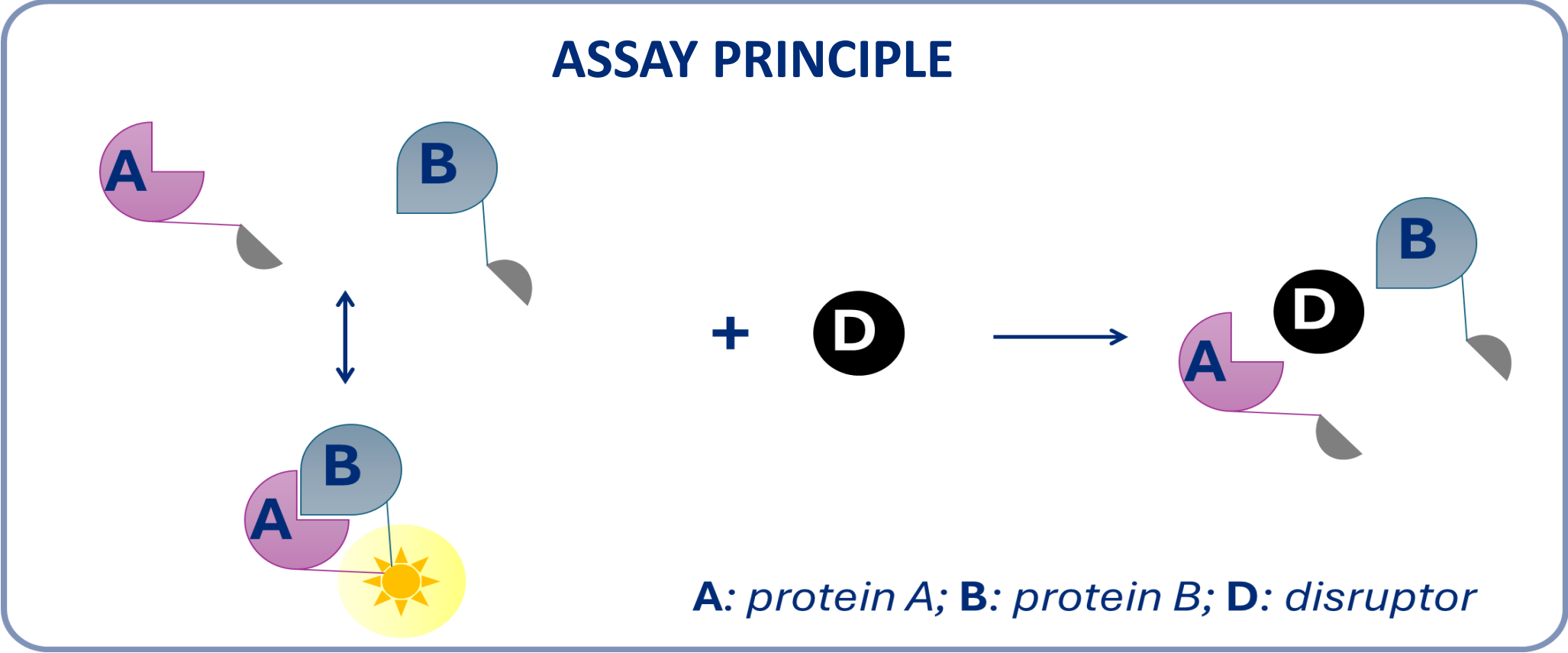


A High-Throughput Screening and Triage Platform for Identifying PPI Disruptors in Cancer

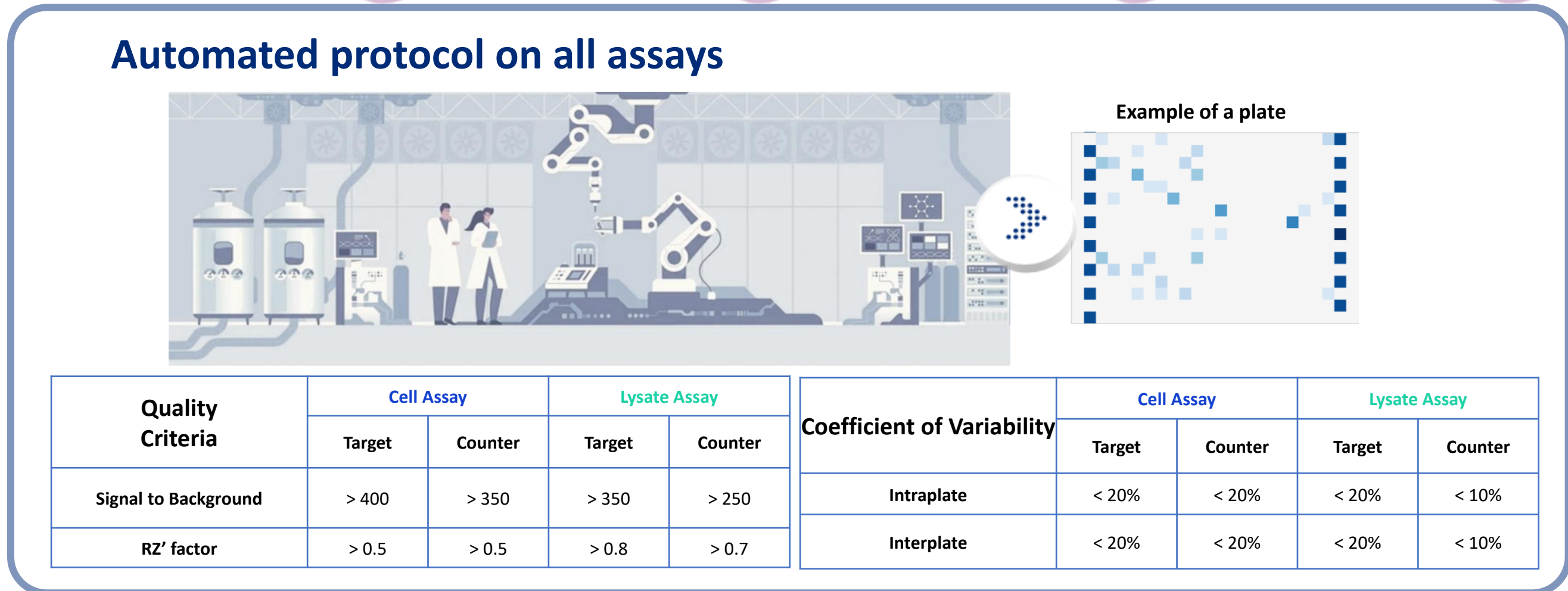
Mariantonietta Rubino, Aurelie Dos Santos, Roberta De Gioia, Alice Segnali, Debora Zian, Giovanna Maresca, Angela Molteni, Jean-Francois Rolland and Maria Pia Catalani.
Axxam S.p.A. OpenZone, Via Meucci 3, 20091 Bresso (Milan), Italy

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.

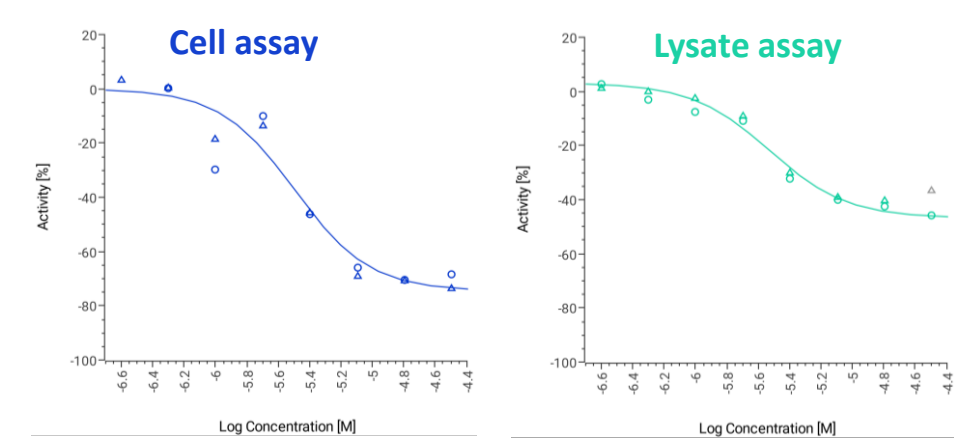


Hit discovery workflow

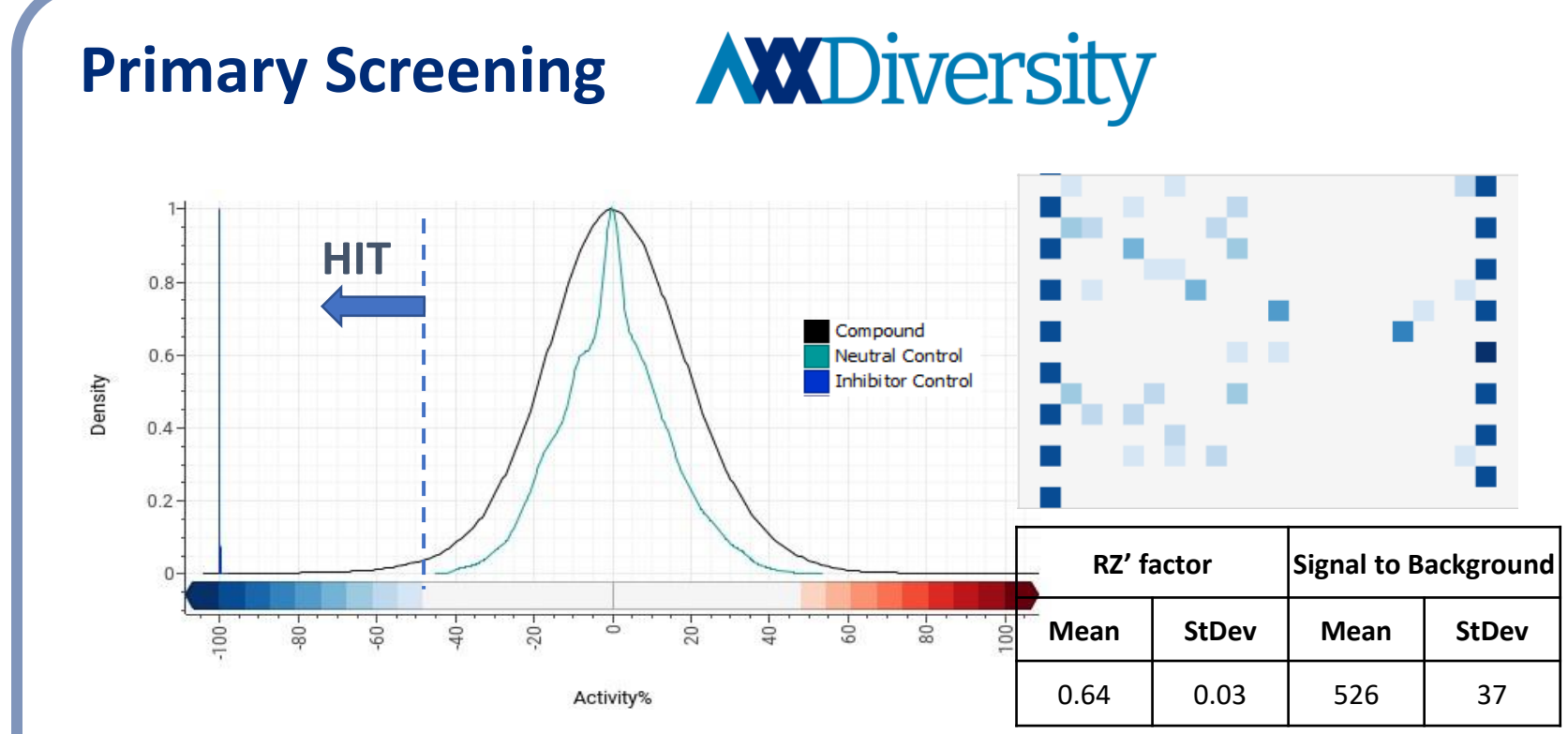


Tool Compound Pharmacology on Target Assays

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



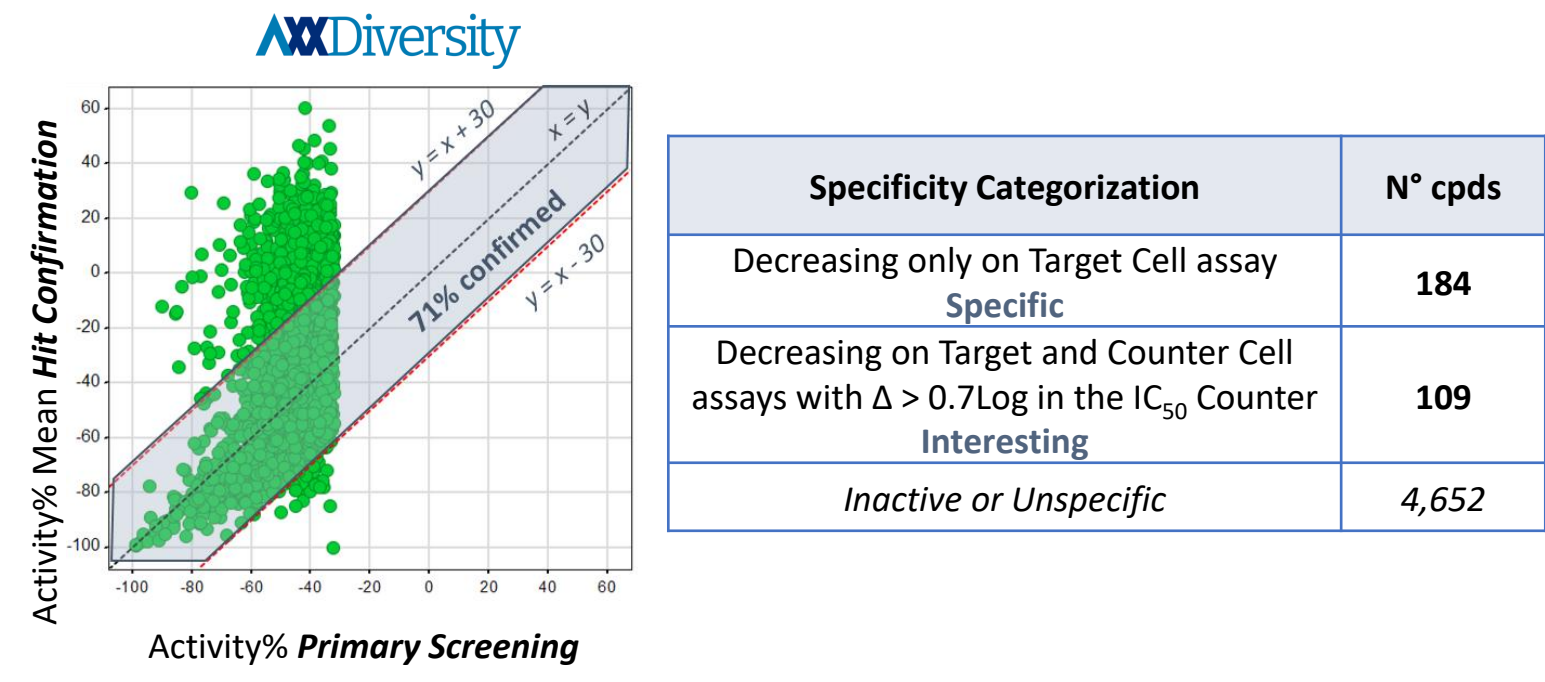
	Assay Transfer Cell	Assay Transfer Lysate	Adaptation to Automation Cell	Primary Screening Cell	Hit Confirmation Cell	Potency Determination Cell	Potency Determination Lysate
Log IC ₅₀ Mean	-5.19	-5.22	-5.16	-5.27	-5.49	-5.48	-6.04
Log IC ₅₀ SE	0.14	0.27	0.09	0.15	0.32	0.35	0.31
IC ₅₀ Mean (M)	6.8E-06	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



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)

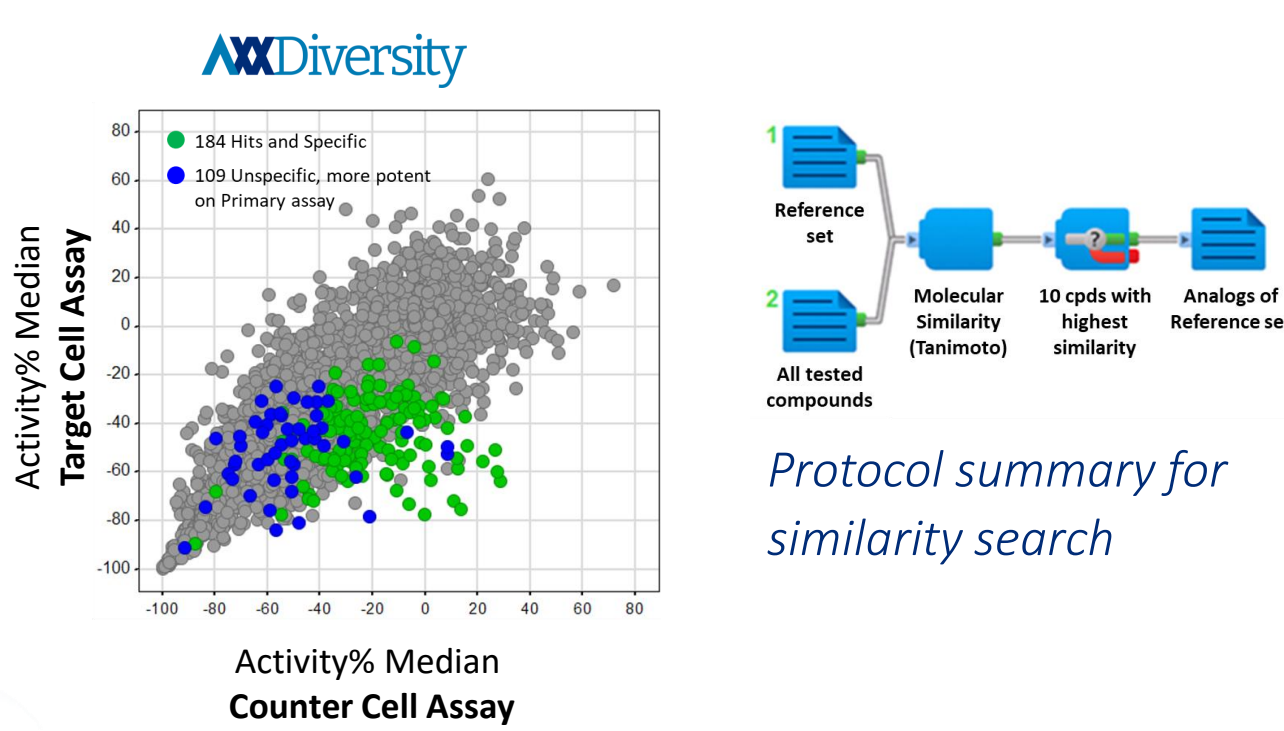
Compounds were tested on Target and Counter Cell assays



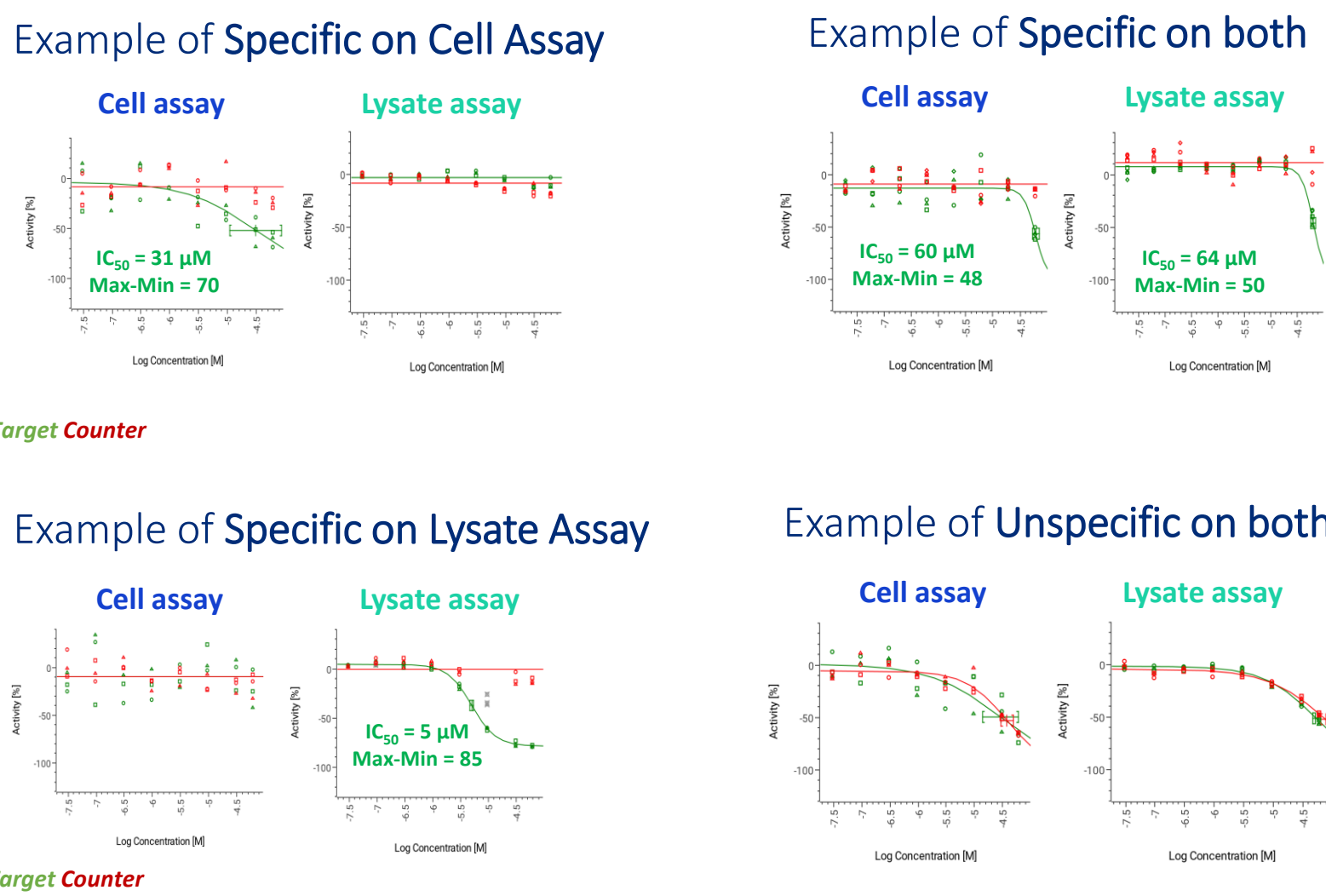
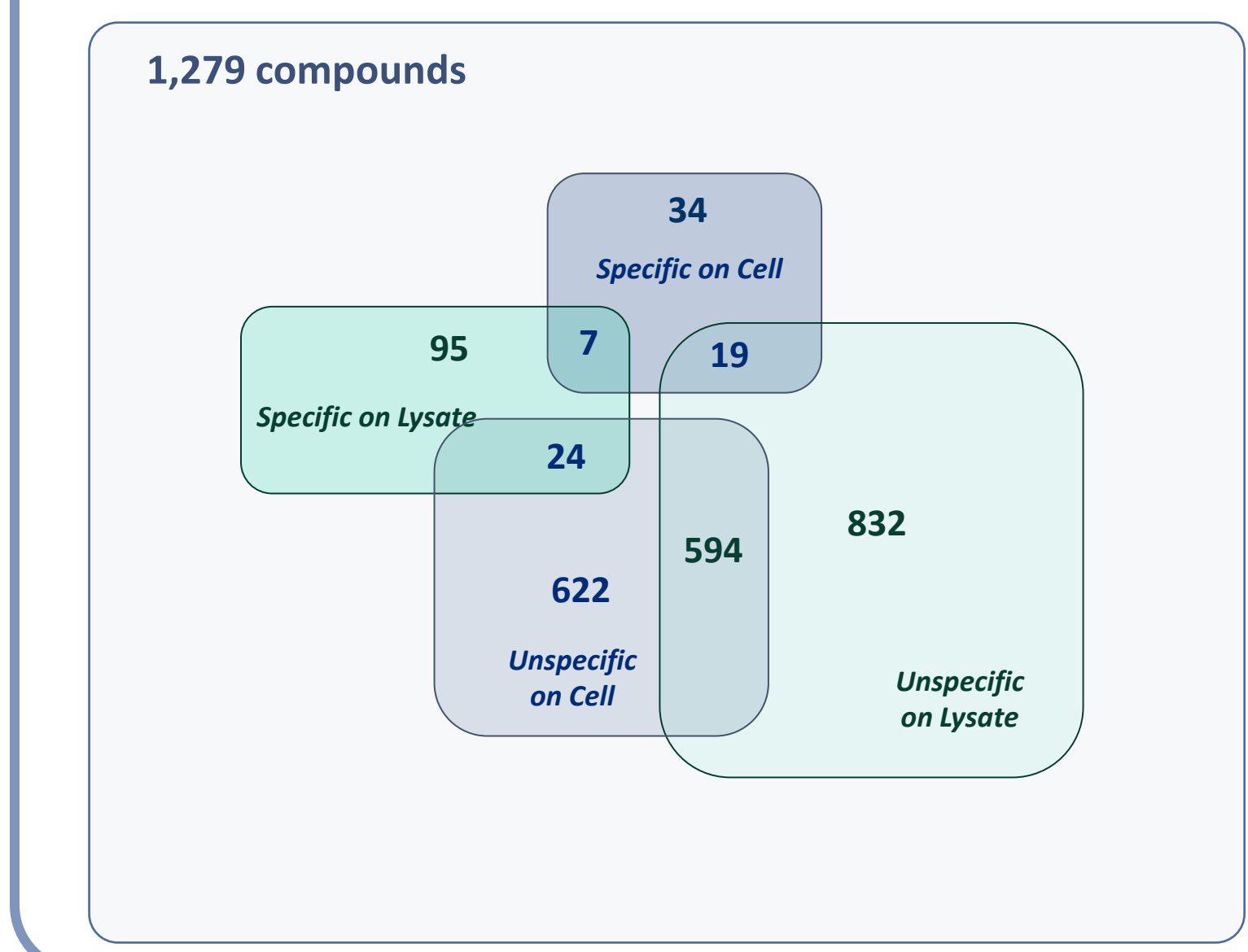
Confirmation rate of 60 to 71% of the hits
➤ Selection of **293** compounds for Rescue Analysis

Compound Rescue Analysis

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



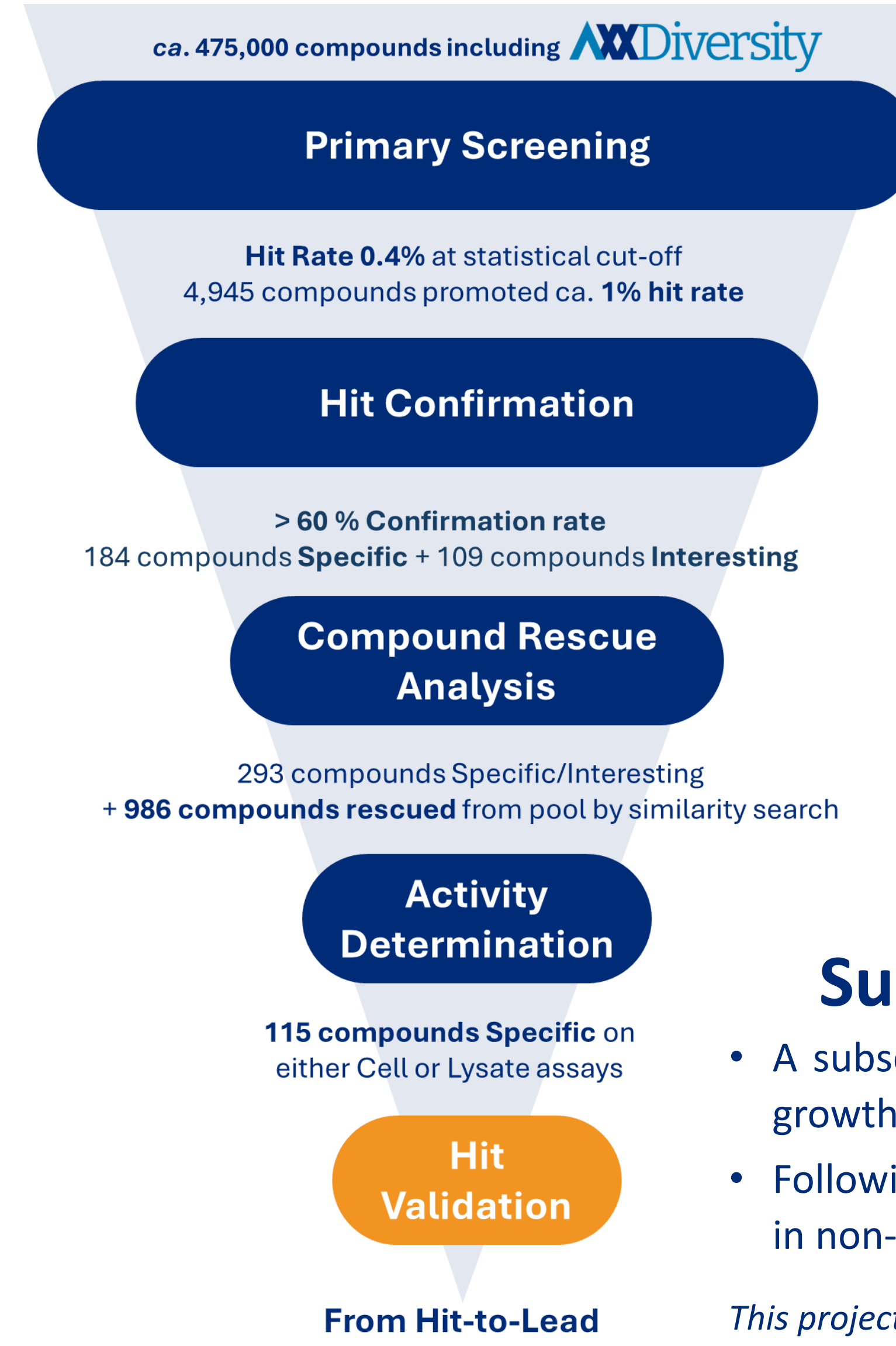
Potency Determination on Target and Counter Cell and Lysate Assays (8 doses, n=3)



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

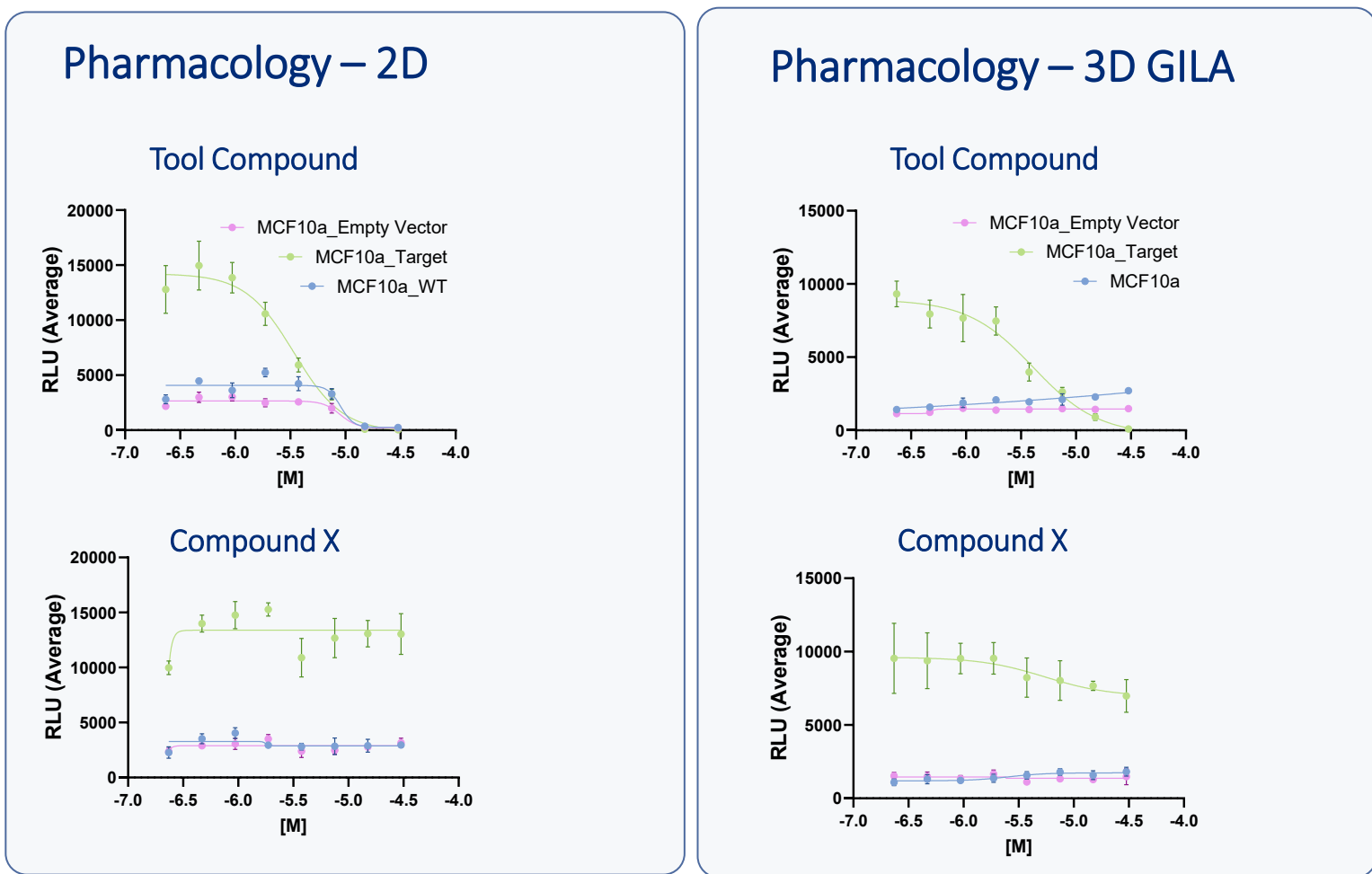
➤ Identification of **115** compounds specific for preliminary SAR and Hit Validation



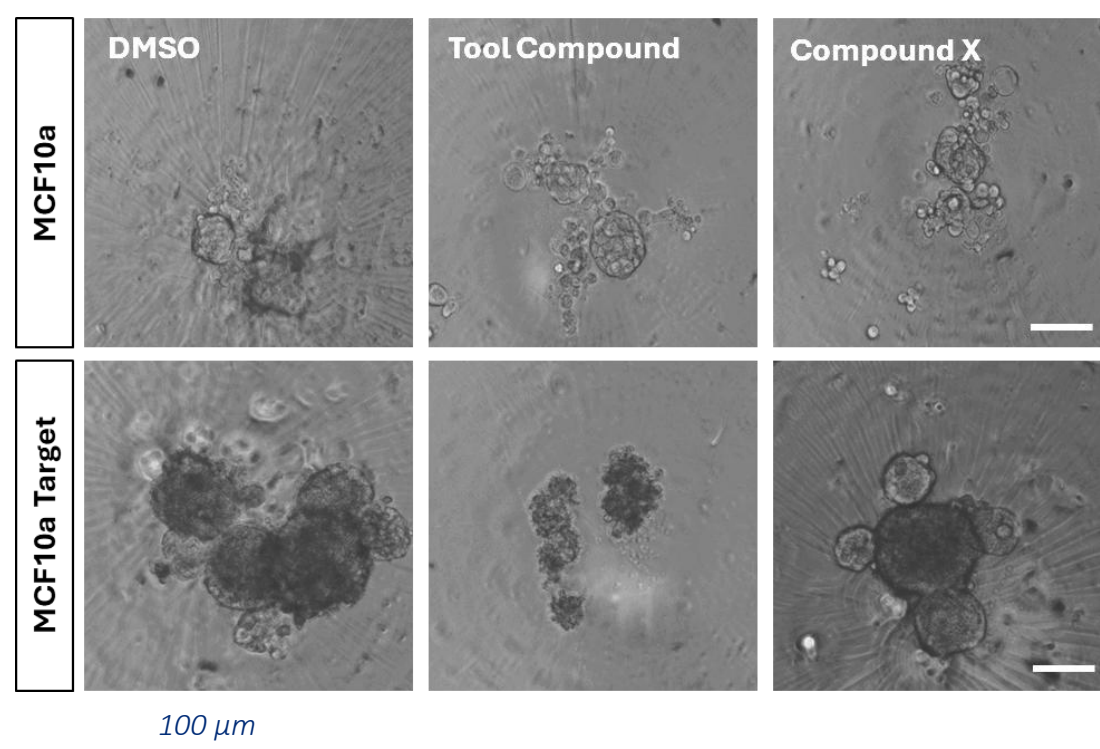
Hit Validation

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 Representative pictures at day 5 in vitro (50 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.

