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POSTER ABSTRACTS

1. Neoadjuvant intensified chemotherapy vs Standard Therapy in Locally Advanced Rectal Cancer

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Background

Standard therapy for locally advanced rectal cancer includes concurrent chemoradiotherapy (CRT) followed by surgery and adjuvant chemotherapy. An alternative strategy - neoadjuvant intensified chemotherapy (NIC) involves administration of neoadjuvant chemotherapy (FOLFOX4) before surgery plus concomitant chemoradiation (in those only who did not achieve MRF (neg.)) with the goal of delivering optimized systemic therapy to eradicate micrometastases. A comparison of these 2 approaches was the aim of study.

Objective

To determine the differences in rates of pathologic complete response (pCR), mesorectal fascia (MRF) involvement, disease-free survival (2 year DFS) between patients receiving NIC vs standard CRT.

Material and Method

This is a prospective single institution clinical trial (ClinicalTrials.gov Identifier: NCT05378919). The study included patients with locally advanced stage II-III rectal cancer. Patients were randomized 1:1 for neoadjuvant concomitant chemoradiation or neoadjuvant intensified chemotherapy (FOLFOX4 regimen, a total of 8 cycles). 4-6 weeks after completion of treatment radiological examination was performed and the patients underwent surgery. For those from NIC arm who did not achieve MRF (neg.) additional concomitant chemoradiation was given before surgery.

Results

85 patients (pts.) were included into the study and analyzed. The median follow-up of patients is 36 months (1-77 months). Both groups are well balanced: by age, sex, disease stage, MRF status. At baseline, MRF was involved in 21/42 patients (pts) (50%) in the NIC arm and in 25/43 pts (58%) in CRT arm. The pelvic MRI was performed after neoadjuvant treatment. Radiologically, MRF remained involved after initial treatment in 13/42 pts (31%) NIC group and 11/43 pts (26%) in the CRT group.

Surgery was not performed in 5/42 pts. (12%) from NIC arm due to disease progression (1) or early deaths during neoadjuvant treatment (thromboembolism (2), stroke (1), covid-19 infection (1) and in 6/43 pts. (14%) in the CRT arm (1 pts. remained not resectable, 2 cases of disease progression, 3 refused surgery but one of them achieved a complete response). Additional neoadjuvant CRT was given to 7 / 42 pts. in the NIC arm. After this treatment, surgery was performed 6/7 pts. and R0 surgery was achieved. Surgery was not performed for only one pts due disease progression.

After surgery, circumferential resection margin (CRM) was involved in 2/30 pts. (7%) in NIC and in 3/33 pts. (9%) CRT groups with no statistically significant difference between these groups (p=0.6). pCR was achieved in 9/30 pts (30%) NIC group and in 12/33 pts. (36%) CRT group (not sig. difference between groups). After treatment in NIC arm, a reduction in the tumor stage (evaluated by radiologist) was observed in 5/42 (12%) pts, and in pathologists report – in 20/30 pts (67%). In CRT arm, radiological down staging was achieved in 12/43 pts. (28%) and pathologically in 24/33 (73%), but no statistical difference was observed. Two-year DFS was 66.7% and 80% in NIC and CRT groups, respectively (p = 0.2). Two-year overall survival (OS) did not differ statistically significantly between groups to.

14 patients have died during the follow-up period: 10/42 pts. (24%) in the NIC group, of whom 6/42 (14%) due to disease progression, 4/42 have died due to other reasons (thromboembolism (2), stroke (1), covid-19 infection (1)); 4/43 pts. (9%)have died in the CRT group: 2/43 (5%) due to disease progression, 2 have died due to other reasons (thromboembolism (1), pneumonia (1)); respectively (p = 0.071).

Conclusions and Recommendations
The preliminary findings of this ongoing prospective clinical trial did not show statistically significant difference in 2 year DFS and OS between neoadjuvant intensified chemotherapy and neoadjuvant concomitant chemoradiation arms but numerically chemoradiation arm was more benefitial.

2. PJ-34 Confers Radiosensitizing Effect on MDA-MB-231 Cells | *Best Poster Award Received*

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Background and Objectives
Radiotherapy is one of the common approaches for cancer therapy, however, the radioresistance remains a major obstacle for the effectiveness of treatment. Previous studies have reported that various poly (ADP-ribose) polymerase (PARP) inhibitors demonstrate anti-cancer activity as a single agents and in combination with chemotherapeutic agents or radiotherapy. These findings suggest that PJ-34, one of the PARP inhibitors, could be the potential agent for radiosensitizing breast cancer cells. Thus, the aim of the study was to analyze the radiosensitizing effect of PJ-34 on human triple negative breast cancer cells MDA-MB-231. To achieve this objective, the activation of the Ataxia Telangiectasia Mutation (pATM) and the one of the variants of histone H2A.X (pH2A.X), as the early markers of a cell's response to DNA damage, and cell survival were studied.

Material and Method
MDA-MB-231 cells were cultured under sterile conditions at 37˚C in a humid environment containing CO_2 (5%) and the culture medium comprised Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin with 100 µg/mL streptomycin and 2 mM L-glutamine. Cells were treated with PJ-34 (SigmaAldrich) one hour before irradiation to the single dose of 2 and 4 Gy (Clinac 2100C/D linear accelerator). To conduct the quantitative analysis of the pATM and pH2A.X positive cells, Muse Multi-Color DNA Damage kit (Merck Millipore) was used one hour following irradiation. The survival of cells was evaluated by clonogenic assay. Statistical analyses were performed using Student's t test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results
The percentage of pATM and pH2A.X positive cells significantly increased following 10 µM of PJ-34 and 2 Gy irradiation compared to irradiation alone. Interestingly, the greatest amount of pH2A.X positive cells was following 4 Gy irradiation (0 µM of PJ-34) and then decreased to a lower level with PJ-34. The percentage of pATM positive cells decreased in the similar way following 4 Gy irradiation. Moreover, we observed that survival fraction significantly decreased in dose-dependent manner. The significant differences were obtained between 0 µM and 10 µM of PJ-34 in 2 Gy group.

Conclusions and Recommendations
The level of pATM and pH2A.X, and surviving fraction are the important factors in assessing the radiosensitivity of cells. Our results suggest that PJ-34 at concentration of 10 µM may be appropriate agent for enhancing the effect of 2 Gy radiotherapy.

3. Association of TIRAP rs8177376 and rs8177374 Polymorphisms with Cervical Cancer Phenotype and Prognosis

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Background and Objectives
One of the most common cancers in woman worldwide is cervical cancer, which is usually caused by HPV, mostly high-type risk HPV16 and HPV18. Toll-like receptors (TLRs) signaling pathway is responsible for removing virus from the body. However, in some cases persistent infection can develop. Although the mechanism is still not fully understood, studies have been showed that signal transduction between TLRs and other components could be disrupted and become uncontrollable due to single nucleotide polymorphisms (SNPs) in genes that are involved in

TLRs signaling pathway. Therefore, the aim of this study was to analyze associations between *TIRAP* rs8177376, rs8177374 polymorphisms and cervical cancer phenotype and prognosis.

Material and Method
A total of 153 female patients with cervical cancer were enrolled in this study. Patients were selected based on inclusion criteria: age at the time of diagnosis, tumor size and grade, lymph node involvement, metastasis, disease progression and death. Incomplete medical documentation, other malignancies and significant comorbidities were considered as exclusion criteria. The data was collected from medical records. For SNPs analysis blood samples were collected and subsequently used for genomic DNA extraction. PCR-RFLP assay was done. The study was approved by Kaunas Regional Biomedical Research Ethical Committee (protocols No. BE-2-10 and No. P1-BE-2-10/2014).

Results
In this study more than half of patients were older than 50 years (60.8%). It was determined that the mean age at the time of diagnosis was 55.4 ± 13.5 years (range 22 – 83 years). For most of the patients, tumor size was smaller (T1 + T2) and more differentiated (G1 + G2) (63.4% and 74.5%, respectively). Moreover, lymph node involvement was found for 45.1% of patients. A study also showed that only 5.2% of patients had distant metastasis, while disease progression and death were confirmed for 29.4% and 23.5% of patients, respectively. The distribution of genotypes of *TIRAP* polymorphisms among studied patients (n = 153) were: TT – 50.3%, TG – 39.9%, GG – 9.8% for rs8177376; CC – 75.8%, CT – 22.9%, TT – 1.3% for rs8177374. In association analysis, the results by Pearson Chi-square test showed that rs8177376 T allele was associated with death ($p = 0.023$), however the significance was not found after logistic regression analysis ($p > 0.05$). The analysis also revealed that an association between rs8177374 and death was significant ($p = 0.029$). The carriers of CT genotype had a 2.85 times higher risk for death (95% CI 1.254-6.469, $p = 0.029$) than those with CC genotype. Unfortunately, after multivariate logistic regression analysis an association was in loss of significance, suggesting that other factors, such as age at the time of diagnosis, tumor size, tumor grade and lymph node involvement could be more important for an association. On the contrary, some associations were observed after logistic regression analysis even though a significant association was not determined by Pearson Chi-square test. Univariate logistic regression analysis revealed that rs8177376 T allele was more prevalent in the group of patients older than 50 years (OR = 1.654, 95% CI 1.172-2.334, $p = 0.004$). Moreover, the carriers of rs8177376 T allele had a lower risk for G3 tumor grade (OR = 0.340, 95% CI 0.232-0.499, p = 0.000) compared with non-carriers. In contrast, patients having CT genotype of rs8177374 had a higher probability for G3 tumor grade (OR = 2.427, 95% CI 1.081-5.445, p = 0.032) in comparison to CC genotype. The significance between mentioned associations were observed even after multivariate logistic regression analysis. In survival analysis, statistically significant associations between *TIRAP* polymorphisms and OS or PFS were not determined.

Conclusions and Recommendations
In conclusion, the results of our study showed that *TIRAP* rs8177376 T was associated with tumor size and tumor grade, while rs8177374 was associated with tumor grade. Thus, we suggest that T allele of rs8177376 and CT genotype of rs8177374 might be useful in the future as genetic biomarker for prognosis of cervical cancer.

4. Predictors of the Development of Infectious Complications During Post Cytostatic Cytopenia in Patients with Acute Leukemia

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Background and Objectives
Patients with hematological malignancies undergoing chemotherapy (CT) have high incidence of infections which profile is affected by various factors including neutropenia. The initial stages of the disease (screening and induction therapy) are very often accompanied by the development of infections complications in adult patients with acute leukemia. The aim of our study was to determine in patients with newly diagnosed acute myeloid leukemias (AML) and acute lymphoblastic leukemias (ALL) of the possible influence of clinical and laboratory parameters at the screening stage on the development of infectious complications during post-cytostatic cytopenia.

Material and Method
The prospective study (2021) included 49 adult patients diagnosed with acute leukemia who received the induction stage of therapy: 33 patients with AML, 2 patients with a mixed-cell variant with myeloid predominance, 6 patients with AML with changes associated with myelodysplasia, 8 patients with ALL. In the study sample, 30 patients received an induction course of polychemotherapy with high doses ("7+3", FLAG-Ida, Hyper-CVAD/HMA), 19 received courses with standard doses of chemotherapy drugs ("7+3", CALGB, ALL-2009). The paired Student's t-

test and Pearson's goodness-of-fit test (chi-square) were used to compare qualitative traits. The Mann–Whitney U-test was used to compare quantitative traits. The study of the relationship of the influence of screening factors on the development of an infectious complication during the period of cytopenia was studied by the Spearman's rank correlation coefficient. Differences were considered statistically significant if the probability of an error-free prediction was 95% ($p \leq 0.05$).

Results

Among total study subjects, 30 patients experienced infectious episodes during cytopenia. According to the results of the study, the presence in patients with a newly diagnosed AML and ALL at the screening stage of such phenomena as a temperature reaction (U= 178,0; p= 0,0289), the presence of an infectious process during the attack of the disease (U= 164,5; p= 0,0138), the results of microbiological studies of the material from the posterior pharyngeal wall (U= 176,5; p= 0,0267) or anus (U= 167,0; p= 0,0159) with the presence of etiologically significant microflora (Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae spp, Candida spp) are statistically significant predictors of the development of infectious complications during post-cytostatic cytopenia. These results are confirmed by a quantitative assessment of the statistical analysis of the relationship between the phenomena by the Spearman's rank correlation coefficient. At the same time, there was no statistically significant effect of the input parameters of peripheral blood and bone marrow of patients ($p > 0,05$).

Conclusions and Recommendations

The presence in patients with newly diagnosed AML and ALL during the period of the disease attack of such factors as an infectious process, temperature reaction, the result of a microbiological study of material from the posterior pharyngeal wall or anus with an etiologically significant microflora can be predictors of the development of infectious processes in the period of post cytostatic cytopenia, and how as a result, they form a high-risk group of patients for the development of infectious complications.

5. Impact of Hematological Malignancy and Type of Therapy on COVID-19 Severity and Mortality

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Aim

To evaluate the impact of hematological malignancy and type of therapy on COVID-19 severity and mortality.

Objectives

1. To evaluate the clinical characteristics (age, sex, co-morbidities, type of hematologic malignancy and therapy) impact on the severity of COVID-19 disease in adult patients with hematologic malignancies.
2. To evaluate the clinical characteristics (age, sex, co-morbidities, type of hematologic malignancy and therapy) impact on the outcomes of COVID-19 disease in adult patients with hematologic malignancies.
3. To evaluate the vaccination impact on the severity of COVID-19 disease and outcomes in adult patients with hematologic malignancies.

Material and Method

A retrospective study was made by collection of medical data of patients with hematological malignancies that were found to have a positive SARS-CoV-2 RT-PCR test. Data about patients' age, sex, co-morbidities, medications that were used as treatment against hematological malignancies, vaccination status, symptoms, stem-cell transplantation status, intensive care unit (ICU) admission status, survival status, given treatment for COVID-19 disease, hospitalization time and laboratory tests were collected from the Hospital Information System. Patients were divided into "asymptomatic and mild-moderate" and "severe and critically ill" groups. Asymptomatic patients were found to have a positive RT-PCR result. Mild - moderate patients that showed symptoms and were treated for COVID-19 in the ward and received symptomatic and supportive treatment. Severepatients were transferred to the COVID-19 department and required treatment with dexamethasone, convalescent plasma and remdesivir. Critical patienst were transferred to the ICU in order to get advanced treatment, were intubated and/or died during the course of the disease. The data of the 2 groups was compared.

Results

The "asymptomatic and mild-moderate" had 21 patients, 6 (28.5%) of which were male and 15 (71.5%) were female, with a median age of 73. The "severe and critically-ill" group consisted of 31 patients, 21 (67.74%) of them were male and 10 (32.26%) females, with a median age of 61 years. Male sex (p=0.006) was found to be statistically significant factor that predicted a severe and critical course of COVID-19. The type of haematological malignancy

was found to be a statistically significant factor (p=0.027) affecting the severity of disease, 14 (45.15%) acute leukemia patients had severe-critical COVID-19 course, while 7 (33.33%) multiple myeloma patients and 11(52.38) patients with lymphomas were in the “asymptomatic and mild-moderate” group. Out of all the laboratory tests that were assessed only C-reactive protein (CRP) was found to be a statistically significant factor (p=<0.001) to predict severe/critical course of disease. A binary logistic regression analysis revealed that CRP level (p= 0.002) and older age (p= 0.017) are predicting factors of severe outcomes of disease.
Vaccination status was also found to be a statistically significant factor to predict the course of COVID-19 disease, 15 (71.42%) of the patients in the “asymptomatic and mild-moderate” group were vaccinated, whereas 20 (62.52%) of the “severe-critical” group were not vaccinated.

Conclusions and Recommendations
1. Our study had confirmed that demographical characteristics such as male sex and older age, as well as having acute leukemia and increased level of CRP had a great impact on the severity of COVID -19 disease.
2. Vaccinations had a significant impact on lowering the severity and improving outcomes of COVID-19 disease.

6. **Results of Allogeneic Hematopoietic Stem Cell Transplantation, Taking into Account the Characteristics of Unrelated Donors**

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Background and Objectives
In most European centers, the gold standard is to find a donor matching HLA-A, -B, -C, -DRB1 and -DQB1, the so-called 10/10 match. An alternative matching algorithm recommended by the NMDP is to find a donor compatible with HLA-A, -B, -C and -DRB1 (match 8/8). If a patient has several donors with 10/10 compatibility, further selection of donors consists in selecting a patient-donor pair based on other factors not related to the HLA system: cytomegalovirus (CMV) status, gender and age of donors. Objective is to analyze the characteristics of donors associated with better patient survival after transplantation.

Material and Method
The analysis included the results of 53 allogeneic transplantations from an unrelated donor performed by adult (over 18 years old) patients diagnosed with acute leukemia on the basis of the State Institution "Minsk Scientific and Practical Center for Surgery, Transplantology and Hematology" since January 2015 to December 2021, among them there were 30 patients with acute myelogenic leukemia and 23 with acute lymphoblastic leukemia.

Patients were divided into subgroups based on diagnosis, disease status, age, gender and CMV status. Donors were divided into subgroups according to HLA compatibility, age, gender and CMV status. Each of the factors was considered separately.

Overall survival was the primary endpoint. Death for whatever reason was considered an event. Relapse was defined as morphological, cytogenetic, or molecular recurrence of the disease and was considered an event. The selection of an unrelated HSC donor was carried out taking into account the degree of compatibility with HLA, donor age and infectious status. The median follow-up was 20 months.

Results
We did not obtain statistically significant differences in the duration of overall survival in allo-HSCT from unrelated donors, differing by gender (patient's gender p=0.953, donor's gender p=0.826), age (patient's age p=0.761, donor's age p=0.677), CMV status (p=0.681) and the degree of compatibility according to the HLA system (10/10 and 9/10, p=0.181), diagnosis: ALL vs AML p=0.696. An important factor influencing the duration of overall survival is the disease status: 3 remission (+relapse) vs 1 remission p<0.001. Duration from diagnosis to transplantation (>730 days, p<0.059).

We did not find statistically significant differences in the duration of event-free survival in allo-HSCT from unrelated donors, differing by gender (patient's gender p=0.528, donor's gender p=0.289), age (patient's age p=0.938, donor's age p=0.268), CMV status (p=0.197) and degree of compatibility according to the HLA system (10/10 and 9/10, p=0.069), diagnosis (ALL vs AML p=0.18), duration from diagnosis to transplantation (>730 days, p<0.081). Statistically significant differences in the duration of event-free survival depending on the status of the disease were revealed: 3 remissions (+relapse) vs. 1 remission, p<0.001.

Conclusion and recommendations
At the current level of HLA typing, differences in donor-recipient pairs with 9/10 compatibility versus donor-recipient pairs with 10/10 compatibility are not statistically significant, which increases the number of potential donors, but requires more careful selection of donors according to other characteristics.
The most important when performing allo-HSCT is the status of the disease (95% CI, 15.9 (3.2 –79)) and the timing of transplantation (95% CI, 4.7 (0.94-23)).

7. Idiopathic Thrombocytopenic Purpura: Epidemiology in Kaunas County and Treatment Standard

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Aim
To evaluate epidemiology of patients who have idiopathic thrombocytopenic purpura in Kaunas County and evaluate the treatment and its effectiveness.

Objectives
1. To evaluate patients with idiopathic thrombocytopenic purpura epidemiologic data in Kaunas County.
2. To evaluate thrombopoietin's dose, treatment time in patients who have idiopathic thrombocytopenic purpura.
3. To evaluate how demographic, clinical and laboratory findings have impact on resistance to glucocorticosteroids.

Material and Method
The retrospective study was made by using medical histories of patients who have idiopathic thrombocytopenic purpura. All patients were divided into two groups: patients who are without treatment and patients who are still getting treatment with thrombopoietins. Data of gender, age, contagious, chronic and autoimmune diseases, appearance of hemorrhagic syndrome at time when the disease was diagnosed and during the treatment, medications dose, response to treatment of glucocorticoids and thrombopoietins, period of time when patients got treatment, remission time was compared between the groups.

Results
Patients who are in remission consist of 21 male and 22 female. 21 of these people were under 65 years old and 22 people were ≥65. The group of people that are getting thrombopoietin's consists of 10 male and 15 women and there were 19 patients <65 and 6 patients who are ≥65. Appearance of having autoimmune disease and hemorrhagic syndrome is higher in the group where patients are treated with thrombopoietins, when p= 0.020 and p=0,010 respectively. Response time in the control group was 46,0 weeks, while in other group it was 15,0 weeks, when p= 0,241. Response to treatment with glucocorticoids most of the time was early, while in other group there were some patients who do not got any response, when p= 0,013. Bleeding during treatment was not noticed in 39 patients in control group and 14 patients in research group, when p< 0,001. Eltrombopag as treatment method most of the time was chosen as 2^{nd} or 3^{rd} line treatment, while Romiplostinum was chosen as 4^{th} line treatment. Medium dose of Eltrombopag, which the patient got, was 50 mg and it was used for approximately 88,0 weeks. This medication was prescribed after 84,0 weeks from diagnosis. Medium dose of Romiplostinum was 10 mcg/ kg and period of using this medication was 14,0 weeks. This medication was prescribed 276,5 weeks from diagnosis. On a binary logistic regression analysis age, autoimmune disease, bleeding episodes during treatment were wound to be as predicting factors of resistance for glucocorticosteroids.

Conclusions and Recommendations
1. Patients who get trombopoetins are more often under 65 years, have autoimmune disease, more often have haemorrhagic syndrome before treatment starts and during treatment and get late response to gliucocorticosteroids or there is no response, while in other group dominate early response to treatment.
2. The average dose of Eltrombopag is 50 mg, which is usually used by 88,0 weeks, while Romiplostinum average dose is 10 mcg/kg and the period of treatment is 14,0 weeks.
3. The resistance to gliucocorticosteroids is caused by younger than 65 years, having at least one autoimmune disease and appearance of bleeding during treatment.

8. The Radiosensitizer Potential of Sulforaphane on Breast Cancer Cells

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Background and Objectives

Radiation therapy is commonly applied in the treatment of breast cancer. However, radioresistance and side effects are limiting factors of this practice. Therefore, studying substances that can enhance the radiation effect and, at the same time, protect normal cells is very relevant. One of these is sulforaphane (SFN). Sulforaphane is an herbal isothiocyanate that typically occurs in cruciferous vegetables like broccoli and cauliflower. In recent years, it gained scientific popularity for its cancer preventive attributes as well as its antitumor effects. Thus, the **aim** of this work is to evaluate the response of the breast cancer cell line to the impact of sulforaphane and the effects of this substance in combination with radiotherapy

Material and Methods

Cell viability and colony formation assay were used to test the anticancer efficiency of sulforaphane and to confirm the ability of SFN to sensitize MCF-7 and MDA-MB-231 breast cancer cells to radiotherapy. Colony formation assay. Cells were plated at a cell density of 300 cells per well in 6-well tissue culture plates. After attachment overnight, the medium was replaced and cells were treated with different concentrations of SFN (0, 1, 3, 5, 10, 25, 50 and 80 μM) for various incubation times (24, 48, 72 h). Subsequently, the cells were cultured for 10–14 days until colonies were visible. Then the cells were fixed with ethanol (70%) for 10 min and stained with crystal violet. Colonies containing at least 50 cells were scored. Irradiation (IR). Cells were seeded into culture plates, incubated overnight, treated with SFN as described above. After 48 h the media was removed and replaced with fresh SFN-free culture medium. Cells were irradiated with 2 or 4 Gy using high energy X-rays, which were generated using an X-rays instrument *Clinac 2100C/D*. Fourteen days after irradiation colonies were fixed and calculated as described above.

Statistical analyses. All data are represented as the means ± SD. Statistical significance was determined using Student's t-tests. $P<0.05$ was considered to indicate a statistically significant result.

Results

We examined the effect of SFN on viability of two breast cancer cell lines: MDA-MB-231 and MCF-7. Our study suggests that the inhibition of cell viability was significantly increased in MCF-7 cell line in response to SFN in a dose- and time-dependent manner compared with the control group (0 μM SFN) ($P<0.05$). The results also indicated that MDA-MB-231 cells have higher SFN tolerance than MCF-7 cells. Further the radiosensitizing effect of SFN at different concentrations (1, 3, 5, 10 μM) in 48 h cell culture irradiated at 0, 2, and 4 Gy were evaluated. The combination of SFN and radiation treatment produced significantly greater antitumor effects on the breast cancer cells than either treatment alone.

Conclusions

Our study results revealed that SFN is a potential radiosensitizer of MCF-7 and MDA-MB-231 breast cancer cells.

9. Pyruvate Kinase Deficiency – A Long Way to a Correct Diagnosis

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Introduction and Aim

Pyruvate kinase deficiency (PKD) is the most commonly encountered glycolytic enzymopathy associated with anemia. Anemia, jaundice and splenomegaly are regularly present in PK deficiency. The anemia may be profound, occurring in utero or in early infancy, and require regular blood transfusions for survival. The differential diagnosis includes the heterogeneous group of congenital and acquired hemolytic disorders. We present a clinical case of congenital hemolytic non-spherocytic anemia with a correct diagnosis of pyruvate kinase deficiency. Aim is to describe a case of a patient with PKD.

Case report
The girl was born in June 2016. She had suffered severe anemia since the neonatal period. After birth complete blood count showed Hb 65 g/L, red blood cell count (RBCs) 1.68×10^9/L, mean corpuscular volume 106.8 fL, mean corpuscular hemoglobin 36.9 pg, mean corpuscular hemoglobin concentration 34.2 pg, red cell distribution width 24.1% and reticulocytes 16.8% . A blood film examination showed marked anisopoikilocytosis, polychromasia, rare elliptocytes, ovalocytes, and spherocytes. She received first erythrocyte transfusion after birth, then she needed transfusions every 1-2 months. Complete hematological examination, differential diagnosis of anemia was performed at the age of 4 months. Direct Coombs' test and Parvovirus B19 PCR analysis were negative, G6PD activity, hemoglobin electrophoresis was normal. Bone marrow aspiration showed erythroid hyperactivity together with a small number of double and multinucleated erythroid precursors, which may indicate CDA. Pyruvate kinase activity we were able to do only at the age of 11 months. The PK enzyme level was normal, which was 1 month after the last transfusion. Unfortunately, we could not analyze hereditary haemolytic anemia panel for 5 years of the disease. Finally, in May 2021, molecular analysis of the PK-LR gene revealed the presence of heterozygous c.1529G> A (p.Arg.510 Gln) mutation. However, no genetic mutation detected concerning CDA (SEC23B, CDAN1).

Discussion
PKD is an autosomal recessive disorder. The diagnosis of PKD is based on the presence of clinical signs and symptoms and laboratory markers of chronic haemolytic anaemia, on reduced PK enzymatic activity, and on the detection of compound heterozygous and homozygous mutations in the PKLR gene.

Conclusions
The diagnostic approach to transfusion-dependent hereditary hemolytic anemia could be challenging related to false normal erythrocyte enzyme studies. Genetic analysis of PK-LR should be performed in patients with regular transfusions.

10. Harnessing Genetically Engineered Feeder Cell Line for Ex Vivo Expansion of Human Natural Killer Cells with Increased Production of Ifn-Y

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Background and Objectives
Natural killer (NK) cells belong to the group of innate immune cells. The main functions of NK cells are to protect the body from viruses and malignant cells. Numerous clinical studies are currently underway on the use of NK cells in anticancer immunotherapy. Interferon-γ (IFN-γ) is a cytokine that has antitumor activity and can be effectively used in immunotherapy of oncological diseases. Various cell types are responsible for the production of IFN-γ, including activated T cells and NK cells. However, the level of IFN-γ production by NK cells can be altered by various cytokines. We have previously shown that the use of a K562-mbIL21-41BBL feeder line (further FD21) leads to a significant expansion and activation of NK cells.
Thus, the aim of the study is to obtain genetically engineered K562-mbIL21-mbIL12-41BBL (further FD21_12) feeder cell line based on FD21 with the expression of a membrane-bound recombinant variant of human IL-12 for the expansion of natural killer cells with increased production of IFN-γ and antitumor activity.

Material and Method
Whole blood of 5 healthy donors was used in the work. Mononuclear cells were isolated from the donor's peripheral blood on a density gradient by centrifugation and the number of isolated cells was counted. The expansion of NK cells was performed by culturing peripheral blood mononuclear cells of donors in the presence of irradiated (100 Gy) feeder cells and IL-2 (50 IU/ml) in complete RPMI-1640 medium for 12 days in G-Rex 24-well plate. On the 12th day, the number of NK cells were determined and the level of their activation, as well as the number of NK cells, producing IFN-γ, expression CD107a and cytotoxic activity. Two variants of feeder cell lines were tested. First – FD21; the second – FD21_12.

Results
Previously received line FD21 was transduced with lentiviral particles to obtain stably modified variants expressing the recombinant cytokine IL-12. Expression of the introduced transgenes was confirmed at the mRNA level by quantitative PCR and at the level of protein products by flow cytometry.
The level of expansion of NK cells through 12 days of cultivation, when using the obtained feeder lines, was 392 (193-843) and 528 (234-1227) for FD21 and FD21_12 lines. Cytotoxic activity in a ratio 10:1 against for K562 cells for the first line was 89.7% (87.7 – 91.6 %) and the second line 90.7% (82.2 – 91.1%). The percentage of CD107a+

cells was 64.3 % (52.5 – 72.4 %), 71.6 % (56.0 – 84.6 %), accordingly. The number of cells producing IFN-γ - 20.6% (9.3-62.2%), 31.7% (29-63.1%), accordingly.

Conclusions and Recommendations
As a result of the work, a new feeder cell line FD21_12 was obtained, which makes it possible to obtain a NK cell with high level of expansion, cytotoxic activity, IFN-γ production and expression CD107a.

11. The Constraints During Treatment in Pediatric Oncology: Tough Dialogue - a Simple Decision

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Background and Objectives
Communication in pediatric oncology serves several functions for family members: establishing relationship with the medical team, information exchange, confirmation of information, making decisions, creating an algorithm of actions and its implementation. The implementation of these functions maintains emotional balance, hope for success, trust in physicians, emotional support and a sense of recognition and need, consolation in emotional trauma. When parents or official guardians of a pediatric cancer patient are faced with poor quality information or misunderstanding of the content received - in most cases, family members will have difficulties with decision making, doubt the veracity of the decision already made, and express a lower level of trust in physicians. Therefore, it will lead to the failure to follow the recommendations and prescriptions in full. Identification and analysis of communication barriers in communication between: physician - parents (guardians) or medical staff - parents (guardians) of a pediatric cancer patients.

Material and Method
A retrospective cohort multicenter study was conducted among parents of pediatric cancer patients who cared for a child while receiving scheduled chemotherapy. Information was collected through questionnaires indirectly, the information was provided by respondents anonymously, remotely, using Google-forms.

Results
106 couples of family members of pediatric cancer patients who received treatment in medical institutions of Ukraine specializing in the treatment of pediatric cancer took part in the questionnaire survey. Categories of questions used in the questionnaire survey: barriers that arose during communication with physicians and medical staff during the period of undergoing the therapeutic protocol; a source for providing information to parents (guardians) of pediatric cancer patients; time for communication with the attending physician and its adequacy in relation to the required scope of information received.

The research showed that 66% of respondents (n=70) noted difficulties in communicating with physicians. The comments to the question what exactly made the barriers for respondents shown the following points: respondents' misunderstanding of medical terminology, inadequate perception of information by parents due to significant emotional trauma, superficial behavior, and irritability of doctors in the case of a request for re-provision of oral information. Some parents noted that they were embarrassed to initiate a conversation on their own to specify issues that concerned them, due to uncertainty and fear of negative criticism from physicians and medical staff. Regarding communication barriers in contact with nurses and staff, 85% (n=90) of respondents reported difficulties in contact with middle grade medical staff and junior staff, which were related to ignorance of parents and the difficulty of perceiving and reproducing new medical information about features of child care during chemotherapy, rude and superficial behavior of some health professionals, non-compliance of nurses and medical staff with deontological aspects of the work - discussion of personal data obtained from medical records during communication. The respondents indicated the most convenient source of information about the disease of a pediatric patient (features of the pathology, treatment options and features of care during and after the main therapy of the disease) as follows: attending the physician - 79.5%, Internet resources - 11.4%, and middle grade medical staff - 5.7%. Analysis of the answers in the section of questions classified as "Time of communication with the attending physician and his adequacy in relation to the required scope of the information to be received" revealed the following: the vast majority of physicians communicate with parents (guardians) of patients up to 10 minutes a day, at the same time 41.5 % of doctors use only working hours for this, 17% - only morning examinations of patients. 60.4% of cases have shown that there was enough time for communication with the physician to address the main issues, the vast majority of

respondents (56.6%) noted that physician who were directly involved in the treatment of pediatric cancer patients were available throughout the day, using additional communication tools (telephone, e-mail, messengers of social platforms, etc.). On the other hand, 39.6% (42 respondents) indicated a lack of time for doctors to communicate, which is why 9.4% of respondents still have unresolved questions about certain aspects of their child's cancer.

Conclusions and Recommendations
The research revealed the following problems: lack of adequate communication links between patients' parents and physicians and medical staff; insufficient readiness of parents for the situation and emotional trauma; lack of time for physicians and medical staff to establish communication. In the course of the research, a tool for elimination of these communication errors was found – creation of a video channel with visual thematic and clearly structured content for parents of pediatric cancer patients.

12. *DDIT4* mRNA Level Determines Aspirin Effect on *NDRG1* Expression in MDA-MB-468 Human Breast Cancer Cell Line

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Background and Objectives
NDRG1 (N-myc downstream-regulated gene 1) is overexpressed in approximately 25% of cases in TCGA (The Cancer Genome Atlas) breast cancer data set. Elevated *NDRG1* mRNA amount in breast tumor has been associated with poor prognosis in a large meta-analysis of 23 cohorts. *NDRG1* has been suggested to contribute to breast cancer aggressiveness through regulation of fatty acid metabolic fate.
We previously found that knockdown of *DDIT4* (DNA damage-inducible transcript 4) facilitates aspirin-mediated dephosphorylation of mTORC1 target 4E-BP1 in breast cancer cells. In the present study, we aimed to test whether *DDIT4* mRNA amount affects the expression of *NDRG1*, which is downstream of mTORC2, after aspirin exposure.

Material and Method
MDA-MB-468 human breast cancer cell line was used in this study. Down-regulation of *DDIT4* mRNA level was carried out by siRNA transfection. Non-targeting siRNA was used to generate control cells. Cells were exposed to aspirin 24 hours after reverse transfection. Expression of *NDRG1* and *DDIT4* genes was analyzed by quantitative reverse transcription-PCR following 24-hour aspirin treatment.

Results
siRNA knockdown of *DDIT4* attenuated *NDRG1* mRNA expression 1.36-fold following aspirin exposure in MDA-MB-468 cell line.

Conclusions and Recommendations
DDIT4 mRNA level determines *NDRG1* expression following aspirin treatment in breast cancer cells. This data provides a rationale to assess whether *DDIT4* expression in breast tumors predicts response to aspirin treatment in clinical setting.

Funding
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13. Natural Killer Cell Therapy in Children with Refractory Acute Myeloid Leukemia

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Background and Objectives
The use of natural killer (NK) cells as a cellular immunotherapy has increased over the past decade, especially for patients with hematologic malignancies. The advantages of NK cells for adoptive therapy are the high innate anticancer activity and low incidence of toxicity reactions. Today, there are different technologies and sources to obtain NK cells: immunomagnetic isolation from peripheral blood, differentiation with cytokines from umbilical cord blood or induced pluripotent stem cells. Another way to get NK cells is expansion with stimulatory/feeder cells such as irradiated peripheral blood mononuclear cells (PBMCs), EBV-transformed lymphoblastoid cell lines or genetically engineered feeder cells. We have previously reported the creation of K-562-based feeder cell line

transduced to express 4-1BBL and membrane bound Interleukin-21 (mbIL-21) [Vashkevich E., 2020]. Here we describe four cases of NK immunotherapy for children with refractory acute myeloid leukemia (AML). Registered at www.clinicaltrials.gov as NCT04327037 and NCT05272293.

Material and Method

A total of 4 children with a median age of 16 (range, 11-17) with refractory AML were enrolled in the study. Peripheral blood samples of 4 haploidentical donors (3 mothers and 1 father) with a median age of 42.5 years (range, 40.6–50.4) were the source of PBMCs for NK cells expansion. NK expansion was induced by co-culturing of donor PBMCs with feeder line K562-mbIL-21-41BBL obtained in our laboratory.

Course of immunotherapy consisted of a block of FLAG-based chemotherapy followed by NK cells infusions (from 1 to 3) during the period of cytopenia. NK cells were administered intravenously on day 0. Two patients received six dose of IL-2 ($1x10^6$ IU/m^2, Roncoleukin, LLC NPK BIOTECH, Russia) every second day starting on day -1, one patients received one dose of IL-2, another one was treated without IL-2.

Results

Totally 4 patients received 9 infusions of NK cells. The purity of NK cells was 95.5 % (77.3-98.3), median dose of infused NK cells was 4.1 (1.2-8.8)*10^7/ kg, CD3+ cells – 0.9 (0.8-11.5)*10^6 / kg. The majority of obtained NK cells had the phenotype of immature activated cells (NKG2A+, double bright CD56++CD16++, CD57-) expressing NKp30, NKp44, NKp46, NKG2D, CD69, HLA-DR and CD96.

Patient #1 received two courses of immunotherapy with one NK cells infusion per course. This patient achieved complete morphological remission (CRm) and received matched unrelated HSCT, is alive (+667 days).

Patient #2 received one course of immunotherapy with two NK cell infusions. After immunotherapy the level of blast cells in bone marrow decreased from 47.7 to 9.5%. However, the patient did not get the second course of immunotherapy because of infection status. The patient died from progression (+260 day).

Patient #3 received one course of immunotherapy with two NK cell infusions, achieved CRm and received matched related HSCT, is alive (+464 days).

Patient #4 received one course of immunotherapy with three NK cell infusions, achieved CRm. Further treatment was delayed because of infection complications. The patient is alive (+87 days).

Conclusions and Recommendations

Infusions of haploidentical ex vivo expanded NK cells were safe and well tolerated, provided clinical response in 3 out of 4 poor prognosis patients with refractory AML. Encouraging results stimulate us to continue the investigation of NK immunotherapy for patients with AML.

14. VRd Regimen for Treatment of Vertebral Plasmacytoma with Spinal Cord Compression

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Introduction and Aim

Patients with relapsed multiple myeloma (MM), who have received several lines of chemotherapy (CT), have limited specific treatment options and low probability to achieve a complete response. MM relapse increases the risk of secondary infiltration of the body's organs and systems due to soft tissue extramedullary tumors. Aimis to present the results of patient's management with relapsed MM, which clinically manifested by spinal cord compression on the background vertebral plasmacytoma development at the level of Th5-Th7, the effectiveness of combined CT with bortezomib, lenalidomide and dexamethasone was evaluated.

Case report

Our case report involves a patient born in 1971, with light Kappa chains Multiple myeloma stage III A (Durie, Salmon), with bone lesions of the skull, ribs, spine (wedge-shaped vertebrae deformation C6, Th3, 5, 7, 8, 9), pelvic bones, femurs, vertebral plasmacytoma at the level of Th5-Th7 with secondary acute spinal cord compression, ischemic myeloneuropathy and senestopathic syndrome.

In August 2020 she was hospitalized to the hematology department of PE "Poltava Regional Clinical Hospital n.a. M.V. Sklifosovsky PCR" with complaints of pronounce sensitivity decrease, paresthesia in the lower extremities, gait disorders, loss of pelvic functions control.

From the anamnesis it is known that patient was diagnosed with multiple myeloma in September 2012 based on changes in the myelogram: 44% of plasma cells. The patient had received 3 lines of chemotherapy (CT): 1) VAD (vincristine, doxorubicin, dexamethasone) in 2012 with a partial response; 2) TCD (thalidomide, cyclophosphamide, dexamethasone) in 2015; 3) and a similar course of TCD in 2018 with further maintenance therapy with thalidomide until December 2019.

The progression of MM was recorded in August 2020. Clinically, MM relapse manifested by progressive neurological symptoms, pelvic dysfunction. The level of free Kappa light chains in the serum was 344.0 mg/l (norm up to 19.4 mg/l). MRI with contrast of the thoracic and lumbar spine diagnosed an intradural extramedullary plasmacytoma of the vertebral canal at the level of Th5-Th7 with spinal cord compression.

In order to treat the third MM relapse, the patient was offered the 4 line of CT: VRd regimen (bortezomib 1.3 mg/m^2 1, 4, 8, 11 days, lenalidomide 25 mg per day 1-21 days, dexamethasone 20 mg per day 1, 2, 4, 5, 8, 9, 11, 12 days). After the third course of CT, the general condition of our patient significantly improved, sensitivity in the lower extremities and control of pelvic organ functions were restored.

After 8 courses of VRd regimen, a very good partial response was achieved according to the criteria of the International Myeloma Working Group (IMWG): free Kappa light chains in the blood serum decreased to 33.8 mg/l by 90.2% lower. According to MRI of the thoracic and lumbar spine, the extramedular neoplasm on the Th6 vertebrae level significantly reduced, there are myelopathic changes in the spinal cord at this, which occurred after the spinal cord compression by plasmacytoma. After completing the VRd in April 2021, the patient was followed by lenalidomide maintenance. To date, no progression of MM was recorded during the 12-month period.

Discussion and Conclusions
Combined chemotherapy with bortezomib, lenalidomide, and dexamethasone may be the treatment of choice for relapsed patients with soft tissue extramedullary plasmacytomas.

15. *CALR* 52 bp Mutation Impairs Oxidative Stress Response and Increases Oxidative Stress-Induced Apoptosis Level in UT-7 Cell Line

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Background and Objectives
BCR-ABL1-negative classic myeloproliferative neoplasms (MPN) include primary myelofibrosis, polycythemia vera, and essential thrombocythemia. Calreticulin (*CALR*) 52 bp deletion and 5 bp insertion were discovered to be involved in MPN pathogenesis, particularly in *JAK2* and *MPL* unmutated essential thrombocythemia and primary myelofibrosis. Calreticulin, a Ca^{2+}-binding chaperone, is implicated in Ca^{2+} homeostasis, protein folding, and response to oxidative stress. It is well known that oxidative stress induces the accumulation of reactive oxygen species (ROS) that damage membrane lipids, proteins, and DNA. Moreover, several studies demonstrated that MPN patients show high serum levels of intracellular ROS, which can lead to chronic inflammation and genomic instability. However, there is not much data on how mutated calreticulin affects oxidative stress response and oxidative stress-induced apoptosis. Therefore, we aimed to investigate the response to oxidative stress and apoptosis induction in UT-7 cells expressing either *CALR* WT or *CALR* 52 bp deletion.

Material and Method
The UT-7 cell line was used in our study. CRISPR/Cas9 system was chosen for *CALR* 52 bp deletion initiation in cells. After the selection of potential DNA targets in gDNA, corresponding tabs were cloned into a vector (pSpCas9(BB)-2A-Puro (PX459) V2.0) that is optimized for Cas9 and RNA-guided expression in eukaryotic cells. Transfection of plasmid construct and HDR template into UT-7 cells was performed by electroporation. The following electrotransfection parameters were applied: 1 HV pulse of 1600 V/cm with 500 µs pulse duration and the electroporation system BTX T820 was used. The transfected cells were selected in puromycin (1 µg/ml). Further, UT-7 cells expressing WT and *CALR* 52 bp deletion were treated with H_2O_2 for 24 hours. The intracellular oxidative

stress and apoptosis induced by H_2O_2 were measured by means of Muse® Oxidative Stress Kit and Annexin V & Dead Cell Kit, respectively. Cells were examined using the Muse® Cell Analyzer and at least 5000 events were detected for each sample.

Results
To assess whether *CALR* 52 bp deletion can impact the response to oxidative stress and oxidative stress-induced apoptosis, UT-7 cells expressing either the WT or 52 bp deletion variants of *CALR* were treated with H_2O_2 for 24h. Our results showed that UT-7 cells expressing *CALR* 52 bp deletion exhibit higher levels of oxidative stress (i.e., ROS-positive cells) compared to cells expressing wild-type *CALR* (49.07% ± 2.96 *vs* 39.02% ± 4.38). These differences are more evident after cells were given 24 additional hours to reduce ROS accumulation induced by H_2O_2. After 24 h of repair, cells expressing *CALR* 52 bp deletion were unable to reduce ROS level. UT-7 cell line with mutated calreticulin exposed 51.53% ± 6.06 ROS positive cells. Whereas UT-7 cells with *CALR* WT were able to efficiently counteract the ROS accumulation (29.49% ± 1.65).
Further, we analyzed whether the different level of oxidative stress has an impact on different ability to induce apoptosis. Our results revealed an increase in oxidative stress-induced apoptosis levels in UT-7 cells with *CALR* 52 bp deletion (27.5% ± 4,97) compared to *CALR* WT cells (25.00% ± 3.43).
These *in vitro* data demonstrated that *CALR* 52 bp deletion impairs cell ability to respond to oxidative stress. Moreover, *CALR* Del52 cells can be characterized by a higher apoptosis level compared to *CALR* WT.

Conclusions and Recommendations
Our results demonstrated that the UT-7 cell line with *CALR* 52 bp deletion can be characterized by a greater increase of intracellular oxidative stress and have a higher apoptosis level compared to cells expressing wild-type *CALR* following the exposure to H_2O_2.

16. Baseline Evaluation of Cardiac Function and Volumes in Patients Undergoing Hematopoetic Stem Cell Transplantation and Relation to Prior Use of Doxorubicin | *Best Poster Award Received*

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Background and Objectives
Cardiovascular disease and cardiovascular complications are common complications associated with HSCT. These complications can occur both acutely within the first 100 days as well as many years after the initial transplantation period. Many components of HSCT like the use of myeloablative chemotherapy, cardiotoxicity of dimethyl sulfoxide, volume overload, sepsis is a huge load for the heart. Therefore, it is very important to evaluate the baseline cardiac function before starting HSCT, to know risk factors and to stratify patients which need special attention. We aimed to find a relation between left ventricle (LV) function, end diastolic volume (EDV) and prior use of doxorubicin.

Material and Method
Data of 21 patient undergoing autologous or allogenic hematopoetic stem cell transplantation at the Department of Oncology and Hematology in the Hospital of Lithuanian University of Health Sciences Kaunas Clinics between 2021 October and 2022 April was evaluated. Bioethics approvement for prospective study was obtained (No BE-2-96). Cardiac evaluation including ECG, echocardiography, cardiovascular magnetic resonance (CMR), troponin I and BNP levels was performed before the stem cell mobilisation. CMR was performed using 3T MRI Siemens Magnetom Skyra, volumetric analysis was evaluated using Medis Suite 3.2. The patients were divided into two groups: one with prior chemotheraphy regimens including doxorubicin and the other without doxorubicin. SPSS statistics 20 was used for statistical analysis. Qualitative data is presented as absolute value (N) and percentage (%), quantitative parameters are given as average (m ± standard deviation). We used Student t test to compare averages of quantitative parameters. Statistically significant difference was considered when $p<0.05$.

Results
Data of 21 patient was evaluated. 7 patients (33,3%) received prior chemotherapy with doxorubicin and 14 (66,7%) without doxorubicin. We found that in the first group left ventricle end diastolic volume corrected to body surface area (LV EDV/BSA) was bigger than in the second group (83,6ml/m2 ± 15,5 vs 74,2ml/m2 ± 14,7). LV ejection fraction (EF) was smaller in the first group receiving chemotherapy including doxorubicin (57,1% ± 9,3 vs 62,4% ± 6,5). Both values were statistically not significant ($p = 0,193$ and 0,149 respectively).

Conclusions and Recommendations

Patients before HSCT with prior chemotherapy regimens including doxorubicin tended to have bigger LV EDV/BSA and lower LV EF compared to patients with prior chemotherapy without doxorubicin. We assume that statistical significance was not obtained due to small amount of patients tested.

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