

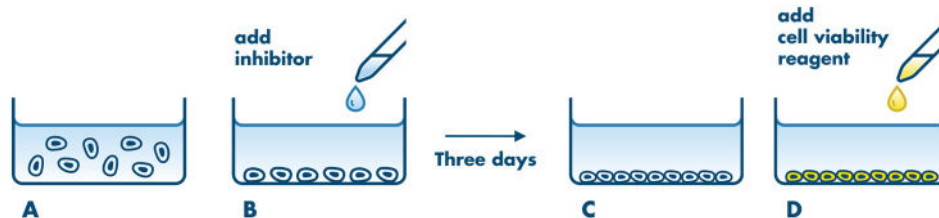
➤ Economic panel screening with the Cell Proliferation Assay

Use the ProLiFiler to screen your compound on a panel of 140 tumor cell lines.

- Performed on a regular basis (starting every 6 weeks)
- Huge cost-savings
- Fast turnaround time: get your results in 5 to 6 weeks
- Semi-automated processing for highly reproducible data
- Single drug or combination drug testing
- Option for in-depth bioinformatics analysis

➤ Assay procedure of the Cell Proliferation Assay

The ProLiFiler assay will be performed with a contact-free, state-of-the-art, nano-dispensing system (Tecan D300E) requiring small amounts of your compound.



Assay procedure. Cells are seeded into 384 multi-well plates (A). The next day, compounds are added (B), and cells are incubated for 72 h (C). Subsequently, the cell viability dye CellTiter-Glo is added, and the luminescence is measured as a parameter for cell viability (D). IC_{50} values are determined from 8 concentrations in duplicates.

➤ Bioinformatics Analysis

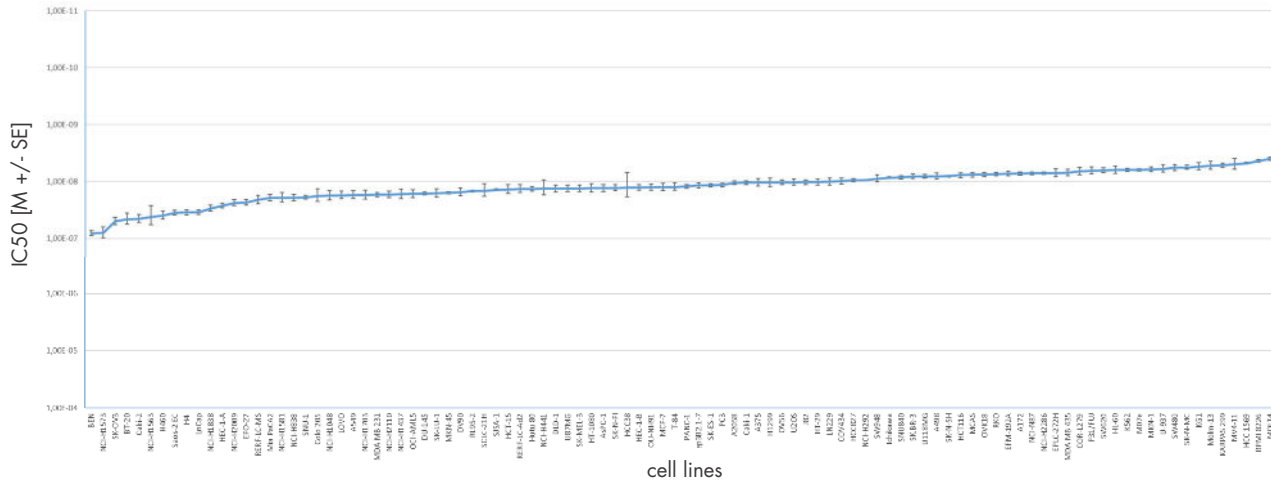
Our bioinformatics analysis service is available to identify the mode of action of your test compound and to detect suitable biomarkers for stratification of responders vs. non-responders.

Our partner's comprehensive bioinformatics database integrates proprietary and public data comprising multiple cancer datasets with over 1,800 preclinical samples and 1,000 reference drugs into a single platform for visualization and statistical analysis.

The report includes both raw data for additional analysis and data mining results from bioinformatics scientists available to help answer your key questions.

➤ High reproducibility

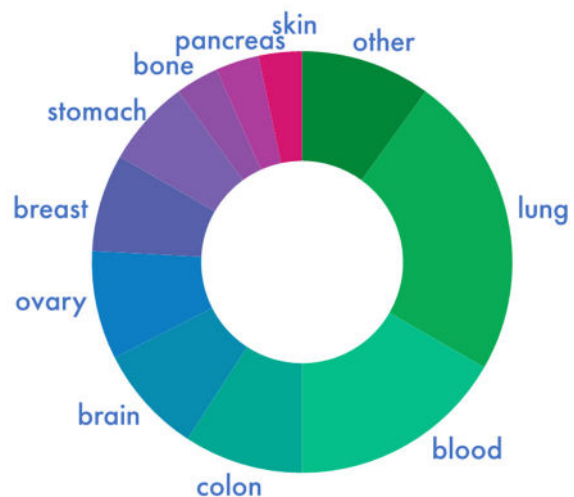
The use of semi-automated pipetting systems ensures for highest reproducibility. The IC50 values of the reference compound bortezomib across 4 independent runs of the ProLiFiler cell panel screen are shown in the figure below.



➤ Origin of cell lines

The panel currently consists of 140 human tumor cell lines derived from 18 tumor types.

| Tumor type | # | Tumor type | # |
|------------|----|--------------|---|
| lung | 31 | pancreas | 4 |
| blood | 24 | endometrium | 4 |
| colon | 11 | prostate | 3 |
| brain | 11 | kidney | 4 |
| ovary | 11 | liver | 2 |
| breast | 13 | fibrosarcoma | 1 |
| stomach | 9 | head/neck | 1 |
| skin | 5 | bladder | 1 |
| bone | 4 | duodenum | 1 |



➤ MoA Finder

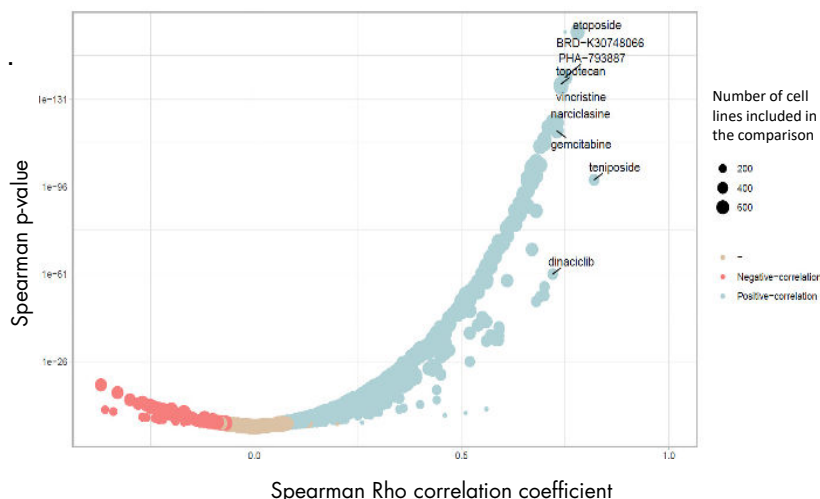
The MoA Finder tool aims to identify the mode of action (MoA) of your test compound based on the results obtained in the ProLiFiler cell panel screening. To this end, we compare the sensitivity profile of your drug candidate to more than 1000 drugs for which the MoA is known.

- The MoA Finder tool allows the confirmation of the mode of action of drugs.
- The data help to identify potential off-targets and side effects.
- The MoA of compounds with unknown modes can be identified and gives suggestions for target deconvolution in a phenotypic approach.

Database: The drug sensitivity profile, which defines the mode of action of more than 1000 anti-cancer compounds, was collected from both literature and internal results.

Approach: The drug sensitivity profile (IC50 values of ProLiFiler screen) of your test compound will be compared to the reference compounds' drug sensitivity profiles. The correlation of the two datasets is evaluated by two statistical values, the Spearman Rho correlation value, and its p-value.

Deliverables: Graphical presentation of the correlation analysis. Excel table with raw data.



Example of doxorubicin mode of action analysis.

Shown is a graphical presentation of the correlation of the drug sensitivity profile of doxorubicin to 732 reference compound's drug sensitivity profiles. Each dot represents one correlation.

The dots' size is related to the number of cell lines used to define the reference drug's sensitivity profile. A positive correlation of doxorubicin and the drug is blue; a negative correlation is shown in red.

Those reference drugs with a Spearman Rho correlation higher than 0.7 are named.

➤ Biomarker Analysis Tool

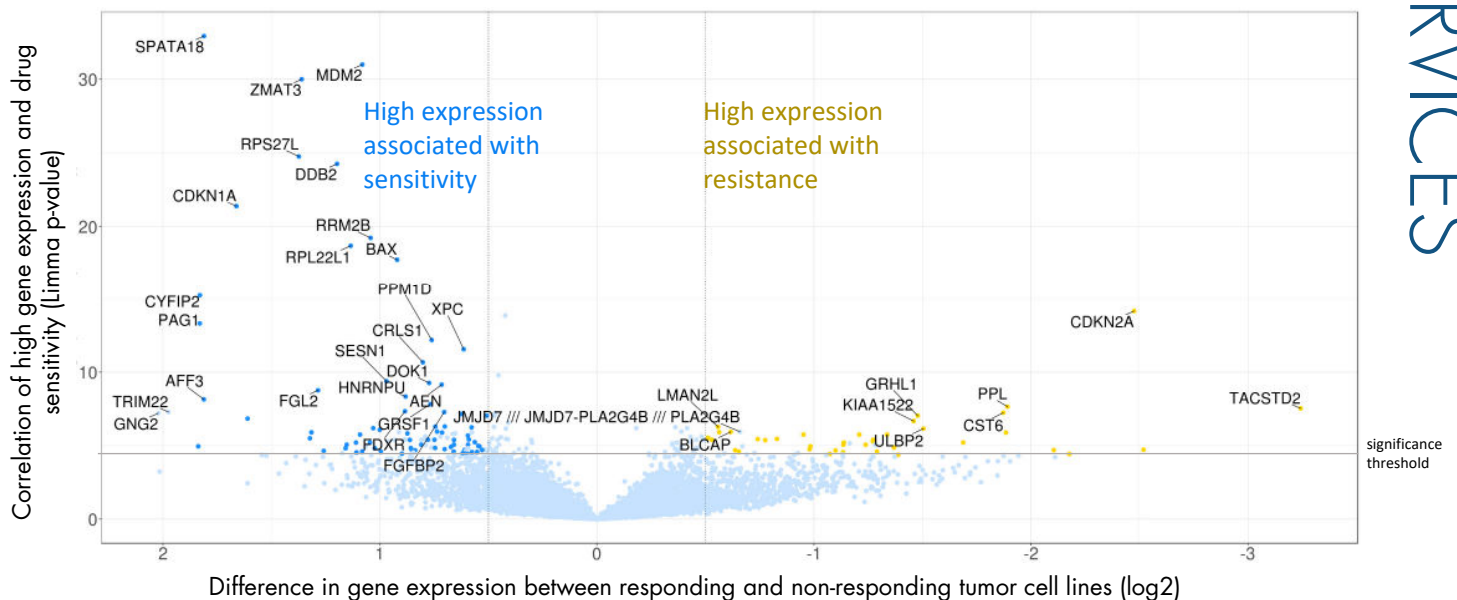
The Biomarker Analysis Tool reveals the specific genomic signature of tumor cell lines that are sensitive to your test drug. To this end, we correlate the drug sensitivity profile (IC50 data of ProLiFiler screen) of your test drug with data sets of gene expression and genetic alterations (mutations, deletions) known for the tumor cell lines.

Use the Biomarker Analysis Tool to identify genes or genetic alterations whose presence is indicative of the efficacy of the test compound and may serve for predictive biomarkers in disease models and patient cohorts.

Approach: The ProLiFiler results are correlated with the gene expression, whole-exome sequencing data (mutations), and somatic copy number alteration data of the tumor cell lines. An analysis is performed for every cell line to determine the correlation of its drug sensitivity to each gene with regards to the expression level, mutation status, and somatic copy number.

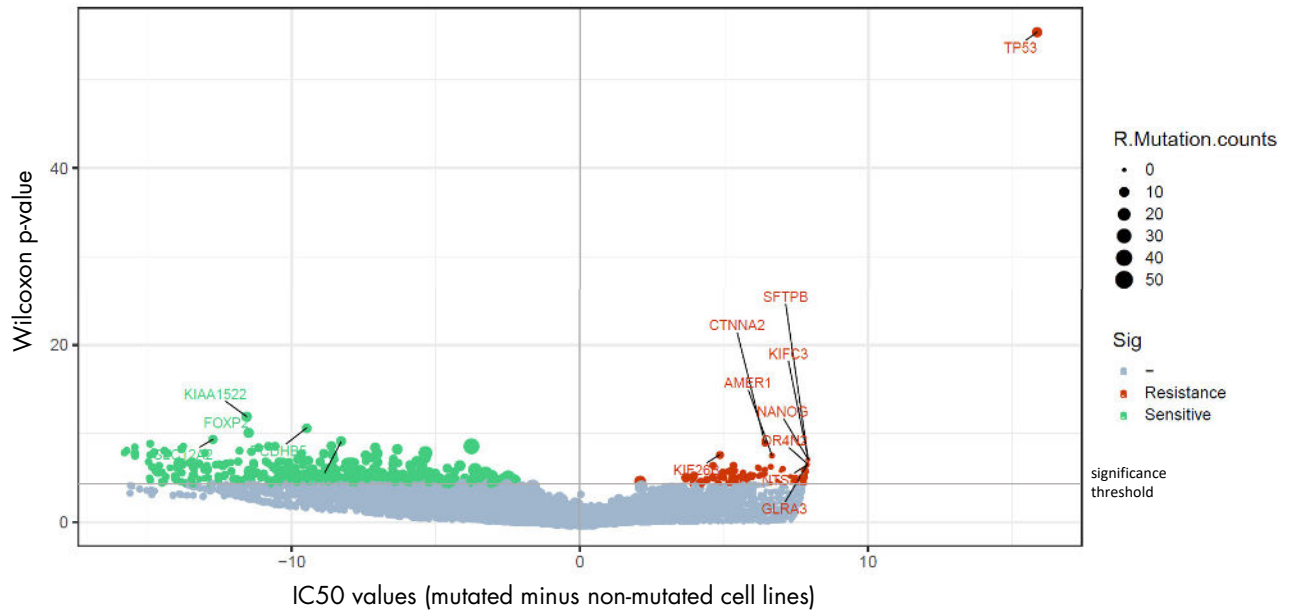
➤ Biomarker Analysis Tool - Case Study Nutlin-3

Nutlin-3 is a small molecule compound that inhibits the interaction of mdm2 and tumor suppressor p53 resulting in the stabilization and increased action of p53.



Example of nutlin-3 gene expression analysis. Graphical presentation of Limma test results show which genes are highly expressed in drug-resistant and drug-sensitive cell lines. MDM2 and other p53 pathway-related genes were highly expressed in tumor cell lines, which were sensitive to nutlin-3 treatment.

MDM2 expression is a predictor of sensitivity to nutlin-3 treatment.



Example of nutlin-3 mutation analysis. Nutlin-3 was tested for dose-response in a panel of tumor cell lines. These cell lines contain a total of 16,998 mutated genes. The volcano blot shows for each gene (one dot) the Wilcoxon test p-value for the correlation of the drug response in proportion to the existence of mutants. For example, p53 mutations were found mostly in tumor cell lines that did not respond to nutlin-3. KIAA1522, on the other hand, was found the most probable mutation that exists in cell lines responding to nutlin-3 treatment.

| Gene symbol | Mutation counts in the Responder group | Mutation counts in the Non-Responder group | No Mutation counts in the Responder group | No Mutation counts in the Non-Responder group | Wilcoxon test p-value | Log2 of Wilcoxon test p-value | Mean absolute IC50 in non-mutated group | Mean absolute IC50 in mutated group |
|-------------|--|--|---|---|-----------------------|-------------------------------|---|-------------------------------------|
| TP53 | 10 | 152 | 53 | 22 | 2.25E-17 | 55 | 11.6 | 27.5 |
| KIAA1522 | 10 | 5 | 53 | 169 | 0.0002 | 12.3 | 23.2 | 11.6 |
| FBXL8 | 6 | 1 | 57 | 173 | 0.0006 | 10.7 | 22.9 | 6.7 |

The top three mutated genes correlated with nutlin-3 efficacy. P53 was found mutated in 10 cell lines, which responded to nutlin-3 treatment and in 152 cell lines, which did not respond to nutlin-3 treatment. The mean IC50 value of nutlin-3 in tumor cell lines with p53 wild type was about 11.6 μ M compared to 27.5 μ M in cell lines with p53 mutation resulting in a difference of 16 μ M between mutated and non-mutated cell lines which was used as a measure for resistance.

➤ Laying the fundament for successful drug discovery

The ProLiFiler identifies which tumor cell lines of which origins are most susceptible to drug treatment. Many of the cell lines included in the panel can directly be used for in vivo testing. The In Vivo Hollow Fiber Model is an excellent follow-up tool for testing of up to 12 tumor models in vivo to find the most suitable tumor model for subsequent xenograft studies.

The MoA Finder provides information for the interpretation of data and enables informed choices of the next steps in drug development. Biomarker data allows to identify the signature of responders and to ultimately steer the development of the drug towards the cohort of patients that are to receive the treatment.