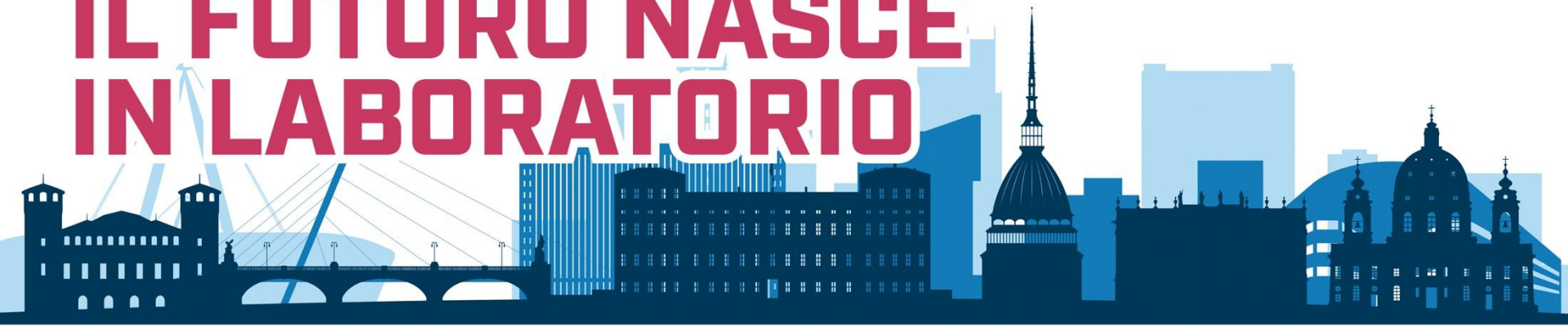
YOUNG SCIENCE FORUM: IL FUTURO NASCE IN LABORATORIO



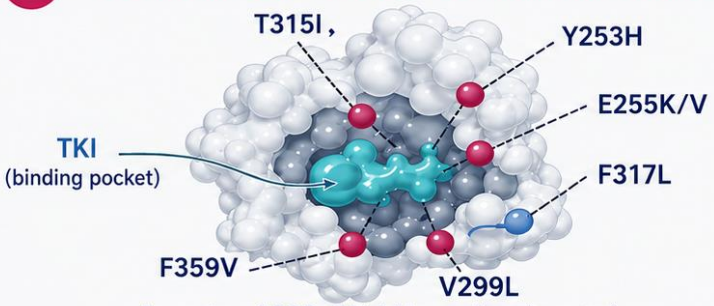
Targeting epigenetic and metabolic crosstalk in CML via butyrate induced HDAC inhibition

Rita Vacca

University of Turin, Department of Clinical and Biological Sciences

TORINO, ACCADEMIA DI MEDICINA | 4-5 GIUGNO 2026

1 *BCR::ABL1* kinase-domain mutations



Examples of BCR::ABL1 kinase-domain mutations

- Mutations within the BCR::ABL1 kinase domain impair TKI binding (reduced affinity / steric hindrance)
- Selected under TKI pressure and associated with TKI-specific cross-resistance patterns
- Representative categories: P-loop mutations, gatekeeper mutation T315I, ATP-binding site changes
- Asciminib resistance is often linked to myristoyl-pocket mutations (e.g., A337V/T, P465S, V468F).

On-target resistance

2 Other mechanisms *(BCR::ABL1-independent or non-kinase-domain)*

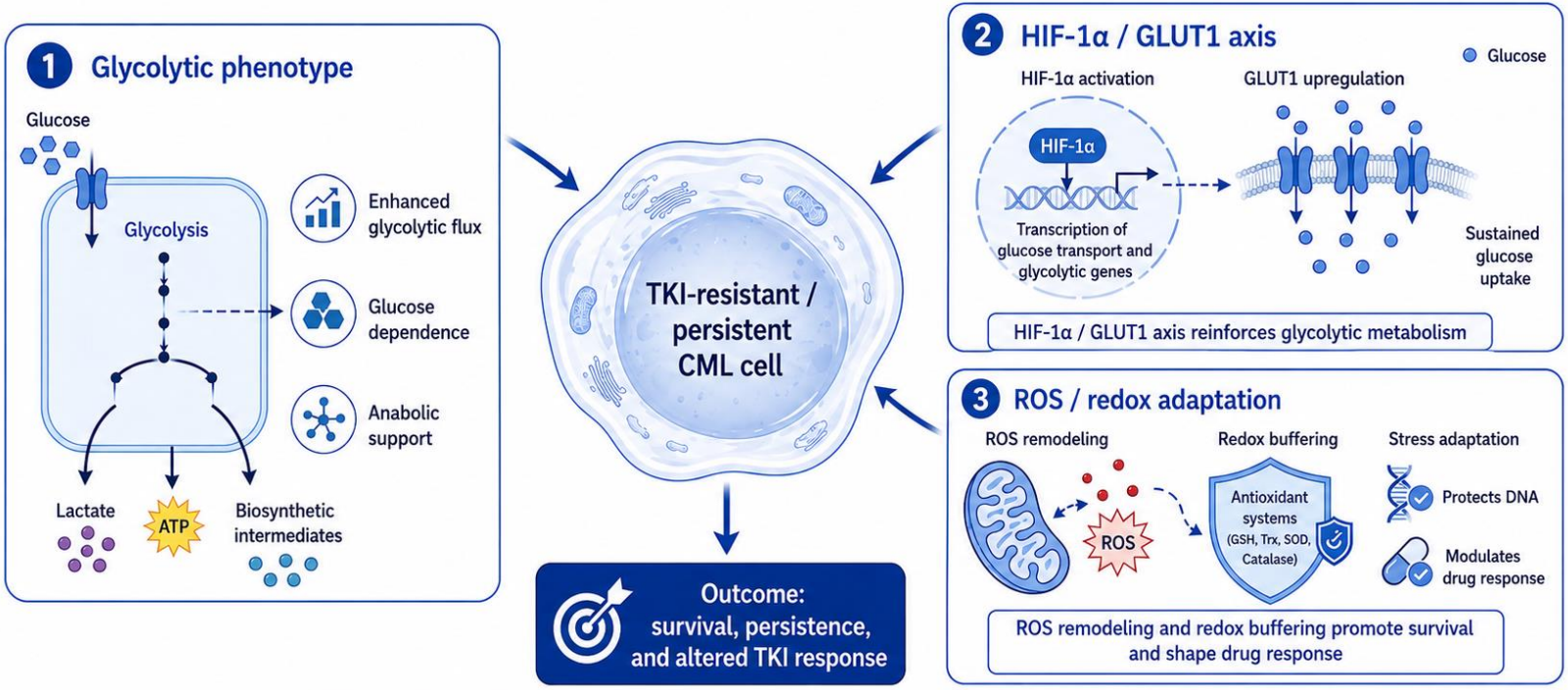
1. BCR::ABL1 overexpression / amplification
2. Activation of alternative pathways (SRC, PI3K-AKT-mTOR, JAK-STAT)
3. Immune system dysregulation / immune evasion
4. LSC quiescence and protection by the bone marrow niche
5. Metabolic and redox remodeling (glycolysis, OXPHOS, HIF-1α, ROS)
6. Additional somatic lesions / clonal evolution

Non-on-target persistence and resistance

Clinical outcome: persistence, suboptimal response, or TKI resistance

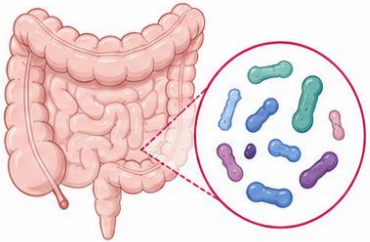
BCR::ABL1 kinase-domain mutations explain on-target resistance; residual persistance and treatment failure also arise from BCR::ABL1-independent mechanisms, clonal evolution, and the LSC compartment.

Metabolic determinants of TKI persistence in CML



Integrated metabolic rewiring supports persistence under TKI pressure.

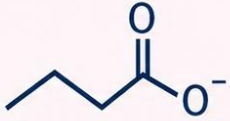
1 Origin



Short-chain fatty acid generated by microbial fermentation

Microbiota-derived SCFA

2 Butyrate



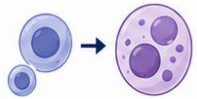
Short-chain fatty acid (SCFA)

3 Pleiotropic biological effects

Chromatin regulation

Metabolism

Differentiation



Butyrate exerts pleiotropic effects on chromatin regulation, metabolism, and differentiation.

4 Relevance to CML

CML cell TKI

- Potential to modulate TKI response
- Its impact in CML remains insufficiently defined

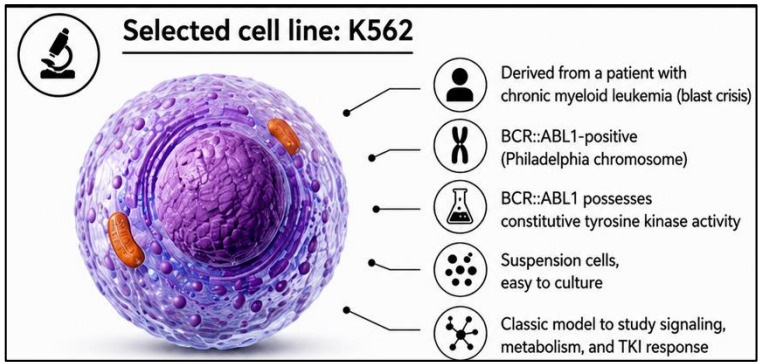
Open question: Does butyrate reshape TKI sensitivity through metabolic and epigenetic remodeling?

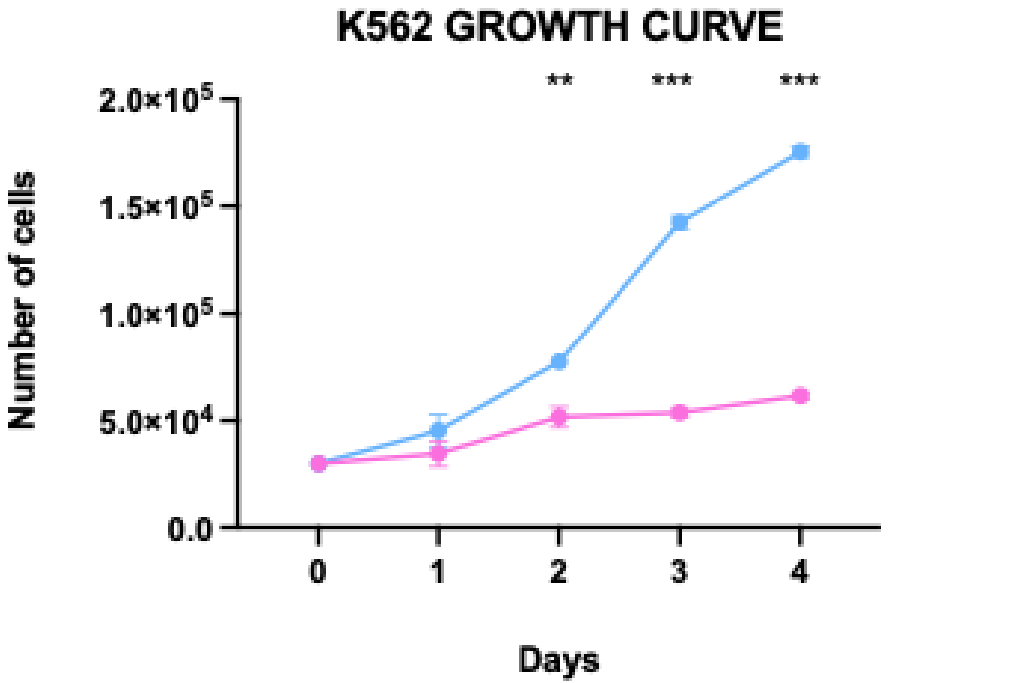
Butyrate may connect the microbiota to metabolic and epigenetic rewiring relevant to TKI response in CML.

How butyrate modulates TKIs response?

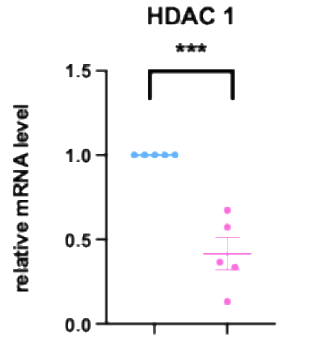
Preclinical evidence suggests that combining HDAC inhibitors with imatinib may enhance leukemic cell apoptosis and target resistant stem-like populations through the modulation of key cell survival pathways.

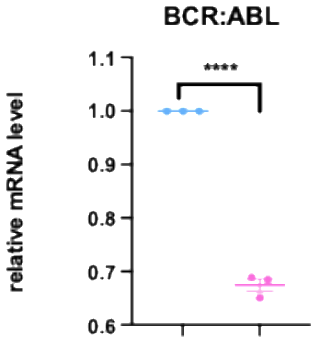
Topic	Condensed Key Findings	Reference
Butyrate-Induced Histone Acetylation in K562	Butyrate induces H3/H4 acetylation; resistant K562 variants show defective acetylation and abnormal HDAC activity	Ohlsson-Wilhelm, Am J Hum Genet, 1984
Leukemia Stem Cell Targeting	SAHA + imatinib target CD34+/CD38− leukemic stem cells and reduce stemness pathways	Bamodu, Exp Cell Res, 2018
Synergy Mechanisms	HDAC inhibitors + imatinib induce synergistic apoptosis via suppression of RAF/MEK/ERK, AKT, and STAT5 signaling	Yu, Cancer Res, 2003
MAP Kinase Pathway Modulation	Butyrate inhibits ERK and activates p38 MAPK, promoting differentiation	Witt, Blood, 2000


Selected cell line: K562
Derived from a patient with chronic myeloid leukemia (blast crisis)
BCR::ABL1-positive (Philadelphia chromosome)
BCR::ABL1 possesses constitutive tyrosine kinase activity
Suspension cells, easy to culture
Classic model to study signaling, metabolism, and TKI response


K562 GROWTH CURVE
**
***
***
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0.0
Number of cells
0
1
2
3
4
Days
K562 Untreated
K562 Butyrate 1 mM

Butyrate mediates epigenetic rewiring of K562

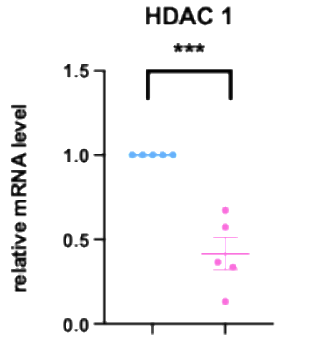


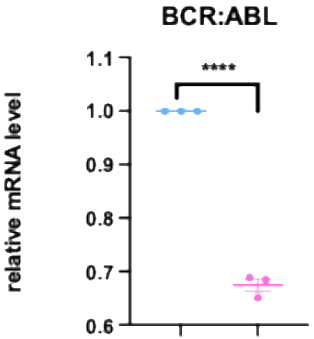


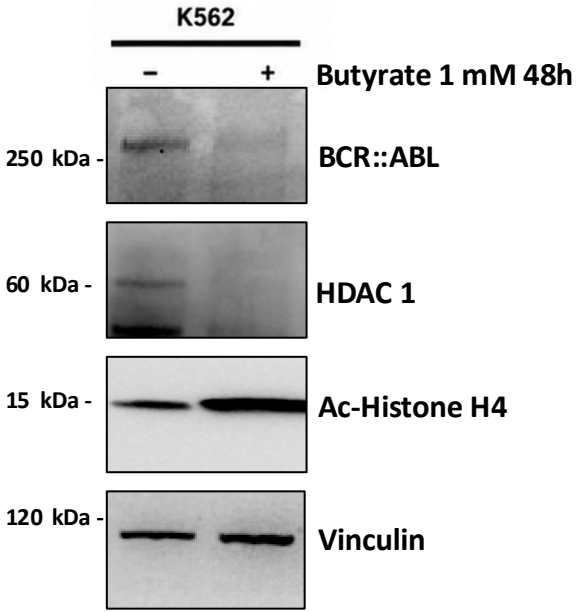
K562 Untreated

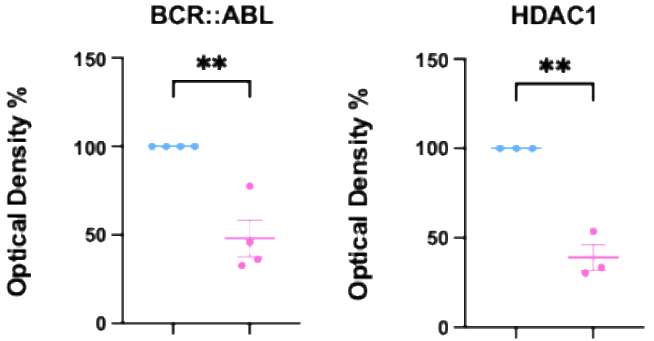
K562 Butyrate

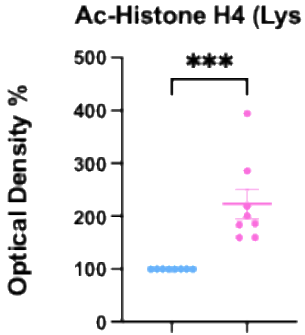
Butyrate mediates epigenetic rewiring of K562







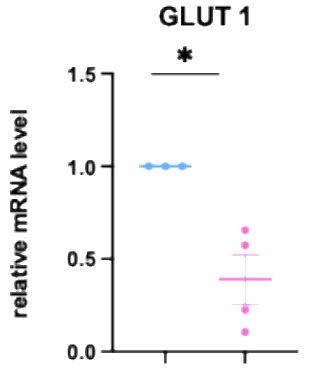


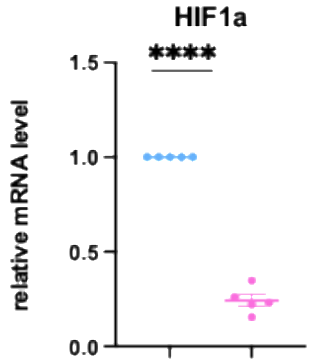


K562 Untreated

K562 Butyrate

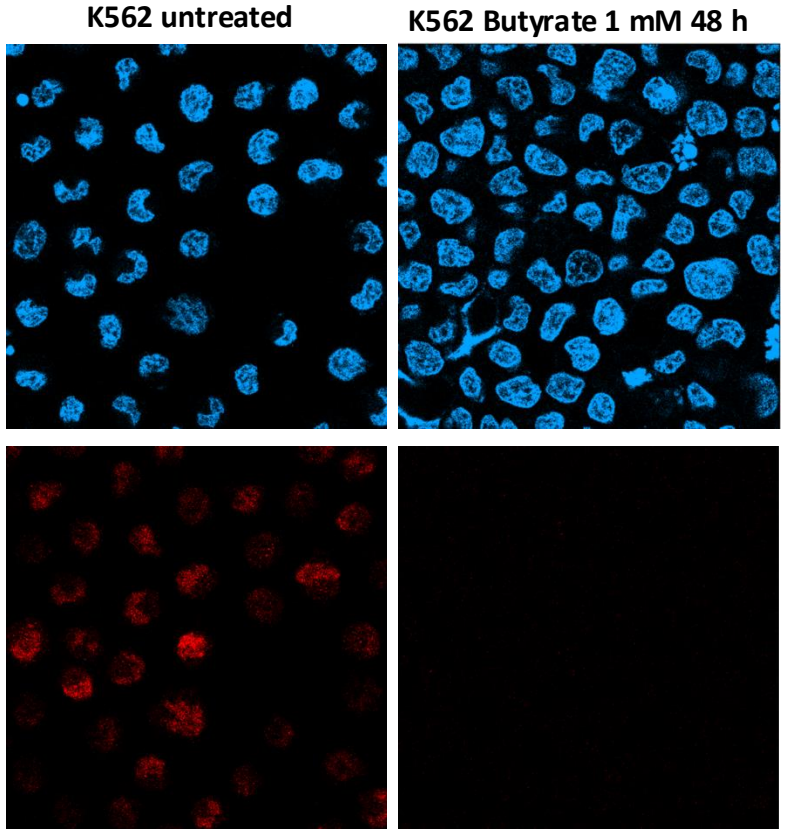
Butyrate drives metabolic rewiring in K562 cells



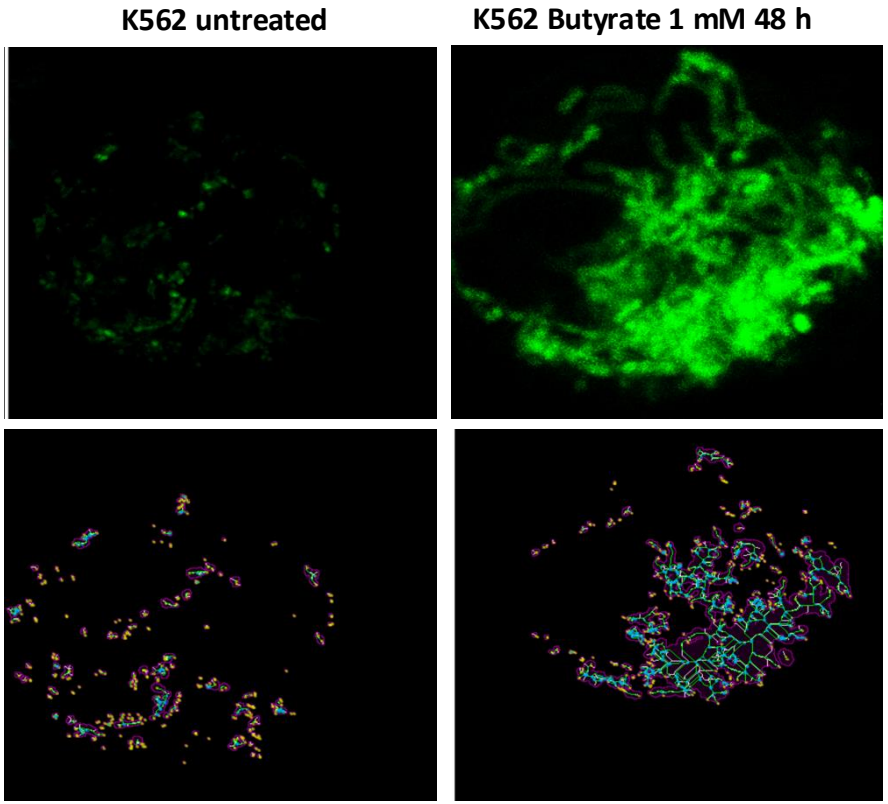


K562 Untreated

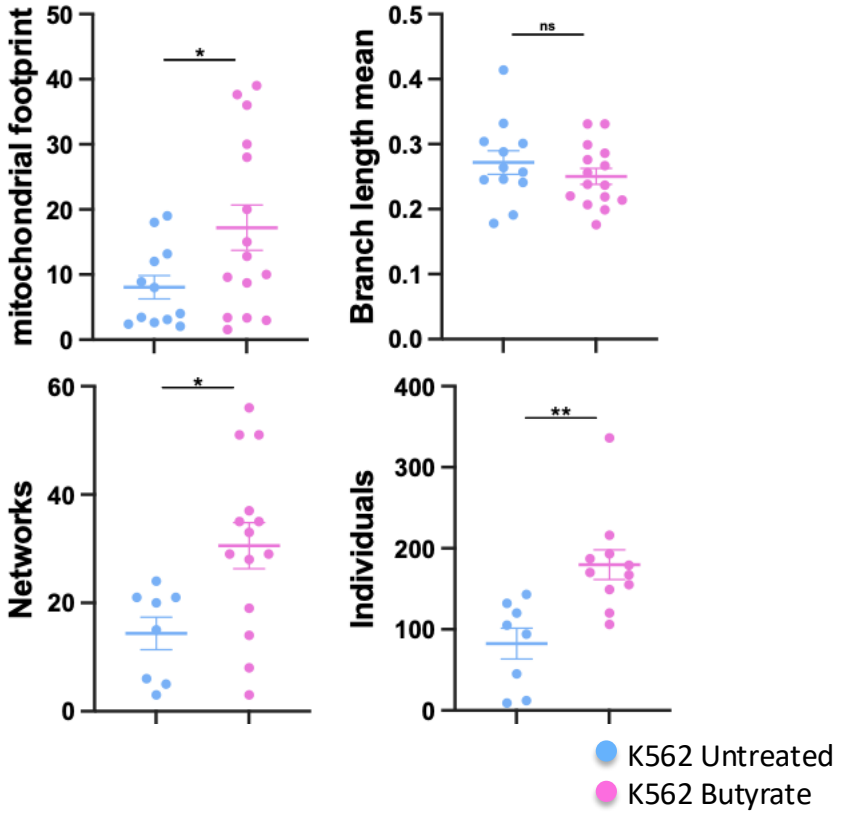
K562 Butyrate



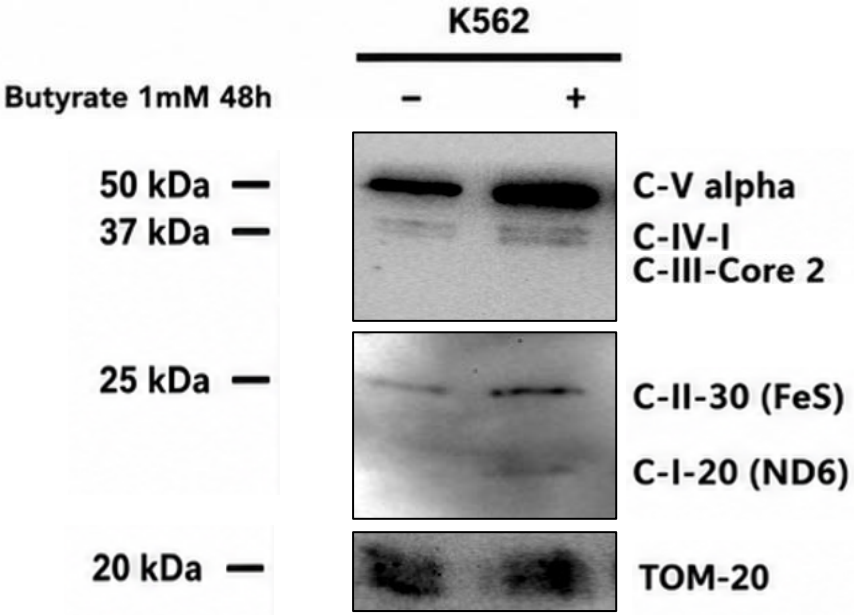
Butyrate drives metabolic rewiring in K562 cells

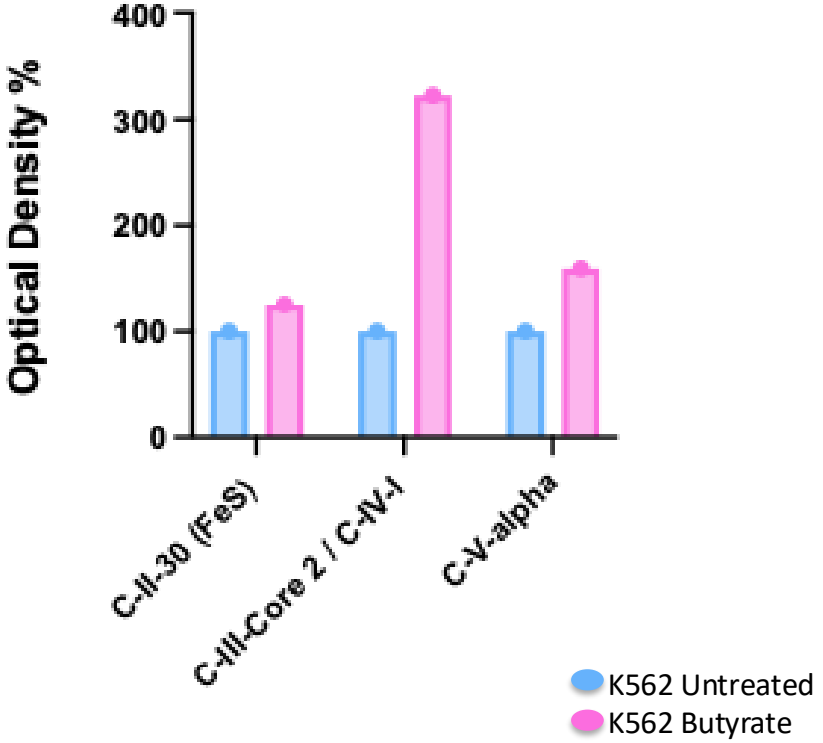


Representative MitoTracker images



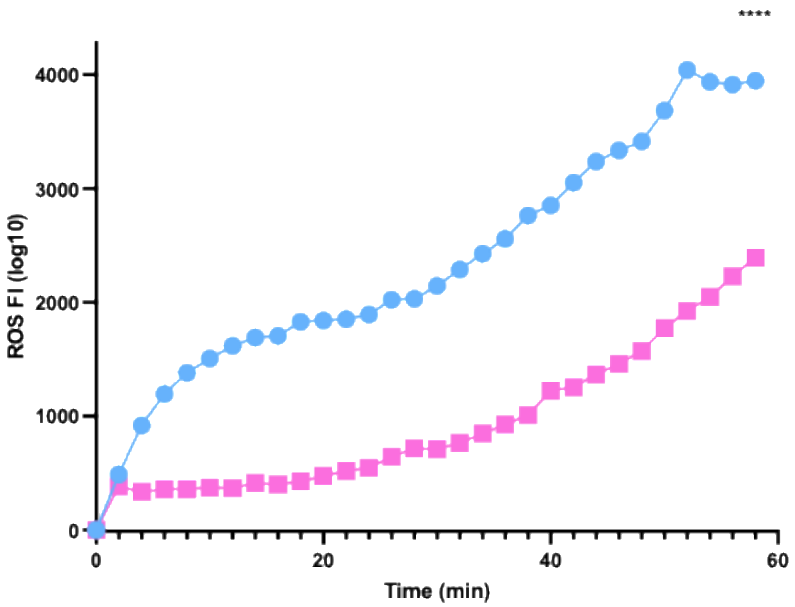
Butyrate drives metabolic reprogramming in K562 cells



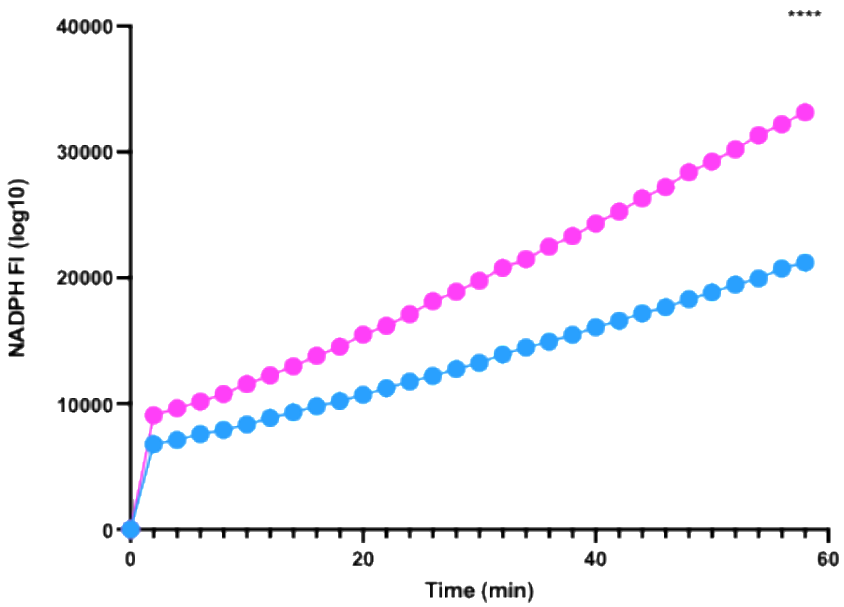


Butyrate reprograms redox homeostasis

ROS quantification

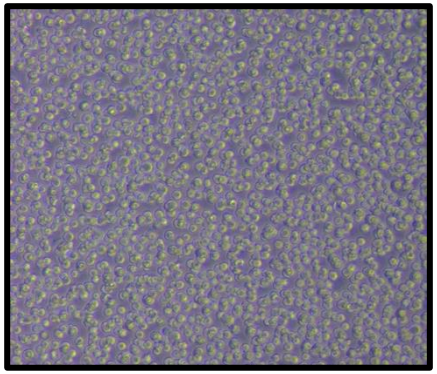


NADPH quantification

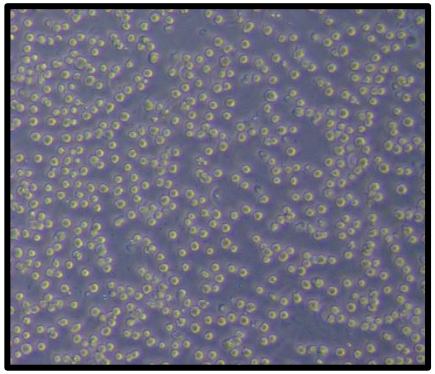


K562 Untreated
K562 Butyrate

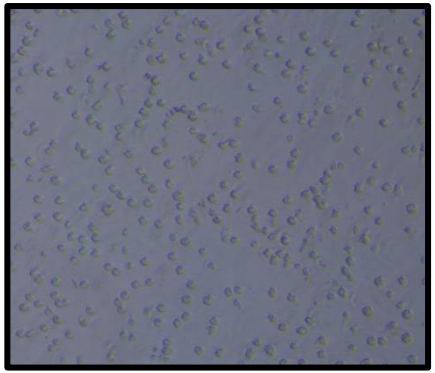
+ GLC/-NaBu



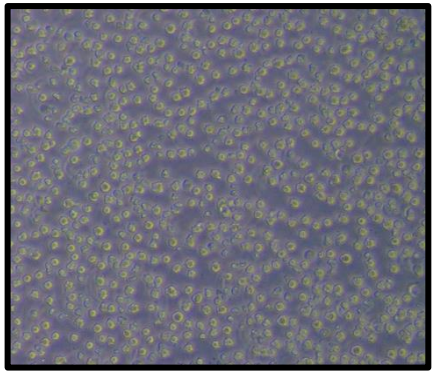
+GLC/+NaBu

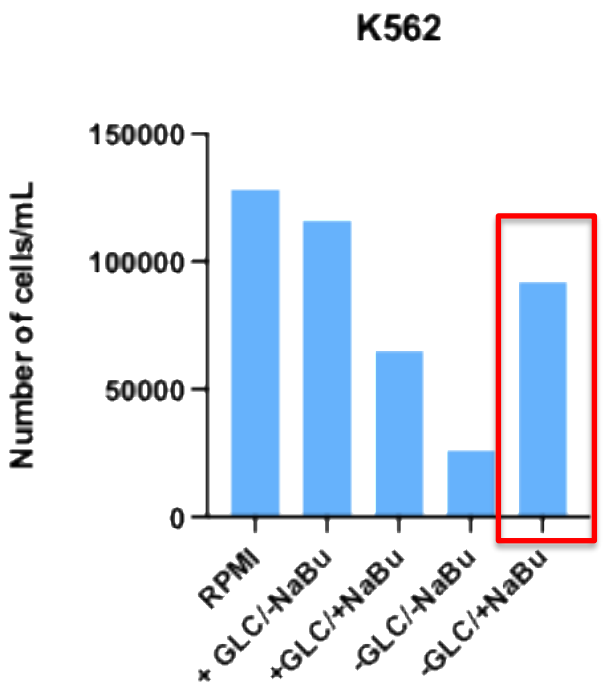


- GLC/- NaBu

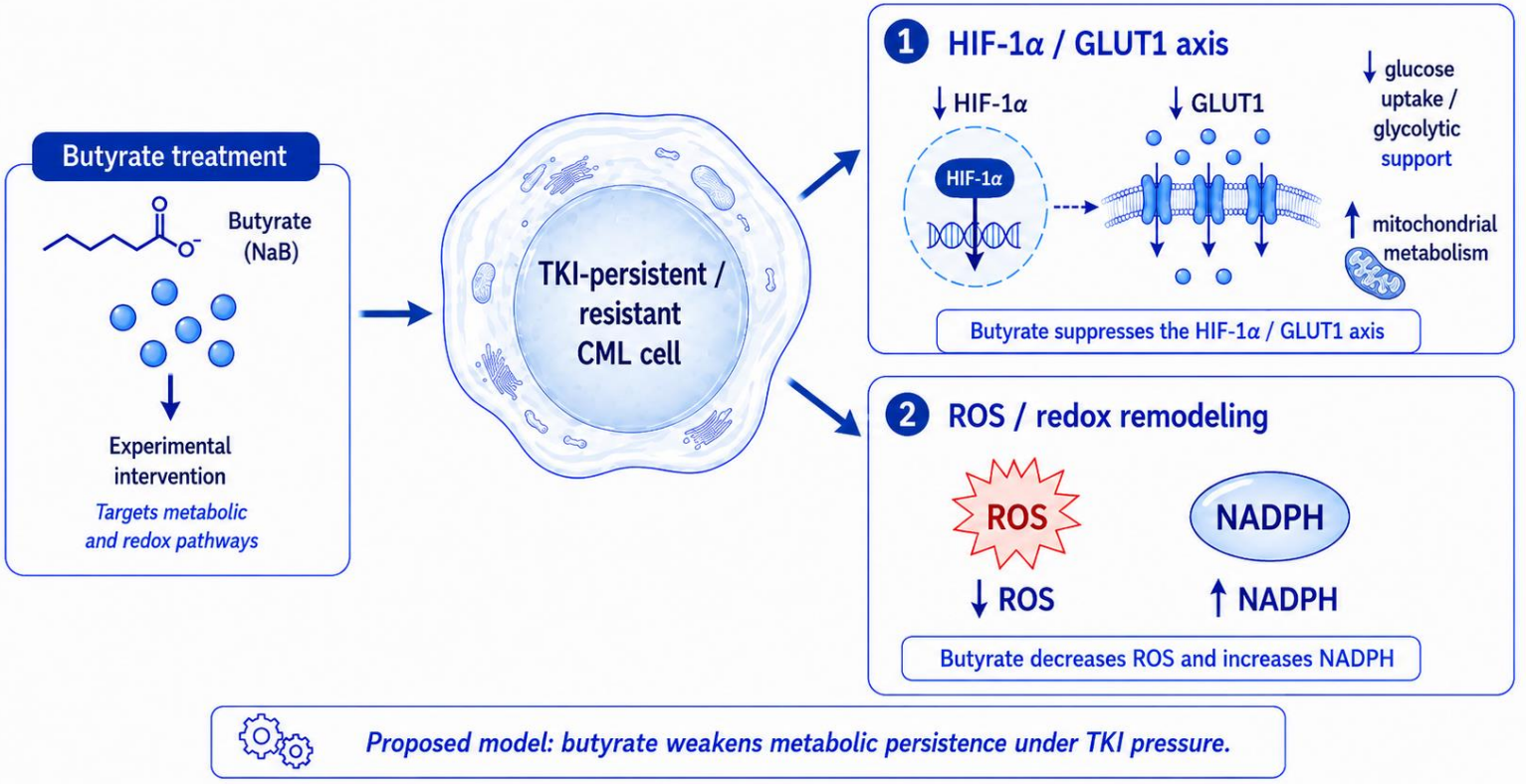


- GLC/ + NaBu





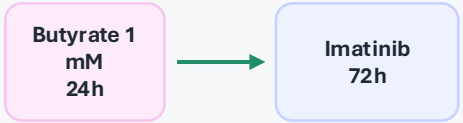
Butyrate disrupts the HIF-1α / GLUT1–redox axis in persistent CML



Proposed model: butyrate weakens metabolic persistence under TKI pressure.

Butyrate pre-treatment modulates TKI response

Pre-treatment workflow

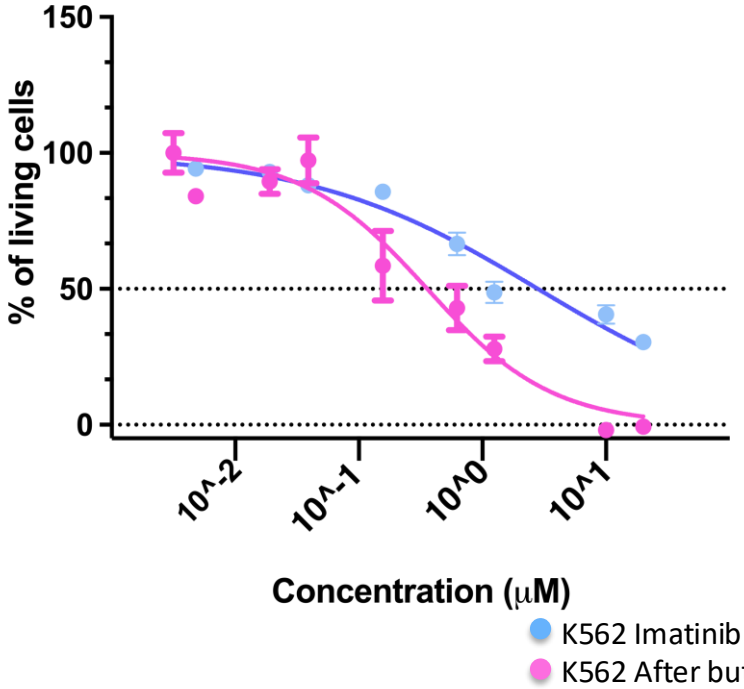


IC50 summary

	Imatinib 72h	After butyrate priming
K562	1.90 µM	0.3575 µM

IDR: 5.3

Dose–response after butyrate priming



Conclusions and future perspectives

Butyrate primes K562 cells through epigenetic + metabolic reprogramming, increasing vulnerability to imatinib.

- **Butyrate induces an epigenetic and metabolic reprogramming in K562 cells**
- **Enhances imatinib sensitivity**

Butyrate pre-treatment reduces imatinib IC50, suggesting a priming effect that increases TKI response.

- **Reduces BCR::ABL protein abundance**

Butyrate treatment decreases the level of the main oncogenic driver of CML.

- **Promotes metabolic rewiring**

Butyrate reduces glycolytic/hypoxic markers, including GLUT1 and HIF1α, and increases mitochondrial engagement.

- **Reduces glucose dependency**

Under glucose deprivation, butyrate-treated cells better tolerate metabolic stress, suggesting a shift toward alternative energy pathways.

- **Supports a less glycolytic, more mitochondria-oriented state**

This remodeled state may contribute to increased vulnerability to imatinib.

Thank you for your attention

Acknowledgements

<u>Carmen Fava (Associate Professor)</u>

Daniela Cilloni (Full Professor)

Barbara Pergolizzi (Associate Professor)

Cristina Panuzzo (RTDA)

Alessandro Ferrando (Postdoc)

Simone Rocco (Postdoc)

Alice Danzero (Ph.D. student)

Afshin Bagherzadeh (Ph.D. student)

Antonio Frolli (Ph.D. student)