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Accelerated high-dose radiotherapy by target splitting for inoperable non-small cell lung cancer: a phase I trial

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Background: To determine toxicity of accelerated high-dose radiotherapy (RT). Second endpoint: survival, locoregional control.
Material and Methods: 04/2002 – 04/2003: 30 consecutively referred, not selected patients with histologically/cytolocically proven inoperable, locoregionally confined NSCLC. Stage I: 7 pts., II: 3 pts., IIIA: 12 pts., IIIB: 8 pts. Squamous cell carcinoma: 16 pts., adenocarcinoma: 9 pts., NSCLC not otherwise specified: 5 pts. M/f = 21/9. Only 2 Patients have been staged by FDG-PET scan.
The majority of the patients have been treated by the conformal technique of target splitting. Dose to primary sites: 84.6 Gy ICRU median (75.6 – 90.0 Gy), nodes 63.0 Gy median (59.4 – 72.0 Gy), elective nodes 45.0 Gy. Single fraction size 1.8 Gy, twice daily, 5 days/week, interval 11h (10–12h), treatment duration 35 days median (30–43). For planning 'slow' CTs have been used (4 sec/slice), patients freely breathing, 7 mm margins from GTV to PTV.
In 19 patients' chemotherapy before RT was given, 2 cycles median; no concurrent chemotherapy. Median follow-up of patients alive: 33.5 months (26–37 m).
Results: Acute non-hematologic toxicity (until 6 months after the end of RT): esophagus grade 1: 13 pts., grade 2: 2 pts.; lung: no patient >grade2. No chronic toxicity >grade1. No fatal pulmonary hemorrhage. Overall actual 1-, 2-year survival rate: 73%, 63%, respectively, median 26.3 months. Respective figures for 11 patients IIIA-N2: 82%, 73%, median survival not reached. 5 patients died of intercurrent diseases (3 of these were stage I – patients) 9 patients recurred locally (8 of these presented initially with tumor-caused atelectasis). 4 regional recurrences (2 of them in supraclavicular sites, initially not treated).
Conclusions: Accelerated RT with doses up to 90 Gy in twice daily fractions of 1.8 Gy, delivered mostly by the conformal technique of target splitting shows low toxicity. Survival and locoregional tumor control are encouraging. A trial studying tumorsize-dependant doses has been started.

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Phase II trial of topotecan plus gemcitabine in previously treated small cell lung cancer (SCLC) patients: an Alpe-Adria Thoracic Oncology Multidisciplinary group study (ATOM 012)

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Background: Single-agent topotecan has proven active in the treatment of second-line small cell lung cancer (SCLC) with response rates up to 14% in chemotherapy-refractory patients and up to 38% in chemotherapy-sensitive patients. The role of topotecan in combination with another active agent may be still clarified and may provide further improvement.
Methods: In this phase II trial we evaluate the activity and toxicity of topotecan (1 mg/sqm iv d1–5) plus gemcitabine (1250 mg/sqm iv d1) in relapsed or progressing SCLC patients. Treatment is repeated every 4 weeks up to a maximum of 6 cycles. Eligibility criteria include: histologically or cytologically confirmed SCLC; documented progressive disease after at least 1 prior chemotherapy regimen; age >18 yrs; ECOG PS 0–2; measurable disease (RECIST); no prior treatment with topotecan or gemcitabine; adequate hematologic, hepatic and renal function; written informed consent. Brain metastases are allowed.
Results: To date, 37 patients have been enrolled. Patient characteristics: median age, 64 yrs (range 35–77); male/female, 29/8; ECOG PS 0/1/2, 10/17/10 patients; 61% patients had sensitive disease (recurrence >3 months after first-line chemotherapy) and 39% patients had refractory disease (failure <3 months after first-line chemotherapy). Thirty-six patients had received prior platinum-based therapy involving etoposide and either cisplatin or carboplatin. Ninety-nine cycles have been delivered (median 2, range 1–6). All patients are evaluable for toxicity. Grade 3–4 toxicities include 54% neutropenia, 16% anemia, 46% thrombocytopenia, 13% neutropenic fever, 27% fatigue. One toxic death was observed. One patient (3%) obtained complete response and 8 patients (22%) obtained partial response, for an overall response rate of 24%; SD was observed in 7 patients (19%), PD in 15 patients (40%). Four early deaths were reported; 2 patients are currently on treatment. At the time of this analysis median time to progression is 7.7 weeks. Median survival time is 16.3 weeks, and 1-year survival rate is 11%.
Conclusions: Based on these preliminary results, the combination of topotecan and gemcitabine is active and has an acceptable toxicity profile in previously treated SCLC patients. However, it is unlikely that the addition of gemcitabine improves the outcome compared to single agent topotecan.

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Telomere biology and DNA-repair systems in non-small cell lung cancer

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Background: Telomeres and telomerase perform important roles in suppressing or facilitating malignant transformation. Moreover, recent "in vitro" observations propose that telomere-binding factors may be ancient and general DNA-repair factors that have been exploited by the cell to protect telomeres. Our aim consists of investigating telomere biology in non-small cell lung cancer (NSCLC) in relation to genome integrity.
Material and Methods: Sixty-two NSCLCs were evaluated to establish telomere status. Thus, we studied terminal restriction fragment (TRF) length and telomerase activity. Following, we performed expression analyses by using a human microarray in which 96 genes related to the different DNA repair systems have been included. This study was established by comparing gene expression in two subgroups of cancers, the first one maintaining telomeres, and the second one showing a significant telomere shortening.
Results: Overall, telomere length in NSCLCs was 8.12±0.37, with no significant differences to values detected in non-tumor samples (7.84±0.21) (P = 0.401). Telomerase activity was detected in 54 (87%) tumors, and no significant differences were found in relation to telomere length. As comparing with control samples, 32 tumors (51.6%) showed telomere maintenance, and 20 (48.4%) telomere shortening (>20%). Kaplan-Meier survival curves indicated important prognostic differences between both groups, the group of cancers displaying telomere shortening conferred a significant poorer clinical evolution (P = 0.02). Results from microarray expression analyses suggested significant changes for a number of genes regarding to telomere status. The major differences seems to concern to *PARP-1* and *PMS6*. Whereas *PARP-1* is overexpressed in most of NSCLCs with telomere maintenance, *PMS6* overexpression seems to be associated with telomere shortening.
Conclusion: These observations lead us to draw an interaction between DNA repair and telomere function.

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Involvement of Akt/PKB signalling in survival of cisplatin-sensitive and -resistant human pulmonary mesothelioma cells

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Background: It is shown that Akt/Protein kinase B (PKB) is constitutively active and promotes survival and resistance to chemotherapy in several non-small cell lung cancer cell lines. PI3-kinase can activate Akt/PKB, and Akt/PKB can exert antiapoptotic effects through phosphorylation of members of the apoptosis-regulating Bcl-2 family. We determined the importance of Akt/PKB in survival of the human pulmonary mesothelioma cell line P31 wt (wt) and the cisplatin-resistant subline P31 res1.2 (res1.2).
Methods: The cell lines were exposed to 25 or 50 μM LY294002 (LY), a PI3-kinase inhibitor, for 30 min, 2 or 6 h. The phosphorylation of Akt 1 at S473 (P-Akt) was determined by Western blot (WB), and effects on apoptosis induction was determined by TUNEL staining and caspase activity assays. Preliminary WB analysis of Bcl-X(L) and Bcl-2 expression was also performed. All experiments were performed in medium with 10% serum.
Results: Both cell lines expressed Akt 1 and P-Akt under control conditions at all times examined. Exposure to 25 μM LY did not inhibit P-Akt in either cell line, and 50 μM was inefficient in res1.2 cells. In wt cells, 50 μM LY inhibited P-Akt after 30 min, but not after 2 or 6 h exposure. An increase in the number of TUNEL positive wt cells was found after 30 min and 2 h, but not 6 h, exposure to 25 and 50 μM LY. No changes in number of TUNEL positive cells were found in res1.2 cells at any exposure. Caspase-3 activity but not caspase-8 or -9 activities increased after 6 h exposure to 25 and

50 μM LY in both cell lines. Both cell lines expressed Bcl-X (L) and Bcl-2, but the effect of LY was ambiguous.
Conclusions: Effective inhibition of P-Akt corresponded to an increased apoptosis in wt cells, but not to caspase activation. In res1.2 cells, efficient inhibition of P-Akt never occurred, and there was no change in apoptosis. The inefficient inhibition of P-Akt by LY indicated that PI3-kinase is either strongly overactivated or that PI3-kinase is not the main phosphorylator of Akt 1 in P31 cells. The role of the anti-apoptotic proteins Bcl-X (L) and Bcl-2 in survival of P31 cells needs to be further investigated. To conclude, P31 res1.2 cells appeared to be more resistant to LY inhibition of P-Akt than P31 wt cells. In P31 wt cells, P-Akt was important for survival and affected caspase-3 activity. The involvement of PI3-kinase and Akt/PKB in the cisplatin-induced apoptosis signalling pathways of P31 wt and res1.2 cells remain to be elucidated.

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EGFR mutations in NSCLC: Genotypic analysis and implementation of complementary screening tests for detection purposes

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Background: Somatic mutations of the epidermal growth factor receptor (*EGFR*) gene in non-small cell lung cancer (NSCLC) predict responsiveness to the EGFR tyrosine kinase inhibitors. These mutations are commonly identified using DNA sequencing methods. Although considered the gold standard, this approach requires a high ratio of tumor to normal tissue DNA for optimal results which is not often available in biopsies obtained from these patients. Due to this limitation, we have applied selected screening tests to enhance the sensitivity of DNA sequencing.
Materials and methods: Clinical specimens from 50 NSCLC patients were analysed for *EGFR* mutations in exons 18, 19, and 21. After DNA extraction and PCR, mutations were examined by sequencing genomic DNA. Additionally, PCR products were screened for exon 19 deletions using a fragment analysis strategy.
Results: Sequencing revealed 5 mutations: 3 missense mutations in exon 21 and 2 deletion mutations in exon 19. Fragment analysis of the samples detected the original 2 deletion mutations and an additional 4 new exon 19 deletion mutations that were further confirmed by direct sequencing with re-designed PCR primers. In our hands, fragment analysis was able to detect mutations in samples containing as little as 10% mutated DNA whereas direct sequencing requires at least 30%.
Conclusion: Clinically relevant mutations in the *EGFR* gene may not be detected using sequencing techniques because of insufficient tumor DNA in biopsy samples. The application of additional rapid and more sensitive screening tests may be able to overcome this limitation. Fragment analysis is a quick and reliable method for the detection of *EGFR* exon 19 deletion mutations in lung cancer that may be missed by standard DNA sequencing methods. Fragment analysis to detect deletion mutations and other more sensitive screening tests to detect missense mutations should be implemented as complimentary methods for detection of *EGFR* mutations.

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Identification of prognostically significant subsets of stage IIIA N2 non-small cell lung cancer patients by hierarchical clustering analysis of tissue microarray immunostaining. An Alpe-Adria Thoracic Oncology Multidisciplinary group study (ATOM 014)

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Background: Based on gene expression profiling, prognostically relevant subsets of patients have been identified for breast cancer, lung cancer and lymphoma. In the future, gene expression profile of each individual patient might provide support for tailored therapeutic decision making.
Methods: We performed a hierarchical clustering analysis of tissue microarray (TMA) immunostaining data of 87 patients with stage IIIA pN2 non small cell lung cancer (NSCLC), treated with radical surgery between 1985 and 1997. The expression of the following markers was evaluated: EGFR, ErbB-2, c-kit, COX-2, survivin, bcl-2, cyclin D1, cyclin B1, MMP-2, MMP-9 and univariate, multivariate analyses and unsupervised hierarchical clustering analysis by using these 10 markers were performed.
Results: Bcl-2 ($p < 0.0001$) and cyclin D1 ($p = 0.0036$) are more expressed in squamous cell carcinoma (SCC), while MMP-2 ($p = 0.0115$), MMP-9 ($p = 0.0075$) and survivn ($p = 0.02$) display increased expression levels in histological subtypes other than SCC. In univariate analysis, only squamous cell histology, bcl-2 and cyclin D1 expression were favorable prognostic factors ($p = 0.0149$, $p = 0.0013$, $p < 0.0001$, respectively), while MMP-2 expression was associated with worse prognosis ($p = 0.013$). In multivariate analysis, cyclin D1 and MMP-2 were the only positive and negative prognostic factors, respectively ($p < 0.0001$, $p = 0.06$). Unsupervised hierarchical clustering analysis of TMA immunostaining data produced 5 distinct cluster groups and the deduced tree identified 2 prognostically significant subsets of patients, with better (groups 1–2) and worse (groups 3–4–5) prognosis in terms of median survival (51 vs. 10 months, $p < 0.0001$). Notably, groups 1–2 were mostly composed of SCC (80%).
Conclusions: These results suggest that hierarchical clustering of TMA immunostaining data by using a limited set of markers might provide a useful tool for the identification of radically resected NSCLC patients at high risk of recurrence, likely to benefit from more aggressive treatment.

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Expression of hypoxia-inducible factor-1 alpha and its prognostic significance in small-sized adenocarcinomas of the lung

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Objective: To analyze the prognostic value of hypoxia-inducible factor-1 alpha expression and its correlation with clinicopathologic variables and expression of vascular endothelial growth factor-A, -C, and R-2 in patients with lung adenocarcinomas of small size.
Methods: The expression of hypoxia-inducible factor-1 alpha was immunohistochemically determined in 78 cases of small-sized adenocarcinoma (maximum dimension is less than 2 cm) using polyclonal antibody against a recombinant protein corresponding to amino acids 575–780 of hypoxia-inducible factor-1 alpha. Data regarding patient survival, clinicopathologic factors, and immunohistochemical studies of vascular endothelial growth factor were also collected.
Results: Strong expression of hypoxia-inducible factor-1 alpha was observed in 29 (37%) of 78 cases; no expression was found in the bronchioalveolar carcinomas. Strong expression of hypoxia-inducible factor-1 alpha was significantly higher in cases with vascular invasion, lymphatic permeation, lymph node involvement, and advanced pathological stage. Strong expression of hypoxia-inducible factor-1 alpha was correlated with strong expression of vascular endothelial growth factor-A, -C, and R-2. The 5-year survival rate was 69% if expression of hypoxia-inducible factor-1 alpha was strong and 84% if expression was weak. Multivariate analysis revealed that pathological N status and pleural invasion were independent prognostic factors and strong expression of hypoxia-inducible factor-1 alpha was marginal significance.
Conclusions: Strong expression of hypoxia-inducible factor-1 alpha was associated with vascular invasion, lymphatic permeation, nodal involvement, pathological stage, and strong expression of vascular endothelial growth factor-A, -C, and R-2. Strong expression of hypoxia-inducible factor-1 alpha was a poor prognostic factor for patients with small-sized adenocarcinoma of the lung.

1158 POSTER

Can 18FDG-PET/CT scan be used to define a biological target volume (BTV) for IMRT treatment planning of non-small cell lung cancer patients?

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Purpose: To test the feasibility of FDG based PET/CT data on target volume delineation in radiotherapy treatment planning of NSCLC patients, and impact of these outlined biological target volumes (BTV) for IMRT treatment.
Materials and methods: Patient diagnosed with non-operable NSCLC in the right upper lobe had a 3D conformal planning based on CT data with our hypo-fractionated regimen of 52.5 Gy in 15 fractions. Planning was