

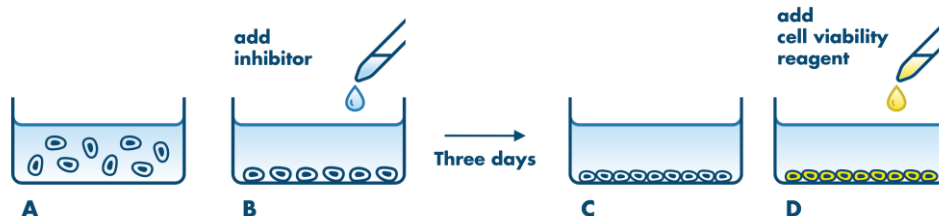
➤ Economic panel screening with the Cell Proliferation Assay

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

- Continuous screening set-up, 6-8 runs annually
- Huge cost savings; up to 15 compounds are processed in each run
- Determination of IC_{50} values for the inhibitory capacity of a drug candidate
- Uncover the mechanism of action of your test compound
- Determine biomarkers using genetic annotation profiles available for all cell lines tested
- Identify cell lines for subsequent testing as in vivo tumor models

➤ 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

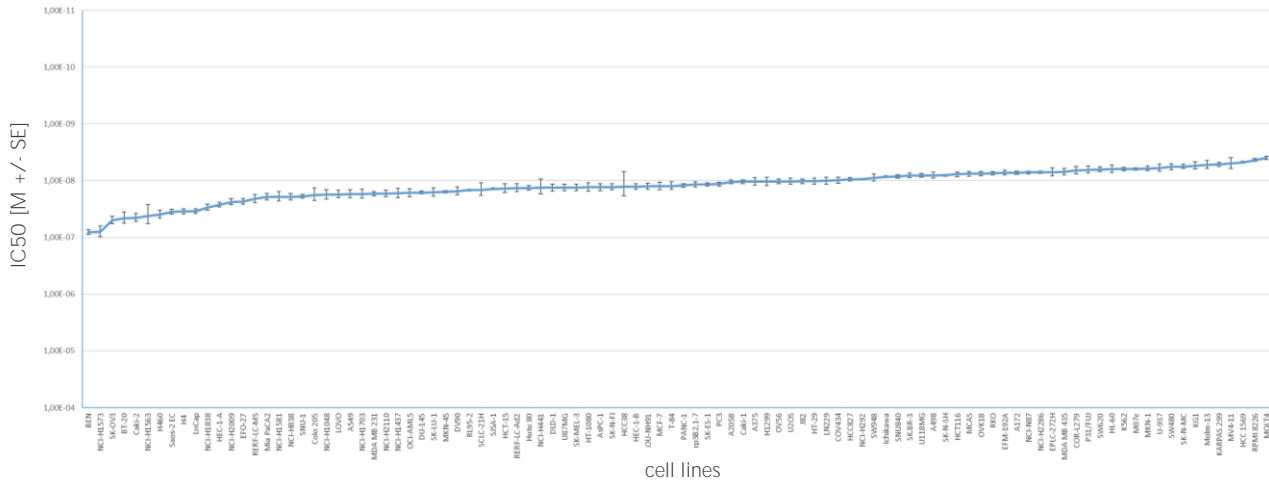
Our bioinformatics analysis tool is available to identify the mechanism of action of your test compound and to detect suitable biomarkers for stratification of responders vs. non-responders.

For this purpose, we have partnered with 4HF Biotech GmbH, a bioinformatics firm specializing in cancer data mining to discover new anti-cancer drugs. 4HF Biotech build a comprehensive database, including proprietary and public data of more than 1,800 preclinical samples and 800 reference drugs, comprising multiple cancer datasets that are integrated into a single platform for visualization and statistical analysis.

4HF Biotech provides hands-on support with a high focus on client communication. The report comprises raw data for your own analysis and data mining results performed by 4HF Biotech scientists who are also available for personal communication to help to answer your key questions.

➤ High reproducibility

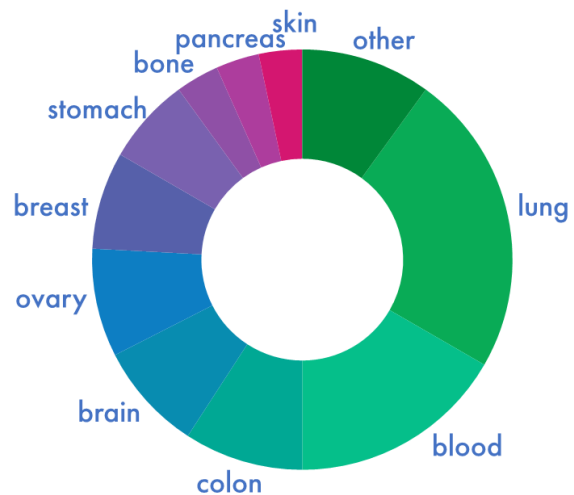
The use of semi-automated pipetting systems ensures for highest reproducibility. The IC50 values of the reference compound bortezomib across 4 independent runs of the ProLiFiler cell panel screen are shown in the figure below.



➤ 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

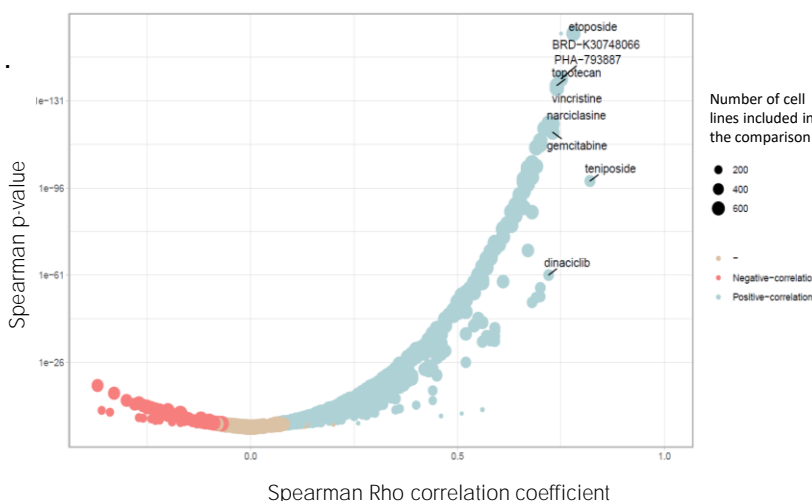
The MoA Finder tool aims to identify the mechanism of action (MoA) of your test compound based on the results obtained in the ProLiFiler cell panel screening. To this end, we compare the sensitivity profile of your drug candidate to more than 700 drugs for which the MoA is known.

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

Database: The drug sensitivity profile, which defines the mechanism of action of more than 700 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 mechanism 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

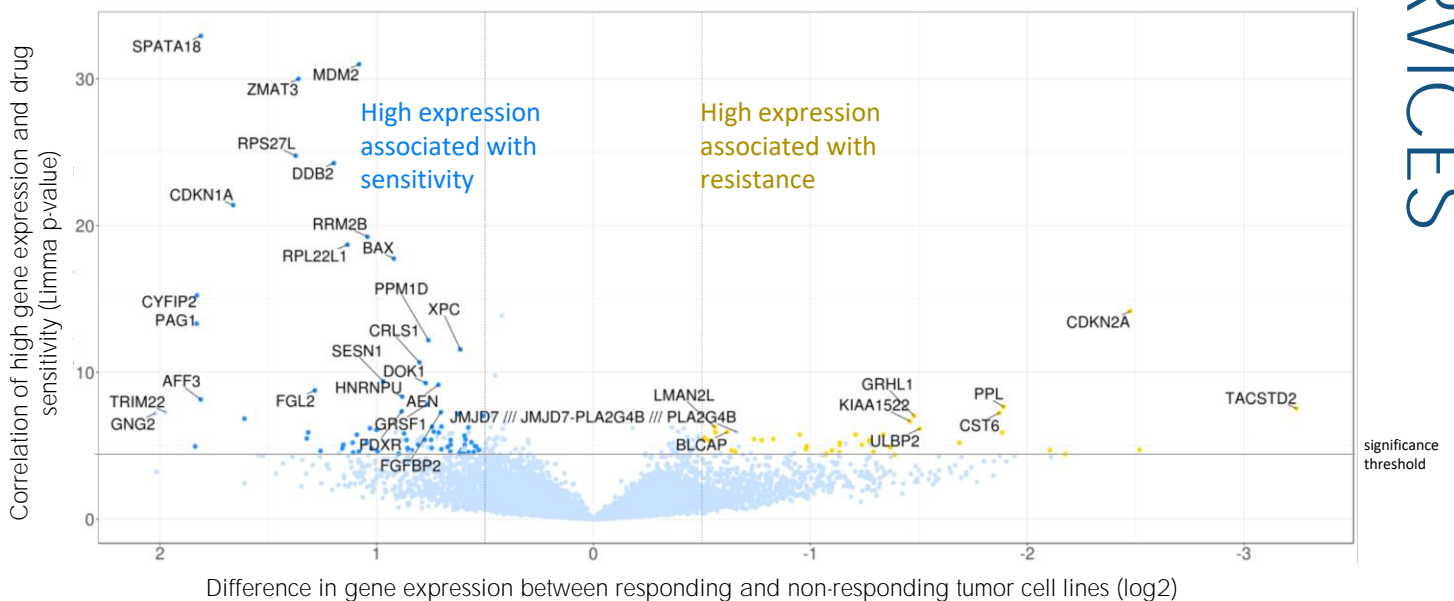
The Biomarker Analysis tool reveals the specific genomic signature of tumor cell lines that are sensitive to your test drug. To this end, we correlate the drug sensitivity profile (IC50 data of ProLiFiler screen) of your test drug with data sets of gene expression and genetic alterations (mutations,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

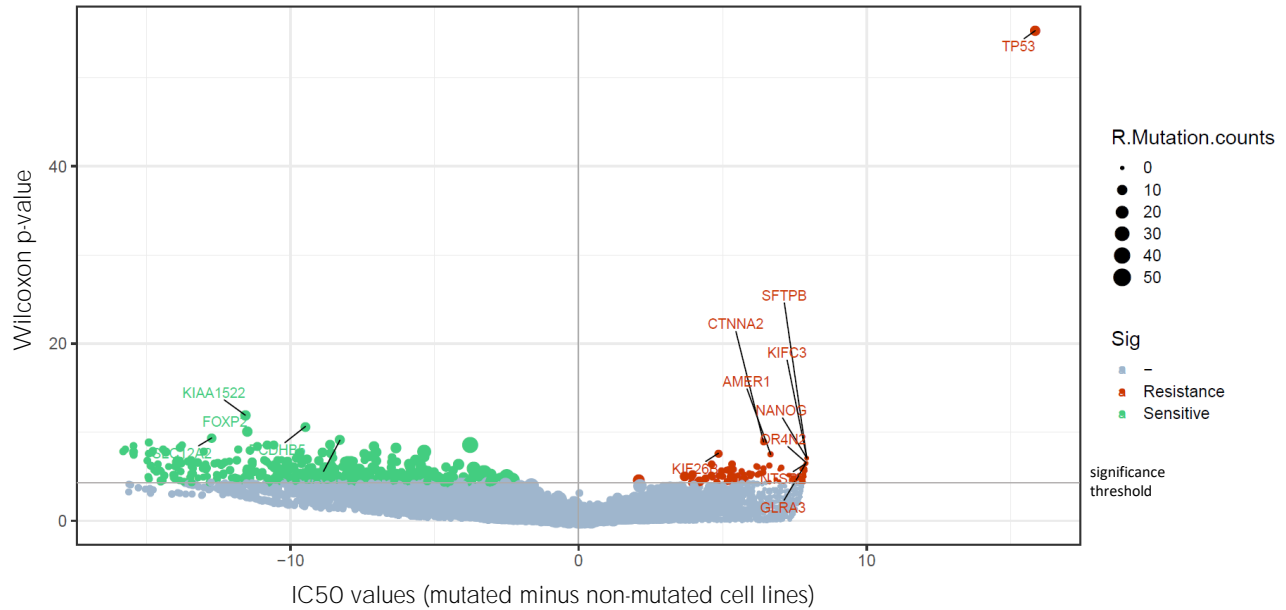
➤ Biomarker Analysis - Case Study Nutlin-3

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 μM compared to 27.5 μM in cell lines with p53 mutation resulting in a difference of 16 μM 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.