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Cooperative phosphorylation including the activity of polo-like kinase 1 regulates the subcellular localization of cyclin B1

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The cyclin-dependent kinase 1 (Cdc2)/cyclin B1 complex performs cardinal roles for eukaryotic mitotic progression. Phosphorylation of 4 serine residues within cyclin B1 promotes the rapid nuclear translocation of Cdc2/cyclin B1 at the G2/M transition. Still, the role of individual phosphorylation sites and their corresponding kinases remain to be elucidated. Polo-like kinase 1 (Plk1) shows a spatial and temporal distribution which makes it a candidate kinase for the phosphorylation of cyclin B1. We could demonstrate the interaction of both proteins in mammalian cells. Plk1 phosphorylated wild-type cyclin B1 expressed in bacteria or in mammalian cells. Ser-133 within the cytoplasmic retention signal (CRS) of cyclin B1, which regulates the nuclear entry of the heterodimeric complex during prophase, is a target of Plk1. In contrast, MAPK (Erk2) and MPF phosphorylate Ser-126 and Ser-128 within the CRS. Phosphorylation of CRS by MAPK (Erk2) prior to Plk1 treatment induced enhanced phosphorylation of cyclin B1 by Plk 1. In addition, pretreatment of cyclin B1 by MAPK (Erk2) altered the phosphorylation site of Plk 1 suggesting a synergistic action of both enzymes towards cyclin B1. An immunofluorescence study revealed that a mutation of Ser-133 reduced the nuclear import rate of cyclin B1. Still, multiple serine mutations are required to prevent nuclear translocation completely indicating that orchestrated phosphorylation within the CRS triggers rapid import of cyclin B1.

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Preliminary results of an ongoing phase I and pharmacokinetic study of CYC202, a novel oral cyclin-dependent kinases inhibitor, in patients with advanced malignancies

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CYC202, (enantiomerically pure R-roscovitine), inhibits cdk1, cdk2 and cdk5, modulates cell cycle with accumulation of sub-G1 population of cells indicative of apoptosis induction. CYC202 have shown antiproliferative and anti-tumor effects against a broad range of human tumor models including those with cisplatin- and doxorubicin-resistant phenotypes. This phase I trial was aimed at defining the toxicity profile, the maximal tolerated dose (MTD), and pharmacokinetics of oral CYC202 administered at fixed doses twice daily (bid) for 5 consecutive days every 3 weeks. Based on animal toxicology and a previous trial, the starting dose was 100 mg bid in 3 patients. Further dose escalation was based on toxicity at cycle#1 with 1-2 patients per dose level and 100% dose-escalation until toxicity < grade 2 and a 25% dose escalation in at least 3 patients in case of grade 2 toxicity. If dose-limiting toxicity (DLT, grade > 3 toxicity) occurred, 6 patients were entered. As of June 2002, 19 patients (male/female: 7/12, median age 53, range 30-64) with good performance status (WHO 0, 1, and 2 in 10, 7, and 2 patients, respectively) were entered. Tumor types include gastrointestinal (6 patients), prostate (3 patients), breast (3 patients), sarcoma (3 patients), lung (1 patient), parotid (1 patient), ACUP (1 patient), and adrenal cortical carcinoma (1 patient). Eighteen patients were previously treated with chemotherapy (median number of prior regimens: 4; range, 2-9). Doses of CYC202 bid 100 mg (3 patients), 200 mg (2 patients), 400 mg (1 patient), 800 mg (4 patients), 1000 mg (6 patients), and 1250 mg (3 patients) were explored. No DLT was observed up to the dose of 800 mg bid. At the dose of 1000 mg bid, grade 3 nausea/vomiting (1/6 patient) and asthenia (1/6 patient) were observed, dose escalation then continued to 1250 mg bid. Other toxicities observed were mild to moderate and include asthenia, nausea/vomiting, mucositis, myalgia, anemia, and skin reactions. Preliminary pharmacokinetic data showed good oral bioavailability with dose proportional increases in blood levels with some interpatient variability. At 800 mg bid: Cmax=2630 ng/ml, AUC=12722 h*ng/ml, VD=225 L, half-life=3 h. Sustained 6-month tumor stabilization was observed in 2 patients. In summary, CYC202 was well tolerated up to 2000 mg/day. Further dose escalation will define the MTD and longer treatment duration will be explored.

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A phase I study of Ro 31-7453, a novel oral cell cycle inhibitor, in combination with paclitaxel: final results

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Ro 31-7453 is a novel, oral M-phase cell cycle inhibitor, with CYP3A4-dependent metabolism, and anti-tumor activity in many preclinical, including multidrug resistant models. Preclinical data have demonstrated synergistic anti-tumor activity between Ro 31-7453 and paclitaxel (P). A phase I study of Ro 31-7453 (po q12 hr days 1-4) and P (IV 3hrs, day 1) every 3 weeks (wk) in patients (pts.) with advanced solid tumors was conducted to determine the maximum tolerated dose (MTD), toxicity profile, and pharmacokinetic (PK) profile of this regimen. Plasma samples were collected on days 1 + 4 of cycle 1 to determine PK for P and Ro 31-7453 + its 2 active metabolites. Patients: A total of 30 pts. were treated, 10 F, 20 M; median age 58 (42-75); median KPS 90 (70-100); median prior chemotherapy regimens 1 (0-4); prior taxane therapy 8, tumor types - non-small cell lung cancer (NSCLC) 15, head and neck cancer (H&N) 9, mesothelioma 5, thymoma 1. Toxicity: Treatment-related grade 3/4 events (grade-number pts.): neutropenia (G3/4-1/3), fatigue (G3-4), nausea (G3-1), emesis (G3-1), mucositis (G3-1), dyspnea (G3-1), constipation (G3-1), and arthralgia (G3-1). There were no treatment-related deaths. The MTD [recommended phase II dose (RPTD)] was Ro 31-7453 220 mg/m^2 po q 12hr, days 1-4 and P 150 mg/m^2 day 1 Q 3 wk. DLT (cycle 1 G4 neutropenia) was noted in 1/6 pts. in this cohort; unacceptably severe neutropenia prohibited paclitaxel dose escalation to 175 mg/m^2. PK (25 pts.): Day 1 Ro 31-7453 + metabolites interpatient AUC variability averaged 44%, consistent with previous single agent data. In contrast to previous studies, no accumulation of Ro 31-7453 was seen on day 4 (mean day 4/day 1 AUC ratio = 1.03). PK for P (AUC) was dose-proportional, similar to historic data, suggesting no PK interaction with Ro 31-7453. Response data: Median no. of treatment cycles 5 (range 1-8), 1CR (head and neck cancer, free of disease after 8 cycles) and 12 SD (lasting > 4 cycles) (6 NSCLC, 4 H&N, 1 mesothelioma, 1 thymoma) were observed.
Conclusions: The q 3 wk combination regimen of Ro 31-7453 and P demonstrates an acceptable safety profile, and RPTD is Ro 31-7453 220 mg/m^2 po q12hr d 1-4 and P 150 mg/m^2 d 1 Q 3 wk. CYP3A4 induction due to dexamethasone premedication for P might account for he strikingly low day 1-4 intrapatient variability of Ro 31-7453 AUC. Evidence of antitumor activity suggests a rationale for phase II studies in H&N and lung malignancies.

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Phase I study of flavopiridol (HMR1275) in combination with paclitaxel and carboplatin in non-small cell lung cancer (NCSLC) patients

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Flavopiridol is a synthetic flavonoid and a potent cyclin-dependent kinase inhibitor which has anti-tumour effects *in vitro* at a clinically achievable concentration. Administration of other antineoplastic agents such as the platinums and taxanes prior to flavopiridol resulted in cytotoxic synergy *in vitro*. The primary objective of this study was to determine the safety and tolerability of increasing doses (30-160 mg/m^2) of flavopiridol in combination with paclitaxel and carboplatin in stage IIIB-IV non-small cell lung cancer (NSCLC) patients. The secondary objectives were to investigate the pharmacokinetics of flavopiridol in combination with paclitaxel and carboplatin, and its efficacy. Eighteen patients (6 women, 12 men) with previously untreated advanced NSCLC were enrolled (12 evaluable for efficacy based on RECIST and WHO). On Day 1, patients received intravenously (i.v.) 175 mg/m^2 paclitaxel in 3 hr and carboplatin (AUC=5 mg/ml*min, Chatelut formula) in 1 hr, and on Day 2, flavopiridol i.v. in 24 hr. The treatment cycle was repeated every 21 days for 3 cycles. The flavopiridol dose was escalated in 4 steps from 30 mg/m^2 to 85 mg/m^2, until dose limiting toxicity of flavopiridol in combination with paclitaxel and carboplatin was reached. Seventeen patients reported adverse events at least possible related to treatment. Most of

them (including diarrhoea) were grade 1 or 2, except for leukopenia. There was no clear relationship between flavopiridol dose level and the occurrence or severity of these adverse events. One subject at 85 mg/m^2 died of cardiac arrest and myocardial infarction during the first cycle and was judged to be possibly related to administration of flavopiridol. Pharmacokinetics of flavopiridol showed a non-linear increase in concentrations at the end of the infusion. The mean half-life of flavopiridol was 27 hr [range 7-66 hr]. Most patients exhibited a secondary peak in plasma concentrations between 24 hr and 48 hr after initiation of dosing. Of the 12 patients (evaluable for response), 6 had an overall partial response and 6 had stable disease (WHO criteria). The results of this study indicate that flavopiridol in combination with paclitaxel and carboplatin can be given to cancer patients safely up to a dose of 70 mg/m^2 as a 24-hr infusion. Future clinical trials will investigate whether 1-hr infusion of flavopiridol in combination with chemotherapy is superior to chemotherapy alone in the treatment of advanced NSCLC.

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Phase II study of E7070 in patients with metastatic melanoma (stage IV)

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Background: E7070 (N-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide) is a novel sulphonamide derivative which delays the G1/S transition by inhibiting cyclin E expression and cdk2 phosphorylation.
Patients and methods: Patients received 700 mg/m^2 of E7070 as a 60-min infusion every 3 weeks. A two stage Gehan study design was applied on the basis that if the results of the trial were compatible with a response rate of 15% in the studied population, the drug should be further investigated in malignant melanoma. Blood samples were taken during cycles 1 and 3 for population pharmacokinetic analysis. Paired skin biopsies were collected from 3 patients for micro-array analysis within 14 days prior to the 1st administration and 48h (±6h) following the 1st administration of E7070. Results The study was conducted in 8 EORTC institutions in 7 countries. 24 patients were eligible out of 28 recruited in 6.5 months. Demographic data were as follows: median age 56 years (range 26-75); M/F - 18/10; PS 0/1/2-15/12/1. 25 patients showed normal ECG at baseline with only 3 judged to be abnormal with no clinical significance. 27 patients were chemo-naive and one had received (neo)adjuvant chemotherapy. 6 patients had prior immuno/BRM/vaccination treatment. Site of the primary was leg (11), head (7), trunk (5), neck (1), arm (1) and 3 unknown. Data are available for 28 patients who received 71 cycles (median-2; range: 1-14). Safety profile: 3 patients experienced Grade (G) 3 leucocytopenia; 4-G3 neutropenia; 3-G3 anemia and 3-G3 thrombocytopenia. Non-hematological G3 toxicity was: supraventricular arrhythmia; infection; thromboembolism; injection site reaction; rash; vomiting; (one case each); fatigue (3 cases) and G4: dyspnea. Reasons for stopping treatment were PD (22), toxicity (2) and other reason (4). Central objective response review was performed and, in eligible patients, 5 NC, 13 PD, 1 early death were validated and 5 not assessable.
Conclusion: E7070 at the dose of 700 mg/m^2 as a 60-min infusion does not produce objective responses in metastatic melanoma stage IV. Toxicity profile was as expected from previous preclinical and Phase I clinical data and appears to be acceptable, reversible and easily manageable. Full data analysis will be available at the time of presentation.

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Gene expression profiling of the cyclin-dependent kinase inhibitor CYC202 (R-Roscovitine)

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Components of the cell cycle are crucial for regulating proliferation and are frequently deregulated in cancer. In particular, loss of negative regulators of the cell cycle, such as p16INK4A or amplification of positive regulators such as cyclin D1, leads to an increased proliferative capacity. Therefore, inhibitors of cell cycle progression are of interest as anticancer therapeutics. The cyclin-dependent kinases phosphorylate the retinoblastoma protein (RB), displacing E2F transcription factors and lead to progression through the cell cycle. Inhibition of CDK activity blocks the phosphorylation of RB and prevents cell cycle progression, retarding cellular proliferation. In addition, specific inhibition of CDK2 has been shown to induce tumour cell-specific apoptosis. As a consequence, CYC202, a selective inhibitor of CDK2 is currently undergoing clinical trials. Gene expression profiling of human colon adenocarcinoma cell lines (KM12 and HT29) treated with CYC202 was performed in order to further explore the mechanism of action of the compound and how it may be utilised most effectively. Cells were treated with 20-50uM CYC202 for time course experiments of up to 72h, concentrations that inhibit RB phosphorylation and block cellular proliferation. Analysis of treated cells by flow cytometry showed that CYC202 caused a loss of cells in G1 phase and an increase in the number of cells in G2/M. RNase protection assay demonstrated a loss of cyclins D1, B1 and A which was confirmed by Western blotting. The levels of CDK1, 2 and 4 protein were unchanged in response to CYC202. Loss of the cyclins is a potential mechanism through which cell cycle arrest is maintained in addition to inhibition of CDK activity by CYC202. CYC202 was compared with equitoxic doses of flavopiridol and alsterpaullone to determine any common trends associated with CDK inhibition. Preliminary analysis indicates that CYC202 induced changes in the expression of genes involved in cell cycle control and apoptosis. The significance of these responses is to be investigated and results will be discussed.

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A novel class of pyridopyrimidine tyrosine kinase inhibitors blocks cancer cells in the S-phase of the cell cycle

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A novel family of pyrido [2,3-d]pyrimidines has been identified on the basis of their selective inhibition of Src-related tyrosine kinases (TKs) (Kraker A.J. et al, 2000, Biochem. Pharmacol. Oct. 1; 60(7):885-98). Further work has shown that individual members of the family also have activity against PDGF receptor and c-Abl. We have examined the effects of this family on cancer cell lines and have previously shown that a subset of compounds, including PD173955, block cells in the prophase stage of mitosis. We now report that another subset inhibits S-phase progression. The M-phase and S-phase activities are mutually exclusive in that individual compounds inhibit progression of the cell through mitosis or S phase, but not both. Paradoxically, the S-phase inhibitor PD179483 caused activation of cyclin A, cdk2 and cdc2-associated kinase activity. No such activation was elicited by the M-phase inhibitors. Phosphorylation of the Tyr-15 residue of cyclin-dependent kinases (cdks) results in their inhibition and is catalyzed by the Myt1 and Wee1 tyrosine kinases. Activation of cdk kinases in PD179483 treated cells was associated with dephosphorylation of Tyr-15 and with failure to rephosphorylate this tyrosine in cells progressing from mitosis to early G1. Furthermore, PD179483 causes the accumulation of both Wee1 and Myt1 kinases in hyperphosphorylated state that correlates with their inhibition. These data suggest that PD179483 selectively affects a target upstream of Wee1 and Myt1 that is required for their activation. This results in inability to phosphorylate Tyr-15, dysregulated cdk activation and is associated with S-phase arrest.

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A novel class of cdc25 phosphatase inhibitors shows potent anticancer activity by blocking cell cycle and inducing apoptosis

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The dual specificity phosphatase cdc25 regulates cell cycle progression through activation of cyclin/cyclin-dependent kinase (cdk) complexes by removing inhibitory phosphate groups from cdks. Of three human cdc25 homologs, cdc25A and cdc25B are considered potential oncogenes because overexpression of these genes is found in up to 50% of all major human cancers and is associated with oncogenic transformation. MAXIA has developed a new class of small molecule compounds that inhibit cdc25. MX7306 and other compounds of this series exhibited selective inhibition of cdc25A activity in *in vitro* phosphatase assays as compared to the dual specificity phosphatase MKP-1 and the tyrosine phosphatase PTP1B. Treatment of PC-3 prostate cancer cells with cdc25 inhibitors enhanced cdk2 phosphorylation at Tyr15 and diminished the cdk2 kinase activity without changes in protein levels of cdks 1, 2 and 4 as well as p21 (waf1) and p27 (kip1), which is consistent with the direct inhibition of cdc25. Cell cycle analysis revealed that the phosphatase inhibitors blocked G1/S transition in synchronized PC-3 cells. MX7306 or its analogs also inhibited DNA replication and S phase progression of asynchronously growing cells as assessed by BrdU incorporation. Furthermore, extended exposure to inhibitors led to cell apoptosis. MX7306 and its analogs exhibited efficacious and broad anticancer activi-