9° WORKSHOP
IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Bologna, Aula "G. Prodi", 19-20 maggio 2025

Strategies to overcome resistance to FLT3 inhibitors in AML

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Disclosures di Samantha Bruno

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
N.A.							

Activating FLT3 mutations in AML

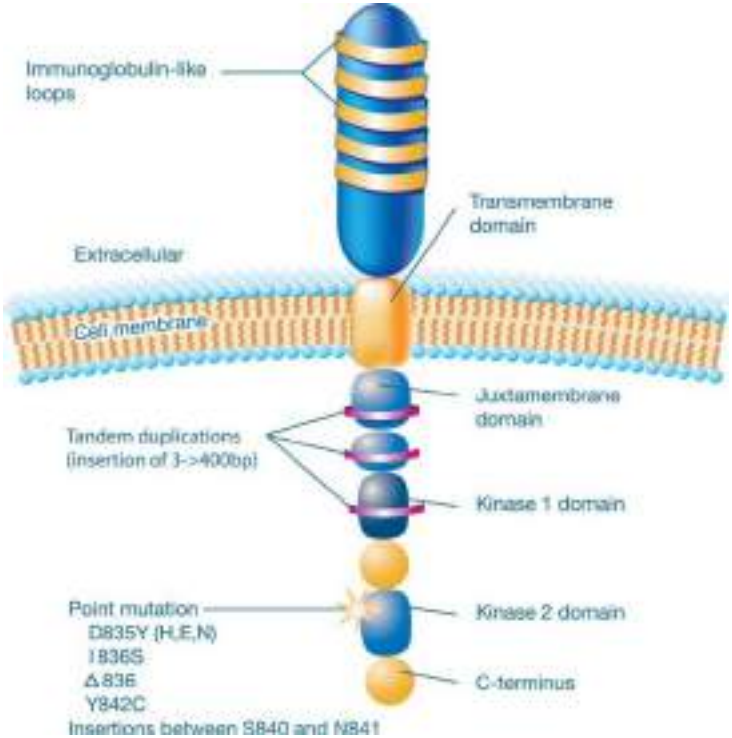
» **ITD (20-25%)**

-Distrupt the autoinhibitory function of JM region; are associated with a higher rate of relapse and inferior OS

» **TKD point mutations (5-10%)**

-Activate FLT3; the prognostic impact is less clear

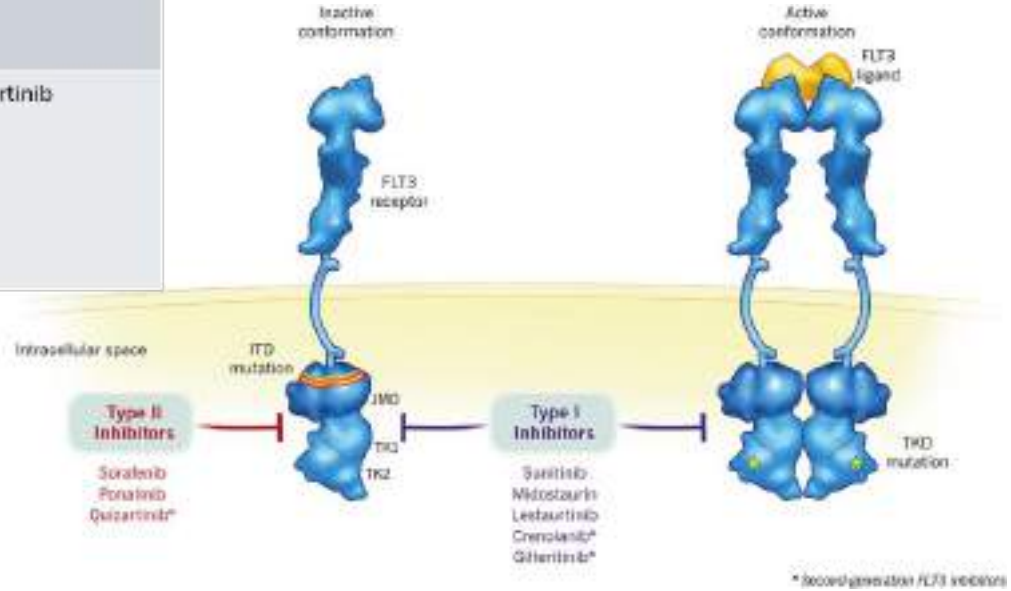
The uncontrolled receptor activation induces cellular proliferation and survival and inhibits differentiation



Pemmaraju N., Cancer 2011

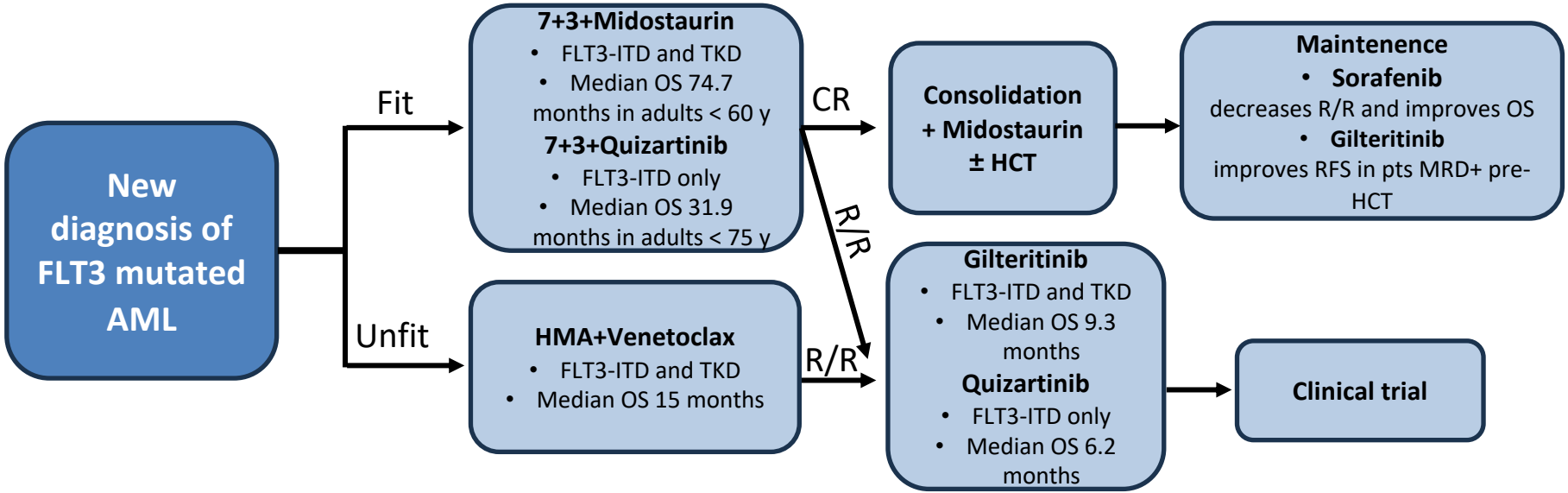
Classification of FLT3 inhibitors (FLT3i)

Type	Activity	1st Generation Less specific	2nd Generation More Specific and potent
Type I	Pure ATP-competitive regardless of receptor conformation. Active on ITD- and TKD-positive cells.	Sunitinib Midostaurin	Gilteritinib Crenolanib
Type II	Bind to the hydrophobic region directly adjacent to the ATP- binding domain when the protein is in inactive conformation. Active only against ITD-positive cells.	Sorafenib	Quizartinib

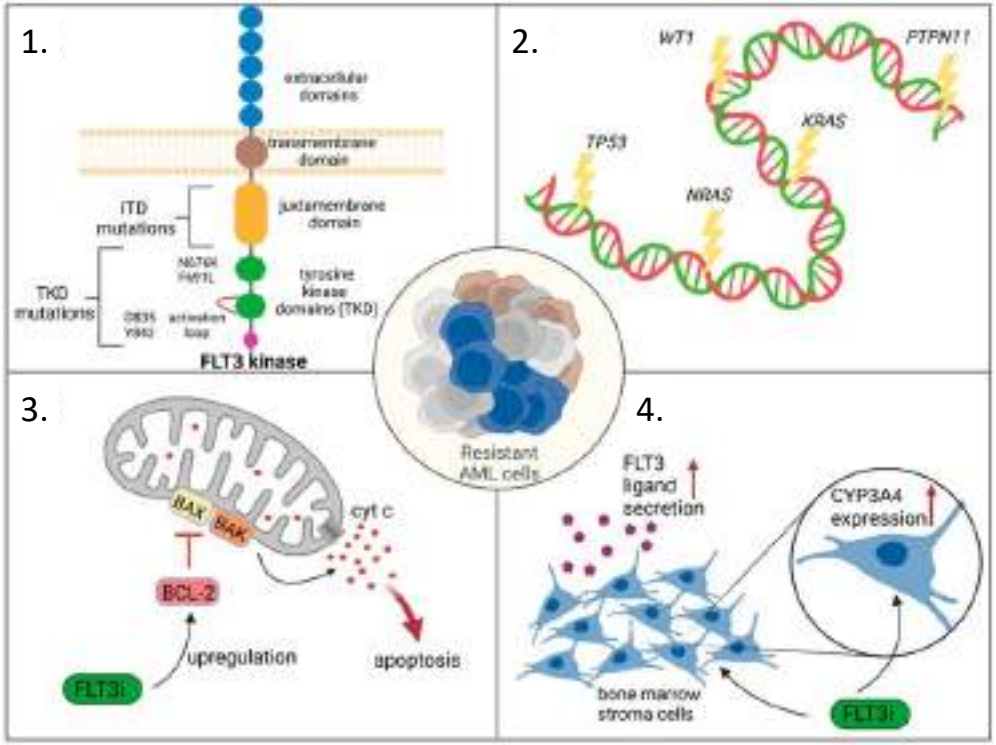


N. Daver, Leukemia, 2019

Standard of care treatment of patients with *FLT3*-mutated AML

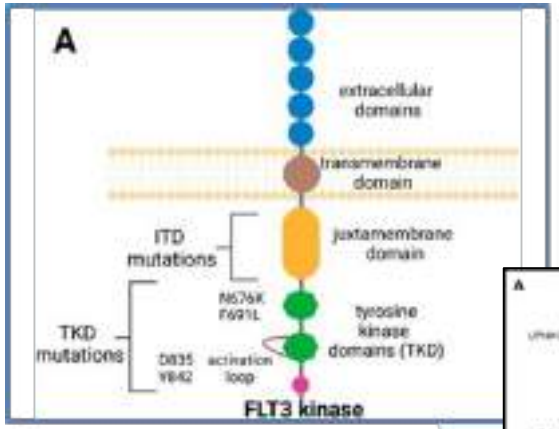


Mechanisms of resistance to FLT3i

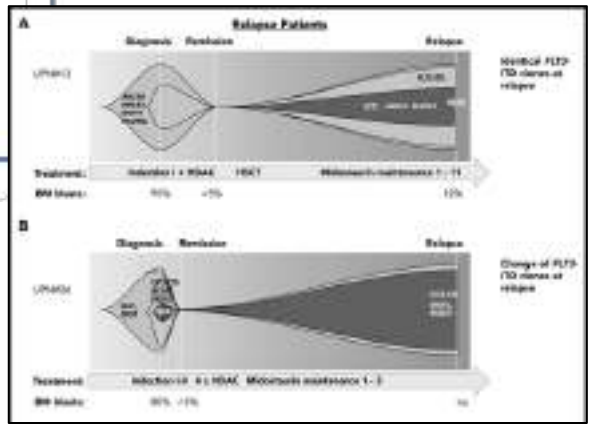


T. Lang, Cancers, 2023

1. On-target resistance



T. Lang, Cancers, 2023



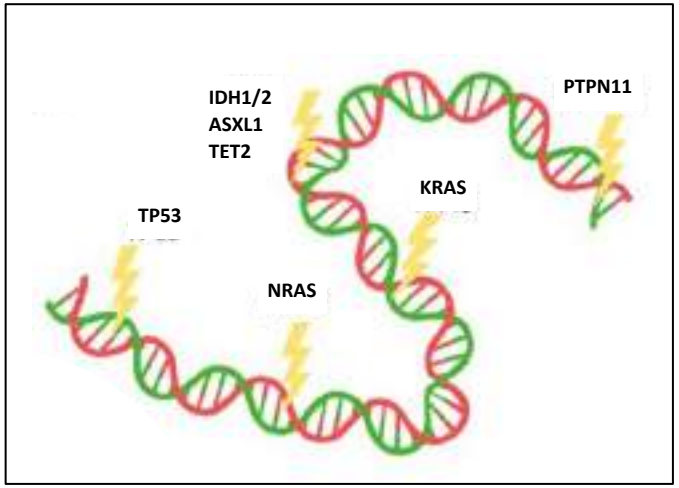
L. K. Schmalbrock, Blood, 2021

FLT3 inhibitor	Mutations mediating resistance to FLT3 inhibitors
Crenolanib	K429E (extramembrane domain)
	F691L (gatekeeper residue)
Midostaurin	F691L (gatekeeper residue)
	N676K (tyrosine kinase domain I)
Gilteritinib	F691L (gatekeeper residue)
Quizartinib	D835Y/V/F (activation loop)
	Y842C/H (activation loop)
	F691L (gatekeeper residue)
Sorafenib	D835Y/V (activation loop)
	Y842C/H (activation loop)
	F691L (gatekeeper residue)

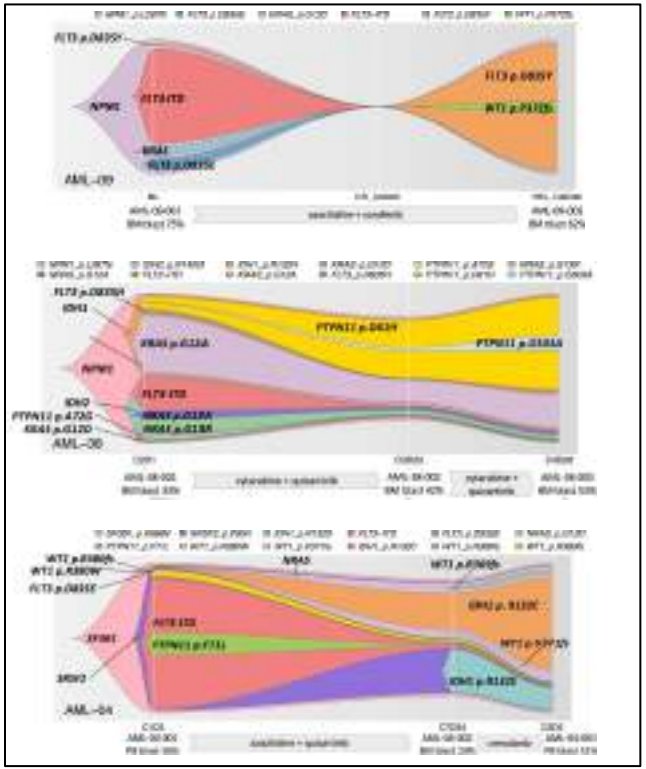
Select trials of novel FLT3-directed therapies to overcome on-target resistance

Clinical Trial ID	FLT3i	Phase	Target	Sensible Mutations
NCT02390752	Pexidartinib	I	FLT3-ITD, cKIT, CSFR	FLT3-ITD, FLT3 F691L
NCT03194685	FF-10101-01	I	FLT3	FLT3-ITD, FLT3 D835, F691,Y842
NCT03426605	LAM-003	I	FLT3, HSP90, KDM6A	FLT3-ITD, FLT3 D835, F691
NCT00783653	SU11248	I/II	FLT3	FLT3-ITD, FLT3 D835
NCT05947344	STI-8591	I	FLT3	FLT3-ITD, FLT3 D835Y, F691L
NCT05918692	BMF-500	I	FLT3	FLT3-ITD, FLT3 D835V-H-Y, F691L
NCT03922100	NMS-03592088	I/II	FLT3, KIT, CSF1R	FLT3-ITD, FLT3 D835, F691L
NCT04842370	PHI-101	I	FLT3	FLT3-ITD, D835E, N676K

2. Off-target resistance

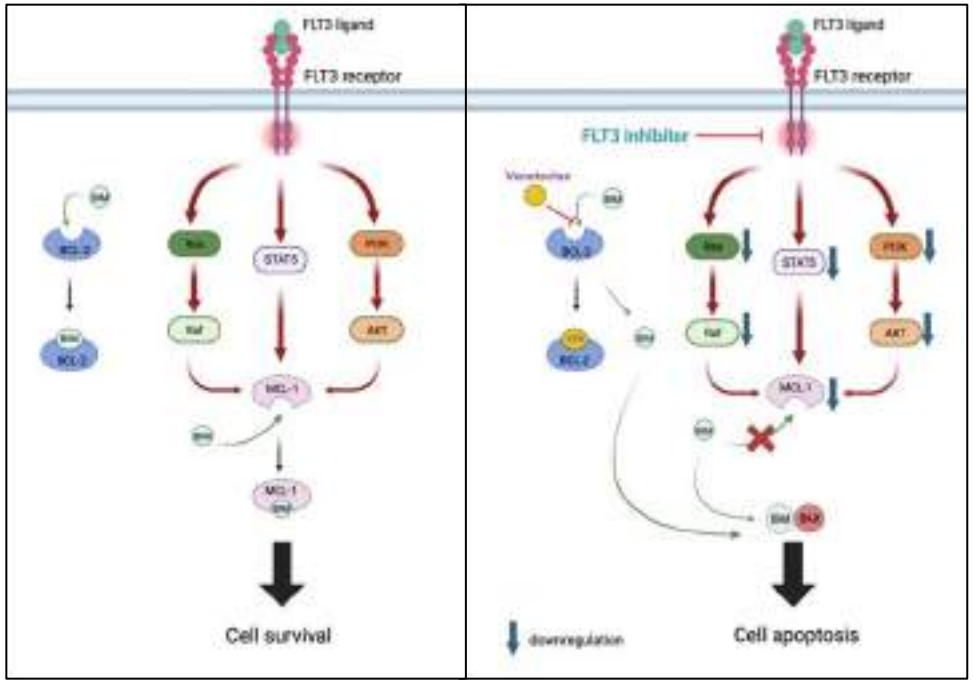


T. Lang, Cancers, 2023



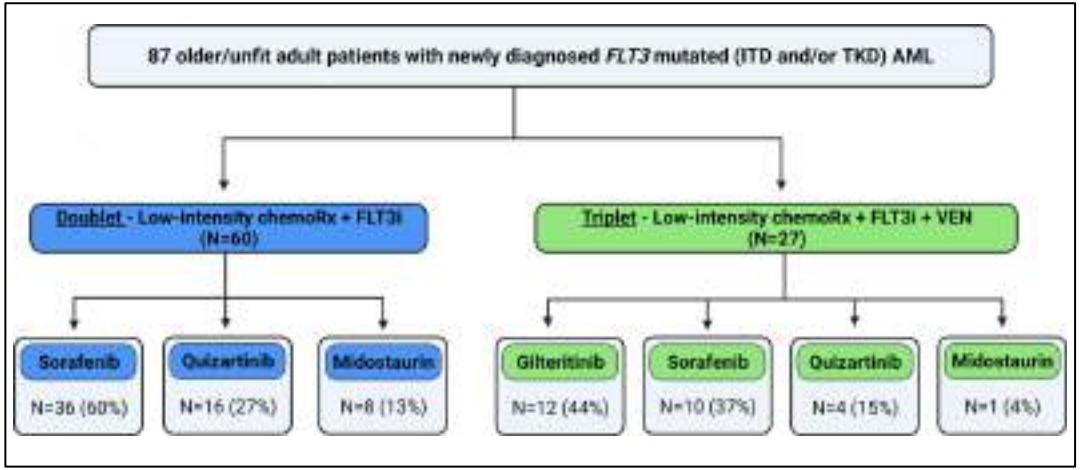
K. Morita et al. Nature Comm. 2020

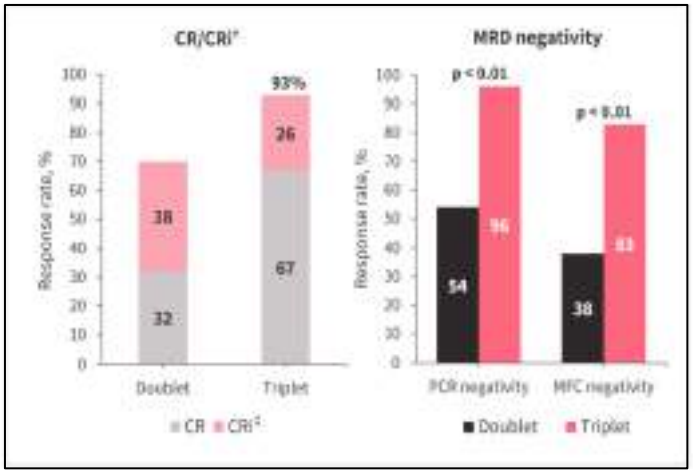
3. Up-regulation of antiapoptotic BCL2



A. Milnerowicz et al. Int. J Mol. Sci. 2023

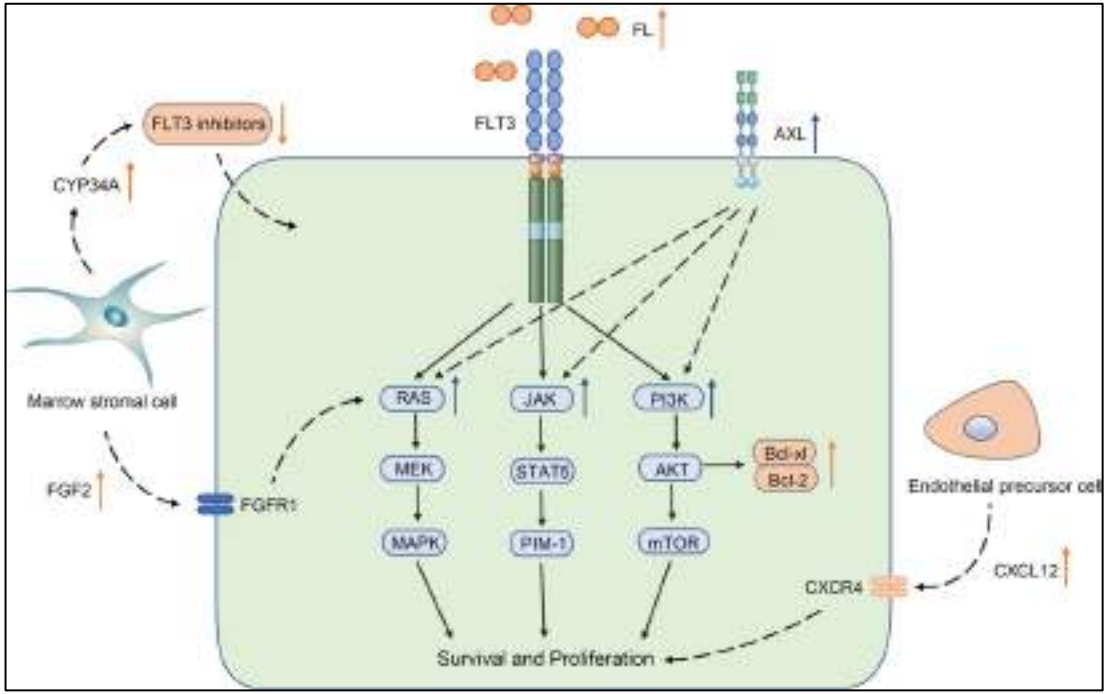
HMA and venetoclax with FLT3i: “triplet” therapy to overcome resistance mediated by BCL2





M. Yilmaz et al. Blood Cancer Journal. 2022

4. Microenvironment and cytokines alterations



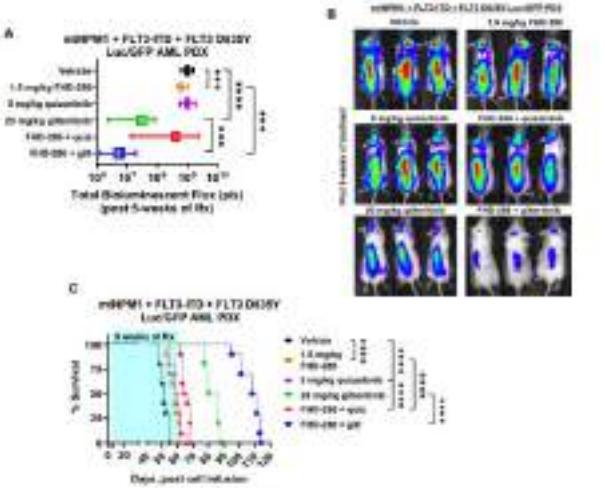
S. S. Ge, Front. Onc., 2022

Preclinical studies to overcome FLT3i resistance

Blood Cancer Journal

ARTICLE

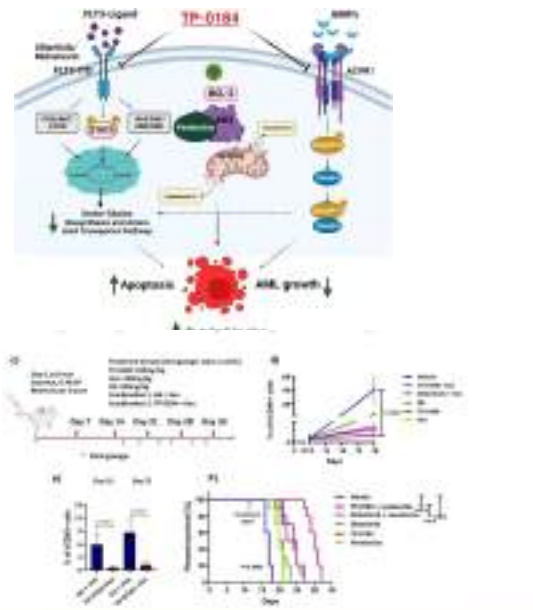
Superior preclinical efficacy of co-treatment with BRG1/BRM and FLT3 inhibitor against AML cells with FLT3 mutations



Leukemia

ACUTE MYELOID LEUKEMIA

TP-0184 inhibits FLT3/ACVR1 to overcome FLT3 inhibitor resistance and hinder AML growth synergistically with venetoclax

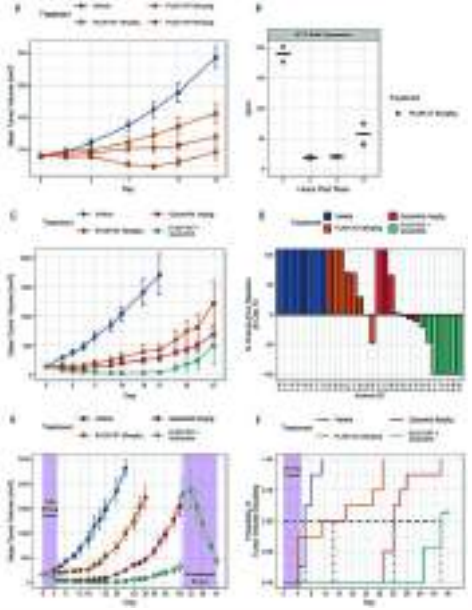


Haematologica 2021
Volume 106(4):1022-1033

A novel combination regimen of BET and FLT3 inhibition for FLT3-ITD acute myeloid leukemia

Lauren Y. Lee,[1] Yoshiyuki Hizukuri,[2] Paul Severson,[3] Benjamin Powell,[3] Chao Zhang,[3] Yan Ma,[3] Maiko Narahara,[3] Hiroyuki Sumi,[3] Daniela Hernandez,[1] Trivikram Rajkhowa,[1] Gideon Bollag[3] and Mark Levis[1]

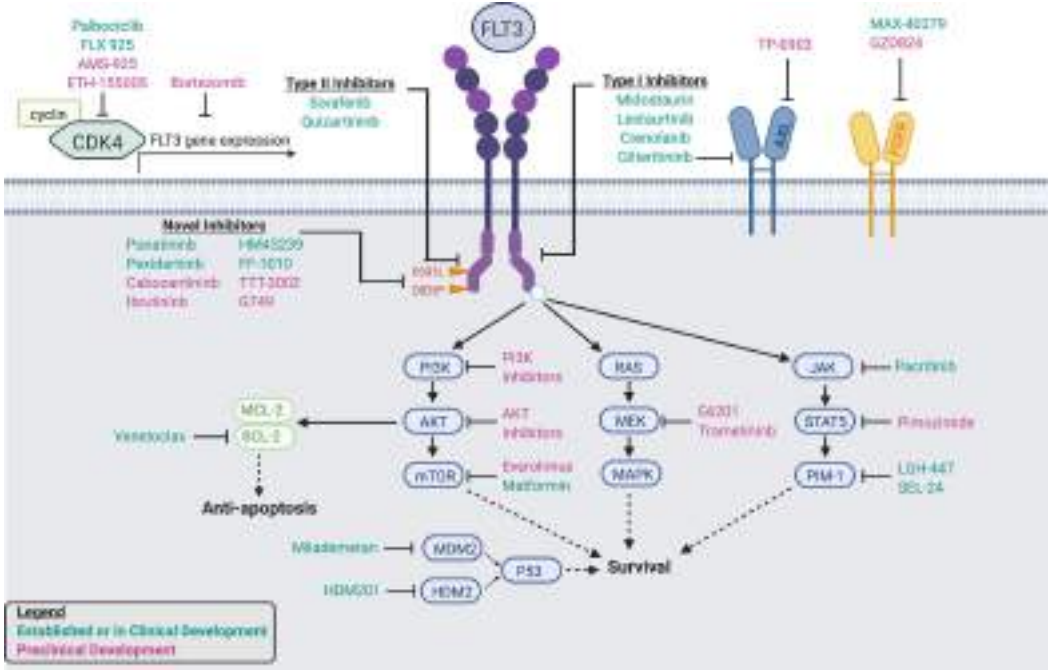
[1]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA;



Select trials of novel combination strategies to overcome microenvironment mediated resistance

Clinical Trial ID	Drugs Combination	Phase	Mechanism of action
NCT03642236	BTK inhibitor+ CT±FLT3 inhibitor	II/III	BTKi reduces the cyto-protection provided to FLT3-ITD AML cells by bone marrow stromal cells
NCT05028751	SYKi (Lanraplenib)+ Gilteritinib	Ib/II	disturbed mitochondrial biogenesis and suppression of oxidative metabolism (OXPHOS) in LSCs
NCT02532010	IRAK1i (Pacritinib)+De citabine or Cytarabine	II	inhibits the secretion of various inflammatory cytokine IL1 and NF-kappa B signalling

Established and in-development FLT3 inhibitors, dual inhibitors, and combination agents

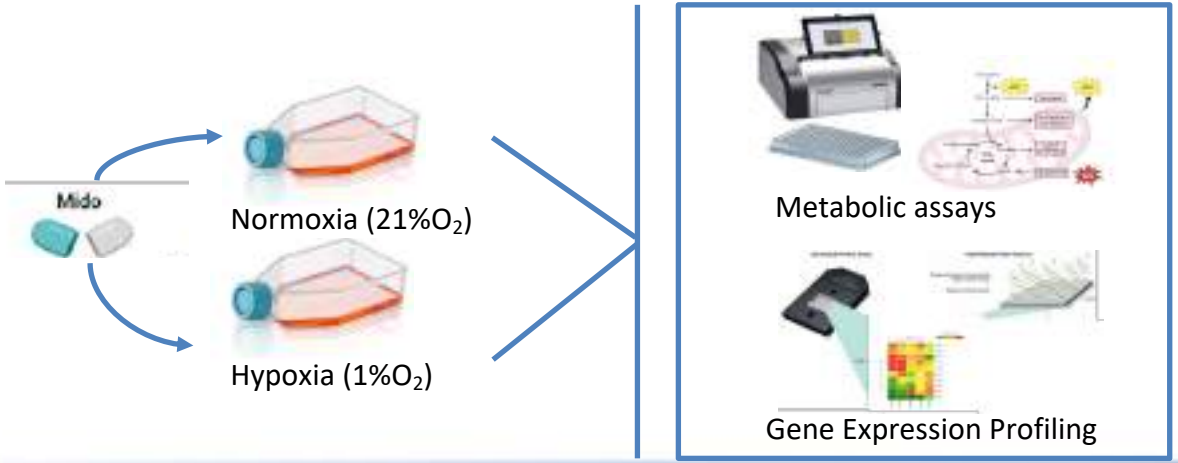


V. E. Kennedy, Front. Oncol., 2020

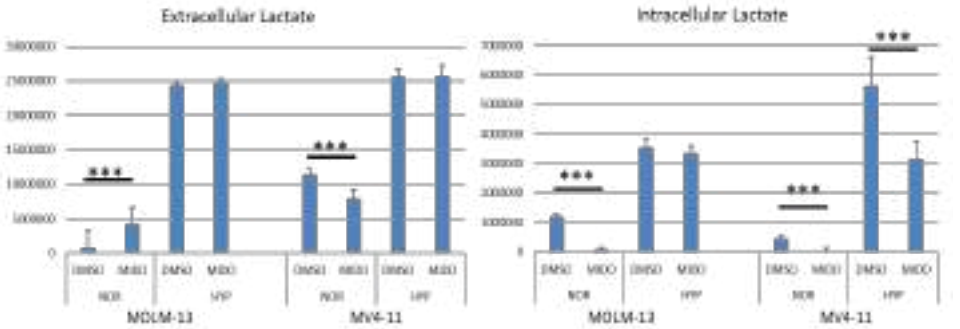
Ongoing Study

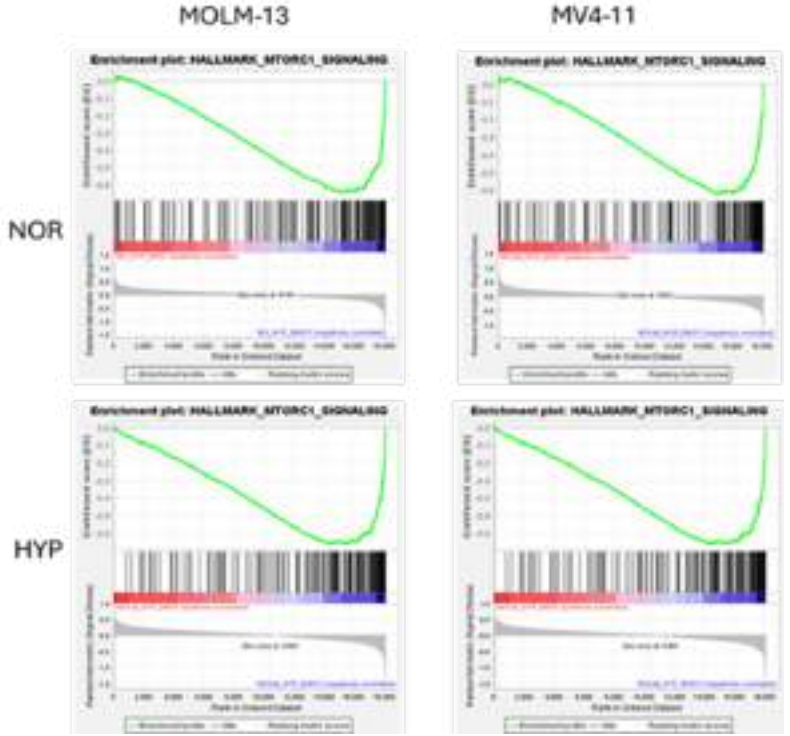
Autophagy inhibition as a novel combination strategy to target FLT3-mutated leukemic stem cells under conditions mimicking the hypoxic bone marrow microenvironment.

Bruno et al., EHA 2025



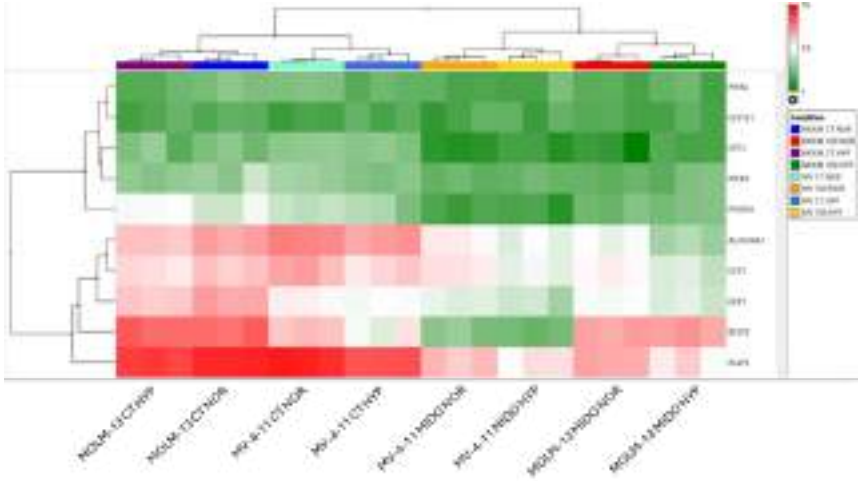
Mido treatment and hypoxia alter metabolic features and down-regulates mTORC1 signalling

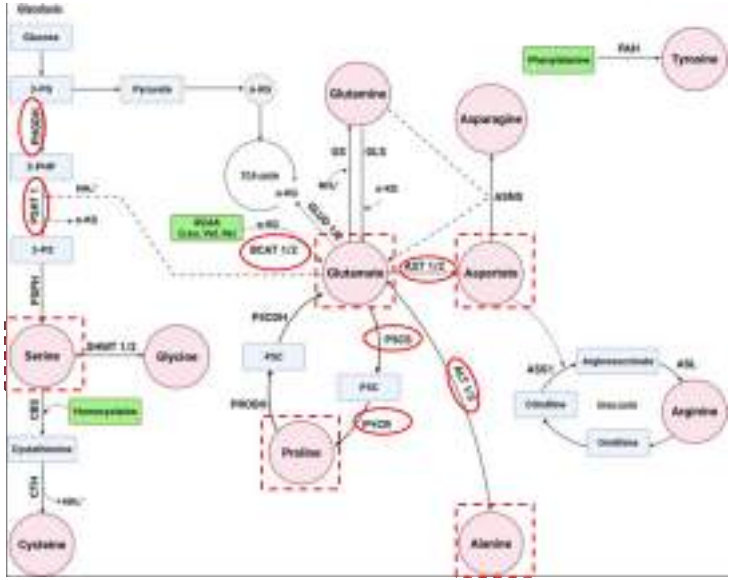




Bruno et al., EHA 2025

Mido suppresses the expression of biosynthetic enzymes crucial for synthetizing non-essential amino acids (NEAA)

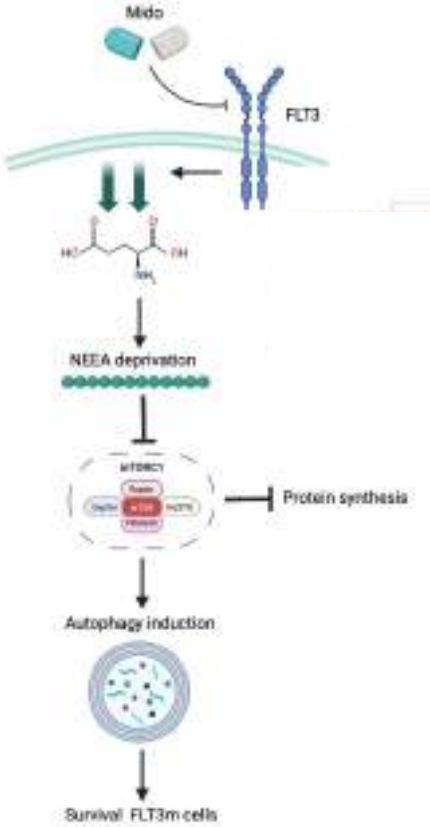




Bruno et al., EHA 2025

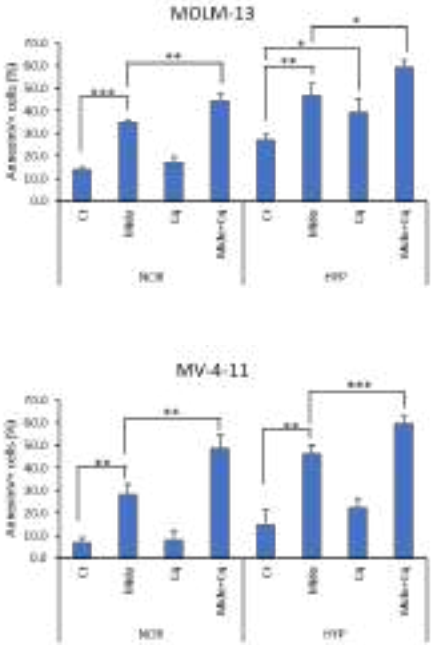
Metabolic and transcriptomic results suggest the activation of autophagy network as a prosurvival mechanism adopted by *FLT3*mut cells treated with Mido

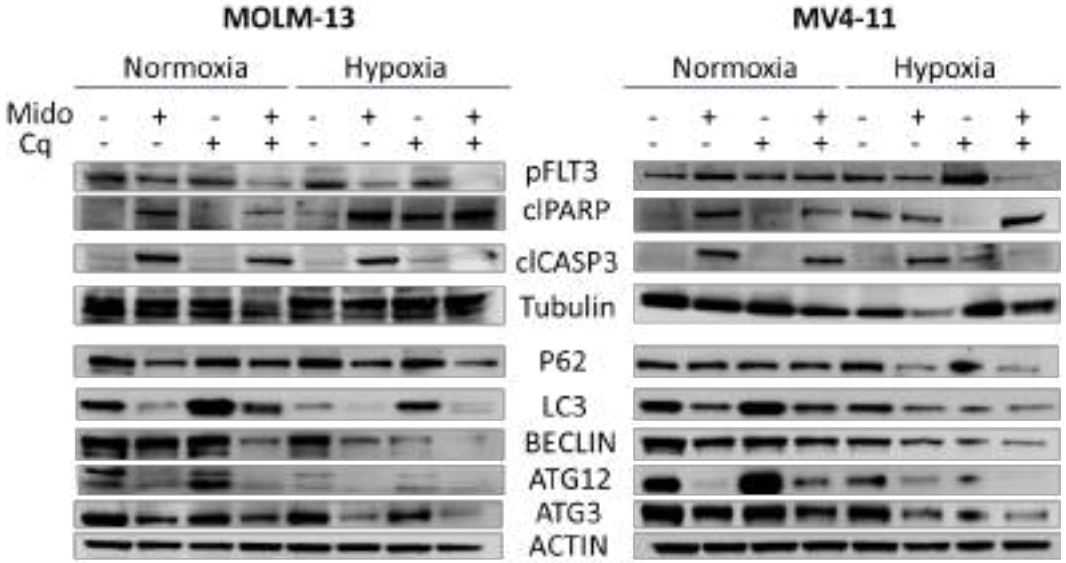
Can autophagy be considered as a new therapeutic target to increase the efficacy of mido??



Bruno et al., EHA 2025

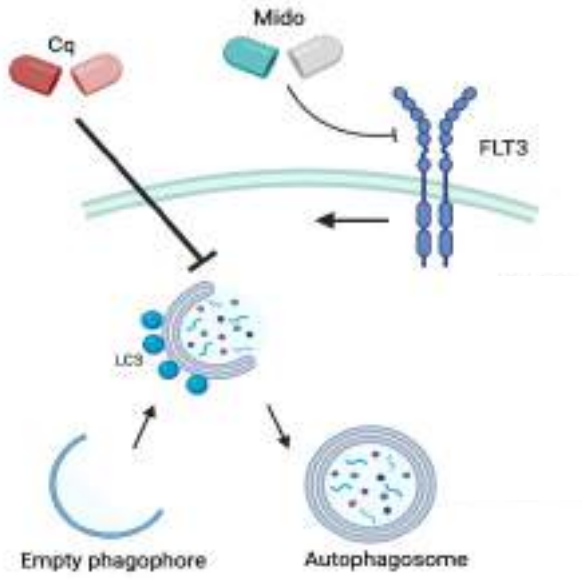
Dual inhibition of autophagy and FLT3 triggers apoptosis and downregulates autophagy-related proteins

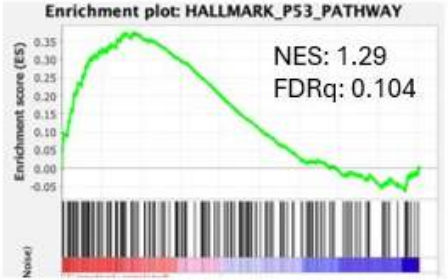


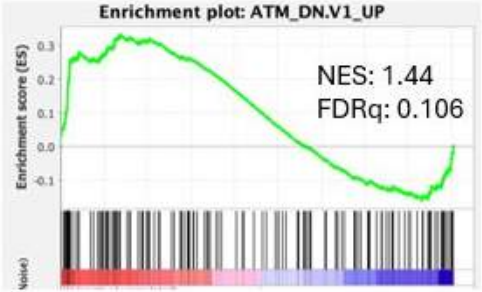


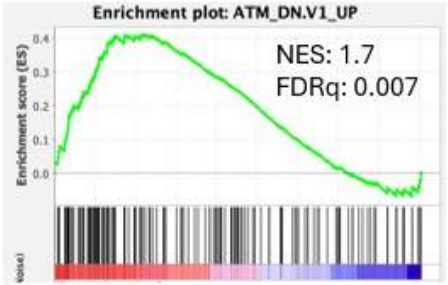
Bruno et al., EHA 2025

Autophagy inhibition enhances Mido efficacy through up-regulation of P53 pathway and activation of DNA repair mediated by ATM









Conclusions

- ✓ Acquisition of “on-target” or “off-target” mutations under FLT3i treatment can mediate resistance and disease relapse in *FLT3m* AML patients.
- ✓ Acquisitions of secondary mutations can occur through a linear evolution, if they arise in the original *FLT3m* clone, or through a branching evolution, if they arise in a clone different from the original *FLT3m* leukemic clone.
- ✓ Stromal factors could bypass FLT3 ‘silencing’, activating its downstream signaling or stimulating the antiapoptotic protein BCL2 and AXL gene.

- ➢ Novel double or triplet regimens can increase the depth of response and avoid persistence or reappearance of leukemic subclones.
- ➢ Administration of oral target agents and hypomethylating agents can spare chemotherapy and improve quality of life and the psychological impact of the disease by reducing adverse events.

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Thank you
for your attention!