Sequential phase II Southwest Oncology Group studies (S0112 and S0301) of daunorubicin and cytarabine by continuous infusion, without and with ciclosporin, in older patients with previously untreated acute myeloid leukaemia

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Summary

Attempts to overcome multi-drug resistance in acute myeloid leukaemia (AML) have been limited by toxicities. To investigate the effect of reducing peak drug levels, we performed sequential phase II studies using continuous infusion daunorubicin and cytarabine without (AD) and then with ciclosporin (ADC) in older patients with AML. Untreated patients (age 56+ years) received daunorubicin (45 mg/m² per day for 3 d) and cytarabine (200 mg/m² per day for 7 d), both by continuous infusion, without (S0112, 60 patients) and then with (S0301, 50 patients) the addition of ciclosporin. Complete response (CR) rates were 38% on S0112 and 44% on S0301. Fatal induction toxicities occurred in 17% and 12% respectively, arising primarily from infection and haemorrhage. Median overall and relapse-free survival was 7 and 8 months for AD respectively, and 6 and 14 months for ADC. Patients with phenotypic or functional P-glycoprotein had somewhat higher CR rates with ADC than AD, although confidence intervals overlapped. In these sequential trials, continuous infusion AD produced CR rates comparable to those with bolus daunorubicin. The addition of ciclosporin did not cause undue toxicities, produced a similar CR rate, and possibly improved relapse-free survival. Further correlate analyses did not identify a subpopulation specifically benefitting from the addition of ciclosporin.

Keywords: acute myeloid leukaemia, P-glycoprotein, multi-drug resistance, ciclosporin, chemoresistance.

The median age of patients with acute myeloid leukaemia (AML) exceeds 60 years, and despite advances in therapy for younger patients, older patients continue to have poor outcomes with little improvement over time. Induction outcomes in older patients are often complicated by more frequent co-morbidities, and by fundamental differences in disease biology (Leith *et al*, 1997, 1999; Godwin *et al*, 1998; Goldstone *et al*, 2001; Anderson *et al*, 2002; Appelbaum *et al*, 2006; Farag *et al*, 2006; Wilson *et al*, 2006; van den Heuvel-Eibrink *et al*, 2007; van der Holt *et al*, 2007). These biological differences include, among others, an increased frequency of expression of P-glycoprotein (Pgp) and other drug efflux

proteins that share homology with the ATP-Binding Cassette (ABC) transporters (Ross *et al*, 1993, 1994; van den Heuvel-Eibrink *et al*, 2001, 2002; Mahadevan & List, 2004; Schilthuizen *et al*, 2007). Compounds, such as ciclosporin and its derivative PSC-833, as well as unrelated compounds such as zosuquidar have been investigated in younger and older patients with AML in attempts to overcome resistance and improve treatment outcomes (Solary *et al*, 1996; Advani *et al*, 1999; Lee *et al*, 1999; Tallman *et al*, 1999; Chauncey *et al*, 2000; List *et al*, 2001, 2002; Liu Yin *et al*, 2001; Baer *et al*, 2002; Solary *et al*, 2003; van der Holt *et al*, 2005; Greenberg *et al*, 2004; Burnett *et al*, 2009; Becton *et al*, 2006; Lancet *et al*, 2009). To date, however, none of these strategies have been consistently successful in improving outcome in younger or older patients, and many trials have been complicated by increased chemotherapy-related toxicities owing to higher blood levels of antineoplastics, such as anthracyclines and etoposide, when administered with these Pgp-modulating agents.

Based on the hypothesis that anthracycline-related toxicity in these regimens may be ascribed to higher peak drug concentrations rather than total drug exposure, we previously conducted a trial in patients with recurrent or untreated secondary AML testing the effect of ciclosporin with continuous infusion daunorubicin and high-dose cytarabine (List *et al*, 2001). In that prospective randomized trial (S9126), the addition of ciclosporin did not significantly improve the complete response rate, but the incidence of induction failure due to resistant disease (RD) was significantly reduced, and both relapse-free and overall survivals were significantly prolonged. The benefit of ciclosporin was greatest in patients whose blasts expressed a multi-drug resistant (MDR) phenotype.

Considering the high frequency of Pgp expression in older AML patients, we performed sequential phase II feasibility studies to assess the tolerance of infusional daunorubicin and cytarabine administration in older patients with AML, either alone (study S0112) or with the addition of ciclosporin (study S0301). The second study would proceed only if continuous infusion daunorubicin had no untoward impact on toxicity or efficacy. The results of S0112 were, within the limits of study size, similar to those in previous Southwest Oncology Group (SWOG) trials in similar patients using bolus daunorubicin, allowing S0301 to proceed. The addition of ciclosporin to the infusional induction regimen was poorly tolerated in the aged and those patients with very poor performance status. After exclusion of those patients from study eligibility, the addition of ciclosporin did not appear to increase toxicities.

Patients, materials and methods

Eligibility

Patients 56 years of age or older with a morphologically confirmed diagnosis of AML, except for acute promyelocytic leukaemia (M3), were eligible (Cheson *et al*, 2003). Diagnosis of AML was by French-American-British (FAB) criteria, i.e.

required ≥30% marrow blasts (≥20% if the peripheral blast percentage was ≥30%). Patients with blast crisis of chronic myeloid leukaemia were excluded. Prior systemic chemotherapy for acute leukaemia was not permitted, although treatment for hyperleucocytosis with hydroxycarbamide, or intrathecal therapy for suspected or proven central nervous system involvement was allowed. Patients with secondary AML, defined as AML arising after a morphological diagnosis as myelodysplasia (MDS) (Cheson *et al*, 2003) or AML arising after prior chemotherapy or radiotherapy, were not excluded. Prior treatment for MDS with cytarabine (≤100 mg/m^2) or azacitidine administered more than 30 d before administration was allowed. Patients were initially required to have performance status (PS) of 0–3 by SWOG criteria. However, in S0301, the permissible range of PS was modified after the initial 14 patients as described below. The modified criteria permitted PS 0–3 for patients aged ≤60 years, 0–2 for age 61–70 years and 0–1 for age ≥71 years. Adequate solid organ function was required, as defined by (i) serum bilirubin ≤2 times institutional upper limits of normal (IULN); (ii) aspartate transaminase and/or alanine transaminase ≤4 × IULN; (iii) serum creatinine ≤1·5 × IULN and/or creatinine clearance ≥0·67 ml/s; (iv) ejection fraction ≥50% as measured by either multigated cardiac blood pool (MUGA) scan or echocardiography; and (v) absence of unstable cardiac arrhythmia or unstable angina. Informed consent in accordance with local institutional guidelines and federal regulations was required of all patients.

Treatment plans

For S0112, patients received cytarabine 200 mg/m^2 daily by 24-h continuous infusion on days 1–7 and daunorubicin 45 mg/m^2, also as 24-h continuous infusion on days 1–3. For S0301, ciclosporin A was added as a loading dose of 6 mg/kg over 2 h followed by 16 mg/kg per day by continuous infusion for 3 d. A bone marrow aspirate and biopsy was obtained on day 14 and if the marrow blast percentage was more than or equal to 5%, a second cycle of induction of identical chemotherapy was administered upon the earliest recovery of marrow cellularity to more than 20%. Once the marrow blast percentage of <5% was documented, patients received either granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), administered intravenously once daily until the absolute neutrophil count (ANC) reached 1·5 × 10^9/l (Godwin *et al*, 1998). The dose and drugs administered for cycle two were the same as cycle one except daunorubicin was reduced by 50% for serum bilirubin between 2–4 × IULN, or discontinued for higher serum bilirubin concentrations.

Supportive care

All patients received antimicrobial prophylaxis as follows: (i) ciprofloxacin 500 mg orally or 200 mg intravenously twice

daily until administration of broad spectrum antibiotics for febrile neutropenia; (ii) fluconazole, to begin 48 h after completion of chemotherapy at 200 mg orally or intravenously daily or until administration of broad spectrum antifungal therapy and (iii) among patients who were seropositive for herpes simplex virus, acyclovir 800 mg orally or 250 mg/m^2 intravenously twice daily, to begin 48 h after completion of chemotherapy. If patients developed febrile neutropenia, ciprofloxacin was discontinued and parenteral broad-spectrum antipseudomonal antibiotic coverage was instituted. Antibiotics were continued until the ANC was over 0.5×10^9/l, and clinical signs of infection resolved. Guidelines for transfusion support included prophylactic platelet transfusion at a threshold of 10.0×10^9/l in asymptomatic patients and red cell transfusion at a haemoglobin threshold of 80 g/l. Early institution of hyperalimentation was recommended for patients with poor oral intake.

Post-remission therapy

Patients on either study who achieved a complete response (CR) after one or two cycles of induction chemotherapy were registered to receive two cycles of post-remission therapy. For S0112, this consisted of daunorubicin (45 mg/m^2) intravenously as 24-h continuous intravenous infusion on days 1 and 2, and cytarabine (200 mg/m^2) daily as 24-h continuous infusion on days 1–5. On S0301, an identical regimen was used with the addition of ciclosporin at a loading dose of 6 mg/kg over 2 h, followed by 16 mg/kg per day by continuous intravenous infusion on days 1–2. Performance status 0–2 by SWOG criteria and serum bilirubin less than or equal to twice the IULN were required for post-remission therapy, as was a MUGA scan demonstrating an ejection fraction more than or equal to 45%, for patients with cardiac toxicity after induction therapy. Use of G-CSF or GM-CSF with post-remission therapy was left to the discretion of the treating physician.

Definitions of outcomes

Response criteria were adapted from National Cancer Institute (NCI)-sponsored workshop guidelines (Cheson *et al*, 1990). CR required the following for ≥28 d: marrow with >20% cellularity, <5% blasts and no Auer rods; peripheral blood with neutrophil count $>1.5 \times 10^9$/l, platelet count $>100 \times 10^9$/l, and no blasts; and absence of extra-medullary disease. Responses meeting CR criteria but without evidence of ≥28-d duration were designated as 'unconfirmed CR.' Patients who failed to achieve CR after induction therapy were classified according to type of failure: resistant disease (RD); death during aplasia; or indeterminate, defined as death <7 d after completion of induction therapy or with no persistent leukaemia in the peripheral blood, but no post-induction marrow examination (Cheson *et al*, 2003). Overall survival (OS) was measured from the

date of registration until death from any cause, with observation censored at the date of last contact for patients last known to be alive. Relapse-free survival (RFS) was measured from the date of CR until relapse or death from any cause, with observation censored at the date of last contact for patients last known to be alive without report of relapse. Toxicities were defined and graded according to the NCI Common Toxicity Criteria version 2.0 (http://ctep. cancer.gov/protocolDevelopment/electronic_applications/docs/ ctcv20_4-30-992.pdf) for S0112 and Common Terminology Criteria for Adverse Events version 3.0 (http://ctep.cancer. gov/protocolDevelopment/electronic_applications/docs/ctcaev3. pdf) for S0301.

Laboratory and cytogenetic data

Multi-drug resistance (MDR)-1 expression by the leukaemic blasts was measured using MRK-16 and three colour flow cytometric assays for blasts co-staining with CD34 (hemato-poietic stem/progenitor cell antigen) and CD33 (panmyeloid antigen) as previously described (Leith *et al*, 1999). To assess ciclosporin-inhibited functional drug efflux of the leukaemia blasts, two fluorescent dyes, diethyloxacarbocyanine iodide [Di(OC)$_2$] and rhodamine 123 (Rh123), were measured with and without exposure to ciclosporin (CSA) in a single-colour flow cytometric assay as described in previous reports (Leith *et al*, 1999). MRK-16 expression and CSA-inhibited Di(OC)$_2$ and Rh123 efflux were measured as continuous values using the Kolmogorov-Smirnov statistic, denoted D (Young, 1977). Cytogenetic studies were performed at previously approved laboratories and centrally reviewed by the SWOG Cytogenetics Committee. Cytogenetic abnormalities were grouped according to published criteria adopted by SWOG as favourable, intermediate, unfavourable, or unknown (Slovak *et al*, 2000).

Statistical considerations

Each trial was conducted in two steps to permit termination if early results proved unfavourable. In S0112, if ≥9 of the first 30 patients achieved CR, 25 more would be enrolled; if ≥23 of the 55 achieved CR, results would be considered favourable. This design had critical (α) level 4% if the true CR rate with daunorubicin and cytarabine (AD) is 30%, and power 91% if the true CR rate is 50%. S0301 tested both response and the following indicator toxicity: any Grade 4+ non-haematological toxicity except (i) hyperbilirubinemia or (ii) mucositis assigned Grade 4 due solely to ulceration (not requiring enteral/parenteral nutrition or prophylactic intubation). Initially S0301 would accrue 25 patients; if <7 achieved CR or >10 experienced indicator toxicity, the trial would end, otherwise another 25 would be enrolled. Results would be favourable for ADC (AD + ciclosporin) if ≥16 of 50 achieved CR and <18 experienced indicator toxicity. S0301 had α level <5% if the true CR rate is ≤25% or the true risk of indicator

toxicity is ≥45%, and power ≥89% if the true CR rate is ≥45% and the true risk of indicator toxicity is ≤25%. Neither trial was stopped early, although the S0301 eligibility criteria were modified as described below. Logistic regression analysis and Fisher's exact test (for CR and RD) and proportional hazards regression (for OS and RFS) were used to test the effects on treatment outcomes of patient and disease characteristics including MDR. Distributions of OS and RFS were estimated by the method of Kaplan and Meier (1958). Confidence intervals (CIs) were calculated at the 95% confidence level. Results are based on data available as of March 23, 2009.

Results

Patient accrual and characteristics

Study S0112. A total of 71 patients from 24 institutions were enrolled on study S0112 between August 2001 and April 2003. Ten were ineligible because of non-AML diagnosis ($N = 8$), PS = 4 ($N = 1$), or prestudy MUGA <50% ($N = 1$). An additional patient died before treatment and was excluded from the analysis. Demographic and disease characteristics for the 60 included patients are summarized in Table I. Sixteen patients (27%) had AML secondary to prior MDS ($N = 14$) or

Table I. Demographic and disease characteristics at baseline, by study.

		S0112 (N = 60)	S0301: original eligibility criteria (N = 14)	S0301: revised eligibility criteria (N = 50)
Age (years)		68 (56, 85)	69 (57, 80)	65 (56, 81)
Sex (Males)		29 (48%)	11 (79%)	30 (60%)
Performance Status	0	20 (33%)	2 (14%)	18 (36%)
	1	25 (42%)	7 (50%)	28 (56%)
	2	11 (18%)	2 (14%)	4 (8%)
	3	4 (7%)	3 (21%)	0
Race	White	57 (95%)	14 (100%)	43 (86%)
	Black	2 (3%)	0	3 (6%)
	Asian	1 (2%)	0	0
	Unknown	0	0	4 (8%)
AML onset	*De novo*	44 (73%)	10 (71%)	42 (84%)
	MDS-related	14 (23%)	4 (29%)	7 (14%)
	Treatment related	2 (3%)	0	0
	Unknown	0	0	1 (2%)
Laboratory values*				
WBC count($1·0 \times 10^9$/l)		8·5 (0·6, 152·4)	8·4 (0·7, 91·6)	10·4 (0·6, 172·6)
Platelet count ($1·0 \times 10^9$/l)		57 (5, 438)	47 (12, 159)	57 (13, 643)
Neutrophils (%)		16 (0, 75)	7 (0, 31)	12 (0, 67)
Haemoglobin (g/l)		94 (56, 146)†	90 (75, 106)	92 (57, 124)
Blasts(%)–blood		16 (0, 91)†	35 (0, 88)	23 (0, 98)
Blasts(%)–marrow		51 (13, 97)‡	54 (23, 90)	62 (17,98)
FAB classification	M0	4 (7%)	0	4 (8%)
	M1	15 (25%)	5 (36%)	10 (20%)
	M2	15 (25%)	4 (29%)	10 (20%)
	M4	14 (23%)	3 (21%)	15 (30%)
	M4eos	1 (2%)	0	2 (4%)
	M5	8 (13%)	2 (14%)	6 (12%)
	M6	2 (3%)	0	2 (4%)
	M7	1 (2%)	0	1 (2%)
Cytogenetic Risk classification	Evaluable	51	11	31
	Unfavourable§	19 (37%)	2 (18%)	12 (39%)
	Intermediate§	29 (57%)	7 (64%)	16 (52%)
	– Normal§	25 (49%)	4 (36%)	11 (35%)
	Favourable§	3 (6%)	2 (18%)	3 (10%)

AML, acute myeloid leukaemia; WBC, white blood cell; FAB, French-American-British.
*Median (min, max).
†Not reported for one patient.
‡Not reported for two patients.
§Percentages based on number with evaluable cytogenetics.

prior cytotoxic chemotherapy or chemoradiotherapy ($N = 2$). Pre-treatment cytogenetic results were not available for 9 (15%) eligible patients, either because specimens were not submitted to approved laboratories ($N = 2$) or evaluable studies were not obtained ($N = 7$). Among 51 patients with cytogenetic data, 37% had unfavourable karyotypes. MRK16 expression was detected in 64% of patients, and CSA-inhibited efflux of $Di(OC)_2$ and Rh123 occurred in 73% and 72% respectively, of patients tested for MDR-1 expression or function (Table II).

Study S0301. Study S0301 was activated in October 2003, but was temporarily closed in March 2004 after accruing 14 patients at six institutions due to concern about possible excessive toxicity and early mortality, particularly among older patients with poor performance status. At the time, six patients had died within 30 d of study entry, an additional patient had grade 4 non-haematological toxicities, and median survival was only 2 months, though for the 6 (43%) who achieved CR, the median RFS was 8 months. Because of these results, study S0301 was amended to exclude patients of age 61–70 with PS 3, and patients of age 70+ with PS 2–3. The two-stage design was reinitiated after this modification, so that 25–50 patients meeting the amended eligibility criteria would be enrolled. The results for study S0301 presented below focus primarily on patients registered after the eligibility criteria modification, although results for the first 14 patients are included in some summaries for completeness. After the modification, 55 patients were accrued between August 2004 and December 2006, at 16 institutions. Three patients were ineligible because of non-AML diagnosis ($N = 2$), or presence of concomitant malignancy ($N = 1$) at the time of registration. Two additional patients who did not receive study treatment are excluded from the analysis. Among the 50 eligible patients, 42 (84%) had *de novo* disease, 7 (14%) had prior MDS and 1 (2%) had unknown prior disease status. Pre-treatment cytogenetic results were not available for 19 (40%) eligible patients

because specimens were not submitted to approved laboratories ($N = 7$) or evaluable studies were not obtained ($N = 12$). Among 31 patients with cytogenetic data, 39% had unfavourable karyotypes. MRK16 expression was found in 50% of patients, and CSA-inhibited efflux of $Di(OC)_2$ and Rh123 was seen in 44% and 36% respectively (Table II).

Response to induction therapy

Study S0112. As shown in Table III, 23 (38%) of the 60 patients on S0112 achieved CR (CI 26–52%). Nineteen patients (32%) achieved CR after a single course of induction therapy. An additional 20 patients received a second course of identical induction therapy, and four (20%) of these achieved CR. Nineteen patients (32%, CI 20–45%) had RD following one ($N = 7$) or two ($N = 12$) courses of induction therapy. Responses of nine patients (15%) were indeterminate due to death within 7 d of completing induction therapy or during subsequent aplasia, and the response of nine others was not adequately assessed. Twenty of the first 55 patients achieved CR, fewer than the 23 required for the primary test of effectiveness. Fifty-six (93%) patients experienced non-haematological toxicities of grade 3 or higher. These included 10 (17%) with fatal induction toxicities, most commonly infection ($N = 6$), haemorrhage ($N = 4$) and/or cardiac toxicity ($N = 2$) and 12 others (20%) with grade 4 non-haematological toxicities.

Study S0301. Twenty-two (44%) of the 50 patients on S0301 achieved CR (CI 30–59%), all after a single cycle of induction chemotherapy (Table III). Seven patients received a second course of induction, but none achieved CR. Thirteen patients (26%) had RD (CI 15–40%) following one ($N = 10$) or two ($N = 3$) courses of induction therapy. Responses of six patients (12%) were indeterminate due to death within 7 d of completing induction therapy or during subsequent aplasia, and responses of nine others were not adequately assessed.

Table II. MDR1 Expression and functional efflux at baseline, by study.

		S0112 (N = 60)	S0301: original eligibility criteria (N = 14)	S0301: revised eligibility criteria (N = 50)
MRK16*	Negative	13 (26%)	1 (7%)	21 (42%)
	Weak positive	5 (10%)	4 (29%)	4 (8%)
	Positive	32 (64%)	9 (64%)	25 (50%)
$Di(OC)_2$ efflux*	Negative	9 (24%)	1 (10%)	17 (47%)
	Weak positive	1 (3%)	1 (10%)	3 (8%)
	Positive	27 (73%)	8 (80%)	16 (44%)
	Not done	13	4	14
Rh123 efflux*	Negative	8 (25%)	2 (22%)	20 (56%)
	Weak positive	1 (3%)	0 (0%)	3 (8%)
	Positive	23 (72%)	7 (78%)	13 (36%)
	Not done	18	5	14

*Negative: Kolmogorov-Smirnov $D < 0.15$; Weak positive: $0.15 \leq D < 0.20$; Positive: $D \geq 0.2$.

Table III. Response to induction chemotherapy, by study.

		S0112 (N = 60)	S0301: original eligibility criteria (N = 14)	S0301: revised eligibility criteria (N = 50)
Complete response	Confirmed	6 (10%)	4 (29%)	10 (20%)
	Unconfirmed	17 (28%)	2 (14%)	12 (24%)
	Total	23 (38%)	6 (43%)	22 (44%)
Resistant disease		19 (32%)	2 (14%)	13 (26%)
Died during aplasia		5 (8%)	2 (14%)	2 (4%)
Died <7 d after 1st course		4 (7%)	3 (21%)	4 (8%)
Died without bone marrow exam		2 (3%)	1 (7%)	4 (8%)
Assessment inadequate		7 (12%)	0 (0%)	5 (10%)

Forty patients (80%) had non-haematological toxicities of grade 3 or higher. Six patients (12%) suffered fatal induction toxicities including infection (N = 3), pulmonary haemorrhage (N = 2) and asystole (N = 1) and eight others (16%) experienced grade 4 non-haematological toxicities. Consequently, the ADC regimen met both the response and toxicity criteria defined for study S0301.

Post-remission therapy

Study S0112. On S0112, 3 patients who achieved a CR were not registered for consolidation. Thirty-one patients were registered for consolidation, though 11 were ineligible and excluded from the analysis. Of the 20 evaluable patients on S0112 who were registered and received protocol post-remission therapy, 19 completed the two cycles of consolidation therapy specified in the protocol. There were no fatal toxicities, and one patient experienced grade 4 non-haematological toxicities including lung infection, acute respiratory distress syndrome, central nervous system haemorrhage, confusion, anxiety and hepatotoxicity.

Study S0301. On S0301, 19 patients were registered for consolidation. One patient was excluded due to incomplete blood count recovery following induction therapy. Of the 18 patients, there was one fatality from sepsis, and another patient experienced grade 4 non-haematological toxicity (high glucose). Three patients did not receive the second cycle of protocol-specified consolidation therapy.

Overall and relapse-free survival

Study S0112. Of the 60 evaluable patients on S0112, 57 have died. The remaining patients were last known to be alive 73, 75 and 81 months after entry into the study. The median OS was 7 months (CI 3–10 months), and the estimated probability of survival was 33% (CI 21–45%) at 1 year and 15% (CI 6–24%) at 2 years (Fig 1).

Of the 23 patients who achieved complete remission on S0112, 19 have relapsed and one other died without report of disease status. The estimated median RFS was 8 months (CI
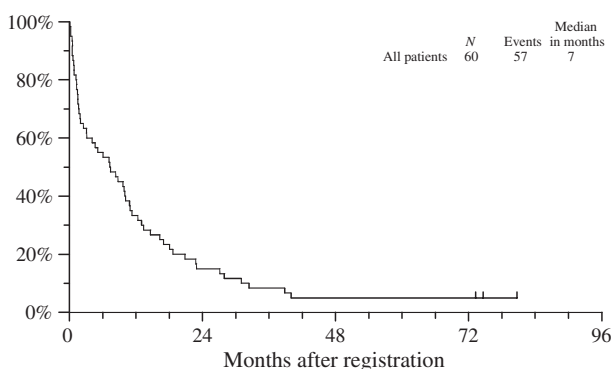
Fig 1. S0112 overall survival.

5–11 months), and the estimated probability of RFS was 35% (CI 15–54%) at 1 year, and 22% (CI 5–39%) at 2 years (Fig 2).

Study S0301. Of the 50 evaluable patients on S0301, 40 have died, and the remaining 10 patients were last known to be alive between 22 and 34 months (median 27 months). The estimated median OS was 6 months (CI 2–11 months), and the estimated probability of survival was 38% (CI 24–51%) at 1 year, and 28% (CI 16–40%) at 2 years (Fig 3).

Of the 22 patients who achieved CR on S0301, eleven have relapsed and four others have died without report of relapse.
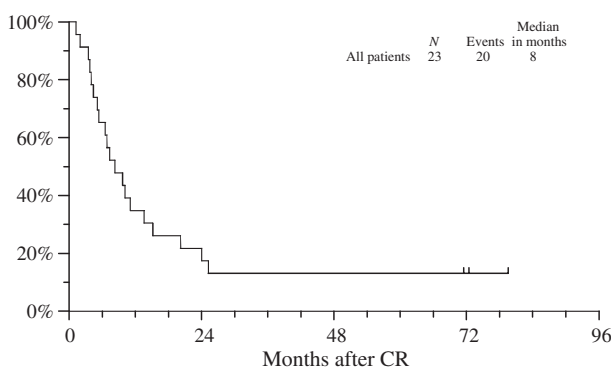
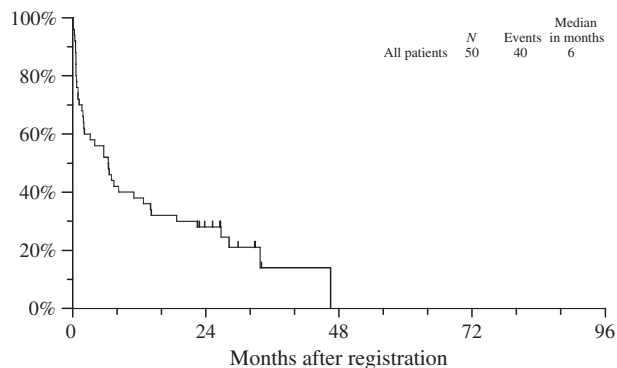**Fig 2.** S0112 relapse-free survival.

Fig 3. S0301 revised eligibility: overall survival.

The estimated median RFS was 14 months (CI 7–19 months) and the estimated probability of RFS was 55% (CI 34–75%) at 1 year and 36% (CI 16–56%) at 2 years (Fig 4).

Effect of MDR and other demographics on treatment outcomes

Analyses of cytogenetics, phenotypic drug efflux, CD34 expression, white count, performance status and history of prior antecedent myelodysplastic syndrome did not identify a population of patients specifically benefitting from the addition of ciclosporin (Tables SI–SIII).

The impact of MDR phenotype on treatment outcome was analysed separately for S0112 (without ciclosporin) and S0301 (with ciclosporin). In S0112, MDR as measured by Rh123 efflux was associated with a lower CR rate ($P = 0.036$), but neither MRK16 expression nor $Di(OC)_2$ efflux was statistically significantly associated with remission rates (Table IV). RD as a cause of induction failure tended to increase with CSA-inhibited efflux of both $Di(OC)_2$ ($P = 0.48$) and Rh123 ($P = 0.54$). None of the measures of MDR were significantly associated with OS or RFS, a finding that is not unexpected given the small number of remitting patients. In S0301, the CR rate did not vary significantly according to any of the three measures of MDR, although the RD rate tended to increase
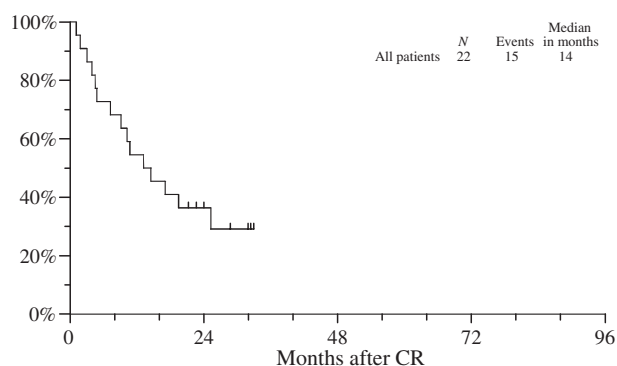

Fig 4. S0301 revised eligibility: relapse-free survival.

with increasing $Di(OC)_2$ efflux ($P = 0.049$). None of the other associations of MDR phenotype and treatment outcome were significant.

Discussion

In these sequential phase II studies, the administration of daunorubicin by continuous infusion along with cytarabine did not significantly alter the toxicity profile or the efficacy of the standard '7 + 3' regimen in older adults with newly diagnosed AML. In contrast, the addition of ciclosporin to the continuous infusion regimen increased toxicity in very old patients with poor performance status. This finding is consistent with earlier SWOG studies that have shown the interaction of age and performance status in determining outcome for this older AML population (Appelbaum *et al*, 2006). Once this subgroup of patients was excluded from enrollment, the toxicities in S0301 were not markedly different from those seen in S0112. Although study S0112 did not meet its planned test criterion for efficacy, the CR rate for patients on both studies were consistent with past experience for older AML patients; 38% (CI 26–52%) with AD on S0112, and 44% (CI 30–59%) with ADC on S0301.

These phase II studies were not designed to formally compare the effectiveness of these two regimens. Nevertheless, it is noteworthy that the addition of ciclosporin in S0301 did not produce a markedly higher CR rate or lower RD rate than seen in S0112, even though the S0301 patients had a better overall performance status. Still, RFS appeared to be somewhat longer in S0301 (with ciclosporin, 14 months) compared to S0112 (without ciclosporin, 8 months). Nevertheless, this was not a prospective randomized study and the confidence intervals for RFS and OS overlap between studies. Such comparisons must be interpreted cautiously in two sequential trials. Indeed, after the eligibility criteria were revised for S0301, restricting enrollment for older patients with poor performance status, baseline patient characteristics became slightly more favourable on S0301 than S0112. Nonetheless, the possibility of improved remission maintenance with the addition of ciclosporin cannot be excluded. Such an effect is consistent with the outcome of the phase III randomized trial, S9126 (List *et al*, 2001), where the addition of ciclosporin to a regimen of continuous infusion daunorubicin plus high-dose cytarabine did not improve complete response rates, but did lead to a significantly lower rate of RD and prolonged RFS and OS in younger patients with recurrent or secondary AML. The current phase II studies suggest that ciclosporin as a modulator of MDR in older patients may impact remission duration rather than remission induction.

Various measures of the MDR phenotype were studied in S0112 and S0301 with the hypothesis that their association with outcome would be seen most clearly in S0112, but attenuated in S0301 with the addition of ciclosporin. The baseline assessment of phenotypic and functional Pgp appeared to differ substantially between the two studies

Table IV. Effects of MDR1 expression and functional efflux on response to induction chemotherapy, by study.

		Study S0112			Study S0301: revised eligibility criteria		
		Patients	Complete response	Resistant disease	Patients	Complete response	Resistant disease
MRK16	Negative	13	5 (38%)	4 (31%)	21	10 (48%)	3 (14%)
	Weak positive	5	4 (80%)	1 (20%)	4	1 (25%)	3 (75%)
	Positive	32	10 (31%)	11 (34%)	25	11 (44%)	7 (28%)
	Trend*		$P = 0.15$	$P = 0.098$		$P = 0.30$	$P = 0.94$
Di(OC)$_2$ efflux	Negative	9	6 (67%)	1 (11%)	17	9 (53%)	2 (12%)
	Weak positive	1	1 (100%)	0 (0%)	3	1 (33%)	0 (0%)
	Positive	27	8 (30%)	11 (41%)	16	6 (38%)	7 (44%)
	Not done	13	4 (31%)	4 (31%)	14	6 (43%)	4 (29%)
	Trend*		$P = 0.34$	$P = 0.48$		$P = 0.52$	$P = 0.049$
Rh123 efflux	Negative	8	5 (63%)	2 (25%)	20	12 (60%)	2 (10%)
	Weak positive	1	1 (100%)	0 (0%)	3	0 (0%)	1 (33%)
	Positive	23	6 (26%)	10 (43%)	13	4 (31%)	6 (46%)
	Not done	18	7 (39%)	4 (22%)	14	6 (43%)	4 (29%)
	Trend*		$P = 0.036$	$P = 0.54$		$P = 0.49$	$P = 0.12$

*Two-sided P-value based on logistic regression, treating MRK16 expression or efflux D-value as a continuous variable, excluding patients with assay not done.

(Table II). Whether this difference was a chance occurrence or the result of an unidentified change in methodology is unknown. Although there were trends in association for several measures of MDR with lack of CR and persistent disease in S0112, these patterns were not strikingly altered by the addition of ciclosporin in S0301 (Table IV). However, because of the differences in baseline measures, the proportion of patients not evaluable for MDR function in S0301 (28%), and the limited number of complete responders, comparisons of MDR expression and treatment responses between studies are difficult.

Other studies have investigated Pgp-modulation in patients with relapsed and refractory AML. In the UK Medical Research Council (MRC) AML-R study, patients with relapsed or refractory AML were randomized to ADE (cytarabine, daunorubicin, etoposide) with and without ciclosporin at 5 mg/kg per day (Liu Yin *et al,* 2001). There was no apparent benefit, though the ciclosporin dosing was arguably inadequate for reversing Pgp-mediated efflux (List *et al,* 2001; Mahadevan & List, 2004; Qadir *et al,* 2005). A salient feature of many of the Pgp-efflux inhibitors is their pharmacokinetic effects on other cytotoxic drugs, such as anthracyclines and etoposide. This has typically required dose-attenuation of cytotoxic drugs with the goal of achieving equitoxic regimens with and without the modifying agent. This strategy, in turn, creates a complex interaction of cytotoxic drugs with a spectrum of toxicities and risks underdosing of patients without pharmacokinetic changes. Other Pgp-inhibitors, such as zosuquidar, show very little pharmacodynamic effects on cytotoxic agents, although a recent Eastern Cooperative Oncology Group (ECOG) study in older patients with newly diagnosed AML using zosuquidar by prolonged infusion with conventionally administered

daunorubicin and cytarabine (E3999) was also ineffective at improving outcomes (Cripe *et al,* 2006).

Other investigators have found similar results using the ciclosporin analogue, PSC-833. In the Cancer and Leukaemia Group B (CALGB) study of patients over 60 with untreated AML (CALGB 9720), daunorubicin was given by conventional bolus infusion along with etoposide and cytarabine in parallel prospective comparative studies with and without PSC-833 (Baer *et al,* 2002). Prior to this study, in separate phase I studies, daunorubicin was dose-adjusted to achieve equivalent toxicity in those patients receiving PSC-833 to those not receiving PSC-833. The use of PSC-833 required a 30% dose reduction of daunorubicin and 40% reduction in etoposide dose. Despite this dose adjustment, the arm receiving PSC-833 was closed early due to excessive toxicity. Though there were trends toward improved response and disease-free survival (DFS) for patients receiving PSC-833, they did not reach statistical significance. Similar results were seen with patients younger than 60 years of age in CALGB 9621 (Kolitz *et al,* 2004). In this set of phase II trials, PSC-833 appeared to improve DFS and first round induction efficacy, as well as improve OS in patients younger than 45 years, however, a confirmatory phase III study (CALGB 19808) was stopped early when PSC-833 became unavailable (Kolitz *et al,* 2005).

The Dutch-Belgian Haemato-Oncology Cooperative Group (HOVON) and MRC performed a parallel prospective comparative study with cytarabine and daunorubicin by bolus infusion at attenuated doses with and without PSC-833, in patients aged 60 years and over. Patients received two induction cycles followed by a single cycle without PSC-833 as consolidation (van der Holt *et al,* 2005). Again, there was a trend toward improved DFS for patients who received

PSC-833, though other outcomes were not improved. A later MRC trial, LRF AML14, showed increased induction mortality with PSC-833, with no apparent benefit to remission duration. (Burnett *et al*, 2009). Similarly, the ECOG performed a prospective comparative study utilizing PSC-833 along with conventional bolus infusion of mitoxantrone, etoposide and cytarabine in patients with relapsed and refractory disease in E2995 (Greenberg *et al*, 2004), again without improvement in any outcome parameters assessed, though PSC-833 decreased clearance of mitoxantrone and etoposide in predictable fashion.

SWOG also performed a phase I dose-escalation study (S9617) using mitoxantrone and etoposide by conventional bolus administration along with PSC-833 by continuous infusion, and determined a maximum tolerated dose (MTD) consistent with other studies in patients with AML over 55 years-old (Chauncey *et al*, 2000). However, after completion of the study, the regimen was not further pursued based on results of S9333, which showed that mitoxantrone and etoposide regimen given as first-line therapy for older patients with AML, was not superior to conventional daunorubicin and cytarabine (Anderson *et al*, 2002). Of note, in the AML11 prospective comparative trial of three different induction regimens, the MRC showed that their etoposide-containing regimen was less effective at achieving remission than the combination of anthracyclines and cytarabine in older AML patients (Goldstone *et al*, 2001).

In contrast, the GOELAMS (Groupe Ouest Est d'Etude des Leucémies et Autres Maladies du Sang) study in patients with *de novo* AML aged between 15 and 60 years old, used quinine by continuous infusion along with cytarabine by continuous infusion and idarubicin by conventional bolus administration (Solary *et al*, 2003). Patients with persistent disease after this regimen received high-dose cytarabine and mitoxantrone, and patients in CR received consolidation with an amsacrine and cytarabine regimen. After that, patients underwent ablative allogeneic transplant from a sibling donor or chemotherapy consolidation with high-dose cytarabine and mitoxantrone followed by amsacrine and etoposide with and without quinine. In this study, quinine improved response, EFS and OS for those patients with demonstrated Rh123 efflux, though this subgroup analysis did not reach statistical significance for EFS and OS.

MDR-1 expression may serve as a marker for other mechanisms of resistance that parallel the induction and/or selection of MDR-1 expression. In addition to Pgp, other ATP-binding cassette (ABC)-efflux mediators such as MRP and BCRP, as well as LRP, correlate with resistance in various clinical settings, and PSC-833 and ciclosporin may be clinically less effective at modulating drug-efflux by these other transporters. Because the presence of other transporters was not assessed in the current study, their significance in this dataset is unknown.

It is likely that many mutations exist in leukaemia cells in older patients with AML, as well as redundant mechanisms of resistance, complicating any therapeutic approach. While the increased expression of an MDR phenotype in the blasts of older patients continues to represent a theoretically attractive target, attempts to reverse that phenotype as a single approach have not had the anticipated success in this patient population.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table SI. Immunophenotype at baseline, by study.

Table SII. Effects of immunophenotype on response to induction chemotherapy, by study.

Table SIII. Effects of demographic and disease characteristics at baseline on response to induction chemotherapy, by study.

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