CLL Study Group, especially greatest thanks to Prof. Dr. Michael Hallek, the Head of the GCLLSG and the 1st Department of Internal Medicine, Uniklinik Köln, who gave me the opportunity to work in Germany and supported my work and life in all details in Germany, and to Dr. Barbara Eichhorst, Dr. Anna-Maria Fink and Dr. Sebastian Böttcher, who had an indispensable role in the creation and preparation of our joint research work.

Thanks to the support of my German colleagues: Dr. Valentin Goede, Dr. Kirsten Fischer, Dr. Paula Cramer, Dr. Julia von Tresckow and Dr. Christian Maurer, and in carrying out the statistical analyzes to Sandra Kluth and Jasmin Bahlo.

At last but not at least, thanks to my entire family for supporting me persistently to finalize this work.

8. Publication list

8.1. Publications related to the theses

1 **Kovács G**., Molnár L., Dávid M., Nagy Á., Szomor Á., Pajor L., Méhes G., Kajtár B., Jáksó P., Lacza Á., Magyarlaki T., Losonczy H.: Új prognosztikai faktorok vizsgálata krónikus lymphoid leukaemiában. Hemat Transzf 2005; 38: 218-224

2 Méhes G., **Kovács G**., Kajtár B., Lacza Á., Várnai A., Losonczy H., Pajor L: Karyotype complexity and VH gene status in B-cell chronic lymphocytic leukemia. Haematologica/the hematology journal 2006; 91, 1430-1431

3 Kajtár B., Jáksó P., Kereskai L., Lacza Á., Méhes G., Bodnár MA., Dombi JP., Gasztonyi Z., Egyed M., Iványi JL., **Kovács G**., Marton É., Palaczki A., Petz S., Tóth P., Sziládi E., Losonczy H., Pajor L.: Prognosztikai faktorok komplex vizsgálata krónikus lymphocytás leukémiában. Orv Hetil 2007; 148, 16.: 737- 743

4 Losonczy H., **Kovács G**., Kajtár B., Méhes G., Szendrei T., Potó L., Molnár L., Nagy Á., Dávid M., Szomor Á., Kosztolányi Sz., Csalódi R., Tóth O., Pajor L.: Új prognosztikai faktorok diagnosztikai és klinikai jelentősége krónikus lymphocytás leukaemiában. Magyar Belorvosi Archívum 2009; 62: 187- 195

5 **Kovacs G**, Robrecht S, Fink AM, Bahlo J, Cramer P, von Tresckow J, Maurer C, Langerbeins P, Fingerle-Rowson G, Ritgen M, Kneba M, Döhner H, Stilgenbauer S, Klapper W, Wendtner CM, Fischer K, Hallek M, Eichhorst B, Böttcher S. Minimal Residual Disease Assessment Improves Prediction of Outcome in Patients With Chronic Lymphocytic Leukemia (CLL) Who Achieve Partial Response: Comprehensive Analysis of Two Phase III Studies of the German CLL Study Group. J Clin Oncol. 2016 Aug 29. pii: JCO671305. [Epub ahead of print] PubMed PMID: 27573660.

Impact factor: ***20.982***

8.2. Abstracts related to the theses

1 Deák L., Kalász V., Jáksó P. **Kovács G**., Losonczy H., Méhes G.: Citogenetikai vizsgálatok krónikus lymphocytás leukaemiában (CLL). Magyar Belorvosi Archivum 2003, 1. Suppl., 56:13

2 **Kovács G**., Méhes G., Magyarlaki T., Losonczy H.: Prognosztikai faktorok vizsgálata chronicus lymphoid leukmiában. Magyar Onkológia 2004, 1. Suppl., 48: 20

3 **Kovács G**., Méhes G., Kajtár B., Pajor L., Deák L., Kalász V., Moilnár L., Dávid M., Nagy Á., Szomor Á., Losonczy H.: Genetikai vizsgálatok chronicus lymphoid leukemiában. Magyar Belorvosi Achivum 2005, 1. Suppl., 58: 139

4 **Kovács G**., Pajor L., Méhes G., Kajtár B., Lacza Á., Deák L., Kalász V., Pótó L., Molnár L., Dávid M., Nagy Á., Szomor Á., Szendrei T:, Losonczy H.: Genetikai vizsgálatok klinikai jelentősége krónikus lymphoid leukemiában. Hemat Transzf 2006, 1. Suppl., 39: 31

5 Losonczy H., **G. Kovács**, B. Kajtár, G. Méhes, L. Molnár, M. Dávid, Á. Nagy, Á. Szomor, T. Szendrei, L. Potó, L. Pajor: Influence of favourable and unfavourable genetic prognostic markers and CD 38 expression in chronic lymphocytic leukemia on treatment free interval and overal survival. Single center experience between 2002-2006 Blood Reviews 2007, 21: Suppl 1 Abstract No: P171

6 **Kovács G**, Pajor L, Méhes G, Kajtár B, Alpár D, Lacza Á, Deák L, Kalász V, Kiss R, Hermesz J, Molnár L, Dávid M, Nagy Á, Szomor Á, Kosztolányi Sz, Csalódi R, Tóth O, Szendrei T, Potó L, Losonczy H.: Új prognosztikai faktorok klinikai jelentősége krónikus lymphoid leukemiában. Hemat Transzf 2008;41:50

7 **Kovács G**, Losonczy H, Molnár L, Dávid M, Nagy Á, Szomor Á, Kosztolányi Sz, Csalódi R, Tóth O, Szendrei T, Pajor L, Méhes G, Alpár D, Kalász V, Kiss R, Kajtár B.: A del(11q) klinikai jelentősége krónikus lymphoid leukemiában. Hemat Transzf 2010;43:35

8 **Kovacs G**, Boettcher S, Bahlo J, Kluth S, Ritgen M, Fink Am, Stodden I, Cramer P, Von Tresckow J, Maurer C, Doehner H, Stilgenbauer S, Kneba M, Fischer K, Hallek M, Eichhorst B: Value of Minimal Residual Disease (MRD) Negative Status at Response Evaluation in Chronic Lymphocytic Leukemia (CLL): Combined Analysis of Two Phase III Studies of the German CLL Study Group (GCLLSG). American Society of Hematology 56th Annual Meeting 2014, San Francisco.

8.3. Publications not related to the theses

1 Molnár L., Nagy Á., Dávid M., Szomor Á., Méhes G, **Kovács G**., Losonczy H.: Imatinib kezelési eredmények krónikus myeloid leukaemia késői krónikus fázisában, interferon-α kezelés után. Orv Hetil 2004; 145(17): 901-907

2 **Kovács G**., Gasztonyi B., Battyány I., Nagy Á., Buzogány I., Kassai M., Hunyady B., Losonczy H.: Fiatalkori penisthrombosis. LAM 2005 15(12): 933-936

3 Szendrei T., Magyarlaki T., **Kovács G**., Nagy Á., Szomor Á., Molnár L., Dávid M., Tőkés Füzesi M., Rideg O., Pótó L., Pajor L., Kajtár B, Losonczy H.: Multidrogrezisztencia-vizsgálatok krónikus lymphoid leukaemiában. Orv Hetil 2008; 149: 161-167

4 Tóth O., Dávid M., Nagy Á., Szomor Á., Lima N., Kosztolányi Sz., **Kovács G**., Csalódi R., Szendrei T., Réger B., Losonczy H.: Rotációs thrombelastographia alkalmazása a familiáris thrombophilia diagnózisában. Hemat Transzf 2008; 41: 155-163

5 Cramer P, Isfort S, Bahlo J, Stilgenbauer S, Döhner H, Bergmann M, Stauch M, Kneba M, Lange E, Langerbeins P, Pflug N, **Kovacs G**, Goede V, Fink AM, Elter T, Fischer K, Wendtner CM, Hallek M, Eichhorst B. Outcome of advanced chronic lymphocytic leukemia following different first-line and relapse therapies: a meta-analysis of five prospective trials by the German CLL Study Group (GCLLSG). Haematologica. 2015 Nov;100(11):1451-9. doi: 10.3324/haematol.2015.124693. PubMed PMID: 26315931; PubMed Central PMCID: PMC4825288.

6 Fischer K, Bahlo J, Fink AM, Goede V, Herling CD, Cramer P, Langerbeins P, von Tresckow J, Engelke A, Maurer C, **Kovacs G**, Herling M, Tausch E, Kreuzer KA, Eichhorst B, Böttcher S, Seymour JF, Ghia P, Marlton P, Kneba M, Wendtner CM, Döhner H, Stilgenbauer S, Hallek M. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016 Jan 14;127(2):208-15. doi: 10.1182/blood-2015-06-651125. PubMed PMID: 26486789.

7 Eichhorst B, Fink AM, Bahlo J, Busch R, **Kovacs G**, Maurer C, Lange E, Köppler H, Kiehl M, Sökler M, Schlag R, Vehling-Kaiser U, Köchling G, Plöger C, Gregor M, Plesner T, Trneny M, Fischer K, Döhner H, Kneba M, Wendtner CM, Klapper W, Kreuzer KA, Stilgenbauer S, Böttcher S, Hallek M; international group of investigators.; German CLL Study Group (GCLLSG).. First-line chemoimmunotherapy with

bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. Lancet Oncol. 2016 Jul;17(7):928-42. doi: 10.1016/S1470-2045(16)30051-1. PubMed PMID: 27216274.

8 Maurer C, Langerbeins P, Bahlo J, Cramer P, Fink AM, Pflug N, Engelke A, von Tresckow J, **Kovacs G**, Stilgenbauer S, Wendtner CM, Müller L, Ritgen M, Seiler T, Fischer K, Hallek M, Eichhorst B. Effect of first-line treatment on second primary malignancies and Richter's transformation in patients with CLL. Leukemia. 2016 Oct;30(10):2019-2025. doi: 10.1038/leu.2016.113. PubMed PMID: 27133817.

Impact factor: ***57.125***

Cumulative impact factor: ***78.107***

8.4. Abstracts not related to the theses

1 Kecskés M., Dávid M., Molnár L., Nagy Á., Szomor Á., **Kovács G**., Vidra T., Losonczy H., Meyer M.: Autoszomális domináns öröklődésmenetet mutató, enyhe vérzékenységgel járó familiáris macrothrombocytopenia. Magyar Belorvosi Archivum 2001, 1. Suppl., 54: 24

2 Szomor Á., Molnár L., Nagy Á., Dávid M., Hussain A., Kecskés M., Vidra T., **Kovács G**., Kereskai L., Pajor L., Losonczy H.: Granulocytás sarcoma (extramedullaris myeloid tumor) akut myeloid leukaemiában. Magyar Belorvosi Archivum 2001, 1. Suppl., 54: 43.

3 Szomor Á., Molnár L., Nagy Á., Dávid M., Alizadeh H., Kecskés M., Vidra T., **Kovács G**., Losonczy H.: Philadelphia-negatív krónikus myeloproliferativ betegségek kezelése alfa-interferonnal. Magyar Belorvosi Archivum 2001, 2. Suppl., 54: 66.

4 **Kovács G**., Kereskai L., Molnár L., Nagy Á., Szomor Á., Losonczy H.: Akut myeloid leukaemia extramedullaris relapszusa – esetismertetés. Magyar Belorvosi Archivum 2002, 1. Suppl., 55: 70.

5 Molnár L., Dávid M., Nagy Á., Szomor Á., **Kovács G**., Losonczy H.: Fludarabin kezeléssel szerzett tapasztalataink CLL-ben és indolens lymphomában. Magyar Onkológia 2002, 1. Suppl., 46: 99.

6 Molnár L., Losonczy H., Dávid M., Nagy Á., Szomor Á., **Kovács G**.: Hazai tapasztalatok STI571 (Imatinib) kezeléssel CML krónikus fázisában. Magyar Belorvosi Archivum 2002, 3. Suppl., 55: 97.

7 Molnár L., Jáksó P., Méhes G., **Kovács G**., Szomor Á., Losonczy H., Pajor L.: Klonalitásvizsgálatok myelodysplasiás szindrómában citogenetiai és X-kromoszóma-inaktivációs módszerekkel. Magyar Belorvosi Archivum 2003, 1. Suppl., 56: 25

8 **Kovács G**., Molnár L., Kereskai L., Losonczy H.: Krónikus myeloproliferativ betegség kapcsán észlelt fatális hypercalcaemia esete. Magyar Belorvosi Archivum 2003, 1. Suppl., 56: 52

9 Vidra T., Dávid M., Szomor Á., Kosztolányi Sz., **Kovács G**., Szalontay Cs., Losonczy H.: Kemoterápiás score alkalmazása a perifériás őssejtgyűjtés sikerének prognosztizálására. Magyar Onkológia 2004, 1. Suppl., 48: 19

10 Molnár L., Nagy Á., Dávid M., Szomor Á., Kosztolányi Sz., **Kovács G**., Kajtár B., Losonczy H.: Imatinib – kezeléssel szerzett tapasztalataink CML-ben első és második vonalbeli alkalmazás során. Hemat Transzf 2005, 1. Suppl., 38: 56

11 Molnár L., Szomor Á., Dávid M., Nagy Á., Kosztolányi Sz., **Kovács G**., Losonczy H.: Köpenysejtes lymphoma kezelésével szerzett tapasztalataink. Hemat Transzf 2006, 1. Suppl., 39: 41

12 Nagy Á., Csalódi R., Kereskai L., Kajtár B., Molnár L., **Kovács G**., Kosztolányi Sz., Szomor Á., Dávid M., Losonczy H.: Folliculáris lymphomás betegeink immunokemoterápiája. Hemat Transzf 2006, 1. Suppl., 39: 43

13 Nagy Zs., Szomor Á., Kereskai L., Benkő I., **Kovács G**., Molnár L., Losonczy H.: Bronchus–asszociált lymphoma (BALT) kezelésében szerzett tapasztalataink. Hemat Transzf 2006, 1. Suppl., 39: 44

14 Szendrei T., Magyarlaki T., **Kovács G**., Pótó L., Nagy Á., Szomor Á., Molnár L., Dávid M., Losonczy H.: Multidrog rezisztencia vizsgálatok krónikus lymphoid leukémiában. Hemat Transzf 2006, 1. Suppl., 39: 55

15 Vidra T., Dávid M., Szomor Á., Kosztolányi Sz., **Kovács G**., Szalontay Cs., Pótó L., Losonczy H.: Össejtgyűjtést megelőző kemoterápia toxikus hatásának jóslása pontrendszerrel. Hemat Transzf 2006, 1. Suppl., 39: 64

16 Szendrei T., T. Magyarlaki, **G. Kovács**, Á. Szomor, Á. Nagy, L. Molnár, M. Tőkés-Füzesi, O. Rideg, L. Potó, H. Losonczy: Multidrog resistance in chronic lymphocytic leukemia. Blood Reviews 2007, 21: Suppl 1 Abstract No: P202

17 Pammer J., Nagy Á., Molnár L., Kosztolányi Sz., **Kovács G**., Csalódi R., Vidra T., Losonczy H., Dávid M., Szomor Á.: Autológ hemopoetikus őssejtátültetés Hodgkin-lymphomában: pécsi eredmények. . Hemat Transzf 2008, 1. Suppl., 41: 19

18 **Kovács G**., Szomor Á., Nagy Á., Kosztolányi Sz., Csalódi R., Szőts M., Illés Z., Tóth M., Gömöri É., Kereskai L., Kravják A., Dávid M.: Klinikánk intravaszkuláris lymphomás esetei. Magyar Belorvosi Archivum 2008, 3. Suppl., 61: 76.

19 Kosztolányi Sz., Nagy Á., Szomor Á., Csalódi R., **Kovács G**., Melegh B., Dávid M.: BRCA-gén mutációs vizsgálat 3 primer tumorral rendelkező 50 éves nőbetegben. Magyar Belorvosi Archivum 2008, 3. Suppl., 61: 75-76.

20 Csalódi R., Szomor Á., **Kovács G**., Kosztolányi Sz., Kereskai L., Dávid M.: Többszöri extranodális relapszus diffúz nagy B-sejtes lymphomában. Magyar Belorvosi Archivum 2008, 3. Suppl., 61: 47-48.

21 Dávid M., Szomor Á., Alpár D., Kajtár B., Nagy Á., Kosztolányi Sz., **Kovács G**., Csalódi R., Hermesz J., Tábori J., Szalontay Cs., Pajor L., Losonczy H.: A plazmasejt-daganatok autológ őssejt-transzplantációjával szerzett tapasztalatok a PTE I. sz. Belgyógyászati Klinikáján. Hemat Transzf 2008. Suppl 1: 49-50.

22 Szendrei T, Magyarlaki T, **Kovács G**, Nagy Á, Szomor Á, Dávid M, Tőkés-Füzesi M, Rideg O, Pótó L, Losonczy H.: Multidrogrezisztencia-vizsgálatok krónikus lymphoid leukaemiában. Magyar Belorvosi Archívum. 2008; 61:113.

23 Szomor Á, Nagy Á, **Kovács G**, Kosztolányi Sz, Vidra T, Kereskai L, Csalódi R, Losonczy H, Dávid M: T-sejtes non hodgkin lymphomás betegek autológ hemopoetikus őssejt-transzplantációs eredményei. Magyar Belorvosi Archívum 2008;61:115

24 David M., Kosztolanyi Sz., Szomor A., Alpar D., Kajtar B., Nagy A., **Kovacs G**., Csalodi R., Hermesz J., Tabori J., Szalontay Cs., Losonczy H., Pajor L.: Genetic prognostic

factors and the outcome of autologous stem cell transplantation in plasma cell disorders. Bone marrow transplantation. 2009; 1. Suppl.: 43:149.

25 Nagy Á., Losonczy H., **Kovács G**., Csalódi R., Tóth O., Kosztolányi Sz., Szomor Á., Dávid M.: Esszenciális thrombocytás betegek Thromboreductin-kezelésével szerzett tapasztalataink. Hemat Transzf 2009. Suppl. 1:48.

26 Szomor Á., Szendrei T., Nagy Á., Kosztolányi Sz., **Kovács G**., Molnár L., Csalódi R., Tóth O., Vidra T., Kereskai L., Losonczy H.: Autológ haemopoeticus őssejt-transzplantáció (ASCT) agyi érintettséggel járó non-Hodgkin-lymphomás betegeknél. Hemat Transzf 2009. Suppl. 1:53.

27 **Kovács G**., Nagy Á., Szomor Á., Losonczy H., Csalódi R., Tóth O., Kosztolányi Sz., Dávid M.: A thromboticus thrombocytopeniás purpura rituximab kezelsével szerzett tapasztalataink 3 eset kapcsán. Hemat Transzf 2011. Suppl. 1. 44:96.

28 Kosztolányi Sz., **Kovács G**., Tóth O., Szomor Á., Nagy Á., Molnár L., Losonczy H., Dávid M.: Essentialis thrombocytaemia anagrelid-kezelése, eredményeink. Hemat Transzf 2011. Suppl. 1. 44:45-46.

29 Farkas L., Szabó E., Kajtár B., Kereskai L., Bátai Á., Reményi P., Molnár L., **Kovács G**., Csalódi R., Nagy Á., Kosztolányi Sz., Tóth O., Losonczy H., Dávid M., Szomor Á.: Késői relapszus autológ haemopoeticus őssejt—traszplantáció után anapláziás nagysejtes lymphomában. Hemat Transzf 2011. Suppl. 1. 44:86.

30 Szomor Á., Kosztolányi Sz., Csalódi R., **Kovács G**., Nagy Á., Tóth O., Pammer J., Losonczy H., Dávid M.: Autológ haemopoeticus őssejt-transzplantáción átesett lymphomás betegeink kondícionáló kezelésével szerzett tapasztalataink. Hemat Transzf 2013. Suppl. 1. 46:120-121.

31 Losoczy H., **Kovács G**.: A CLL naprakész kezelési lehetőségei. Hemat Transzf 2013. Suppl. 1. 46:82.

32 Eichhorst B, Fink Am, Busch R, **Kovacs G**, Maurer C, Lange E, Köppler H, Kiehl Mg, Soekler M, Schlag R, Vehling-Kaiser U, Köchling Gra, Plöger C, Gregor M, Plesner T, Trneny M, Fischer K, Döhner H, Kneba M, Wendtner Cm, Klapper W, Kreuzer Ka, Stilgenbauer S, Böttcher S, Hallek M: Frontline Chemoimmunotherapy with Fludarabine (F), Cyclophosphamide (C), and Rituximab (R) (FCR) Shows Superior Efficacy in Comparison to Bendamustine (B) and Rituximab (BR) in Previously Untreated and Physically Fit Patients (pts) with Advanced Chronic Lymphocytic Leukemia (CLL): Final Analysis of an International, Randomized Study of the German CLL Study Group (GCLLSG) (CLL10 Study). American Society of Hematology 56th Annual Meeting 2014, San Francisco.

33 **G Kovacs**, J Bahlo, S Kluth, P Cramer, AM Fink, K Fischer, C Gross-Ophoff-Mueller, P Langerbeins, C Maurer, J Tresckow, CM Wendtner, S Stilgenbauer, M Hallek, B Eichhorst, V Goede: Prognostic Impact and Risk Factors of Reducing Prescribed Doses of Fludarabine, Cyclophosphamide and Rituximab (FCR) during Frontline Treatment of Chronic Lymphocytic Leukemia (CLL). American Society of Hematology 57th Annual Meeting 2015, Orlando.