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Abstract Book

IASLC

Conquering Thoracic Cancers Worldwide
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

organized by the
Czech Lung Cancer Cooperative Group
Central European Lung Cancer Board

under the auspices of
International Association for the Study of Lung Cancer (IASLC)

Smoking cessation workshop
Epidemiology and tobacco control in Central Europe – IASLC workshop to tobacco control and smoking cessation

Smoking and lung cancer, smoking cessation among lung cancer patients

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Background: Smoking is the most important risk factor for lung cancer. 85% of lung cancer patients have a history of smoking and 40–50% of patients report smoking at the time of diagnosis. Smoking however is not merely a risk factor for lung cancer, but continued smoking impairs therapeutic effectiveness, quality of life and survival. Smoking creates hypoxia in the tissues which has an adverse effect on wound healing and increases the rate of postoperative complications. Continued smoking also deteriorates the therapeutic effectiveness of radio- and chemotherapy, and increases the probability of metastases. Tobacco smoke promotes tumor development and enhanced vascularization of tumor tissues. Continued smoking increases mortality from second primary tumors, COPD and cardiovascular diseases.

Methods: We recorded data from 929 lung cancer patients in our research. 53% of patients were smoking at the time of diagnosis, 25% quit previously and 22% never smoked. 57% of smokers quit after the diagnosis, while 3% of them relapsed. During the 30-month follow-up, the survival rate was significantly higher for those who quit, than those who continued to smoke (54% vs. 42% [HR: 1.29; $p<0.001$]). The beneficial effects of quitting were explicit regardless of whether they had surgery or not.

Results: The aim is to motivate and support smoking lung cancer patients to quit. Our Institute provides individual, group and telephone cessation counseling. During 2014 and the first half of 2015, 2200 new lung cancer patients were detected. 900 patients were current smokers at the time of diagnosis. 330 patients (37%) were referred to the cessation counseling clinic, 135 (15%) of them joined the cessation program lasting several months. 99 patients (73%) quit successfully. At the

6-month follow-up, 125 patients were reached, 78 of whom did not smoke (62%). At the 12-month follow-up, 24 of the 40 patients remained smoke free (60%).

Conclusions: In the hope of better chance of survival and quality of life, patients are encouraged to quit smoking even when they are diagnosed with lung cancer. For this purpose, facilities treating lung cancer patients should develop cessation counseling clinics.

Intensive treatment of tobacco dependence: 10 years Czech experience

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Background: Treatment of tobacco dependence could be marked as a kind of chemoprevention of lung cancer. Unfortunately, it is not broadly available in Eastern Europe. There are about 40 centres offering this intensive treatment (intervention and pharmacotherapy) across our country.

Methods: Description of 2005–2014 activities of the Centre for Tobacco-Dependent in Prague, analysis of 12-months CO validated abstinence.

Results: During 10 years approx. 500 new smokers yearly visited our clinic with average no. of 6 visits during one year follow-up period. We analysed data of 4,355 patients, from them 3,368 with the complete record. From total number of patients including those who did not pass the intervention, 34.6% were abstinent after 12 months (9.9% of those without intervention solely), but from those passing the intervention 38.8% (1,307/3,368) did not smoke after one year. Patients not using pharmacotherapy (646) were successful in 16.1%, but among those using any kind of the first-line pharmacotherapy (nicotine, varenicline, bupropion and/or combination) 44.2% were abstinent. Duration of pharmacotherapy up to 3 months, 3–6 months, and 6–9 months led to abstinence in 30.2%, 63.4% and 73.0%, respectively.

Conclusions: Intensive treatment of tobacco dependence including intervention and long-term pharmacotherapy use is effective.

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Smoking and Oncology Nurses

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Background: Use of tobacco products contributes to one third of cancer cases worldwide. Oncology nurses are uniquely positioned to deliver evidence-based interventions for tobacco dependence including to patients already diagnosed with cancer. Nurses in the growing number of countries are taking leadership in promoting tobacco control (e.g. preventing uptake, helping patients quit, promoting a smoke-free environment, etc.). Those should be the leading example for other countries that recognize this large gap in lacking standard nursing care in smoking patients.

Methods: Outline responsibilities and impact oncology nurses have in smoking cessation within the professional role. Describe the existing wide differences in nursing practice with regards to smoking prevention and intervention across Europe with relation to the current situation in the Czech Republic, Hungary, Slovakia, Slovenia and Romania. Summary of officially accepted recommendations.

Results: Even after lung cancer diagnosis, smoking cessation is essential for improving quality of life and clinical outcome. Nurses in Eastern Europe, as well as oncologists, mostly poorly recognize this fact and they have different levels of knowledge equipping them to include this into routine nursing practice (plus often missing nursing guidelines). Adequate standardized training is essential but not naturally part of education curriculums here. Moreover, nurses themselves need to be leading non-smoking role models for patients and the wider society. Literature shows that nurses' smoking status is negatively connected with the use of intervention in patients who smoke.

Conclusions: Oncology nurses must realize their potential impact on the patients' health. By engaging in tobacco prevention and cessation, namely with oncology patients and families, they can contribute to improved quality of life and lung cancer diagnosis outcomes leading to years of saved life.

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Opening session

History of Central European Lung Cancer Conference – CELCC

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Central Europe (archaically "Middle Europe") – region lying between the variously defined areas of the Eastern and Western parts of the European continent. Central European Lung Cancer Conference has a long and successful history. The tradition of CELCC was established after the downfall of communism in Central Europe opening the doors for the real international scientific cooperation. Central Europe is the region with world-wide highest incidence rates of lung cancer in some of the countries. CELCC was first established to be a scientific forum for sharing current knowledge and research on lung cancer. The aim was to develop strategies for decreasing the burden of lung cancer in the Central Europe focused on improvement in prevention, early diagnosis, multimodality therapies, chemotherapy and radiotherapy with future research cooperation. CELCC was founded in the year 1992 in Prague.

The founders of CELCC:

- Anna Gregor, UK (born in Czechoslovakia)
- Jean Klastersky, Belgium (born in Czechoslovakia)
- Lubos Petruzelka, Czech Republic
- Petr Zatloukal, Czech Republic

International co-founders were Heine Hansen (Denmark), Jacek Jassem (Poland), Robert Pirker (Austria), and Guyla Ostoros (Hungary). The Central European Lung Cancer Conferences have a long academic tradition for over two decades and have regularly been held in various cities of Central Europe since 1992. These multidisciplinary conferences focused on both education and scientific developments in the field of lung cancer by offering symposia, oral sessions, poster sessions and satellite symposia. The first CELCC, under the auspices of IASLC, was held in May of 1992, at Prague with large attendance by participants from

throughout the world. The continuing CELCC were held yearly or every two or three years in the following locations: Ljubljana (1993), Prague (1995), Gdansk (1996), Prague (1998), Budapest (1999), Prague (2001), Vienna (2002), Gdansk (2004), Prague (2005), Ljubljana (2007), Budapest (2009), Prague (2011), Vienna (2014).

The list Central European Lung Cancer Board Members in period 1992–2015

- Rafal Dziadziuszko, Poland
- Wilfried Eberhardt, Germany
- Martin Filipits, Austria
- Anna Gregor, UK
- Heine Hanssen, Denmark
- Jacek Jassem, Poland
- Jean Klastersky, Belgium
- Manfred Manegold , Germany
- Gyula Ostoros, Hungary
- Lubos Petruzelka, Czech Republic
- Robert Pirker, Austria
- Gunta Purkalne, Latvia
- Rolf Stahel, Switzerland
- Johan Vansteenkiste, Belgium
- Milada Zemanova, Czech Republic
- Petr Zatloukal, Czech Republic

Basics of lung cancer immunotherapy

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Lung cancer immunotherapy is any interaction with the immune system to treat that cancer. In order to understand how different immunotherapies may bring benefits to patients, we need to understand the generation and regulation of the immune response in health and what goes wrong in cancer.

When encountering a foreign attack, e.g. bacterial, the body reacts with two different lines of immune defense, the innate and the adaptive immune response.

The *innate response* is the first-line defense by natural killer (NK) cells and phagocytes. NK cells recognize and attack host-unfriendly organisms, phagocytes such as macrophages and dendritic cells (DCs) then help to digest these pathogens.

In the *adaptive response*, there are several important steps. First, by digesting the pathogens, the DCs are able to present their digested antigens as peptides on their

surface in association with the major histocompatibility complex (MHC) receptors. In locoregional lymph nodes, this presentation – if in the presence of the required co-stimulatory signals (such as B7.1 and B7.2) – results in priming of different subtypes of immune cells (*priming phase*). CD4+ T helper cells augment the immune response by secreting interleukins 2 and 12 and interferon gamma, which enhances the activation of CD8+ T cells into cytotoxic T lymphocytes.

The latter cells then move to the periphery (*effector phase*), where they recognize the antigens of offending pathogens, which leads to the initiation of different processes, such as the release of pore-forming granzymes and perforins, resulting in destruction of the offender.

Once the offender is cleared, there are two next steps. One is creation of memory, by the generation of memory T cells, and by antigen-specific antibodies, produced by as B cells maturated into plasma cells under the influence of activated CD4+ T helper cells. The other is ending the inflammatory immune attach in order to protect normal tissue (e.g. avoid that viral infection of the liver continues into chronic hepatitis). This gatekeeper process is predominantly mastered by inhibitory immune cells such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and by expression of inhibitory checkpoints in the cascade, such as the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and the programmed cell death-1 (PD-1) receptor on the T-lymphocyte and other immune cells.

The process of ending the immune response is abused by cancers to evolve in a previously healthy individual, in a process of immune tolerance and immune escape. The cancer may downregulate of antigens or MHC molecules, may produce immunosuppressive compounds such as Transforming Growth factor beta (TGFb), iNOS or IDO, or cytokines that promote influx of Tregs and MDSC. But above all, the tumor induces checkpoints that favor tolerance, such as CTLA-4 and PD-L1.

Two types of lung cancer immunotherapy are discussed in an accompanying abstract. One is antigen-specific immunotherapy, aiming at specific priming of the immune system to recognize the tumor as foreign, thereby generating specific antibodies and/or cytotoxic T cells. This is attempted by the administration of tumor antigen(s) in combination with strong adjuvant(s) (*therapeutic cancer vaccines*). The other is promotion of anti-tumor responses by inhibiting gatekeeper mechanisms of the immune system such as negative T-cell regulators. Acting on T-cell immune checkpoints has delivered remarkable success for lung cancer therapy in recent years (*immune checkpoint inhibitors*).

Zatloukal Memorial Lecture
Lung cancer: Future strategies to decrease its world-wide burden

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Lung cancer remains the leading cause of cancer deaths with 1.6 million deaths worldwide in 2014. Lung cancer is divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC comprises about 80% of all cancers. World-wide management of lung cancer requires a broad approach involving primary prevention, early detection, accurate diagnosis and advanced care. Advances were achieved in all of these areas during recent years. Nevertheless, management of lung cancer remains challenging, mainly due to its both high numbers and complexity in terms of diagnosis and treatment. In addition, some areas such as prevention and early detection will require greater attention in the future.

The focus on primary prevention must be increased in order to successfully counteract the current worldwide epidemic of lung cancer. Tobacco control measures such as the "WHO Tobacco Free Initiative" that includes "WHO Framework Convention on Tobacco Control" and "MPOWER" will have to be more efficiently implemented and more strictly enforced in the future, particularly also in Central European countries (1, 2). The benefits of stopping smoking have clearly been proven. The United Kingdom Million Women Study demonstrated that quitting smoking even at the age of 50 years significantly reduced the relative risk of dying from lung cancer (3). The general public must also be better informed that the majority of lung cancers could be avoided simply by non-smoking.

An increasingly promising area is early detection of lung cancer. Screening with low dose CT in heavy smokers has recently been shown to reduce lung cancer mortality by 20% and overall mortality by 6.9% (4). Based on these findings, early detection by low dose CT in persons at high risk for lung cancer should reduce lung cancer mortality in the future and recommendations for screening have been published (5). An important challenge is the high rate of false positive scans. Research must focus on both better selection of persons at high risk for lung cancer and better techniques for the discrimination of pulmonary nodules. Regular follow-up of screened persons and screening registries must be part of screening programs. Therefore, CT screening by means of low-dose CT should initially be done in major cancer centers. After successful establishment and proper training of doctors, screening should be a more widely implemented. Screening should always be combined with tobacco cessation programs.

Major advances have occurred in the understanding of the biology of lung cancer. As a consequence, a more detailed classification of lung cancers has

become available. The term NSCLC should be replaced by the exact histological subtype, e.g. adenocarcinoma and squamous cell carcinoma. In addition, molecular classification of tumors has become routine standard. Molecular analysis currently involves determination of EGFR mutations and ALK status for all patients with advanced adenocarcinomas. Analyses of further molecular aberrations are expected to enter clinical practice in the near future.

Therapy of lung cancer requires a multidisciplinary approach. Treatment of lung cancer is based on tumor histology, tumor stage, performance status of the patients and other parameters. Therefore, accurate determination of both tumor histology (including molecular aberrations) and tumor stage is crucial for selecting the right treatment.

Major advances have been achieved in the systemic therapy of patients with advanced NSCLC. Systemic treatment consists of chemotherapy and also of targeted therapies, either alone or in combination with chemotherapy. The type of systemic treatment depends on patient parameters (age, performance status, organ functions and co-morbidity), tumor histology, presence or absence of driver mutations (EGFR mutations, ALK-translocations), toxicity and other parameters.

In the absence of driver mutations, patients with advanced NSCLC receive palliative chemotherapy which decreases cancer-related symptoms and prolongs survival of the patients (6, 7). Patients with good performance status and adequate organ functions receive first-line chemotherapy with platinum-based doublet that contains a third generation anticancer drug. Cisplatin-based protocols are preferred in patients with good performance status and adequate organ functions. Elderly patients and patients with reduced performance status also benefit from single agents or well tolerated doublets. Customized chemotherapy based on molecular alterations in the tumors has been attempted but until now failed to improve outcome within clinical trials and, therefore, remains experimental.

Advances have occurred by combining palliative chemotherapy with targeted therapies. Bevacizumab improved outcome of first-line chemotherapy in patients with advanced non-squamous NSCLC (8, 9) and has been approved in combination with platinum-based first-line chemotherapy for these patients. Cetuximab added to chemotherapy increased survival, particularly in patients with high EGFR expression in their tumors (10–12). Necitumumab increased survival when added to cisplatin plus gemcitabine in squamous NSCLC (13).

Maintenance therapy with pemetrexed or erlotinib is a treatment option for selected patients. At the time of disease progression, patients receive second-line therapy with docetaxel, pemetrexed or erlotinib. Recently, angiogenesis inhibitors (nintedanib, ramucirumab) added to second-line therapy with docetaxel have been shown to improve outcome compared to second-line chemotherapy alone (14, 15). Afatinib was shown to increase survival compared to erlotinib in patients with advanced squamous cell NSCLC (16).

Patients with driver mutations in their tumors receive treatment based on these molecular aberrations. Patients with EGFR mutation-positive tumors are preferentially treated in the first-line setting with EGFR-directed tyrosine kinase inhibitors (afatinib, erlotinib, gefitinib). In phase III trials, either of these drugs increased progression-free survival and improved quality of life compared to first-line chemotherapy (for review see ref.17). The pooled analysis of the LUX-Lung 3 and LUX-Lung 6 trials demonstrated a survival benefit for afatinib compared to cisplatin-based chemotherapy in patients with common mutations (18). This survival benefit was shown for patients with exon 19 deletions, while no difference in survival was seen in patients with L858R mutations. Third-generation EGFR tyrosine kinase inhibitors (AZD9291, rociletinib, HM61713) are in clinical development. They target EGFR-activating mutations and the T790M mutation, while sparing wild-type EGFR. Results from early trials look promising (19, 20) and corresponding phase III trials are ongoing. In patients with ALK-positive advanced NSCLC, crizotinib was shown to be superior to chemotherapy, both in the second-line and, more recently, also in the first-line setting (21, 22). Thus crizotinib has been approved for the treatment of patients with ALK-positive advanced NSCLC. Further therapeutic progress will be achieved by the second-generation ALK inhibitors (23).

Recent therapeutic advances have been achieved by immune checkpoint inhibitors (24). These include anti-cytotoxic T lymphocyte antigen-4 antibodies and antibodies directed against either the programmed death (PD)-1 receptor or the PD-ligand 1. PD-L1 is produced by tumor cells and binds to PD-1 receptors on T cells. This results in apoptotic death of T cells, thereby leading to reduced T cell activity. Immune checkpoint inhibitors inhibit PD-L1-mediated death of T cells. While anti-CTLA4 antibodies such as ipilimumab resulted in increased inflammatory and autoimmune toxicities, the latter antibodies are better tolerated. Several anti-PD1 or anti PD-L1 antibodies are in clinical development. Anti-PD1 antibodies include nivolumab and prembrolizumab. Both antibodies have already been studied in patients with lung cancer. Nivolumab added to docetaxel has resulted in increased survival compared to docetaxel alone in with chemotherapy pre-treated patients with advanced squamous cell carcinomas or adenocarcinomas (25, 26). Anti-PD-L1 antibodies also evaluated in patients with advanced NSCLC. Characterization of predictive biomarkers is ongoing but has turned out to be more challenging than anticipated. Challenges of biomarker characterization include source of the tissue (archived versus fresh tumor; tumor versus lymphocytes), differences in expression between primary tumor and metastases, standardization and validation of tests, cut-off levels for positivity, changes in expression levels over time or during treatment, and the potential use of a combination of markers.

Patients with early stage non-small cell lung cancer (NSCLC) undergo surgery with curative intent. Many patients, however, will relapse within 5 years. Adjuvant

cisplatin-based chemotherapy has been shown to increase survival in three randomized phase III trials (IALT, JBR.10, ANITA). In these trials, the increase in the 5-year survival rates ranged from 4% to 15%. The Lung Adjuvant Cisplatin Evaluation (LACE) meta-analysis, which was based on five cisplatin-based trials, demonstrated a survival benefit of 5.3% ± 1.6% at 5 years (27). A slightly higher improvement in survival was seen with vinorelbine-based chemotherapy (28). Thus adjuvant chemotherapy with a cisplatin-based doublet, preferentially cisplatin plus vinorelbine, has been established as a standard treatment for patients with completely resected NSCLC stages II and III. The establishment of adjuvant chemotherapy has been one of the major therapeutic advances within the last two decades.

Strategies to improve the outcome of adjuvant treatment for early-stage NSCLC are customized chemotherapy, targeted therapies and cancer immunotherapy. Great interest has been in several molecular features as potential biomarkers (29–31). Both ERCC1 and p237 were shown to predict outcome of adjuvant chemotherapy (29, 30). However, validation of these markers failed (32). Customized chemotherapy based on molecular tumor features is currently evaluated within clinical trials. Targeted therapies, either as single modality or in combination with chemotherapy, also have the potential to improve outcome of adjuvant chemotherapy. Bevacizumab added to adjuvant chemotherapy did not improve outcome in terms of progression-free or overall survival in the ECOG 1505 trial. Two trials (NCIC CTG BR19, RADIANT) also failed to demonstrate a survival benefit for adjuvant therapy with gefitinib or erlotinib in patients unselected for the presence of EGFR mutations. Phase III trials with EGFR tyrosine kinase inhibitors in patients with EGFR mutation-positive tumors are currently ongoing. The MAGRIT trial failed to show a benefit for the vaccination with MAGE-A3 vaccine after complete tumor resection plus/minus adjuvant chemotherapy. Immune checkpoint inhibitors hold promise but have yet to be evaluated in clinical trials in the adjuvant setting.

A major research area is the characterization of predictive biomarkers for targeted therapies. Predictive biomarkers will define those patients who will derive a benefit from targeted therapies. EGFR-activating mutations have been established as predictive biomarkers for EGFR tyrosine kinase inhibitors and ALK rearrangements for crizotinib. Important challenges of the characterization of predictive biomarkers are heterogeneity of biomarker expression, insufficient tumor tissue for molecular analysis, determination of the most appropriate laboratory test including proper cut-off levels, and priorization of biomarker assessments. Liquid biopsies will gain importance in the future. They use circulating cell-free DNA derived from tumor cells or circulating tumor cells for the assessment of biomarkers. Systemic treatments based on predictive biomarkers will become increasingly important in the future and development of targeted agents will be accompanied by biomarker development.

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Scientific session I
Immunology for clinicians

Immunotherapy and lung cancer

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Lung cancer a tobacco related disease is one of the most frequent human cancers and the leading cause of cancer related deaths. Although significant improvements both in the local management and systemic therapy results of treatment of both small cell and non-small cell lung cancer remains unsatisfactory for major group of patients diagnosed of this disease. Two solutions to that situation emerged recently. First one is to diagnose and cure cancer before metastatic spread by implementing LDCT screening to common practice. The second one is employing self immune system to eradicate or at least control cancer.

Immunotherapy was used for the first time at the end of 19th century by William Coley. After observation of bacterial infection causing regression of facial sarcoma he developed mixture of bacterial cultures called the Coley's vaccine. Some positive results of its use were described. Another observation of lower incidence of tumors among patient with active tuberculosis led to implementing BCG vaccine to cancer treatment. Local use of BCG is still one of the therapeutic options in bladder cancer.

There are two major types of immunotherapy, antigen specific (vaccination) and non antigen specific (immunomodulation).

There were numerous trials implementing many different vaccines and therapeutic approaches but recently several phase III trials revealed their results. Those trials included patient in early, locally advanced and metastatic disease.

In early stage NSCLC MAGRIT trial was performed. It was double blind randomized placebo controlled study of MAGE-A3 vaccine. It consists of a recombinant fusion protein of melanoma associated antigen (MAGE) A3 with protein D of H. influenzae and AS15 as immunostimulating adjuvant. Patient after

radical resection in stage Ib-IIIA and adjuvant chemotherapy were screened for MAGE-A3 presence on tumor cells in resected material. After confirmation of antigen positivity and completion of standard treatment with no signs of tumor relapse patients were randomized to receive vaccination or placebo. The trial showed no difference in disease free survival and was recognized as negative.

In locally advanced setting there was a signal of positive reaction for tecemotide (L-BLP25) (MUC1 based vaccine delivered in liposomal system) in phase II open label study. Mucinous glycoprotein-1 (MUC 1) a highly glycosylated transmembrane protein is normally found on the cell surface of many tissue types, but based on results showing trend to better response in stage IIIb NSCLC large phase III double blind placebo controlled trial was performed (START) in stage III NSCLC after concurrent or sequential chemoradiation. In overall population median OS was 25.6 months for vaccination arm and 22.3 for control arm (HR 0.88 p=0.123) and was recognized negative but in prespecified subgroup analysis significant difference was observed in concomitant chemoradiation part. Median OS was 30.8 months for tecemotide vs 20.6 months for placebo (HR 0.78 p=0.016). Vaccination was safe and well tolerated. The idea of tecemotide vaccination after the concurrent chemoradiation was planned to be tested in INSPIRE and START 2 trials but results of EMR 63325-009 study led to decision of termination of development tecemotide in NSCLC.

Another MUC1 targeting vaccine (TG 4010) was used in advanced NSCLC. This vaccine is based on genetically modified attenuated Ankara virus expressing both whole MUC-1 protein and IL-2. In phase II studies TG 4010 was added to standard chemotherapy (cisplatin + vinorelbine or cisplatin + gemcitabine) in treatment of patients with advanced NSCLC. In retrospective analysis it was found that best results is associated with low level of NK cells and further specified as triple positive activated lymphocyte (TrPAL – CD16+, CD56+, CD69+). During 16th World Congress of Lung Cancer results of phase IIb part of TIME study were presented showing significant improvement of both PFS and OS in non-squamous low TrPAL group. Phase III of the trial is ongoing and results are awaited.

Another vaccine tested in advanced stetting was belagenpumatucel-L an allogenic whole tumor cell vaccine. It was derived from 4 NSCLC lines (2 adenoca, 1 squamous and 1 large cell). Those cells were irradiated and transfected with plasmid containing TGF-b2 antisense transgene. In phase II study promising results were noted in group of patient with high dose comparing with those with low dose of vaccine. Base on that phase III study was initiated (STOP trial). The trial was generally negative but in the subgroup of patients randomized within 12 weeks after chemotherapy and treated with radiotherapy median OS was 40.1 for vaccination arm vs. 10.3 for control arm (HR 0.45 p=0.014).

There are also two vaccines from Cuba: EGF vaccine and racotumomab (1E10). The first one is recombinant human EGF combined with P64K Neisseria meningitidis protein with aluminium hydroxide and ISA 51 immunoadjuvants. There

was small phase II study published showing some trend to better survival, and the vaccine was licensed for use in Cuba. Actually phase III trial is recruiting patients in UK and Malaysia.

The second compound is racotumomab an anti-idiotype NeuGc-GM3 ganglioside vaccine, formed to target neoplastic cells as that variant of ganglioside was identified almost only on surface of those cells. Results of phase II/III data were presented on ESMO 2012 but not yet published. They showed significant improvement of median OS by 2 months (8.3 vs. 6.3 p=0.02). Based on those results vaccine was approved for use in Argentina and Cuba. Phase III study is recruiting patients in South America and Asia.

The other way of influencing immune system is using non antigen specific agents which targets different steps of immune response. Among those compounds called immunomodulators appeared very promising group named immune check-point inhibitors. The mostly advanced in development are agents influencing T-cell activity by inhibiting CTLA-4, PD-1 or PD-L1. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) plays the role in giving inhibitory signal to T-cells when binding to dendritic cell presenting antigen. It prevents activation of T cell and further immune response. When anti CTLA-4 antibody mute suppressive signal then activation and proliferation of T cells is possible.

Ipilimumab, a humanized IgG1 directed against CTLA-4 was tested in lung cancer in two different manners. It was added to first 4 courses of chemotherapy (concurrent sequence) or started with 3rd cycle (phased sequence) and compared with placebo. This phase II study (CA184-041) showed significant improvement of irPFS (immune related progression free survival) of phased ipilimumab and not the concurrent one for both non small cell and small cell lung cancer. There was also similar trend in OS improvement. Following those results phase III trials were initiated and are ongoing.

Another way of T cell activity modulation is influencing on binding programmed death-1 (PD-1) receptor with its ligand. This action take place both in contact of T cell with dendritic cell and mainly when T cell approaches tumor cells and limits the activity of effector cells leaving tumor cells intact. Disrupting this binding is connected with restoration of antitumor immune system activity. There are both anti-PD-1 (nivolumab, pembrolizumab) and anti-PD-L1 (atezolizumab, durvalumab) antibodies.

Nivolumab a fully human IgG4 antibody was found to be active in NSCLC during phase I study (CA209-003) with refractory solid tumors showing both activity and durable responses. That efficacy was confirmed in two second line phase III trials comparing nivolumab with docetaxel in squamous and non-squamous histology (CheckMate 017, CheckMate 057). This compound reduces risk of death by 41% in SQ-NSCLC and 27% in non-SQ-NSCLC. In both trials significant prolongation of PFS and OS was shown as well as increasing 1-year survival. Analysis of survival curves shows that there is durable response in about 25% of patient´s population

expanding over 18 months. Based on those results nivolumab was registered in second line in US and Europe.

Another anti PD-1 antibody is pembrolizumab (humanized IgG4 Ab) was tested in phase I study (KEYNOTE 001) showing promising activity (ORR 18.4%, mOS 12 months). The trial also looks for biomarker of efficacy and found that PD-L1 positive staining with cut-point of 50% is a valid one. The drug was approved by FDA in PD-L1 positive NSCLC and other trials are ongoing.

For anti PD-L1 antibodies (atezolizumab, durvalumab) some data was presented on recent meetings showing also promising activities. In the process of atezolizumab development a new strategy of evaluation of PD-L1 positivity not only on tumor cells but also on immune cells was presented (POPLAR study).

In the further development of immune check point inhibitors the other strategies are currently tested. They include first line and adjuvant setting, combination of anti-PD-1 or anti-PD-L1 with anti-CTLA-4, duration of treatment and using in SCLC treatment.

Toxicity of modern lung cancer immunotherapy

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Introduction

Lung cancer is a leading cause of death worldwide, namely non-small cell lung cancer (NSCLC); over the last 30 years, standard therapy for NSCLC was based on chemotherapy with platinum-based doublets, with overall survival (OS) rarely reaching 12 months (1).

Tyrosine kinase inhibitors targeting epidermal growth factor mutations or anaplastic lymphoma kinase translocation have improved median OS in NSCLC up to 24–36 months in limited and selected populations (2). These newer therapies present with a wide spectrum of adverse events, different from those associated with chemotherapy; the adverse effects of which targeted therapies have been reviewed recently (3).

Currently, efforts are under way to develop new immunotherapies namely the checkpoints inhibitors which make now immunotherapy a reality for the treatment of NSCLC (4). The new approaches, as well as older therapies, are associated with specific types of adverse events; these aspects have been reviewed recently but that analysis was not focused on lung cancer (5).

The present review focuses on immunotherapies which have been used recently in NSCLC; so far, relatively few studies have been published under a form allowing

to comprehensively evaluate the side effects, although many such investigations are under way and will be available in a near future (6).

Material and methods

We reviewed the available information published in peer-reviewed papers as phase 2 or phase 3 studies of immunotherapy using checkpoints inhibitors in NSCLC. The therapies evaluated were pembrolizumab (7), nivolumab (8, 9) (2 anti-PD antibodies) and BMS 936559 (10) (an anti PD-L1 antibody); although in this latter case, there were only 75 patients with NSCLC among a total of 207 patients evaluated. Because anti CTLA-4 inhibitors such as ipilimumab and tremelimumab have not been comprehensively evaluated in patients with NSCLC, we used the extensive experience accumulated for ipilimumab in melanoma (11). It should be stressed, however, that tremelimumab, a monoclonal antibody directed against CTLA-4 has been used in 87 patients with NSCLC; the overall incidence of AE grade 3/4 was 20%, mostly consisting of diarrhea and colitis (9%) (12).

Similarly, because no solid information is available so far on the combination of anti-PL and anti CTLA-4 antibodies in lung cancer, we used the only available study, performed in melanoma (13).

We analyzed the frequency in those different studies for reported adverse effects using the published tables, comments and supplementary appendices, when available. Those adverse events observed with a frequency < 2% (overall) were not included in the overall analysis (dry mouth, dysgeusia, dizziness, erythema, back pain, ↑ bilirubin, dry skin, hypocalcemia, insomnia, pain in the extremities, ↑ LDH, conjunctivitis, uveitis, dyspepsia, eczema, hair color change, muscle spasms, bone bain, flushing, memory impairment, neutropenia, pharyngeal pain, peripheral edema, URT infection, hypoglycemia, anemia, myocarditis, sarcoidosis, myasthenia gravis). However, we analyzed separately events of special of interest with potential immune related causes and the incidence of infusion-related complications.

Results

As shown in Table 1, the most common adverse effects (AE) observed in ≥ 5% of patients with NCLC receiving pembrolizumab were fatigue, asthenia, pruritus, skin rash, decreased appetite, diarrhea, nausea, arthralgia and hypothyroidism; the frequency of such AE with a grade > 3 was less than 1%. This spectrum of AE is almost identical to that observed in melanoma patients receiving pembrolizumab (14). Patients with NSCLC receiving nivolumab showed a similar pattern of AE; fatigue, myalgias, pruritus, rash, decreased appetite, nausea, loss of weight, transaminitis, vomiting and arthralgia were observed in ≥ 5% of the patients with no grade > 3 or in less than 1%. BMS-336559 (an anti PD-L1 antibody) showed the same pattern of AE; fatigue, pruritus, rash, diarrhea, nausea and arthralgia were seen in ≥ 5% with no or very rare grades > 3.

Table 1 – Frequency of adverse effects > 2% (% of any grade; % of grades > 3)

	Pembro-lizumab		Nivolumab		Nivolumab + Ipilimumab (Melanoma)		Ipilimumab (Melanoma)		BMS-936559 (≠ tumors)	
N° patients	459		389		94		256		207	
Fatigue	19	< 1	9	1	39	5	15	1	16	1
Asthenia	5	< 1	4	0	9	0	6	< 1	–	–
Pyrexia	4	< 1	2	0	20	3	2	0	3	0
Myalgia	3	< 1	5	1	10	0	2	< 1	1	0
Pruritus	11	0	5	1	35	1	25	< 1	6	0
Rash	10	0	5	0	41	5	14	< 1	7	0
Acneiform rash	3	< 1	–	–	16	3	< 1	0	–	–
↓ Appetite	10	1	5	1	15	0	8	0	3	0
Diarrhea	8	2	3	0	45	11	23	3	9	0
Nausea	7	< 1	13	0	22	1	9	< 1	6	0
↓ Weight	4	0	9	0	–	–	2	< 1	–	–
Aspartate amino-t	3	< 1	9	0	–	–	2	< 1	–	–
Colitis	–	–	–	–	23	17	7	6	2	0
Pancreatitis	1	0	2	0	11	5	6	0	–	–
Vomiting	3	< 1	5	0	14	1	5	0	1	1
Dyspnea	4	< 1	4	0	10	3	1	< 1	1	0
Pneumonitis	4	2	2	2	11	2	< 1	0	2	1
Arthralgia	9	< 1	5	0	11	0	5	< 1	7	0
Hypothyroidism	7	< 1	4	0	16	0	< 1	0	3	0
Other immunologic complications*	3	< 1	–	–	12	2	2	< 1	–	–

*see Table2 + Hypophysitis

There is no yet reported large experience in NSCLC with ipilimumab; the experience gained in patients with melanoma with the compound, shows a similar pattern of AE, with fatigue and asthenia, skin manifestations, digestive symptoms and arthralgia being seen in ≥ 5% of the patients. Most of these manifestations were more frequent than with anti-PD antibodies but were not more severe, as grade > 3 were not seen in more than 1% of the patients, with the exception of diarrhea (3%). This has perhaps to do with the higher frequency of colitis: 7% of the patients with grade > 3 in 6%; pancreatitis was observed in 6% of the patients. These 2 AE, presumably of auto-immune origin, are seen only occasionally in patients receiving anti-PD or anti-PDL antibodies but, as already mentioned. Tremilimumab, another anti CTLA-4, was associated with a high frequency of diarrhea and 9% of colitis.

Table 2 – Frequency of death and of discontinuation of therapy and endocrine – autoimmune complications (in addition to those indicated in Table 1)

	Pembrolizumab (NSCLC)	Nivolumab (NSCLC)	Nivolumab + Ipilimumab (melanoma)	Ipilimumab (melanoma)	BMS 936559 (NSCLC Et a)
N° of patients	459	389	94	256	207
Died (%)	0.2	2	3	0.3	1
Discontinued therapy because AE (%)	0.2	4	45	9.0	6
Infusion problem (%)	3.0	0	0	0.0	10
Endocrine-autoimmune manifestations in ≤ 2%	Thyroid ↑	Nephritis Colitis Adrenal ↓	Hypophysitis Adrenal ↓ Tyroid ↑ Vitiligo	Hypophysitis Nephritis Tyroid ↑ Myositis Adrenal ↓	Hepatitis Sarcoidosis Endophtalmitis Diabetes Myosthenia Tyroid ↑

A very striking increase of frequency and severity of these AE is observed in patients receiving anti PD and anti CTLA-4 antibodies (though our experience is based only on melanoma patients). General manifestations (fatigue, asthenia, pyrexias), skin disorders and gastro-intestinal symptoms are more frequent and more severe than in patients receiving anti-PD or anti CTLA-4 antibodies alone. In particular diarrhea was present in 45% of the patients (with 11% having grade > 3); colitis was seen in 23% of the patients with 17% with grade > 3. Pancreatitis was observed in 11% with 5% severe cases.

Pneumonitis, presumed to be due to auto-immunity, has been reported with a < 5% incidence in patients receiving pembrolizumab, nivolumab, ipilimumab or BMS 936559 with occasionally a severe case; as will be discussed later, pneumonitis is the most common fatal AE reported with these agents in our review, an observation already made by others (14). In case of a combination of nivolumab and ipilimumab, the frequency of pneumonitis was 11% with 2% of cases with grade > 3; clearly higher than the rates observed with nivolumab or ipilimumab alone.

Another type of AE, presumably of auto-immune origin are a series of endocrinologic manifestations, the most common being hypothyroidism, which was present in < 10% of the patients receiving monotherapy but which frequency was 16% in those receiving nivolumab + ipilimumab; no severe case was reported. Other endocrine AE (such as hyperthyroidism, adrenal insufficiency, hypophysitis) are less frequent (< 5% of the patients) but their frequency, namely hypophysitis, which was seen only in patients receiving ipilimumab as already reported in the literature (15), strikingly increased in patients receiving nivolumab + ipilimumab. Other auto-immune manifestations that are seen occasionally are listed in Table 2.

As shown in Table 2, the mortality associated with the use of checkpoint inhibitors was relatively low (0.2–3%). Pneumonitis was the most frequently reported AE associated with a fatal outcome and this was observed with all regimens, except ipilimumab alone; in that case the only reported death was due to cardiac arrest caused by severe ionic troubles related to severe diarrhea. Discontinuation of therapy because of AE was rare with pembrolizumab (0.2–0.4%) and was due to infusion reactions and renal failure. The discontinuation rate was higher for nivolumab (3–12%), ipilimumab (9%) and BMS 936559 (6%); for the latter, infusion problems were the most common cause for discontinuation but for the other drugs, it was clearly pneumonitis. The rate of discontinuation, because of AE, rose to 45% with the combination nivolumab + ipilimumab; the most common causes for discontinuation were severe skin reactions and pneumonitis; nonetheless, more than 40% of these patients could be continued on nivolumab alone. A discussion of the management of immune-related toxicities associated with checkpoint inhibitors are beyond the scope of this review but recommendations are available from the literature (16, 17).

To summarize our review, it can be stated:

- overall, AE of checkpoints inhibitors are relatively unfrequent. The most common manifestations are fatigue, pruritus, rash, loss of appetite, nausea, and arthralgia. Most are not severe. Adequate therapy (corticoids mainly) and/or replacement treatment are often effective
- the most threatening AE is pneumonitis, which can be responsible for drug discontinuation and, occasionally cause death
- hypothyroidism is the most common endocrinologic AE followed by hypopituitarism, that is clearly associated with ipilimumab; replacement therapy is effective
- AE are to some extent drug-specific but do not appear to be tumor-specific
- the wide variety of symptoms and signs associated with the AE linked to checkpoint inhibitors requires an approach based on a solid knowledge of internal medicine

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Ongoing and future trials in lung cancer immunotherapy

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In an accompanying abstract, we discussed the underlying biology leading to two main types of immunotherapy for lung cancer. One is antigen-specific immunotherapy, aiming at specific priming of the immune system to recognize the tumor as foreign, thereby generating specific antibodies and/or cytotoxic T cells. This is attempted by the administration of tumor antigen(s) in combination with strong adjuvant(s) (*therapeutic cancer vaccines*). The other is promotion of anti-tumor responses by inhibiting gatekeeper mechanisms of the immune system such as negative T-cell regulators. Acting on T-cell immune checkpoints has delivered remarkable success for lung cancer therapy in recent years (*immune checkpoint inhibitors*).

The clinical benefit from therapeutic cancer vaccines remains disappointing until now.

In the largest therapeutic study ever performed, the *MAGE-A3 vaccine* was tested in a population that has long been considered to be optimal candidates: patients with minimal residual disease, in case patients with resected stage I-IIIA non-small cell lung cancer (NSCLC)[1]. Almost 14,000 surgical patients were screened, 4210 patients were MAGE-A3 positive (33%), and 2312 patients were randomized. The median DFS (primary endpoint) was slightly better with MAGE-A3 (60.5 versus 57.9 months), but the difference was unfortunately not significant (Hazard Ratio (HR) 1.02, 95%CI: 0.89, 1.18, *P*=0.74). No subgroups with potential benefit could be identified. Based on this disappointing result, further development of the MAGE-A3 vaccine in NSCLC was abandoned.

The mucin MUC1 expression, altered mainly by aberrant glycosylation, is another target for NSCLC vaccination. *Tecemotide* (a tandem repeat MUC1-peptide in a liposomal formulation) was studied in a large phase III, double blind, randomized controlled trial (RCT) comparing maintenance therapy with Tecemotide (n=829) or placebo (n=410) in patients with unresectable stage III NSCLC who did not progress after sequential or concurrent chemoradiotherapy[2]. The primary endpoint – OS – was not significantly different between the vaccine and placebo group (25.6 and 22.3 months). However, pre-planned subgroup analysis showed that the patients treated with concurrent chemoradiotherapy (N=829) had a 10.2-month improvement in OS (30.8 versus 20.6 months, adjusted HR 0.78, *P*=0.016). The consequential trial was START 2, a similar large RCT in patients who completed concurrent chemoradiotherapy for unresectable stage III NSCLC (NCT02049151). However, this RCT and further development of Tecemotide was abandoned after disappointing results of a smaller trial in Japanese patients with stage III NSCLC and concurrent chemoradiotherapy.

Table

Target	Agent	Class	Current status
PD-1	Nivolumab (MDX 1106, BMS-936558)	IgG4	FDA approved relapsed squamous & non-squamous NSCLC EMA approved relapsed squamous NSCLC
	Pembrolizumab (MK-3475)	IgG4 engineered/ humanized	FDA approved relapsed sq & non-sq with biomarker PD-L1 positive+
PD-L1	Atezolizumab (MPDL3280A)	IgG1 Fc engineered	Randomized data available [POPLAR]
	Durvalumab (MEDI4736)	IgG1	Randomized trials ongoing
	Avelumab (MSB0010718C)	IgG1	Randomized trials ongoing

MUC1 continues to be evaluated in the clinical trial program of *TG4010*. This is a vaccine based on a recombinant viral vector (attenuated strain of vaccinia virus) expressing both the tumor-associated MUC1 and interleukin-2. It is explored in an ongoing phase IIB/III RCT (TIME trial, NCT01383148) in the setting of first-line therapy of NSCLC. In the phase IIB part, a potential biomarker – level of activated Natural Killer (NK) cells – was reported to be predictive based on a PFS endpoint[3]. The phase III part of the trial continues in patients with non-squamous NSCLC.

In contrast, a quite impressive sequence of positive results was noted with *checkpoint inhibitors* over the last 5 years. The publication of the very first phase I results with nivolumab goes back to just 2010![4] The agents in more advanced development for NSCLC are listed in the Table. These agents can directed against the programmed cell death-1 (PD-1) receptor or the programmed cell death-1 ligand (PD-L1); they can be IgG4 or IgG1 antibodies, sometimes engineered; they are in various phases of approval or clinical testing.

The Anti-PD-1 antibodies are the most advanced in development. *Nivolumab* (MDX 1106, BMS-936558) now is approved for relapsed NSCLC in the US and the EU (squamous histology only). Nivolumab has been approved without any predictive biomarker based on two phase III trials, one in squamous and one in non-squamous relapsing NSCLC[5,6]. *Pembrolizumab* (MK-3475) was studied in a large phase I expansion study with special emphasis on the role of PD-L1 immunohistochemistry to predict patient benefits[7].

Of the anti-PD-L1 antibodies, only one RCT has been reported until now[8]. At the last ECC meeting, the primary analysis showed in a significantly benefit overall survival of *Atezolizumab* versus docetaxel in patients with relapsed NSCLC. A confirmatory phase III trial is expected to be reported soon. From the other two compounds in this class, Durvalumab and Avelumab, no RCT data have been reported yet.

Ongoing and future trials will be further discussed at the meeting.

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Predictive biomarkers of immune response and immunotherapy

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There are not many areas of oncology with such fast development in the last decade as diagnostics, classification and treatment of non-small cell lung cancer. Some while ago, the basic differentiation between small cell- and non-small cell carcinoma was fully sufficient as in the group of NSCLC there were no differences in therapeutic approach between e.g. adenocarcinoma and squamous cell carcinoma. Thus, any additional subclassification of NSCLC was beyond the therapeutic needs. Only discovery of novel treatment options, which do work best in certain subgroups of NSCLC required distinction of heterogeneous NSCLC category into adenocarcinoma, squamous cell carcinoma, large cell carcinoma etc. However, even this precise morphological typing is not sufficient anymore. The emerging biologic treatment targeting various molecular signaling pathways is efficient only in patients with neoplasms bearing certain molecular changes, most often one of the so called driver mutations – typically EGFR activating mutation or EML4/ALK gene rearrangement. Thus the classical morphologic diagnosis must be – at least in certain tumor types – accompanied (supplemented) by result(s) of molecular test(s). According to our current knowledge, individual driver mutations are usually mutually exclusive, therefore EGFR positive NSCLC virtually never shows e.g. ALK rearrangement. Therefore, in addition to morphological classification, tumors can be classified also on the basis of their molecular characteristics. This approach helps the physician in decision making what particular drug(s) should be considered for treatment.

Based on these new data, the diagnostic guidelines have been updated in many countries (including Czech Republic) and molecular testing is nowadays a diagnostic procedure implemented as routine step in complex diagnostics of NSCLS.

Unfortunately, majority of clinical studies focused on use of targeted therapy, such as TKI, show promising results regarding prolongation of progression free survival, but only very few studies were successful in demonstration of prolongation of overall survival. Thus, majority of patients on targeted treatment do benefit of it for certain period, however, sooner or later does the neoplasm progress and the treatment fails. The mechanisms of development of resistance to treatment are still not fully understood. For example, in case of TKI treatment, secondary resistance mutations such as T790M do develop and are responsible for overgrowth of neoplastic clone resistant to original therapy. Despite the fact that new generations of TKI are on the way and these can more or less effectively target even recurring neoplasms bearing these resistance mutations, the fact that even these drug inevitably will fail and the cancer will progress is highly probable. The reason for development of resistance is quite clear. Malignant neoplasms are extremely plastic "organisms" and as such, they are able to adapt in accordance to the changes of the environment. Thus, any targeted treatment represents a selective pressure on neoplastic population and cells with signaling alternative bypassing the blocked molecular pathway can survive, grow and sooner or later replace the original tumor mass.

Are there any chances how to overcome or eliminate this principal limitation of our therapeutic efforts? To be able to answer this question, one has to return back to our understanding of tumor biology. The most important point which we ignore in the current targeted approach is the fact that malignant tumor is not just a cluster of neoplastic cells. These cells are able to grow, multiply and survive only thanks to the neoplastic stroma, which represents an integral part of the tumorous lesion and as such has strong influence on the biological behavior of the disease. Stroma does not only serve as a "skeleton" of the neoplasm, it modifies its properties, supplies via network of capillary vessels oxygen and various nutrients, stromal fibroblasts produce signaling and regulatory molecules such as cytokines and growth factors, which are distributed to the neoplastic cells both via the microvessels as well as by diffusion through the extracellular matrix. Another essential component of the tumor stroma are various types of inflammatory elements – granulocytes, histiocytes and lymphoid cells. Each component plays a crucial role and only successful orchestration of all factors together gives the neoplasm full invasive potential.

The current strategies in systemic therapy are mostly targeted on the neoplastic population itself. The only exception is blockage of microvessels by the use of antiangiogenic therapy. However, there remains significant – so far not fully harnessed – potential in the influencing of other components of tumor stroma. The currently most promising are of novel approaches is influence of anti-tumor immune response by immune checkpoint inhibitors.

For the tumor to become clinically significant, neoplastic population must escape from the immune surveillance of the organism. This is usually achieved by active blockage of the immunity using both suppression of effector populations of immune cells (such as NK lymphocytes) and stimulation of regulatory T-cells (Tregs) with inhibiting effect on anti-tumor immunity. The idea of unblocking of immune response and thus helping the organism to fight the cancer by own means have proven to be effective in the clinical trials with new therapeutic antibodies targeted against different molecules regulating the immune response, such as anti-CTLA4 ipilimumab or anti-PD1 nivolumab or pembrolizumab. Other molecules are emerging and the results from additional clinical studies should be available quite soon.

Unfortunately, these quite promising molecules prove to be efficient only in minority of patients. At this moment, there are no evidence based clinical or molecular markers which could be used to predict the effect of the treatment in individual patients. Therefore, there is an enormous need for identification of such markers to make the treatment more efficient.

While there are no putative predictive markers available so far for the ipilimumab therapy, there has been done a lot in the research of predictive markers for anti-PD1 treatment (pembrolizumab and nivolumab). At least in certain tumor types, one of the ligands for PD1 receptor, namely PD-L1 molecule is evaluated as a potential predictor of anti-PD1 treatment efficacy. This ligand is expressed in many neoplasms and as such can be detected by immunohistochemistry in the samples of tumor tissue. Unfortunately, only little is known about the pattern of expression of the molecule – both temporal and spatial heterogeneity exists and it is not established, what is the optimal way for evaluation of expression regarding the distribution of the molecule (center of the tumor vs. its periphery), significance of expression in different cell types (positivity in neoplastic cells vs. expression by the lymphoid infiltrate) or threshold of positivity. In addition, only very little is known about the dynamics of the expression during the course of the disease, how much is the expression influenced by the coincident treatment by other medicaments modifying the immune reaction (such as cytotoxic chemotherapy or use of corticosteroids). Yet another variable not solved so far is the fact that different clinical studies used different methods for detection of the PD-L1 expression in the tissue and so far, there exists no study comparing the pattern and intensity of expression of PD-L1 detected by various primary antibodies and detection kits.

Another puzzling issue is the fact that whereas PD-L1 is strongly predictive of treatment effect in certain neoplasms (such as lung non-squamous carcinoma), it has not been predictive in other neoplasms (such as melanoma or squamous cell lung carcinoma). Therefore, although it is not fully clear if the expression of PD-L1 can be used in the latter mentioned tumors, it is highly probable that this marker will be used as a predictor required for starting the treatment in the lung

adenocarcinoma. Therefore, a network of histopathological laboratories, which are currently testing predictive markers for other drugs (such as EGFR mutations for the use of TKI or EML4/ALK rearrangement for the use of anti-ALK therapy) will need to introduce standardized immunohistochemical protocols for these tumors. To guarantee constant optimal performance of the testing, all laboratories have to use appropriate internal controls as well as participate in the external quality assurance program.

In conclusion, the testing of predictive markers enabling us to select those patients who will with highest probability benefit from the immunotherapy has to be established. However, there are several key questions which have to be answered before the predictive value of these markers can be considered as fully evidence based.

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Scientific session II
Lung cancer in Central Europe

TULUNG – clinical registry of patients with non-small-cell lung cancer (NSCLC)

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Introduction: TULUNG is a clinical registry of NSCLC patients. It is a joint project of the Czech Pneumological and Phthiseological Society, the Czech Society for Oncology, and the Institute of Biostatistics and Analyses of the Masaryk University. The registry contains clinical data on patients who have been treated with afatinib, bevacizumab, erlotinib, gefitinib, crizotinib, nintedanib, and pemetrexed. From September 2005 to March 2015, a total of 4,983 patients have been treated with the above-mentioned preparations and recorded in the registry. The analysis was performed on data on patients who have a record on treatment with the above-mentioned preparations, and the following parameters have been recorded: sex, date of birth, smoking status, histological and/or cytological type of primary tumour, performance status at the time of treatment initiation, date of treatment initiation.

Results: The registry contains the highest number of records (3,537) on patients treated with erlotinib: it has been used in 371 patients as first-line treatment, 1,786 patients as second-line treatment, 1,330 patients as third-line treatment, and 50 patients as fourth- and higher-line treatment. Pemetrexed is the second most commonly administered drug, as it has been used in a total of 1,963 patients with complete records: 812, 959, 168 and 24 patients as first-line, second-line, third-line and fourth-line treatment respectively. A total of 201 patients treated with bevacizumab in first-line treatment have been recorded. There are 227 complete records on patients treated with gefitinib. Recently, there have been records on patients treated with afatinib, crizotinib, and nintedanib.

Conclusion: Results of the analysis as defined by the subpopulation from the TULUNG registry differ from data obtained in randomised controlled trials in many aspects, and their interpretation is determined by certain limitations. However, the registry provides a reliable illustration of costly treatment administration in real clinical practice. In some parameters, the difference from published results of randomised controlled trials is probably also caused by a less strict methodology. The frequency of adverse events should be interpreted with caution. In clinical trials, patients are actively encouraged to report even relatively minor adverse events; in real clinical practice, however, only adverse events obviously linked to therapy are usually considered to be reportable: severe laboratory toxicities, unexpected adverse events, or non-laboratory adverse events which make patients complain spontaneously. From the methodology point of view, overall survival is the relatively least vulnerable parameter. Despite the above-mentioned shortcomings, it is clear which drugs have been used in specific patients and how frequently they have been used; this knowledge is subsequently very useful in talks within expert medical societies, and in negotiations with health care payers.

Oncogene-directed therapies in non-small-cell lung cancer (NSCLC)

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Conflict of interest
Author has no conflict of interest to declare.

Introduction
In the last decade, major progress has been made in the treatment of advanced non-small cell lung cancer (NSCLC) through a better understanding of the molecular biology of lung cancer, identification of some oncogene drivers and development of oncogene-directed drugs. At present, there are at least ten known molecular biomarkers that characterize NSCLC transformation and may represent critical oncogenic drivers amenable to targeted therapy (1). Oncogene-directed therapies with EGFR and ALK TKIs in conjunction with well-validated methods for the detection of activating EGFR mutations or ALK rearrangements are now standard practice in approx. 20% of advanced NSCLC Caucasian patients harboring those genetic alterations (2). Treatment with targeted agents improved median survival rates of advanced NSCLC patients with oncogene-driven NSCLC up to an unprecedented 35 months, with major improvements in quality of life and symptom control (reviewed by Cufer & Knez, 3). Encouragingly, in the recent years a number of additional oncogenic drivers beyond EGFR mutations and ALK rearrangements

have emerged as novel molecular targets with potential therapeutic implications, such as ROS1 and RET rearrangements, BRAF and HER2 mutations and MET amplification (Table 1).

EGFR-directed therapy

The discovery of somatic mutations in the tyrosine kinase-domain of the EGFR gene, recognized as an oncogenic driver of NSCLC, has been followed by intensive research, which confirmed EGFR activating mutations as strong predictors for response to EGFR TKIs (4). Activating mutations in EGFR are present in 10–35% of NSCLC patients and are more frequently in non-smokers, women, individuals of Asian ethnicity and those with adenocarcinoma histology. In the last few years, seven large prospective randomized clinical trials uniformly confirmed the superiority of first- (gefitinib, erlotonib) or second-generation (afatinib) EGFR TKIs over chemotherapy in the first-line therapy of EGFR-mutated NSCLC (3). In all seven trials significant improvements in response rate and progression-free survival were obtained by EGFR TKIs compared to chemotherapy. Treatment with EGFR TKIs led to high response rates (from 56% to 83%) and long median PFS (from 9.2 to 13.1 months), never before observed in advanced NSCLC. In both arms remarkably long median OS rates (from 19.3 up to an impressive 35.5 months) were achieved. However, probably due to a high, up to 94.6%, cross-over to EGFR TKI after chemotherapy failure in the individual trials no significant differences in survival were observed. Most recently, a significant OS benefit of first-line EGFR TKI over chemotherapy was reported in a large pooled analysis of two, LUX-Lung 3 and the LUX-Lung 6 trials. Despite a high, 70% crossover, afatinib treatment resulted in a significant OS advantage (HR 0.81, 95% CI 0.66–0.99, p = 0.037) over chemotherapy in the pooled analysis (5). In addition, the pre-planned subset analyses of PFS and OS according to the type of EGFR-mutations performed in these two trials pointed out to a possible role of Del19 as the most EGFR TKIs sensitizing mutation (5). Treatment with EGFR TKIs was uniformly associated with a more favorable toxicity profile compared to chemotherapy and improved quality of life in the pivotal trials.

As a result of this data EGFR determination by RT-PCR and EGFR-directed therapy with first- or second-generation EGFR TKIs nowadays, represents the standard first-line therapy for advanced NSCLC with activating EGFR mutations (6). This practice has been successfully implemented through international lung cancer collaboration (INSIGHT registry) also in our region (7).

ALK-directed therapy

Yet another important development in the oncogene-directed therapy of NSCLC is the discovery of ALK rearrangements that led to the development and fast approval of a new class of agents, the ALK TKIs (8). ALK rearrangements are present in approximately 4% of NSCLC patients, which are typically

adenocarcinoma, younger, male and never- or light-smokers. Patients with ALK rearrangements demonstrated an extraordinary response to the MET and ALK oral inhibitors crizotinib and ceritinib. In an extended phase I trial, enrolling patients with ALK-positive advanced NSCLC, a 60.8% objective response rate (ORR) was observed, with a median PFS of 9.7 months in the overall group of patients and an enthusiastic median PFS of 18.3 months in the 24 patients receiving crizotinib as first line therapy (9). The superiority of crizotinib over chemotherapy was shown in two phase III trials, PROFILE 1007 and PROFILE 1014 comparing crizotinib versus chemotherapy in second and first-line treatment of ALK-positive disease. Both studies reported significant improvements in PFS and RR; again, probably due to a high 87% of crossover to crizotinib after chemotherapy failure no significant differences in OS were observed (reviewed by Cufer & Knez, 3). In addition, the next generation ALK inhibitor ceritinib has already shown strong antitumor efficacy. In the extended phase I (ASCEND-1) trial a high response rate of 60% and a median PFS of 7.0 months was achieved in the whole group of ALK-positive patients, with a notable efficacy observed also in ALK-pretreated patients (10). Based on this data, nowadays, ALK determination by FISH followed by ALK-directed therapy in biomarker-positive patients represent a gold standard for advanced NSCLC (6).

Treatment beyond progression on EGFR and/or ALK-directed therapy

Despite the marked initial responses to EGFR TKIs and/or crizotinib, patients inevitably develop resistance to first-line EGFR TKI and/or crizotinib, mostly in one to two years' time. In half of the patients it is attributed to the development of secondary genetic alterations of the target, such as the EGFR T790M resistant mutation or the ALK L1196M gatekeeper mutations, while in the rest of the patients the resistance appears through the activation of alternative signalling pathways via aberrant oncogenes, such as MET amplification, PIK3CA mutation and HER2 amplification, etc. (1). To overcome resistance several new generation TKIs and drug combinations are being developed. The first results for some of the next generation EGFR TKIs, such as the AZD9291, CO-1686, indicate extremely promising efficacy and good tolerability in patients with T790M mutation and are under the process of registration by FDA and EMA (11).
A different approach to overcome EGFR TKI resistance is the dual inhibition with EGFR TKI and anti-EGFR antibody. The combination of afatinib and cetuximab which led to highly promising results in a phase II trial is now being evaluated in two large prospective phase III randomized trials. To address crizotinib resistance multiple, new-generation ALK TKIs such as ceritinib, alectinib, AP26113 and HSP90 inhibitors, such as ganetespib are under development. As already mentioned, first results are encouraging; the ALK TKI ceritinib led to high 55.4% RR and a PFS of 6.9 months in the subgroup of 121 crizotinib pre-treated patients (10). In addition, some of these agents, namely alectinib showed a

remarkably high response rate in CNS metastases (56%), which are quite common in ALK-positive disease (12).

Emerging oncogene-directed therapies

Besides EGFR mutations and ALK rearrangements, numerous novel genetic alterations have been recognized as oncogenic drivers, with some of them being sensitive to already available therapies. First in the line are ROS1 rearrangements, present in 1–2% of NSCLC patients with characteristics very similar to the ALK-positive patients. In ROS1-positive patients remarkable responses (80%) and mPFS (9.7 months) were noted with crizotinib treatment that besides ALK inhibits also ROS1 tyrosine kinase (13). In addition, as a dual ALK and MET inhibitor, crizotinib appears to have antitumor activity in cMET amplified NSCLC, which as opposed to MET protein expression seem to be good molecular target. Several commercially available TKIs were found to possess activity against RET rearrangements, present in about 1–2% of NSCLC patients, including vandetanib and cabozantinib (14). Both of these drugs are being currently evaluated in multiple ongoing prospective clinical trials. Mutations in HER2 represent another candidate for targeted therapies, since observational studies reported good responses with HER2-directed therapies as trastuzumab and afatinib (15). However, the frequency of HER2 mutations is still to be defined, as well as which molecular characteristic: protein expression, gene amplifications or HER2 mutations, is the best prognostic factor for disease course and the best predictive factor for response to targeted therapies. The mutation V600E in BRAF exon 15 is a possible target for BRAF-directed therapy with vemurafenib and dabrafenib (16). To date, despite early-phase evidence suggesting that patients with those genetic alterations may derive benefit from targeted drugs, the lack of formal screening recommendations for those particular genetic alterations as well as a formal approval of targeted agents may restrict access to the drugs in NSCLC patients. In addition, there are numerous novel agents targeting these genetic alterations under the evaluation. Therefore patients with advanced NSCLC should be enrolled in a master protocols screening for genetic alterations and providing targeted therapies, such as SPECTALung (Screening Platform for Efficient Clinical Trials Access) platform, currently being initiated by the two European academic groups EORTC and ETOP.

A peculiar molecular biomarker are KRAS mutations, predominantly found in non-squamous NSCLC (11–26%) and usually mutually exclusive with other molecular events, as EGFR mutations or ALK rearrangements. Unfortunately, although KRAS mutations are the most frequent mutations found so far in NSCLC, their prognostic value is yet unresolved and no effective targeted therapy is yet available. The large heterogeneity of KRAS positive NSCLC may hinder advances in this field. In fact, KRAS mutation not only differ in the affected gene codon, with mutations most frequently occurring in codons 12 and 13, but differing also in the affected base pair, with different outcomes observed in mutations involving a purine

or a pyrimidine base pair (17). In addition, concurrent activation of other signalling pathways, such as MEK and PI3K, was described in some instances. Recently, MEK inhibitors have been evaluated in early phase clinical trials. Among these, selumetinib and trametinib showed an interesting activity in KRAS-mutant NSCLC patients when combined with chemotherapy, the schema that is being further evaluated in ongoing phase 3 trials.

Conclusions

During the last few years, we witnessed major improvements in individualizing therapy for advanced NSCLC. Much effort is being oriented towards better understanding of resistance mechanisms to existing therapies, identification of new oncogene-drivers and development of novel targeted therapies. While recent discoveries are making difference in mostly non-squamous NSCLC there is optimism that through further characterization of lung cancer genome, biomarker-driven therapies for squamous NSCLC will be developed as well. The introduction of oncogene-directed therapies with agents targeting FGFR1, such as brivanib, and DDR2 mutations, such as dasatinib will hopefully move the oncogene-directed therapy in the field of squamous NSCLC in as well. In addition, new technologies, such as next generation sequencing and liquid biopsies including circulating tumour DNA (ctDNA) hold promise not only in the identification of new oncogenic drivers but also in allowing us to follow the tumour clonal evolution over the treatment and to adopt treatment accordingly , thus welcoming us into the era of "precision medicine".

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Epidemiology of *EGFR* gene mutations in Poland

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Molecular testing in the course of cancer treatment planning is becoming a standard procedure. Patients with tumors harboring genetic alterations have proven to be more sensitive for novel, molecularly targeted drugs. It is also the case with non-small cell lung cancer patients and mutations in epidermal growth factor receptor (*EGFR*) gene. Constant tyrosine kinase activation results in permanent proliferation of cancer cells, thus tumor growth, and the alterations in *EGFR* gene became the anchor point of tumor growth inhibition with *EGFR* tyrosine kinase inhibitors (TKIs) – reversible (erlotinib and gefitinib) or irreversible (afatinib). Since the first studies on EGFR TKIs, it was proven that patients with particular mutations show better drug efficacy and present longer progression-free survival (PFS) and overall survival (OS) than those treated with standard chemotherapy (1, 2).

EGFR gene mutations have been described in patients with non-small cell lung cancer (NSCLC), predominantly with adenocarcinoma (AC) subtype, so as the

efficiency of the inhibitors. Mutations occur in exons from 18 to 21 of the *EGFR* gene, and can be divided into frequent and rare. The most frequently detected are those among exon 19 (mostly deletion) and exon 21 (L858R; L858M; L861Q; L861R). These activating mutations are considered to be sensitizing mutations and are responsible for the effectiveness of EGFR TKIs. Mutations in exons 18 and 20 are less frequent and some of them are considered to be resistance mutations, decreasing the effectiveness of EGFR TKIs (3, 4).

Although, *EGFR* mutations have been described more frequently in non-smoking women, it is not an inclusion factor when treatment decisions are to be made, without previous mutation analysis. These mutations are also predominantly described in patients of East-Asian origin, thus studies from East-Asia present far higher percentages of detected mutations than studies on Caucasian patients (5).

Since the beginning of *EGFR* mutation analyses we are involved in molecular testing of NSCLC patients in qualification to molecularly targeted agents in Poland. Our laboratory used various techniques (from so called 'home-made' self-designed PCR-based methods to CE-IVD real-time PCR tests) to, at first, detect only exon 19 and 21 mutations and presently using certified test to detect most of the described mutations (exons 18–21) in regular molecular diagnostics. We are currently diagnosing a large part of Polish NSCLC patients in course of lung cancer treatment qualification to EGFR TKIs, hence our experiences with detecting *EGFR* gene mutations in Polish patients are quite extensive.

Experiences we obtain during day-to-day diagnostics are resulting in preparation of Polish recommendations for not only *EGFR* gene mutation analysis, but also *ALK* gene rearrangement testing in lung cancer patients (6).

EGFR gene mutations are detected in around 10% of Caucasian NSCLC patients. In a large multicenter study on Polish NSCLC patients, mutations were detected in 9% of all studied cases. The study included 2450 patients from 10 Polish cancer treatment centers, among whom 123 (5%) exon 19 deletions and 98 (4%) exon 21 mutations have been detected. The mutations were significantly more frequent among women and AC patients (13.9% and 10%, respectively) and among patients who had TTF-1 positive histological samples (13%). The study focused also on the types of material that is sent for molecular testing and its cellularity, as many molecular methods require large percentage of tumor cells for proper results. Small biopsy samples gave significantly lower DNA yields than surgically resected primary tumor samples, hence giving less sensitive mutation testing results (7). Recent report on mutation diagnostics in Poland between 2011 and 2014 presents fast rising number of studied NSCLC cases, with predominance of AC subtype. In 2011 there were 287 cases studied (13.3% had detected mutations), whereas in 2014 there were 4307 cases analyzed with 10,2% of detected mutations. Exon 18 mutations were observed in 5.2% of cases, exon 19 in 52.5%, exon 20 in 10.2% and exon 21 in 32.1% of studied samples. The percentage of AC cases also rises when compared to NOS cases (from 85.9% to 93.2% with AC, and from 10% to 5.3%

with NOS diagnoses), which increases the chance of proper diagnosing and further treatment qualification. The majority of studied material had high percentage of tumor cells (>50%), but there also was 7.5% of <10% tumor cellularity cases (8).

Our studies also focused on detection of *EGFR* gene mutations in lung cancer metastases, particularly bone and CNS metastases, as lung AC frequently presents metastases to these regions. Qualification to EGFR TKIs is possible based on analysis of metastatic tissue if primary tumor sample is not available. Retrospective analysis of 143 CNS lung cancer metastases, along with 32 corresponding primary tumor samples, revealed 9 cases with *EGFR* mutations in CNS metastases, of which two cases of *EGFR* gene mutation were in both primary and metastatic sample (one deletion in ex 19 and one L858R substitution in ex 21) (9). In another study 6 out of 8 AC patients with bone metastases had *EGFR* gene mutations (4 in ex 19 and 2 in ex 21) detected in metastatic material, but in only one case the coexistence of primary and metastatic mutation presence was confirmed, due to lack of corresponding primary tumor samples. It is also noteworthy that all the patients with mutations detected in bone metastatic material benefited from EGFR TKI treatment (10).

Because *EGFR* testing in course of treatment qualification became a standard in current clinical proceedings, there are more and more reports on differences in effectiveness of EGFR TKIs when different mutations are detected. The methods used are also more sensitive and detect more kinds of mutations, diversifying treatment options due to different efficacy of TKIs. Therefore, there is more focus on the incidence of rare mutations and their role in disease relapse and resistance to molecularly targeted therapies (11, 12). Our laboratory has conducted few studies on the incidence of these rare mutations and their role in NSCLC treatment with EGFR TKIs.

The most often diagnosed rare *EGFR* gene mutation in EGFR TKIs pretreated patients is the T790M substitution in ex 20. It is postulated that this mutation may be the reason for TKIs failure in patients already treated with these drugs, as it may be a secondary mutation occurring in some clones of tumor cells, causing their resistance to reversible EGFR TKIs. But it might also be observed along 'popular' activating mutations in primary tumors. The study conducted in our laboratory revealed T790M substitution in 25 (175%) cases of CNS metastatic tumors among 143 analyzed samples, in chemotherapy and TKI naïve patients. The study also assessed the content of mutated DNA necessary for proper detection of this rare mutation. It was observed that in all cases the percentage of T790M mutated DNA was lower than 1% of all analyzed material, in 80% (20) of these cases it was less than 0.1%. It shows that it is possible to detect rare and low-content mutations primarily before TKI treatment, even though they appear in a very small percentage of tumor cells (13).

In 2014 Faller-Beau et al. presented their results of the largest study in Europe on *EGFR* gene mutation status of NSCLC patients. The study focused on the

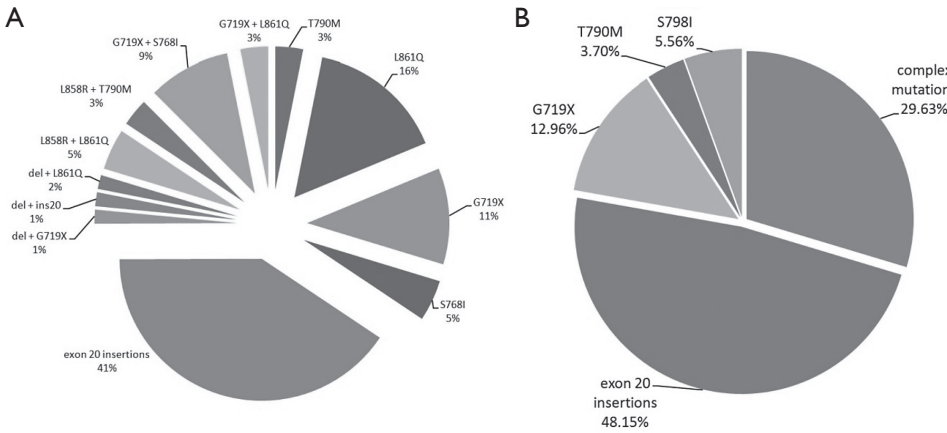

Figure 1. A – Occurrence of rare EGFR mutations in Polish NSCLC patients. B – Occurrence of rare mutations in EGFR exon 18 and 20. Figure 1B matches Figure 1 of Beau-Faller study and presents only these mutations which were analyzed in French ERMETIC-IFCT study (14).

occurrence of rare mutations and their role in lung cancer treatment effectiveness. *EGFR* gene mutations were diagnosed in 10.35% of 10 117 cases, of which 108 were rare mutations in exons 18 and 20 (14). In relationship with this publication, we are presenting our own experiences in this field, showing *EGFR* mutation status analysis in 3856 Polish NSCLC patients and discussing the frequency of particular rare mutations in Polish NSCLC population. Our study presents higher percentage of exon 18 and 20 rare mutations than the French study (1.4% vs 0.89% respectively; p=0.0075). Significantly higher percentage of complex mutations (p=0.0057) and lower percentage of G719X substitution (p=0.029) was also observed (15) (Figure 1).

Our findings support the importance of *EGFR* gene mutation analysis in course of NSCLC treatment qualification and show that exon 18 and 20 mutations are not as rare as they were thought to be. They should be the objective of further, a lot larger studies, as role of these mutations in treatment effectiveness is not exactly understood.

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Central Data Collection of Management NSCLC stage III patients – ongoing study – first results

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Introduction: The project is a multinational, multicentre, prospective, observational, non-interventional registry of patients in Central and Eastern Europe, inspired by Central European Initiative against Lung Cancer. The aim of the study is to determine the actual standard management (diagnostic and therapeutic procedures) of patients with stage III NSCLC in Central European centres/ countries.

Methods: Patients diagnosed with morphologically confirmed (cytology or histology) NSCLC in stage III according to UICC7 were included in web-based registry organised by Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic. After ethical committee approval in each centre and signing inform consent the data about diagnostic and therapeutic procedures of all consecutive patients diagnosed in stage III NSCLC were collected.

Results: 22 centres from 8 countries were registered for this project. With cut-off 22 September 2015, 242 patients from 6 countries (11 centres – 50% of registered) were enrolled, median number of subject per centre being 14 (range 2 – 71). There were 88 (36.4%) women, 16 (6.6%) never smokers, 41 (17%) subjects with weight loss more than 10%. Performance status distribution was as follows: 0=82 (34%), 1=137 (57%), 2=18 (7%), 3=4 (2%). Squamous cancer was the most frequent histological finding (120 cases = 50%), followed by adenocarcinoma 102 (42%) of subjects. Genetic mutations were examined in 57 cases (24%), predominantly the EGFR in 48 subjects with 2 positive findings, while the ALK mutation was examined in 24 patients with no positive finding. Rarely tested RAS mutation was positive in 7 from 8 tested patients. Regular staging procedures were X-Ray scan (97%), chest CT (97%) and bronchoscopy (88%). Staging was completed by abdominal CT scan in 80% patients, abdominal US in 25%, PET/CT scan in 23%, bone scan in 19% and brain CT or MRI in 10%, respectively. Morphological mediastinal lymph-nodes positivity was confirmed in 91 (38%) patients, 10% with EBUS, 2.5% with VATS and 2% with mediastinoscopy, respectively, as 23% of patients had mediastinal positivity confirmed at surgery. Stage IIIA was found in 141 (58%), stage IIIB in 99 (41%) patients, 2 subjects were not specified. Median time from (morphological) diagnosis to first treatment was 22 days with maximum 321 days. Treatment procedures were: surgery 56 (23%), chest radiotherapy 108 (45%), chemotherapy 213 (88%) subjects, respectively. Platinum compounds were most often combined with

vinorelbine, N=82 (40%). Concurrent chemoradiotherapy was done in 27 (11%) and surgery plus radiotherapy in 18 (7%) of patients, respectively. Combination of surgery plus chemotherapy was used in 46 (19%) patients and 15 (6%) patients had tri-modality treatment. In time of data cut-off only 126 patients were evaluated for survival, with 13 confirmed deaths and 38 progresive diseases.

Conclusion: The most prevalent histology in Central European countries is still squamous cancer. Morphological confirmation of mediastinal lymph-nodes was done in 38%, mostly during surgery. Driving mutations were examined in 24% of patients and PET/CT scan was done in 23%. Majority of patients were treated with chemotherapy, combined modality treatment is much less frequent. Study is ongoing.

Hormesis-threshold model for individualized lung cancer risk assessment in smoking and low-dose radiation exposure

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Background: The depleted uranium (DU) repeatedly military use, approximately every four years since 1991 (Iraq 1991, Bosnia 1994–1995, Kosovo, Serbia, and Montenegro 1999, Afghanistan 2001–2003, Iraq 2003–2011), has induced the low dose radiation (air pollution easily transferable to the remote distances from the place of explosion), slow doses (DU ammunition remnants can be fully oxidized into corrosion products twenty-five to thirty-five years after impact) and its further prolonged contribution to the maintenance of alpha particles radiation. Inhalation is dominant way of DU entering the body. After embedding into the tissue, DU micro or nanoparticles exert radiotoxic effect of hard metal as well as radiogenotoxic effect of mixed alpha, beta, gamma radiation. Nanoparticles can produce irreversible damage of cells by oxidative stress or/and organelle injury, preceding tissue inflammation and altered cell death mechanisms. Cigarette smoking is one of the strongest risk factor for lung cancer. Smoking exposure is proportional to internal dose from tobacco delivered radionuclides and additionally from inhaled air radionuclides which are adsorbed to tobacco smoke particles overlying the bronchial tree. Change in apoptotic regulation is one of earliest responses to radiation events leading to radioadaptive/radioprotective tissue response.

Methods: Apoptotic parameters in cytocentrifuge preparations of bronchoalveolar lavage cell suspensions were evaluated by light microscopy using TUNEL in situ

cytochemical method in healthy nonsmokers and smokers and hard smokers with non-small-cell lung cancer. Based on apoptotic index (ratio yielding by apoptotic cells) and apoptotic capacity (reflects generation of apoptotic bodies and their clearance by alveolar macrophages) the graph was carried out by a neural network method.

Results: The graph obtained by neural network method can be used for the assessment of dose-response relationship regarding smoking and radiation effects at low-doses. Both approaches provide clear distinction of nonsmokers, smokers and smokers with lung cancer according to apoptotic parameters and smoking exposure. Interpretation: The findings fit with hormesis-threshold model of tissue response to low-dose radiation. The method represents a step toward individualized screening and lung cancer risk assessment. Proposed model does not require an exact measuring of tissue doses in conditions of exposure to low doses of radiation, especially alpha emitting radioisotopes. This method enables quick orientation about the extent of damage of complex tissue regulatory mechanisms in situ, and indirectly, it may indicate the existence of adaptive, premalignant or malignant lesions.

Exhaled breath condensate pH in lung cancer, the impact of clinical factors

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Background: Lung cancer may be associated with airway acidification due to enhanced airway inflammation and oxidative stress. Exhaled breath condensate (EBC) pH is a non-invasive indicator of airway acidity; however, it is still unclear how EBC pH changes in lung cancer. The aim of the study was to investigate EBC pH in lung cancer together with clinical variables.

Methods: Thirty-five patients with lung cancer and 37 control subjects (21 patients with stable COPD and 16 non-COPD smokers) were enrolled. EBC was collected for pH, which was determined with the argon-purging method, compared among the groups and correlated with clinical variables of patients with lung cancer.

Results: No difference was found in EBC pH between patients with lung cancer and control subjects. However, endobronchial tumour localisation, squamous-cell carcinoma subtype and gastro-oesophageal reflux were associated with low EBC pH values. No relationship was observed between EBC pH and the presence of COPD, lung function variables or smoking history.

Conclusions: Although, EBC pH is unchanged in lung cancer, lower EBC pH values are associated with distinct phenotypes. Our findings could facilitate further research on airway acidity in lung cancer.

Discontinuation of bevacizumab in non-progressing NSCLC

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Background: Bevacizumab is in general well-tolerated treatment in patients with locally advanced or metastatic non-squamous NSCLC. However, permanent discontinuations of bevacizumab occur also before progressive disease (PD), either due to undesirable side effects of therapy or due to other reasons. Purpose of this study was to find out the causes of permanent discontinuation of bevacizumab before PD in patients with NSCLC treated in two centres in Slovakia.

Methods: Retrospective study approved by the Ethics Committee of the Specialised Hospital of St Zoerardus Zobor. The institutional databases were searched for patients with advanced NSCLC who started treatment with bevacizumab between 2007 and 2013. Response to treatment was evaluated using RECIST version 1.1. MedCalc Statistical Software (https://www.medcalc.org) was used for the data analyses.

Results: Altogether 161 patients were included. Patient's characteristics: M/W: 99/62, age: median 61 years (32–83), histologically and/or cytologically confirmed NSCLC: adenocarcinoma/large cell/adenosquamous: 158/2/1. Number of cycles with bevacizumab (induction and maintenance): median 8 (1–52), PFS: median 7 months (1–42). Bevacizumab was permanently discontinued before PD in 28 of 161 patients (17.4%), in 18 of 161 (11.2%) due to undesirable effects (cavitation: 3, pneumothorax: 3, cerebrovascular events: 2, gastrointestinal perforations: 2, hypertensive crises: 2, pneumonias: 2, proteinuria: 2, thrombotic events: 2), and in 10 of 161 (6.2%) due to other reasons (molecular testing and start of TKI: 2, patients' preference: 2, car accident and death: 1, planned surgery: 1, traumatic fractures: 1, family reasons: 1, lost to follow-up: 2). Comparison of two rates (Group I: 18/161 and Group II: 10/161) showed Group I incidence rate 1:9 (95%CI: 1:15 to 1:6), Group II incidence rate 1:16 (95%CI: 1:34 to 1:9), the incidence rate ratio: 1.8 (95%CI: 0.79 to 4.36). The difference between the rates of undesirable

effects (Group I) and others causes (Group II) leading to the bevacizumab discontinuation before PD was not significant (p = 0.13).

Discussion: The rates of bevacizumab permanent discontinuation ranged in clinical trials between 14% and 30+%. Some of recent trials reported higher rate of bevacizumab discontinuation due to other reasons than due to adverse events (Patel JD et al. J Clin Oncol 2013, 31:4349–57). In our study these rates did not differ significantly.

Conclusions: As the management of undesirable effects of bevacizumab has improved, other reasons for treatment termination before PD are becoming more important and deserve more attention.

Scientific session III
Case disscussion workshop

Successful treatment with afatinib (case report)

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60-year-old non-smoking male patient presented with right upper lobe mass. His previous medical history were significant for in situ resection of melanoma and hyperthyreoidism. In Sept 2013 he underwent a right upper lobe lobectomy for a NSCLC. The pathological stage was T2aN1M0 adenocarcinoma. Three cycles of vinorelbine plus cisplatin was administered as an adjuvant chemotherapy between Nov 2013 and Jan 2014. In Sept 2014 elevated serum CEA level was observed. PET CT scan revealed pathological FDG accumulation in bilateral pulmonary small nodules. The multidisciplinary tumor board estimated these findings as an early recurrence of pulmonary malignancy. The molecular analysis of the surgical specimen was done, which revealed EGFR 20 exon mutation. The treatment with afatinib started in Oct 2014. Chest CT scan revealed almost complete response, which is maintained until now (12 months). The patient tolerates well the treatment. Treatment related adverse event includes only grade 2 diarrhea. The patient's quality of life is improved, he can continue his work. Afatinib is associated with prolongation of PSF, good tumor response, and reduction of lung cancer related symptoms.

Successful treatment with Erlotinib after initial failure to find EGFR activating mutation: case report

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Background: Treatment with tyrosine kinase inhibitors (TKIs) provides a substantial benefit for NSCLC patients with sensitizing EGFR mutations. Response rate for single-agent TKI therapy ranges from 55 to 80% with a median progression

free survival (PFS) of 9.2 to 13 months. Testing for mutations in EGFR is therefore an important step in the treatment-decision pathway. Different approaches in mutation analysis are available today. Direct DNA sequencing is a historical standard, able to detect more than only pre-defined mutations, but this method is complex, time-consuming and has low sensitivity (i.e. only detects mutations when sufficient amount of mutant DNA is present in a biopsy). Detection sensitivity was increased with development of mutation screening assays, mostly based on real-time polymerase chain reaction (PCR). These commercially available diagnostic kits are able to detect mutations in cytology samples or even in plasma ctDNA, but identify only the most common and clinically relevant alterations discovered to date. Still some limitations exist also for this technology. Although PCR-based methods can in theory detect mutations from a single cell, a low copy number DNA template can generate sequence artefacts, mainly guanine to adenine transitions, due to stochastic occurrence of polymerase errors early in the PCR process. In addition, the DNA damage caused by formalin fixation can also lead to sequence artefacts. There are also literature data that an extra mutation (P848L) in the EGFR gene, located 10 residues away from L858R can hide this mutation from detecting by Cobas 4800 EGFR Mutation Test. Another big problem is heterogeneity of tumor sample.

Case report: A 67 year old never smoker woman with histologically confirmed right lung stage IV adenocarcinoma. Biopsy acquired from pleural metastasis during video-assisted thoracoscopic surgery and talc pleurodesis procedure. Tumor sample from formalin-fixed, paraffin-embedded (FFPE) tissue block was examined with Cobas 4800 EGFR Mutation Test and no mutations were detected. Patient started Cisplatin/Paclitaxel chemotherapy in clinical trial and the same FFPE tissue block and the same Cobas test were used for reassurance that no EGFR mutations are present. Surprisingly, Exon 21 L858R mutation was found, most probably due to heterogeneity of tumor sample. After finishing 4 cycles of chemotherapy partial response was lasting for 14 months. At progression Erlotinib therapy was started and stable disease still ongoing after 20 months of therapy.

The effect of tyrosine kinase inhibitor erlotinib in patients with squamous cell lung cancer in the second and third line of treatment: Case reports

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Backround: The tyrosine kinase inhibitor erlotinib is indicated as first-line treatment for patients with non-small cell lung cancer (NSCLC) with locally

advanced or metastatic disease with proved activating mutations of endothelial growth factor receptor (EGFR), or as second- and third-line treatment in EGFR negative NSCLC patients after failure of chemotherapy.
Methods: Three case reports are presented of patients with squamous cell lung cancer who were treated with erlotinib in the second and third line of treatment. All patients were verified by histological samples. The EGFR mutation status was negative in one patient, other samples were not suitable for genetic screening. All patients were in a good clinical status at the time of starting erlotinib therapy.
Results: The therapeutic response lasted for 52, 40 and 13 months, respectively. The patient with the longest therapeutic response is still continuing erlotinib therapy. One of the patients died of an unknown cause without any signs of progression of the disease on CT scan. In one case we continued with erlotinib despite a small progression of the tumour on CT scans. After 8 more months, sustained clinical progression occurred and the treatment was terminated.
Discussion: All patients had benefit from erlotinib therapy. In one case the dosage of erlotinib was reduced due to severe side effects, in another case side effects were under control. Two of the patients were remaining in a very good clinical state over a relatively long period of time.
Conclusion: The beneficial effect of erlotinib in our patients is evident. Erlotinib in the second or third line of treatment can have very satisfying results. We should consider this type of treatment even for squamous cell lung cancer patients, especially those in a good clinical state.

Clinical case presentation: Management of the EGFR-mutated patient with acquired resistance to EGFR TKI

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Introduction: 57-year old, healthy never smoker with a history of operable breast carcinoma (28 mm in diameter, SLNB 0/1, GIII, mitoses 3, ER 0%, PR 0%, HER-2 positive (IHC 3+, FISH 5.9) radically treated with surgery, radiation therapy, chemotherapy and anti-HER2 therapy in 2009, presented in 2013, after 4 years of disease free interval, with generalized urticaria, dry cough and progressive fatigue, PS1.

Diagnostic work-up: Chest CT revealed single 16 mm mass in left upper lobe (LUL) with enlarged left hilar lymph node (LN). CA 15-3 was 74 (normal value <35). CT-guided core biopsy of LUL tumor confirmed adenocarcinoma,

TTF-1+, ER+, PR-, KRAS-, EGFRmu+ (exon19 deletion), HER-2 unknown. PET-CT showed distant metastases in the spleen, bone and left axillary LNs up to 10 mm in diameter. Following this finding and history of breast carcinoma left axillary dissection of 6 lymph nodes was performed, pathology revealed adenocarcinoma with the same immunophenotype as in lung carcinoma (Q1).

Treatment: Treatment with EGFR TKI erlotinib 150 mg/day and bone modifying agent (BMA) once monthly was initiated in October 2013. Four weeks after the start of erlotinib, substantial symptom and PS improvement was observed and evaluation CT of the thorax and abdomen confimed PR. Erlotinib was well tolerated with no major adverse events. After 20 months of progression-free survival she presented with new symptoms of increasing pain in the upper abdomen, PS 1. Control CT of the thorax and abdomen revealed disease progression in bones and new lesion in the liver (36x56 mm), while primary tumor and spleen lesion remained unchanged (Q2). Core biopsy of the liver mass revealed metastasis of adenocarcinoma, TTF-1+, ER-, PR-, EGFRmu+ (exon19 deletion + T790M). Additionally, cDNA confirmed the coexistence of exon19 deletion and resistance mutation T790M (Q3). Erlotinib was switched for cisplatin-based chemotherapy, BMA was continued. Evaluation CT after 3 cycles of chemotherapy revealed PR of measurable lesions in the liver and chest, while several new lesions appeared in the brain (Q4).

Discussion points: Q1: Are activating EGFR mutations lung adenocarcinoma specific? Q2: Should a re-biopsy/liquid biopsy (cDNA) be performed at progression? Q3: What is the most appropriate treatment beyond progression on first-line EGFR TKI nowadays? Q4: What is the most appropriate management of CNS-only progression in case of coexistence of activating and resistance EGFR mutations?

22-year old patient with EML4-ALK positive non-small lung cancer and multiple allergies

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Introduction: The connection between allergies and risk of cancer is uncertain. Several immunological processes seem to play a role in this connection. A hyperactive immune system present in allergic people is supposed to work against cancer development. On the other hand, persistent inflammation associated with allergic diseases increases the risk of development of cancer. At present, no objective allergy tests can predict an individual's risk of developing cancer.

Case Report: A 22-year old female, never-smoker, veterinary student was hospitalized in January 2011 due to angioedema after clindamycin was administrated to treat a festering angina. From August till December 2011 she developed 3 festering anginas. There was no serious prior illness or cancer family history. In May 2012 she developed urticaria of unknown cause. By the end of the month breathing problems, headaches, dry cough, appetite and weight loss developed. Diagnostic tests revealed EGFR and k-RAS negative poorly differenced adenocarcinoma of the lung T2N3M1b (bone metastases). Fluorescent in situ hybridization showed ALK translocation (p21, p23). Chemotherapy (pemetrexed/cisplatin) was administered to the patient. After one cycle, no clinical benefit was seen so in July 2012 an oral treatment of 250 mg crizotinib twice daily was started. At the start of crizotinib therapy the patient's weight was 47 kg and performance status WHO 4. After two weeks of crizotinib, performance status improved to WHO 1 and the patient gained weight (4 kg). The only reported side effect was nausea grade 1. After two months of crizotinib PET-CT revealed a significant improvement but MR of the head showed asymptomatic progression of the disease, with multiple CNS metastases. Whole brain radiotherapy was administered (25Gy in 10 fractions). In February 2013 imaging revealed disease progression. Crizotinib was temporary suspended but then restored to slow down disease progression. Due to poor performance status she was unable to receive other treatments. She died in August 2013 at the age of 23. The patient developed several other allergies short before and during the treatment. Allergy to amoxicillin, clindamycin, ciprofloxacin, paracetamol, naproxicillin, metamizole, ketoprofen, azytromycin, citrus and tomato were discovered. Based on this, an immunologist was consulted and tests performed. Flow cytometry revealed no abnormalities in the concentration of lymphocytes B (CD9), T helper cells (CD4), cytotoxic T cells (CD8) and natural killer cells (CD56). What caused the total immunological collapse seen in this patient that resulted in multiple allergies and lung cancer remains unknown.

Adie's pupil and ANNA-1 associated paraneoplastic neurologic syndromes predict complete response in limited-stage SCLC: a case report

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Background: Paraneoplastic sensorimotor neuropathy with cerebellar ataxia (PSN CA) is an extremely rare, rapidly progressive, autoimmune disease associated

with the development of antibodies against neuronal-specific Hu proteins that are abnormally expressed in small cell lung cancer (SCLC).

Methods: A comprehensive chart and literature review was performed. Results: We present the unique case of a 55-year-old obese woman, previous heavy smoker, who, during treatment with standard cisplatin-etoposide chemotherapy (total of six courses) for limited-stage SCLC (T1aN2M0, clinical stage IIIA), developed unilateral Adie pupil of the right eye and worsening symptoms consistent with PSN CA that led to significant neurological disability with severe axonal electrophysiological pattern on nerve conduction studies. Serology confirmed the presence of low-titre anti-neuronal nuclear antibodies (ANNA-1), previously named anti-Hu antibodies. Following plasmapheresis (five cycles), intravenous then oral corticosteroids, cyclophosphamide, and antiepileptic medication for myoclonus, the neurological symptoms remarkably abated. She is now sitting independently, feeding herself, mobile with the assistance of others, but with subsisting speech difficulties. The tumour has completely regressed with no recurrence on subsequent radiological examinations.

Conclusions: Although neuronal damage is reported in the literature to be irreversible, this case highlights the importance of early recognition and rapid treatment of paraneoplastic neurologic syndromes (PNS) as key to achieving significant recovery and marked improvement of neurologic deficit in some patients. In addition, this report extends the literature by confirming earlier studies showing that the presence of serum ANNA-1 in SCLC, an aggressive and challenging type of pulmonary carcinoma to treat, portends a more favourable prognosis and response to chemotherapy. ANNA-1 may be a useful biomarker for early SCLC diagnosis given that symptoms of PNS often precede tumour detection. Furthermore, all patients with SCLC, even in the absence of a neurological disorder, should be tested for serum ANNA-1. A multimodality approach to treating PNS composed of a combination of plasmapheresis, immunosuppressive therapy, and physical rehabilitation, is necessary to ensure beneficial clinical and oncologic outcomes.

Secondary malignant neoplasm in the lung and the low-grade liver cancer

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Background: Diagnostic of secondary malignant neoplasm of the lung in the patient with epithelial hemangioendothelioma.

Methods: CT scan, bronchoscopy, thoracic surgery intervention, imunohistochemistry CD31 and CD34

Results: 40 years old woman with epithelial hemangioendothelioma verified in 2005 was admitted to our department for diagnostic pulmonary lesion with non-specific symptoms. According to CT scan, we supposed the similarity of the lesion with low-grade liver cancer. Bronchoscopic biopsy was compared with the earlier bioptic material of the liver. On the recommendation of the pathologist, the lesion was finally verified by the biopsy from the thoracic surgery intervention, using the imunohistochemistry CD31 and 34.

Conclusion: There is important to beware of rare diagnosis metastasis in the lung in patient with hemangioendothelioma.

Migratory thrombophlebitis as a first sign of lung adenocarcinoma

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Introduction: Thrombophlebitis migrans is an acquired coagulopathy characterised by the development of recurrent (migratory) thrombophlebitis which is approximately in 50% of cases linked to an underlying malignancy, especially solid tumours of the adenocarcinoma type.

Case presentation: 34 year old male patient presented with the symptoms of deep venous thrombophlebitis of the left leg on April 2015. He was admitted to surgical ward and treatment with low-molecular-weight heparin was introduced. Three months later color-duplex scan examination confirmed thrombophlebitis of the right femoro-popliteal vein. After recurrence of thrombophlebitis surgical team suspected on Trousseau's syndrome. Further examinations confirmed lesion of the right lung, mediastinal and cervical lymphadenomegaly, lesions of thoracolumbal segment of the spine, multiple lesions of the pelvis. Biopsy of the supraclavicular lymph node and bone marrow was done and confirmed adenocarcinoma with neuroendocrine differentiation. Molecular profile of tumour tissue confirmed ROS-1 translocation, sensitive to crizotinib and EGFR and ALK testing was negative. As in our country crizotinib is not registered medicine it takes time to find it and family bought it in E.U. In September 2015 the patient developed symptoms of

acute abdomen and become blind. CT scan of the abdomen and brain confirmed renal and splenic infarction and ischemic lesions of the brain. Higher dose of low-molecular-weight heparin was introduced and after stabilisation of those symptoms therapy with crizotinib was introduced. This is first patient with ROS-1 translocation in our institution. Patient is now one week on crizotinib therapy and his general condition is improved. We are waiting for further analysis.

Conclusion: Thrombophlebitis migrans is easier to recognize in patients with an established diagnosis of malignancy than in situations where the thrombophlebitis is first diagnosed. Any patient with characteristic migratory thrombotic event should be fully investigated to exclude malignancy.

Key words: lung adenocarcinoma, thromophlebitis migrans

Multimodality treatment of squamous cell lung cancer. Case report

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We report 61 year old patient with the history of squamous cell carcinoma of the distal esophagus diagnosed 2004 staged T4N1M0 and treated with concomitant neoadjuvant chemo radiotherapy followed by radical resection of the esophagus. During the summer 2013 he has been examined because of the elevation of SCCA marker. Chest and upper abdomen CT scan showed expansive process in the area of right hilum without regional lymphadenopathy or distant metastatic disease. Bronchoscopic examination revealed tumorous granulations in distal part of the trachea, both main bronchi and right bronchus intermedius narrowed lumen to 1/3 of the norm. Interventional endoscopic recanalization was carried on. Squamous cell carcinoma with low differentiation has been verified from endobronchial biopsy. Because of the inoperable and previous radiotherapy (overall dose 45Gy) the treatment decision was uneasy. As there was a risk of the loosing ventilation of the main bronchus we decided for intraluminal brachytherapy during which 8 Gy has been applied into tumorously obstructed bronchus. Chemotherapy followed composed of gemcitabine plus cisplatin 6 cycles with stable disease result. During the restaging (February 2014) there was newly apparent right sided fluidothorax with the atelectasis of the right lower lobe. Bronchoscopy showed complete combined obstruction of the right middle and lower lobes with patent bronchus intermedius. There was no indication for interventional bronchoscopy procedure so the second line of palliative chemotherapy (docetaxel)

was introduced followed by 3rd line with vinorelbine. By February 2015 CT examination showed new metastatic lesions and chemotherapy was finished. After nivolumab therapy has been accessible in Expanded Access Program the patient started this treatment on August 2015 and is on immunotherapy till now.

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Parallel session: Minisympozium
Molecular and Cell Biology of Lung Cancer: The Implications for Prevention, Diagnostics and Therapy

Chromosomal damage as markers of genotoxic effect and carcinogenesis

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Background: Human cancers often arise from cells unable to maintain genomic and chromosomal stability, mainly due to altered DNA repair mechanisms. Chromosomal instability (CIN) and alteration in the number of chromosomes are consistently observed in virtually all cancers. Recurrent CAs arise through a clonal growth of cells with specific translocations, deletions or amplifications of chromosomal regions or whole chromosomes and many specific CAs are believed to be causative events in malignant transformation (Mitelman et al. 2007). In some cancers, individual chromosomes have experienced chromothripsis, a catastrophic parsing of illegitimate chromosomal segments together (Zhang et al. 2015 Nature). Non-specific chromosomal aberrations (CAs) may arise as a result of direct DNA damage by ionizing radiation or replication on a damaged DNA template; the former lesions would be detected as chromosome-breaks (CSAs), whereas the latter may be CSAs or chromatide-breaks (CTAs). These CAs remain in lymphocytes for their lifetime. Conventionally, CSAs are thought to arise as a result of direct DNA damage by e.g., ionizing radiation which causes double-stranded breaks. A suggested alternative mechanism is replication of a damaged DNA template, resulting in CSAs or CTAs, the latter being preferentially produced by chemical carcinogens and mutagens (Natarajan et al. 2008; Durante et al. 2013). More recently it has been realized that telomere biology is intimately connected to

CAs (Xu, et al. 2013). Shortening of telomeres at each cell division leads ultimately to replicative crisis. Eroded telomeres may be poorly end-capped and they may be recognized by DNA repair systems as double-stranded breaks which are joined to non-homologous chromosomes (Artandi and DePinho 2010).

CAs have been used in monitoring of radiation exposure and exposure to genotoxic compounds and, together with sister chromatid exchanges and micronuclei, CAs have offered the only available method for human biomonitoring for genotoxic exposures and they represent a sequential consequence of altered DNA repair mechanisms (base and nucleotide excision, mismatch, non-homologous DNA end joining and non-conservative homologous recombination repair). Chromosomal aberrations (CAs) in peripheral blood lymphocytes reflect inter-individual sensitivity to exogenous and endogenous genotoxic substances and can be used as biomarkers of an early effect of genotoxic carcinogens and markers of carcinogenic risk (Musak et al. 2013).

Only a limited number of reports analyzed effects of genetic predispositions on inter-individual variability in DNA and chromosomal damage by studying variants in genes encoding xenobiotic-metabolising enzymes, enzymes of DNA repair or folate metabolism and DNA repair capacity (Vodicka et al. 2004; Naccarati et al. 2006; Musak et al. 2008; Skjelbread et al. 2011).

Several epidemiologic prospective studies provide convincing data on the association of CA frequency with subsequent risk of several malignancies (Bonassi et al. 2008). Interestingly, CA frequency could be predictive of cancer risk irrespective of either exposure to carcinogens or main confounders, such as smoking, sex, age and the period between cytogenetic analysis and cancer detection (Bonassi et al. 2008; Rossi et al. 2009). The strongest association was found for respiratory, gastrointestinal and genitourinary cancers (Rossi et al. 2009; Rossner et al. 2005; Boffetta et al. 2007). Regarding the types of CAs, both CTAs and CSAs were predictive for cancer in study of Hagmar et al. (Hagmar et al. 2004), whereas Rossner et al. (Rossner et al. 2005) and Boffetta et al. (Boffetta et al. 2007) found significant association only for the CSAs, but not for CTAs.

In order to complete the chain of evidence linking CAs to the risk of cancer we demonstrated increased frequencies of non-specific structural chromosomal aberrations in several types of cancer at diagnosis, such as breast, prostate and head and neck cancers, but not in patients with gastrointestinal cancers (Vodicka et al. 2010). Recently, significantly higher frequency of micronuclei was observed in colorectal cancer (CRC) patients than in controls (Maffei et al. 2014).

Cancers represent complex genetic and epigenetic diseases that are, despite intensive research, still at the forefront of human morbidity and mortality. Development of cancer is associated with genome instability (Abbas et al. 2013), resulting in both numerical and structural chromosomal abnormalities in cancer cells (Futreal et al. 2004; Rajagopalan et al. 2004; Burrell et al. 2013). Present study was aimed at the detection of CA frequencies in peripheral blood lymphocytes

in newly diagnosed patients with the currently most frequent malignancies, such as colorectal, lung and breast cancers. In addition, the attempt to relate CA frequency to the clinico-pathological characteristics is addressed for the first time. Additionally, genetic factors modulating chromosomal damage in healthy subjects have been addressed as well.

Methods: The case-control study among breast, colorectal and lung cancer patients was conducted between 2006 and 2013. The first group comprised 101 incident patients with sporadic CRC, the second one 87 patients with lung cancer and the third one 158 patients with breast cancer. The control groups enrolled healthy control subjects of similar age, sex and socio-economical background; the former comprised 300 healthy individuals, the latter 158 healthy women for comparison with breast cancer patients. For all subjects included in the study clinico-pathological characteristics have been available. Blood samples for cytogenetic analysis were collected only from patients with newly diagnosed cancer. Only those patients, who did not undergo any radiotherapy or chemotherapy to date and who had primary cancer disease, were included in the study. Other anamnestic data were also collected (family history of cancer, occupational history, smoking and other diseases such as hypertension, diabetes mellitus, cardiovascular disease, including their treatment). Individuals, who have quit smoking five or more years ago, were included among non-smokers and those quitting smoking less than five years ago, were classified as smokers. To evaluate chromosomal damage in relation to clinico-pathological characteristics we have collected data on TNM (Tumor Nodus Metastasis) stage, histopathological grade, histological classification (non-invasive/invasive and ductal/lobular types of breast tumors, non-small/small cell and bronchogenic/pulmonary types of lung tumors), laterality of tumors in all three groups of patients and the presence of estrogen and progesterone receptors in breast tumors.

The group of studied healthy volunteers (more than 2100) with measured frequencies of CAs were recruited between 2002 and 2011 in eastern Bohemia and 1997–2006 in Slovakia and consisted of unexposed controls as well as subjects with defined occupational exposures, such as small organic compounds, cytostatics, anesthetics, metals, asbestos, mineral fibre and ionizing radiation.

All individuals completed a questionnaire regarding the job category, mode and duration of exposure, various exogenous factors (such as smoking, drug usage, exposure to X-ray radiation, alcohol consumption and dietary habits) prior to blood collection and provided a written consent. The present study adheres to all principles of the Helsinki Declaration and its design was approved by the appropriate Local Ethical Committees.

Cytogenetic analyses were carried out by using conventional cytogenetic method (microscopical analysis by two independent scorers in a double-blind fashion of 100 mitoses per person), as described earlier (Vodicka et al. 2010; Musak et al.

2013). We detected the frequencies of aberrant cells (ACs), total CAs and individual types of aberrations – CTAs (including chromatid breaks and chromatid exchanges) and CSAs (including chromosome breaks, terminal deletions, interstitial deletions, dicentric chromosomes with difragments, ring chromosomes with difragments and abnormal chromosomes). Gaps were scored, but excluded from total CAs and from the statistical evaluation. Individual values of chromosomal damage were expressed as means ± standard deviation and medians.

Variants in DNA repair genes were taken into the study on the basis of predicted functional effects (SIFT and PolyPhen databases) and relevant published literature. Genotyping of DNA repair gene polymorphisms *XPD* Lys751Gln (rs13181; T>G), *XPG* Asp1104His (rs17655; C>G), *XPC* Lys939Gln (rs2228001; A>C), *XPA* 5'UTR (rs1800975; G>A), *XRCC1*, Arg194Trp (rs1799782; C>T), Arg280His (rs25489; G>A) and Arg399Gln (rs25487G>A), *OGG1* Ser326Cys (rs1052133; C>G), *XRCC2* Arg188His (rs3218536; G>A), *RAD54L* Ala730Ala (rs1048771; C>T) and *XRCC3* Thr241Met (rs861539; C>T), was carried out using primers and conditions previously described. The amplified fragments were digested with appropriate restriction endonucleases and the digested polymerase chain reaction (PCR) products resolved on 2% agarose gel and visualized under UV light after staining with ethidium bromide. Genetic polymorphisms in *APE1* Asn148Glu (rs1130409; G>T) and *NBS1* Glu185Gln (rs1805794; C>G) were analysed using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, Assay-on-demand, SNP Genotyping products: C 26470398 10 for *NBS1* and C 8921503 10 for *APE1*).

Regarding genes encoding XME, the functional evidence appears to indicate that all the variant genotypes tested either decrease enzyme activity or completely abolish it (EPHX1, NQO1, GSTM1, GSTT1). In CYP1B1 two SNPs were covered (CYP1B1/432 being Leu432Val, dbSNP: rs1056836 and CYP1B1/453 being Asn453Ser, rs1800440) by restriction fragment length polymorphism and GSTM1 (gene deletion) and GSTT1 (gene deletion) were assayed by allele-specific multiplex PCR,. Polymorphisms in GSTP1 (Ile105Val, rs1695), NQO1 (Pro187Ser, rs1800566) and EPHX1 (His113Tyr, rs1051740 and Arg139His, rs2234922 *EPHX1* Tyr113His (rs1051740) and His139Arg (rs2234922) were assayed by allelic discrimination using the TaqMan technology. The results were regularly confirmed by random re-genotyping of more than 10% of the samples for each polymorphism analysed.

Data were analysed using the statistical program SPSS analytical package version 16.0 (SPSS Inc, Chicago, IL, USA). Descriptive statistical analysis for individual groups was carried out. Differences in frequencies of cytogenetic end points of interest between patient and control subjects were tested by non-parametric Mann-Whitney U-test and Kruskal-Wallis test. The effects of the cytogenetic end points on the risk of cancer were evaluated by using binary logistic regression. Odds ratios (aOR) adjusted for potential confounders (age and smoking) are

reported with 95% confidence intervals. CRC and lung cancer patients were compared with a general control group (N=300) and breast cancer patients with female controls (N=158). All statistical tests were performed at the P-value ≤ 0.05. Regarding the effects of gene variants in healthy subjects, odds ratios (ORs) from multivariable logistic regression analysis were employed to consider simultaneous effects of particular occupational exposures, age, gender and smoking habits on the frequencies of CAtots, CTAs and CSAs. For each SNP, adjusted ORs were calculated regarding their effect on CAtot, CTA and CSA. Irrespective of whether or not a SNP appeared to be individually significant, all possible pairs of two SNPs were considered for the SNP-SNP-interaction analysis. In particular, the following genetic models were tested for each pair. 'Likelihood ratio (LR) tests were performed to assess whether including the SNP-SNP-interaction term yielded a significantly better fit of the data. For each best model the corresponding ORs and the Wald estimates for their confidence intervals and p-values were calculated. To assess the contribution of all genetic components (both SNPs and interaction term) to the model, LR based p-values were computed.

Results: Based on the assumption that increased chromosomal aberrations in peripheral blood lymphocytes may predict cancer risk or even to be causative phenomenon in malignant transformation, we sought for chromosomal aberrations in newly diagnosed 101 colorectal, 87 lung and 158 breast cancer patients and corresponding healthy controls. Strong differences in distributions of aberrant cells (ACs), chromosomal aberrations (CAs), chromatid (CTAs) and chromosome-type aberrations (CSAs) were observed in lung and breast cancer patients as compared to healthy controls. The frequency of CAs was significantly higher in all three groups of cancer patients (2.9±1.5 for lung; 2.7±1.6 for breast and 2.3±1.6 for colorectal cancer, resp.) compared to both control groups (1.8±1.5 and 1.7±1.2, respectively, P<0.001). In colorectal cancer patients, only CTAs were significantly elevated. Binary logistic regression, adjusted for main confounders, indicates that all the analyzed cytogenetic parameters along with smoking were significantly associated with breast and lung cancer risks. Significant differences in terminal deletions between breast cancer patients and corresponding female controls were recorded (0.39 vs. 0.18; P ≤ 0.05). We did not find any association of CAs with TNM stages or histopathological grade in either cancer type. Chromosomal aberrations were neither associated with additional tumor characteristics – invasivity, ductal and lobular character, estrogene/progesterone receptors in breast tumors nor with non-small/small cell and bronchogenic/pulmonary types of lung tumors.

In a search for intermediary cancer biomarker we assayed for CAs in 1028 healthy subjects, exposed to various potentially carcinogenic compounds, in comparison with 751 unexposed healthy subjects; frequencies of chromosomal damage were significantly higher in exposed individuals. Interestingly,

polymorphisms in *EPHX1* (a gene coding for epoxide hydrolase) and *XPD* (helicase involved in NER) modulated significantly frequencies of CAs. Analysed individually, we observed significantly lower CTA frequencies in association with *XPD* Lys751Gln homozygous variant genotype (OR 0.64, 95%CI 0.48–0.85, P=0.004; n=1777). A significant association of heterozygous variant genotype in *RAD54L* with increased CSA frequency (OR 1.96, 95%CI 1.01–4.02, P=0.03) was determined in 282 subjects with available genotype. By addressing DNA repair gene-gene interactions, we discovered 14 interactions significantly modulating CAs, 9 CTAs and 12 CSAs frequencies. Highly significant interactions included always pairs from 2 different pathways. In all genotype combinations involving XME genes with increased odds ratio for CAs a GST variant was involved.

Polymorphisms in genes involved in mitotic apparatus (*BUB1B*, *PTTG*, *ZWINT*) further modulated CAs. The results for total CAs showed significant effects of occupational exposure (OR 1.68) and CCND1 AA genotype (OR 1.85). In the separate analysis of CTAs and CSAs, the only significant effect of OR 1.99 (P=0.003) was on CSAs. The G870A genotype differentially influences the splicing of CCND1 mRNA. The G870 allele creates an optimal splice donor site at the exon 4/intron 4 boundary, resulting in the cyclin D1a transcript, whereas the A870 allele partially hinders splicing and allows read-through into intron 4 resulting in the cyclin D1b transcript. Cyclin D1 participates in DNA DSB repair by binding to RAD51, the main recombinase involved in homologous recombination. The induction of the DNA damage response is mediated by the cyclin D1a, whereas cyclin D1b lacks this activity. Thus, the present findings of the AA genotype preferentially inducing CSAs are consistent with these CAs being markers of double-stranded breaks. The shortening of telomeres in each cell division may lead to telomere crisis and complex CAs. Relative telomere length (RTL) was determined in 187 individuals based on their CA count in peripheral lymphocytes. The median RTL was 1.28 for 48 subjects showing no CAs. The median was 1.19 for 47 individuals with a total of more than 2 CAs (p=0.03). The median was 1.12 for 68 individuals with CSAs (p=0.00). The results were confirmed in logistic regression analysis adjusted for potential confounders.

Conclusion: Our studies demonstrate that chromosomal aberrations serve as a predictive marker for breast and lung cancer, whereas only CTAs were elevated in incident colorectal cancer patients. The results on healthy subjects provide strong novel evidence that telomere biology contributes to CA formation. Apart from the effect of the cyclin D1 splice site polymorphism on increased frequency of lymphocyte CAs, variants in genes coding for metabolic enzymes interact and are associated with CA frequencies in peripheral lymphocytes of healthy volunteers, so are interactions between DNA repair gene variants. Apparently, CAs accumulation requires complex interplay between different metabolic, DNA repair and cell cycle pathways.

The state of knowledge suggests a biological basis for the link between CAs and cancer risk. Moreover, they provide the first evidence on a genetic control of the overall CA frequency.

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Transcriptional Regulation of Survivin Gene Expression in Lung Cancer

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Survivin, an important anti-apoptotic protein is expressed in tumors, whereas in normal tissues the expression of this protein is absent or extremely low, defining a role for survivin as a bona fide cancer protein. However, why survivin expression is sharply and invariably restricted to tumor tissue, remains unclear. Here, we identified 11 putative consensus binding sites for GLI transcription factors in the survivin promoter and characterized the promoter activity. Inhibitors of the Hedgehog/GLI pathway, cyclopamine and GANT61, decreased the promoter activity in reporter assays in human lung cancer cell line A549. ΔNGLI2 (a more active GLI2 truncated mutant which lacks the repressor domain) was the most potent vector in activating the survivin promoter-reporter. Moreover, GANT61, a GLI1/2 inhibitor, repressed endogenous survivin protein and mRNA expression in most cells across a large panel of tumor cell lines including lung cancer. Chromatin immunoprecipitation showed GLI2 binding to the survivin promoter. The ectopic GLI2-evoked expression of endogenous survivin was observed in normal human fibroblasts. GANT61 reduced the growth of melanoma xenografts and decreased survivin level in nude mice tumors, mimicking the activity of GANT61 in cultured cells. The immunohistochemistry of human lung adenocarcinomas and other tumors revealed a correlation between the tissue regions showing GLI2 and survivin positivity. Thus, these results demonstrated that survivin is a classical transcriptional target of GLI2, a Hedgehog pathway signaling effector, potentially reflecting the high expression in human tumor cells. As the Hedgehog pathway is upregulated in virtually all types of cancer cells, this finding substantially contributes to the explanation of uniform survivin expression in tumors. Survivin thus constitutes a potential target for the development of a more effective treatment of cancers through the inhibition of GLI2 to restrain survivin activity.

Background: The Hedgehog signaling pathway is a phylogenetically conserved cascade activated by Sonic Hedgehog (Shh), a morphogen which stimulate cells in a paracrine or autocrine manner. The canonical activation of the Sonic Hedgehog (HH/GLI) cascade involves the binding of the ligand (Shh) to the 12-transmembrane protein PATCHED (PTCH), which, upon stimulus, releases the activity of adjacent transmembrane protein Smoothened (SMO). Thus, PTCH inhibits SMO rendering the pathway inactive in the absence of Shh. Upon activation, the effector proteins GLI (1–3) are released from the inhibitor SuFu (Suppressor of Fused) and translocated to the nucleus to activate target genes (Varjosalo et al., 2008). HH/GLI is active during normal embryonic development. Aberrant activation of this signaling pathway has been associated with many human cancers. The activation of HH/GLI increases cancer cells proliferation and survival and induces cancer stem cell (CSC) marker expression (Marini et al., 2011; Ryan et al., 2012). HH/GLI is important during normal embryonic development, and the aberrant activation of this signaling pathway has been associated with many human cancers. The HH/GLI pathway is important for multiple tumor types, although this signaling route was initially suggested as necessary for only basal cell carcinoma and medulloblastoma (Teglund et al., 2010). Deregulated HH/GLI signaling is now recognized to play a critical role in commonly occurring tumors, such as non-small-cell lung cancers (Rodriguez-Blanco et al., 2013) and many others (Yang et al., 2010). The critical role of this signaling pathway in general tumor maintenance brought about clinical studies utilizing several HH/GLI inhibitors (Atwood et al., 2012). Several studies have implicated a non-canonical Hh signaling pathway in regulating HH/GLI signaling, thus substituting the necessity of upstream ligand signaling. Pathways such as AKT (Riobo et al., 2006) MAPK (Stecca et al., 2007) and RAS (Lauth et al., 2010) can directly activate GLI factors in tumors. Survivin (BIRC5) is an anti-apoptotic protein highly expressed in the vast majority of human cancers and is associated with chemotherapy resistance, increased tumor recurrence and shortened patient survival. Survivin is abundantly and ubiquitously expressed during development and this expression is consistently recapitulated in tumor tissue (Altieri, 2008). We show here that survivin is a transcriptional target of the Hedgehog effector factor GLI2. We found that survivin promoter harbors 11 potential GLI binding sites in the promoter. GLI2 is a pro-invasive protein present in most tumor cell lines and mainly GLI2 substantially contributes to the stably elevated survivin levels observed in tumors.

Methods: 40 tumor cell lines of various origin including 12 lung cancer cell lines were utilized. In reporter-luciferase assays, cell lines were treated with HH/GLI inhibitors 20 μM GANT61, 20 μM cyclopamine. Quantitative real time PCR to detect survivin mRNA expression after treatment with 0, 10 and 20 μM GANT61 was conducted using Taqman system QuantiTect Probe PCR Kit (Qiagen) on ViiA^{TM}7 Real-Time PCR system (Life Technologies) according to the manufacturer's

instructions. Similar results were obtained in two independent experiments. Actin was used as an internal standard control. Whole-cell extracts prepared in RIPA buffer were used for immunoblotting analysis. W. blots were incubated with primary and horseradish peroxidase-conjugated secondary antibodies. Chemiluminescent detection was used. The antibodies for Western blots: anti-survivin (D-8) from Santa Cruz Biotechnology, anti-SRC and anti-BCL2 from Cell Signaling Technology and β-actin (AC-74) from Sigma-Aldrich. Anti-GLI2 (C-10) antibody was from Santa Cruz Biotechnology or Biorbyt. Transient cell transfections of the promoter-reporters were performed by using transfection reagents LipoJet or PolyJet (SignaGen). The pGL3basic vector (Promega) was used as a control. Expression vectors were contransfected in some experiments. Cell lysates were used for dual luciferase assays (Promega) performed as recommended by supplier's instructions. Several versions of the survivin promoter and its mutants have been cloned as XhoI-HindIII inserts in the pGL3basic plasmid. The following survivin promoter-reporter plasmids were generated (numbering is related to the start of translation, +1): –1814+57, –990+57, –1814–10, –990–10, –1814–10Δ(–319–60), and –990–10Δ(–319–60). Chromatin immunoprecipitation: lung carcinoma cells A549 were transfected with the pcDNA3-ΔNGLI2 expression plasmid. After 2 days cells were fixed with 1% formaldehyde, incubated with glycine solution, and washed four times with PBS. The cell extracts were isolated and processed according instructions of the ChIP-IT High Sensitivity Kit (Active Motif, Carlsbad, CA, USA). As a positive control, anti-acetylated histone H4 antibody was used (Millipore). Negative controls were buffer, rabbit or mouse non-immune IgG. To detect GLI2 bound on the promoter, mouse anti-GLI2 (C-10) (Santa Cruz Biotechnology, sc-271786) and rabbit anti-GLI2 (Abcam, ab26056) were used. For the detection of ChIP-generated DNA, real-time PCR was performed by the QuantiTect SYBR Green PCR Kit (Qiagen). In vivo xenografts: melanoma cell lines SK-MEL-3 and 501mel were engrafted subcutaneously into athymic-nude mice. GANT61 was administered 3-times a week intratumorally for 2 weeks. After ending the experiment, the fresh tumor tissue was used for Western blot and analysed by immunochemistry. For immunochemistry of archival samples, parallel tissue sections from 20 tumors (lung and ovarian carcinomas and melanomas) were stained with survivin and GLI2 primary antibodies purchased from GeneTex. The detection of antigen-antibody complexes was performed using EnVision+ avidin-biotin detection system (Dako). Mice tumors were immunochemically stained only for survivin.

Results: We observed that the survivin promoter contains 11 sites for binding GLIs, effectors of the HH/GLI signaling pathway; however, none of these sites are full consensus sequences. Non-consensual sites with two or three mismatches can also activate the transcription of other GLI-regulated genes, such as BCL2 (Regl et al. 2004). The proximal promoter (–990 to –10) was sufficient to activate luciferase activity and only slightly less efficiently than the longer (–1814–10) promoter. The

distal portion of the promoter contains 4 GLI sites (no. 1–4), and the mutations at these sites appreciably decrease promoter activity (approximately 2.5-fold, Figure 2). Intriguingly, after the addition of a short downstream region (–10 to +57 nt) containing sites 10 and 11, the strong repression of promoter activity appeared. However, when site 10 was mutated the activity was reverted to normal, indicating that the site 10 is an inhibitory site. We further deleted the central region of the proximal promoter (–390 to –60). After the deletion, the activity was completely abrogated. Because this region comprises all Sp sites, this result is consistent with the known role of Sp1 and Sp3 as factors necessary for the basic promoter activity. The role of GLI sites no. 6–9 in the deleted region cannot be precisely established because some of these areas overlap the Sp sites. A SMO inhibitor cyclopamine significantly diminished the activity of the survivin promoter in several cell lines to various. GANT61, which specifically inhibits GLI1/2 activity consistently inhibited the promoter. These results further suggest that survivin expression is regulated through the HH/GLI pathway. To stimulate cells with GLIs, an empty vector (control) or expression vectors for GLI1, GLI2, ΔNGLI2 (an active form of GLI2) and GLI3 were cotransfected into A549 cells. Among all activators, ΔNGLI2 exhibited the highest activation potential. Western blot analysis revealed that GANT61 inhibited or attenuated survivin expression in most cell lines. In some cell lines survivin expression was dramatically decreased (SK-MEL-3, WM-35, SW-13) whereas some cells responded weakly. Further experiments with MG132 (MG), a proteasome inhibitor, showed that the survivin protein level protection in tumor cells is mediated through both GLI-dependent and -independent mechanisms. Consistently, real-time PCR revealed that the inhibition of survivin protein levels was accompanied with the downregulation of survivin mRNA to various extents, confirming the predominantly transcriptional repression of this gene after GANT61 treatment. Taken together, these results suggest that the decrease of endogenous survivin in the presence of GANT61 is predominantly a transcriptional event, whereas the specific cellular context might also modify protein degradation. To confirm the direct interaction of GLI2 with the endogenous survivin promoter, we performed a quantitative chromatin immunoprecipitation assay (ChIP) in A549 cells using two different anti-GLI2 antibodies. The GLI2 enrichment on the promoter was quantified using SYBR green real-time PCR. The results confirmed the significant enrichment with GLI2 suggesting that GLI2 was indeed recruited to the survivin promoter in cells. Further, GLI1, GLI2 and ΔNGLI2 were transfected into the normal diploid human fibroblast cell line IMR90 which does not express survivin or GLI2. The strongest signal was obtained with the most potent activator ΔNGLI2, suggesting that GLI2 is a direct survivin promoter activator in cells.
To determine whether the in vitro effects of GANT61 are recapitulated in vivo, SK-MEL-3 and 501mel cells were subcutaneously engrafted into athymic nude mice. Whereas the tumor masses with GANT61 remained as controls in 501mel cells (reflecting the resistance of 501mel cells in vitro), SK-MEL-3 tumors were

markedly diminished. Expectedly, in all tumors from GANT61-treated animals, survivin expression estimated by Western blot was markedly decreased. The immunochemistry of animal tumors showed positive survivin staining in controls, while only scarce positive cells remained in tumors from GANT61 treated animals. Taken together, these data showed that the in vivo cell growth was broadly reflective of the growth observed in vitro. If GLI2 is indeed an activator of survivin transcription in cell models, then the positive areas for both proteins should correlate in authentic human tumor sections. We examined parallel sections of 20 randomly selected human tumors and examined the GLI2 and survivin staining. Indeed, positively stained GLI2 areas were correlated with survivin-positive areas in all tumors. Some tumor cells showed perfectly correlated positivity for both proteins in parallel sections, as exemplified in 2 cases of lung adenocarcinomas. Thus, these data showed the overall correlation between GLI2 and survivin staining in tumors, strongly supporting that the HH/GLI pathway is an activator of survivin expression.

Conclusions: Here, we have partly revealed a mysterious mechanism resulting in high survivin levels in tumors, which has not been anticipated previously. More than half of the tumor cell types analysed showed at least slightly downregulated survivin expression after treatment with 20 μM GANT61. Several tumors manifested nearly complete inhibition. As some tumors were resistant to the GANT61-mediated reduction of survivin expression, other mechanisms likely maintain the high survivin level observed in tumor cells. We dissected and mutated the survivin promoter, revealing many GLI binding sites, and demonstrated that the promoter-reporter is substantially inhibited through the HH/GLI inhibitors cyclopamine and GANT61. The increased number of GLI binding sites made it difficult to precisely determine the combination of sites critical for survivin activity mediated via HH/GLI. We propose that GLIs are associated with the promoter in each tumor cell but other (co)factors dictate survivin expression. We previously demonstrated epigenetic mechanism important for survivin expression. The knockdown of BRG1, an ATPase of the SWI/SNF chromatin remodeling complex, dramatically reduced the expression of survivin RNA and protein in melanoma cells (Ondrušová et al., 2010). Consistent with the present study, Brun et al. revealed that survivin is a critical therapeutic target in medulloblastoma cells, where HH/GLI signaling in invariably increased. The inhibition of survivin expression through the specific inhibitor YM155 profoundly affected the viability of tumor cells and sensitized tumors to radiation. Taken together, we revealed ubiquitous expression mechanism of the tumor protein survivin, although it is not functional in all tumors. These results show the direct activation of survivin expression through the HH/GLI signaling mediated by GLI2. Importantly, anti-GLI2 therapy or combined anti-GLI2 and anti-survivin therapies might decrease survivin in tumors and markedly improve the treatment of cancer.

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Granzyme B-induced apoptosis and its regulation in lung cancer cells

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Background: To kill cancer cells, human cytotoxic lymphocytes (CTLs) and natural killer (NK) cells use the multistep and complex granzyme-perforin mechanism.

This cell death mode functions via the secretory immunological synapse (SIS) that is formed between the immune killer cell(s) and the tumour cell that had been recognized as a target (Galandrini et al., 2013; Voskoboinik et al., 2015). Human CTLs and NK cells express five different granzymes: A, B, H, K and M. These serine proteases are vectorially released into SIS from polarized cytotoxic granules (secretory lysosomes) of synapsing CTLs and NK cells. The released granzymes are subsequently internalized into the cytosol of target tumour cells via a process dependent on the Ca^{2+}-binding glycoprotein perforin, which is co-secreted from synapsing CTLs and NK cells into SIS (Voskoboinik et al., 2015). The entry route of granzymes into the cytosol goes through the polyperforin pores formed in the plasma membrane and/or in the non-acidifying endosomes (gigantosomes) of target tumour cells, which engulf the granzymes together with perforin monomers via endocytosis. Although cytotoxic activities have been ascribed to all human granzymes, only granzyme B has been unequivocally proven to exert multiple cell death activities (Roušalová and Křepela, 2010b; Joeckel and Bird 2014a).

Proteolytic targets of granzyme B within tumour cells during the granzyme B-induced apoptosis comprise several tens of proteins, which have different subcellular localization and belong to different functional groups (Roušalová and Křepela, 2010b; Joeckel and Bird 2014b; Jacquemin et al., 2015). The multiplicity of the granzyme B-mediated proteolytic attacks is important to overcome the apoptotic threshold of cancer cells since it can lead to significant amplification and robustness of the apoptotic process. The intracellular proteolytic targets of granzyme B that contribute to amplification of its cell death activities include procaspase-3 and -7, Bid and Mcl-1 proteins, and the inhibitory/chaperone subunit A of DNA fragmentation factor. Recent developments in the recombinant human granzyme B-based biopharmaceuticals suggest that these proapoptotic drugs could be promising tools for specific killing of tumour cells (Caldas et al., 2006; Kurschus and Jenne, 2010; Oberoi et al., 2013; Lu et al., 2015).

Tumour cells use several mechanisms to prevent cytosolic entry of granzymes from CTLs and NK cells. They include inhibition of the chemokine-mediated lymphocyte trafficking into the tumours (da Silva et al., 2015), extracellular acidification that blocks the formation of polyperforin pores (Praper et al., 2010), autophagy of gigantosomes during hypoxia leading to degradation of endocytosed granzyme B (Viry et al., 2014), and the emperitosis- and activation -induced death of immune killer cells (Wang et al., 2013; Bird et al., 2014). Importantly, many tumour cell types are also capable to abolish the cytosolic and nuclear death activities of granzyme B via its inactivation by a specific, fast-reacting and irreversible protein inhibitor serpinB9 (Roušalová and Křepela, 2010b). There is evidence that hypoxia and the extracellular acidic stress, which often occur in the microenvironment of many solid tumours, can induce overexpression of *SERPINB9* gene in both stem and non-stem cancer cells (Li et al., 2009; Hjelmeland et al., 2011). Due to frequent overexpression of serpinB9 in lung cancer cells and

tumours, this serpin could be a significant protectant of lung cancer cells against the immune-mediated death (Roušalová and Křepela, 2010a; Soriano et al., 2012).

In the present study we investigated the expression of serpinB9 (SB9) and its involvement in the inhibition of granzyme B (GrB) in human lung cancer cell lines and non-small cell lung carcinoma (NSCLC) tumours and matched lungs from surgically treated patients. We analysed the GrB-mediated activation of procaspase-3 and the cytosolic inhibition of the GrB-generated caspase-3 activity, the expression status of SB9 in NSCLC and small cell lung carcinoma (SCLC) cells and other human cancer cell lines, the role of cytosolic SB9 in the inhibition of GrB-mediated induction of caspase-3-like activity, and the formation of a stable GrB·SB9 complex. In order to get insight into the regulation of *SERPINB9* gene expression in NSCLC cells we examined the relationship between the expression of SB9 and EGFR mRNAs and we studied the effect of 5-aza-2'-deoxycytidine (ADC) on the level of SB9 mRNA expression.

Methods: The samples of NSCLC tumours were obtained from surgically treated lung cancer patients and were histopathologically classified according to the WHO criteria 2004. The study was approved by the Ethical Committee of the Hospital Bulovka, Prague. NSCLC cell lines (CALU-1, SKMES-1, NCI-H520, A549, SKLU-1, COLO-699, LXF-289, COR-L23, LCLC-103H, NCI-H1299), SCLC cell lines (NCI-H69, NCI-H82, NCI-H146, NCI-H209, NCI-H345, NCI-H378, NCI-H446), glioblastoma cell lines (U87, U118, U138, U251, U373, T98G) and breast cancer cell lines (T47D, MCF-7, MDA-MB-231, SK-BR-3) were grown in culture as described (Roušalová et al., 2010a; Bušek et al., 2012). The indicated NSCLC cells were also cultured in the presence of 10 mM of ADC for 72 hours. The expression of SB9 and EGFR mRNAs and β-actin mRNA (a reference transcript; Roušalová et al., 2010a), were quantitated by coupled real-time RT-PCR. In the assays of SB9 mRNA and EGFR mRNA expression, the used forward and reverse primers and TaqMan probe had the following sequences, respectively: 5´-GGAATGAACCGTTTGACGAA-3', 5'-TTTCCACCGTGCTGA GCT-3´, and 5´-(6-FAM)CGCACCTCGCCCACGTGG(TAMRA)-3´, and 5´-CAGCGCTAC CTTGTCATTCAG-3´, 5´-GGTTGCACTCAGAGAGCTCAG-3´, and 5´-(6-FAM)ATGAGG TACTCGTCGGCATCCACCA(TAMRA)-3´. The expression of procaspase-3 (PC-3) and SB9 proteins was assessed by Western blot-ECL analysis using specific antibodies and the GrB-mediated induction of caspase-3-like activity was measured with Ac-DEVD-AFC as the fluorogenic caspase substrate (Křepela et al., 2004; Roušalová et al., 2010a). The formation of a stable GrB·SB9 complex in cytosol from NSCLC cells was examined by Western blot-ECL analysis using recombinant human GrB and specific antibodies (Roušalová et al., 2010a).

Results: The full GrB-mediated PC-3 activation in cytosolic extracts from NSCLC cells and tissues and lungs required both the proteolytic processing

of PC-3 and the thiol-assisted reduction of PC-3 and/or caspase-3 (CS-3). At higher protein concentrations, the GrB-generated CS-3-like activity was lower in NSCLC tumours as compared to matched lungs, suggesting increased level of a GrB inhibitor in the tumours, which had significantly higher PC-3 protein expression. Although all tested NSCLC cells expressed SB9 mRNA and protein, these tumour cells could be classified according to their SB9 level as high and low SB9 expressors. Certain analysed tumour cell types have substantially different expression of SB9, e.g. lung cancer cells, which mostly showed high SB9 mRNA expression, and glioblastoma cells, which expressed extremely low SB9 mRNA levels. On the other hand, among tumour cells of the same type both the high and low SB9 expressors could be found, e.g. in NSCLC cells and breast cancer cells. In NSCLC cells, the level of SB9 protein showed a significant inverse correlation with the GrB-mediated induction of CS-3-like activity. Human recombinant GrB formed with SB9 in the cytosol from NSCLC cells a denaturation-resistant complex GrB·SB9, indicating that the endogenous SB9 is a functional GrB inhibitor. The levels of SB9 mRNA and EGFR mRNA showed a positive correlation in NSCLC cell lines, suggesting that the increase of EGFR expression may promote the expression of SB9. Treatment of cultured NSCLC cells lines with 5-aza-2'-deoxycytidine, an inhibitor of DNA methyltransferases, induced high increase of SB9 mRNA expression in 8 of 10 tested NSCLC cells lines. This suggests that DNA methylation could be involved in silencing of *SERPINB9* gene expression in NSCLC cells.

Conclusions: The present study suggest that the majority of lung cancer cells is endowed with high expression of the functional granzyme B inhibitor serpinB9. This phenotypic property may significantly increase their apoptotic threshold against the apoptotic attack of immune killer cells such as CTLs and NK cells. The high expression of serpinB9 in lung carcinomas may represent an obstacle for their effective immunotherapy and possibly also for targeted biomolecular therapy using recombinant granzyme B-based biopharmaceuticals.

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Prognostic and predictive biomarkers of lung cancer

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In the recent years, significant progress has been made in the understanding of the biology of lung cancer which had a direct impact on translational research. Investigation of the molecular landscape of lung tumors provides new insight into a broad range, from genetic diagnostics to therapy stratification, early recurrence detection and drug resistance monitoring. Various approaches including high-throughput and high-resolution technologies, have been tested to find reliable molecular markers of cancer.

Genomics studies conducted using tumor samples (lung), often including comparison with blood in order to distinguish germline mutations, revealed somatic point mutations (*EGFR, KRAS, BRAF*), translocations (*ALK, ROS1, RET*) and amplifications (*MET, FGFR1*), which provided significant clinical benefits (e.g., fusion genes became new molecular targets). Moreover, comprehensive molecular profiling of lung adenocarcinoma (by the Cancer Genome Atlas Research Network) identified novel loss-of-function *MGA* mutations which are mutually exclusive with focal *MYC* amplification, as well as many tumor suppressor gene abnormalities (*TP53*, *STK11*, *CDKN2A)* which were also common, but currently do not have clinical significance. Unfortunately, there is still a question related to driver oncogenes in most lung adenocarcinomas.

Transcriptomic analysis of lung cancer was not followed by milestone steps in the field of genetic diagnostics as it played a role in breast cancer studies using microarray analysis and resulting in a 70-gene expression signature. Nevertheless, several gene expression signatures were proposed that allowed prediction of disease recurrence risk in patients with non-small cell lung carcinoma (NSCLC). Further studies narrowed the number of genes of interest and showed that even a single analysis of *ERCC1* expression may bring prognostic and predictive information. Gene expression can be regulated by epigenetic effects, such as DNA methylation and histone modifications, and can be controlled by the spatial organization of chromatin and miRNAs expression. The miRNAs are short noncoding RNAs (19–23 nucleotides in length) which are aberrantly expressed in the lung, which indicates their potential as biomarkers that may stratify patients into distinct molecular groups with high and low risk of recurrence. For example, genome-wide miRNA-sequencing in primary lung adenocarcinoma allowed identification of miRNA-31 whose high expression predicts the presence of lymph node metastases and low expression is associated with excellent survival.

In this presentation, I would like to highlight our results obtained using molecular tools (Real-Time PCR, MLPA-based assays, FISH) for the detection of small-size somatic mutations (*EGFR*), amplification in lung cancer (*EGFR, MET, ERBB2*), chromosome rearrangements (*ALK*), as well as somatic variations of the number of copies of 14 miRNA genes consistently found as either over- or underexpressed in lung cancer. Our recent observations support the idea of high genomic instability of miRNA gene regions and the contribution of copy number variations to the regulation of miRNA expression in cancer. In particular, I will discuss the correlation between copy number variations of miRNA biogenesis genes with the survival of lung cancer patients. Last but not least, I will show the preliminary results of a pilot study in which almost 200 miRNAs were evaluated in plasma derived from a diversified group of adenocarcinoma patients.

There have been several studies evaluating lung cancer genome, transcriptome, epigenome and microRNome. This has significantly expanded the pool of prognostic and predictive diagnostic markers which are already in routine use or RUO. Nevertheless, not only single markers, but also molecular profiling of the whole tumor using NGS strategy, together with liquid biopsy, seem to be a new challenge and constitute novel diagnostics tools.

Proton pump inhibitors; a paradigm for a new strategy against cancer

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From the first preclinical evidences showing that PPI may work either as chemosensitizing agent (1) or highly cytotoxic anti-tumor agents (2, 3), to the clinical evidences that PPI chemosensitize either human (4) or pets tumor patients (5, 6), the proof of principle is becoming solid and convincing. PPI were able to chemosensitize human tumor cells of different histologies, through a normalization of extracellular pH, both in vitro and in vivo (1–3). Actually, one common feature of tumors is that they are acidic (7, 8) and the way tumors become acidic is not entirely made clear. However, an interesting hypothesis is that during the primary tumor growth malignant cells develop what is also called "Warburg Effect", that is the ability of cancer cells to fermentate sugars with lactate production, independently on the oxygen levels within the tumor mass (9, 10). The condition of H+ accumulation within the tumor tissues progressively selects tumor cells armed to survive in this hostile microenvironment (7, 8). One of the most recognized mechanism allowing cancer cells to survive in the acidic milieu are a series of proton exchangers (11), that help the tumors cells

in avoiding intracellular acidification. Between these exchangers there are some proton pumps, such as vacuolar ATPases, that are extremely active in tumor cells by pumping H+ both from the cytosol to internal vacuoles and from the plasma membrane to the extracellular microenvironment (11, 12). Thus, the first idea was to inhibit V-ATPases in order to deprive cancer cells of this mechanism, but direct inhibition of these proton pumps was toxic, being V-ATPases ubiquitous into the body (12). We focused our attention on a family of proton pump inhibitors (PPI) that are used worldwide as very potent antiacidic drugs against peptic diseases or as gastro-protectors, (i.e. omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole) (13), that did not show relevant systemic toxicity, even in prolonged treatments and at very high dosages, as in patients with Zollinger and Ellison syndrome, but also in other disease conditions (14). PPI specifically target gastric H+/K+ ATPases, but VATPases as well (11–13). PPI are prodrugs needing protonation in acidic milieu to be transformed into the active molecule, while chemical drugs are mostly weak bases, undergoing neutralization outside the tumor cells by protonation (15). Thus, while acidity represents a potent mechanism of tumor resistance to drugs, PPI exploit tumor acidity to become functional (15–17). We thus started with a series of preclinical investigations showing that PPI sensitize tumor cells and tumors to the action of chemotherapeutics (1, 18). However, we also showed that PPI per se exert a potent antitumor activity, through an in vivo modulation of tumor pH (2, 3). Lastly we showed that acidity represents a potent mechanism of tumor immune escape and PPI increase the immune reaction against tumors (19). These preclinical data represented the background for a series of clinical studies aimed at supporting the use of PPI as chemosensitizers. Up to now the results of two clinical trials in humans are published in either osteosarcomas or metastatic breast cancer patients (MBC) (4, 20). The results showed that pre-treatment with PPI increased the effectiveness of neoadjuvant chemotherapy in osteosarcoma patients, particularly in the chondroblastic variant (4) and the time to progression (TTP) or overall survival (OS) in MBC patients maintained under PPI treatment for one year after the stop of chemotherapy (20). Moreover, two clinical studies in companion animals with spontaneous tumors, ,highly supported the efficacy of PPI in increasing the efficacy of standard chemotherapy and significantly improving the quality of life of treated pets, in either standard treatment (5) or metronomic regimens (6). More recently, a meta-analysis in head and neck tumor patients confirmed an increased response in patients receiving anti-acidic drugs, particularly those treated with PPI (21). These results should induce to sit down and think to new anti-tumor strategies in which PPI should be included.

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Scientific session IV (1st part of Best of WCLC) News in diagnostic and treatment modalities

New proposals for TNM staging

W. Eberhardt (Germany)
Abstract missing

News in pathology: Histological classification, pheno- and genotypic markers' WCLC 2015

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WHO classification (1)

In 2015 WHO classification of lung carcinomas changed significantly due to the development of diagnostic in the past decade and to the development of a kind of precision therapy. These changes include new classification of differentiated and undifferentiated carcinomas. Concerning adenocarcinomas, new features are definition of the preinvasive lesions as AAH and the novel AIS as defined by a lesion smaller than 3 cm, establishment of the minimally invasive ADC as defined by a size less than 3 cm and the invasion zone is smaller than 5 mm, no lymphovascular invasion and lack of necrosis. Equally important is the grading of the invasive ADCs, where G1 tumors are characterized by lepidic growth pattern, G2 is the acinar and papillary forms and G3 tumors are the solid or micropapillary variants. Since ADC is almost always a heterogenous tumor, diagnosis must be based on the predominant histological type. The new classification affects also TNM staging since AIS is equal to Tis, MIA is equal to Tmi and T size must be based on the size of the invasive component exclusively.

Squamous cell carcinomas are composed of keratinizing and non-keratinizing forms as well as the novel basaloid carcinoma, all must be p40/p63 positive and TTF1 negative. Large cell carcinoma definition is also changed based on pheno- (IHC) and genotypes and now is a mucin negative, IHC-null tumor. All the other subtypes

of LCC is either eliminated or moved to different categories. May be the major change of the new classification is the establishment of the neuroendocrine subclass which now contains carcinoid tumors, SCLC as well as neuroendocrine large cell carcinomas all characterized by positivity for at least one neuroendocrine marker.

It is now well defined the extent of diagnostic possibilities of the small samples. In case of presence of morphological criteria ADC and SQCC can be diagnosed but in the absence of those IHC testing is obligatory. However, in the positive cases the diagnosis can only be NSCLC in favour of the ADC or SQCC. In case of inconclusive IHC data NSCLC, NOS can be assigned. As far as the markers concerned, p40 is preferred over p63 in SQCC and TTF1 is preferred over napsin in ADC. The novel classification is not entirely new since 2011 most of the components have been introduced gradually and tested in real time.

TNM staging (2)

IASLC collected a novel lung cancer patient database of more than 77000 patients and evaluated their clinical and pathological data to improve the 8th TNM classification. When patient survival was analyzed according to tumor T size data indicated that decreased survival can be observed by 1 cm increment from 1 to 7 cm which is relevant irrespective of the nodal involvement (either N0M0, R0 or N+ cases). Novel data confirmed that T1a and T1b patients survival is significantly different from each other (1–2 cm versus 2–3 cm) and from tumors of less than 1 cm. This last finding can be important from the point of view of lung cancer screening programs. Also, data shown that T2a tumors has worth prognosis as compared to T1b even in the same smaller than 3 cm range. On the other hand, 5–7 cm tumors has similar prognosis to T3 rather than T2b and tumors of larger than 7 cm have a similar survival as T4 patients. These novel observations are valid in both pathologic and clinical staging. It is a further study, whether the measurement of the invasive component exclusively, as recommended by UICC, is the standard procedure to define T category.

Analysis indicated that endobronchial tumors of less than (T3) or more than 2 cm (T2) from the carina have similar survival in both pathological and clinical staged patients. Complications of the endobronchial tumors are atelectasis and pneumonitis and these are current descriptors in T2/T3. The analysis of the patients' data indicated that partial or total atelectasis/pneumonia are not a sensitive predictor in T2/T3. On the other hand, visceral pleural invasion (VPI) is a sensitive strong descriptor in tumors of less than 7 cm sizes and the PL0-2 invasion categories are sensitive T2 descriptors. However, VPI can only be used in pathological staging. Invasion of the diaphragm was revealed to be a strong descriptor of the T4 category but not of the T3. Based on careful analysis of patient survival according to mediastinal pleural invasion this T category descriptor cannot be used further.

When these novel information were used to T stage lung cancer patients, in all T categories significant survival differences have been found indicating that

improved or selective use of the descriptors improve T staging, especially in T3 and T4.

New pathology

Since adenocarcinoma is a heterogenous tumor and the histological subtype variant carry prognostic value it is now mandatory to define the predominant type. Novel study indicated that the second-predominant histological subtype may also has a prognostic significance: in case of a lepidic second component the incidence of lymphovascular invasion is lower and the DFS is longer as compared to a non-lepidic second component (A1070). A novel 57-gene lung subtyping gene expression signature (LSP) was tested on a large frozen lung cancer database and validated on a FFPE cohort using histological classification as standard. LSP specificity to define squamous, adenocarcinoma or neuroendocrine variant was 78–91% on frozen samples and 82% on FFPE samples suggesting that it can be used when classical approach is failed or the sample size is not enough. **(3)** The large cell neuroendocrine cancer (LCNEC) is an established histological entity but genomic studies identified two variants, an SCLC-like and an AD-SQ-like ones. (A1667). Squamous cell cancer in the lung raises frequently the question of whether it is a primary or metastasis of a head and neck cancer. Using CK19, MMP3, ZNF830 and PI3 proteins as IHC markers it is possible to distinguish between the two options (A1525). Pathological staging of lung cancer is critical in case of surgically resected cases, where beside the T size N status also have a strong impact. However, standardization of the intrapulmonary recollection of the lymph nodes is missing, which critically affect the effectiveness of this procedure. Novel data indicated that due to non-standardized collection of intrapulmonary nodes results in understaging of the lung cancer (Ostaogiagbon A).

Biomarkers

It seems that in lung cancer molecular diagnostics the tissue is still an issue. There is a consensus that all type of materials must be collected for a comprehensive differential- and molecular ones. Although the cytologic smear was discredited before, there is a revival of its parallel use since frequently it contains a significant amount of tumor DNA. Furthermore, although in ALK testing its use is not recommended, in case no other option it is an appropriate alternative. Using small biopsies it is very important to produce unstained slides economically (i.e. in one trimming step) for IHC, molecular analysis (5-10 slides) and FISH. (A2011)

Targeting Pemetrexed beyond adenocarcinoma is an open issue and several mRNA and protein markers have been tested before without producing a clinically validated marker. Though P-resistant tumors differ in gene expression signature that cannot be translated to an IHC one (A2793). Using MS technology two enzymes DHFR and GARFT involved in folate metabolism have been tested and found to be a promising marker combination for P-prediction (A1685).

Though MET gene mutation or amplification is a relatively rare event in adenocarcinoma, using IHC about half of the tumors demonstrate protein overexpression. However, it is a question if the gene alteration of MET or the protein overexpression or both are predictive markers for sensitivity to novel MET targeting agents (antibodies or TKIs). (A2114) The detailed analysis indicated that high MET copy number rather than MET amplification is associated with increased MET protein expression. However, it is very important that various thresholding is possible for IHC evaluation of MET protein and H score (>200 or 250), or >30% of strong positivity seem to best correlate with increased copy numbers. This type of stratification might help to better target anti-MET drugs in lung cancer. (A2155).

Anti-EGFR antibody treatment of NSCLC is a long issue involving Cetuximab and the novel Necitumumab both tested in phase III trials. It is evident without appropriate predictive marker(s) no of the two agents can produce robust clinical efficacy. In case of a Cetuximab containing regime of the SWOG0819 trial there was no efficacy detected in unselected NCSLC patients. However, in SQCLC subgroup especially in case of EGFR FISH+ patients a highly significant PFS and OS benefit was recorded. (A3612) In case of the SQUIRE trial where necitumumab was added to chemotherapy EGFR IHC positivity and EGFR FISH+ was required to detect clinical benefit of anti-EGFR antibody treatment of SQCLC. (A2651)

Check-point inhibitors are registered in NSCLC but the patient selection is under debate. PDL1 immunohistochemistry is under investigation as a promising immunotherapy predictive marker. Detection of PDL1 gave various results in case of various tumor types and agents which is most probably due to the various technologies (Dako, Ventana and Merck) and the scoring systems used. In some cases tumor cell expression is scored using a proportion score where intensity levels have also been incorporated, H-score (% of positivity x intensity levels 1–3), or tumor and/or immune cell scores 1–3. In case of Pembrolizumab (**4**) a proportion score >50% was established as clinically validated cut off level of tumor cell PDL1 expression predictive for PFS and OS using the Dako IHC system. This high treshold can be confronted with other studies where a check-point inhibitor efficacy was linked to any (>1%) PDL1 positivity. In an ongoing anti-PDL1 antibody trial (FIR) Ventana system is used and the TC/IC scoring 2/3. Data indicate that intrapatient heterogeneity of PDL1 IHC expression is low and is maintained in metastases as well. (A1609, 3226) RAS mutation status seems to be a factor behind elevated PDL1 expression (A2496) or PDL1 amplification (A2257).

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News in screening

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Deaths from lung cancer exceed those from any other type of malignancy with 1.5 million deaths in 2010. I tis why there has been substantial interest in the studies dealing with lung cancer screening. The reason behind this fact is clear-cut. With no screening approach which we apply nowadays there is 5 years survival rate less than 18% with operability rate less than 25%. Screening logically increases operability and now we have clear data that it also increases cancer- related and overall survival.

Historical chest X-ray studies from the end of last century clearly showed that plain chest X- ray is not a useful screening test.

Largest of modern LDCTstudies is the National Lung Cancer Screening Trial (NLST), which has reported a reduction in lung cancer deaths among screened individuals. This involved patients with a significant risk of lung cancer due to age and smoking history. There are however many shortcomings of screening – mainly abundant amount of false positive results. In NLST overdiagnosis was 19% which poses significant both medical and ethical issues in the screening process. The number of cases of overdiagnosis found among the 320 participants who would need to be screened in the NLST to prevent 1 death from lung cancer was 1.38. Newer methods of LDCT evaluation as volumetry might be the way forward however variability of both the results and evaluative techniques remains obstacle in the screening process. Screening participants of the NELSON low-dose CT study underwent screening followed by rescreening in the case of indeterminate nodules (volume50–500 mm3) by a follow-up CT performed 3 months after baseline. All malignant fast-growing lung nodules referred after the 3-month follow-up CT in the baseline lung cancer screening round had VDT ≤232 days. That documented that by lowering of the VDT cutoff the number of false-positive referrals may be reduced. Type of the lesion is the important determinant of its biological behaviour. Subsolid nodules (divided into part solid a GGO- ground glass opacities) are growing at the low rate. Invasive evaluation and surgery is indicated when ground glass CT pathology is greater than 2 cm (representing minimally invasive adenocarcinoma) or when subsolid nodule incorporates solid component bigger than 5 mm signalling invasive adenocarcinoma.

Due to current recommendation of the European Society of Radiology and the European Respiratory Society it is recommended to screen lung cancer in comprehensive, quality-assured, longitudinal programmes within a clinical trial or in routine clinical practice at certified multidisciplinary medical centres. Minimum requirements include: standardized operating procedures for low-dose image

acquisition, computer-assisted nodule evaluation, and positive screening results and their management; inclusion/exclusion criteria; expectation management; and smoking cessation programmes. Further refinements are recommended to increase quality, outcome and cost-effectiveness of lung cancer screening: inclusion of risk models, reduction of effective radiation dose, computer-assisted volumetric measurements and assessment of comorbidities (chronic obstructive pulmonary disease and vascular calcification). All these requirements should be adjusted to the regional infrastructure and healthcare system, in order to exactly define eligibility using a risk model, nodule management and a quality assurance plan. The establishment of a central registry, including a biobank and an image bank, and preferably on a European level, is strongly encouraged.

There are new data showing that prolongation of the screening interval as in Nelson trial (1yr, 2 yrs, 2,5 yrs) brings stable cancer incidence per round (~0.8%) and also significant stage shift towards worse stage after 2,5 years, so 2.5 years interval appears to be too long.

Another problem is that most research is focused on lung nodules detected during baseline screening. There are however new nodules reports of which are usually quite inconsistent (different definitions, limit comparison) and in which cancer rates are rarely explicitly reported. Trials as ELCAP, I-ELCAP, PLuSS and Mayo trial showed annual incidence of new nodules 3.4-13%. New nodules are logically expected to be fast growing and previous trials showed cancer rate in participants with new nodules 1.6–7.5%. Contrary to baseline nodules, new nodules develop within a known time-frame and the growth rate can likely be quantified. At incidence LDCT lung cancer screening, newly detected solid nodules show different volume for malignant and benign lesions. It appears that lower volume cut-off will be needed for follow-up of incident nodules.

There are many models attempting to discern between benign and malignant nodules. In 2013, McWilliams et al. published 4 prediction models for the probability of lung cancer in pulmonary nodules on first screening CT, taking subject characteristics and morphological features of a nodule into account.

In 2014, the American College of Radiology published the Lung-RADS Assessment Categories, using 5 categories to determine nodule management, based on nodule type, size and growth. However it is unknown which of these systems performs best to help radiologists select the subgroup of nodules that need more invasive follow-up. As the Lung-RADS model does not incorporate clinical data and has been approved to report from screening centres so it is most widely used.

CT examinations with semi-automated volume measurements of a lung nodule diagnosed as being malignant after four subsequent CT examinations. This nodule grew from 17.0 mm^3 at baseline to 88.3 mm^3 at time of referral after follow-up examination in the second screening round, 1.5-years after baseline.

In summary it is clear that the selection of individuals with a high risk of developing lung cancer is key to the successful implementation of national

screening programmes. The future management of screen-detected pulmonary nodules will probably be based on volumetric assessment. 3D volumetric measurements are superior to 2D diameter measurements in terms of accuracy and reproducibility.

Lung cancer CT screening is challenging for surgeons because of the large number of non-malignant CT-detected nodules identified. Multidisciplinary session including thoracic surgeon should therefore have a key role in the planning of CT screening programmes.

If lung cancer screening is judged to be appropriate for national health services, cost effectiveness will be a major issue, especially the frequency of screening.

Best of WCLC 2015: Lung cancer prevention, smoking cessation, and tobacco control

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Treatment of tobacco dependence must be an essential part of standard oncology care. It would improve survival, effect of chemotherapy, surgery and radiotheprapy, and psychics, as well as lower adverse reactions to oncolgy treatments and comorbidities including secondary tumors.

The detrimental effects of smoking on the whole body, but mainly respiratory system, was clearly demonstrated years ago. Despite the fact that about one third of all cancers are caused by smoking, during cancer conferences we see very few presentations about tobacco. Conference presentations mostly mention smoking in relation to **prevention.** During this conference, there were several tens of presentations aimed at **treatment of tobacco dependence,** which is a very cost-effective and essential part of standard oncology care.

Global rules for tobacco control are sumarized in the WHO Framework Convention on Tobacco Control of the WHO which advocates effective policies including plain packaging legislation, widespread smoking bans and control of advertising. Tobacco control policies, despite apparent simplicity, are complex to implement and vulnerable to attack. The tobacco industry anticipates and undermines most policy change. To match this, tobacco control needs to be sophisticated, robust and anticipate tobacco industry tactics. Article 5.3 of the FCTC calls for protection of tobacco control against attack. Clinicians may improve smoking cessation with an understanding of current tobacco control.

Tobacco tax increases are likely to have a significant effect on reducing tobacco consumption, prevalence and initiation among young people, as well as on reducing the chances of young people moving from experimentation to addiction. According to the studies referenced in the WHO technical manual on tobacco tax administration and IARC Handbooks of Cancer Prevention: Tobacco Control. Volume 14, the relationship between real prices and tobacco consumption is generally inelastic, meaning that the decline in consumption is less than proportional to the increase in real price. Most estimates of the price elasticity of demand lie between –0.2 and –0.8 and consumption will fall even more in the long term. Tobacco tax increases are the single most effective strategy to lower tobacco consumption.

Based on the Doll and Peto studies of smoking related mortality and life expectancy, one presenter provided evidence that there is 12.6 minutes of life lost per cigarette or 4 hours per pack. He concludes, that taken into account also the passive smoking effect, the smoker may be seen as a mini-suicide-nano-terrorist (12). Less known is the fact that, more pro-inflammatory diets are associated with increased risk of lung cancer, particularly for former and current smokers, suggesting that dietary-mediated inflammation plays an important role in lung carcinogenesis (8).

Interesting changes have been observed in lung cancer trends. There is an increase in the incidence of lung cancer in never-smokers, especially non-small cell lung carcinoma (NSCLC). This is an absolute increase in number and not due to a change in the ratio of never-smokers to current and ex-smokers.

Also the incidence of adenocarcinoma of the lung has continued to increase to such an extent that it comprises a clear majority of all lung cancers in the US. Adenocarcinoma currently represents 55% of lung cancers in the US. It is the most common histology in men and women, in whites, blacks, and other-races, and in all age groups. In the early 1950s, adenocarcinoma comprised about 5% of lung cancers and appeared to be unrelated to smoking. In the 1960s and 1970s, adenocarcinoma increased sharply, and became strongly related to cigarette smoking. The percentage of lung cancers that were adenocarcinomas has increased from 29% (in 1973–1974) to 55% (in 2010–2011). During this 38-year period, the percentage of lung cancers that were squamous cell carcinomas decreased from 41% to 26%. Adenocarcinoma surpassed squamous cell in 1990–1994 in men, while it was already most common in women by 1973–1974.

Adenocarcinoma rose 77% in men from 1973–1974 to 1990–1994, while it rose 197% in women between 1973–1974 and 2005–2006. Among whites, adenocarcinoma surpassed squamous carcinoma by 1985–1989, while this occurred among blacks by 1990-1994. It was already the most common form of lung cancer among other race individuals in 1973–1974. Adenocarcinoma was already most common among patients <50 years of age by 1973–1974, while adenocarcinoma

rapidly increased and surpassed squamous carcinoma in all other age groups by 1990–1994 (10).

The 1981 Surgeon General Report recommended to smokers unable to quit to switch to filtered and low tar cigarettes. It was not until the analysis of Brown & Williamson internal documents in 1994 and other previously secret Tobacco Industry documents after the Master Settlement Agreement in the 1998 that it became abundantly clear regarding the extent to which the Tobacco Industry had knowingly deceived both the public and federal government about the safety of cigarette design changes for decades.

Big Tobacco intentionally and extensively deceived the public during the second half of the 20th century. Trends in the rising incidence of adenocarcinoma of the lung correlate with the wide-scale adoption by smokers of filtered and low-yield cigarettes. Actions of Big Tobacco were predominantly responsible for the current epidemic of smoking-related lung adenocarcinoma (11).

Smoking cessation before the initiation of chemotherapy is associated with a better median overall survival, 16 vs 10 months ($p=0.007$). This is even seen in heavy smokers, with a median OS of 15 vs 8 months ($p=0.008$). The multivariable analysis confirms that active smoking is an independent negative factor on survival (51% increase in the risk of death) after adjustment for gender, heart or vascular disease, diabetes, high blood pressure, ECOG performance status, histology, site of metastases (brain, liver, adrenals, lungs and bones) (1).

Many onclogists believe it is too late to matter, or perceive that patients will not be receptive to smoking cessation. However, a growing body of literature has identified substantial health benefits from smoking cessation in cancer patients including improved general health, improved all-cause and cancer-specific mortality, reduced toxicity, greater response to treatment and decreased risk of disease recurrence and secondary tumors.

Based on this evidence, Cancer Care Ontario (CCO) undertook an initiative to support smoking cessation for new ambulatory cancer patients in its Regional Cancer Programs (RCPs) in 2013. The initiative was based on the Ottawa Model for Smoking Cessation, and piloted in all 14 health regions in Ontario in 2014 (4).

It is well known that quitting smoking improves the prognosis of cancer patients. Among chronic disease populations (NHIS 2006 vs 2012), 15.2% of lung cancer survivors continue to smoke, compared to 20.9% in 2006. Among other smoking-related cancers, 33.8% of survivors continue to smoke, compared to 38.8% in 2006. Among persons with no chronic disease, the comparable percents of current smokers were 16.6% in 2012 and 19.3% in 2006. The Tobacco Treatment Program (TTP) was established in 2006 at no cost to participants, including family members in the MD Anderson Cancer Center, Texas, USA. The programme provides a range of treatment options that become progressive more intense, to match the needs of each participant: Self-help educational packet and follow-up call;

Motivational intervention, education and follow-up call; Telephone counseling only; and Comprehensive, individualized counseling involving in-person counseling and both in-person and telephone follow-up. This component includes pharmacotherapy and the assessment and treatment of psychiatric co-morbid disorders. In 2012, MD Anderson began automatic referral to the TTP of all patients who currently smoke or recently quit smoking for proactive assistance. In 2014, 4,613 patients had a motivational interaction with program staff, including 3,639 current smokers and 974 recent quitters. Psychiatric co-morbidities included: 12% alcohol abuse, 13% major depression, 11% other depression, 13% anxiety, and 8% panic disorder; 61% no psychiatric disorder. Response rates were high – 89% at 3 months, 83% at 6 months, and 76% at 9 months. Among respondents the 7day point prevalence abstinence rates were at 3 months – ITT (intention to treat) 41.1%, RO (respondents only) 46.0%, at 6 months – ITT 39.1%, RO 47.2%, and at 9 months – ITT 35.1%, RO 46.2% (6).

Electronic cigarettes (EC) or nicotine vaping pressurized aerosol nicotine products, and heat no-burn tobacco products represent a new paradigm for tobacco contro. These products ostensibly offer smokers an opportunity to obtain nicotine in ways that does not cause the extreme risks for such a broad spectrum of smoking-caused diseases that make tobacco smoke the leading cause of premature death in high-income nations. The rapidly growing demand for EC seen in many countries suggests that these products are already having an impact on cigarette consumption today. Despite this unpromising history of harm reduction products, vaping products, of which e-cigarettes are the best know, represent a new generation of alternatives that show some promise for eventually displacing cigarettes and possibly offering real harm reduction (2).

In contrast to lung cancer treatment, treatment of tobacco dependence is one of the most cost-effcetive interventions in the whole of medicine. Terms such as life-years gained (LYG), quality-adjusted life year (QALY), or the incremental cost-effectiveness ratio (ICER) are commonly used. A review of economic evaluations of drugs used for advanced non-squamous NSCLC suggests that ICERs are progressively rising: the ICER for erlotinib as a 3[rd] line therapy was only $39,000/LY when compared to best supportive care (BSC) (3). However, the ICER for pemetrexed used as a 1st line treatment in tumours with no known mutations was $142,500 US dollars (2013) per QALY when compare to best supportive care (BSC) and $164,000 per life year (LY) gained when compared to erlotinib. Estimates of the ICER for afatinib based on the pan-Canadian Oncology Drug Review (pCODR) ranged from $39,000 to 211,000/QALY when compared to gefitinb reflecting the uncertainty in the clinical benefit in the absence of a head-to-head comparative trial. The ICER for crizotinib as first-line therapy in ALK +ve patients ranged from $173,570 (CDN) to $285,299, reflecting uncertainty in economic model assumptions related to the incremental benefit and the time horizon selected. ICERs above $100,000 per QALY are generally not considered

"cost-effective" in Canada. Concerning smoking cessation, one study examined the cost-effectiveness of a pre-operative smoking cessation program for patients with early-stage NSCLC in the United States, and reported an ICER of $2,609/QALY and $2,703/LY at 5-years post-surgery. The cost-effectiveness of smoking cessation programs could be more dramatic over longer time horizons. (3).

To conclude, during oncology care, even after oncology diagnosis, during hospitalization and follow-up – any kind of complex treatment of tobacco dependence should be offered and available to oncology patients, as well as programs to avoid exposure to passive smoking.

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Scientific session V (2nd part of Best of WCLC) Multidisciplinary therapy of thoracic cancers

Adjuvant and neoadjuvant chemotherapy in NSCLC

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Surgery remains the basic treatment for patients with localized non-small cell lung cancer (NSCLC). Nonetheless, even after an apparently complete resection procedure the risk of recurrence remains substantial. For patients with pathological stage II the 5-year survival rate after surgery alone is under 50% (stage IIA 46%, and IIB 36%) and it drops as low as to 24% for stage IIIA. Trials present in WCLC 2015 discussed relevant biomarkers, toxicity and survival of adjuvant and neoadjuvant chemotherapy of NSCLC.

Adjuvant chemotherapy

Adjuvant chemotherapy (AC) plays a significant role in the treatment of resected NSCLC patients and has become standard in clinical practice. Adjuvant cisplatin-based chemotherapy is recommended in patients with stages IB (≥ 4 cm), IIA, IIB, and IIIA of NSCLC after radical resection. Vinorelbine with cisplatin are preferable drugs in this indication and usually four cycles of therapy are recommended.

Neoadjuvant chemotherapy

Cancer shrinkage of primary site and lymph node by neoadjuvant chemotherapy (NAC) may make surgical resection easier. NAC event. combined with radiotherapy might make possible the complete resection of a locally advanced cancer (i.e. down staging of stage IIIA-N2 or IIIB disease). NAC may also destroy micrometastases and prevent the spread of cancer cells during the surgery.

Comparison of adjuvant and neoadjuvant chemotherapy

A large number of trials have evaluated the efficacy of adding AC or NAC to radical resection for NSCLC, but there is limited data directly comparing AC and NAC. Pignon conducted LACE meta-analysis. The results favored AC with HR for OS of 0.89 (95% CI, 0.82–0.96, P=0.005) and HR for DFS of 0.84 (95% CI,

0.78–0.91, P<0.001). Survival advantage of 5.4% at 5 years was achieved. Burdett's in 2014 meta-analysis indicated the benefit from NAC on OS with HR of 0.87 (95% CI, 0.78–0.96, P=0.007) and on RFS with HR of 0.85 (95% CI, 0.76–1.0.94, P=0.002). Absolute improvement of 5% at 5 years was achieved. The two individual patient data meta-analyses yielded almost compatible HRs and survival benefit. Both meta-analyses had 5 year survival <50% in chemotherapy groups.

WCLC 2015 adjuvant studies.

H.A. Wakelee et al conducted a large phase 3 study (E1505) to evaluate the addition of bevacizumab to adjuvant chemotherapy in early stage resected NSCLC. 21 day cycles all with Cisplatin given at 75 mg/m^2 on day 1 in doublet with vinorelbine 30 mg/m^2 day 1, 8 or docetaxel 75 mg/m^2 day 1 or gemcitabine 1200 mg/m^2 day 1,8 or pemetrexed 500 mg/m^2 day 1. Bevacizumab was given in active arm 15 mg/kg IV q 3 weeks for up to 1 year. 1501 patients were enrolled. In the arm with chemotherapy only 82% patients finished the treatment, in the arm with bevacizumab only 37% of patients completed the protocol due to adverse event, withdrawal or death. There were no statistical differences in survival parameters between both arms: OS hazard ratio 0.99, 95% CI: (0.81–1.21), p=0.93, DFS hazard ratio: 0.98, 95% CI: (0.84–1.14), p=0.75. The addition of bevacizumab to adjuvant chemotherapy failed to improve survival for patients with surgically resected early stage NSCLC. This is another negative large adjuvant trial with biological treatment after Radiant study with erlotinib. Paul Bunn as a discussant raised a skeptical question if similar AC trials are needed? Should we abandon trials without good biomarkers and more robust evidence for complete responses and survival prolongation? Should we focus more on neoadjuvant trials to hasten drug development (immunotherapy) and achieve better results of survival?

Side effects of adjuvant chemotherapy

Side effects of AC were not negligible in large adjuvant trials. Usually toxicity is manageable and transient, but toxicity related death appeared when cisplatin was used. Neutropenia is the main problem. Only few studies with less toxic carboplatin exist and direct comparison of cisplatin and carboplatin based AC is missing. Kolek et al presented a phase II prospective multicentre study of AC with oral vinorelbine and carboplatin (Switch 1). Four cycles of 21 days regimens were planned. Patients received carboplatin AUC 5 on the day 1, vinorelbine 25 mg/m^2 intravenously on the day 1 switched to 60 mg/m^2 orally on the day 8. Mean number of applied cycles was 3.77, four planned cycles finished 82.4% patients. Median of disease specific survival was 7.63 y (95% CI: 4.57 to NR), median of overall survival (MOS) was 5.9 y (95% CI, 3.7 to, NR) and median of disease free survival (DFS) 4.43 y. Five-year survival of 56.2% was reached. AC with carboplatin and vinorelbine given intravenously on the day 1 and orally on the day 8 in 21 day regimen appears to be a comfortable and tolerable therapy in radically resected NSCLC. It provides

higher dose intensity and more of accomplished treatments compared to large adjuvant trials and LACE meta-analysis.

Biomarkers

Significant efforts have been made to refine prognostic information with molecular markers. At the present time, molecular predictive factors and gene signatures are only investigational and need to be confirmed in prospective trials which are currently active (EGFR, VEGF, ALK, PDL-1, circulating progenitor cells, microRNA, ERCC1, TS).

Conclusion

Both adjuvant and neoadjuvant chemotherapy improve survival for operable NSCLC compared with surgery alone. Although the current consensus recommends the use of AC, both strategies should be regarded as the first choice treatment options. Use of molecular targeted drugs for more sophisticated peri-operative chemotherapy seems to be promising, but reliable predictive biomarkers are still missing.

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Chemoradiotherapy in locally advanced non-small cell lung cancer: the selection of presentations from WCLC 2015

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Concurrent chemoradiotherapy (CHT-RT) has a beneficial effect on overall survival (OS) compared to sequential CHT-RT or radiotherapy alone in patients with locally advanced non-small cell lung cancer (LA-NSCLC) (1, 2). Nonetheless, the optimal CHT-RT scheme (dose, schedule, technique, type and doses of CHT) still needs to be identified. Dose escalation above 60 Gy up to a maximum of 74 Gy with concurrent CHT-RT initially showed to be promising in terms of improved local control and OS (3, 4). However, the RTOG 0617 trial recently showed that patients

receiving a conventional high dose radiation scheme (37 x 2 Gy) had a significantly shorter median OS (20.3 months) compared to patients receiving a conventional (30 x 2 Gy) radiation scheme (28.7 months), p=.004 (5).

In the 16th World Conference on Lung Cancer hosted by the International Association for the Study of Lung Cancer (IASLC) in Denver (Colorado) from 5th to 9th September 2015, several presentations addressed pertinent issues of CHT-RT in LA-NSCLC.

Belderbos et al. (6) presented mature results of Raditux, a randomized phase II trial that between 2009 and 2011 randomized 102 patients to compare hypofractionated accelerated CHT-RT (66 Gy in 24 fractions, 5 times a week with daily low dose of cisplatin: 6 mg/m^2) with or without addition of weekly Cetuximab. Earlier publication of these results revealed that there was no improvement of disease outcome in terms of objective responses and survival with addition of Cetuximab to CHT-RT (7). Most (92%) patients had stage III disease. Median follow-up for living patients was 60 months. Median OS was 32 months and five-year OS rate was 37%. Squamous histology, better WHO performance status, and lack of comorbidities at baseline were prognostic for improved survival in multivariate analysis. This surprisingly good outcome in terms of survival incited authors to compare patients' and treatment characteristics with RTOG 0617 trial in which higher RT dose of 72 Gy was related to worse survival than the conventional dose 60 Gy arm and additionally, both arms had lower OS than that obtained in Raditux. Moreover, patients from RTOG 0617 trial received aggressive concurrent and consolidation CHT. This raises a question if difference of results between Raditux and RTOG 0617 is related to baseline patients characteristics or we may generate the hypothesis that hypofractionated accelerated RT with low radiosensitizing CHT doses is better than high dose conventionally fractionated RT with full dose CHT. Patient's baseline characteristics were very similar in both trials (about 90% of PET-CT staged; 92% and 100% of stage III cases in Raditux and RTOG 0617, respectively). Biologically Effective Dose (BED) in Raditux trial was estimated at 78 Gy and was higher even than in the dose escalation arm of RTOG 0617 trial. Thus it is very probable that acceleration of RT with hypofractionation overcomes a loss of efficacy with prolonged treatment time of physical dose escalation with conventional fractionation. In the latter scheme, addition of full dose CHT did not compensate for prolonged RT time. Obviously, such an indirect comparison of two CHT-RT schedules has its limitations, because we cannot exclude that other not accounted factors influenced an outcome. However, a comparison of conventionally fractionated RT combined with full dose CHT and hypofractionated accelerated high dose RT with or without CHT in randomized study is warranted. A recent review of the radical intent hypofractionated RT schedules with or without CHT has demonstrated a potential of such an approach for further improvement of outcome of definitive RT of NSCLC (8).

Chun et al. (9) evaluated how RT techniques used in discussed above RTOG 0617 influenced treatment outcome. The intensity-modulated RT (IMRT) was compared with 3-dimensional conformal RT (3D-CRT). The use of any technique was at the discretion of treating physician. Both RT techniques were equally distributed in both (higher and lower dose) treatment arms, 3D-CRT 53% and IMRT 47% of cases. Patients treated with IMRT had significantly larger PTV than patients treated with 3D-CRT, 486 cc vs. 427cc, respectively, p=.005. Overall survival, progression free survival and local control rates were not different between patients treated with compared techniques. More patients in IMRT than in 3D-CRT group completed consolidation CHT, 37% vs. 29%, respectively, p=.05. Rate of grade III and higher pneumonitis was lower in IMRT group, 3.5% vs. 8%, p=.046. IMRT did not reduce esophageal toxicity. It was concluded that similar outcome of both techniques despite treatment of more challenging tumors (larger PTV) with IMRT confirms the value of IMRT in the treatment of NSCLC. Certainly, this study shows that we may use IMRT for thoracic malignancies, however, its benefit in comparison with 3D-CRT cannot be confirmed by data provided in this trial.

Choice of CHT schedule for combined treatment of NSCLC varied between countries and even between centers. General agreement is that platinum compounds are the basis of such treatment, however, the exact drugs' combination is a subject of controversies. Steuer et al. (10) conducted a systematic review of published trials to compare outcomes and toxicities between CE (Cisplatin/Etoposide) and CP (Carboplatin/Paclitaxel) combined with RT for stage III NSCLC. There were 3194 patients from 32 studies in the CE arm and 3789 patients from 51 studies in the CP arm included. Authors concluded that there was no significant difference in efficacy between CE and CP. Rate of esophagitis and pneumonitis was similar for both schedules. However, hematological and gastric toxicity was higher in CE arm. Thus both schedules are acceptable standards for combined treatment of NSCLC. Prospective data is needed to determine the optimum regimen.

Smeltzer et al. (11) evaluated the validity of guidelines for post-operative adjuvant therapy in patients with R1 resection of NSCLC. There are no level one evidence based for this category of patients, because they were not included in randomized trials. Consensus-based recommendations indicate postoperative RT (PORT) alone for stage I, and CHT-RT for stage II-IIIA. Authors selected from National Cancer Data Base (NCDB) 4132 patients with positive margins out of 98176 surgically resected pathologic stage I-IIIA NSCLC. Radiotherapy had harmful effect on OS in R1 stage IA patients, p=.0006. For higher stages in R1 resections, OS was significantly improved with CHT or CHT-RT, but not with RT alone. These findings challenge clinical guidelines for use of adjuvant radiotherapy in margin positive resections of NSCLC. Also, there are at some extent against conclusions from a recent analysis of NCDB data in which a longer OS in patients

completing a full regime of PORT at 50–74 Gy was demonstrated (12). PORT for R1 resections represents a clinical scenario for which we don't expect to have the level I evidences. Thus all such published data should be helpful for guiding clinical practice.

Some other aspects of combined treatment for NSCLC, as the futility of the use of consolidation CHT will be discussed at the meeting, in the context of presented toxicity of Proclaim trial (13).

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The important advances in systemic treatment of Small-Cell Lung Cancer (SCLC)

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Substantial progress has recently been made in non-small cell lung cancer (NSCLC) with the discovery of molecular targets leading to the targeted drug development. Only limited therapeutic progress has been achieved in the recent decades and despite multiple mutations no targeted therapy for SCLC has been available by now. The number of randomized trials studying new drugs in SCLC trials lag far behind NSCLC (1). Almost all of the studies focused on the molecular targeted therapy for second-line treatment of SCLC were failed. However, the discovery of molecular targets leading to the targeted drug development have shown promising activity in pre-clinical models and in early clinical trials also against SCLC.

Targeting NOTCH3

NOTCH3 IHC staining showed expression in most SCLC cases, with high NOTCH3 trending towards worse survival in extensive stage. This supports the rationale of targeting NOTCH3 by tarextumab (TRXT, OMP-59R5, anti-Notch2/3) in SCLC patiens. TRXT, a fully human IgG2 antibody targeting Notch2 and 3 receptors, has shown efficacy in SCLC in combination with etoposide and platinum. (2).

Targeting DLL3

A delta-like protein 3 (DLL3) is a ligand involved in the hedgehog/notch pathway. DLL3 is highly expressed in 70% of SCLC patients. The early results a phase I trial of a delta-like protein 3 (DLL3)-targeted monoclonal antibody conjugated to cytotoxic agent rovalpituzumab tesirine (Rova-T) showed a strong signal of efficacy. The antibody drug conjugate should be prospectively tested in randomized trials (3).

Targeting the tumor microenvironment

Lurbinectedin (PM01183) inhibits transactivated transcription and acts on the tumor microenvironment. It lacks cross-resistance with platinum. The PM01183 and DOX combination showed compelling clinical activity as 2nd line treatment in SCLC. A randomized study is planned to help define the role of this combination in relapsed SCLC patients (4).

Targeting PARP1

PARP1 a poly (ADP-ribose) is expressed at high levels in SCLC. PARP inhibitors (olaparib, rucaparib, talazoparib), have shown promising activity against SCLC in

pre-clinical models and in early clinical trials (5). Clinical trials of PARP inhibitors (veliparib) and other molecules targeting DNA damage response in combination with chemotherapy has been initiated for SCLC (6).

Targeting PI3K/AKT/mTOR

PI3K/AKT/mTOR pathway is the promising therapeutic target in SCLC . It was shown that the dual inhibition of PI3K and mTOR might improve the response over the single inhibition of mTOR. A novel potent dual inhibitor of PI3K and mTOR is PF-05212384. Initiated phase II study of PF-05212384 in advanced recurrent SCLC patients harboring molecular alterations in PI3K/AKT/mTOR pathway is planned to be started in January 2016 (7).

Imunotherapy of SCLC

Results with immune checkpoint inhibitors were certainly the most anticipated during year 2015. The results of second-line trials of PD-1 and PD-L1 inhibitors vs docetaxel in patients with nonsquamous and squamous NSCLC were particularly exciting. The potential for meaningful progress was seen in small-cell lung cancer. Preliminary results in small-cell lung cancer (associated with an extremely high mutational burden) showed favorable rates of response to immune checkpoint inhibition, although the number of treated patients was low and follow-up short. The results of two phase II studies of immunotherapy in previously treated SCLC are provocative. There have been no practice-changing advances in a long time. Now it seems that immunotherapy may change that, with studies of pembrolizumab and nivolumab with or without ipilimumab .These data are extremely encouraging for heavily pretreated patients with SCLC. Response rates were 35% for pembrolizumab in PD-L1-positive patients, 17% for nivolumab monotherapy, and 18% for nivolumab plus ipilimumab in all comers. Several responses appear to be durable. These are all phase I studies, and it is still too early to determine if the combination is better than single-agent therapy (8,9). The results from 16th World Conference on Lung cancer 2015 will be presented.

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News in surgery

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The 16th IASLC World Conference on Lung Cancer (WCLC) was held from September 6th–9th 2015 in Denver, Colorado. During this meeting, which was also attended by many thoracic surgeons, several surgical aspects and recent advances were presented and discussed. Despite several poster presentations more than 25 oral scientific sessions were covering surgical oncological issues during the WCLC 2015.

With regard to early stage non-small cell lung cancer, several abstracts on interesting topics were presented at the conference. In particular many abstracts on 2 highly debated areas, namely the role of sub-lobar resections and the outcome comparison of stereotactic body radiation therapy (SBRT) vs. surgery for early stage disease were presented.

In a study by Yang et al, the efficacy and safety of wedge resection vs. segmentectomy in patients with stage T1a N0 NSCLC using the USA National Cancer Data Base in over 8000 patients was investigated. They found no significant differences between wedge resection and segmentectomy regarding 30-day mortality (1.6% vs. 1.5%, respectively; p=0.94). However, segmentectomy was associated with significantly better long-term survival than wedge resection (median survival of 88.1 months and 5-year survival of 64.9%).

The role of surgery vs. SBRT in operable NSCLC was also lively discussed during the first Pro/Con session. From a surgical point of view, surgical treatment remains the main option for functionally operable patients with early-stage NSCLC. SBRT may be valid option for patients who cannot tolerate lobectomy due to functional reasons. However during this session both, radiation oncologists and surgeons agreed on the fact that further evidence is warranted and is achievable by close cooperation between thoracic surgeons and radiation oncologists. Only this way, designing comparative trials with precise inclusion criteria and strict definitions of endpoints is scientifically valid and provides proper comparison between SBRT and surgical resection.

Another spotlight on surgery in NSCLC was the multimodality treatment of Stage III disease. Many abstracts were dealing with use of surgery as part of multimodality protocols. In the most important study by Behera et al, authors compared the outcomes and predictors associated with trimodality therapy vs. chemoradiotherapy (CRT) alone in 29,584 patients with stage IIIA/N2 disease from the National Cancer Database. Median OS was 44.5, 25.6 and 15.7 months in CRT + lobectomy, CRT + pneumonectomy and CRT alone ($p<0.0001$), with 5-year

survival rates of 44%, 33% and 14%, respectively. 30-day mortality was higher in pneumonectomy vs. lobectomy (7% vs. 2.6%; OR 0.26 [95%CI 0.16, 0.45]); p<0.001). Authors concluded that trimodality therapy demonstrated better outcomes compared with CRT alone in patients with stage IIIA-N2 NSCLC.

The use of surgery in small cell lung cancer patients is not common, however in the recent years a series of publications have highlighted the potential benefit from surgery in the multimodality setting in limited stage SCLC patients. In Denver, an intriguing study by Yang et al was presented with the intention to test the hypothesis of whether surgery offers a survival advantage among patients with node-positive SCLC compared with non-operative management. Patients were identified in the National Cancer Data Base between years 2003-2011 and had to have pT1–2 N1–2 M0 SCLC. All patients underwent chemotherapy ± radiotherapy (≥45 Gy) or surgery with adjuvant chemotherapy ± radiotherapy (≥45 Gy). In total, 712 (66.5%) underwent non-operative management and 359 (33.5%) patients underwent surgery with adjuvant chemotherapy ± radiation. Authors were able to show that the addition of surgery was associated with a significantly higher OS than non-operative management (5-year OS 28.1% vs 18.3, log-rank p<0.01). Patients with pN1 and pN2 had significantly better median OS in the surgery cohort compared to no surgery. In conclusion these results clearly support the re-evaluation of the role of surgery within multimodality protocols for node positive limited-stage SCLC.

Various sessions on Malignant Pleural Mesothelioma (MPM) were also held during the WCLC 2015. Besides the presentation of the initial analyses of the IASLC MPM database with implications for the upcoming 8th edition of the AJCC/UICC staging manuals, data from a large institutional series on risks and benefits of extended pleurectomy decortication (EPD) for MPM were shown. In a retrospective analysis of 266 patients who underwent EPD within the time period of 15 years, overall median survival was 12.2 months for all patients. However, epithelioid pN0 disease was the most favourable subgroup with longer survival rate at 1, 3 and 5 years (64.9%, 17.5%, and 5.2% respectively) and longer overall median duration of survival (23.1 months). 30-days Post-operative mortality was 3.8% and 9% at 90 days, respectively. Median length of hospital stay was 13 (5–70) days. The most common post-operative complications were: persistent air leak (31%) patients and atrial fibrillation (16.7%). Duration of chest drainage time was significantly associated with the development of pleural empyema (p<0.001) and dehiscence of the neodiaphragm (p=0.042). Re-operation was required in 11.3% of patients. Complications prevented chemotherapy use in 28% of patients with empyema and 22.7% of those with neodiaphragm dehiscence. The authors concluded that EPD remains a palliative procedure with the exception of a selected subgroup which benefits more from this procedure.

The 16th IASLC-WCLC in Denver provided news in surgery in many areas of thoracic oncology and the current presentation intends to cover the most important abstracts of the conference on surgical oncology.

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Chinese – Central European Sympozium
East-West cooperation in managing of lung cancer

The application of Traditional Chinese Medicine (TCM) in treatment for lung cancer

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There has been a consensus of a strategy of comprehensive treatment for lung cancer among most oncologists. As a part of comprehensive treatment, TCM has got some achievements in synergy with chemotherapy & radiotherapy, prolonging life span, reducing adverse effect, improving QOL, et al. As an emerging therapy, immunotherapy has many concept alike with TCM. It's a new direction for TCM research to combine with immunotherapy.

In recent years, there have been more and more fundamental and clinical researches for TCM treatment in lung cancer. More and more oncologist have become interested in TCM, for its unique concepts and philosophies, such as holism, multi-target and multi-stage treatment.

Improve short-term effect

The mechanisms of TCM treatment for lung cancer involves increasing immune function, inhibiting proliferation, inducing apoptosis, intervention of signaling pathway, reversion of multidrug resistance, et al. The most researched medicines are as follows.

Kanglaite(KLT) Injection/Capsule

KLT is a Chinese Patent Medicine. In TCM theory, KLT has the function of Tonifying Qi and Yin, Removing Cumulation and Stasis. Its effective constituent is Semen coicis triglycerides, which is extract of Coix lacryma. KLT has effect of synergy with chemotherapy & radiotherapy, anti-cachexia, and pain killing for some advanced patients.

A fundamental research from Johns Hopkins University suggested KLT could inhibit tumor cell proliferation by Inhibits NF-kB and Protein Kinase C Signaling [1]. Another research suggested KLT combined with chemotherapy drug could improve effect of tumor-inhibition.[2]

A clinical research suggested KLT combined with PVM regimen could improve the effect for NSCL. Meanwhile, KLT could improve KPS and reduce hematotoxicity of chemotherapy drug [3]. Another research suggested KLT combined with chemotherapy could prolong MTTP & MST, and improve one year survival rate.

Shenyi Capsule

Shenyi Capsule is also a Chinese Patent Medicine. Its effective constituent is Ginsenoside-Rg3, which is extract of Panax ginseng. Shenyi Capsule could improve the symptoms of Qi deficiency, enhance body immune function, and synergize the effect of chemotherapy & radiotherapy. Research suggested Ginsenoside-Rg3 has functions of pro-apoptotic, proliferation & metastasis inhibition, anti-angiogenesis, et al.

Elemene Injection

Elemene Injection is another Chinese Patent Medicine. Its effective constituent is β-Elemene, which is extract of Curcuma zedoaria. Many researchers suggested Elemene Injection is effective for malignant pleural effusion & malignant and some solid tumor. The mechanism is related to pro-apoptotic, proliferation & metastasis inhibition, reversion of multidrug resistance, et al.

Reduce adverse Effect of Chemotherapy & Radiotherapy

Chemotherapy-induced peripheral neuropathy (CIPN)

CIPN is a common dose-limiting side effect in peripheral nervous by chemotherapy, many chemotherapy drugs for lung cancer can induce CIPN, such as platinum, paclitaxel, vincristine.

A clinical research suggested Chinese medicine LC07 for external use could effectively reduce the pain of CIPN & systematic with good safety [4]. Other interrelated experiments found, external use of Chinese medicine LC07 could improve the changes in behavioristics under the temperature and mechanical stimulation, as well as accelerate the sensory nerve conduction velocity (SNCV) of coccygeal nerve & the contents of nerve growth factors (NGF) in plasma of rats with peripheral nerve toxicity induced by oxaliplatin. Moreover, LC07 could reduce the neuropathic pain, through inhibiting harmful signal transmission induced by activation of astrocyte in spinal dorsal horn.

Targeted therapy-induced skin injury

Targeted therapy of lung cancer is a treatment with development prospect. However, its side effects are depressing. The most common one is skin injury (acne rash, skin dryness, etc.).

A clinical research suggested Chinese medicine ZY-06 for external use has significant curative effect on EGFRIs related acne rash, in alleviating acne rash and

relieving pruritus, with little adverse reactions [5]. Another research suggested TCM Moistening Cream had better efficacy than urea ointment in alleviating skin rhagadia, desquamation and pruritus. Moreover, TCM Moistening Cream took effect more quickly, healed and repaired skin in shorter time.

Radiation-induced pulmonary injury

Radiation-induced pulmonary injury is an inflammatory reaction of lung after Radiotherapy. A clinical suggested Pingfei oral liquid (PFOL) could reduce the incidence of Radiation pneumonia and pulmonary fibrosis [6]. Fundamental research showed that PFOL could inhibit proliferation and induce apoptosis of lung fibroblasts, regulate immune activity on Th1/Th2 type cytokines, and reduce the aggregation of mast cells, those may contribute to its protective effect on radiation—induced lung injury.

Joint treatment of TCM & immunotherapy

Shenqi fuzheng Injection

Shenqi fuzheng Injection (SFI) is extracted from Codonopsis (or ginseng) and Radix Astragali. SFI could nourish Qi supplement Zheng. It is usually used in lung cancer patients, with TCM syndrome of physically and mentally fatigued, weak breath, disinclination to talk, spontaneous perspiration, dizziness. SFI could regulate immunity of NSCLC patients, increaCD3+, CD4+, CD41/CD8+, and improve curative effects of anti-cancer treatment.

Ginseng polysaccharides Injection

Randomly controlled trails reported effects of Ginseng polysaccharides Injection (GPS) plus dendritic cells (DC) in treating NSCLC patients. The results showed PFS (Progression-Free-Survival) in DCs+GPS group (16.39±2.60 months) was significantly longer than PFS in DCs control group (14.68±2.85 months). Th1 cytokines (INF-γ, IL-2) and the ratio of Th1/Th2 cytokines (INF-γ/IL-4, IL-2/IL-5) were significantly higher in the DCs+GPS group than in the DCs control group [7]. Conversely, FACT-L scores and Th2 cytokines (IL-4, IL-5) were significantly higher in the control group than in the DCs+GPS group. In vivo administration of ginseng berry extract (GB) promoted up-regulation of CD86, MHC class I and MHC class II and production of IL-6, IL-12 and TNF-α in mouse spleen DCs. GB also promoted the generation of Th1 and Tc1 cells. Ginseng polysaccharides (NGP) could stimulate the maturation of murine BMDCs, increase expression of MHC II, CD80, CD86, CD83, CD40 and secretion of higher level of IL-12 and low level of TNF-α.

Astragalus polysacharin

Astragalus polysacharin (APS) could accelerate the mature of human peripheral blood mononuclear cells (PBMCs) and increase expression of CDla, CD80 and

CD86. APS induced mature DCs could stimulate the proliferation of allogeneic T lymphocytes and significantly increased the levels of IL-12 and IFN-γ in co-culture supernatant. Dendritic cell potently inhibit the tumor growth in vivo and improve the survivals of tumor bearing mice. The mechanism is possibly related to the generation of the antitumor cytokines IL-12 and TNF-α [8]. A Clinical study entitled on "optimization of TCM herbs based on constitution plus DC in treating NSCLC and CRPC" is ongoing in integrative oncology department, China-Japan friendship hospital. This study has included 20 NSCLC patients. Preliminary results show SFI plus DC treatment has excellent clinical effects and is safety in use.

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The influence of perioperative allogeneic blood transfusions to the prognosis of lung cancer patients – yes or no, why?

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Most data display differentiation stage and post-operation adjuvant treatment were found to be the significant affecting the prognosis, whereas the blood transfusion was not significant. Perioperative allogeneic blood transfusion may cause post-

transfusion infection, cancer recurrence, even decrease the disease-free survival in non-small lung cancer patients.

From transfusion professional perspective, we discuss the reason for the "NO", or why does perioperative allogeneic blood transfusions just may influent the prognosis of lung cancer patients. And why there is no definite conclusion until now. What did we miss?

We focus on only one type of lung cancer: non-small cell lung cancer patients.

The following are these questions from transfusion medicine area we will discuss.

First of all, why did we select lung cancer? Are there any prognosis differences between non-small cell lung cancer and other carcinomas? Yes, the prognosis of lung cancer with curative pulmonary resection is worse than others, especially blood transfusion during operation.

1. What is the definition of perioperative allogeneic blood transfusions? No answer: three months, weeks, or days before and after operation definitely include during operation. We think it is worse during operation and after three days.
2. Different blood components (red cells suspending, depleted white cells, plasma, platelets). Transfused red cells is worse than other products because one unit red cells suspending contains more large number cells. Blood products with white cells may increase the weight of influence patients' prognosis.
3. The amount and volume of blood products transfused. Here we should correct different patients' whole blood volumes. The more blood products transfused the more effect the influence to patients.
4. The storage period of red cells products. Blood products storage lesion exists, especially for red cells.
5. Difference between autologous transfusion and allogeneic blood transfusions.

All facts above may affect the influence. So less patient data cannot show the significant affecting the prognosis unless we strictly select patients in treatment groups. Or we may enlarge the patient numbers to make up for the study even it does work very limited. The fifth sentence may provide the clue to resolve the question whether specific immune regulation or non-specific immune regulation in transfusion practice.

Common, uncommon, rare and complex EGFR mutations in Czech NSCLC patients and therapeutic effect of tyrosinkinase inhibitors

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Introduction: EGFR mutations can be categorized as common, uncommon, rare and any combination thereof, termed as complex. It is known that most EGFR mutations are positive predictors of TKI sensitivity and some, in exon 20, denote TKI resistance. However a detailed information on the effect of uncommon, rare and complex mutations remains to be confirmed.

Patients and methods: We analyse a cohort of Czech EGFR positive NSCLC patients from the National Registry TULUNG with respect to the EGFR mutation type, tumor characteristics and evaluate their effect on the outcome of TKI therapy. In total, 305 EGFR-positive NSCLC patients treated subsequently by gefitinib were evaluated. The aim was to obtain a distribution of mutation types across clinical data (sex, age, PS, stage of disease and adverse effects of first line) and time to progression and overall survival for common, uncommon, rare and complex mutations.

Results: When comparing exons, the most frequently found mutations were various exon 19 deletions found in a total of 179 patients (58.6%). The exon 21 point substitutions were found in 84 patients (27.5%), with L858R in 65 (21.3%) and L861Q in 5 (1.6%) as the most prevalent. Then, mutations in exon 18 were found in 26 patients (8.5%) with G719X as the most frequent one in 19 patients (6.2%). Mutations in exon 20 were found in 28 patients (9.1%), including T790M in 5 (1.6%), S768I in 3 (1%), and insertions 3 mutations on exon 20 were found in 16 patients.

Discussion: No differences in clinical data or therapy response were observed between the two dominant mutation types (exon 19 deletions and exon 21 L858R substitution) or when comparing patients with uncommon mutations. On the other hand, patients with uncommon mutations were more frequently smokers, duration of gefitinib therapy was shorter (p=0.08), response rate (p=0.024), DCR (p=0.016), PFS p=0.001 and OS (p=0.017) were worse compared to a group with frequent mutations. Uncommon mutations, such as G719X or L861Q, were associated with moderate TKI sensitivity. Special awareness should be given to patients with complex mutations to be individually considered to the type of therapy. Negative predictive role of mutations in exon 20 due to the resistance to the first and second generations of TKI was confirmed.

Conclusion: Patients with common as well as uncommon EGFR sensitive mutations have benefit from gefitinib therapy. The G719X mutation yield moderate PFS and OS benefit. As expected, exon 20 mutations (including the insertions) did not show any benefit from targeted therapy.

Western medicine and Traditional Chinese Medicine: is there any space for collaboration in lung cancer treatment?

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Background: TCM (Traditional Chinese Medicine) is a broad range of medicine practices sharing common concepts which have been developed in China and are based on a tradition of more than 3,000 years, including various forms of herbal medicine, acupuncture, massage (Tui na), exercise (qigong), and dietary therapy.

Methods: University Hospital Hradec Kralove created partnership with Shanghai University of Traditional Chinese Medicine to build the first TCM University Center and Department in the Czech Republic. The objective of mutual collaboration is to set up a system for delivery of treatment, preventive measures, training and research in the Czech Republic and in Central and Eastern European region. We will explore possible positive aspects of integrated medicine approach.

Discussion: Chemotherapy or biological therapy remains the golden standard for treatment of lung. TCM may however bring some added value. There are some herbs like astragalus – a common medicinal herb in China, which may ease side effects, increase tumor response, reduce chemotherapy toxicity and improve survival.

Conclusion: TCM in combination with western medicine may reduce chemotherapy toxicity and improve survival even in lung cancer. TCM could improve patients' Quality of life and clinical symptoms during adjuvant chemotherapy.

Scientific session VI (3rd part of Best of WCLC) Future strategies in lung cancer

Perspectives of targeted therapy

F. Hirsch (USA)
Abstract missing.

Next generation EGFR inhibitors

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After a decade of intensive research on EGFR inhibitors in lung cancer, first-line treatment of patients with tumors harboring *EGFR* mutations is now a common standard of care world-wide, based on the results of several phase III clinical trials documenting progression-free survival (PFS) benefit of these agents as compared to chemotherapy. Erlotinib and gefitinib, first generation reversible EGFR inhibitors, are commonly used in this setting. Recently, a second generation irreversible EGFR inhibitor, afatinib, became available in these patients, with documented overall survival benefit as compared to chemotherapy in pooled, post-hoc analysis of two randomized phase III clinical trials (1). This benefit was confined to patients with tumors harboring *EGFR* exon 19 deletion.

After a median of approximately 10–12 months, patients treated with first or second generation EGFR inhibitors develop acquired resistance, with radiological signs of disease progression. The kinetic of radiological progression in asymptomatic progressing patients is variable, and some patients are still treated with EGFR inhibitors for a period of several months until symptoms occur (2). There are multiple mechanisms of resistance identified to gefitinib or erlotinib, with acquired resistance mutation T790M being most frequent, observed in approximately 50–60% of patients and associated with more favorable outcomes once resistance is found (3). Other, clinically significant mechanisms of resistance include *MET* (hepatocyte growth factor receptor) amplification, *HER2* amplification, *PIK3CA*

mutation, AXL activation, transformation to small-cell lung cancer or epithelial-to-mesenchymal transition (4). More than two mechanisms can occur in one patient, with individual special and temporal resistance pattern. Far less is known about the mechanisms of resistance to afatinib, however T790M appears to play dominant role also in progressing tumors in patients treated with this compound.

Third generation EGFR inhibitors were designed to overcome limitations of first and second generation inhibitors, with key features of mutant vs. wild-type receptor protein selectivity and activity against T790M mutant EGFR protein. Four agents are currently in clinical development, including CO-1686 (rociletinib), AZD9291 (osimertinib), HM61713 and EGF816. Rociletinib was evaluated in patients treated in TIGER X phase 1–2 trial, mostly with confirmed T790M resistance mutation from a biopsy at progression on first or second generation EGFR inhibitor. In overall study population of patients treated at clinically relevant drug doses, objective response rate was 59% with long response duration (5). Osimertinib was tested in a phase 1 clinical trial program (AURA), with expanded cohort of 222 patients who were previously treated with EGFR inhibitor and developed acquired resistance. Patients were treated in cohorts of five different doses of the study drug, ranging from 20 to 240 mg once daily (6). Both patients with and without centrally confirmed T790M resistance mutations were included, with response rates of 61% and 51%, and PFS of 9.6 and 2.8 months, respectively. These results indicate that osimertinib is active primarily in patients whose tumors show T790M mutations, although some activity in a cohort of the patients without T790M mutations was also observed. The most significant all-cause adverse events of the study drug were diarrhea, rash, nausea and decreased appetite, indicating that its selectivity against mutant protein may not be perfect. Other agents mentioned above are currently tested in phase I trials.

2015 World Lung Cancer Conference included several important presentations on the subject of third generation EGFR inhibitors. Dr. Jonathan Goldman presented TIGER X data in cohorts of patients who progressed on gefitinib, erlotinib or afatinib, had T790M resistance mutation, and were treated in cohorts of three different doses of rociletinib (500mg, 625mg and 750mg twice daily). Antitumor activity was independent of the dose, whereas adverse events were more frequent with increasing the dose of rociletinib. Therefore, 500mg twice daily is an optimal dose of the drug for further development. The most significant, dose-dependent adverse event of rociletinib was hyperglycemia. In another presentation, rociletinib activity in brain metastases from lung adenocarcinoma was shown to be 41%, with progression-free survival data similar to the lung cancer population of patients without CNS involvement. Expansion cohort of patients with T790M resistance mutation, treated with osimertinib at 80mg daily was presented by Dr. Yang and colleagues, with excellent toxicity profile, response rate of 61% and median PFS not reached by the time of analysis. In a small cohort of patients with *EGFR* mutations treated up-front with 80mg or 160mg of osimertinib, response

rate was 60% and 80%. Progression-free survival data were very promising at data cut-off, with 52 out of 60 patients still on treatment. Preliminary data on resistance mechanisms to osimertinib show acquired C797S *EGFR* mutation, *HER2* amplification, and other by-pass signaling mutations in genes such as *BRAF* or *PIK3CA*.

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Molecular biomarkers

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Individualized therapy based on predictive molecular biomarkers is among the most promising strategies to further improve outcome of patients with non-small cell lung cancer (NSCLC). The use of clinical factors or molecular tests to accurately differentiate responding from non-responding patients should lead to higher response rates, reduced treatment-related toxicity, and improved cost effectiveness.

In early-stage NSCLC, a variety of candidate biomarkers has been investigated in retrospective analyses including the International Adjuvant Lung Cancer Trial Biologic Program (IALT-Bio) and Lung Adjuvant Cisplatin Evaluation (LACE)-Bio Program. In IALT-Bio, molecular biomarkers have been investigated for their potential prognostic and predictive values in patients who were treated within the IALT trial and it has been suggested that proteins such as ERCC1 might be useful to customize chemotherapy. Other promising predictive factors such as gene expression profiles have also been reported in retrospective analyses but, up

to now, none of these biomarkers is ready for implementation in clinical routine.

In patients with advanced NSCLC, customized chemotherapy appears feasible but comparable to early-stage NSCLC still remains experimental. Improvements of the outcome of first-line chemotherapy have been achieved by the addition of EGFR-directed monoclonal antibodies in patients with EGFR-positive NSCLC and of bevacizumab in selected patients with non-squamous cell NSCLC.

The impact of biomarkers for patient selection has now been well established for EGFR tyrosine kinase inhibitors (TKIs) with EGFR mutations as the most reliable predictor for improved response rate and survival. Despite initial responses to EGFR TKIs, the majority of patients will relapse within 1 to 2 years (acquired resistance). In approximately two thirds of these patients, the mechanism of acquired resistance is the development of a resistance mutation, the *EGFR* T790M mutation. This mutation leads to an enhanced affinity for ATP, thus reducing the ability of ATP-competitive reversible EGFR TKIs to bind to the tyrosine kinase domain of EGFR. One strategy to overcome this mechanism of resistance is through the use of irreversible EGFR inhibitors. Recent clinical trials showed that these third-generation EGFR TKIs are highly active in patients with advanced *EGFR*-mutated NSCLC who relapsed after treatment with EGFR inhibitors. A major limitation in the advancement of these third-generation EGFR TKIs in patients with NSCLC is the challenge of tumor re-biopsy to detect the T790M mutation. Thus, non-invasive techniques may be additionally needed to fully realize the potential of this treatment. Analysis of cell-free plasma DNA from blood samples (liquid biopsy) has the potential to enable non-invasive assessment of the EGFR mutation status in patients with advanced NSCLC. Continuous monitoring of the tumor genotype would also be important for the early identification of emerging changes in tumor biology leading to acquired resistances against initially effective TKIs. Several studies have now suggested that highly sensitive genotyping assays such as digital droplet PCR (ddPCR) can indeed detect mutations in cell-free plasma DNA. However, the detection of low-prevalence mutant alleles remains a challenge.

Other important biomarkers are the presence of oncogenic fusion genes consisting of EML4 and anaplastic lymphoma kinase (ALK) or ROS1 translocations which are observed in a small subgroup of patients. NSCLCs with ALK or ROS1 rearrangement are highly sensitive to ALK kinase inhibition.

Immune checkpoint inhibitors have recently shown promising activity in patients with advanced NSCLC. Objective response rates of approximately 20% and improved PFS and OS have been reported in patients with advanced NSCLC treated with drugs targeting programmed cell death 1 (PD1) or its ligand, programmed cell death ligand 1 (PD-L1). Response rates appear to be higher in patients with tumor PD-L1 expression assessed by immunohistochemistry. The role of PD-L1 expression as predictive biomarker for anti-PD1/PD-L1 therapy in NSCLC is currently evaluated in clinical trials.

In summary, biomarkers for molecular targeted therapy have already found their way into daily clinical practice and will continue to improve outcome of NSCLC patients in the future.

Immunotherapy of lung cancer. Best of WCLC 2015

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In order to fully exploit the recently introduced immune check point inhibitors, there is a new challenge to find the most effective combination with the traditional treatment modalities (surgical resection, radiation therapy, cytotoxic treatment) and the. Currently the CTLA4 and PDL axis inhibitors are delivering promising results in the treatment of various solid tumors. The PD-1 (nivolumab and pembrolizumab) and PDL-1 (atezolizumab, durvalumab and avelumab) inhibitors show relevant activity in melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), renal and bladder cancer, thymoma and mesothelioma. There are several ongoing trials investigating the potential and optimal role for immune check point inhibitors in different tumor types in various indications. There is now increasing evidence that the efficacy of immune check point inhibitors may depend on the somatic mutation frequencies of solid tumors. The highest mutation frequency was found in melanoma. Lung cancer – especially smoking associated tumors – has a high frequency of mutations as well. Accordingly, PD1 and PDL1 inhibitors are likely to be more effective in smoker's lung cancer.

The latest data of **CheckMate017** (phase 3, global, randomized trial of nivolumab vs. docetaxel in advanced squamous NSCLC) with longer follow-up shows that nivolumab continues demonstrating survival benefit versus docetaxel in previously treated patients (18-month OS: 28% vs 13%, 18-month PFS: 17% vs 2.7%, mOS: 9.2 vs 6.0 mo.). Nivolumab benefit was independent of PD-L1 expression and was seen across clinical subgroups. Safety profile of nivolumab was favorable versus docetaxel and consistent with prior studies (*Ref 1*).

Evaluation of disease-related symptoms from the **CheckMate017** showed more favorable safety profile for nivolumab than docetaxel. In addition, major symptom benefits were noted with nivolumab when compared with docetaxel, as shown by a lung cancer-specific symptoms scale (LCSS). Patients who remained on treatment showed greater symptom improvement on nivolumab, whereas patients on docetaxel remained stable. While on treatment, most symptoms showed a significant improvement in the nivolumab group. Nivolumab patients were significantly slower to deteriorate than docetaxel patients (*Ref 2*).

An update for the safety and overall survival data was reported from CheckMate063, a phase 2, single-arm study of nivolumab in patients with squamous NSCLC who progressed during or after prior platinum based chemotherapy and ≥1 additional systemic regimens. The OS data from June 2014 versus July 2015 was compared. In this trial with longer follow-up in patients with advanced SQ NSCLC, nivolumab continues to demonstrate clinically meaningful efficacy with no new safety concerns (mOS: 8.1 mos., 12-mo OS: 39%, 18-mo OS: 27%). Consistent with randomized phase 3 trials, most treatment-related AEs were of low grade and manageable with established guidelines. Most patients who experienced a treatment-related AE reported their first event within 3–6 months of treatment initiation. Clinical benefit with nivolumab was observed independent of PD-L1 expression (*Ref 3*).

Another trial presented the safety and the efficacy of first-line nivolumab and ipilimumab in NSCLC. First-line therapy with these two drug combination demonstrated a high level of clinical activity characterized by deep and durable responses in advanced NSCLC. Confirmed ORR: 13–39% and mPFS: 4.9–10.6 mos. Nivolumab plus ipilimumab is associated with a favorable safety profile with low frequency of treatment-related grade 3–4 AEs leading to discontinuation. Clinical activity was observed regardless of tumor PD-L1 expression. Preliminary evidence suggests greater activity in ≥1% PD-L1 expressing tumors (*Ref 4*).

A phase Ib trial of atezolizumab combined with platinum-based chemotherapy in NSCLC was presented. Atezolizumab was combined with carboplatin/paclitaxel or pemetrexed/carboplatin or carboplatin/nabpaclitaxel. Atezolizumab demonstrated no unexpected toxicities in combination with standard first-line chemotherapy regimens for advanced NSCLC. This preliminary analysis showed high response rate (63% for the combined NSCLC cohort) supporting potential synergy between atezolizumab and chemotherapy. Other endpoints of the trial, including duration of response and PFS, are still immature and will be presented at a later date. Several phase III studies looking at atezolizumab monotherapy and combinations with other agents in NSCLC are underway (*Ref 5*).

There is data available that prior tyrosine kinase inhibitor therapy in EGFR mutant patients associates with lack of response to anti PD-1 treatment. No current trials are evaluating front-line PD-1/PD-L1 inhibition in EGFR mutant patients. Retrospective analysis of EGFR mutant NSCLC patients enrolled in the **KEYNOTE-001** trial showed a strong correlation between response and lack of prior EGFR TKI treatment, especially in patients with a sensitizing mutation. PD-L1 levels decrease in response to an EGFR TKI in cell lines sensitive to the TKI. This trial data suggest that PD-1/PD-L1 inhibition prior to an EGFR TKI may be more efficacious than a strategy in which the PD-1/PD-L1 inhibitor follows or is given concurrently with, an EGFR TKI. It seems that EGFR mutations upregulate PD-L1 expression and EGFR TKIs downregulate oncogene driven PD-L1 expression (*Ref 6*).

The efficacy and safety profile of pembrolizumab (2 mg/kg Q3W) for previously treated PD-L1–positive advanced NSCLC was investigated with the primary endpoints ORR and safety. Pembrolizumab demonstrated a manageable toxicity profile with robust and durable antitumor activity (ORR: 15.4 %, in all patients, 30.4 % with PD-L1 ≥50%patients). No apparent difference in antitumor activity between pembrolizumab 2 mg/kg and 10 mg/kg in previously treated NSCLC was seen. Data supports that pembrolizumab 2 mg/kg Q3W is an effective dose in NSCLC (*Ref 7*).

In another presentation the efficacy of pembrolizumab was analyzed in key subgroups of patients with advanced NSCLC from the **KEYNOTE001** trial stratified by histology, PDL-1 level, smoking history and EGFR/KRAS mutation status was analyzed. In all subgroups examined, improved response was observed in patients with PD-L1 TPS ≥50% compared to those with less PD-L1 expression. PD-L1 and smoking status independently associated with increased odds of response. Across all PD-L1 subgroups, patients with *EGFR*-mutant tumors had lower ORR than patients with *EGFR*–wild-type tumors. Patients with or without *KRAS* mutation had similar ORR. Patients with no PD-L1 expression who had squamous histology, never smoked, or had *EGFR*-mutant tumors showed no response to pembrolizumab (*Ref 8*).

The efficacy of pembrolizumab for extensive stage small cell lung cancer (SCLC) was also investigated with the relationship with PD-L1 expression. Promising antitumor activity in a pretreated, PD-L1–positive SCLC population was found (ORR: 29.2%). Safety profile was consistent with previous experience for pembrolizumab in other tumor types. There was no relationship between higher PD-L1 expression on tumor and inflammatory cells and frequency of response. There are several ongoing trials of pembrolizumab for extensive-stage SCLC (*Ref 9*).

The potential efficacy of immune check point inhibitors is also studied in malignant pleural mesothelioma (MPM). The single-agent pembrolizumab treatment for MPM patients showed 28.0% ORR and 76.0% DCR better than historical response rates for second-line chemotherapy. Some responses were already observed at first imaging assessment. No relationship was found between higher PD-L1 expression on tumor and inflammatory cells and frequency of response. 5.8-month median PFS with 50.0% 6-month PFS rate was encouraging. Further evaluation of pembrolizumab in mesothelioma is planned (*Ref 10*).

How can we manage the immune-mediated adverse events during the immune check point inhibitor treatment? The incidence of immune-mediated AEs and use of corticosteroids for their management was investigated with pembrolizumab treatment in the **KEYNOTE001** study. Immune-mediated AEs with pembrolizumab were seen in 14.5% of patients with advanced NSCLC. Most events were of grade 1 or 2 severity and did not require steroids. Most severe events were successfully managed by temporary pembrolizumab interruption and

use of corticosteroids. Limited data suggest no clear relationship between steroid use and continued efficacy of pembrolizumab (*Ref 11*).

An exploratory responder analysis of best RECIST response and survival in patients with metastatic squamous NSCLC treated with nivolumab based on **CheckMate017** and **CheckMate063** trial was analyzed. This analysis showed the correlation between response and survival but did not evaluate the treatment effect. However, the analysis does suggest that patients who attain RECIST response are likely to derive most benefit from anti-PD1 therapy. Small subset of patients with PD might derive some benefit with treatment beyond progression. There is a need for further development of biomarkers to identify those most likely to respond (*Ref 12*).

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Posters

Concomitant rosuvastatin-induced rhabdomyolysis and maprotiline-associated Ogilvie syndrome in an 80-year-old female lung cancer survivor

Poster Number: P1

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Background: Adverse drug reactions (ADRs) are rare events that pose a clinical challenge. Multiple ADRs may occur in the same patient as a result of polypharmacy. Rosuvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitor, is a commonly prescribed drug for the treatment and prevention of atherosclerotic diseases. Maprotiline is a routinely used tricyclic antidepressant (TCA), also indicated for symptomatic relief of insomnia. Both medications have been reported to infrequently cause rhabdomyolysis. In addition, insomnia is a known but unusual side effect of rosuvastatin, while atypical findings of acute intestinal pseudo-obstruction (Ogilvie syndrome) have been attributed to maprotiline.

Methods: A comprehensive chart and literature review was performed.

Results: We present a unique case of synchronous rhabdomyolysis and Ogilvie syndrome in an 80-year-old female with a history of recent stroke, who six years earlier had undergone right superior lobectomy due to lung adenocarcinoma (EGFR and ALK negative), followed by two lines of adjuvant chemotherapy and finally, radiation therapy. She was prescribed rosuvastatin as a secondary prevention measure after her stroke, and as a side-effect of this therapy developed insomnia for which she was treated using maprotiline. Upon removal of the aforementioned offending agents, and initiation of conservative treatment, her condition

progressively and rapidly improved. Pharmacogenetic testing of drug-metabolizing enzymes (CYP2C9 *2,*3; CYP2C19 *2,*17; CYP2D6 *3,*4,*5,*6) and transporters (SLCO1B1 [OATP1B1] 388A>G and 521T>C; ABCG2 421C>A) was undertaken and revealed significant susceptibility to ADRs of HMG-CoA reductase inhibitors. The patient's genetic profile (CYP2C9 1/1 extensive or normal metabolizer, CYP2C19 1/17 ultra-rapid metabolizer, and CYP2D6 1/4 intermediate metabolizer, SLCO1B1 G/G and C/C rapid and poor transporters, respectively, and ABCG2 C/A moderate transporter) was subsequently translated into clinical practice to personalize her drug palette and appropriately adjust dosages.

Conclusions: Tailoring therapies using commercially available pharmacogenomic tools should be the mainstay of everyday medical practice to optimize treatment response and prevent ADRs. This integrative approach is especially important in elderly patients with various co-morbidities requiring concurrent prescriptions. It provides risk information that helps predict and reduce harmful drug-drug interactions which may consequently improve daily medication adherence.

Less frequent cause of recidive pleural effusion at higher age: a case report

Poster Number: P2

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Background: Relapsing pleural effusion, either bilateral or unilateral occurs commonly at higher age. Its etiology is often combined on the cardio-respiratory basis. Less frequently, malignant pleural mesothelioma can be the causative factor. Due to its difficult diagnostics, rare occurrence and non-specific symptoms the diagnostic process is often prolonged. Therefore, the options for effective therapeutic intervention are limited. The aim of this study is to present a case report of this interesting issue and a review of diagnostic and therapeutic options in the light of current knowledge.

Case report: Malignant pleural mesothelioma (MPM) is an uncommon tumor that is difficult to diagnose. It is a locally invasive and rapidly grown malignancy which can be associated with asbestos exposure. Etiopathology of this mechanism is still not completely clear. MPM is a local malignancy that diffusely involves the pleural cavity. Extrathoracic metastases either endogenic, but mainly iatrogenic are not usual. In our case we report the follow up of the 82 years old male patient with repetitive unilateral fluidothorax with unclear genesis. He was admitted to our department due the progressive shortness of the breath, cough and fever.

The imaging methods (X-ray, Ultrasound, CT-scan) revealed fluid (800 ml) in the thorax, with no signs of solid infiltration of the lung parenchyma or pleural cavity. In a period of six months the patient underwent several times thoracocentheses and chest drainages. The cytopathological examination of the fluid has still shown only inflammatory type of the effusion, without neoplastic cells. Diagnosis was established 4 months later from biopsy of the infiltration in previous place of the chest tube insertion. Surprisingly, the pathologist confirmed the incision-site metastasis of the epiteloid type of the MPM.

Conclusion: In the clinical and diagnostic expectations of the etiology of the repeated pleural effusions is important to think also about this rarely occurring diagnosis. The tissue biopsy is best made by mini-invasive techniques such as CT navigated biopsy or thoracoscopic (VATS) approach and the aid of immunohistochemistry. The poor prognosis of this disease is probably due the common clinical signs, late diagnostics and an advanced disease in the time of diagnosis confirmation. In selected group of patients in good condition the radical surgery or palliative chemoradiotherapy could be method of the choice. The diagnosis and treatment of patients with MPM requires a multidisciplinary approach.

Salivary gland-type tumours of the lung: a two centre experience

Poster Number: P3

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Background: Salivary gland-type tumours are very rare primary lung tumours, representing only 0.1–0.2% of all lung cancers. Morphological distinction from the head and neck salivary gland tumour metastasis is impossible, therefore careful and detailed clinical workup is obligatory. They develop from tracheobronchial, submucosal glands, and are most often slow growing, low grade tumours. Surgery is recommended treatment option, and when complete bears very good prognosis. Most common types are mucoepidermoid (MC) and adenoid cystic carcinoma (ACC), followed by epithelial-myoepithelial carcinoma. The aim of this study was to compare occurrence and epidemiological characteristics in two central European University Hospitals.

Methods: Electronical pathological archives of the Institute of Pathology Medical University of Graz (MUG), and Institute of Pathology University of Zagreb School

of Medicine (UZSM) were searched for salivary gland-type tumours, for the period between January 2002 and December 2011. Data analysed were diagnosis, sex and age.

Results: In 10-year period, at the MUG 13 patients with salivary gland-type tumours were found, 7 with mucoepidermoid carcinoma, and 6 with adenoid cystic carcinoma. In the same period at UZSM 16 patients were diagnosed, 8 with mucoepidermoid carcinoma, and 8 with adenoid cystic carcinoma. In patients with MC at MUG 4 were males, and 3 females, while at UZSM 6 were males and only 2 females. In ACC group at MUG 3 were male, and 3 female patients, and at UZSM 3 were male and 5 female patients. Concerning age, MC at MUG presented at age range from 34 to 83, with median value 74, while in Zagreb median value was 44 (range 35 to 70). Similar, in ACC median age at presentation at MUG was 63.5 (range 41 to 82), and at UZSM 47.5 (range 43 to 70).

Conclusion: In retrospective analysis of pathological archives of two big central European institutions occurrence of salivary gland-type tumours in both was similar, as well as distribution according to gender. Although the numbers are low, it was clear that median age at the diagnosis at UZSM was significantly lower in both histological types when compared to MUG. Another interesting fact is that in both institutions no other, even rarer, histological types of salivary gland-type tumours were diagnosed in selected period.

The frequency of pulmonary thromboembolism in patients with lung cancer

Poster Number: P4

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Background: Malignant diseases including lung cancer are the risk for development of pulmonary thromboembolism (PTE). Objective: To show the number of PTE in patients with lung cancer treated in Clinic for pulmonary diseases and TB "Podhrastovi" in three-year period: from 2012–2014.

Material and methods: This is the retrospective study in which we present the number of various types of lung cancer treated in three-year period, number and per cent of PTE in different types of lung carcinoma, number and per cent of PTE of all diagnosed PTE in lung carcinoma according to the type of carcinoma.

Results: In three-year period (from 2012 to 2014 year) 1609 patients with lung cancer were treated in Clinic for pulmonary diseases and TB "Podhrastovi" Clinical Centre of Sarajevo University. 42 patients: 25 men middle-aged 64.4 years and 17 women middle-aged 66.7 or 2.61% of all patients with lung cancer had diagnosed PTE. That was the 16.7% of all patients with PTE treated in Clinic "Podhrastovi" in that three-year period. Of all 42 patients with lung cancer and diagnosed PTE 3 patients (7.14%) had plano-cellular cancer, 4 patients (9.53%) had squamo-cellular cancer, 9 (21.43%) had adenocarcinoma, 1 (2.38%) had NSCLC, 3 (7.14%) had microcellular cancer, 1 (2.38%) had neuroendocrine cancer, 2 (4.76%) had large cell-macrocellular and 19 (45.24%) had histologically non-differentiated lung carcinoma.

Conclusion: Malignant diseases, including lung cancer, are the risk factor for development of PTE. It is important to consider the including anticoagulant prophylaxis in these patients and so to slow down the course of diseases in these patients.

Improved overall survival in patients with NSCLC treated to 36 Gy with hypofractionated radiation therapy

Poster Number: P5

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Background: Despite advances in oncological treatment, outcomes in the treatment of non-small cell lung cancer are still disappointing. We analyzed the effect of two different schemes of hypofractionated radiation therapy for NSCLC. We aimed to compare the treatment outcome of 36 Gy regimen administered in 12 fractions/daily and 30 Gy regimen in 10 fractions/daily.

Methods: We retrospectively reviewed medical charts of all patients treated in the period between July 2005 and July 2012 for lung cancer in the institutional database of the Copernicus' Memorial Regional Oncology Centre. All available therapeutic protocols of individuals treated for inoperable stage IIIa and IIIb NSCLC given external beam radiation therapy to alleviate thoracic symptoms were reviewed. Details on radiotherapy protocol were available in 741 individuals. Treatment with the 36 Gy regimen was performed in 80 individuals. Out of that number, complete clinical data was collected from 67 patients. The reference group was constituted by individuals treated with standard 30 Gy dose, age-matched to the 36 Gy in a 2:1 ratio. Clinical data collected from the multivariate Cox' proportional hazard

regression was used to estimate survival. To adjust for the effect of diagnosis period, we additionally stratified the analysis by the year of diagnosis.

Results: The final study groups numbered 67 and 138 patients in the 36 Gy and 30 Gy group respectively. Median age was 65.03 (+/– 8.67) in the 36 Gy group and 64.95 (+/– 8.71) in the 30 Gy group. Proportion of female patients was similar in both groups (21/67 vs 28/138; p=0.09). The median overall survival time was 10.92 months, which was significantly longer than in patients treated with the 30 Gy regimen (8.04 months; p=0.04;). The effect of the therapeutic protocol remained significant after adjustment for covariates (HR 0.58 CI 95% 0.38–0.88; p=0.01). The impact of haematocrit at treatment completion was significant (HR 0.97 95%CI 0.94–1.00; p=0.04) while age at diagnosis was at borderline significance (HR 1.02 95%CI 1.00–1.03; p=0.05). Survival did not differ significantly between males and females (p=0.59) nor depending on the year of diagnosis (p=0.52).

Conclusions: The clinical results of radiation therapy with 36 Gy delivered in 12 fractions reveal a clinically-relevant advantage over the standard, 30 Gy protocol in the treatment of patients with NSCLC.

Change in serum lactate dehydrogenase in patients with advanced-stage NSCLC treated with erlotinib

Poster Number: P6

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Background: Molecular targeted therapy based on low-molecular weight tyrosine kinase inhibitors directed at epidermal growth factor receptor (EGFR-TKIs)

represents one of the novel effective strategies in management of advanced non-small cell lung cancer (NSCLC). Activating *EGFR* gene mutations are a strong predictive factor of response and survival to EGFR-TKIs. The efficacy of EGFR-TKIs, particularly erlotinib, in the predominant patient group harbouring wild-type *EGFR* gene is low and there is no available predictive biomarker.

Methods: Clinical data of 309 patients with locally-advanced (IIIB) or metastatic stage (IV) NSCLC treated with erlotinib were analysed. Serum samples were collected and the measurements were performed within one week before the initiation and after one month of erlotinib treatment. Absolute difference in lactate dehydrogenase (LDH) was defined as: Δ (delta) = value after one month of therapy – value at erlotinib therapy initiation, thus negative delta means decrease in LDH value and positive delta means increase in LDH value.

Results: The disease control rate (DCR) for Group I ($\Delta < -0.3$ ukat/l) was 86.7% (104/120), for Group II (-0.3 ukat/l $\leq \Delta \leq 0.3$ ukat /l) 85.2% (98/115) and for Group III ($\Delta > 0.3$ ukat/l) 73.0% (54/74), respectively. The difference in DCR was statistically significant (p=0.006). The median progression-free (PFS) and overall survival (OS) for Group I was 3.7 and 13.2 months compared to 3.0 and 16.4 months for Group II compared to 2.1 and 11.4 months for Group III (p=0.010 and p<0.001).

Discussion: High serum lactate dehydrogenase (LDH) levels have been reported as a poor prognostic biomarker in various malignant diseases including NSCLC. However, very little is known about the dynamics of serum LDH levels during the systemic treatment of patients with advanced-stage NSCLC. The aim of our retrospective study was to evaluate the association of changes in serum lactate dehydrogenase with outcome of 309 patients with advanced-stage NSCLC treated with erlotinib.

Conclusion: The results of the conducted retrospective study suggest that the change in LDH serum level during the first month of erlotinib treatment was independently associated with DCR, PFS and OS. LDH is a commonly used serum biomarker which is easy and cheap to detect and thus feasible for the use in routine clinical practice.

This study was supported by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.

Parenchyma sparing surgery for lung cancer in a heart transplant recipient with poor respiratory reserve

Poster Number: P7

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Background: Malignancies occur more frequently in solid organ transplant patients and they seem to be characterized by rapid progression and a worse prognosis. Permanent immunosuppressive therapy, a history of heavy smoking and advanced age are the major risk factors in the development of lung cancer in heart transplant recipients. Treatment of lung carcinoma in such patients depends on the type, stage and performance status similarly to non-transplant patients. Unfortunately, the majority of such cases are far advanced at the time of diagnosis, median survival of non-resectable patients is about 3 months.

Methods: The authors present one case of centrally located squamous cell lung carcinoma in a cardiac transplant recipient. Definitive diagnosis was obtained by bronchoscopy in 2013. The tumor originated at the ostium of the right main bronchus, which is why the high-risk procedure of a right upper sleeve bi-lobectomy was indicated. PET CT scan showed a tumor mass in the right upper lobe with a satellite lesion in the middle lobe, but no mediastinal lymphadenopathy (cT4N1M0). Inductive chemotherapy was terminated after seven cycles. After re-staging with a non-progression finding, a right upper sleeve lobectomy with complete lymphadenectomy was performed because of limited respiratory functions. Reconstruction of the bronchial tree was difficult due to the inability to release the pulmonary hilum after prior cardiac surgery. The postoperative course was complicated by a persistent air leak, which was treated by prolonged chest drainage.

Results: 7 years after heart transplantation, 25 months after diagnosis of lung cancer and 13 months after bronchoplastic lung resection, our patient still remains in follow up.

Conclusion: The long-term results of such borderline surgery still remain unclear.

This study was supported by grant IGA MZCR NT 14406/2013.

Initiary experience with crizotinib in the treatment of NSCLC in the Czech Republic

Poster Number: P8

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Background: Crizotinib is a highly selective drug used in the treatment of anaplastic lymphoma kinase (ALK) gene re-arrangement positive non-small cell lung cancer (NSCLC). In the Czech Republic it was used in frame of compassionate cases program and now is reimbursed in pre-treated tumors with EML 4/ALK gen translocation verified by FISH and/or IHC testing. The recommended dose is 250 mg bid/ day. Crizotinib is used since 2011, data are evaluated according to the National Reference Centre Registry.

Material and Methods: Present study evaluates 35 pts, 20 males, 15 females with mean age 69 (31–75) years. Out of them 13 (37.1%) were non-smokers, 9 (25.7%) ex-smokers and 13 (37.1%) smokers. All of them had NSCLC, 27 adenocarcinoma, 4 NOS, one adenosquamous and one epidermoid cancer. Stage in the time of treatment was IIIB in 5 and IV in 30 pts. Crizotinib was applied in 2nd line in 23 pts, 3rd line in 8 pts, 4th line in 3 pts, 5th in one patient. PS was 0 in 6 pts, 1 in 23 pts and 2 in 6 pts.

Results: On the date of evaluation, 18 pts continued the treatment, 14 died and 3 stopped treatment. Crizotinib effectiveness was assessed in 27 pts: CR in 3 (8.4%) pts, PR in 6 (17.1%) pts, SD in 11 (31.4%) pts, PD in 7 (20.0%) pts. CR was associated with long response duration (10.7, 31.8, 34.1 months). Grade 3 adverse events (gastrointestinal discomfort and liver disease) were observed in six (17.1%) pts. Median (m) PFS was 4.9 months, mOS was 22.2 months.

Conclusion: Interim analysis of present series shows, that crizotinib has very good tolerability and promising effectiveness even in heavily pre-treated patients with EML4/ALK gene translocation. Long term survival analysis is running.

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Experience with radical (chemo) radiotherapy in the treatment of lung cancer at Teaching Hospital Comenius University of Hradec Králové

Poster Number: P9

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Background: The definitive and best treatment of NSCLC in stages IIIA N2 and IIIB in patients with PSO-1 is primary radical chemo-radiotherapy dosed concomitantly. In patients with large tumours, chemotherapy is applied sequentially with the intention to initially reduce the primary tumour mass and then in the second stage radical radiotherapy is applied. In patients who for internal diseases are inoperable, radical stereotactic radiotherapy is suitable, allowing us to achieve similar results as the surgical procedure.

Methods: It is standard at Teaching Hospital Hradec Kralove to use the hypofractionated radiotherapy, when 55 Gy/20 fr daily is applied with concomitant chemotherapy usually based on a platinum derivative, etoposide. For stereotactic radiotherapy the regime we use is 50Gy/5 fr daily without concomitant chemotherapy.

Results: Our group of 89 patients treated with radical (chemo) radiotherapy, of which 47 patients in stage III, had 2-year overall survival of 51%, 3-year overall survival of 20%, while in stage IIIA 2-year overall survival was 36% and in stage IIIB it was 49%, further 2-year local progression-free survival in stage IIA of 46% and stage IIIB 58.4 %. Comparing normo and hypofractionated radiotherapy, 2-year overall survival was 41% for hypofractionated and 53% for normofractionated radiotherapy, which is basically comparable for such a small group when comparing acute toxicity and only slightly worse late toxicity. A significant effect (p 0.03) was caused by the application of chemotherapy and radiotherapy in comparison with radiotherapy itself in stage III, which forms the largest part of our patients. Applying chemotherapy concomitantly with radiotherapy has a significant impact on local progression-free survival (p 0.05), but does not affect overall survival (p 0.98). 2-year survival of our group with stereotactic radiotherapy is 83 % and 2-year interval to progression is 88.9%.

Conclusion: We were able to prove the theory that the application of stereotactic radiotherapy in stages I and II in serious internal diseased patients has results comparable to surgery. Further, in stages IIIA N2 and IIIB radical

chemo-radiotherapy is primary treatment, preferably concomitantly, if the primary volume of the tumour tissue so allows. It is important to start treatment as fast as possible, as the smaller the tumour the better the result (the tumour size influences overall survival as well as local progression-free survival p 0.0003). Using normofractionated and hypofractionated radiotherapy, has the same effect, with tolerable toxicity. It is clear that using modern techniques is an advantage for precise targeting of the tumour and protection of the surrounding tissues.

Dosimetric parameters as predictive factors for radiation pneumonitis and esophagitis in patients with locally advanced Non-Small Cell Lung Cancer

Poster Number: P10

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Purpose: To report about the role of dosimetric parameters as predictive factors for pulmonary and esophageal toxicity of radiation treatment in patients with locally advanced Non-Small Cell Lung Cancer (NSCLC).

Material and methods: Thirty nine consecutive patients (all stages IIIA and IIIB) were treated with radical radiotherapy (RT). Among them 82% were men; 82% presented squamous cell carcinoma, 18% adenocarcinoma. Of them, 77% received sequential chemotherapy while 23% were treated with radiotherapy alone. Dose prescription ranged from 40 to 70.2Gy. No elective radiotherapy of mediastinal lymph nodes was used. Analysis included toxicity profiles of lung and esophagus. The following dosimetric parameters were studied: mean lung dose (MLD) and percentage of volume of lung receiving >20Gy (V20), mean dose to the entire esophagus, maximal point dose to the esophagus, percentage of volume of esophagus receiving >55Gy (V55).

Results: Twenty three percent of our patients had lung toxicity and among them V20 did not show tendency to be predictive factor, as well as MLD (p=1). Twelve patients (31%) developed treatment-related acute esophagitis: 4 patients (10%) grade 2 and 8 patients (21%) grade 3. Twenty seven (69%) did not develop radiation-induced esophagitis associated with their course of radiotherapy. Mean dose to the entire esophagus >34 Gy were associated with a risk of esophageal toxicity and there were very statistically significant difference between groups of patients who developed and who did not develop the radiation-induced esophagitis (p=0.0013). Also, there was statistically significant difference between two groups

of patients regarding maximal point dose to the esophagus (<60Gy versus >60Gy, p=0.0030). Mean V55 was 63% (min=40%, max=100%) for patients who developed acute esophagitis and 16% (min=0%, max=60%) for those who were without acute esophageal toxicity (p=0.0001, Fisher's exact test, two-tail).

Conclusion: In our analysis, in patients who developed acute toxicity of lung there was not power of V20 and MLD as predictive factors. However, among patients who received acute radiation esophagitis this occurrence was expected according to the dosimetric parameters.

Therapy-related myeloid neoplasms after lung cancer chemotherapy

Poster Number: P11

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Background: Therapy-related leukemia defined by the World health Organization 2008 classification scheme of hemato-lymphoid tumors including therapy-related acute myeloid neoplasms (t-AML), myelodysplastic syndrome (t-MDS). They occur as late complication of cytotoxic chemotherapy, radiation therapy and molecular targeted agent therapy against primary neoplasms. Recently, for the lung cancer chemotherapy, new anti-cancer agent and molecular targeted agents are increased and more intensification chemotherapy performed. We report that we reviewed t-AML cases who survived from lung cancer and suffered t-AML.

Methods: We intended for multiple neoplasms 298 cases including hematological malignancy. We reviewed 39 multiple neoplasms including the lung cancer. In 39 cases, second neoplasms that were t-AML cases were 4 cases, t-MDS case was 1 case. All patients were followed up until death or until December 2014. Survival was measured from the diagnosis of multiple cancer to time of death or last contact. We investigated cytogenetic abnormality, therapy, clinical outcome, prognosis, and cause of death.

Results: In 5 cases, 4 cases were diagnosed t-AML, 1 case was t-MDS. 5 of cases were 4 male and 1 female, primary diagnosis were small cell carcinoma, squamous carcinoma and adenocarcinoma 3 cases. One case (male case) was t-APL, he was treated by all-trans retinoic acid and he reached complete response. T-M2 type was treated by chemotherapy included daunorubicin and Ara-C (DC3-7), she did not achieve complete response. About prognosis, t-APL case, he lived 1 month

after complete response, he died by lung cancer, t-AML cases, one female case, she lived 25 months after partial response, she died by t-AML relapse and refractory for salvage CTx. Other 3 cases, 1 case death by t-MDS, 2 cases sill received chemotherapy.

Conclusion: As the number of lung cancer survivors increased due to improvement in chemotherapy, clinician must more take attention of therapy-related leukemia and myelodysplastic syndrome by previous treatments.

Multiple neoplasms consist of lung cancer and hematological malignancies

Poster Number: P12
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Background: The lung cancer is a cancer of the most in Japan and first place in cause of death. Lung cancer is still poor prognosis disease that cure only in early clinical stage. Recently, new anti-cancer agent and molecular targeted agents are increased but clinical outcomes are not satisfied. We report that we reviewed 39 cases of multiple neoplasms with lung cancer and the hematological malignancy.

Methods: We intended for multiple neoplasms 312 cases including hematological malignancy. We reviewed 42 multiple neoplasms including the lung cancer. All patients were followed up until death or until August 2015. Survival was measured from the diagnosis of multiple cancer to time of death or last contact. Definition of the multiple neoplasms was in compliance with Warren & Gates. Also we determined the synchronous type and metachronous type in accordance with the definition of Moertel, so within less than 6 months was synchronous type, more than 6 months was metachronous type. About statistical examination, we used IBM SPSS statistics version 21.

Results: All cases are 42 cases, consist of male 33 cases, female 9 cases, type of multiple neoplasms, synchronous type 10 cases, metachronous type 32 cases. Number of multiple neoplasms, double neoplasms 22 cases, triple neoplasms 11 cases, quad neoplasms 2 cases. The median age was 70 years (range, 47–86y ears). The counterpart of malignancies, non-Hodgkin´s lymphoma 22 cases, myelodysplastic syndrome 1 case, acute myelogenous leukemia 6 cases, Hodgkin's lymphoma 2 cases, macroglobulinemia 1 case, chronic lymphocytic leukemia

2 cases, chronic myelogenous leukemia 1 case, acute lymphoblastic leukemia 1 case, monoclonal gammapathy undetermined significance 2 cases. Other solid cancer were 18 cases. In double neoplasms, the median age of first diagnosis was 69 years, the second cancer was 71 years. About interval between lung cancer and hematological malignancies, lung cancer precedence case was 34 moths (M), hematological malignancy precedence case was 51M. The median overall survival was 13M.

Discussion and conclusion: Diagnosis of lung cancer within 5 years were 8 cases out of 17 cases. The important point is that 5 years are required for careful observation at the time of hematological malignancy diagnosis. We think that a prognosis is improved.

The clinical, pathological and prognostic significance of cancer stem cell markers CD44 and ALDH1 expression in adenocarcinoma of the lung

Poster Number: P13

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Background: Adenocarcinoma is the most common histologic type of non-small cell lung carcinomas. The existence and role of lung cancer stem cells (CSCs) in human tissue is controversy. The aim of this study is to investigate the expression of CD44 and ALDH1 and evaluate their relationships with clinical and pathological parameters including the survival in lung adenocarcinoma.

Methods: Immunohistochemistry for CD44 and ALDH1 was performed in 77 curative surgical resection cases with primary lung adenocarcinoma using tissue microarray.

Results: High expression of CD44 and ALDH1 were found in 64.9% and 33.8% in the study group, respectively. High CD44 expression was statistically associated with female gender (p=0.010), no pleural invasion (p=0.017), and smaller tumor size (p=0.043). High ALDH1 expression was statistically associated with female gender (p=0.003), N0 LN (p=0.006), low pathologic stage (p=0.019), and smaller tumor size (p=0.011). The high expression of CD44 had better survival compared with the low expression (p=0.014). In multivariate analysis, high expression of CD44 and early pathologic stage were the independent favorable prognostic factors for overall survival.

Conclusions: Our results showed that the expression of CSCs marker CD44 was associated with a good prognosis. There is a need for more research about CSC in human lung adenocarcinoma.

Salvage surgical treatment of advanced lung adenocarcinoma patient after Gefitinib therapy

Poster Number: P14

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Introduction: First-line treatment with a tyrosine-kinase inhibitors (erlotinib or gefitinib), is an option in patients with advanced non-small cell lung cancer, harbouring an activating EGFR mutation according to the guidelines for the non-small cell lung cancer.

Case presentation: 63 year old female patient performed brain CT scans due to neurological symptoms, which showed three metastatic lesions in the brain. MR scan of brain was the same. Patient underwent craniotomy and the biggest metastatic lesion was extirpated on 22th March 2012. Histological findings of brain tumor confirmed metastatic adenocarcinoma. Chest radiogram showed left lower lobe shadow. Chest CT findings showed tumorous lesion 48x35mm of the left basal region of the lung, nodular, micro-nodular lesions 7mm on both side of the lung, and enlarged mediastinal lymph nodes. Biopsy obtained from the bronchoscopy confirmed adenocarcinoma of the left lower lobe. EGFR molecular testing of material obtained by bronchoscopy was positive – mutation on exon 19 of the EGFR gene was found. Stage of the diseases was T2bN3M1b. Patient underwent palliative radiotherapy of the brain in April 2012 (total dose 20 Gy/5 fractions). Gefitinib (first line) treatment started on 4th April 2012 in dose of 250 mg once daily. On November 2013 the radiological findings of the CT scans of the chest and abdomen was stabile after achieving partial regression of the tumor lesions, and CT findings of the brain was without radiological signs of metastatic lesions. Institutional Council indicated left thoracotomy and left pneumonectomy was done on 19th November 2013. Postoperative histological staging was ypT1bN0M0 and patient continued gefitinib therapy after operation. Patient underwent control CT scans of the chest, abdomen and head every three months and the radiological progression of the diseases was confirmed in May 2014. Therapy with gefitinib was stopped and Oncological Council indicated second-line chemotherapy with Cisplatin/Vinorelbine and re-irradiation of the brain due to progression of the brain metastases. Patient completed fourth cycles of the second-line chemotherapy in October 2014 and brain re-irradiation in April 2015.

Conclusion: World literature on neoadjuvant administration of the tyrosine-kinase inhibitors is very meager. Ongoing trials may herald an era of individualized therapy including neoadjuvant gefitinib in at least some NSCLC patients.

Key words: non-small cell lung cancer, geftinib, surgery

The platelet-to-lymphocyte ratio as a predictor of distant metastasis in lung cancer

Poster Number: P15

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Background: Recent studies have shown that the presence of systematic inflammation correlates with poor survival in various cancers. Platelets are also part of inflammatory response and thrombocytosis is common in patients with tumors. Platelets can protect tumor cells from natural killer cells-mediated lysis thereby capability of tumor cells to induce platelet aggregation correlates with metastatic potential. Knowing that low lymphocyte counts may also be associated with shorter survival, the platelet-to-lymphocyte ratio (PLR) has been investigated as a prognostic factor associated with poor survival with various cancers.

Methods: The aim of this study was to examine whether PLR could be a marker to differentiate lung cancer patients with distant metastasis from lung cancer patients without distant metastasis at the time of first presentation. Retrospectively 50 lung cancer patients with brain metastasis at the time of diagnosis and 50 lung cancer without distant metastasis were included in the study. Patients with hypertension, hematological, renal and serious cardiovascular disease were excluded from the study. PLR was defined as the absolute platelet count divided by the absolute lymphocyte count. The platelet and lymphocyte counts of peripheral blood were measured with hematology analyzer before chemotherapy.

Results: PLR values were significantly higher in patients with brain metastasis (PLR: 245 vs 170, p=0.007). No statistically significant relationship was determined between platelet count and occurrence of brain metastasis at the time of first presentation. Discussion: Recently, studies have shown that elevated pretreatment PLR represents significant prognostic indicator of survival in various cancers including lung cancer. The elevation of the PLR has been associated with poor prognosis, although published data is controversial. To our knowledge this is the

first study investigating association of PLR with occurrence of distant metastasis in lung cancer patients.

Conclusion: The PRL is broadly available and cheap marker which could be used to highlight patients that should be screened for distant metastases. Longer prospective studies are required to confirm these findings.

Pemetrexed in maintenance therapy of 134 patients with non-small-cell lung cancer (NSCLC)

Poster Number: P16

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Introduction: The effectiveness and safety of continuation maintenance therapy with pemetrexed versus the watch-and-wait approach was proved by a large randomised phase III trial (Paz-Ares et al., 2013). We focused on continuance maintenance therapy with pemetrexed in routine clinical practice in the Czech Republic.

Methods: The primary objective of our analysis was to evaluate the overall survival, defined as the length of time from the start of maintenance therapy to the date of death. Data was summarised using the standard descriptive statistics, absolute and relative rates for categorial variables, averages for continuous variables, 95% confidence intervals, as well as median, minimum and maximum values. Kaplan-Meier survival curves were used to display the patient survival. All analyses and graphical outputs were performed in the SAS 9.4 Software. The analysed cohort of NSCLC patients treated with pemetrexed maintenance therapy in the Czech Republic as on 22 June 2015 involved 134 patients. The median age was 64.8 (24.0; 79.0) years; stage IV was the predominant clinical stage (85%), 50.0% of patients were men, and 50.0% were women. Adenocarcinoma was the morphological diagnosis in 133 patients, and large-cell carcinoma in one patient.

Results: From a total of 134 patients, treatment response was assessed in 105 patients. Among the assessed patients, 25 of them (23.8%) showed partial

response (PR). Stable disease (SD) was the most frequent response, reported in 56 patients (53.3%); progression occurred in 19 patients (18.1%). As for treatment safety, a total of 23 adverse events were reported (8 different adverse events) in 15 patients (11.2%). Life-threatening adverse events were reported in 3 patients, serious adverse events were reported in 8 patients, and moderate adverse events were reported in 8 patients. Adverse events led to the termination of treatment in only 2 patients. In the registration trial, adverse events were reported in 86 patients (24%). The duration of pemetrexed administration has confirmed a good treatment tolerance: the median number of cycles of maintenance therapy in our study was 5.0 (1.0; 24.0), and the median duration of maintenance therapy was 15.7 (3.0; 62.1) weeks. In the registration trial, the median number of cycles was 4.0 (1.0; 44.0). The primary objective, median overall survival (median OS), was 23.5 months (95% CI: 14.4; not reached).

Conclusion: The continuation maintenance therapy with pemetrexed has been shown to be effective and well tolerated in the Czech population. Treatment had to be terminated only in 3 patients (2.9%) due to adverse events, whereas in the registration trial, adverse events were reported in 86 patients (24.0%). In the registration trial involving 359 patients (Paz-Ares et al., 2013), the continuation maintenance therapy with pemetrexed led to the median OS of 13.9 months, whereas in the Czech Republic, the median OS has been 23.5 months.

The prognostic role of KRAS mutation in patients with advanced NSCLC treated with 2nd or 3rd line chemotherapy

Poster Number: P17

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Background: The role of prognostic and predictive value of KRAS mutation in non-small cell lung cancer (NSCLC) patients is the aim of investigation mainly for EGFR-TKI treatment. However there is only little of evidence for higher lines of chemotherapy. The present study is aimed at an elucidation of the role of specific KRAS mutation types in prediction of outcome of patients with advanced NSCLC receiving pemetrexed or docetaxel as 2nd or 3rd line therapy.

Methods: The outcome of 127 patients with advanced NSCLC who received pemetrexed or docetaxel at 2nd or 3rd line therapy was retrospectively analyzed. Statistical significance of the differences in progression-free (PFS) and overall survival (OS) was assessed using the log-rank test (p=0.05).

Results: Considering the distribution of KRAS mutation types we divided patients into three groups: patients with KRAS wild-type, patients with KRAS G12C mutation and patients with other KRAS mutation. PFS was not significantly different between our 3 groups. OS was significantly longer for KRAS wild-type vs. KRAS mutated patients (median 16.1 vs. 7.2 months, p=0.008). We also observed longer OS for non-G12C KRAS mutation vs. G12C KRAS mutation group of patients (median 6.4 vs. 10.3 months, p=0.011).

Discussion: Prognostic and predictive value of KRAS is shown in some publications in relation to EGFR-TKIs as well as to first line chemotherapy, however another authors did not confirm these conclusions. Significance of specific KRAS mutation type is also mentioned in some publications. The data for higher lines of chemotherapy at KRAS positive patients are very rare.
Conclusion: KRAS status (especially KRAS G12C mutation) correlated with adverse prognosis in patients treated with 2nd or 3rd line pemetrexed or docetaxel. Further validation in prospective study is needed.

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The biological and clinical significance of alpha-1 antitrypsin in non-small cell lung cancer

Poster Number: P18

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Objective: Lung cancer progression is generally associated with extensive tissue remodeling to provide a suitable environment for tumor growth, invasion and metastasis, and it is known that proteinases expressed by cancer cells and/or host cells play a key role in this process. However, the biological role of alpha-1 antitrypsin (AAT) in lung carcinogenesis is not clear.

Methods: Serum and FFPE tissue samples from 206 NSCLC patients (stages I-IV) were analyzed for AAT and CRP blood concentration, AAT phenotype and AAT protein expression in tumor cells. Reference groups consisted of 183 PiMM COPD patients and 23 PiMM patients with benign lung nodules (positive chest radiograph).

Results: Only 10/206 (5%) NSCLC patients carried deficient AAT allele (mean AAT blood concentration 150 mg/dl). In the PiMM NSCLC patients mean AAT serum concentration (195.5 mg/dl) was significantly higher than in the PiMM COPD group (171 mg/dl) and the patients with benign lung nodules (154 mg/dl; $p<0.0001$). AAT concentration was significantly higher in SQC type (202 mg/dl) than ADC (175 mg/dl; $p<0.029$) patients, and in advanced (IIIb-IV, 247 mg/dl) versus early stage disease (I-IIIa, 190 mg/dl, $p<0.0001$). The AAT levels significantly correlated with CRP ($R=0.6$; $p<0.0001$), however CRP level did not differentiate NSCLC from COPD. Importantly, the strong AAT expression observed in tumor tissue was positively associated with the higher AAT blood levels, while weak or no AAT expression directly correlated with the lower AAT blood levels ($p<0.0079$).

Conclusions: Our results evidenced that local production of AAT by tumor cells significantly contribute to high levels of AAT in blood of NSCLC patients reflecting an active role of this anti-protease in lung carcinogenesis. The study is on-going.

Abbreviations: ATT (alpha-1 antitrypsin), FFPE (formalin-fixed paraffin embedded), NSCLC (non-small cell lung cancer), SQC (squamous-cell carcinoma), ADC (adenocarcinoma), COPD (Chronic Obstructive Pulmonary Disease), CRP (C-reactive protein)

Correlation of Gefitinib (G) efficacy and skin rash severity in locally advanced and metastatic EGFR mutated (EGFRm) non-small cell lung carcinoma (NSCLC)

Poster Number: P19

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Background: The problem of correlation of skin toxicity severity and efficacy of some TKI drugs is now widely discussed. This study was performed to evaluate the efficacy of G and specific G-associated skin toxicity as clinic marker of efficacy in patients with advanced and metastatic EGFRm NSCLC.

Methods: Eligibility criteria included measurable advanced and metastatic NSCLC T2-4N1-2M0-1, ECOG≤2, adequate liver, kidney and bone marrow function, no

brain metastases. G was administered 250 mg per day. Endpoints include response rate (RR) by RECIST criteria, OS, PFS and toxicities (CTCAE 3.0).

Results: 32 patients (6 men, 26 women), mean age 58.1±4.7 years were recruited. The deletion in exon 19 were detected in 90.6% (29) samples and mutation L858R in 9.4% (3). All patients were non-smokers. 50.0% (16) pts received G in the 1st line, 34.4% (11) in the 2nd line, 15.6% (5) in the 3rd line after chemotherapy. All pts had the toxicities: skin rash 2/3Gd 75.0% (24), diarrhea 1/2Gd 56.3% (18), interstitial pneumonia 2Gd 3.1% (1). RR was 7CR (21.9%), 17PR (53.1%) and 8SD (25.0%). RR 24 pts with skin rash was 75.0% (18), 8 pts without skin rash 25.0% (2), p=0.042. Med OS for pts with skin rash = 15.8±2.1 months (mos), without skin rash = 6.5±1.8 mos (p=0.032). Med PFS for pts with skin rash = 11.0±2.1 mos, without skin rash = 5.4±1.9 mos (p=0.27).

Conclusions: G demonstrated well tolerability for pts with advanced and metastatic EGFRm NSCLC. Representation of G-specific toxicity (skin rash) could be used as clinic marker of efficacy G in EGFRm NSCLC.

Bristol-Myers Squibb Satellite Symposium 15th CELCC, Prague, November 28, 2015
Summary

Lung cancer is the leading cause of cancer deaths globally, resulting in more than 1.5 million deaths each year, according to the World Health Organization. NSCLC is one of the most common types of the disease and accounts for approximately 85 percent of cases. Survival rates vary depending on the stage, histology and subtype of lung cancer. The majority of NSCLC patients have advanced stage disease at the time of diagnosis. Globally, the five-year survival rate for Stage I NSCLC is between 47 and 50 percent; for Stage IV NSCLC, the five-year survival rate drops to two percent.

The European Commission has approved nivolumab for the treatment of locally advanced or metastatic squamous (SQ) non-small cell lung cancer (NSCLC) after prior chemotherapy. This approval marks the first major treatment advance in SQ NSCLC in more than a decade in the European Union (EU). Nivolumab is also the first and only PD-1 immune checkpoint inhibitor to demonstrate overall survival (OS) in previously-treated metastatic SQ NSCLC.

The approval is based on data from two studies (Phase III CheckMate -017 and Phase II CheckMate -063). Together, the trials investigated nivolumab at a dose of 3 mg/kg every two weeks, which has been well-established across the Phase III nivolumab clinical development program for various tumors. The study's primary endpoint was OS and secondary endpoints included progression-free survival (PFS) and overall response rate (ORR). The trial included patients regardless of their PD-L1 expression status.

Results from CheckMate -017 showed a 41% reduction in the risk of death with a one-year survival rate of 42% for nivolumab (42.1% [95% CI: 33.7, 50.3]) versus 24% (23.7% [95% CI: 16.9, 31.1]) for docetaxel (HR 0.59, 96.8% CI: 0.43, 0.81; p=0.0002). Median OS was 9.2 months versus 6 months for nivolumab and docetaxel, respectively. Nivolumab also demonstrated consistent, statistically significant and clinically meaningful improvements across secondary endpoints, ORR and PFS, versus docetaxel in patients with previously treated advanced SQ NSCLC. Survival benefit was observed independent of PD-L1 expression across all pre-specified expression levels (1%, 5% and 10%).

The safety profile of nivolumab in CheckMate –017 was consistent with prior studies and favorable versus docetaxel. Treatment-related adverse events (AEs) occurred less frequently with nivolumab (any grade, 58%; grade 3–4, 6.9%; no grade 5 events) than docetaxel (any grade, 86%; grade 3–5, 55%; grade 5, 2.3%), including both hematology and non-hematology toxicities. Treatment-related AEs led to discontinuation in 3.1% of patients in the nivolumab arm compared to 10.1% for docetaxel.

With the EU approval of nivolumab, patients in Europe have for the first time in more than ten years access to an entirely new treatment modality for advanced squamous non-small cell lung cancer, which has the potential to replace the current standard of care.

 Prague Medical Report / Vol. 116 (2015) Suppl., p. 136–138

Boehringer Ingelheim Satellite Symposium 15th CELCC, Prague, Czech Republic November 29, 2015 Overall survival in NSCLC: translating evidence into practice

Summary

The EGFR mutant lung adenocarcinoma is a distinct entity. On basis of the results of IPASS and EURTAC trials both first generation EGFR TKIs, gefitinib and erlotinib have significant PFS benefit compared to chemotherapies in first line, however in overall survival (OS) none of them shown significant survival difference.

Afatinib is a second generation EGFR TKI with blockade of the whole ErbB family, including EGFR, HER2 and HER4 receptors. Its bindings to the ErbB dimers are covalent and irreversible with high receptor affinities. In LUX-Lung 3 registration trial the median progression-free survival (PFS) with afatinib was 13.6 vs 6.9 months with cisplatin plus pemetrexed (HR: 0.47, p<0.0001) in patients with common EGFR mutations (Del19/L858R), in first line treatment of EGFR mutant lung adenocarcinoma. The safety profile of afatinib was well defined and manageable. The two most important AEs were rash and diarrhoea.

The starting and maintaining dose of afatinib is 40mg per day. In absence of its drug-related events (> grade 1 within the first three weeks of treatment) the daily dose can be increased up to 50mg per day. In case of ≥ grade 3 AEs the daily dose should be decreased to 30 or 20mg per day. The dose titration with afatinib is quite easy due to its four presentation forms (50mg, 40mg, 30mg and 20mg p.o., film-coated tablets). Due to the lack of its CYP-related interactions afatinib may offer a number of theoretical advantages in concomitant drug uses.

According to the results of LUX-Lung 3 and 6, afatinib is the first EGFR TKI having significant OS benefit in first line treatment of EGFR mutant lung adenocarcinoma, compared to chemotherapy in common EGFR mutations (exploratory combined analysis from LUX-Lung 3 and LUX-Lung 6: 27.3 month vs 24.3 months, p=0.0374). However there is no significant difference in OS of patients with L858R mutations, individually, or in exploratory combined analysis, but in del19 patients afatinib significantly improved OS vs chemotherapy (LUX-Lung 3: median 33.3 vs 21.1 months, HR=0.54, p=0.0015, LUX-Lung 6: median 31.4 vs 18.4 months, HR=0.64, p=0.0229) Treatments beyond first line were balanced between the two treatment arms. These OS data with afatinib mean a great progress compared to first generation TKIs. The del19 and L858R patients are two distinct populations and should be studied separately in the future. First-

line afatinib should be the standard of care for EGFR del19 patients and remains a treatment option for EGFR L858R patients.

LUX-Lung 2, 3 and 6 provide the largest prospective dataset in patients with uncommon EGFR mutations (n=75). There is a high heterogeneity within the subgroup with uncommon EGFR mutations. Afatinib was active in NSCLC tumours that harboured certain types of uncommon EGFR mutations, especially T790M+L858R, G719X, L861Q, and S768I that are known to be less responsive to reversible EGFR TKIs. Activity was in the range of efficacy observed with afatinib in common EGFR mutations. Clinical benefit was lower in patients with de novo T790M and exon 20 insertion mutations. These data could help inform clinical decisions for patients with NSCLC harbouring uncommon EGFR mutations.
In patients with brain metastases, results indicate a trend for improved PFS with afatinib compared with chemotherapy (median 11.1 vs 5.4 months, HR=0.52, *P*=0.13) Prospective evaluation of optimal timing and sequence of systemic therapy and cranial radiotherapy in patients with oncogene-dependent lung cancers and asymptomatic brain metastases is warranted.

Treatment of advanced lung adenocarcinoma after first line chemotherapy has no significant improvement in overall survival reported in second line in nearly a decade (median OS is less than 1 year). Till 2014 basically three therapies were used in second and later lines: pemetrexed, erlotinib and docetaxel. In 2014 on the basis of the results of LUME-Lung 1 clinical trial a new combination was registered for the treatment of lung andenocarcinoma after first line chemotherapy: nintedanib plus docetaxel.

Nintedanib is a triple angiokinase inhibitor, targeting VEGF, PDGF and FGF receptors. Due to its unique mechanism of action both endothelial cells (expressing VEGFR and FGFR), smooth muscle cells (FGFR and and PDGFR) and pericytes (PDGFR) are also being inhibited during the treatment. Nintedanib has no drug-drug interaction liability via CYP-450, and demonstrated manageable safety profile in combination with commonly used chemotherapies like docetaxel, pemetrexed, platinum doublet or cisplatin/gemcitabine. The starting and maintaining dose of nintedanib is 2x200mg p.o./day.

In LUME-Lung 2 trial nintedanib in combination with pemetrexed was studied in non-squamous NSCLC compared to pemetrexed after first line chemotherapy, however a preplanned futility analysis by the Data Monitoring Committee recommended the premature termination of the study. Considering the fact, that there were no safety concerns, the study became unblended, and follow-up continued per protocol for enrolled patients. Primary endpoint was met despite being halted prematurely, showing a clear PFS benefit for the combination arm. LUME-Lung 2 did not lead to registration.

In LUME-Lung 1 registration trial nintedanib in combination with docetaxel was studied in squamous and non-squamous NSCLC after first line chemotherapy compared to docetaxel. LUME-Lung 1 met its primary endpoint and demonstrated

significant prolongation of PFS regardless of histology (mPFS 3.4 vs 2.7 months, HR=0.79, 95% CI: 0.68–0.92, p=0.0019), and significant improvement in OS (key secondary endpoint) in patients with adenocarcinoma (mOS 12.6 vs 10.3 months, HR=0.83, 95% CI: 0.70–0.99, p=0.0359). Post study treatments were balanced between the two arms. Commonly reported AEs included gastrointestinal events and reversible liver enzyme elevations. There was no compromise to patients' self-reported QoL.

There was an even more convincing difference in the median overall survival data in patients, who progressed in less than 9 months after start of first line therapy (mOS 10.9 vs 7.9 months, HR = 0.75, 95% CI: 0.60–0.92, p=0.0073).

LUME-Lung 1 is a milestone in the treatment of lung adenocarcinoma, because it was the first prospective clinical trial showing significant OS benefit for a therapy compared to docetaxel having median overall survival beyond 1 year for nintedanib plus docetaxel combination.

Authors' Index

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