XIX CONGRESSO NAZIONALE SIES 2026

FLT3 AS A NOVEL THERAPEUTIC TARGET IN PRECLINICAL MODEL OF PEDIATRIC PAX5-REARRANGED BCP-ALL

Alessia Curto, PhD Student

University of Milano-Bicocca, Fondazione Tettamanti

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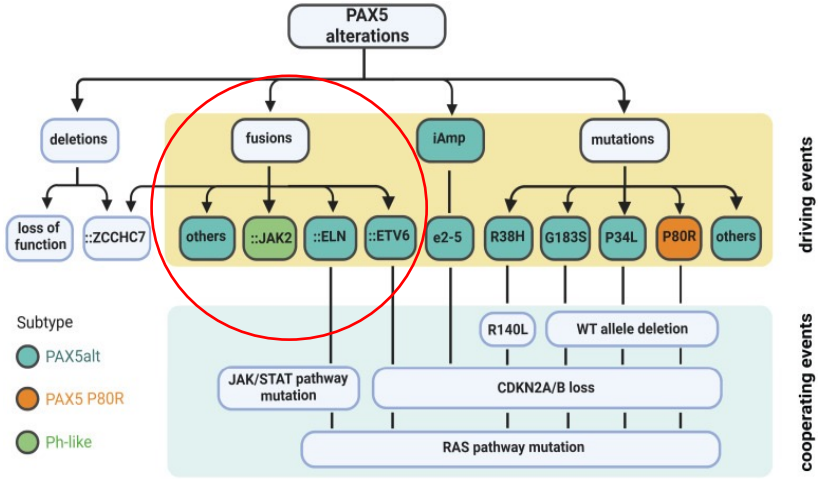
Palazzo degli Affari

Disclosures of Alessia Curto

Not applicable

Introduction

- **B-cell Acute Lymphoblastic Leukemia** (B-ALL) is the most common pediatric malignancy
- **Characterized by molecular alterations** that define the different subgroups



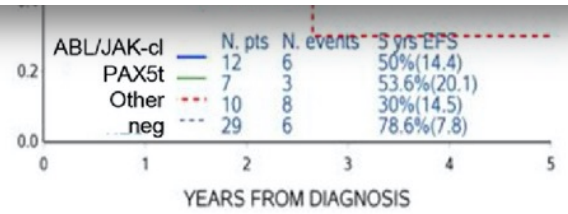
Jia and Gu, Frontiers in Oncology 2022

- **PAX5-alt** is linked to poorer prognosis and a lower chance of survival and complete remission

C051

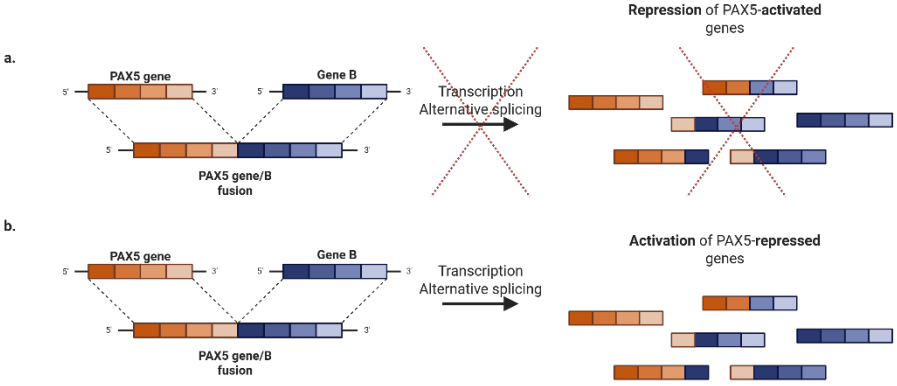
LA LEUCEMIA LINFOBLASTICA ACUTA (LLA) PEDIATRICA PAX5-R È UN SOTTOGRUPPO MOLECOLARE AD ALTO RISCHIO DI LLA A CELLULE B CARATTERIZZATO DA UN PROFILO PROGNOSTICO SFAVOREVOLE MRD-DIPENDENTE

N. Peccatori, A. Curto, D. Silvestri, S. Rebellato, Ž. Antić, S. Bhatia, A.K. Bergmann, A. Borkhardt, M. Zimmermann, S. Strehl, K. Nebral, C. Saitta, M. Quadri, C. Palmi, M. Bardini, D. Guardo, L. Lo Nigro, R. Parasole, M.C. Putti, F. Locatelli, J. Alten, M. Stanulla, M.G. Valsecchi, B. Buldini, V. Conter, A. Biondi, A. Balduzzi, M. Schrappe, A. Attarbaschi, G. Cario, C. Rizzari, G. Cazzaniga, G. Fazio *(Monza-MB, Milano, Hannover-DE, Würzburg-DE, Düsseldorf-DE, Vienna-AT, Genova, Catania, Napoli, Padova, Roma, Kiel-DE)*

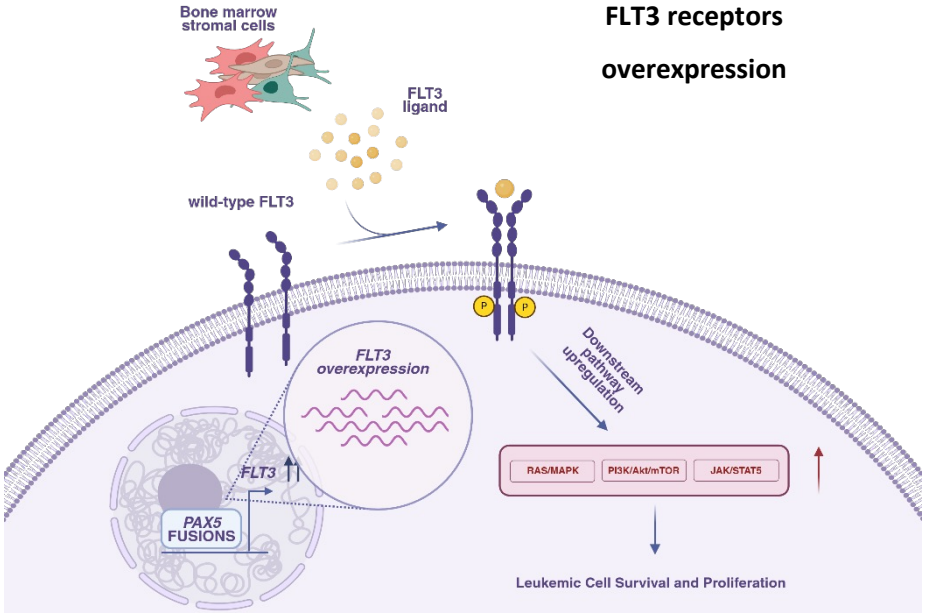


Fazio G, Bresolin S, Silvestri D, et al. PAX5 fusion genes are frequent in poor risk childhood acute lymphoblastic leukaemia and can be targeted with BIBF1120

Hypothesis of the study



FLT3 gene



FLT3 receptors overexpression

Study objectives

- Study of the *Flt3* gene expression profile in patients with *PAX5*r B-ALL to identify a potential innovative therapeutic target
- Evaluation of cell viability following treatment with FLT3 inhibitors *in-vitro*, *ex-vivo* e *in-vivo*

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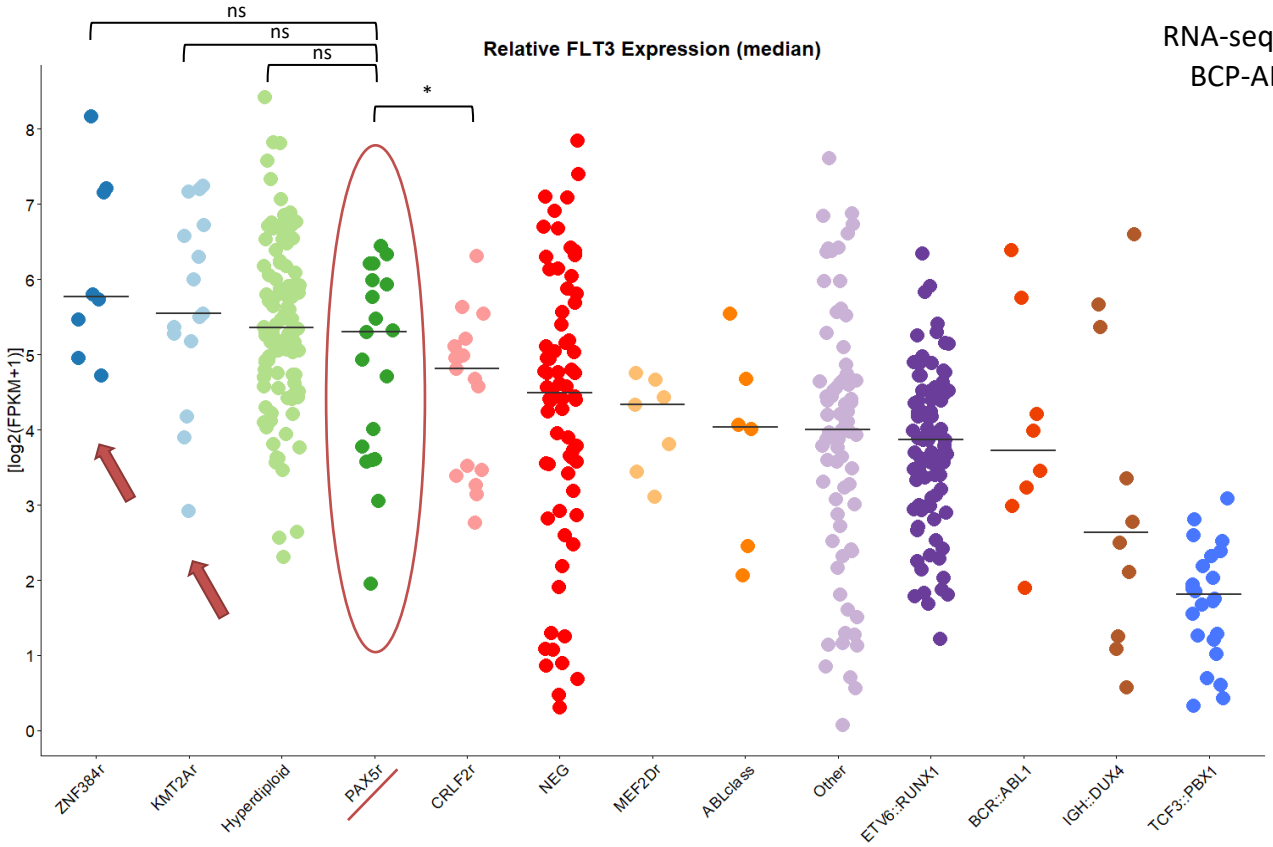
Results (I): high levels of *FLT3* expression in *PAX5*r patients

RNA-seq in 465 consecutive BCP-ALL AIEOP patients



Wilcoxon-signed-rank test

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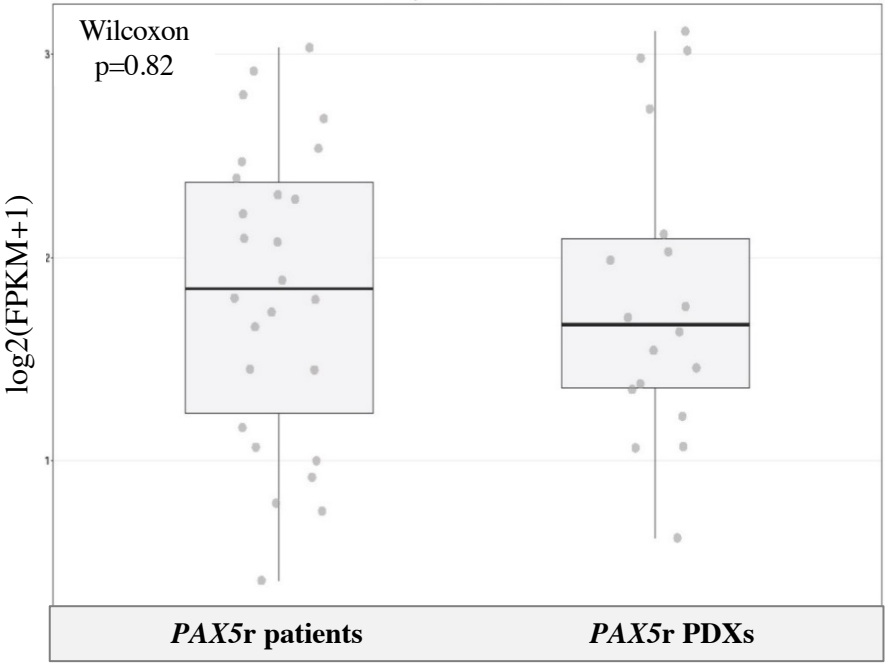
Results (II): HTP Drug screening in PAX5r PDXs

FLT3 expression levels from RNA-SEQ



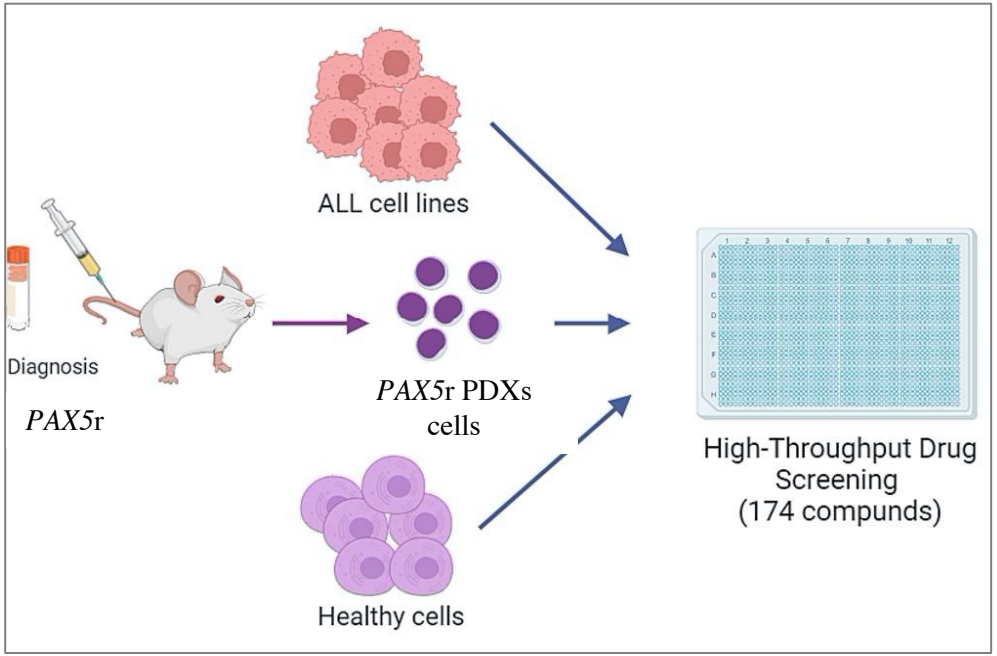
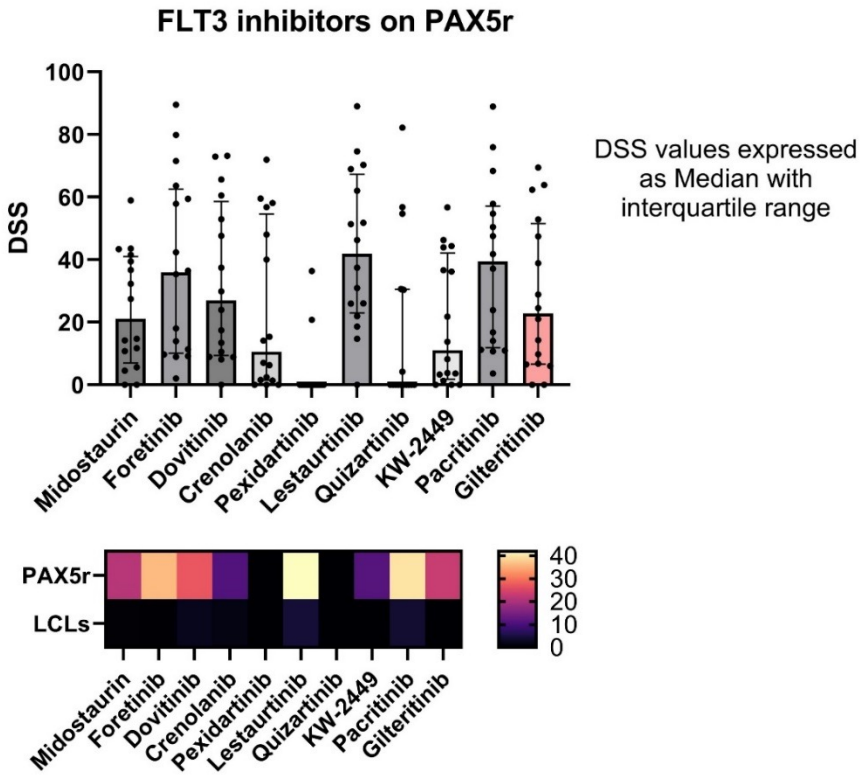


Image adapted from Oikonomou A., Valsecchi L., Quadri M., et al.High-throughput screening as a drug repurposing strategy for poor outcome subgroups of pediatric B-cell precursor Acute Lymphoblastic Leukemia

Results (II): Gilteritinib has a potential new drug for *PAX5*r in pediatric B-ALL

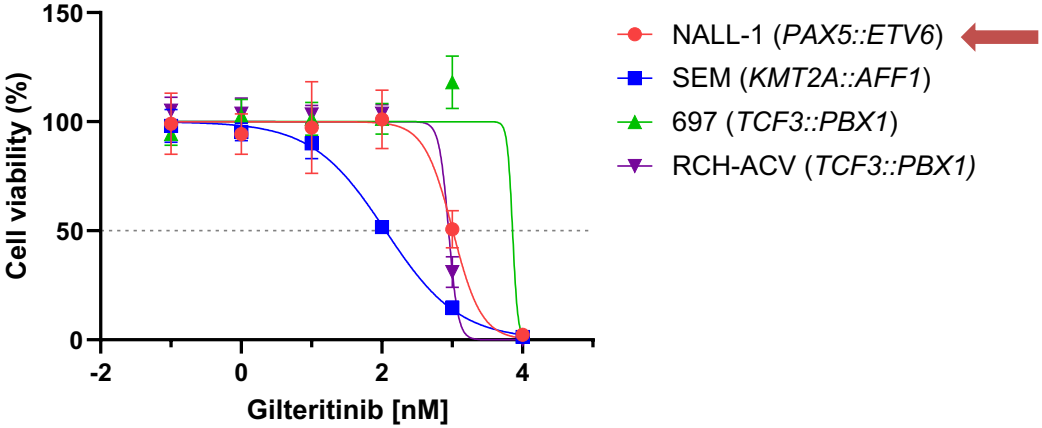


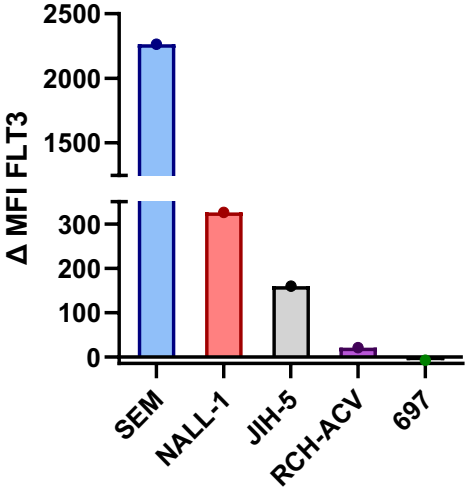
DSS values expressed as Median with interquartile range

WHY GILTERITINIB?

- **Selective** for FLT3
- **Safe** for patients
- **FDA-approved** for adult patients with FLT3-mutated relapsed/refractory acute myeloid leukemia (AML) and in **pediatric patients** with relapsed/refractory FLT3-ITD AML

Results (II): Gilteritinib is efficient in *PAX5*r cell line



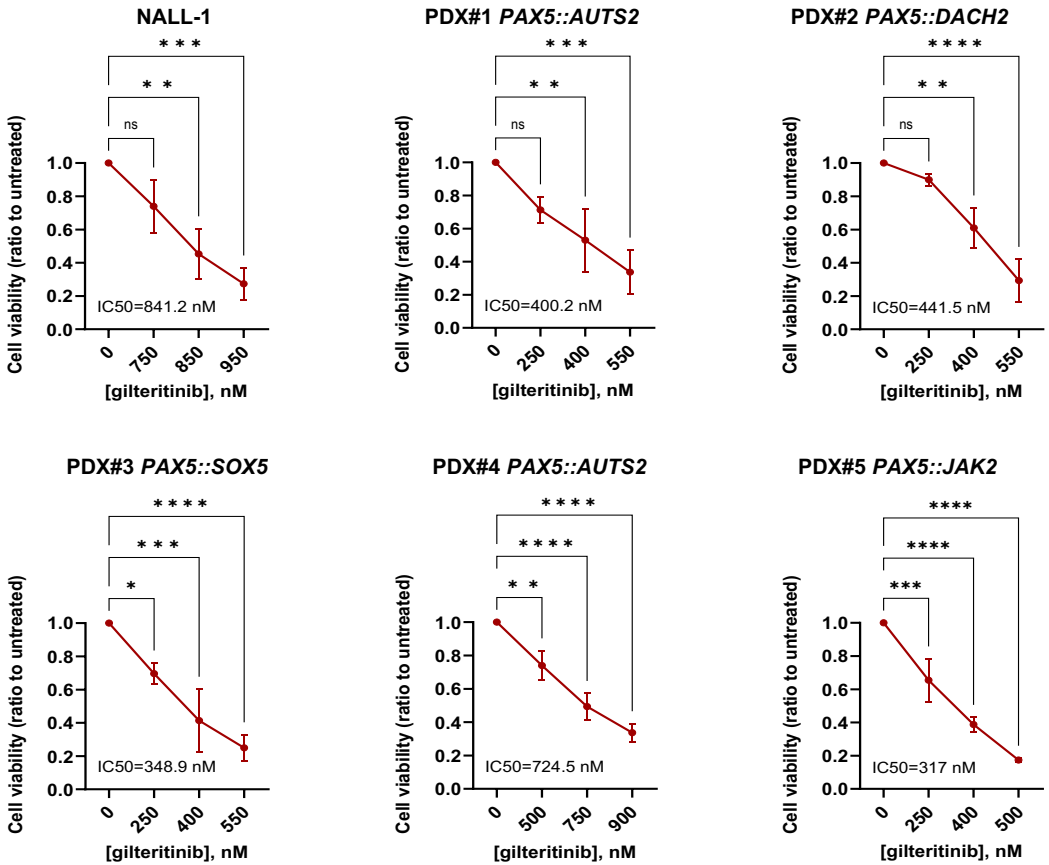


Cell line	IC50 Gilte (nM)
NALL-1	1014
SEM	112,4
697	7170
RCH-ACV	884,3

*The evaluation of gilteritinib efficacy in JIH-5 (*EP300::ZNF384*) is ongoing..

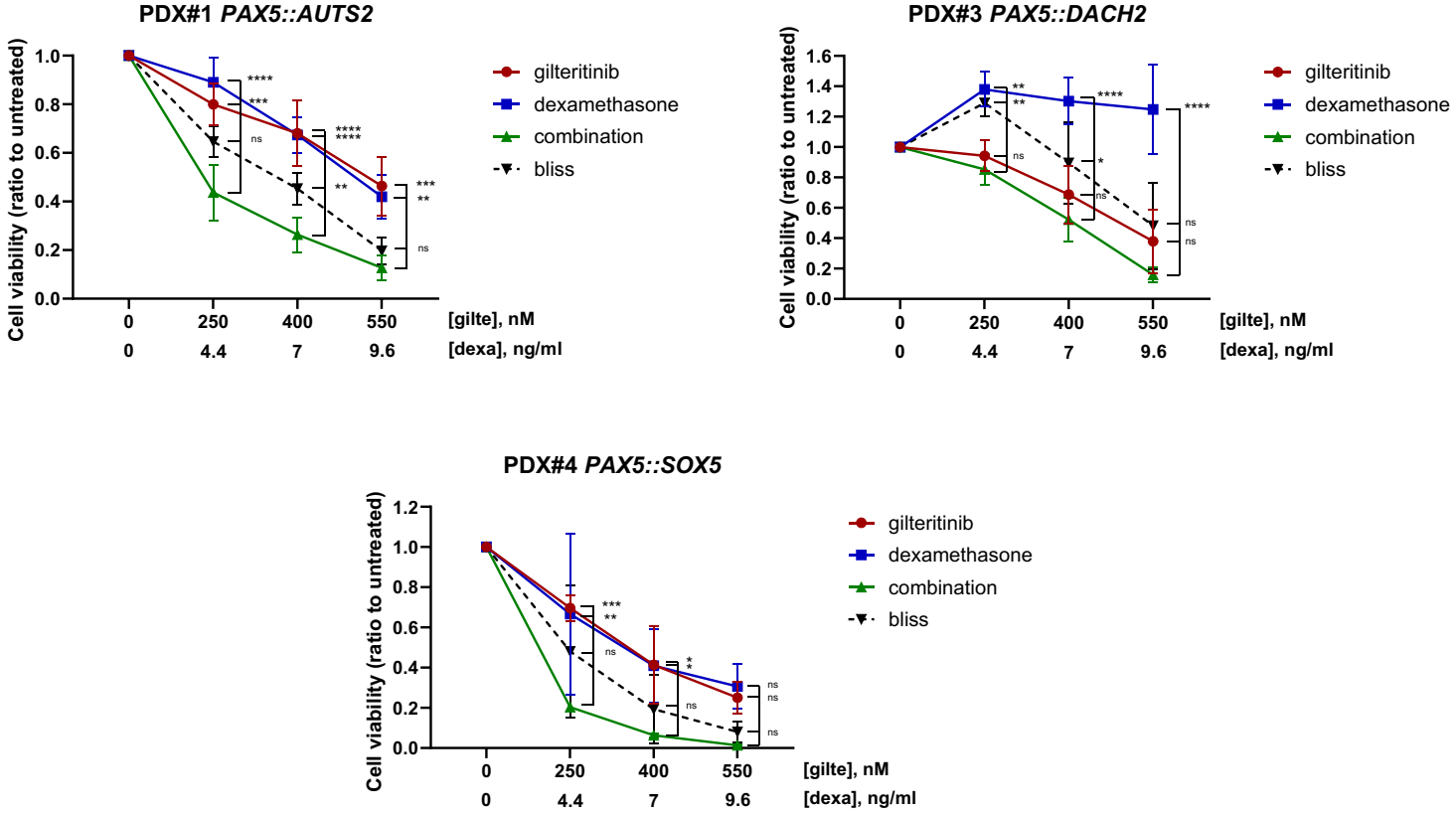
Results (II): Gilteritinib reduces cell viability in *PAX5*r PDX cells in a dose-dependent manner

Number of live cells (Ann V-/7-AAD-) in treated vs untreated condition
n=3 experiments

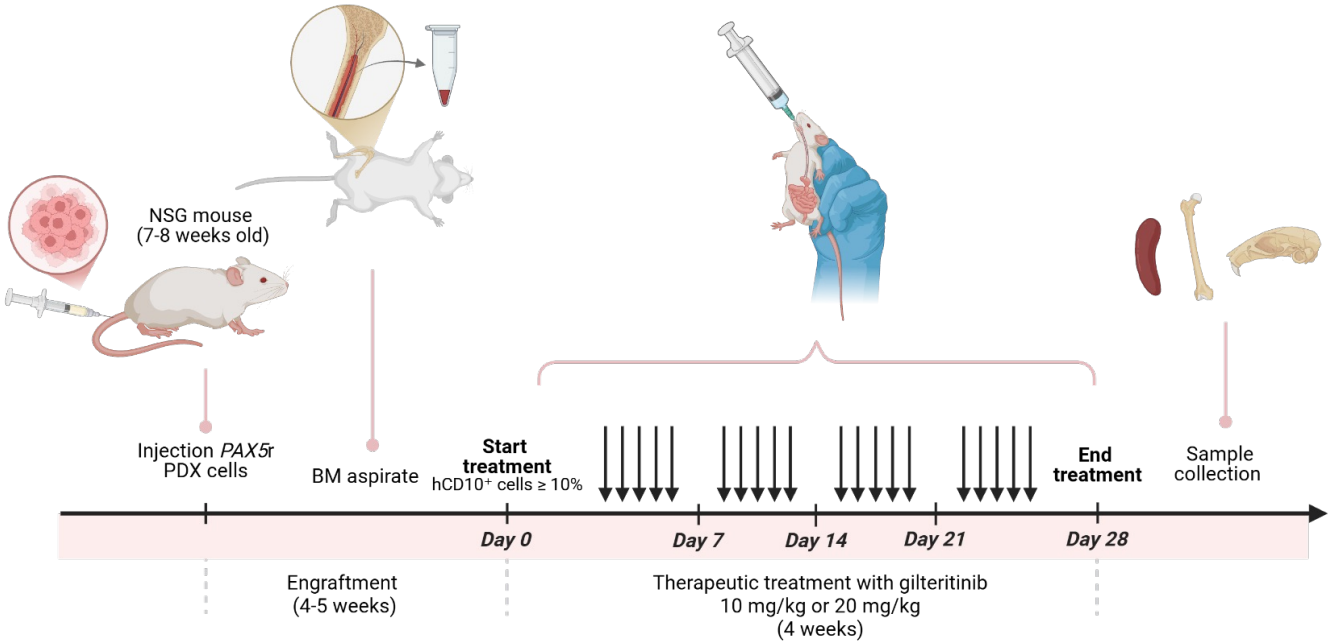


One-way ANOVA with the Dunnett post-test for multiple comparisions using untreated as the comparator

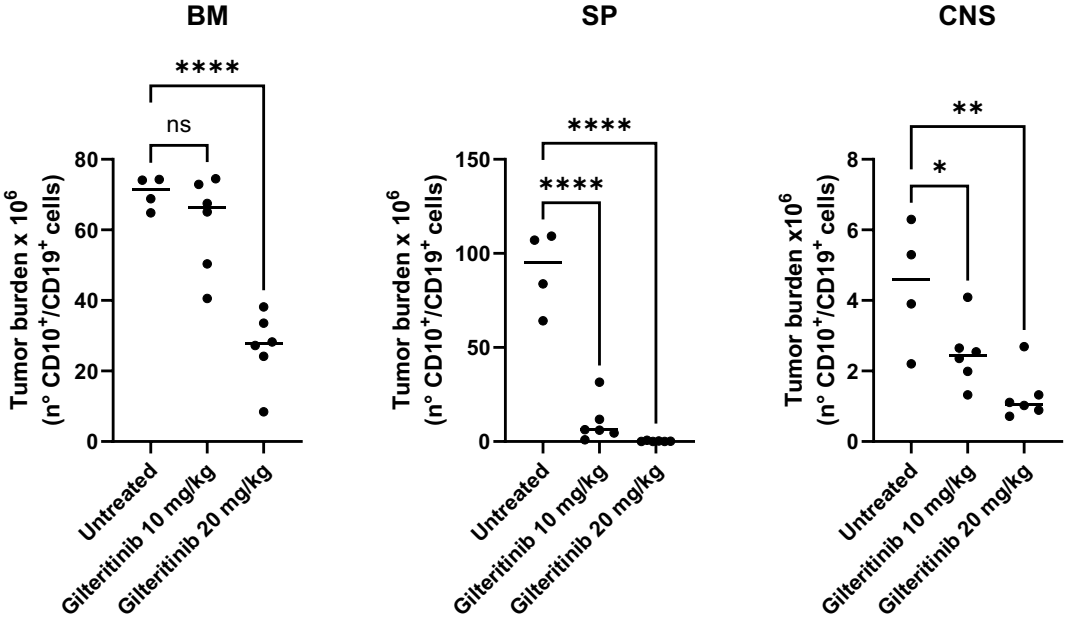
Results (II): Gilteritinib exerts a synergistic effect in combination with dexamethasone

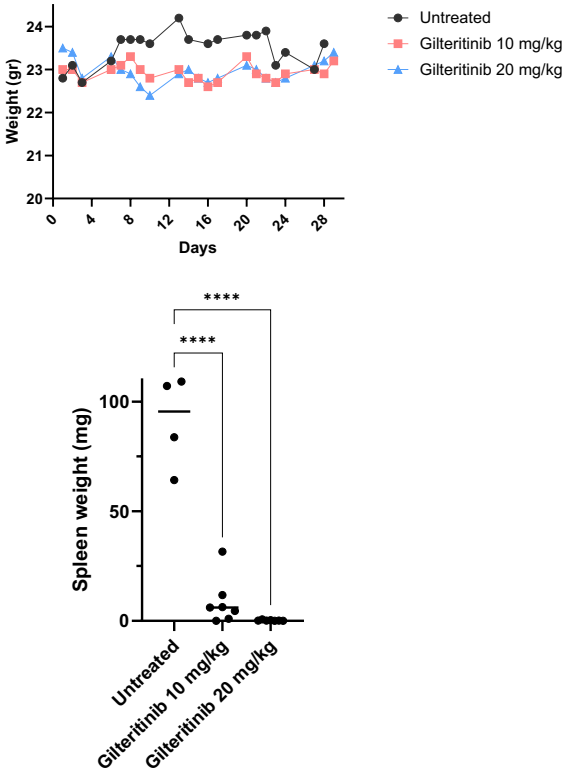


Results (II): *in-vivo* experimental design



Results (II): gilteritinib demonstrates potent antileukemic activity *in-vivo*





Peripheral blood (PB) was also evaluated, but it showed poor engraftment (0%-0,4%)

One-way ANOVA with the Dunnett post-test for multiple comparisions using untreated as the comparator

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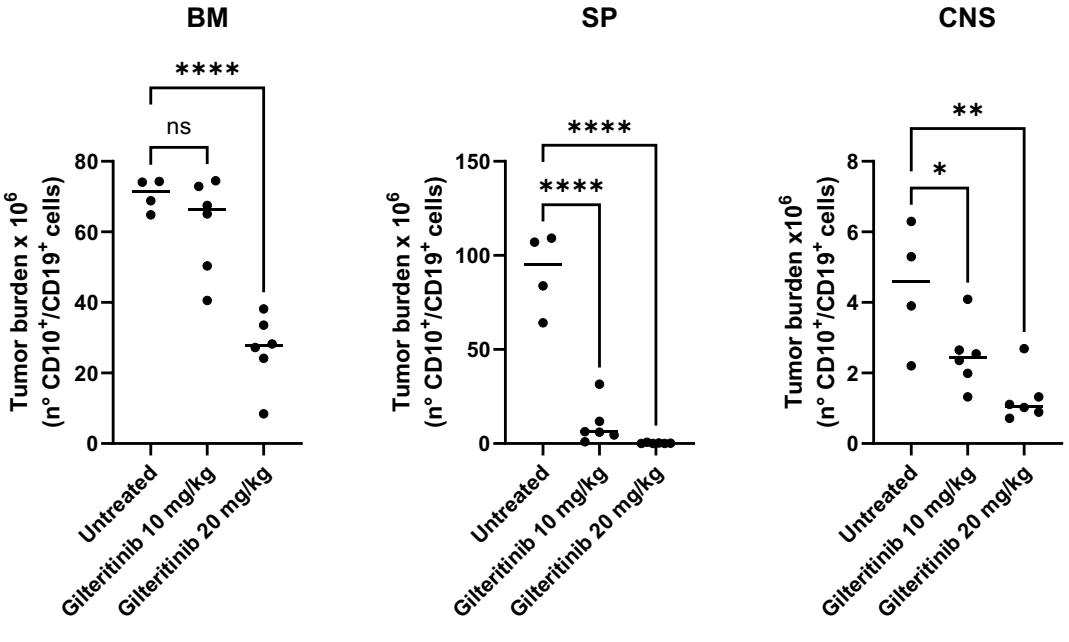

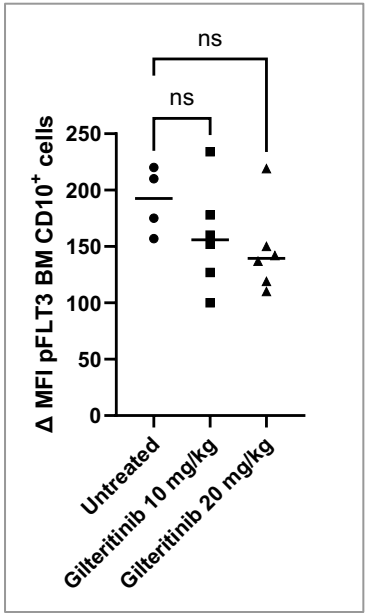

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Conclusions and future perspectives

- ✓ *FLT3* expression is increased in *PAX5*r patients
- ✓ Gilteritinib is effective in *in-vitro*, *ex-vivo* and *in-vivo* in preclinical *PAX5*r models, and its anti-tumor activity is enhanced in the presence of standard chemotherapeutic drugs → **additive/synergistic effect**

- ➢ Study the expression of **FLT3 protein** and its active form, P-FLT3, in *PAX5*r patients
- ➢ Evaluate the efficacy and synergy of gilteritinib *in-vitro* and *in-vivo* in **PDX mouse models** of gilteritinib with other drugs, such as venetoclax

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