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Late-breaking abstract: Relationship of aquaporin 1, 3 and 5 expression in lung cancer cells to cellular differentiation, invasive growth and metastasis potential

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An oncogenic capacity of aquaporins (AQPs), transmembrane channels for water, was recently proposed. This study seeks to elucidate the involvement of AQP1, 3 and 5 in the development and progression of lung cancer. Expression analyses of AQP1, 3 and 5 by immunohistochemistry, western blot and real time RT-PCR in 160 lung cancers showed that AQP1, 3 and 5 were expressed in tumor cells of 71, 40 and 56%, of lung cancers, respectively. AQPs expressions were frequent in adenocarcinomas (ADCs), while AQP1 and 5 were negative in squamous cell carcinomas. Bronchioloalveolar carcinoma (BAC) cells exhibited an apicolateral AQP1 and apicolateral or basolateral AQP3 localization in non-mucinous type, and apical AQP1 and 5 and basolateral AQP3 expression in mucinous type, which corresponded to AQPs expression of non-neoplastic lung tissue. Basolateral AQP5 expression was acquired during tumorigenesis of non-mucinous BAC. In contrast, invasive ADC tumor cells, either with fibroblastic reaction or papillary growth in the alveolar space, overexpressed AQP1 and 5 with loss of subcellular polarization and with an intracytoplasmic distribution. Overexpression of AQP1 correlated with high postoperative ADC metastasis ratios and unfavorable disease-free survival rates (p=0.031). We conclude that expression patterns of AQP1, 3 and 5 in lung cancer cells are mostly associated with cellular differentiation. However, the expression of AQP1 and 5 is up-regulated in invading lung cancer cells, particularly in ADCs, and the overexpression of AQP1 with loss of subcellular polarization is suggested to be involved in their invasive and metastatic potential.

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A phase III randomised trial assessing the activity of first-line ifosfamide-gemcitabine (IG), a non-platinum chemotherapy (CT), in patients with advanced non-small cell lung cancer (NSCLC)

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Rationale: Few data showed similar survival for platinum-free and platinum-based CT regimens. Our primary endpoint was to determine if cisplatin-based chemotherapy, GIP or DP, will improve survival in comparison to the IG combination in patients with advanced NSCLC.

Methods: After central randomisation, patients received either GIP (gemcitabine 1g/m^2 D1-8, ifosfamide 3g/m^2, cisplatin 50 mg/m^2), IG (gemcitabine 1g/m^2 D1-8, ifosfamide 3g/m^2) or DP (docetaxel 75 mg/m^2, cisplatin 50 mg/m^2). According to the ELCWP experience, an increase of the 1 year survival rate from 35% in the IG arm to 50% in the two cisplatin arms will require to observe 360 deaths.
Results: From 12/2003 to 03/2009, 693 eligible patients were randomised. Main characteristics were: median age 58 years, male/female 523/170, squamous/adenocarcinoma/other 152/386/155, Karnofsky PS 60-70/80-100 157/536, stage IIB-III/IV 129/564. The main results are:

	IG	GIP	DP	p
Response rate (95% CI)	27% (21–32)	33% (27–39)	26% (20–31)	0.29
Median PFS (95% CI)	3.6 m (2.8–4.4)	4.1 m (3.4–4.8)	3.6 m (3.2–3.9)	0.66
1-year survival (95% CI)	38% (31%–45%)	35% (29%–41%)	35% (29%–41%)	0.82
Median survival (95% CI)	8.9 m (7.2–10.6)	9.0 m (7.7–10.2)	8.3 m (6.9–9.7)	

GIP was associated with ($p < 0.05$) more neutropenia, thrombopenia, vomiting, while more cardiotoxicity, diarrhea and peripheral neuropathy was observed with DP and encephalopathy with IG.
Conclusion: In this large phase III trial, a non-platinum CT regimen (ifosfamide-gemcitabine) had similar activity to cisplatin-based CT in terms of survival, PFS and response rates with a favourable toxicity profile.

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Gemcitabine sensitizes lung cancer cells to Fas/Fas ligand system-mediated killing

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Gemcitabine (GEM) is an agent commonly used in the treatment of non small cell lung cancer (NSCLC). GEM induces apoptosis in NSCLC cells indirectly by increasing functionally active Fas expression. To further explore the mechanisms involved in the activation by GEM of apoptosis extrinsic pathway in lung cancer cells, we evaluated the ability of GEM to upregulate the expression of FasL in NSCLC H292 (mucoepidermoid carcinoma) cell line and to increase the sensitivity of these cells to Fas-mediated killing of cytotoxic lymphocytes. Cells were cultured with and without GEM (0.05 μM) for 24, 48, 72 and 96 hrs, and FasL mRNA and protein were evaluated by real-time PCR, and by western blot and flow cytometry, respectively. Apoptosis of cells expressing FasL was evaluated by flow cytometry. Cytotoxicity of LAK and malignant pleural fluid (PF) lymphocytes against H292 cells was analyzed in presence and absence of neutralizing anti-Fas antibody (clone ZB4), by a flow cytometry-based assay. Expression of FasL mRNA and protein in H292 cells after incubation with GEM was increased at all time-points and this increase was higher after 72 hrs. Accordingly, the percentage of apoptotic H292 cells expressing FasL was higher after 72 hrs. Cytotoxicity of LAK and PF lymphocytes was significantly increased after incubation of H292 cells with GEM and was partially inhibited by neutralizing anti-Fas antibody.
These data demonstrate that: 1) GEM induces an up-regulation of FasL in NSCLC cells triggering cell apoptosis via an autocrine/paracrine loop; 2) GEM is able to increase the sensitivity of NSCLC cells to cytotoxic activity of LAK and PF lymphocytes by activation of Fas/FasL signalling system.

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Non-small cell lung cancer treatment by inhalation of Erbitux and gemcitabine in murine model

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We evaluate the efficacy of chemotherapy delivered by inhalation in murine model of lung cancer.
Methods: Mouse models of human (H226) and murine (3LL) non-small cell lung cancer (NSCLC) were use. Cultivated human NSCLC cells were inoculated orthotopically into the lung (H226) or intravenously (3LL) producing advanced disseminated lung cancer. Aerosols were delivered daily from day 5 to day 30 (after tumor cell inoculation) using an ultrasonic nebulizer in a cage specially constructed to in total dosage 30mg/kg (Erbitux) and 0.5mg/kg (Gem). The effect of nebulization on Erbitux was assessed in terms of its affinity for membrane EGFR (using ELISA), inhibition of cell growth (XTT assay) and inhibition of EGFR phosphorylation (by immunoprecipitation).
Results: Significant inhibition of tumor growth by Erbitux alone or in combination with Gem was observed. Inhalation of Erbitux in 3LL resulted in 50% reduction of lung weights and number of tumor nodules. Nebulized Gem demonstrated 80% decrease in the lung weights and tumor foci whereas combination therapy resulted in complete disappearance of tumor masses. Inhalation in H226 with Erbitux demonstrated 10% reduction of lung weight, 40% reduction with Gem and 50% with combination therapy. Inhalations were well tolerated without toxicity to lungs, kidney, colon, skin, liver or spleen.
Conclusions: We demonstrated an efficacy of the aerosol treatment by Erbitux and Gem in animal model of NSCLC without any pulmonary toxicity. The study demonstrated therapeutic perspective of aerosol delivery of Erbitux/Gem in the treatment of patients with advanced disseminated lung cancer.

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Use of erlotinib in management of advanced non-small cell lung cancer (NSCLC): The Czech Republic experience with the treatment of a non-selected population of 1735 patients (pts)

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Erlotinib is the first EGFR TKI to be approved in Czech Republic for use in advanced NSCLC. This analysis presented examines data of all pts treated in Czech republic between 12/2005 and 12/2010.
Data of all 1735 patients were summarised and retrospective analyses were carried out to determine if any associations were seen between specific characteristics and either mPFS or mOS.
Of the 1735 pts with advanced NSCLC 35,6% were female and 64,4% male, 21,6% non-smokers, 39,5% former smokers and 38,9% current smokers. Median age was 65 years. Erlotinib was used as 1st line therapy in 14,4%, as 2nd line in 47,3% and as 3rd line in 38,3% pts. PS 0 was in 7,6%, PS 1 in 56,3%, PS 2 in 33,5% and PS 3 in 2,6% pts. Adenocarcinoma was confirmed in 45,9%, squamous-cell carcinoma in 39,2%, large–cell carcinoma in 4,0% and non specified carcinoma in 9,4% pts. CR was confirmed in 0,6%, PR in 123 7,1% pts, SD in 44,1% of pts; 23% of pts progressed and 25,2% pts were not evaluated. Skin toxicity was in 56,7%. Median survival (95% CI) was 7,5 months, PFS (95% CI) was 2,9 months. The differences between groups of pts according to PS (0+1 vs. 2+3) were statistically significant ($p < 0,001$). The best median survival (17,6 month) was in the group of pts with PS 0. Statistically significantly longer ($p < 0,001$) was mOS and mPFS survival in patients with skin toxicity, in female pts, in non-smokers and in pts with adenocarcinoma.
Erlotinib in this non-selected group of pts with advanced NCCLC was well tolerated and the results from Czech Republic are better then the data from BR.21 study and comparable with the results of TRUST.

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Gefitinib selectively targets non-small cell lung cancer cells through inhibition of Forkhead box M1

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Rationale: Gefitinib shows dramatic treatment effects in non-small cell lung cancer (NSCLC) patients. But only about 20% of such patients are benefited. Why the remainder derives no such effect is only partly understood. FoxM1 has been shown to be overexpressed in NSCLC. Therefore, we assessed the effect of FoxM1 on the response of lung cancer cell line to gefitinib and examined the possible mechanism.
Method: Expression levels of FoxM1 and downstream targets were measured by RT-PCR and immunoblotting in gefitinib sensitive and resistant NSCLC cells lines H292 and SPC-A-1. A H292 cell line with constitutively active FoxM1 mutant (N-FoxM1 group)was established. MTT assay and FCM were used to detect the cell survival and apoptosis in N-FoxM1 and vehicle groups.
Results: The mRNA levels of FoxM1 in SPC-A-1 cells at 24, 48 and 72 hours after incubation of gefitinib increased by 38%, 53%, 72%, while reduced by 23%, 46%, 54% in H292 cells with 10 uM and 1 μM of gefitinib. The survival rates of N-FoxM1 and vehicle groups with incubation of 1, 0.5 and 0.1μM of gefitinib for 3 days are 65.34%, 73.8%, 93.17%, and 50.62%, 60.59%, 80.53%. The apoptosis rates of both groups are 7.39%, 3.84% and 18.97%, 13.85% with treatment of 1uM and 0.1 μM gefitinib. The mRNA expression level of FoxM1 downstream targets aurora kinase B, SKP2, PLK1, CDC25B, survivin and cyclinB1 were increased in SPC-A-1 cells with gefitinib treatment. Moreover, the increased protein level of survivin and cyclinB1 were further confirmed by immunoblotting.
Conclusion: These data suggest that FoxM1 confers the resistance of lung cancer cells to gefitinib. Thus, FoxM1 could be used as a therapeutic target of gefitinib resistance.

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Carboplatin-paclitaxel alone or with bevacizumab in stage III-IV lung adenocarcinoma

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Introduction: Bevacizumab, a monoclonal antibody against vascular endothelial growth factor, has been shown to benefit lung adenocarcinoma patients when added to standard chemotherapy.
Aims and objectives: To assess the clinical efficacy of Bevacizumab in lung adenocarcinoma stage III-IV when added to standard chemotherapy with carboplatin and paclitaxel in a pragmatic tertiary healthcare setting.
Materials and methods: Two groups of consecutive patients with inoperable lung adenocarcinoma were investigated. Group A consisted of 20 patients that received initially carboplatin-paclitaxel with bevacizumab as first line chemotherapy and thereafter bevacizumab alone as maintenance therapy until progression. Group B consisted of 22 patients that received carboplatin-paclitaxel alone. Both groups had similar smoking history and comorbidities. Treatment options were assessed in terms of time to progression and side effects.
Results: Our patient groups consisted of 13 females and 29 males with a mean age of 61.8 years (range 42-82). Twenty seven patients were in stage IV. All patients were ex smokers with equivalence in regard with the smoking habit, the disease stage and the application of radiotherapy between groups A and B. The side effects were similar to both groups of patients ($p>0.10$). Time to progression was strongly associated with the therapeutic regimens; Group B presented with longer time to progression than Group A ($p=0.002$).
Conclusions: The addition of bevacizumab to carboplatin-paclitaxel in the treatment of consecutive patients with inoperable lung adenocarcinoma does not seem to confer a significant improvement in progression free survival and response rate.

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Intraoperative photodinamic therapy as a part of combined radical treatment for stage III NSCLC

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Background: Incomplete resections for lung cancer remain an important problem. To increase the radicalism we proposed an intraoperative photodynamic therapy (PDT) of resection margins.
Methods: 16 patients with central NSCLC (12 men, 4 women) were prospectively included in the study (10 – IIIb, 6 – IIIa). Hystological types were: squamous cell – 10, adeno – 4, large cell – 2. At the time of diagnosis all patients were considered as unresectable or inoperable, reasons were tracheal involvement (7), functional intolerance to pneumonectomy (7) and N3 disease (2). Preoperative treatment included three cycles of chemotherapy and two courses of endobronchial PDT. During operation, after lung resection (pneumonectomy – 11, lobectomy – 5) intraoperative PDT of resection margins (bronchial and vascular stumps, mediastinum) was done. Chlorine e6 complex was used as a photosensitizer in dose of 2 mg/kg. The interval between the injection and illumination was 2 hours. Red light at 662±1 nm wavelength was used to achieve a total illumination dose of 250 j/cm^2.
Results: After preoperative treatment partial response of tumor was achieved in all cases and patients underwent surgery with radical intent. There was no post PDT complication. 14 operations were R0, 2 – R1. No major postoperative complications noted except cardiac arrhythmia in 3 patients (19%). Average period of follow-up was 16 months (4 to 30 months), all patients are alive without any signs of recurrence.
Conclusion: The first experience of the combined treatment including intraoperative PDT for locally advanced NSCLC demonstrates safety and effectiveness. Additional studies are needed to proof the value of intraoperative PDT.