SH2017-0144:
Differential response to FLT3 inhibition (using quizartinib/AC220) in acute myeloid leukemia is affected by baseline molecular genetics and cytogenetics

Siddharth Bhattacharyya, MD*, Grant E. Nybakken, MD+, Darshan Roy, MD*;
Jennifer Morrissette, PhD*, Christopher Watt, MD PhD*; Alexander Perl, MD*;
Martin Carroll, MD*, Jonathan Canaani, MD*, **Adam Bagg, MD***

* University of Pennsylvania
+Kaiser Permanente, California

Introduction

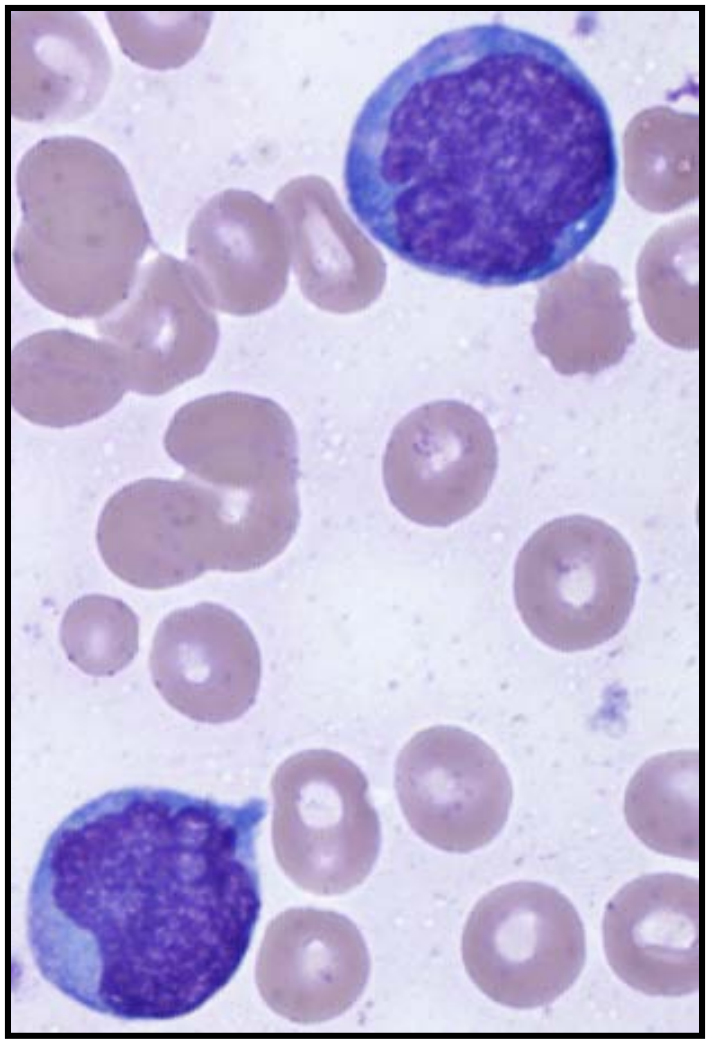
- Cohort of 19 patients with relapsed/refractory acute myeloid leukemia, all known to be *FLT3*-ITD mutation positive
- Clinical trial of FLT3 inhibitor, Quizartinib (AC220) monotherapy
- Bone marrow studies pre- and post-therapy (interval 29 days)
- Highlight two prototypical cases illustrating different therapeutic responses to AC220 therapy

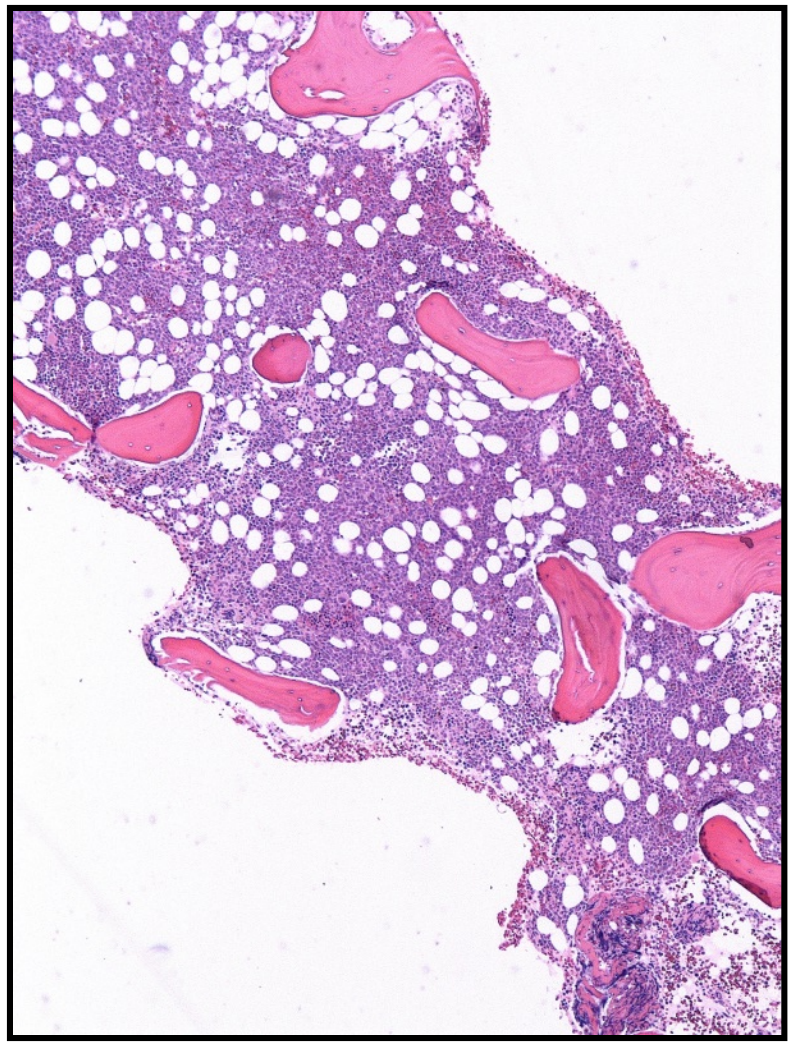
Patient A

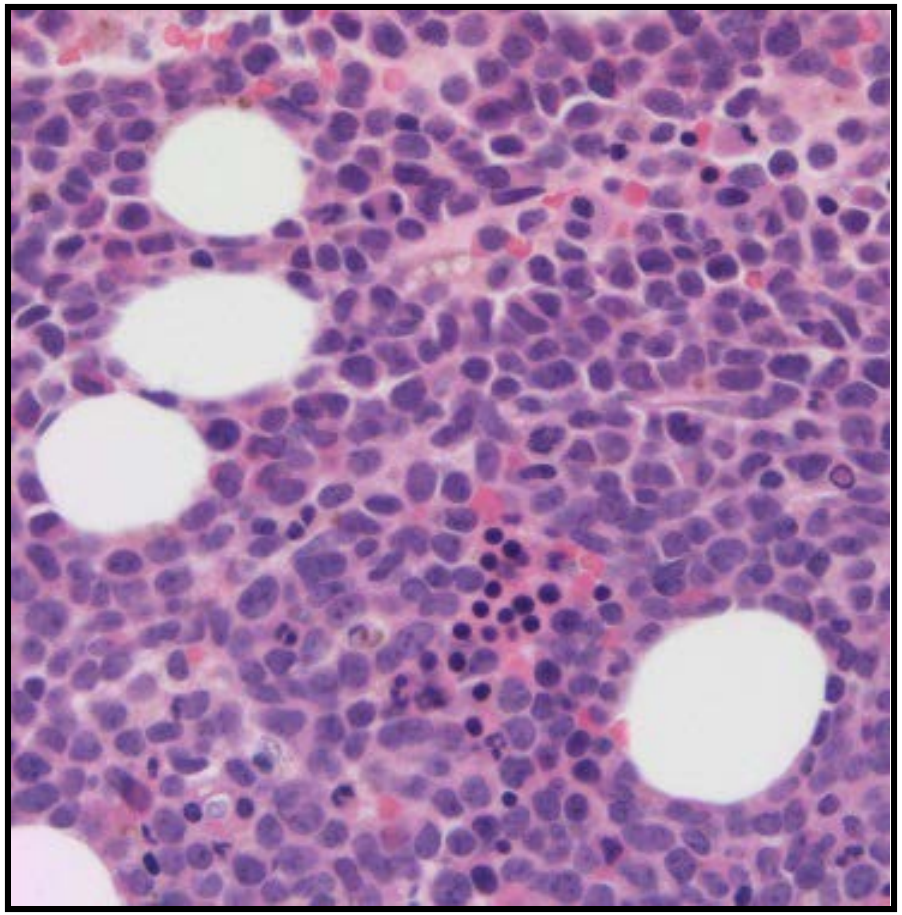
- A 33-year-old woman with a history of relapsed acute myeloid leukemia (AML) with mutated *NPM1*
- Known to be *FLT3*-ITD positive
- Peripheral Blood:
  - WBC: 13.6 K/µL; Hgb: 11.9 g/dL; Plt: 96 K/µL; MCV: 93 fL
    - 53% neutrophils, 6% lymphocytes, 3% monocytes, 0% eosinophils, 0% basophils, **38% blasts**

Patient A

Pre-Therapy Bone Marrow



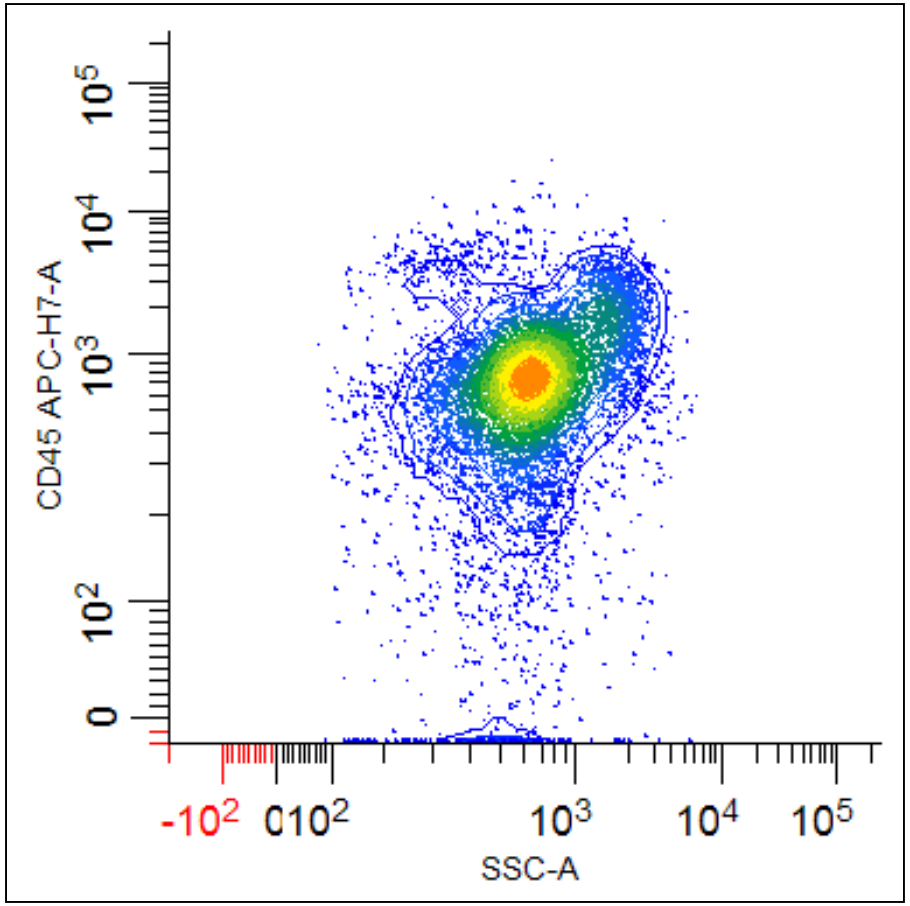




Patient A

Pre-Therapy Laboratory Results

Flow Cytometry: Blasts (74%): CD13+ CD33+ CD34+ CD64(s)+ CD117+ HLA-DR+

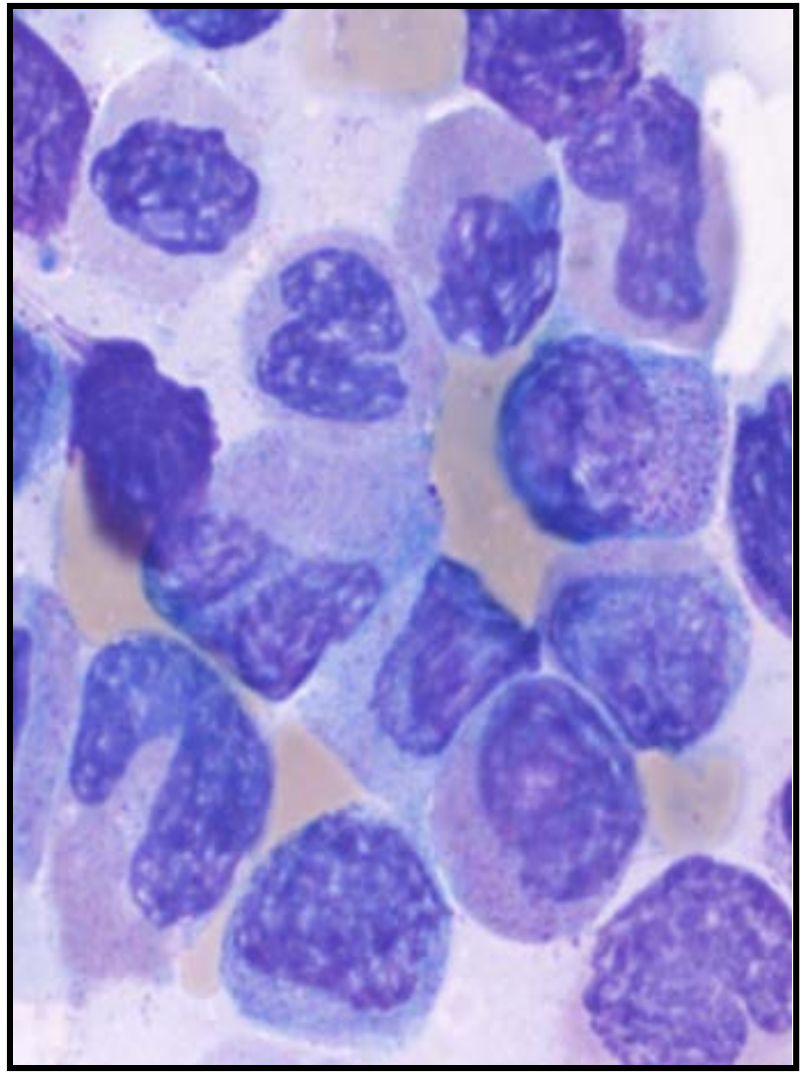


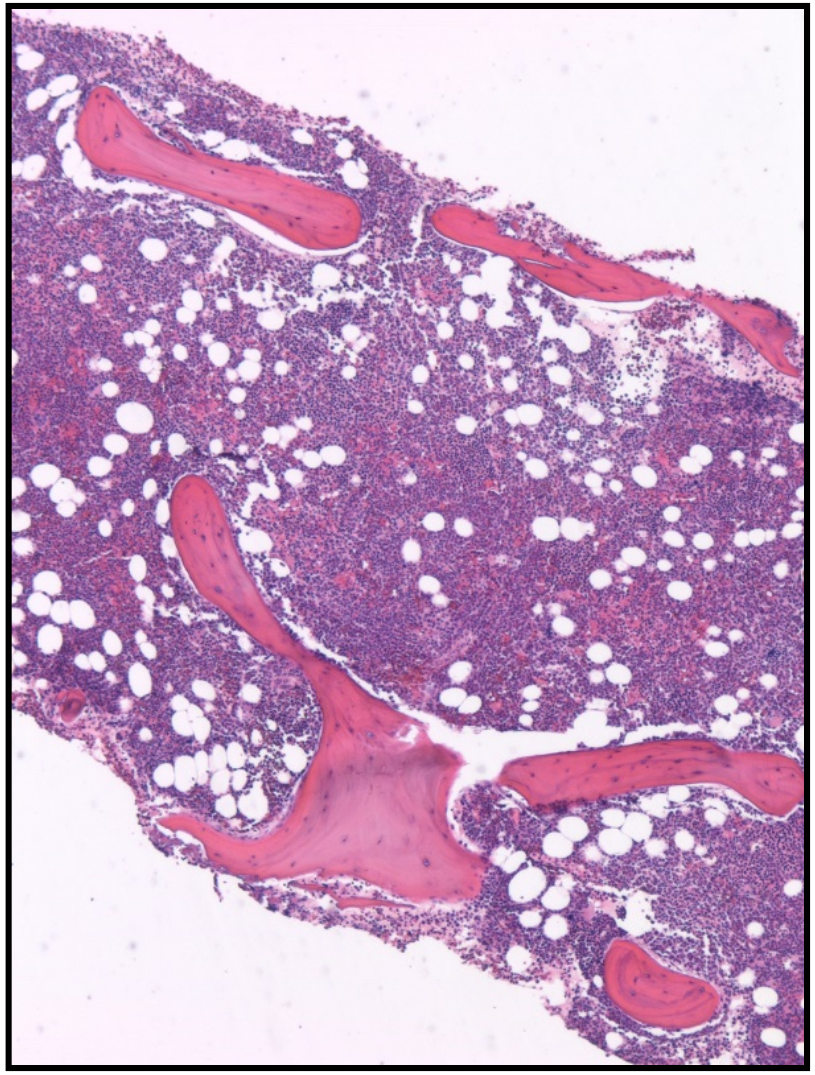
Genetic Studies

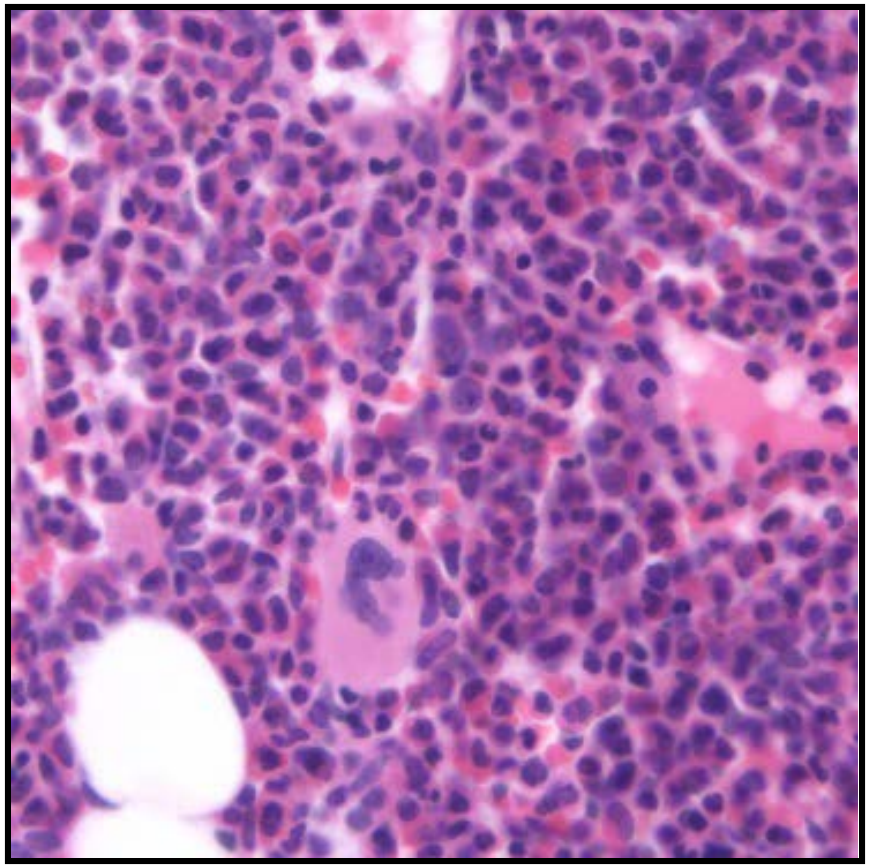
- **Cytogenetics**: 46,XX[20]
- ***FLT3*-ITD**:
  - **ITD size**: 165 bp
  - **Mutant-WT ratio**: 0.45
- **NGS studies**:
  - *DNMT3A* (VAF: 51%)
  - *NPM1* (VAF: 45%)
  - *TET2* (VAF: 47%)

Patient A

Post-Therapy Bone Marrow



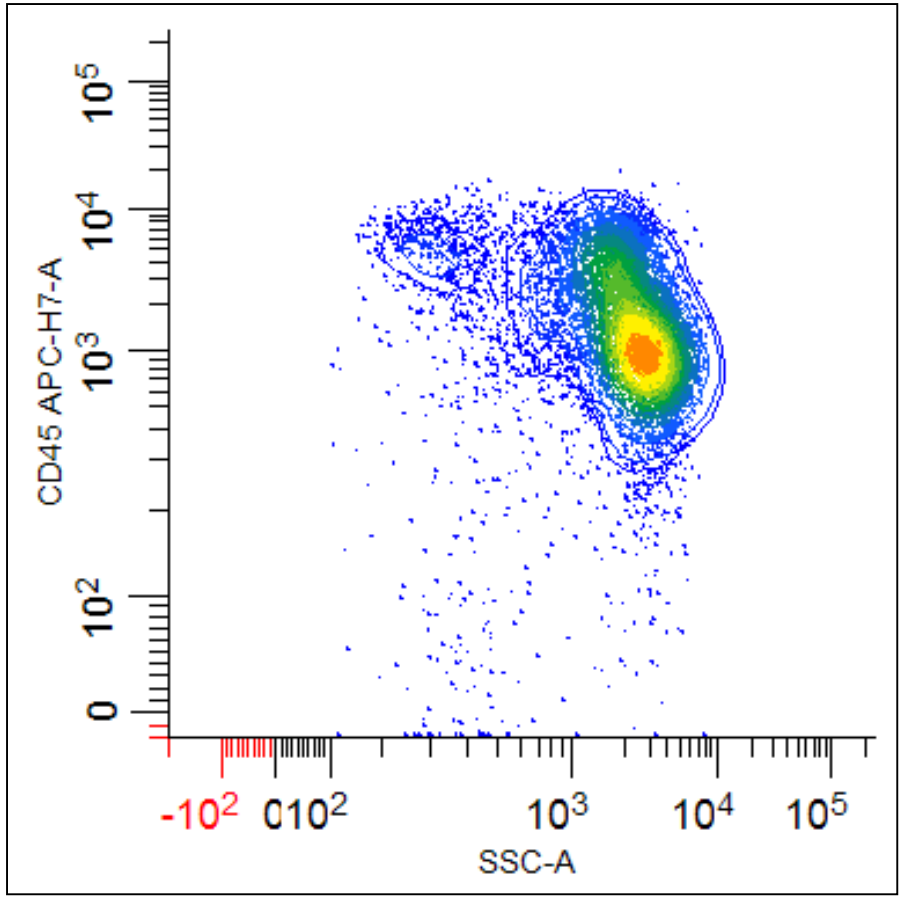




Patient A

Post-Therapy Laboratory Results

Flow Cytometry: Blasts (<5%): Spectrum of myeloid differentiation



Genetic Studies

- **Cytogenetics**: 46,XX[20]
- ***FLT3*-ITD**:
  - **ITD size**: 165 bp
  - **Mutant-WT ratio**: 0.56
- **NGS studies**:
  - Previously detected variants still present
  - No new pathogenic variants

Peripheral Blood

- Normal CBC and differential count

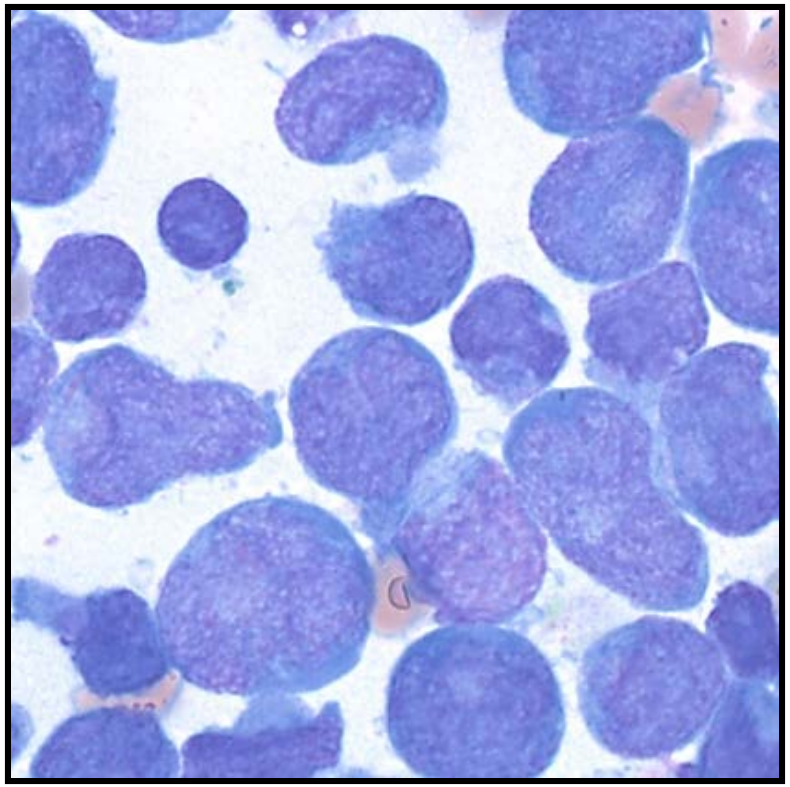
Patient B (Contrast Case)

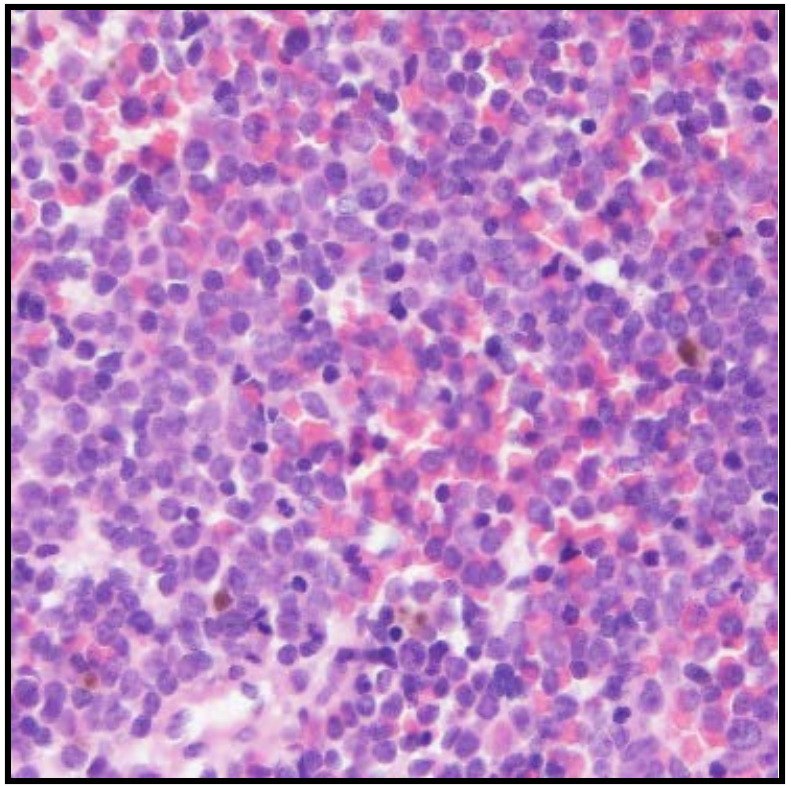
- 70-year-old man with history of relapsed AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
- Known to be *FLT3*-ITD positive
- CBC: WBC 12.5 K/µL; Hgb 9.4 g/dL; Plt 50 K/µL
    - 6% neutrophils, 49% lymphocytes, 7% monocytes, 0% eosinophils, 0% basophils, **38% blasts**

Patient B

Pre-Therapy Laboratory Results

- **Flow Cytometry**: Blasts (95%): CD33+ CD34+ CD56+ CD117+ HLA-DR+
- **Cytogenetics**45,X,-Y,add(2)(p12),t(6;19)(p21;q11.2),**t(8;21)(q22;q22)**, add(16)(q13),add(20)(q?13.1)[20]
- ***FLT3*-ITD**:
  - **ITD size**: 33 bp
  - **Mutant-WT ratio**: 0.43
- **NGS studies**: *TET2**

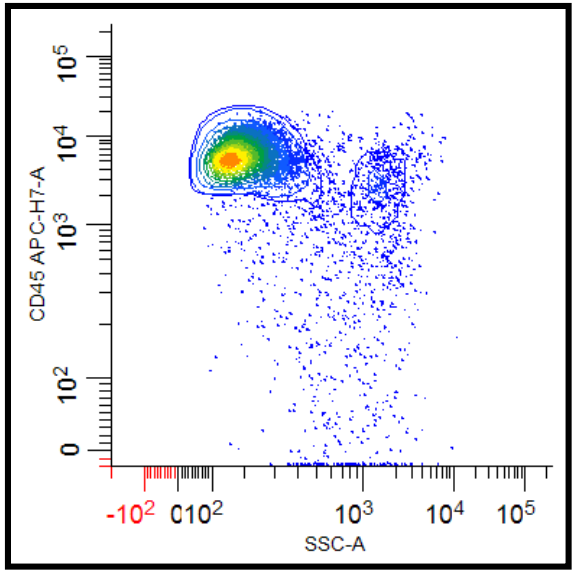


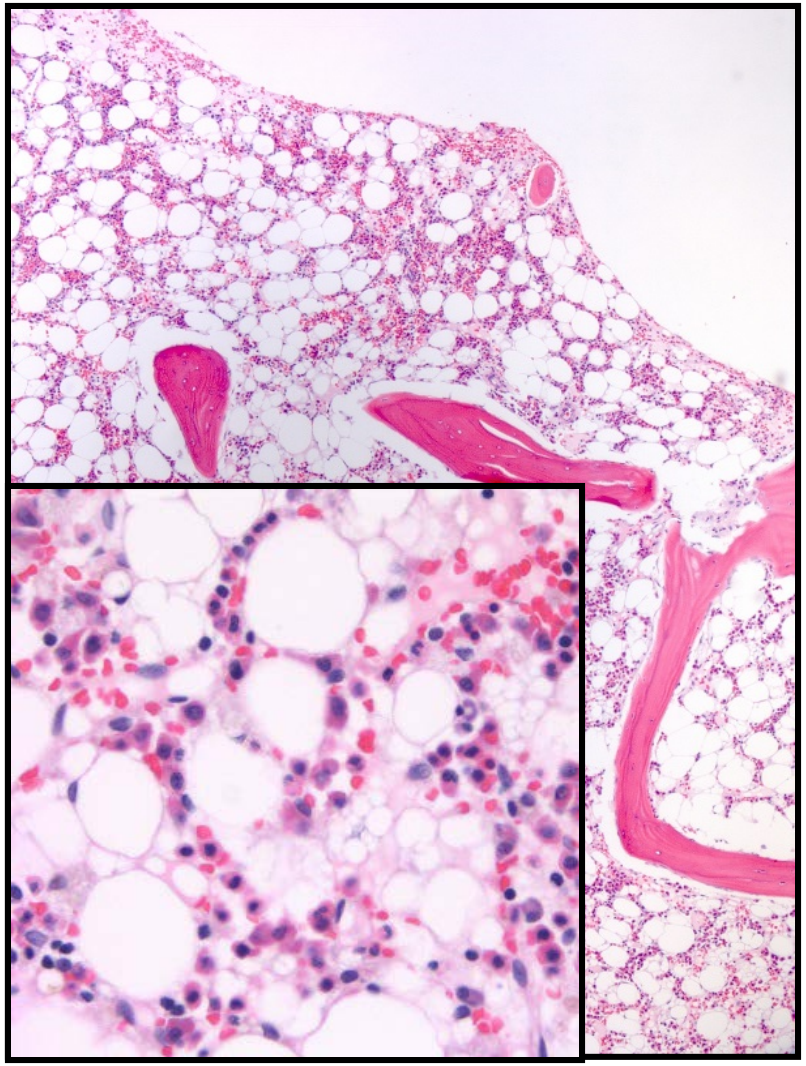


Patient B

Post-Therapy Laboratory Results

- **Flow Cytometry**: <5% Blasts
- **Cytogenetics**: similar
- ***FLT3*-ITD**:
  - **ITD size**: 33 bp
  - **Mutant-WT ratio**: 0.05
- **NGS studies**: No new variants
- **Peripheral blood**: pancytopenia, no circulating blasts





Summary of Findings

- **Case A** shows a **differentiation response**:
  - maintenance of marrow cellularity
  - return of trilineage hematopoiesis, with myeloid maturation
  - Sustained peripheral blood neutrophil recovery
  - **essentially unchanged M:WT ratio**
- In contrast, **Case B** shows a **cytotoxic response**:
  - marked reduction in marrow cellularity
  - marked decrease in hematopoiesis
  - **decreased FLT3-ITD M:WT ratio**

Morphology vs ITD Mutant Fraction?

- Relatively constant/increased *FLT3*-ITD mutation fraction suggests a drug-induced maturation of the leukemic clone
- *FLT3*-ITD fraction was similar in peripheral blood and bone marrow specimens
- This finding is similar to ATRA therapeutic response in APL with *PML-RARA* and more recently with IDH2 inhibitors in AML[2-3]
- Bone marrow biopsies may be reported descriptively to address apparent discordance

FLT3 ITD Biology

- FLT3:FMS-like tyrosine kinase 3
  - Member of class III receptor tyrosine kinase family
- *FLT3*-ITD mutations leads to terminal block in myeloid differentiation by inhibition of *CEBP*α by phosphorylation [4-6].
- Pharmacologic inhibition of *FLT3* (AC220 and CEP-701) overcomes differentiating block in leukemic cell lines [6].
- Concomitant *CEBPα* mutations may negate this effect [4].

Why the differential responses?

- AML with *FLT3*-ITD, *DNMT3A*, and *NPM1* mutations: patients tend to be younger and female, with high blast counts and perhaps an overall worse prognosis [7].
  - *DNMT3A* mutation → hypomethylation of hematopoietic enhancers
  - *NPM1* mutation → cytoplasmic localization of protein, suppression of ARF-p53 pathway [8]
- *RUNX1-RUNX1T1*: transcriptional repressor → repress microRNA miR-223 → block myeloid maturation [9-10].

Clinical Followup

- **Patient A:**
  - Rapid clearance of leukemic blasts in peripheral blood and bone marrow, with sustained neutrophilic recovery
  - Eventual withdrawal from study for allogeneic HSCT.
  - Post-transplant bone marrow biopsy *FLT3*-ITD negative (>99% donor)
  - At last account, patient is alive and well, with no relapse of AML.
- **Patient B:**
  - Withdrawal from study due to leukemia progression

Summary of Cohort Data

- Our analysis of a cohort of 19 patients shows that baseline genetic studies affect responses
- In particular, *NPM1* and *DNMT3A* mutational status and cytogenetics are useful in predicting the type of response
- Cases that undergo differentiation responses tend to be cytogenetically normal and possess *DNMT3A* and/or *NPM1* mutations
- Those that undergo cytotoxic responses tend to be cytogenetically abnormal and complex and tend to lack these mutations

Summary of Cohort Data

Patient A

Patient B

	Subject	FLT3 genotype	Best marrow blast %	Baseline Karyotype response	Karyotype at response	Cooperating mutations
Differentiation response	1009-01	FLT3-ITD	10%	46, XY	46, XY	DNMT3a*,NPM1,ASXL1,IDH1
	1009-02	FLT3-ITD	15%	46, XX	46, XX	DNMT3a,NPM1,TET2
	1009-14	FLT3-ITD	<5%	46, XY	46, XY	DNMT3a,NPM1
	1009-04	FLT3-ITD	<5%	46, XX	46, XX	DNMT3a,NPM1,WT1,ATM*
	1009-07	FLT3-ITD	<5%	46, XX	46, XX	DNMT3a,NPM1
	1009-09	FLT3-ITD	<5%	46, XY	46, XY	DNMT3a,NPM1,TET2
	1009-11	FLT3-ITD	<5%	46, XX	46, XX	DNMT3a,NPM1,TET2
	1009-10	FLT3-WT	<5%	46, XY	46, XY	TET2
	1009-21	FLT3-ITD	10%	46, XY, +11	46, XY, +11	DNMT3a,ASXL1
Cytotoxic response	1009-06	FLT3-ITD	<5%	complex	46, XY	No sample available
	1009-03	FLT3-ITD	<5%	hyperdiploid/complex	46, XX	DNMT3a,RUNX1
	1009-13	FLT3-WT	<5%	complex	46, XY	TP53, NOTCH1
	1009-12	FLT3-WT	<5%	complex	46, XY	No sample available
	1009-17	FLT3-WT	<5%	complex	complex	TP53,JAK2
	1009-19	FLT3-ITD	<5%	complex	no growth	No mutations
	1009-08	FLT3-ITD	<10%	46, XY, del(12)(p11.2)	46, XY, del(12)(p11.2)	RUNX1
	1009-18	FLT3-ITD	<5%	46, XY, t(8;21),(q22;q22) with multiple additional abnormalities	46, XY, t(8;21),(q22;q22) with multiple additional abnormalities	TET2*
	1009-16	FLT3-ITD	15%	46, XY, del(5)(q23q33)	46, XY, del(5)(q23q33)	ATM*
	1009-15	FLT3-ITD	70%	47, XX, +8, del(16)(q13)	47, XX, +8, del(16)(q13)	DNMT3a,NPM1

References

1. Nybakken, G.E., Canaani, J., Roy, D., Morrissette, J.D., Watt, C.D., Shah, N.P., Smith, C.C., Bagg, A., Carroll, M. and Perl, A.E., 2016. Quizartinib elicits differential responses that correlate with karyotype and genotype of the leukemic clone. *Leukemia*, *30*(6), pp.1422-1425.
2. Mueller, B.U., Pabst, T., Fos, J., Petkovic, V., Fey, M.F., Asou, N., Buergi, U. and Tenen, D.G., 2006. ATRA resolves the differentiation block in t (15; 17) acute myeloid leukemia by restoring PU. 1 expression. *Blood*, *107*(8), pp.3330-3338.
3. Wang, F., Travins, J., DeLaBarre, B., Penard-Lacronique, V., Schalm, S., Hansen, E., Straley, K., Kernytsky, A., Liu, W., Gliser, C. and Yang, H., 2013. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science*, *340*(6132), pp.622-626.
4. Sexauer, A., Perl, A., Yang, X., Borowitz, M., Gocke, C., Rajkhowa, T., Thiede, C., Frattini, M., Nybakken, G.E., Pratz, K. and Karp, J., 2012. Terminal myeloid differentiation in vivo is induced by FLT3 inhibition in FLT3/ITD AML. *Blood*, *120*(20), pp.4205-4214.
5. Zheng, R., Friedman, A.D., Levis, M., Li, L., Weir, E.G. and Small, D., 2004. Internal tandem duplication mutation of FLT3 blocks myeloid differentiation through suppression of C/EBPα expression. Blood, 103(5), pp.1883-1890.
6. Radomska, H.S., Bassères, D.S., Zheng, R., Zhang, P., Dayaram, T., Yamamoto, Y., Sternberg, D.W., Lokker, N., Giese, N.A., Bohlander, S.K. and Schnittger, S., 2006. Block of C/EBPα function by phosphorylation in acute myeloid leukemia with FLT3 activating mutations. *Journal of Experimental Medicine*, *203*(2), pp.371-381.
7. Loghavi S, Zuo Z, Ravandi F, Kantarjian HM, Bueso-Ramos C, Zhang L, Singh RR, Patel KP, Medeiros LJ, Stingo F, Routbort M, Cortes J, Luthra R, Khoury JD. Clinical features of de novo acute myeloid leukemia with concurrent DNMT3A, FLT3 and NPM1 mutations. J Hematol Oncol. 2014 Oct 4;7:74.
8. Chauhan, P.S., Ihsan, R., Singh, L.C., Gupta, D.K., Mittal, V. and Kapur, S., 2013. Mutation of NPM1 and FLT3 genes in acute myeloid leukemia and their association with clinical and immunophenotypic features. *Disease markers*, *35*(5), pp.581-588.
9. Hyde, R.K. and Liu, P.P., 2010. RUNX1 repression-independent mechanisms of leukemogenesis by fusion genes CBFB–MYH11 and AML1–ETO (RUNX1–RUNX1T1). *Journal of cellular biochemistry*, *110*(5), pp.1039-1045.
10. Lam, K. and Zhang, D.E., 2012. RUNX1 and RUNX1-ETO: roles in hematopoiesis and leukemogenesis. *Frontiers in bioscience: a journal and virtual library*, *17*, p.1120.

Final/Panel Diagnosis

- **Patient A**: Acute myeloid leukemia with mutated *NPM1*
- **Patient B**: Acute myeloid leukemia with t(8;21)(q22;q22:1);*RUNX1-RUNX1T1*