VARIANT FLT3 MUTATIONS CAN BE ERADICATED BY CYTARABINE/ANTHRACYCLINE/CRENOLANIB INDUCTION IN ADULT PATIENTS WITH NEWLY DIAGNOSED FLT3 (ITD/TKD) MUTANT AML

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Abstract

Background: Patients (pts) with FLT3-internal tandem duplication (ITD) and FLT3-D835 mutant AML have a high relapse rate. These relapses are typically due to outgrowth of mutant FLT3 clones. Previously available PCR-based tests only checked for presence of FLT3-ITD and FLT3-D835/I836 mutations. Whole genome sequencing of 799 pediatric AML samples from COG trials have shown novel FLT3 variants in not only the tyrosine kinase domain but also the juxtamembrane (JM) and transmembrane domains in 7.6% of these samples (Tarlock et al. ASH 2015). Some of these mutations result in autophosphorylation of FLT3 and therefore may be oncogenic.

Aims: Identify novel FLT3 mutations in pts with FLT3 mutant AML and further investigate whether these novel clones are sensitive to induction chemotherapy plus a potent pan-FLT3 inhibitor, crenolanib.

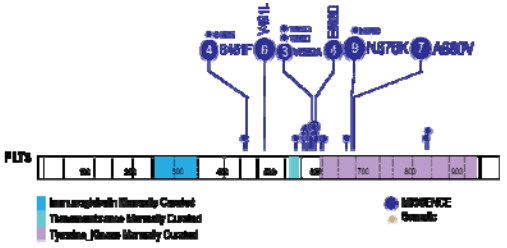
Methods: Pts with newly diagnosed FLT3 mutant AML were enrolled and treated with cytarabine/anthracycline/crenolanib induction followed by high dose cytarabine (HiDAC) consolidation. Crenolanib 100 mg TID was started on day 9 of induction and continued till next chemotherapy. Crenolanib was given following consolidation and allogeneic stem cell transplantation. Bone marrow samples were collected at baseline and at the time of remission assessment. Sequencing of the entire FLT3 gene was performed through FoundationOne Heme panel (n=18) and MSKCC multigene panel (n=5). Sequencing of exons 14,15,16, and 20 was performed through the Rapid Heme Panel at Dana-Farber Cancer Institution in additional 6 pts.

Results: Out of 29 newly diagnosed FLT3 mutant AML patients with full/partial FLT3 gene sequencing performed, 4 pts were found to have novel variant FLT3 mutations consisting of V491L, V592L, D593H, A680V, and N841I/T/K (Table 1). The majority of these novel mutations were located at the JM, kinase domain 1 and the activation loop (kinase domain 2). The allele fractions of these FLT3 variants ranged as high as 29% (higher than that of FLT3-ITD in pt3), suggesting that some of these clones may have been potentially driving clinical leukemia progression in some pts. All 4 pts had NPM1 mutations, and two also had DNMT3A mutations. All 4 pts achieved CR with full count recovery (3/4 pts achieved CR after just one cycle of cytarabine/anthracycline/ crenolanib induction). The pt with FLT3-D835Yand N841T achieved a CR after cytarabine/ anthracycline/ crenolanib induction and one cycle of HiDAC consolidation. All pts became FLT3-ve and have remained FLT3-ve.

3 out of 4 pts received 1-4 cycles of HiDAC consolidation followed by crenolanib maintenance. Only one pt underwent allo SCT. With a median follow up of 13 months, one pt relapsed (at 8.4-month following treatment). This 61F pt was found to have FLT3-ITD, D593H and I836del FLT3 abnormalities at the time of diagnosis. A full FoundationOne gene panel done at the time of relapse, showed no residual FLT3 mutant clones.

FLT3 Variant Mutations

FLT3 variants detected in pediatric AML



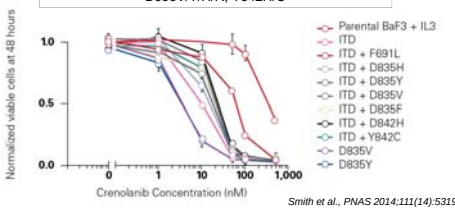
Tarlock et al., EHA 2017, abstract P184

1. Novel FLT3 variants were found in 7.6% pediatric AML samples (799 samples). Tarlock et al., Blood 2015;126(23):87
2. Mutations throughout the FLT3 gene were detected, including the extracellular domain, juxtamembrane domain, kinase domain 1, and kinase domain 2 (activation loop) of FLT3.
3. These variants have ability to auto-phsophorylate and activate downstream signaling pathway.

Tarlock et al., EHA 2017, abstract P184

Crenolanib is a Type I, Pan- FLT3 TKI

Crenolanib has activity against activation loop mutations: D835V/Y/H/R, Y842H/C



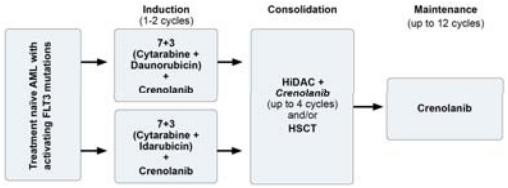
Smith et al., PNAS 2014;111(14):5319-24

Crenolanib Inhibits FLT3 phosphorylation at low nM concentrations in all variant FLT3 mutations

FLT3 Mutation		IC_{50} of Crenolanib (nM)
Juxta-membrane domain	E573D	1.3
	L576R	1.4
	V592A	6.7
	F594C	6.4
	F594Y	6.7
	Y599C	2.1
Kinase 1	M664I	2.1
	N676K	15.9
	A680V	1.3
Kinase 2 (activation loop)	D835Y	2.4
	D839E	3.2
	N841I	2.5

Tarlock et al., EHA 2017, abstract P184

Study Design



Induction:	Cytarabine 100mg/m²/CIV for 7 days Anthracycline: Daunorubicin 90mg/m² (<60y) or 60mg/m² (≥60y) or Idarubicin 12 mg/m² x 3 days Crenolanib 100mg TID starting day 9
Consolidation:	Cytarabine 3g/m² (<60y) or 1g/m² (≥60y), q12h x 6 doses Crenolanib 100mg TID starting day 7
Maintenance:	Crenolanib 100mg TID continuously

Methods

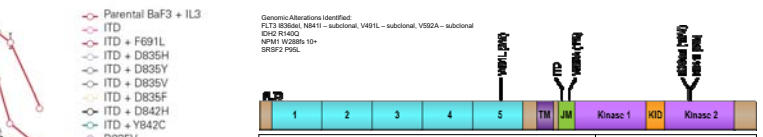
Previously available PCR-based tests only checked for presence of FLT3-ITD and FLT3-D835/I836 mutations. Herein, we reported a subset of patients treated with 7+3 and crenolanib with FLT3 sequencing results available on juxtamembrane domain (JM, exons 14 and 15), kinase domain 1 (exon 16), and kinase domain 2 (activation loop, exon 20). FLT3 sequencing was performed at baseline and at the time of remission assessment. Sequencing coverage may vary among laboratories, as summarized below.

	# of patients	Sequencing coverage on FLT3				
		Full gene	Exon 14	Exon 15	Exon 16	Exon 20
FoundationOne Heme	18	Yes	Yes	Yes	Yes	Yes
MSK Multigene Panel	5	Yes	Yes	Yes	Yes	Yes
Rapid Heme Panel	6	No	Yes	Yes	Yes	Yes

Case 1: ECD, JM and Activation Loop Mutations

54/F

Subject with FLT3-ITD and 4 additional mutations, including 2 TKD mutations



FLT3 Mutation and Allele Frequency			IC_{50} of Crenolanib (nM)
Extracellular domain	V491L	3%	N/A
Juxtamembrane domain	ITD	N/A	1.3*
Juxtamembrane domain	V592A	1%	6.7
Kinase 2 (activation loop)	I836del	18%	N/A
Kinase 2 (activation loop)	N841I	8%	2.5

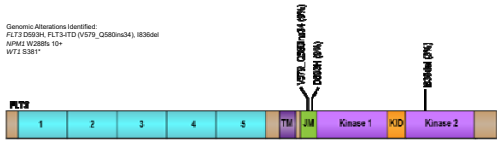
*Galanis et al., Blood 2014 123(1):94-100; N/A: not available

54/F, normal karyotype, WBC count at presentation 233,290/µL
Response to induction 1: CR with MRD negativity (by flow)
FLT3 status after treatment: negative
Status: Alive, in crenolanib maintenance, leukemia-free

Case 2: JM and Activation Loop Mutations

61/F

Subject with FLT3-ITD and 2 additional mutations including 1 TKD mutation



FLT3 Mutation and Allele Frequency			IC_{50} of Crenolanib (nM)
Juxtamembrane domain	ITD	9%	1.3*
Juxtamembrane domain	D593H	9%	N/A
Kinase 2 (activation loop)	I836del	3%	N/A

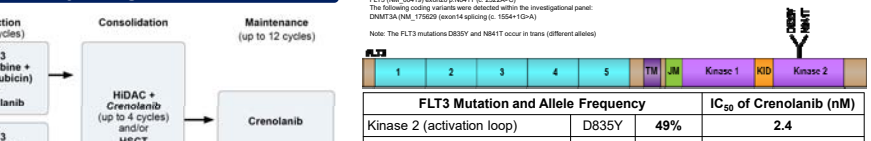
*Galanis et al., Blood 2014 123(1):94-100; N/A: not available

61/F, normal karyotype
Response to induction 1: CR with MRD negativity (by flow)
FLT3 status after treatment: negative
Status: Alive, relapsed with non-FLT3 clones (NRAS, JAK3+ve), remains FLT3-ve

Case 3: Activation Loop Mutations *in trans*

36/F

Subject with 2 TKD mutations occurring *in trans*



FLT3 Mutation and Allele Frequency			IC_{50} of Crenolanib (nM)
Kinase 2 (activation loop)	D835Y	49%	2.4
Kinase 2 (activation loop)	N841T	N/A	N/A

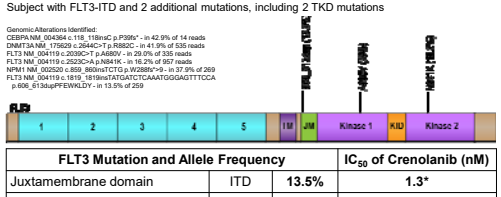
N/A: not available

36/F, normal karyotype
Response to induction 1: PR with FLT3 negativity (DNMT3A+ve)
FLT3 status after treatment: negative
Status: Alive, leukemia-free

Case 4: TKD1 and Activation Loop Mutations

54/F

Subject with FLT3-ITD and 2 additional mutations, including 2 TKD mutations

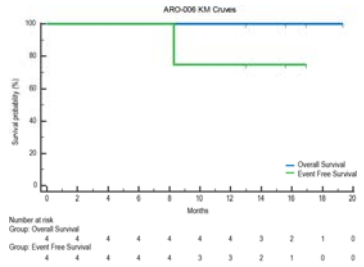


FLT3 Mutation and Allele Frequency			IC_{50} of Crenolanib (nM)
Juxtamembrane domain	ITD	13.5%	1.3*
Kinase 1	A680V	29%	1.3
Kinase 2 (activation loop)	N841K	16.2%	N/A

*Galanis et al., Blood 2014 123(1):94-100; N/A: not available

54/F, normal karyotype
Response to induction 1: CR
FLT3 status after treatment: negative
Status: Alive, in crenolanib maintenance, leukemia-free

Conclusions



With a medium follow-up of 16.3 months, 3 of 4 patients are still in remission. All 4 patients remain FLT3 negative.

- Novel FLT3 variant mutations can be found in adult AML patients.
- The allelic burden of these FLT3 variant mutations can sometime be higher than that of FLT3-ITD.
- Crenolanib in combination with standard induction chemotherapy has the ability to eradicate variant FLT3 clones.
- All 4 pts treated with chemotherapy followed by crenolanib showed clearance of FLT3-ITD, TKD, as well as other novel variants.
- To achieve maximal clinical benefit, a potent pan-FLT3 inhibitor with the ability to inhibit ITD, D835, as well as other activating mutations maybe beneficial.

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