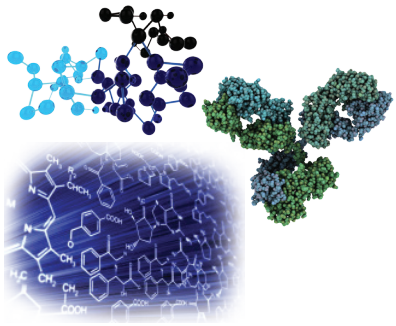
eurofins | Pharma Discovery Services

OncoPanel™

Cell-Based Profiling Service



Profile *YOUR* molecule **with *INSIGHTFUL* assays**

to select the *BEST* drug and stratify *PATIENTS*

300 Human Cancer Cell Lines

The OncoPanel™ Cell-Based Profiling Service provides drug response data across a panel of 300 genomically diverse cancer cell lines that span 19 different tissue types. Both small and large molecule therapeutics may be tested in the entire panel of 300 cell lines or researchers may select any subset of cell lines to test their therapeutic(s) of interest. Depending upon data requirements, therapeutics may be run in 3, 5, or 10-day assays. Using high-content imaging, simultaneous detection of anti-proliferative activity and other biological responses in a single, multiplexed assay may be performed. Genomic analysis of OncoPanel™ data enables the discovery of predictive biomarkers. This helps determine which genomic features may predispose patients to therapeutic response or identify potential resistance mechanisms that can inform combinations therapies.

Cell Type	2D Culture
Bladder	11
Breast	18
Central Nervous System	24
Colon	30
Endocrine	7
Eye	1
Female GU	20
Haematopoietic	57
Head and Neck	12
Kidney	10
Liver	7
Lung	23
Pancreas	13
Placenta	3
Prostate	6
Skin	25
Soft Tissue	25
Stomach	7
Testis	1
	300

OncoPanel™ Cell Lines

- All cell lines are licensed from ATCC or other commercial vendors
- The identity of all cell lines is confirmed by STR analysis
- Extensive genomic characterization: mRNA expression and mutation/DNA copy number data
- Rigorous cell culture quality control to provide consistent responses and reproducibility
- Meticulous tracking of cell line performance since 2009

Data Options

The Singleplex and Multiplex data options listed below are available for 2D culture, long-term culture (LT), combination and long-term combination studies. 3D culture, with or without a 2D comparison, is available in the Singleplex format. Data for cluster analysis are also available for greater than 50 standard of care and tool compounds.

Singleplex

- Cell proliferation: IC_{50}, EC_{50}, GI_{50}, GI_{100}, integrated area above the dose-response curve (activity area) values and intra-assay doubling time

Multiplex

- Cell proliferation: IC_{50}, EC_{50}, GI_{50} and GI_{100}, integrated area above the dose-response curve (activity area) values and intra-assay doubling time
- Apoptosis: concentration required for significant caspase-3 induction (5-fold and 6SD over vehicle) and maximum fold induction (Emax)
- Cell cycle: concentration required for significant increase or decrease (2-fold relative to vehicle) in histone H3 phosphorylation
- Custom markers

Univariate Genomic Analysis

- Identify and prioritize predictive genomic biomarkers from drug response data
- >175,000 genomic features analyzed
- Genomic features: mRNA over expression, amplifications, deletions, coding and non-coding mutations and gain/loss of function lesion categories
- Analysis in the context of cancer subtypes and tissue classification
- Rigorous statistical analysis

Gene-specific Panels

Aberrations in kinase and epigenetic targets have been shown to be responsible for cancer development and progression. Many of these targets are currently being investigated to identify novel cancer therapeutics. Based on the genomics of OncoPanel™ cell lines, specific kinase and epigenetic target panels are available. These panels are useful to:

- Confirm the activity found with in vitro kinase and epigenetic assays in cells
- Provide in vitro efficacy studies and selectivity evaluation
- Confirm in vitro efficacy based on gene aberration(s)
- Identify potential sensitivity and resistance biomarkers
- Assist lead optimization studies to identify best compounds for development

Genomic aberrations present in the gene-specific panels include:

- Amplification
- Amplification and Mutation
- Amplification and Overexpression
- Deletion
- Deletion and Underexpression
- Mutation
- Mutation and Overexpression
- Mutation and Underexpression
- Overexpression
- Underexpression

Kinase and Epigenetic Aberrations

Kinase Genes	Number of Gene Aberrations
ABL	31
ACVR	37
AKT	16
ALK	29
ALPK	147
ATM	51
ATR	35
BRAF	42
CDK	60
CHK	8
CRK	5
EGFR	53
EPHA	21
ERBB	56
FGF	150
FGR	35
FLT	34
HGF	52
IGF	21
JAK	26
KDR	49
KIT	58

Kinase Genes	Number of Gene Aberrations
LIMK	10
MAPK	306
MERTK	19
MET	39
MSTR	20
MTOR	31
NRG	47
NTRK	110
PAK	11
PDGFR	94
PI3CK	56
PRKC	112
RAF	12
RET	39
RICK	19
ROCK	20
ROS	46
SGK	4
STK	7
SYK	22
TGFBR	18
VEGF	6

Epigenetic Genes	Number of Gene Aberrations
BRD	86
DNMT	83
DOT	16
EED	8
EHMT	20
EZH2	14
HDAC	139
KDM	89
L3MBTL3	18
MLL	43
PRMT	10
SETD	19
SIRT	39
SMYD	6
SUZ12	18
WHSC1	27

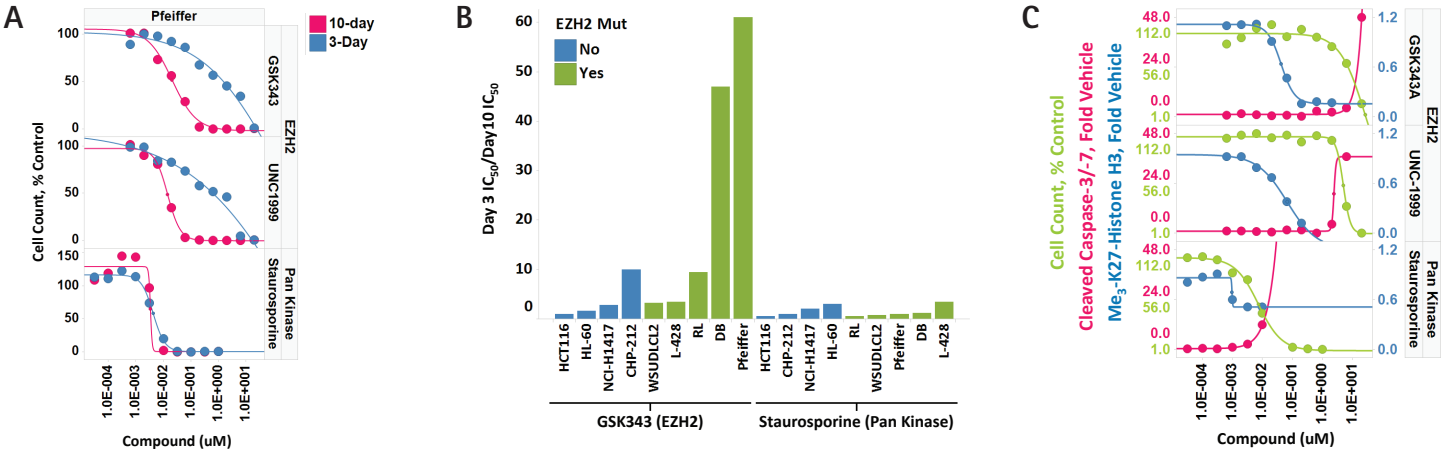
*Some cell lines contain multiple genomic aberrations

Long-term Culture

OncoPanel™ LT provides multiparametric, long-term (up to 10 days), drug response data across a large panel of genomically-defined human cancer cell lines that cover a wide range of cancer subtypes. The EZH2-selective inhibitor, GSK343, elicits a drug response that is dependent both on extended assay duration and EZH2 mutational status; whereas other less targeted inhibitors show little or no preference for either condition, illustrating the utility of this assay.

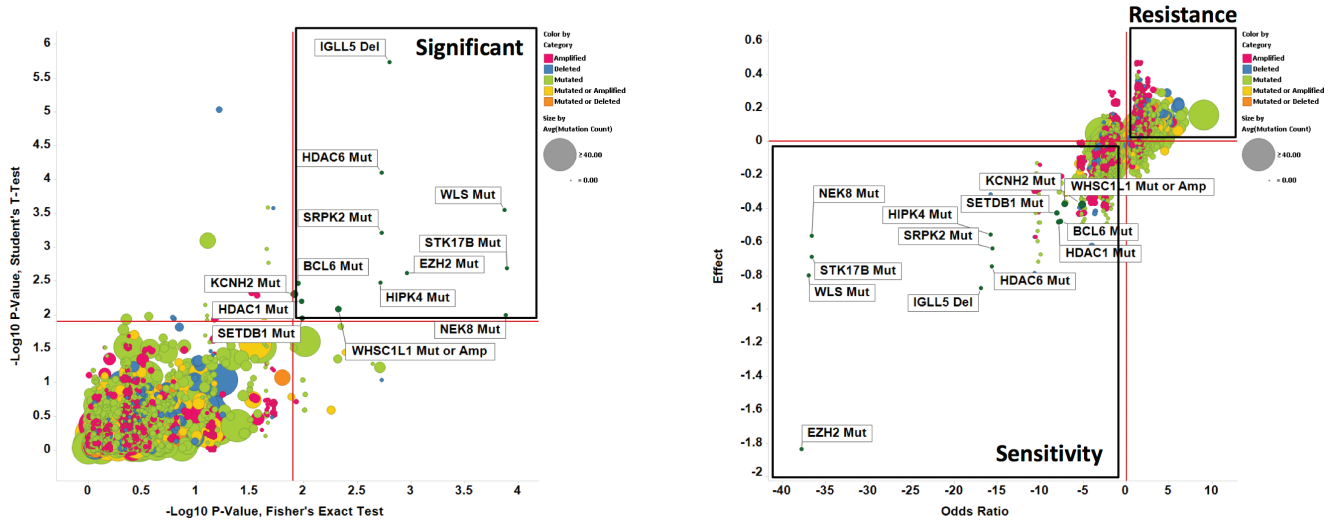
Detection of growth inhibition by EZH2 inhibitors requires an extended assay duration

A) Representative data from 3 and 10-day cell growth assays measuring the activity of distinct EZH2 inhibitors in the Pfeiffer (EZH2 mutant) cell line. B) Sensitivity of EZH2 mutant cell lines to the EZH2 inhibitor, GSK343, is increased with increased exposure time, whereas the non-specific kinase inhibitor, staurosporine, shows no preference for EZH2 mutational status or assay duration. C) High-content imaging and quantification of cell count (DAPI, green), cleaved caspase-3/-7 (apoptosis, pink), and tri-methylation of histone H3 on lysine 27 (Me3-K27-histone H3, blue).



OncoPanel™ genomics analysis can help target clinical trials to the patients that will derive the most benefit from treatment

Genes influencing cancer cell sensitivity or resistance to epigenetic therapeutic GSK343 were discovered using the Eurofins genomics analysis. Orthogonal statistical tests identify interesting genetic mutations or copy number alterations (left) and categorize them by effect (right).

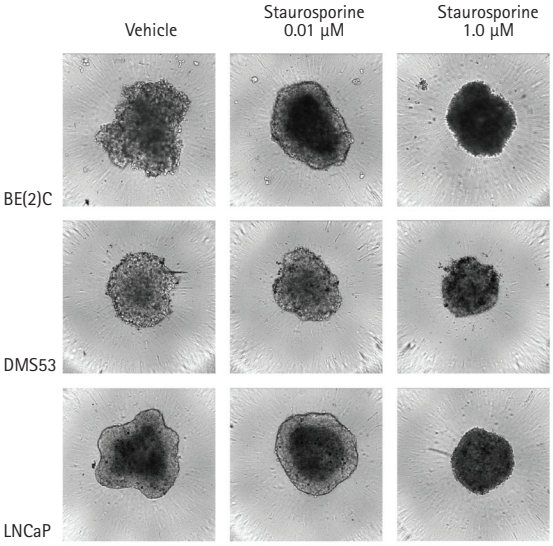


3D Culture

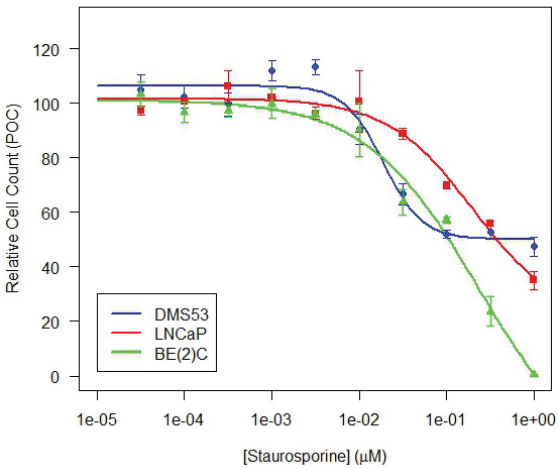
To study solid tumors OncoPanel™ 3D contains approximately 100 cancer cell lines, which grow as three-dimensional (3D) tumor spheroids, or loose aggregates, that phenocopy key attributes of human tumors. To pass quality control, spheroid diameter is 100-200µM and >80% of cells in a well must exist as spheroids or loose aggregates. Drug testing is initiated only after spheroid formation is confirmed through bright-field imaging. Tumor spheroid growth inhibition is measured using a homogeneous, luminescence-based assay, (CellTiter-Glo 3D) and cell viability is provided with EC50, IC50, GI50 and integrated area above the dose-response curve (activity area). This platform provides a means to compare the tumor-penetrating properties of anti-cancer therapeutics or a means to compare therapeutic activity in 2D versus 3D cell culture.

Cell Type	3D Culture
Bladder	6
Breast	6
Central Nervous System	7
Colon	15
Endocrine	1
Female GU	14
Head and Neck	8
Kidney	5
Liver	5
Lung	11
Pancreas	7
Prostate	3
Skin	8
Soft Tissue	8
	104

Staurosporine Treatment of 3D Spheroid Cultures



A) Cells were seeded into Corning 384-well, U-bottom Spheroid plates and were incubated at 37°C for four days to allow spheroid formation, which was verified by bright-field, high-content imaging of the plates. The medium was replenished, and the spheroids were treated with a 10-point staurosporine concentration curve, with DMSO as the vehicle, for three days. The spheroids were imaged again with the bright field objective at 20% transmittance on an ImageXpress Micro high-content Imager.



B) Cells were plated at established seeding numbers for each cell line and grown for four days to allow spheroid formation. Medium was replenished, and staurosporine was added over a 10-point concentration curve in half-log dilutions, starting at 1 µM. The spheroids were incubated for an additional three days, after which, they were lysed with CellTiter-Glo 3D reagent (Promega), and the luminescent signal was measured in triplicate wells for each test concentration. The data were plotted, using a custom R script, as a sigmoidal dose-response to the percent of untreated vehicle control wells versus staurosporine concentration (µM) as mean ± SEM.

Large Molecule Biologics

The anti-cancer efficacy of biologics may be assessed with OncoPanel™. In addition to multiparametric endpoints (cell proliferation, apoptosis, cell cycle and custom markers) OncoPanel™ for biologics can also report quantitative therapeutic antibody binding parameters. Univariate genomic analysis may also be conducted to prioritize genomic biomarkers associated with sensitivity and resistance to the tested biologic.

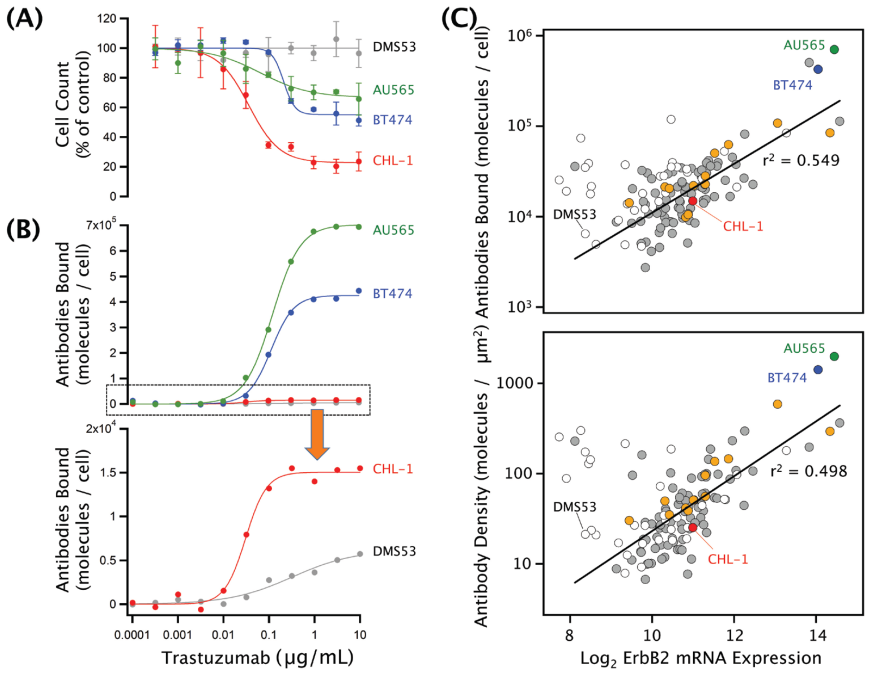
The data below describe high-throughput parallelization of quantitative, comparative therapeutic antibody binding measurements and drug response profiling using high-content imaging. Therapeutic antibodies, trastuzumab and cetuximab, were profiled, providing a rank ordering of cell surface expression for ErbB2/EGFR and for defining thresholds of antibody binding that are needed to inhibit cell proliferation. Compared with mRNA expression, quantitative antibody binding was a superior predictor of biological response. With OncoPanel™, targeted studies may be conducted to determine the most relevant cancer types in which the activity of a monoclonal antibody is likely to be most effective.

Trastuzumab Data

A) 120-hour proliferation curves showing 4 selected OncoPanel™ cell line examples after treatment with trastuzumab.

(B) Corresponding binding curves showing total antibodies bound per cell vs. concentration. The area surrounded by the dotted rectangle is expanded below, showing binding to CHL-1 (specific binding) and DMS53 (probable non-specific binding).

(C) Scatter plots showing total antibodies bound per cell (top), or antibody density (bottom) vs. ErbB2 mRNA expression. Gray circles indicate cell lines that showed specific binding by trastuzumab. Open circles indicate cell lines that showed anomalous, or nonspecific, binding. Yellow circles indicate cell lines where trastuzumab caused >30% growth inhibition. The black line represents the correlation between protein and mRNA levels, only among those cell lines classified as specific binders.

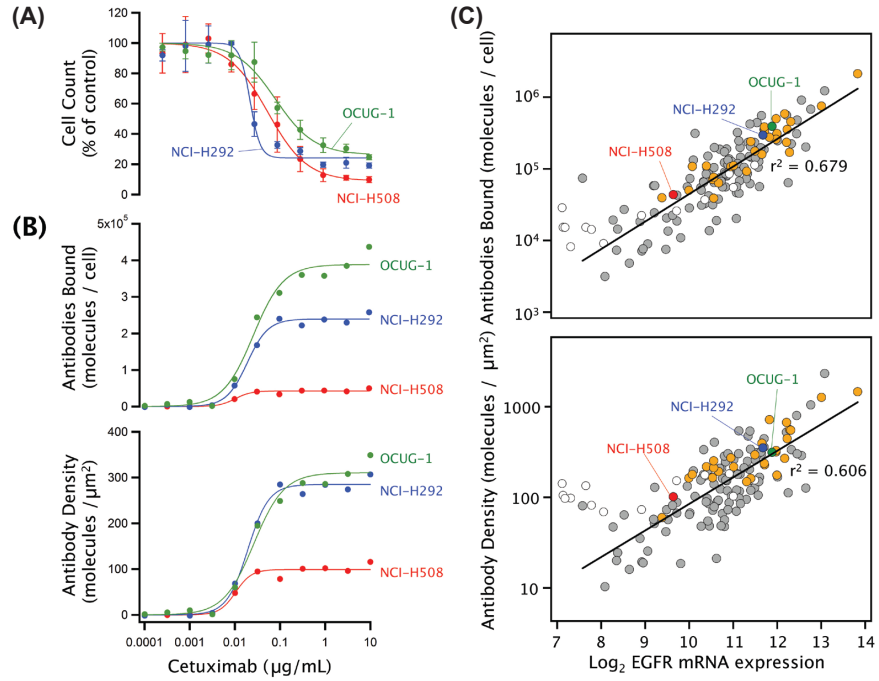


Cetuximab Data

A) 120-hour proliferation curves showing 3 selected OncoPanel™ cell line examples after treatment with cetuximab.

(B) Corresponding binding curves showing total antibodies bound per cell (top) or antibody density (bottom) vs. concentration.

(C) Scatter plots showing total antibodies bound per cell (top), or antibody density (bottom) vs. EGFR mRNA expression. Gray circles indicate cell lines that showed specific binding by cetuximab. Open circles indicate cell lines that showed anomalous or non-specific binding. Yellow circles indicate cell lines where cetuximab led to >30% growth inhibition. The black line represents the correlation between protein and mRNA levels, only among those cell lines classified as specific binders.



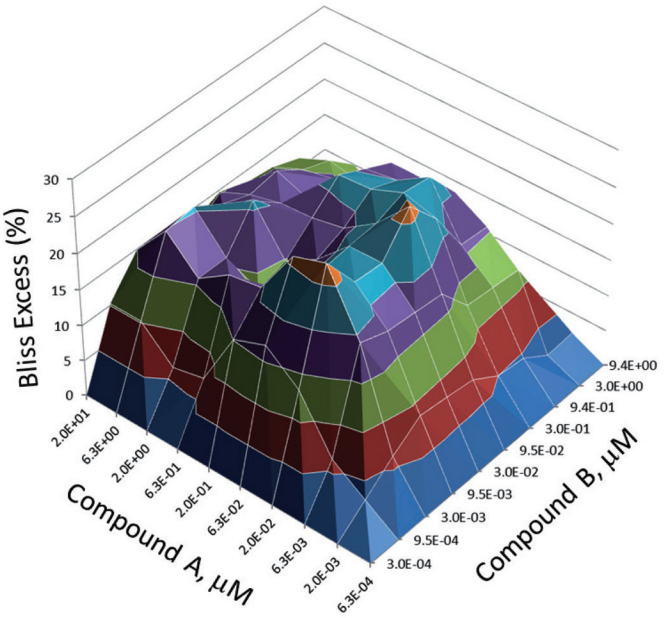
Drug Combination Studies

Cell signaling pathways can have redundancies within tumors that create the potential for cell signal compensation when a single anti-cancer therapeutic is administered, which may lead to resistance. Combinations of therapeutics (small or large molecules) may be tested using OncoPanel™ in a dose-response matrix that provides multiparametric endpoints. These data are ideal for a variety of downstream drug-drug combination analyses, including calculation of combination index, isobolographic analysis and determination of excess over Bliss independence.

0 15 30 Bliss Excess

Compound B, µM \ Compound A, µM	2.0E+01	6.3E+00	2.0E+00	6.3E-01	2.0E-01	6.3E-02	2.0E-02	6.3E-03	2.0E-03	6.3E-04
3.0E-04	0	0	0	0	0	0	0	0	0	0
9.5E-04	10.2	10.2	6.1	7.1	15.1	16.8	13.2	6.1	3	0
3.0E-03	14.9	19.9	15.7	18.6	19.3	25.9	27.4	19.2	6	0
9.5E-03	13.5	20.4	15.7	13.7	15.8	21.2	22	17.8	8.6	0
3.0E-02	13.1	18	20.8	14.8	17.4	21.6	21.7	17.4	8.1	0
9.5E-02	14.7	16.6	17.7	18	18.9	21.9	26.5	17	7.5	0
3.0E-01	13.3	17.2	15.6	21.2	21.5	23.3	22.1	12.5	5	0
9.4E-01	12.8	14.7	15.6	19.3	20	22.7	15.7	12.1	4.5	0
3.0E+00	11.4	13.3	12.5	17.5	17.5	17.9	15.4	11.7	1.9	0
9.4E+00	8.1	10	6.4	9.8	11.6	9.3	5.8	4	2.2	0

Dose-response matrix of two agents, Compound A and Compound B, with excess over Bliss independence shown using cell proliferation values, expressed as a percentage. Values ≥15 represent dose combinations that are considered significantly synergistic.

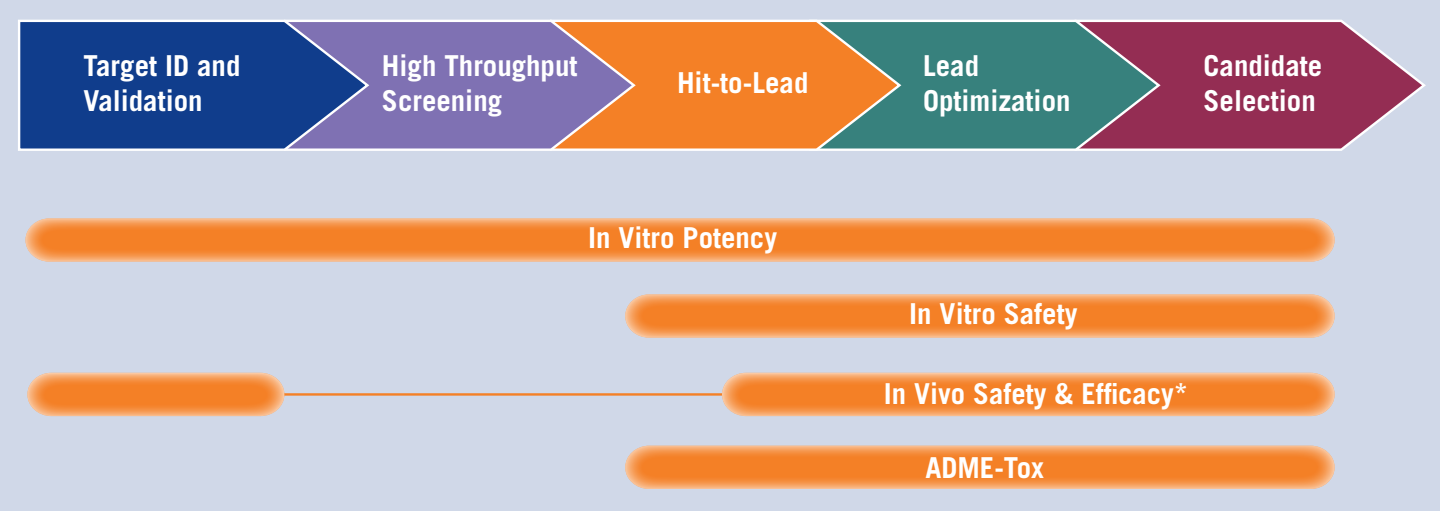


The same data displayed as a surface plot; dose combinations at or above the green band indicates significant synergy.

Comprehensive Products and Services

Built upon the expertise of Eurofins Discovery Services, Cerep & Panlabs

Eurofins Pharma Discovery Services offers a comprehensive package of drug discovery products and services. The extensive range of products and services aids in the decision making process, starting with Target ID and Validation through to Candidate Selection. Key services for drug discovery include:



In Vitro Potency

Target-based in vitro binding and functional assays for preliminary assessments of efficacy.

In Vitro Safety

Early assessment across a range of targets for safety and toxicity, including: CardiacProfiler™, SafetyScreen44™ and SafetyScreen87™

In Vivo Safety & Efficacy

240+ disease-relevant in vivo models to evaluate drug efficacy, along with models for early PK and toxicity assessment. *In vivo services are provided by our partner lab Pharmacology Discovery Services.

ADME-Tox

140+ ADME-Tox assays to help clients characterize and better understand the DMPK properties of a drug candidate.

For more information visit: eurofins.com/epds

Built upon the expertise of Eurofins Discovery Services, Cerep, Panlabs & Villapharma

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