A Rational Approach for the Discovery of NSD2 Inhibitors for the Treatment of Cancer

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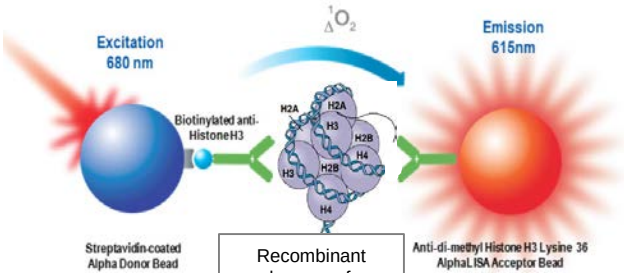
1-INTRODUCTION

Multiple myeloma (MM) is a plasma cell malignancy which accounts for approximately 10% of hematologic malignancies. Despite the introduction of new therapeutic agents, MM remains incurable and nearly all patients ultimately relapse. About 20% of MM are due to a chromosomal translocation t(4;14) leading to overexpression of the NSD2 histone methyltransferase. NSD2 catalyzes dimethylation of lysine 36 on histone H3 (H3K36me2) and is associated with transcriptionally active regions. Several studies have shown that in MM harboring the translocation t(4;14), oncogenic programming is dependent on the methyltransferase activity of NSD2. Thus, NSD2 is a potential therapeutic target for MM for which no curative treatment is available to date. In addition, the NSD2 overactivity is also observed in prostate and lung cancers. To address these medical needs, Inventiva has started a drug discovery program on NSD2 inhibitors.

2- ASSAY PRINCIPLE

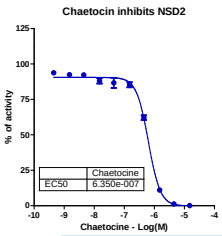
The assay is based on AlphaLISA technology and relies on the detection of H3K36me2 marks on nucleosome by a specific antibody.



Recombinant nucleosome from *E. coli*

Assay in 384-well plate format

3- VALIDATION OF THE ASSAY WITH REFERENCE COMPOUNDS



Chaetocin, a non specific inhibitor, is able to block NSD2 activity in a dose dependent manner, with an IC50 of 635 nM, similar to the ones described in the literature.

This result validates the assay and the automation

4- INVENTIVA LIBRARY: IVALib

- **240,000** Compounds
- **2/3rd Original** compared to the Zinc library[1]
- Designed over years for drug discovery programs
- Available as liquid solutions and 70% as powders
- Stored in controlled environment
- Regular quality control
- Collection enrichment to improve diversity and maintain originality
- **Good hit rate** on internal screening programs

Library available for external partnerships

1) Irwin J. et al *J. Chem. Inf. Model. 2012*

5- SCREENING AND HIT SORTING

- Inventiva library
- Primary screening at 10 µM in single point (Alphalisa assay) hit selection > 60% inhibition
- Confirmation: Alphalisa Assay + Truhit in duplicate hit selection > 70% inhibition
- DR IC50 :AlphaLisa & Counter screen (HeLa Nucleosomes) + Redox + QC + resynthesis
- Orthogonal confirmation by a radioactivity assay using ^{3}H SAM
- Binding assay by SPR *on going*
- Mini SAR / selection of series

From Inventiva's collection IVALib, 6 hits have been identified with distinct and new chemical matters.

6- HIT CONFIRMATION BY SPR

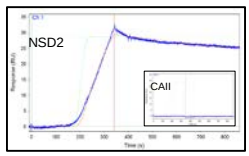
The binding of our hits is being addressed by SPR technology using the SensiQ Pioneer FE.

Experiment design:

- The full length NSD2 was immobilized on channel 1 through amine coupling on the COOHV chip.
- No immobilization on channel 2 (reference channel).
- A non relevant protein, carbonic anhydrase II (CAII), was immobilized on channel 3 to control the binding specificity of the compounds

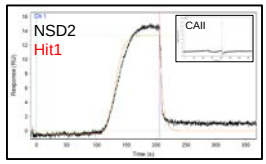
Proper immobilization and functionality was confirmed by SAH binding for NSD2.

SAH
Gradient: 0 to 200 µM
Onestep injection

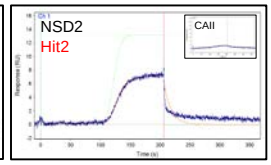


Ka (S^{-1})	Kd ($M^{-1}S^{-1}$)	KD (M)
31	3,40E-04	1,10E-05

First results showed that some hits directly bind to NSD2 and not to CAII.



Ka (S^{-1})	Kd ($M^{-1}S^{-1}$)	KD (M)
1.19E+04	0.19	1.65E-05



Ka (S^{-1})	Kd ($M^{-1}S^{-1}$)	KD (M)
1.77E+03	0.08	4.68E-05

The study is still on going and will include competition experiments with SAM .

7 - CONCLUSIONS

In addition to the biochemical inhibition of NSD2, we are able to show direct binding for some of our hits to the human full length NSD2 via the Pioneer FE from SensiQ. This direct interaction strengthen our confidence in our hits and further SPR testing will provide more information on the mechanism of action.
In parallel, we are developing secondary cellular assays based on the H3K36me2 methylation and proliferation to further confirm hit activity.
To our knowledge, no specific NSD2 inhibitor have been identified to date despite several screening effort performed by other groups. Our library has already produced new chemical starting points for other KMTs, and we believe that our hits are promising starting points to generate potent and selective NSD2 inhibitors.