A "Designed" High-Throughput Drug Screening Strategy Identifies Aurora Kinase A Inhibitors as Promising Preclinical Candidates for the Treatment of *NPM1*-Mutated AML

Roberta Ranieri[1], Martin Neuenschwander[2], Sabrina Kleissle[2], Federica Mezzasoma[1], Serenella Silvestri[1], Alessio Ferrari[1], Sara Pierangeli[1], Serena Donnini[1], Francesca Milano[1], Marcella Sabino[1], Valentina Tini[1], Giulio Spinozzi[1], Brunangelo Falini[1], Jens Peter von Kries[2], Ilaria Gionfriddo[1], Maria Paola Martelli[1]

[1]Hematology and Clinical Immunology, University of Perugia, Perugia, Italy. [2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany

Abstract

NPM1-mutated AML accounts for about one-third of all adult AML. Despite recent improvements in treatment, relapses still commonly occur highlighting the need for new therapeutic approaches. To identify novel targets in AML we performed a high throughput screen (HTS) in *NPM1*-mutated AML models. Through a "designed" strategy, we identified the Aurora kinase A inhibitors (AURKAi) as an effective anti-leukemic class of drugs with an enhanced effect in the *NPM1*-mutated AML.

Background & Objective

Despite its chemo-sensitivity and relatively favorable prognosis, *NPM1*-mutated AML lacks any specific therapies. Because NPM1 mutant protein is not druggable, targeting its non-oncogene addictions and its specific vulnerabilities can represent an alternative option for identifying a tailored therapy for *NPM1*-mutated AML.

To unravel vulnerabilities and possible new targets for therapy of *NPM1*-mutated AML we have developed a two-step screening strategy to find compounds specifically active on the *NPM1*-mutated AML entity and thus would represent ideal hits to be further developed as drugs.

Materials & Methods

A collection of 38,720 compounds belonging to different chemical libraries were sourced from Leibniz-FMP Screening Unit (Berlin) organized in 384-well plates and screened at 1mM on the IMS-M2 cell line (*NPM1*^{mut}AML).

Results

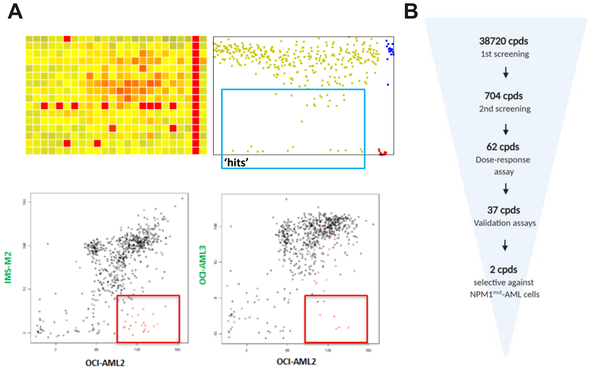


Fig.1A) *Some steps of the selection process for the list of primary actives (Example of 384-well screening plate and resulting selected compounds plot are shown).* ***B)*** *HTS workflow allows the identification of 62 compounds that met qHTS threshold criteria and then 37 selected both for their selective activity on the mutated cells. From rescreen, 2 compounds are considered true hits.*

The resulting 704 'actives' molecules were measured in triplicate at concentrations of 1, 0.1 and 0.01 µM on OCI-AML3 and IMS-M2 (*NPM1*^{mut}AML) and OCI-AML2 (*NPM1*^{wt}AML) in order to search for compounds that selectively kill *NPM1*-mutated cells (**Fig.1**).

62 of these compounds were followed by up IC50 testing from a top concentration of 20 µM in an 8-point curve.

The assay readout was cellular metabolism/viability by CellTiter Blue (CTB) assay at 48 hours.

From dose-response assay analysis, we obtained 37 'hit' compounds chosen both for their selective activity cells and for the lowest effective dose on the mutated cells.

By exploring their signaling pathways as belonging to selected classes of drugs, we identified the highly represented pathway of Aurora kinase A.

Based on target selectivity and clinical status we selected two resulting AURKAi (MK-5108, MK-8745) and two additional AURKAi (Alisertib and ENMD-2076), not included in the screening library.

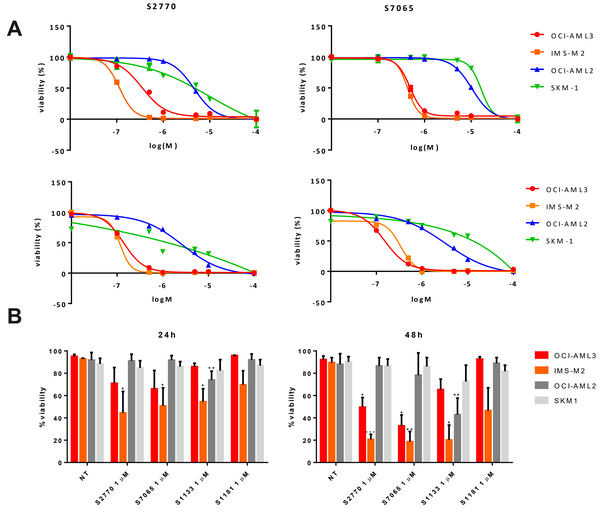
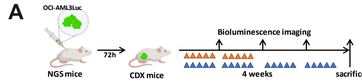


Fig.2A) *Dose-response curve for NPM1^{mut} OCI-AML3 and IMS-M2 cells versus NPM1^{wt} OCI-AML2 and SKM1 cells treated for 48h with serial dilutions of the AURKAi S2770, S7065, S1133 and S1181 (MK-5108, MK-8745, Alisertib, and ENMD-2076, respectively) using CTB and CyQ assays.* ***B)*** *Apoptosis assay upon 24h and 48h treatment (1 µM). Percentage of apoptotic cells at each time point is shown (*P<0.05;**P<0.01; ***P<0.005).*

These drugs were confirmed more active against *NPM1*-mut than *NPM1*-wt AML cells by CellTiter Blue and CyQUANT (a cell number-based assay), **Fig.2A**. These effects were also confirmed in terms of apoptosis (AnnexinV/7AAD- positive cells), **Fig.2B**



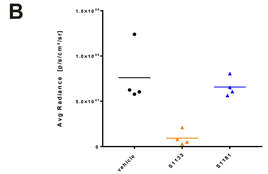


Fig.3A) *NGS mice were injected with luciferized OCI-AML3 cells and treated with Alisertib (10 mg/kg, 5d/w) and ENMD-2076 (100 mg/kg, 5d/w).* ***B)*** *Tumor burden was monitored by bioluminescent imaging.*

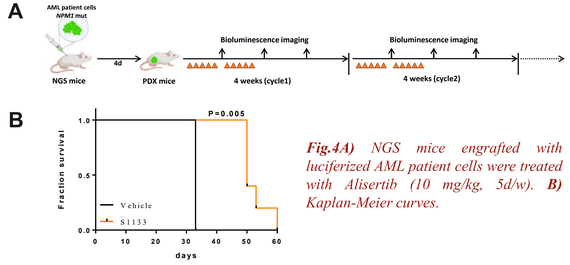


Fig.4A) *NGS mice engrafted with luciferized AML patient cells were treated with Alisertib (10 mg/kg, 5d/w).* ***B)*** *Kaplan-Meier curves.*

We next validated the anti-leukemic effect of these inhibitors *in vivo*. Our results demonstrated that Alisertib and ENMD-2076 were effective in reducing tumor burden in *NPM1*-mutated CDX mice (**Fig.3**). Alisertib confirmed its activity in PDX models providing a survival advantage compared with vehicle-treated mice (60 *vs* 32 days, *P*=0.005) (**Fig.4**).

Conclusions

Here, we demonstrated that our "designed" HTS strategy can rapidly lead to the identification of promising lead compounds. We also provide preclinical evidence of the antileukemic activity of AURKAi, with a particular focus on *NPM1*-mutated AML.

contact information: Prof. Martelli Maria Paola (MD, PhD): maria.martelli@unipg.it, Dr. Ranieri Roberta (PhD): roberta.ranieri@unipg.it; CREO (Center for Research in Hemato-Oncology), Piazzale Menghini 8/9, Perugia, Italy