NIH NATIONAL CANCER INSTITUTE

Refining PRECISION THERAPEUTICS

With DNA Damaging Agents

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Center for Cancer Research,
Building 37, Room 5068
Bethesda, Maryland

Precision therapeutics can be defined as the ability to:

- **prescribe effective therapies only to those patients who will respond effectively (cure),**
- **while limiting toxicity to normal tissues and minimizing side effects.**

- Synthetic lethality beyond BRCA and PARP inhibitors
  - TOP1 inhibitors
- Cancer Cell Line genomics as model systems
- SLFN11 as a highly penetrant determinant of response
- Practical implications: example of temozolomide

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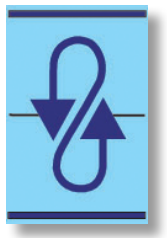
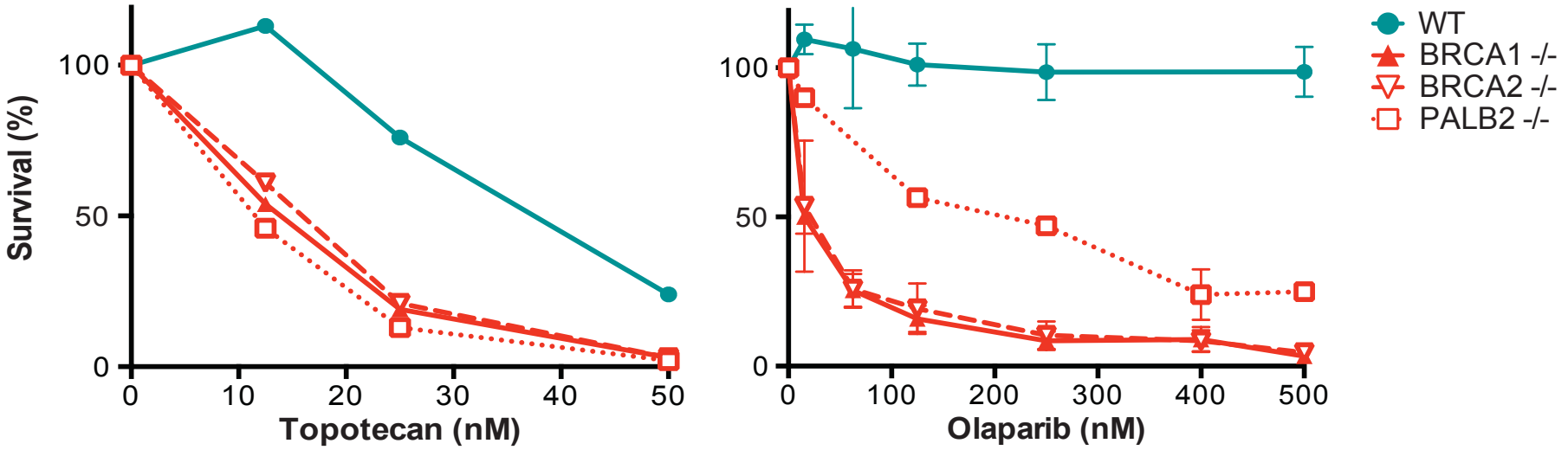


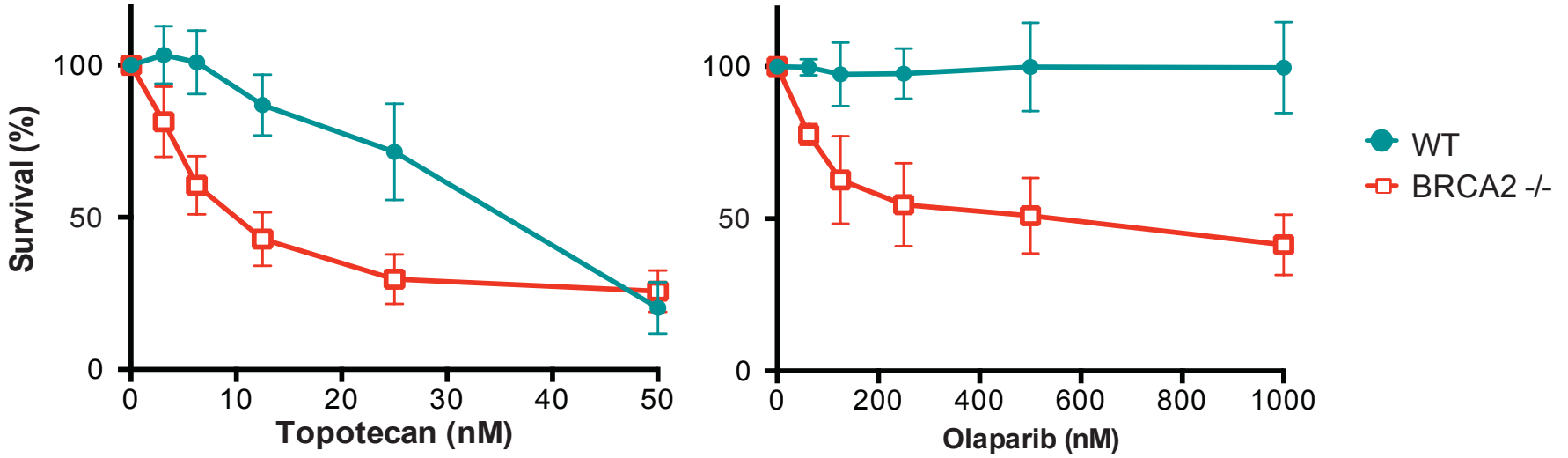
Synthetic lethality for BRCA cells beyond PARP inhibitors

TOP1 inhibitors

DT40 cells



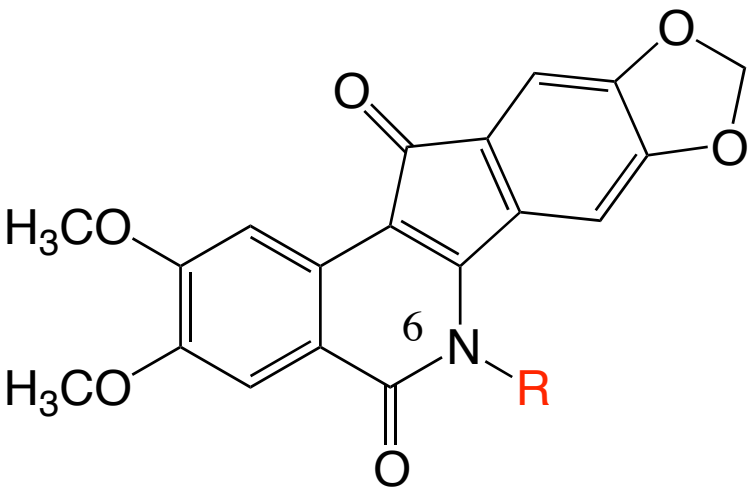
DLD1 cells

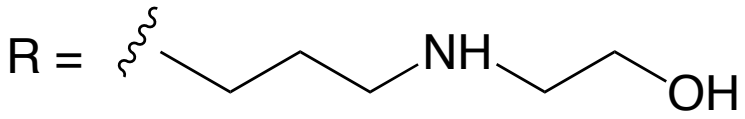


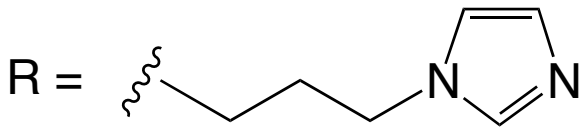
Rationale for the development of non-camptothecin TOP1 inhibitors

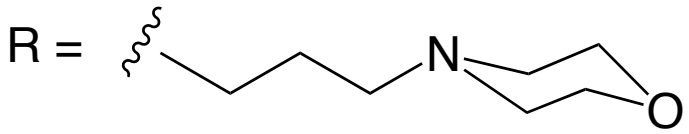
- Camptothecin derivatives (Irinotecan and Topotecan) are potent anticancer agents and highly selective TOP1 inhibitors
- Camptothecins are selective for HR (BRCA) deficient tumors
- Camptothecins are the only chemical class of TOP1 inhibitors (many tubulin, TOP2…)
- Camptothecins have well-established limitations
  - ✓ Chemically unstable (inactivated within minutes in plasma)
  - ✓ Reversibly block TOP1-DNA complexes (long exposure required to maximize effect)
  - ✓ Eliminated from cancer cells by ABC drug efflux transporters (ABCG2 – ABCB1)
  - ✓ Short plasma half-life (2-3 hours due to rapid clearance)
  - ✓ Dose-limiting bone marrow toxicity
  - ✓ Severe diarrhea (Irinotecan)

Non-camptothecin TOP1 inhibitors developed by the NCI: the Indenoisoquinolines: the LMPs









NSC 706744
LMP744

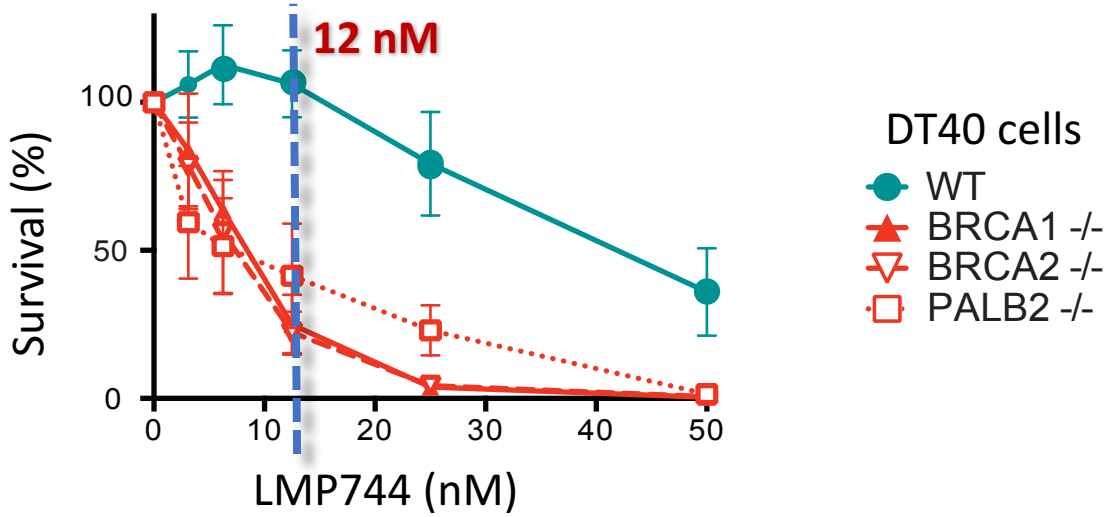
NSC 725776
Imidotecan
LMP776

NSC 724998
Indotecan
LMP400

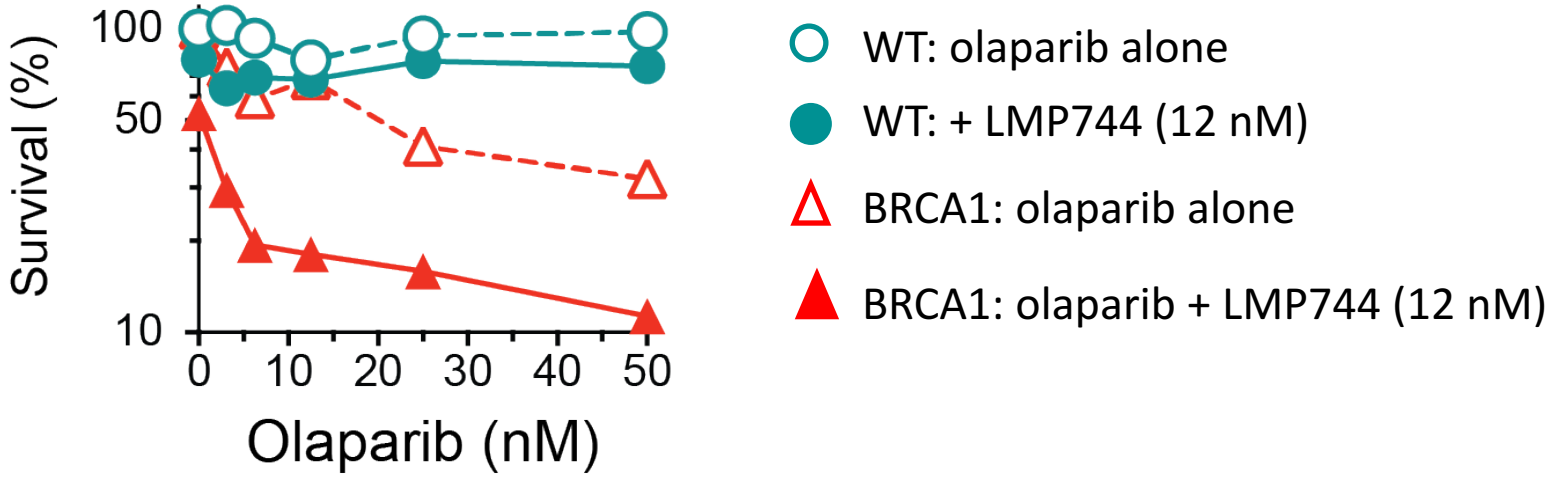
LMP400 (Indotecan) and LMP776 (Imidotecan) completed Phase 1
LMP744 is beginning phase 1

Synthetic lethality of the indenoisoquinolines for BRCA cells beyond PARP inhibitors

Indenoisoquinoline TOP1 inhibitors are potent as single agents at nanomolar concentration in HR deficient cells



Indenoisoquinolines synergize with olaparib in BRCA1-deficient cells



Unpublished

The indenoisoquinoline TOP1 inhibitors are in Phase 1-2 clinical development

- As the TOP1 inhibitor camptothecin derivatives (Irinotecan and Topotecan), the indenoisoquinolines are potent anticancer agents
- ~~Camptothecins are the only chemical class of TOP1 inhibitors (many tubulin, TOP2…)~~
- The indenoisoquinolines are selective for HR (BRCA) deficient tumors
- The Indenoisoquinolines overcome the limitations of camptothecins
  - ✓ Chemically ~~un~~stable (no lactone E-ring)
  - ✓ More stable block of TOP1-DNA complexes than camptothecins
  - ~~✓ Eliminated from cancer cells by ABC drug efflux transporters (ABCG2 – ABCB1)~~
  - ~~✓ Short~~ Long plasma half-lifes (12-17 hours vs. 2 hours)
  - ✓ Dose-limiting bone marrow toxicity
  - ~~✓ Severe diarrhea (Irinotecan)~~

- Synthetic lethality beyond BRCA and PARP inhibitors
  - TOP1 inhibitors
- Cancer Cell Line genomics as model systems
- SLFN11 as a highly penetrant determinant of response
- Practical implications: example of temozolomide

The NCI CellMiner Genomic and Bioinformatics facility (CGBF)

Our mission is to integrate pharmacological, genomics, proteomic and metadata to:

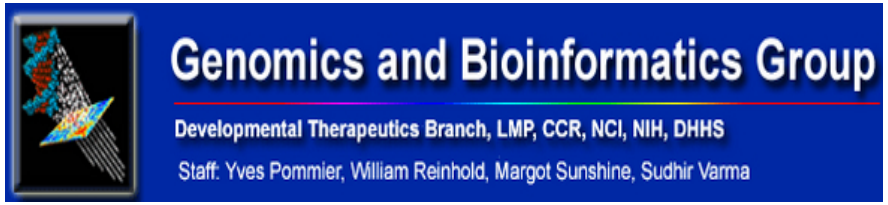
1. Discover new drug response determinants (sensitivity <-> resistance; signatures)
2. Enable others to make new discoveries through user friendly interface across multiple cancer cell lines databases

CellMiner is a unique facility open world wide with over thousands of user monthly since its inception.
It can be accessed through: http://discover.nci.nih.gov/cellminer
http://discover.nci.nih.gov/cellminercdb

Augustin Luna

The publicly available cancer cell line databases and the CellMiner website

Developmental Therapeutics Program NCI/NIH



NCI-60

http://discover.nci.nih.gov/cellminer/
http://discover.nci.nih.gov/cellminercdb/

wellcome trust sanger institute

MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER

GDSC (CGP)

http://www.cancerrxgene.org/ (Genomics of Drug Sensitivity in Cancer Project)

BROAD INSTITUTE

CCLE Cancer Cell Line Encyclopedia

CCLE

http://www.broadinstitute.org/ccle/ (Broad-Novartis Cancer Cell Line Encyclopedia)

BROAD INSTITUTE

Cancer Therapeutics Response Portal v2

CTRP

http://www.broadinstitute.org/ctrp/ (Stuart L. Schreiber Research Laboratory)

CellMiner CDB (Cross Data Base): a new online tool for the community of biomedical researchers, biologists and pharmacologists

Source	# Lines	# Lines (Median	# Drugs	DNA Variant	mRNA Exp	DNA Copy	DNA Meth.	Mir Exp	Protein Exp	# Molecular
NCI-60	**60**	57	21768	9307	25040	23232	17553	417	162 (RPPA)	75711
GDSC	**1080**	900	297	**16532**	19562	*	**17580**	NA	NA	53674
CCLE	**1036**	491	24	1667	19851	23316	*	*	*(RPPA)	44834
CTRP	**823**	751	481	1667	19851	23316	*	*	*(RPPA)	44834
NCI-SCLC	**67**	66	526	NA	17804	NA	NA	NA	NA	17804

~ 80,000 genomic parameters

Gaps

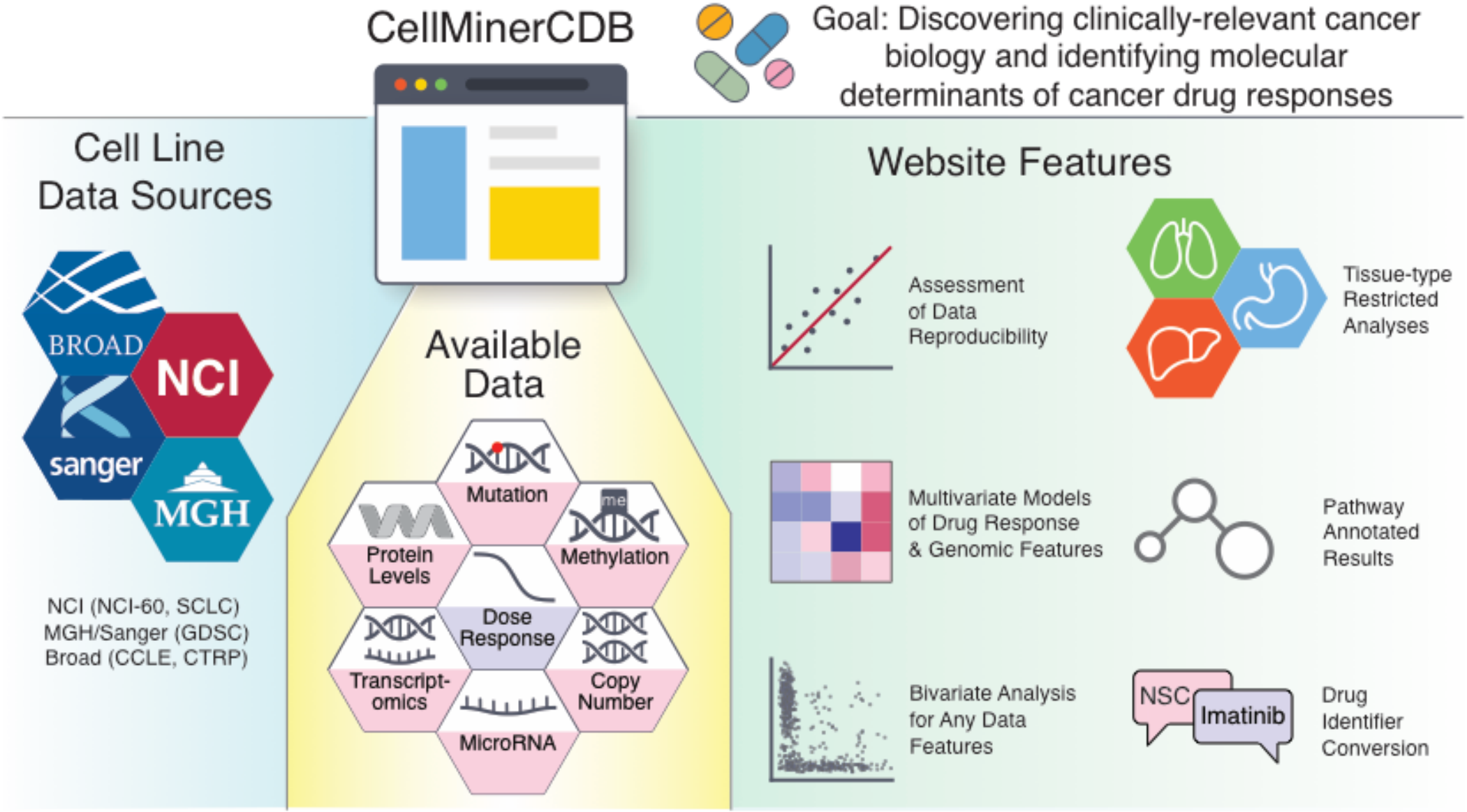
CELL LINES	NCI-60	GDSC	CCLE	CTRP
NCI-60	60	**55**	**44**	**41**
GDSC		1080	**671**	**597**
CCLE			1036	**823**
CTRP				823

Cell lines overlap

DRUGS	NCI-60	GDSC	CCLE	CTRP
NCI-60	21768	**57**	**12**	**63**
GDSC		256	**13**	**74**
CCLE			24	**16**
CTRP				481

Drugs overlap

http://discover.nci.nih.gov/cellminercdb



http://discover.nci.nih.gov/cellminercdb/

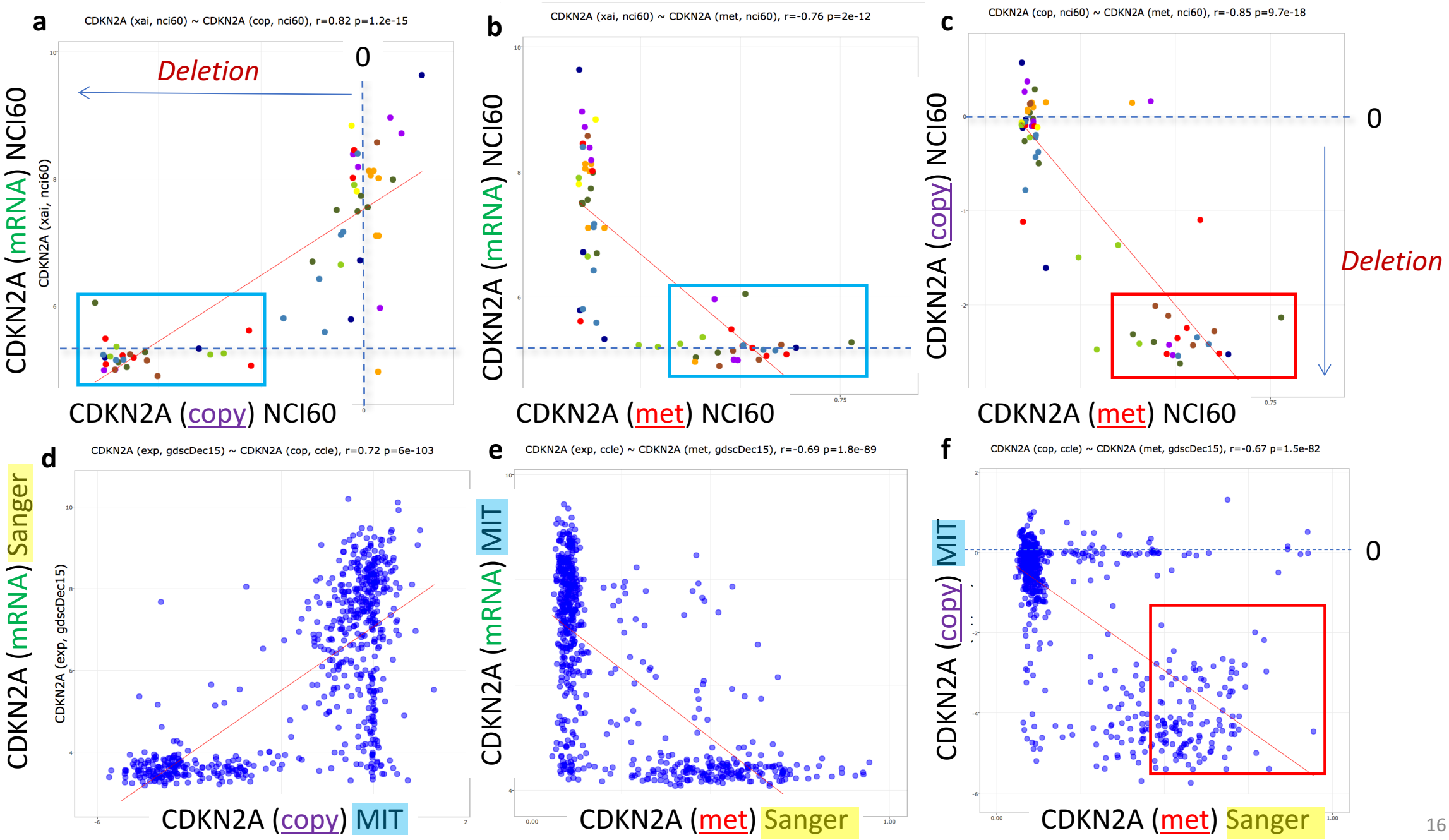
CellMiner CDB: the power of Cross DataBase analyses (SLFN11 as test run)
Reproducibility is high across databases (cell lines are comparable at the genomic level)

NCI60

GDSC (Sanger)

http://discover.nci.nih.gov/cellminercdb

CellMiner CDB: Exploring gene expression determinants: CDKN2A – p16 tumor suppressor



RESEARCH ARTICLE SUMMARY

CANCER THERAPY

Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15

Ting Han, Maria Goralski,* Nicholas Gaskill,* Emanuela Capota, Jiwoong Kim, Tabitha C. Ting, Yang Xie, Noelle S. Williams, Deepak Nijhawan†

INTRODUCTION: Indisulam is an aryl sulfonamide drug that inhibits the proliferation of certain human cancer cell lines. Its mechanism of action and the mechanism underlying its selectivity are poorly understood. On the basis of its anticancer activity in vitro and in mice, indisulam has been extensively tested in patients with advanced-stage solid tumors. No unacceptable toxicities were reported in patients receiving indisulam monotherapy, but fewer than 10% of patients showed a clinical response.

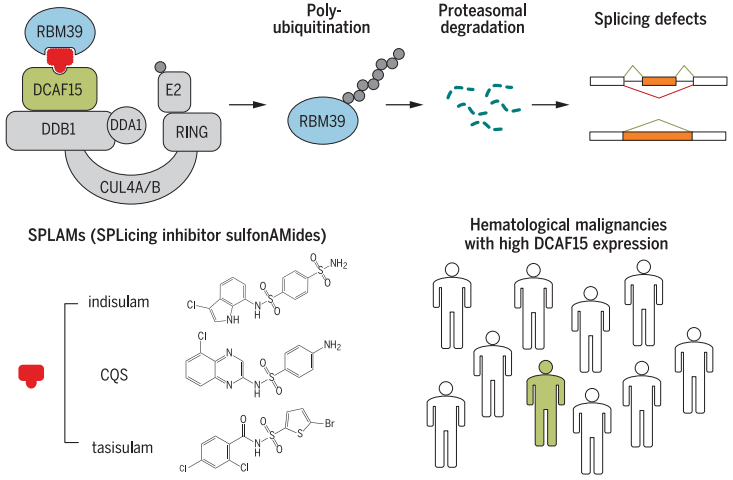
RATIONALE: At present, there is no way to predict which cancer patients are most likely to benefit from indisulam treatment. We reasoned that a better understanding of the molecular mechanism underlying indisulam's anticancer activity might reveal why only a subset of tumors respond to it. This in turn might lead to more effective clinical use of the drug. To study indisulam's mechanism of action, we identified genetic mutations that confer resistance to its cytotoxic effect.

RESULTS: Using a forward genetic strategy, we discovered that several single amino acid substitutions in a nuclear protein called RBM39 (RNA binding motif protein 39) conferred resistance to the toxic effects of indisulam in cultured cancer cells and in mice with tumor xenografts. In the presence of indisulam, RBM39 associated with the CUL4-DDB1-DDA1-DCAF15 E3 ubiquitin ligase complex (CUL4-DCAF15), leading to polyubiquitination and proteasomal degradation of RBM39. Mutations in RBM39 that cause indisulam resistance impeded the formation of this complex. Interestingly, we found that two other clinically tested sulfonamides with structural similarity to indisulam—tasisulam and chloroquinoxaline sulfonamide (CQS)—share the same mechanism of action as indisulam. RBM39 is a nuclear protein that is involved in precursor mRNA (pre-mRNA) splicing. Biochemical isolation of RBM39 revealed an association with numerous splicing factors and RNA binding proteins. We found that degradation of RBM39 by indisulam led to aberrant pre-mRNA splicing, including intron retention and exon skipping, in hundreds of genes.

In a large survey of indisulam sensitivity across more than 800 cancer cell lines, we found that cancer cells derived from the hematopoietic and lymphoid (HL) lineages were more sensitive than cancer cells derived from other lineages. In HL cancer cell lines, *DCAF15* mRNA expression levels and *DCAF15* gene copy number variation directly correlated with indisulam sensitivity.

CONCLUSION: Cancer genome–sequencing studies have highlighted the importance of pre-mRNA splicing in tumorigenesis. Drugs such as indisulam, tasisulam, and CQS—which we collectively refer to as SPLAMs (splicing inhibitor sulfonamides)—provide a strategy to target RBM39-dependent pre-mRNA splicing in cancer. Many of the earlier clinical trials of indisulam focused on patients with solid tumors. Our findings suggest that indisulam may be most effective in patients with leukemias and lymphomas that express relatively high levels of DCAF15.

The activity of SPLAMs resembles that of IMiDs (immunomodulatory drugs). IMiDs are anticancer drugs that act as a "molecular glue," bringing together the E3 ubiquitin ligase receptor cereblon and a variety of neosubstrates. In an analogous manner, SPLAM derivatives potentially could be used to target DCAF15 to novel neosubstrates that, like RBM39, are otherwise undruggable. ■



SPLAMs target the splicing factor RBM39 for proteasomal degradation. A class of clinically tested anticancer sulfonamides, including indisulam, tasisulam, and CQS, functions by promoting the interaction of the RBM39 splicing factor and the CUL4-DCAF15 E3 ubiquitin ligase, leading to polyubiquitination and proteasomal degradation of RBM39. Cancer cell lines from hematopoietic and lymphoid lineages that show high expression levels of DCAF15 are more sensitive to the cytotoxic effects of SPLAMs, suggesting that DCAF15 is a potential biomarker to guide future clinical trials of SPLAMs.

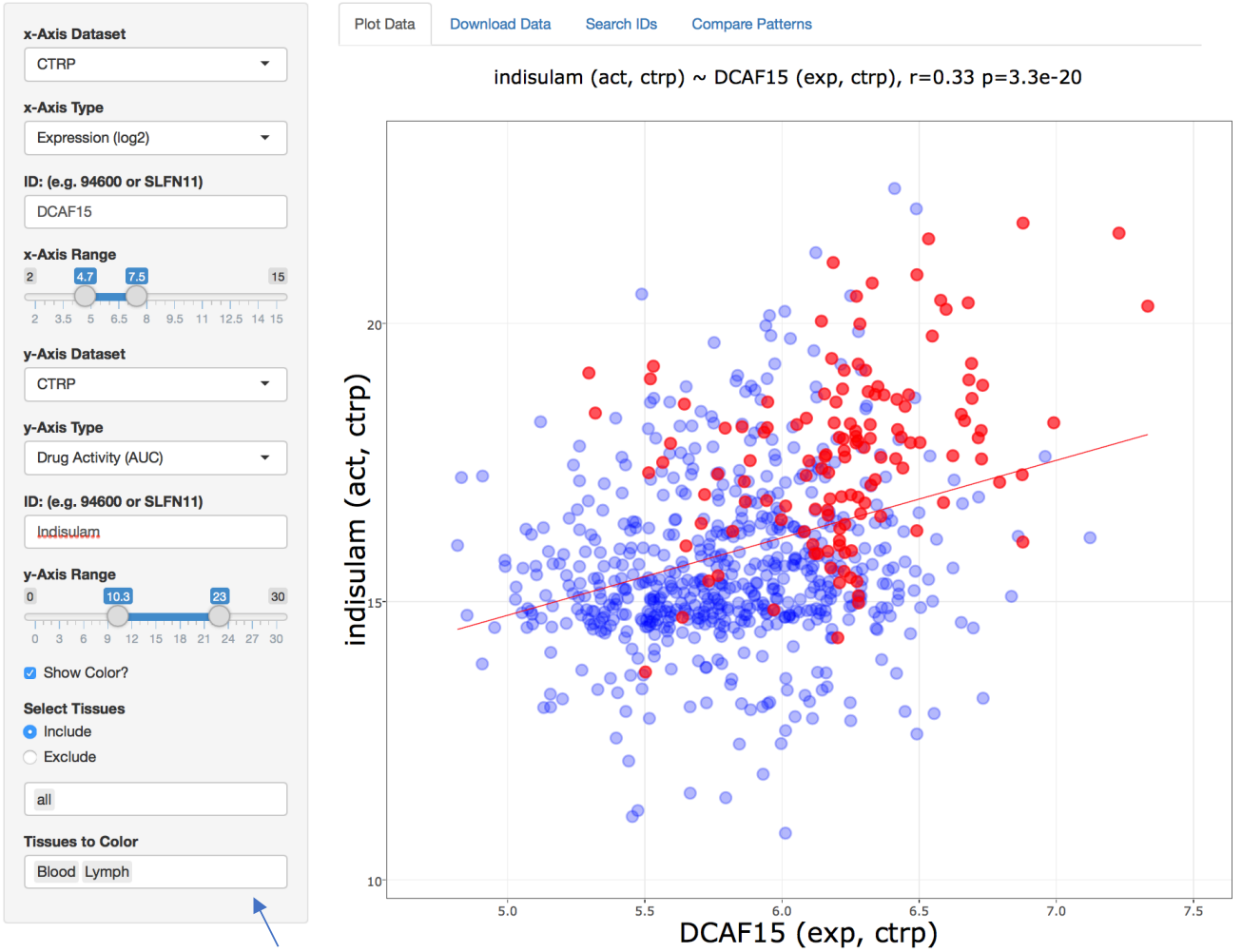
ON OUR WEBSITE

Read the full article at http://dx.doi.org/10.1126/science.aal3755

The list of author affiliations is available in the full article online.
*These authors contributed equally to this work.
†Corresponding author. Email: deepak.nijhawan@utsouthwestern.edu
Cite this article as T. Han *et al.*, *Science* **356**, eaal3755 (2017). DOI: 10.1126/science.aal3755

Han *et al., Science* **356**, 397 (2017)
28 April 2017

Validation of Exceptional Responders in Cancer Cell Lines (CTRIP-CCLE cancer cell line encyclopedia (Stuart Schreiber)



Blood and Lymph cell lines colored in red

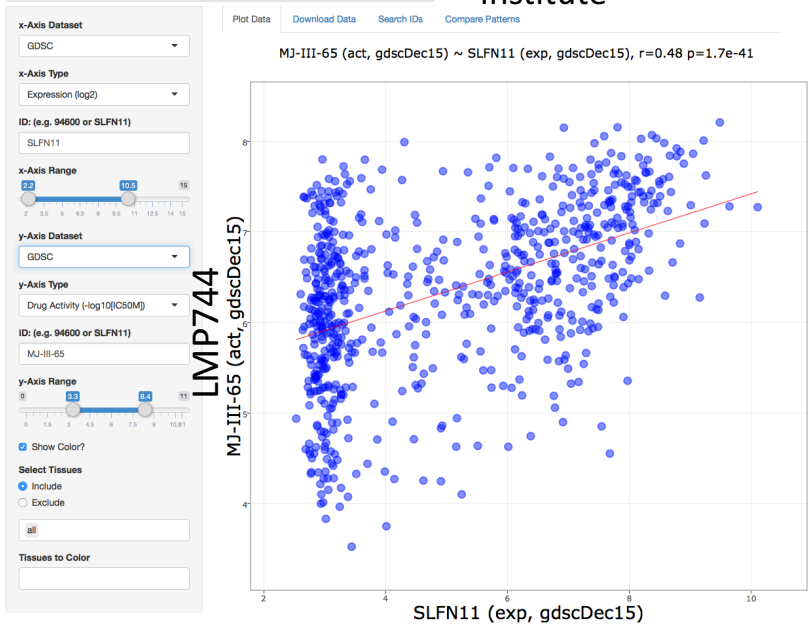
http://discover.nci.nih.gov/cellminercdb

Other synthetic lethal interactions and genomic signatures to determine rational indications and combinations

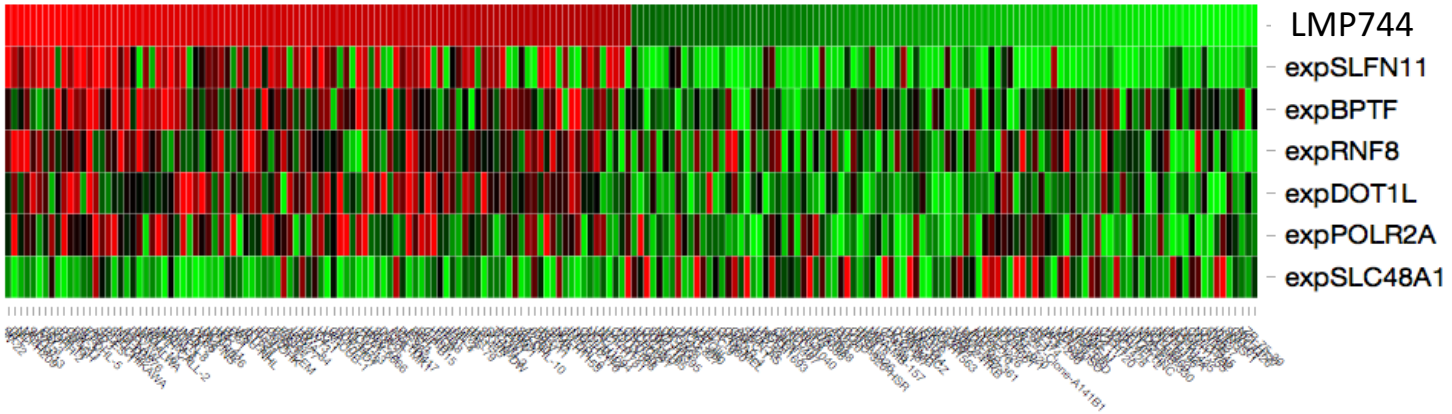
CellMiner CDB (http://discover.nci.nih.gov/cellminercdb)

Univariate Analysis

Collaboration Sanger Institute



Regression Analysis - Multivariate Analysis - Lasso Regression Analyses



(see CellMiner website)

100 most sensitive (green) and most resistant (red) cell lines are displayed above

- Synthetic lethality beyond BRCA and PARP inhibitors
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- SLFN11 as a highly penetrant determinant of response
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Putative DNA/RNA helicase Schlafen-11 (SLFN11) sensitizes cancer cells to DNA-damaging agents

Gabriele Zoppoli[a,b,1,2], Marie Regairaz[a,1], Elisabetta Leo[a,1], William C. Reinhold[a], Sudhir Varma[a], Alberto Ballestrero[b], James H. Doroshow[c], and Yves Pommier[a,2]

[a]Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; [b]Department of Internal Medicine, University of Genova and Istituto di Ricovero e Cura a Carattere Scientifico Azienda Ospedaliera Universitaria San Martino, Instituto Nazionale per la Ricerca sul Cancro, 16132 Genoa, Italy; and [c]Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Edited* by Allan H. Conney, Rutgers, State University of New Jersey, Piscataway, NJ, and approved July 27, 2012 (received for review April 23, 2012)

Top1 & Top2 inhibitors, cisplatin, carboplatin, gemcitabine, cytarabine

The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity

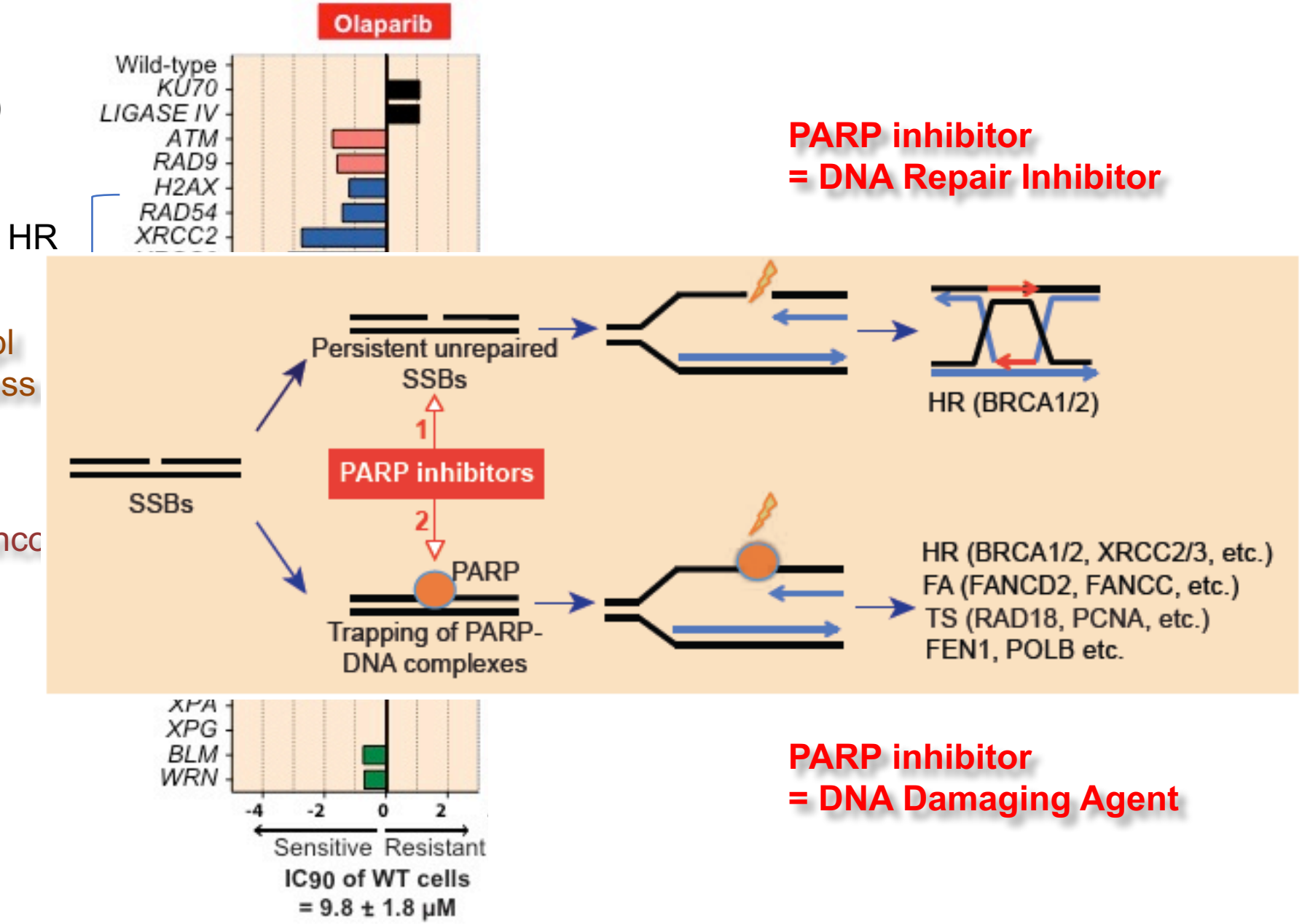
Jordi Barretina[1,2,3]†*, Giordano Caponigro[4]*, Nicolas Stransky[1]*, Kavitha Venkatesan[4]*, Adam A. Ma
Christopher J. Wilson[4], Joseph Lehár[4], Gregory V. Kryukov[1], Dmitriy Sonkin[4], Anupama Reddy[4], Ma
Michael F. Berger[1]†, John E. Monahan[4], Paula Morais[1], Jodi Meltzer[4], Adam Korejwa[1], Judit Jané-Val
Joseph Thibault[5], Eva Bric-Furlong[4], Pichai Raman[4], Aaron Shipway[5], Ingo H. Engels[5], Jill Cheng[6], C
Peter Aspesi Jr[4], Melanie de Silva[4], Kalpana Jagtap[4], Michael D. Jones[4], Li Wang[4], Charles Hatton[3], E
Supriya Gupta[1], Scott Mahan[1], Carrie Sougnez[1], Robert C. Onofrio[1], Ted Liefeld[1], Laura MacConaill[3],
Michael Reich[1], Nanxin Li[5], Jill P. Mesirov[1], Stacey B. Gabriel[1], Gad Getz[1], Kristin Ardlie[1], Vivien Cha
Barbara L. Weber[4], Jeff Porter[4], Markus Warmuth[4], Peter Finan[4], Jennifer L. Harris[5], Matthew Meyer
Michael P. Morrissey[4]*, William R. Sellers[4]*, Robert Schlegel[4]* & Levi A. Garraway[1,2,3]*

29 MARCH 2012 | VOL 483 | NATURE | 603

The systematic translation of cancer genomic data into knowledge of tumour biology and therapeutic possibilities remains challenging. Such efforts should be greatly aided by robust preclinical model systems that reflect the genomic diversity of human cancers and for which detailed genetic and pharmacological annotation is available[1]. Here we describe the Cancer Cell Line Encyclopedia (CCLE): a compilation of gene expression, chromosomal copy number and massively parallel sequencing data from 947 human cancer cell lines. When coupled with pharmacological profiles for 24 anticancer drugs across 479 of the cell lines, this collection allowed identification of genetic, lineage, and gene-expression-based predictors of drug sensitivity. In addition to known predictors, we found that plasma cell lineage correlated with sensitivity to IGF1 receptor inhibitors; *AHR* expression was associated with MEK inhibitor efficacy in *NRAS*-mutant lines; and *SLFN11* expression predicted sensitivity to topoisomerase inhibitors. Together, our results indicate that large, annotated cell-line collections may help to enable preclinical stratification schemata for anticancer agents. The generation of genetic predictions of drug response in the preclinical setting and their incorporation into cancer clinical trial design could speed the emergence of 'personalized' therapeutic regimens[2].

Determinants of response to PARP inhibitors beyond BRCA and MDR

Panel of DT40 Single Knockouts (Shunichi Takeda)



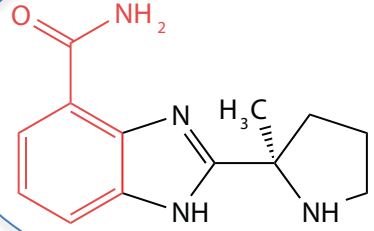
PARP inhibitor
= DNA Repair Inhibitor

PARP inhibitor
= DNA Damaging Agent

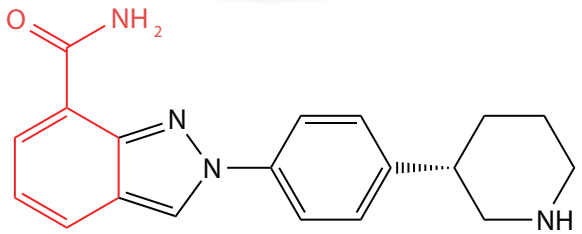
Murai, J…Doroshow, J…Pommier, Y (2012) Cancer Res

Clinical PARP Inhibitors that trap PARP have most extended and rigid chemical structures

Least Trapping
Good catalytic inhibition

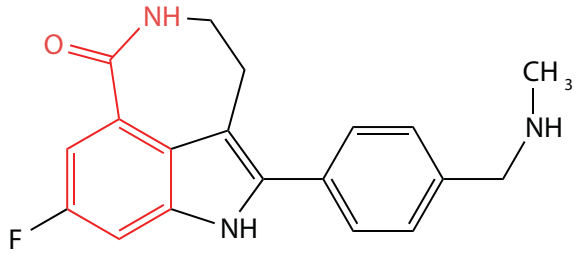


Veliparib
ABT-888



Niraparib
MK-4827

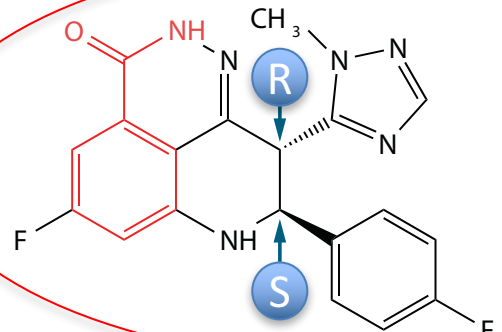
Zejula
FDA Approved
March 2017



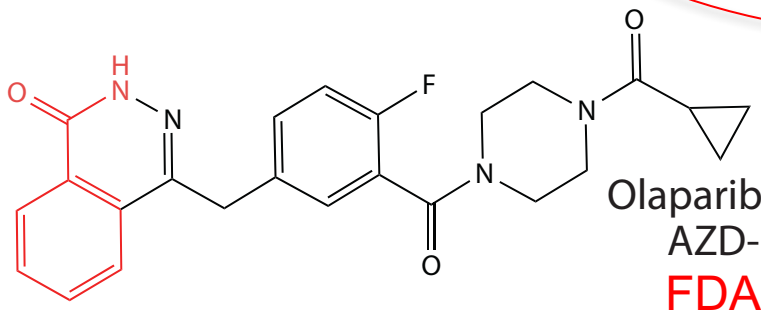
Rubraca
Accelerated FDA Approval
Dec 2016

Rucaparib
AG-014699

Most trapping



Talazoparib
BMN-673



Olaparib: Lynparza
AZD-2281
FDA Approved
Dec 2014

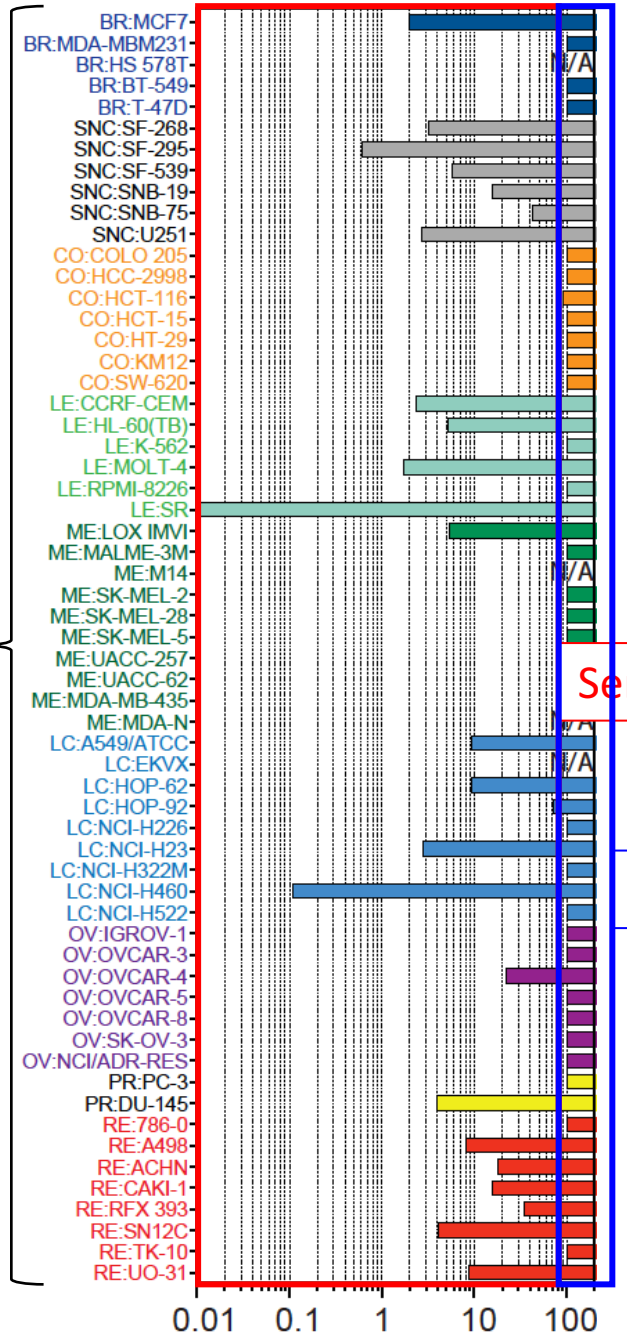
Pommier, O'Connor, de Bono 2016 Science TM

BRCA-independent determinant of talazoparib sensitivity

Homozygous mutation of BRCA1: 0/60
Homozygous mutation of BRCA2: 1/60
(Sousa et al., DNA repair, 2015)

NCI-60

Sixty cancer cell lines



IC_{50} for Talazoparib (=BMN674) (μM)

Sensitive to talazoparib

Resistant to talazoparib

Very potent
C_{max}: 50 nM

=> CellMiner (cdb) COMPARE analysis)

Developmental Therapeutic Program

Sanger database 1000 cancer cell lines

PARP inhibitor Talazoparib

CellMinerCDB | Univariate Analyses | Regression Models | Metadata | About

x-Axis Dataset: GDSC

x-Axis Type: Drug Activity (-log10[IC50M])

ID: (e.g. 94600 or SLFN11): BMN-673

x-Axis Range: 0 | 3 | 8.2 | 11 (0 1.5 3 4.5 6 7.5 9 10.51)

y-Axis Dataset: GDSC

y-Axis Type: Expression (log2)

ID: (e.g. 94600 or SLFN11): PARP1

y-Axis Range: 2 | 4.3 | 9.9 | 15 (2 3.5 5 6.5 8 9.5 11 12.5 14 15)

Show Color?

Select Tissues

Include

Exclude

all

Tissues to Color

Plot Data | Download Data | Search IDs | Compare Patterns

- cop: Copy Number
- mut: Mutation
- exp: Expression (Z-Score)
- xai: Expression (Avg. log2 Int.)
- pro: Protein (RPLA)
- mir: MicroRNA
- mda: Metadata
- swa: Protein (SWATH-MS)

Pattern Comparison: Molecular Data

With Respect to: x-Axis Entry

Show 10 entries

Search:

Data Type	Gene	Location	Correlation	P-Value	Annotation
All	All	All	All	All	All
exp	SLFN11	17q12	0.438	9.48e-45	DNA Damage Response (DDR)
exp	AASS	7q31.3	0.29	1.94e-19	lysine catabolic process;L-lysine catabolic process
met	RAB17	2q37.3	0.287	8.73e-19	protein transport;small GTPase mediated signal transduction
exp	HS3ST4	16p11.2	0.286	7.26e-19	heparan sulfate proteoglycan metabolic process
exp	UGT3A2	5p13.2	0.281	3.46e-18	metabolic process
exp	CMTM3	16q21	0.28	3.83e-18	EMT (Mesenchymal)
exp	QKI	6q26	0.273	2.67e-17	EMT (Mesenchymal)
exp	VIM	10p13	0.271	4.6e-17	EMT (Mesenchymal)
exp	LINC00632	Xq27.1	0.267	1.38e-16	
met	CLDN7	17p13.1	0.261	1.02e-15	EMT (Epithelial)

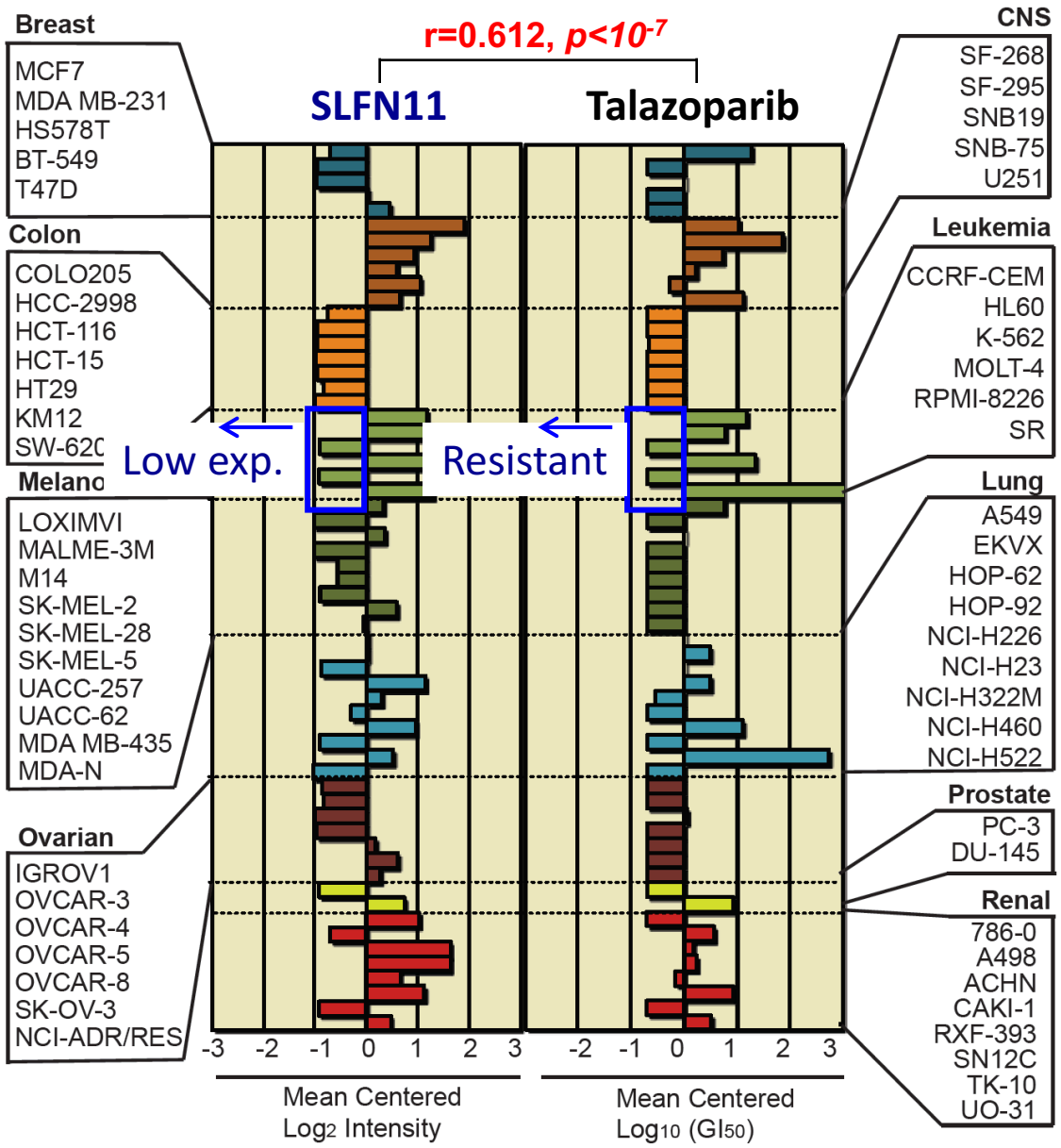
Showing 1 to 10 of 53,678 entries

Previous | 1 | 2 | 3 | 4 | 5 | ... | 5368 | Next

Drug discovery signatures

http://discover.nci.nih.gov/cellminercdb

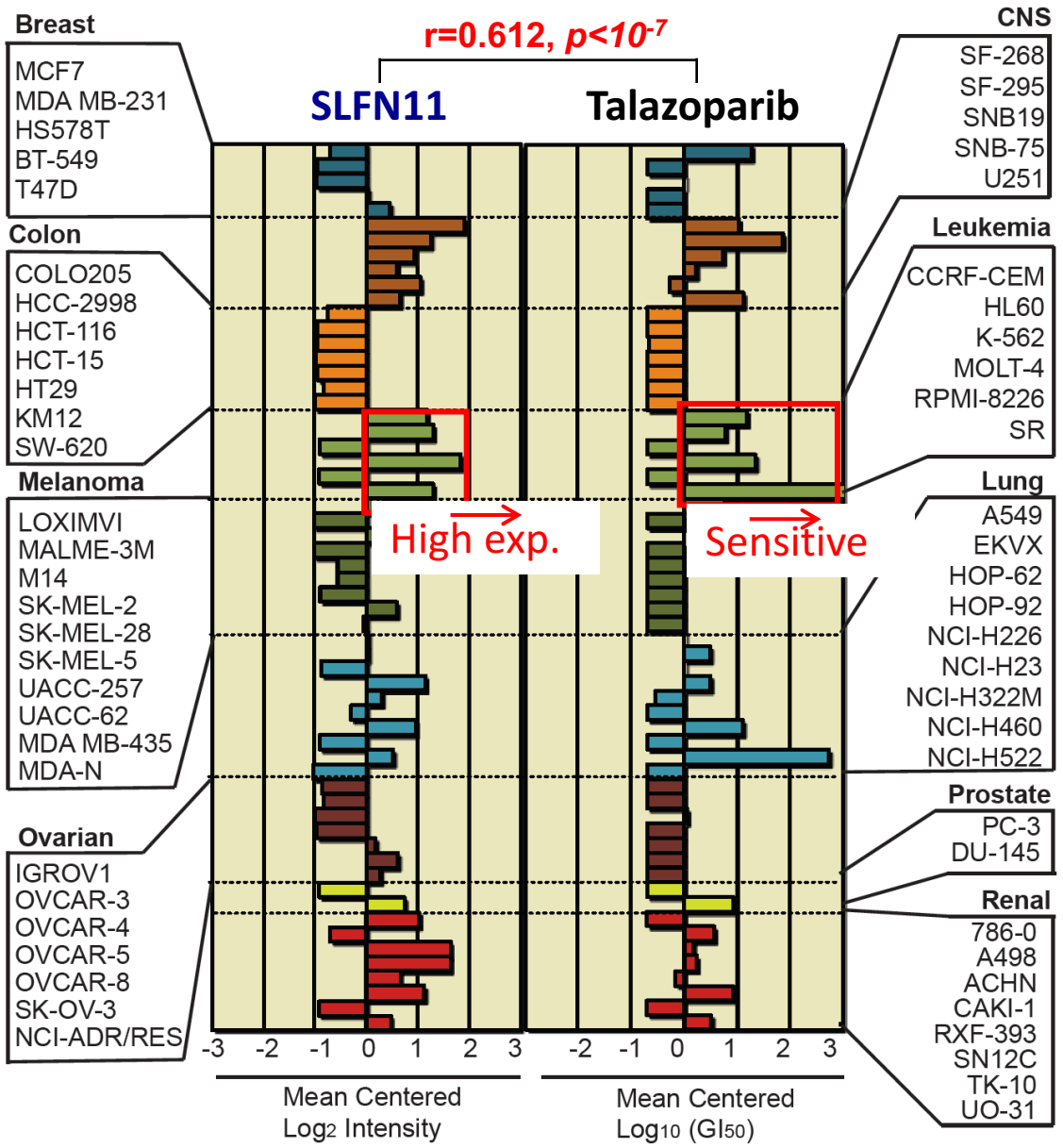
High correlation between expression of *Schlafen 11* (*SLFN11*) and cellular response to talazoparib



CellMiner
http://discover.nci.nih.gov/

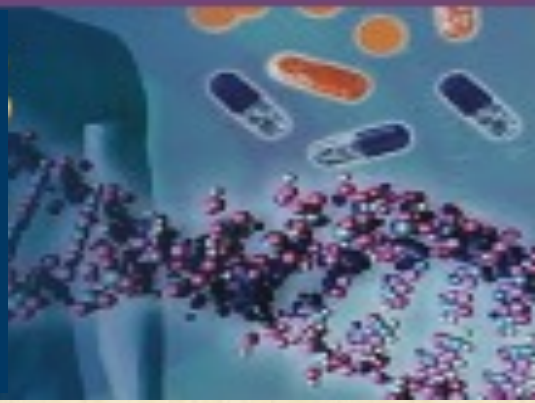
Developmental Therapeutic Program

High correlation between expression of *Schlafen 11* (*SLFN11*) and cellular response to talazoparib



CellMiner
http://discover.nci.nih.gov/

Molecular biology: SLFN11

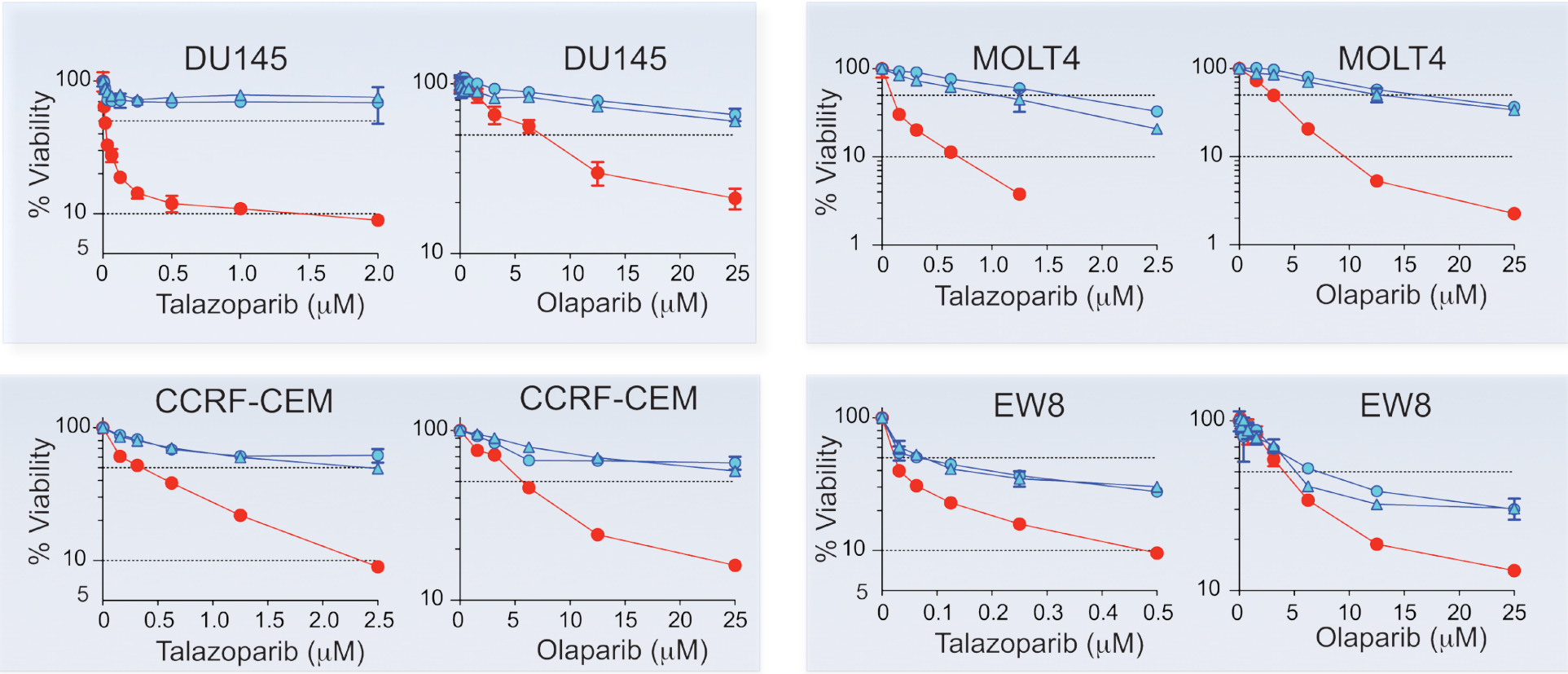


- **A member of the Schlafen (SLFN) family, found only in mammals;**
- **Located in the nucleus;**
- **A putative DNA/RNA helicase;**
- **Binds to chromatin, RPA at damage sites, tRNA…**
- **Transcriptionally regulated by:**
  - ***ETS transcription factors* (EWS-FLI1 in Ewing's)**
    (Clin Cancer Res 2015)
  - ***Promoter methylation***
    (Oncotarget 2015; Cancer Res 2017)
- **Determines sensitivity to *PARP inhibitors* (*Oncocotarget 2016*)**

Schlafen
= To sleep in German

SLFN11 inactivation in 4 different isogenic cell lines confers high resistance to PARP inhibitors

=> SLFN11 inactivation is a novel mechanism of resistance to PARP inhibitors

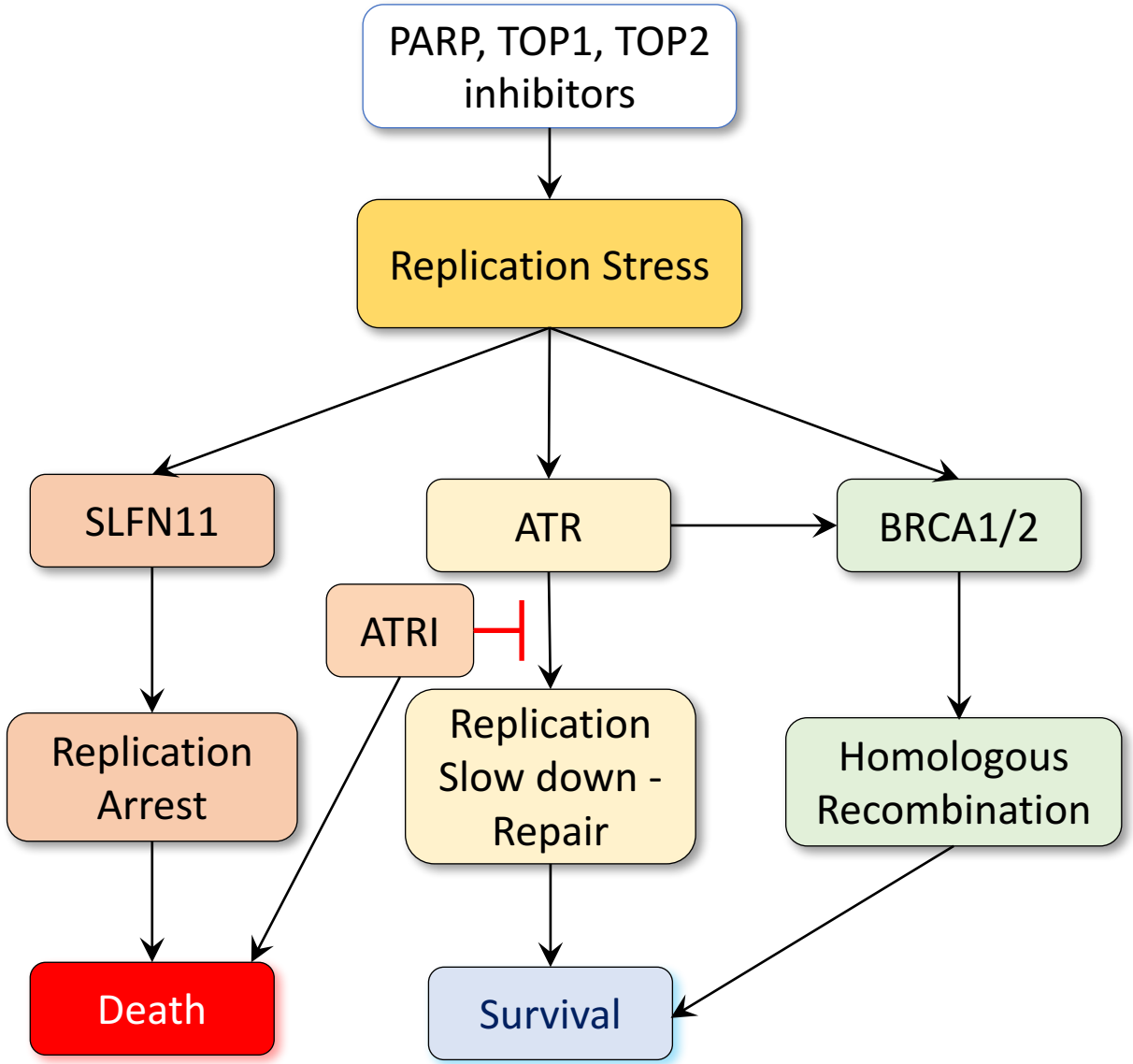


DU145: Prostate cancer
MOLT4 and CCRF-CEM: Leukemia
EW8: Ewing's sarcoma
(CRISPR/Cas9)

Junko Murai

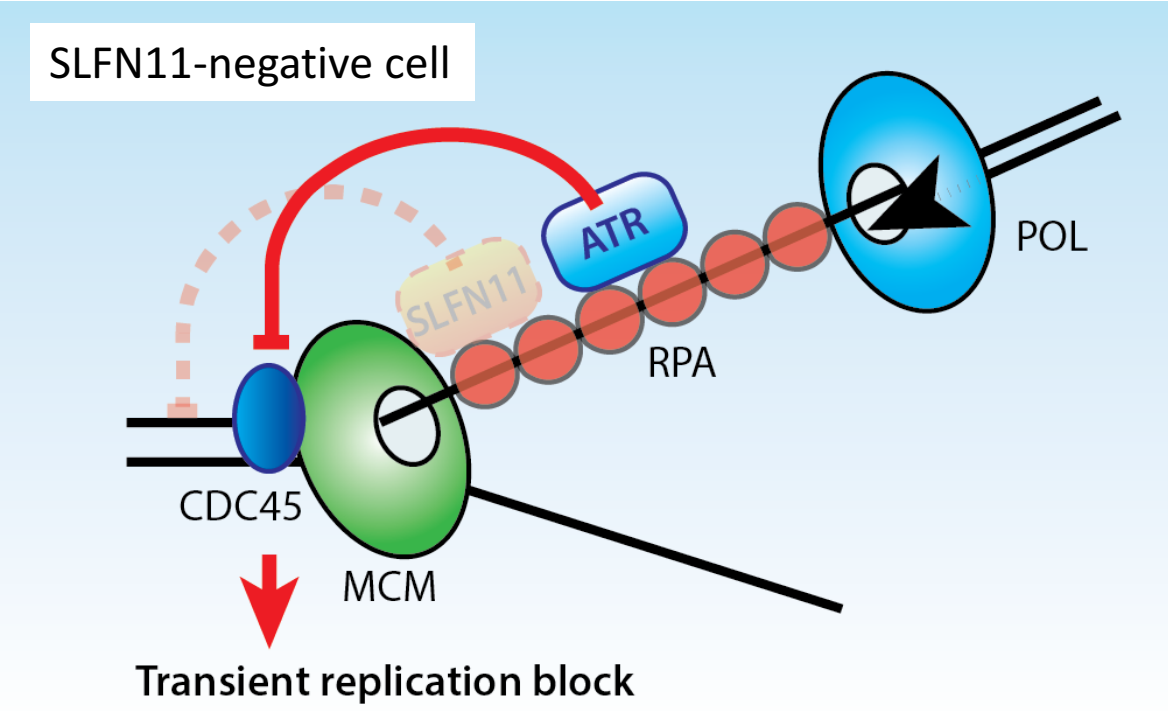
SLFN11 determines response to a broad range of DNA-targeted agents:
TOP1, TOP2, PARP inhibitors, cisplatin, carboplatin, gemcitabine, hydroxyurea…

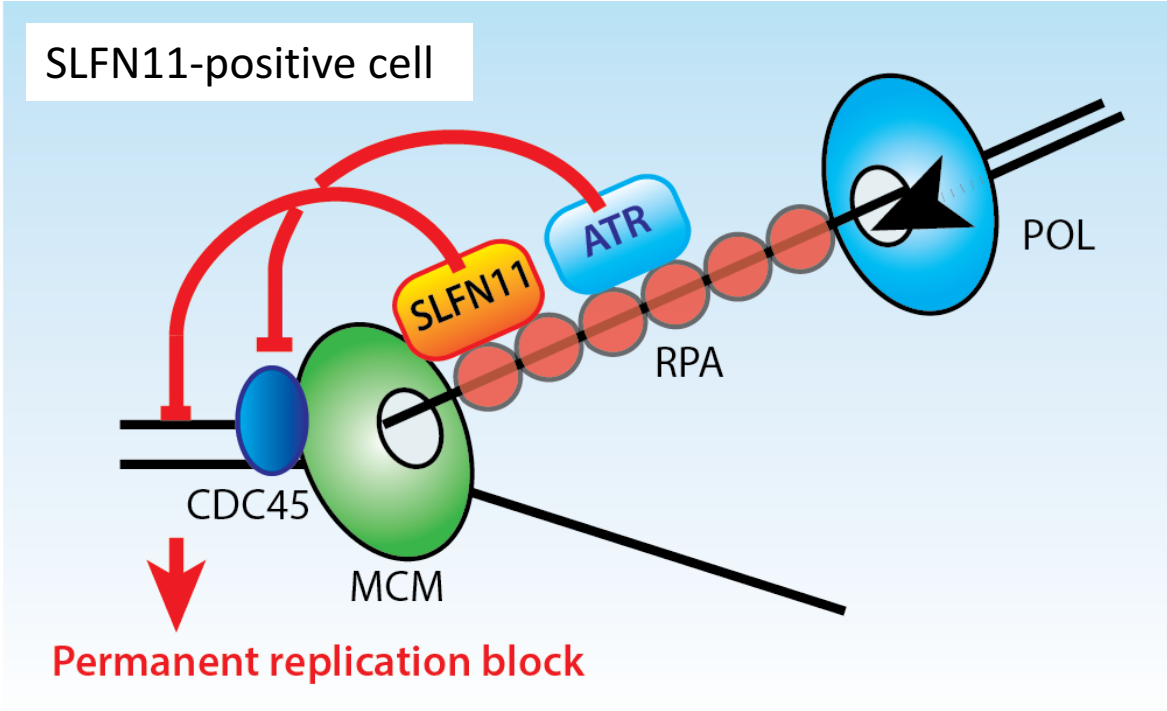
SLFN11 induces lethal replication arrest independently of ATR and BRCA1/2



Murai…Pommier Oncotarget 2016

Working model for replication block by SLFN11

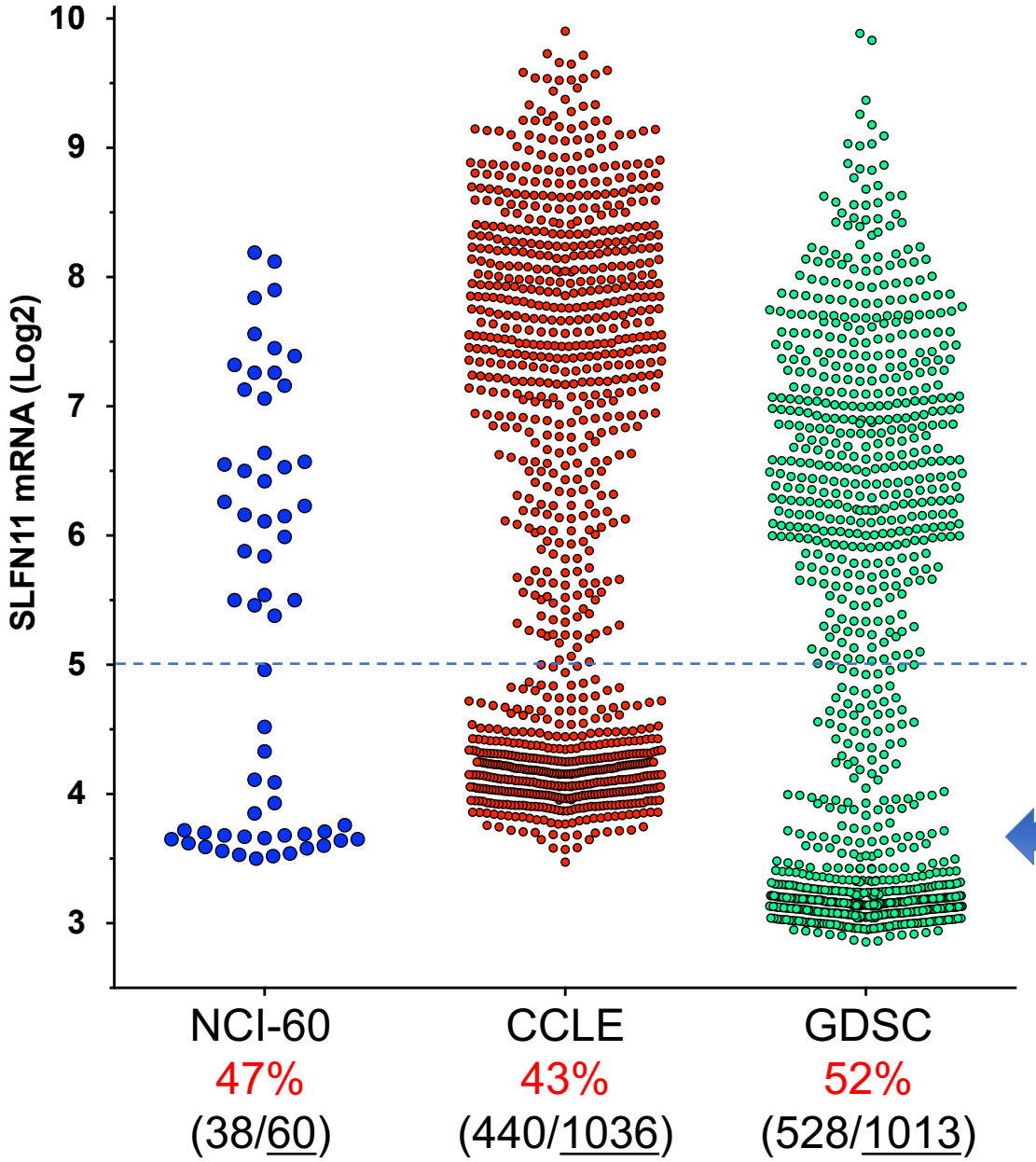




In the absence of SLFN11
(≃ 50% cancer cell lines: HeLa, U2OS, HCT116, RKO, MCF7, MDA-MB231…), ATR-CHK1 transiently arrests replication to allow DNA repair

SLFN11 binds to stressed replication forks through RPA, and arrests replication by blocking the replicative helicase complex

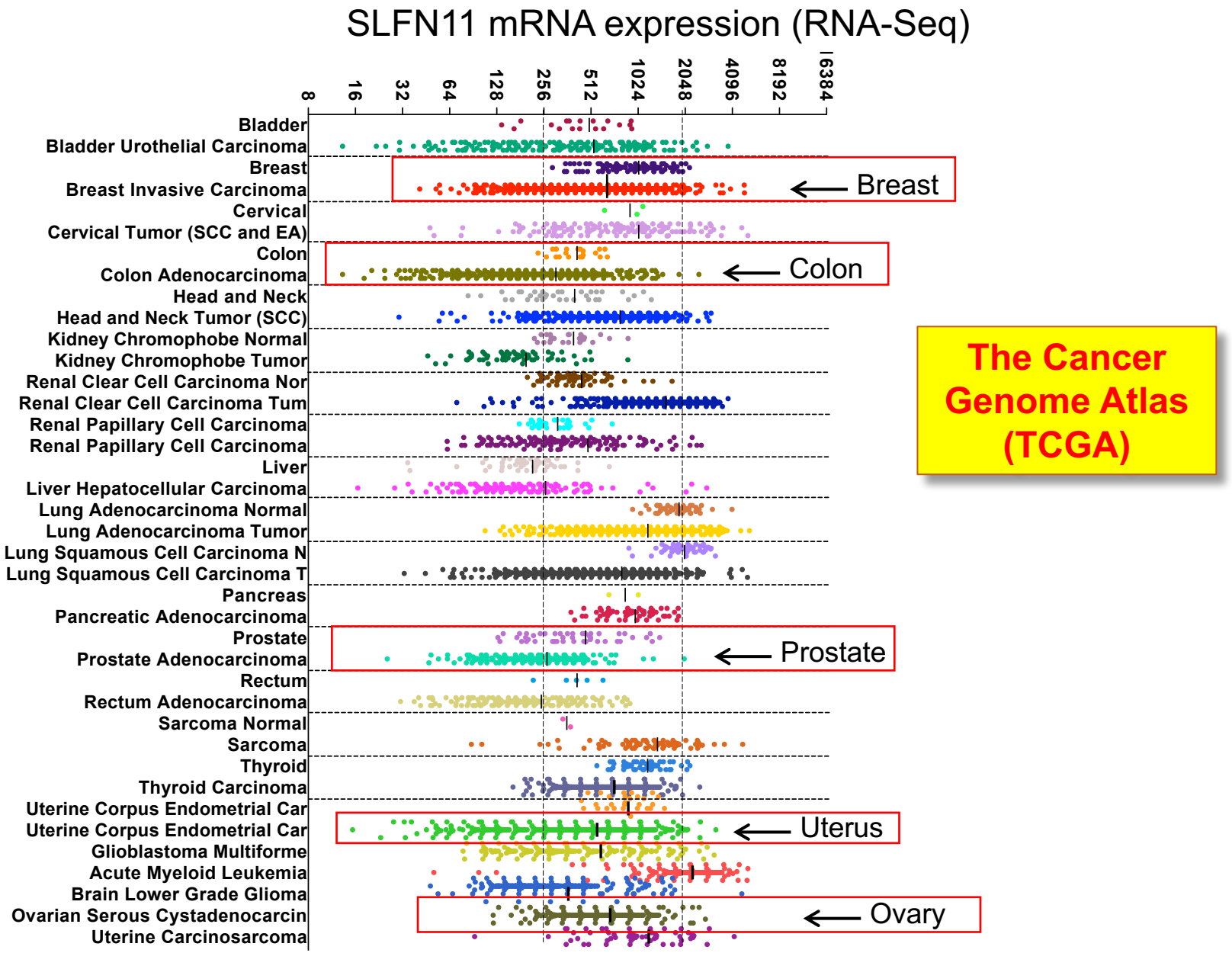
SLFN11 is inactivated in ≈ 45% of cancer cell lines



Especially in the cancer cell lines that are commonly used for screening resistance to PARP inhibitors

HeLa
U2OS
HCT116
RKO
MCF7
MDA-MB231
…

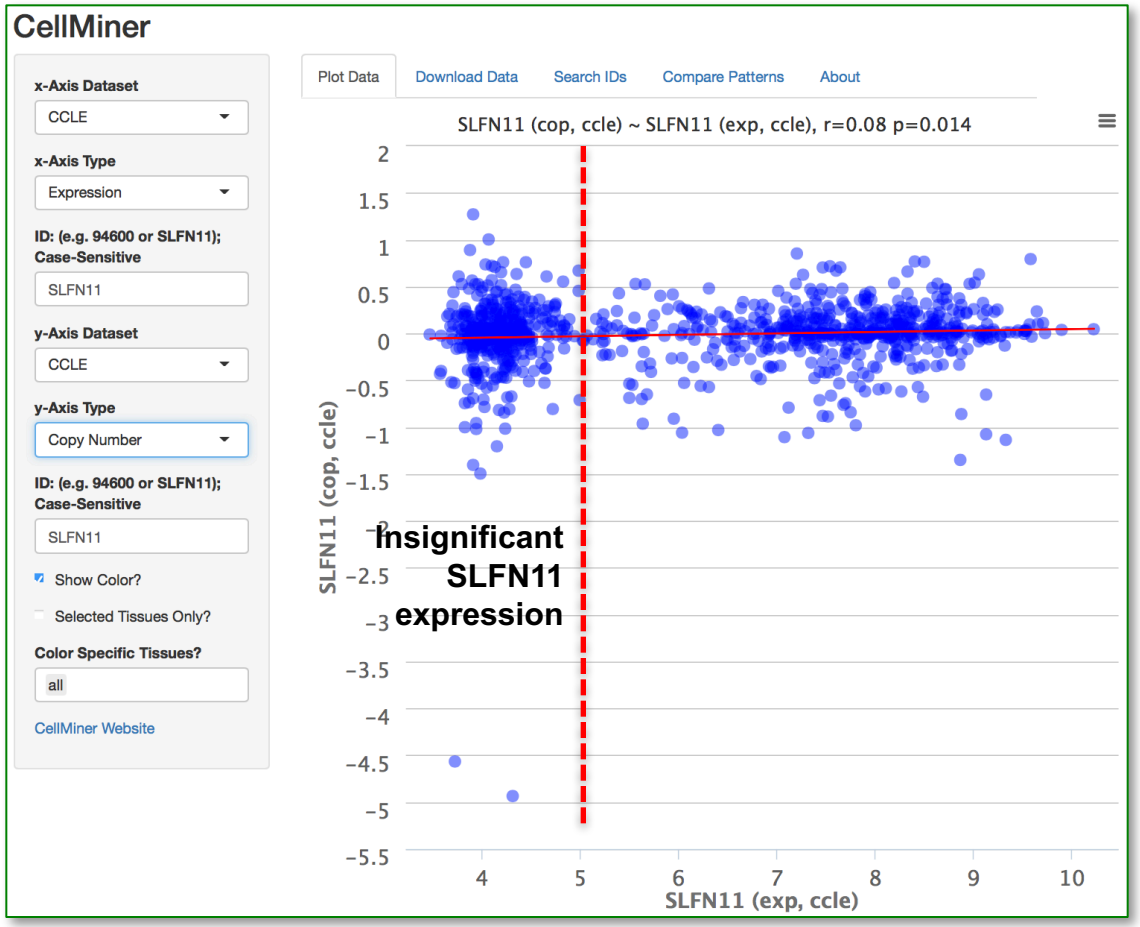
Broad range of SLFN11 expression in tumor tissue



The Cancer Genome Atlas (TCGA)

The regulation of SLFN11 in cancers:

- Transcriptional target of FLI1 and ETS (Ewing's) (Tang, S. 2015)
- Inactivation in about 40% of cancer cell lines (NCI-60 and CCLE) (not by gene deletion)



CellMiner

x-Axis Dataset
NCI-60

x-Axis Type
Expression (Z-Score)

ID: (e.g. 94600 or SLFN11); Case-Sensitive
SLFN11

y-Axis Dataset
NCI-60

y-Axis Type
Methylation

ID: (e.g. 94600 or SLFN11); Case-Sensitive
SLFN11

☑ Show Color?

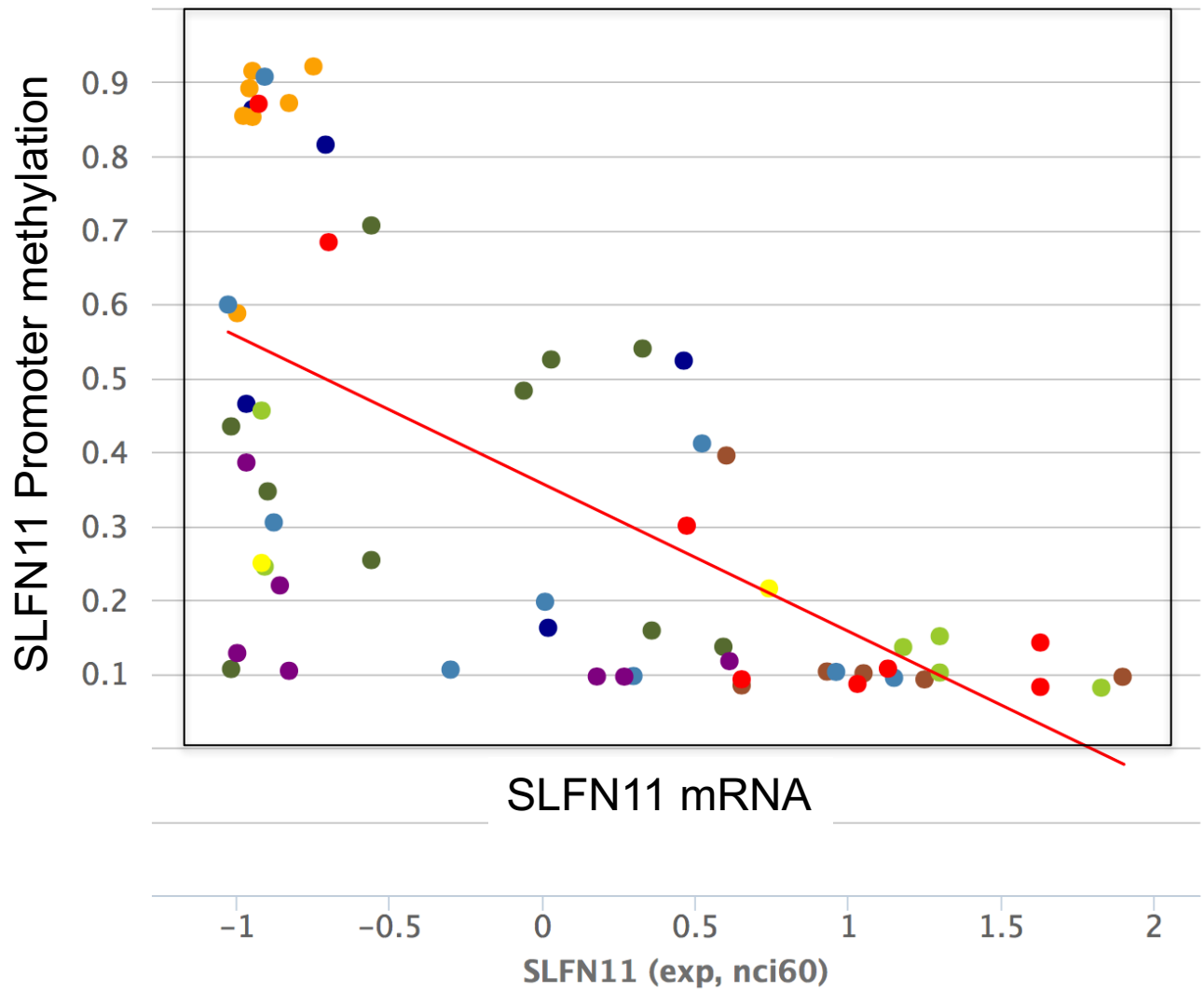
☐ Selected Tissues Only?

Color Specific Tissues?
all

CellMiner Website

Plot Data | Download Data | Search IDs | Compare Patterns | About

SLFN11 inactivation in about 40% of cancer cell lines is in part due to epigenetic imprinting by promoter methylation



SLFN11 inactivation in about 40% of cancer cell lines is in part due to epigenetic imprinting by promoter methylation

CCLE and GDSC
Cross database
Analysis with
Cellminer cdb

x-Axis Dataset
CCLE

x-Axis Type
Expression (log2)

ID: (e.g. 94600 or SLFN11)
SLFN11

x-Axis Range
2 3.1 10.6 15
2 3.5 5 6.5 8 9.5 11 12.5 14 15

y-Axis Dataset
GDSC

y-Axis Type
Methylation

ID: (e.g. 94600 or SLFN11)
SLFN11

y-Axis Range
0 1
0 0.5 1

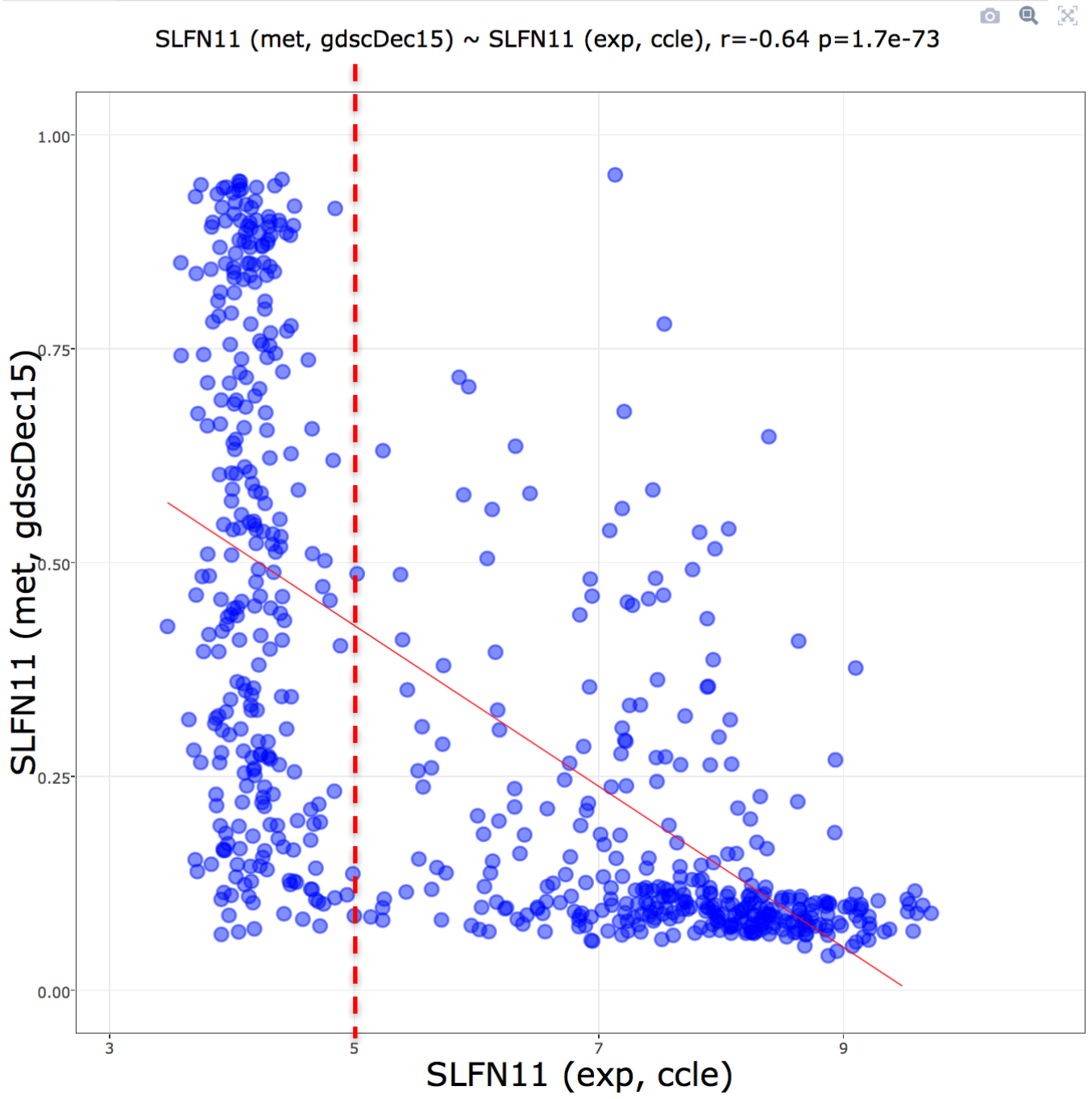
Show Color?

Select Tissues
Include
Exclude

all

Tissues to Color

Plot Data | Download Data | Search IDs | Compare Patterns



SLFN11 inactivation by promoter methylation correlates with resistance to a broad range of DNA damaging agents (NCI-60 database)

	Target/MOA	NSC	Correlation	p-value
Cisplatin	DNA alkylation	119875	-0.59	5.7E-07
Carboplatin	DNA alkylation	241240	-0.55	6.1E-06
Melphalan	DNA alkylation	757098	-0.46	4.7E-04
Topotecan	Top1	759263	-0.42	8.0E-04
Topotecan	Top1	609699	-0.45	2.6E-04
Camptothecin	Top1	94600	-0.48	1.2E-04
LMP-400	Top1	724998	-0.34	8.9E-03
Etoposide	Top2	141540	-0.29	2.8E-02
Gemcitabine	Antimetabolite	613327	-0.41	1.2E-03
Fludarabine	Antimetabolite	312887	-0.42	8.6E-04
Cytarabine	Antimetabolite	63878	-0.31	1.4E-02
Hydroxyurea	Antimetabolite	32065	-0.32	1.3E-02
Bleomycin	DNA	125066	-0.43	6.6E-04
Talazoparib	PARP1/2	767125	-0.30	2.4E-02
Olaparib	PARP1/2	747856	-0.23	9.5E-02
Paclitaxel	microtubules	758645	0.14	2.8E-01
Docetaxel	microtubules	628503	0.16	2.7E-01
Erlotinib	EGFR	718781	-0.08	5.3E-01
Crizotinib	ALK	756645	0.15	2.7E-01
Vemurafenib	BRAF V600E	753082	0.05	7.0E-01

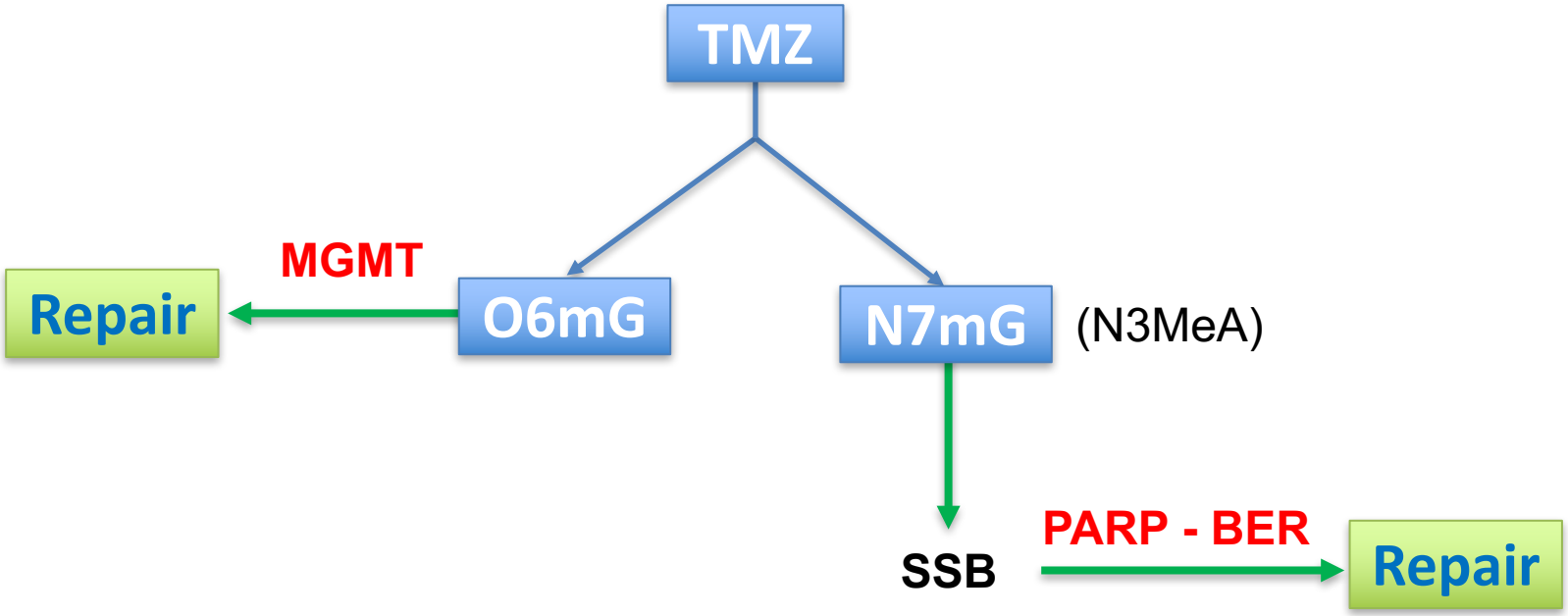
Reinhold, W....Doroshow, J...Pommier 2017 Cancer Res

- Synthetic lethality beyond BRCA and PARP inhibitors
  - TOP1 inhibitors
- Cancer Cell Line genomics as model systems
- SLFN11 as a highly penetrant determinant of response
- Practical implications: example of temozolomide

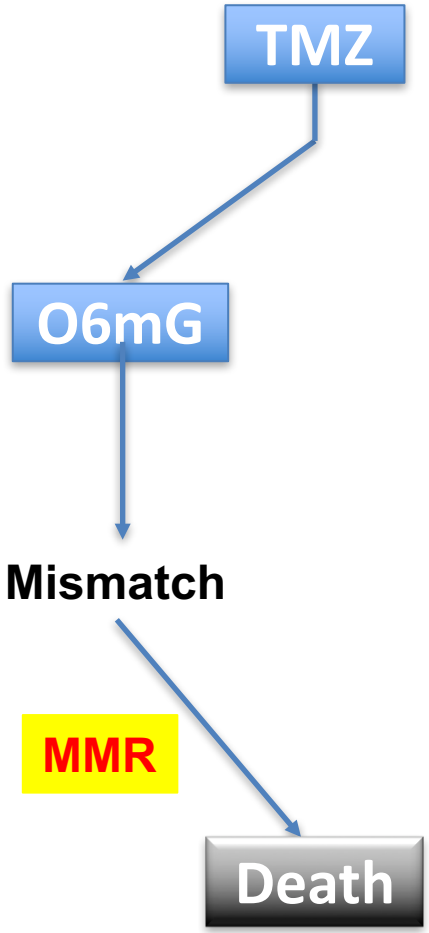
Temozolomide (TMZ) is an oral DNA methylating prodrug approved for glioblastomas based on:

- **its selective cytotoxicity in methylguanine methyltransferase (MGMT)-deficient cells (which is frequent in glioblastomas)**
- **its liposolubility and blood-brain barrier (CNS) penetration.**
- **its relatively low cytotoxicity to normal cells (dose limiting toxicity: bone marrow)**

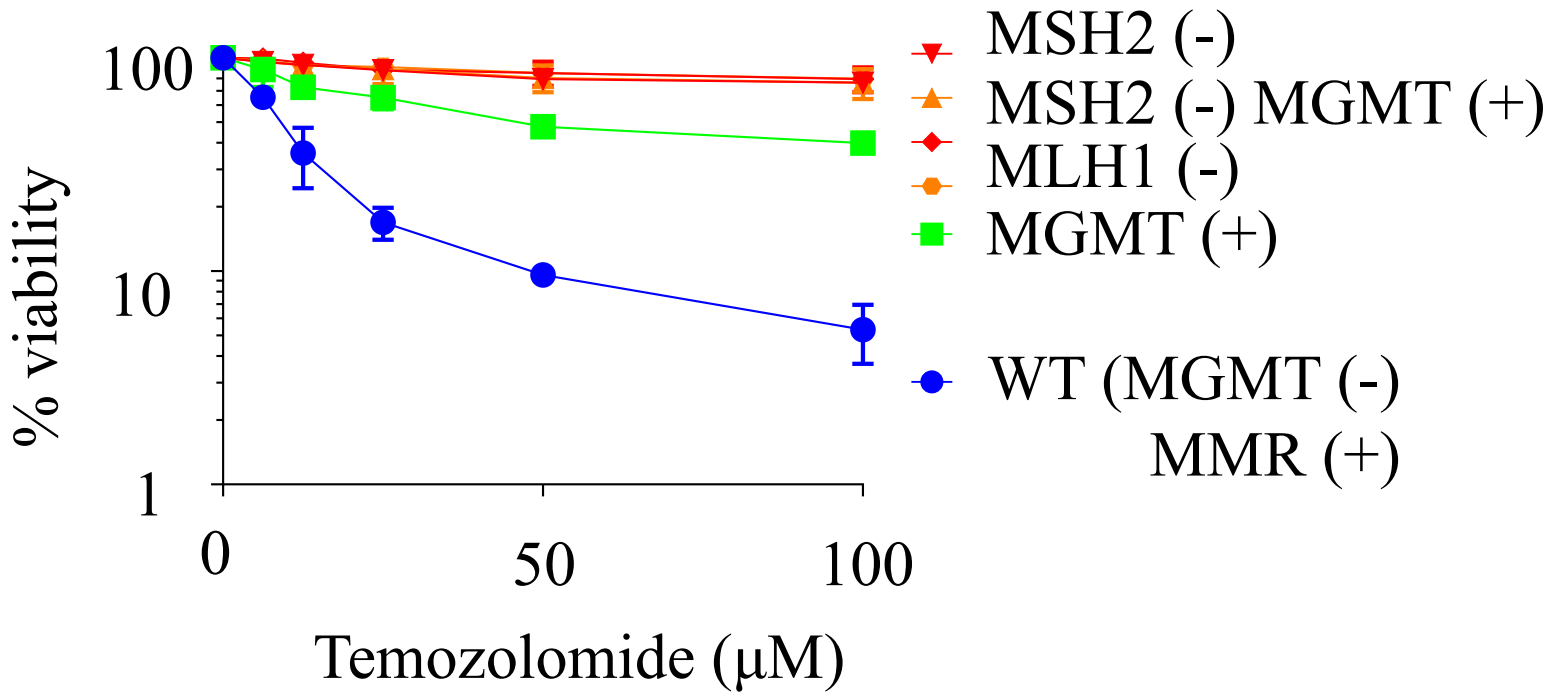
In normal cells TMZ tends to be non-cytotoxic



In MGMT-deficient cells TMZ is cytotoxic by MMR



Both MGMT and MMR determine resistance to temozolomide



MGMT determination and staging based on cancer cell lines (NCI-60)

- MGMT deficiency is frequent (1/3 of NCI-60) and not limited to CNS cancer cells.
- High correlation between protein expression (measured by RPPA – reverse phase protein array - Gordon Mills)
  => transcripts or protein are reliable.
- Poorer performance for promoter methylation ⇔ methylation misses many cell lines such as the CNS, which have no protein (and transcript).

CellMinerCDB | Univariate Analyses | Regression Models | Metadata | About

x-Axis Dataset

CCLE

x-Axis Type

Expression (log2)

ID: (e.g. 94600 or SLFN11); Case-Sensitive

SLFN11

y-Axis Dataset

CCLE

y-Axis Type

Expression (log2)

ID: (e.g. 94600 or SLFN11); Case-Sensitive

MGMT

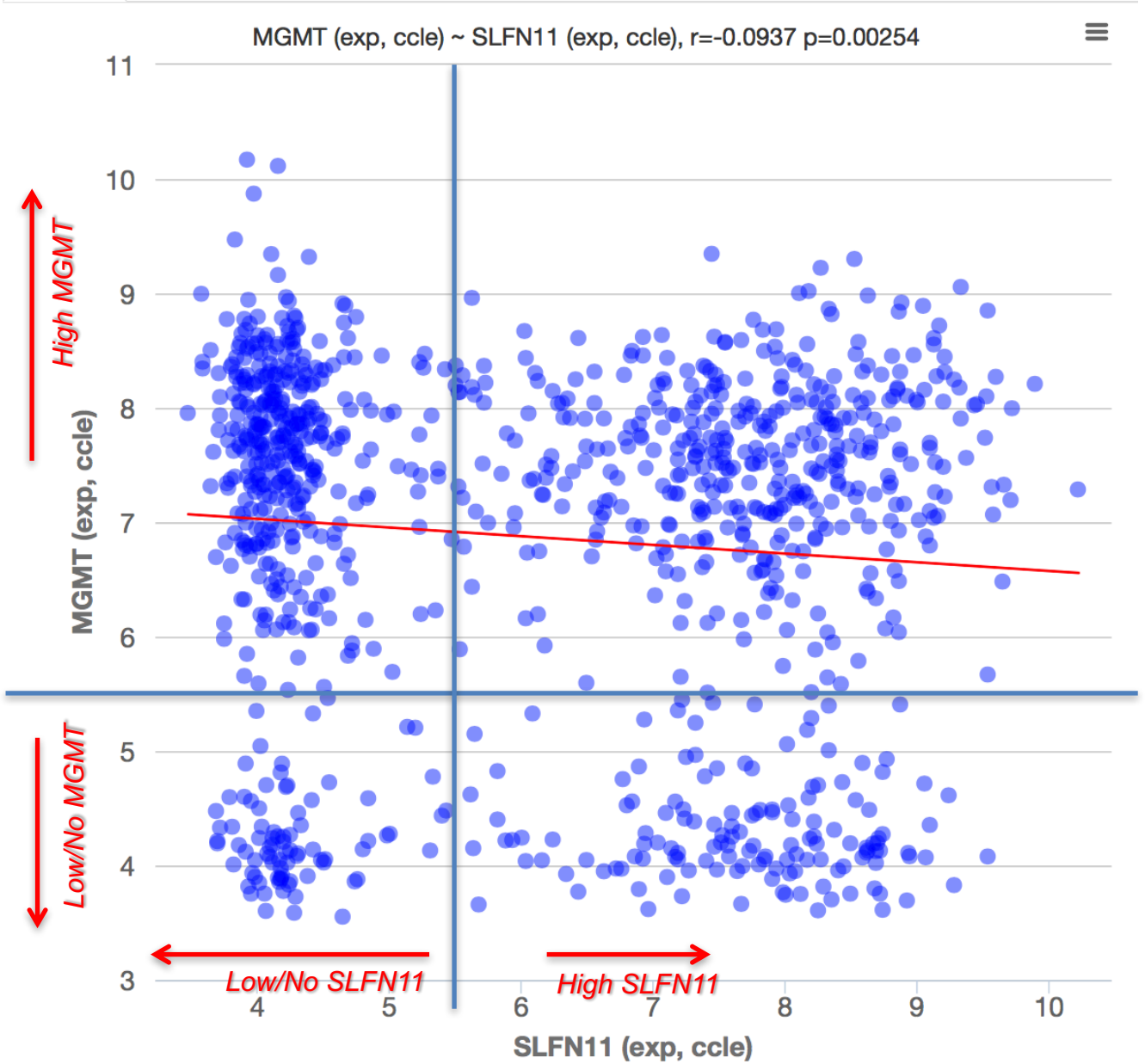
☑ Show Color?

☐ Selected Tissues Only?

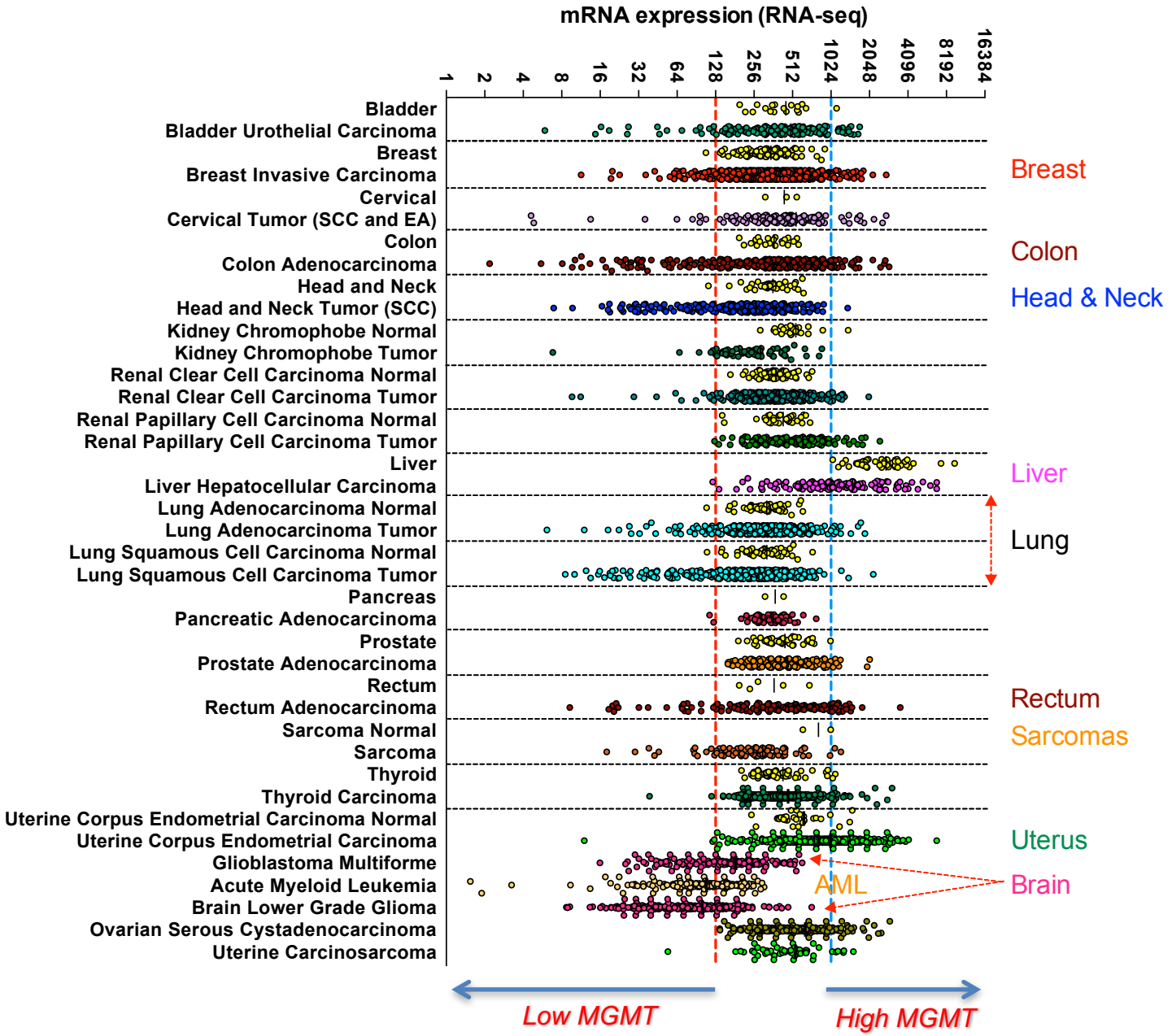
Color Specific Tissues?

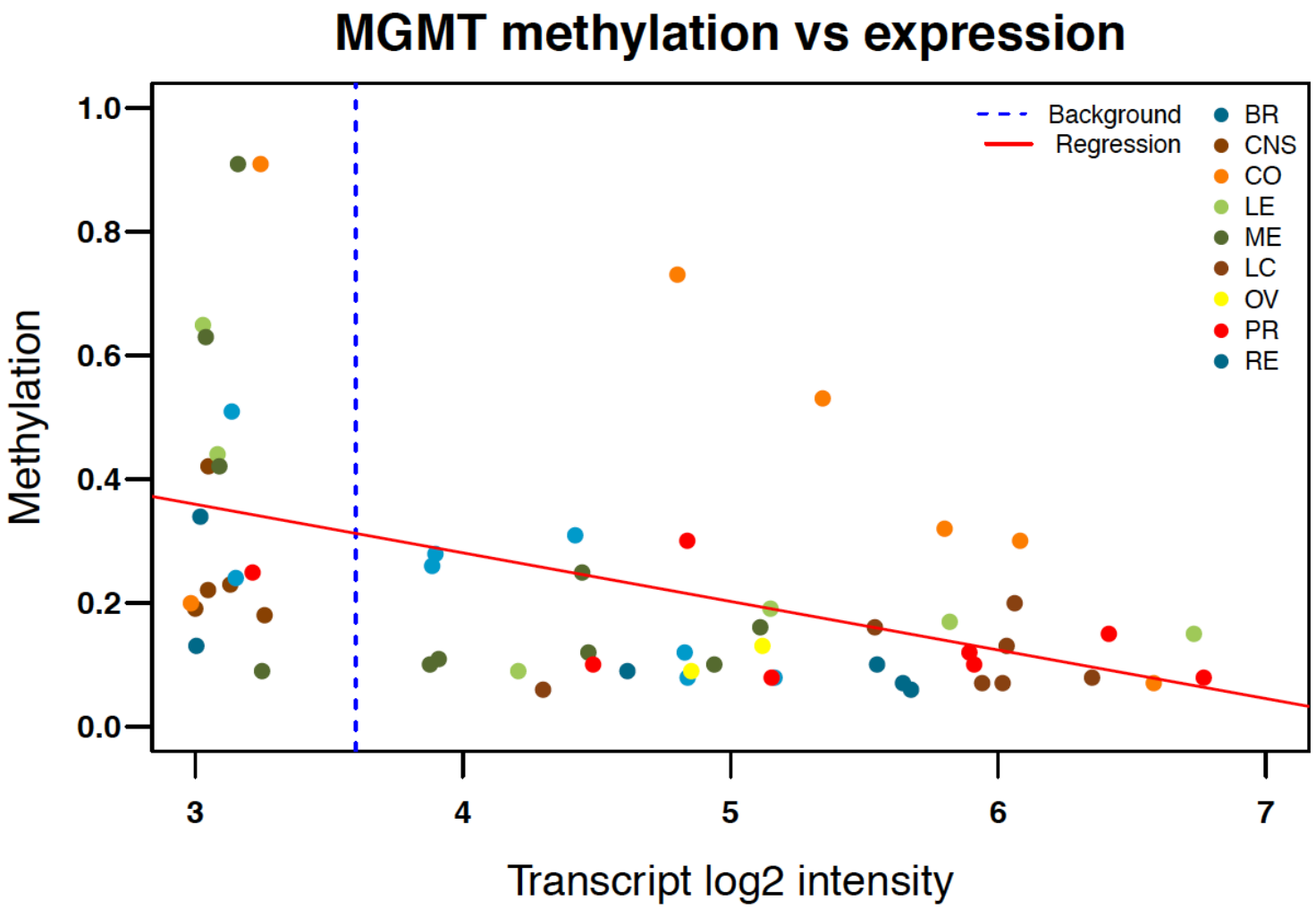
all

Plot Data | Download Data | Search IDs | Compare Patterns



MGMT expression TCGA



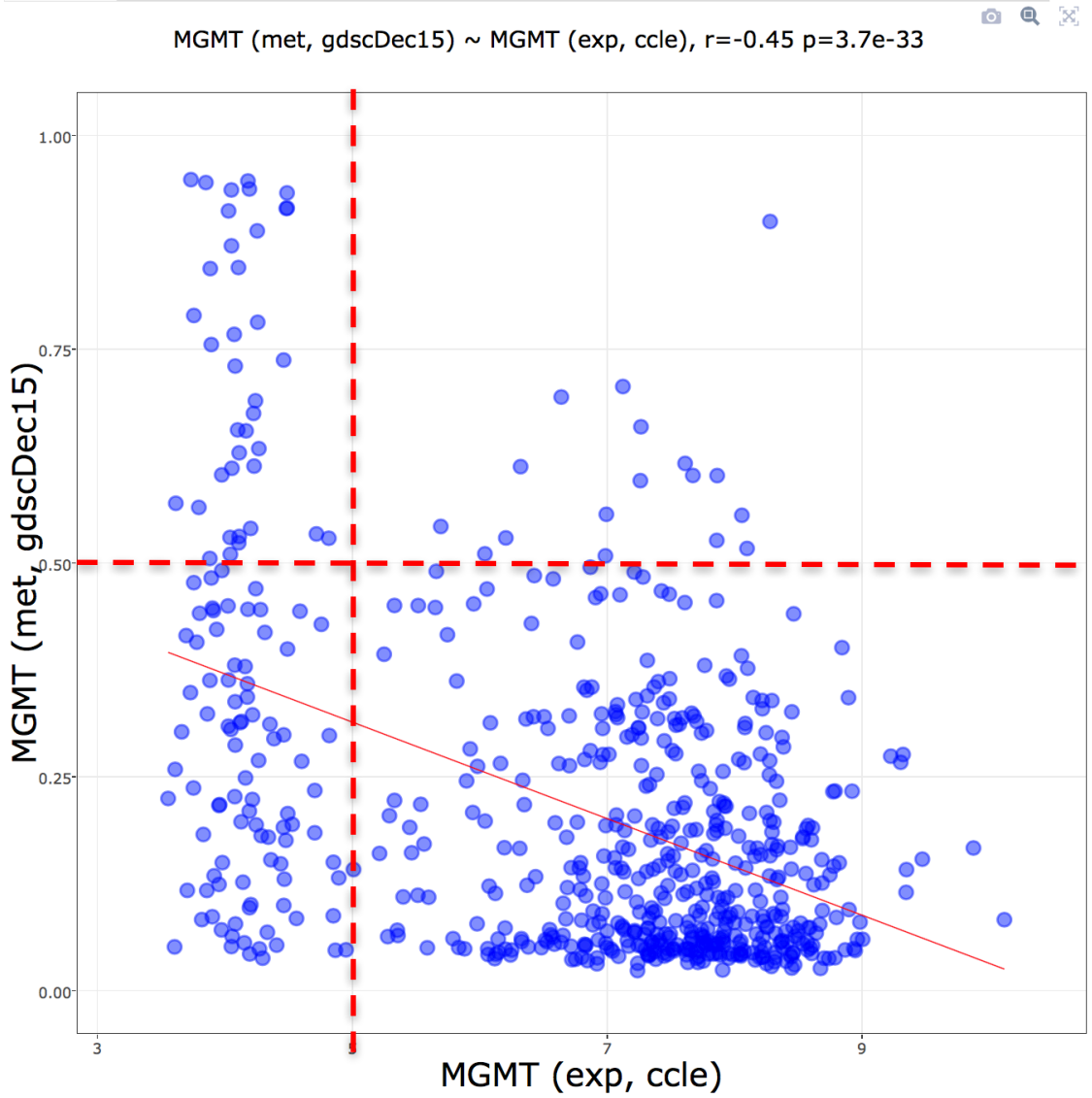


MGMT is r for
temozolo ffect
MGMT exp se cell
lines. In ad ressed
cell lines. e cell
lines is as pr d in the
presence of >40% methylation) as it is for the glioblastomas (CNS).

MGMT promoter methylation is not a “precise” measure of MGMT status (transcripts or protein)

x-Axis Dataset
CCLE
x-Axis Type
Expression (log2)
ID: (e.g. 94600 or SLFN11)
MGMT
x-Axis Range
2 3.2 10.4 15
2 3.5 5 6.5 8 9.5 11 12.5 14 15
y-Axis Dataset
GDSC
y-Axis Type
Methylation
ID: (e.g. 94600 or SLFN11)
MGMT
y-Axis Range
0 1
0 0.5 1
Show Color?
Select Tissues
Include
Exclude
all
Tissues to Color

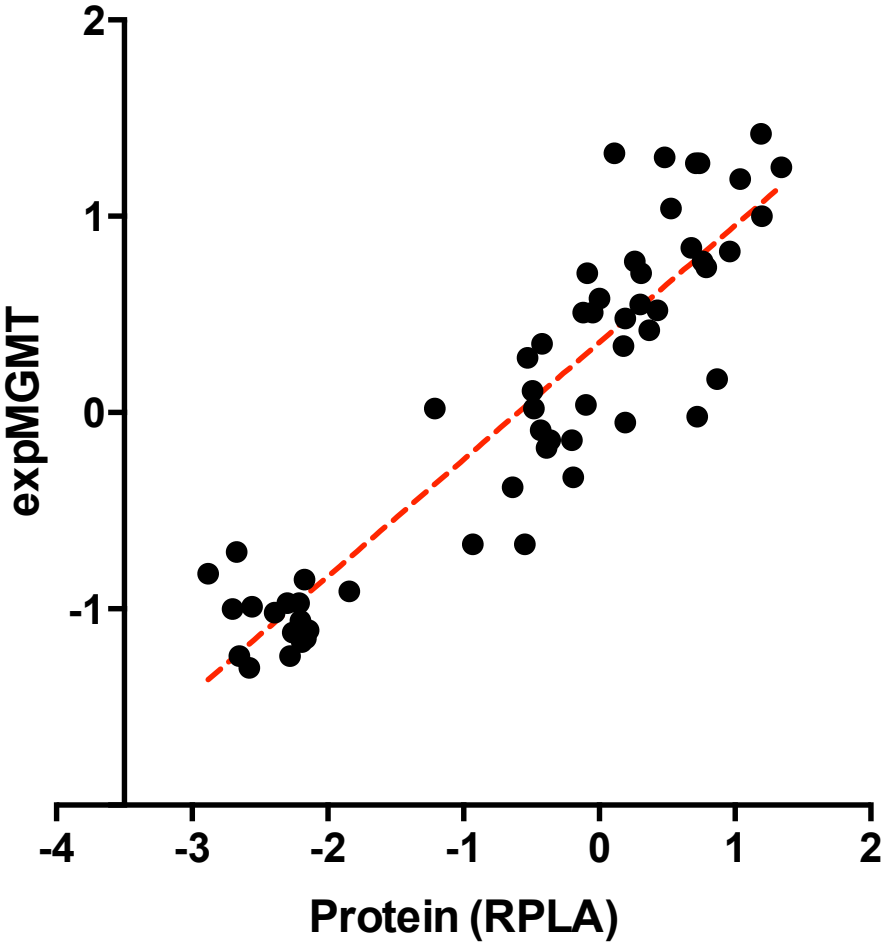
Plot Data | Download Data | Search IDs | Compare Patterns



CCLE and GDSC Cross database Analysis with Cellminer cdb

MGMT promoter methylation is not a “precise” measure of MGMT status (transcripts or protein)

High correlation between transcripts and protein for MGMT across the NCI-60



https://dtp.cancer.gov/mtweb/targetinfo?moltid=MT18300&moltnbr=374563

- Synthetic lethality beyond BRCA and PARP inhibitors
  - TOP1 inhibitors
- Cancer Cell Line genomics as model systems
- SLFN11 as a highly penetrant determinant of response
- Practical implications: example of temozolomide
- DNA repair alterations are frequent in cancers

Testable genomic signatures
Matching DNA targeted drugs and genes

	Genomic Biomarker (mRNA expression)												
Drugs	**SLFN11**	**ABCG2**	**ABCC3**	**ABCB1**	**LMNA**	**TOP1**	**TOP2A**	**MGMT**	**MMR**	**MYC**	**MYCL**	**MYCN**	**TP53 (mut)**
TOP1 inhibitors (camptothecins, indenos)	1	1	1	0	1	1	0	0	0	?	?	?	0
TOP2 (Daunorubicin, Etoposide)	1	0	?	1	?	0	1	0	0	?	?	?	0
PARP inhibitors (olaparib, talazoparib, niraparib)	1	0	?	1	?	0	0	0	0	?	?	?	0
Temozolomide	0	?	?	?	?	0	0	1	1	?	?	?	1
ATR inhibitors (VE-970; AZD6738)	0	?	?	?	?	0	1	0	0	?	?	?	1
Wee1 inhibitor (AZD1775)	0	?	?	?	?	0	1	0	0	?	?	?	1
Chk1/2 inhibitor (LY-2606368; Prexasertib)	0	?	?	?	?	0	1	0	0	1	1	1	1
DNA-PK inhibitor (VX-984)	0	?	?	?	?	0	?	0	0	?	?	?	?

Genomic Biomarkers

SLFN11 exp
ABCG2 exp
ABCC3 exp
ABCB1 exp
LMNA exp/mut
TOP1 exp
MGMT exp
MMR (MLH1, MLH3, MSH2, MSH3, MSH6, PMS1 and PMS2) exp/mut
MYC exp
MYCL exp
MYCN exp
TP53 mut

- Synthetic lethality beyond BRCA and PARP inhibitors
  - TOP1 inhibitors
- Cancer Cell Line genomics as model systems
- SLFN11 as a highly penetrant determinant of response
- Practical implications: example of temozolomide
- DNA repair alterations are frequent in cancers

Precision therapeutics can be defined as the ability to:

- **prescribe effective therapies only to those patients who will respond effectively (cure),**
- **while limiting toxicity to normal tissues and minimizing side effects.**

Second Generation Camptothecins with Targeted Delivery

Name	Company	Active Derivative (Payload)	Formulation (Conjugate; Target)
Onivyde™ = MM398*	Merrimack	Irinotecan	Liposome
CRLX101	Cerulean Pharma Inc.	Camptothecin	PEG
NKTR-102	Nektar Therapeutics	Etirinotecan (20 position)	PEG (Pegol)
PLX038	ProLynx	SN-38	PEG
IMMU-132 = Sacituzumab govitecan **	Immunomedics (Seattle Genetics)	SN-38 (20 position)	ADC - TROP2 (TACSD2)
IMMU-130 = Labetuzumab govitecan	Immunomedics	SN-38	ADC-CEACAM5
DS-8201a ***	Daichi Sankyo	DXd (Exatecan)	ADC – HER2
PEN-866	Tarveda Therapeutics	SN-38 (10 position)	Conjugate Hsp90
NK012	Nippon Kayaku	SN-38	Polymeric micelles (PEG-polyglutamate)
ALOS4-CPT	Ariel University	Camptothecin	HDC – ALOS-4

* FDA Approved, October 2015

** FDA Breakthrough, February 2016

*** FDA Breakthrough, August 2017 (Breast)

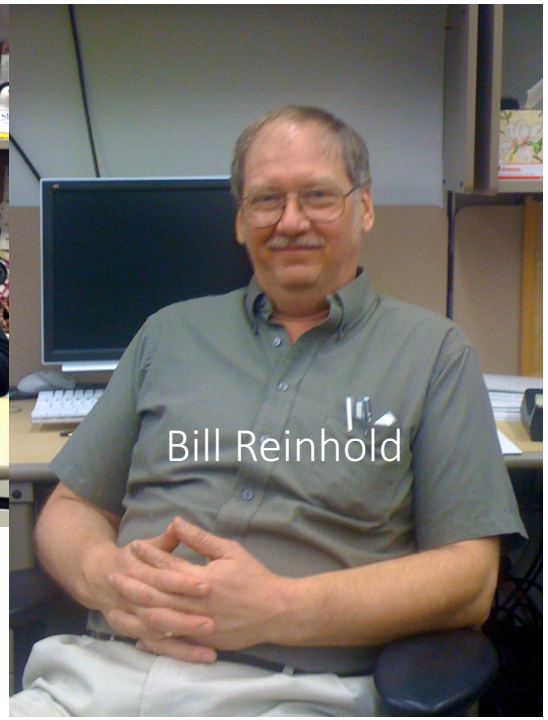
Camptothecins as warheads

Tumor-specific delivery

Acknowledgements (present lab members):

PARPi
SLFN11
HR TOP1

CellMiner



Augustin Luna

http://discover.nci.nih.gov/cellminer

http://discover.nci.nih.gov/cellminercdb

James Doroshow

PARPi
TOP1 inhibitors clinical trials
NCI-60