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Mapping combinatorial drug effects to DNA damage response kinase inhibitors

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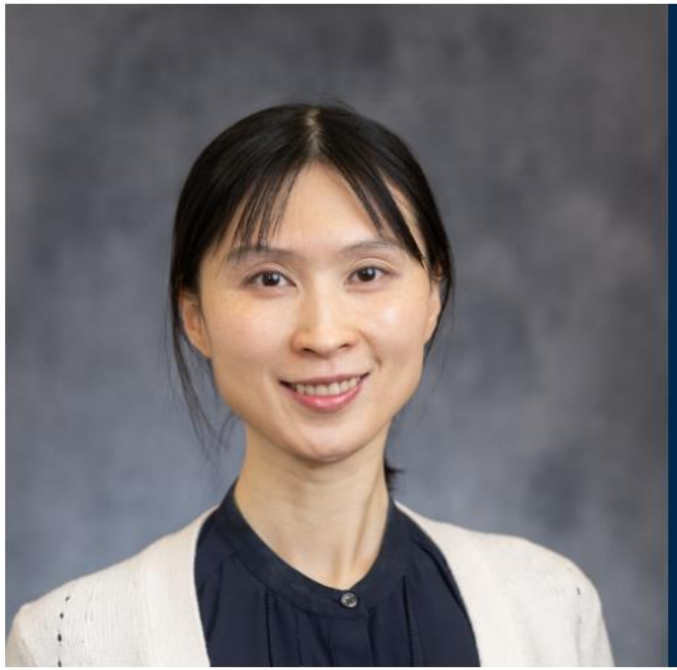

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Michigan Medicine

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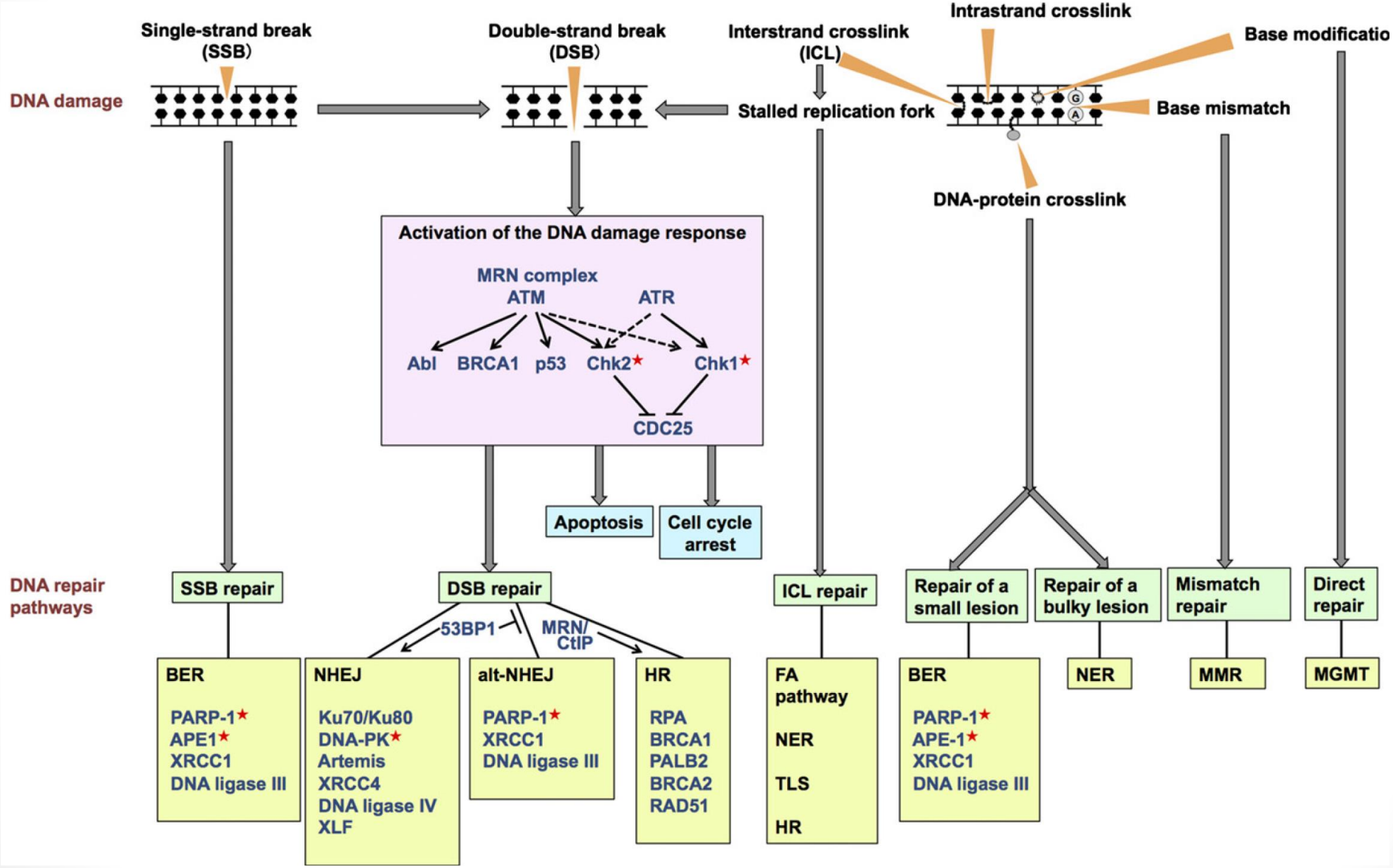
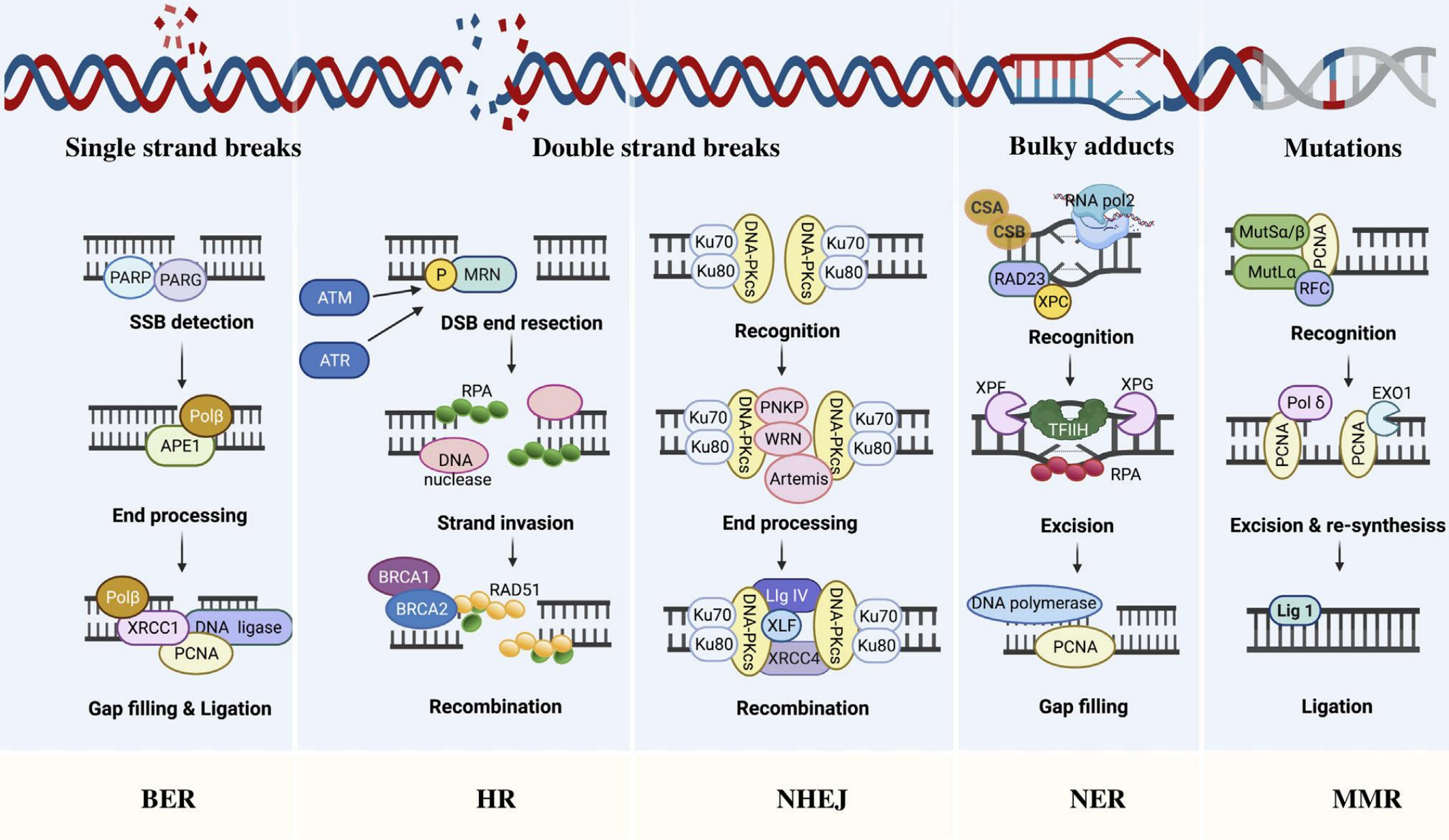
DNA damage response (DDR)




Single strand breaks
Double strand breaks
Bulky adducts
Mutations
PARP
PARG
SSB detection
Polβ
APE1
End processing
Polβ
XRCC1
DNA ligase
PCNA
Gap filling & Ligation
ATM
ATR
P
MRN
DSB end resection
RPA
DNA nuclease
Strand invasion
BRCA1
BRCA2
RAD51
Recombination
Ku70
Ku80
DNA-PKcs
Recognition
PNKP
WRN
Artemis
End processing
LIg IV
XLF
XRCC4
Recombination
CSA
CSB
RNA pol2
RAD23
XPC
Recognition
XPF
XPG
TFIIH
RPA
Excision
DNA polymerase
PCNA
Gap filling
MutSα/β
MutLα
PCNA
RFC
Recognition
Pol δ
EXO1
PCNA
PCNA
Excision & re-synthesiss
Lig 1
Ligation
BER
HR
NHEJ
NER
MMR

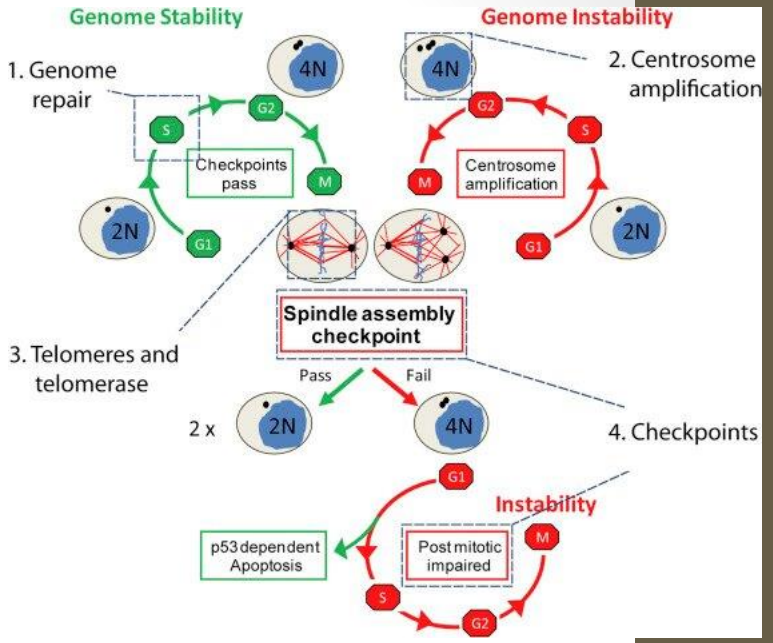
DNA damage response (DDR)

- DNA damage response (DDR): Cells have evolved a complex network of biochemical pathways.
- DDR prevents detrimental mutations from being passed on to their progeny.
- A set of **450 proteins involved in different pathways** of the DNA damage response has recently been mapped.

https://www.mdanderson.org/documents/Labs/Wood-Laboratory/human-dna-repair-genes.html#Human%20DNA%20Repair%20Genes

- DDR **coordinates** DNA repair with **cell-cycle checkpoint** activation and other global cellular responses.

Genomic instability and cancer



DDR genes are frequently mutated in cancer

Genomic instability:

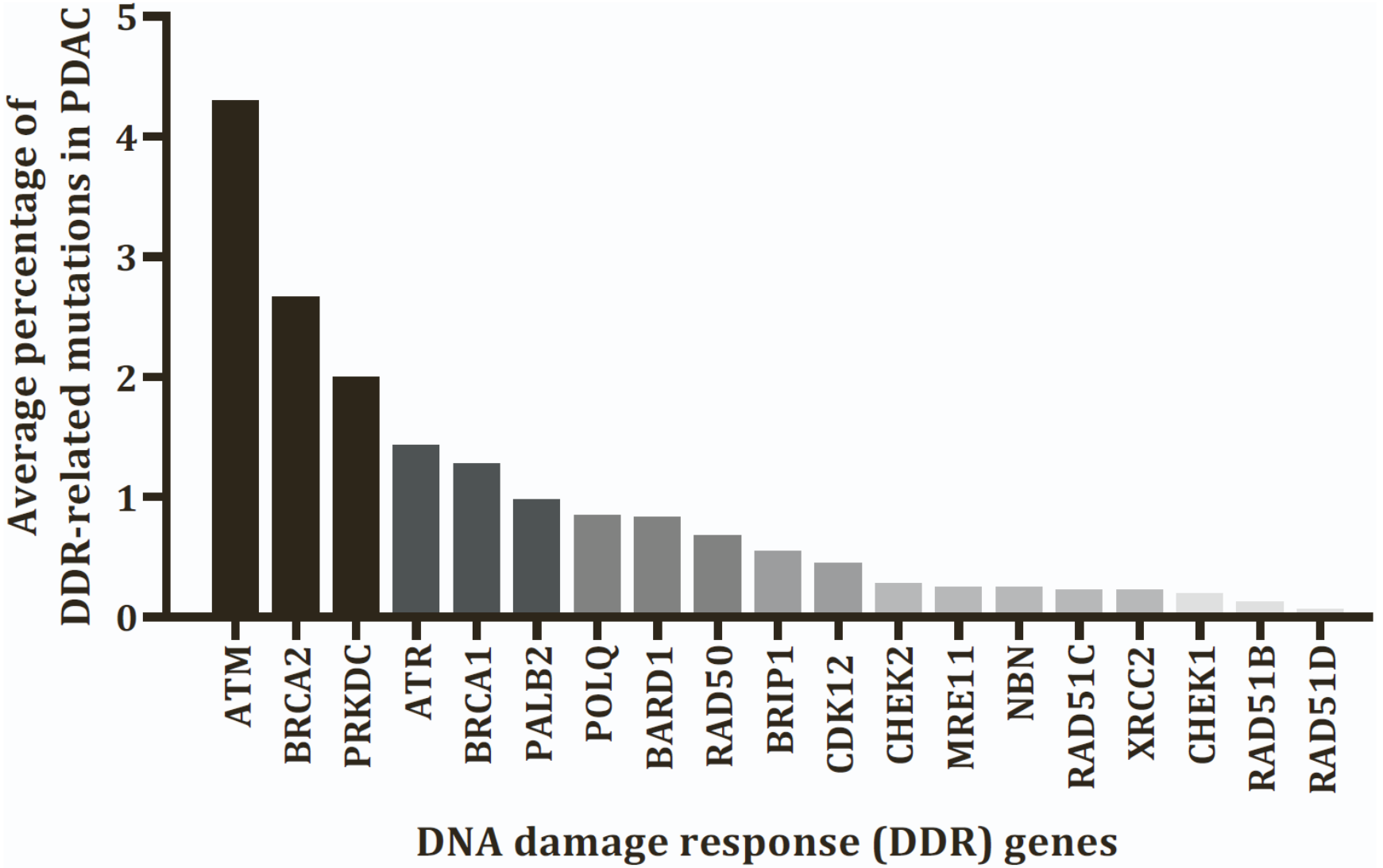
An intrinsic feature of cancer that underlies their ability to grow

DDR genes are frequently mutated in cancer

Tumors respond to treatments that inflict DNA damage

- DDR mutations make cancer cells more **susceptible** than normal tissues to DNA damage.

- Taking advantage of this **vulnerability**, DNA-damaging treatments such as ionizing radiation and platinum-based antineoplastic have long been used as anti-cancer treatments.



- Moosavi et al., 2024

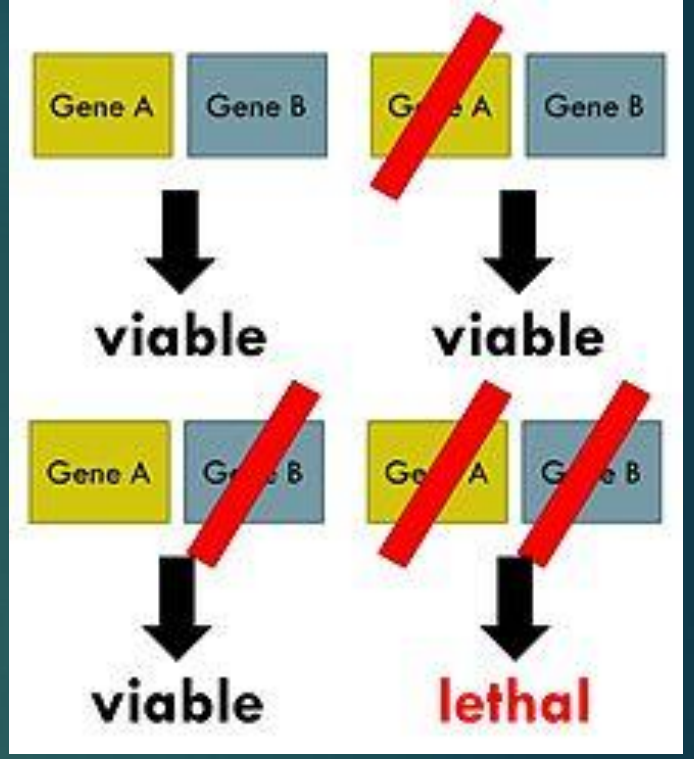
- Therapeutic agents **targeting DNA damage response (DDR)** pathways has been developed that specifically exploits this susceptibility, promising reduced side effects compared to nontargeted treatments.

- It is commonly assumed that specific pathways exist that address different types of DNA damage, e.g., for SSBs, DSBs, or mismatch repair.

BUT **loss of function of a DDR pathway can be compensated by parallel repair pathways**.

- Therefore **simultaneous inhibition of multiple complementary DDR pathways** is necessary as a promising therapeutic strategy in cancer treatments.

Synthetic lethality

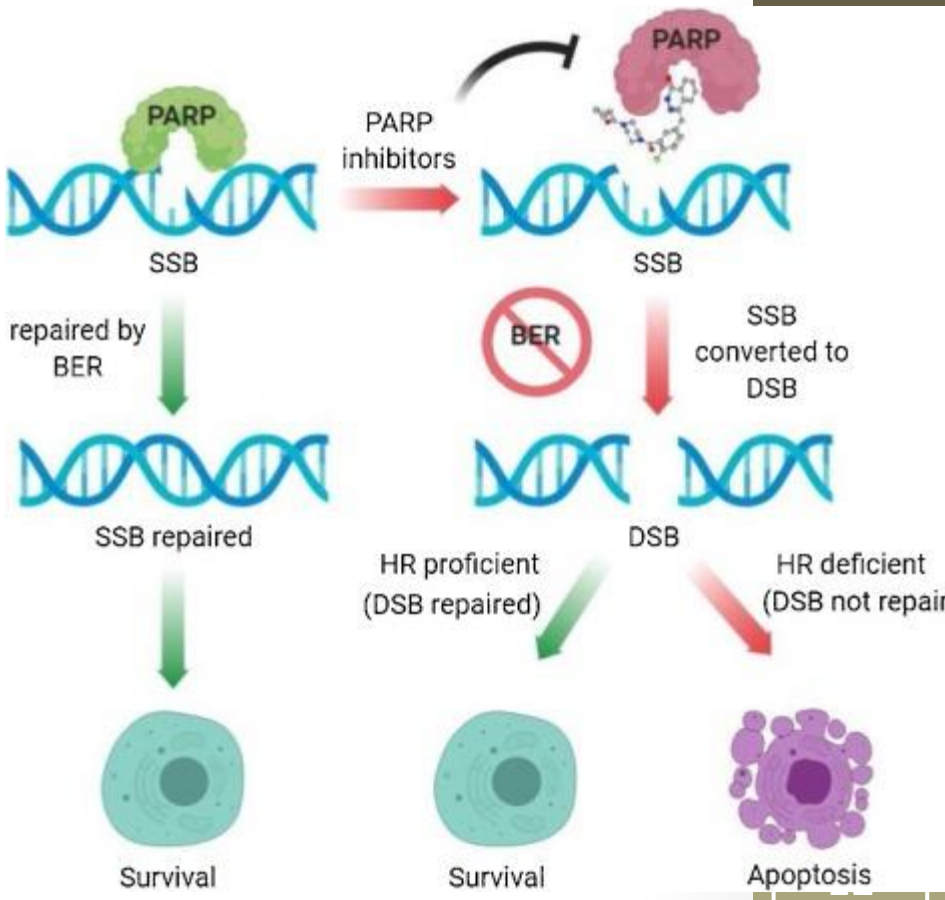


- Two genes or proteins are synthetic lethal when inactivation of either one is compatible with cell viability but inactivation of both genes or proteins results in cell death.

Example of synthetic lethality: BRCA1/2 and PARP

- Tumors with mutated *BRCA1* or *BRCA2* (compromised homologous recombination repair) rely on alternative repair mechanisms and are susceptible to poly(ADP-ribose) polymerase (PARP) inhibitors.

- This has become a **paradigm** for targeted cancer therapy.



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Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy

[PDF] researchgate.net

H Farmer, N McCabe, CJ Lord, ANJ Tutt, DA Johnson, TB Richardson, M Santarosa…

Nature, 2005 • nature.com

Abstract

BRCA1 and BRCA2 are important for DNA double-strand break repair by homologous recombination, and mutations in these genes predispose to breast and other cancers. Poly(ADP-ribose) polymerase (PARP) is an enzyme involved in base excision repair, a key pathway in the repair of DNA single-strand breaks. We show here that BRCA1 or BRCA2 dysfunction unexpectedly and profoundly sensitizes cells to the inhibition of PARP enzymatic activity, resulting in chromosomal instability, cell cycle arrest and subsequent

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Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase

[PDF] academia.edu

HE Bryant, N Schultz, HD Thomas, KM Parker, D Flower, E Lopez, S Kyle, M Meuth…

Nature, 2005 • nature.com

Abstract

Poly(ADP-ribose) polymerase (PARP1) facilitates DNA repair by binding to DNA breaks and attracting DNA repair proteins to the site of damage[1]. Nevertheless, $PARP1^{-/-}$ mice are viable, fertile and do not develop early onset tumours. Here, we show that PARP inhibitors trigger γ-H2AX and RAD51 foci formation. We propose that, in the absence of PARP1, spontaneous single-strand breaks collapse replication forks and trigger homologous recombination for repair. Furthermore, we show that BRCA2-deficient cells

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Other synthetic-lethal relationships

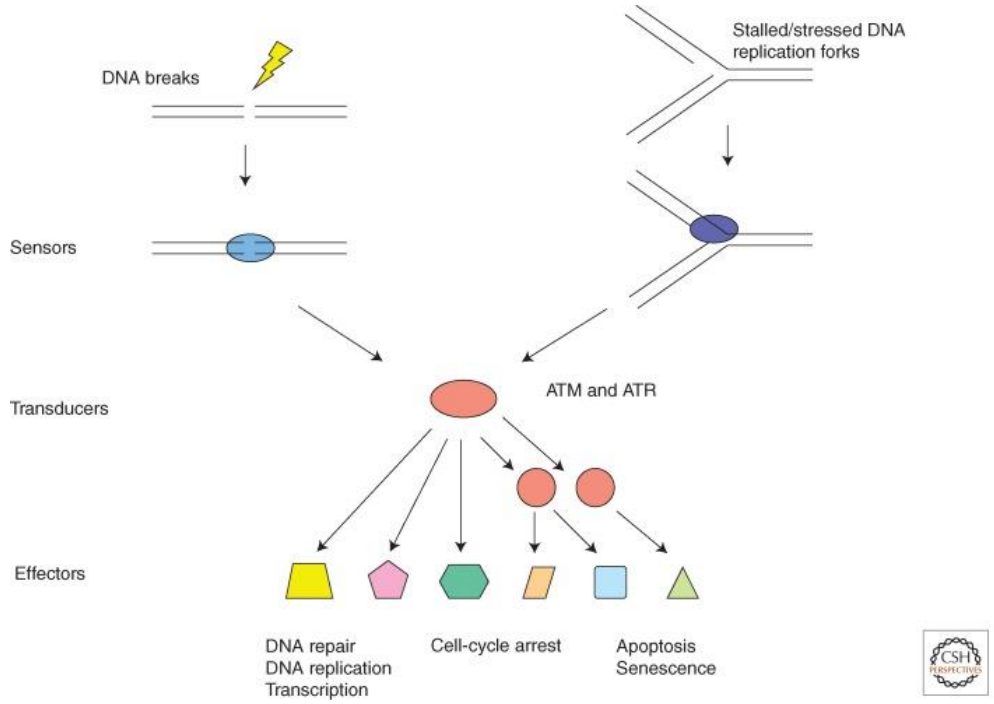
- **Many other synthetic-lethal** relationships exist between DDR genes.

Synthetic lethality also inspired the development of **combination treatments of multiple DDR inhibitors** to:

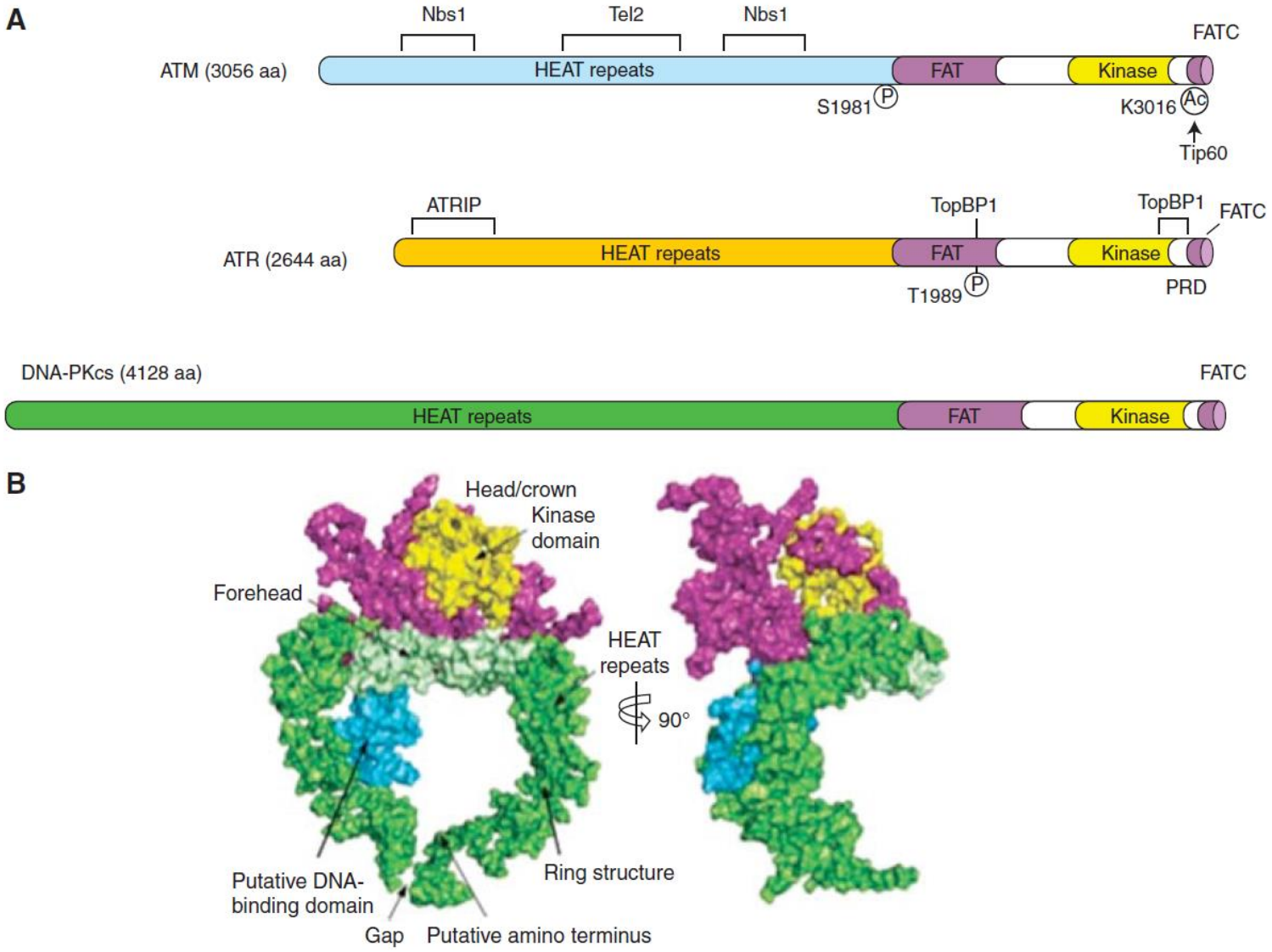
- overcome resistance to single drugs
- achieve synergistic effect
- expand DDR drugs' **usage to other indications beyond BRCA-deficient cancers.**

Three canonical **DNA damage-sensing kinases** that are central to the human DDR:

- Ataxia telangiectasia mutated (ATM)
- Ataxia telangiectasia and Rad3-related (ATR)
- DNA-dependent protein kinase (DNA-PK), (or protein kinase, DNA activated, catalytic subunit (PRKDC))



- ATM (ataxia-telangiectasia mutated) and ATR (ATM- and Rad3-Related) and DNA-PKcs (DNA-dependent protein kinase) kinases are the most upstream DDR kinases and the **master transducers** of DNA damage signals.
- These large serine/threonine kinases are members of the phosphatidylinositol-3-kinase-like kinase family (PIKKs).
- ATM is primarily activated by double-stranded DNA breaks; ATR responds to a broader spectrum of DNA damage.
- In response to DNA damage, hundreds of proteins are phosphorylated at Ser/Thr-Glu motifs and additional sites in an ATM- or ATR-dependent manner.
- DNA-PKcs regulates a smaller number of targets and play a role primarily in nonhomologous end joining (NHEJ)


A
Nbs1
Tel2
Nbs1
FATC
ATM (3056 aa)
HEAT repeats
FAT
Kinase
S1981 P
K3016 Ac
Tip60
ATRIP
TopBP1
TopBP1
FATC
ATR (2644 aa)
HEAT repeats
FAT
Kinase
T1989 P
PRD
DNA-PKcs (4128 aa)
FATC
HEAT repeats
FAT
Kinase
B
Head/crown
Kinase
domain
Forehead
HEAT
repeats
90°
Putative DNA-
binding domain
Ring structure
Gap
Putative amino terminus

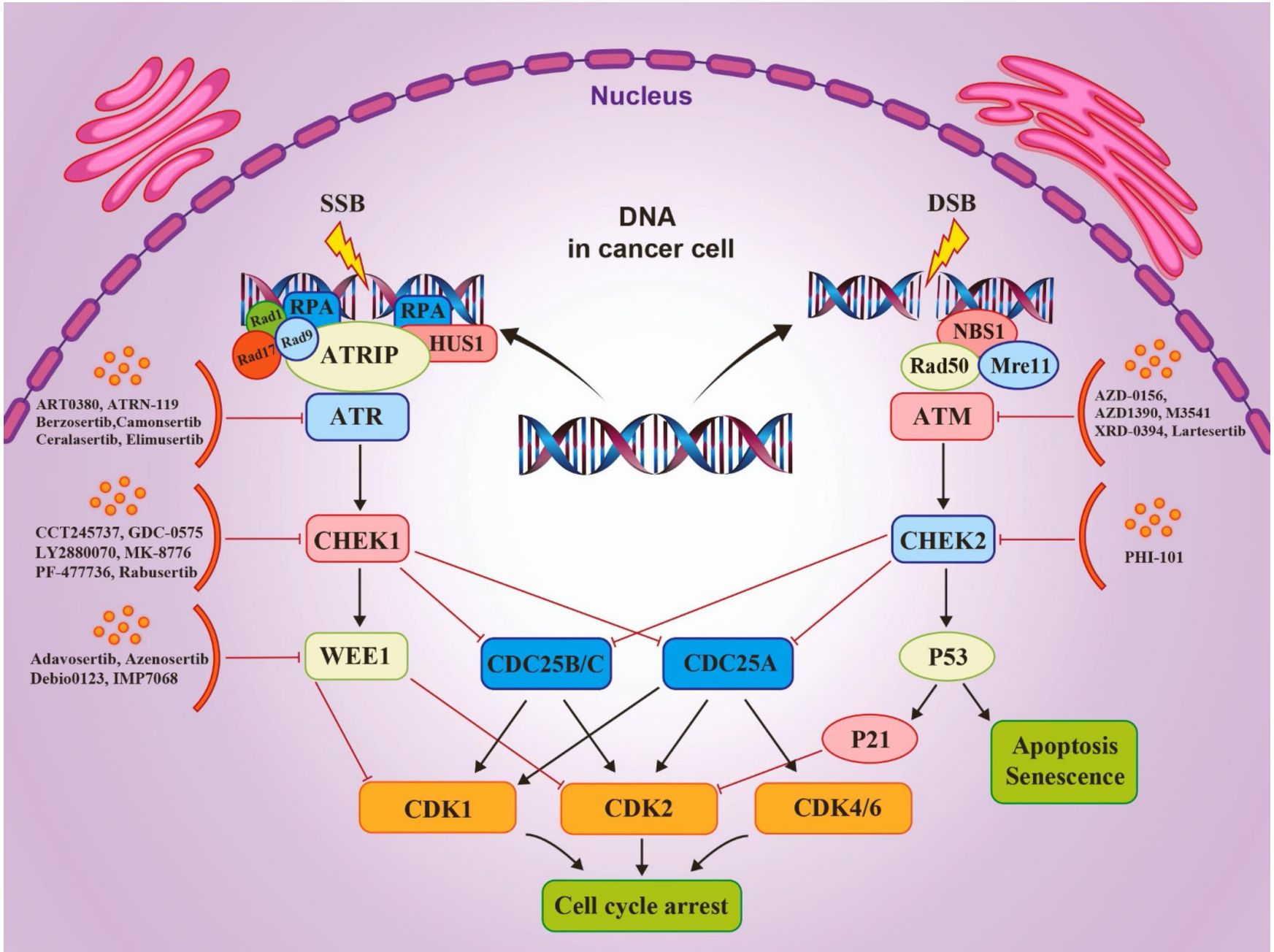
ATM and ATR are the kinases responsible for orchestrating cellular responses to DNA damage:

- DNA repair, checkpoint activation, apoptosis, senescence, and alterations in chromatin structure, transcription and pre-mRNA splicing.

- The downstream cell-cycle checkpoint kinases **CHK1** and **CHK2** are major substrates for ATR and ATM, respectively, and are responsible for downregulating the activity of cyclin-dependent kinases (CDKs) to halt cell-cycle progression in response to genotoxic stress.

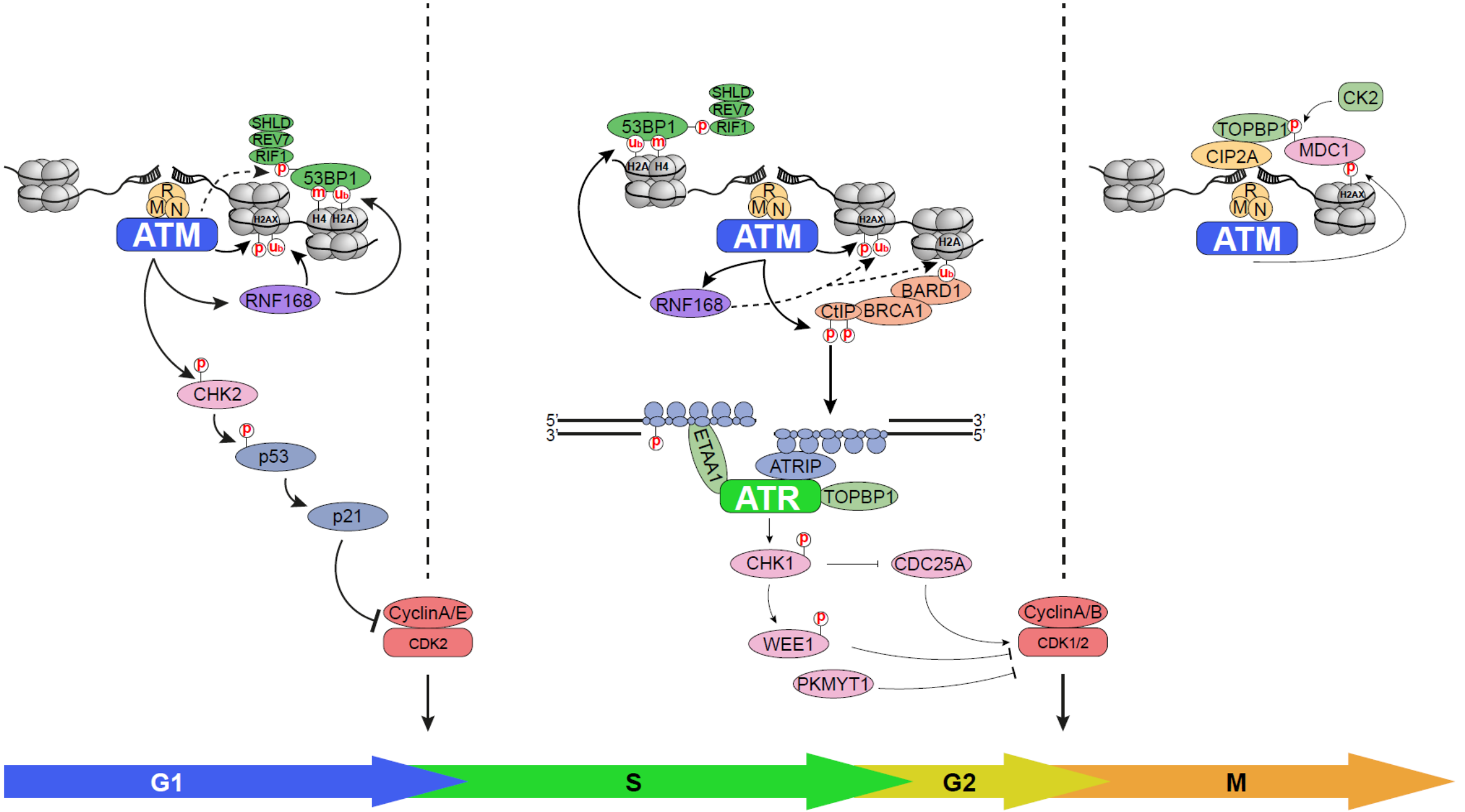
- This is achieved in G1 phase cells by ATM and CHK2 through phosphorylation and stabilization of p53.

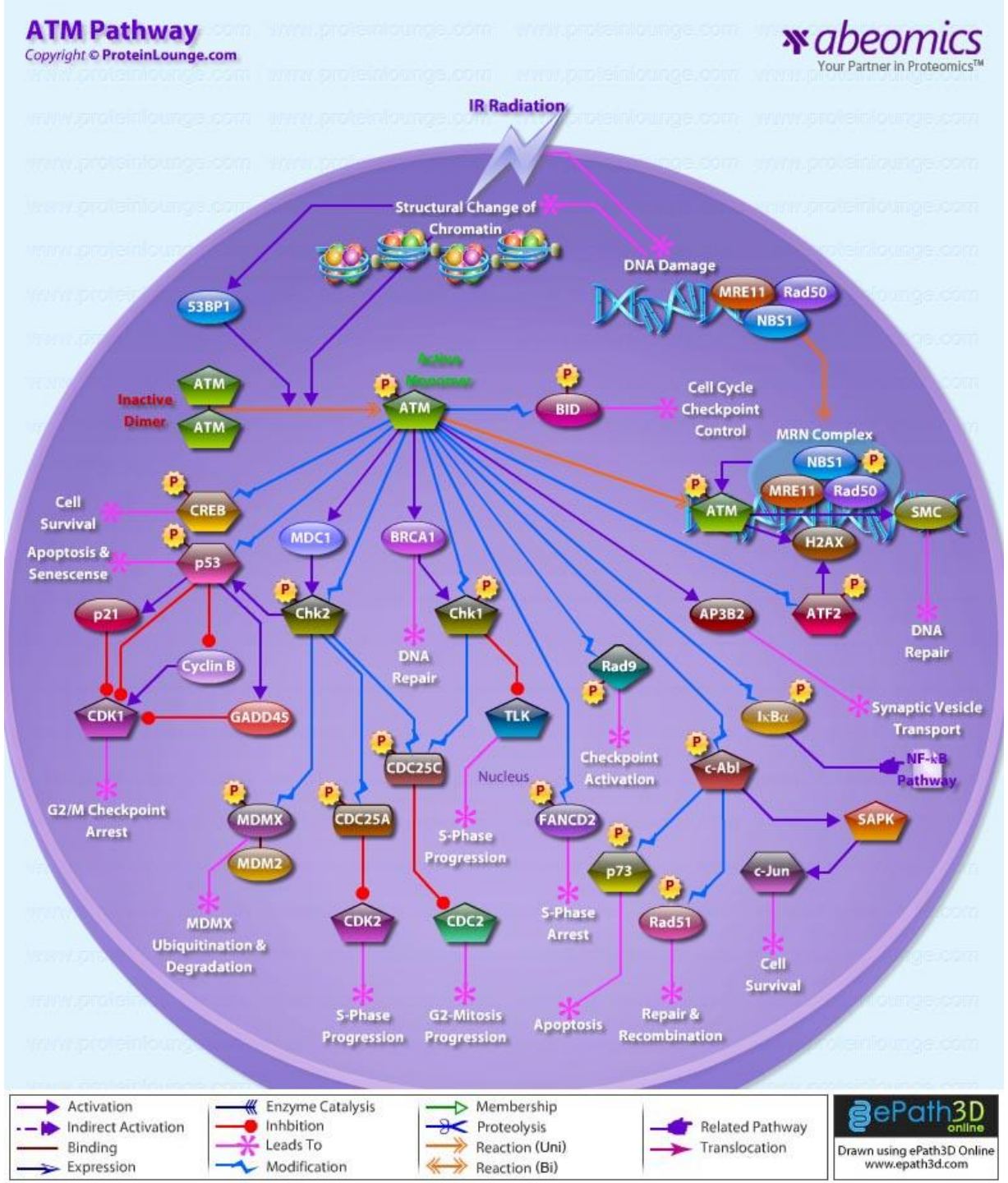
ATM and ATR are the kinases responsible for orchestrating cellular responses to DNA DSBs and SSBs



- Moosavi et al., 2024

Groelly et al. - Figure 1




ATM Pathway
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abeomics
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IR Radiation
Structural Change of Chromatin
DNA Damage
53BP1
MRE11
Rad50
NBS1
Inactive Dimer
ATM
Active Monomer
BID
Cell Cycle Checkpoint Control
MRN Complex
Cell Survival
CREB
Apoptosis & Senescense
p53
MDC1
BRCA1
SMC
H2AX
p21
Chk2
Chk1
AP3B2
ATF2
DNA Repair
Cyclin B
Rad9
CDK1
GADD45
TLK
IκBα
Synaptic Vesicle Transport
CDC25C
Nucleus
Checkpoint Activation
c-Abl
NF-κB Pathway
G2/M Checkpoint Arrest
MDMX
CDC25A
FANCD2
SAPK
S-Phase Progression
MDM2
p73
c-Jun
CDK2
CDC2
S-Phase Arrest
Rad51
MDMX Ubiquitination & Degradation
Cell Survival
S-Phase Progression
G2-Mitosis Progression
Apoptosis
Repair & Recombination
Activation
Indirect Activation
Binding
Expression
Enzyme Catalysis
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Modification
Membership
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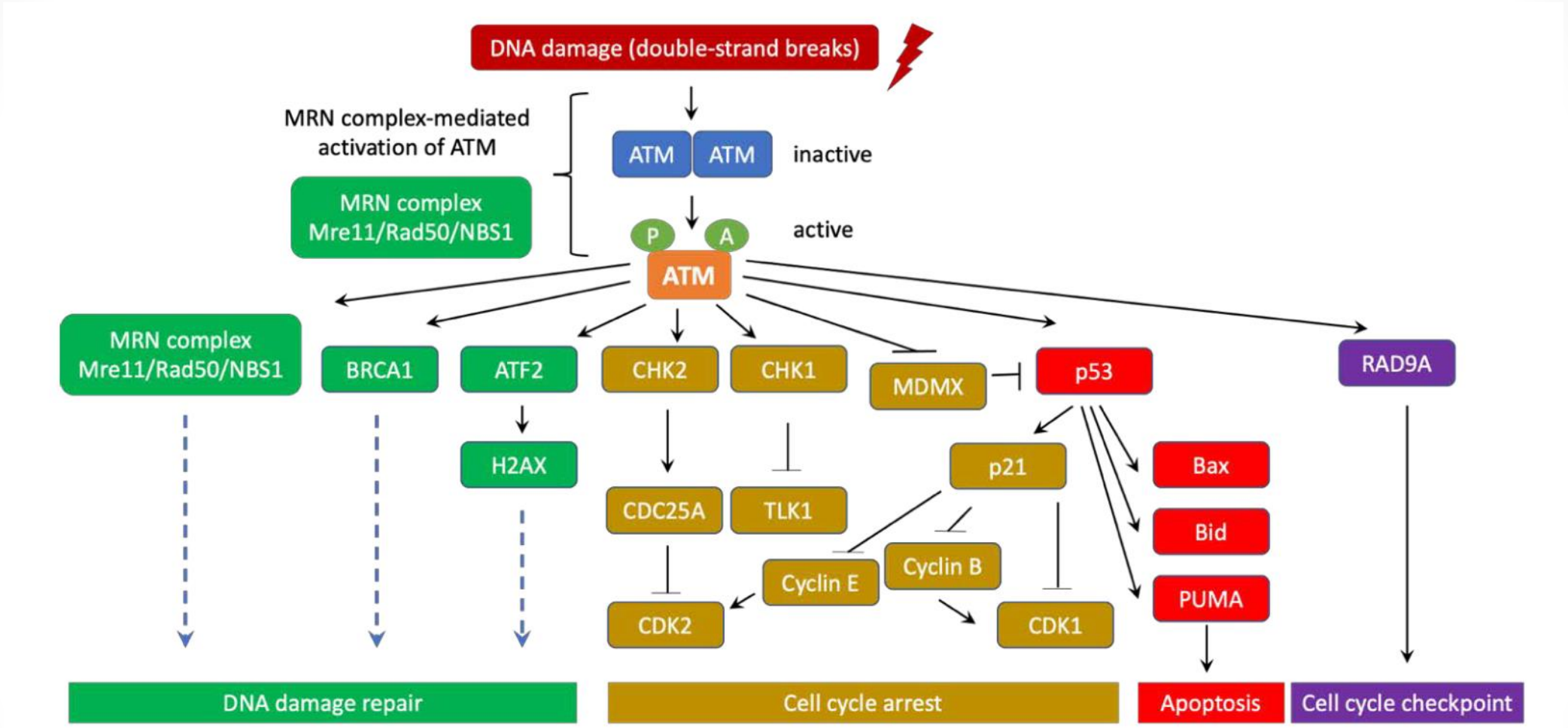


- Phan 2022, Genes.

6 kinase inhibitors:

Two ATM inhibitors (M3541 and M4076)

Three ATR inhibitors (berzosertib, gartisertib, M1774)

One DNA-PK inhibitor (peposertib)

87 anti-cancer drugs of a wide range of mode-of-actions

- 22 ~ 62 cancer cell lines across 12 tumor types
- A total of **17,912 combination** treatments.

- So far, studies that comprehensively map the synergistic effects between small molecule inhibitors of these key DDR kinases and other anti-cancer drugs are lacking in both coverages across tumor types and the number of combination therapy partners.

- In order to characterize tissue-specific patterns of DDR inhibitor combination treatments, we carried out **full-genome and transcriptomic profiling of all 62 cell lines** and statistically associated dose responses with genomic and transcriptomic readouts.

Methods: Cell culture and drug response detection

- All dose-response experiments were conducted at Oncolead GmbH & Co. KG (Karlsfeld, Germany).
- Cell lines were purchased directly from the ATCC, NCI, CLS and DSMZ cell line collections.
- The cell lines were grown in the media recommended by the suppliers in the presence of 100 U/ml penicillin G and 100 µg/ml streptomycin supplied with 10% FCS.

SRB experiments

- All cells growing without anticancer agents were **sub-confluent by the end of the treatment**, as determined by **visual inspection.**

- Cell growth and treatment were performed in **96-well** microtiter plates CELLSTAR® (Greiner Bio-One, Germany). Cells were allowed to stay for another **48 h prior to compound treatment**.

- The treatment was performed for **120 h** and stopped by the addition of trichloroacetic acid followed by using a total protein staining protocol (Sulforhodamine B (SRB) staining).

- In the case of combination, both agents were **mixed together in DMSO** at equal volumes so that the final concentration of DMSO was 0.2%.

- All experiments contained a few plates with cells that were analyzed immediately after the 48 h recovery period. These plates contained information about **the cell number, Tz, at time zero**, i.e., before treatment, and served to calculate the cytotoxicity.

- The calculation nomenclature used was introduced by DTP of the NCI 61.

- The first step in data processing was calculating an **average background value** for each plate, derived from plates and **wells containing mediums without cells.**

The average background optical density was then subtracted from:

- Appropriate control values (containing cells without the addition of a drug)
- Values representing the cells treated with an anticancer agent
- Values of wells containing cells at time zero.

- Thus, the following values were obtained for each experiment:
- C: control cell growth
- Ti: cells in the presence of an anticancer agent
- Tz (or T0): Cells prior to compound treatment at time zero

- A **four-fold dilution** and **5 data points** were sufficient to cover the complete activity range for most of the agents (**Supplementary Figs. 8 and 9**).

Supplementary Figure 9. Demonstration of the dose-response matrices of M4076-berzosertib combination treatment. The responses (growth inhibition rate, GI) in cell lines at different doses of M4076 (mol) and berzosertib (mol) were shown by heatmaps.

Dose-response evaluation measures

- The non-linear curve fitting calculations were performed using algorithms and visualization tools using four-parameter log-logistic regression.

To obtain an estimate of treatment efficacy that encompasses **both potency and maximum effect**:
Relative area over the curve (AoC):

- **the area under the fitted dose-response curve** by the trapezoidal rule
- within ranges of relative growth rates compared to untreated controls between 0% and 100%
- within ranges of drug concentrations between 1 nM and 1 mM
- **dividing the estimated area** by the **sum of areas below and above the curve**.

- Relative AoC measure used in this work thus captures both the potency of a compound combination (usually measured by IC50 or GI50) as well as the maximum effect on cellular growth (efficacy).

- Relative AoC is of particular usefulness for capturing the efficacy of DDR inhibitors: many have a **low maximum effect** less than 50% growth inhibition at realistic concentrations, which makes IC50 and GI50 less practically relevant.

Combination effects

- Combination effects for the different compound combinations are calculated using the **Bliss independence model** under the assumption of **independent modes of action** of the combination partners.

- Bliss excess was calculated as the average **excess of the observed effect EOBS** (i.e., the relative reduction of growth rate compared to untreated controls) over the calculated linear combination of the monotherapy treatments effects ($E_{1+2} = E_1 + E_2 - E_1 E_2$) **for all concentrations used:**

$$\text{Bliss}_{\text{excess}} = \frac{1}{n}\sum_{i=1}^{n} E_{\text{OBS}_i} - E_{1+2_i}$$

In this formulation, the Bliss excess is a continuous value between −1 and 1

- Values higher than 0.2 : Synergistic
- Values below −0.2 : Antagonistic.

Statistics and reproducibility

- The reproducibility of measured response (i.e. AoC and Bliss score) are measured by Pearson's correlation within the replicated experiments. No data were excluded from the analyses.

Quantification and statistical analysis for drug response variance test

- For hierarchical clustering based on drug responses, we used **heatmap. 2 function of gplots module (3.1.3) from R (4.2.3)** for hierarchical clustering using Euclidean as the distance function and ward.D2 as the cluster function.

- We used Python (≥3.8) module *scipy* (1.11.3) to carry out the Kruskal–Wallis test to test if a drug has different responses between different cancer types.
- Kruskal–Wallis test is especially suitable for this situation as a **non-parametric test**, so it won't be affected by the different sample sizes of the subsets.
-
- For the significantly tissue-specific drugs ($p<0.01$), we also used *scipy* to carry out post hoc tests, including Dunn's test, Mann–Whitney Pairwise test, Conover–Iman test and bootstrapping for 10,000 times to locate the significantly different tissue types. Bonferroni correction was performed to adjust the above multiple comparisons.

Data availability

- The DDR combination in vitro screening data collected in this study are shared at and can be freely downloaded from: https://osf.io/8hbsx/. Source data are provided with this paper.

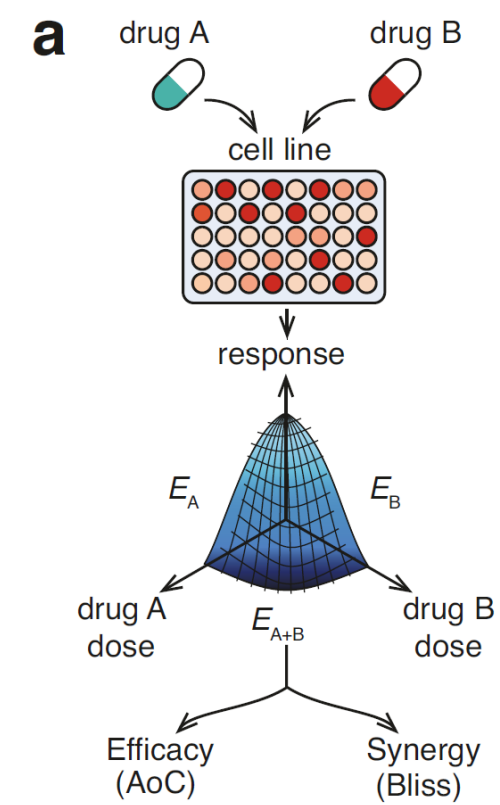
Results: The experimental dose-response screen of three DDR inhibitors across a wide range of anti-cancer combination treatments

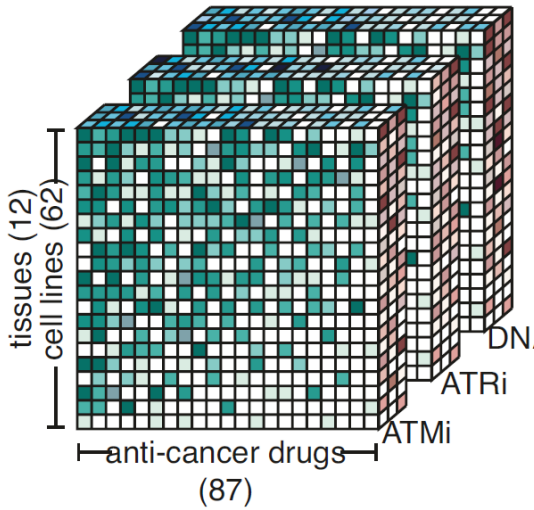
- Two ATM inhibitors (M3541 and M4076), three ATR inhibitors (berzosertib, gartisertib, M1774), and one DNA-PK inhibitor (peposertib)
- 87 anti-cancer drugs
- 62 cancer cell lines covering 12 tissues or tumor types

[SEE DRUG LIST in SUPPLEMENT]

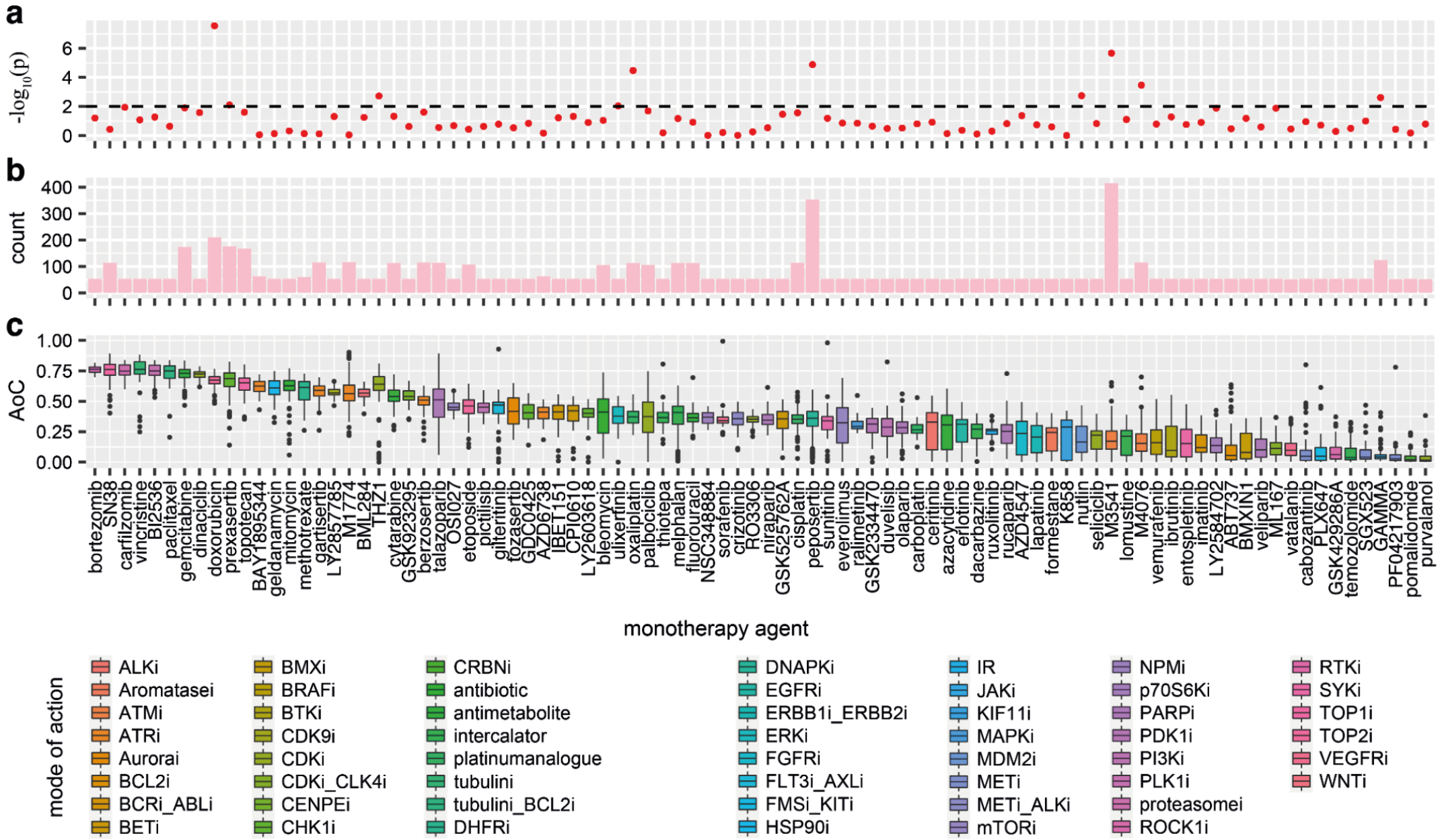
For each of the cell lines, we carried out:

- RNA- and whole-genome DNA sequencing
- Genome-wide readouts covering gene expression, copy-number profiling, and loss-of-function mutation both for single genes as well as biological pathways.





Supplementary Figure 1. Monotherapies



Supplementary Figure 1. Overview of all monotherapies used in this study.

(**a**) the significance (-log10(*p*)) from the Kruscal-Wallis variance test across all cancer types for each monotherapy. A dashed line marks the significance threshold (p=0.01, two-sided).

(**b**) The total count of experiments of monotherapy.

(**c**) boxplot shows the efficacy of all anti-cancer drugs used in this study. The color of the boxplot indicated the mode-of-actions. Drugs were ordered by average efficacy in all experiments in descending

Combination treatment responses were quantified on the level of both efficacy and synergy

- The efficacy of treatment: relative AoC score
- The synergy: the Bliss score

- Hierarchical clustering based on responses in different cell lines shows **treatments with the same mode-of-actions tend to cluster together** (Supplementary Figs. 3–7).

- For example, for monotherapy, ATM inhibitors (M3541 and M4076), CHK1 inhibitors (GDC0425 and LY2603818), and BET inhibitors (IBET151, CPI0610, and GSK525762A) are located adjacent to each other (Supplementary Fig. 1).

- The same pattern, i.e., combinations with the same or similar mode-of-actions are more likely to cluster together, also appears in combination response in terms of efficacy (Supplementary Figs. 4 and 5) and synergy (Supplementary Figs. 6 and 7).

- 17,912 combination treatment experiments
- 7081 monotherapy experiments

- With reproducibility of Pearson's correlation = 0.8380 ($p < 1e{-}22$) in AoC score for monotherapy and 0.7611 ($p < 1e{-}22$) in Bliss score for combination treatment. Is comparable with previously reported combination treatment screening datasets including **DREAM 23, ALMANAC 23,24, and O'Neil 25**.

Mapping the global interaction relationships between DDR inhibitors and combination treatment partners

- Due to the **complex relationships between DDR pathways**, finding optimal drug combinations that **show broad efficacy across multiple tumor types and genomic contexts of tumors is particularly challenging.**

Should we look for **broad efficacy across multiple tumor types?**

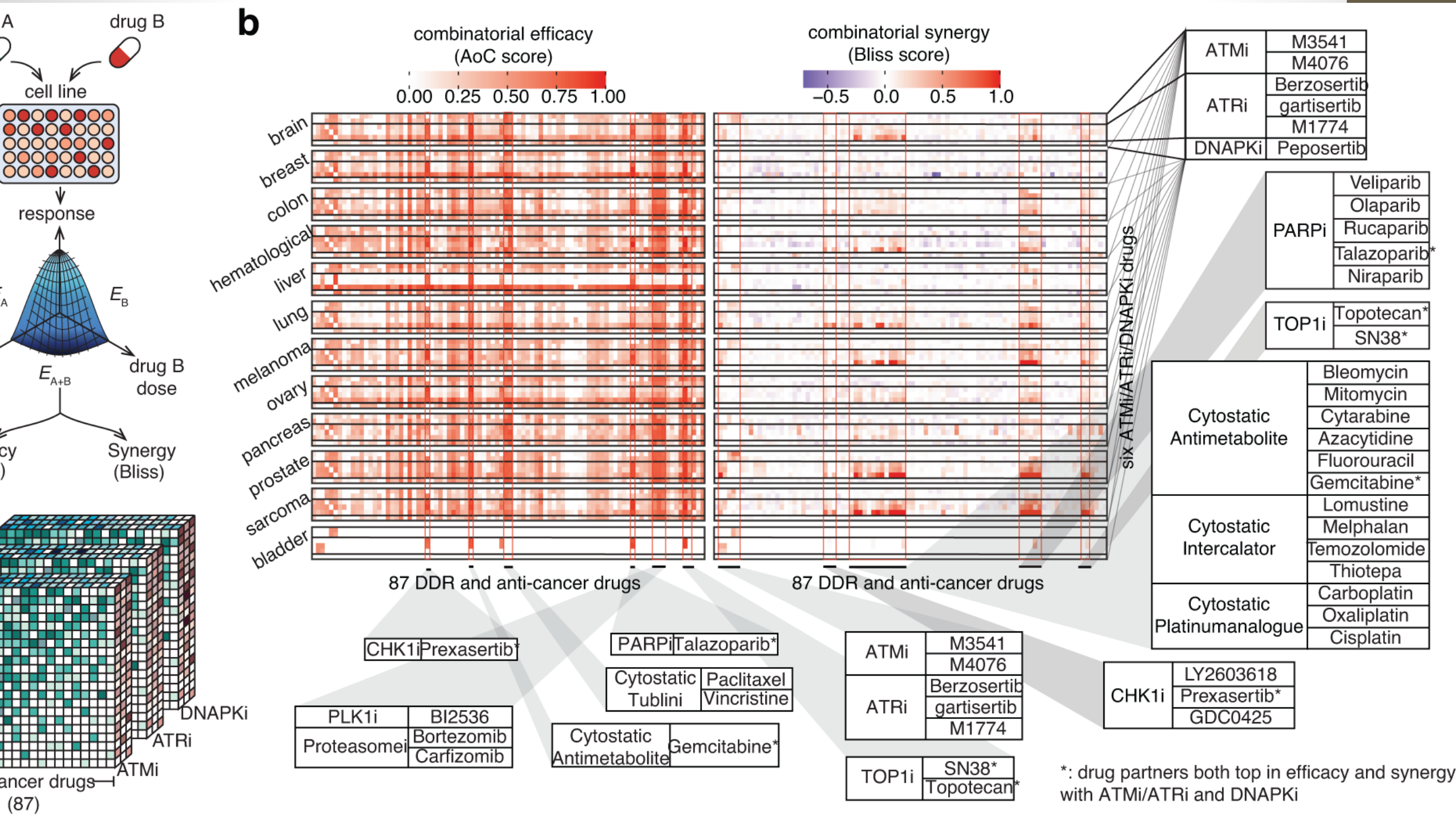

A
drug B
cell line
response
E_B
drug B dose
E_{A+B}
Synergy (Bliss)
DNAPKi
ATRi
ATMi
ancer drugs
(87)
b
combinatorial efficacy (AoC score)
0.00 0.25 0.50 0.75 1.00
combinatorial synergy (Bliss score)
−0.5 0.0 0.5 1.0
brain
breast
colon
hematological
liver
lung
melanoma
ovary
pancreas
prostate
sarcoma
bladder
87 DDR and anti-cancer drugs
87 DDR and anti-cancer drugs
six ATMi/ATRi/DNAPKi drugs
ATMi
M3541
M4076
ATRi
Berzosertib
gartisertib
M1774
DNAPKi
Peposertib
PARPi
Veliparib
Olaparib
Rucaparib
Talazoparib*
Niraparib
TOP1i
Topotecan*
SN38*
Cytostatic Antimetabolite
Bleomycin
Mitomycin
Cytarabine
Azacytidine
Fluorouracil
Gemcitabine*
Cytostatic Intercalator
Lomustine
Melphalan
Temozolomide
Thiotepa
Cytostatic Platinumanalogue
Carboplatin
Oxaliplatin
Cisplatin
CHK1i
LY2603618
Prexasertib*
GDC0425
CHK1i Prexasertib*
PARPi Talazoparib*
Cytostatic Tublini
Paclitaxel
Vincristine
PLK1i
BI2536
Proteasomei
Bortezomib
Carfizomib
Cytostatic Antimetabolite
Gemcitabine*
ATMi
M3541
M4076
ATRi
Berzosertib
gartisertib
M1774
TOP1i
SN38*
Topotecan*
*: drug partners both top in efficacy and synergy with ATMi/ATRi and DNAPKi

Fig. 2 | Top DDR inhibitor combination treatments that achieve the highest efficacy and synergy across all cell lines.

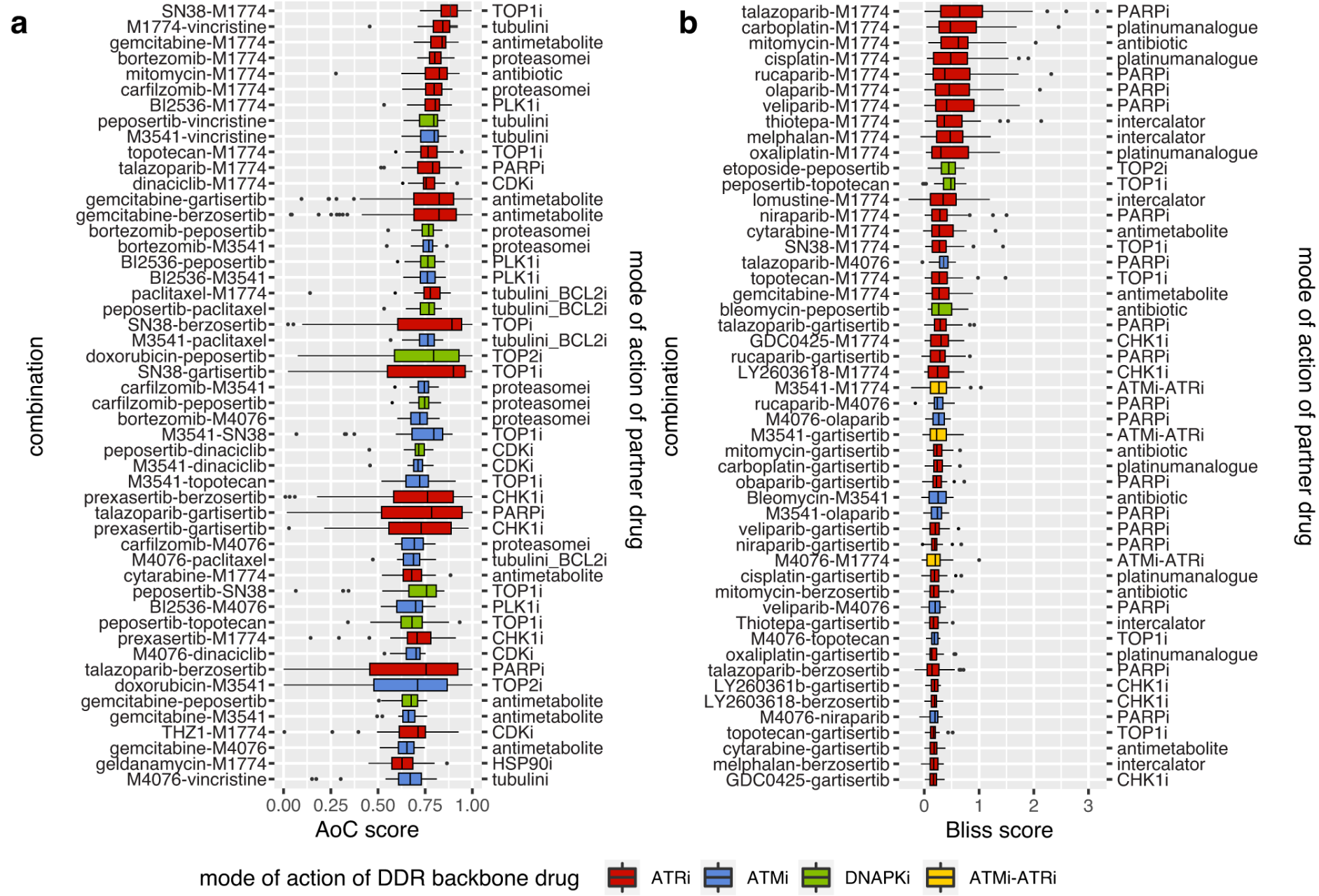


Fig. 2 | Top DDR inhibitor combination treatments that achieve the highest efficacy and synergy across all cell lines.

Boxplots showing the treatment responses of drug combinations with the **top 50 averaged** (a) **efficacy** and (b) **synergy** responses in all 62 cancer cell lines (n = 62).Drug combinations are shown on the left side. Mode-of actions of the DDR inhibitors are denoted by red (ATR inhibitor), blue (ATM inhibitor), green (DNA-PK inhibitor), and yellow (ATR inhibitor-ATM inhibitor combination) in the box plot.

The **interquartile range (25th to 75th percentile**) and median lines are show, with whiskers extending to **1.5 times the interquartile range**.

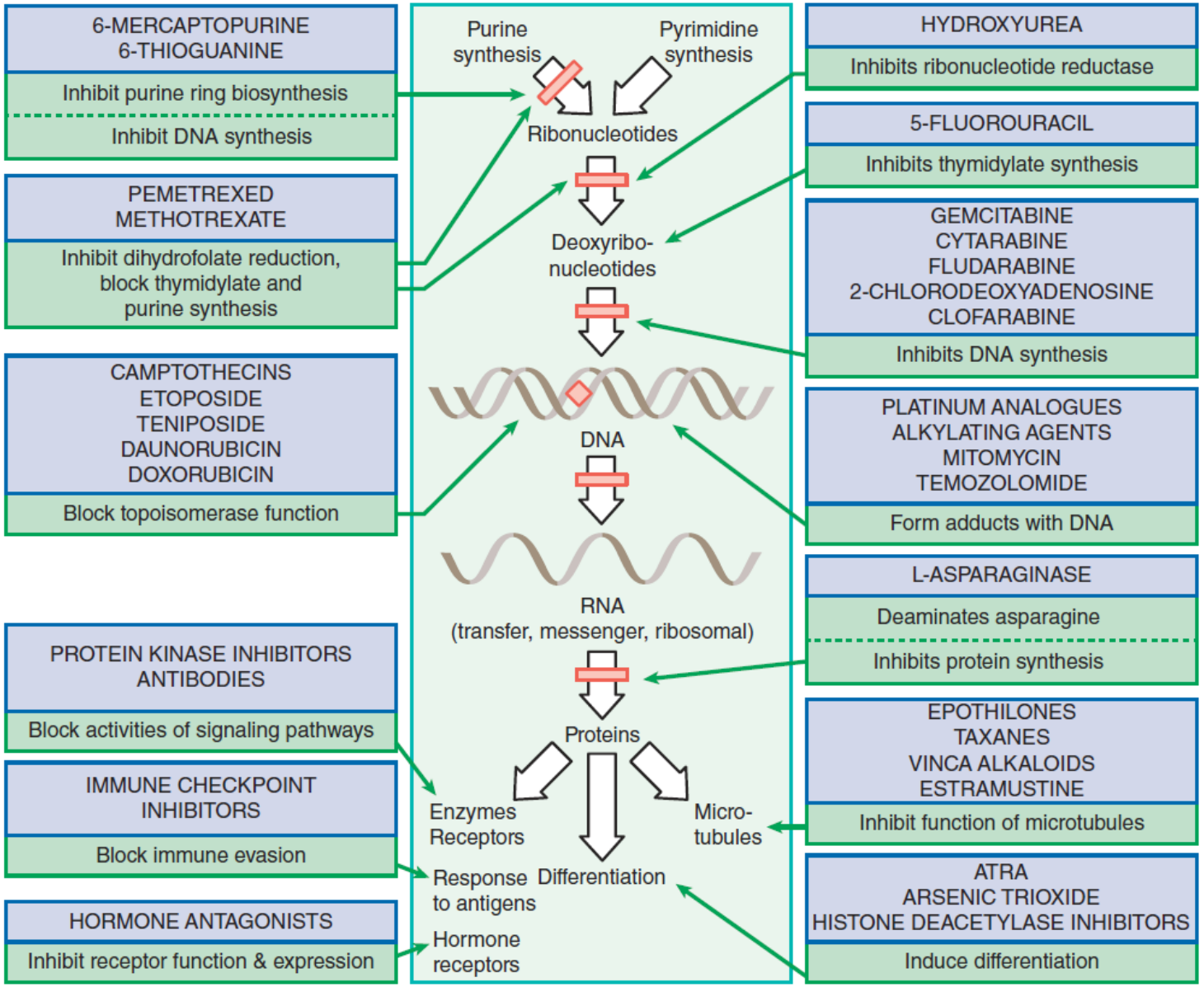
Successful drug combinations

PARP	ATM, ATR, DNA-PK
Platinum agents	ATR
Alkylating agents (Thiothepa, Melphalan, Lomustine)	ATR
TOPO1i and TOPO2i (Topotecan, Etoposide)	DNA-PK
Bleomycin	DNA-PK
CHK1i	ATR
Cytarabine, Gemcitabine	ATR
TOPO1i (SN-38, Topotecan)	ATR
Mitomycin	ATR

PARP	ATM, ATR, DNA-PK
Platinum agents	ATR
Alkylating agents (Thiothepa, Melphalan, Lomustine)	ATR
TOPO1i and TOPO2i (Topotecan, Etoposide)	DNA-PK
TOPO1i (SN-38, Topotecan)	ATR
CHK1i	ATR
Cytarabine, Gemcitabine	ATR
Mitomycin	ATR
Bleomycin	DNA-PK

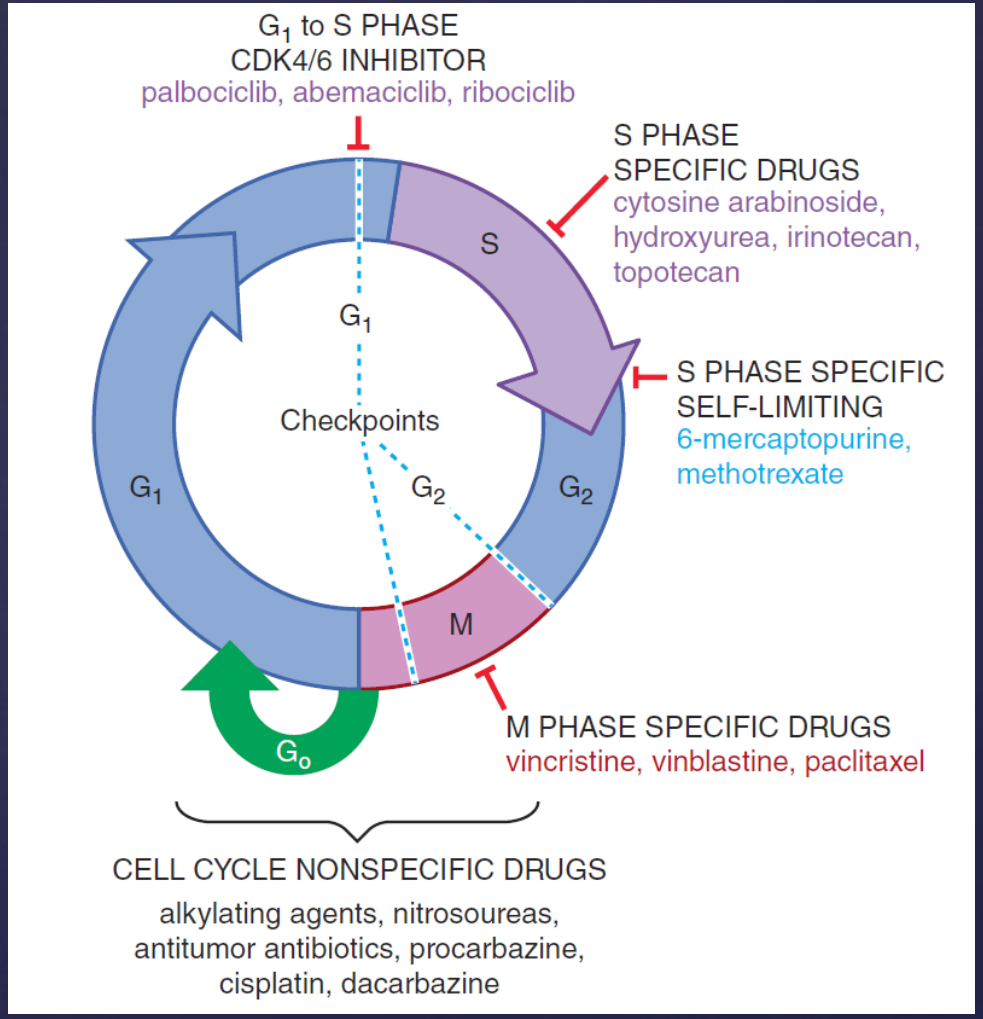
Sites of action of drugs used for treatment of cancer

(Anticancer drugs are quite varied in structure and mechanism of action)



Cell cycle specific and nonspecific drugs

- Many cytotoxic agents act by damaging DNA.
- Some anticancer drugs act at specific phases in the cell cycle, mainly at the S and M phases; other drugs are cytotoxic at any point in the cell cycle and are termed *cell cycle phase nonspecific*.



Findings on drugs

- In general, **ATR inhibitors have stronger synergy** and efficacy compared to other DDR inhibitors in all combinations tested.

- **PARP inhibitors** (veliparib, talazoparib, rucaparib, olaparib, and niraparib):

Highest synergy with ATM and ATR inhibitors across multiple cancer types.

The TOP1/2 (DNA topoisomerase 1/2) inhibitors (SN-38 (the active metabolite of irinotecan), topotecan, etoposide, and doxorubicin):

- High efficacy and synergy with ATM/ATR/DNA-PK inhibitors (DNA-PK > ATR > ATM), as previously reported in preclinical studies19,28,29.

- Synergistic relationship between ATRi and gemcitabine has been reported before. However, Synergy between **gemcitabine and either DNA-PKi or ATMi have not been reported before**.

- **Tubulin inhibitors** achieved high **efficacy** but **low synergy** with DDR inhibitors, possibly due to the high cytotoxicity of tubulin inhibitors alone that may result in a plateau effect in cell growth inhibition which could not be further increased by combination with DDR inhibitors.

- Overall, the dataset shows a **low Pearson's correlation of 0.2 ($p < 1e{-}22$) between efficacy and synergy**, which, while well within the range of values observed in previous studies31,32, highlights the need of analyzing both measures of response independently.

Findings on pathways

- Five pathways that displayed strong co-therapeutic efficacy and synergy globally:

1. DNA topoisomerase pathway (TOP1 and TOP2 inhibitors)
2. Serine/threonine protein kinase PLK1 pathway (PLK1 inhibitors) ?

[THE ONLY PLK1 INHIBITOR IS BI2536, WHICH IS INACTIVE]

1. p53-inducible ribonucleotide reductase pathway (gemcitabine and cytarabine)
2. PARP pathway
3. Cell cycle checkpoints (in particular, CHK1 inhibitors).

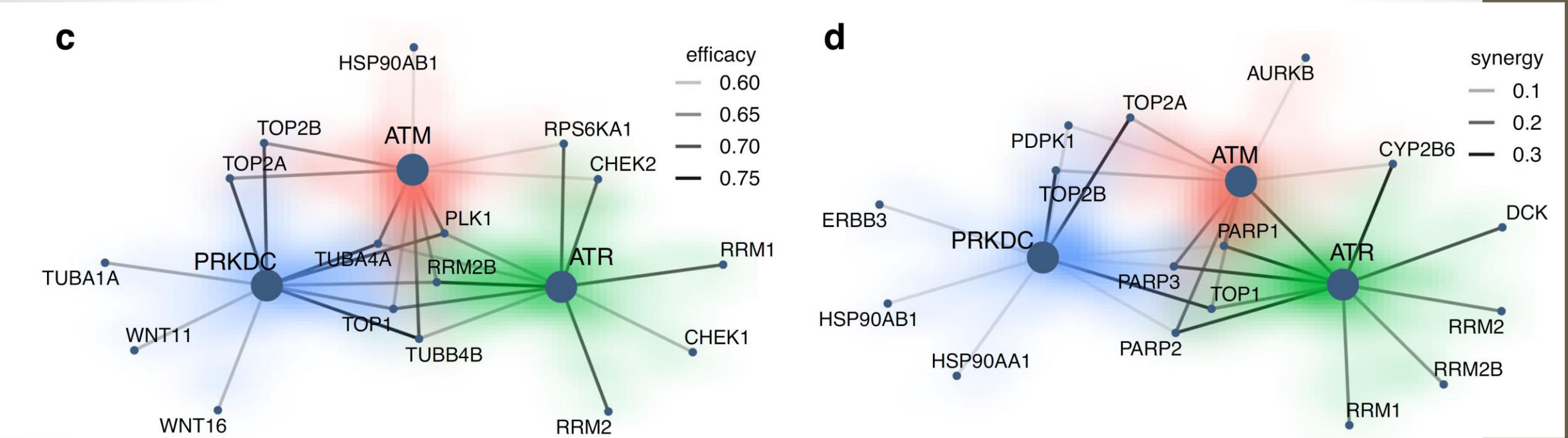


Fig. 2 c, d show the top 10 target genes with the highest average (c) efficacy and (d) synergy in combination with ATR, ATM, and DNA-PK (PRKDC) inhibitors.

Each target gene of a partner drug is denoted by a node in the diagram, and the combination response (efficacy or synergy) is denoted by the relative strength of the connection.

- RRM 1/2 pathway is only synergistic in combination with ATR, but not ATM and DNA-PK inhibition,
- TOP pathway is synergistic with all ATR, ATM and DNA-PK inhibition.
- PARP inhibitors: strongly and broadly synergistic in combination with ATRi/ATMi, but not DNAPKi.

Four monotherapy and two DDR inhibitor combinations show significant variability in response between different cancer types

- For investigating whether general biological backgrounds, such as cancer or tissue types, influence treatment response, we carried out statistical comparisons of the efficacy and synergy responses between different cancer types covering the 87 monotherapy agents and 465 combination treatments screened in our study.

- As the number of cell lines covering each of the 12 cancer types varies, we chose the non-parametric Kruskal-Wallis test to analyze the variance of treatment response of each treatment across all cancer types in this study.

For all monotherapy and combination therapies that showed significant differences in responses across cancer types, we carried out statistical post hoc analysis including Dunn's test, to identify individual cancer types with variable responses to individual drugs and drug combinations (Fig. 3d–f, Supplementary Fig. 2 and Supplementary Data 4 and 5).

variable mono-therapeuties

Of the four significantly **variable mono-therapeutic** agents:

- **Doxorubicin** showed significantly higher efficacy in hematological cancers than other cancer types.
- **M3541** demonstrated lower efficacy in both pancreas and melanoma cancers than other cancer types (Supplementary Fig. 2b).
- For **peposertib** and **oxaliplatin**, the difference of efficacy was only significant between bladder and ovary/hematological cancers, as well as between sarcoma and hematological cancers (Supplementary Fig. 2b).

Cross-cancer type variance

Fig. 3 |

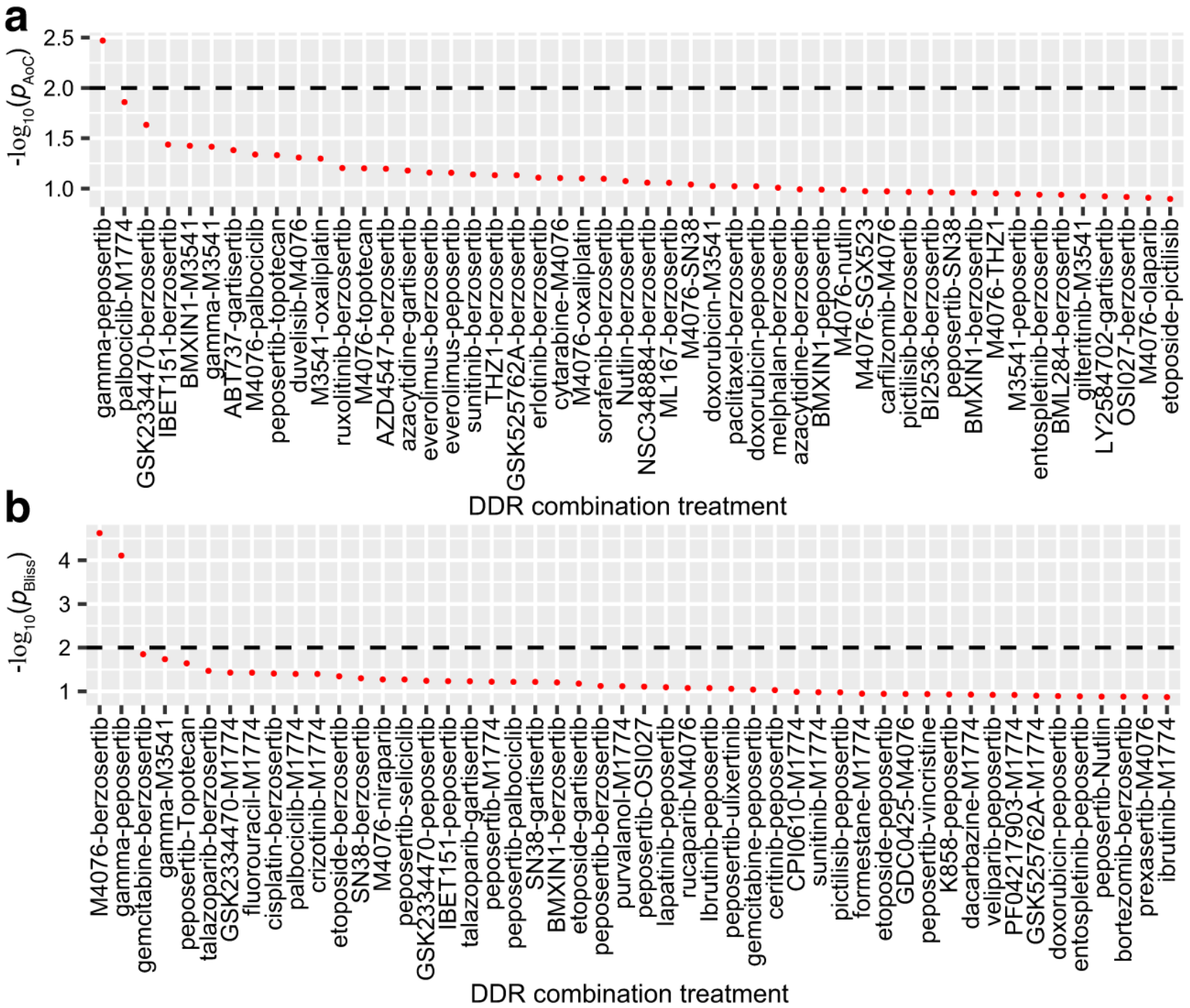
Results from cross-cancer type variance test of DDR inhibitor combination treatment response.

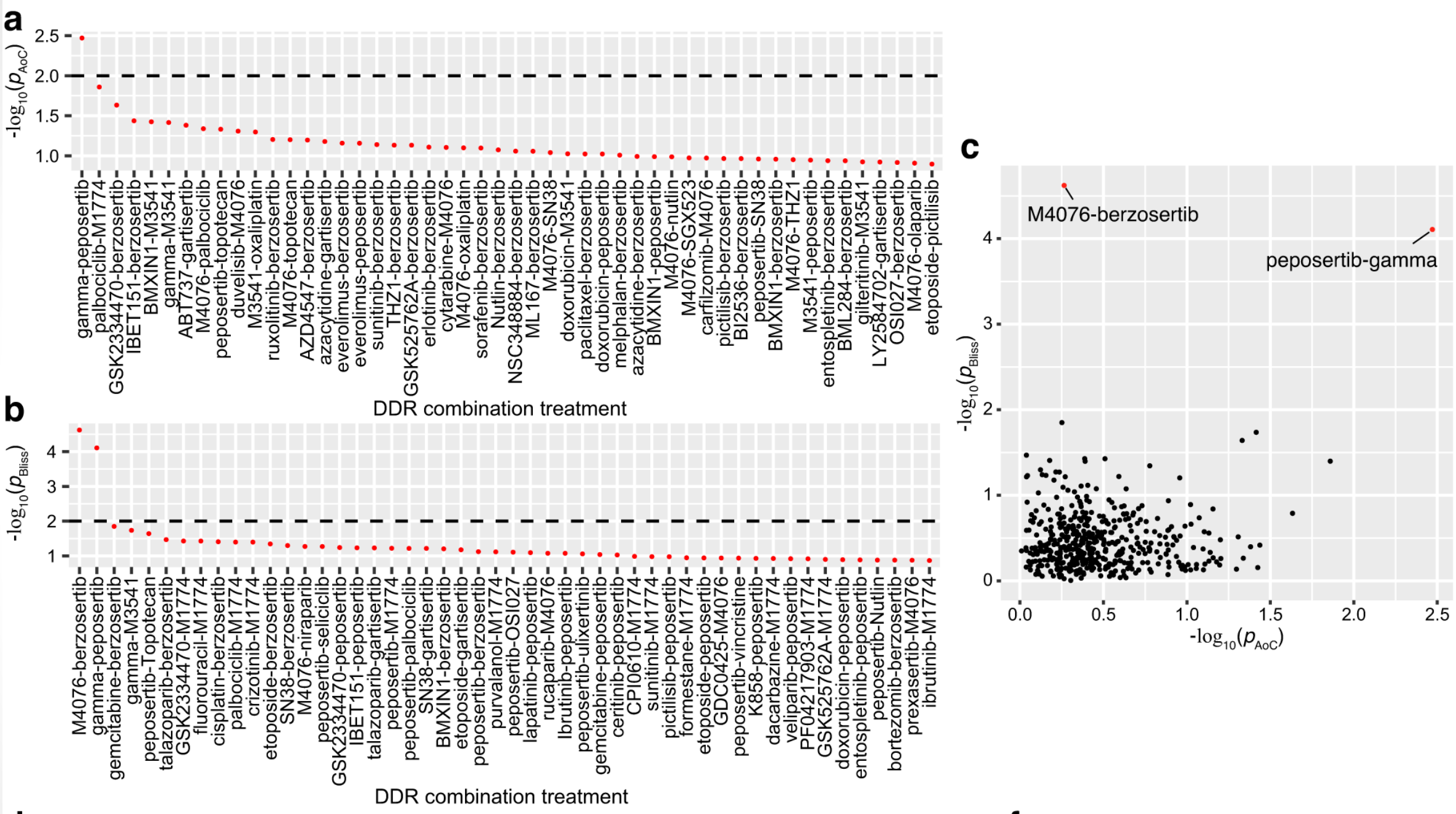
a, b Kruskal–Wallis test shows the significance of cross-cancer type variance of DDR inhibitor combinations tested in this study.

−log10(p) from the cross-tissue variance test for (a) efficacy (AoC score) and (b)

synergy (Bliss) of the top 50 combinations are shown

The significance threshold (p = 0.01) is marked by a dashed line.

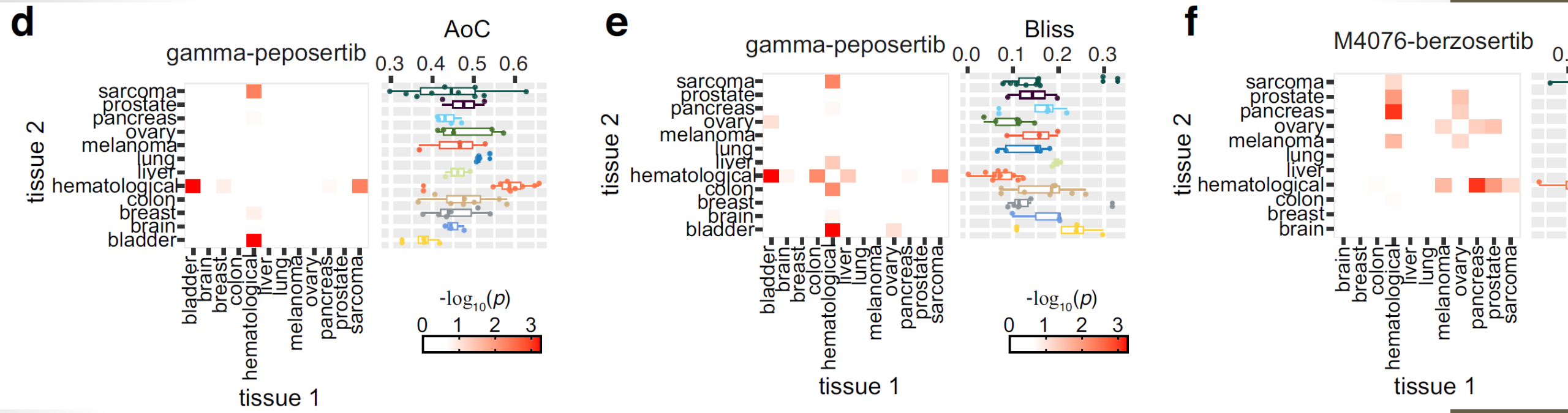



a
-log10(pAoC)
2.5
2.0
1.5
1.0
gamma-peposertib
palbociclib-M1774
GSK2334470-berzosertib
IBET151-berzosertib
BMXIN1-M3541
gamma-M3541
ABT737-gartisertib
M4076-palbociclib
peposertib-topotecan
duvelisib-M4076
M3541-oxaliplatin
ruxolitinib-berzosertib
M4076-topotecan
AZD4547-berzosertib
azacytidine-gartisertib
everolimus-berzosertib
everolimus-peposertib
sunitinib-berzosertib
THZ1-berzosertib
GSK525762A-berzosertib
erlotinib-berzosertib
cytarabine-M4076
M4076-oxaliplatin
sorafenib-berzosertib
Nutlin-berzosertib
NSC348884-berzosertib
ML167-berzosertib
M4076-SN38
doxorubicin-M3541
paclitaxel-berzosertib
doxorubicin-peposertib
melphalan-berzosertib
azacytidine-berzosertib
BMXIN1-peposertib
M4076-nutlin
M4076-SGX523
carfilzomib-M4076
pictilisib-berzosertib
BI2536-berzosertib
peposertib-SN38
BMXIN1-berzosertib
M4076-THZ1
M3541-peposertib
entospletinib-berzosertib
BML284-berzosertib
gilteritinib-M3541
LY2584702-gartisertib
OSI027-berzosertib
M4076-olaparib
etoposide-pictilisib
DDR combination treatment
b
-log10(pBliss)
4
3
2
1
M4076-berzosertib
gamma-peposertib
gemcitabine-berzosertib
gamma-M3541
peposertib-Topotecan
talazoparib-berzosertib
GSK2334470-M1774
fluorouracil-M1774
cisplatin-berzosertib
palbociclib-M1774
crizotinib-M1774
etoposide-berzosertib
SN38-berzosertib
M4076-niraparib
peposertib-seliciclib
GSK2334470-peposertib
IBET151-peposertib
talazoparib-gartisertib
peposertib-M1774
peposertib-palbociclib
SN38-gartisertib
BMXIN1-berzosertib
etoposide-gartisertib
peposertib-berzosertib
purvalanol-M1774
peposertib-OSI027
lapatinib-peposertib
rucaparib-M4076
ibrutinib-peposertib
peposertib-ulixertinib
gemcitabine-peposertib
ceritinib-peposertib
CPI0610-M1774
sunitinib-M1774
pictilisib-peposertib
formestane-M1774
etoposide-peposertib
GDC0425-M4076
peposertib-vincristine
K858-peposertib
dacarbazine-M1774
veliparib-peposertib
PF04217903-M1774
GSK525762A-M1774
doxorubicin-peposertib
entospletinib-peposertib
peposertib-Nutlin
bortezomib-berzosertib
prexasertib-M4076
ibrutinib-M1774
DDR combination treatment
c
M4076-berzosertib
peposertib-gamma
-log10(pBliss)
-log10(pAoC)
0.0
0.5
1.0
1.5
2.0
2.5
d
AoC
gamma-peposertib
0.3
0.4
0.5
0.6
sarcoma
e
Bliss
gamma-peposertib
0.0
0.1
0.2
0.3
sarcoma
prostate
f
M4076-berzosertib
Bliss
0.0
0.1
0.2
sarcoma
prostate

Variable combinations

Only two combination treatments showed significant variation in response across different cancer types:

- Peposertib-gamma-ionizing-radiation combination: both efficacy ($p= 3.38e{-3}$) and synergy ($p=7.82e{-5}$)),
- M4076-berzosertib (ATMi-ATRi combination): only in terms of synergy ($p= 2.39e{-05}$)) (Fig. 3a, b).



- d–f : Heatmap shows the results from post hoc analysis by Dunn's test on the significantly **variant combination treatments** (peposertib-gamma-ionizingradiation and M4076-berzosertib) from the Kruskal–Wallis test, and the right lane shows the distribution of responses
- In the case of M4076-berzosertib, shows a significantly lower synergy in hematological cancers compared to pancreas, prostate, melanoma, and sarcoma cancers were observed (Fig. 3f).

- It is essential for future studies to meticulously evaluate the **toxicity and adverse events linked to such combined treatment** approaches, ensuring patient safety and precise dosage calibration.

- The concept of synthetic lethality, which forms the foundation of DDR-targeted combination therapy, inherently enhances efficacy while concurrently **increasing the risk of toxicity and adverse events48,49**.

- For example: The simultaneous administration of the PARP inhibitor olaparib with the ATR inhibitor ceralasertib, has been correlated with the onset of anemia, neutropenia, and thrombocytopenia54,55.

- Furthermore, certain combinations elucidated in our current study have previously been reported to increase the incidence of toxicity and adverse events. The ATR inhibitor berzosertib, usually well-tolerated as a single-agent therapy, has shown an increased prevalence of adverse events and hematological toxicities, including anemia, nausea, and neutropenia, when combined with carboplatin56, gemcitabine57,58, or topotecan59 in early-phase clinical trials.

- https://lincsproject.org/