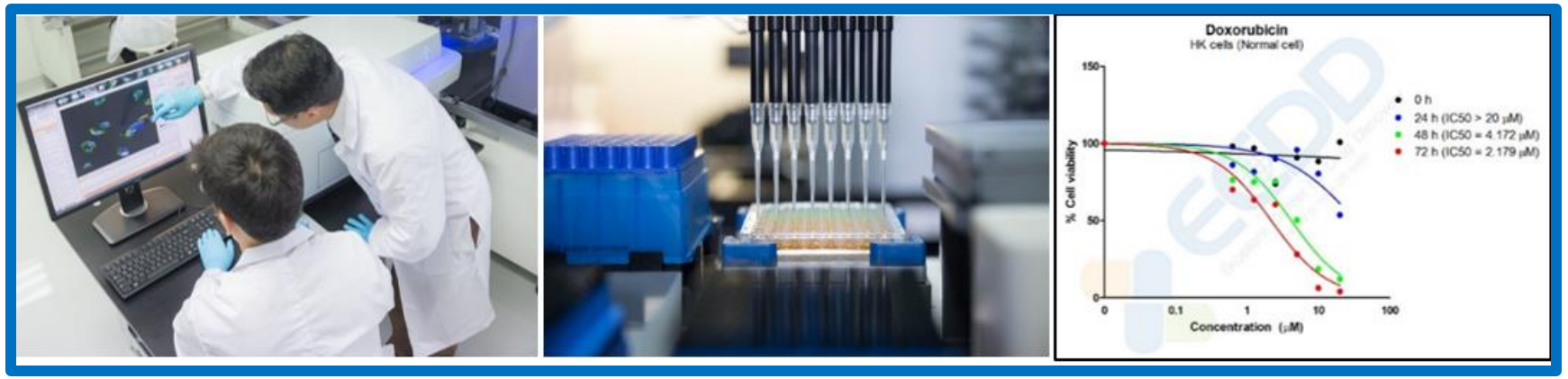
# A Role of ECDD in Leading Natural Products for Drug Discovery and Development in Thailand




**Suparerk Borwornpinyo**, Founder and Director
Excellent Center for Drug Discovery (ECDD), Mahidol University

**13th April 2021**

Approximately more than 60% of drugs available on the market today are derived from chemical structures found in nature.

Yet over the past few decades,
the influence of natural products on drug discovery has notably reduced.

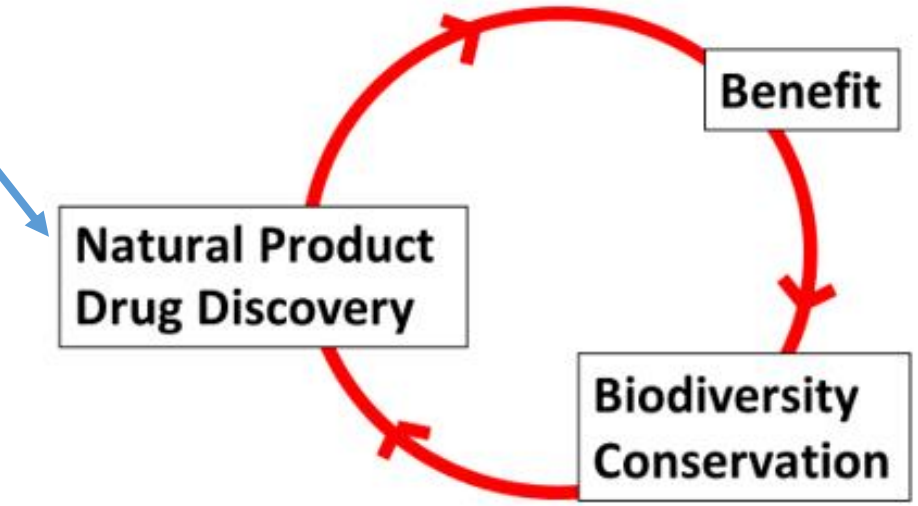
The perceived difficulties of isolating and synthesizing these complex molecules

The challenges associated with screening them using high throughput assays

A renewed interest in natural products,
the urgent need for new drugs.

# NPDD Ecosystem in Asia: An Open Innovation Platform

ECDD, HTS/HCS platform in Thailand




Thailand: Top 20 countries in megabiodiversity

**New Technology**

Screening technology for extracts
Synthetic biology using microbes
Analog synthesis using microbes
Other s

**New Platform**

Construct isolated compound library
Construct mid size compound library
Construct DB for Dereplication
Others

**Drug Discovery Capability of Pharma Industry in Japan**

# APAC DA-EWG: Pillar 5
# Drug Discovery using Natural Product


## Preparation for Pilot Project

**Internship for technology transfer**
**(Bilateral communication)**

- 4 month (February-May, 2019) internship at Takeda Shonan Research Center (tech-transfer & capacity building)
- Mr. Phongthon Kanjanasirirat
  (ECDD, Mahidol University)

# Overall scheme of the NPDD initiative with Thai institutions
## Capacity building through phenotypic screening

iPark/Japan

MN cell death assay
MNP stocks

1.Tech transfer for HTS/HCS
2. Drug discovery knowledge

Takeda

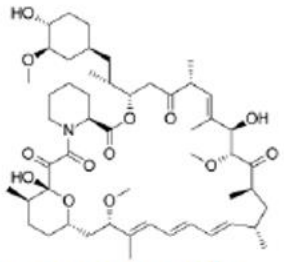
Masaaki-san Keiko-san

1.Receive the results
2.Make decision
for further collaboration

Thailand

After 3-6 months
Return to Thailand

1st June

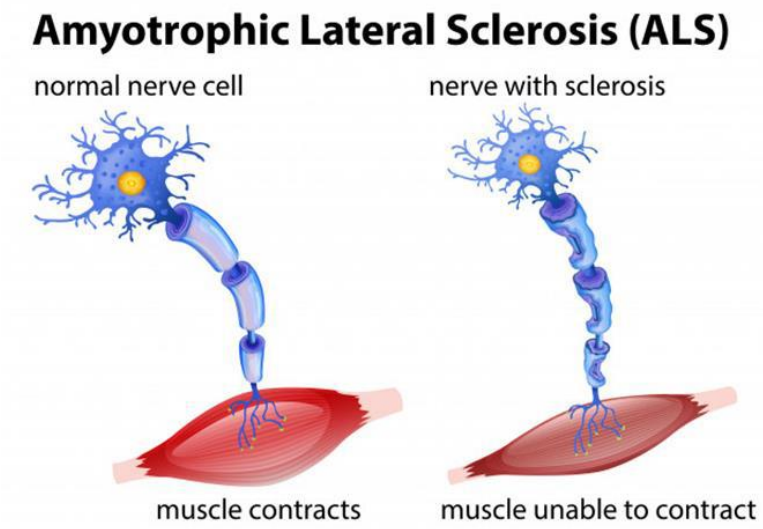


Hit compounds

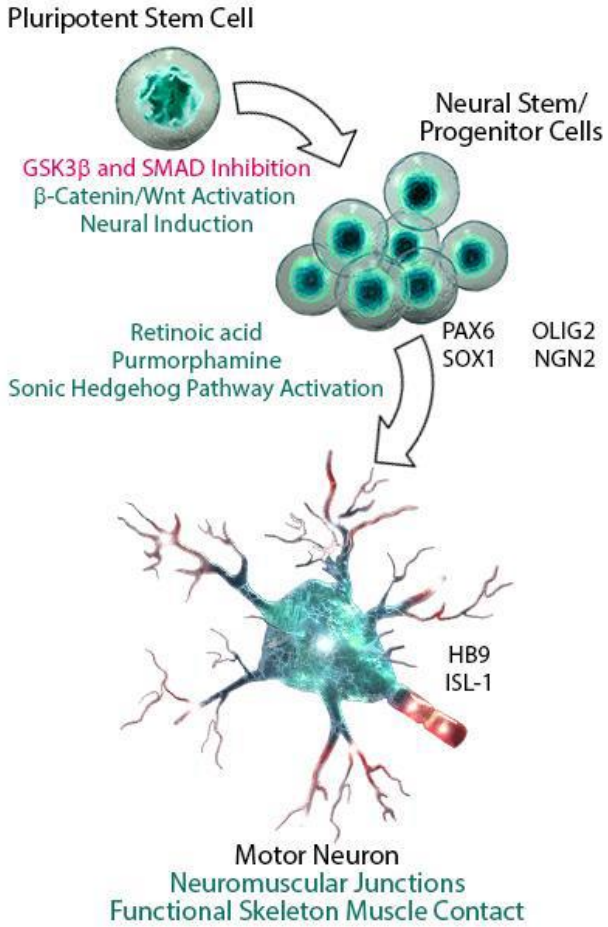
Perform HTS/HCS for
Natural Product(NP) lib

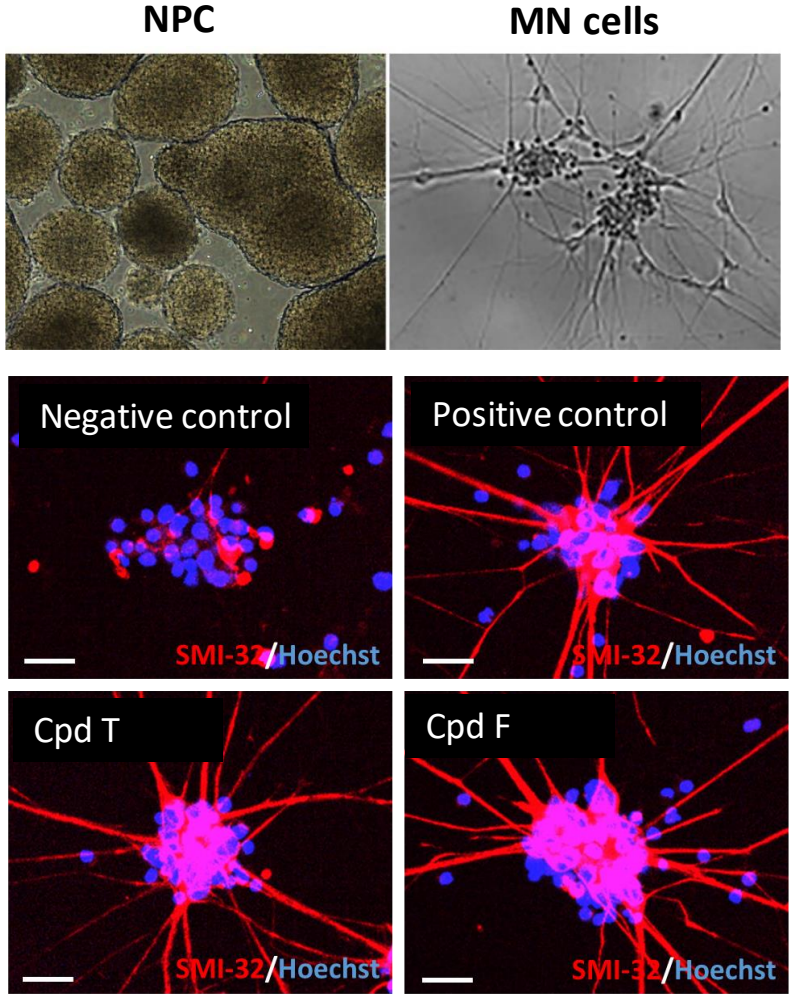
August – October 2019
Assay optimization

# iPSC-derived MN cell death assay project




**Motor neuron (MN) deficiency**



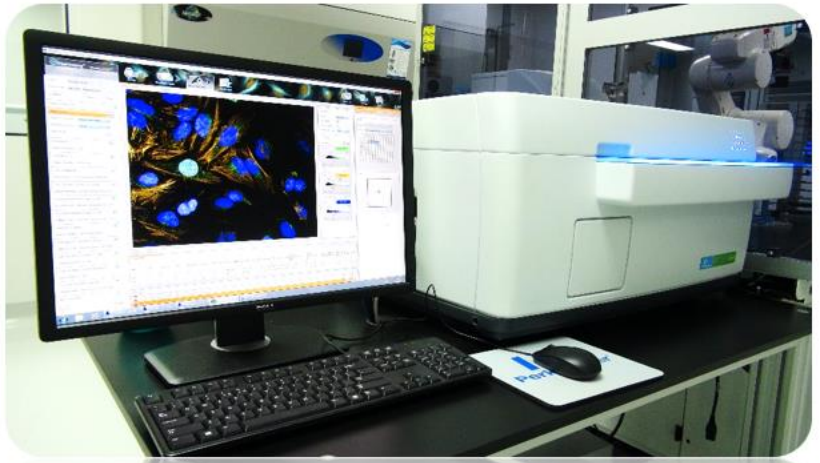

Note: SMI-32 = Neurofilament H
Hoechst = Nucleus
Scale bar = 25 um

# ECDD High-Throughput and High-Content Screening Platform

Robotic system

Compound plate

Assay plate (Cell plate)



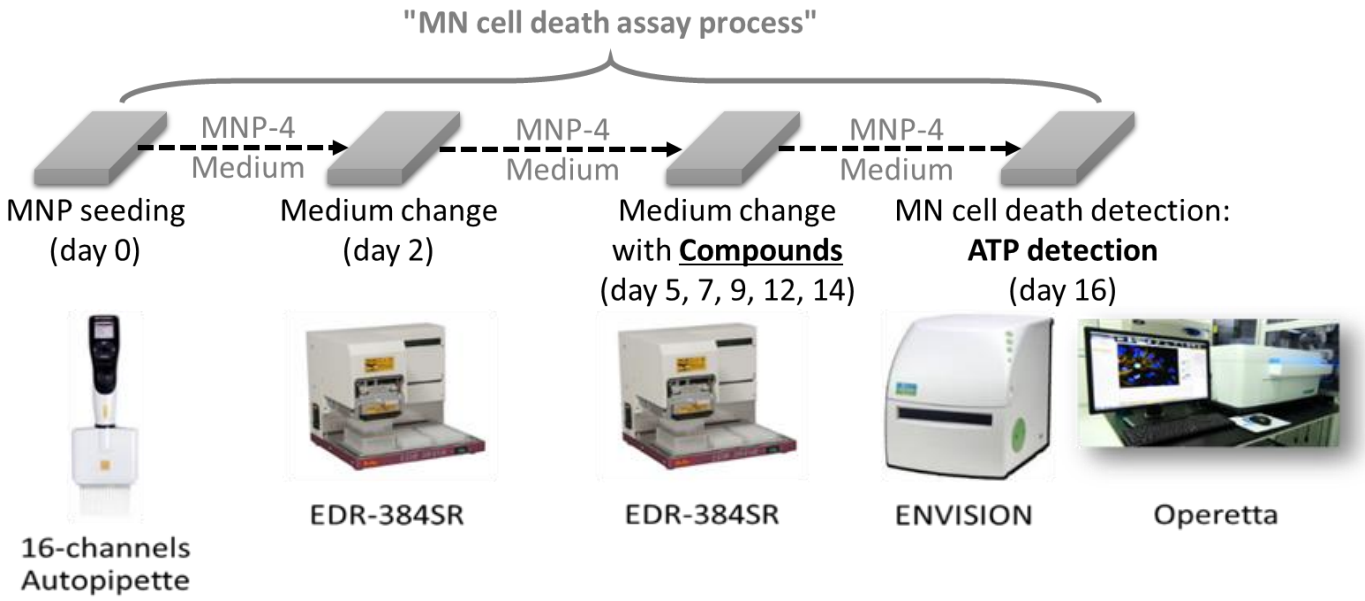
High-Content Imaging system

established in 2016:
TCELS-Mahidol University
(SC and MD Ramathibodi Hospital)

# Motor neuron cell death assay

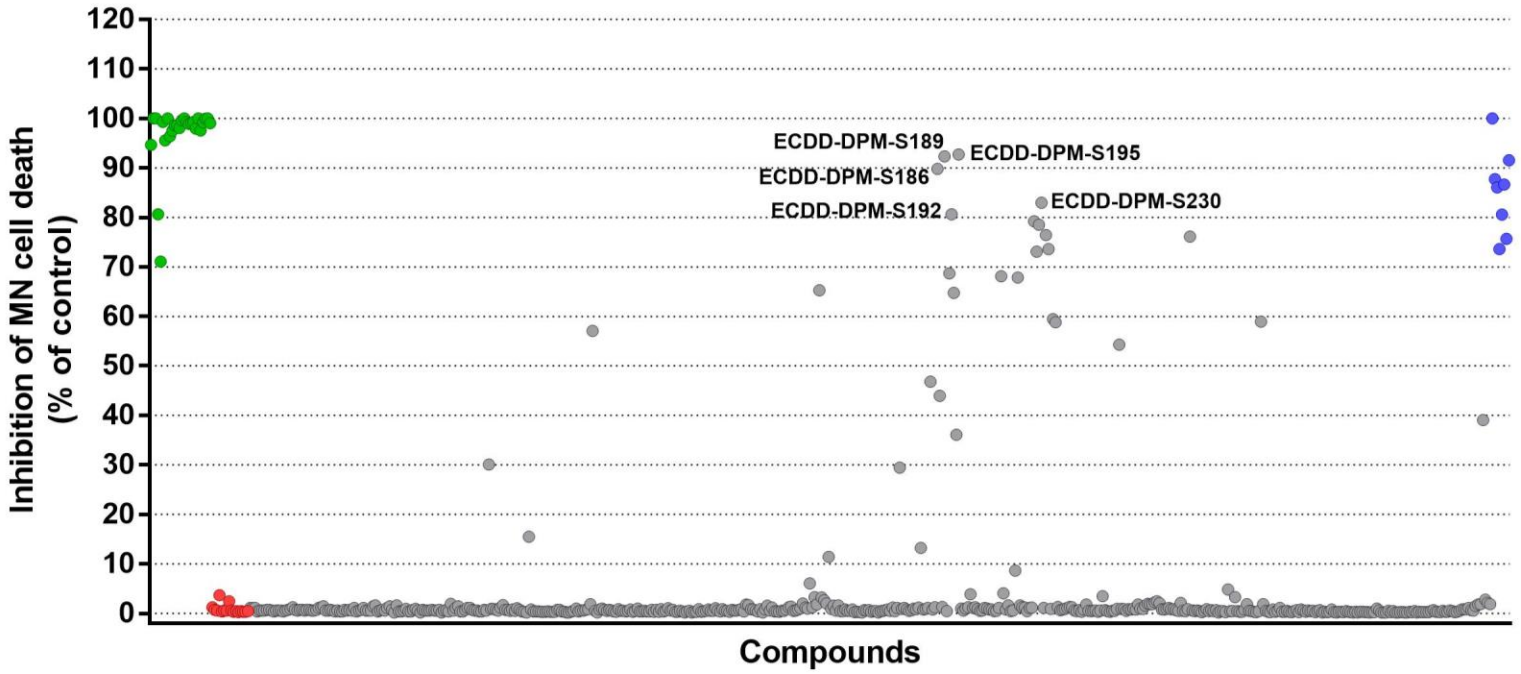
## Methodology

- Cell numbers: 7,500 cells/well
- Concentration of compounds for screening:
  - Crude extract = 10 µg/ml
  - Purified compound = 10 µM
  - Synthesis compound = 10 µM

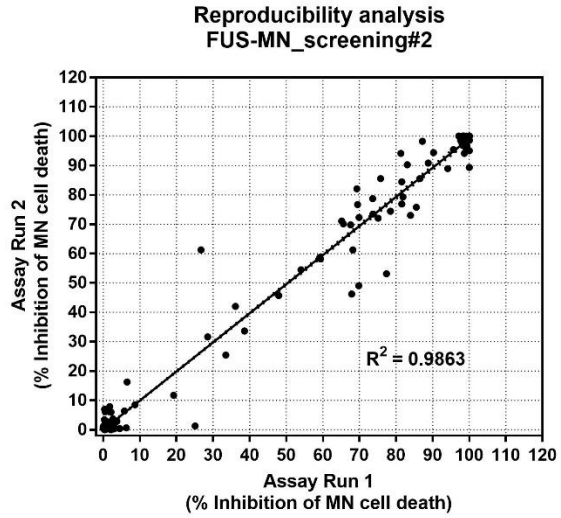


# Motor neuron cell death assay

## The Results of an anti-MN cell death screening



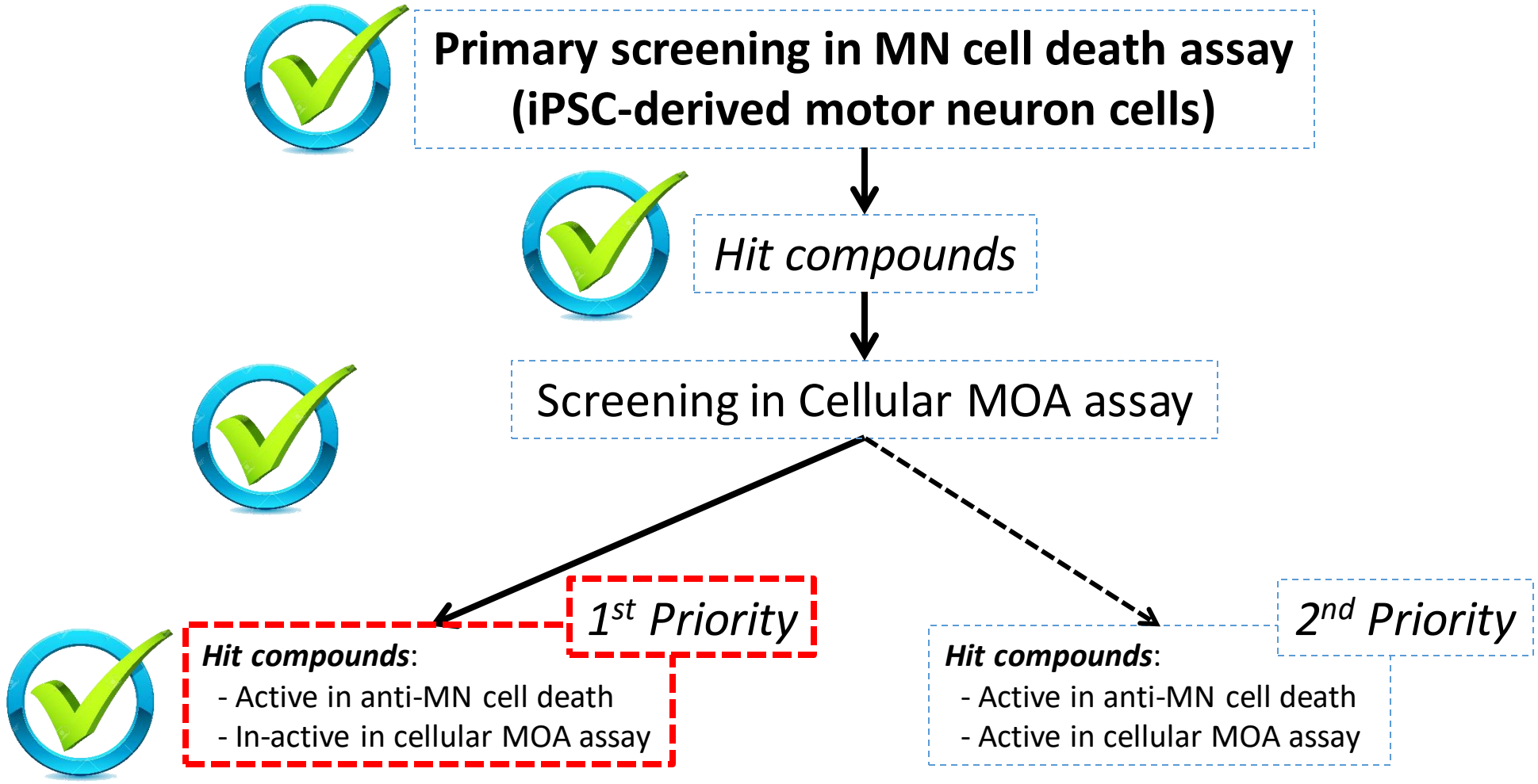

- Positive control
- Negative control
- Compounds
- Ferrostatin-1



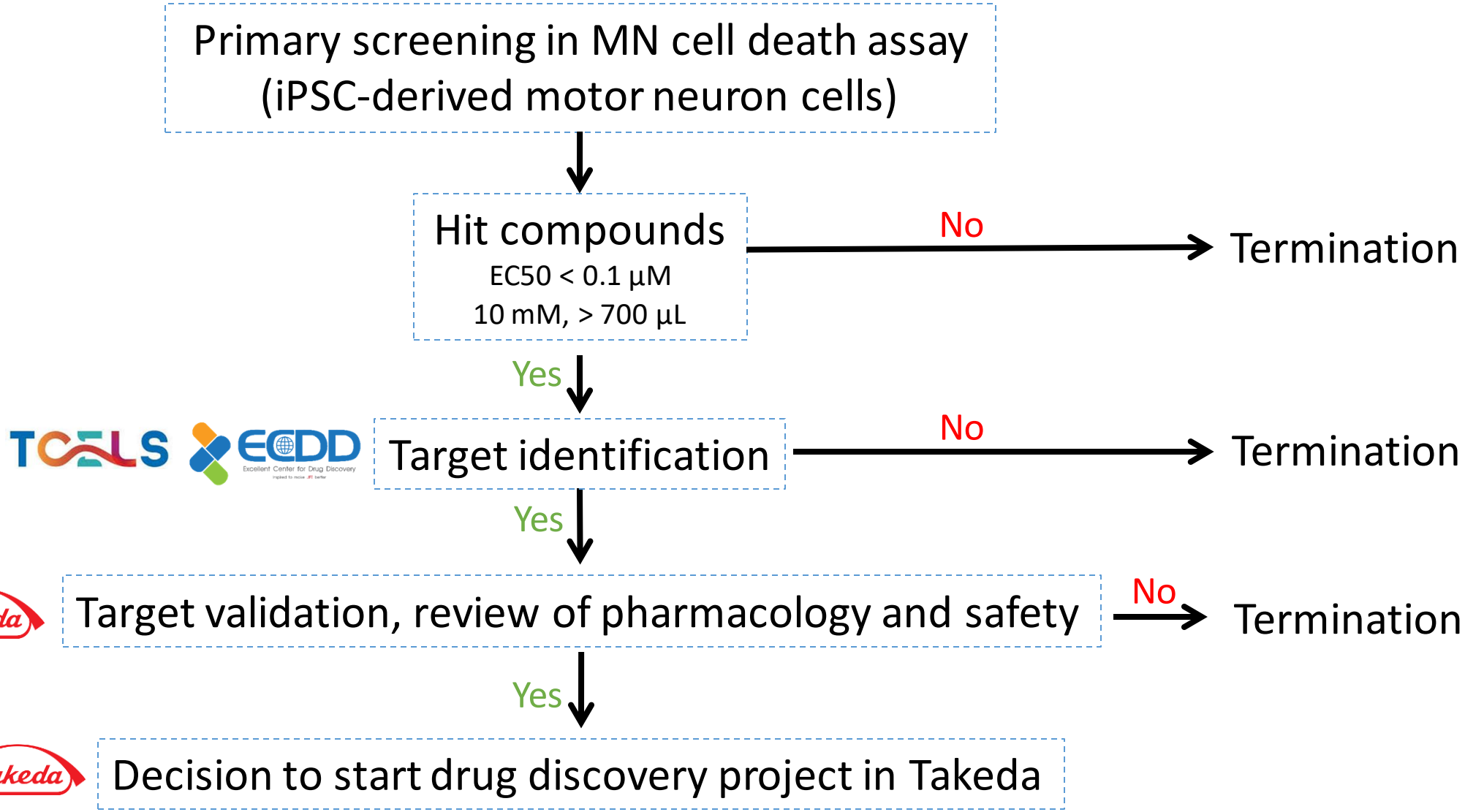

**Z-factor = 0.688**

# iPSC-derived MN cell death assay project

*Screening Process*



# Flow of collaboration, decision, plans and actions



# iPSC-derived MN cell death assay project

## ☆ First priority selection (IC50 < 0.1 for cellular MOA assay)

| No. | Compounds | IC50 MN | IC50 MOA assay |
|---|---|---|---|
| 1 | ECDD-DPM-N924 | 6.77 | non-effective |
| 2 | ECDD-DPM-N937 | 3.06 | non-effective |
| 3 | ECDD-DPM-S186 | 3.06 | 6.56 |
| 4 | ECDD-DPM-S189 | 5.95 | 4.87 |
| 5 | ECDD-DPM-S192 | non-effective | 16.97 |
| 6 | ECDD-DPM-S195 | 1.2 | 4.45 |
| 7 | ECDD-DPM-S230 | 3.32 | non-effective |
| 8 | ECDD-DPM-E971 | non-effective | non-effective |
| 9 | ECDD-DPM-E1095 | 5.69 | non-effective |
| 10 | ECDD-DPM-E1168 | 1.85 | non-effective |
| 11 | ECDD-DPM-E1171 | 0.05 | non-effective |
| 12 | ECDD-DPM-E1183 | 1.46 | 9.26 |
| 13 | ECDD-DPM-E1204 | 0.38 | non-effective |
| 14 | ECDD-DPM-E1211 | 3.53 | non-effective |
| 15 | ECDD-DPM-E1215 | 9.52 | non-effective |
| 16 | ECDD-DPM-S685 | non-effective | 7.57 |
| 17 | ECDD-DPM-S777 | 3.13 | non-effective |
| 18 | ECDD-DPM-S443 | 0.08 | 3.25 |
| 19 | ECDD-DPM-S461 | 0.63 | non-effective |
| 20 | ECDD-DPM-S465 | 0.33 | non-effective |

| No. | Compounds | IC50 MN | IC50 MOA assay |
|---|---|---|---|
| 21 | ECDD-DPM-N131 | non-effective | non-effective |
| 22 | ECDD-DPM-N190 | 0.2 | 1.47 |
| 23 | ECDD-DPM-N197 | 3.54 | 6.11 |
| 24 | ECDD-DPM-N664 | 3.54 | non-effective |
| 25 | ECDD-DPM-N745 | 0.12 | non-effective |
| 26 | ECDD-DPM-E278 | 0.07 | 0.52 |
| 27 | ECDD-DPM-E326 | 0.24 | non-effective |
| 28 | ECDD-DPM-E329 | 0.06 | non-effective |
| 29 | ECDD-DPM-E651 | 5.15 | non-effective |
| 30 | ECDD-DPM-E691 | 10.44 | non-effective |
| 31 | ECDD-DPM-E707 | 0.66 | non-effective |
| 32 | ECDD-DPM-E760 | non-effective | non-effective |
| 33 | ECDD-DPM-E824 | 2.91 | 4.44 |
| 34 | ECDD-DPM-E825 | 5.05 | non-effective |
| 35 | ECDD-DPM-E829 | non-effective | non-effective |
| 36 | ECDD-DPM-E831 | 2.86 | non-effective |
| 37 | ECDD-DPM-E865 | non-effective | non-effective |
| 38 | ECDD-DPM-E868 | 7.31 | non-effective |
| 39 | ECDD-DPM-E869 | 3.64 | non-effective |
| 40 | ECDD-DPM-E873 | 3.51 | non-effective |

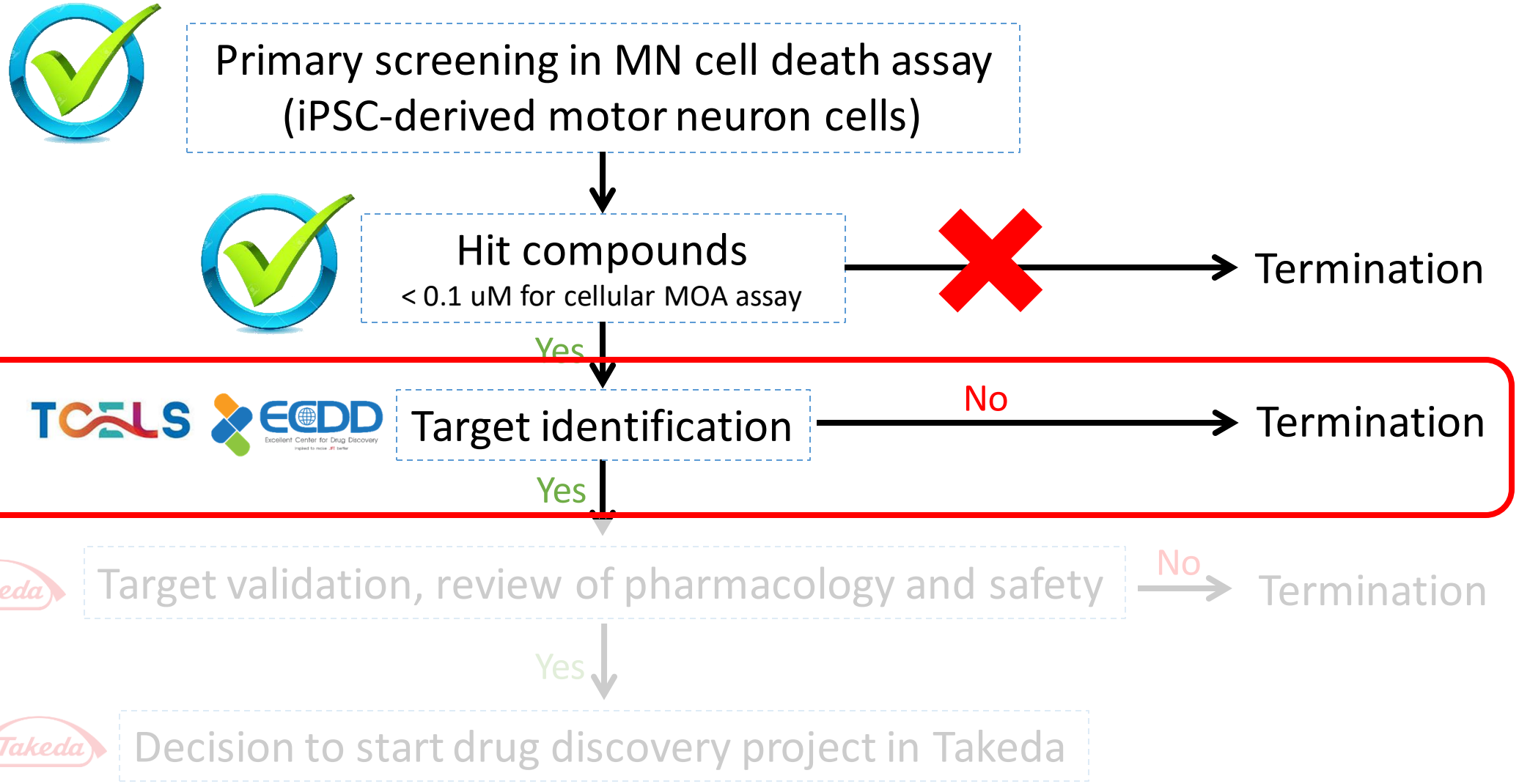
Prof. Wanchai De-Eknamkul

Dr. Lily Eurwilaichitr

Assoc. Prof. Dr. Surat Laphookhieo

Prof. Patoomratana Tuchinda

# Flow of collaboration, decision, plans and actions



# Flow-chart for Initiation of target deconvolution

**To plan the experiments for Target deconvolution**

↓

*Assay optimization & validation*

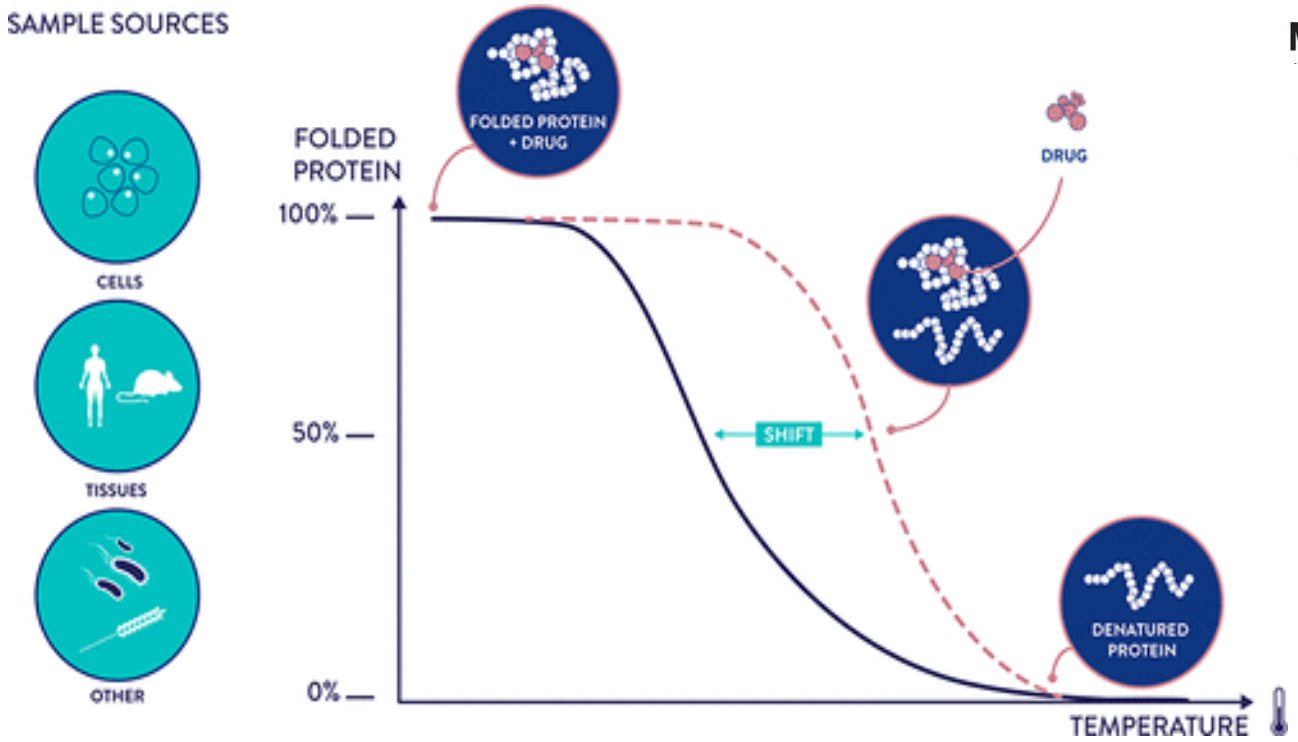
↓

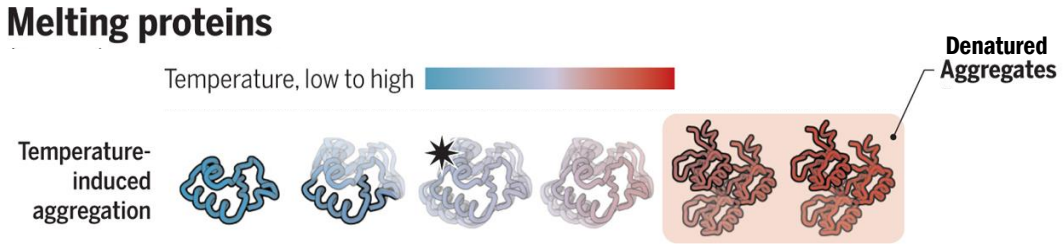
*Potential targets of hit compounds*

**Dr. Sitthivut Charoensutthivarakul**
School of Bioinnovation, Mahidol University

# Plan the experiment for target deconvolution

- **Thermal shift assay**

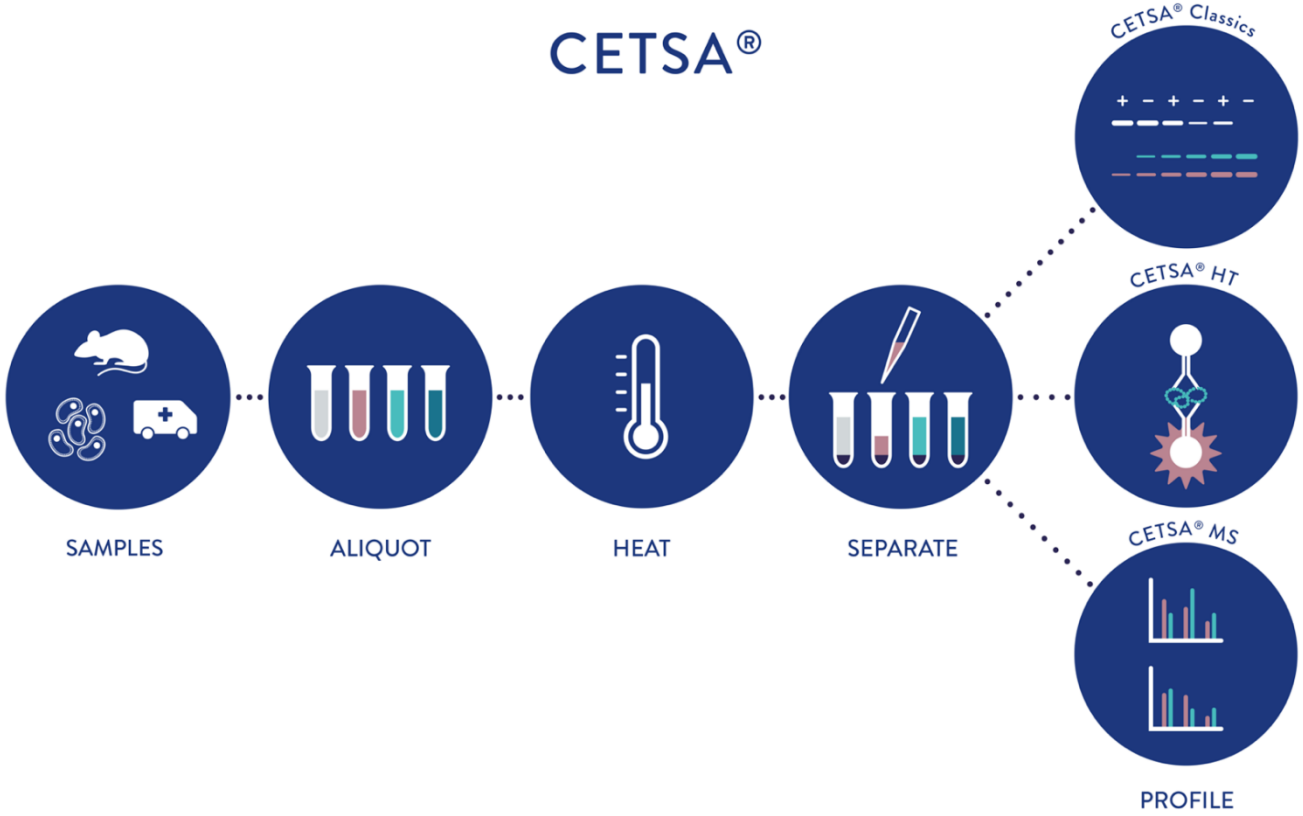



A **thermal shift assay** (**TSA**) measures changes in the thermal denaturation temperature. Upon heating a protein will encounter a temperature at which it denatures, referred to as the melting point. This melting temperature is a physical property and a constant for any given set of conditions. Compounds that interact with a protein will change the melting temperature (thermal shift).

# Plan the experiment for target deconvolution

- **Experimental workflow**




The **CE**llular **T**hermal **S**hift **A**ssay is a method that allows the quantification of a compound's target engagement within living cells or in disrupted cells.

The CETSA principle is based on the change in thermal denaturation profile of the target protein that occurs following the binding of a compound. However, in contrast to traditional Therma Shift Assay, that are carried out in highly purified and isolated systems monitoring a single protein species, CETSA can be performed in complex protein samples and in live cells.

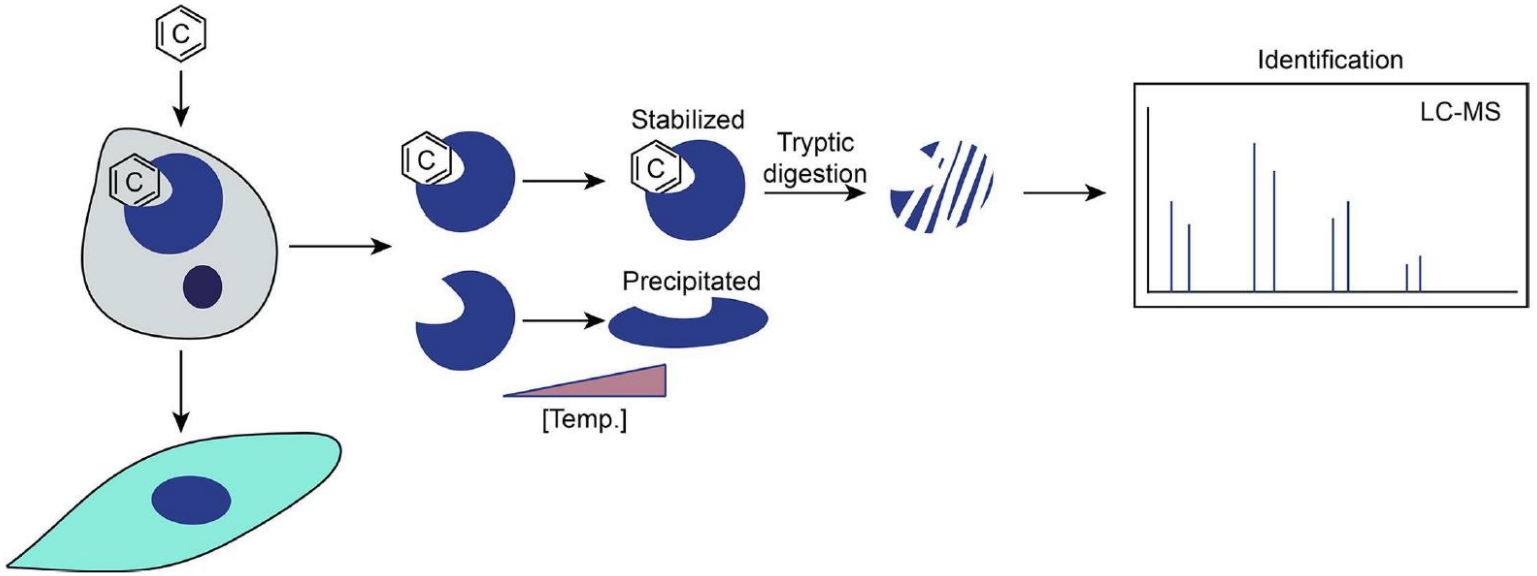
The CETSA is performed by incubating the cells with the test compound, followed by heating of the compound-treated cells, and then by measuring the remaining soluble target protein.

There are three main formats in the CETSA technology platform. Two of the formats, CETSA Classics and CETSA High Throughput (HT) are both targeted CETSA methods for confirming target engagement of a single known protein target using antibodies for the quantification. The third format, CETSA MS, is proteome-wide measurement of cellular target engagement using mass spectrometry.

# Plan the experiment for target deconvolution

- **CETSA-MS**

MS-based CETSA® for target deconvolution in phenotypic drug discovery




https://www.sciencedirect.com/science/article/pii/S0968089619309174

The antibody-based readout enables CETSA to measure the stability shifts of different target proteins in a protein mixture, while this method requires prior knowledge of interested targets. Thus, it is not suitable for unbiased drug target discovery and also cannot be conducted at proteome scale.

To solve these problems, The Cellular thermal shift assay followed by MS (CETSA-MS) allows for an unbiased search of drug targets and can be applied in living cells without requiring compound labeling.

To date CETSA-MS has been used in several studies for target deconvolution, which have both confirmed previously known compound – target interactions and discovered new ones.

# Flow of collaboration, decision, plans and actions

Primary screening in MN cell death assay
(iPSC-derived motor neuron cells)

↓

Hit compounds
EC50 < 0.1 µM
10 mM, > 700 µL

No → Termination

Yes ↓

Target identification

No → Termination

Yes ↓

Target validation, review of pharmacology and safety

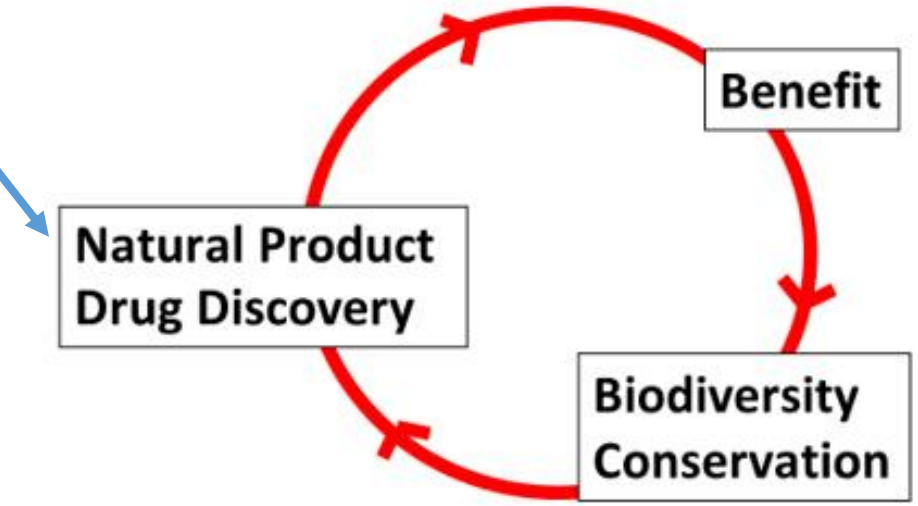
No → Termination

Yes ↓

Decision to start drug discovery project in Takeda

# NPDD Ecosystem in Asia: An Open Innovation Platform

ECDD, HTS/HCS platform in Thailand




Thailand: Top 20 countries in megabiodiversity

**New Technology**

Screening technology for extracts
Synthetic biology using microbes
Analog synthesis using microbes
Other s

**New Platform**

Construct isolated compound library
Construct mid size compound library
Construct DB for Dereplication
Others

**Drug Discovery Capability of Pharma Industry in Japan**

Thank you