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Frequency of EGFR Mutations in Greek Non-Small-Cell Lung Cancer (NSCLC) Patients

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Background: First line treatment of NSCLC patients harboring activating somatic mutations within the tyrosine kinase (TK) domain of the epidermal growth factor receptor (*EGFR*) with Iressa (Gefitinib) has recently received licence. *EGFR* targeted therapy with gefitinib leads to improved response and survival outcomes in patients carrying such mutations; therefore screening for *EGFR* mutations has entered routine clinical practice. Several clinico-pathological factors correlate with these mutations including gender, smoking history, and histology. The frequency of *EGFR* mutations is also ethnicity-dependent, wherein the incidence in Asian populations is ~30%, while in Caucasians (Whites) it is lower, ~15%. However, limited data is available on intra-ethnic differences throughout Europe.
Aim: The aim of this study was to determine the frequency and spectrum of *EGFR* mutations in an unselected group of Greek NSCLC patients and investigate technical aspects of analysis.
Methods: We set up High Resolution Melting Analysis (HRMA) assays to identify mutations in exons 18−21 of the *EGFR* gene and validated their analytical sensitivity by making serial dilutions of samples with known mutations and tumour cell content (TCC). A total of 342 NSCLC patients were screened with HRMA for somatic *EGFR* mutations in exons 18−21 and mutation status was verified by bi-directional sequencing. ME-PCR (mutant enriched-PCR) was used in conjunction with standard bi-directional sequencing in a further 300 patients. Pathological review was obtained for all samples and macro-dissection was used to ensure a TCC of >75% in all possible cases.
Results: The sensitivity of our HRM assays was found to be $\leqslant$1.5% Using HRMA and bi-directional sequencing a frequency of 18.4% was obtained; 47 x exon 19, 12 x exon 21 and 4 x exon 20. Using ME-PCR the mutational frequency was 16.3%; 21 x exon 19, 22 x exon 21, 4 x exon 20 and 2 $\times$ exon 18.
Conclusions: Applying a very sensitive mutation detection technique in a large cohort of unselected Greek NSCLC patients in routine diagnostic practice, we obtained an overall mutation frequency of **18.4%**. This mutation frequency is similar to that found by the SLADB and EURTAC studies in European populations. Differences in sensitivity between techniques suggest that more than one technique should be advised in routine diagnostic practice.

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Association Between +61 A/G Polymorphism in the EGF Gene and Non-Small Cell Lung Cancer Risk in Male Caucasians

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Background: Epidermal growth factor (EGF) and its receptor may play critical role in non-small cell lung cancer (NSCLC) carcinogenesis steps. Our group previously demonstrated the impact of the EGF+61 A/G polymorphism in EGF expression levels and its association with increased susceptibility to glioma. This study was conducted to evaluate the +61A/G polymorphism in the EGF gene as risk factor in Portuguese NSCLC patients.
Design and settings: Case-control study. Exposure was defined as EGF+61A/G genotype.
Laboratory tests and participants: EGF+61 A/G gene polymorphism was analyzed at ICVS, University of Minho, Braga, Portugal, by PCR-RFLP of DNA samples from peripheral blood of NSCLC patients treated in Hospital São João, Porto, Portugal, between February 2010 and March 2011.
Statistical analysis: Logistic regression analyses were used to calculate odds ratio (OR) and 95% confidence intervals (CI 95%).
Results: In this preliminary analysis, we enrolled fifty-two Caucasian Portuguese patients with NSCLC and 150 healthy Caucasian Portuguese blood donor from Braga Hospital. The EGF+61 genotypes frequencies in controls were: AA (29.3%), AG (42.7%), GG (28%); and in NSCLC: AA (25%), AG (44.2%), GG (30.8%). No statistically significant associations were found between EGF+61 genotypes and overall risk for NSCLC development: +61AG (OR = 2.289, CI 95%: 0.793–6.607) and +61GG (OR = 2.012, CI 95%: 0.641–6.316). However, stratification by gender revealed an increased risk of males carrying +61AG genotype for developing NSCLC when compared to AA and GG genotypes (OR = 4.563, CI 95%: 1.106–18.818).
Conclusion: This preliminary study in a small population suggests an increased risk to develop NSCLC in males carrying the EGF +61 AG genotype. Further studies in a larger population are ongoing to access the potential impact of EGF +61 polymorphism in NSCLC susceptibility in the Portuguese population.

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Procoagulant and Inflammatory Mediators in Small Cell Lung Carcinoma − Potential Role in Thromboembolic Complications

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Introduction: Small Cell Lung Carcinoma (SCLC) patients exhibit a higher prevalence of thromboembolic complications. We hypothesized that in this malignancy, procoagulant and inflammatory mediators contribute to the pathogenesis of such complications and warfarin may down regulate these levels.
Methods: In a prospective, randomized, controlled study, patients with inoperable lung cancer (n = 100) were randomized to receive chemotherapy and radiation with and without warfarin (INR 1.5−2.5). Blood samples were drawn prior to and after the 2nd treatment cycle with warfarin or control and retrospectively analyzed for microparticles and thrombin generation markers such as fibrinopeptide A (FPA), thrombin-antithrombin complex (TAT) and prothrombin fragment F1.2 (F1.2). In addition, biochip array for C-reactive protein (CRP), D Dimer, neuron specific enolase (NSE), neutrophil gelatinase associated lipocalin (NGAL), tumour necrosis factor receptor 1 (TNFR1), and thrombomodulin (TM) were measured. The results were compared with a normal population (N = 50).
Results: The microparticles were markedly increased in the SCLC patients (3 fold increase) at baseline. Similary, the thrombin generation markers showed variable increase (1.5−3.2 fold increase). In the biochip array analysis, variable increase was noted. CRP (2.4 fold), D DIMER (11.6 fold), NSE (1.8 fold), NGAL (1.7 fold), TNFR1 (2 fold) and TM (1.3 fold) were all increased as compared to normal controls. All of the markers exhibited a decrease after warfarin treatment with a most pronounced decrease in the D Dimer and TNFR1.
Conclusions: These results validate the hypothesis that SCLC patients exhibit a hypercoagulable state that is associated with simultaneous upregulation of inflammatory mediators. Warfarin treatment results in a down regulation of these mediators. Our results provide a rationale for prophylactic anticoagulant therapy in this group of patients.

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Phase I/II Trial of Vorinostat (V) in Combination With Erlotinib (E) in Advanced Non-small Cell Lung Cancer (NSCLC) Patients (pts) With EGFR Mutations After Erlotinib Progression − the TARZO Trial (NCT00503971)

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Background: EGFR-mutant NSCLC pts ultimately overcome resistant to tyrosine kinase inhibitors (TKIs). V is a histone deacetylase (HDAC) inhibitor with antitumour activity in vivo and in vitro. Inhibition of HDAC by V increases levels of E-cadherin, p21 and downregulates phospho-AKT/ERK1−2. A synergistic antiproliferative effect of V and TKIs has been observed in vitro. We aim to demonstrate if the addition of V could reverse the sensitivity to E in mutated NSCLC pts.
Material and Methods: Pts with advanced NSCLC with EGFR mutations (Exon 19 and 21), >18 years old, ECOG $\leqslant$2, measurable disease, adequate bone marrow, liver and renal functions after E progression ($\geqslant$12 weeks) were eligible. The primary objective was to determine activity and safety of treatment. Pts received the MTD reached at phase I with oral E 150 mg PO daily plus oral V 400 mg QD on days 1−7 and 15−21 in a 28-day cycle. All pts were treated until progression disease or intolerable toxicity.
Results: Twenty-four pts have been included up to date, 16 woman, 16 never smokers and 21 adenocarcinomas. Median age was 60 years (range