Poster Discussion Presentations (Sun, 25 Sep, 11:00−12:00)

Genitourinary Malignancies − Other

7105 POSTER DISCUSSION

Belinostat in Combination With Carboplatin and Paclitaxel (BelCaP) for Treatment of Bladder Cancer − A Pharmacokinetic Study of Exposure to Belinostat and Its Metabolites

R.J. Jones[1], J. Tjørnelund[2], K.D. Erichsen[3], L. Sengeløv[4], J. De Bono[5]. [1]*Cancer Research UK Beatson Laboratories, Centre for Oncology and Applied Pharmacology, Glasgow, United Kingdom;* [2]*Topotarget, Clinical Pharmacology, Copenhagen, Denmark;* [3]*Topotarget, Medical Affairs, Copenhagen, Denmark;* [4]*Herlev Hospital, Department of Oncology, Copenhagen, Denmark;* [5]*Royal Marsden Hospital, Institute for Cancer Research, Sutton, United Kingdom*

Background: Belinostat (Bel, PXD101) is a class I and II Histone DeACetylase (HDAC) inhibitor. A single arm Ph II study was conducted to evaluate the safety and activity of Belinostat, Carboplatin and Paclitaxel (BelCaP) in patients (pts) with Transitional Cell Carcinoma of the Bladder (TCCB) (n = 15). A part of the study was a pharmacokinetic study of plasma exposure to Bel and its metabolites. The *in vitro* efficacy of belinostat and its metabolites were compared and related to plasma exposure in pts.
Materials and Methods: Pts with TCCB were treated with BelCaP every third week; Bel was given as a 1000 mg/m^2 30-min i.v. inf. on days 1−5 with P (175 mg/m^2) and subsequently Ca (AUC5) administered 2−3 hrs after Bel on day 3. The plasma exposure (AUC) of Bel and its metabolites were determined. The *in vitro* pharmacological effect of Bel and its five major metabolites: belinostat glucuronide (BelGlcU), 3-(Anilinosulfonyl)benzene carboxylic acid (3-ASBA), methylated belinostat (Metbel), belinostat amide (Belam) and belinostat acid (Belac) were examined in a HeLa HDAC enzyme inhibition assay (HDAC-i), in WST proliferation assays and in clonogenic assays (CA). Fold differences in exposure of metabolites and belinostat (10 pts on day 3) and fold differences in *in vitro* efficacy of belinostat and metabolites were compared.
Results: The exposure of each metabolite relative to Bel was evaluated. The increases (molar $AUC_{0-\infty}$) relative to Bel were 16- (BelGlcU), 3- (3-ASBA), 1- (Metbel), 1- (Belam) and 0.5-fold (Belac).
Bel metabolites did not inhibit HDAC-i activity or cell WST proliferation *in vitro*. In the CAs the IC_{50} for Bel were 0.4 to 1.3 μM. Three metabolites had weak effect relative to Bel. The fold increase in IC_{50} relative to Bel was: >65 (BelGlcU), >42 (Metbel) and >114 (Belam).
Conclusions: Five major human Bel metabolites (BelGlcU, 3-ASBA, Metbel, Belam and Belac) were identified in a Ph II study of BelCaP in pts with TCCB.
Bel metabolites were inactive in HDAC-i assays and in WST assays and had weak activity in CA. The metabolite with highest fold exposure compared to Bel was BelGlcU (16-fold), which was 65 fold less effective *in vitro* than Bel. The present study finds that Bel metabolites do not have significant biological effect at therapeutic relevant plasma exposure in cancer pts.

7106 POSTER DISCUSSION

The Proteasome-inhibitor Bortezomib is Active in Human Urothelial Cancer Cell Lines in Combination With the Tyrosine-kinase Inhibitor Sunitinib or Cisplatinum

U.M. Vogl[1], W. Berger[2], M. Bojic[1], W. Lamm[1], A. Haitel[3], G. Kramer[4], C.C. Zielinski[1], M. Schmidinger[1]. [1]*Medical University Vienna, Medicine I and Cancer Center Clinical Division of Oncology, Vienna, Austria;* [2]*Medical University Vienna, Medicine I and Cancer Center Institute of Cancer Research, Vienna, Austria;* [3]*Medical University Vienna, Department of Pathology, Vienna, Austria;* [4]*Medical University Vienna, Department of Urology, Vienna, Austria*

Background: The outcome of advanced muscle-invasive urothelial cell carcinoma (UCC) is generally modest. Cisplatinum-based chemotherapy is considered the standard of care, but novel strategies are urgently required. The proteasome-inhibitor bortezomib has shown activity in UCC in vitro and in vivo by inducing apoptosis through the tumour-necrosis-factor-related apoptosis-inducing-ligand (TRAIL). The aim of this study was to investigate whether induction of apoptosis and/or cytotoxicity can be enhanced by combining bortezomib with cisplatinum or the tyrosine-kinase inhibitor sunitinib.
Materials and Methods: HTB-2, HTB-3 (squamous) and HTB-5 cell lines were treated with increasing concentrations of bortezomib alone and in combination with sunitinib or cisplatinum for 72 hours. Cytotoxicity was determined by an MTT-based vitality assay (EZ4U, Biomedica). Apoptosis was detected by flow cytometry (PI-staining) using FACS calibur (Becton Dickinson). Protein analysis was performed using Western blots.
Results: In all three urothelial carcinoma cell lines bortezomib showed a significant reduction in cell-proliferation with an IC50 of 5 to 8 nanomolar (nM). Bortezomib alone induced significant DNA-fragmentation in up to 50% of viable cells in all cell lines, mostly at concentrations below 10 nM. This led to a consecutive reduction of cells in the G2/M and S-Phase. On the protein basis bortezomib induced apoptosis through down-regulation of pSTAT3, PARP and pS6 at concentrations starting at 5nM. The combination of bortezomib and sunitinib mainly induced an anti-proliferative effect through cell cycle arrest in the G0/G1 phase. P-ERK, PARP and pS6 were significantly downregulated when the two drugs were combined. Bortezomib (5, 10 nM) and sunitinib (5 μM) showed synergistic activity at low concentrations that are achievable in vivo, while cisplatinum and bortezomib showed additive activity through induction of apoptosis.
Conclusions: At concentrations feasible in vivo, bortezomib induced apoptosis in urothelial cancer cell lines. Sunitinib and cisplatinum enhanced the cytotoxic and apoptotic effects of bortezomib. These combinations warrant further clinical investigation.

7107 POSTER DISCUSSION

Prognostic Impact of the Expression of Putative Cancer Stem Cell Markers ALDH1 and SOX2 in Urothelial Cancer of the Upper Urinary Tract

H. Kitamura[1], T. Torigoe[2], Y. Hirohashi[2], H. Asanuma[2], S. Nishida[1], T. Tanaka[1], N. Masumori[1], N. Sato[2], T. Tsukamoto[1]. [1]*Sapporo Medical University School of Medicine, Department of Urology, Sapporo, Japan;* [2]*Sapporo Medical University School of Medicine, Department of Pathology, Sapporo, Japan*

Background: The aim of this study was to elucidate the prognostic impact of the putative cancer stem cell markers aldehyde dehydrogenase 1 (ALDH1) and sex determining region-Y (SRY)-related high mobility group box 2 (SOX2) in urothelial cancer of the upper urinary tract.
Material and Methods: Immunohistochemical staining for ALDH1 and SOX2 was carried out on archival specimens from 125 patients with urothelial cancer of the upper urinary tract who underwent radical nephroureterectomy from April 1995 to August 2010. The expression of ALDH1 and SOX2 was compared with clinicopathologic features, cancer-specific survival (CSS) and recurrence-free survival (RFS).
Results: In univariate analysis, grade, T stage, N stage, lymphovascular invasion, ALDH1 and SOX2 were associated with a poor prognosis and disease recurrence. In multivariate analysis, the independent factors of prognosis and recurrence were grade ($p = 0.0378$; $p = 0.0029$), pN ($p = 0.0276$; N.S.), and ALDH1 expression ($p = 0.0005$; $p = 0.0377$). When using subgroup analysis, the subgroups with two positive, one positive, or no positive immunohistochemistry in ALDH1 and SOX2 expression had estimated 5-year CSS of 79.7%, 46.6%, and 22.2%, respectively ($p < 0.0001$).
Conclusions: Expression of ALDH1 and SOX2 correlates with patient survival and recurrence in urothelial cancer of the upper urinary tract. ALDH1 expression is an especially strong independent predictive factor. The data suggest that cancer stem cells may play an important role in the progression of urothelial cancer.

7108 POSTER DISCUSSION

Surveillance in Stage I Testicular Cancer − Safety of Low Dose CT Scans

P. Warde[1], M. O'Malley[1], M. Jewett[1], T. Panzarella[1], D. Hogg[1], M. Moore[1], L. Anson-Cartwright[1], M. Haider[1], M. Gospodarowicz[1], P. Chung[1]. [1]*University Health Network, Radiation Medicine, Toronto − Ontario, Canada*

Background: Surveillance is accepted as the optimal management strategy for stage I testicular seminoma and is an accepted option for stage I non-seminoma. However, there is concern that the cumulative radiation exposure associated with multiple CT scans of the abdomen and pelvis (CT A/P) used in follow-up may result in an increased risk of second malignancy. The purpose of this study was to assess the safety of using low-dose CT scans in detecting retroperitoneal nodal relapse.
Methods: Between 2005 and 2011, 244 patients (198 seminoma, 46 non-seminoma) with stage I testicular germ cell tumours were enrolled into a phase II study. All patients initially underwent standard dose CT A/P and low dose CT A/P (40−60% dose reduction) and those with acceptable image quality continued with low dose CT alone. Relapse in the retroperitoneum detected on low dose CT was confirmed with standard dose CT. A single radiologist prospectively compared nodal size between the 2 imaging techniques.
Results: One patient had images unsuitable for surveillance with low dose CT. At a median follow up of 26 months there were 34 relapses: 32 in retroperitoneal lymph nodes and 2 with raised serum tumour markers. Of the 32 nodal relapses, 30 pairs of CT images were assessed for nodal size. Mean size of retroperitoneal nodal relapse (short axis) was 16.7 mm and 16.6 mm for standard and low dose CT, respectively ($p = 0.48$). The