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Phase I Trial of Pemetrexed and Cisplatin Combination Chemotherapy With Concurrent Thoracic Radiotherapy in Japanese Patients With Locally Advanced Non-Small-Cell Lung Cancer

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Background: Pemetrexed (PEM) was found to have a radiosensitizing potential when evaluated *in-vitro* in combination with platinum-containing compounds and radiation. The purpose of this study was to determine the recommended dose (RD) of PEM and cisplatin (CDDP) combination chemotherapy with concurrent thoracic radiotherapy in Japanese non-small-cell lung cancer (NSCLC) patients (pts).
Materials and Methods: Eligible pts were histologically or cytologically confirmed non-squamous NSCLC with unresectable IIIA or IIIB disease. Written informed consent was obtained from all patients. The study treatment consisted of 2 phases: a chemo-radiation therapy phase (CRT) and a consolidation phase (CONS). In CRT, the first 6 pts received the level 1 dose (L1D), PEM 500 mg/m^2 plus CDDP 75 mg/m^2, on day 1 of a 21-day cycle for 3 cycles. Thoracic radiotherapy was given concurrently at a total dose of 60 Gy. If the dose limiting toxicity (DLT) occurred in <2 pts at L1D, the radiation dose was escalated to a total dose of 66 Gy (L2D). In CONS, PEM 500 mg/m^2 was administered in 3 cycles beginning 4 to 6 weeks after CRT. DLT was defined as any grade 3/4 hematological or non-hematological toxicity which resulted in delay or omission of study treatment, or any other grade $\geqslant$3 therapy-related adverse event except nausea or vomiting.
This study was sponsored by Eli Lilly Japan K.K.
Results: Eighteen pts (14 male, 4 female), median age 61 years were enrolled. In CRT, 1 patient experienced 2 DLTs (Grade 3 anorexia and diarrhea) at L1D, however no DLT was seen in the 6 pts at L2D. Therefore, L2D was determined as RD and an additional 6 pts were treated L2D. All 18 pts completed CRT. Twelve pts (5 at L1D and 7 pts at L2D) completed the CONS phase. No deaths occurred during the study. Common grade $\geqslant$3 toxicities were lymphocytopenia, neutropenia and leukopenia with frequencies of 89, 67 and 67%, respectively. At L1D, 2 pts with radiation pneumonitis (grade 3 and 2, 1 each) and 2 pts with radiation oesophagitis (grade 2 and 1, 1 each) were observed. There were 11 pts with radiation pneumonitis (6 grade 2 and 5 grade 1) and 11 pts with radiation oesophagitis (4 grade 2 and 7 grade 1) at L2D. Of 18 pts, 15 achieved partial response; 2 had stable disease and 1 progressive disease.
Conclusions: Combination chemotherapy of pemetrexed 500 mg/m^2 plus cisplatin 75 mg/m^2 with concurrent thoracic radiotherapy at a total dose of 66 Gy was well-tolerated; further development is warranted.

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E-cadherin Expression in Lung Cancer and Its Clinical Importance

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Background: Cadherins are considered to be the most important adhesion molecules necessary for the maintenance of normal tissue architecture. E-cadherin is a basic factor in the connection adhesions between epithelial cells. Reduced expression of these molecules correlates well with increased invasiveness, metastases, and poor prognosis in neoplasms.
Material and Methods: Patients with clinically operable primary lung cancer were included in the present study. The tissue samples of the tumours (paraffin cubes) were analysed using the tissue microarrays method. This was followed by immunohistochemistry study of E-cadherin and Ki-67 cell proliferation factor. The image analysis and processing were accomplished using special software. Finally, a database was created with all the clinical and histological data of the patients.
Results: In total, 108 patients (81 men and 27 women) with a mean age of 62 years were assessed. The histology types were: 44% adenocarcinoma, 31% squamous cell carcinoma, 9% large cell carcinoma and 16% other types. Associations between variables were analyzed by the application of Univariate Analysis Of Variance with SPSS v15.0 software (SPSS Inc., Chicago, IL, v.15.0). Two tailed p values $\leqslant$ 0.05 were considered to be statistically significant. Statistical significance was identified correlating E-cadherin lower-expression to grade III of the tumours (p-value 0.011), to stage IV (p-value 0.045) and in a positive correlation between E-cadherin and Ki-67 (p-value 0.040). In contrast, protein expression was not strongly associated to tumour size, to histological type, to patient age or to gender.
Conclusions: The most important conclusions of this study are that there is a low-expression of E-cadherin protein mainly in grade III and also in stage IV lung cancers and that there is a positive correlation between E-cadherin and Ki-67 expression.

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Development of a Novel RT-PCR Assay for the Detection of EML4-ALK Fusion Genes in FFPE Speciments

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Background: Echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) is a fusion-type protein tyrosine kinase identified recently in a subset of human lung carcinomas and seems to be a promising candidate for a therapeutic target as well as for a diagnostic molecular marker in NSCLC.
Indeed, ALK kinase inhibitors (Crizotinib (PF0234–1066, Pfizer) have already been developed and have been reported to be efficient only in patients positive for the EML4-ALK fusion.
To date, several *EML4-ALK* variants (1, 2, 3a, 3b, 4, 5a, 5b, 6, 7, "4" and "5") have been identified in lung cancer samples. A variety of methods have been used for the detection of these fusions, including immunohistochemistry, fluorescent in situ hybridization, and reverse transcriptase polymerase chain reaction (RT-PCR), which is the only method that can distinguish between different variants.
Existing RT-PCR methods though, are designed to amplify large cDNA fragments and are inadequate for the analysis of formalin-fixed paraffin-embedded (FFPE) tissues which produce cDNA fragments of limited size. Thus, we designed an RT-PCR assay that can detect all published *EML4-ALK* variants and is suitable for use with this commonly available material.
Methods: The study included FFPE speciments from NSCLC patients without EGFR and K-RAS mutations. Detection of all EML4-ALK fusions was achieved using a multiplex reverse transcription-PCR (RT-PCR). For this reason specific primers that enhance specifically EML4-ALK transcripts 1, 2, 3a, 3b, 4, 5a, 5b, 6, 7, "4" and "5" were deisigned. Synthetic DNA fragments for each variant were cloned using the pCR2.1 cloning vector and used as positive controls. DNA sequencing analysis was performed to confirm the specificity of the obtained PCR products.
The sensitivity of the method was calculated by adding to 1 μg RNA serial dilutions of the synthetic DNA fragments. It was found that up to 22 copies of the translocation can be detected per μg of RNA.
Results: None of the 26 FFPE speciments tested so far, was positive for the EML4-ALK fusion. The study is in process and we plan to test 100 FFPE speciments.
Conclusions: We designed a robust multiplex RT-PCR assay that permits the sensitive detection of all published EML4-ALK variants and is suitable for use with commonly available materials such as FFPE speciments and spuntum samples.

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Pilot Results for the Detection of Minimal Residual Disease in Lung Cancer

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Background: Lung cancer is one of the leading causes of cancer death and has become an increasingly urgent worldwide health problem. The term minimal residual disease (MRD) in solid tumours refers to the presence of tumour cells in the body of a patient who has undergone surgery without further clinical signs of the disease. These isolated tumour cells are considered to be the precursors of micrometastases. Testing MRD lung cancer patients can eliminate complicated surgical procedures in patients with systemic molecular dissemination of the disease and can improve the prognosis.
Material and Methods: The basic principle for the detection of MRD by methods based on quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) is the detection of biomarkers of epithelial tumour cells in compartments of mesenchymal origin. We validated the QRT-PCR method for 4 selected biomarkers for MRD detection in lung cancer − carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR),