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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

42−77). Fifteen pts had mutations in exon 19 and 9 in exon 21. Eight pts had ECOG 0, 14 ECOG 1 and 2 ECOG 2. Most common sites of metastases were bone (37.5%), liver (33.3%) and brain (20.8%). Median time since diagnosis was 26.9 months and the median of previous administered treatments was 3. The median number of cycles of E and V administered per pt were two (range 1−9). No objective antitumour responses were observed. Seven pts experienced stable disease, of whom two lasted more than 9 months. Median TTP was 2.0 months and 29% of pts were free of disease progression at 12 weeks. The most common toxicities were mild or moderate (grade I-II) and include anaemia (75.0%), diarrhoea (62.5%), rash (45.9%), asthenia (45.9%), nausea (41.7%), vomiting (37.5%), anorexia (37.5%), dry skin (33.3%), xerostomia (20.8%). The most common grade III-IV adverse events were asthenia (20.9%), diarrhoea (12.5%) and anorexia (8.3%).
Conclusions: Concurrent administration of E 150 mg PO daily plus oral V 400 mg QD on days 1−7 and 15−21 is feasible. No objective antitumour activity was detected with the addition of V to E treatment; however, some prolonged stabilizations have been observed in this group of advanced NSCLC pts with EGFR mutations after E progression.

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MRNa Levels and Genetic Status of Genes Involved in the Epidermal Growth Factor Receptor (EGFR) and the Nuclear Factor kB (NF-kB) Pathways in Metastatic Non-Small-Cell Lung Cancer (NSCLC) Patients (P)

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Background: Little is known about the potential effect of genetic alterations in the NFkB and Notch pathways on NSCLC p. Musashi 2 activates HES-1 in the Notch pathway, and HES-1 can abrogate CYLD. A20, AEG-1, EZH2 and TRAF6 are also involved in NFkB activation. BRCA1 and RAP80 are modulators of cisplatin-based chemotherapy. Mutations in NFKBIA and DUSP22, which prevent NFkB activation, were described in the sequencing exome of a single NSCLC p, together with K-ras mutations.
Material and Methods: mRNA expression of Musashi 2, CYLD, HES-1, A20, EZH2, AEG-1, TRAF6, NFKBIA, RelA, BRCA1 and RAP80 was analyzed by quantitative RT-PCR in tumour samples from 60 advanced NSCLC p. Expression levels by terciles were correlated with clinical characteristics and outcome to chemotherapy. Mutations in NFKBIA and DUSP22 were sequenced in 28 and 21 patients, respectively, and in 12 cancer cell lines.
Results: Patient characteristics: 36 male; 39 adenocarcinomas; 22 smokers; 23 bone metastases; 9 EGFR mutations; 10 K-ras mutations. No NFKBIA or DUSP22 mutations were observed in any of the p or cell lines. PFS was 12.3 months (m) for p in the lowest tercile of AEG-1 expression vs 9.3 m for p in the intermediate tercile and 4.8 for p in the highest tercile (P = 0.002). The multivariate analysis showed that only AEG-1 expression was associated with shorter PFS (HR, 1.43; P = 0.006). Expression levels of the other genes did not correlate with outcome. In patients with low levels of both BRCA1 and AEG-1, PFS was 13.02 months, compared to 5.4 months in those with high levels of both genes and 7.7 months for those with other combinations (P = 0.025). The multivariate analysis for PFS confirmed the role of high BRCA1/AEG-1 expression (HR, 3.1; P = 0.01).
Conclusions: The present study helps to improve our understanding of the clinical relevance of genetic factors in metastatic NSCLC. AEG-1 and BRCA1 mRNA expression could be a useful prognostic model for the management of NSCLC p.

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Expressions of IGF-1R and IGFBP3 in Advanced Non-small Cell Lung Cancer (NSCLC)

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Background: The insulin-like growth factor (IGF) pathway plays an important role in cell proliferation, differentiation, and apoptosis, and IGF induces those effects mainly through IGF receptor-1 (IGF-1R). The activities of IGF are strictly regulated by a family of IGF binding proteins (IBFBP), especially IGFBP3, a major serum carrier protein for IGF. To date, however, insufficient data have been accumulated concerning the expressions of IGFR and IGFBP3 in advanced NSCLC.
Material and Methods: Between January 2006 and February 2009, 191 patients were histologically diagnosed as having non-small cell lung cancer (NSCLC) in our hospital, and 74 patients were treated by chemotherapy alone. We examined immunohistochemical expression of both IGF-1R and IGFBP3 in 68 patients who were definitively diagnosed as having adenocarcinoma or squamous cell carcinoma among the 74 patients. We also investigated the association of IGF-1R and IGFBP3 expression and clinical background, including histology, and survival. Staining of each antibody was considered positive if >10% of the tumour cells were stained.
Results: Clinical characteristics of the included patients were as follows: median age was 68 (range, 29−86), male/female=40/28, stage III/IV=18/50, PS 0−1/2−4=58/10, smoker/non-smoker=44/24, Sq/Ad=13/55. Expression of IGF-1R and IGFBP3 was observed in 37 (54%) and 11 patients (11%), respectively. IGF-1R expression was detected more frequently in Sq patients (100%) than Ad patients (44%, p < 0.001), while IGFBP3 expression was not significantly associated with histology (p = 0.356). Both IGF-1R and IGFBP3 expression were not significantly associated with the response to chemotherapy (p = 0.196 and p = 0.846, respectively). Among all factors, including IGF-1R and IGFBP3 expression, only PS was significantly associated with OS (p < 0.001).
Conclusions: IGF-1R expression, not IGFBP3 expression, was significantly associated with histology; however, neither of these was correlated with chemo-sensitivity or survival in advanced NSCLC patients treated by chemotherapy.

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EGFR Mutation Testing and First Line Treatment of Patients With Advanced NSCLC and Positive EGFR Mutation Status − Results From a German Registry

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Background: In patients diagnosed with advanced non-small-cell lung cancer (NSCLC), somatic mutations in the epidermal growth factor receptor (EGFR) gene are predictors of sensitivity to EGFR tyrosine kinase inhibitors (TKIs). With effective TKIs available the EGFR mutation analysis is becoming increasingly integrated into the diagnostic routine.
Methods: REASON is an AstraZeneca sponsored registry (ClinTrials ID: NCT00997230) being conducted in Caucasian patients in Germany. Between Nov 2009 and Mar 2011, 150 sites recruited 4,300 patients with histologically confirmed locally advanced/metastatic NSCLC stage IIIB/IV for whom an EGFR mutation analysis was planned. Data collected include demographic information, tumour anamnesis, result of EGFR gene mutation analysis and therapeutic agents selected for the intended first-line palliative therapy. Furthermore, clinical outcomes for patients with positive EGFR mutation status (EGFR mut+) were correlated with the applied treatment regimen.
Results: To date, information covering the period up to the first-line treatment is available on 3,155 patients. The rate of sensitizing EGFR mutations was 9.8% (all histologies, 12.8% adenocarcinoma), with the majority analysed in the primary tumour tissue (84%). The median turnaround time for testing was 11 days. Among those patients with EGFR mutations, there was a similar proportion of non-smokers and smokers (47% vs 53%), and approx. twice as many females as males (62% vs 38%). The rate of female EGFR mutation positive patients was twice as high as the respective rate of male patients (62% vs 38%). 274 out of all 310 EGFR mutation positive patients had adenocarcinoma histology (88%). Mutation analyses were performed locally at 67 pathology laboratories. 88% of EGFR mutation positive patients (n = 273) received a first-line therapy, with either gefitinib (45%), platinum-based combinations (39%), Bevacizumab containing triple combinations (9%), Erlotinib (3%) other combinations (4%).
Conclusion: The REASON data base allows a thorough analysis of epidemiological parameters in correlation with clinico-pathological characteristics in patients with advanced NSCLC and will contribute further insight into this frequent disease. Providing information on EGFR mutation status influences treatment decisions regarding first-line TKI application.