

# Tecan Innovation Day

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## Nerviano Medical Sciences S.r.l. (NMS)

- Nerviano Medical Sciences (NMS) is a research-based Italian company dedicated to the discovery and development of new drugs for the treatment of cancer.



- NMS is the Drug Discovery branch of NMS Group.



- NMS Group affiliates provide preclinical development, manufacturing and clinical CRO services to Academia, Hospitals, Biotechs and Pharmaceutical Companies worldwide.



Preclinical development



Pharmaceutical development  
Manufacturing

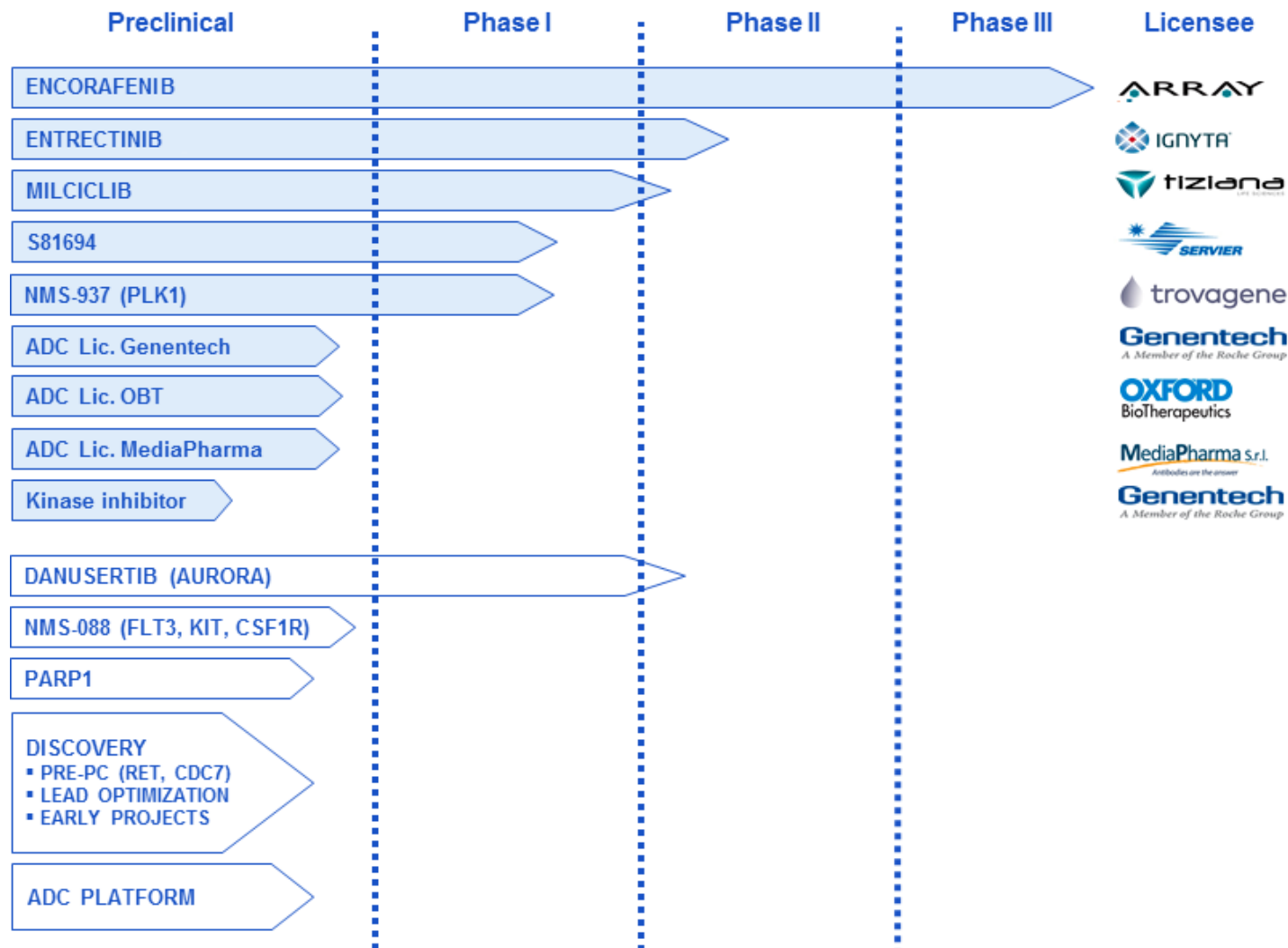


Clinical development



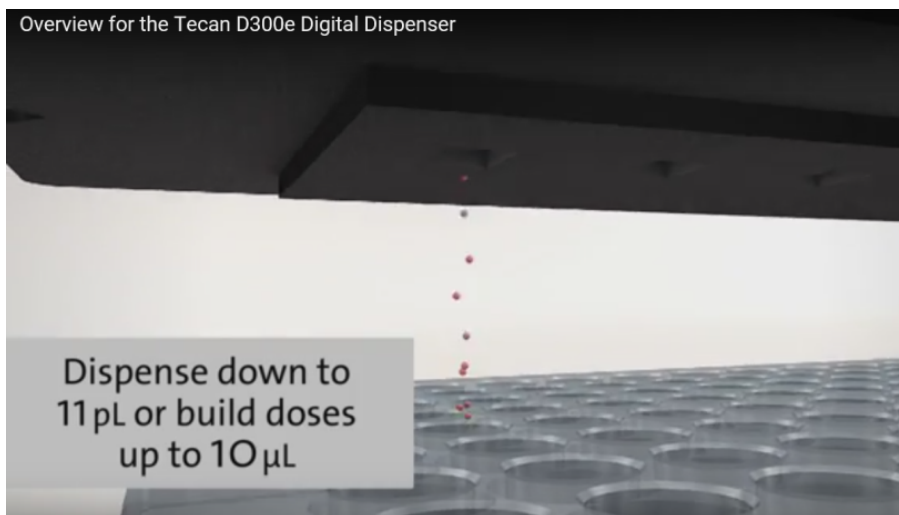
## NMS pipeline

The current portfolio of projects includes a number of anticancer compounds in Phase I-III clinical trials, others in advanced preclinical development, as well as target identification and early discovery activities. The pipeline includes several programs in collaboration with third parties.





## Overview of the Tecan D300e Digital dispenser



 **TECAN.**



# D300e Digital dispenser: technical specifications

## DISPENSING FEATURES



T8+ Dispensehead Cassette



D4+ Dispensehead Cassette

Fluid type	DMSO	Aqueous + surfactant	Aqueous + surfactant + glycerol	Master mix	DMSO, Aqueous + surfactant, Aqueous + surfactant + glycerol, Master mix
Use for	Titration, Low volume dispensing and normalization of master mixes, aqueous fluids (+ surfactants) and DMSO				
Dispenseheads per cassette	8			4	
Minimum dispense volume	13 pl	11 pl	12 pl	11 pl	1 nl
Minimum dispense volume (Total dispense volume >10 µl)	1 nl	1 nl	1 nl	1 nl	1 nl
Maximum dispense volume per well	10 µl	10 µl	10 µl	10 µl	10 µl
Minimum fill volume	2 µl	4 µl	4 µl	4 µl	50 µl
CV	< 8%*	< 8%**	< 8%**	< 8%***	< 8%****

\* for volumes >100 pl using 100 % DMSO

\*\* for volumes >100 pl using aqueous test fluid containing 0.1 % Brij™-35

\*\*\* for volumes >100 pl using Master mixes included in the fluid class list

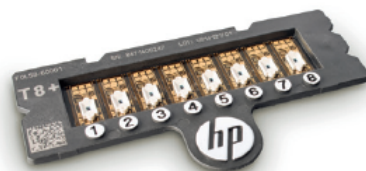
\*\*\*\* for volumes >1 nl using test fluids listed above and Master mixes included in the fluid class list





## D300e Digital dispenser: fluid specifications

### FLUID REQUIREMENTS



T8+ Dispensehead Cassette



D4+ Dispensehead Cassette

Maximum total dispense volume	10 µl	20 µl	200 µl
Small molecules	Max. 800 Da at 10 mM	Max. 800 Da at 10 mM	Max. 800 Da at 10 mM
Biomolecules	300 kDa, 3 mg/ml	300 kDa, 1 mg/ml	300 kDa, 0.3 mg/ml
	10,000 bp, 3 mg/ml	10,000 bp, 1 mg/ml	10,000 bp, 0.3 mg/ml
Nanoparticles	Stable suspension of < 1 µm diameter particles at max. 0.5 % w/v		
Total buffer components <150 mM			

### FLUID CLASSES

Aqueous solutions require the addition of a surfactant to reduce surface tension and enable priming. In addition, the presence or absence of glycerol impacts dispensing – there are separate fluid classes dependent on glycerol content.

Fluid class	Surfactant	Surfactant concentration in stock solution	Glycerol
a + B	Brij-35	0.1 %	0-5 %
a + B + g	Brij-35	0.1 %	5-20 %
a + TX	Triton® X-100	0.1 %	0-5 %
a + TX + g	Triton X-100	0.1 %	5-20 %
a + Tw	Tween™ 20	0.3 %	0-5 %
a + Tw + g	Tween 20	0.3 %	5-20 %



# D300eControl

D300eControl

Home Advanced Current Protocol

Run Undo Cut Wells Copy Wells Copy All Wells Paste Paste Special Clipboard

Set Value Titration Targeted Titration Synergy Quick Plate Enzyme Profile PCR Normalize Randomize One Plate All Plates View

Fluids +

Plates +

1 of 1

Plate 1

Additional volume: 100  $\mu$ L DMSO limit: 1% ☐

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Show

All fluids



## D300eControl: define fluids and plates

**D300eControl**

Home | Advanced | Current Protocol

Run | Undo | Cut Wells | Paste | Copy Wells | Paste Special | Copy All Wells | Clipboard

Set Value | Titration | Targeted Titration | Synergy | Quick Plate | Enzyme Profile | PCR | Normalize | Randomize | One Plate | All Plates | View

**Fluids** + **Plates** + 6 of 6

**Fluid Edit**

1 of 1 +

Name: Fluid 1

Class: DMSO-based

Concentration: 10 mM

Dispense by: Concentration

Source plate:

Source well:

Color: 1

Dispense: Yes

OK Cancel

**Plate Edit**

1 of 1 +

Plate type: 96 well

Additional volume (μL): 100

DMSO limit (%): 1

Name:

☐ Don't shake

OK Cancel

**Plates**

Plate 1: Additional volume: 800 μL DMSO limit: 1%  
1 1 2 3 4  
A  
B  
C

Plate 2: Additional volume: 400 μL DMSO limit: 1%  
2 1 2 3 4 5 6  
A  
B  
C  
D

Plate 3: Additional volume: 200 μL DMSO limit: 1%  
3 1 2 3 4 5 6 7 8  
A  
B  
C  
D  
E  
F

Plate 4: Additional volume: 100 μL DMSO limit: 1%  
4 1 2 3 4 5 6 7 8 9 10 11 12  
A  
B  
C  
D  
E  
F  
G  
H

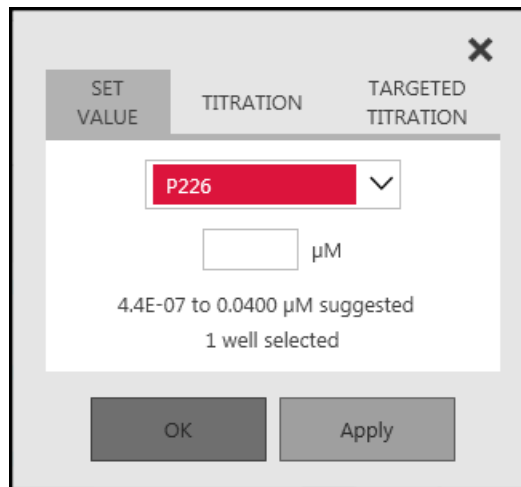
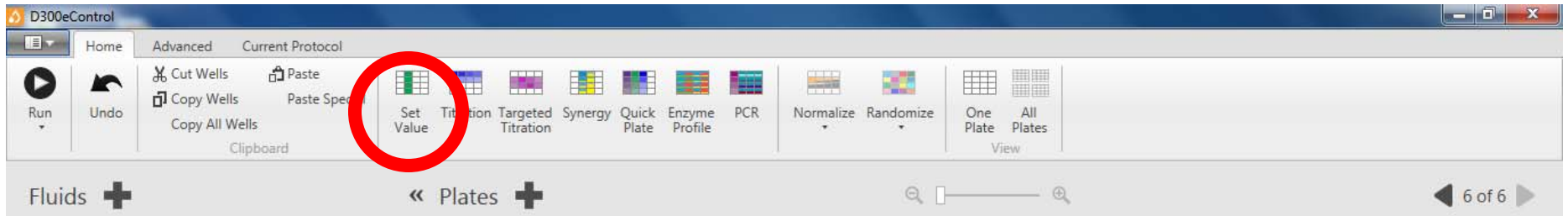
Plate 5: Additional volume: 50 μL DMSO limit: 1%  
5 1 2 3 4 5 6 7 8 9 10 11 12  
A  
B  
C  
D  
E  
F  
G  
H

Don't Shake: Additional volume: 10 μL DMSO limit: 1%  
6 1 2 3 4 5 6 7 8 9 10 11 12  
A  
B  
C  
D  
E  
F  
G  
H



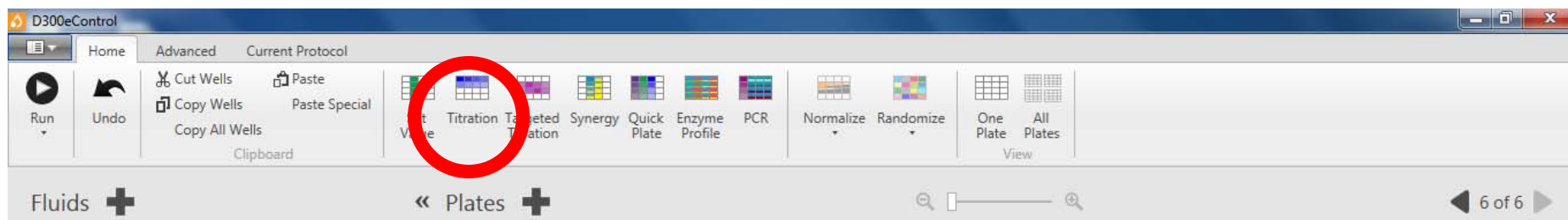


## D300eControl: single concentration





## D300eControl: titration



SET VALUE TITRATION TARGETED TITRATION

Specify titration using

- ☒ Highest and lowest concentrations
- ☐ Highest concentration
- ☐ Lowest concentration

Fluid: P226

Highest concentration:   $\mu\text{M}$

Lowest concentration:   $\mu\text{M}$

Distribution: Logarithmic

Replicates per level: 2

Titration pattern:

Paste range is 4.4E-07 to 0.0400  $\mu\text{M}$  20 wells selected

OK Apply

SET VALUE TITRATION TARGETED TITRATION

Specify titration using

- ☐ Highest and lowest concentrations
- ☒ Highest concentration
- ☐ Lowest concentration

Fluid: P226

Highest concentration:   $\mu\text{M}$

Distribution: 1:2 (50%)

Replicates per level: 1/2 Log

Titration pattern: 1:2 (50%)

Paste range is 4.4E-07 to 0.0400  $\mu\text{M}$  20 wells selected

OK Apply

SET VALUE TITRATION TARGETED TITRATION

Specify titration using

- ☐ Highest and lowest concentrations
- ☐ Highest concentration
- ☒ Lowest concentration

Fluid: P226

Lowest concentration:   $\mu\text{M}$

Distribution: 1:2 (50%)

Replicates per level: 1/2 Log

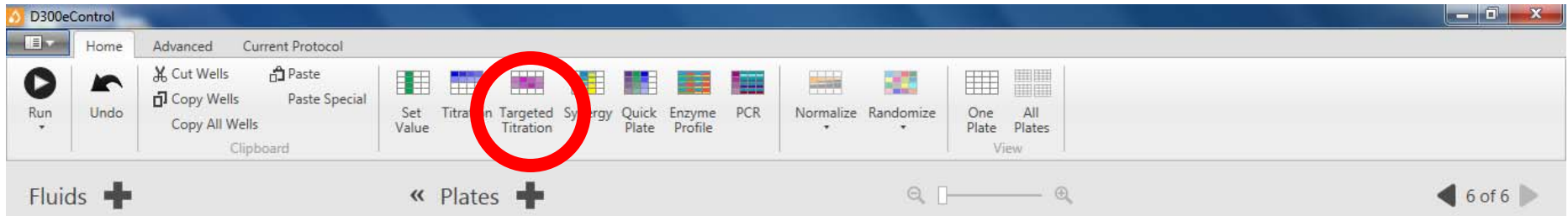
Titration pattern: 1:2 (50%)

Paste range is 4.4E-07 to 0.0400  $\mu\text{M}$  20 wells selected

OK Apply



## D300eControl: targeted titration



SET VALUE TITRATION TARGETED TITRATION

Fluid **P226** ▼

Highest concentration   $\mu\text{M}$

Lowest concentration   $\mu\text{M}$


Distribution Logarithmic ▼


Target   $\mu\text{M}$

Target region  levels

$\pm$  Target range  log

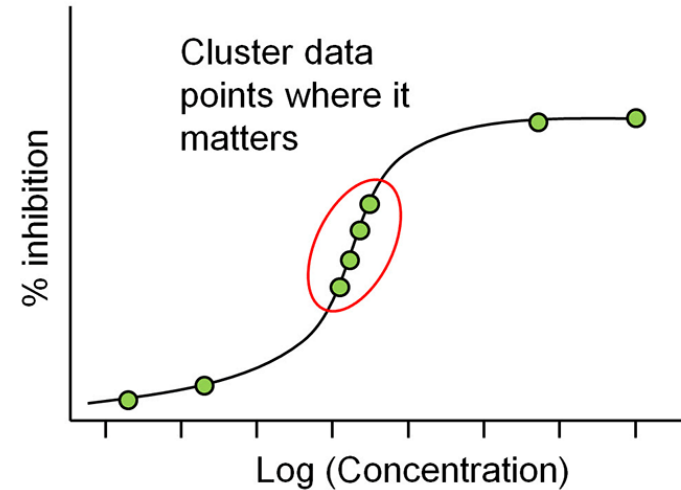
Replicates per level  2

Titration pattern  ▼

Paste range is 4.4E-07 to 0.0400  $\mu\text{M}$  

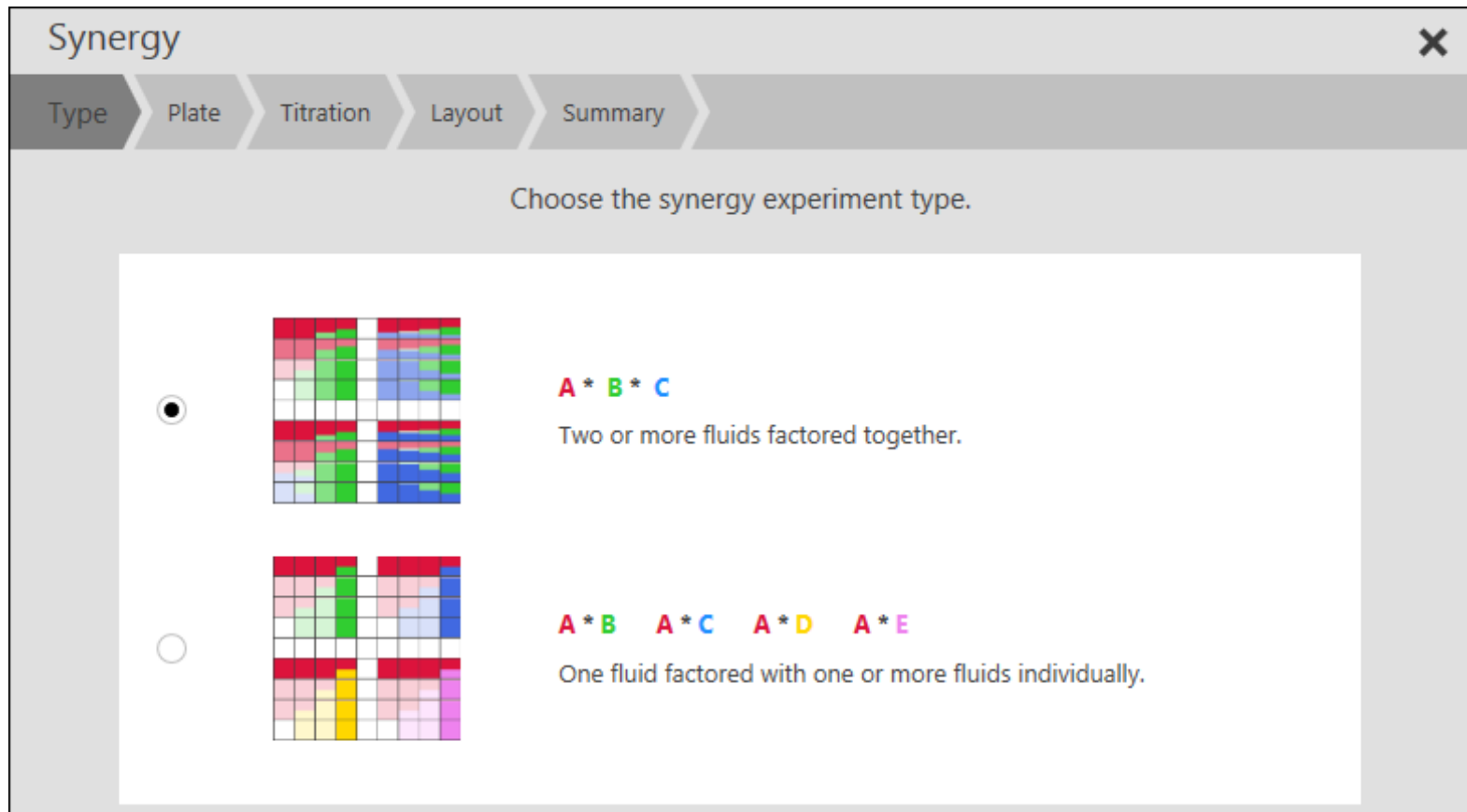
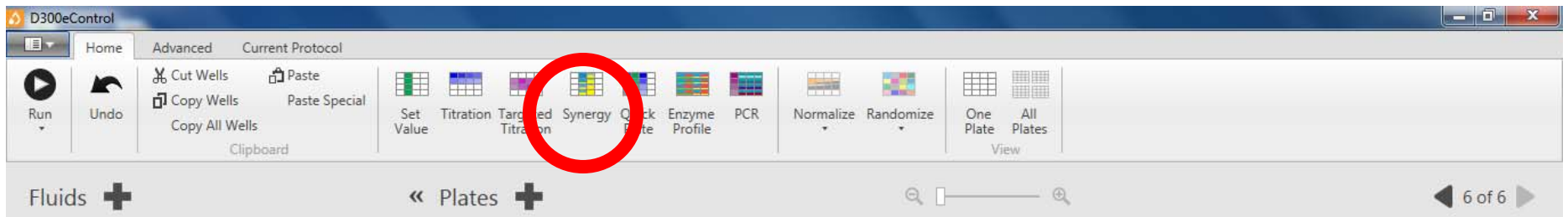
20 wells selected

OK Apply



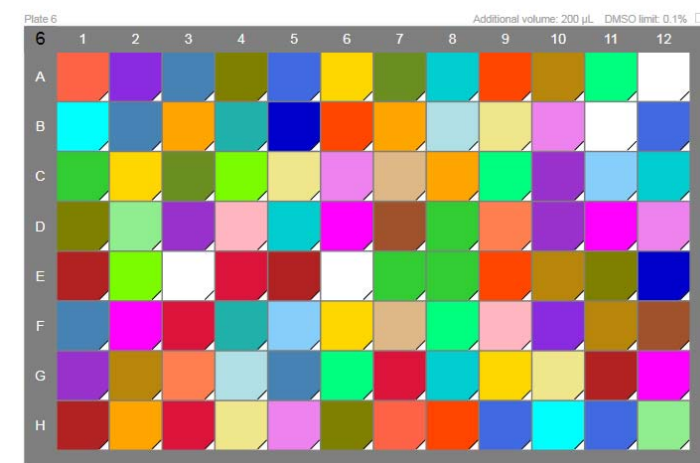
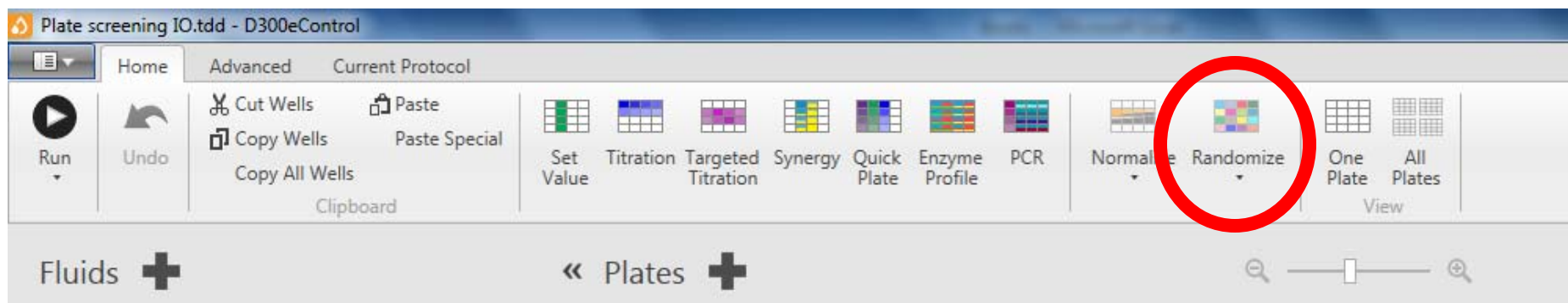


## D300eControl: synergy





## D300eControl: randomization





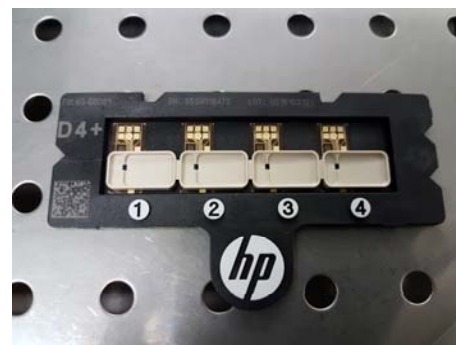


## Tecan Digital dispensers available at NMS (since 2015)

- Instruments installed under laminar flow cabinet for sterile treatments
- Multiuser environment
- D4+ and T8+ cassettes
- Dispensing of aqueous and DMSO solutions in 96-w and 384-w plates

D4+ Dispensehead cassettes

from 1 nL to 10  $\mu$ L



T8+ Dispensehead cassettes

from 11 pL to 10  $\mu$ L



D300 Digital dispenser

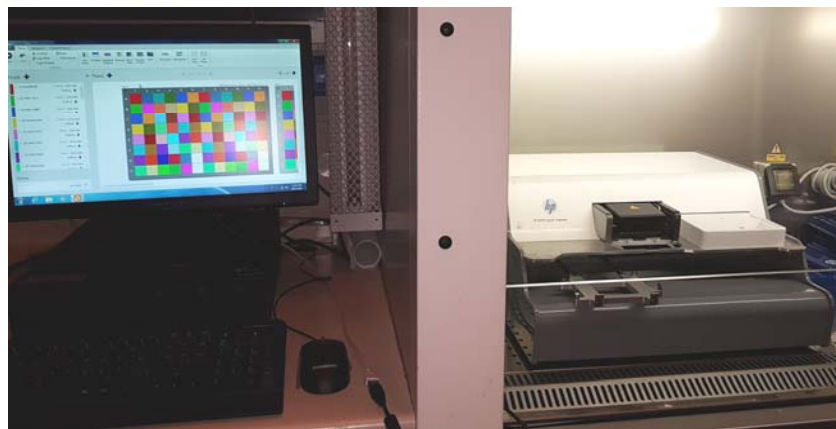


D300e Digital dispenser





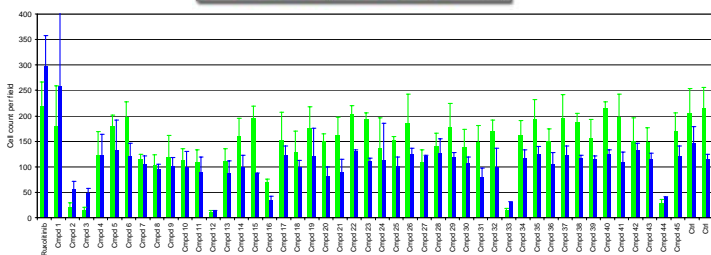
## Tecan Digital dispensers: common applications at NMS



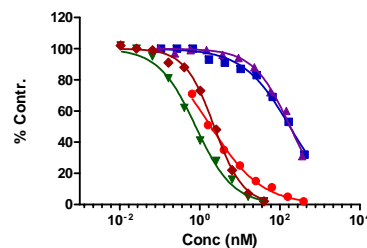
Treatment of cell cultures with compound solutions, as single treatment or in combinations:

- Small molecules
- Small molecule-drug conjugates
- Antibodies and Antibody-Drug Conjugates (ADC)
- siRNA oligos

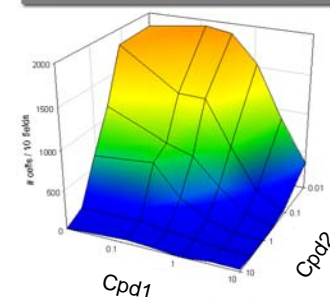
### Compound screening



### Dose-response single treatment



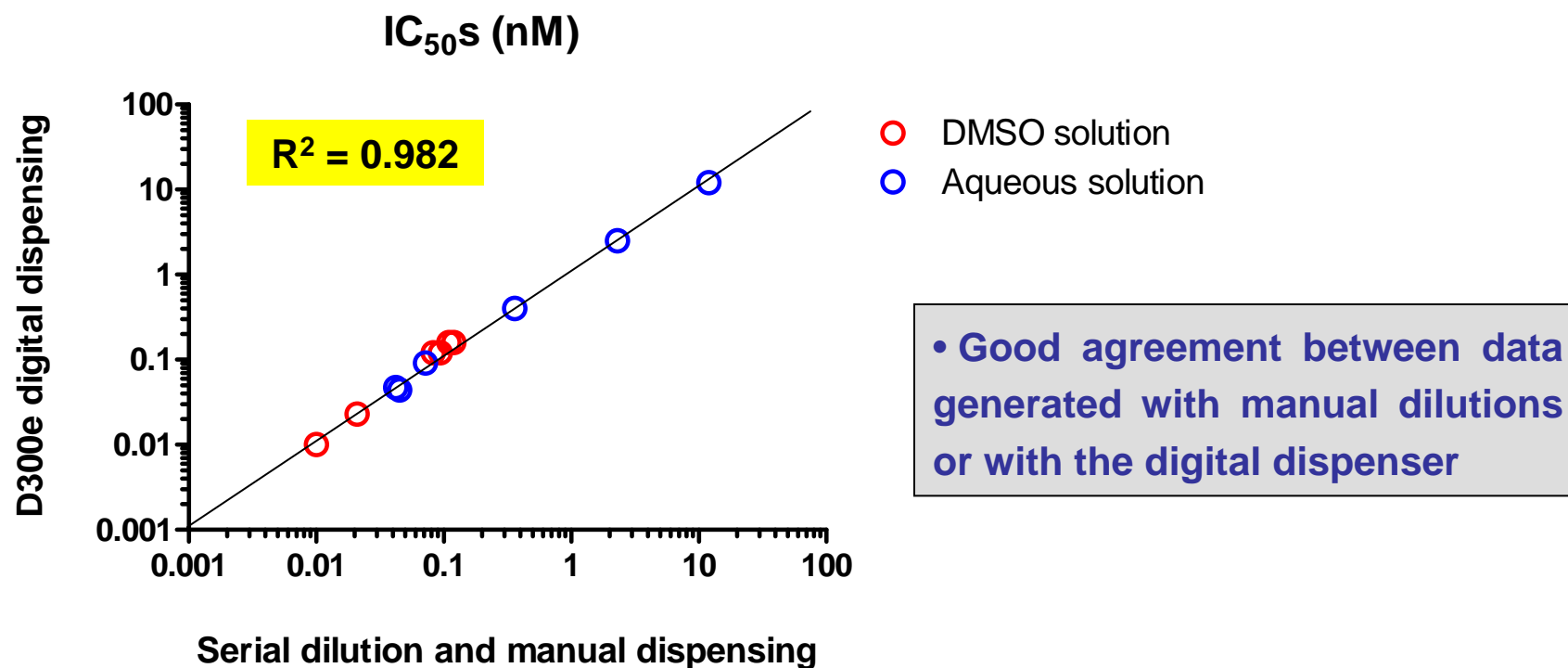
### Compound combinations





## Dose-response treatment: manual Vs. digital dispensing

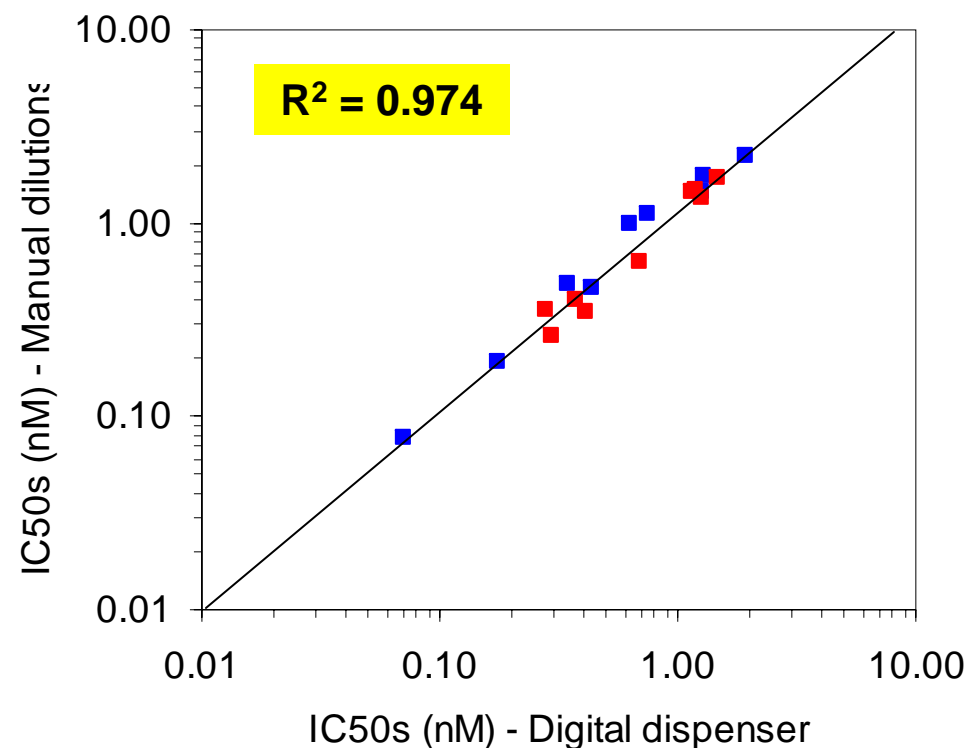
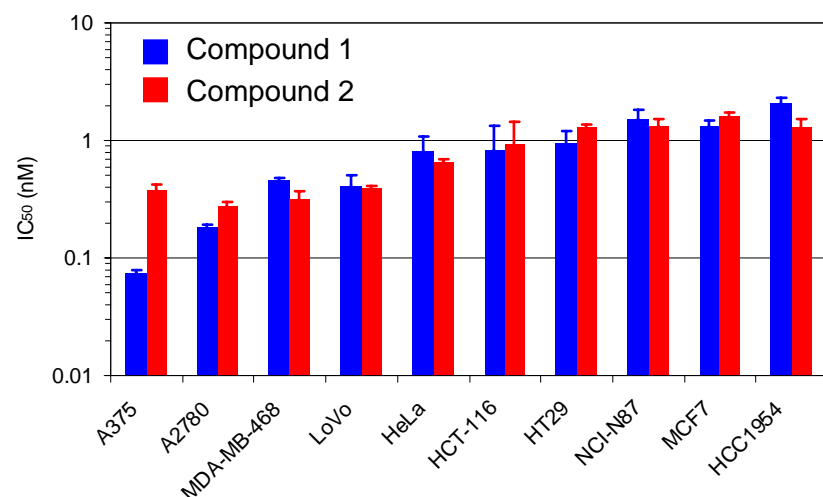
12  $IC_{50}$  values were calculated from dose-response curves generated as indicated  
(HeLa, 72 h treatment, CellTiter Glo, luminescence)





## Proliferation assays with sub-nanomolar cytotoxic drugs

IC<sub>50</sub> values were calculated from dose-response curves generated as indicated  
(144 h treatment, CellTiter Glo, luminescence)



- Two sub-nanomolar cytotoxic drugs showed similar activity profile on a panel of 10 cell lines
- Good agreement between data generated with manual dilutions or with the digital dispenser

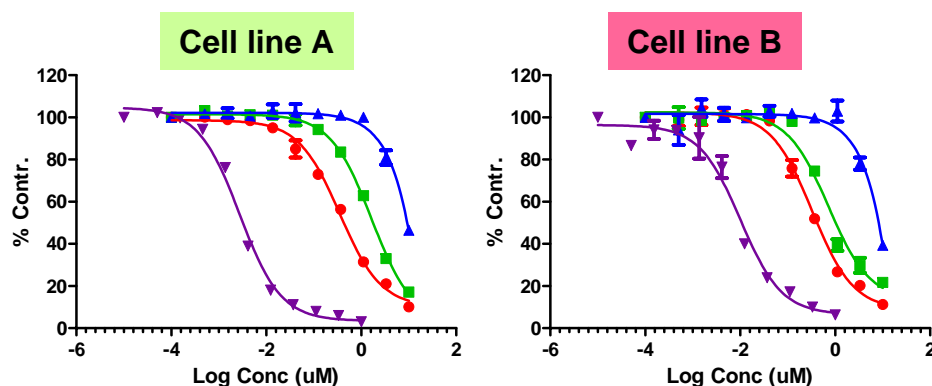


## Proliferation assays with compounds diluted in different solvents

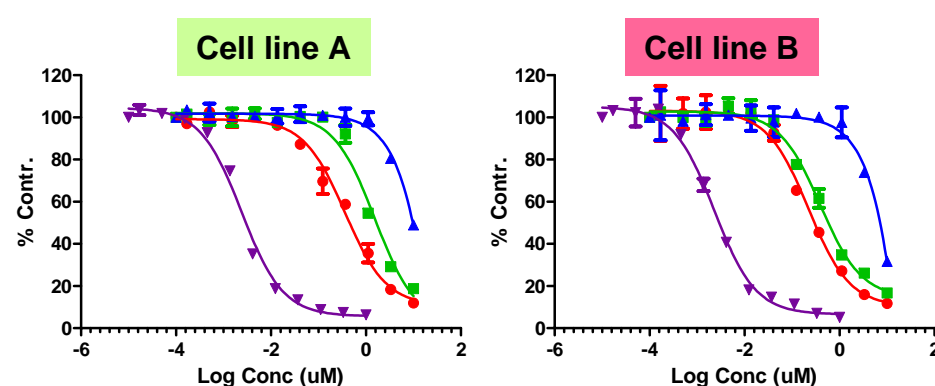
- Reliability of D300e dispensation is not affected by compound dilution in different solvents (DMSO or water)

- Compound 1 (DMSO)
- Compound 1 (Water)
- Compound 2 (DMSO)
- Paclitaxel

Manual dispensing



Digital dispenser D300e (Tecan)



Manual  
dispensing

IC <sub>50</sub> (nM)	Compound 1 (DMSO)	Compound 1 (Water/EtOH)	Compound 2 (DMSO)	Paclitaxel
Cell line A	351.8	1653	10000	2.75
Cell line B	329.2	722	9700	10.1

Digital  
dispenser  
D300e (Tecan)

IC <sub>50</sub> (nM)	Compound 1 (DMSO)	Compound 1 (Water/EtOH)	Compound 2 (DMSO)	Paclitaxel
Cell line A	362.0	1546	10000	2.42
Cell line B	221.9	390.4	9500	2.31

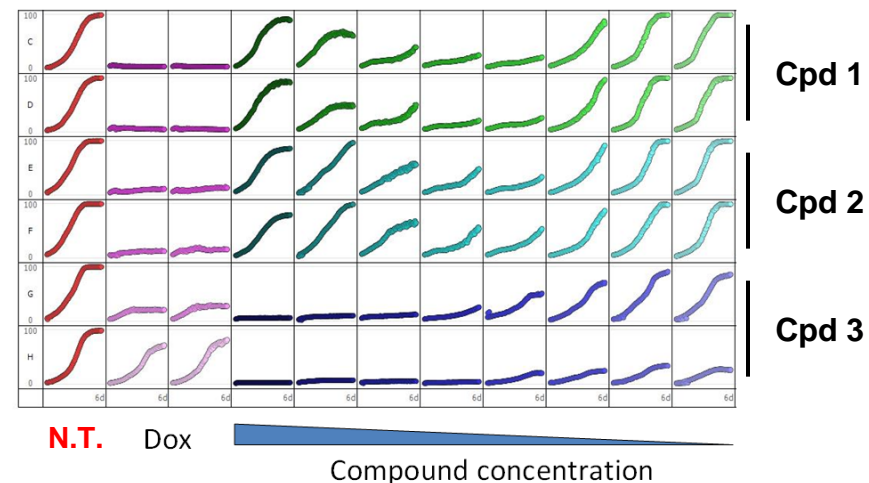




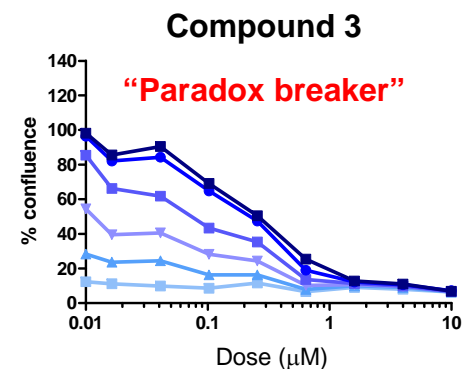
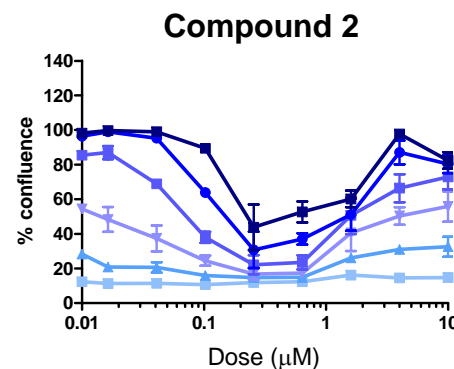
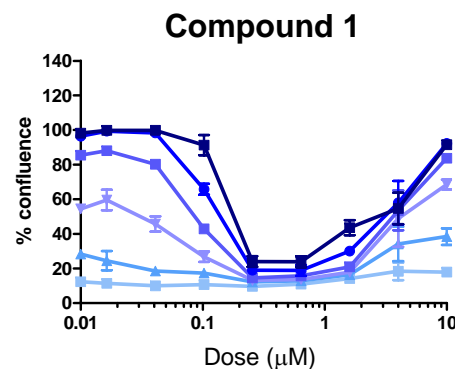
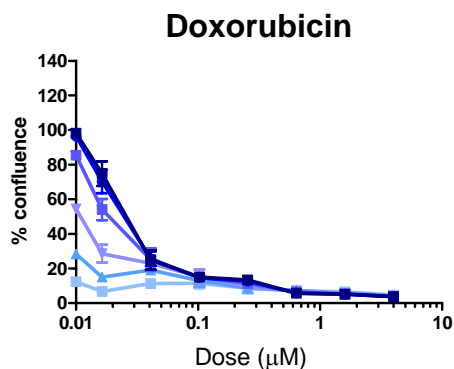
# Live-cell kinetic proliferation assays (6 days, IncuCyte)

- Investigating the “paradoxical” proliferative effect of different BRAF inhibitors in the CJM melanoma cell line (NRASmut)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	N.T.	Doxo	1.60	Compound 1 (uM)								
C	N.T.	(uM)	0.64	10.00	4.00	1.60	0.64	0.26	0.10	0.04	0.02	
D	N.T.		0.26	Compound 2 (uM)								
E	N.T.		0.10	10.00	4.00	1.60	0.64	0.26	0.10	0.04	0.02	
F	N.T.		0.04	Compound 3 (uM)								
G	N.T.		0.02	10.00	4.00	1.60	0.64	0.26	0.10	0.04	0.02	
H												



T = 24 h  
 T = 48 h  
 T = 72 h  
 T = 96 h  
 T = 120 h  
 T = 144 h





## From our hands-on experience with D300e: advantages

- Significant reduction of treatment time (from hours to few minutes)
- Minimization of the amount of compound solution needed (no dead volume)
- Costs saving for disposable materials (no need of intermediate compound dilutions)
- Particularly effective for treatments of many plates with few compounds
- Easy and intuitive design of complex plate schemes (e.g., compound combinations, randomization)
- Possibility of targeted titrations in the dose range of interest
- Precise and accurate dosing of compounds, especially important for ultracytotoxic drugs (pM - fM potency range)



## From our hands-on experience with D300e: pay attention to...

- Poorly soluble compounds cannot re-solubilize at lower concentrations, as it often happens with serial dilutions
- Evaporation (aqueous solutions) or hydration (DMSO solutions) before dispensation may change both the concentration and the surface tension
- The MW and the concentration of small molecules / biomolecules must meet definite criteria
- Surfactants must be added to aqueous solutions to decrease surface tension (e.g. Brij-35, TX-100 or Tween 20)
- If anything goes wrong (cassette loading, bubbles, precipitates, etc.) it is difficult to identify the problem: you do not actually see the compound being dispensed
- The instrument is not designed to be used at high-throughput: do not ask him more than it can do, but make the most of what it can do!



## Acknowledgements



### **Cell bank – Cell Proliferation Unit**

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Dario Fiorentino

