

Poster session 5: Erb receptors and signalling

P.501 EFFECT OF THE EGFR TYROSINE KINASE INHIBITOR GEFITINIB ON PROSTATE CANCER CELL LINES

J. Limpens, P. Van Duijn, C.M.A. De Ridder, S. Erkens, S. Reneman, I. Sprengers, F.H. Schröder, J. Trapman, W.M. Van Weerden
Erasmus MC, Rotterdam, The Netherlands

The selective EGFR tyrosine kinase inhibitor gefitinib ('Iressa', ZD1839) has shown a clear benefit as a second or third line monotherapy in 11–28% of the patients with NSCLC. Although EGFR is (over-)expressed in most prostate cancers (PC) and appears to play a role in tumor initiation and progression, preliminary results from PC phase II trials suggest limited activity of gefitinib as a monotherapy in hormone refractory PC (F.H. Schröder 2004; M. Moore 2002). In accordance, we found no tumorinhibitory effect of gefitinib (daily oral doses of 50, 100 and 200 mg/kg) in the orthotopic human PC-346C xenograft model, whether given to established tumors (>200 mm³) or directly after tumor inoculation.

To investigate whether the androgen-sensitive PC-346C cell line, used in this study, was inherently insensitive to gefitinib, we compared its *in vitro* sensitivity with that of three other human PC cell lines, LNCaP, PC-3 and DU-145, and with A431 cells, known to be very sensitive to gefitinib. All PC-cell lines were found to be markedly less sensitive (10–100 fold) than A431 cells (IC50: 0.1–0.3 μM). To gain more insight into the low sensitivity of PC-cells we screened a total of 19 PC cell lines and xenografts for activating mutations in exons 19 and 21 of the EGFR-TKI domain, known to harbour most EGFR mutations that confer sensitivity in NSCLC. We detected only wild type sequences and one silent mutation, suggesting that activating EGFR mutations are rare in PC. We also started experiments to investigate the effects of gefitinib on downstream targets of EGFR. Our preliminary findings indicate that gefitinib effectively abrogated EGFR-phosphorylation in all cell lines. However, whereas it also effectively blocked AKT phosphorylation in sensitive A431 cells, it failed to substantially decrease the AKT activity in 3 PC cell lines having a non-functional Pten (PC-346C, LNCaP and PC-3). This may be relevant since Pten loss and subsequent persistent activity of AKT is a frequent event in PC. In conclusion, PC cells appear to have a low sensitivity to gefitinib. Possible explanations include (1) the lack of activating EGFR-mutations in PC and (2) the frequent persistent activity of downstream EGFR targets (e.g. by Pten-loss) counteracting antitumor action of gefitinib in PC.

P.502 GEFITINIB AFFECTS THE ACTIVITY OF THE CELLULAR TOPOISOMERASE I AND MODULATES THE RESPONSE OF PC3 PROSTATE CARCINOMA CELLS TO ETOPOSIDE

D. Bobilev¹, S. Ariad¹, K. Regev², E. Priel²
¹Soroka Medical Center, Beer-Sheva, Israel; ²Ben-Gurion University, Beer-Sheva, Israel

Gefitinib (Iressa, ZD1839) is a known epidermal growth factor receptor (EGFR)-tyrosine kinase (TK) inhibitor that demonstrates antitumor activity alone or in combination with other drugs. Although, gefitinib is a specific EGFR-TK inhibitor, accumulating data suggest that no clear relationship exists between the expression level of EGFR and the tumor response to gefitinib. Therefore, it is possible that gefitinib exerts its antitumor activities by also affecting other essential cellular targets. In previous studies, we showed that certain tyrphostin derivatives, known protein TK antagonists, act as potent inhibitors of DNA topoisomerase I (topoI) *in vitro* and *in vivo*. In the present study we investigated the effect of gefitinib on topoI activities *in vitro* and *in vivo* in prostate carcinoma cells (PC3). A significant decrease in topoI DNA relaxation activity was observed in gefitinib treated cells. This reduction was not due to a decrease in the level of topoI protein. However, the activity of topoI, purified or derived from nuclear extracts of untreated PC3 cells, was not affected by the addition of gefitinib to the reaction mixture. These data suggest that the decrease in topoI activity, in gefitinib treated PC cells, is probably due to, yet unclear, posttranslational modifications of the topoI protein. To investigate the influence of a combined treatment with gefitinib and topo inhibitors on the growth of PC3 cells, we chose etoposide, a topoII inhibitor as a conventional anticancer drug. An additive inhibitory effect on the growth of PC3 cells was observed when a combined treatment with gefitinib and etoposide was used. To elucidate the mechanism that leads to this additive effect, we examined the influence of these drugs alone or in combination, at different concentrations and intervals, on the cell cycle progression of PC3 cells. Gefitinib alone caused cell cycle arrest at G1 and etoposide at G2, while the combined treatment arrests the cell cycle at S-phase, in a dose and time dependent manner. Examination of the effect of gefitinib and etoposide on topoI activity in PC3 cells revealed that treatment with gefitinib alone decreased the activity, while etoposide alone enhanced the activity of topoI, and the combination of these drugs prevented the inhibition of this enzyme observed by gefitinib treatment alone.

The results of this study suggest that gefitinib in PC3 cells exhibited its anticancer properties not only by its anti-EGFR activity but also by affecting the activity of an essential nuclear enzyme such as topoI, and by modulating the effects of topo inhibitors such as etoposide. The arrest in the S-phase following the combined treatment with etoposide and gefitinib, suggests that the addition of a third compound that specifically acts in S-phase might be effective in the treatment of highly drug resistant tumors.

P.503 TRANSLATIONAL RESEARCH IN A PHASE II TRIAL OF GEFITINIB IN SECOND-LINE TREATMENT OF ADVANCED ESOPHAGEAL CANCER

M.L. Janmaat¹, M.I. Gallegos-Ruiz¹, J.A. Rodriguez¹, C. Van Groeningen¹, D. Richel², G. Giaccone¹
¹VU University Medical Center, Amsterdam, The Netherlands; ²AMC, Amsterdam, The Netherlands

Background: Gefitinib (IressaTM) is an orally available selective EGFR tyrosine-kinase inhibitor. This phase II trial evaluated the efficacy and safety of gefitinib second-line in patients with advanced esophageal cancer. Assessment of the molecular pathways involved in EGFR inhibition was performed on patient's material.

Methods: 38 esophageal cancer patients have been enrolled to receive gefitinib 500 mg/day until disease progression or withdrawal. Analyses of tumor material included EGFR, k-ras, and PI3K mutation analysis, and IHC expression analysis of EGFR, phosphorylated Akt and phosphorylated Erk.

Results: 38 patients were enrolled from February 2002 to February 2004. 37 patients received gefitinib and are fully assessable, of whom 25 were male and 12 were female. The assessment at 6 weeks determined a non-progression rate of 22%. Best response was: 1 PR, 7 SD, 24 PD, 6 NE. No EGFR and PI3K mutations were found in 20 patients so far assessed, but 1 patient with PD had a K-ras mutation. Preliminary data suggest that high EGFR and phospho-Erk expression is associated with higher disease control rates, whereas high phospho-Akt expression seems to correlate with PD. The responder was a female, with no mutations in EGFR, PI3K, or K-ras, and showing high expression of EGFR and phospho-Erk.

Conclusions: Gefitinib was well tolerated as second-line monotherapy in advanced esophageal cancer. One partial response was seen in a female, which showed high EGFR expression. Further analysis of material from this study is ongoing. Final evaluation of the biological material will be presented.

P.504 ERLOTINIB PLUS RADIOTHERAPY IN PATIENTS WITH LOCALLY ADVANCED RECTAL CANCER

C.H. Smorenburg, B. Van Triest, J.R.M. Van der Sijp, C.J. Van Groeningen, G. Giaccone
VU University Medical Center, Amsterdam, The Netherlands

Background: In patients with locally advanced rectal cancer (LARC), preoperative radiotherapy (RT) improves local control of disease. The majority of colorectal cancer overexpresses the epidermal growth factor receptor (EGFR), which is associated with worse prognosis. Preclinical and clinical studies demonstrate radiosensitization after EGFR blockade. This phase I study investigates the safety and efficacy of combining erlotinib (E), a selective inhibitor of EGFR tyrosine kinase, with RT for locally advanced (LARC) or recurrent rectal cancer.

Methods: Patients with LARC (T3 or T4 Nx M0) or any recurrent rectal cancer are treated with RT for 5.5 weeks (50.4 Gy in 28 fractions, day 8–45, 3D confirmation technique) in combination with E. Prior RT or chemotherapy for rectal cancer is not allowed. Tumors are staged using MRI of the pelvis and CT of thorax and abdomen. Treatment with E starts on day 1 in escalating doses of 50 mg once daily on day 1–28 (3 patients), 50 mg once daily on day 1–45 (3 patients) and 100 mg once daily on day 1–28 (1 patient). Surgical resection is performed 4–6 weeks after RT. Patients are enrolled according to a traditional phase I accrual design.

Results: So far, 6 patients with LARC and 1 patient with recurrent RC have been treated. No dose limiting toxicities (DLTs) nor any grade 3–4 toxicity were observed in the first 3 dose levels. Toxicity consisted of acneiform skin rash (grade 1: 3 patients, grade 2: 2 patients) and diarrhea (grade 1: 1 patient, grade 2: 1 patient). Four out of 5 patients had a radiological partial response. Pathological examination of the surgical specimen demonstrated downstaging in 3 out of 5 patients, without any pathological complete remission. Two patients are scheduled for surgery.

Conclusions: Preliminary data show that the preoperative combination of E and RT is feasible in patients with LARC or recurrent rectal cancer. Updated results will be presented at the meeting.

P.505 EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) TYROSINE KINASE INHIBITORS: IS DOSE RELEVANT?

L. Seymour, G. Goss
NCIC Clinical Trials Group, Kingston, Canada

Background: In non small cell lung cancer (NSCLC), the presence of EGFR mutation may predict for tumour shrinkage; the predictive value in terms of survival benefit is not known. Laboratory models demonstrate differential sensitivity of mutated vs. non-mutated cells, suggesting dose may be relevant for patients without mutation. In NCIC CTG BR.21 a significant survival benefit was seen for patients treated with daily erlotinib (erlot) 150 mg despite low response rates, suggesting a subset of patients may have a survival benefit in the absence of objective response. Recently, ISEL, a trial similar in design to BR.21, failed to demonstrate a survival benefit for patients treated with daily gefitinib (gefit). Although the earlier IDEAL trials did not demonstrate significant differences in response or progression free survival between 250 mg and 500 mg gefitinib, it remains unknown whether the failure of ISEL to demonstrate a survival benefit reflects patient selection, an inadequate

dose, or other reasons. NCIC CTG has conducted studies with both erlotinib and gefitinib at varying doses using the same centres, investigators and methods of toxicity assessment.

Methods: One published NCIC CTG phase I and 3 phase II studies of gefitinib or erlotinib were selected and common drug related toxicity rates compared across studies.

Results: See table.

N	Erlot: 150 mg		Gefit: 225–250 mg		Gefit: 400–500 mg		Gefit: 750–800 mg	
	gr: all	gr: 3–4	gr: all	gr: 3–4	gr: all	gr: 3–4	gr: all	gr: 3–4
diarrhea	58%	12%	60%	0%	53%	0%	69%	19%
stomatitis	24%	0%	10%	0%	9%	0%	17%	0%
rash	88%	3%	20%	0%	52%	0%	81%	3%
nail changes	12%	0%	0%	0%	4%	0%	14%	0%

Conclusions: Gefitinib at a dose of 750–800 mg appears comparable to erlotinib 150 mg in terms of expected toxicities. At these doses, objective responses were noted in the erlotinib study (chemotherapy naïve) but not in the gefitinib studies (heavily pretreated colorectal cancer) although minor responses were seen. Further studies exploring gefitinib dose response and comparing erlotinib and gefitinib at equitoxic doses are needed.

P.506 A SINGLE ARM, MULTICENTRE, OPEN-LABEL PHASE II STUDY OF ORALLY ADMINISTERED LAPATINIB (GW572016) AS SINGLE-AGENT, SECOND-LINE TREATMENT OF PATIENTS WITH LOCALLY ADVANCED OR METASTATIC TRANSITIONAL CELL CARCINOMA

Marc-Oliver Grimm, Jean-Pascal Machiels, Christian Wülfing, Dirk Richel, Uwe Treiber, Marco de Groot, Philippe Beuzebob, John Farrell, Jessica Colman, Iman El-Hariry,

Lapatinib is an oral, dual tyrosine kinase inhibitor of ErbB1 and ErbB2 receptors. This multicentre, phase II trial assessed lapatinib as a single-agent in relapsed advanced urothelial tumours. Objective response rates (RECIST) and toxicity were primary endpoints.

Fifty-nine patients which had progressed on platinum-based therapy, received 1250 mg lapatinib once daily until disease progression or withdrawal. Safety and efficacy assessments (independent review) were carried out at 4- and 8-week intervals, respectively. Patients were also assessed at withdrawal, and followed every two months until death.

Mean age was 62.5 years. Most patients (78 %) had visceral metastases. Except one, all patients had confirmed expression of ErbB1 and/or ErbB2 (1+, 2+ or 3+ by immuno-histochemistry). There was no complete response. One patient (2%) had a partial response according to independent review and two patients (3%) had a partial response according to investigator evaluation. By independent review 18 subjects (31%) had stable disease for at least 8 weeks. Median time to progression was 8.6 weeks. Median overall survival time was 17.8 weeks. WHO Grade 3 or 4 toxicity occurred in 21 (36%) of patients. Overall toxicity was mild with diarrhoea 23 (39%), rash 16 (27%), nausea 16 (27%), vomiting 13 (22%), asthenia 7 (12%), and fatigue 6 (10%) being most frequent. There was no relationship between ErbB1 or ErbB2 tumour expression and response.

Conclusion: Second-line treatment with single-agent lapatinib had minimal activity in an unselected population and was generally well-tolerated in this patient population.

P.507 INHIBITION OF BOTH EGFR AND HER2 INCREASES THE SENSITIVITY TO DOCETAXEL IN HUMAN OVARIAN CANCER CELL LINES

M.N.A. Bijman, M.P.A. Van Berkel, M.L. Janmaat, M.C.A. Duyndam, E. Boven VU University Medical Center, Amsterdam, The Netherlands

Possible targets in ovarian cancer to be explored for therapeutic usefulness are members of the Human Epidermal growth factor Receptor (HER) family. Recombinant human monoclonal antibodies (mAbs) directed against EGFR (Cetuximab) and HER2 (Herceptin) are known to inhibit growth of EGFR- or HER2-overexpressing cells. Omnitarg is a new HER2-targeting antibody that is potentially effective in tumor types, not overexpressing HER2. Docetaxel, a member of the taxane family, is active in ovarian cancer. We studied the possible influence of EGFR and HER2 inhibition on the antitumor effects of docetaxel in this type of disease. A panel of five human ovarian cancer cell lines was characterized for expression of HER family members by Western blot. OVCAR-3 (moderate EGFR, low HER2, moderate HER3), IGROV-1 (low EGFR, moderate HER2, moderate HER3) and SKOV-3 (high EGFR, high HER2, no HER3) were selected for further experiments. From the

MTT assay it was evident that the docetaxel-induced antiproliferative effects (1-h exposure, IC50) could be increased by a 2-h pretreatment with 5 mg/ml Cetuximab (relative gain in cell growth inhibition +22% in OVCAR-3, +13% in IGROV-1, +18% in SKOV-3), 20 nM Herceptin (+6%, +10%, +18%) or 20 mg/ml Omnitarg (+23%, +14%, +9%). Antiproliferative effects of docetaxel further increased upon pretreatment with Cetuximab combined with Herceptin (+22%, +24%, +25%) or Omnitarg (+19%, +31%, +34%). The caspase-3 activity assay, as a measure of apoptosis, reflected the data of the MTT assay: docetaxel-induced caspase-3 activity was further increased by pretreatment with Cetuximab combined with Herceptin or Omnitarg resulting in a relative gain of, respectively, +32% and +31% in OVCAR-3, +33% and +60% in IGROV-1, +17% and +29% in SKOV-3. To further examine the molecular consequences of EGFR/HER2 inhibition, cells were stimulated with EGF or Heregulin after they were treated or not with either of the mAbs or a combination. Upon stimulation with EGF, increased protein levels of phosphorylated EGFR and ERK1/2 in all three cell lines could almost completely be inhibited by Cetuximab, but were not affected by Herceptin or Omnitarg. Early immunoprecipitation studies in IGROV-1 and SKOV-3 revealed that EGF could induce HER2 phosphorylation, which could be inhibited by Omnitarg. In conclusion, 1) human ovarian cancer cells frequently express EGFR, HER2 and HER3, 2) pretreatment of OVCAR-3, IGROV-1 and SKOV-3 cells with a combination of Cetuximab plus either Herceptin or Omnitarg resulted in increased antitumor effects of docetaxel, 3) although Omnitarg could inhibit EGF-induced HER2 phosphorylation, it was not able to downregulate EGF-induced ERK1/2 phosphorylation. Current studies are running to reveal the importance of HER2 as the preferred dimerization partner of HER3 after stimulation with Heregulin.

P.508 CETUXIMAB PLUS CAPECITABINE (CAP) AND OXALIPLATIN (LOHP) AS SALVAGE TREATMENT FOR PATIENTS WITH METASTATIC COLORECTAL CANCER (CRC) RELAPSING AFTER COMBINATION CHEMOTHERAPY INCLUDING OXALIPLATIN, IRINOTECAN (CPT-11), AND 5-FLUOROURACIL (5-FU) OR CAPECITABINE

J. Souglakos, N. Androulakis, A. Kalykaki, Z. Saridakis, N. Vardakis, K. Kalbakis, S. Agelaki, L. Vamvakas, N. Kentepozidis, S. Giassas, D. Mavroudis, V. Georgoulas University General Hospital of Heraklion, Heraklion, Greece

Background: Cetuximab is an Ig1 monoclonal antibody targeting EGFR which has shown to be active in irinotecan-refractory metastatic CRC. This phase II trial evaluated the safety and efficacy of cetuximab combined with CAP and LOHP (XELOX) as salvage treatment for patients with metastatic CRC.

Methods: Patients with metastatic CRC who had progressed on prior treatment including CPT-11, LOHP, 5-FU and CAP, with ECOG PS ≤ 2 were enrolled into the study. Cetuximab was given at an initial dose of 400 mg/m², then 250 mg/m² iv weekly; LOHP was administered on d1 at the dose of 85 mg/m² and CAP on d1-7 at the dose of 2000 mg/m²/d in a two week cycle.

Results: Until December 2004 22 patients have been enrolled into the study. Median age was 65 years, and median PS (ECOG) was 1. For two patients it was 2nd line treatment while for the 20 it was 3rd. Sixty-seven cycles have been administered with a median of 3 cycles/patient. Twenty-one patients are evaluable for toxicity and 11 for response. No grade 3–4 hematologic toxicity has occurred while non-hematologic toxicity included grade 3 vomiting in 5% of the patients, grade 3 diarrhea in 5%, grade 3 fatigue in 5%, grade 2 neurotoxicity in 5% and grade 2 allergy in 9%. Among the 11 patients evaluable for response one experienced a PR and two SD. The median TTP was 2 months.

Conclusions: Salvage treatment with cetuximab plus XELOX of patients with metastatic CRC is safe and feasible. More data are needed in order to further evaluate its efficacy in patients with LOPH/5FU or Xeloda-resistant CRC.

P.509 A NOVEL MODIFIED CPG AGONIST OF TLR-9 INHIBITS EGF RECEPTOR SIGNALLING AND SYNERGISTICALLY ENHANCES ANTI-TUMOR ACTIVITY OF CETUXIMAB AND IRINOTECAN ERADICATING COLON CANCER XENOGRAFTS

G. Tortora¹, R. Bianco¹, R. Caputo¹, F. D'Armiento¹, D. Melisi¹, A.R. Bianco¹, S. De Placido¹, S. Agrawal², F. Ciardiello³, V. Damiano¹
¹University Federico II, Napoli, Italy; ²Hybridon Inc, Cambridge, Ma, Italy; ³Seconda Università di Napoli, Napoli, Italy

CpG dinucleotides act as agonists of Toll-like Receptor 9 (TLR9), inducing potent Th1-type immune responses, and have recently shown antitumor activity and enhancement of the effect of topoisomerase I inhibitor topotecan. Moreover, CpG oligos have shown ability to enhance the antibody-dependent cell-mediated cytotoxicity (ADCC) of monoclonal antibodies (MAbs) and clinical studies are ongoing to evaluate their cooperative activity with the MAb Rituximab. Recently, second-generation immunomodulatory CpG oligonucleotides (IMOs) containing 3'-3'-attached novel structures and synthetic immunomodulatory dinucleotides have been synthesized. An IMO has shown potent preclinical antitumor activity in a broad spectrum of tumor models, enhancing the activity of different cytotoxic agents, and is now undergoing a clinical evaluation in a phase II study in cancer patients.

In recent years, inhibition of EGF receptor (EGFR) expression by selective targeted agents, including the MAb Cetuximab or small molecules, has become a successful therapeutic strategy in different types of cancer. In particular, recent clinical studies have demonstrated a relevant activity of Cetuximab, alone or in combination with irinotecan, in colorectal cancer patients refractory to former chemotherapy regimens. We have hypothesized that CpG oligos may interfere with EGFR-related signalling. If this is the case, they could enhance the activity of a MAb, such as Cetuximab, with a mechanism additional to ADCC. Therefore, we have investigated the effect of the IMO, alone and in combination with Cetuximab, on the growth of human colon cancer xenografts and the expression of several proteins critical for cell proliferation, apoptosis and angiogenesis.

IMO exhibited significant antitumor activity in colon cancer xenografts that was accompanied by a marked inhibitory effect on the expression of EGFR, pMAPK, pAkt, bcl-2, COX-2 and VEGF. The combination of IMO with Cetuximab produced a potent and long lasting antitumor effect on tumor xenografts, together with a potent inhibition of the expression of the analyzed signalling proteins and of microvessels formation. Moreover, when IMO was added to Cetuximab plus the cytotoxic agent irinotecan, the regimen shown active in colorectal cancer patients, a dramatic antitumor effect was obtained, resulting in the eradication of the tumors in over 90% of mice, paralleled by inhibition of the above signalling proteins.

These results demonstrate for the first time an interference of the novel TLR9 agonist IMO with the EGFR-related signalling and a potent cooperative effect with Cetuximab and irinotecan. Since all these agents are under clinical evaluation, our study provides a strong rationale for the translation of this therapeutic strategy in patients affected by colorectal cancer.