

of common cold, headache and asthma. However, its role in cancer growth has not been elucidated yet. The present study is aimed at investigating the cytotoxicity of the aqueous extract of the seeds of *Datura innoxia* against cervical cancer HeLa cells.

**Materials and Methods:** The effect of aqueous extract of the seeds of *Datura innoxia* on cell death and intracellular targets that affect angiogenesis (VEGF), inflammation (IL-12, TNF- $\alpha$ ), apoptosis (caspase-3, -8 & -9) and antioxidant (superoxide dismutase & catalase) were determined by MTT assay, ELISA and enzymatic activity assay. In addition, Anti-VEGF neutralization effect was evaluated alone and in combination with aqueous extract, to assess whether it could result in augmented anticancer efficacy than the single agent.

**Results:** Aqueous extract of the seeds of *Datura innoxia* inhibited growth of cancer cells in a dose and time-dependent manner. Experiments aiming to investigate the anti-angiogenic activity against HeLa, revealed that following the treatment, a dose-dependent decrease ( $p < 0.001$ ) in the levels of VEGF secreted by the cells was recorded. In another set of experiments Aqueous extract potentiated ( $p < 0.001$ ) the cell death induced by anti-VEGF antibody. VEGF and its receptors are established as major mediators of tumor cell growth and invasiveness; taken together, the results of these experiments suggest that *datura* possesses antiangiogenic activity. Although it appeared to decrease the levels of tumorigenesis factor, TNF- $\alpha$  ( $p < 0.05$ ), it did not alter IL-12 level significantly. The pro-apoptotic effects were confirmed by significant ( $p < 0.001$ ) increase in caspases-3 and -9 but not 8 activity. Significant increase in antioxidant enzymes (SOD, catalase) activity was also recorded.

**Conclusion:** Aqueous extract of the seeds of *Datura innoxia* acts via multiple albeit specific molecular targets to elicit anti-carcinogenic activity thus might be a candidate for developing multifunctional anti-cancer agent through its inhibitory activity on several aspects of tumor growth and angiogenesis.

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##### Correlation between cancer stem cell like cells markers and clinical assessment in breast cancer patients

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**Background:** Recent literature and experimental data have pointed out the predictive value of circulating tumor cells (CTCs), while other studies have demonstrated that cancer stem cell-like cells (CSCs) are included in the vast majority of CTCs. The aim of the present study is to find out the correlation between clinical assessment and CSCs' markers (Nanog, Oct3/4, SOX2, Nestin, CD34).

**Materials and Methods:** For the realization of this study have been identified and isolated CTCs from patients with breast cancer in different stages according TNM classification system. It has been followed quantification of cancer stem cell-like cells in CTCs cultures and molecular analysis of the above cells with RT-qPCR, by using gene-specific primers for each marker and for the housekeeping gene (18s rRNA) that has been used. The comparative Ct method ( $2^{-\Delta\Delta Ct}$ ) has been used for the calculation of relative quantitation. Finally, has been requested the clinical assessment from physicians of each patient so far.

**Results:** The expression of the transcription factors was dependent on the clinical status of patients. It has been observed that SOX2 is overexpressed in cases of complete response, while Nestin is expressed much more than the other factors in cases of stable disease. In stable disease it has been also observed the decrease of CD34 transcription factor. High levels of Nestin combined with overexpression of Oct3/4, SOX2 have been noted when patients had progress of disease. The expression of Nanog transcription factor also seems to vary, depending of the clinical status.

**Conclusion:** This study is an attempt of correlation of clinical evaluation with the transcription factors responsible for cancer stem cells in patients with breast cancer. The results show that the expression pattern of transcription factors is proportional to the clinical assessment of patients. However, worth noting is that the CSCs which were studied, were isolated before starting treatment. Therefore, might be able to avoid cases of relapse which are not seemed *prima facie*. However, it is necessary to perform numerous studies to a greater range and number of samples in order to be applied at clinical level.

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##### Chromosomal aberrations in group of PTCL, NOS – own experience

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**Background:** Cytogenetic investigations play important role in the diagnosis of lymphomas, however T-cell neoplasms remain poorly characterized by genetic alterations. The low availability of diagnostic material for testing

and its intractability in obtaining cell divisions during in vitro culture is one of the major research problem.

**Materials and Methods:** We investigated group of 32 cases of PTCL, NOS (peripheral T-cell lymphoma, not otherwise specified), diagnosed in M. Skłodowska-Curie Memorial Cancer Centre and Institute in 2007–2010. Lymphoma cells were obtained mostly by fine needle aspiration biopsy (FNAB) of lymph nodes or extranodal tumours, peritoneal fluid, and from bone marrow and peripheral blood. In order to identify cytogenetic aberrations G-banding and FISH analyses were performed (commercial unique probes for TRA@/TRB@, TRG@, TRB@ and CDKN2A/CEP9 genes).

**Results:** In analysed group we detected aberrant karyotypes in 6 and normal karyotypes in 7 cases. No metaphases were obtained in 8 cultures, while other materials (11 cases) was not diagnostic. Among the aberrant karyotypes we found the presence of 3 cases with hiperdiploid karyotype, 2 cases with pseudodiploid and 1 with hypodiploid set of chromosomes. Structural changes affecting a number of chromosomes were detected, but the only recurrent aberration was: der(11)del(11)(p13)del(11)(q21). We also revealed two repeatable breakpoints: 6p21 and 10p13. T-cell receptor rearrangements were sporadic in FISH analysis. We detected only one case with rearrangement of TRA@/TRD@ (5.2%), while the changes in copy number of TRG@ and TRB@ were found thrice (20%). In one case we identified three copies of TRG@ and TRB@, probably because of trisomy 7, while in second – four copies of TRG@ was visible. Third patient had loss of one copy of TRG@. Monoallelic deletion of CDKN2A was detected in two cases (12.5%).

**Conclusion:** A random chromosomal aberrations were seen in few cases, but only a few were recurrent: der(11)del(11)(p13)del(11)(q21) and breakpoints: 6p21 and 10p13. Both rearrangements of T-cell receptor genes and CDKN2A are sporadic in PTCL, NOS.

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##### Integration of clinical, histopathological, radiological and biomolecular data for prediction of oral squamous cell cancer (OSCC) recurrence: the NeoMark project's interim results

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**Background:** Neoplastic diseases are at large the second cause of death in the western world. In the last decade, the continuous improvement in treatment protocols has substantially increased the number of patients who achieve a complete disappearance of the disease (remission) after treatment. NeoMark Project – ICT Enabled Prediction of Cancer Reoccurrence performs research in the integration of heterogeneous clinical, laboratory, biomolecular and imaging data to develop a data integration environment facilitating multiscale and multilevel modeling, aimed at advancing models and methods currently in use to predict neoplastic reoccurrences, and to apply it to the study of oral cancer.

**Materials and Methods:** The NeoMark concept will be tested and validated through a clinical observational study in a relevant number (100–150 patients) of OSCC cases treated with conventional surgery  $\pm$  radiation and followed up for at least 12 months. Clinical and imaging data, as well as blood cell genomics will be collected at the time of diagnosis, after treatment, and during follow-up. Cancer specimen will be collected intra-surgery for histological and gene expression analysis. All patients showing a complete remission of the disease from a clinical and laboratory point of view after first line treatment will be selected for the follow-up phase. Primary end-point of the study will be the clinical evidence of recurrence during follow-up.

**Results:** The presented interim results of the Neomark Project obtained from the Baseline Data Analysis with the current state of the dataset are quite encouraging; however, as more and more patients' records become available, the data will be analyzed again in order to infer more reliable results.

**Conclusion:** The main contributions that NeoMark expects to bring to research is a better understanding of the correlations between biological factors (personal for each patient and specific for OSCC) that are characteristic for oral cancer and fostering reoccurrences. The emphasis given by NeoMark partners to the evaluation and processing of integrated data from each patient and from many patients with similar tumours is expected to lead us to the identification of some prominent markers which will be experimentally analysed on a prototype medical diagnostic device based on RNA micro-array techniques and on chip-based RT-PCR technologies.