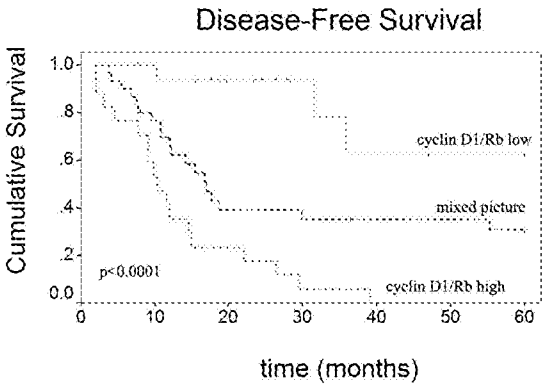
classified into 3 risk strata (high cyclin D1 and Rb, low cyclin D1 and Rb, and mixed). Tumors in the high-risk group had a 5-year disease free survival of 0% versus 63% in the low risk group (p<0.0001).



Conclusion: Our results demonstrate the power of AQUA in identifying molecular targets for cancer therapy. The association of low levels of p16 and high levels of cyclin D1 with poor survival indicates that CDK inhibitors may have a role in treatment of patients with oropharyngeal tumors. Additionally, the association of elevated p16 levels with low pRb expression may indicate a causal role of Human Papillomavirus (HPV) in this tumor subset. It is known that oncoprotein E7 of high-risk HPVs binds and degrades Rb protein. Experiments in our laboratory are underway to explore the association between HPV positivity and pRb /p16 levels. If this holds true, then HPV targeted therapies can be used in oropharyngeal tumors with p16 overexpression.

420 POSTER

The combination of Indisulam (E7070) with cisplatin, oxaliplatin and 5-fluorouracil are synergistic in vitro and in vivo

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Indisulam is an experimental anticancer agent that is presently completing phase II studies in solid tumours. DNA array analysis indicates that indisulam down-regulates several genes that are related to cytotoxic drug resistance, including cyclin H and general transcription factor IIH 62kDa-subunit (GTF2H1) [involved in nucleotide excision repair], glutathione synthetase (GS) [related to detoxification of platinum], thymidylate synthetase (TS) [target molecule of 5-fluorouracil (5-FU)], and so on. To determine the importance of these events in establishing combination therapies with indisulam, we investigated the efficacy of indisulam in combination with cisplatin, carboplatin, oxaliplatin and 5-FU *in vitro* and *in vivo*. *In vitro* studies were performed using HCT15 colorectal cancer cells. Combination effects were analyzed by MTT assay and isobologram analysis. Initial exposure to indisulam followed by cisplatin, carboplatin, oxaliplatin or 5-FU were synergistic. *In vivo* studies were performed using an HCT15 xenograft model (cisplatin and 5-FU), a Lu99 (NSCLC) xenograft model (cisplatin) and a Colo320D. M.(colorectal) xenograft model (5-FU). In the HCT15 xenograft model (simultaneous administration), indisulam + CDDP and indisulam + 5-FU demonstrated tendency of synergy, even as CDDP alone and 5-FU alone showed no effect. In Colo320D. M. xenograft model, indisulam + 5-FU had tendency of synergy in the indisulam pre-administration schedule, but not in other administration schedules. In the Lu99 xenograft model, indisulam + cisplatin also showed synergistic effects only in the indisulam pre-administration schedule. The present data suggest that the optimal combination schedule in combination of indisulam with platinum-containing drugs or 5-FU is the administration of indisulam preceding platinum or 5-FU. The synergistic efficacy of Indisulam with platinum and 5-FU in the schedule may be attributable to increasing the chemosensitivity to platinum-containing drugs and 5-FU, at least partially, due to cyclin H, GTF2H1, GS and TS suppression caused by Indisulam. From these data, clinical trials of indisulam in combination with carboplatin, oxaliplatin and 5-FU are ongoing.

421 POSTER

Repression of cell cycle-related proteins by oxaliplatin but not cisplatin in human colon cancer cells

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Oxaliplatin (*cis*-[(1*R*,2*R*)-1,2-cyclohexanediamine-*N, N'*] oxalato (2-)-*O, O'* platinum; Eloxatin) is a third-generation platinum which has an *in vitro* and *in vivo* spectrum of activity distinct from that of cisplatin, especially in colon cancer cells. Here, we studied the molecular basis of this difference on the human colon carcinoma HCT-116 cell line. Oxaliplatin inhibited HCT-116 cell proliferation with greater efficacy than cisplatin (IC_{50} at 0.4 μM and 1.2 μM for oxaliplatin and cisplatin, respectively). At equicytotoxic concentration, oxaliplatin activated the G1 and G2/M checkpoints while cisplatin activated the S and G2/M checkpoints. To search for oxaliplatin-specific target genes and mechanism different from those of cisplatin, we established the transcriptional signatures of both products on HCT-116 cells using the microarray technology and found from hierarchical clustering that i) much more genes were modulated by oxaliplatin compared with cisplatin, ii) among the 117 modulated genes, 79 genes were similarly regulated by both drugs and in sharp contrast, 38 genes were dose-dependently down-regulated by oxaliplatin and conversely up-regulated or unaffected by cisplatin. Interestingly among these latter group is a number of cell cycle-related genes, encoding proteins involved in DNA replication (CDC6, primase, cyclin A, replication protein A and replication factor C), and G2/M cell cycle progression (cyclin B1, Cdk1, and CDC25C). RNA modulations, confirmed at the protein level, were in accordance with the oxaliplatin effects on the cell cycle. Furthermore, given the modulations of cyclins and cdks, we examined the nucleolus using an electron microscopic approach and evidenced that oxaliplatin dramatically affected the nucleolar architecture. Interestingly cisplatin, which did not repress these genes, had no effect.
Beyond the identification of target genes that are affected by both drugs, the identified oxaliplatin-specific target genes could be additionally useful as predictive markers to evaluate and compare the efficacy of platinum drugs.

422 POSTER

The mode of action of CYC202 (R-roscovitine) a cyclin dependent kinase inhibitor currently in phase II clinical trials

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CYC202 is a potent Cyclin Dependent Kinase (CDK) inhibitor, competing with ATP for its binding site on the kinase. It has greatest in vitro activity against CDK2/Cyclin E, CDK7/Cyclin H and CDK9/Cyclin T. CYC202 inhibits both cell cycle progression and transcription by RNA polymerase II. CYC202 induces apoptosis from all phases of the cell cycle in tumour cell lines and reduces tumour growth in human tumour xenografts in nude mice. Two Phase I studies have been completed and three Phase II trials are ongoing: in combination with gemcitabine/cisplatin for NSCLC; in combination with capecitabine for breast cancer; and as a single agent in haematological B-cell malignancies
In order to identify potential biomarkers that may be useful for clinical development of CYC202, cellular mode-of-action experiments have been performed in both multiple myeloma and solid tumour cell lines. In cell lines from both tumour types, CYC202 induced rapid dephosphorylation of the retinoblastoma protein and the C-terminal domain (CTD) of RNA polymerase II. Phosphorylation of RNA polymerase II is crucial for its role in transcription and is controlled by CDKs 2, 7, 8 & 9 while Rb is phosphorylated by CDKs 2 & 4; thus in cells, CYC202 appears to be inhibiting several different CDKs. Inhibition of CDKs 7 amp; 9 would block transcription and would be predicted to exert its greatest effect on gene products where both mRNA and protein have short half-lives; one such gene product is the anti-apoptotic factor Mcl-1 which inhibits pro-apoptotic proteins and is essential for survival of a range of cell types including multiple myeloma. As hypothesised, treatment of multiple myeloma cells with CYC202 caused rapid down regulation of Mcl-1, this correlated with the induction of apoptosis as determined by TUNEL and PARP cleavage. A significant reduction in Mcl-1 was also seen in solid tumour cell lines.
Results will be presented detailing these ongoing experiments examining the cellular mode-of-action of CYC202.