

## Introduction

- Secondary AML (sAML), AML following myelodysplastic syndromes (MDS) or prior chemotherapy, is commonly associated with the multidrug resistant (MDR) phenotype through overexpression of P-glycoprotein (Pgp) and other efflux transporters<sup>1</sup>
- MDR mechanisms are a cause of treatment failure in sAML patients receiving standard anthracycline-based remission-induction regimens such as daunorubicin plus cytarabine<sup>2</sup>
- Two studies that utilised daunorubicin and cytarabine induction therapy reported complete response rates of 24% and 26% in patients with sAML<sup>3,4</sup>
- This response rate is significantly lower than that for *de novo* AML and consequently improved treatment options are needed for patients with sAML<sup>2</sup>
- Amonafide (AS1413) is an anti-leukaemic agent that induces apoptosis by intercalating into DNA and that is unaffected by multidrug resistance mechanisms<sup>5,6</sup>

## Objectives

- To evaluate the efficacy of amonafide in combination with cytarabine in patients with previously untreated sAML
- To assess the safety and tolerability of amonafide in combination with cytarabine
- To assess whether clinical responses observed following treatment with amonafide were related to the ability of the drug to evade MDR mechanisms

## Methods

### Phase II study design

- Multicenter, open-label, phase II study evaluating amonafide plus cytarabine in patients with secondary AML
- Patients aged ≥ 18 yrs with:
  - Histologic diagnosis of AML according to WHO diagnostic criteria (at least 20% myeloid blasts in the bone marrow or blood), with FAB classification other than M3 (acute promyelocytic leukaemia)
  - Known and documented exposure to prior leukaemogenic chemotherapy or radiotherapy, or diagnosis of MDS for at least 3 months prior to study entry with prior bone marrow aspirate and biopsy documenting MDS available for central pathology review
- Cytarabine was administered at a dose of 200 mg/m<sup>2</sup>/day i.v. as a continuous infusion on days 1–7
- Amonafide was administered at a dose of 600 mg/m<sup>2</sup>/day i.v. over 4 hours on days 1–5
- A re-induction cycle was permitted if the day 14 bone marrow demonstrated persistent leukaemia
- Post-remission therapies (PRT) were stem cell transplant (SCT) or high dose (HiDAC)/intermediate dose (IDAC) cytarabine, according to age

### Safety assessments

- Standard clinical and laboratory safety assessments
- Adverse events

### Efficacy assessments

- All bone marrow aspirates and biopsies underwent independent pathology review
- Complete remission (CR) was defined as all of the following:
  - Peripheral blood counts:
    - ANC ≥ 1,000/μl (≥ 1.0 × 10<sup>9</sup>/L)
    - Platelet count ≥ 100,000/μl (≥ 100.0 × 10<sup>9</sup>/L)
  - Bone marrow:
    - < 5% blasts cells in a 200-cell differential
    - No Auer rods
    - No extramedullary leukaemia
- Complete remission with incomplete haematopoietic recovery (CRi) was defined as:
  - Satisfying all criteria for CR except
    - ANC < 1,000/μl and/or
    - Platelet count < 100,000/μl, but not requiring platelet transfusion

### Blast cell assessments

- Pre-treatment AML blasts from a subset of patients were analysed retrospectively for Pgp-mediated transport
- Pgp-mediated transport (efflux) was assessed by comparing uptake of drug (amonafide or daunorubicin) in the presence and absence of the Pgp inhibitor cyclosporin A (CSA)<sup>7</sup>
- Efflux (Efflux<sub>app</sub>) was calculated as (MFI2-MFI1 / MFI2 × 100), where MFI1 is the mean fluorescence index (MFI) of drug measured by flow cytometry and MFI2 is the MFI of the drug in the presence of CSA<sup>7</sup>

### Study variables

- Pharmacokinetic parameters
- Primary safety outcomes: adverse events, laboratory abnormalities
- Efficacy endpoints
  - CR+CRi rate
  - Duration of remission (time to relapse for responders)
  - Overall survival (OS)
- Pgp-mediated transport of daunorubicin and amonafide in patient cells

## Results

### Patient characteristics

Table 1: Baseline characteristics of patients

Total number	88
Male / female, n (%)	41 (47%) / 47 (53%)
Median age, years (range)	62.5 (23 – 87)
ECOG performance status, n (%)	
0	21 (24%)
1	51 (58%)
2	14 (16%)
3	2 (2%)
Type of secondary AML, n (%)	
Prior leukaemogenic therapy*	45 (51%)
Prior MDS only	43 (49%)
Treatment for prior MDS†, n (%)	
Yes	27 (31%)
No	21 (24%)
Cytogenetic risk group‡, n (%)	
Favourable	1 (1%)
Intermediate	36 (41%)
Unfavourable	42 (48%)
Unknown	9 (10%)

\* Five of those who had prior leukaemogenic therapy also had prior MDS  
 † A total of 48 patients had prior treatment for MDS  
 ‡ As defined by SWOG-ECOG criteria<sup>8</sup> with the modification that cytogenetics of unknown prognostic significance are classified as "intermediate"

### Safety

Table 2: Frequent non-haematologic AEs of NCI-CTCAE ≥ grade 3\* (n (%))

Preferred Term (event)	Grade 3		Grade 4		Grade 5	
	All	Related	All	Related	All	Related
Fatigue	7 (8.0%)	4 (4.5%)	1 (1.1%)	1 (1.1%)	0	0
Diarrhoea	7 (8.0%)	6 (6.8%)	0	0	0	0
Rash/rash generalised	10 (11.4%)	7 (8.0%)	0	0	0	0
Pneumonia	8 (9.1%)	3 (3.4%)	3 (3.4%)	3 (3.4%)	1 (1.1%)	1 (1.1%)
Pyrexia	8 (9.1%)	4 (4.5%)	1 (1.1%)	1 (1.1%)	0	0
Bacteraemia	9 (10.2%)	6 (6.8%)	0	0	1 (1.1%)	1 (1.1%)
Sepsis/septic shock	2 (2.3%)	1 (1.1%)	5 (5.7%)	3 (3.4%)	5 (5.7%)	1 (1.1%)
Hypotension	8 (9.1%)	3 (3.4%)	3 (3.4%)	1 (1.1%)	3 (3.4%)	2 (2.3%)
Respiratory failure	2 (2.3%)	1 (1.1%)	3 (3.4%)	2 (2.3%)	3 (3.4%)	2 (2.3%)

\*Number of patients (%) for events occurring at ≥ grade 3 in more than 5% of patients; 'related' - assessed by the investigator as possibly, probably or definitely related to amonafide

- Deaths on study:
  - Deaths within 14 days of first dose were 6/88 (6.8%)
  - Deaths within 28 days of first dose were 18/88 (20.4%)
  - Total deaths on study are 69/88 (78.4%) at 18 months follow up
- The safety profile of the combination of amonafide and cytarabine is predictable and manageable in this poor-risk patient population

### Efficacy

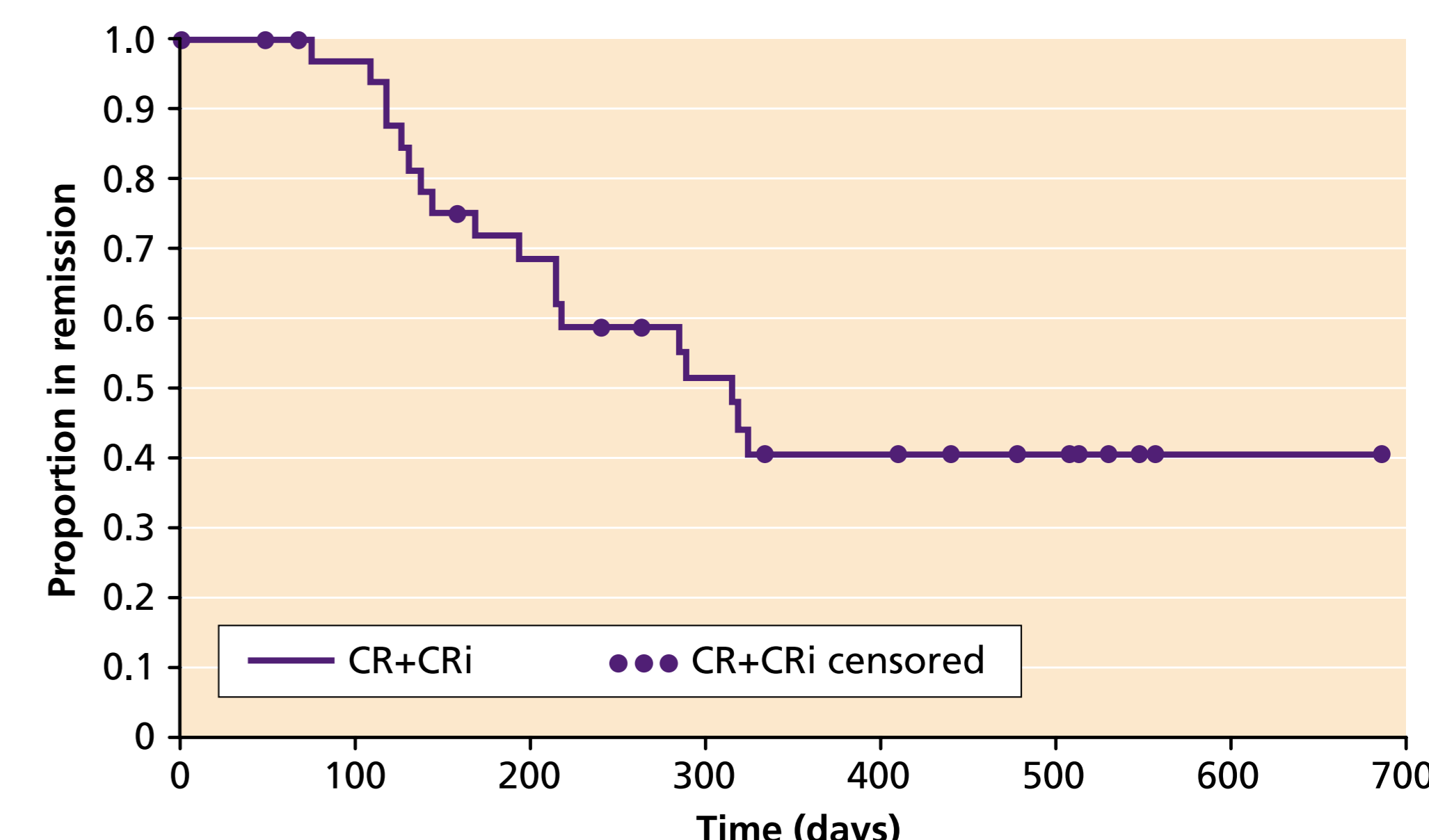
Table 3: Response rates\* (n/total (%))

CR + CRi	37/88 (42.0%)
CR	34/88 (38.6%)
CRi	3/88 (3.4%)
CR+CRi by sex	
Male	16/41 (39.0%)
Female	21/47 (44.7%)
CR+CRi by age	
<60	13/33 (39.4%)
≥60	24/55 (43.6%)
CR+CRi by ECOG performance status	
0	13/21 (61.9%)
1	21/51 (41.2%)
2	3/14 (21.4%)
3	0/2 (0.0%)
CR+CRi by type of secondary AML	
Prior leukaemogenic therapy	18/45 (40.0%)
Prior MDS only	19/43 (44.2%)
CR+CRi by treatment for prior MDS	
Yes	10/27 (37.0%)
No	9/21 (42.9%)
CR+CRi by cytogenetic risk group	
Favourable	1/1 (100%)
Intermediate	22/36 (61.1%)
Unfavourable	10/42 (23.8%)
Unknown	4/9 (44.4%)

\*Subgroup percentages are based upon number in subgroup at baseline

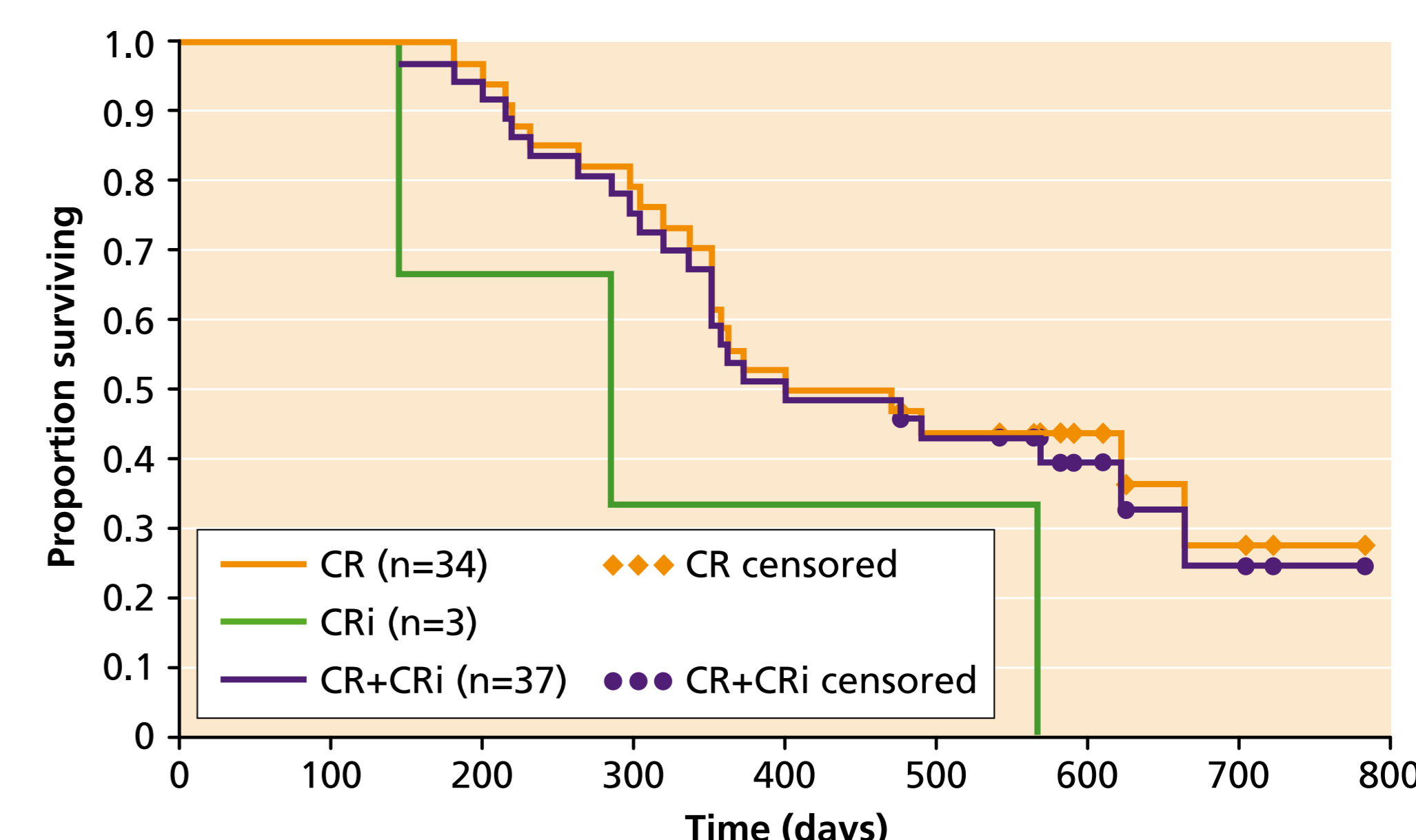
- A CR+CRi rate of 42.0% was observed in this study, with 38.6% of patients achieving a CR
- The CR+CRi rate was maintained among subsets of sAML patients with additional poor risk factors

Figure 1: Duration of remission among responders



- The proportion of responders in continuous CR/CRi at 18 months by Kaplan-Meier estimate was 40%

Figure 2: Overall survival (responders)



- The overall survival of all responders at 18 months by Kaplan-Meier estimate was 43% (44% for patients with CR and 33% for patients with CRi)

### Drug efflux from leukaemic blasts

- Pre-treatment blasts from patients with sAML showed greater efflux of daunorubicin than amonafide
- This difference was also observed in blasts from those patients who achieved remission following amonafide + cytarabine and those harbouring an unfavourable cytogenetic risk karyotype

Table 4: Mean efflux of amonafide and daunorubicin from blasts of patients with sAML

	n	Mean amonafide efflux <sub>app</sub> (s.e.m.)	Mean daunorubicin efflux <sub>app</sub> (s.e.m.)	Significance
All	15	5.21 (3.2)	16.2 (2.1)	p=0.0083
Unfavourable cytogenetics	10	0.13 (3.7)	16.1 (2.2)	p=0.0015
CR patients	7	3.97 (6.7)	21.3 (2.9)	p=0.035

## Conclusions

- The overall remission rate of 42% (38.6% CR; 3.2% CRi) in patients with secondary AML treated with amonafide and cytarabine compares favourably with historical data from similar multi-institution and cooperative group studies in which patients were treated with daunorubicin and cytarabine
- Efficacy of the amonafide-cytarabine combination is maintained in elderly and other poor-risk subsets of patients
- Secondary AML is often associated with overexpression of Pgp and other efflux transporters that confer MDR
- The lower efflux of amonafide vs. daunorubicin from blasts of sAML patients in this study is consistent with evasion of MDR mechanisms by amonafide
- Evasion of MDR by amonafide may contribute to observed differences in response rates between studies using amonafide and those using daunorubicin in sAML
- ACCEDE, a pivotal phase III study evaluating the combination of amonafide and cytarabine vs. a standard daunorubicin and cytarabine regimen is ongoing and includes further, prospective evaluation of MDR parameters

## References

- Greenberg PL, et al. Mitoxantrone, etoposide and cytarabine with or without valspodar in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome: a phase III trial (E2995). *J Clin Oncol.* 2004;22:1078-86
- Visani G, et al. Chemotherapy of Secondary Leukemias. *Leuk Lymphoma.* 2000;37:543-9
- Leith CP, et al. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group Study. *Blood.* 1999;94:1086-99
- Anderson JE, et al. Outcome after induction chemotherapy for older patients with acute myeloid leukemia is not improved with mitoxantrone and etoposide compared to cytarabine and daunorubicin: a Southwest Oncology Group study. *Blood.* 2002;100:3869-76
- Otake Y, et al. Amonafide interferes with topoisomerase II binding to DNA and induces chromatin disorganization. *AACR Meeting Abstracts* 2008;647
- Chau M, et al. Amonafide, a topoisomerase II inhibitor, is unaffected by P-glycoprotein-mediated efflux. *Leukemia Res.* 2008;32:465-73
- Burcu M, et al. Amonafide L-malate is not a substrate for multidrug resistance proteins in secondary acute myeloid leukemia. *Leukemia.* 2008;22:2110-5
- Slovak ML, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood.* 2000;96:4075-83