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A phase I dose escalation study of oral bexarotene in combination with intravenous decitabine in patients with AML

John S. Welch[1], **Haixia Niu**[1], **Geoffrey L. Uy**[1], **Peter Westervelt**[1], **Camille N. Abboud**[1], **Ravi Vij**[1], **Keith E. Stockerl-Goldstein**[1], **Meagan Jacoby**[1], **Iskra Pusic**[1], **Mark A. Schroeder**[1], **John F. Dipersio**[1], and **Amanda F. Cashen**[1]

[1]Division of Oncology, Department of Internal Medicine. Washington University, St Louis, Missouri

Abstract

The response rate of non-M3 AML to all trans retinoic acid (ATRA) has been limited. Using Affymetrix expression arrays, we found that in diverse AML blasts *RXRA* was expressed at higher levels than *RARA* and that mouse *Ctsg-PML-RARA* leukemia responded to bexarotene, a ligand for RXRA. We therefore performed a phase I study of combination bexarotene and decitabine in elderly and relapsed AML patients. We found that this combination was well tolerated, although outcomes were modest (1 CRi, and 3 PR among 19 patients). Correlative studies found that patients with clinical response had increased differentiation to bexarotene both *in vivo* and *ex vivo,* suggesting that pre-treatment analysis might identify a more susceptible subgroup of patients.

Introduction

Current therapy for AML consists of cytotoxic chemotherapy followed by consolidation chemotherapy or stem cell transplantation. Two patient populations remain difficult to treat: patients with relapsed disease and patients who are over the age of 60. Salvage therapy successfully achieves a remission in less than 50% of relapsed patients and in the absence of consolidative allogeneic stem cell transplantation, remissions and survival are typically brief.(1) Compared with younger patients, AML patients who are older than 59 years have response rates that are lower, remissions that are briefer, and they are more likely to experience toxicities.(2–10) Therefore, more effective and less toxic therapies are needed.

Initial Affymetrix expression data of diverse AML samples demonstrated that *RXRA* was expressed at higher levels than *RARA*, suggesting that RXRA might be a better target for differentiation therapy than RARA, and might explain why clinical response of non-M3 AML to ATRA have been so limited.(11, 12)

Therefore, we investigated whether bexarotene can be combined with decitabine in elderly and relapsed acute myeloid leukemia (AML) patients. Both drugs have been well tolerated

Corresponding author: John S. Welch, MD/PhD, Washington University, 660 South Euclid Ave, Box 8007, St Louis, MO 63110, jwelch@dom.wustl.edu, phone: 314-362-2626, fax: 314-362-9333.

Conflicts of Interest.
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in AML patients as single agents,(13, 14) and have non-overlapping mechanisms and side-effects. Bexarotene activates transcriptional effects of RXRA through hetero- and homodimers, while decitabine induces DNA hypomethylation.(15–20)

We found that this combination was well tolerated, but lead to only modest responses in this population. We also observed that patients with clinical response tended to exhibit greater AML blast differentiation when exposed to bexarotene either *in vivo* or *ex vivo*, suggesting a possible future approach to identify patients more likely to gain benefit from therapy.

Methods

Clinical trial

We enrolled 19 subjects on a phase I, dose escalation study. The clinical trial was approved by the Washington University institutional review board, conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practice, and listed on Clinicaltrials.gov (NCT01001143).

Enrollment

Patients with acute myeloid leukemia were either ≥60 years old or had relapsed disease. Major exclusion criterion were: white blood cell count (WBC) > 10,000/μl, bilirubin > 1.5 x upper limit of normal (ULN), AST/ALT > 2.5 x ULN, creatinine > 2 ULN, triglycerides > 1,000 mg/dl, active graft vs host disease, central nervous system involvement with leukemia, and performance status > 3. The exclusion of patients with WBC > 10,000/μl was because we have observed reduced response to decitabine in these patients.(13)

Treatment schedule

Patients were treated in 3+3 dose-escalating cohorts (Table 1). Patients received oral bexarotene in one of three cohorts: 100, 200, and 300 mg/m^2/day, for all days of all cycles. All patients also were treated with decitabine 20 mg/m^2 IV on days 1–5 of 28 day cycles. During cycle 1, decitabine was initiated after 3 days of bexarotene and following a repeat bone marrow collection for correlative studies.

Supportive care

Supportive care and symptom management were provided according to institutional standards of care. In addition, all patients were monitored for hypertriglyceridemia, hypercholesterolemia, and hypothyroidism, and treated accordingly.(21)

Response and outcomes definitions

The maximum tolerated dose (MTD) was defined as the dose level immediately below the dose level at which 2 patients of a cohort (of 2 to 6 patients) experience dose-limiting toxicity during the first cycle. Myelosuppression, infection, differentiation syndrome, hypertriglyceridemia, hyperlipidemia, hypothyroidism, nausea, weight loss and reversible electrolyte abnormalities were not considered dose limiting. Toxicity grading during the first and all subsequent cycles was performed according to the revised National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, published

May 29, 2009 and available at http://ctep.cancer.gov/reporting/ctc.html. Response was assessed according to the IWG criteria.(22)

Expression analysis

Affymetrix, Nanostring nCounter, and RNA-Seq methods have been described elsewhere. (23–25)

Methylcellulose analysis of mouse leukemia

Cryopreserved leukemic spleen cells were thawed, plated at 2×10^6/ml in RPMI with 15% FCS, 100 ng/ml SCF, 6 ng/ml IL-3, 10 ng/ml IL-6 (Peprotech, Rocky Hill, NJ) ± 1 μM ATRA (Sigma, St. Louis, MO) or 1 μM bexarotene (LC Laboratories, Woburn, MA) and maintained at 3% oxygen and 5% CO_2 in a humidified chamber (Billups-Rothenberg, Del Mar, CA) for 48 hours. Cells were plated at 8.3×10^3/ml (MethoCult M3534 Stem Cell Technologies, Vancouver, Canada) and maintained in 3% oxygen and 5% CO_2. After seven days, colonies were counted.

In vivo analysis of bexarotene response in mice

Leukemic sample arising in mouse 13341 was selected because this leukemic sample was the least immunophenotypically differentiated of the samples assessed at baseline based on CD117 and Gr1 expression.(26) 1×10^6 leukemia cells were injected by retro-orbit into recipient mice. Indicated mice received either 1 mg bexarotene dissolved in DMSO and corn oil, or placebo corn oil by gavage on days 8–10 (~300 mg/m^2/day). Mice were sacrificed and analyzed on day 14. All of the mice were cared for in the experimental animal center of Washington University School of Medicine. The Washington University Animal Studies Committee approved all animal experiments.

Ex vivo analysis of human AML samples

Cryopreserved leukemia samples were thawed and co-cultured as previously described.(27) Bone marrow samples collected on day 0 and day 3 of bexarotene therapy were grown for 72 hours on MS5 murine stromal cells in media containing IL3, IL6, SCF, and TPO ± 1 μM bexarotene or ATRA and assayed by flow cytometry (anti-CD11b-APC, clone ICRF44, eBioscience, San Diego, CA) or EdU staining, performed per manufacturers recommendations, after culturing cells with EdU for 1 hour (Life Technologies, Grand Island, NY).

Results

Human expression of RARA and RXRA

We used Affymetrix expression arrays to determine the relative level of retinoid receptors in diverse, unselected FAB AML cases. We observed elevated levels of *RXRA* relative to *RARA* in primary AML cells, regardless of FAB (Figure 1A). We observed no differences in either *RARA* or *RXRA* expression in AML cases with WBC > 10,000/μl vs. those with WBC < 10,000/μl, when controlled for FAB (data not shown). We validated these findings in a

smaller set of AML and MDS cases using a different technology, Nanostring nCounter (Figure 1B). Each of these cases presented with WBC < 10,000/μl.

Response of murine AML to bexarotene

In order to determine whether an RXRA ligand (e.g. bexarotene) might be effective at differentiating AML blasts, we treated 9 unique mouse *Ctsg-PML-RARA* acute promyelocytic leukemia (APL) samples with either ATRA or bexarotene *ex vivo*. We found that bexarotene was able to decrease the number of colony forming units in *Ctsg-PML-RARA* leukemia cells, but not as effectively as ATRA (Figure 1C).

To determine whether bexarotene might be effective *in vivo*, secondary transplantation of the leukemia arising in mouse 13341 was performed and mice were treated with bexarotene or placebo. We observed an increase in the expression of CD11b and a decrease in the spleen weight, consistent with an *in vivo* response to bexarotene (Figure 1D – E). Based on these results, we established a phase I study of bexarotene and decitabine in patients with AML.

Clinical trial

The primary object of this trial was to determine the safety and tolerability of this combination. Secondary objectives were to determine the response rate and to correlate outcomes with *in vivo* and *ex vivo* differentiation response to bexarotene.

Patients were treated with oral bexarotene in three escalating dose cohorts, and with decitabine at the same dose in all cohorts (Table 1 and Figure 2).

Patient characteristics are described in Table 2. The average age was 74. The median performance status was 1. An adverse karyotype was observed in 9 patients (48%). And 12 patients (63%) had relapsed after prior therapy.

The combination was well tolerated. Only one patient experienced a study-defined dose limiting toxicity (grade 3 fatigue, cohort 2) and 9 patients were treated with the maximum bexarotene dose, 300 mg/m^2/day. Grade 3 – 5 toxicities noted during additional cycles are described in Table 3.

The overall response rate was 21%: 1 complete remission with incomplete count recovery (CRi), and 3 patients achieved blast reduction greater than 50% and less than 25% bone marrow blast cells (partial response, PR). Patients with CRi, PR, or SD completed an average of 4.2 cycles, while other patients completed an average of 1.2 cycles. The median survival was 118 days and 5 patients survived more than 1 year. One patient remains alive under continued follow-up (patient 18). Of note, 3 patients successfully transitioned to allogeneic transplant following therapy (patients 6, 8, and 18; average age 68). Unlike Tsai *et al.*,(14) we did not observe improved platelet counts during bexarotene treatment (data not shown), possibly due to decitabine co-treatment.

Response of patient bone marrow cells to bexarotene in vivo and ex vivo

We correlated *ex vivo* bexarotene sensitivity with clinical response. Bone marrow cells were collected on day 0 and day 3 of bexarotene and co-cultured with irradiated MS5 stromal cells with or without further bexarotene treatment. We compared CD11b expression in cells treated *ex vivo*, and we compared expression between samples collected on day 0 vs day 3 (*in vivo* treatment). Bexarotene increased CD11b expression greater in the 4 responding patients vs non-responders (fold increase in CD11b: *in vivo* 1.6 ± 0.3 vs 0.7 ± 0.2, $p < 0.03$, and *ex vivo* 2.1 ± 0.3 vs 1.1 ± 0.1, $p < 0.003$; increase in absolute percentage of CD11b+ cells: *in vivo* 13.6% ± 4% vs −3.6% ± 2.2%, $p < 0.002$, and *ex vivo* 24% ± 2.6% vs 0.7% ± 1%, $p < 0.001$, Figure 3A – B). We also observed an equivalent or modestly increased induction of CD11b when cells were treated *ex vivo* with ATRA vs bexarotene (Figure 3C – D) (the fourth responding patient did not have adequate samples for this experiment).

One case with clinical response had sufficient bone marrow cells collected on day 0 and day 3 to also determine the extent of cell cycle kinetics by EdU staining. We observed a decrease in EdU staining following *in vivo* and *ex vivo* bexarotene (Figure 3E).

To determine transcriptional effects of *in vivo* bexarotene, we performed expression array profiling on 10 cases, 4 of which had clinical responses. Bone marrow samples were collected on day 0 and day 3 of oral bexarotene and total bone marrow cells subjected to Affymetrix expression array profiling. We observed no statistically significant differences in expression at baseline or in response to bexarotene in responders vs non-responders (expression difference >2 fold and FDR < 0.05, data not shown). We again compared the expression of *RXRA* vs *RARA* in this dataset and found no difference in expression levels, either between groups of patients, or within individual patients (Figure 4A). We reviewed the recently available RNA-Seq data from the TCGA AML project (Figure 4B),(28) which also was not consistent with increased absolute *RXRA* expression vs *RARA* expression across diverse FAB subtypes.

Discussion

Initial expression analysis, using both Affymetrix and nCounter data, suggested that AML blasts express higher levels of *RXRA* compared with *RARA* (Figure 1A – B). We further found that bexarotene, a retinoid which selectively binds to and activates RXRs, but not RARs, lead to loss of self-renewal of mouse APL tumors *ex vivo* and increased differentiation and decreased growth of a selected mouse leukemia *in vivo* (Figure 1C – E). Consistent with these findings, other investigators have observed differentiation effects of bexarotene in leukemia cells lines.(15–17) These data suggested that a ligand specific for RXR may be more effective to induce retinoid-dependent differentiation in non-M3 AML than the RARA ligand ATRA.(12, 29) We therefore performed a phase I, dose-escalation study of combination decitabine and bexarotene, two drugs with non-overlapping mechanisms and non-overlapping side-effect profiles.

We found that bexarotene can be safely combined with decitabine in elderly and relapsed AML patients. This combination was well tolerated to a maximum bexarotene dose of 300 mg/m^2/day, with toxicities similar to single agent decitabine.(13) The toxicities we

observed, across all cohorts and cycles, were primarily cytopenias, infections, and hypertriglyceridemia, as expected (Table 3).

Combination bexarotene and decitabine lead to modest responses in a subset of patients, and could bridge elderly patients to allogeneic transplant. However, survival outcomes were limited, with a median survival in this cohort of only 118 days. These results are not significantly different from historical cases treated with single agent decitabine, which typically results in response rates of 22–30%,(13, 18, 30–38) or historical cases treated with single agent azacitidine, with response rates of 7–20%.(39–41) Therefore, from this small cohort of relapsed, pre-treated, and high risk AML, we cannot conclude that the combination of agents has improved clinical outcomes compared with decitabine alone.

We did find that in patients with clinical response, bexarotene lead to differentiation with increased CD11b expression and decreased cell proliferation, both following 3 days of oral bexarotene *in vivo*, and when bone marrow blasts were treated with bexarotene *ex vivo* (Figure 3).

However, expression analysis of bone marrow blasts from ten cases collected on day 0 and day 3 of bexarotene did not reveal statistically different expression, including differences in the expression of *RARA* vs *RXRA* (Figure 4A and data not shown). This is likely due to high variability in the bone marrow blast percentage and in the diversity of FAB classification present in the data set. Review of the recently published RNA-Seq data from TCGA also showed no difference in absolute expression of *RARA* vs *RXRA.* The differences we observed in our original dataset therefore may have been technical artifacts related to the Affymetrix U133 (Figure 1A) vs Exon arrays (Figure 4A) or probe selection.

Our original hypothesis that *RXRA* is more highly expressed in AML blasts than *RARA* and that RXRA may therefore be a better target for differentiation therapy is not supported by our observed modest response rates or by subsequent RNA-Seq and array analysis of expression levels.

Our data does suggest that a minority of AML patients respond to retinoid-based therapy *ex vivo* (e.g. ATRA or bexarotene) and that in this small cohort, this correlated with clinical response to the combination of bexarotene and decitabine. In light of the limited clinical success of retinoid therapy in non-M3 AML,(12, 29) these data suggest that cell intrinsic processes may facilitate retinoid-sensitivity in a subset of AML patients. This does not appear to correlate with *RARA* or *RXRA* expression levels. However, because these patients can be identified by *ex vivo* retinoid treatment, further studies of retinoids in non-M3 AML patients might benefit by selecting patients who display *ex vivo* sensitivity.

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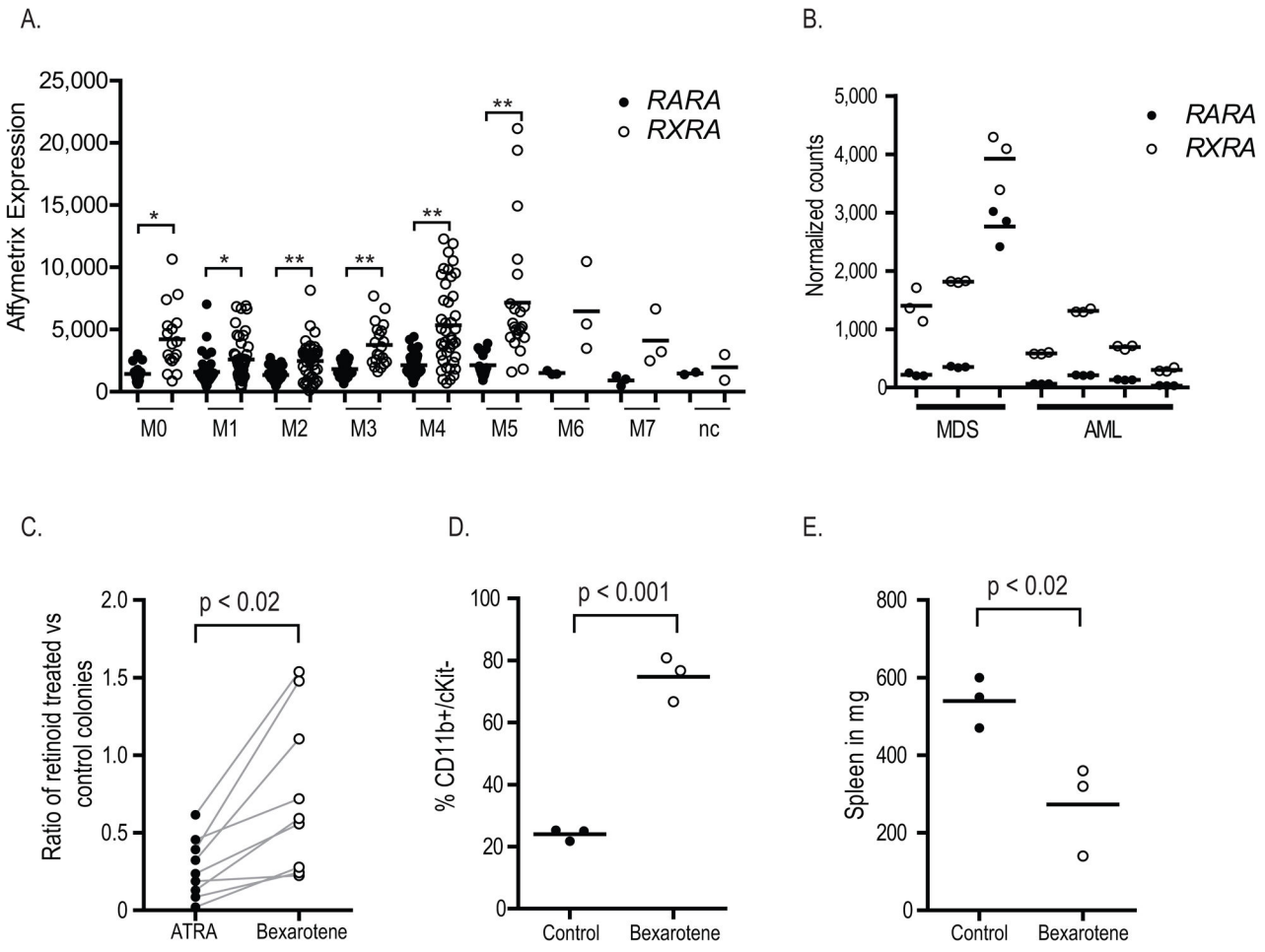

Figure 1.

Expression profile of *RARA* (closed circles) and *RXRA* (open circles) in total bone marrow cells from 197 human AML cases using Affymetrix Human Genome U133 Plus 2.0 GeneChip Arrays (* $p < 0.002$; ** $p < 0.001$).(23) B. Expression profile of *RARA* (closed circles) and *RXRA* (open circles) in total bone marrow cells from 3 MDS patients and 4 AML patients using Nanostring nCounter (each sample analyzed in technical triplicates, two way ANOVA, $p < 0.001$). C. Reduction in methylcellulose colony forming by 9 unique leukemia samples derived from *Ctsg-PML-RARA* mice when treated for 48 hours with 1 μM of indicated retinoid and then assayed in myeloid methylcellulose. D. and E. *In vivo* differentiation of leukemia arising in mouse 13341. Following secondary transplantation of 1×10^6 leukemia cells, mice were treated with either placebo or bexarotene days 8–10, sacrificed and analyzed on day 14.

Study Schema:

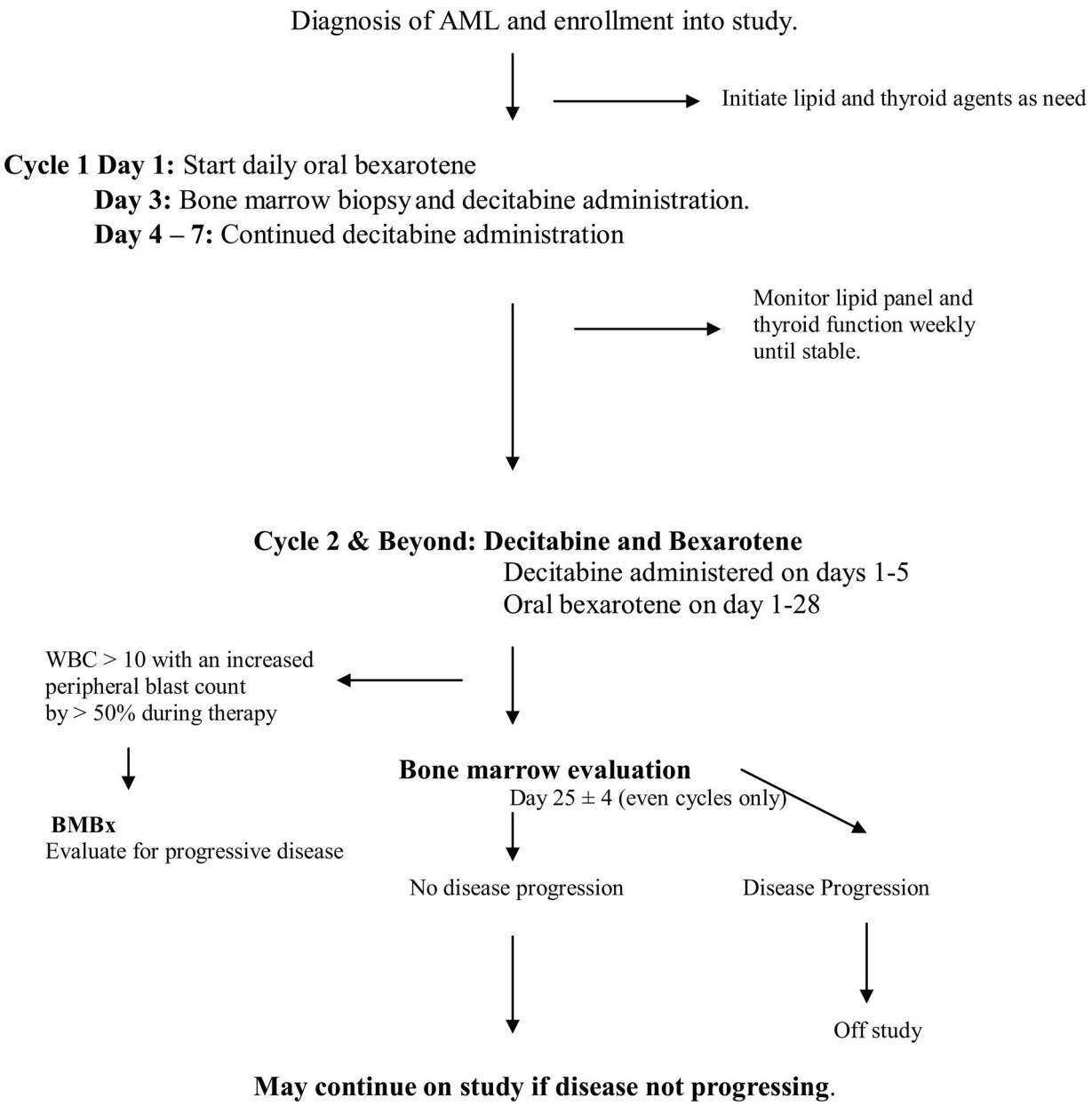

Figure 2.
Study schema.

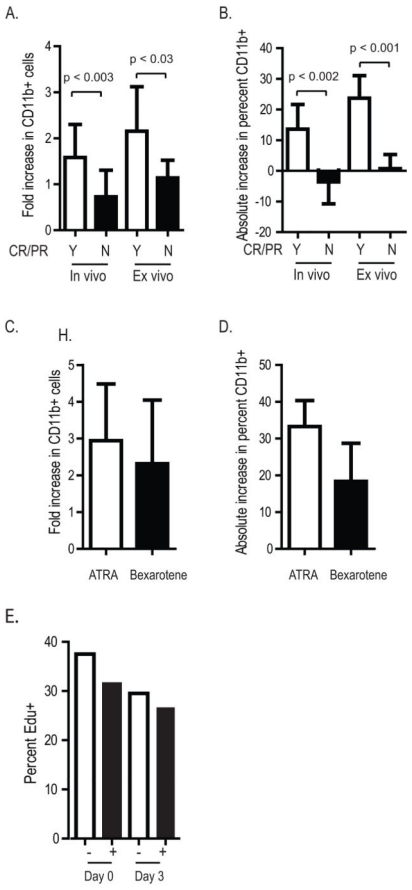

Figure 3.

Ex vivo analysis of patient samples. A-B. Bone marrow cells collected on day 0 and day 3 of bexarotene therapy were grown for 72 hours on MS5 murine stromal cells and treated with and without 1 μM bexarotene and assayed by flow cytometry for CD11b expression. C-D. Bone marrow cells collected on day 0 from patients 2, 5, and 6 were cultured on MS5 cells without treatment or with 1 μM ATRA or 1 μM bexarotene and assayed for CD11b expression 72 hours later. E. Cell cycle analysis by EdU staining of patient 6 bone marrow cells grown *ex vivo* as in A.

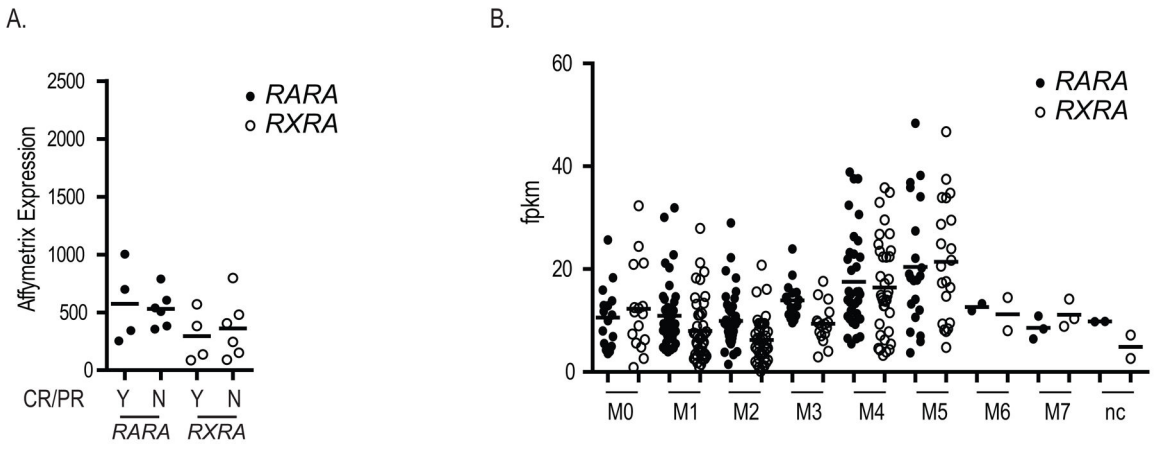

Figure 4.

Expression analysis. A. Expression array analysis of *RARA* (closed circles) and *RXRA* (open circles) in total bone marrow cells collected on day 0 from 10 cases analyzed by Affymetrix Human Exon 1.0 ST arrays. B. Expression analysis of *RARA* (closed circles) and *RXRA* (open circles) in 179 human AML samples by RNA-Seq.(28) All arrays normalized to chip mean of 1,500.

Table 1

Dose level	Decitabine (mg/m2) IV Days 1–5	Bexarotene (mg/m2) PO daily
1	20	100
2	20	200
3	20	300

Table 2

ID	Age	Gender	Prior Tx History	WHO/FAB	Karyotype	ECOG @ Baseline	Cohort	Dose Level	# Cycles Completed	DLT	Best Response	Survival (Days)
A01	79	M	No prior treatment	M1	Complex	1	1	1	1	No	PD	38
A02	83	M	**1** Revlimid (x5) **2** Vidaza (x2)	AML with MLD	Complex	1	1	1	3	No	PR	125
A03	81	M	**1** Vidaza (x8)	M2	Complex	2	1	1	3	No	SD	118
B04	76	M	**1** Vidaza (40+ cycles	AML with MLD	XY, +8	2	2	2	2	No	PD	299
B05	79	F	No prior treatment	M0	XX, +8, +19	1	2	2	2	Yes	PR	240
B06	64	F	no prior treatment	AML, NOS	Complex	1	2	2	5	No	PR	428
B07	82	F	**1** Aurora kinase inhibitor on clinical trial -22 cycles	M2	XX	1	2	2	< 6	No	SD	554
B08	67	M	**1** 7+3 **2** cytarabine-1 cycle **3** mito EC	AML, NOS	Complex	1	2	2	2	No	CR	543
B09	68	M	**1** 7 + 3 **2** Oral clofarabine (x5)	M2	XY, del(20)(q11.2q13.1)	0	2	2	< 1	No	N/A	10
B10	79	M	**1** 7+3 **2** reinduction **3** Vidaza (x7)	AML with MLD	XY. NPM1 exon 12 insertion.	1	2	2	< 3	No	SD	84
C11	72	F	**1** CYPT JAK2 inhibitor clinical trial	M2	Complex. JAK2 V617F.	1	3	3	1	No	PD	27
C12	80	M	**1** Vidaza (x12)	M2	XY	1	3	3	1	No	N/A	38
C13	39	F	**1** 7+3 **2** FLAG-IDA **3** MEC **4** Cy-TBI MUD	AML	Complex	1	3	3	1	No	N/A	17

ID	Age	Gender	Prior Tx History	WHO/FAB	Karyotype	ECOG @ Baseline	Cohort	Dose Level	# Cycles Completed	DLT	Best Response	Survival (Days)
D14	69	M	**1** 7+3 **2** 5+2 **3** cytarabine consolidation **4** clofarabine/Ara-C **5** decitabine	AML	Complex.	1	Ex	3	12	No	SD	427
D15	82	M	**1** Hydrea	AML	XY	2	Ex	3	<1	No	PD	84
D16	77	M	**1** Hydrea	M2	XY. FLT3-ITD+.	1	Ex	3	<1	No	PD	19
D17	71	M	**1** Hydrea	AML with MLD	XY.	2	Ex	3	3	No	SD	109
D18	72	M	**1** GCSF+Vidaza+Plerixafor	AML with MLD	XY, -7q, del(12)(q13q15)	0	Ex	3	4	No	SD	673
D19	81	M	No prior treatment	AML	92, XXYY[2]/46, XY[18]	2	Ex	3	4	No	SD	303

Table 3

	Grade 3–4 Toxicity during any cycle
Cardiac Arrest	1 (5%)
Intracranial hemorrhage	1 (5%)
Anemia	17 (89%)
Neutropenia	10 (53%)
Pneumonia	9 (47%)
Purpura	1 (5%)
Anorexia	1 (5%)
Nausea/Vomiting	2 (11%)
Hypertriglyceridemia	7 (37%)
Hyperglyceridemia	2 (11%)
Hypocalcemia	3 (16%)
Hyponatremia	1 (5%)
Pleural effusion	1 (5%
Hypoalbuminemia	1 (5%
Fatigue	2 (11%)
Prolonged PTT/INR	2 (11%)