PROLONGED XPO1 INHIBITION IS ESSENTIAL FOR OPTIMAL ANTI-LEUKEMIC ACTIVITY IN *NPM1*-MUTATED AML

Abstract AML-099

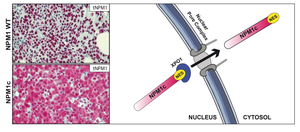
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soho22 TENTH ANNUAL MEETING

BACKGROUND & AIM

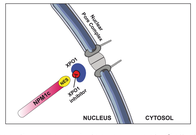
Acute myeloid leukemia with mutated *NPM1* (NPM1c AML) is the most frequent AML subtype in adults[1]. The most distinguishing feature of *NPM1*-mutated cells is the aberrant localization of mutant NPM1 (NPM1c) in the cytoplasm[1], which is caused by the loss of a nucleolar localization signal and the gain of a nuclear export signal (NES) within the C-terminal end of NPM1. Both nuclear export and cytoplasmic accumulation are dependent on NPM1c interaction with the nuclear exporter Exportin-1 (XPO1), which is mediated by the C-terminal NES[1].



NPM1c AML is also characterized by a consistent overexpression of HOX/MEIS1 genes[2], which are critical to maintain the undifferentiated state of AML cells and the leukemic phenotype[3].

Pharmacological inhibition of XPO1 by Selinexor (Seli) leads to NPM1c nuclear relocalization, loss of HOX genes expression, terminal differentiation and growth arrest of *NPM1*-mutated cells[3].

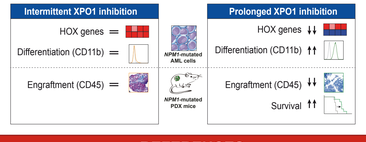
HOX genes ↓↓
Differentiation ↑↑
Cell growth ↓↓



Despite promising preclinical result, once or twice per week Seli did not result into clinical benefit for NPM1c AML patients in early-phase clinical trials[4,5]. We hypothesized that such intermittent administration of Seli used in clinical trials may not stably disrupt the NPM1c-XPO1 interaction, limiting its efficacy.

The second-generation XPO1 inhibitor Eltanexor (Elta) is currently being administered 5 days/week (5d/w) in clinical studies[6] and may therefore represent an attractive alternative to Seli to elicit a more pronounced anti-leukemic activity in NPM1-mutated cells

The aim of this study is to demonstrate that prolonged XPO1 inhibition (5d/w) by Seli or Elta is significantly more effective than intermittent XPO1 inhibition (2d/w) in NPM1c AML *in vitro* and *in vivo* models.

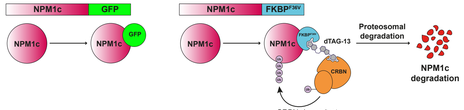


REFERENCES

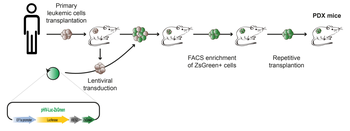
[1] Ranieri R et al. **Leukemia**. 2022 Aug 25. | doi: 10.1038/s41375-022-01666-2.
[2] Alcalay M et al. **Blood**. 2005;106(3):899-902. | doi: 10.1182/blood-2005-02-0560.
[3] Brunetti L et al. **Cancer Cell**. 2018 Sep 10;34(3):499-512.e9. | doi: 10.1016/j.ccell.2018.08.005.
[4] Garzon R et al. **Blood**. 2017;129(24):3165-3174. | doi: 10.1182/blood.2020007231.
[5] Sweet K et al. **Leuk Lymphoma**. 2021 Dec;62(13):3192-3203. doi: 10.1080/10428194.2021.1950706.
[6] Lee S et al. **J Clin Oncol.** 2022;39(15). | doi:10.1200/JCO.2021.39.15_suppl.e19037.

MATERIALS & METHODS

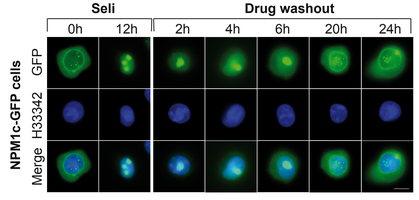
In vitro experiments:
- Parental OCI-AML3 cells;
- Patient-derived xenograft of two highly aggressive *NPM1*^{mut}/*DNMT3A*^{mut}/*FLT3*$^{ITD/ITD}$ AML cells;
- CRISPR-edited OCI-AML3 cells with endogenous NPM1c fused to GFP (NPM1c-GFP) and to FKBP (F36V) degron tag (NPM1-Degron).



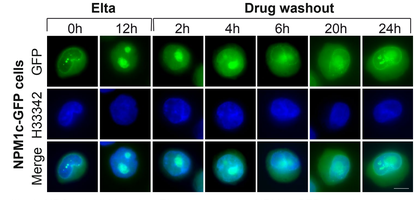
In vivo experiments:
- Patient-derived xenograft of two highly aggressive *NPM1*^{mut}/*DNMT3A*^{mut}/*FLT3*$^{ITD/ITD}$ AML engineered to express ZsGreen protein and Luciferase (PDX mice).



DYNAMICS OF NPM1c LOCALIZATION UPON INTERMITTENT XPO1 INHIBITION



12h incubation of NPM1c-GFP cells with Seli resulted in successful NPM1c-GFP nuclear relocalization. Drug washout led to progressive relocation of NPM1c-GFP into the cytoplasm in less than 24h, demonstrating quick recovery of the NPM1c-XPO1 interaction following transient XPO1 inhibition.

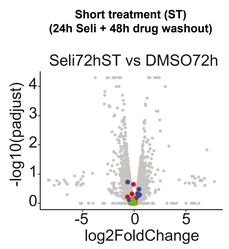


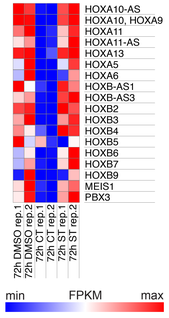
Intermittent XPO1 inhibition by Elta resulted in NPM1c-GFP localization dynamics overlapping with those observed with Seli.

CONTINUOUS SELI TREATMENT IS NECESSARY TO INDUCE A PERSISTENT DOWNREGULATION OF HOX GENES

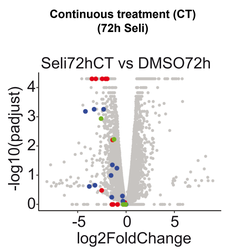
OCI-AML3 cells

Short treatment (ST) (24h Seli + 48h drug washout)





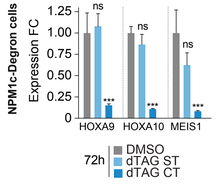
Continuous treatment (CT) (72h Seli)

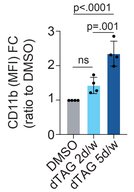


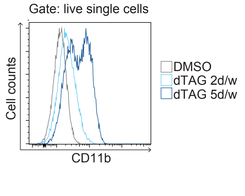
Short treatment caused only transient downregulation of HOX genes expression, but did not result in significant expression changes at 72h.

Continuous treatment caused persistent downregulation of HOX genes, combined with upregulation of genes related to myeloid differentiation, p53 activation and oxidative phosphorylation.

CONTINUOUS SELI TREATMENT IS NECESSARY TO INDUCE A PERSISTENT DOWNREGULATION OF HOX GENES



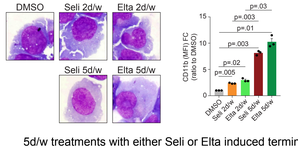


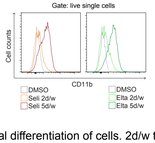


Only prolonged NPM1c degradation caused stable HOX genes downregulation and cells differentiation (increased CD11b expression).
↳ Specific degradation of NPM1c perfectly mimicked what observed upon XPO1 inhibition.

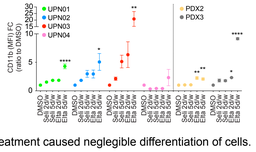
2d/w vs 5d/w XPO1 INHIBITION: CELL DIFFERENTIATION

OCI-AML3 cells





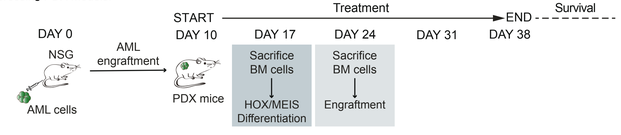
***NPM1*-mutated primary and PDX AML cells**

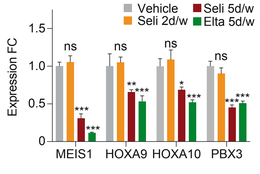


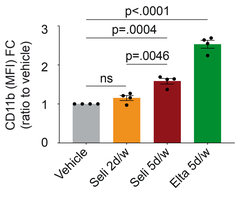
5d/w treatments with either Seli or Elta induced terminal differentiation of cells. 2d/w treatment caused neglegible differentiation of cells. Elta showed signals of higher efficacy, particularly in terms of differentiation induction.

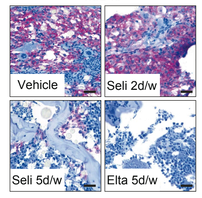
2d/w vs 5d/w XPO1 INHIBITION *in vivo*: BM ENGRAFTMENT, HOX/MEIS EXPRESSION, CELL DIFFERENTIATION

Intermittent and prolonged XPO1 inhibition was compared *in vivo* using two highly aggressive *NPM1/FLT3/DNMT3A* triple-mutated luciferase-expressing PDX models.

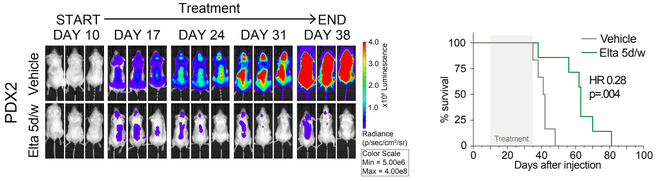








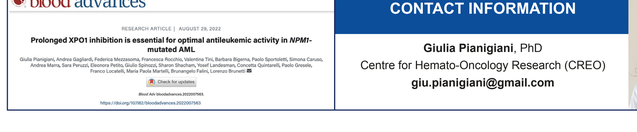
In contrast to Seli 2d/w, both 5d/w regimens treatment caused remarkable HOX/MEIS downregulation, blasts differentiation, coupled with a significant engraftment reduction (hCD45) in the bone marrow.



The effect of prolonged XPO1 inhibition on PDX survival was established by treating mice for four consecutive weeks with Elta 5d/w. Elta resulted in significant reduction of bioluminescence and prolonged survival compared to vehicle.

CONCLUSIONS AND FUTURE PERSPECTIVES

- **Intermittent XPO1 inhibition does not elicit robust anti-leukemic activity in *in vitro* and *in vivo* NPM1c AML models.**
- **Prolonged XPO1 inhibition stably downregulates HOX genes and induces terminal differentiation and is necessary to achieve optimal anti-leukemic activity in NPM1c AML.**
- ***In vitro* experiments on cell lines, PDX cells and primary samples demonstrate that Elta has higher efficacy, particularly in terms of differentiation induction.**
- **5 days/week Elta results into prolonged survival of NPM1c PDX mice.**



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