

**SHORT THESIS FOR THE DEGREE OF DOCTOR OF  
PHILOSOPHY (PHD)**

**Novel prognostic markers in B cell lymphoproliferative disorders**

**by Ildikó Pál, MD**

**Supervisor: László Váróczy, MD, PhD**



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Prof. Dr. Takács István, MD, PhD

The Examination takes place at the Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 14<sup>th</sup> of July, 2020. 12:00 am.

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 14<sup>th</sup> of July, 2020. 14:00 pm.

## 1. Introduction

B cell lymphomas can arise at any stage of normal B cell development. This essay focuses on diffuse large B cell lymphoma and multiple myeloma among B-cell disorders.

Diffuse large B cell lymphoma (DLBCL) is the most common histologic subtype of non-Hodgkin-lymphoma (NHL) accounting for approximately 25-40% of NHL cases. The median age at presentation is 64 years. Patients with DLBCL typically present with a rapidly enlarging symptomatic mass, most usually nodal enlargement in the neck or abdomen, or in the case of primary mediastinal large B cell lymphoma, the mediastinum. Systemic „B” symptoms (fever, weight loss, drenching night sweats) are observed in 30% of patients. The diagnosis of DLBCL is best made based on by excisional tissue biopsy, most commonly a lymph node. The pathologic diagnosis of DLBCL is based on morphology and immunophenotyping. Laboratory findings, such as LDH and  $\beta$ 2-microglobulin levels may help to establish the diagnosis. In case of bone marrow involvement pancytopenia may be detected in the peripheral blood as well. Imaging techniques, X-rays and ultrasound are performed to determine the stage of the disease. Although CT and MR can be performed also, PET-CT is the most sensitive technique for staging. The Ann Arbor staging system is commonly applied which was modified by Lugano classification. Disease prognosis is determined by International Prognostic Index (IPI). Diffuse large B cell lymphoma should be treated as soon as possible with systemic chemotherapy plus immunotherapy with the recombinant anti-CD20 antibody rituximab. R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristin, prednisone) therapy is the gold standard in DLBCL. Primary refractory or relapsing DLBCL may respond to autologous stem cell transplantation after salvage protocols. New therapeutical tools, such as the bcl-2 inhibitor venetoclax, PD-1 inhibitors, CAR-T cells (chimera antigen receptor T cell therapy), brentuximab vedotin, blinatumomab could be also considered in the treatment of refractory cases. Metabolic enzymes like cytochrome P450 (CYP), glutathione-S-transferase (GST), and N-acetyl-transferase (NAT) are involved in the elimination of carcinogenes and drug metabolism. Consequently, these metabolic enzymes may play a role not just in the lymphomagenesis, but may also influence the therapeutic response and survival of lymphoma patients.

Multiple myeloma (MM) accounts for 1 to 2 percent of all cancers and 17 percent of hematologic malignancies. MM is a disease of older adults. The median age at diagnosis is 66 years, only 10 and 2 percent of patients are younger than 50 and 40 years. Most patients with MM present with signs or symptoms related to the infiltration of plasma cells within the bone or other organs. The disease is characterized by the CRAB symptoms, which include hypercalcaemia, renal failure, anaemia and bone lesions. Besides these symptoms, neuropathy and infections are also common. In case of multiple myeloma high erythrocyte sedimentation rate, anaemia, renal failure, hypercalcaemia are typical in the laboratory findings. Serum LDH, total protein, uric acid,  $\beta$ 2-microglobulin levels are elevated, but albumin level is low. Monoclonal protein can be detected with serum and / or urine electrophoresis methods. Bone marrow examination is necessary to establish the diagnosis. Flow cytometry and FISH examination should be performed from the bone marrow sample. Imaging is a key part of the evaluation of all patients with multiple myeloma. First X-ray should be done to detect lytic bone lesions and plasmocytomas. However CT, MR and PET-CT are much more sensitive in detection of the bone lesions. Diagnosis is based on the IMWG criteria. The stage is determined by International Staging System (ISS). In terms of multiple myeloma treatment, clinicians have to decide patients' transplant eligibility. Patients who are eligible for autologous stem cell transplantation should receive 4-6 cycles of proteasome inhibitor containing triplets. After the induction treatment stem cell collection and autologous stem cell transplantation could be performed. In high risk patients consolidation therapy is administered and most of the patients receive maintenance treatment. If the patient is not eligible for transplantation, proteasome inhibitor or immunomodulatory drug containing induction therapy is offered. New therapeutic agents, monoclonal antibodies such as anti-CD38 antibody, daratumumab and SLAMF7 inhibitor, elotuzumab can be further treatment options. Bcl-2 inhibitors (in case of t(11,14) translocation), check point inhibitors such as pembrolizumab and CAR-T cells promise good therapeutic response in refractory multiple myeloma patients. In multiple myeloma treatment efficacy is influenced by several factors. Imids act via the cereblon- $\beta$ -catenin pathway. Thalidomide binds to cereblon and inhibits its E3 ubiquitin ligase function. Moreover, thalidomide facilitates upregulation of p21 and downregulation of interleukine-8, that results in an arrest in the G0/G1 transition of the cell cycle. However, myeloma cells are able to become resistant against Imids which process is still not clear in details. Lenalidomide and thalidomide increase the intracellular concentration of  $\beta$ -catenin, but also facilitate the expression of c-myc and other anti-apoptotic factors, and treatment may become ineffective consequently.

## 2. Aims

1. Our aim was to investigate whether drug metabolism gene polymorphisms have any effect on the treatment responses and survival data in DLBCL
2. Our purpose was to investigate if CRBN, CTNNB1 and GSPT1 gene polymorphisms have any effect on treatment response and survival data of a large group of Hungarian MM patients and if administration of personalized treatment can be considered in this disease
3. The objective of the third study was to describe presenting features and outcomes of multiple myeloma patients  $\leq 40$  years and to compare their survival data to the older MM population

### 3. Patients and methods

1. Fifty-one DLBCL patients were involved in the study. They were all diagnosed with DLBCL between February 2011 and November 2016. The clinical files of DLBCL patients were reviewed with particular reference to age, gender, IPI, response to treatment, and survival. Examining the survival rates, overall survival was determined by consideration of death events due to any reason, while event-free survival was determined by consideration of death events, relapses, or disease progression that indicated further treatment. Genotyping of the single nucleotide polymorphisms (SNPs) was conducted by real-time polymerase chain reaction (PCR). High molecular weight DNA for genotyping was extracted from peripheral blood samples according to the manufacturer's recommendation using a QiaAmp DNA Blood Mini Kit (Qiagen GmbH, Germany). DNA was quantitated by UV absorption at 260 and 280 nm. Genotyping of the single nucleotide polymorphisms (SNPs) was conducted by real-time polymerase chain reaction (PCR). We used a real-time PCR method that proved to be faster than the conventional methods based on restriction enzyme digestion. PCR primers and TaqMan probe specific for the polymorphisms were purchased from Applied Biosystems (Foster City, CA, USA). The assay enables scoring of both alleles in a single well. Real-time PCR was performed using Corbett Rotor-Gene RG-3000 (Qiagen, Hilden, Germany) equipment, which was set to detect FAM and VIC reporter dyes simultaneously. The PCR reaction was carried out in a 20 µl reaction volume containing TaqMan Universal Master Mix (2X, 4331182, Applied Biosystems), TaqMan genotyping Assay (20X), and optimized quantities of genomic DNA. The Universal Master Mix contained AmpliTaq Gold DNA Polymerase, AmpErase UNG, dNTPs with dUTP, passive reference, and optimized buffer components. Reactions were set up in duplicate. Thermal cycling was initiated by incubation at 95°C for 10 minutes for optimal AmpErase UNG activity and activation of AmpliTaq Gold DNA polymerase. After this initial step, 40 cycles of PCR were performed. Each PCR cycle consisted of heating to 92°C for 15 seconds for melting, and to 60°C for 1 minute for annealing and extension.

2. In terms of 97 multiple myeloma patients cereblon-beta-catenin gene polymorphisms were determined. Patients were diagnosed between January 2012 and December 2016. The clinical files of multiple myeloma patients were reviewed with particular reference to age, sex, clinical stage, response to treatment and survival. ISS stages were determined using the International Myeloma Working Group criteria. FISH results of unfavourable prognosis included t(4;14), t(14;16) and del(17p). Examining the survival rates,

overall survival (OS) was determined by consideration of death events due to any reasons, while progression-free survival (PFS) was determined by consideration of relapses, deaths or disease progression that indicated further treatment. DNA for genotyping was extracted from peripheral blood samples obtained into K3-EDTA Vacutainer tubes (Becton Dickinson, San Jose, CA, USA) using QiaAmp DNA Blood Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's recommendations. LightSNip typing assays were applied to determine CRBN (rs121918368C>T), CTNNB1 polymorphisms (rs4135385 A>G, rs4533622 A>C) and GSTP1 105 (rs1695A>G) and 114 (rs1138272C>T) (TIB-MolBiol, Berlin, Germany) on a LightCycler 480 Real-time PCR Instrument (Roche Diagnostics, Mannheim, Germany). Amplifications were performed based on the recommendations of the manufacturer. PCR reaction was carried out in 20 µl volume containing the Light Cycler Fast Start DNA Master HybProbe (Roche Diagnostics). Samples were run in duplicates.

3. Patients who were 40 years or less and diagnosed with multiple myeloma at our institute between 01 January 2006 and 31 December 2015 were included in this study. The diagnosis of multiple myeloma was established according to the relevant International Myeloma Working Group (IMWG) criteria. Those patients who had monoclonal gammopathy, smouldering myeloma or solitary plasmacytoma were excluded from the trial. Genetical alterations were examined with the fluorescence in situ hybridization (FISH) method. The treatment they received was administered according to the current recommendations of the national myeloma working group. Response to treatment and progression were assessed using the IMWG criteria.

### **Statistical analysis**

Examining the survival rates, overall survival (OS) was determined by consideration of death events due to any reasons, while progression-free survival (PFS) was determined by consideration of relapses or disease progression that indicated further treatment. Descriptive statistical analysis was used to characterize the patient populations. Normality of the parameters were examined applying the Wilk-Saphiro test. Comparing two groups, F probe and t test were administered by normal distribution of the parameters, otherwise the nonparametrical Mann-Whitney test was applied. Differences were significant if probability level was less than 5 % ( $p < 0.05$ ). Survival rates were calculated using the Kaplan Meier's method, while the survival data were compared using the log-rank test.

#### 4. Results

1. Altogether 51 patients, 32 males, 19 females, were involved in the study. Their mean age was 53.1 years. They were all diagnosed with DLBCL between February 2011 and November 2016 and received 1–8 cycles (6.2 cycles as average) of R-CHOP-14 or R-CHOP-21 chemotherapies. The data collection was finished in May 2016, the mean follow-up time was 3.78 years. Of the patients, 42% had activated B-cell type, 24% had germinal center type disorder, and the rest were not classified. Therapeutic response and survival data in terms of the four drug metabolism gene polymorphisms (CYP2E1, GSTP1, NAT1, and NAT2) resulted in the following outcomes.

In terms of the CYP2E1 gene, encoding an important member of cytochrome P450 enzyme network, TT genotype was found to be dominant. There was no significant difference in clinical features and therapeutic response data between patients bearing AA, AT, or TT genotypes. Patients with AA genotype were found to have a bit more favorable overall and event-free survival results than those with either AT heterozygosity or TT homozygosity.

With respect to the GSTP1 gene, the homozygous AA genotype was found to be most common when examining GSTP1 gene polymorphisms and only five patients bore two G alleles. Clinical features and therapeutic responses were quite similar in all three groups. There were no significant differences found in the overall and event-free survival results.

In terms of the NAT1 gene, no AA homozygous patients were found. Overall response rate and complete remission data were more favorable in AG heterozygous than in GG homozygous patients; however, the differences were not significant. Survival results were very similar in the two groups.

The distribution of A and G alleles was quite steady when examining the NAT2 gene. GG homozygosity seemed to be more favorable in terms of therapeutic response and survival results; however, the differences were not statistically significant.

2. Ninety-seven multiple myeloma patients were involved in the study who were treated in our institution between January 2012 and December 2016. Their mean age was 62.47 years at diagnosis, male-female ratio was 43:54. The distribution of ISS1, 2 and 3 stages were 27.8%, 42.3% and 29.9%, respectively. FISH tests were performed in 67 patients and 42 (62.7%) of them had a result of unfavourable prognosis. Treatment modalities included thalidomide-

based (59 cases), lenalidomide-based (18 cases), and alkylating-agent based (57 cases) regimens. Thirty-eight patients received two or more treatment lines. In this cohort, the distribution of CTNNB1 (rs4135385) AA, AG and GG genotypes were 48.5%, 47.4% and 4.1%, respectively. Patients with AA genotype were older than those who carried G allele (64.5 vs. 61.0 years of age,  $p=0.05$ ). However, the presence of the CTNNB1 (rs4135385) AA genotype did not show association with ISS stage or FISH test results. Regarding the rs4533622 polymorphism of the CTNNB1, the different genotypes were detected in the following ratio: AA genotype in 18.5%, AC in 50.5% and CC in 31% of these patients. MM was diagnosed at significantly younger age in those carrying the CC genotype (59.1 vs. 65.7 years,  $p=0.015$ ). Additionally, females were more likely to bear the AA genotype. Similarly to the other polymorphism of  $\beta$ -catenin, there was no relationship between allele distribution and clinical stages or FISH results. In terms of the CRBN (rs121918368) polymorphism, all patients carried the CC genotype, thus, no further analysis was performed in regard to clinical features, treatment results and survival ratio. For the GSTP1 105 polymorphism, 54 (55.7%) patients carried AA (Ile/Ile), 33 (34%) had AG (Ile/Val) and 10 (10.3%) had GG (Val/Val) genotype. When GSTP1 114 polymorphism was tested among these MM individuals, CC (Ala/Ala) genotype was found in 85 (87.6%) subjects, while CT (Ala/Val) genotype was found in 12 (12.4%) patients. No association was observed between these polymorphic variants in GSTP1 and demographical data. We also investigated the effect of CTNNB1, CRBN and GSTP1 polymorphisms on response to chemotherapy. We found that response to Imids-based therapies ( $p<0.05$ ) and PFS ( $p=0.032$ ) were significantly more favourable in AA homozygous group. Cox univariable (HR: 2.371 [1.026–5.477],  $p=0.043$ ), and multivariable analysis tests (HR: 2.554 [1.099–5.933],  $p=0.029$ ) for PFS proved that CTNNB1 (rs4135385) polymorphism can be considered as an independent prognostic factor besides ISS stages and FISH results. In patients with stage II and stage III disease, G carriers were expected to have more unfavourable survival results than AA homozygous individuals. In contrast, the other polymorphism (rs4533622) of  $\beta$ -catenin gene did not markedly influence the effectivity of thalidomide and lenalidomide-based therapies. Progression-free survival was markedly favourable in the AA group; however, the survival curves did not differ significantly from each other.  $\beta$ -catenin gene polymorphisms did not have any impact on the occurrence of common side effects of the Imid-based therapies. Interestingly, when GSTP1 105 and 114 polymorphisms were investigated, no significant association was observed between the response to chemotherapy or survival ratio of these MM patients.

3. Among 312 multiple myeloma patients there were sixteen (5,1%), ten males and six females who were 40 years old or younger at the time of diagnosis. Eight patients had IgG, three patients had IgA-type disease, three had light-chain myeloma and two of them had non-secretory disease. FISH test was performed in eleven patients, hyperdiploidy was detected in six, t(4;14) in three and del(17p) in two cases, respectively. The most common 'CRAB' symptom was bone disease (fourteen cases), followed by hypercalcaemia (three cases), anaemia (two cases) and kidney failure (two cases). Five patients had two or more CRAB symptoms. The distribution of the ISS disease stages was the following: seven patients had stage 1, five patients had stage 2 and four patients had stage 3 disease at the time of diagnosis. Regarding the induction treatment, twelve patients received bortezomib-containing regimens including VTD (bortezomib, thalidomide, dexamethasone), PAD (bortezomib, doxorubicine, dexamethasone), CyBorDex (cyclophosphamide, bortezomib, dexamethasone) or VTDPACE (bortezomib, thalidomide, dexamethasone, cisplatin, doxorubicine, etoposid, cyclophosphamide), two patients received thalidomide-dexamethasone and two patients received VAD (vincristine, doxorubicine, dexamethasone) protocol. Two patients died because of progressive disease during the period of induction treatment, one of them had primary plasma cell leukaemia. Fourteen patients underwent autologous peripheral stem cell transplantation (APSCT), the conditioning regimen was high-dose melphalan (200 mg/m<sup>2</sup>) in all cases. Ten patients were administered consolidation or maintenance therapy after the APSCT, that included either bortezomib-based combinations or monotherapies (thalidomide or interferon). Altogether 294 patients underwent APSCT in our institute within the 10 years' period, 132 of them (45%) were diagnosed and treated in other hospitals before hand. We compared our young patients' treatment results to those patients' data who received APSCT but were older than 40 years at the time of diagnosis. There were no significant differences found between treatment results, however, young patients were more likely to receive maintenance or consolidation therapies. We also compared the survival data of patients  $\leq$ 40 years and  $>$ 40 years old. The 5-year overall survival were 83% and 53%, the 5-year progression-free survival were 48% and 35%, respectively.

## 5. Discussion

Prognosis of B cell lymphoproliferative disorders is influenced by several factors. Genetical studies of drug metabolising gene polymorphisms and other disease modifying factors are highlighted nowadays as these factors may help us to treat these patients more effectively and in this way, progression-free and overall survival of patients may be prolonged.

In our study we were searching the role of different prognostic factors in diffuse large B-cell lymphoma and multiple myeloma patients to determine if personalized medicine can be implemented.

In our first study the therapeutic response and survival data of DLBCL patients treated with R-CHOP therapy were reviewed in terms of drug metabolizing gene polymorphisms. Metabolic enzymes like cytochrome P450 (CYP), glutathione-S-transferase (GST), and N-acetyl-transferase (NAT) are involved in the elimination of carcinogenes and drug metabolism. Consequently, these metabolic enzymes may play a role not just in the lymphomagenesis, but may also influence the therapeutic response and survival of lymphoma patients. In our study, therapeutic response and survival data of 51 diffuse large B-cell lymphoma patients were investigated in terms of the polymorphisms of four drug metabolism genes (CYP2E1, GSTP1, NAT1, and NAT2). CYP2E1, a member of the CYP enzyme family, is expressed mainly in the liver and participates in the metabolism of nitrose amins and several other small-sized toxic molecules entering the body. The polymorphism of the CYP2E1 gene may play a role in the development of NHL. Moreover, the efficacy of R-CHOP treatment may vary in patients having different genotypes, as CYP2E1 contributes to the metabolic process of both cyclophosphamide and rituximab. In our experience, complete remission rates and event-free survival data were slightly more favorable in patients being homozygous for the T allele; however, the overall survival results were similar in all groups. Interestingly, other researchers found that individuals bearing the T allele had an elevated risk for the development of DLBCL. Glutathione-S-transferase has an important role in the metabolization of several drugs, such as prednisolon and vincristin, and consequently it influences the efficacy of treatment and the development of any toxicities. Others reported that the AG genotype of GSTP1 AG is associated with a lower risk for NHL development. Chinese authors declared that markedly favorable survival results can be expected in those NHL patients in which the deletion of GSTT1 isoenzymes were detected. In the presence of

the G allele of GSTP1 isoenzyme, markedly higher myelo- and gastrointestinal toxicities can be observed. The enzymes NAT1 and NAT2 also play an important role in the elimination of toxic agents. Earlier studies reported that the polymorphism of NAT1 influences cell growth and may contribute to the development of etoposide resistance. We found GG genotype in most of our DLBCL patients; however, the presence of the A allele was associated with a markedly favorable survival result. Our results also highlighted that the NAT1 gene polymorphism may have a role in the therapeutic response to R-CHOP treatment, as complete remission was more commonly achieved in GA heterozygous patients. In terms of the NAT2 gene, the results were quite similar, as bearing the G allele was associated with a markedly favorable prognosis and therapeutic response.

Other part of our study was based on multiple myeloma. The gene polymorphisms of the cereblon-beta-catenin pathway and glutathione-S-transferase were investigated in multiple myeloma patients and the other objective of this study was to describe presenting features and outcomes of multiple myeloma patients  $\leq 40$  years and to compare their survival data to the older MM population.

The gene polymorphisms of the cereblon-beta-catenin pathway and glutathione-S-transferase were also investigated in multiple myeloma patients. Recently, it has been becoming a trend to administer personalized treatment modalities in cancer patients to improve survival data. Molecular profiling can be a good tool for effective personalized care. In our study, the gene polymorphisms of the cereblon-beta-catenin pathway and glutathione-S-transferase were investigated in multiple myeloma patients. Several publications have highlighted the importance of CRBN gene expression in the effectivity of Imid-based therapies. Thalidomide resistance may occur in case of a low CRBN activity, while high levels of CRBN mRNA were found to be associated with better responses to thalidomide therapy. Our patient population was homogenous in terms of the CRBN gene polymorphism as each of them carried the CC genotype. On the other hand, we could find some links with the CTNNB1 (rs4533622) polymorphism. In CC homozygous patients, the onset of the disease occurred at a significantly younger age and A alleles were significantly less common in males. These associations have never been published as yet.

CTNNB1 (rs4533622) polymorphism was reported to influence the ISS stages as patients carrying an A allele had more advanced disease. Formerly other authors also described better treatment responses and more neutropenic events in AA homozygous patients. However, we

could not confirm such associations in our cohort. A few studies investigated the role of CTNNB1 (rs4135385) polymorphism in the pathogenesis of malignant disorders. On the other hand, no predisposing role of any CTNNB1 (rs4135385) polymorphism could be confirmed in terms of multiple myeloma. However, cyclophosphamide – thalidomide – dexamethasone (CTD) combination therapy was more effective in MM patients carrying the AA genotype. Our results were in accordance with this finding as therapeutic responses and progression-free survival results were more favourable in AA homozygous patients after Imid-based therapies. Moreover, CTNNB1 (rs4135385) polymorphism were found to be an independent prognostic factor in terms of PFS results besides ISS stages and FISH results. No associations were detected between beta-catenin gene polymorphisms and the severity of any Imid-related side effects. Being aware of this polymorphism, personalized treatment strategies can be suggested for multiple myeloma patients. Glutathione-S-transferase enzyme plays an important role in the metabolism of carcinogens and cytostatic agents as well. GSTP1 105 variant of this enzyme has less ability for detoxification, therefore the effectivity of cytostatic agents is increased in such multiple myeloma patients. In patients carrying the GSTP1 105Ile homozygous genotype, event-free and overall survival results were reported to be more favourable after alkylating agent or anthracyclin-based treatment. In our cohort, neither GSTP1 105, nor GSTP1 114 polymorphisms had any impact on the treatment results and survival data. Our results highlighted that beta-catenin gene rs4135385 gene polymorphism may influence the clinical features of multiple myeloma patients and have some impact on the treatment results and survival data after Imid-based therapies. Determination of molecular patterns and gene polymorphisms in multiple myeloma patients is an important aspect of personalized medicine which may improve patients' survival.

We found sixteen young patients in the whole population diagnosed with multiple myeloma at our institute within a 10 years' period and this ratio (5.1%) is higher than in other centers. Young MM patients used to be thought to have more indolent courses of the disease presenting with multiple solitary or extramedullary plasmocytomas, more osteolytic lesions, but fewer infiltrating plasma cells within the bone marrow. However, other authors reported a cohort of 72 patients younger than 40 years old who had the very same clinical features as the older population. Polish researchers published a multi-institutional case-control study in which they compared the characteristics and outcomes of MM patients aged 21–40 versus 41–60 years. In their large cohort, they could analyze the data of 173 young patients and found a higher incidence of lytic bone lesions among them than in the older group. The same working

group found 52 patients who were  $\leq 30$  years of age, 22% of them presenting with light chain-only disease. In the Mayo Clinic cohort, higher rates of plasma cell leukaemia and renal failure were found under 40 years of age. In our patients, the ratio of ISS stages was more or less of equipartition and the most common CRAB finding was bone lesion, however, no statistical analysis could be performed due to the low number of cases. Only one patient presented with primary plasma cell leukaemia. Austrian researchers were the first who analyzed cytogenetical alterations in multiple myeloma patients younger than 50 years old and found no difference from older population in the frequency of any cytogenetic abnormality. However, the Polish group detected higher incidence of adverse genetical alterations in patients aged 21–40 years. We performed FISH tests in only ten patients and as a result both standard and high-risk alterations were available. All of our young patients were considered as potential candidates of autologous stem cell transplantation, however two of them passed away shortly after the establishment of diagnosis because of primary refractory disease / plasma cell leukaemia. The induction treatment they received was concordant with the current recommendations of the IMWG. VAD protocol was administered until 2007 then thalidomide and bortezomib-based regimens were preferred. Former publications reported on only conventional chemotherapies administered in young MM patients before APSCT. The response to primary treatment (induction + APSCT) was not significantly different between the younger and older population. However, both progression-free and overall survival results were more favorable in the cohort of patients  $\leq 40$  years. Authors from the Mayo Clinic reported same survival results after APSCT among young and older myeloma patients, on the other hand, in European cohorts older age was found as an adverse risk factor in terms of the life expectancy of newly diagnosed MM patients. Thus, we have to underline that both of the latter studies were published before the era of novel therapies. A more recent publication reported on similar overall response rates in younger and older patients (79 vs 83%) after novel agent-based therapies, however patients aged 21–40 years were found to have significantly more favorable five-and ten-year overall survival results (83% vs 67% and 56% vs 39%, respectively) than the group aged 41–60 years. Treatment results and survival data of multiple myeloma patients have significantly improved since the introduction of new drugs including proteasome inhibitors, immunomodulatory agents and monoclonal antibodies. However, despite the encouraging results, myeloma is still considered as an incurable disease characterized by remissions and relapses. While the prolongation of lives of elderly people seems to be a good compromise, clinicians may not accept anything but complete cure for young patients. Still, there is no specific, widely-accepted treatment protocol available for

young MM patients, studies rather focus on high-risk cases. There are several approaches targeting this population that include tandem autologous transplant, several generations of total therapies and administration of new drugs. Consolidation and maintenance therapies also seem to be reasonable approaches to prolong remission periods. Unfortunately, unlike from chronic myeloid leukaemia, there is no perfect maintenance treatment in multiple myeloma as tolerability and side effects are strong limiting factors. Recently, the most accepted maintenance therapy is the administration of lenalidomide. Seven of our long-term survival patients received some kind of maintenance therapy after APSCT that included thalidomide, bortezomib or interferon as lenalidomide was not available in our institute. Allogeneic stem cell transplantation is considered as the only curative treatment method in multiple myeloma, however, its role is still controversial. Due to the high mortality rates, it is still offered as a kind of 'end-of-the-road' option for refractory-relapsing patients. None of our young MM patients have undergone allogeneic transplantation so far, the main reasons were either the lack of consent or non-eligibility. However, we are planning to consider this treatment modality for high-risk patient who relapse shortly after the autologous stem cell support. Our study's main limitation is that we processed the data of a relatively small number of patients within a single institution. However, our results may highlight that though the ratio of young cases is small, there is an unmet need for new therapies that provide complete cure or at least long-term remission for fit multiple myeloma patients. The introduction of novel drugs to the early treatment line may result in increasing number of minimal residual disease (MRD) negative cases and markedly prolonged survival.

Our studies reflect that personalized medicine may have an important role in diffuse large B-cell lymphoma and multiple myeloma patients as the detailed gene polymorphisms can improve treatment efficacy and patients survival.

## 6. Summary and new results

1. Our results could not confirm that genetic polymorphism in metabolic pathways has any predictive role in diffuse large B-cell lymphoma but other studies reported on the importance of genetic polymorphism in metabolic pathways in DLBCL and these studies showed the role of genomic tests in lymphoma treatment.
2. Our results highlighted that beta-catenin gene rs4135385 gene polymorphism may influence the clinical features of multiple myeloma patients and have some impact on the treatment results and survival data after Imid-based therapies. In terms of CTNNB1 (rs4135385) polymorphism AA genotype, the disease is diagnosed in older age and thalidomid or lenalidomid treatment is significantly more effective which improve patients' event-free survival. According to CTNNB1 (rs4533622) polymorphism, A allele is more frequent in older age and it is not typical in men.
3. However, our results may highlight that though the ratio of young cases is small, there is an unmet need for new therapies that provide complete cure or at least long-term remission for fit multiple myeloma patients. Young patients were more likely to receive maintenance or consolidation therapies and their overall survival was more favourable.

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### List of publications related to the dissertation

1. Pál, I., Szilágyi, B., Nagy, B. J., Pál, T., Hódosi, K., Illés, Á., Váróczy, L.: The Impact of Beta-Catenin and glutathione-S-transferase Gene Polymorphisms on the Treatment Results and Survival of Multiple Myeloma Patients.  
*Pathol. Oncol. Res. [Epub ahead of print]*, 1-6, 2019.  
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2. Pál, I., Illés, Á., Váróczy, L.: Multiple Myeloma of the Young: a Single Center Experience Highlights Future Directions.  
*Pathol. Oncol. Res. [Epub ahead of print]*, 2018.  
DOI: <http://dx.doi.org/10.1007/s12253-018-0526-1>  
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3. Pál, I., Illés, Á., Gergely, L., Pál, T., Radnay, Z., Szekanecz, Z., Zilahi, E., Váróczy, L.: The Impact of Drug Metabolism Gene Polymorphisms on Therapeutic Response and Survival in Diffuse Large B-Cell Lymphoma Patients.  
*Isr. Med. Assoc. J.* 20 (4), 217-221, 2018.  
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### List of other publications

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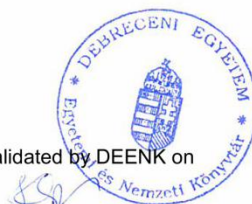


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9. Pál, I., Illés, Á., Pál, T., Váróczy, L.: Refrakter-relabáló plazmasejtes myeloma kezelésével szerzett tapasztalataink.  
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**Total IF of journals (all publications): 9,493**

**Total IF of journals (publications related to the dissertation): 5,694**

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.



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