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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

Y. Minegishi[1], A. Inoue[2], K. Kobayashi[3], A. Gemma[1], M. Maemondo[4], S. Oizumi[5], Y. Saijo[6], S. Morita[7], K. Hagiwara[3], T. Nukiwa[2]. *[1]Nippon Medical School, Japan; [2]Tohoku University, Japan; [3]Saitama Medical University, Japan; [4]Miyagi Cancer Center, Japan; [5]Hokkaido University, Japan; [6]Hirosaki University, Japan; [7]Yokohama City University, Japan*

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.

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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

A. Mueller[1], S. Schwarz[1], S. Biesterfeld[1], S. Kanzler[1], T. Trarbach[2], T. Seufferlein[3], S. Kubicka[4], F. Lordick[5], P.R. Galle[1], M. Moehler[1]. *[1]University of Mainz, Germany; [2]University of Essen, Germany; [3]University of Ulm, Germany; [4]University of Hannover, Germany; [5]University of Heidelberg, Germany*

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.

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

V. Musella, P. Miodini, M. Callari, A. Carbone, M.G. Daidone, V. Cappelletti. *Fondazione IRCCS Istituto Nazionale Tumori, Italy*

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.

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Immunohistochemistry in cancer medicine: our experience in Cameroun

B. Nkegoum[1], V.J. Ama Moor[2], M.J. Atanga[3], T. Mbah[3]. *[1]University Hospital Center, Cameroon; [2]Erasmus Hospital, Belgium; [3]Universite Des Montagnes, Cameroon*

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