problem.The increased expression of: (a) ER and PgR (70–75% of all cases of breast cancer) is an indication for hormone therapy, which is one of the simplest and most effective methods of systemic treatment of breast cancer; (b) receptor HER2/neu is a marker of highly aggressive form of breast cancer and indication for the use of targeted therapy Gertseptin; (c) proliferative factor Ki-67 reflects the ability of a tumor to metastasize.

Today, the "gold standard" of gene expression diagnostic of ER, PgR, HER2/neu and Ki-67 in breast cancer is immunohistochemistry (IHC) using foreign test systems (Ventana, Dako Inc, USA) . However, IHC diagnostics has some significant drawbacks. It leads up to 15–17% of cases of incorrect choice of drug therapy, which based on incorrect results of IHC studies. As a result, the significant group of patients do not receive effective treatment. Aim of the study Aim: the development a prototype of diagnostic test system for detection the receptor status of breast cancer, based on RT-PCR.

**Materials and methods:** Breast cancersamples consisted of 45 fresh-frozen tissue (FFT) samples of breast cancer (sites of malignant transformation and normal tissue from the same patients) and 59 FFPET sampleswere collected. All the samples had the IHC characteristic of receptor status of ER, PgR, HER2/neu and proliferation factor Ki-67 (Dako Inc., USA). Samples were submitted by SBIH NR "Novosibirsk Regional Oncology Center" (Novosibirsk, Russia). The experimental part of the study was divided into several stages: separation of mRNA from cells, obtaining cDNA (reverse transcription reaction), PCR in real-time and validation of the test system. Isolation of total RNA from FFT samples was performed using a set of "SV Total RNA Isolation system" according to the manufacturer's instructions (Promega, USA). Isolation of total RNA from FFPET samples was performed using a set "ReliaPrep FFPE Total RNA Miniprep System" (Promega, USA) according to the manufacturer's instructions. The concentration of total RNA was determined using a NanoDrop 1000 microspectrophotometer (Thermo Bioscience, USA) (RNA concentration were 15–660 ng/ml).

**Results:** For the reverse transcription reaction (RT) and PCR, the main parameters were selected. For RT: RNA incubation time and temperature of the reaction, enzyme concentration in the reaction mix. For PCR: the amount of DNA template, the number of primers, the concentration of magny ions, the concentration of the polymerase. Validation of the developed test system was carried out by comparing the results obtained by RT-PCR with the results of the IHC analysis of the same samples. Statistical processing of the research results was performed using the MedCalc program v.14.12.0 (Microsoft Corp.).

**Conclusion:** We have considered modern advanced methods of diagnostic of breast cancer receptor status. It was concluded that the use of RT-PCR in real time technology is the best alternative technology of the IHC, which currently is the standard definition of the expression of ER, PgR, HER2/neu and Ki-67. Prototype of test system for the detection of ER, PgR, HER2/neu and Ki67 proliferative factor in breast cancer samples was designed and optimized by RT-PCR.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.058

**T25**

### Selective glucocorticoid receptor agonists as novel anti-cancer agents

E. Lesovaya[a,*], L. Tilova[a], O. Zadorozhnaya[a], A. Savinkova[a], K. Kirsanov[a], A. Ogloblina[a], G. Belitsky[a], G. Baida[b], I. Budunova[b], M. Yakubovskaya[a]. [a] *Institute of Carcinogenesis, N.N. Blochin Cancer Research Center, Moscow, Russian Federation,* [b] *Northwestern University, Feinberg Medical School, Department of Dermatology, Chicago, USA* * Corresponding author.

Glucocorticoids (GCs) are widely used in treatment of many cancer types due to its ability to induce apoptosis in malignant cells in blood cancer therapy, and to prevent nausea, emesis and chemotherapy-associated hepatotoxicity in case of solid tumors. However, severe dose-limiting side effects occur, including osteoporosis, diabetes and other metabolic complications. Moreover, in therapy of solid tumors GCs strongly affect microenvironment which could be associated with poor prognosis, risk of metastasis and high frequency of relapses.

Biological response to GCs is mediated by glucocorticoid receptor (GR), a well-characterized transcription factor. GR controls gene expression via (1) transactivation, which requires binding of GR homodimers to glucocorticoid-responsive elements (GRE) in gene promoters and enhancers, and (2) dimerization-independent transrepression mediated via negative interaction between GR and other transcription factors including major effectors of inflammation and proliferation. Transrepression plays an important role in anti-inflammatory and anti-cancer effects of GR, including normalizing influence on microenvironment, while side effects are associated with GR transactivation. In particular, GCs induce insulin resistance in adipocytes, a major component of the mammary microenvironment, which secrete pro-inflammatory cytokines and growth factors, implicated in tumor progression. Selective GR agonists (SEGRA) that preferentially activate GR transrepression could be a better option for treatment of cancer.

Dozens of candidate SEGRAs were identified, synthesized and tested by industry and academia, with some having reached clinical trials. One of the novel GR modulators is 2-(4-acetoxyphenyl)-2-chloro-N-methylethylammonium-chloride, or CpdA, synthetic analogue of aziridine precursor isolated from Namibian shrub Salsola tuberculatiformis Botschantzev. It was shown that CpdA acts as "dissociated" GR ligand: it competes with GCs for GR binding and efficiently induces GR transrepression but not transactivation. We and other authors reported recently that CpdA inhibits survival of prostate cancer cells as well as blood cancer cells in GR-dependent fashion. Furthermore, primary leukemia cells from T-ALL patients appeared to be equally sensitive to GCs and CpdA.

Our further studies were concentrated on three directions:

(1) GC/SEGRA-based chemotherapy. We screened biological effect of CpdA in combination with traditional agents (doxorubicin, vincristine) and newer therapeutics (Bortezomib, Carfilzomib, MLN-4924, Rapamycin). Pretreatment of lymphoma cells with proteasome inhibitor Bortezomib resulted in GR accumulation and enhanced ligand properties of CpdA. We also

revealed remarkable GR-dependent cooperation between CpdA and Bortezomib in suppressing survival of lymphoma cells in vitro and in vivo. Also surprising findings were substantial cooperation in anti-cancer effect of immunosuppressant Rapamycin and CpdA in vitro, and unexpected "dissociated" effect of Rapamycin on GR signaling realized through down -regulation of REDD1, mTORC1 inhibitor. These data suggested high clinical potential of Rapamycin/GC combination in cancer treatment.

(2) SEGRA list extention

We used two approaches to extend SEGRA list: (1) synthesis of CpdA enantiomers and (2) its chemical derivatives. Chemical analogues of CpdA were designed by appending of bulky substituent into benzene ring, alkylation of carbon atom adjacent to chlorine atom or appending of substituents to nitrogen atom. Evaluation of biological properties of enantiomers revealed higher GR-dependent anti-cancer potential of S-CpdA. Cytotoxic and proapoptotic effects of CpdA analogues were comparable with precursor.

(3) Selection of tumor types acceptable for SEGRA treatment.

CpdA was selected for NCI-60 in Vitro Cell Line Screening Project providing direct support to anticancer drug discovery program. It was shown that CpdA affect viability of some adherent cancer cell lines. We demonstrated that CpdA unlike GCs did not modify microenvironment and disintegrate tight junctions between cells decreasing risk of metastasis in case of solid tumors. It demonstrates reasonability of further investigations.

Overall, our data provide the rationale for novel therapy of cancer based on combination of non-steroidal GR modulators with classic and modern chemotherapeutics. Approaches to obtain more SEGRAs were elaborated.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.059

**P77**

### Clonal evolution of breast tumor during neoadjuvant chemotherapy and metastasis

N. Litviakov[a,b,c,*], M. Ibragimova[a], M. Tsyganov[a,b,c], P. Kazantseva[d], E. Slonimskaya[d], N. Cherdyntseva[b,c]. [a] *Laboratory of Oncovirology, Tomsk Cancer Research Institute, Tomsk, Russian Federation,* [b] *Laboratory of Translational Cell and Molecular Biomedicine, National Research Tomsk State University, Tomsk, Russian Federation,* [c] *Laboratory of Molecular Oncology and Immunology, Tomsk Cancer Research Institute, Tomsk, Russian Federation,* [d] *Department of General Oncology, Tomsk Cancer Research Institute, Tomsk, Russian Federation* * Corresponding author.

**Background:** There are numerous evidences suggesting that tumor evolution follows the laws of Darwinian evolution, whereby individual tumor cell clones have private genetic aberrations, including chromosomal abnormalities. The combined effect of genetic instability and differential selective pressures of the microenvironment and chemotherapy can result in the creation of new tumor clones (Navin et al., 2011; Ng et al., 2012). The aim of this study is to show breast tumor clonal evolution during neoadjuvant chemotherapy (NAC) using microarray analysis.

**Material and methods:** Breast cancer patients ($n = 26$) with stage IIA to IIIC (T1-4N0-3M0), were treated with NAC (FAC or CAX regimens). DNA was extracted from 26 samples of tumor tissue derived before or after NAC using QIAamp DNA mini Kit (Qiagen, Germany). Copy Number Aberrations (CNA, deletions and amplifications, or Loss and Gain, respectively) and number of mutant clones were detected in pre- and post-NAC tumor samples using the high density microarray platform Affymetrix (USA) CytoScanTM HD Array. This study was approved by Tomsk Cancer Research Institute review board.

**Results:** We have revealed that 19% (5/26) of patients during the NAC showed the decrease in the number of mutant clones and CNA frequency right up to their complete elimination (genetic regression) at one case. In 7 (27%) cases chemotherapy had no any effect on number of mutant clones and the frequency of CNA in tumor. In the tumors of 10 patients the elimination of some mutant clones as well as the formation of new clones with deleted genetic material occurred under the influence of chemotherapy. 6 patients have demonstrated the appearance of new tumor clones with gene amplifications which were associated with the development of metastases in 83% of cases (5/6). All other patients ($n = 21$) who has not acquired the new tumor clones with Gain function mutation after NAC did not manifest distant metastasis in 5-year follow-up (Kaplan–Meier, $p = 0.00001$ Log-rank test).

**Conclusion:** The first time evidence is presented that the formation of new tumor clones may occur during the NAC. Metastasis of breast cancer is associated with the appearance of new clones with DNA amplifications. Detection of these clones allow getting new prognostic factor in breast cancer.

The study was supported by the Russian Foundation for Basic Research – Russia (Project 15-04-03091).

http://dx.doi.org/10.1016/j.ejcsup.2015.08.060

**A87**

### Proliferative activity, lymphatic and blood vessel density in different clinical stages of melanoma

A. Lomakin[a,*], S. Fursov[a], N. Bgatova[b], I. Kachesov[a], S. Chepko[a], N. Isakova[a], Yu. Borodin[b], V. Voytsitsky[a], V. Konenkov[b]. [a] *Novosibirsk Regional Oncology Center, Novosibirsk, Russian Federation,* [b] *Federal State Budgetary Scientific Institution "Scientific Institute of clinical and experimental lymphology", Novosibirsk, Russian Federation* * Corresponding author.

Cutaneous melanoma is one of the most aggressive human neoplasms that can quickly metastasize to regional lymph nodes. Currently, prognosis is determined by measuring tumor thickness but more reliable markers for metastatic spread are urgently needed. It is well known that tumors require a microvasculature development in order to grow and metastasize. Angiogenesis and lymphangiogenesis play an important role not only in the tumor growth, but also in the tumor metastasis. As malignancy is a disorder of cellular growth control, the assessment of cell proliferation rates in tumors has intuitive appeal as a prognostic marker. One such biomarker is Ki-67, a cell cycle dependent protein. Recent reports related to its role as a prognostic factor