556 POSTER

Post-radiation reaction includes induction of fluoropyrimidine metabolizing enzymes – a concept supporting enhancement of capecitabine anabolism regardless of timing administration

M. Kahle[1], L. Frencl[2], P. Holeckova[2]. [1]*Insitute of Experimental Medicine, Czech Academy of Science, Prague, Czech Republic;* [2]*Insitute of Radiation Oncology, Univ. Hospital Na Bulovce, Prague, Czech Republic*

Background: Chemoradiotherapy employing capecitabine became common though not standard treatment approach for rectal cancer as well as for other gastrointestinal tumors. The efficacy seems at least comparable to chemoradiotherapy with continuous infusion of 5-FU. However the plasma levels of main capecitabine anabolites (5-FU, F-dUMP) basically do not simulate a continuous infusion of 5-FU. Presumably the induction of flouropyrimidine anabolizing enzymes by radiation is responsible for enhanced capecitabine anabolism to active forms. This phenomenon is more decisive for the efficacy of chemoradiation with capecitabine than is the administration schedule. In a previous series of experiments we have proved the enhanced levels of (TP), thymidine kinase (TK), thymidine synthetase (TS) and dihydropyrimidine dehydrogenase (DPD) 24 hours and later after single radiation (200 cGy) in HeLa cells. The experiments were continued to define the development of cellular levels of TP, TK, TS, DPD 8–120 hours after radiation.
Material and Methods: HeLa cells were irradiated by a dose of 200 cGy followed by an array of assessments TP, TK, TS and DPD. A Western blot analysis was performed using specific commercially available antibodies. The time intervals between radiation and onset of increased enzyme concentration were established.
Results: The protein levels of TP, TS and DPD increase from 24 hrs. after the radiation. The increase was up to 5−6 fold and lasted for more than 96 hrs. after the radiation in the first series of experiments. In the following series the increase is much less apparent, not more than 2 fold, however lasts beyond 120 hours. A TP/DPD ratio may be roughly established, in a range of relative values 2−3 in the initial series of experiment and decreased to relative values 1−2 in the following series. Thus the domination of anabolism is much less apparent.
Conclusion: The protein assessments confirm a stable enhancement of fluoropyrimidine anabolism to active forms. The enhancement period far exceeds the intervals between single fractions of radiation and between single doses of capecitabine. The TP/DPD ratio confirms slight excess of anabolism over catabolism. The enhanced anabolism of capecitabine is presumably more important factor than any possible timing of administration related to radiation fractions. Favorable efficacy of capecitabine for chemoradiation does not consist in any simulation of continuous 5-FU infusion

557 POSTER

Viral markers as prognostic factors of cervical lesions progression

G. Anton[1], D. Socolov[2], C. Bleotu[3], A.C. Anton[2], M. Anton[4], C. Goia[5], A. Botezatu[5]. [1]*Stefan S. Nicolau Institute of Virology, Viral genetics, Bucharest, Romania;* [2]*UMF, Gynecology, Iassy, Romania;* [3]*Stefan S. Nicolau Institute of Virology, Genetics, Bucharest, Romania;* [4]*UMF, Genetics, Bucharest, Romania;* [5]*Stefan S. Nicolau Institute of Virology, Molecular biology, Bucharest, Romania*

The etiological role of high-risk HPV in preinvasive and invasive cervical cancer has been demonstrated by epidemiological and molecular studies. Taking into account this association, by using viral load and E6/E7 viral mRNA as clinical markers for progression, we try to identify subjects at risk for the development of cervical lesions. Case patients with ASCUS and LGSIL were selected from a cohort of 200 women enrolled in a study on HPV prevalence in different areas in Romania. Subjects were examined at 6 months interval and their cytological and colposcopy data and virological tests (HPV DNA type and load) were monitoring. HPV typing, viral mRNA levels and viral load were determined in cervical-brush specimens at base line and in the samples obtained at 6 month interval.
HPV genotypes were determined by a hybridization assay (Innolipa HPV) and viral load was quantified according to number of copies/ng of β globin. 43 patients (24−48 years) presenting specific viral types (16, 18 and 45 in single or mixed infections) were subjected to viral load testing and viral mRNA detection. As negative controls, cervical specimens from 8 patients without HPV infection and normal cytology were used.
From the 16 patients with ASCUS cytology, 4 subjects presented significant viral loads in both samples; only in one case, the viral load was higher in the second sample. 27 LGSIL subjects presented higher viral load than ASCUS patients (especially in patients with HPV 16 and 16 and18 mixed infections).
E6/E7 mRNA levels were higher in 69.76% cases which presented infection with HPV16 type (alone or coinfections). From this, only 3 cases were patients with ASCUS (significant or moderate viral load). In 2 of the 7 cases presenting LGSIL and HPV 18 infections, the viral load and viral mRNA were higher. Both cases came from a Romanian area were this HPV type is found with a higher incidence. Our results indicated that viral persistence, an important factor in cervical lesion evolution, is associated with higher viral loads and mRNA levels but the patients monitoring must be extended for a longer period of time and perhaps for other markers.

558 POSTER

Measurement of DPD and TS transcripts aimed to predict clinical benefit from fluoropyrimidines: confirmation of the trend in Russian colorectal cancer series and caution on the gene-referee.

E.N. Suspitsin[1], A.G. Iyevleva[1], K.G. Buslov[2], A.F. Erofeev[2], A.V. Togo[1], D.E. Matsko[3], V.P. Filimonenko[4], A.R. Prochukhanov[1], V.M. Moiseenko[4], E.N. Imyanitov[1]. [1]*N.N. Petrov Institute of Oncology, Group of Molecular Diagnostics, St-Petersburg, Russian Federation;* [2]*Pediatric Medical Academy, Department of Medical Genetics, St-Petersburg, Russian Federation;* [3]*N.N. Petrov Institute of Oncology, Department of Pathology, St-Petersburg, Russian Federation;* [4]*N.N. Petrov Institute of Oncology, Department of Biotherapy and Bone Marrow Transplantation, St-Petersburg, Russian Federation*

Background: Measurement of intratumoral expression of dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS) may have some value in predicting the response to fluoropyrimidine-containing therapy.
Materials and Methods: We attempted to validate this association in a series of Russian metastatic colorectal cancer cases. While replicating already published protocols, we unexpectedly found that the use of commonly utilized gene-referees, GAPDH and b-actin, may lead to artifacts due to pseudogene-driven amplification from the genomic DNA template. We have developed a real-time PCR protocol, which amplifies short PCR fragments thus allowing an efficient analysis of archival formalin-fixed paraffin-embedded tumor samples, and relies on SDHA as a gene-referee therefore avoiding an amplification from genomic DNA.
Results: Low content of DPD transcripts was observed in 13/20 (65%) patients with the disease control (tumor response or disease stabilization) as compared to only 3/9 (33%) subjects with progressive disease ($p = 0.11$). Despite the low number of patients, this association reached the level of statistical significance when similar analysis was done for TS expression (11/20 (55%) versus 1/9 (11%); $p = 0.03$).
Conclusions: Thus our data confirm that low DPD and TS expressors have higher chances for success of fluoropyrimidine-containing regimens.

559 POSTER

Small interfering RNA administration against alpha-fetoprotein inhibits proliferation and anti-apoptotic properties of hepatocellular carcinoma cell lines

N. Mitsuhashi[1], F. Kimura[2], H. Shimizu[2], H. Yoshidome[2], M. Ohtsuka[2], A. Kato[2], H. Yoshitomi[2], S. Nozawa[2], K. Furukawa[2], M. Miyazaki[2]. [1]*Chiba University Center for Frontier Medical Engineering, Medical Nanotechniques, Chiba, Japan;* [2]*Chiba University Graduate School of Medicine, General Surgery, Chiba, Japan*

Background: Hepatocellular carcinoma (HCC) is the 5th most commonest malignant tumorsworldwide. Its incidence is the highest in Southeast Asia and Africa, but the number of reported cases of HCC has been steadily increasing in the United States and Europe because of the spread of hepatitis C virus infection. Current options for the treatingment of this cancer consist of include surgical resection, orthotropic liver transplantation, transcatheter arterial embolization, chemotherapy, and percutaneous ablation therapy. Although early diagnosis and treatment improve survival, HCC is rarely cured after these therapies, because multicentric tumor development or intrahepatic metastasis results in frequent recurrence. Therefore, there is a continuing need for new therapeutic strategies are continuously required to impact improve outcome. On the other handAFP is one of the most important markers in the diagnosis of primary HCCand many other. More than a few Some authors reported AFP as an independent predictor of poor prognosis for HCC. Furthermore, other organ cancers expressing AFP showed aggressive characteristics. It has been demonstrated that AFP ishas been demonstrated to be involved in pleiotropic activities affecting the processes of cell differentiation, cell growth, apoptosis, cytokine production, immunosuppressionve activity, and tumorigenesis. These results have an important implication strongly imply that AFP may function as an HCC growth stimulator; thus, the suppression of AFP gene expression and its biological activities may become an attractive strategy for HCC.
Materials and Methods: We analyzed the correlation between serum AFP levels and clinicopathological findings in 37 HCC patients who received curative operation about correlation of serum AFP level and

clinicopathological findings. Next, we used human HCC cell lines to know estimatethe endogenous AFP secretion and response to exogenous AFP. We applied introduced AFP siRNA in HuH7 and HepG2 cells to examine whether it can could inhibit AFP secretion on HuH7 and HepG2 cells.
Results: Post-operative serum AFP correlates to both disease free and overall survival. Further, that value is an independent prognostic factor in HCC patients. Five HCC cell lines, including HepG2 and HuH7, secreted AFP. Human cord blood and AFP can make could induce HCC cells proliferatione on a dose-dependently in vitro manner. AFP inhibited apoptosis induced by 5-fluorouracil (5-FU) on in several cell lines. When we introduced AFP specific siRNA in HepG2 and HuH7, AFP mRNA and protein secretion were significantly inhibited. AFP siRNA could inhibited the proliferation of HCC lines significantly. Furthermore, AFP siRNA induced apoptosis on in these cells co-culture with 5-FU.
Conclusions: These results indicate that AFP inhibition of AFP by siRNA may be effective to in inhibiting the HCC progression.

Drug Development

Poster presentations (Thu, 27 Sep, 08:00−11:00)
Drug development

700 POSTER

Outcome of the Clinical Trials Directive on clinical cancer research in Europe: a 3-years'-follow-up analysis

M. Hartmann[1], F. Hartmann-Vareilles[2]. *[1]European Consulting & Contracting in Oncology, Clin Reg Affairs, Trier, Germany; [2]Academy of European Law, Section I: European Private Law, Trier, Germany*

Background: The implementation of the Clinical Trials Directive 2001/20/EC (CTD 2001/20) between 2004 and 2006 in each EU Member State marks a watershed in European clinical research. The Directive, which set-up a mandatory framework for clinical trial registration, ethical review and regulatory approval by national authorities, requires sponsors of commercial like non-commercial trials to respect GCP requirements and to follow a meticulous set-up, supervision and reporting scheme for any drug trial. Perceived as a particular obstacle for non-commercial trials due to rising administrative complexity and increased costs, investigators, sponsors and analysts have forecasted a sharp decline in clinical research. Nevertheless, no accurate data have been published so far allowing to quantify the impact of the CTD 2001/20 on clinical cancer research at EU level.
Methods: This study constitutes a follow-up analysis to results presented in November 2005 at ECCO 13 (Abstract 528), aimed to provide reliable figures on the impact of the CTD 2001/20 on clinical cancer research three years after its coming-into force on 1 May 2004. For this purpose, clinical trial application charts from European (EMEA) and national authorities in half a dozen relevant EU Member states were evaluated. Whenever possible, data for commercial and non-commercial clinical trials were tracked separately as well as figures for oncology and paediatric oncology studies. In addition, heads of coordinating ethics committees in the respective EU member states as well as cancer research associations were contacted in order to collect comparative data. Descriptive statistics were applied for data presentation. Standard methods were used to test for statistical significance of differences or means.
Results: The CTD 2001/20 formally changed the categorisation of clinical trials. Hence, the authorities have revised their monthly/annual reporting. As a result, the pre- and post-implementation statistics are not fully comparable. The most reliable data arise from Italy, where an official trial reporting system is in place since 2000. Like for Italy, data from other countries confirm a marked decline of academic clinical cancer research activity of around one-third, although the overall figures for the set-up of cancer trials remained rather stable between 2000 and 2006 with a short post-implementation nadir in each country. Paediatric oncology, much affected by the CTD 2001/20, may be revived by the Paediatrics Regulation, which came in force in January 2007.
Conclusion: The CTD 2001/20 has resulted into a drop of non-commercial clinical cancer research within the EU. Representing 25% of all clinical trials prior to implementation, actually only 18% of new trials are conducted by academic sponsors. Efforts of all stakeholders are necessary to prevent a lasting cutback of interest in, commitment to and funding for clinical research.

701 POSTER

Phase I study of oral LBH589 in advanced solid tumours and non-Hodgkin's lymphoma

H.M. Prince[1], D. George[2], A. Patnick[3], M. Mita[3], P. Atadja[4], M. Dugan[5], D. Butterfoss[5], K. Culver[5], H.S. Burris[6], J. Beck[7]. *[1]Peter MacCallum Cancer Centre, Haematology and Medical Oncology, Melbourne, Australia; [2]Duke University, Urologic Surgery, Durham, USA; [3]Institute for Drug Development, Cancer Therapy and Research, San Antonio, USA; [4]Novartis Institutes for Biomedical Research Inc, Oncology Clinical Development, Cambridge, USA; [5]Novartis Pharmaceuticals, Oncology Clinical Development, East Hanover, USA; [6]Sarah Cannon Cancer Center, Hematology/Oncology, Nashville, USA; [7]Johannes-Gutenberg-Universitat, Medical and Tumor Immunology, Mainz, Germany*

Background: LBH589 is a highly potent deacetylase inhibitor. We evaluated the safety, tolerability, and preliminary efficacy of oral LBH589 in a phase 1 study in patients with refractory advanced solid tumors or non-Hodgkin's lymphoma (NHL).
Material and Methods: LBH589 was given orally every Monday, Wednesday, and Friday (MWF) until there was disease progression or unacceptable toxicity. Histone acetylation (HA) was studied using Western blots on total cell lysates from peripheral blood lymphocytes. Plasma pharmacokinetic (PK) profiles were analyzed on days 1 and 15. Noncompartmental analysis was used to determine PK parameters derived from plasma concentration time curves. Thirty-two patients (pts) received either the initial dose level of 15 mg MWF weekly, dose-limiting toxicity (DLT) level of 30 mg MWF weekly, or maximum tolerated dose (MTD) of 20 mg MWF weekly for cutaneous T-cell lymphoma (CTCL), renal cell carcinoma (RCC), melanoma, rhabodomyosarcoma, mesothelioma, prostate, hepatic, colon, bladder, or other malignancies.
Results: Three dose-limiting toxicities (DLTs) were reported: G3 diarrhea and transient G4 thrombocytopenia in the 30 mg cohort and G3 fatigue in the 20 mg cohort. Anorexia, nausea, fatigue, diarrhea, and transient thrombocytopenia were the most common adverse events. Of 1057 ECGs analyzed, 1 patient in the 20 mg cohort had a QTcF >500 ms (503 ms), which was an isolated event after the first dose with no recurrence with continued LBH589 therapy. Mean change in QTcF from baseline was 2-fold increase in HA was seen in the 15 mg cohort, but HA increased in 50% of pts for 72 h postdose in the 20 mg and 30 mg cohorts. After dosing, LBH589 was rapidly absorbed in plasma (T_{max} 1.5 h) then decreased with a mean terminal half-life of 16 h. At doses between 15−30 mg, C_{max} and AUC increased linearly with an estimated bioavailability of 49%. Two CTCL pts had complete response and 4 CTCL pts attained partial response; stable disease was attained in 7 pts with CTCL, RCC, melanoma, mesothelioma, or parotic gland cancer; 15 pts progressed on treatment; 4 pts were not evaluable.
Conclusions: In the 20 mg oral MWF weekly cohort, LBH589 produced a sustained effect on HA in 50% of patients. No clinically significant effect on QtcF was seen. Objective evidence of tumor response was seen in CTCL patients.

702 POSTER

Phase I pharmacokinetic and pharmacodynamic study of once-weekly IV LBH589

S. Sharma[1], N.J. Vogelzang[2], J. Beck[3], A. Patnaik[4], M. Mita[4], M. Dugan[5], A. Hwang[5], K. Culver[5], P. Atadja[6], H.M. Prince[7]. *[1]Nevada Cancer Institute, Gastrointestinal Oncology, Las Vegas, USA; [2]Nevada Cancer Institute, Director, Las Vegas, USA; [3]Johannes-Gutenberg-Universitat, Medical and Tumor Immunology, Mainz, Germany; [4]Institute for Drug Development, Cancer Therapy and Research, San Antonio, USA; [5]Novartis Pharmaceuticals, Oncology Clinical Development, East Hanover, USA; [6]Novartis Institutes for Biomedical Research Inc., Oncology Clinical Development, Cambridge, USA; [7]Peter MacCallum Cancer Centre, Hematology, Melbourne, Australia*

Background: LBH589 is a highly potent deacetylase inhibitor. We tested the safety and tolerability of IV LBH589 once weekly for 3 of 4 weeks in patients with advanced solid tumors or lymphoma.
Material and Methods: LBH589 was given IV on days 1, 8, and 15 of a 28-day cycle. Histone acetylation (HA) was studied using Western blots on total cell lysates from peripheral blood lymphocytes (PBLs). Plasma pharmacokinetic (PK) profiles, derived from plasma concentration time curves, were analyzed on days 1 and 8. Noncompartmental analysis was used to determine PK parameters. Thirty-five patients (pts) were treated at 3 dose levels (10 mg/m^2, 15 mg/m^2, and 20 mg/m^2) for cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL), mesothelioma, and prostate, colon, or other malignancies.