A High-Throughput Screening and Triage Platform for Identifying PPI Disruptors in Cancer

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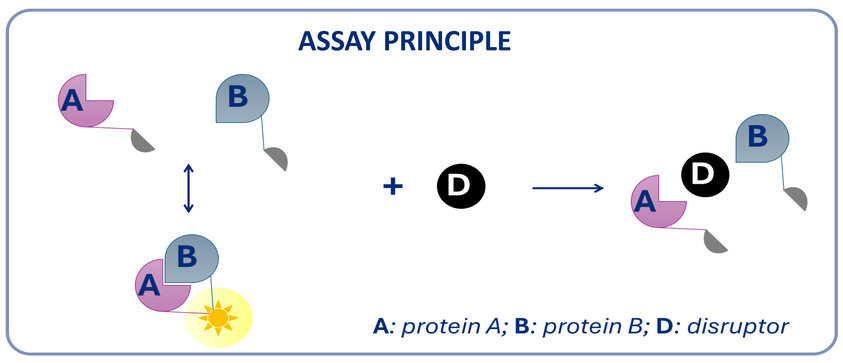
Introduction

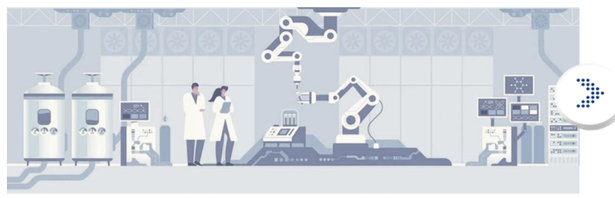
- **Epigenetic and transcriptional dysregulations** play pivotal roles in cancer development, progression aggressiveness, and recurrence of malignancies
- Using a **luminescence complementation assay** with the truncated protein targets, small molecules capable of selectively disrupting the target **Protein-Protein Interaction (PPI)** within cells (native conformation, *i.e.* target assay) as well as disrupting the interacted proteins in cellular extracts were tested.
- **Over 400,000 compounds** (AXXDiversity + Client library) were screened using the target assay, successfully adapted to a 384-well plate format, in the search of a specific compound disrupting the native conformation of the PPI.



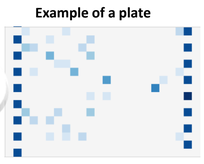
Hit discovery workflow

Assay Transfer → Assay Adaptation to Automation → Pilot Screening → Primary Screening → Compound Rescue Analysis → Hit Confirmation → Potency Determination → Hit Validation

Automated protocol on all assays



Example of a plate

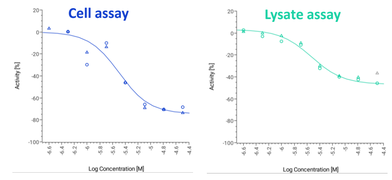


Quality Criteria	Cell Assay		Lysate Assay	
	Target	Counter	Target	Counter
Signal to Background	> 400	> 350	> 350	> 250
RZ' factor	> 0.5	> 0.5	> 0.8	> 0.7

Coefficient of Variability	Cell Assay		Lysate Assay	
	Target	Counter	Target	Counter
Intraplate	< 20%	< 20%	< 20%	< 10%
Interplate	< 20%	< 20%	< 20%	< 10%

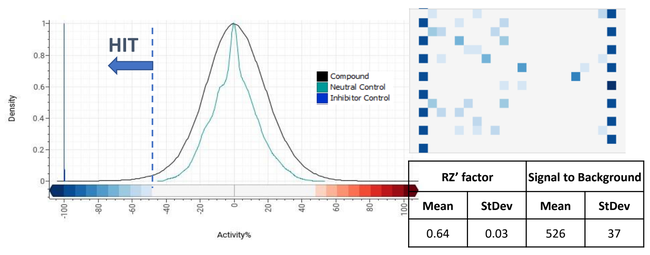
Tool Compound Pharmacology on Target Assays

Reproducible pharmacology of reference compound across phases, in line with Client's results



	Assay Transfer Cell	Assay Transfer *Lysate*	Adaptation to Automation Cell	Primary Screening Cell	Hit Confirmation Cell	Potency Determination Cell	Potency Determination *Lysate*
Log IC_{50} Mean	-5.19	-5.22	-5.16	-5.27	-5.49	-5.48	-6.04
Log IC_{50} SE	0.14	0.27	0.09	0.15	0.32	0.35	0.31
IC_{50} Mean (M)	6.8E-06	6.9E-06	6.9E-06	5.4E-06	3.2E-06	3.3E-06	9.0E-07
Min Value (mean)	-54	-34	-60	-51	-64	-66	-42

Primary Screening AXXDiversity



RZ' factor		Signal to Background	
Mean	StDev	Mean	StDev
0.64	0.03	526	37

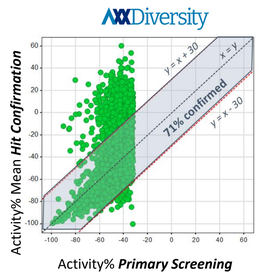
Hit rate of **0.4%** (cut-off ranging between 46%-57% inhibition) obtained on compounds' libraries screening tested

➢ Selection of **4,945** compounds for Confirmation

Hit Confirmation *(6 doses, n=3)*

Compounds were tested on Target and Counter **Cell** assays

AXXDiversity



Specificity Categorization	N° cpds
Decreasing only on Target Cell assay **Specific**	**184**
Decreasing on Target and Counter Cell assays with Δ > 0.7Log in the IC_{50} Counter **Interesting**	**109**
Inactive or Unspecific	*4,652*

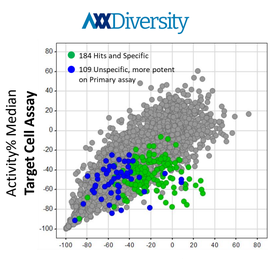
Confirmation rate of 60 to 71% of the hits

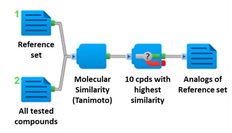
➢ Selection of **293** compounds for Rescue Analysis

Compound Rescue Analysis

Similarity search using Tanimoto coefficient led to the selection of analogs from Pool

AXXDiversity



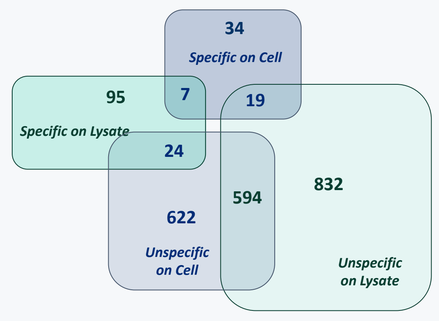


Protocol summary for similarity search

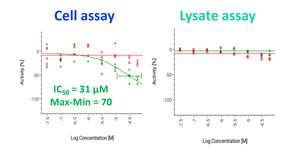
➢ Selection of **1,279** compounds for dose response

Potency Determination on Target and Counter Cell and Lysate Assays *(8 doses, n=3)*

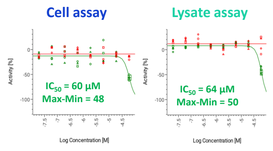
1,279 compounds



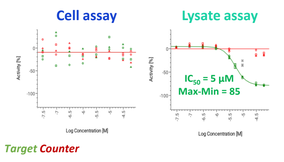
Example of **Specific on Cell Assay**



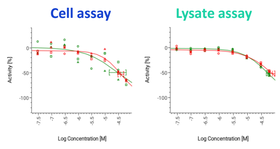
Example of **Specific on both**



Example of **Specific on Lysate Assay**



Example of **Unspecific on both**



Potency range of Specific compounds

IC_{50}	Specific on Cell	Specific on Lysate
<10 µM	-	3
10-30 µM	-	7
30-50 µM	6	8
50-100 µM	24	14
>100 µM	4	63
Total	***34***	***95***

➢ Identification of **115 compounds specific** for preliminary SAR and Hit Validation

ca. 475,000 compounds including AXXDiversity

Primary Screening

Hit Rate 0.4% at statistical cut-off
4,945 compounds promoted ca. **1% hit rate**

Hit Confirmation

> 60 % Confirmation rate
184 compounds **Specific** + 109 compounds **Interesting**

Compound Rescue Analysis

293 compounds Specific/Interesting
+ **986 compounds rescued** from pool by similarity search

Activity Determination

115 compounds Specific on either Cell or Lysate assays

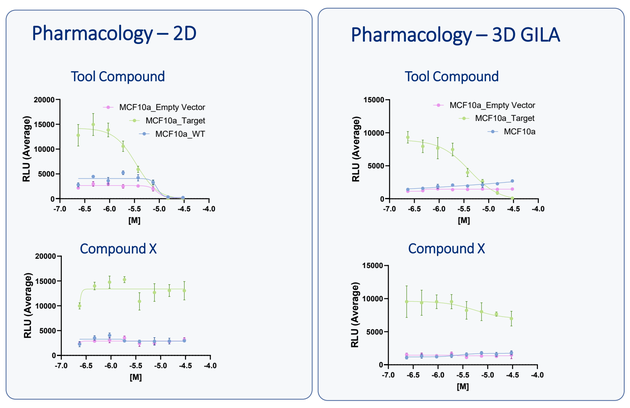
Hit Validation

From Hit-to-Lead

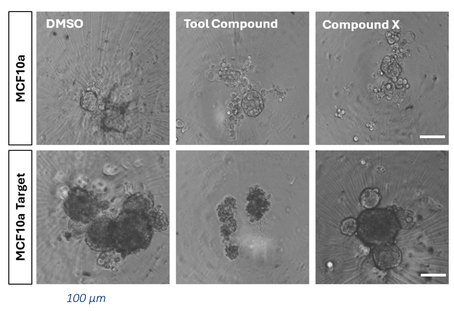
Hit Validation

The **3D GILA** (Growth in Low Attachment) assay, used with MCF10A cells (expressing the Target), is an *in vitro* assay to evaluate tumorigenicity (5 days treatment)

*Assessment of compounds in **2D** and **3D** culture in parallel*



3D GILA Rappresentative pictures at day 5 in vitro (50 µM)



100 µm

3D GILA can be used to screen for drugs that selectively inhibit or increase transformation, as seen with the specificity of the tool compound.
Additional assays to further validate the activity and selectivity of the compounds are under development.

Summary & Prospects

- A subset of compounds has undergone further validation through co-immunoprecipitation and target-specific *in vitro* growth arrest models, **showing promising results**.
- Following a well-defined screening cascade that integrates bioactivity in cancer-specific cell lines with cytotoxicity assays in non-transformed cells, the compounds will be further triaged **to select the best qualified hits**.

This project was performed in collaboration with a pioneering stealth company sprouted from the academic world.

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