Ki 67 or Bax, Bcl-2 and the morphological growth pattern or patient clinical profile.
Conclusion: The histogenesis and morphogenesis of these neoplasms demonstrated wide spectrum of histologic features depends on their biologic behavior. Salivary gland neoplasms are clinically diverse group of neoplasms and the frequency of these neoplasm outcomes is probably not recognized when analyzing short term survival results in patients to whom the disease has a long natural history.

102 Large melanoma of skin: level of invasion or tumour volume what is superior?

I. Galaychuk[1]. [1]*Ternopil State Medical University, Department of Oncology, Ternopil, Ukraine*

Background: The main prognostic factors for malignant melanoma are Breslow and Clark criteria, ulceration, growth patterns and localization of tumour [Balch, 2001; Cascinelli et al., 2009]. But, on the other hand, the exophytic nodular part of large melanoma often is ignored. Purpose: to access prognostic significance of nodular melanoma tumour burden in comparison with its Clark level of invasion.
Material and Methods: 107 patients of 28−70 years of age (M – 35, F – 72) with melanoma T4N0M0 were enrolled in the retrospective study. The tumour volume of exophytic nodular part of melanoma was calculated in vivo by formulas for spherical and hemispherical patterns after measurement of tumour's thickness and base. All patients underwent surgery – wide local excision of melanoma. Survival analysis estimated by using the Kaplan-Meier method and the log-rank test. Spearman and Kendall's correlation coefficients (τ) were used for comparison of prognostic signification between Clark's levels and tumour mass.
Results: The 5-year overall survival rates in 15 patients with nodular melanoma 5−10 mm of thickness (or tumour volume about 0.5 cm^3) were 86.6%; in 40 patients with nodular melanoma 11−20 mm (tumour volume 0.5−4.0 cm^3) − 52.5%; in 33 patients with nodular melanoma 21−30 mm (tumour volume 4.1–14.0 cm^3) − 36.4%; and nobody of 19 patients with tumour more than 31 mm thickness (>14.0 cm^3) lived out 5 years. Equation, which shows dependence of survival rate of patients on tumour volume, was made on the base of regressive analysis of received data. It estimates that in case of large melanoma tumour volume is significantly superior ($\tau = 0.67$) in prognosis of patients' overall survival than Clark's levels of invasion ($\tau = 0.10$).
Conclusion. The assessment of tumour volume in the nodular exophytic part of melanoma could be the additional diagnostic and prognostic criterion, which will determine the strategies of patient's treatment.

103 KRAS mutational spectra in routine diagnostic analysis of metastatic colorectal cancer

K. Jakovljevic[1], E. Malisic[1], J. Dobricic[1], M. Spasic[1], R. Jankovic[1]. [1]*Institute for Oncology and Radiology of Serbia, Experimental Department, Belgrade, Serbia*

Background: *KRAS* tumour mutational status is now widely recognized as a predictive biomarker in patients with colorectal cancer (CRC). Large clinical trials have shown that only CRCs with wild-type *KRAS* respond to epidermal growth factor receptor (EGFR) targeted antibody treatment. Seven different DNA base pair substitutions within codons 12 and 13, each leading to an amino acid substitution in the protein, are the most frequently observed genetic events within *KRAS* gene in CRC. Therefore, analysis of these hotspot clustered mutations is compulsory before treatment and for large routine diagnostic tests reliable frequency and types of *KRAS* mutations have yet to be established.
Material and Methods: Formalin-fixed paraffin-embedded tissue blocks were collected and DNA was extracted from tissue sections from 259 cases of metastatic CRC. Mutation analysis of *KRAS* codons 12 and 13 was performed by allele-specific real-time polymerase chain reaction.
Results: *KRAS* point mutations in codons 12 and 13 were present in 96 cases (37.07%) of 259 analyzed CRCs. All seven tested mutation types were observed; among them the frequencies of mutations in codon 12 were GAT (p.G12D, 11.58%), GTT (p.G12V, 10.42%), GCT (p.G12A, 4.25%), TGT (p.G12C, 3.47%), CGT (p.G12R, 1.16%), AGT (p.G12S, 1.16%) and the only one in codon 13 was GAC (p.G13D, 5.02%). Furthermore, the rate of transversions (52.08%) was found to be higher than the transitions' rate (47.92%). All the transitions were of the G>A type (the most prevalent alteration in our study) affecting the first base of codon 12 or predominantly the second base of both codons. Two types of transversions (G>T, G>C) occurred only in codon 12, mainly at the second base.
Conclusions: According to the previous studies G12V and G12R mutations may predispose to more aggressive biological behavior in advanced CRC. Though, G12D and G12S mutants have less aggressive transforming phenotypes. In addition, G>A nucleotide substitutions score the second place among the point mutations in human cancers in the Human Gene Mutational Database for all human somatic missense mutations and in our study they represent the most common alterations. Since the performed analysis detected all tested *KRAS* mutation types, which may have different impacts on the outcome of CRC patients, we want to emphasize as a conclusion the need for defining individual mutational spectra for developing therapeutic strategies targeting *KRAS* mutations in clinical practice.

104 HPV infection in epithelial ovarian cells of women at high risk of developing ovarian cancer

O. Bilyk[1], T. Pejovich[2], N. Pande[2], L. Buchynska[1]. [1]*IEPOR of NAS of Ukraine, Oncogenetic, Kyiv, Ukraine,* [2]*Oregon Health and Science University, Oncogynecologic, Portland, USA*

Background: The causal role of high-risk (HR) HPV has been well established for cervical and other anogenital cancers but our understanding of the role of HPV in ovarian cancer etiology is controversial. Attempts to identify molecular risk factors for ovarian cancer in women at high risk of developing ovarian cancer which include family history of ovarian or/and breast cancer as well as personal history of breast cancer lead to the discovery that their ovarian epithelial cells are characterized by cross-linking-agent-induced chromosomal instability analogous to Fanconi Anemia phenotype and therefore can be susceptible to environmental risk factors including HPV infection.
The objective of the study is to investigate the frequency of HR HPV in women at high risk of developing ovarian cancer.
Materials and Methods: 20 ovarian samples were obtained from women undergoing risk reducing oophorectomy because of significantly elevated risk of developing ovarian cancer. The patients considered to be at high risk for ovarian cancer are defined as women with two or more first degree relatives with ovarian and/or breast cancer, or a personal history of breast cancer and a first-degree relative with breast and/or ovarian cancer. HPV testing was performed using polymerase chain reaction (PCR) with type-specific primers for E6 fragment of high-risk HPV type 16, 18 and 33. Real-time quantitative PCR was performed with ABI 7500 Fast Real-time PCR system and SYBR-Green master mix to calculate HPV copy number. Immunohistochemistry by using polyclonal anti-HPV 16 E6/18 E6 antibody (clone SC-460, Santa Cruz Biothechnology) to detect the E6 expression in the ovarian sections was performed..
Results: In the tissues derived from women at high risk of developing ovarian cancer, 30% of the samples were positive for HPV-16, 35% positive for HPV-18 and 10% positive for HPV-33. About 20% of the studied samples had a mixed infection. HPV copy number varied from 0.04 to 851 copies/reaction in HPV-16 positive samples and from 0.27 to 2793.51 copies/reaction in HPV-18 positive samples. By immunohistochemistry we determined that E6 HPV16/18 protein is expressed in follicular and surface ovarian epithelium. It is necessary to note that some vessel endothelium of the ovaries was focal positive for E6 protein.
Conclusion: Our study suggests that HR-HPVs ascending from the cervix to the ovaries might have a role in susceptibility of ovarian surface epithelium to neoplastic changes in women at high risk of developing ovarian cancer. Our broader goal is to define tools and methods to better understand the environmental risk factors that account for ovarian cancer risk and to translate these mechanisms into strategies for early ovarian cancer prevention.

105 Role of IL-1RN VNTR polymorphism in host immune susceptibility to viral associated neoplasias

H. Sousa[1], A.M. Santos[1], E. Breda[2], R. Catarino[1], D. Pinto[1], J. Moutinho[3], R. Medeiros[1]. [1]*Instituto Português de Oncologia do Porto FG EPE, Grupo Oncologia Molecular, CI Lab 4º Piso, Porto, Portugal,* [2]*Instituto Português de Oncologia do Porto FG EPE, Serviço de Otorrinolaringologia, Porto, Portugal,* [3]*Instituto Português de Oncologia do Porto FG EPE, Serviço de Ginecologia, Porto, Portugal*

Background: A common 86bp VNTR polymorphism within intron 2 of the IL-1 receptor antagonist (IL-1RN) gene seems to be associated with the balance between host immunity and viral infections. Several authors have referred that the Il-1RN A2 allele is responsible for shorter immune responses to viral infections and therefore might be associated with increased risk of viral associated cancers development.
Methods: We have developed a cross-sectional study to analyse the role of this 86bp VNTR polymorphism on the development of viral associated neoplasias: cervical and nasopharyngeal carcinoma. Genotyping of Il-1RN VNTR polymorphism was performed by PCR in DNA extracted from peripheral blood samples from both healthy individuals (n = 446), individuals with cervical lesions (n = 346) and nasopharyngeal carcinoma (n = 122) from the Northern Region of Portugal.
Results: Our study revealed that the IL-1RN A2 allele was significantly increased in both patients with cervical and nasopharyngeal carcinoma when compared with healthy individuals. Statistical analysis revealed that it is associated with increased risk for both invasive cervical cancer ($p = 0.027$; OR = 1.84) and undifferentiated type of nasopharyngeal carcinoma ($p < 0.001$; OR = 3.73).

Conclusions: This study reveals that the IL-1RN A2 allele seems to be involved in genetic susceptibility for the development of viral associated neoplasias. We assume that the mechanism trough which it increases the risk is by increasing the predisposition to shorter immune responses that predispose the host to develop easily viral infections. Therefore, oncogenic viruses can infect cells efficiently and promote cancer development.

106 Lung cancer risk and air pollution in an industrial region of Northern Spain: a hospital-based case-control study

T.G. Adonina[1], M.F. Lopez-Cima[1], J. García-Perez[2], B. Perez-Gómez[2], N. Aragones[2], G. Lopez-Abente[2], M. Pollán[2]. *[1]Universidad de Oviedo, Instituto Universitario de Oncologia, Oviedo-Asturias, Spain, [2]Instituto de Salud Carlos III, Centro Nacional de Epidemiología, Madrid, Spain*

Background: Asturias, an Autonomous Region in Northern Spain with a large industrial area, registers high lung cancer incidence and mortality. While this excess risk of lung cancer might be partially attributable to smoking habit and occupational exposure, the role of industrial and urban pollution also needs to be assessed. The objective of this abstract was to ascertain the possible effect of air pollution, both urban and industrial, on lung cancer risk in Asturias.
Material and Methods: This study will be undertaken within the wider context of the Asturian Lung Cancer (*Cáncer de Pulmón en Asturias – CAPUA*) study, a hospital-based case-control study conducted in Asturias with the aim of ascertaining the influence of environmental and genetic factors on the development of lung cancer. This analysis included 626 lung cancer patients and 626 controls matched individually by ethnicity, hospital, age, and sex. Distances from the respective participants' residential locations to industrial facilities and city centers were computed. Using logistic regression, odds ratios (ORs) and 95% confidence intervals (95% CIs) for categories of distance to urban and industrial pollution sources were calculated, with adjustment for sex, age, hospital area, tobacco consumption, family history of cancer, and occupation.
Results: Whereas individuals living near industries displayed an excess risk of lung cancer (OR = 1.49; 95% CI = 0.93–2.39), which attained statistical significance for small cell carcinomas (OR = 2.23; 95% CI = 1.01–4.92), residents in urban areas showed a statistically significant increased risk for adenocarcinomas (OR = 1.92; 95% CI = 1.09–3.38). In the Gijon health area, residents in the urban area registered a statistically significant increased risk of lung cancer (OR = 2.17; 95% CI = 1.25–3.76), whereas in the Aviles health area, no differences in risk were found by area of exposure.
Conclusions: This study provides further evidence that air pollution, both urban and industrial, is a moderate risk factor for lung cancer, which varies according to histologic type and health area.

107 LNA™ based universal RT microRNA PCR system – a new generation high throughput QPCR platform optimized for development microRNA based molecular diagnostic assays on clinical FFPE and blood serum and plasma

B. Nielsen[1], S.J. Nielsen[1], I.K. Dahlsveen[1], A. Baker[1]. *[1]Exiqon, Diagnostics, Vedbæk, Denmark*

Background: Using a Locked Nucleic Acid (LNA™) based miRNA detection technology we have developed a high throughput QPCR system for genome wide detection of miRNAs in clinical paraffin-embedded tissue as well as blood derived plasma or serum. The use of the LNA™ bases adds critical specificity and sensitivity creating a more robust system for more rapid assay development in the clinical and diagnostic assay development.
Material and Methods: Blood derived serum or plasma are important bio-fluids that potentially hold critical biomarker information about disease diagnosis and prognosis. We have developed the advantages of our PCR system to provide a truly sensitive miRNA genome wide screening technology from extremely small volumes of blood derived serum or plasma. In addition the system is ideally suited for screening laser captured and macro-dissected tissue specimens allowing us to build extremely accurate and sensitive miRNA expression profiles from critical tumour biopsies.
Results and Conclusion: We have used the PCR system to screen miRNAs in colorectal cancer patient plasma samples and their matching tumour samples. We have been able to identify miRNAs in both the blood derived plasma and tumours that are differentially expressed between patients and healthy controls.

108 Association between FAS-670A/G polymorphism and ovarian cancer development

L. Lima[1], D. Pereira[2], A.M. Gomes[1], D. Pinto[1], R. Medeiros[1]. *[1]Portuguese Institute of Oncology, Molecular Oncology Group, Porto, Portugal, [2]Portuguese Institute of Oncology, Oncology Department, Porto, Portugal*

Background: Apoptosis is an essential process in malignant cells elimination. One of the characteristics of malignant cells and of tumour development is tumoural cell evasion to apoptotic stimuli and alterations of the apoptotic pathways components.
FAS-670A/G polymorphism in the promoter region of *FAS* gene has been identified, it was proposed that *FAS-670* G allele may reduce Fas expression and might influence apoptosis activation. The aim of this study was evaluate if *FAS-670A/G* have a possible role in ovarian cancer development.
Methods: We performed Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) methodology, for *FAS* gene locus −670 genotyping. It was evaluated DNA samples from 428 women: 189 ovarian cancer patients and 239 healthy control female individuals.
Results: We found that the presence of GG genotype of *FAS-670* A/G represents a significant risk for development of grade III tumours (OR = 3.53; 95% confidence interval (CI): 1.30–9.58). Moreover, we found that individuals carrying *FAS-670* G allele had a higher risk of recurrence after first line chemotherapy with complete response (OR = 5.25; 95% CI: 1.51–18.2). Cumulatively, Kaplan–Meier function plots and probabilities analysis showed that *FAS-670* G allele carriers have a shorter recurrence free survival after first line chemotherapy with complete response (p = 0.017).
Conclusions: Our results indicate that *FAS-670A/G* may have an important role in ovarian cancer development; the study of this polymorphism could help selecting groups at progression risk.

109 Implementation of qPCR and sequencing for KRAS and EGFR mutation detection in Bulgarian patients with colorectal and lung cancer

S. Bichev[1], S. Hristova[2], Y. Slavova[3], R. Vajarova[1], D. Kachakova[4], R. Kaneva[4], I. Kremensky[1]. *[1]Medical University – Sofia, National Genetic Laboratory, Sofia, Bulgaria, [2]Medical University – Sofia, Pathoanatomy, Sofia, Bulgaria, [3]University Hospital of Lung Diseases, Pathomorphology, Sofia, Bulgaria, [4]Medical University – Sofia, Molecular Medicine Center, Sofia, Bulgaria*

Background: Colorectal and lung cancer are among the most common human malignancies both in the United States and Europe. New targeted therapies have been developed in the past decade, such as monoclonal antibodies against epidermal growth factor receptor (EGFR) or KRAS oncogene. Genetic alterations of the intracellular effectors involved in EGFR related signaling pathways may have an effect on response to this targeted therapy Recent data now suggests a differential response to anti-EGFR antibody therapy based on mutational status of a major oncogene called KRAS and 18−21st EGFR's exons for patients with CRS and non-small cell lung cancer (NSCLC) respectively. The aim of this study was to introduce reliable methods for identifying of KRAS/EGFR mutational status in patients with CRC/NSCLC.
Methods and Results: In both groups DNA was extracted from paraffin embedded tissues. Twenty colorectal cancer patients were screened for KRAS mutations by qPCR based on Scorpions technology. Our results showed that five of those patients had mutations in the 12th codon of KRAS oncogene Two patients carried mutation 12 Asp, mutations 12 Ala, 12 Val and 12 Ser were found in the other three patients respectively. No mutations in the 13th codon of KRAS oncogene were found. Eight NSCLC patients were screened for EGFR mutations by qPCR based on high resolution melting technology and subsequent sequencing of aberrant profiles. Among these patients we found only 2 with mutations – one with a deletion (2236−2253del18) and the other a SNP(G719C) in 19th exon of EGFR.
Conclusion: Our study showed that HRM is reliable method for screening of NSCLC patients, however aberrant profiles should be sequenced in order to establish the exact mutation. Scorpion technology used for detection of KRAS status proved to be successful in all 20 patients.

110 Gastric adenocarcinoma development in patients with atrophy or/and intestinal metaplasia: the role of COX-2 polymorphisms in a Northern Portuguese population

A.C. Pereira[1], H. Sousa[1], A.L. Pinto-Correia[1], A.L. Teixeira[1], M. Fragoso[2], L. Moreira-Dias[3], R. Medeiros[1], M. Dinis-Ribeiro[3]. *[1]Portuguese Institute of Oncology, Molecular Oncology GRP, Porto, Portugal, [2]Portuguese Institute of Oncology, Oncology Department, Porto, Portugal, [3]Portuguese Institute of Oncology, Gastroenterology Department, Porto, Portugal*

Background: COX-2 overexpression observed in 69% of gastric cancers (GC) and precancerous tissues is closely intertwined with key mechanisms of gastric carcinogenesis, namely inhibition of apoptosis, tumour growth, angiogenesis, invasion and metastasis. Genetic variations that modify6 the levels of COX-2 protein would be anticipated to have a substantial influence on disease phenotype. Hence, with this study we aimed at understanding the contribution of two functionally expected *COX-2* polymorphisms (−1195A>G and 8473T>C) in the development and progression of gastric lesions.
Material and Methods: A hospital-based case-control study was developed that gathered 134 patients diagnosed with gastric lesions (94 with GC and 40 with atrophy and/or intestinal metaplasia (AIM)) and 255 healthy individuals all from the Northern region of Portugal and recruited at Portuguese Institute