

## 2517 CLINICAL APPLICATIONS OF MOLECULAR PHARMACOLOGY

**Dose finding study of PSC833, a novel MDR reversing agent, with daunorubicin and Ara-C in untreated elderly patients with acute myeloid leukaemia (AML).** P. Sonneveld, B. Lowenberg, P. Vosseveld, J. Malkhandi, A. Covelli, A.W. Dekker, G. Ossenkoppele, D. Milligan, G. Verhoef, A. Ferrant, J. Yin, A. Gratwohl, T. Kovacsovic and A. Burnett. *University Hospital Rotterdam, AZ-VU Amsterdam, AZU Utrecht, The Netherlands; Medical Research Council; Heartlands Hospital, Birmingham, Manchester Royal Infirmary, Manchester, UK; UZ Gasthuisberg, Leuven, UZ St Luc, Brussels, Belgium; Kantonspital Basel, CHUV, Lausanne, Novartis Pharma AG, Basel, Switzerland.*

In elderly de novo AML patients, up to 80% express the Multidrug Resistance phenotype. In AML, CR rates of P-glycoprotein (Pgp) positive vs negative subjects were recently found to be 34% vs 67% respectively, with the probability of attaining CR declining markedly with age. We report the results of a dose finding study to determine the MTD of daunorubicin (DNR) i.v. (days 1-3) given in combination with fixed doses of a P-gp inhibitor, PSC833 10 mg/kg/24 h iv (days 1-4) and Ara-C 200 mg/m<sup>2</sup>/24 h (days 1-7), in patients aged 60 or above with untreated AML. 19 patients, median age 67 (range 60-72) entered cohort 1 (35 mg/m<sup>2</sup> DNR). 11/19 patients completed 2 cycles of chemotherapy, 6/19 patients experienced dose limiting toxicities (DLTs), (cardiac: 2, infection: 2, hypotension: 2) of which 2 were lethal. 20 patients, median age 60 (range 60-83) entered cohort 2 (45 mg/m<sup>2</sup> DNR). 7/20 patients completed 2 cycles, with 8/20 DLTs (ARDS: 1, hyperbilirubinaemia: 1, mucositis: 2, cardiac: 1 haemorrhage: 3), of which 7 were lethal. Prolongation of treatment induced hypoplasia was not observed. Steady state PSC833 blood levels exceeded the level required for inhibition of P-gp (mean 3524 ng/mL days 1-4). The overall treatment results were 48% CR after 1 cycle (16/39) or 2 cycles (3/39), 13% refractory disease, 28% treatment failure (early death or regeneration failure), 10% not assessable. DNR 45 mg/m<sup>2</sup> induced a higher CR rate (60%), but this was associated with increased toxicity with 7/20 patients dead in CR. Thus, DNR 35 mg/m<sup>2</sup> was established as the recommended dose to be administered with PSC833 and Ara-C in this population, giving a CR rate of 42% and a median duration of CR of 9.4 months. AML blast P-gp expression including CD34 subfractions was assessed in 33/39 patients by flowcytometry using MRK16 and UIC2 staining and Rho123 exclusion. 21/33 samples (64%) were P-gp positive. 11/21 (52%) of P-gp positive as compared with 7/12 (58%) Pgp negative AML achieved CR.

PSC833 in combination with 35 mg/m<sup>2</sup> DNR and Ara-C was well tolerated. Based on these results, the efficacy of PSC833 is currently under further investigation in a randomized, controlled phase 3 study.

## 2518

**A phase I dose-finding study of PSC 833, a novel MDR reversing agent, with mitoxantrone, etoposide and cytarabine (PSC-MEC) in poor prognosis acute leukemia (AML).** G. Visani, D. Milligan, F. Leoni, J. Chang, S. Kelsey, R. Marcus, R. Powles, S. Schey and A. Covelli. *Policlinico S. Orsola, Bologna, I, Heartlands Hospital, Birmingham, UK; Policlinico di Careggi, Firenze, I, Christie Hospital, Manchester; Royal London Hospital, London; Addenbrook's NHS Trust, Cambridge; Royal Marsden Hospital, Sutton; Guy's Hospital London, UK; Novartis Pharma AG, Basel, CH, Switzerland.*

The failure of conventional chemotherapy in relapsed or refractory AML and other poor risk groups has been linked to expression of the multidrug resistance gene (MDR-1) product P-glycoprotein (p-gp). PSC 833 (PSC) is a competitive inhibitor of P-gp and has been shown *in vitro* and *in vivo* to restore sensitivity to anticancer drugs (ACDs). Twenty-two patients with poor prognosis AML, i.e. refractory to first-line induction or relapsing within 12 months of attaining complete remission (CR), were enrolled into a phase I study. The median age of these patients was 42 years (range 18-69 years): 8 patients had primary refractory disease, 7 each had relapsed within 6 or 7-9 months after CR, respectively. Six patients had secondary leukemia. The induction regimen consisted of ara-C 1 g/m<sup>2</sup>/day for 6 days in combination with continuous infusion intravenous (CIV) PSC and 6-day courses of escalating doses of mitoxantrone and etoposide: the doses of PSC and ACDs in the three cohorts are reported in the following Table. In all cohorts, CIV PSC was started concomitantly with a 2 mg/kg loading dose given iv in 2 hours. Patients achieving CR were scheduled to receive a 4-day PSC-MEC consolidation cycle.

X	Etoposide (mg/m <sup>2</sup> /d x 6d)	Mitoxantrone (mg/m <sup>2</sup> /d x 6d)	PSC 833 (mg/kg/d)	Patients (n)
1	30	3.0	10 over 168 h	10
2	30	4.5	10 over 168 h	6
3	30	4.5	10 over 144 h	6

Two dose limiting toxicities were observed in cohort 1 (2 grade 4 hyperbilirubinemia >14 days), 2 in cohort 2 (a grade 4 mucositis and a grade 4 hyperbilirubinemia >14 days) and one in cohort 3 (a grade 4 aplasia >14 days). Since the long duration of grade 4 hyperbilirubinemia observed in cohorts 1 and 2 was felt to be secondary to the prolonged infusion of PSC, this was shortened by 24 hours in cohort 3. Pharmacokinetic of PSC was evaluated in all patients: blood levels of PSC after the loading dose ranged from 1200 to 6000 ng/mL (1-6 µM). Overall, 6 patients achieved CR and 18 failed chemotherapy. Causes for failures included: 10 refractory leukemia, 2 marrow regeneration failures, 2 early deaths and 2 not assessable.

Our data indicate that the regimen of PSC-MEC, at the doses outlined above for the third cohort, is well tolerated. Blood levels of PSC are achieved which are

capable of *in vitro* MDR-1 modulation. Finally, the observed CR rate (27%) is higher than what is would be expected based on historical controls (10-19%) in this high risk population. The regimen will be further tested in a randomized phase III trial in refractory/early-relapsing AML patients.

## 2519

**Expression of the lung resistance protein (LRP) predicts poor outcome in de novo acute myeloid leukemia.** R. Pirker, G. Pohl, T. Stranzl, R.W. Suchomel, R.I. Scheper, U. Jäger, K. Geissler, K. Lechner and M. Filipits. *Division of Oncology and Division of Hematology, Department of Internal Medicine I, University of Vienna Medical School, Vienna, Austria; Department of Pathology, Free University Hospital, Amsterdam, The Netherlands.*

The 110 kDa lung resistance protein (LRP) is overexpressed in P-glycoprotein-negative multidrug-resistant cell lines and most likely involved in the multidrug resistance (MDR) of these cell lines. To determine the clinical significance of LRP, we have studied LRP expression of leukemic blasts and its association with clinical outcome in 86 previously untreated patients with de novo acute myeloid leukemia (AML). LRP expression of leukemic blasts obtained from peripheral blood or bone marrow was determined by immunocytochemistry by means of monoclonal antibody LRP-56. LRP expression at diagnosis was detected in 31 (36%) patients. LRP expression was independent of age and sex of the patients. FAB subtype, cytogenetic abnormalities and LDH levels, but correlated with white blood cell count ( $p = 0.01$ ). Eighty-two patients received standard induction chemotherapy that included cytarabine and MDR drugs (daunorubicin in most patients, additional etoposide in the majority of patients). The complete remission rate of induction chemotherapy was 72% (95% CI = 61-82%) for the total study population. The complete remission rate was 81% (95% CI = 67-91%) for patients without LRP expression but only 55% (95% CI = 36-74%) for patients with LRP expression ( $p = 0.01$ ). Overall survival and disease-free survival were calculated according to Kaplan-Meier in 82 and 59 patients, respectively. Overall survival was significantly longer in patients without LRP expression than in patients with LRP expression. At a median follow-up of 16 months, median overall survival was 17 months (95% CI = 12-38 months) for LRP negative patients but only 8 months (95% CI = 4-12 months) for positive patients ( $p = 0.006$ ). Disease-free survival was 9 months (95% CI = 7-11 months) for LRP negative patients and 6 months (95% CI = 5-8 months) for positive patients ( $p = 0.078$ ). Outcome was best in patients lacking both LRP and P-glycoprotein expression. In conclusion, LRP predicts for poor outcome and thus the LRP gene appears to be another clinically relevant drug resistance gene in AML.

## 2520

**Deoxycytidine kinase expression and activity in ara-C resistant cell lines and samples from newly diagnosed acute leukemias.** D. Martincic, V. Kravtsov, M. Koury, V.I. Avramis and J.A. Whitlock. *Division of Pediatric Hematology/Oncology, Vanderbilt University, Nashville, TN; Division of Hematology/Oncology Childrens Hospital Los Angeles, Los Angeles, CA.*

Phosphorylation of ara-C by deoxycytidine kinase (dCk) plays an essential role in ara-C activation. Loss of dCk activity has been associated with resistance to ara-C in leukemic cell lines. The role of decreased dCk activity *in vivo* is not clear. We analyzed five ara-C resistant cell lines and seven samples from newly diagnosed acute leukemias for possible molecular mechanisms of resistance to ara-C. All cell lines and samples were analyzed for resistance to ara-C using microculture kinetic (MiCK) assay. DCK protein activity was determined using [<sup>3</sup>H] deoxycytidine as a substrate in the presence of excess thymidine. DCK mRNA levels were assessed by semiquantitative PCR. DCK point mutations were investigated using dideoxyfingerprinting (ddF). Ara-C resistant cell lines CEM/CHLA1-5 were derived from the ara-C sensitive parental cell line CEM. All 5 cell lines demonstrated resistance to ara-C by the MiCK assay comparing with parental cell line. However, the degree of resistance varied. Five of 7 clinical leukemic samples demonstrated ara-C resistance by the MiCK assay. MDR1 overexpression was not detected by semiquantitative PCR in either the cell lines or four ara-C resistant clinical samples. Semiquantitative PCR revealed decreased levels of dCk mRNA expression in the resistant cell lines compared with parental cell line: relative dCk expression (as determined by densitometry) correlated with the degree of resistance to ara-C. Four of 5 leukemic samples resistant to ara-C express decreased levels of dCk mRNA, compared to an ara-C sensitive cell line. DdF analysis of full length dCk mRNA coding sequence did not reveal any point mutations. DCK activity was reduced in three resistant cell lines compared with the sensitive parental cell line. Studies of hypermethylation in these cell lines showed that downregulation of dCk is due to hypermethylation of the [4] cgcg boxes in the dCk promoter region. These results suggest that a) regulation of dCk expression at the transcriptional level may confer resistance to ara-C; b) transcriptional regulation is due to DNA hypermethylation of the dCk promoter region.

## 2521

**In vitro drug resistance as important prognostic factor in two prospective studies in childhood ALL.** G.J.L. Kaspers, M.L. Den Boer, R. Pieters, G. Janka, E.R. Van Wering, A. Van Der Does-Van Den Berg and A.J.P. Veerman. *Academic Hospital VU, Amsterdam; CoALL Study Group; Hamburg; Dutch Childhood Leukemia Study Group; The Hague, The Netherlands.*

Cellular drug resistance is an important factor in the success or failure of chemotherapy. We have successfully determined cellular drug resistance using the colorimetric 4-days MTT assay in 293 children with untreated ALL: 152 children

treated by protocols in 1996 according to stratified age were each. Combining decrease in profile, via the most independent also found clinical re general p prospective 79% and respective risk (LR) LR group resistant p and 56% independent stratificat be stratifi

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