149

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.

150

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.

151

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.

152

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