Summary of professional accomplishments

Anna Szmigielska-Kapłon

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1. Curriculum vitae

In 1991 I started my studies at Faculty of Medicine of Medical University of Lodz and I got my diploma on 24th June 1997. I attended an individual program of studies for 2 years at the Department of Hematology, Medical University of Lodz. After finishing my post-diploma training I continued my scientific research at the Department of Hematology attending full-time postgraduate studies at Medical University of Lodz. At the same time I was doing the training in internal medicine at the Department of Hematology located in Copernicus Memorial Hospital in Lodz. In April 2002 I presented my doctoral thesis „Interactions of 2-chlorodeoxyadenosine with antracycline antibiotics *in vivo* and *in vitro"* and received my PhD diploma. Since 1st May, 2002 I have been employed as an assisstant in the Department of Hematology. In November 2004 I passed the state exam in internal medicine and gained the internal medicine specialist degree. In 2008 I passed the state exam in hematology and gained the degree of a specialist in hematology. I am a member of two committees in Copernicus Memorial Hospital : A Hospital Committee for Transfusion of Blood Products and Hospital Committee for Infections. In 2007 I joined the Transplantation Team in our Department of Hematology. Apart from bone marrow transplantation, as a scientist I am interested in myelodysplastic syndromes and lymphoproliferative disorders, especially acute lymphoblastic leukemia. In the Department of Hematology I participate in the following activities :

- Examination of patients and preparation of the whole procedure prior to autologous and allogeneic transplantation
- Preparation of the procedure of stem cell separation in healthy donors
- Hematopoietic stem cells separation and transplantation and further care for patients after transplantation in ward and in an out-patient setting
- I coordinate the ward procedures of transfusion of blood products
- I take care of patients with hematological malignancies and non-malignant hematological disorders

2. The list of received diplomas and specialist degrees

- 1997 MD Diploma - Medical University of Lodz
- 2000 Diploma of the first degree specialist in internal medicine
- 2002 PhD Diploma
- 2004 State examination in internal medicine (second degree specialist)
- 2008 State examination in hematology

3. Employment

3.1 Department of Hematology, Medical University of Lodz since 1st May 2002 until now

3.2 Regional Specialist Copernicus Memorial Hospital in Lodz since 1997 until now

4. Scientific achievements in accordance with Act of 14 March 2003 on the Academic Degrees and Titles and on Degrees and Titles in the Arts, (Journal of Laws Art no 65, Item 595)

Title of the achievement : **Influence of bone marrow microenviroment on hematopoietic stem cell mobilization. (Wpływ mikrośrodowiska szpiku na proces mobilizacji macierzystych komórek krwiotwórczych)**

Based on 4 original publications :

1. **Annna Szmigielska-Kaplon** , Janusz Szemraj , Katarzyna Hamara , Marta Robak, Anna Wolska , Agnieszka Pluta , Magdalena Czemerska, Anna Krawczynska A, Krzysztof Jamroziak, Katarzyna Szmigielska, Tadeusz Robak , Agnieszka Wierzbowska . Polymorphism of CD44 influences the efficacy of CD 34+cells mobilization in patients with hematological malignancies. : Biol Blood Marrow Transplant. 2014 Mar 27. doi: 10.1016/j.bbmt.2014.03.019.
   Impact Factor : 3.348
   Ministry of Education Points : 30

2. **Anna Szmigielska-Kapłon**, Anna Krawczyńska, Magdalena Czemerska, Agnieszka Pluta, Barbara Cebula-Obrzut, Marta Robak, Olga Grzybowska-Izydorczyk, Katarzyna Szmigielska, Tadeusz Robak, Agnieszka Wierzbowska. The kinetics of hematopoietic niche cytokines and their influence on mobilization

efficacy and timing in patients with hematological malignancies. Journal of Clinical Apheresis 2014
Impact Factor 1.579
Ministry of Education Points 20

3. **Anna Szmigielska-Kapłon**, Anna Krawczyńska, Magdalena Czemerska, Agnieszka Pluta, Barbara Cebula-Obrzut, Katarzyna Szmigielska, Piotr Smolewski, Tadeusz Robak, Agnieszka Wierzbowska. Circulating endothelial cell kinetics and their potential predictive value during mobilization procedure. Journal of Clinical Apheresis 2013 : Vol. 28, s. 341-348,
Impact Factor 1.579
Ministry of Education Points : 20

4. **Anna Szmigielska-Kapłon**, Anna Krawczyńska, Magdalena Czemerska, Agnieszka Pluta, Barbara Cebula-Obrzut, Katarzyna Szmigielska, Konrad Stępka, Piotr Smolewski, Tadeusz Robak, Agnieszka Wierzbowska. Angiopoietins in hematopoietic stem cell mobilization in patients with hematological malignancies. Blood Transfusion 2014
Impact Factor 1.9
Ministry of Education Points**:** 20

Total impact factor according to Journal Citation Report is 8,4

List of abbreviations used in the text

ANG1, 2- angioepoietin 1 and 2
CEC- circulating endothelial cells)
G-CSF – granulocyte-colony stymulating factor
HCELL - Hematopoietic Cell E/L selectin Ligand
HSC- hematopoietic stem cells
MMP-9 – metalloproteinase 9
OPN-osteopontin
SDF-1-stromal derived factor-1
VCAM-1 - vascular cell adhesion molecule -1
VEGF- vascular endothelial growth factor

VLA4 -very late antigen 4

Nowadays, hematopoietic stem cell transplantation is usually performed with CD34+ cells mobilized to the peripheral blood. In healthy people, who can act as donors for allogeneic transplantation, G-CSF is applied for mobilization of HSC from bone marrow. In patients with neoplastic disorders, G-CSF may be used alone or in combination with chemotherapy to cause the egress of HSC from the bone marrow to the blood. Mechanisms of both types of mobilization are not clear and need careful evaluation . Egress of HSC from bone marrow is a complicated process modulated by different factors. Hematopoietic niche interactions take part in HSC mobilization and may influence the efficacy of the process.

Hematopoiesis

Hematopoiesis is a complex process, which takes place in bone marrow microenvironment, in a so called hematopoietic niche. Close relation of the hematopoietic cells with stromal cells is mediated by interactions of adhesive molecules with respective ligands. Integrins, selectins, cadherins and their counterparts create a complicated network, working to maintain adequate function of hematopoietic progenitors for a lifelong time. Changes in those interactions are crucial in mobilization of hematopoietic stem cells to peripheral blood and reversely in homing of stem cells after bone marrow transplantation. Stromal cell-derived factor 1 (SDF1/CXCL12) is a chemokine secreted by bone marrow stromal cells and interacts with CXCR4 receptor present on hematopietic stem cells. Interactions between SDF1 and CXCR4 play a key role in the processes of hematopiesis. Homing, migration and maintenance of hematopoietic progenitors are regulated by this interaction. It is also responsible for egress of hematpoietic stem cells from bone marrow to peripheral blood after chemotherapy and G-CSF treatment which makes it possible to separate stem cells from the blood for transplantation purposes. Nowadays most hematopietic stem cells transplantation worldwide is performed from peripheral blood apheresis collected stem cells. One of the major problems concerning mobilization of hematopietic stem cells is that some patients can not achieve the number of stem cells in peripheral blood sufficient for their collection. This group of patients, called ‘poor mobilizers’, need several aphereses to gather adequate number of cells. Sometimes another course of mobilization is needed and the whole procedure, including chemotherapy and G-CSF, must be repeated. Several factors are correlated with adequate mobilization of

stem cells, taking into account the underlying disease, the number of previous chemotherapy regimens and age. Common polymorphism of SDF1 gene in 801 position (G-A transition) is correlated with efficacy of stem cells mobilization, making carriers of allele A a group with better mobilizing properties. The axis SDF1/CXCR4 warrants further evaluation in the context of mobilization and engraftment after transplantation.

Hematopoiesis is influenced by processes of angiogenesis, which makes interactions much more complicated. In neoplastic disorders endothelial cells take part in tumor growth and progression but also influence the recovery of hematopoiesis after high dose chemotherapy.

Angiogenesis

Angiogenesis is a complex, multifactorial process leading to new vessel formation. As a multi-step phenomenon it comprises endothelial cell proliferation, differentiation and organization of cells to form tubules. Microvessel formation and spreading is crucial in repair of tissues damaged by ischemia or injury. It is well known that angiogenesis is involved in biology and progression of neoplastic disorders. Levels of anti and proangiogenic cytokines levels correspond with the activity of new vessels development. Another method of evaluation of angiogenesis is the assessment of circulating endothelail cells (CEC). In solid tumors and hematological malignancies CEC were evaluated mainly as non-invasive marker of angiogenesis with an impact on prognosis and survival. In neoplastic disorders angiogenesis takes part in dissemination of cancer cells and progression of the disease. The other spectrum of interest is evaluation of CEC and proangiogenic cytokines in the context of their influence on regeneration of hematopoiesis from damage caused by high dose chemotherapy and stem cells transplantation.

The aim of my study was to evaluate different aspects of hematopoiesis control and processes of angiogenesis during autologous hematopoietic stem cell mobilization. I planned to evaluate the level of different chemokines active in hematopoietic niche responsible for angiogenesis, stem cells development and function. Moreover, I wanted to assess the polymorphisms of genes encoding more important proteins modulating processes of hematopoiesis including SDF1, VCAM-1, and CD44. In order to evaluate angiogenesis, I planned to assess CEC, their subsets and kinetics as well as cytokines levels in peripheral blood, in patients with hematologic malignancies during mobilization of cells for autologous

hematopoietic stem cell transplantation. The samples were collected at different time points: in the course of mobilization of hematopoietic stem cells to peripheral blood. The CEC were evaluated with 4 colour flow-cytometry, peripheral blood levels of different cytokines by ELISA method and gene polymorphisms by PCR. Correlation with clinical data and factors concerning efficacy of mobilization was assessed. Complex evaluation of processes of hematopiesis and angiogenesis together with correlation of different parameters with efficacy of mobilization procedure may enrich our knowledge with important information.

I planned to enroll to the study patients receiving high dose chemotherapy with mobilization of autologous stem cell for transplantation in Department of Hematology , Medical University in Lodz. The mobilization procedures comprised chemotherapy and then G-CSF at a dose of 10μg/kg daily.

Introduction for publication 1 and 2

The HSC niche contains different types of cells, including macrophages, osteoblasts, mesenchymal stem cells and endothelial progenitors. All of these interact and form a unique microenvironment, necessary for the appropriate function and preservation of HSCs in the quiescent state and take a major part in the process of mobilization and homing . The so-called osteoblastic part of the HSC niche is responsible for maintenance of dormant, resting HSCs , while active, dividing HSCs are located mainly near endothelial cells in the vascular part of the niche. The HSC are mobilized from the bone marrow by G-SCF alone or in combination with chemotherapy . The exact mechanisms of mobilization are still not clear . The currently accepted theory explains the mechanism of HSC mobilization by a G-CSF mediated release of proteolytic enzymes from neutrophils, including metalloproteinases, leading to profound changes in the HSC microenviroment.

Publication no 1

SDF1/CXCR4 axis plays a key role in mobilization of neutrophils and other cells from the myeloid lineage as well as the HSCs. A single nucleotide polymorphism of CXCL12 -

CXCL12-801A is associated with a higher number of G-CSF mobilized CD34+ cells in patients. The data concerning CXCL12-801A polymorphism in the context of on HSC mobilization in healthy donors are conflicting. The influence of other polymorphisms on the mobilization outcome in patients with hematological malignancies has not been studied so far.

During G-CSF stimulated mobilization of HSCs in humans a decrease in CD44 expression has been noted. CD44 is a surface glycoprotein receptor which binds different ligands, including hyaluronic acid and osteopontin . Adhesion molecules, responsible for cell to cell and cell to stromal matrix interactions, modulate HSC development in the niche. Vascular cell adhesion molecule -1 (VCAM-1) is expressed by osteoblasts and stromal cells and interacts with very late antigen 4 (VLA-4) present on the HSCs. Disruption of VLA4/VCAM1 results in mobilization of HSC in humans .

The aim of the present study was to thoroughly evaluate constitutive polymorphisms of several genes encoding cytokines and receptors present in the HSC niche, including CD44, VCAM-1 and CXCR4, and to assess their impact on the efficacy of mobilization of CD34+ hematopoietic progenitor cells in patients scheduled for autologous transplantation. To our knowledge, the impact of the polymorphisms of CD44, CXCR4 and VCAM1 on the effects of mobilization in patients with hematological malignancies has not been evaluated so far.

One hundred and ten patients, 60 females and 50 males, were enrolled to the study. Median age of the patients was 55 years. Seventy four patients with multiple myeloma, 19 with no-Hodgkin lymphoma, 15 with Hodgkin lymphoma and 2 with acute myeloid leukemia entered the study. The group of patients (N=108) who achieved minimal threshold for collections (CD34+ at least 10/μl) proceeded to apheresis. Fifteen patients fulfilled the criteria for ‘poor mobilizers’. The ‘poor mobilizers’ group was defined according to GITMO criteria: patients with peak CD34+ in peripheral blood < 20/μL or total yield <2x 10^6 CD34+/kg in a maximum of 3 aphereses.

Results

CD44 polymorphism

The median CD44 mRNA expression in the study group was 0.24 (range 0.08-0.68). TT homozygous genotype resulted in an increased mRNA expression: median 0.52 (range 0.16-0.58), compared to the carriers of allele C (Me=0.27, range 0.17-0.4 in CC and Me=0.15 (0.08-0.68) in CT genotype , $p<0.001$). CD44 mRNA expression strongly correlated with poor mobilization (R=-0.2, p= 0.018).

The group of 'poor mobilizers' had a higher frequency of TT genotype in the rs13347 (CD44) gene (CC+ CT vs TT p=0.047). The difference was even more pronounced in patients with multiple myeloma (N=72, p=0.027). Patients with TT genotype had a lower number of CD34+cells collected during the first apheresis ($0.95x\ 10^6$/kg vs. $3.3x\ 10^6$/kg, p=0.04) and a lower total yield of CD34+/kg than the group with allele C (Median=$3.7x\ 10^6$/kg vs. $5.8x\ 10^6$/kg, p=0.019). The presence of TT genotype correlated with a lower CD34+ total yield (R=0.3 p= 0.01), lower number of CD34+ collected at the first apheresis (R=0.2, p =0.4) and being a 'poor mobilizer' (R=0.23, p= 0.02).

Multivariate logistic regression analysis including age, gender, diagnosis (multiple myeloma vs. others), number of previous treatment lines and CD44 polymorphism (TT vs CT+CC) revealed TT genotype was the only factor associated with a 5-fold higher risk of poor mobilization (p=0.037).

Polymorphic variants of CXCR4 and VCAM-1 did not influence significantly the efficacy of HSC mobilization in our group of patients.

Discussion

The efficacy of mobilization of HSC in healthy donors and in patients with cancer is a problem addressed in numerous publications. Although several trials evaluated the topic, there is still a lack of good predictive factors for successful mobilization. Hence, the hunt is on for new parameters to serve as factors which can predict the mobilizing efficacy of CD 34+.

To my knowledge the influence of polymorphisms in CD44, VCAM1 and CXCR4 genes have not been studied in the context of mobilization efficacy in patients scheduled for autologous transplantation. In our group of patients, we observed a higher frequency of TT genotype in rs13347 (CD44) gene in the 'poor mobilizers' group. The difference was even more pronounced in patients with multiple myeloma. TT homozygous genotype resulted in a higher

CD44 mRNA expression at the time of apheresis in comparison with carriers of C allele. Patients homozygous for T allele had a lower total yield of CD34+/kg than the group with allele C, and a lower number of CD34+cells gathered during the first apheresis. In our group of patients we observed higher CD44 mRNA expression in TT genotype which may result from different mRNA kinetics during mobilization in polymorphic CD44 variants. Our results regarding the influence of CD44 polymorphism on the efficacy of HSC mobilization in patients with hematological malignancies are in line with previous observations concerning healthy donors. Higher CD44 mRNA expression in TT genotype, observed in my study, may explain lower mobilization potential in that group of patients. The SNP location in 3'UTR of CD44 can potentially result in a change in the binding ability of different microRNAs among the two different alleles. MicroRNAs are short, non-coding RNA molecules, which, by targeting mRNAs, cause mRNA degradation or translational repression, thus playing key role in regulating gene expression.

A complex network of different molecules interplay together to maintain HSCs in a quiescent state and take part in mobilization . CD44 is the multifunctional glycoprotein receptor which can bind different ligands including hyaluronic acid and osteopontin. During G-CSF–mediated mobilization neutrophil degranulation occurs leading to upregulation of the matrix metalloproteases, which in turn cause cleavage of CD44. Osteopontin (OPN) is a survival factor for many tissues, on the other hand, it inhibits HSCs proliferation and is involved in the control of the HSC cycle, thus it takes part in HSCs migration and appropriate localization in the bone marrow niche after transplantation. Moreover, OPN also controls angiogenesis, enhances migration and decreases apoptosis of endothelial cells. Angiogenesis is crucial for tissue repair from damage caused by chemotherapy, including the most sensitive hematopoietic cells and takes an important part in HSCs mobilization and homing. The impact on angiogenesis may explain the influence of CD44 polymorphism on the efficacy of mobilization by affecting the CD44/OPN axis. One of the CD44 forms exerts a unique function, being a Hematopoietic Cell E/L selectin Ligand (HCELL). HCELL acts as the most potent E- and L-selectin ligand on human hematopoietic cells with increased expression after G-CSF administration. CD44/OPN as well CD44/hyaluronic acid and HCELL/selectins interactions all take part in HSCs mobilization, and polymorphic variants of the CD44 gene influence different complex pathways of the hematopoietic niche regulatory mechanisms.

In conclusion, my results indicate that among investigated SNPs, only CD44 rs13347 has impact on the efficacy of HSCs mobilization in patients with hematologic malignancies. Furthermore, CD44 SNPs analysis may be helpful in predicting the 'poor mobilizers'. CD44 as multifunctional glycoprotein with several counterparts and can influence the mobilization on different ways.

Publication no 2.

The egress of HSC from bone marrow niche is initiated by a G-CSF mediated release of proteolytic enzymes from neutrophils that leads to cleavage of stromal derived factor (SDF) and in consequence disruption of SDF/CXCR4 interaction. Complex interactions of cytokines and adhesion molecules are key regulatory mechanisms of hematopoiesis and take major part in the process of HSC mobilization. To my best knowledge, HSC niche cytokines have not been evaluated so far in patients during the mobilization procedure in the context of mobilization efficacy.

The aim of my study was to evaluate the levels of VCAM-1, VEGF (vascular endothelial growth factor), MMP-9 (metalloproteinase 9) and SDF during mobilization of CD34+ cells in patients with hematological malignancies.

Thirty four participants, 19 females and 15 males at the median age of 57 years, were enrolled to the study. The group consisted of patients with multiple myeloma (26) and lymphoma (8). All patients achieved minimal threshold for collections (CD34+ at least 10/μl) and proceeded to aphereses. In all cases venous blood samples for the cytokine measurement were collected before administration of chemotherapy. Additionally, in 26 patients, the samples were also taken on the day of first apheresis (when CD 34+ cell count was at least 10/μl). The levels of VEGF, VCAM-1, MMP-9 and SDF were evaluated by enzyme-linked immunosorbent assay (ELISA) technology using commercially available kits (Quantikine, R&D systems.

Results

I observed significant increase in VCAM-1 levels during mobilization. By contrast, VEGF and SDF levels decreased during mobilization procedure. The level of MMP-9 was stable during mobilization.

I divided patients according to baseline cytokines levels below and above median into ’low’ and ‘high’ expressors. Only VEGF baseline levels influenced mobilization efficacy . The group of VEGF ‘low’expressors had longer median time of G-CSF treatment before first apheresis than ‘high’ expressors (12vs10 days, p=0.013). Additionally, I observed that maximal number of CD34+ in peripheral blood occurred later in ‘low’ expressors (Me=13 days) than in ‘high’ expressors (Me=10.5 days, p=0.01). Baseline VEGF levels correlated adversely with duration of G-CSF treatment before first apheresis (R=-0.5, P=0.002) and with the day when maximal number of CD34+cells were present in the peripheral blood (R=-0.53, p=0.001).

Patients were also divided according to median cytokines levels at apheresis into ‘low’ and ‘high’ expressors. ‘High’ VCAM-1 expressors had higher CD34+in peripheral blood than ‘low’ expressors: Me=124 (range 17-560) vs 51 (range 8-195), p=0.003; as well as higher CD34+numbers collected during first apheresis: Me=7.1x10^6/kg (range 0.9-32) than ‘low’ expressors: Me=2.6x10^6/kg (range 0.3-24), p=0.006. Additionally, VCAM-1 levels detected at apheresis correlated with number of CD34+ cells in peripheral blood (R=0.59, p=0.001) and with number of cells collected at that day (R=0.43, p=0.02).

Discussion

In my group of patients VEGF and SDF levels in the peripheral blood decreased at the time of apheresis as compared to baseline, while VCAM-1 levels increased during the mobilization procedure. To my knowledge, changes of those cytokines levels during the mobilization procedure with chemotherapy and G-SCF have not been evaluated so far. VEGF concentration was assessed previously in healthy donors during mobilization. Unfortunately samples were collected in different time points than in my study, what makes comparisons impossible.

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To the best of my knowledge, the potential impact of hematopoietic niche cytokines levels on efficacy and timing of mobilization in patients with hematological malignancies has not been assessed so far. An important issue in clinical practice is the influence of different factors on timing of mobilization. In our present study we observed that higher baseline VEGF levels correlated with shorter time of G-CSF administration.

I observed higher CD34+ number and higher mobilization yield in case of higher VCAM-1 expression in peripheral blood at the day of first apheresis. The majority of studies performed during the course of mobilization in healthy donors evaluated cytokines kinetics and not their influence on effects of mobilization procedure. Evaluation of the cytokines active in mobilization of HSC to peripheral blood is a low-cost method available in a standard apheresis unit, and could optimize the efficacy of CD 34+ collections.

Conclusion: In conclusion, the level of hematopoietic niche cytokines change significantly during mobilization in patients with hematological malignancies. Baseline VEGF can influence timing of mobilization. Higher VCAM-1 corresponds with higher mobilization efficacy.

Publication no 3

The vascular niche plays a key role in the process of HSC mobilization to the peripheral blood. Circulating endothelial cells (CECs) and their subsets are non-invasive markers of angiogenesis and their number can correspond with the proper function of endothelial cells in the bone marrow. CECs were assessed in different hematological malignancies taking into account disease status, progression and response to treatment. The interesting issue is the probable influence of angiogenic processes on the course of mobilization of hematopoietic stem cells to peripheral blood.

To my best knowledge, CECs and their subsets have not been evaluated previously in patients during the mobilization procedure in the context of mobilization efficacy. The aim of the study was to assess the activity of all CEC subsets, their kinetics and their predictive significance in the process of mobilization of CD34+ hematopoietic progenitor cells in patients with hematological malignancies.

Thirty eight patients (19 females and 19 males) were enrolled to the study. The median age of the patients was 56.5 years. The group consisted of patients with multiple myeloma (26), non-Hodgkin lymphoma (4), Hodgkin lymphoma (6) or acute myeloid leukemia (2).

The group of 35 patients who achieved minimal threshold for collections (CD34+ at least 10/µl) proceeded to aphereses. They were included into further analyses regarding collection efficacy. We analyzed the number of aphereses necessary to obtain a minimal amount of CD34+ cells required for transplantation (2 x 10^6 CD34+ /kg) as well as the number of days of G-CSF treatment before first apheresis

Evaluation of CECs

Venous blood samples were collected at different time points: before chemotherapy , 1 day after chemotherapy was completed , on the day G-CSF commenced (G0) , one day after the first dose of G-CSF (G+1), on the day when CD 34+ cells were at least 10/µl – day of first apheresis.

The CECs were evaluated by 4-colour-flow cytometry. Subpopulations of CEC were evaluated : circulating progenitor endothelial cells (CEPC) and apoptotic CEC (ApoCEC)..

Results

1.Analysis of CECs in patients according to number of aphereses required to gather the minimal number of cells .

The patients were divided according to number of aphereses needed to obtain minimal number of cells into two groups: 'highly efficient' mobilizers (1 apheresis was enough to obtain minimal number of cells for transplantation) and 'poorly efficient' (2 or more aphereses were needed to obtain the minimum). It was observed that median ApoCEC assessed at dayG+1 was significantly lower in the 'highly efficient' group (Me=3.1/µl,) than in the 'poorly efficient' group (Me=5.1/µl, p=0.02). Lower ApoCEC at day G+1 was associated with a higher chance of gathering a minimal number of CD34+ cells during one

apheresis (odds ratio=0,34; 95%CI=0,12-0,96, p=0,03). In multivariate analysis lower ApoCEC at day G+1 and diagnosis of multiple myeloma were independent factors associated with increased chance of successful mobilization during 1 apheresis.

Additionally, a correlation was observed between ApoCEC performed at dayG+1 with the number of aphereses required to obtain the minimal number of cells for transplantation (r=0.48, p= 0.038).

2 . Analysis of CECs in patients according to number of days with G-CSF treatment before first apheresis

For the purpose of the study, patients were divided into groups of 'early' and 'late' mobilizers according to the number of days of G-CSF treatment before first apheresis (days of G-CSF): 'early mobilizers' <Me = 11 days, 'late mobilizers' >Me. CECs and their subsets were analyzed in these 2 groups.

A significantly higher baseline CEC count was observed in the group of 'early mobilizers' (Me 12.5/µl,) than in the 'late mobilizers' group (Me =9.9/µl, , p=0.043). CEPC analyzed both before and after the first dose of G-CSF also differed in those two groups significantly. A higher number of CEPC were detected at day G0 among 'early mobilizers' (Me 1.3/µl,) than 'late mobilizers' (Me 0.8/µl, , p= 0.05). CEPC counts at day G+1 were also higher in 'early mobilizers' (Me=1.5/µl,) than in 'late mobilizers' (Me=0.8/µl, p = 0.01).

Additionally, the CEPC numbers at G0 and G+1 correlated adversely with the number of days of G-CSF treatment (r= -0.42, p=0.035 and r= -0.49, p=0.04 respectively). Logistic regression indicated that the factors associated with an increased chance of earlier apheresis were CEPC at day G+1 (odds ratio=12, 95%; CI intervals1,1-124, p=0.03), CEPC at day G0 (odds ratio=5,2; 95% CI intervals 0,9-30, p=0.05) and CEC (odds ratio=0,8; 95% CI intervals 0,7-1,1, p=0,05) Unfortunately, according to multivariate analysis, none of those factors was an independent one.

Discussion

In hematological malignancies CEC are regarded mainly as tumor burden markers. The other area of interest is evaluation of the influence of CECs on the mobilization of hematopoietic stem cells (HSC) to the peripheral blood. I assessed the potential predictive value of CEC and the subsets in this setting. The efficacy of the mobilization procedure was evaluated at different end points, including the number of days of G-CSF treatment required to gain a sufficient number of CD34+ in peripheral blood to begin apheresis, and the number of aphereses needed to gather minimal numbers of CD 34+ cells . I observed that the number of CECs, or their certain subsets, correlate with the duration of G-CSF treatment necessary to begin the collection procedure and with the number of aphereses required to obtain the minimal amount of cells to perform HSCT.

The higher number of endothelial precursors, measured early after chemotherapy cessation and after the beginning of G-CSF treatment, correlated with a shorter time of G-CSF treatment. Moreover, a lower number of apoptotic CECs after the start of G-CSF administration was associated with a lower number of aphereses required to obtain the minimal number of cells for transplantation. The lower number of apoptotic cells reflects better preservation of function of CEC and stresses the role of endothelial cells in the process of effective mobilization of CD 34+ to the peripheral blood. Our results suggest that the angiogenic processes can influence timing of hematopoietic stem cells mobilization. Evaluation of CEC and apoptotic CEC fraction during mobilization procedure, due to their potential predictive significance, may improve aphereses yeld.

Conclusions: All of those observations indicate important function of the microvasculature in the migration of hematopoietic progenitors. The number of endothelial precursors, measured early after chemotherapy cessation correlates with duration of G-CSF treatment The number of apoptotic CEC, measured at the commencement of G-CSF, can predict efficacy of collection of the HSC. CECs and their subsets need further evaluation as low-cost, non-invasive markers of angiogenesis in the context of mobilization efficacy.

Publication no 4

Angiopoietins 1 and 2 (Ang1 and Ang2) both influence different stages of endothelial cell development and play the main role in the disregulated neoplastic angiogenesis. Both cytokines are ligands of endothelial-specific tyrosine kinase receptor Tie2. Apart from being

an endothelial survival factor, Ang1 protects HSC from cellular stress, thus contributing to maintenance of hematopoietic stem cells in a quiescent state in the bone marrow niche. Ang2 promotes apoptosis of endothelial cells acting as a natural antagonist of angiopoietin 1. Osteopontin (OPN), is a survival factor for many tissues which exerts antiapoptotic activity. In addition, osteopontin inhibits HSC proliferation and is involved in the control of the HSC cycle. OPN takes part in HSC migration and proper localization in the bone marrow niche after transplantation. Cytokines active in the bone marrow niche regulate and control the endothelial microenviroment and HSC development and function. By the impact on regenerative angiogenesis, cytokines may influence the efficacy of HSC mobilization.

The aim of the present study was to perform a complex evaluation of Ang1, Ang2 and OPN, during mobilization of CD34+ hematopoietic progenitor cells in patients with hematological malignancies. A second aim was to analyze the relationship between the concentrations of evaluated cytokines and numbers of CEC populations. To my knowledge, the kinetics of both Ang1, Ang2 and OPN during stem cell mobilization procedure in patients with hematological malignancies have not yet been assessed.

Forty-eight patients (24 females and 24 males) were enrolled to the study. The median age of the patients was 56.5 years. The group consisted of patients with multiple myeloma (34), non-Hodgkin lymphoma (7), Hodgkin lymphoma (6) or acute myeloid leukemia (1). The group of 47 patients who achieved minimal threshold for collections (CD34+ at least 10/μl) proceeded to aphereses. The 'poor mobilizers' group was defined according to GITMO criteria: patients with peak CD34+ in peripheral blood < 20/μL or total yield <2x 10^6 CD34+/kg in a maximum of 3 aphereses. Eight out of 48 patients (16.6%) fulfilled the GITMO criteria for 'poor mobilizer'.

Evaluation of cytokines and CEC

In all patients, venous blood samples for the cytokine measurement were collected before administration of chemotherapy (group A). Additionally, in 36 patients, the samples were also collected on the day of first apheresis (when CD 34+ cell count was at least 10/μl). Group B comprises 36 patients from group A in whom cytokines were evaluated twice, and CEC were also assessed. Cytokine levels were evaluated by ELISA technology The CECs were evaluated by 4-colour flow cytometry

Results

Cytokine levels and kinetics

Baseline Ang1, Ang2 and OPN levels were (Median, Me= 7.8 ng/ml , Me=2.8 ng/ml and 115 ng/ml respectively). Additionally a correlation was observed between the level of OPN and Ang2 at baseline (r=0.35, p=0.014) as well as the correlation between Ang1 level and CEC numbers (r=0.34, p=0.04).

The median concentration of Ang1 in the peripheral blood at the time of apheresis was significantly reduced as compared to baseline (2.7 vs 7.8 ng/ml, p <0.001). In contrast, the Me level of Ang2 significantly increased during the mobilization procedure (3.6 vs 2.8 ng/ml, p=0.001). The level of OPN measured at the time of apheresis did not differ from pretreatment values.

Cytokine levels and mobilization efficacy

The baseline Ang2 level in this 'poor mobilizer' group was significantly lower (Me=1.9) than in 'good mobilizers' (Me=3.3, p=0.006). Both OPN and Ang1 levels did not differ between the 'poor mobilizers' and 'good mobilizers' groups .

Multivariate regression analysis was performed to identify factors predictive for poor mobilization. A baseline Ang2 level lower than median and a diagnosis of a disease other than multiple myeloma were factors predicting poor mobilization in univariate analysis (odds ratio 0.1, 95% CI 0.01-0.9, p=0.04 and odds ratio 0.17 (95%CI 0.03-0.9, p= 0.04 respectively). Unfortunately, according to multivariate analysis, none of those factors was independent, although Ang2 level was the last factor excluded from the analysis by the backward stepwise regression model.

Cytokines level and G-CSF administration before first apheresis

For the purpose of the study, the median number of days of G-CSF administration before first apheresis was used to divide patients into groups of 'early' and 'late' mobilizers. Significantly higher baseline Ang1 was observed in 'early mobilizers' (Me=11.6 ng/ml) than in the 'late mobilizers' group (Me=6.0 ng/ml,p=0,046). Ang2 and OPN levels did not differ in 'early' and 'late' mobilizers Moreover, a higher baseline level of Ang1 correlated with significantly shorter time of G-CSF administration (r=-0.39, p= 0.006). Additionally, a significant

positive correlation between the level of Ang2 at the day of first apheresis and the number of CD34+cells in peripheral blood measured at the same time (r=0.39, p=0.03) was observed.

Discussion

In my group of patients, Ang1 level in the peripheral blood decreased at the time of apheresis as compared to baseline, while Ang2 level increased during the mobilization procedure. To my knowledge, changes of angiopoietin levels during the mobilization procedure with chemotherapy and G-SCF have not been evaluated so far. Angiogenic factors including angiopoietins 1, 2 /Tie 2 axis and VEGF play important roles in the process of mobilization of CD34+ to the peripheral blood in healthy people . Angiogenesis is crucial for the repair of tissues, including the most sensitive hematopoietic cells, from damage caused by chemotherapy. The results of my study show a decrease of Ang1 at the time of apheresis as compared to baseline. Additionally, the number of CEC were assessed in our group of patients and their correlation with Ang1 levels was observed. CEC number can correspond with the proper function of endothelial cells in the bone marrow. A correlation was observed between baseline Ang1 and the number of CECs, demonstrating the proangiogenic activity of Ang1. Both Ang1 levels and CEC numbers followed the same trend and were reduced at the time of apheresis. Our observations highlight the important function of the microvasculature in the migration of hematopoietic progenitors.

The study assesses the potential predictive value of angiopoietins in the context of mobilization efficacy. It was observed that apart from type of disease, baseline Ang2 level was the factor predicting failure of mobilization. Patients who fulfilled the criteria for ‘poor mobilizer’ had a lower baseline Ang2 level than ‘good mobilizers’. Additionally, a higher number of CD34+cells in peripheral blood corresponded with higher level of Ang2 on the day of first apheresis. To the best of my knowledge, the potential influence of angiopoietin levels on efficacy and timing of mobilization has not been assessed so far. Studies performed during the course of mobilization in healthy donors evaluated angiopoietin kinetics and not influence of cytokine levels on effects of mobilization procedure.

.In the study I observed that the higher baseline level of Ang1 correlated with shorter time of G-CSF administration. The results indicate the supportive function of bone marrow microvasculature in the mobilization of CD 34+ cells to peripheral blood.

In conclusion, the angiopoietins play a major part in the process of mobilization of the stem cells to the peripheral blood. The vascular niche plays a supportive role in the process of HSC mobilization to the peripheral blood in response to various stimuli including chemotherapy and G-CSF. The baseline angiopoietin 1 level can influence the timing of HSC collection. Angiopoietins, being non-invasive parameters, are attractive markers of bone marrow angiogenesis and are worth evaluating in the context of mobilization efficacy.

Summary

The research presented above was carried out in order to evaluate the influence of mechanisms regulating apoptosis and hematopoiesis on course of HSC mobilization. The bone marrow microenvironment, containing endothelial cells, contributes to proper hematopoietic stem cell function, including regeneration after injury caused by chemotherapy. Myelosupression resulting from cytostatic agents is accompanied by destruction of bone marrow vasculature; microvessels are reconstructed together with recovery of hematopoiesis . Moreover, the angiogenic factors including angiopoietins 1, 2 and VEGF play key roles in the process of mobilization of CD34+ to the peripheral blood. All of those observations indicate important function of the microvasculature in the migration of hematopoietic progenitors. Identification of new prognostic factors may result in optimalization of mobilization procedures.The ELISA assay is an easily available low-cost method of evaluation of cytokines. In routine practice ELISA method could be very useful especially in large centres dealing with many donors for autologous and allogeneic transplantation. In case of identification of potential ‘poor mobilizer’ the addition of plerixafor to chemotherapy regimen seems to be an interesting option.

5 Scientific achievements apart from the ‘thematic cycle’

Bibliometric analysis of scientific achievements

- Total number of MNiSW points granted for publications in scientific journals (without supplements) is 553, including
- 181 points granted for original reports and case reports published as first author
- impact factor is 62,509, including 19,008 for orignal reports published as first author

My scientific achievements, apart from ‘ thematic cycle’ comprise 24 original reports, 3 case reports and 8 review articles published in peer reviewed Polish and international journals. I am first author of 12 of those manuscripts.

Points according to MNiSW list and Impact Factor (without manuscripts, that create ‘thematic cycle’):

Original research: MNiSW=345 points and IF=42,418

Case reports : MNiSW= 21 points and IF=2,498

Reviews : MNiSW=97 points and IF=9,186

5.1 Autologous hematopoietic stem cells mobilization and transplantation

My scientific plans have been concentrated on processes of mobilization and transplantation of autologous HSC since 2008 year. One of the interesting topics involves the influence of angiogenesis in the process of HSC engraftment after transplantation. Indirect methods of angiogenesis evaluation include the assessment of circulating endothelial cells (CEC). I evaluated CEC kinetics and apoptotic profile in the group of patients with hematological malignancies in the course of transplantation. The results of the study were published **(A. Szmigielska-Kapłon** et al. The kinetics and apoptotic profile of circulating endothelial cells in autologous hematopoietic stem cell transplantation in patients with lymphoproliferative disorders. Ann. Hematol 2013).

Infectious complications during autologous HSC transplantation are not as serious as the ones described in the allogeneic setting. I have participated in the study performed in the Department of Hematology in Lodz, evaluating prophylactic use of ciprofloxacin during autologous HSC transplantation. The results were published (A. Wolska, T. Robak, **A. Szmigielska-Kapłon** et al Ciprofloxacin prophylaxis for patients undergoing high-dose chemotherapy and autologus stem cell transplantation (ASCT) - a single-center experience. Adv. Med. Sci.2012)

I have also taken part in evaluation of safety and efficacy of CXCR4 inhibitor- plerixafor (G. Basak, W. Knopińska-Posłuszny, M. Matuszak, E. Kisiel, D. Hawrylecka, **A. Szmigielska-**

Kapłon et al. Hematopoietic stem cell mobilization with the reversible CXCR4 receptor inhibitor plerixafor (AMD 310) - Polish compassionate use experience. Ann. Hematol 2011).

5.2 Evaluation of interactions of 2-chlorodeoxyadenozine with other cytostatics and not-cytostatic substances

At the beginning my scientific research was concentrated on evaluation of different drugs interactions in experimental models in vivo and in vitro. *In vivo* studies were performed in murine leukemias L1210 and P388. I evaluated the influence of arabinoside cytosine and 2-chlorodeoxyadenosine used alone and in combination on the overall survival of leukemic mice. I observed a synergistic effect of the drugs in sequential therapy (**A. Szmigielska** et al**.** Influence of 2-chlorodeoxyadenosine alone and in combination with cytosine arabinoside on murine leukemias L1210 and P388. Cancer J. 1996). The second study, performed on similar *in vivo* model, concerned interactions of gemcitabine and and 2-chlorodeoxyadenosine. An additive effect was observed when drugs were used in combination (E. Marańda, **A. Szmigielska**, T. Robak. Additive action of gemcitabine (2',2'-difluorodeoxycytidine) and 2-chlorodeoxyadenosine on murine leukemias L1210 and P388. Cancer Invest. 1999). Interactions of antracycline antibiotics with cladribine comprised my next scientific project. In the treatment of acute leukemias different cytostatics from antracycline antibiotics group are applied. There were no studies comparing directly antileucemic activity of different antracycline antibiotics either in monotherapy or in combination with 2-chlorodeoxyadenosine. I evaluated interactions of 3 different compounds from antracycline antibiotics group combined with 2-chlorodeoxadenosine on different experimental models. Apart from *in vivo* experiments on murine leukemias, I performed *in vitro* test on those lines utilising MTT method. Additionally, I assessed the influence of the compounds on apoptosis of lymphocytes isolated from patients with chronic lymphocytic leukemia. My doctoral thesis was based on the results of those experiments („Interakcje 2-chlorodeoksyadenozyny z antybiotykami antracyklinowymi w badaniach *in vivo* i *in vitro*"). The results were published **(A. Szmigielska-Kapłon** et al Anthracyclines potentiate activity against murine leukemias L1210 and P388 *in vivo* and *in vitro*. Eur. J. Haematol: 2002 and **A. Szmigielska-Kapłon** et al**.** Evaluation of apoptosis induced *in vitro* by cladribine (2-CdA) combined with antracyclines in lympohocytes from patients with B-cell chronic lympohocytic leukemia.

Ann. Hematol 2002). We planned to analyze potential influence of non-cytostatic compounds on antileucemic activity of 2-chlorodeoxyadenozine. We assessed the efficacy of combined treatment with 2-chlorodeoxyadenosine and dexamethasone *in vivo*. The results indicated no positive influence of addition of steroids on cladribine activity (T. Robak, **A. Szmigielska** Dexamethasone does not enhance antileukemic activity of cladribine in mice with leukemias L1210 and P388. Neoplasma 2000). The next study covered similar topic –influence of cyclosporine A on antileucemic activity of 2-chlorodeoxyadenosine. The results were published (G.Józefowicz-Okonkwo, **A Szmigielska-Kapłon**, T Robak. Cytotoxic effect of cyclosporin A alone and in combination with 2-chlorodeoxyadenosine against P388 murine leukemia *in vivo*. Med. Sci. Monit. 2002).

5.3 Indolent lymphoproliferative disorders – clinical trials and evaluation of apoptosis, gene polymorphisms influencing the course of the disease

5.3.1 Angiogenesis in chronic lymphocytic leukemia (CLL)

I participated in the trials evaluating influence of angiogenesis on clinical course and effects of therapy in CLL. The angiogenesis in patietns with CLL was assessed both by indirect method (circulating endothelial cells, CEC) as well as by direct evaluation of microvessel density in the bone marrow (trephine biopsy). Several publications were based on the results: (original research : 1**. A. Szmigielska-Kapłon**, et al. Influence of cladribine alone and in combinaton with cyclophosphamide or cyclophosphamide and mitoxantrone on bone marrow angiogenesis in chronic lymphocytic leukemia. Leuk. Lymphoma 2007, 2. J. Góra-Tybor, K. Jamroziak, **A. Szmigielska-Kapłon** et al. Evaluation of circulating endothelial cells as noninvasive marker of angiogenesis in patients with chronic lymphocytic leukemia. Leuk. Lymphoma 2009, 3. **A. Szmigielska-Kapłon** et al. Prognostic value of the bone marrow microvessel density in progressive B-cell chronic lymphocytic leukemia. Leuk. Lymphoma 2010 and 1 presentation at ASH 2007 meeting (J. Góra-Tybor et al).

5.3.2 Indolent lymphoproliferative disordes - evaluation of apoptosis, gene polymorphisms influencing the course of the disease

The main stream of scientific research in Department of Hematology in Lodz covers the etilogy and patogenesis of indolent lymphoproliferative disorders. My participation in those scientific topics resulted in several publications: the original research articles: 1. P. Smolewski, **A. Szmigielska-Kapłon et al** . Proapoptotic activity of alemtuzumab alone and in combination with rituximab or purine nucleoside analogues in chronic lymphocytic leukemia cells. Leuk. Lymphoma 2005 , 2. K. Jamroziak, E. Balcerczak, P. Smolewski, R. Robey, B. Cebula, M. Panczyk, M. Kowalczyk, **A.Szmigielska-Kapłon**,et al. MDR1 (ABCB1) gene polymorphism C3435T is associated with P-glycoprotein activity in B-cell chronic lymphocytic leukemia. Pharmacol. Rep. 2006 and 3. E. Lech-Marańda, P Juszczyński, **A. Szmigielska-Kapłon** et al . Human leukocyte antigens HLA DRB1 influence clinical outcome of chronic lymphocytic leukemia: Haematologica 2007. The results were also presented on numerous international conferences : (EHA 2005, ASH 2005, ASH 2008, EHA 2010)

5.3.3. Advanced disease, heavily pretreated patients with indolent lymphoproliferative disorders – a therapeutic challenge

Indolent lymphoproliferative disorders in the advanced stage in heavily pretreated patients are a therapeutic challenge. In the Department of Hematology in Lodz I participated in different retrospective trials evaluating efficacy and safety of treatment options in that group of patients. (1. T. Robak, **Anna Szmigielska-Kapłon** et al. Activity of cladribine combined with etoposide in heavily pretreated patients with indolent lymphoid malignancies. Chemotherapy: 2005, 2. T. Robak, P. Smolewski, B. Cebula, **A. Szmigielska-Kapłon** et al. Rituximab combined with cladribine or with cladribine and cyclophosphamide in heavily pretreated patients with indolent lymphoproliferative disorders and mantle cell lymphoma. Cancer 2006 and case report: T. Robak, **A. Szmigielska-Kapłon**, et al. Hodgkin's type of Richter's syndrome in familial chronic lymphocytic leukemia treated with cladribine and cyclophosphamide. Leuk. Lymphoma: 2003)

5.4. Acute lymphoblastic leukemia treatment and diagnosis

I participated in clinical trials concerning acute lymphoblastic leukemia (ALL) conducted in Department of Hematology in Lodz. I belong to ALL working party in PALG (Polish Adult Leukemia Group). I am responsible for reporting data concerning patients with ALL treated in our Department according to PALG study protocols PALG-5 and PALG-6. The results of prospective, multicenter trial PALG-5 are being summarized now. Publications are being prepared , the preliminary results were presented so far at PTHiT meeting in 2013.

I participated in retrospective, multicenter analysis concerning elderly patients with ALL . Cases diagnosed and treated among different hematology centers in Poland belonging to PALG group were evaluated. Elderly patients with ALL usually carry numerous adverse prognostic factors such as abnormal karyotype and comorbidities, which makes the adequate treatment difficult and worsens the prognosis. The results of the study were published(T. Robak, **A. Szmigielska-Kapłon** et al**.** Acute lymphoblastic leukemia in elderly : the Polish Adult Leukemia Group (PALG) experience. Ann. Hematol.2004).

Atypical clinical presentation needs careful, multidisciplinary diagnosis. We described a case of young male with initial diagnosis of aplastic anemia and finally – acute lymphoblastic leukemia (T. Robak, J. Bartkowiak, H. Urbańska-Ryś, **A. Szmigielska-Kapłon**, et al . Acute lymphoblastic leukemia in adult first manifested as severe aplastic anemia - role of molecular analysis in correct diagnosis. Leuk. Lymphoma: 2002)

5.5 Myelodysplastic syndromes

I am interested in epidemiology, prognostic factors and treatment of myelodysplastic syndromes (MDS). I was particularly concentrated on treatment of this entity **(A. Szmigielska-Kapłon**, T. Robak.: Hypomethylating Agents in the Treatment of Myelodysplastic Syndromes and Myeloid Leukemia. Curr. Cancer Drug Targets 2011)

I have taken part in clinical trial held in our Department concerning treatment of patients with MDS. The results were presented In publication (T. Robak, **A. Szmigielska-Kapłon** et al. Efficacy and toxicity of low-dose melphalan in myelodysplastic syndromes and acute myeloid leukemia with multilineage dysplasia. Neoplasma 2003).

I participate in MDS working party in PALG group. I am responsible for reporting the cases

diagnosed and treated In our Department of Hematology for the Polish retrospective and prospective registry of MDS patients. The epidemiologic data derived from retrospective registry have been summarized and the publication is under revision now. The data were presented so far at numerous congresses : 10th International Symposium on Myelodysplastic Syndromes (MDS) 2009, ASH 2009, Meeting of PTHiT 2011- 4presentations, EHA 2012

5.6 Acute myloid leukemia- biology and treatment

I have taken part in studies evaluating influence of bone marrow microenvirment on biology, course of the disease and effects of treatment of acute myeloid leukemia . The results were published as original articles : 1. A. Pluta, A. Wrzesień-Kuś, B. Cebula-Obrzut, A. Wolska, **A. Szmigielska-Kapłon** et al. Influence of high expression of Smac/DIABLO protein on the clinical outcome in acute myeloid leukemia patients. Leuk. Res. 2010 2. M Czemerska, A.Pluta, **A Szmigielska- Kapłon** et al. Jagged-1: a new promising factor associated with favorable prognosis in patients with acute myeloid leukemia.Leukemia Lymphoma 2014 and congress abstracts; (ASH 2008, EHA 2009, ASH 2009 -2 abstracts , ASH 2012 and ASH 2013). I am also a co-author of review concerning treatment of acute myeloid leukemia (T. Robak, **A. Szmigielska-Kapłon i wsp.** Novel and emerging drugs for acute myeloid leukemia: pharmacology and therapeutic activity. Curr. Med. Chem. 2011)

6. Scientific projects participation

6.1 , Interactions in antileukemic activity of 2-chlorodeoxyadenosine and antracycline antbiotics *in vivo* and *in vitro* " (Interakcje w zakresie przeciwnowotwotowego działania 2-chlorodeoksyadenozyny z antybiotykami antracyklinowymi w badanaich *in vivo i in vitro)*' KBN 4P05B11318

6.2 Optymalization of acute lymphoblastic leukemia treatment in adults according to risk factors and minimal residul disease monitoring – program of Polish Adult Leukemia study Group – PALG (Optymalizacja leczenia ostrej białaczki limfoblastycznej u dorosłych chorych w oparciu o dostosowanie do czynników ryzyka I monitorowanbie choroby restkowej – program Polskiej Grupy d/s Leczenia Białaczek u dorsłych (PALG))

7. Teaching activities, information about international cooperation, rewards

7.1 . Students – didactic supervision and instructive care

A. I teach students at Medical University of Lodz. I run clinical classes and seminars in

- Internal medicine for 3rd year students at Faculty of Medicine
- Hematology for 5th year students at Faculty of Medicine
- Hematology for 5th year students at Faculty of Military Medicine
- Hematology for students at Faculty of Medicine -English Division
- Hematology for students at the Division of Nursing and Midwifery of Faculty of Health Sciences

B. I am a didactic supervisor for hematology course at Faculty of Military Medicine
C. I was a promoter of 2 students and took care of their scientific studies for Bachelor's degree at the Division of Nursing and Midwifery of Faculty of Health Sciences

D. I took care of students who continued their studies at our University in the Individual Course of Studies

E I was a Jury member of *Juvenes pro Medicina* contest of students scientific research

7.2. Post diploma training for physicians

- I am an internal medicine specialization supervisor for two physicians
- I supervise 1 physician during the training course in hematology specialization
- I guide physicians during their post-graduate internship
- I manage and monitor post-graduate training for physicians specializing in internal medicine in our Department. .

7.3. Participation in Polish and international conferences :

1. 18th Conference of Polish Society of Hematology and Transfusion Medicine (Polskiego Towarzystwa Hematologów i Transfuzjologów)Łódź 1999 r. :

2. 6th Annual Meeting of the European Hematology Association Frankfurt, Germany, 2001
3. 9th Conference of Polish Society of Hematology and Transfusion Medicine (Polskiego Towarzystwa Hematologów i Transfuzjologów)- "Hematologia praktyczna i konsultacyjna - transfuzjologia" Jachranka, 2002
4. 7th Annual Meeting of European Hematology Association Florence, Italy, 2002
5. 8th Annual Meeting of the European Hematology Association Lyon, France, 2003
6. 3rd Conference of Polish Society of Hematology and Transfusion Medicine (Polskiego Towarzystwa Hematologów i Transfuzjologów) "Przewlekłe choroby mielo- i limfoproliferacyjne" Kazimierz Dolny, 2006
7. 51st Annual Meeting of the American Society of Hematology, New Orleans, USA, 2009
8. 17th Annual Meeting of the European Hematology Association, Amsterdam, The Netherlands, 2012
9. 54th Annual Meeting of the American Society of Hematology , Atlanta, USA, , 2012
10. 39th Annual Meeting of European Society for Blood and Marrow Transplantation, London, UK, 2013
11. 55th Annual Meeting of the American Society of Hematology, Nowy Orlean, USA, 2013
12. 40th Annual Meeting of European Society for Blood and Marrow Transplantation, Milan, Italy, 2014

7.4. Membership in scientific organizations and societies

- PTHiT -Polish Society of Hematology and Transfusion Medicine
- PALG -Polish Adult Leukemia Group
- Polish Federation of Bone Marrow Transplantation Centers
- EBMT –European Society for Blood and Marrow Transplantation

7.5. Training completed in international scientific centers

- 07-08. 2004 training In Hematologic Department in Leeds, Great Britain,(Leeds Teaching Hospital)

7.6. Article reviewing for international journals

I was a reviewer for the following journals :

- Immunobiology, 2013
- Gene Therapy& Molecular Biology,2014.
- Cellular Physiology and Biochemistry Journal, 2014
- Clinical Interventions in Aging 2014

7.7. Awards for scientific achievements

- 2003 - Rector's Award
  Grade 2 Scientific Award of the Rector of Medical University of Lodz

- 2005- The Minister of Health's Team Award .
  'Ocena skuteczności i toksyczności nowych metod leczenia niektórych nowotworów układu krwiotwórczego'
- 2006 - The Minister of Health's Team Award
  'Badania nad biologią i terapią białaczek'
- 2012- Rector's Award
  Grade 2 Scientific Award of the Rector of Medical University of Lodz
- 2014 - Rector's Award
  Grade 2 Scientific Award of the Rector of Medical University of Lodz granted twice

02.12.2014

A. Supiesha