markers of BRCA1-dysfunction will be essential to translate an understanding of defective DNA repair into targeted treatments for this poor prognosis subtype.

Materials and methods: Twenty-two patients with triple-negative breast cancer (TNBC) were treated with neoadjuvant platinum-based chemotherapy followed by surgery. Pathologic treatment response was assessed in correlation with biomarkers of BRCA1-dysfunction. Pathological response was evaluated according to the Chevallier classification (Ch). All patients were screened for germ-line mutations in BRCA1 (185delAG, 5382insC, 4153delA, 4158A/G, C61G) and BRCA2 (6174delT). All samples negative for germ-line mutations in BRCA1 and BRCA2 then submitted to BRCA1-dysfunction screening. Biomarkers of BRCA1-dysfunction included: BRCA1 somatic mutations (C61G, 185delAG, 5382insC), promoter methylation of BRCA1, low BRCA1 mRNA expression, high ID4 mRNA expression, low RAD51 mRNA expression, PTEN (T4721C) mutation, p53 (Arg72Pro, Pro72Pro) mutations. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP), methylation-specific PCR were used for BRCA1-dysfunction analysis.

Results: Twelve patients (54.5%) achieved a pathological complete response (pCR) (Ch1+ Ch2). Ten patients (45.5%) had a residual disease (Ch3+ Ch4). BRCA1 mRNA expression was absent in 16/22 (72.7%) patients, low in 5/22 (22.7%). 11/21 (52.4%) patients with other than normal BRCA1 mRNA expression achieved a pCR. BRCA1 promoter methylation was detected in 9/22 (40.9%). 6/9 patients with BRCA1 promoter methylation achieved a pCR. RAD51 mRNA levels were low in 14/22 (63.6%), high in 1/22 (4.5%). 8/14 (57.1%) patients with other than normal RAD51 mRNA expression achieved a pCR.

High ID4 mRNA levels were determined in 5/22 (22.7%). 3/5 (60%) patients with high ID4 mRNA expression achieved a pCR. p53 (Arg72Pro, Pro72Pro) mutations were identified in 12/22 (54.5%). No patient had PTEN (T4721C) mutation. No statistically significant correlation was found between the BRCA1 mRNA expression, BRCA1 promoter methylation, RAD51 mRNA expression, high ID4 mRNA levels, p53 (Arg72Pro, Pro72Pro) mutations and pCR to neoadjuvant platinum-based chemotherapy ($p > 0.05$). Eleven patients had BRCA1 somatic mutations: 6/22 (27.3%) – BRCA1 5382insC, 4/22 (18.2%) – BRCA1 185delAG, 3/22 (13.6%) – BRCA1 C61G. 3/4 patients with BRCA1 185delAG mutation had BRCA1 C61G mutation. BRCA1 LOH was detected in 1/22 (4.5%) patient. All patients with BRCA1 5382insC mutation achieved a pCR ($p = 0.01$). All patients with BRCA1 185delAG mutation and BRCA1 C61G mutation had residual disease (0.04 and 0.1, accordingly).

Conclusion: BRCA1 5382insC somatic mutation is associated with good response to neoadjuvant platinum based chemotherapy ($p = 0.01$). BRCA1 185delAG and BRCA1 C61G mutations in the BRCA1 RING domain may predict resistance to neoadjuvant platinum-based chemotherapy (0.04 and 0.1, accordingly).

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P148

CD68 expression in inflammatory cell infiltration of nonspecific invasive breast cancer

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Background: Tumor-associated macrophages play a main role in tumor progression and dissemination. Taking into account the high heterogeneicity of tumor the different clinical impact of macrophages, infiltrating different sites of tumor, could be expected. The aim was to detect the level of CD68+ cells (macrophages) in the different site of stroma in breast tumor in comparison to clinical course.

Materials and methods: One thirty-six women with nonspecific invasive breast cancer T1-4N0-3M0, who were treated in General Oncology Department of Tomsk Cancer Research Institute (Tomsk, Russia), were included in the present study. Patients did not receive preoperative treatment. The material was fixed in 10–12% neutral formalin. Preparation of the histological material was carried out according to standard procedures. Morphological examination of the surgical specimens was performed by the standard method using a light microscope "Carl Zeiss Axio Lab. A1" (Germany) and slidescanner "MiraxMidiZeiss" (Germany). Metastatic lesion was detected in regional lymph nodes. Immunohistochemical study was performed according to standard procedures. Cytoplasmic expression of these markers was determined in the inflammatory cell infiltrate of different tumor segments: (1) in areas with soft fibrous stroma; (2) in areas with coarse fibrous stroma; (3) in the areas of the so-called "maximum stromal-and-parenchymal relationship" where the individual tumor cells, short strands and groups of tumor cells arranged in soft fibrous stroma; (4) among parenchymal elements; (5) in gaps of ductal tumor structures. Double-stained immunofluorescence was performed according to standard procedures using Leica TCS SP2 laser-scanning spectral confocal microscope (Germany). The following primary antibodies were used: mouse monoclonal anti-human CD68 (BD Biosciences) and rabbit polyclonal anti-stabilin-1 or RS1 (marker of M2 macrophages).

Results: The highest expression of CD68 in the inflammatory cell infiltrate was detected more frequently in areas with soft fibrous stroma (54%) or the so-called "maximum stromal-and-parenchymal relationship" (79%) in patients with breast cancer. The lowest expression of CD68 was observed in areas with coarse fiber stroma (23%). The CD68-positive cells of the inflammatory infiltrate were located between parenchymal elements of tumor (88%). Inverse correlation ($R = -0.67$; $p = 0.02$) observed between tumor size and the expression of CD68 in the cells of the inflammatory infiltrate in gaps of tubular tumor structures. The CD68 expression in cells of the inflammatory tumor infiltrate was correlated with the presence of metastatic regional lymph nodes. It was found that in the case of the lymph node metastases the average score of CD68 expression in cells of ductal gaps tumor structures was lower (1.4 ± 0.5) in comparison with the negative lymph nodes case (3.1 ± 1.0; $F = 10.9$; $p = 0.007$). Same time no correlation between the CD68 expression in the inflammatory cell tumor infiltrate and the rate of tumor malignancy was found.

Using confocal microscopy domination of $CD68^+/RS1^+$ cells were found.

Conclusion: So, low CD68 expression level in ductal gaps tumor structures is associated with the presence of metastatic regional lymph nodes.

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A72

Particular qualities of the expression of markers of sensitivity to cytostatics at patients various solid tumours

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Background: To evaluate the expression of markers of sensitivity to chemotherapy in patients various solid tumors.

Materials and methods: The work includes paraffin blocks NSCLC patients ($n = 486$); CRC ($n = 262$); Breast cancer ($n = 55$); cervical cancer ($n = 19$); kidney ($n = 35$); squamous head and neck cancer ($n = 15$); stomach ($n = 51$); ovarian ($n = 25$); melanoma ($n = 58$); soft tissue sarcomas ($n = 52$). On our panel discussed the spectrum of expression of enzymes DPD, TP, TS, ERCC1, β-tubulin. Measurement of the expression of these genes produced by polymerase chain reaction in real time according to the method developed at the Institute of Oncology. NN Petrova.

Results: The combination of markers of sensitivity to fluoropyrimidine (low levels of DPD, TS, and low/high TP) was observed in patients with NSCLC in 30.9%, 41.9% in colorectal cancer, breast cancer in 31.1%, renal cell carcinona 41.1%, head and neck carcinoma in 14.3%, of gastric cancer in 42.5% melanoma in 39.5% of cases. Marker sensitivity to platinum drugs (low ERCC1) occurs in patients with NSCLC in 68.8%, 57.1% in colorectal cancer, breast cancer in 55.0%, renal cancer in 70.8% of squamous cell carcinoma of the head and neck 50, 0% of gastric cancer in 87.9% in melanoma 63.6% of cases. Marker, is an indirect measure of sensitivity to taxane drugs (low β-tubulin) in patients with NSCLC diagnosed in 72.7% of cases, in 75% of colorectal cancer, breast cancer in 66.7%, renal cancer in 92.3% of gastric cancer to 86.6%, melanoma in 73.5%.

Conclusion: The expression of markers in tumor tissue is heterogeneous. Significant heterogeneity of expression of predictive marker indicates on one hand the futility of the empirical approach to the choice of therapy, and on the other the need for their determination in all patients. Information about the molecular and genetic features of the tumor can afford to individualize the choice of drug. Objective data about the informativeness of molecular genetic markers can be obtained on the basis of randomized clinical trials.

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A66

Changes in experimental tumors and surrounding tissue under antitumor influence of magnetite nanoparticles introduced into the peritumoral area

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Background: In previous experiments the self-dependent antitumor effect of magnetite nanoparticles (NPs) injected in peritumoral area in form of magnetic fluid (MF) was shown. Elucidation of the mechanisms of this phenomenon is of theoretical and practical interest. The aim of the study was to investigate the cellular and ultrastructural changes in the tissue tumors regressed under the influence of magnetite NPs, as well as the composition of the cells of the immune system in the peritumoral area.

Materials and methods: The study was carried on white male rats, 180–200 g, with transplanted sarcoma 45 (59) or Pliss lymphosarcoma (50). Special antitumor agents were not used. Magnetite NPs (10 ± 2 nm) were applied in the form of the water-based MF. Original MF (20 kA/m) was diluted with saline in different degree and was injected into peritumoral zone along the tumor borders at a distance of 1.5 cm twice a week in a volume of 0.4–0.9 ml (depending on the animal weight) within 3 weeks. Special anti-tumor agents were not used. At the end of the experiments fragments of the tumor and surrounding tissue were taken for research. The study of changes in the tumor and peritumoral area were performed by the methods of cytology, histology, histochemistry, electron microscopy (microscope JEOL JEM-1011, Japan), flow cytometry, X-ray fluorescence spectroscopy (spectrometer M4 Tornado Bruker).

Results: At a dilution of the original MF in 30 times the treatment was effective in 75% of animals. Complete and partial (more than 2-fold) tumor regression was observed in 2/3 cases. In rats with Pliss lymphosarcoma tumor regression on 70–100% has been reported in 20–40% cases. The results of microscopic examination of sarcoma 45 with partial regression showed significant changes in their immune microenvironment compared with the cases of progressive tumor growth ($p < 0.05$–0.01). This was expressed in the increase in the number of lymphocytes and plasmacytes (respectively, in 12 and 2.5 fold), and in the appearance of macrophages and basophils that were missing in tumors with progressive growth. The results of flow cytometry of tissue from the tumor as well as from peritumoral zone indicate the predominance of plasmacytes in the case of inhibition of tumor growth and increasing the proportion of mature T lymphocytes in the cases of tumor regression (more than 1.5 times, $p < 0.05$). By