Background: According to the "immune-editing" theory, immunocompetent cells being important component of the tumor microenvironment can both show antitumor activity and contribute to the tumor progression. Tumor pathophysiological features affect the structural and functional changes of certain components of tumor microenvironment, in particular, increase the number of T-lymphocytes with regulatory activity (Treg). FOXP3, transcription factor, acts as master regulator for suppressive function of Treg. Recent studies have shown FOXP3 is expressed both in immunocompetent cells and tumor cells; however, the function of this gene in malignant tumors of different genesis is ambiguous (GirdhariLal, et al., 2014). The aim of the study was quantitative assessment of subpopulations CD4+, CD8+, FOXP3+-lymphocytes associated with the tumor, FOXP3+-tumor cells and comparison of these parameters with the clinical and morphological characteristics of endometrial cancer (EC).
Materials and methods: A total of 40 EC patients who did not receive special treatment before surgery with the mean age 56.9 ± 2.8 years were included in the study. Morphological and immunohistochemical methods were used in the study (primary monoclonal antibodies: CD4 – clone 4B12, "Millipore", USA, CD8 – clone RIV – 11, "Millipore", USA, FOXP3 – clone 5H5L12, "Invitrogen", USA, Ki-67 - clone MIB1, "DakoCytomation", Denmark) and Real-Time PCR was also used to determine the DNA methylation status of FOXP3 gene mathematical statistics.
Results: The dependence of the number of intratumoral CD4+-, CD8+- lymphocytes and FOXP3-lymphocytes on such biological characteristics as the degree of differentiation, growth rate and depth of invasion into the myometrium was established. In endometrial adenocarcinomas, low grade content of FOXP3+ lymphocytes increased (27.8 ± 2.6%), number of intratumoral CD4+ (15,3 ± 0,2%), CD8+-lymphocytes (29.6 ± 0.3%) and FOXP3+-tumor cells (15,5 ± 3,3%) decreased in contrast to the same parameters in high grade tumors: FOXP3+-lymphocytes (17.4 ± 3.0%), CD4+ (52.0 ± 2.7%), CD8+ (46.4 ± 5.6%), FOXP3+-tumor cells (27.8 ± 2.6%), $p < 0.05$. DNA analysis of endometrial tumor showed that FOXP3 gene promoter was methylated in 71% of cases. The number of cases with positive methylation status was increasing with lower differentiation grade, that was associated with the low number of FOXP3+-tumor cells. Statistically significant correlation ($p < 0.05$) (Spearman rank correlation) was observed between the deep invasion of tumor in myometrium and the number of FOXP3+-tumor cells ($R = -0.63$), number of FOXP3+- and CD4+-lymphocytes ($R = 0.68$ and $R = -0.55$, respectively) as well as the level of tumor proliferative activity ($R = 0.74$).
Conclusion: Quantitative changes of some components of the tumor microenvironment such as CD4+-, CD8+-, FOXP3+-lymphocytes and content of FOXP3+-tumor cells correlate with the biological characteristics of EC and apparently have a significant role in the progression of this cancer.

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Overall survival of head and neck cancer patients of Tomsk region

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Background: The aim of the study was to identify the factors that determine outcome and overall survival of HNC patients of Tomsk region.
Materials and methods: Clinical data, morphological characteristics of tumors and outcomes were obtained for 91 patients. Data about lifestyle, food preferences, smoking history were obtained from the questionnaire ($n = 35$). All clinical samples were tested by AmpliSens HPV diagnostic kits (Russia) to determine prevalence of 12 high risk HPV types. Statistical analysis was performed using Kaplan–Meier method, Cox regression, Gehan test, Fisher test, Mann–Whitney and Kruskal–Wallis tests.
Results: Gender, age, smoking status, alcohol consumption, distance from harmful factors, professional hazards and duration of its exposure as well as tumor criteria such as T, N, G, the presence of keratinization, invasion into the underlying tissues, HPV-infection, chemotherapy and/or radiation therapy and response to the treatment were assessed for HNC patients. It was shown that the two-year survival rate was about 70%, and the five-year survival rate was about 32%. There was strong correlation between decreased overall survival and increased alcohol consumption ($p = 0.03$) as well as regional lymph nodes status ($p = 0.01$). Patients with early tumor stages and N0 lymph node status as well as patients receiving chemotherapy and/or radiotherapy showed trend towards to better survival ($p = 0.09$, $p = 0.1$, $p = 0.09$, respectively). Overall survival of patients with lymph node metastasis was higher in case of early tumor stages ($p = 0.08$) and in patients who had no alcohol consumption history ($p = 0.06$). Cox regression analysis was used to obtain the model describing overall survival of patients. The model with the highest level of significance includes 3 factors-nodal metastases, the presence of keratinization and radiotherapy. It was shown that the risk of death was 4.2 and 2.6-fold higher in case of lymph node metastases and keratinized cancer, and 2.7-fold lower in case of radiotherapy. It was also shown that metastasis occurred more frequently in cases with invasion into the underlying tissue of a primary tumor ($p = 0.04$) and in cases with a low tissue grade ($p = 0.02$). Association of alcohol consumption with questionnaire data was studied. It was shown that men's preferably smokers consume alcohol more often than other patients ($p = 0.006$, $p = 0.02$, respectively). Our data showed that HPV prevalence was higher in smokers ($p = 0.04$), and in patients with early tumor stages ($p = 0.07$). Furthermore, response to radiotherapy was better in HPV-positive patients compared to HPV-negative cases ($p = 0.09$). Better response to radiotherapy showed the group of patients who received dose higher than 45 Gy ($p = 0.03$) and who had no lymph node metastases($p = 0.05$).

Conclusion: Our data showed that metastasis to lymph nodes and alcohol consumption are the main factors that affect mortality in HNC patients of Tomsk region.

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Immune system contributes to the efficacy of cancer chemotherapy

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Background: Risk of metastasis formation is provided by both tumor cell biological characteristics and the microenvironment features within the primary tumor along with local and systemic conditions for metastatic niche formation. The inflammatory infiltration has been shown to strongly impact on tumor progression (Whiteside, 2013). Dronca et al. (2011) showed that immunosuppressive factors in the tumor microenvironment may impair not only local immune responses but also disturb systemic immunity. Zitvogel et al. anticipate that the comprehension of the mechanisms governing the immunogenicity of cell death will have a profound impact on the design of anticancer therapies.To study the impact of immune system on clinical response to neoadjuvant chemotherapy and metastasis-free survival in breast cancer patients.
Materials and methods: 350 patients with newly diagnosed invasive breast cancer treated with neoadjuvant chemotherapy (NAC) were enrolled into the study. The procedures were made in accordance with the Helsinki Declaration. Clinical response to chemotherapy, the 5-year metastasis-free survival and all major clinical and morphological parameters were determined. The original method of multidimensional data visualization was applied to present the immune system state as integral entirety in visual image for classification of patients with different risk of metastasis (NovoSpark Corporation, Canada). Copy number aberrations (CNA) of cytokine gene regions in tumor specimens were tested using high-density microarray platform CytoScanTM HD Array (Affymetrix, USA). Cytokine gene polymorphism was analyzed. Subpopulations of lymphocytes and macrophages were determined within the primary tumors by IHC.
Results: We found, that favorable clinical immediate response to preoperative chemotherapy was related to the high levels of IL-1beta, TNF-alpha and IL-10 production by peripheral mononuclear cells before the treatment. This correlation was further confirmed by data from the study on association between cytokine gene functional polymorphism and response to NAC. We used NovoSpark Corporation visualization approach allowing the representation the immune system state as integral unit and to discriminate breast cancer patients with high and low risk of haematogeneic metastasis. When estimated before cancer treatment, 95% of breast cancer patients had risk of metastasis. The neoadjuvant chemotherapy and surgical tumor removal reduced the risk of tumor progression to 62–71%. However, in a year after adjuvant chemo- and radiotherapy, the patient group with high risk of metastases increased to 81% again. Thus, the cancer treatment can change the primarily estimated outcome prognosis in breast cancer patients, and the monitoring of immune system is a promising approach to predict the risk of cancer progression or resistance to the therapy. We have found the connection between the profile of intra-tumor inflammatory elements and chemotherapy efficacy.Cytokine gene expression may be influenced by the chromosome anomalies (CNA – Copy Number Aberration) – deletion and amplification – of cytokine gene loci in tumor cells. We found the close relation between the clinical response to NAC and gain of function of IL-10 and CHI3L1 (YKL40) genes. In contrast, loss of TNF-alpha and IL-17 gene function due to corresponding CNA was associated with good response to NAC. Metastasis-free survival of breast cancer patients was shown to be closely related to CNA.
Conclusion: The parameters of the activation of systemic and intra-tumoral immune system by growing tumor and its dissemination have to be validated in order to identify the new prognostic markers for the efficiency of the neoadjuvant chemotherapy.

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DNA inhibits dsRNA-activated NF-kB-based inflammation in tumour cells: The role of Ku protein

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The strong connection between cancer-related inflammation and tumour development with pattern – recognizing receptors (PRRs) activation results in identification of new target molecules that could lead to improved cancer diagnosis and treatment. TLR3, RIG1 and MDA5 synthetic ligand poly(I:C) was shown to trigger apoptosis in cancer cells. However, TLR3 signaling also includes NF-kB transcription factor which has emerged as endogenous tumour promoter via stimulation of pro-inflammatory tumour microenvironment, enhancement of angiogenesis, tumour cell proliferation and metastasis. Thus down regulation of NF-kB-mediated effects after TLR3 activation is needed for implementation of TLR3 ligand-based therapy into clinical trials.

We have earlier demonstrated that DNA and sequence specific ODNs inhibit poly(I:C)-induced production of pro-inflammatory cytokines in human primary fibroblasts and endothelial cells (Cherepanova et al., Immunobiology, 2013). Using these specific ODNs and affinity modification/isolation approach combined with subsequent MALDI-TOF the main cellular targets for these ODNs were identified as Ku protein – heterodimer of KU70 and KU80 (Cherepanova et al., Exp. Opin. Biol. Ther., 2012).

The goals of this study are to reveal whether the ODNs target poly(I:C)-induced activation in tumour cells: cervical carcinoma (Hela) and epidermoid carcinoma (A431) and to confirm Ku