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First line gefitinib versus first line chemotherapy by carboplatin plus paclitaxel in non-small cell lung cancer patients with EGFR mutations: a phase III study (002) by North East Japan Gefitinib Study Group

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Background: Based on our promising results of phase II studies estimating gefitinib in non-small cell lung cancer (NSCLC) patients (pts) with sensitive EGFR mutations (JCO 2006, BJC 2006), this multicenter phase III trial compared progression free survival (PFS) of first line gefitinib versus first line chemotherapy in EGFR mutation positive pts with stage IIIB/IV NSCLC.
Materials and Methods: PNA-LNA PCR clamp test, which had been developed and validated by us (Cancer Res 2005, Cancer Sci 2007), was employed to detect EGFR mutations using cytological samples or histological samples. Pts having sensitive EGFR mutations, measurable site(s), ECOG PS 0−1, age of 20−75 years, and no prior chemotherapy were randomized (1:1 ratio; balanced for institution, sex, and stage) to receive arm A: gefitinib (250 mg/day) orally, or arms B: carboplatin (CBDCA) AUC 6 and paclitaxel (TXL) 200 mg/m^2 in 21-day cycles until disease progression. The primary endpoint was PFS, and the sample size was calculated to be 320 in total ($\alpha = 5\%$, power = 80%) to confirm the superiority of arm A (hazard ratio = 0.69). Per protocol, an interim analysis to investigate PFS was performed 4 months after 200 pts entered to this study.
Results: From April 2006 to December 2008, 200 pts were enrolled, and 198 pts except for 2 ineligible pts were investigated (arm A = 98; arm B = 100). Their characteristics were well balanced between arms: median age 63/63 years; 63%/64% female; 79%/75% Stage IV; 90%/96% adenocarcinoma, respectively. Significantly higher response rate was obtained in arm A (74.5% vs. 29.0%, Fisher's exact test, $P < 0.001$). An interim analysis clearly showed significantly longer PFS by 1st gefitinib than by 1st CBDCA+TXL (10.4 vs. 5.5 months, hazard ratio = 0.357, Logrank test: $p < 0.001$). There were several differences in toxicities between arms (Grade 4 neutropenia: 1% vs. 33%, Grade 3−4 liver dysfunction: 25% vs. 1%, Grade 3 neuropathy: 0% vs. 5%, respectively, $p < 0.01$). There was one toxic death due to interstitial lung disease in arm A.
Conclusion: This is the first phase III study to compare first-line gefitinib with first-line chemotherapy for advanced NSCLC patients harboring EGFR mutations, and the first-line gefitinib to NSCLC patients with EGFR mutations is validated. Therefore, the independent safety committee decided to stop accumulation of patients up to May, 2009. Further analyses including overall survival will be presented.

PP113

Cetuximab with irinotecan/folinic acid/5-FU as first-line treatment inadvanced gastric cancer: A prospective multi-center phase II study and its molecular markers of the Arbeitsgemeinschaft Internistische Onkologie

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Background: Cetuximab combined with irinotecan/folinic acid/5-FU (IF) based therapies demonstrated high efficacy in human metastatic colorectal cancer. In advanced gastric cancer, IF may be an effective and well tolerated alternative to cisplatin-based regimens. We therefore conducted a non-randomized phase II AIO study to evaluate tolerability and efficacy of cetuximab with IF as first-line treatment in patients (pts) with advanced or metastatic gastric cancer. In parallel, we analysed mutation status of KRAS, BRAF, PIK3CA and expression levels of lymphangiogenic markers.
Materials and Methods: Pts were eligible with previously untreated adenocarcinoma of the stomach or oesophagogastric junction, ECOG performance <2, measurable lesions and adequate organ functions. Pts received weekly cetuximab (first 400, subsequently 250 mg/m^2) combined with chemotherapy of irinotecan (80 mg/m^2) + 24 hour continuous infusion of sodium folinic acid (200 mg/m^2) and 5-FU (1500 mg/m^2). Treatment was continued until tumor progression and assessments were performed every 2nd cycle. KRAS, BRAF and PIK3CA were analysed by HRM-PCR and sequencing. EGFR, VEGF-C and VEGF-D were determined by immunohistochemistry (IHC) and correlated with stage, response and survival.
Results: From Aug 2006 to Sep 2007, 49 pts were enrolled: 71% were males, median age was 63 years (33−77), 69% and 31% of pts had gastric and esophagogastric junction carcinomas. Median treatment duration was 15.2 weeks (range 1.1–69.1). Among 48 pts evaluable for response, overall response rate was 43% (CR 4%/PR 39%) and tumour control rate was 77%. Median progression-free and overall survival times were 8.5 months (36.6 weeks; 95% CI 30.1; 48.1) and 16.6 months (71.1 weeks; 95% CI 50; 93.4), respectively. Translational tests of 39 pts significantly correlated IHC expression levels of VEGF-C with PD during study ($p = 0.013$). EGFR expression was associated (0.038) with higher tumor stages. Again, low EGFR levels significantly correlated with non-response ($p = 0.035$). Three of 34 analysed pts had KRAS mutations, 4 were positive for BRAF mutation and 1 pts showed a PIK3CA mutation. None of all pts had concomitant mutations. KRAS mutated pts were all non-responders.
Conclusion: Cetuximab plus IF was well tolerated and encouraging survival data were observed. Even only exploratively analysed, KRAS, BRAF, PIK3CA mutations are rarely seen. Thus, cetuximab combined with chemotherapy in advanced gastric cancer is under further investigation in an ongoing phase III trial.

PP110

Effect of fixative and sample age on success rate in gene profiling studies

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Background: Using RNA extracted from fixed tissue for gene expression studies is technically feasible in the case of formalin fixed paraffin-embedded tissues (FFPE), but is still a problem with other types of fixatives, eg Bouin (BFPE). Fixation induces not only degradation of RNA, but also chemical modifications which interferes with retrotranscription reactions hampering this way gene expression studies. We have attempted a chemical demodification of FFPE and BFPE samples up to 30 years old.
Materials and Methods: Total RNA was extracted with Trizol® (MCF7 cells) or with Recover All® from Ambion (FFPE and BFPE), subjected to chemical demodification and analyzed for 1) amplification yield using a whole transcriptome amplification system; 2) length of cDNA products; 3) performance of qPCR as surrogate technical endpoints for feasibility of gene profiling.
Results: Fixation with Bouin of isolated RNA resulted into worse performances for all the three tested endopoints. Chemical demodification by heat treatment was effective in the case of formalin-fixed RNA, but not of Bouin-fixed RNA whose amplification yields were around 10% of controls (unfixed degraded RNA) and reached 50% of controls after treatment. Size of cDNA obtained from formalin-fixed RNA was 150nt, and was not modified by treatment, while the size cDNA from Bouin-fixed RNA was under 60 and slightly improved with heat. After Bouin fixation Ct values were 12 units higher compared to formalin and the difference dropped to 3 Ct units after treatment.
However, the same heat-treatment applied to 20 RNAs from 30 years-old BFPE blocks did not improve cDNA yields in about 70% of samples. Affymetrix gene profiles gave very low present calls (mean value of 5.26±1.29). FFPE samples had higher present call percentages (16.36–18.30). Among DE genes between ER+ and ER− samples, only GATA3 and SCUBE 2 were observed but with non significant p values ($p < 0.10$, $p < 0.92$). Similarly gene profiling for 502 cancer-related mRNAs with the Illumina DASL assay gave biologically meaningful results in the case of FFPE, but not in the case of BFPE samples despite the heat treatment.
Conclusion: Bouin induces stronger chemical modifications of RNA than formalin reducing the success rate of gene profiling studies. Modifications can be partially reversed by heat treatment but only in freshly-fixed samples. Differently from FFPE, gene profiling studies on archival samples (20 years old) are not feasible for BFPE.

PP137

Immunohistochemistry in cancer medicine: our experience in Cameroun

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Background: Immunohistochemistry (IHC) refers to the process of localizing proteins in cells of a tissue section exploiting the principle of antibodies binding specifcally to antigens in biological tissues. It takes its name from the roots "immuno", in reference to antibodies used in the procedure, and "histo", meaning tissue (c.f. immunocytochemistry). In developed countries, immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specifc molecular markers are characteristic of particular cellular events

such as proliferation or cell death (apoptosis). IHC is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. Visualising an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore, (immunofluorescence).

Materials and Methods: In Cameroon, the above techniques are not available. We established a collaboration with a few laboratories in developed countries; mainly in Switzerland and France. Paraffin blocks of diagnosed cancers have been sent abroad since January 2000. Immunohistochemistry has been performed free of charge.

Results: A series of 103 cancer patients was included in this study. There were 40 malignant lymphomas, 20 cases of early stage Kaposi's sarcoma, 20 soft tissue tumours, 15 breast cancers, 5 brain tumours, 3 urethral cancers. There were no HER-2 cases and and no sentinel node biopsies were performed in this series. The delay of sending specimens and receiving results via internet was one month. Sending specimens and receiving results via the Internet was one month. Apart from classifying and clarifying their diagnosis, none of these patients received specifc treatment after their immunohistochemistry result.

Conclusion: Even performed free of charge, immunohistochemistry does not permit specifc treatment for Cameroonian cancer patients because they can't pay for drugs such as monoclonal antibodies. We hope the situation may change in the future.

PP72

Demonstration of dose-dependent target inhibition using a quantitative biomarker assay for SB939, a potent, orally active HDAC inhibitor, in a Phase I clinical study in solid tumors

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Background: SB939 is an orally active, hydroxamic acid based HDAC inhibitor with very favorable pharmaceutical, pharmacokinetic and pharmacodynamic properties that is currently in Phase I clinical trials. A sensitive Western blot assay to quantitate histone H3 acetylation (acH3) on lysine 9 and 14 was developed to measure target efficacy of SB939.

Materials and Methods: The assay was validated in normal or HCT-116 tumor bearing mice treated with 125 mg/kg t.i.w. SB939. Samples were collected on day 1 and day 15 pre-dose, 3 h and 24 h after dosing, corresponding to the time points of sample collection in the Phase I clinical study. To test the linearity of the acH3 signal mice were treated with doses from 25 mg/kg to 200 mg/kg for 3h. Biomarker analysis of Phase I studies were performed on patients PBMCs, isolated with CPT-tubes. Samples were snap-frozen and lysed in the presence of a HAT inhibitor.

Results: The Western blot assay was sensitive enough to detect as little as 22 ng/ml of SB939 in cultured cancer cells, or 44 ng/ml in PBMCs of healthy volunteers. Signals could be detected in liver, spleen, PBMCs as well as tumor tissue sampled from normal or HCT-116 tumor bearing mice treated with 125 mg/kg SB939 orally t.i.w. The highest signals were detected 3 h post-dose, with no background for vehicle treated mice. AcH3 signals were lower on day 15 than on day 1 in all normal tissues, but increased in tumor tissue, where also the highest absolute acH3 levels were detected. The increase in signal was linear in all tissues tested, except in tumor tissue, which showed maximal saturation already at doses of 100 mg/kg. In PBMC samples from SB939-treated patients with advanced solid malignancies, a dose-dependent increase in relative acetylation values was observed: from 0.8 to 1 to 1.5 for patients treated with 20 mg, 40 mg and 60 mg respectively, correlating well with the proportional increase of SB939 in the plasma. Interestingly, patients which stayed on treatment for the longest time without disease progression showed a sustained enhanced signal on day 15.

Conclusion: Using a sensitive and quantitative Western blot assay, we demonstrate that SB939 induces a dose-dependent increase in acH3 levels in normal and tumor tissues in animal models, as well as in PBMCs from patients with solid tumors in a Phase I trial. Furthermore, a prolonged effect on the acH3 signal on d15 could be indicative for response to SB939 treatment and warrants further investigation.

PP32

A unified approach to define incidence of acute kidney injury (AKI) with serum creatinine as biomarker

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Background: Acute kidney injury (AKI) is a life threatening complex disease associated with mortality, morbidity and length of stay in hospital [Gottlieb SS, 2002]. SCr which is currently a main biomarker for AKI, rise normally in 24−72 hours after the inflammation occurred. Constant and slow rate of production of SCr, among cancer inpatients makes it less reliable for AKI classification. This complexity guides our research in the area of classification and modeling to overcome these challenges. We hypothesize that the rate (gradient change) in the SCr as biomarker will better predict the acute renal disease than simple difference

Materials and Methods: To delineate the extent of AKI using unified criteria, we examined the medical records of 5013 patients admitted to MD Anderson Cancer Center for three months. We run random intercept regression model to estimate the AKI for different baseline SCr. We estimated equation for critical SCr values as: $SCr(crit) = SCr(baseline)^{0.95} * \{[3 - \exp(-t/2SCr^{1.2})]/2\}$, where SCr(crit) is critical SCr level (above which AKI is predicted) in that point in time with reference to the baseline SCr at the first observation time t = 0 days. We internally validated the definition and results are in conformity with the AKIN criteria.

Results: Proposed AKI criteria based on gradient change in SCr performs batter in validation and regression analysis. Sensitivity and specificity remained 93% and 83% respectively. We observed minimal false rejection and improved detection of AKI, with even smaller changes in SCr. AKI is highly associated with length of stay (OR: 2.25; 95% CI: 1.85−2.74), ICU admission (OR: 2.7; 95% CI: 2.0−3.7), PACU admission (OR: 5.0; 95% CI: 3.7−7.0), BMT LLM (OR: 2.59; 95% CI: 2.0−2.74), Med Oncology (OR: 2.8;; 95% CI 2.2−3.6), Surgical Oncology (OR: 2.1; 95% CI 1.4−3.2), Pain Symptom (OR: 2.5; 95% CI 1.6−3.8). An odd of having AKI was fivefold increased for the patients who eventually died in hospital with cancer, in contrast to those remained alive after controlling for other covariates. Similarly, cancer patients who were diabetes mellitus (DM) had about 40% increased risk of odds of AKI compared with those without DM with 95% CI being from 15% to 70% with all P-values <0.0001.

Conclusion: We conclude that by estimating the unified equation for AKI based on the gradient change in SCr, it improves the specificity and early prediction of AKI. Even small serum creatinine SCr increase is independently associated with increased risk of mortality [Smith GL, 2003].

PP120

Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis in resected non-small cell lung cancer

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Background: The impact of host immunity on outcome in lung cancer is controversial. We studied the clinical significance of lymphoid infiltration in resected non-small cell lung cancer (NSCLC) specimens.

Materials and Methods: We analysed 196 NSCLC cases for tumour- and stroma-infiltrating CD3+, CD8+ and FOXP3+ cells by immunohistochemistry to assess the relative proportions of total, cytotoxic and regulatory T-lymphocytes (Tregs), respectively. Enumeration of immune subsets was performed using a novel automated image analysis algorithm. To test the influence of lymphocyte distribution pattern on survival, the data were divided into two groups, based on whether the ratio of intratumoral to intrastromal lymphocyte count was greater or less than the median value.

Results: A high CD8+ tumour/stroma infiltration ratio was associated with an increased overall survival (OS) compared to a low tumour/stroma infiltration ratio (P < 0.001). Conversely, there was an inverse association between survival and tumour islet FOXP3+ Treg density (P < 0.001). Multivariate analysis revealed that CD8+ tumour/stroma ratio emerged as an independent predictor of survival (HR 0.38; 95% CI 0.24−0.61, P < 0.001). The combination of a high tumour islet/stroma CD8+ ratio and low tumour islet/stroma FOXP3+ ratio showed the strongest prognostic effect, being associated with a 3 yr OS rate of 91% (HR 1.58; 95% CI 1.25−2.00, P < 0.001).

Conclusion: Microlocalization of infiltrating T-lymphocytes is a powerful predictor of outcome in surgically resected NSCLC. Immune-based