
**AMONAFIDE:
A Topoisomerase II Inhibitor with
Novel Pharmacological Properties and
Unique Activity for the Treatment of
Secondary AML**

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Amonafide

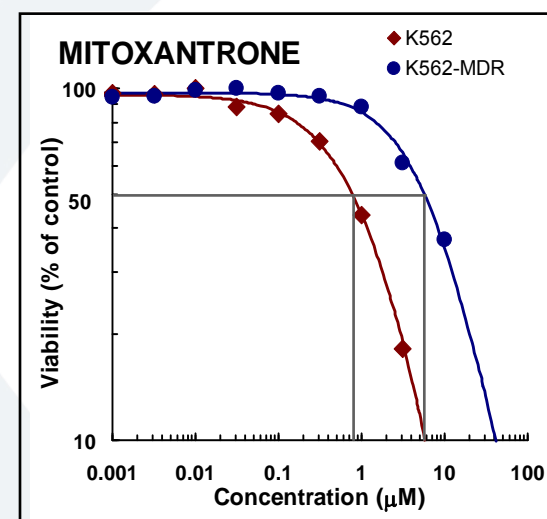
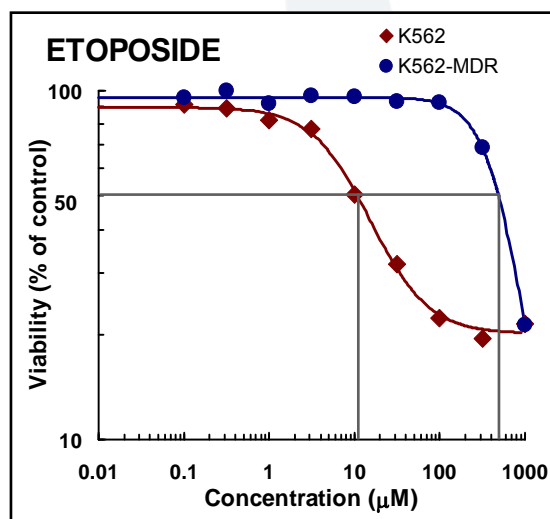
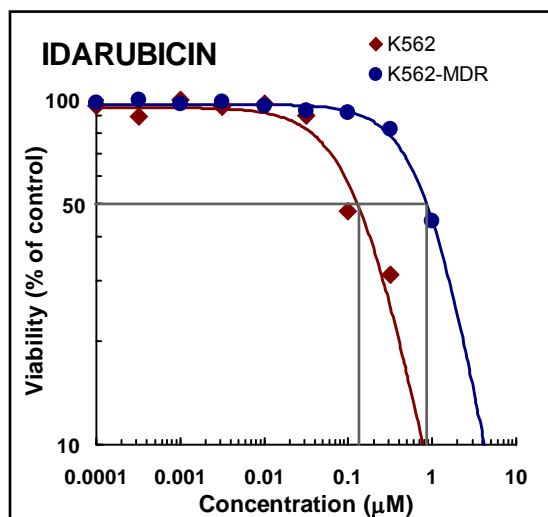
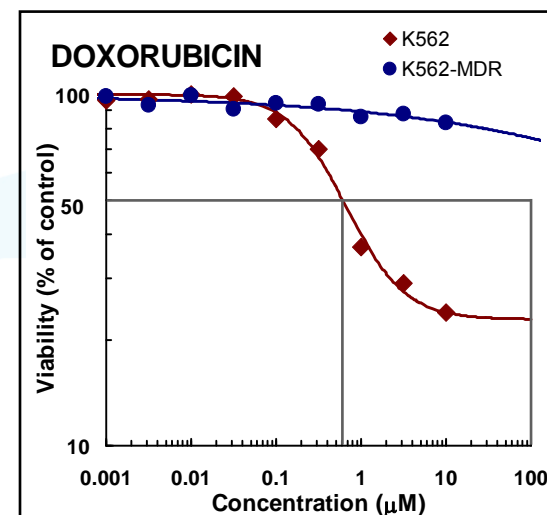
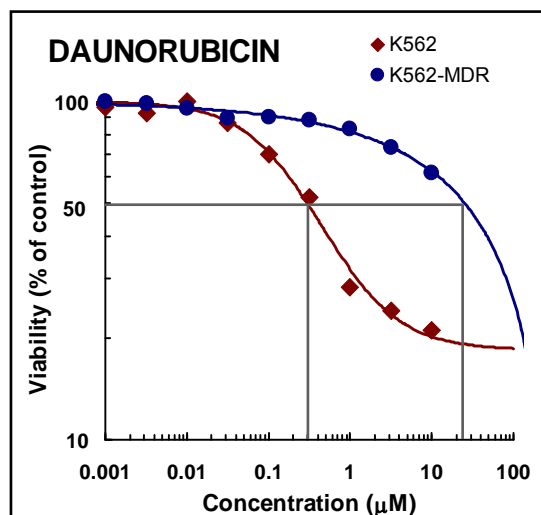
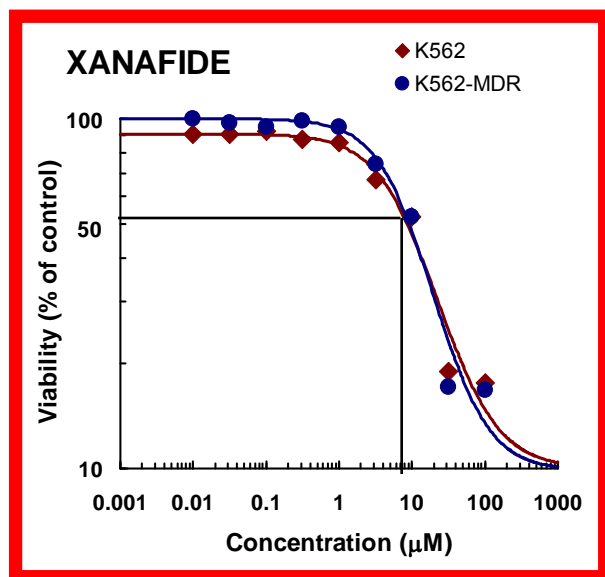
- **Novel mechanism of action**
 - Intercalates into DNA
 - Induces apoptotic signaling by blocking TOPO II binding to DNA
 - Does not affect the DNA/TOPO II cleavable complex nor cause DNA fragmentation
- **Unique Pharmacological Property**
 - Not affected by Multi-Drug Resistance (MDR) phenotype
 - Neither a substrate nor inhibitor of P-glycoprotein (Pgp) and other efflux transporters
 - Retained in leukemia cells that over-express Pgp and other drug efflux proteins
 - Not cross-resistant with daunorubicin, idarubicin, mitoxantrone, or etoposide in leukemia cells that over-express P-glycoprotein
- **Phase 1 clinical trials**
 - Durable Complete Remissions
 - Acceptable safety profile when combined with 7 days of std dose ara-C

K562/DOX leukemia cell line is resistant to doxorubicin due to over-expression of P-glycoprotein.

Other drugs are cross-resistant, i.e., multi-drug resistant phenotype as reflected by 1-2 log increase in IC₅₀ value.

Amonafide evades P-glycoprotein-mediated efflux and does not share the multi-drug resistant phenotype.

IC₅₀ of Drugs vs K562 and K562/DOX (MDR)



There is a high frequency of over-expression of P-glycoprotein in leukemic blasts from elderly populations and other poor risk patients.

MDR1 (Pgp) Multi-drug Resistance in AML

- **Most common cause of clinical treatment failure**
- **Target of significant drug development efforts over past decade**
- **Commonly expressed in AML, including poor-risk AML:**

Trials	Median Age (yrs)	N	% MDR Over-expression
SWOG 9031	68	211	71%
SWOG 9333	68	328	50%
ECOG 2995	58	129	64%
sAML/Hi Risk MDS			71%
CALGB 9720	70	120	65%
SWOG 8600	45	400	30%



Three phase 1 trials defined the maximally tolerated dose of amonafide, alone and in combination with cytarabine (ara-C).

Phase 1 Trials: Responses in Acute Leukemia

Study	N	Agent	Tumor Response
O'Brien Keating (MD Anderson)	24	Single agent	Cleared circulating blasts and bone marrow leukemia cytoreduction
AM-03 (North Shore)	17	Single agent	4 CR
AM-04 (North Shore)	26	Combination with ara-C	10 CR + 2 significant hematologic improvement

Amonafide + ara-C Active in Phase 1: AM04

(Allen, et al. NorthShore Univ. Hospital)

Amonafide (escalating dose) + ara-C

(200mg/m²/d CI x 7)

Response Rates - CR/N

Dose (mg/m ² /d x 5)	Leukemia Diagnosis			Totals	
	2° AML	Relapsed AML	CML BC	Total # Pts.	Complete Remission
600	3/5	0/0	0/1	6	3/6
700	3/8	3/6	1/1	15	7/15
800	1/2	1/2	0/1	5	2/5
	7/15	4/8	1/3	26	12/26

AM04 Remission Duration

(Allen, et al. NorthShore Univ. Hospital)

Diagnosis	Post-Remission Therapy		CR (or response) Duration (months)
Secondary AML	Yes	BMT	>120
		HiDAC	>29
		HiDAC	8
		HiDAC	7.5
	No		10
			5
			<2
Relapsed <i>de novo</i> AML	Yes	BMT	>3*
		ara-C	5.5
	No		2
			2
CML Blast Crisis	No		2
HiDAC, High-Dose ara-C; BMT, Bone marrow transplant * follow-up not documented after bone marrow transplant			

First Line Therapy

**Amonafide + ara-C in
Secondary AML**

***AML200* Phase 2 Trial
N = 88**

AML 200 Trial: Treatment Schema

Remission Induction Chemotherapy (1 or 2 courses)

Amonafide 600 mg/m²/d d 1-5
ara-C 200 mg/m²/d CI d 1-7



Post-Remission Therapy

- Transplant, if eligible
- If not eligible, 3 courses of ara-C:
 - < 60 yo - HiDAC 3 gm/m²
 - ≥ 60 yo - ara-C 1 gm/m²

Study Objectives

- **Primary**

- Rate of complete remission with or without hematopoietic recovery (CR + CRi)

- **Secondary**

- Median duration of CR + CRi
- Proportion of patients in CR + CRi at 6, 12 and 18 months
- Median duration of overall survival
- Safety and adverse events

Eligibility

- AML (non M3) per WHO criteria
- **Either:**
 - **Antecedent MDS \geq 3 months (confirmed by central path review)**
 - OR**
 - **documented prior leukemogenic chemo &/or radiation**
- Age \geq 18 years, ECOG PS 0-2
- No prior AML induction therapy
- LVEF \geq 50%, adequate renal, hepatic function
- No recent cytotoxic therapy
- No serious concomitant illness

Patient Demographics

Male Gender (%)	41 (47)
Median Age, years (range)	63 (23-87)
ECOG PS (%)	
0	21 (23.9)
1	51 (58.0)
2	14 (15.9)
3	2 (2.2)

Characteristics of sAML

Prior MDS Only	40 (45.5%)
Prior treatment for MDS	20
No prior treatment for MDS	19
Treatment Unknown	1
Prior Leukemogenic Therapy	48 (54.5%)
with prior MDS	8
Prior treatment for MDS	2
No prior treatment for MDS	6
Exposure to Prior Leukemogenic Agents	48
Radiation therapy	20
Anti-cancer drug	46
Prior anti-cancer drugs per patient, median (range)	4 (1-29)
Prior treatment regimens per patient, median (range)	2 (1-6)

Cytogenetic Risk Group, n (%)

Cytogenetic Risk Group*	N (%)
Favorable	1 (1.1)
t(8;21)	1 (1.1)
Intermediate	37 (42.0)
Diploid	29 (40.0)
+8	3 (3.4)
Other	5 (5.7)
Unfavorable	41 (46.6)
-5 or-5q	4 (4.5)
-7 or-7q	9 (10.2)
11q23 abnormality	7 (8.0)
Complex**	19 (21.6)
Other	2 (2.3)
Unknown	9 (10.2)

* Modified SWOG:ECOG criteria (Slovak et al 2000 with karyotype of unknown prognostic significance included in “intermediate” category)

** Includes 1 patient with -5/D5, 4 with -7/D7, and 7 with both -5/D5 and -7/D7

Amonafide + Cytarabine Treatment of Secondary AML

SAFETY

Time to Hematopoietic Recovery

- Platelet recovery (median):
 - 20,000/ul = 17 days
 - 100,000/ul = 35 days
- ANC recovery (median):
 - 500/ul = 28 days
 - 1000/ul = 34 days

Deaths on Study

All deaths on study	51 (58%)
Early death (< 14d)	6 (6.8%)
Complications from aplasia	9 (10.2%)
Resistant AML	16 (18.2%)
Relapsed AML	8 (9.1%)
Other	12 (13.6%)
Death \leq 28 days	18 (20.5%)
Early death (< d14)	6 (6.8%)
Complications from aplasia	5 (5.7%)
Resistant AML	3 (3.4%)
Other	4 (4.5%)

Grade 3, 4, 5 AEs

Preferred Term	Grade 3 n (%)		Grade 4 n (%)		Grade 5 n (%)	
	All	Related	All	Related	All	Related
Diarrhea	6 (6.8)	6 (6.8)	0 (0)	0 (0)	0 (0)	0 (0)
Fatigue	7 (8.0)	4 (4.5)	1 (1.1)	1 (1.1)	0 (0)	0 (0)
Rash	6 (6.8)	6 (6.8)	0 (0)	0 (0)	0 (0)	0 (0)
Bacteremia	12 (13.6)	6 (6.8)	0 (0)	0 (0)	1 (1.1)	1 (1.1)
Pneumonia	8 (9.1)	3 (3.4)	3 (3.4)	3 (3.4)	2 (2.3)	1 (1.1)
Resp. failure	2 (2.3)	1 (1.1)	3 (3.4)	2 (2.3)	3 (3.4)	2 (2.3)
Pyrexia	8 (9.1)	4 (4.5)	1 (1.1)	1 (1.1)	0 (0)	1 (1.1)
Hypotension	9 (10.2)	3 (3.4)	2 (2.3)	2 (2.3)	3 (3.4)	2 (2.3)

Amonafide + Cytarabine Treatment of Secondary AML

EFFICACY

Response Criteria

- **Complete remission (CR)**
 - Bone marrow blasts <5%; no Auer rods
 - ANC $\geq 1000/\mu\text{l}$
 - Platelets $\geq 100,000/\mu\text{l}$
- **Complete remission with incomplete hematopoietic recovery (CRi)**
 - Satisfying all criteria for CR except:
 - ANC < $1000/\mu\text{l}$ and/or
 - Platelets < $100,000/\mu\text{l}$

Efficacy – Primary Endpoint

CR + CRi	n=88
Reported by Site¹	45% (40/88)
Assessed per Protocol²	42% (37/88)
CR	34
CRi	3³

- 1 Investigator assessment of CR
2. Protocol requirement of <5% blasts not met for
 - 2 patients with exactly 5% blasts;
 - 1 patient transferred care to non-participating institution prior to day 37 bone marrow assessment; CR confirmed by investigator review and central pathology review of bone marrow.
3. Incomplete hematopoietic recovery:
 - 3 patients with incomplete platelet recovery (13,000, 23,000, 56,000/uL)

CR Maintained in Poor-Risk Subsets

Category	CR + CRi Rate
Age	
< 60	39.4% (13/33)
≥ 60	43.6% (24/55)
Cytogenetics	
Favorable	100% (1/1)
Intermediate	62.1% (23/37)
Unfavorable	22.0% (9/41)
Unknown	44.4% (4/9)
Type of Secondary AML	
Prior leukemogenic therapy (tAML)	40.0% (18/45)
Prior MDS only	44.2% (19/43)
Treatment for Prior MDS*	
Yes	36.0% (9/25)
No	43.5% (10/23)

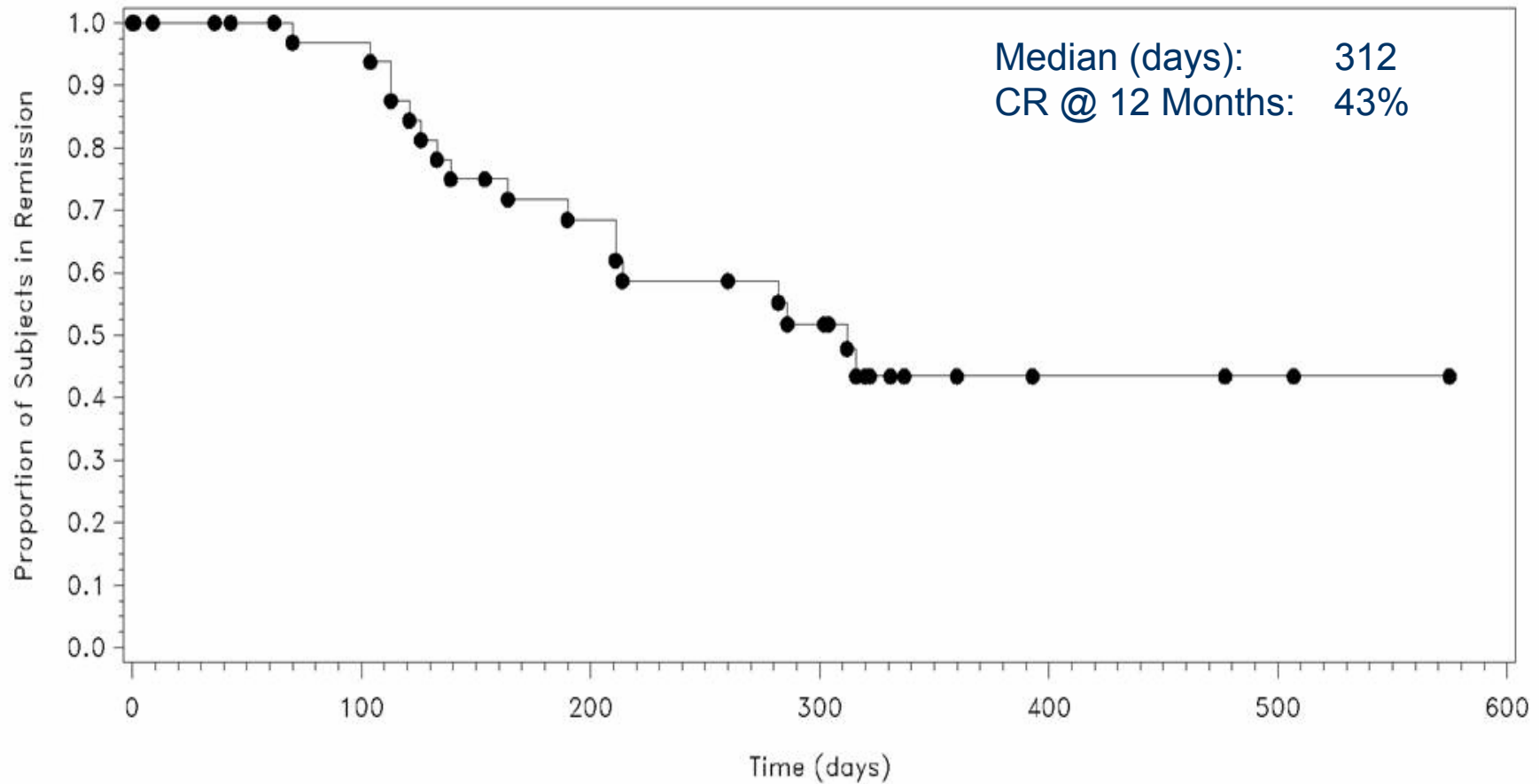
* Includes patients with both MDS and tAML

Cytogenetic Complete Remissions

- Ten CR patients with abnormal baseline cytogenetics and informative post-treatment cytogenetics, 6 of whom achieved Cytogenetic CR

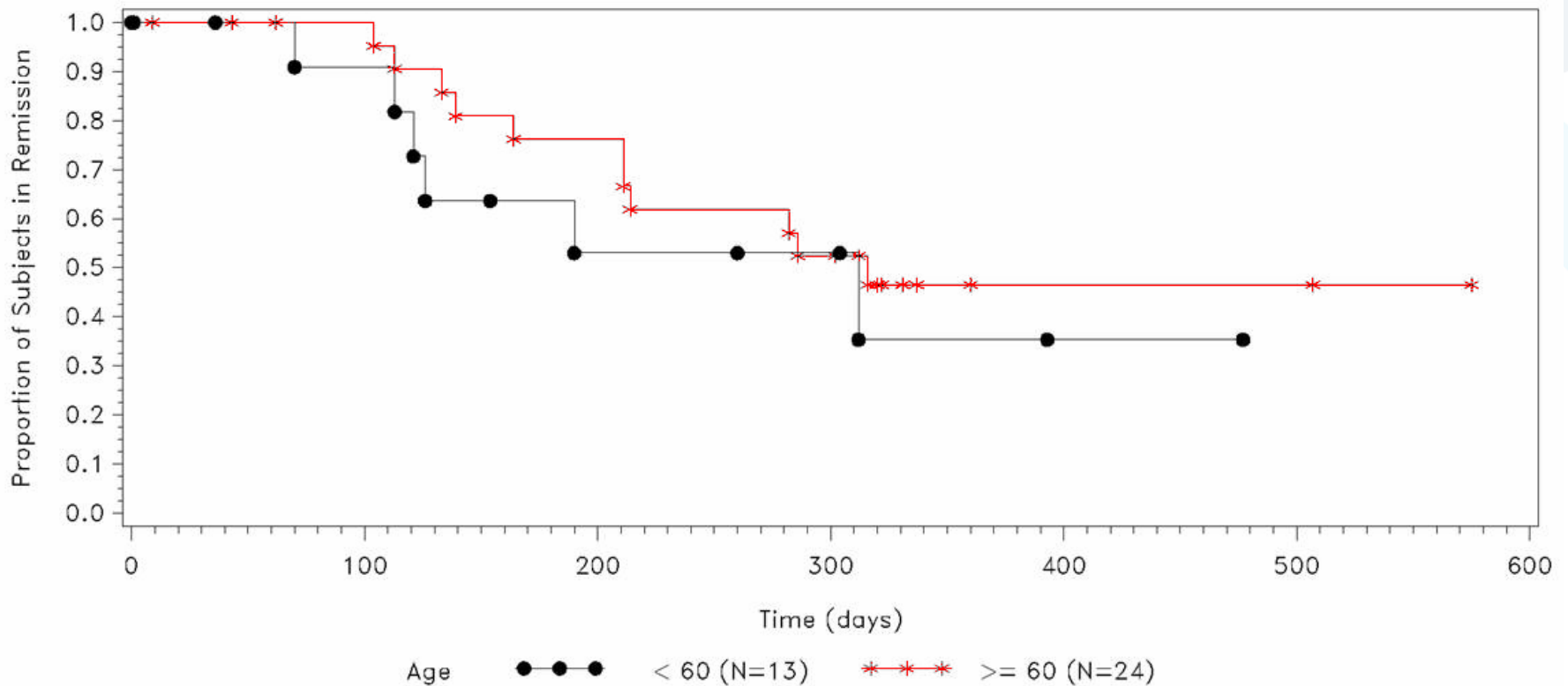
Cytogenetics		Clinical Response
Pre-treatment	Post-treatment	
t(11;19) (q23;p13.1)	Normal	CR
t(11;19) (q23, p13.3)	Normal	CR
+4,+13,+14,+18, i20	Normal	CR
-7	Normal	CR
del(20) (q11.2, q13.3), +mar	Normal	CR
+11q	Normal	CR

Duration of Remission: Overall



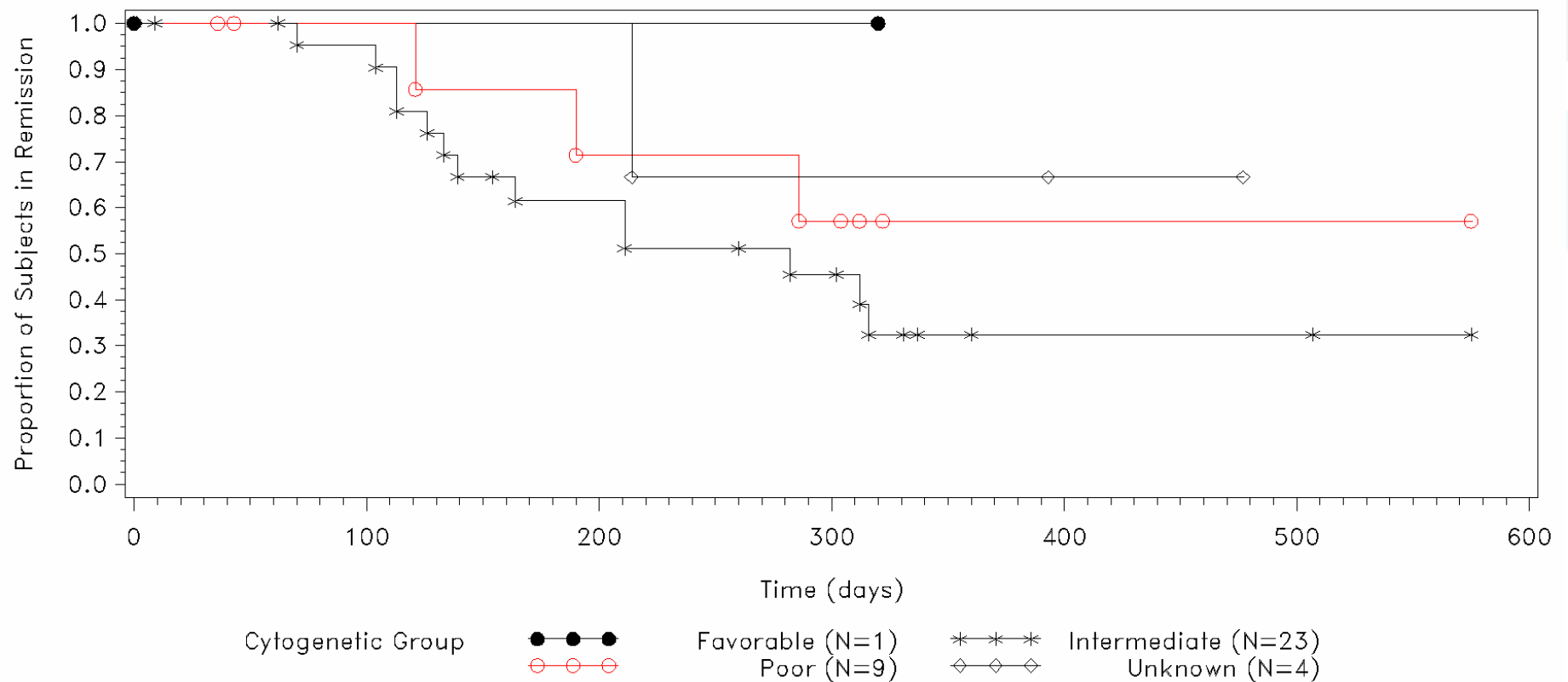
Duration of Remission: By Age

	< 60	≥ 60
Median (days):	312	316
CR @ 12 Months:	35%	47%



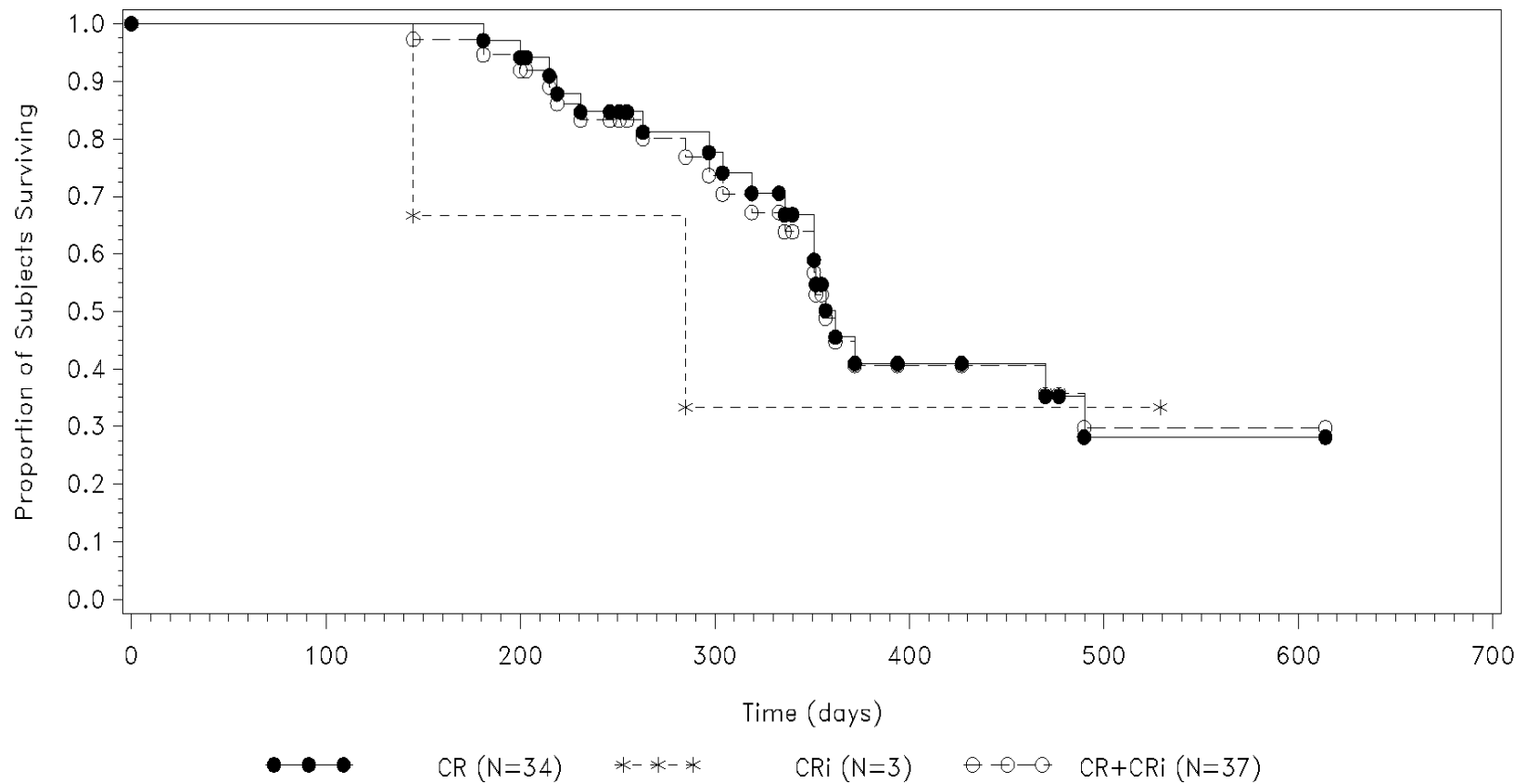
Duration of Remission: By Cytogenetic Risk Group

	Favorable	Intermediate	Poor	Unknown
Median (days):	-	282	-	-
CR @ 12 months:	100%	33%	57%	67%



Overall Survival: CR Patients

	<u>CR</u>	<u>CRi</u>	<u>CR + CRi</u>
Median (days):	362	285	357
OS @ 12 Months:	50%	35%	33%

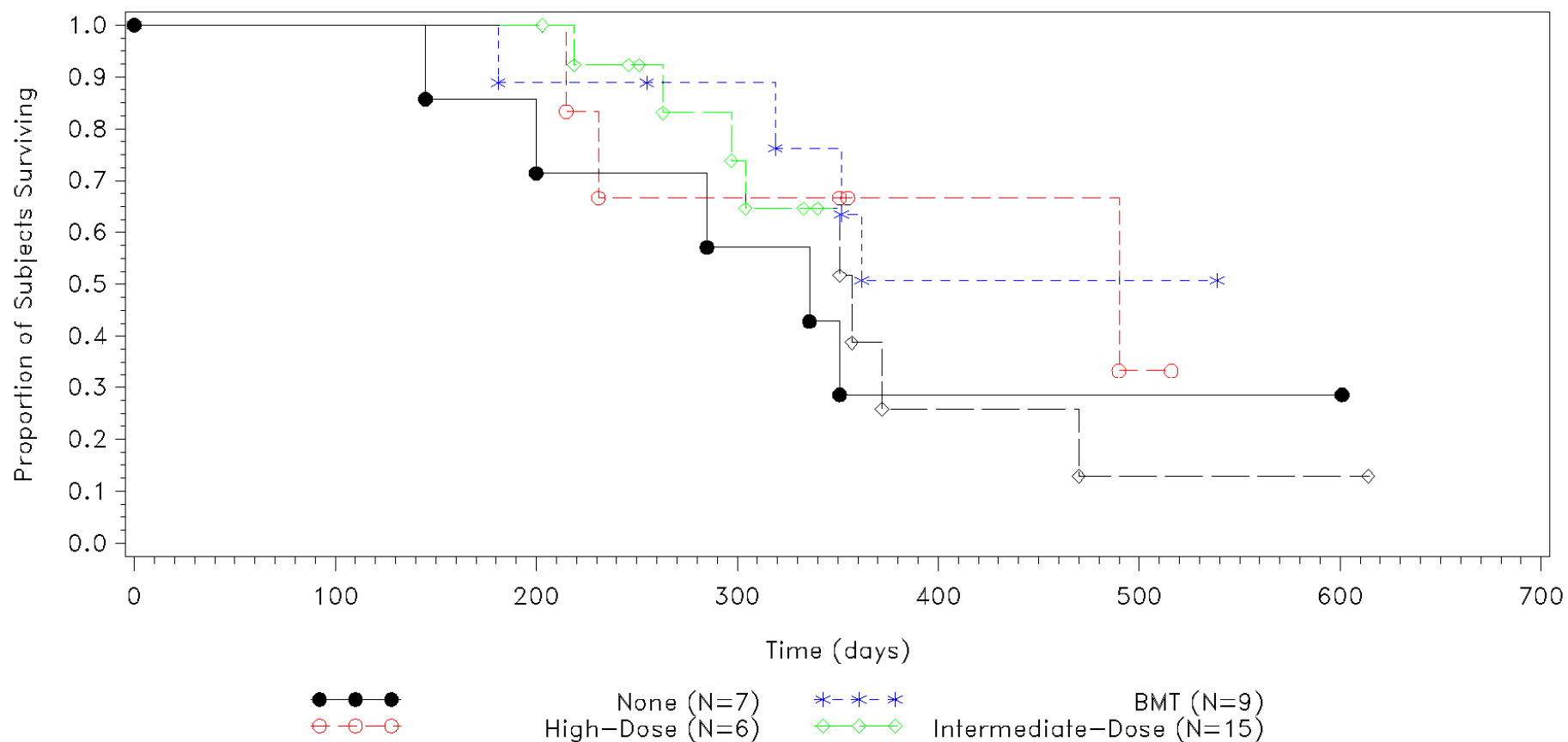


Survival: By Post-Remission Therapy

	BMT	HiDAC	Intermed ara-C	None
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Median (days):	-	490	357	336
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OS @ 12 months:	63%	67%	39%	-
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Summary

- **Novel pharmacologic properties**
 - DNA intercalating agent
 - Induces apoptotic signaling by blocking TOPO II binding to DNA
 - not affected by Multi-drug resistance/Pgp and other efflux pump-mediated resistance mechanisms
 - Not cross-resistant with
 - daunorubicin
 - idarubicin
 - mitoxantrone
 - etoposide
- **Safety**
 - predictable and manageable in this poor-risk population

Summary

- **Clinical efficacy**

- **CR rate** overall (42%) maintained in poor risk subsets of

- Age ≥ 60 43.6%
 - Treatment-related AML40%
 - Prior treatment for MDS36.0%

- **Durable CR**

- Median > 10 months
 - CR @ 12 months: Overall43%;
 - <60 yrs.....35%
 - ≥ 60 yrs 47%
 - Unfavorable cytogenetics 57%

- Randomized Phase 3 trial of amonafide + ara-C in sAML underway