Disclosures

- Grant support to institution for clinical trials from : Astellas, Agios, Abbvie, Daiichi Sankyo, Millennium
- Scientific Advisory Boards: Astellas, Abbvie, Agios, Astra Zeneca, Boston Biomedical, BMS Celgene, Hoffman La Roche, Servier
- Off label usage: Sorafenib, Venetoclax, Gilteritinib for AML.

Penn Medicine

Targeting FLT3 Mutant AML- Past, Present and Future?

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February 11, 2023

A Minute Chromosome in Human Chronic Granulocytic Leukemia

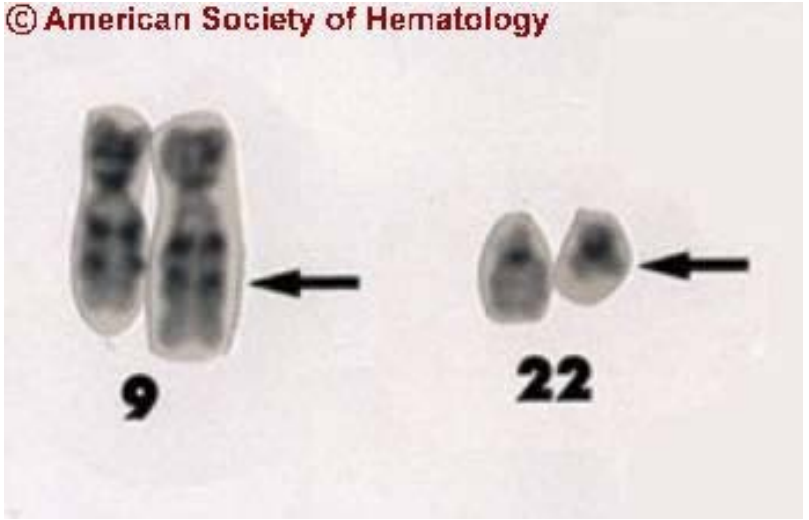
In seven cases thus far investigated (five males, two females), a minute chromosome has been observed replacing one of the four smallest autosomes in the chromosome complement of cells of chronic granulocytic leukemia cultured from peripheral blood. No abnormality was observed in the cells of four cases of *acute* granulocytic leukemia in adults or of six cases of acute leukemia in children. There have been several recent reports of chromosome abnormalities in a number of cases of human leukemia [including two of the seven cases reported here: Nowell and Hungerford, *J. Natl. Cancer Inst.* **25**, 85 (1960)], but no series has appeared in which there was a consistent change typical of a particular type of leukemia.

Cells of the five new cases were obtained from peripheral blood (and bone marrow in one instance), grown in culture for 24–72 hours, and processed for cytological examination by a recently developed air-drying technique (Moorhead, *et al., Exptl. Cell Research*, in press). The patients varied from asymptomatic untreated cases to extensively treated cases of several years' duration in terminal myeloblastic crisis. All seven individuals showed a similar minute chromosome, and none showed any other frequent or regular chromosome change. In most of the cases, cells with normal chromosomes were also observed. Thus, the minute is not a part of the normal chromosome constitution of such individuals.

The findings suggest a causal relationship between the chromosome abnormality observed and chronic granulocytic leukemia.

PETER C. NOWELL
School of Medicine, University of Pennsylvania
DAVID A. HUNGERFORD
Institute for Cancer Research

18 NOVEMBER 1960



Nowell & Hungerford. Science 1960

The New England
Journal of Medicine

VOLUME 344 APRIL 5, 2001 NUMBER 14

EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

BRIAN J. DRUKER, M.D., MOSHE TALPAZ, M.D., DEBRA J. RESTA, R.N., BIN PENG, PH.D., ELISABETH BUCHDUNGER, PH.D., JOHN M. FORD, M.D., NICHOLAS B. LYDON, PH.D., HAGOP KANTARJIAN, M.D., RENAUD CAPDEVILLE, M.D., SAYURI OHNO-JONES, B.S., AND CHARLES L. SAWYERS, M.D.

TABLE 3. HEMATOLOGIC RESPONSES.

DOSE (mg/DAY)	ALL PATIENTS	PATIENTS WITH RESPONSES	PATIENTS WITH COMPLETE RESPONSES
	no.	no. (%)	
25 or 50	6	2 (33)	0
85	4	2 (50)	1 (25)
140	3	3 (100)	1 (33)
200 or 250	16	16 (100)	9 (56)
300–1000	54	54 (100)	53 (98)
Total	83	77 (93)	64 (77)

TABLE 4. CYTOGENETIC RESPONSES.

DOSE (mg/DAY)	ALL PATIENTS	PATIENTS WITH COMPLETE OR MAJOR RESPONSES	PATIENTS WITH MINOR RESPONSES
	no.	no. (%)	
300–350	13	5 (38)	2 (15)
400	6	3 (50)	2 (33)
500	6	1 (17)	1 (17)
600	8	4 (50)	4 (50)
750	6	2 (33)	0 (0)
800	8	1 (12)	2 (25)
1000	7	1 (14)	1 (14)
Total	54	17 (31)	12 (22)

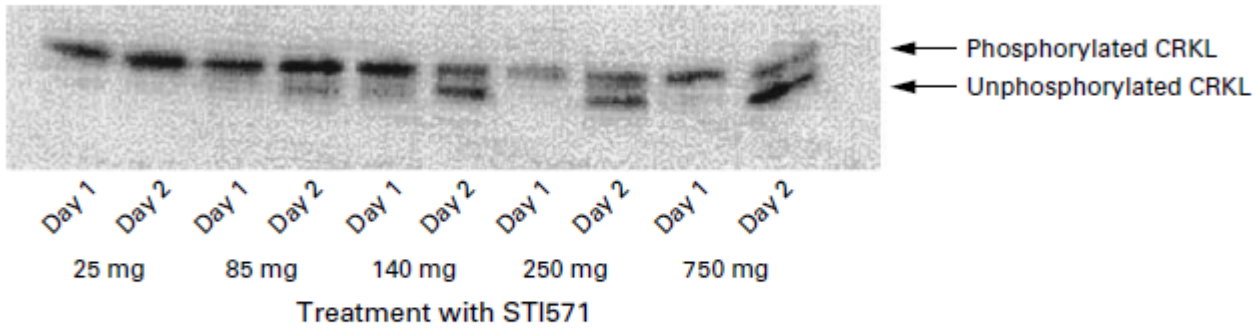


Figure 3. Immunoblot Assays Demonstrating the Degree of Phosphorylation of the BCR-ABL Substrate CRKL in Individual Patients in the Groups Receiving Daily Doses of 25, 85, 140, 250, and 750 mg of STI571.

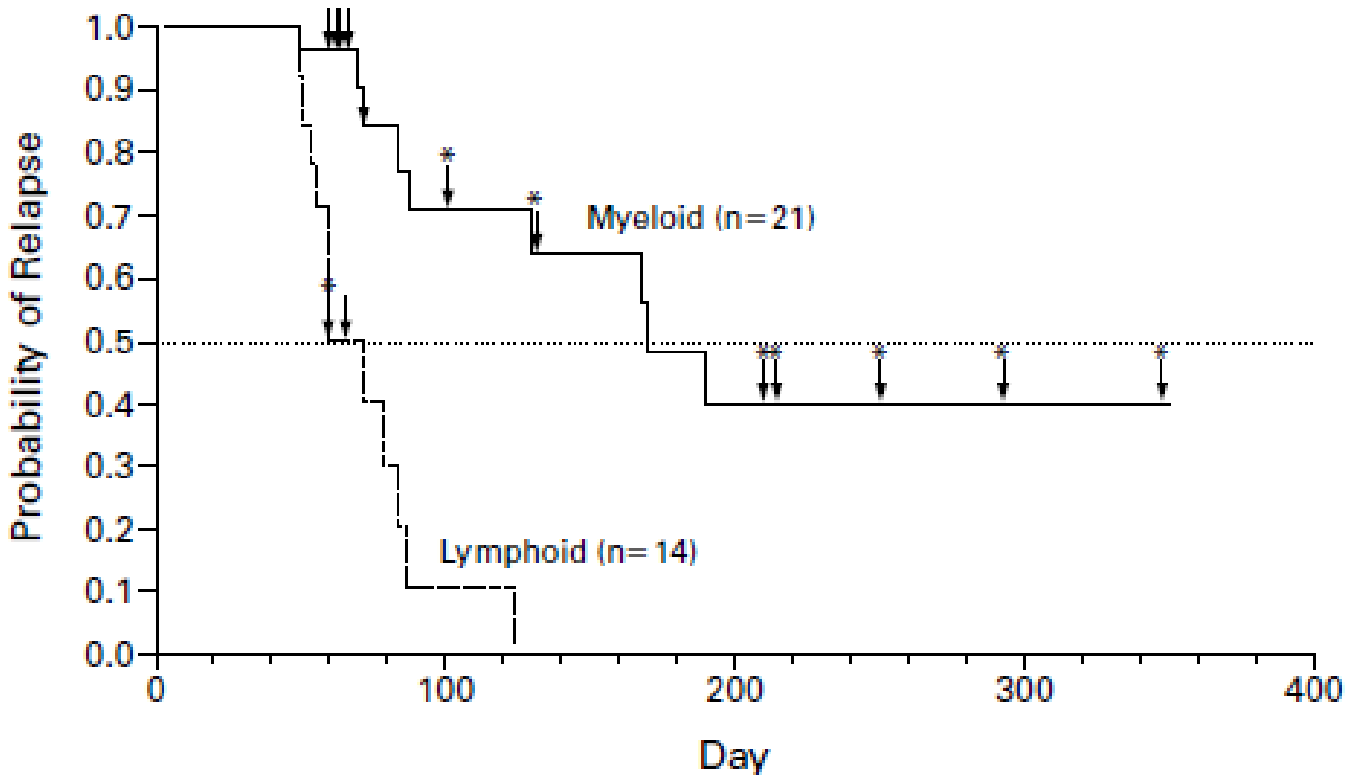


Figure 2. Time to Relapse in Patients with Myeloid or Lymphoid Blast Crisis Who Had a Response to STI571.

Arrows with asterisks indicate patients still enrolled in the study and in remission at the time of the last follow-up; arrows without asterisks indicate the day on which patients were removed from the study.

Druker et al NEJM 2001

Imatinib Post Transplant – Ph + ALL

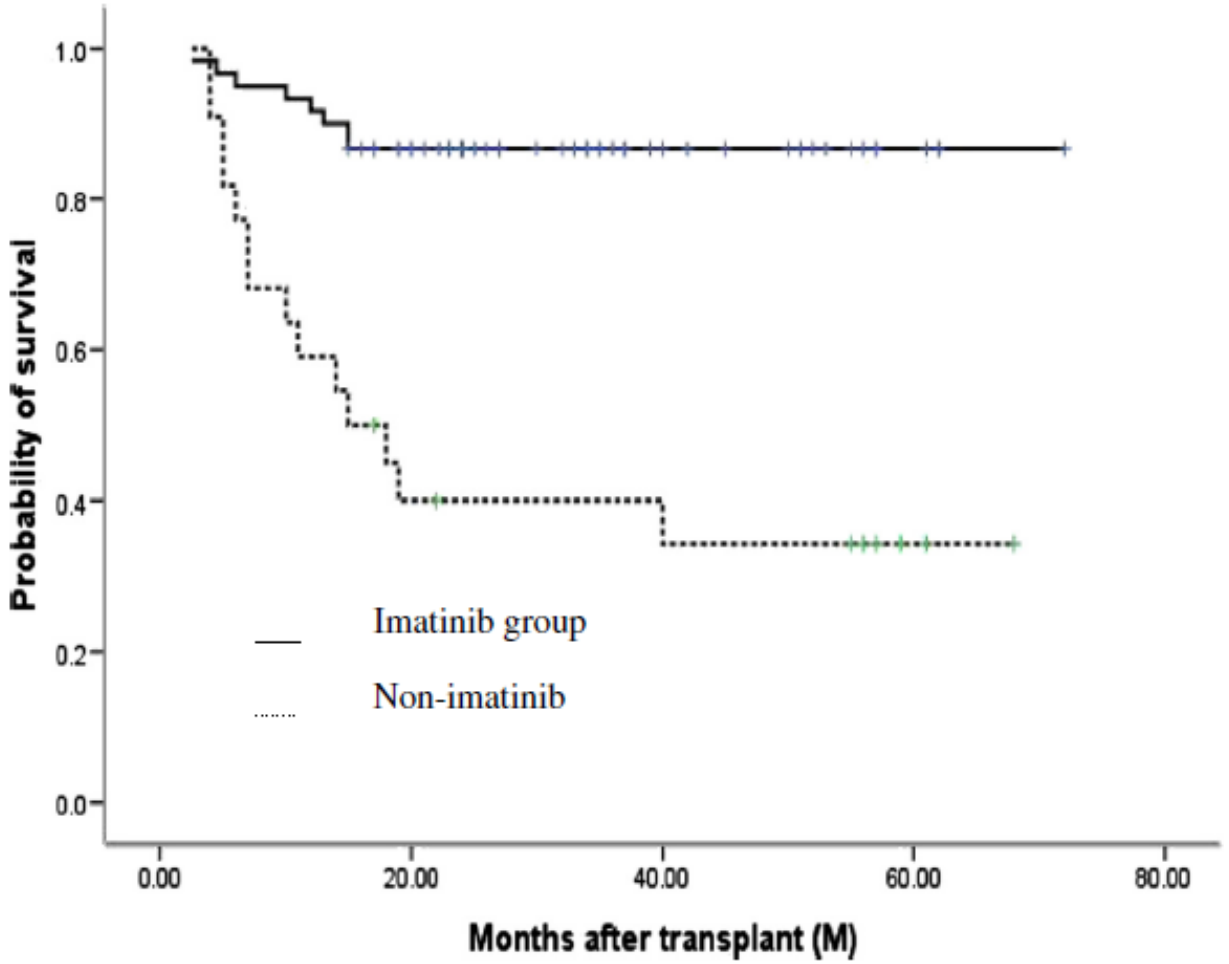


Figure 3 Overall survival (OS) at 5 years in imatinib and non-imatinib groups, post-HCT. Kaplan-Meier analysis showed that the 5-year OS of patients in the imatinib-group was significantly higher than the patients in the non-imatinib group (86.7% *vs* 34.3%, p = 0.000).

Chen et al, J Hem & Onc, 2012

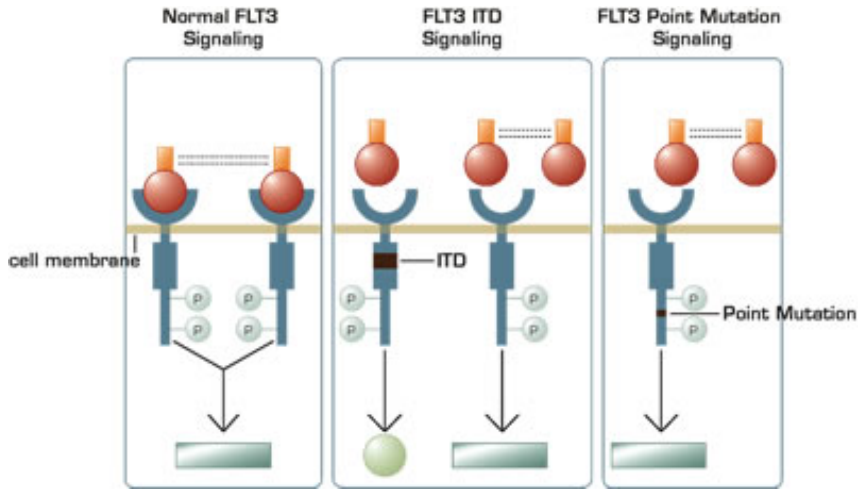
FLT 3 Tyrosine Kinase

- First noted to be over-expressed in AML in 1992(*Birg et al, Blood 1992*)
- Noted to be mutated in AML in 1996 (*Nakao et al, Blood 1996*)
- Type III receptor tyrosine kinase
- Cell surface receptor with normal function in T cell development
- Mutated in ~32% of AML
  - Internal tandem duplications (ITD) in juxtamembrane region are found in ~25% of new AML
  - Point mutations in kinase domain are found in ~7% in new AML

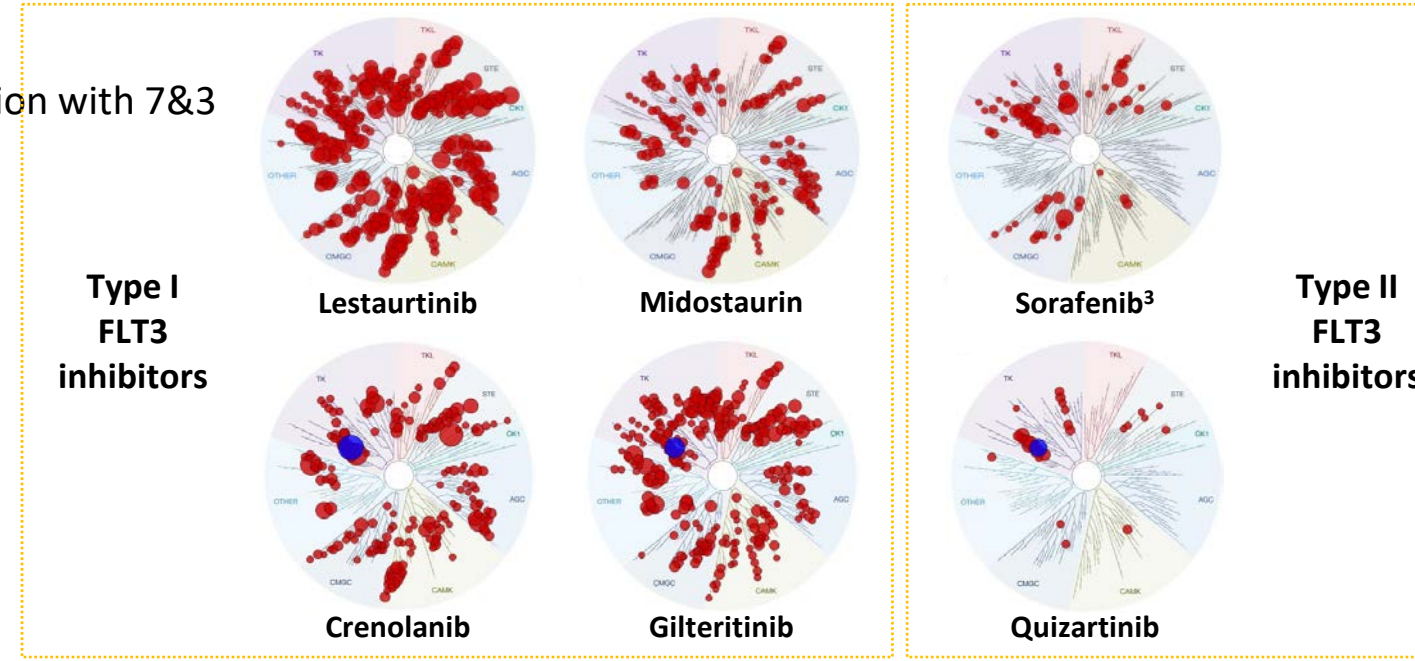
Pratz KW & Levis M. *Blood* 2017;129:565–571

FLT3 as a Target

- Actually three targets
  - Wild type kinase
  - FLT3 ITD mutant
  - Kinase domain mutant
- Compounds in clinical use/development
  - Midostaurin (PKC412)– FLT3 mutant AML to be given with 7&3 based chemo (4/2017 FDA approval)
  - Gilteritinib(ASP2215) – Relapsed and refractory FLT3 mutant AML (11/2018 FDA approval)
  - Sorafenib (BAY 43-9006)– Randomized data for post transplant maintenance
  - Crenolanib- In phase 3 studies in combination with 7&3
  - Quizartinib (AC-220)– Positive phase 3 studies in combination with 7&3
- Type I inhibitors of TKD and ITD
- Type II of ITD only



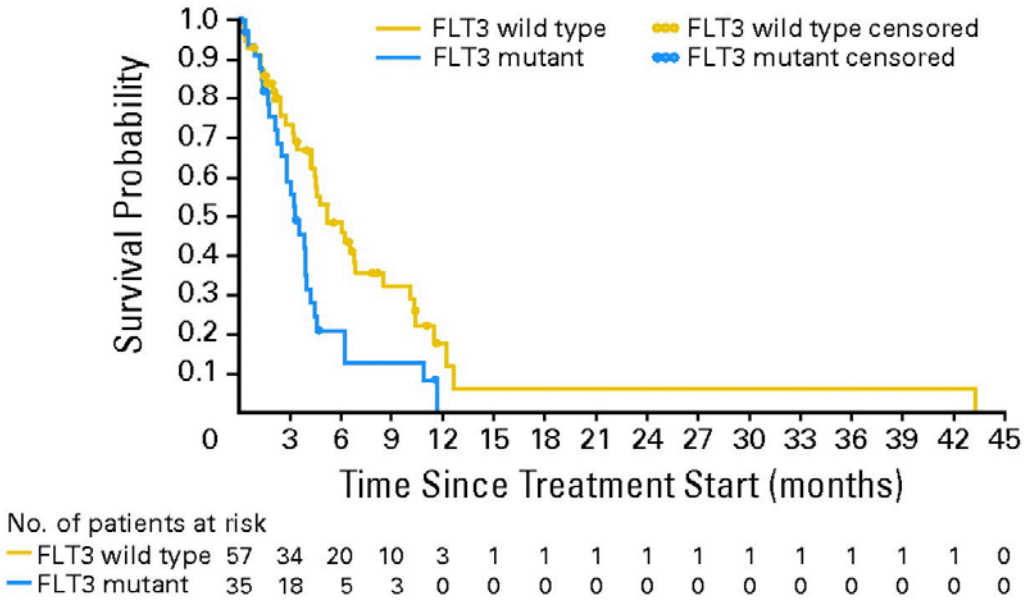
Emanuel P; *2007 Hematologist*

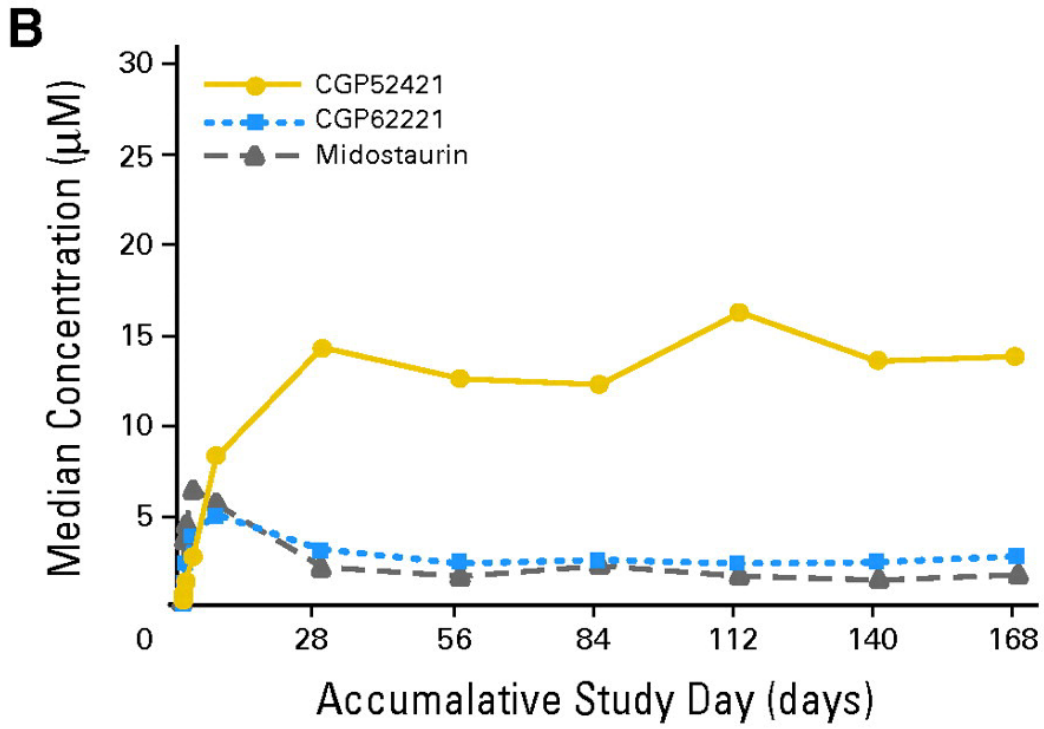


Cortes et al ASH 2018

Midostaurin as single agent

- Phase IIb Study
  - 95 relapsed or refractory patients (60 FLT3 WT, 35 FLT3 mutated)
  - Randomized between 100mg and 50mg Twice daily
  - Standard efficacy endpoints
- Results
  - One PR(1/92), 49 blast reduction(49/92)
  - FLT3 Mutated 25/35(71%) had blast reduction, 1 PR
  - FLT3 WT 24/57(24%) had blast reduction





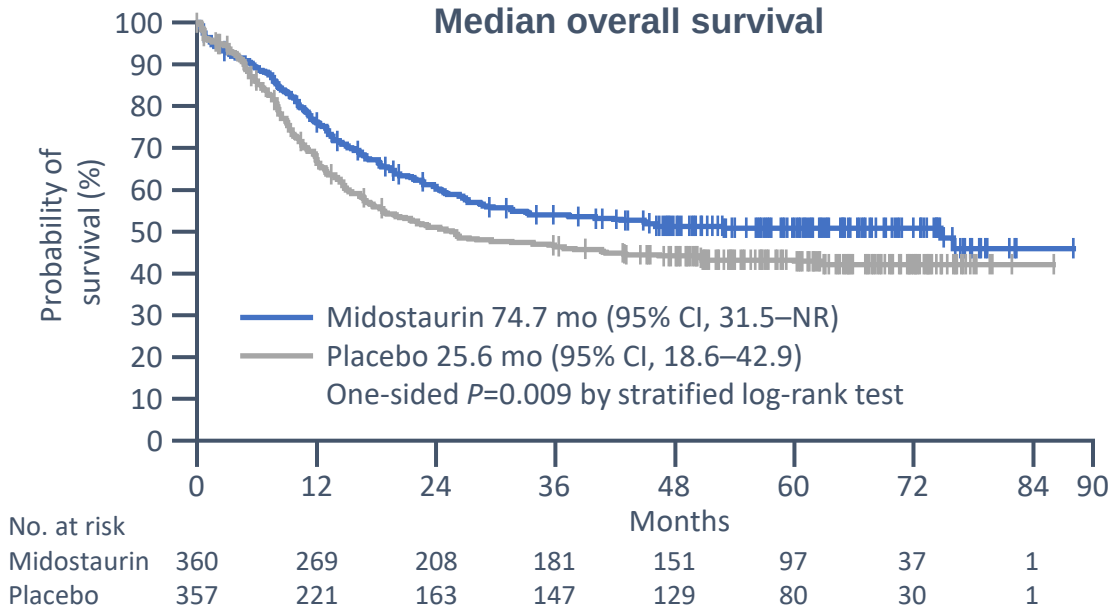
Fischer et al, 2010 JCO

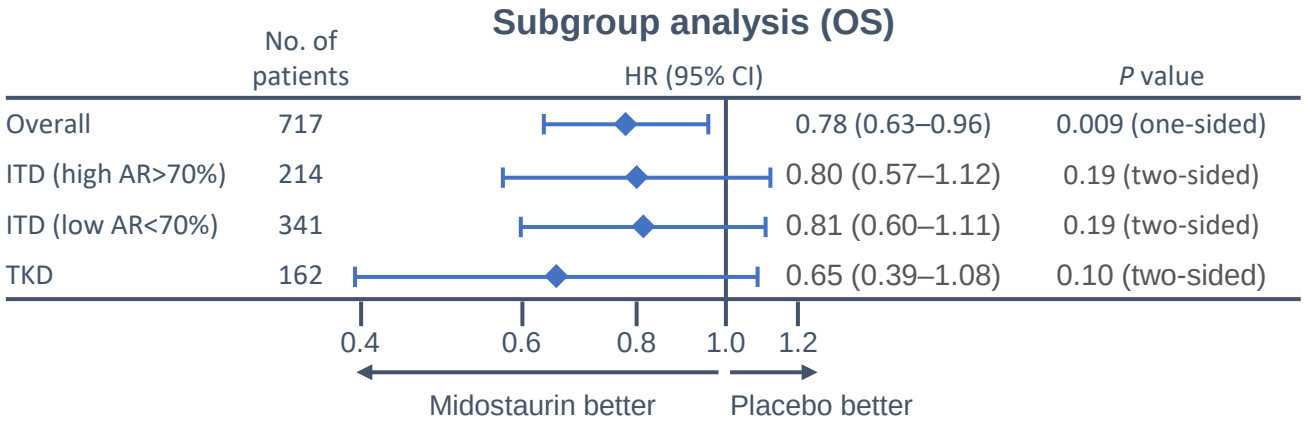
Midostaurin + Chemotherapy - RATIFY trial

- Induction: cytarabine 200 mg/m^2, daunorubicin 60 mg and either
  - Midostaurin 50 mg by mouth twice daily, day 8–21 or
  - Placebo twice daily, day 8–21
- Ages 18–59
- **FLT3 ITD mutant or FLT3 TKD mutant**
- Consolidation: cytarabine 3000 mg/m^2 over 3 hours every 12 hours on day 1, 3, 5 with either
  - Midostaurin 50 mg by mouth twice daily, day 8–21 or
  - Placebo twice daily, day 8–21
- Maintenance midostaurin or placebo x 12 months
- Primary endpoint was overall survival

Results

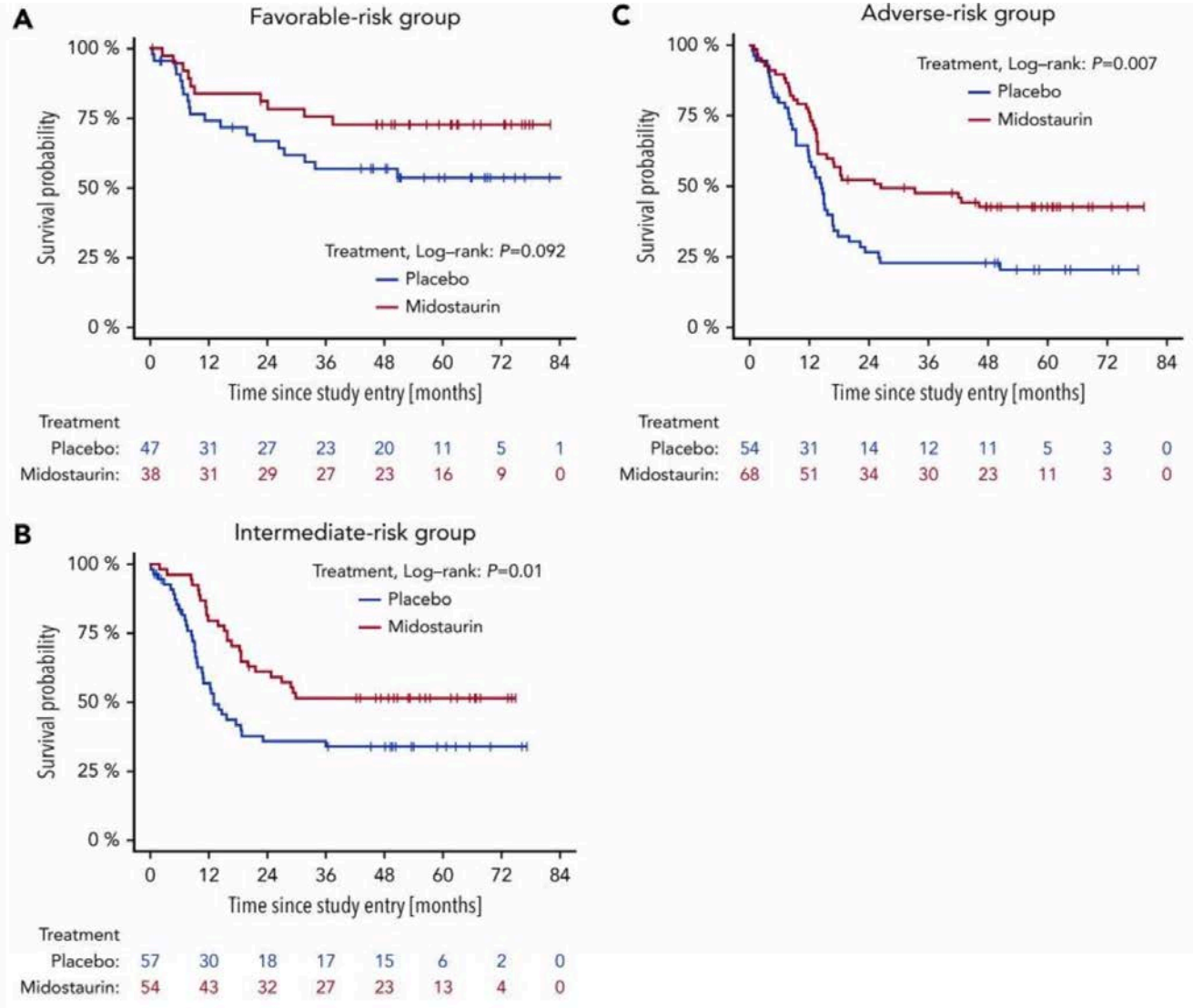
- 717 patients randomized
- Complete remission:
  - Midostaurin arm: 59% (**OS = 74 m**)
  - Placebo arm: 54 % (**OS = 25.6 m**)
- Transplantation: 59% mido vs 55% placebo
- 4-year overall survival 51.4% mido vs 44.3% placebo





Stone RM *et al. N Engl J Med* 2017; doi: 10.1056/NEJMoa1614359

RATIFY study outcomes by ELN 2017 risk groups

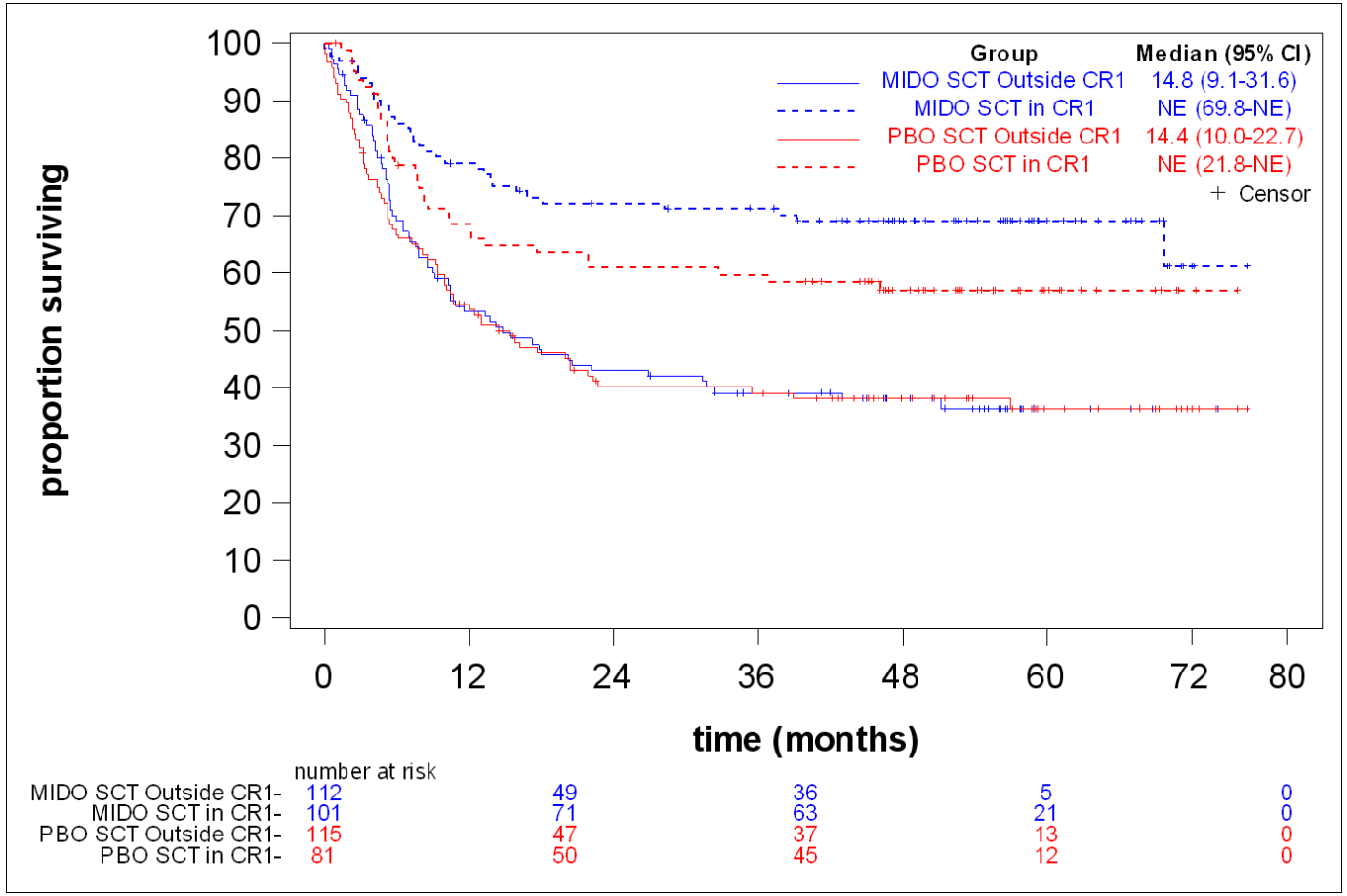


OS of patients with the different *NPM1*/*FLT3*-ITD genotypes by 2017 ELN risk group and by treatment. (A) Favorable-risk group. (B). Intermediate-risk group. (C) Adverse-risk group.

Dohner et al Blood 2020, 135 (5) 371-380

RATIFY trial

- Role for transplant FLT3 ITD AML in CR1 established
- Benefit of midostaurin only in pts who transplant in CR1



Stone RM *et al. N Engl J Med* 2017; doi: 10.1056/NEJMoa1614359

Co-mutation effect on outcome from Ratify

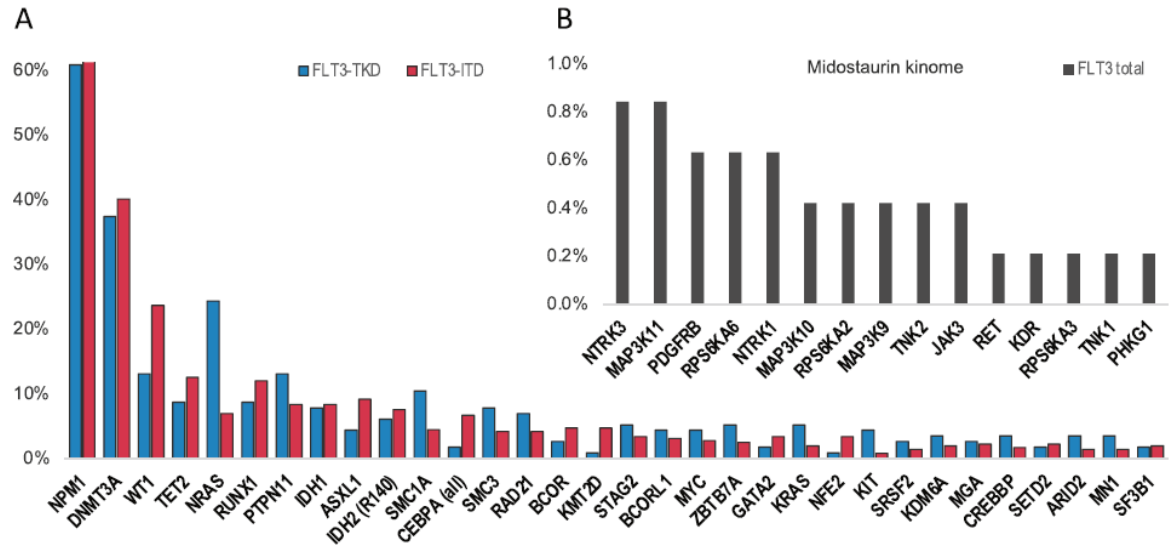
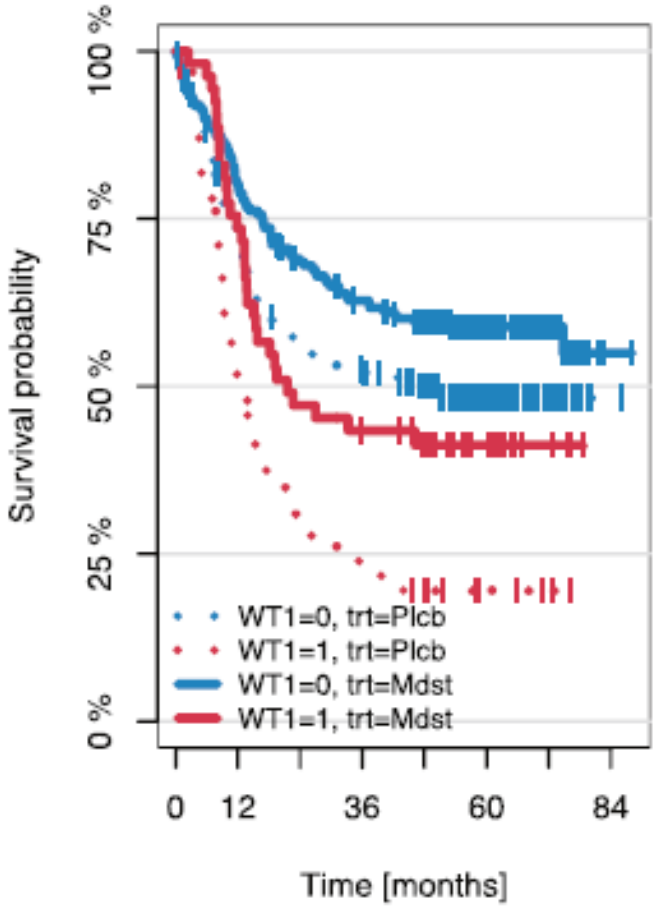


Fig. 2 Mutational landscape of *FLT3*-mutated acute myeloid leukemia. A Mutational landscape of 475 patients stratified according to the *FLT3* mutation type. **B** Incidence of mutations in the midostaurin kinome in the entire cohort of 475 patients.

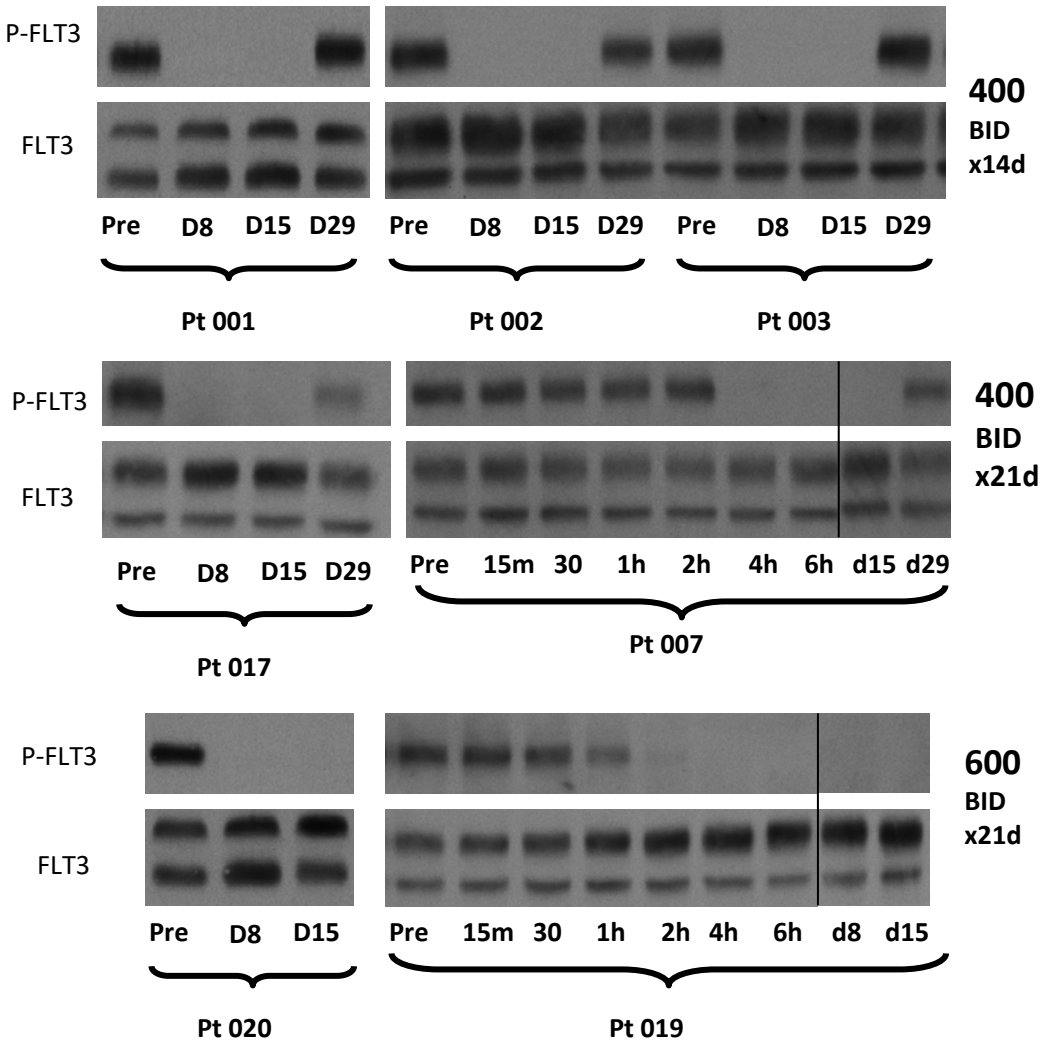
	Overall Surival					Event-free Survival				
	Logrank p-value (raw)	Logrank p-value (adj)	HR	Lower CI	Upper CI	Logrank p-value (raw)	Logrank p-value (adj)	HR	Lower CI	Upper CI
NPM1	<0.001	<0.001	0.60	0.46	0.77	<0.001	<0.001	0.60	0.48	0.74
WT1	<0.001	<0.001	1.83	1.38	2.43	0.018	0.195	1.36	1.05	1.75
ASXL1	0.013	0.125	1.67	1.11	2.52	0.100	0.903	0.72	0.48	1.07
IDH2	0.130	1	0.65	0.37	1.14	0.075	0.748	0.64	0.38	1.05
NRAS	0.097	0.875	0.68	0.43	1.08	0.793	1	1.05	0.71	1.55
DNMT3A	0.984	1	1.00	0.77	1.30	0.275	1	0.88	0.71	1.10
IDH1	0.773	1	1.07	0.68	1.69	0.649	1	0.91	0.61	1.35
PTPN11	0.287	1	0.77	0.48	1.25	0.204	1	0.75	0.48	1.17
RUNX1	0.141	1	1.32	0.91	1.90	0.310	1	0.83	0.58	1.19
SMC1A	0.219	1	0.69	0.37	1.26	0.183	1	1.25	0.90	1.73
SMC3	0.841	1	0.94	0.50	1.77	0.433	1	0.81	0.47	1.38
TET2	0.460	1	0.85	0.56	1.30	0.600	1	1.09	0.79	1.52



Jahn et al Leukemia 2022

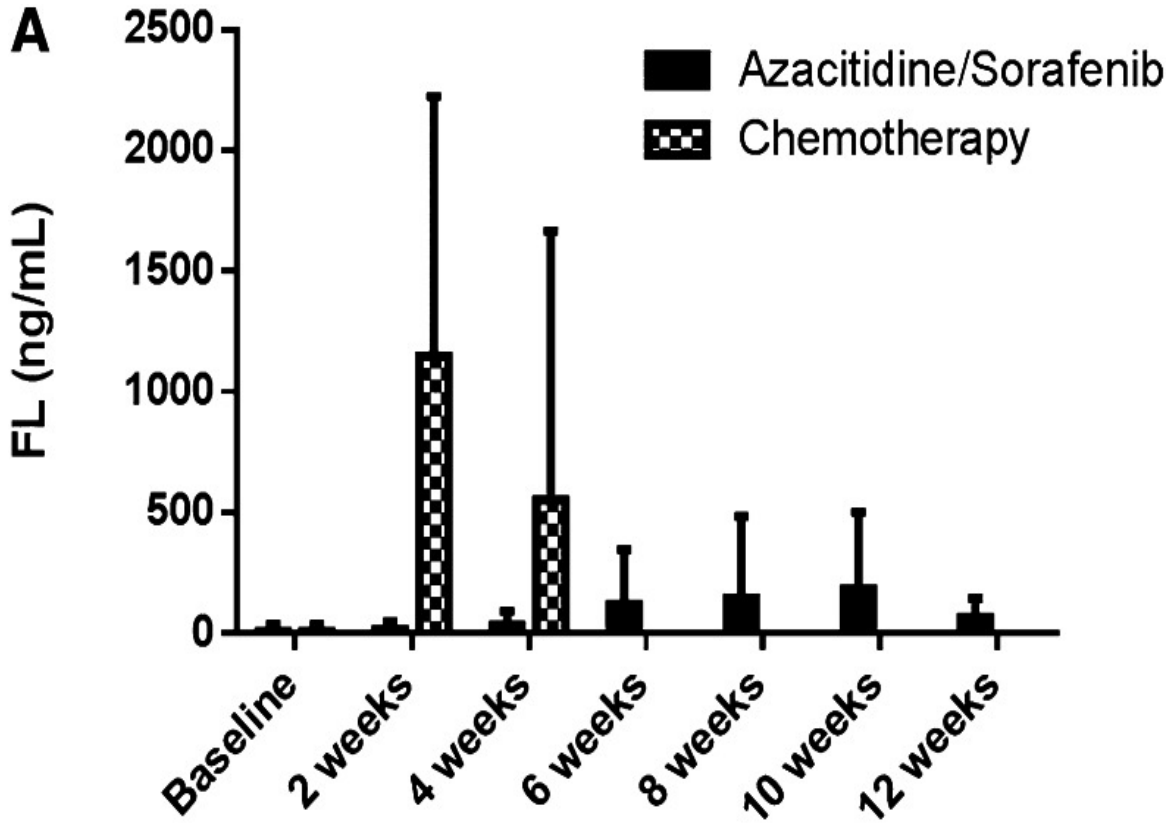
Sorafenib Phase I in refractory leukemias

- 6 of 15 patients demonstrated reduction in BM blasts (2/2 FLT3-ITD)
- 3 of 15 patients had greater than 50% reduction in BM blasts(1/2 FLT3-ITD)
- MTD 400mg BID x 21days
- FLT3 inhibitory dose likely lower than 400mg BID
- Active n-oxide metabolite



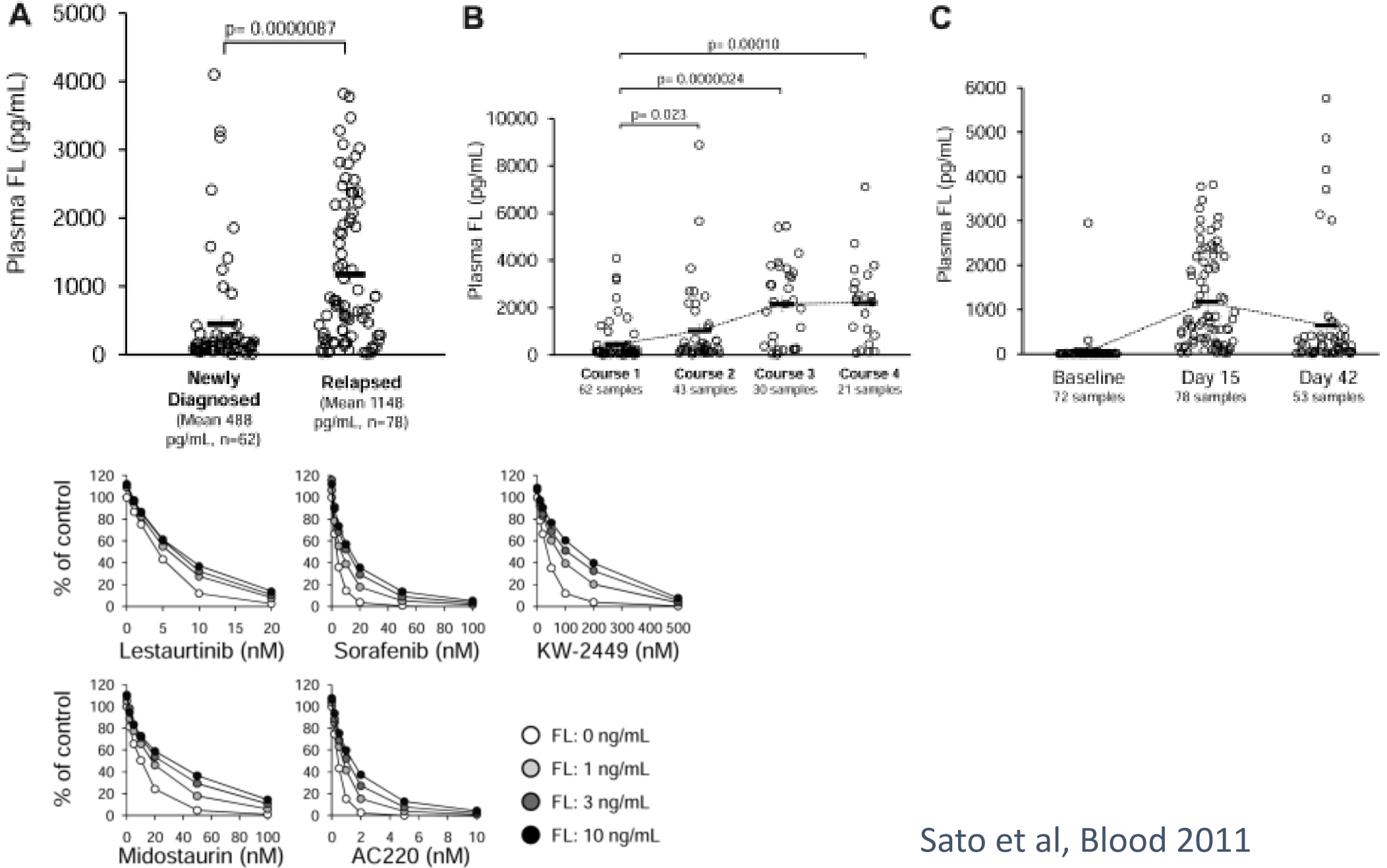
Pratz et al, Leukemia 2010

FL levels in Hypomethylating therapy



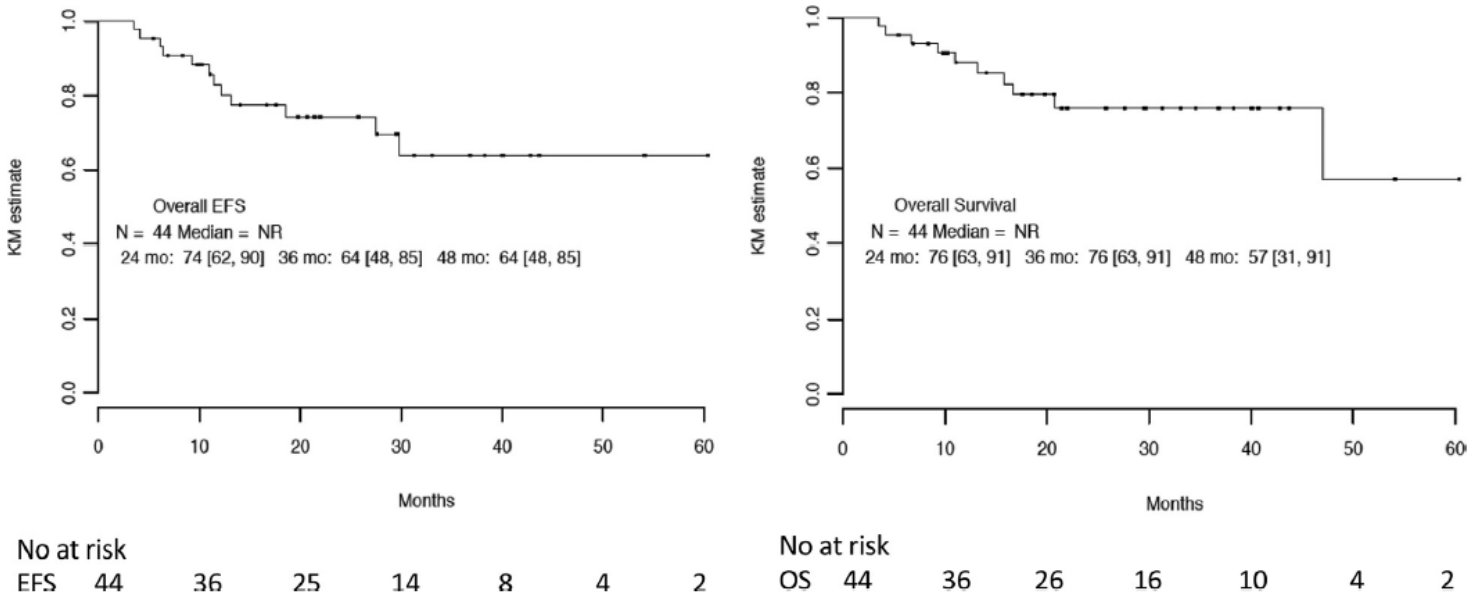
Ravandi et al, Blood 2013

FL impairs the Cytotoxic effects of FLT3 inhibitors

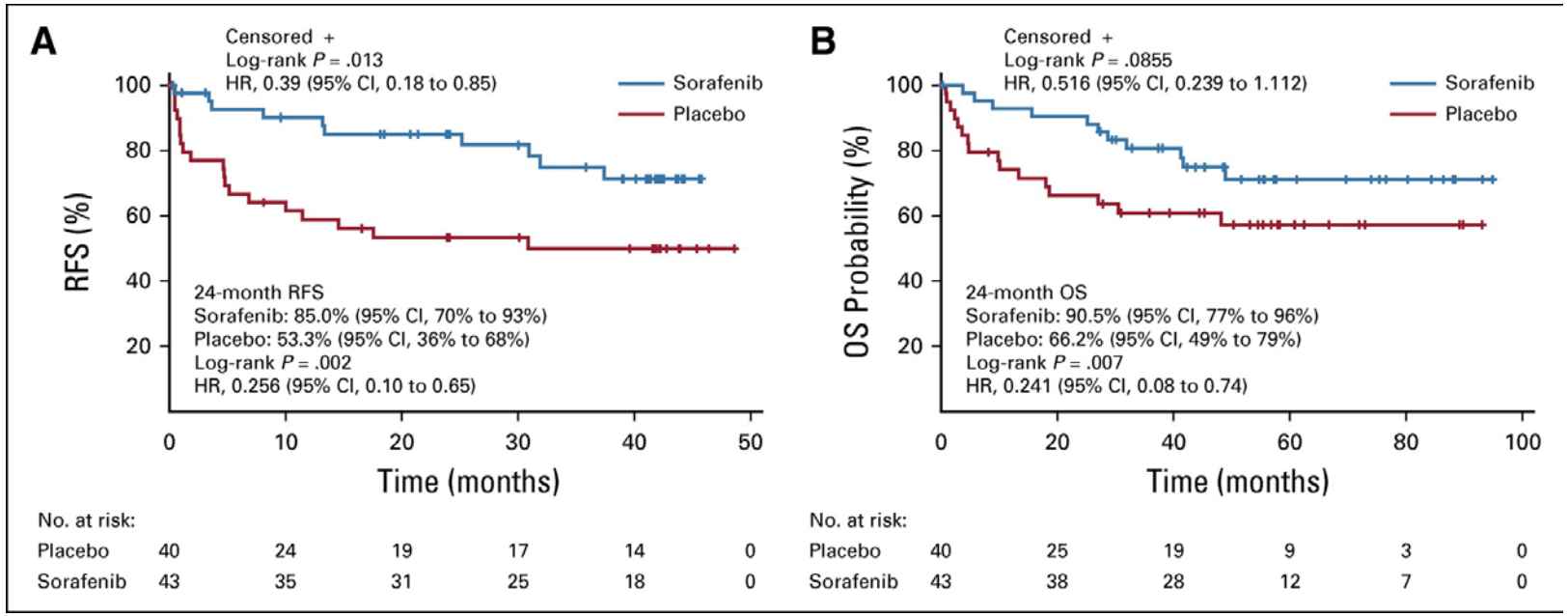


Sato et al, Blood 2011

Sorafenib Post Allogenic transplant



ETCTN-8922
Pratz et al BBMT 2020

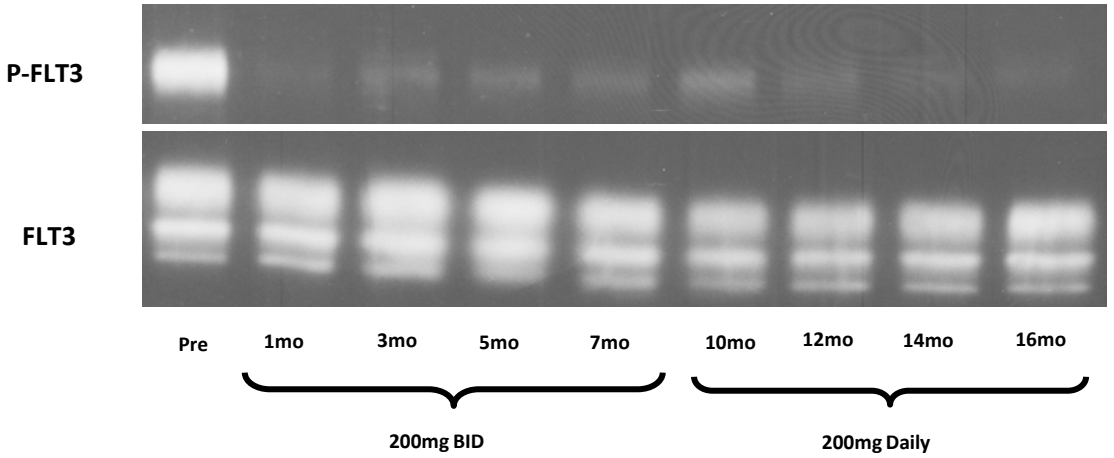


SORMAIN – Burchert et al JCO 2020

Peri-transplant Sorafenib PK/PD analysis

A

Patient #4 PIA example



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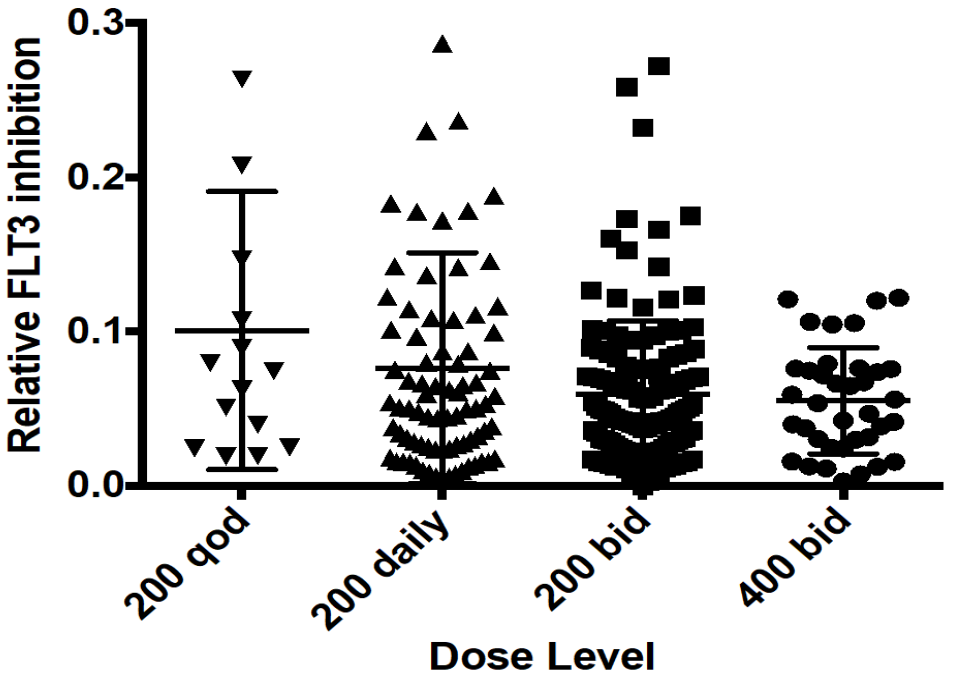
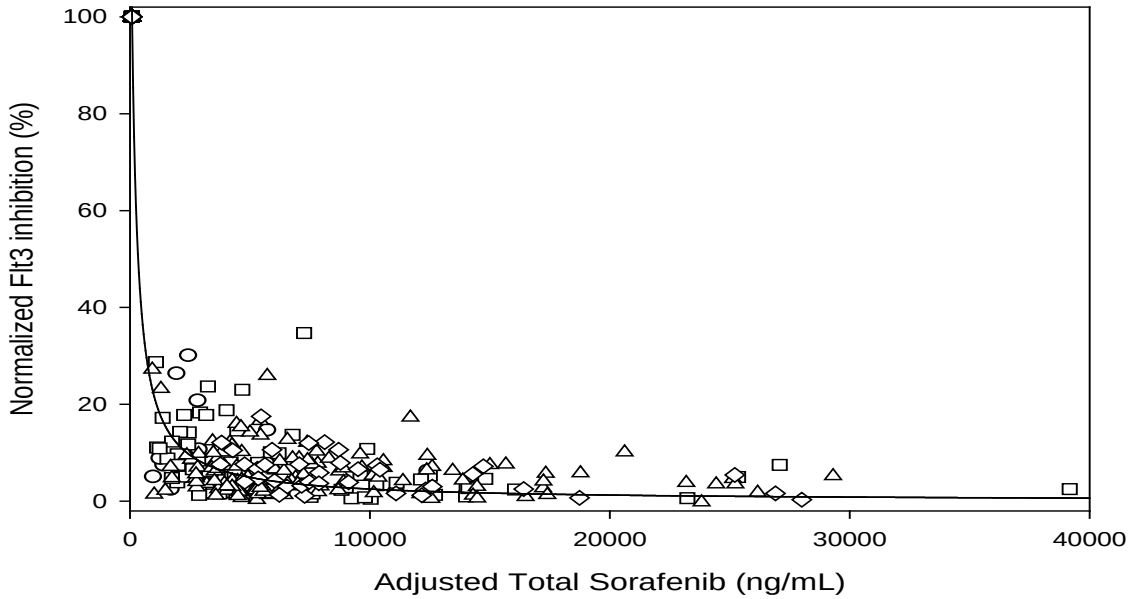


C



Pratz et al BBMT 2020

Sorafenib Tolerance

- 21/44 patients received Pre-transplant sorafenib
  - No cases of primary graft failure.
  - No VOD in pre-transplant sorafenib cohort
- Median restart date post transplant was 65 days(30-119)

Final Sorafenib Dose per Tolerability

400mg BID	200mg BID	200mg daily	200mg Qod
4 patients	22 patients	14 patients	4 patients

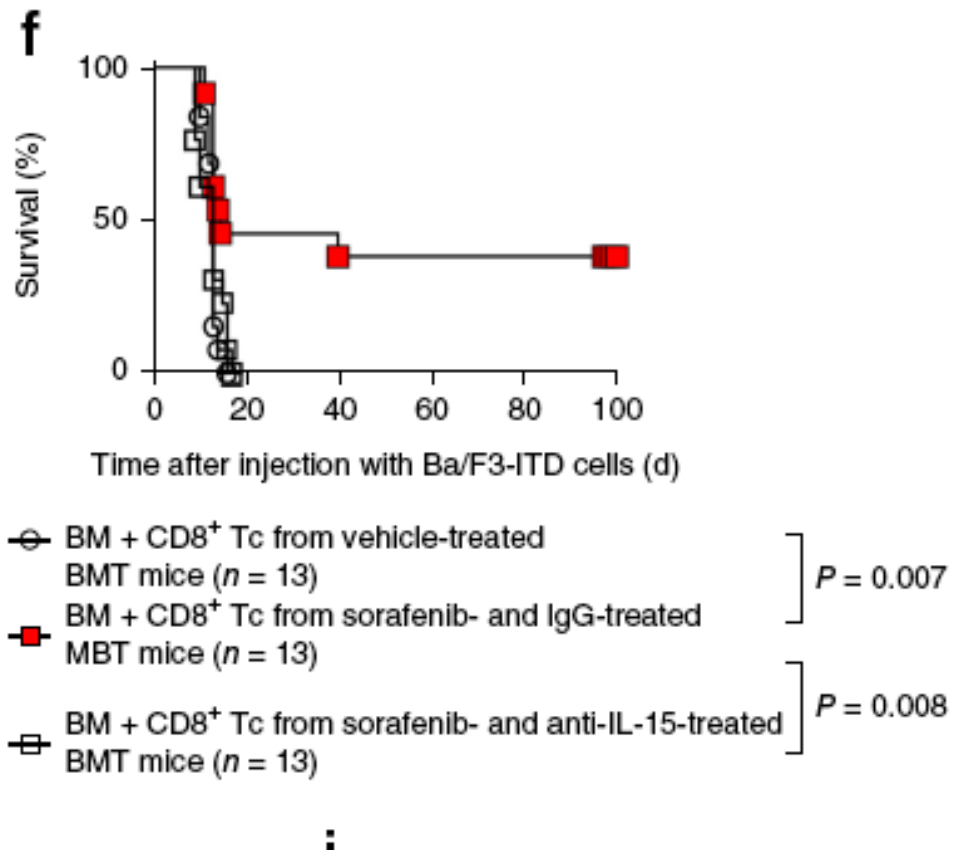
Transplant specific outcomes

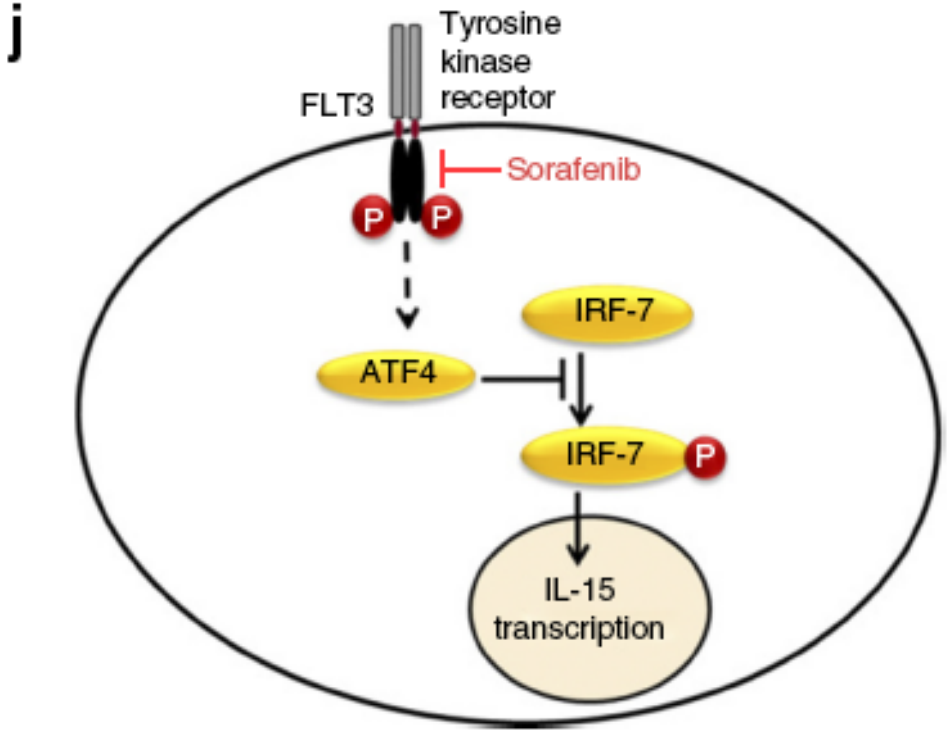
Transplant type	N	GVHD Grade III or IV	Relapsed disease	Non relapse mortality	Event Free
Myeloablative	16	4	3	1	12
Matched Sibling donor	9	1	1	1	7
Matched Unrelated donor	3	2	1	0	2
Haploidentical	4	1	1	0	3
Non-Myeloablative	29	8	5	3	21
Haploidentical donor	16	5	3	1	12
Matched Sibling donor	6	0	1	0	5
Matched Unrelated donor	4	3	0	2	2
Double Umbilical Cord blood	3	0	1	0	2

Pratz et al, BBMT 2020

nature medicine

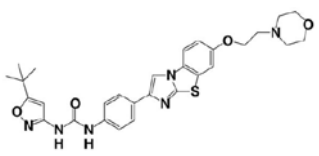
Sorafenib promotes graft-versus-leukemia activity in mice and humans through IL-15 production in FLT3-ITD-mutant leukemia cells



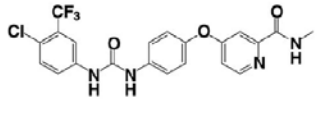


Mathew et al Nat Med 2018 24:8 282-291

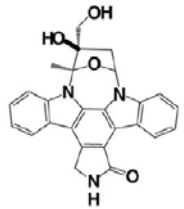
Activity of Quizartinib (AC220) in Primary FLT3-ITD AML Cells



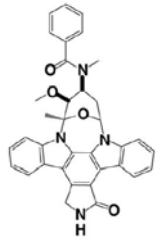
AC220



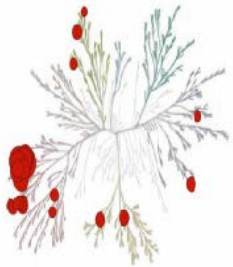
Sorafenib



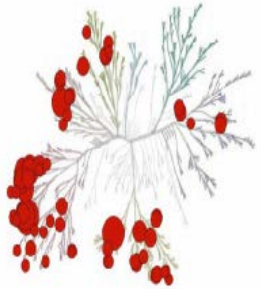
CEP-701



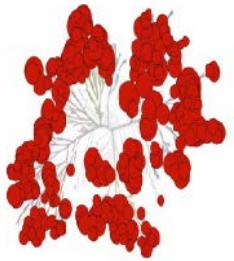
PKC-412



AC220
Quizartinib

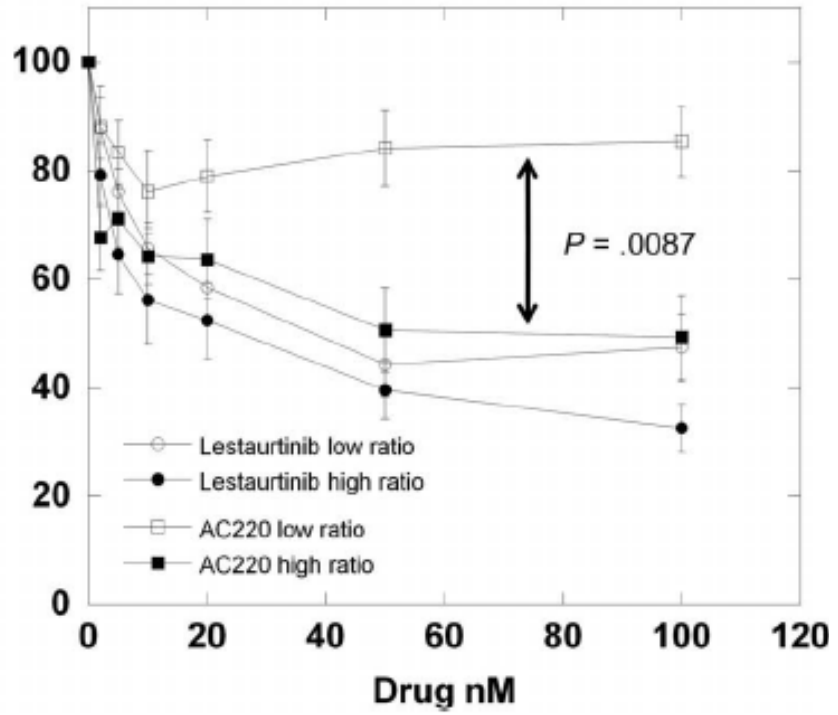


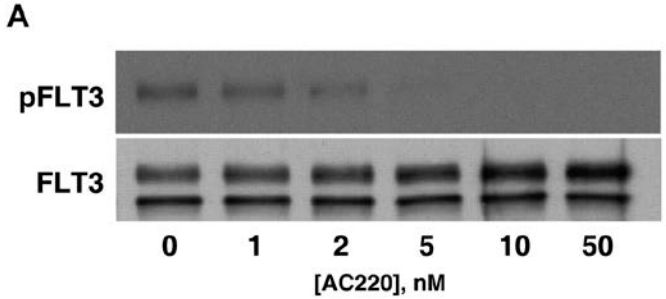
Sorafenib



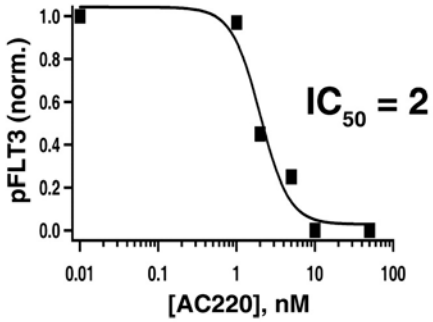
CEP-701
Lestaurtinib

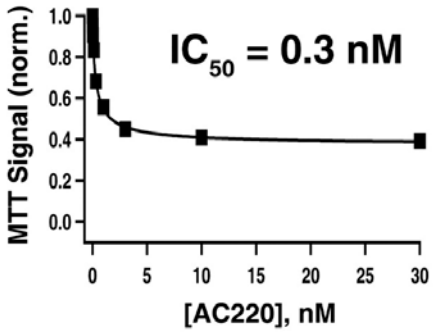
PKC-412
Midostaurin





Zarrinkar et al. Blood 2009;114:2984-2992





Pratz et al, Blood 2010;115:1425-32

Quizartinib Monotherapy Phase I

- Relapsed and refractory AML irrespective of FLT3 mutation status
- 76 total patients total: 51 on 14 day on 14 day off schedule, 24 on continuous schedule. 10 dose levels.
- MTD 200mg Daily due to asymptomatic grade 3 QT prolongation at 300mg dose seen in 3/8 pts.
- QTc prolongation more common in Females and dose dependent
- 200mg continuous selected for Phase II single agent study
- ORR 23/76 pts (30%) including 10 CRc (2CR, 3CRp, 5 CRi)
- FLT3 ITD mutant : ORR 9/17 (53%) including 4 CRc (1CR, 1CRp, 2CRi)
- Median duration of response 13.3 wks.
- PIA assay suggests full FLT3-ITD suppression at all dose levels of 18mg per day and higher.

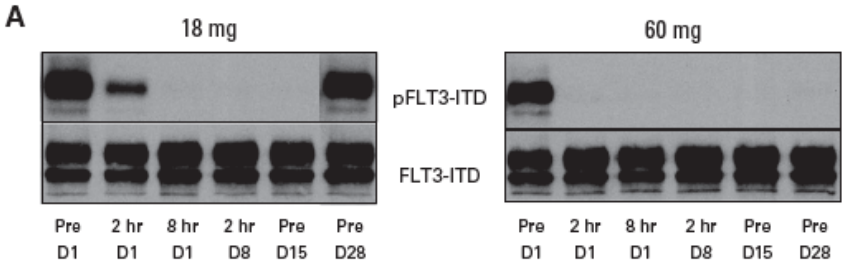


Table 4. Response by Quizartinib Dose and Schedule and FLT3 Status

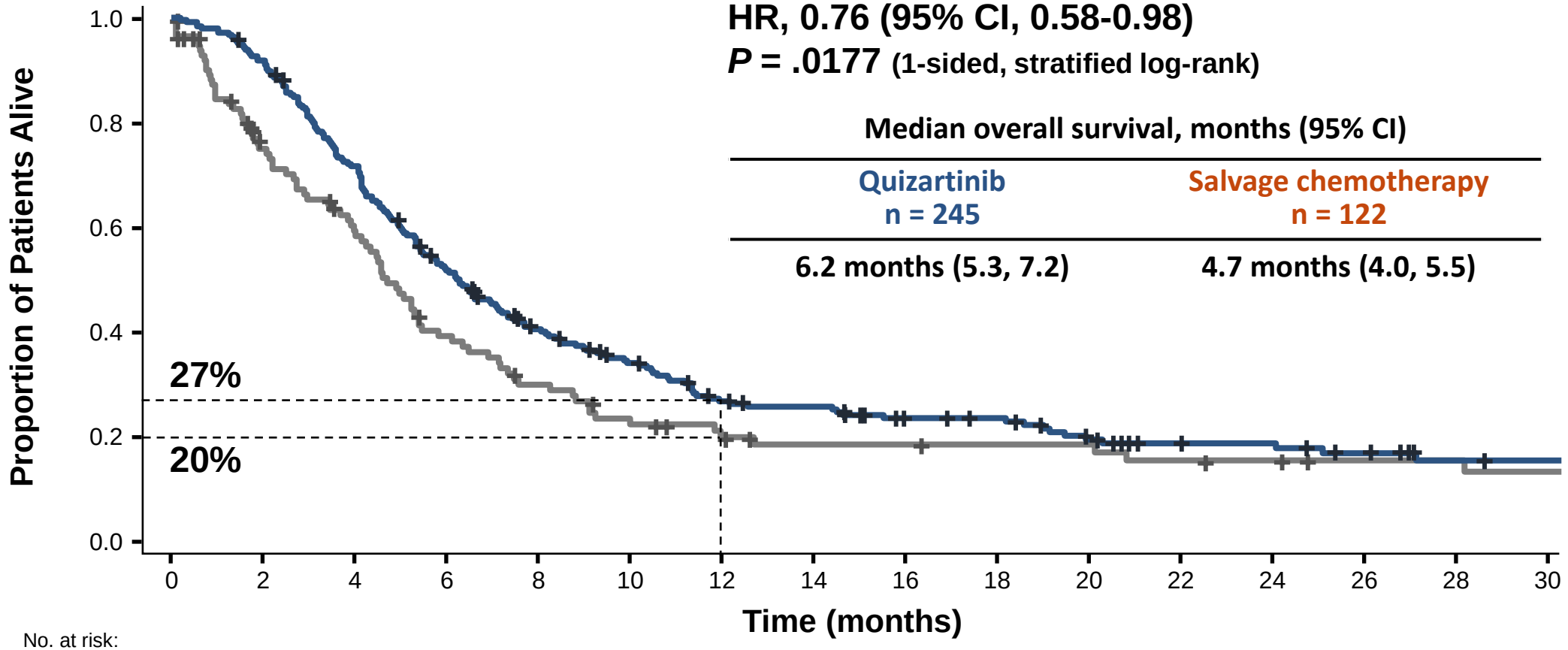
	FLT3-ITD Positive (n = 17)		FLT3-ITD Negative (n = 37)		FLT3-ITD (ind) (n = 22)	
Dose	No.	Responses	No.	Responses	No.	Responses
12 mg ID, n = 3	0		1	0	2	0
18 mg ID, n = 8	0		4	1 PR	4	0
27 mg ID, n = 6	0		3	0	3	2 PR
40 mg ID, n = 5	0		4	1 CRp, 1 PR	1	0
60 mg ID, n = 5	1	1 CRi	2	0	2	1 PR
90 mg ID, n = 3	2	0	1	1 PR	0	
135 mg ID, n = 5	2	0	2	0	1	1 PR
200 mg ID, n = 6	3	1 PR	3	1 CRp	0	
300 mg ID, n = 4	1	1 PR	2	0	1	1 CRi
450 mg ID, n = 6	1	1 PR	4	0	1	1 PR
200 mg CD, n = 17	6	1 CR, 1 CRp, 1 CRi, 1 PR	7	0	4	1 CR, 1 CRi
300 mg CD, n = 8	1	1 PR	4	0	3	1 CRi

Abbreviations: CD, continuous dosing; CR, complete remission; CRp, CR with incomplete platelet recovery; CRi, CR with incomplete neutrophil recovery; FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; ind, indeterminate/not tested; ID, intermittent dosing; PR, partial remission.

Cortes et al, J Clin Oncol. 2013 Oct 10;31(29):3681-7

Quizartinib vs Salvage chemotherapy : Overall Survival

ITT population



- Median follow-up: 23.5 months

Cortes et al ASH 2018

QuANTUM-First Phase 3 Trial (NCT02668653): Quizartinib Plus Standard Induction Chemotherapy and Consolidation Followed by Single-Agent Quizartinib

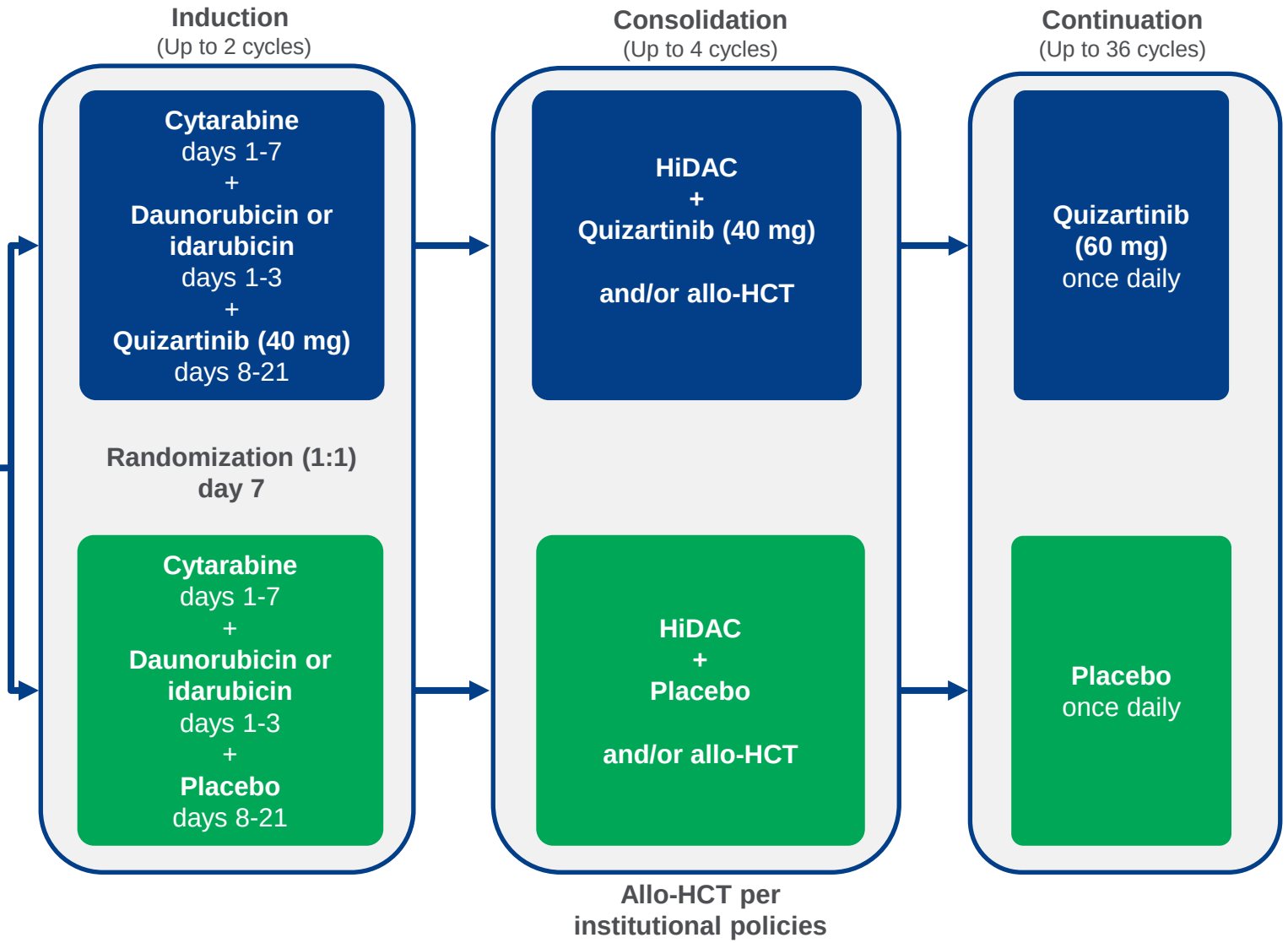
Enrollment dates: September 2016 to August 2019
Data cutoff: August 13, 2021

Stratification factors
- **Region**: NA, EU, and Asia/other regions
- **Patient age**: <60 years, ≥60 years
- **WBC**[a]: <40×10^9/L, ≥40×10^9/L

- Newly diagnosed *FLT3*-ITD+ AML
- 18-75 years of age
- ≥3% *FLT3*-ITD allelic frequency
- Patients begin 7+3 chemotherapy during screening

Selected endpoints
- **Primary endpoint**: OS
- **Secondary endpoints**: EFS, CR/CRc, Safety
- **Exploratory endpoints**: RFS, DoCR

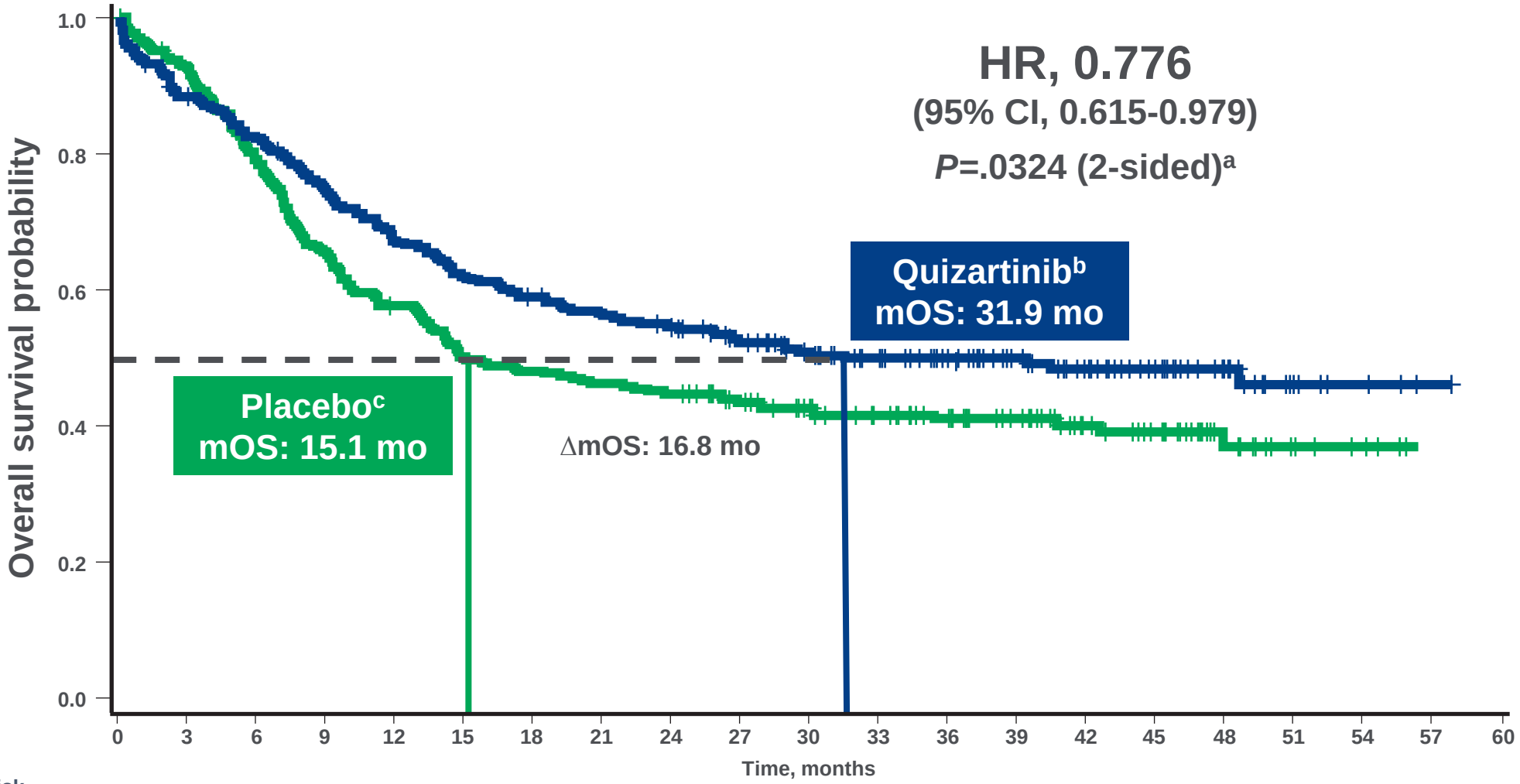
A hierarchical testing procedure was used to test the primary endpoint of OS, followed by EFS, CR and CRc.



AML, acute myeloid leukemia; CR, complete remission; CRc, composite complete remission; DoCR, duration of complete remission; EFS, event-free survival; EU, Europe; HiDAC, high-dose cytarabine; NA, North America, OS, overall survival; RFS, relapse-free survival; WBC, white blood cell.
[a] WBC count was measured at the time of AML diagnosis.

Erba et al, EHA 2022

Primary Endpoint: Overall Survival

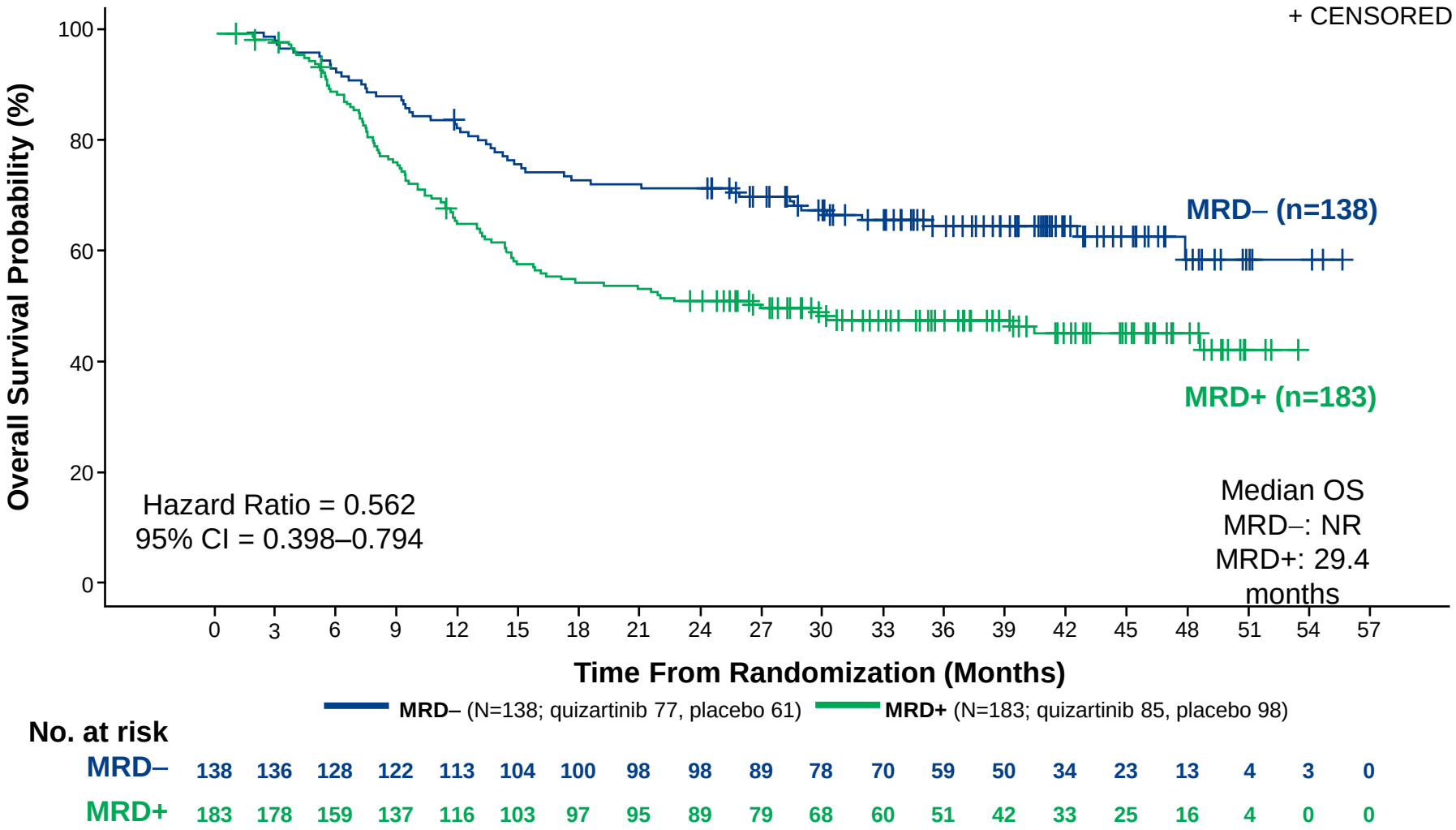


HR, hazard ratio; mOS, median overall survival.

[a] *P* value was calculated using a stratified log-rank test. [b] Median follow-up time for quizartinib arm, 39.2 months. [c] Median follow-up time for placebo arm, 39.2 months.

Erba et al, EHA 2022

Achievement of CRc with MRD Negativity (<10^{-4} Cutoff) by the End of Induction Correlated with Longer OS Regardless of Treatment Arm



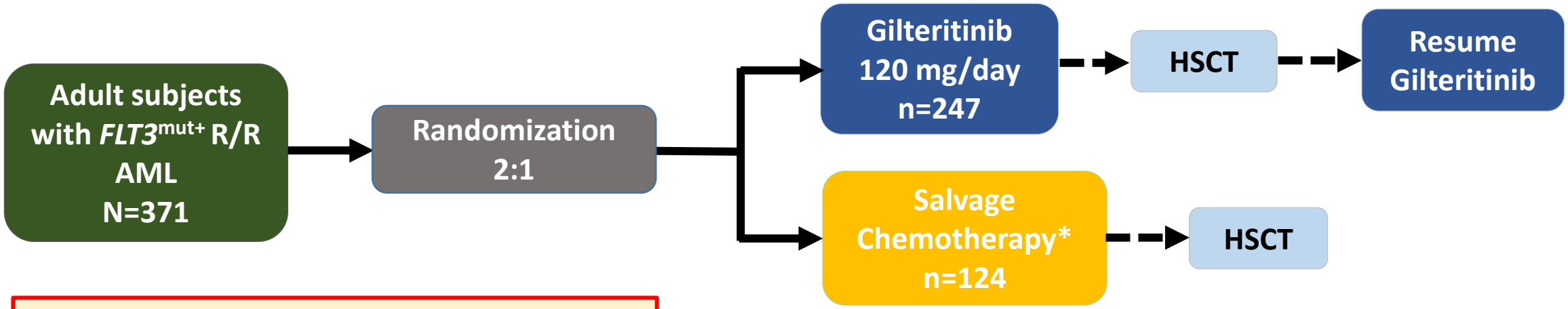
No. at risk

MRD–	138	136	128	122	113	104	100	98	98	89	78	70	59	50	34	23	13	4	3	0
MRD+	183	178	159	137	116	103	97	95	89	79	68	60	51	42	33	25	16	4	0	0

Post hoc analysis.

Levis et al ASH 2022

ADMIRAL Global Phase 3 Randomized Study Gilteritinib vs salvage chemotherapy for relapsed FLT3mut AML



Co-Primary Endpoints: OS, CR/CRh rate
Key Secondary Endpoints: EFS, CR rate

***Salvage chemotherapy regimen was selected prior to randomization**

MEC (mitoxantrone, etoposide, and cytarabine)
FLAG-IDA (fludarabine, cytarabine, idarubicin, and G-CSF)
} High intensity (1-2 cycles)

Low-dose cytarabine
Azacitidine
} Low intensity (given until disease progression or intolerance)

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CRh, complete remission with incomplete hematologic recovery; EFS, event-free survival; G-CSF, granulocyte colony-stimulating factor; HSCT, hematopoietic stem cell transplantation; OS, overall survival; R/R, relapsed/refractory.

Perl et al NEJM 2019

Randomized study of gilteritinib vs salvage chemotherapy in relapsed and refractory FLT3 mut AML

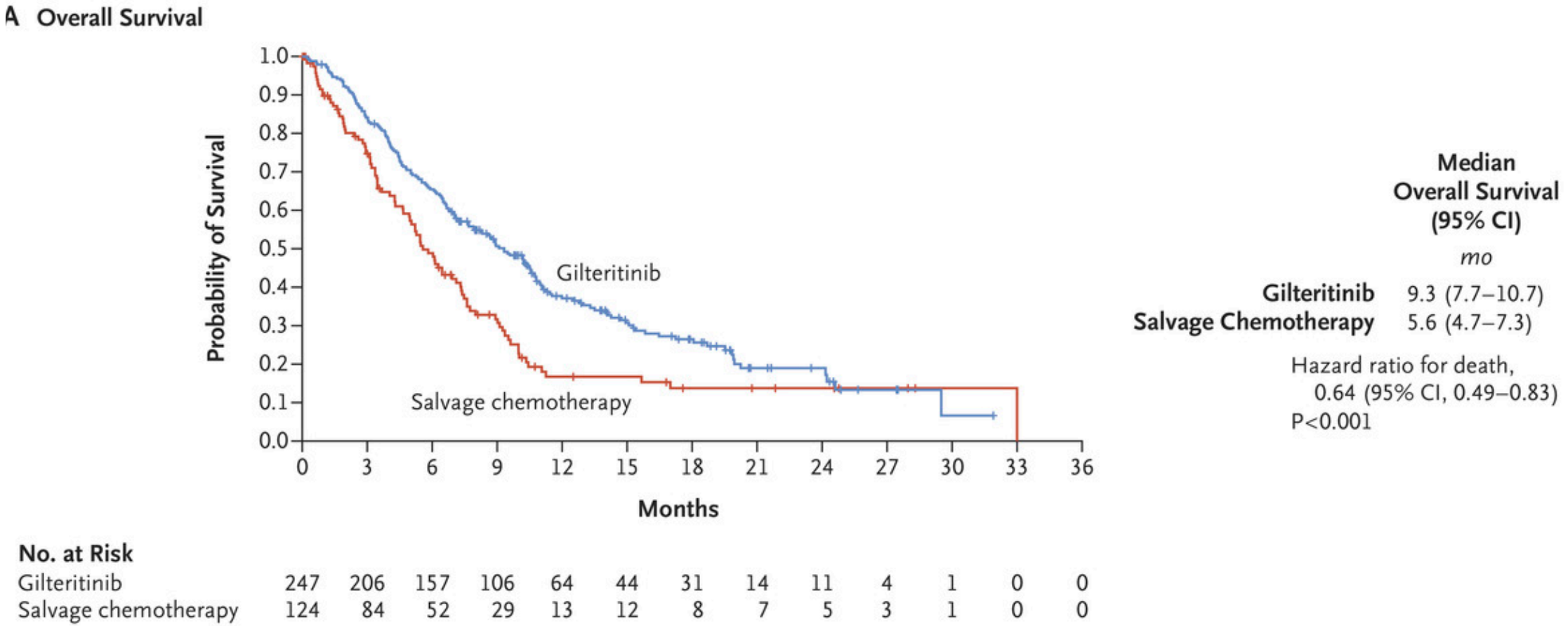
RESPONSE OUTCOMES (ITT POPULATION: N=371)

Parameter*	Gilteritinib (n=247)	Salvage Chemotherapy (n=124)
CR, n (%)	**52 (21)**	**13 (11)**
CRh, n (%)	32 (13)	6 (5)
CRi, n (%)	63 (26)	14 (11)
CRp, n (%)	19 (8)	0 (0)
CRc, n (%)	**134 (54)**	**27 (22)**
CR/CRh, n (%)	**84 (34)**	**19 (15)**
PR, n (%)	33 (13)	5 (4)
ORR, n (%)	167 (68)	32 (26)
NR, n (%)	66 (27)	43 (35)
Median duration of drug exposure (range), months	4.1 (0.1-29.1)	0.9 (0.2-7.1)
Median time to achieve CRc (95% CI), months	1.8 (0.9, 9.5)	1.1 (0.8, 2.9)
Median DoR† (95% CI), months	11.0 (4.6, NE)	NE (NE, NE)
Allogeneic HSCT, n (%)	**63 (26)**	**19 (15)**

AE Perl et al. N Engl J Med 2019;381:1728-1740

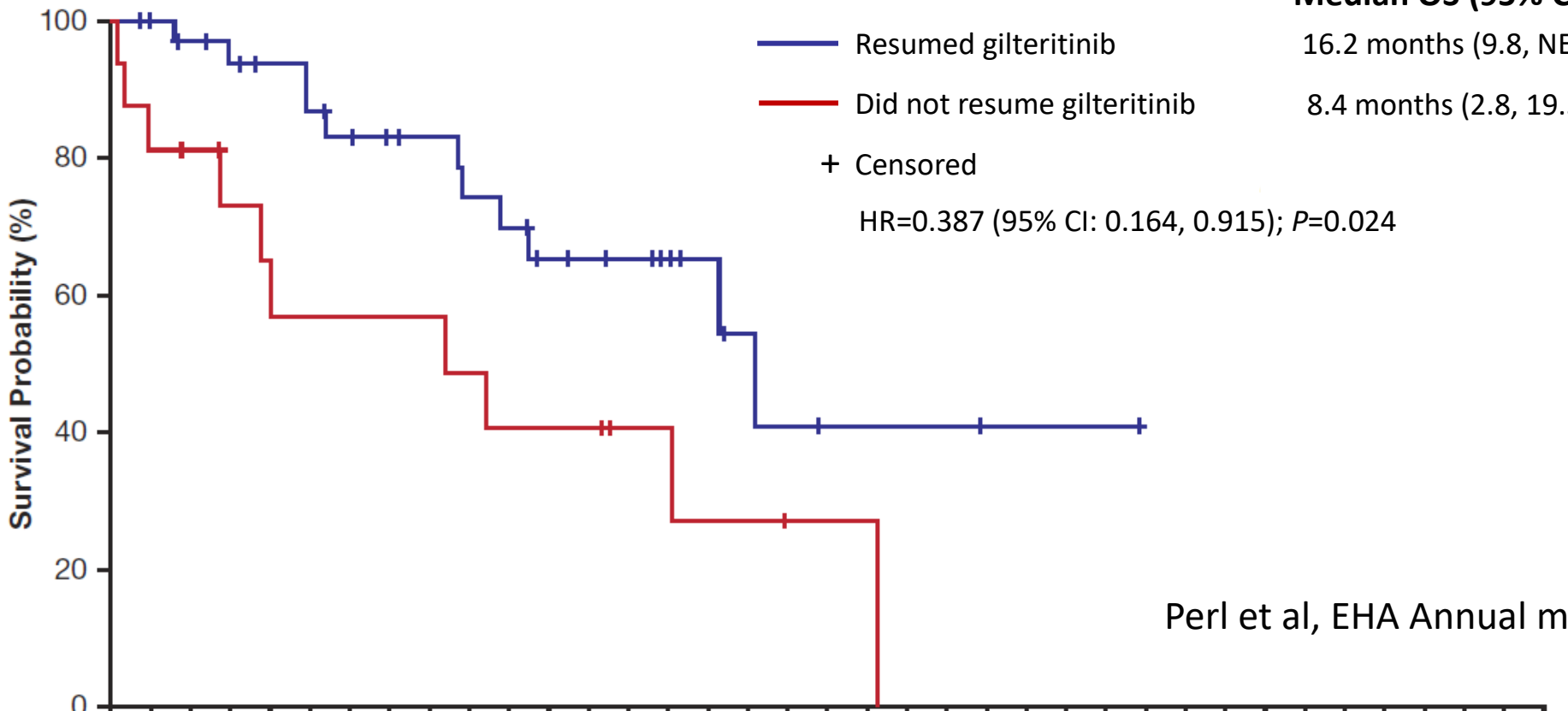
*Response was not evaluable in 14 patients (6%) in the gilteritinib arm and in 49 patients (40%) in the salvage chemotherapy arm.
†Duration of remission was defined as the duration of CR/CRh.
Abbreviations: CI, confidence interval; CR, complete remission; CRc, composite complete remission; CRh, complete remission with partial hematologic recovery; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; DoR, duration of remission; HSCT, hematopoietic stem cell transplantation; ITT, intention-to-treat; NE, not estimable; NR, no response; ORR, overall response rate; PR, partial remission.

Overall Survival among Patients with *FLT3*-Mutated Relapsed or Refractory AML Treated with Gilteritinib or Salvage Chemotherapy (Intention-to-Treat Population).



AE Perl et al. N Engl J Med 2019;381:1728-1740

Post-HSCT Survival in the Gilteritinib Arm: Effect of Maintenance Therapy (Landmark Analysis From Day 60 Post-HSCT; n=51)



Patients at Risk (n)													
Resumed gilteritinib	35	29	22	17	12	6	2	2	1	0	0	0	0
Did not resume gilteritinib	16	9	7	6	5	2	1	0	0	0	0	0	0

Perl et al, EHA Annual meeting 2019

Two-sided *P*-values were determined according to the log-rank test; the Kaplan-Meier method combined with the Greenwood formula were used to determine overall survival and corresponding 95% confidence intervals.
Abbreviations: CI, confidence interval; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; ITT, intention-to-treat; NE, not estimable; OS, overall survival.

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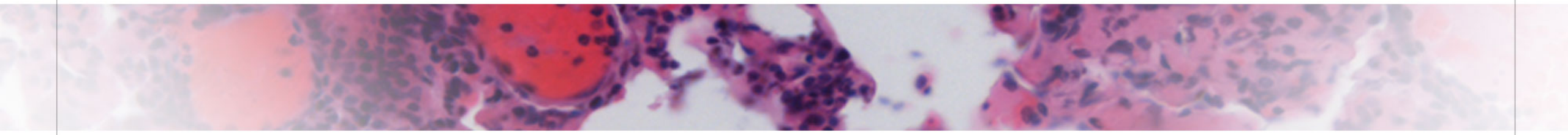

A Phase 1 Study of Gilteritinib in Combination With Induction and Consolidation Chemotherapy in Patients With Newly Diagnosed AML: Final Results

Keith W. Pratz,[1] Mohamad Cherry,[2] Jessica K. Altman,[3] Brenda W. Cooper,[4] Jose Carlos Cruz,[5] Joseph G. Jurcic,[6] Mark J. Levis,[1] Tara L. Lin,[7] Alexander E. Perl,[8] Nikolai A. Podoltsev,[9] Gary J. Schiller,[10] Jason E. Hill,[11] Angela James,[11] Qiaoyang Lu,[11] Ramon V. Tiu[11]

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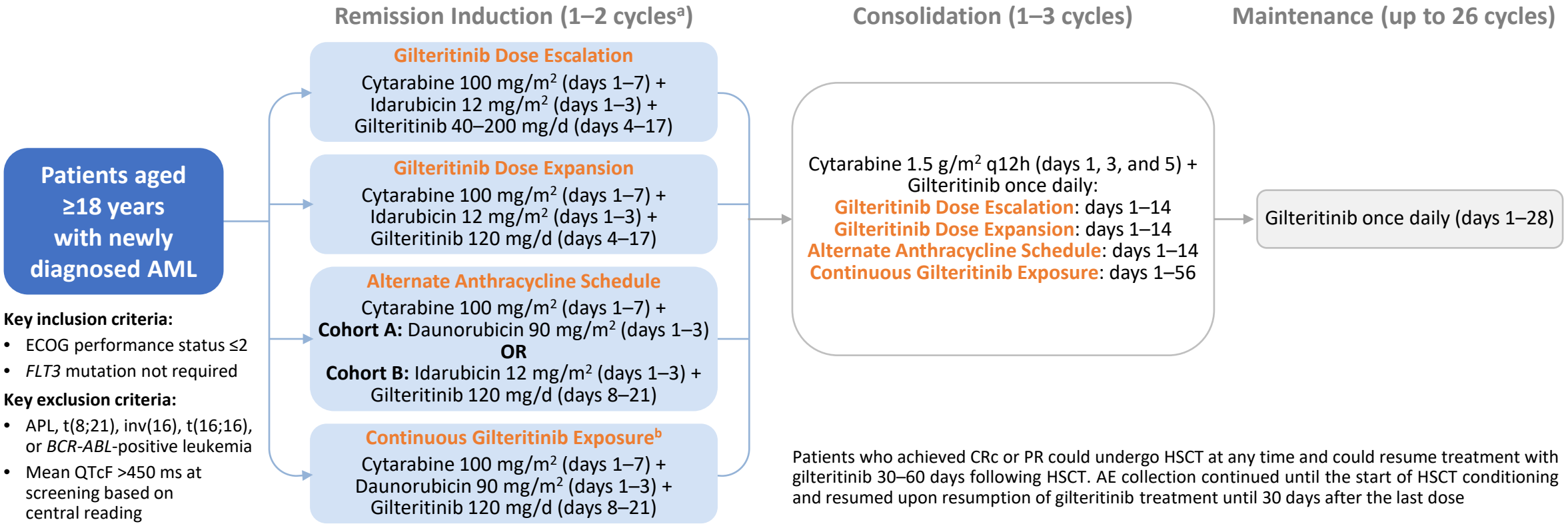
Study Design



Key inclusion criteria:

- ECOG performance status ≤2
- *FLT3* mutation not required

Key exclusion criteria:

- APL, t(8;21), inv(16), t(16;16), or *BCR-ABL*-positive leukemia
- Mean QTcF >450 ms at screening based on central reading

Patients who achieved CRc or PR could undergo HSCT at any time and could resume treatment with gilteritinib 30–60 days following HSCT. AE collection continued until the start of HSCT conditioning and resumed upon resumption of gilteritinib treatment until 30 days after the last dose

[a]If day 21 bone marrow evaluation shows residual blasts and the bone marrow is not aplastic, a second induction cycle with the same regimen could be started ≥7 days after the last dose of gilteritinib but no later than day 28 (gilteritinib dose-escalation and dose-expansion cohorts) or day 35 (alternate anthracycline schedule and continuous gilteritinib exposure cohorts) of the first induction cycle; [b]During the second induction cycle, the dosage of daunorubicin was reduced to 45 mg/m².

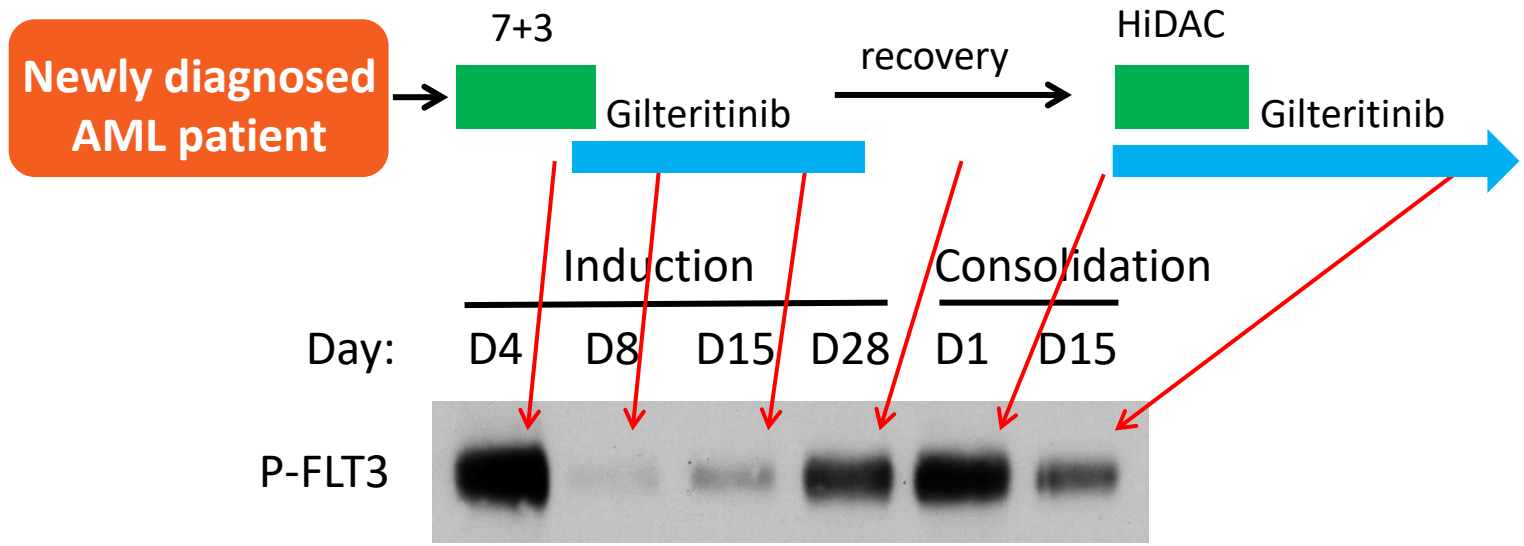
AE, adverse event; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; *BCR-ABL*, breakpoint cluster region-Abelson murine leukemia viral oncogene homolog; CRc, composite complete remission; ECOG, Eastern Cooperative Oncology Group; *FLT3*, FMS-like tyrosine kinase 3; HSCT, hematopoietic stem cell transplant; PR, partial remission; q12h, every 12 hours; QTcF, Fridericia-corrected QT interval.

Antileukemic Response: *FLT3*$^{mut+}$ Patients

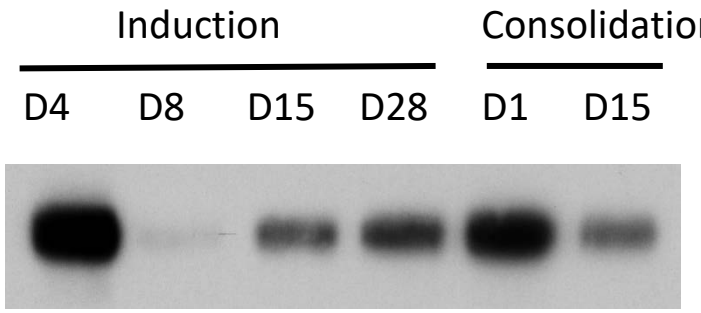
- Median duration of response was 14.1 months
- Median overall survival has not been reached

Response Parameter*, n (%)	*FLT3*$^{mut+}$ (n=44†)
CR	31 (70.5)
CRp	3 (6.8)
CRi	6 (13.6)
PR	0
NR	4 (9.1)
CRc‡	40 (90.9)

*Response parameters were defined according to the International Working Group Criteria for AML (Cheson B, et al. *J Clin Oncol*. 2003;12(24):4642–4649).
†Of the 36 *FLT3*$^{mut+}$ patients, one patient was not included in the response analysis due to good risk cytogenetics, one patient was not included in the response analysis due to refusal to undergo bone marrow evaluation and one patient was not included in response analysis due to withdrawn consent.
‡CRc included patients who achieved CR, CRp, and CRi.
Abbreviations: CR, complete remission; CRc, composite complete remission; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; FLT3, *fms*-like tyrosine kinase 3; mut+, mutation positive; NR, no response; PR, partial remission.



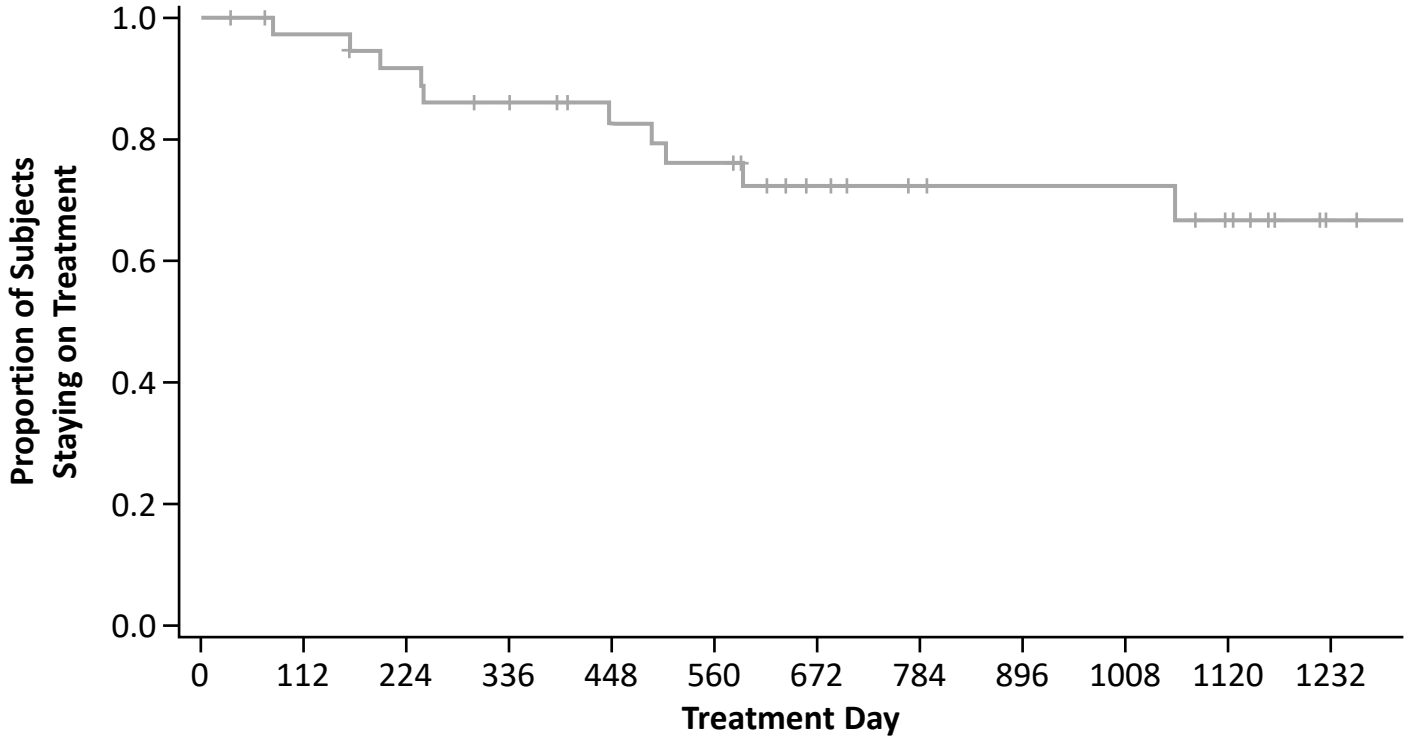
Subject 10009-30004- 120mg Cohort



Subject 10014-30007

Pratz et al, ASH abstract 2019
Pratz et al, SOHO 2022

Survival Probability and 30- and 60-Day Mortality in Patients With *FLT3*$^{mut+}$ AML Who Received Gilteritinib 120 mg (N=38)



Number of subjects at risk	38	37	35	33	32	30	29	27	25	24	23	20	17	15	14	13	13	13	13	12	10	6	4	3

30- and 60-day mortality probability rates for patients *FLT3*$^{mut+}$ AML who received gilteritinib 120 mg were 0.0%

Data are presented from all patients with *FLT3*$^{mut+}$ AML who received at least one dose of gilteritinib at a dose of 120 mg.

AML, acute myeloid leukemia; *FLT3*$^{mut+}$, FMS-like tyrosine kinase 3 mutation-positive.

Pratz et al, ASH 2020

FLT3 ITD Clearance by Ultra-sensitive NGS Assay[a]

- Among patients with *FLT3* ITD-positive AML who received a gilteritinib dose of 120 mg and achieved CRc,[b] high proportions of patients achieved mutational clearance (defined as *FLT3* ITD:total *FLT3* signal ratio $\leq 10^{-4}$)

MRD status	End of induction (N=12)	Beginning of consolidation (N=8)	After consolidation (N=13)
MRD negative	4 (33.3%)	3 (37.5%)	11 (84.6%)
MRD positive	8 (66.7%)	5 (62.5%)	2 (15.4%)

[a]*FLT3* ITD mutation assay was performed as follows: using genomic DNA, *FLT3* exons 14 and 15 were amplified by PCR and *FLT3* ITD and total *FLT3* alleles were subsequently quantified by NGS using an Illumina® MiSeq platform. Read depths of ≥100,000 reads per sample were implemented and operating characteristics were linear to 10^{-4} for the range of ITD lengths using cell lines spiked to normal blood or bone marrow. Data were analyzed using proprietary software. *FLT3* ITD signal ratio was defined as the *FLT3* ITD to total *FLT3* frequency[1]; [b]Analysis set consisted of all patients who were enrolled and received at least one dose of 120 mg gilteritinib, were *FLT3* ITD positive, achieved CRc, and had at least one postbaseline sample with mutational clearance data; [c]Cleared is defined as summed *FLT3* ITD signal ratio of any postbaseline sample $\leq 10^4$; if a patient has multiple mutational clearance samples collected across different time points, the status is cleared if there is at least one mutational clearance sample that is cleared; [d]Not cleared is defined as summed *FLT3* ITD signal ratio of any postbaseline sample $>10^4$; if a patient has multiple mutational clearance samples collected across different time points, the status is not cleared if all available mutational clearance samples are not cleared.

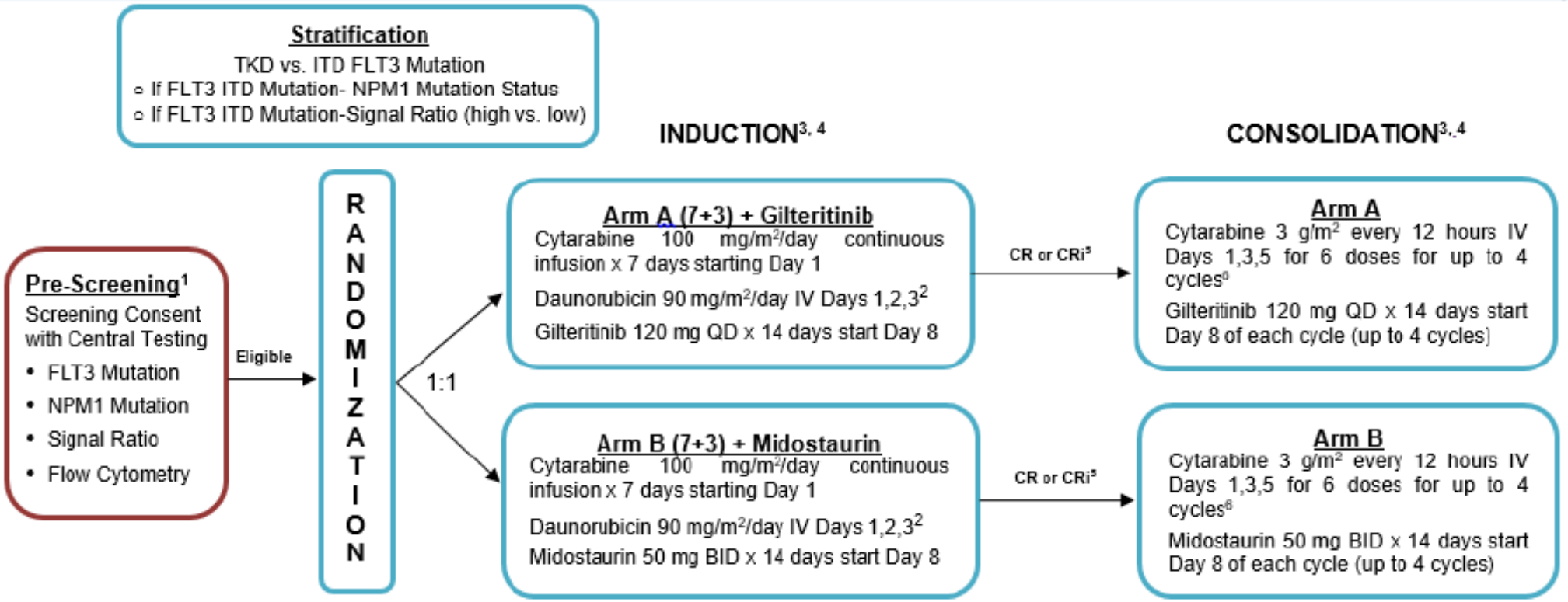
AML, acute myeloid leukemia; CRc, composite complete remission; *FLT3*, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; NGS, next-generation sequencing; PCR, polymerase chain reaction.

1. Levis MJ, et al. *Blood Adv.* 2018;2:825-831.

Pratz et al, SOHO 2022

PrE0905 7&3 Mido vs 7&3 Gilt Phase II

Study Schema

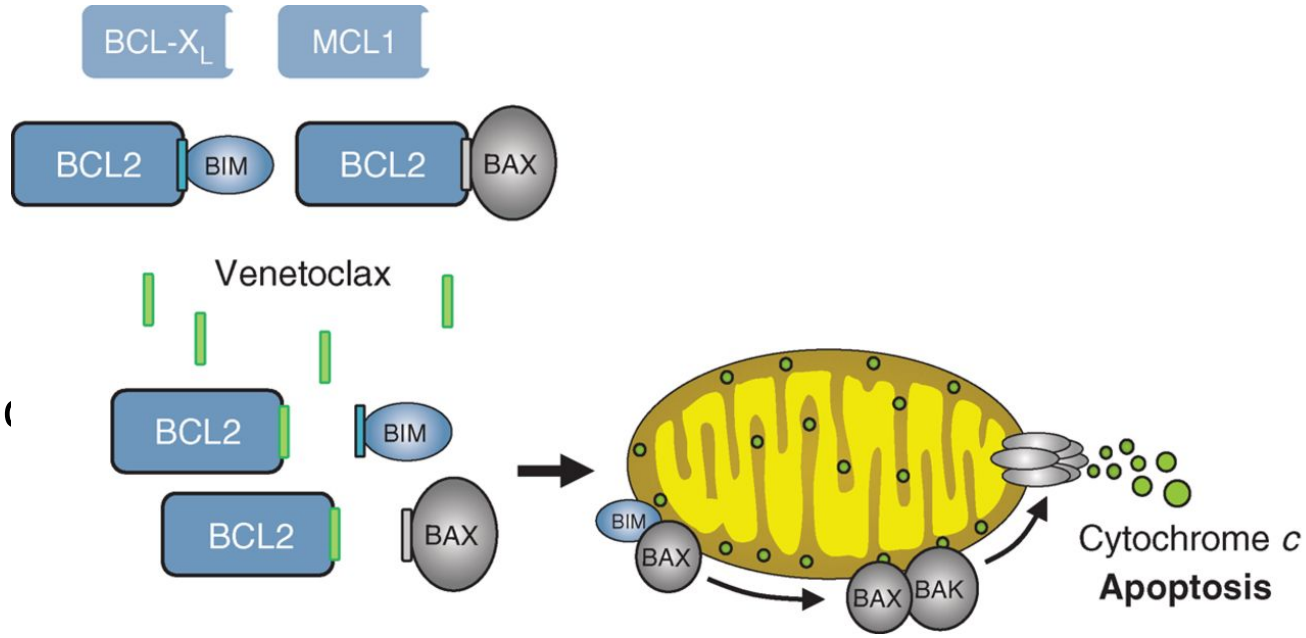


Accrual Goal: 179

1 Any patient undergoing bone marrow biopsy with suspicion of or known diagnosis of AML will be asked to sign a Prescreening Consent in order to confirm diagnosis and determination/confirmation of FLT3 status at central laboratory and obtain research samples for the study prior to randomization.
2 Daunorubicin 90 mg/m^2/day will be administered IV over 30-60 minutes Days 1, 2, 3 (45 mg/m^2/day if receives second cycle of induction).
3 Patients may proceed to allogeneic TRANSPLANT after induction or after 0-4 cycles of consolidation.
4 Patients will go off treatment at the time of transplant or any non-protocol leukemia directed therapy.
5 If Complete Response (CR) or CR with incomplete hematologic recovery (CRi) is not achieved, a second induction cycle of therapy may be administered.
6 For patients age ≥ 55 reduce consolidation cytarabine dose to 1.5 g/m^2.

BCL-2 in AML

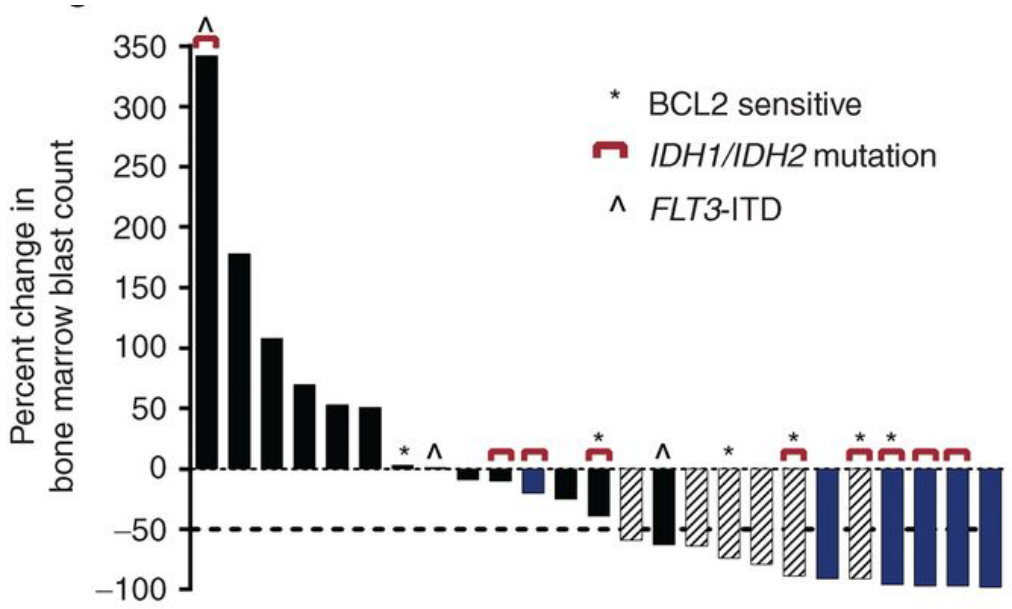
- BCL-2 is a key protein in apoptosis regulation in normal and malignant cells
- BCL-2 is overexpressed in AML cells
- Higher expression is linked to lower rates of CR with conventional therapy.
- Highest BCL2-2 expression is found in leukemia stem cells
- Targeting BCL-2 in the LSC compartment can lea to selective LSC death.
- Early BCL-2 inhibitors(ABT-263) had issues with thrombocytopenia (BCL-XL)
- BCL-2 inhibitor venetoclax (ABT-199) is a BH-3 mimetic without BCL-XL specificity.



Konopleva M, et al. Cancer Discov 2016;6:1106-1117

Phase II study of venetoclax in R/R or unfit AML

- 32 patients total
  - 30 relapsed or refractory
  - 2 unfit for chemotherapy
- Venetoclax 800mg daily Day 1-28
- Results
  - **ORR 19%(6/32),** 2 CR, 4 CRi
  - IDH mutant ORR 33%(4/12), 2 CR, 2CRi
  - Median response duration 2.3 months
  - Median OS 4.7 months



Konopleva M, et al. Cancer Discov 2016;6:1106-1117

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Long-Term Follow-Up of the Phase 3 VIALE-A Clinical Trial of Venetoclax Plus Azacitidine for Patients With Treatment-Naïve Acute Myeloid Leukemia Ineligible for Intensive Chemotherapy

Keith W. Pratz[1], Brian A. Jonas[2], Vinod Pullarkat[3], Michael J. Thirman[4], Jacqueline S. Garcia[5], Walter Fiedler[6], Kazuhito Yamamoto[7], Jianxiang Wang[8], Sung-Soo Yoon[9], Ofir Wolach[10], Jun-Ho Jang[11], Su-Peng Yeh[12], Grace Ku[13], Catherine Miller[14], Ying Zhou[14], Brenda Chyla[14], Jalaja Potluri[14], Courtney D. DiNardo[15]

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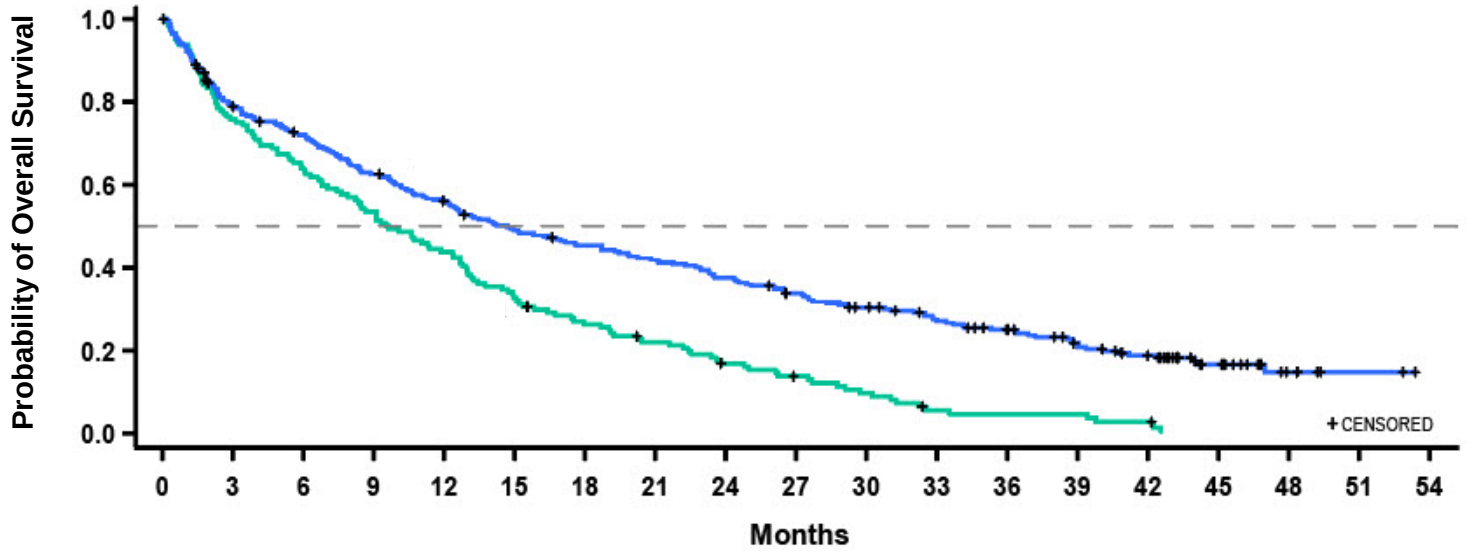
American Society for Hematology 2022, New Orleans, LA, USA

abbvie

Patients treated with Ven+Aza continue to show OS benefit over those on Aza monotherapy

Median follow-up time: 43.2 months (range: < 0.1 - 53.4)



	No. of events/No. of patients (%)	OS (months) median (95% CI)
Ven+Aza	222/286 (77.6)	14.7 (12.1 - 18.7)
Pbo+Aza	138/145 (95.2)	9.6 (7.4 - 12.7)

Hazard ratio: 0.58 (95% CI, 0.465 - 0.723), P < 0.001

HR reduction from 0.66 (95% CI, 0.52 - 0.85) at 75% OS analysis

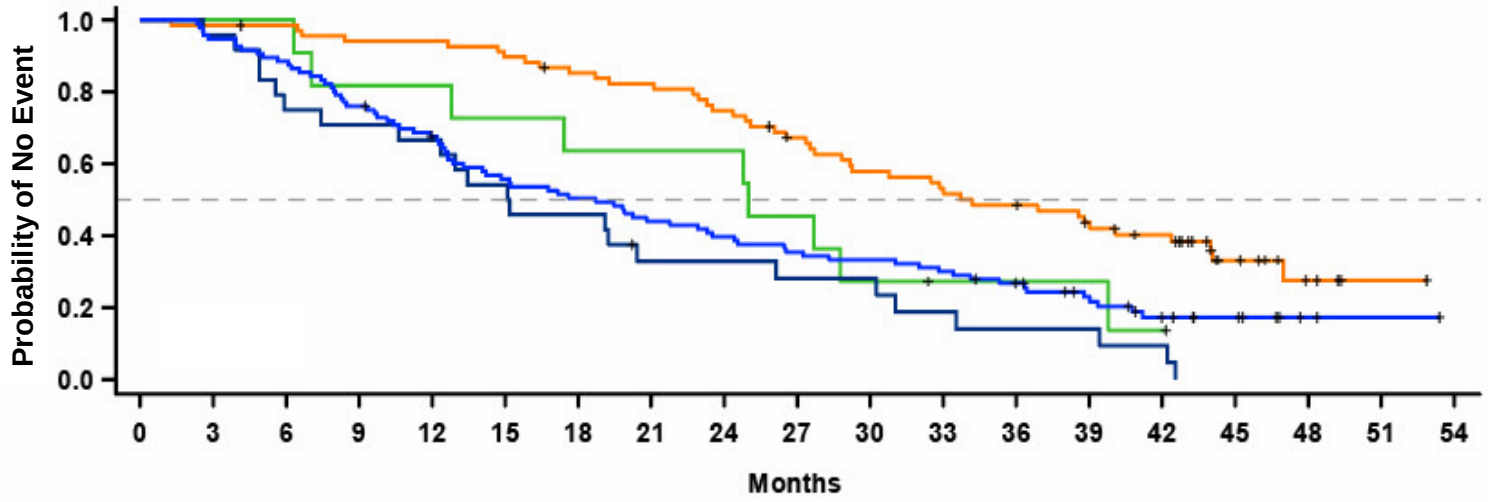
Patients at Risk

Ven+Aza	286	220	199	173	153	133	122	113	101	89	78	67	57	45	34	18	6	2	0
Pbo+Aza	145	109	92	77	63	47	37	30	22	17	12	6	5	5	3	0	0	0	0

Pratz et al ASH 2022

The distributions were estimated for each treatment arm using Kaplan-Meier methodology and compared using the log-rank test stratified by age (18-<75, ≥75 years) and cytogenetic risk (intermediate risk, poor risk); The hazard ratio between treatment arms were estimated using the Cox proportional hazards model with the same stratification factors used in the log-rank test; Data cutoff: 01 Dec 2021
Abbreviations: Aza, azacitidine; Pbo, placebo; Ven, venetoclax

Median OS is longer for MRD < 10^{-3} than MRD ≥ 10^{-3} in patients who achieved CR+CRi on Ven+Aza



	No. of events/No. of patients (%)	OS (months) median (95% CI)
Ven+Aza MRD < 10^{-3}	43/69 (62)	34.2 (27.7 - 44.0)
Ven+Aza MRD ≥ 10^{-3}	76/96 (79)	18.7 (12.9 - 23.5)

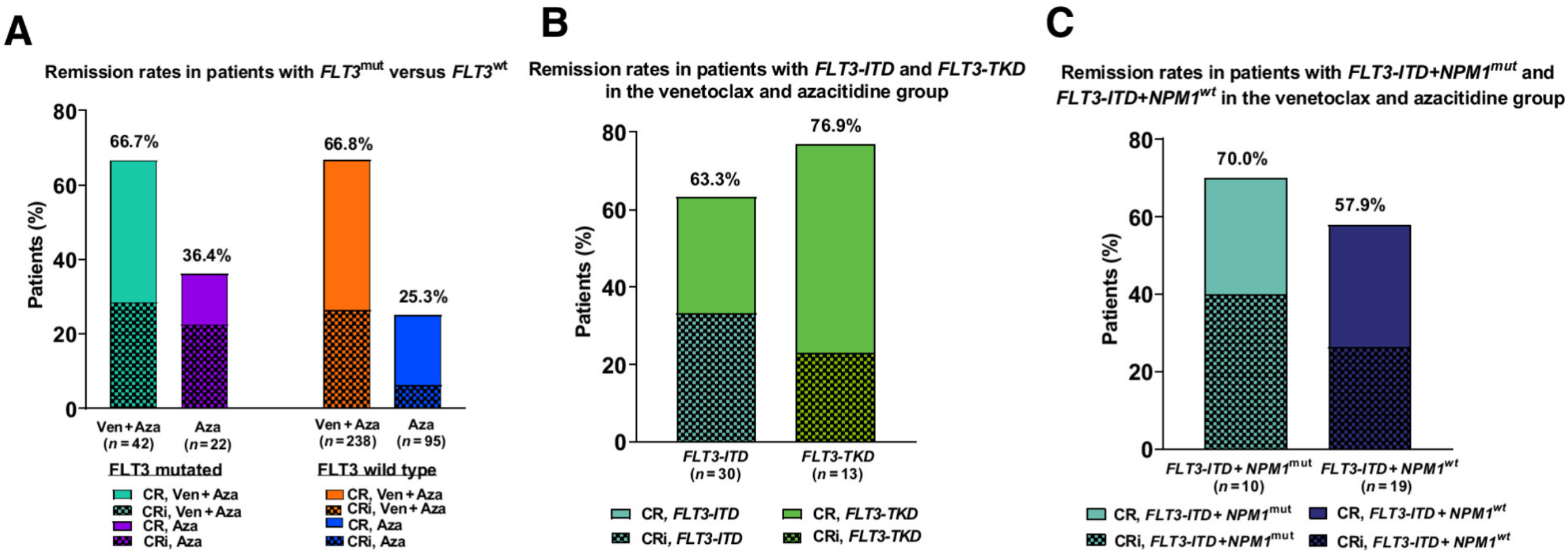
Patients at Risk

Ven+Aza MRD < 10^{-3}	69	68	67	64	64	61	57	55	50	43	37	34	31	26	22	10	4	1	0
Ven+Aza MRD ≥ 10^{-3}	96	91	85	73	63	52	47	41	37	33	31	28	23	17	10	7	2	1	0
Pbo+Aza MRD < 10^{-3}	11	11	11	9	9	8	7	7	7	5	3	2	2	2	1	0			
Pbo+Aza MRD ≥ 10^{-3}	24	23	18	17	16	13	11	7	7	6	6	4	3	3	2	0			

The distributions were estimated for each treatment arm using Kaplan-Meier methodology; Data cutoff: 01 Dec 2021;
Abbreviations: Aza; azacitidine; Pbo, placebo; MRD, minimal residual disease; Ven, venetoclax

Pratz et al ASH 2022

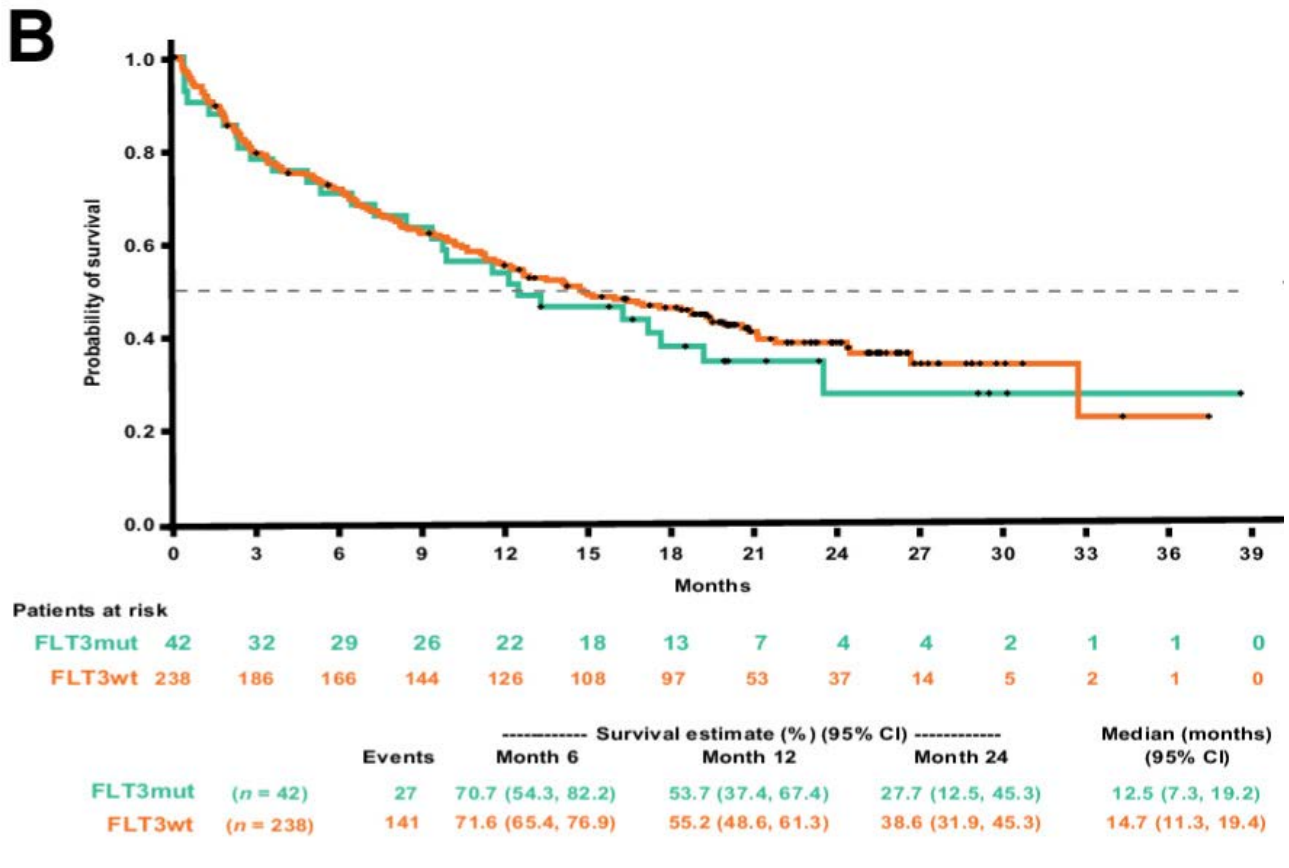
AZA/VEN Response rates in patients with *FLT3* mutation



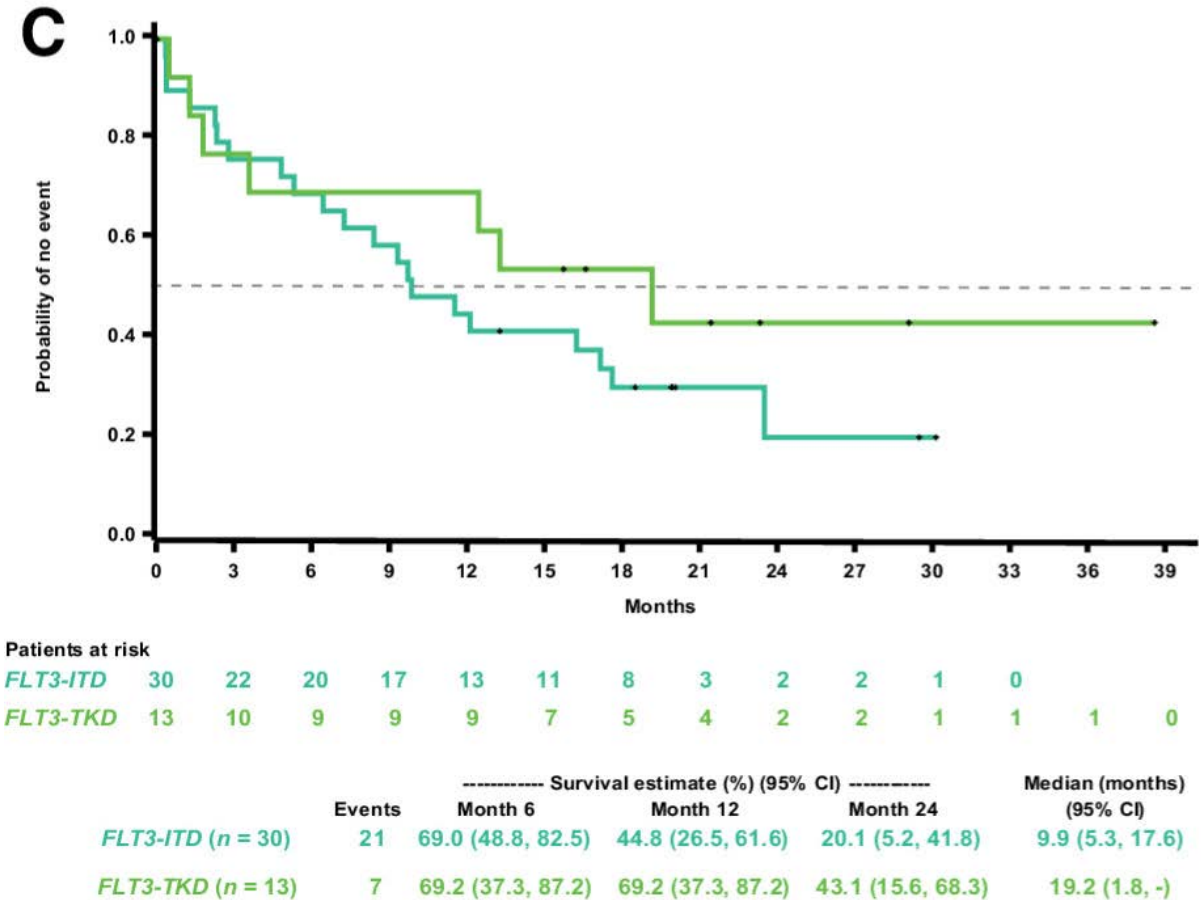
Konopleva et al CCR 2022

Aza: Azacitidine; Pbo: Placebo; Ven: Venetoclax; CR: Complete remission; CRi: CR with incomplete hematologic recovery; NR: not reached; CR was defined as absolute neutrophil count >10^3/µL, platelets >10^5/µL, red cell transfusion independence (TI), and bone marrow with <5% blasts; CRi was defined as all criteria for CR, except for neutropenia ≤10^3/µL or thrombocytopenia <10^5/µL; sample size for time to response analysis included responders only

AZA/Venetoclax in FLT3 AML



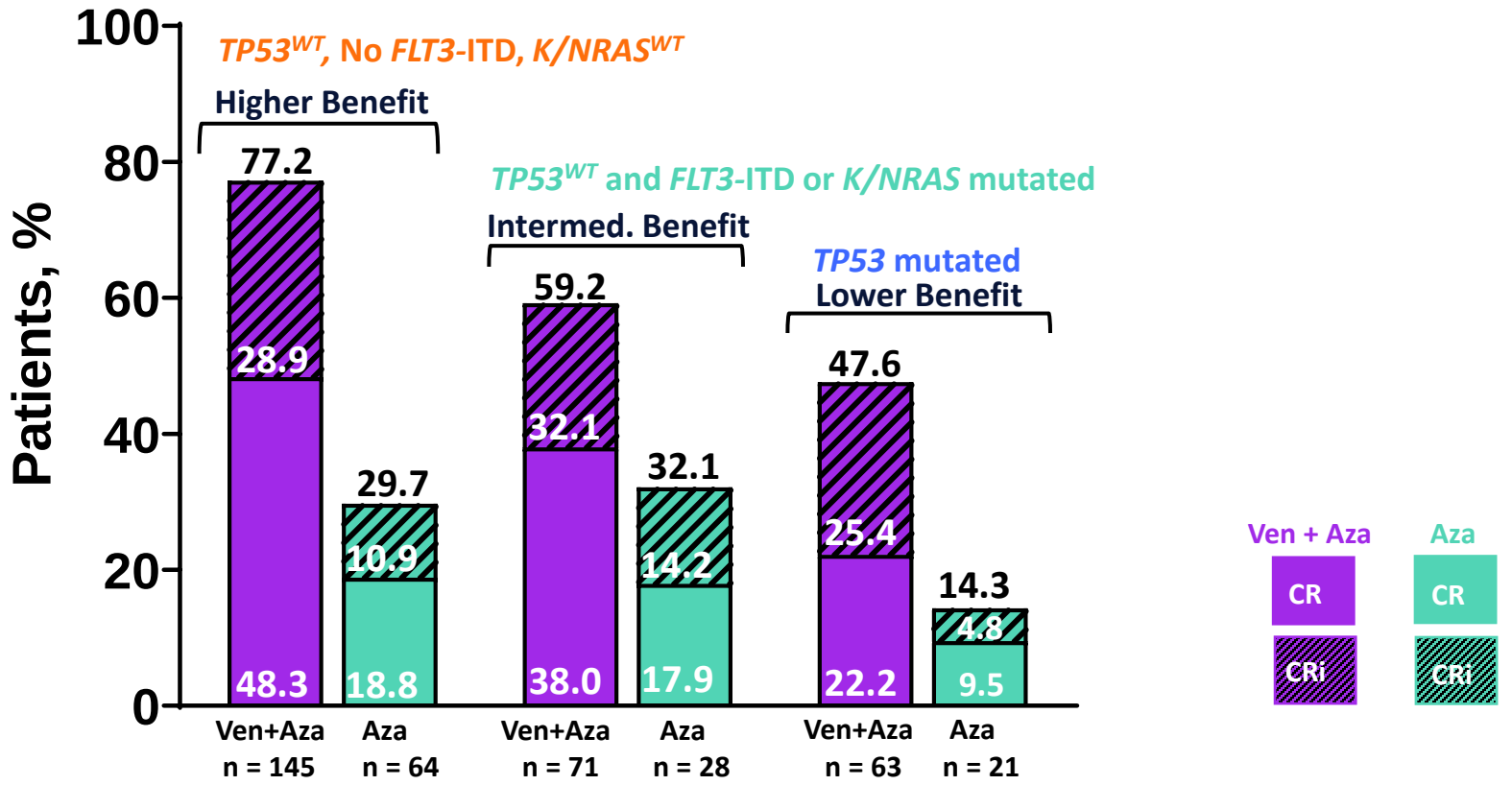
		Events	Survival estimate (%) (95% CI) Month 6	Month 12	Month 24	Median (months) (95% CI)
FLT3mut	(*n* = 42)	27	70.7 (54.3, 82.2)	53.7 (37.4, 67.4)	27.7 (12.5, 45.3)	12.5 (7.3, 19.2)
FLT3wt	(*n* = 238)	141	71.6 (65.4, 76.9)	55.2 (48.6, 61.3)	38.6 (31.9, 45.3)	14.7 (11.3, 19.4)



	Events	Survival estimate (%) (95% CI) Month 6	Month 12	Month 24	Median (months) (95% CI)
FLT3-ITD (*n* = 30)	21	69.0 (48.8, 82.5)	44.8 (26.5, 61.6)	20.1 (5.2, 41.8)	9.9 (5.3, 17.6)
FLT3-TKD (*n* = 13)	7	69.2 (37.3, 87.2)	69.2 (37.3, 87.2)	43.1 (15.6, 68.3)	19.2 (1.8, -)

Konopleva et al CCR 2022

Remission rates were higher with Ven+Aza than with Aza monotherapy across all 3 groups

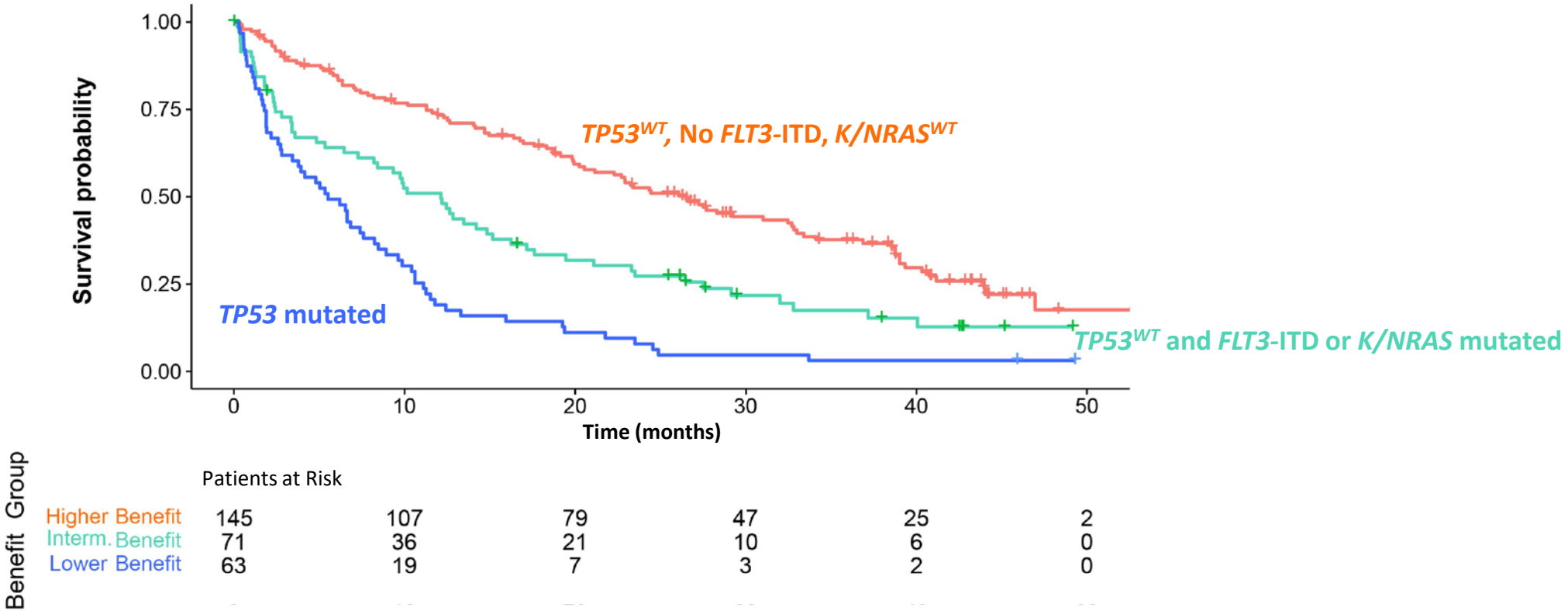


- CR and CR/CRi rates were highest in the higher benefit group
- Higher MRD negativity rates were achieved with Ven+Aza than with Aza monotherapy across all 3 groups

Abbreviations: Aza, azacitidine; CR, complete remission; CRi, CR with incomplete count recovery; Intermed., intermediate; MRD, minimal residual disease; Ven, venetoclax

Dohner, Pratz et al ASH 2022

Three prognostic risk signatures derived to indicate higher, intermediate, and lower benefit from treatment with Ven+Aza



Abbreviations: Aza, azacitidine; OS, overall survival; Ven, venetoclax; WT, wild-type

Dohner, Pratz et al ASH 2022

Leukemia (2008) 22, 808–818

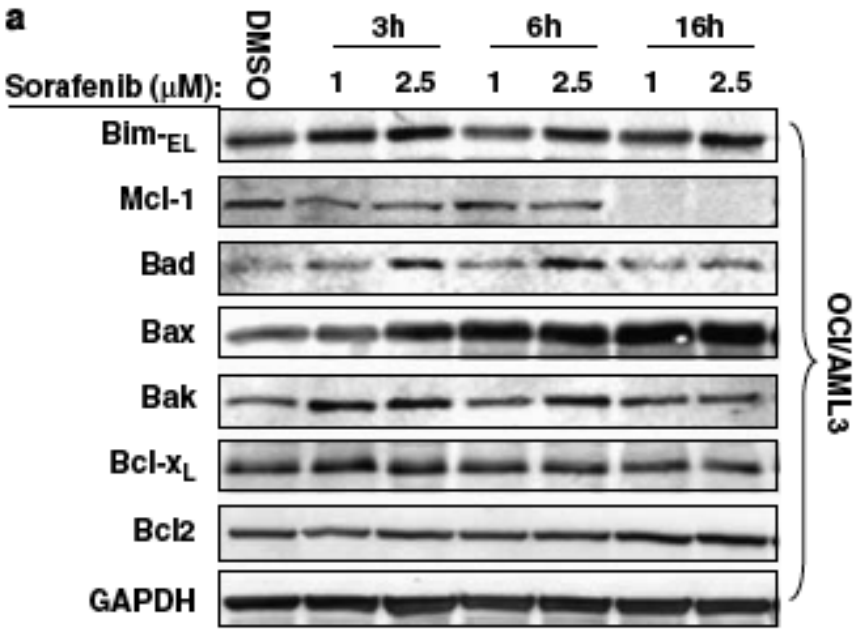
www.nature.com/leu

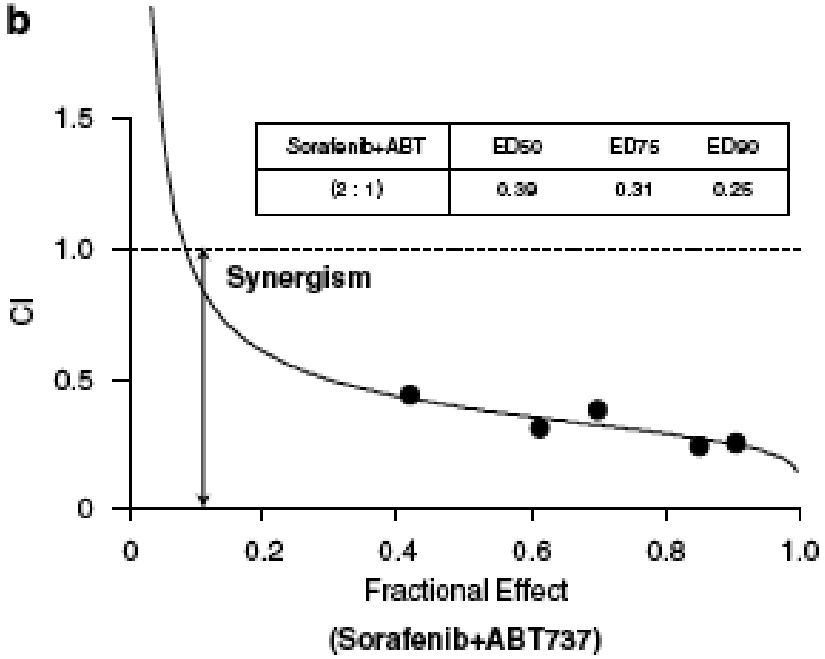
ORIGINAL ARTICLE

Sorafenib induces apoptosis of AML cells via Bim-mediated activation of the intrinsic apoptotic pathway

W Zhang[1], M Konopleva[1], VR Ruvolo[1], T McQueen[1], RL Evans[1], WG Bornmann[2], J McCubrey[3], J Cortes[4] and M Andreeff[1,4]

[1]Section of Molecular Hematology and Therapy, Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; [2]Department of Experimental Diagnostic Imaging, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; [3]Department of Microbiology and Immunology, Brody School of Medicine at East Carolina University, Greenville, NC, USA and [4]Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA





Venetoclax and Gilteritinib in Relapsed Refractory FLT3

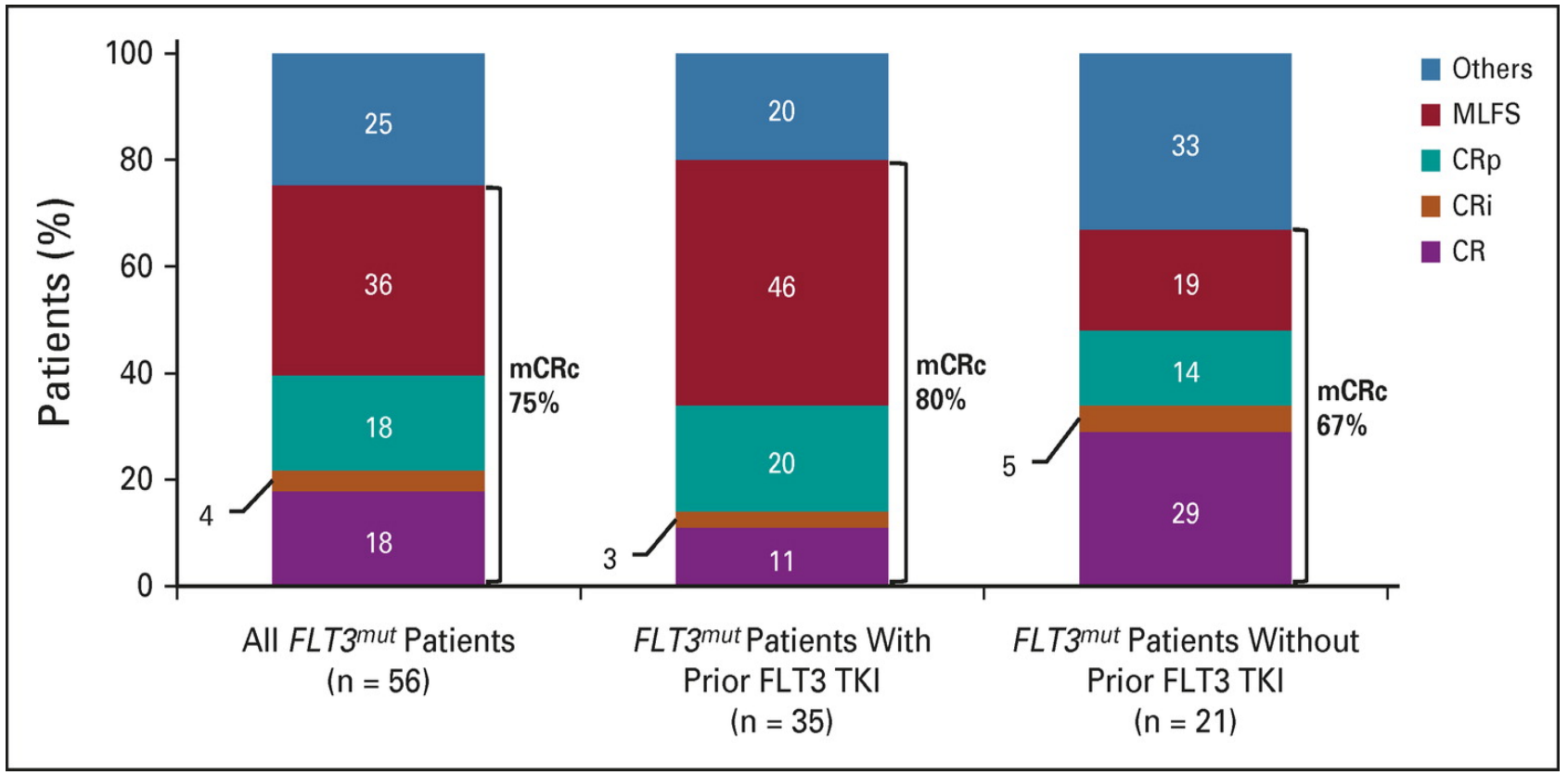
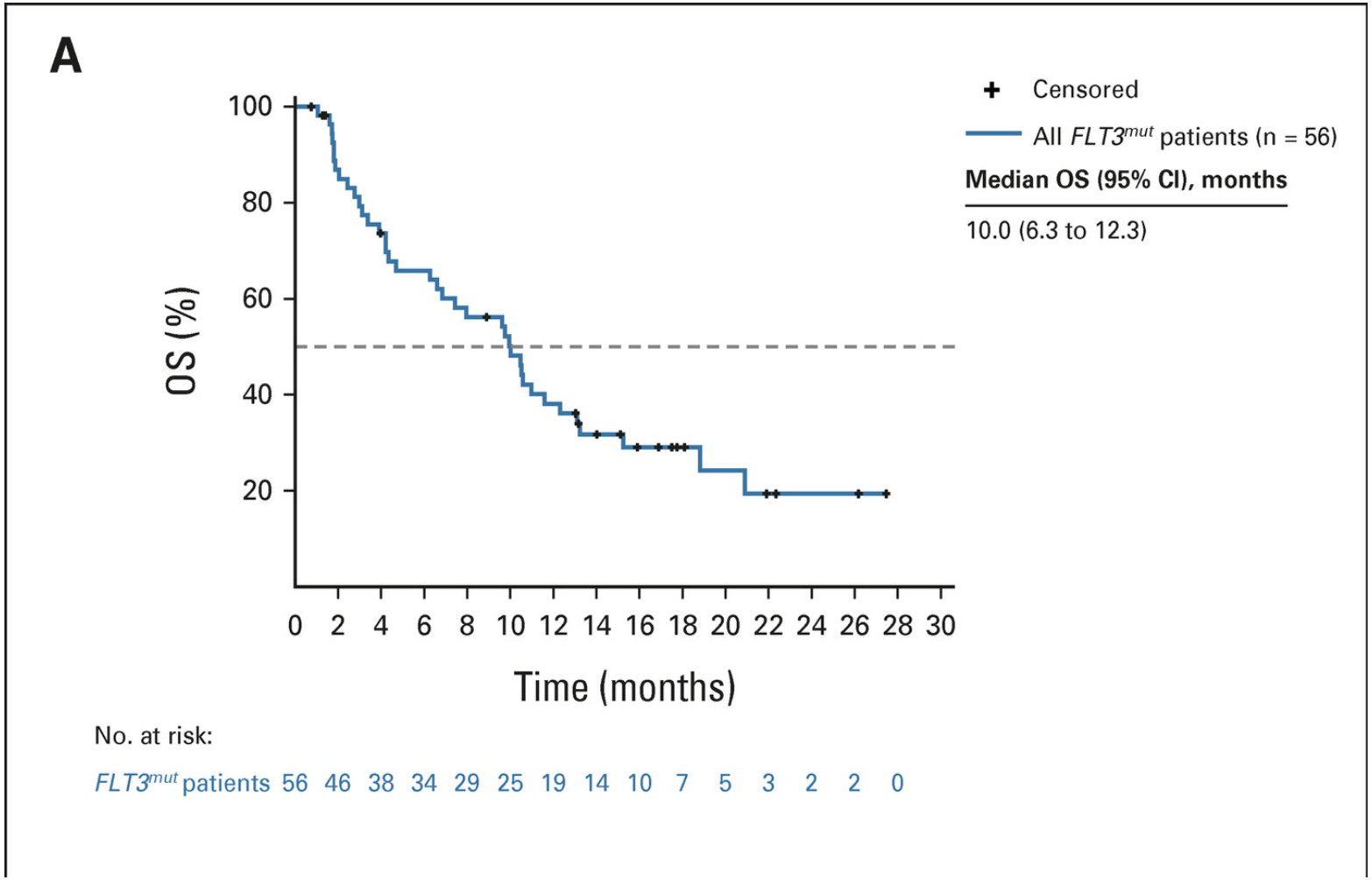


FIG 2. Response rates in all FLT3mut patients treated at any dose (n = 56) and in those who did (n = 35) or did not (n = 21) receive prior treatment with a FLT3 TKI. mCRc was defined as CR + CRi + CRp per criteria used in the ADMIRAL study. CR, complete response; CRi, complete response with incomplete blood count recovery; CRp, complete response with incomplete platelet recovery; mCRc, modified composite complete response; MLFS, morphologic leukemia-free state; TKI, tyrosine kinase inhibitor.

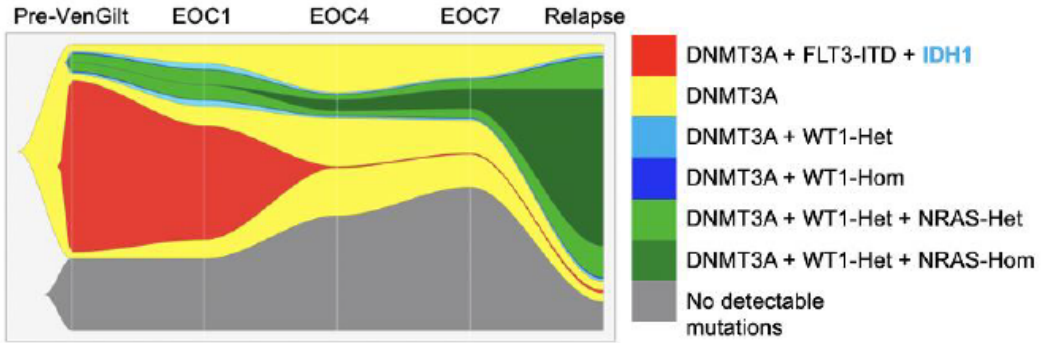
Published in: Naval Daver; Alexander E. Perl; Joseph Maly; Mark Levis; Ellen Ritchie; Mark Litzow; James McCloskey; Catherine C. Smith; Gary Schiller; Terrence Bradley; Ramon V. Tiu; Kiran Naqvi; Monique Dail; Deanna Brackman; Satya Siddani; Jing Wang; Brenda Chyla; Paul Lee; Jessica K. Altman; *Journal of Clinical Oncology* Ahead of Print
DOI: 10.1200/JCO.22.00602

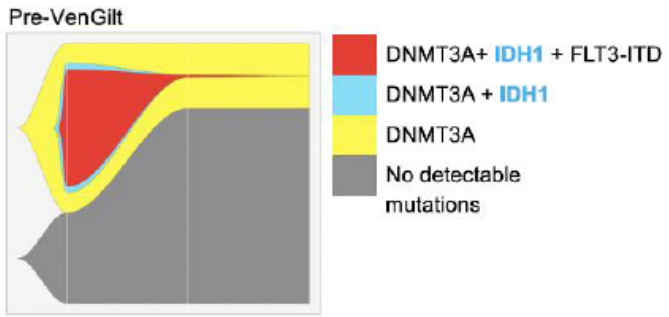
Venetoclax and Gilteritinib in Relapsed Refractory FLT3

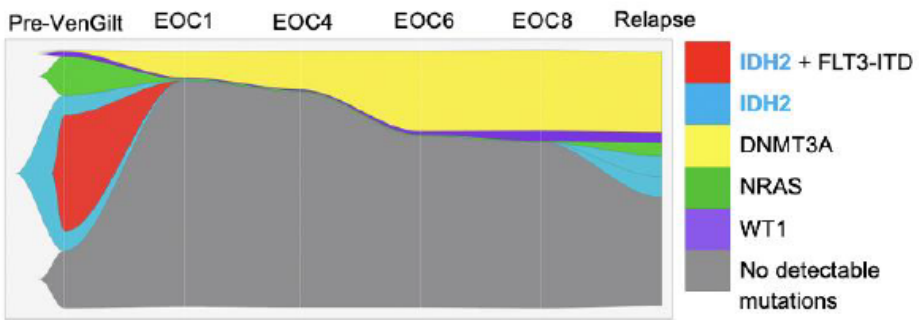


Daver et al, JCO 2022

VenGilt may be active against *IDH1/2-FLT3* co-mutants and non-*FLT3* clones







- In 3 patients, ***FLT3* and *IDH1/2* co-mutant** clones were also markedly decreased on VenGilt
- The presence of an *IDH* mutation in the same cell did not cause these *FLT3*^{mut} clones to be resistant to VenGilt
- Interestingly, 1 patient did develop a dominate *IDH2*^{mut} clone at relapse, but this clone did not have a *FLT3* co-mutation
- 20 **non-*FLT3*^{mut} clones**, including *IDH1-*, *IDH2-*, *DNMT3A-*, *NPM1-*, *TET2-*, *EZH2-*, *WT1-*, and *NRAS*-mutated clones were decreased by >10% in 11 patients
- In 5 patients, 9 clones increased on therapy
  - 4 of these clones expressed only CHIP-associated mutations

Kennedy et al ASH 2022

CHIP, clonal hematopoiesis of indeterminate potential; EOC, end of cycle; Het, heterogeneous; Hom, homogeneous; ITD, internal tandem duplication; VenGilt, venetoclax and gilteritinib.

Aza+Ven+Gilteritinib in FLT3-mutated AML: Regimen

- Relapsed/refractory *FLT3*-mutated* AML or high-risk MDS or CMML

or

- Newly diagnosed *FLT3*-mutated* AML unfit for intensive chemotherapy

Induction

Azacitidine
75 mg/m² IV/SC on D1-7

Venetoclax#
D1-28 (bone marrow on D14)%

Gilteritinib
80-120 mg on D1-28

Consolidation (up to 24 cycles)

Azacitidine
75 mg/m² IV/SC on D1-5

Venetoclax
400mg on D1-7

Gilteritinib
80-120 mg on D1-28

* FLT3-ITD or FLT3 D835 mutations allowed

Venetoclax ramp-up during cycle 1: 100mg on D1, 200mg on D2, 400mg on D3+

% If <5% blasts or insufficient on C1D14, venetoclax held (both cohorts) and gilteritinib held (frontline only)

- Primary endpoints: MTD of gilteritinib in combination (phase I), CR/CRi rate (phase II)
- Secondary endpoints: CR rate, MRD negativity rate, duration of response, OS, safety

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Aza+Ven+Gilteritinib in FLT3-mutated AML: Responses

Response, n/N (%)	Frontline N = 27	R/R N = 20
mCRc (CR/CRi/MLFS)	27 (100)	14 (70)
CR	25 (92)	4 (20)
CRi	1 (4)	3 (15)
MLFS	1 (4)	7 (35)
PR*	0	1 (5)
No response	0	5 (25)
Early death	0	0

* PR in 1 patient with extramedullary-only disease (assessed by PET scan)

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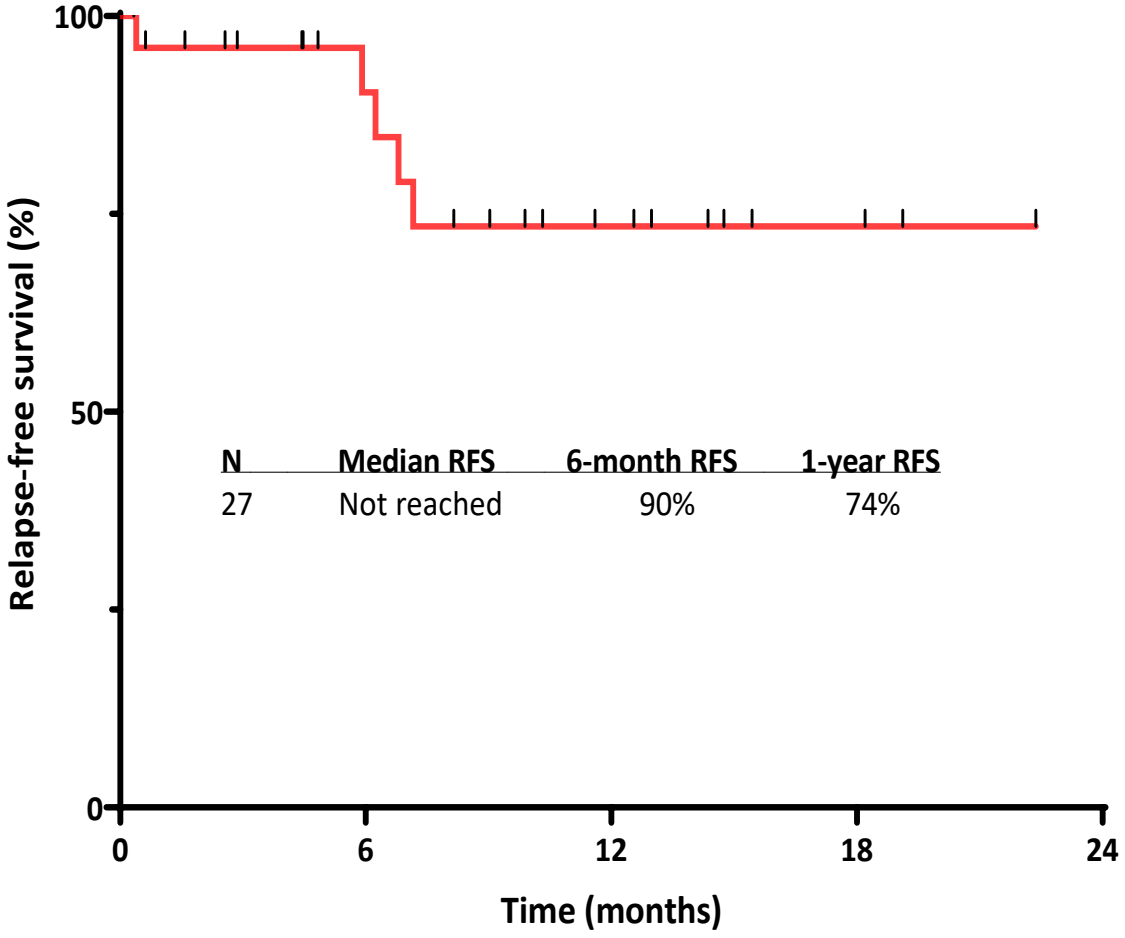
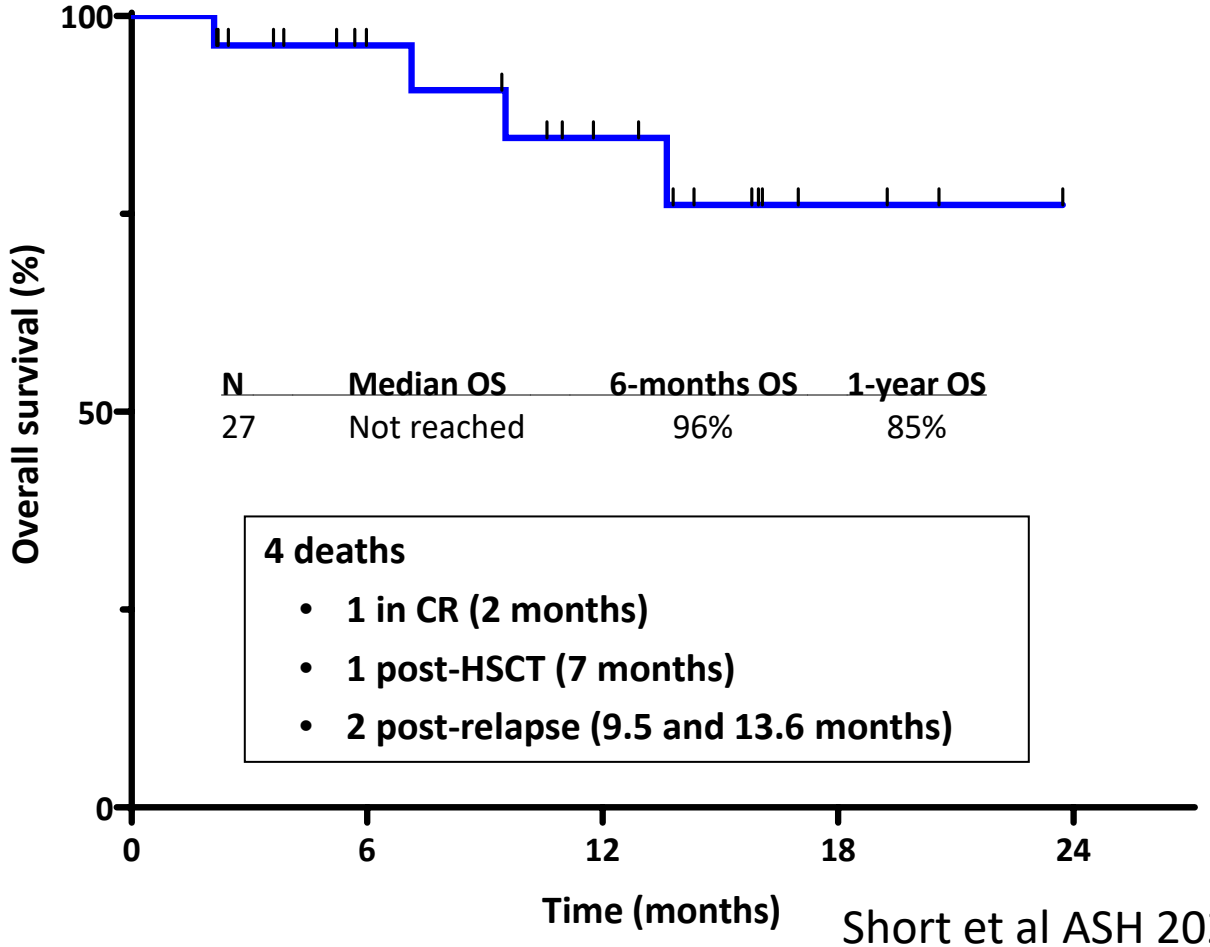
Aza+Ven+Gilteritinib in FLT3-mutated AML: RFS and OS in Frontline Cohort

Median follow-up: 12 months (range, 1.5-24+ months)





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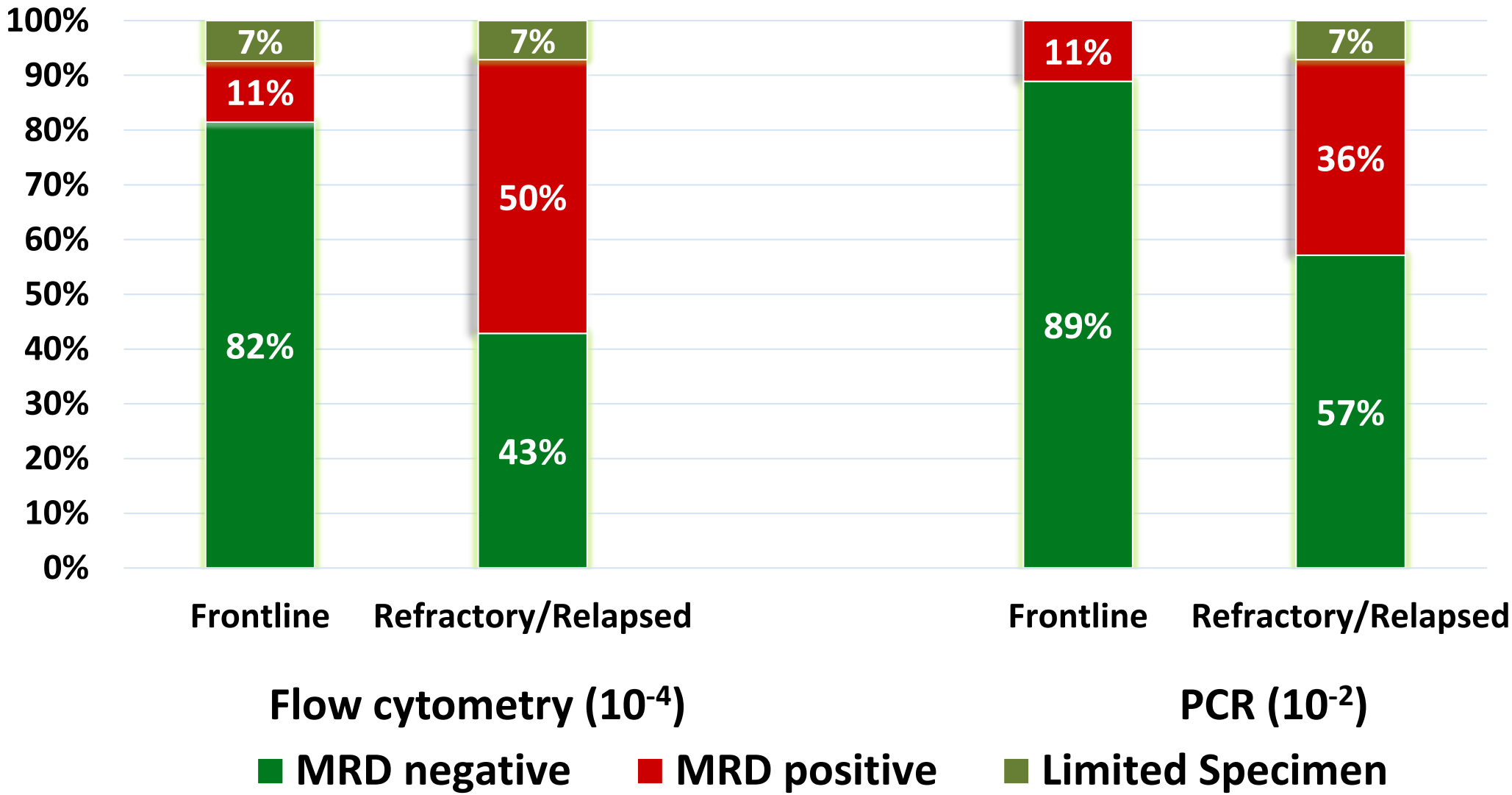
Aza+Ven+Gilteritinib in FLT3-mutated AML: Best MRD Response



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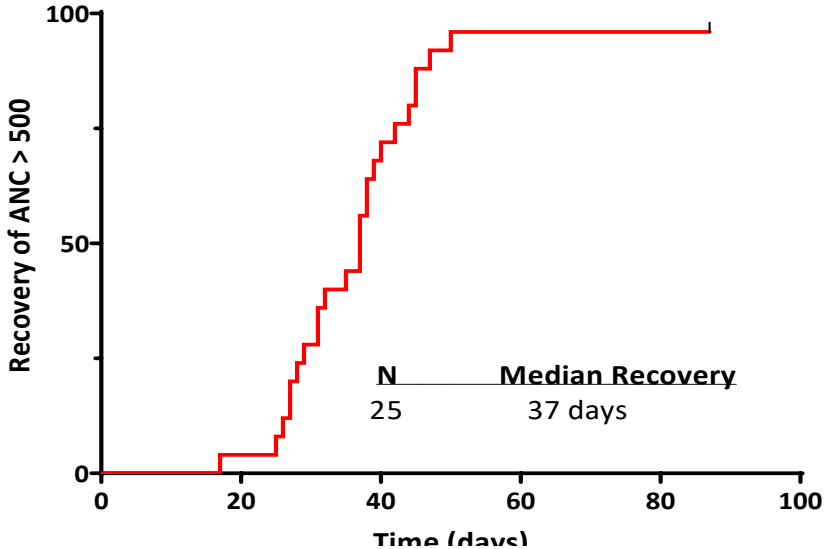
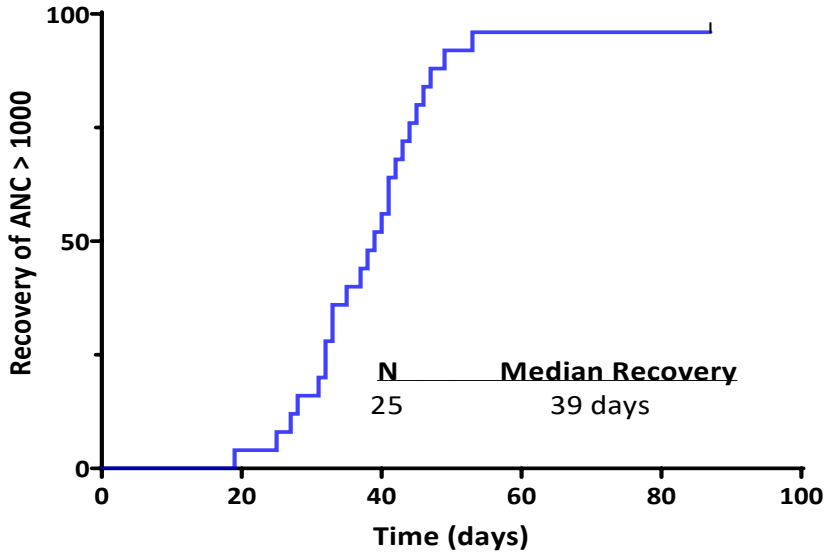
Aza+Ven+Gilteritnib in FLT3-mutated AML: Hematologic Recovery in Cycle 1 (Frontline Cohort)

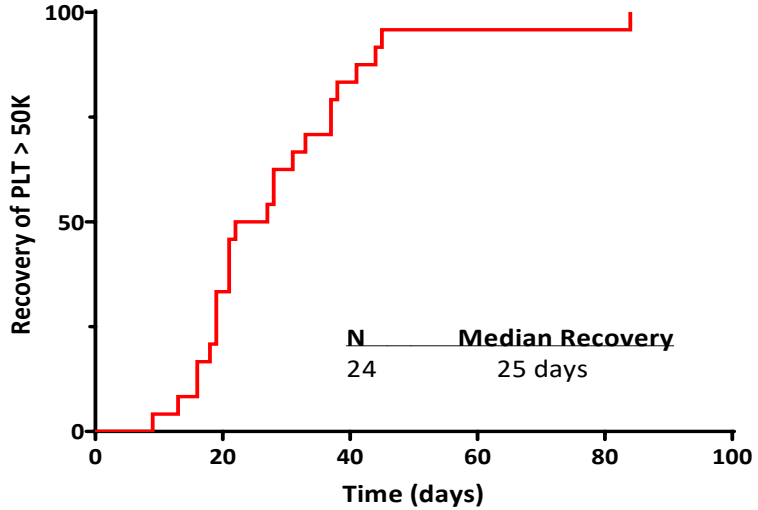
ANC >500



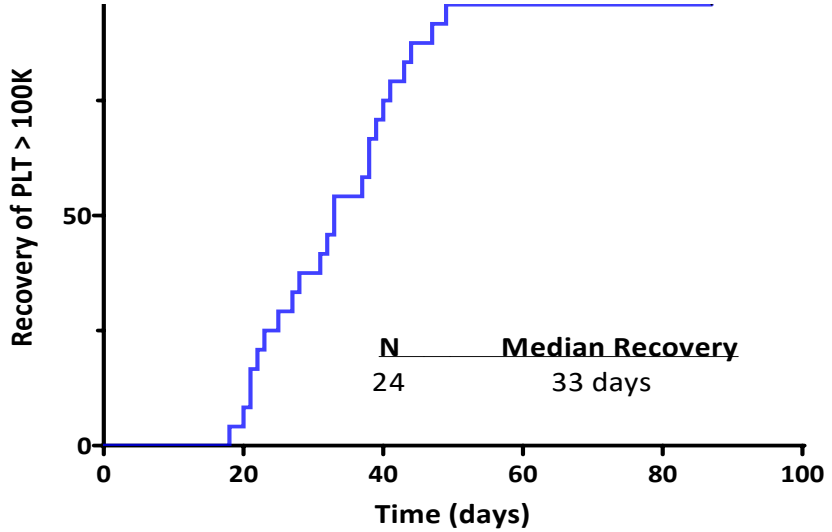
ANC >1000



Platelets >50K



Platelets >100K



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Conclusions

- FLT3 mutant AML is a targetable disease
- Two randomized studies confirm benefit of addition of FLT3i to 7&3
- In the relapsed setting Gilteritinib is associated with improved response rate and survival over conventional salvage therapies
- Combinations of targeted therapies appear active but optimization of dosing is still in process
- Novel agents outside of FLT3 are in development
- Questions: keith.pratz@pennmedicine.upenn.edu