VERTEX

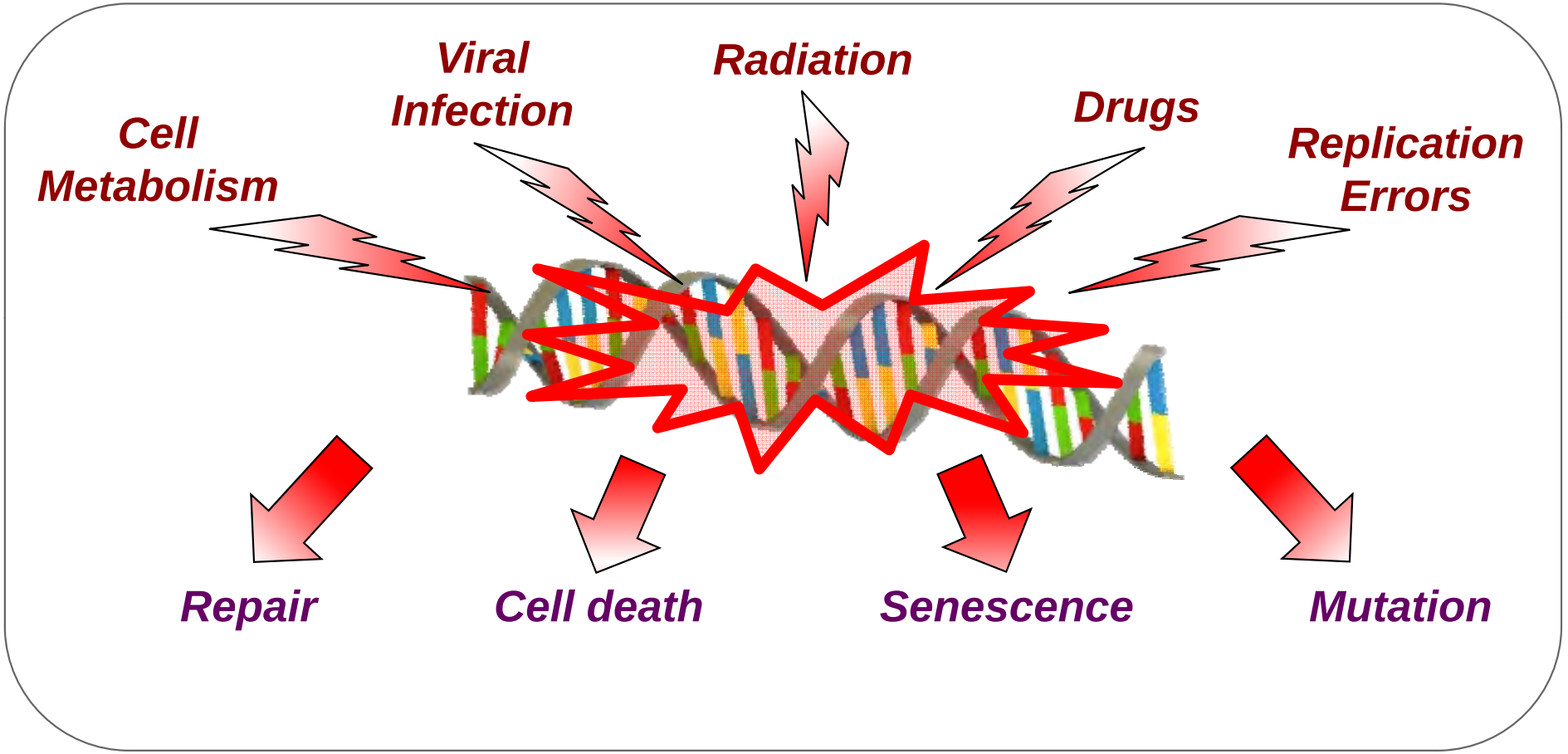
THE SCIENCE *of* POSSIBILITY

John Pollard Ph.D

Discovery and characterisation of potent and selective inhibitors of ATR kinase as anti-cancer agents

Damage to our DNA is relentless

Up to 1 million damage events per cell per day



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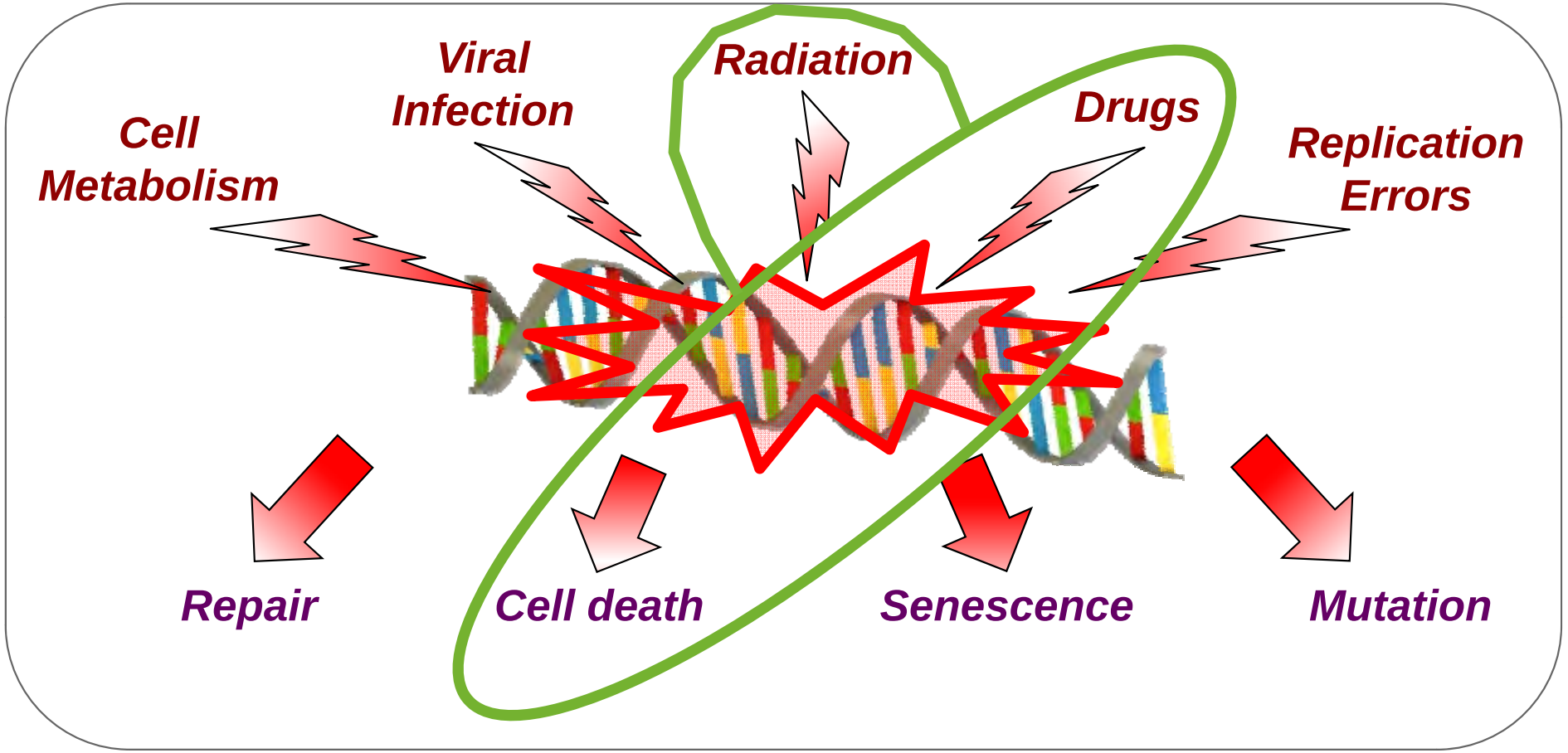
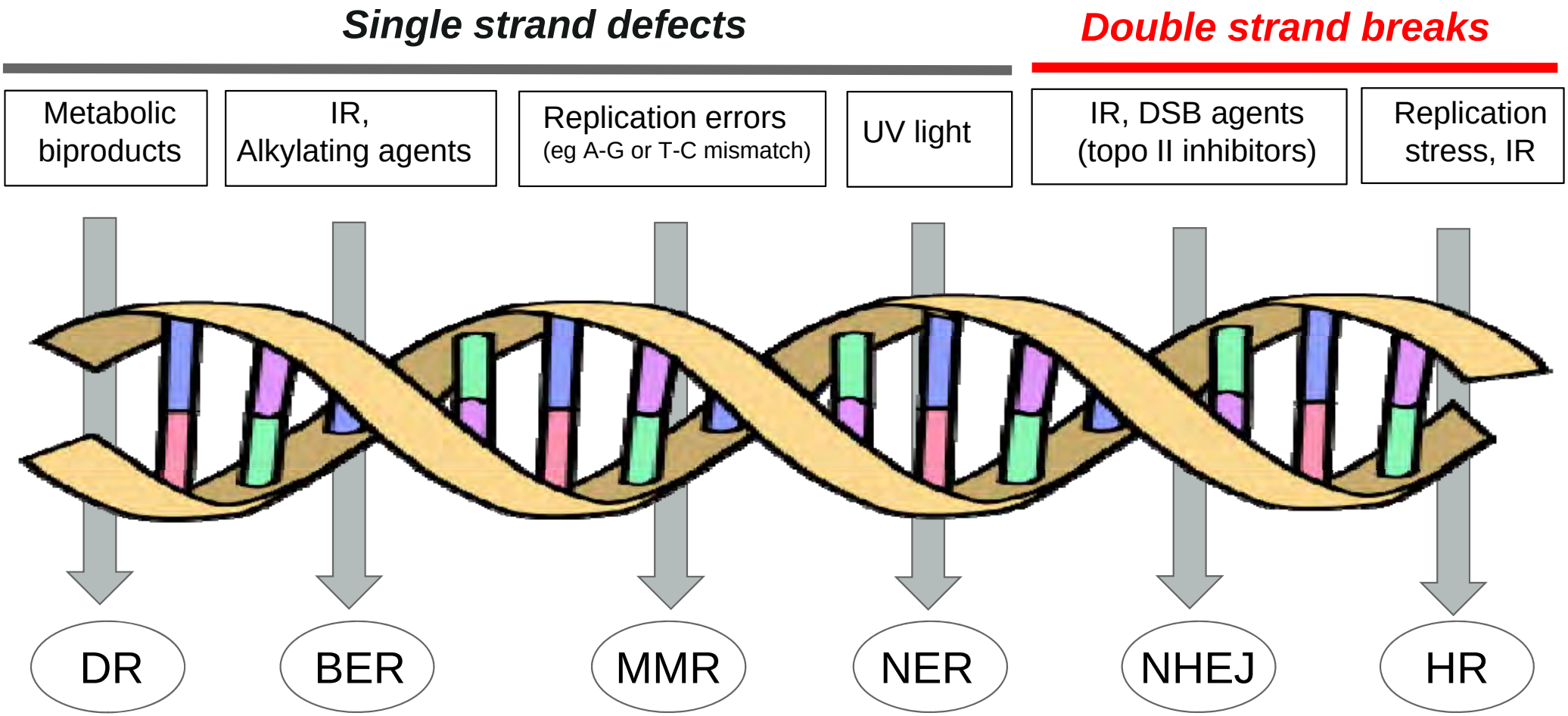


- Many standard-of-care anti-cancer drugs work by inducing DNA damage ….
  …. BUT don't work well because the damage gets repaired

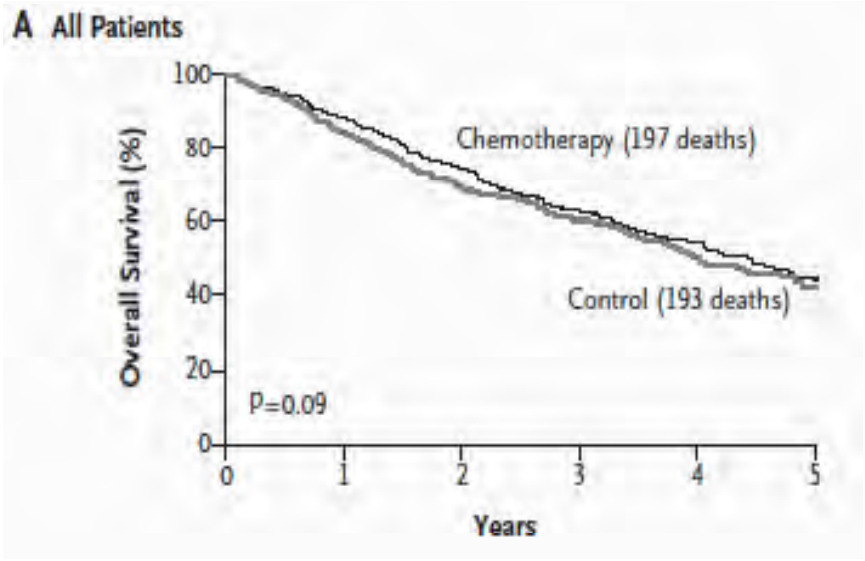
Complex surveillance and repair network in place

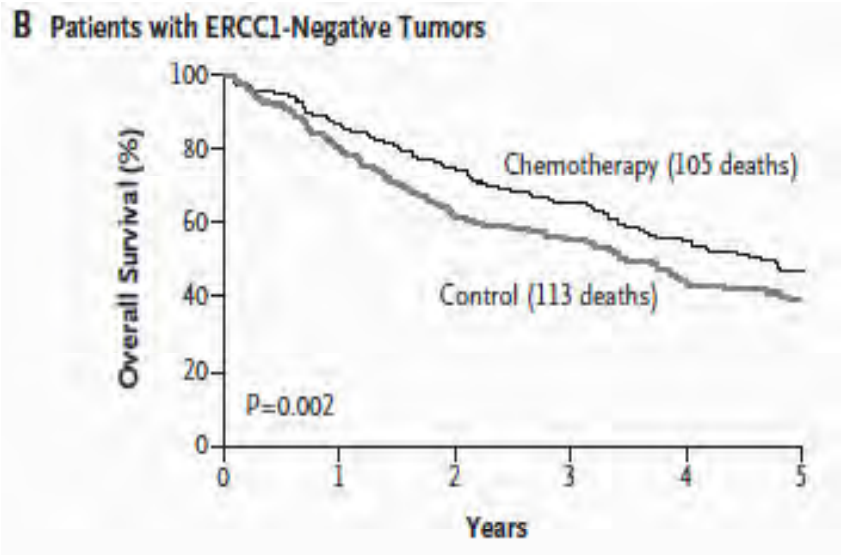


Blocking DNA repair processes will increase toxicity of DNA damage – challenge is to make this specific for cancer cells

Defective DNA damage repair can improve responses to therapy

- NSCLC patients stratified on the basis of ERCC1 status[1]



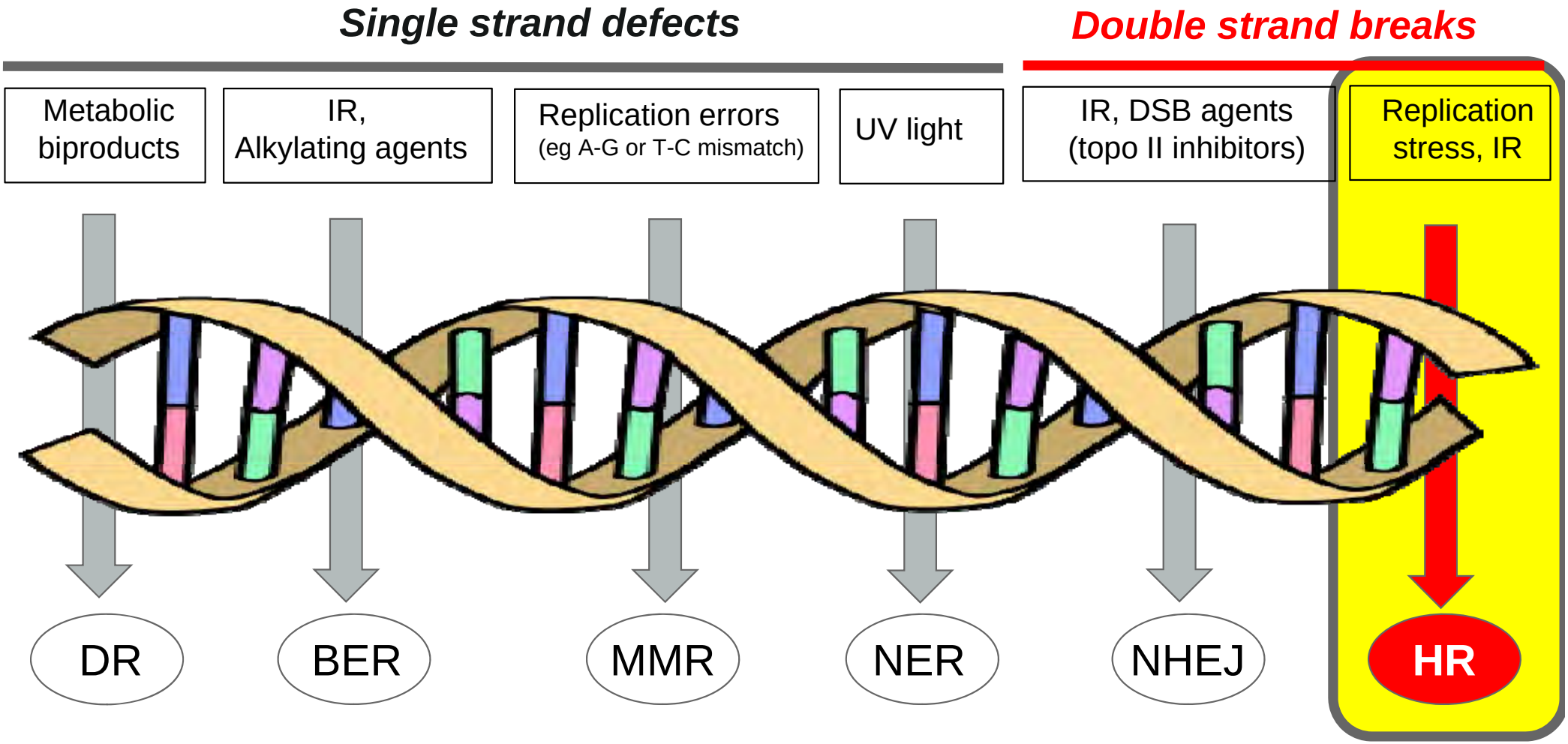


- Testicular cancer has a high response rate to platinum based therapy (>90% cure)[2]
  - Attributed to defects in repair including ERCC1

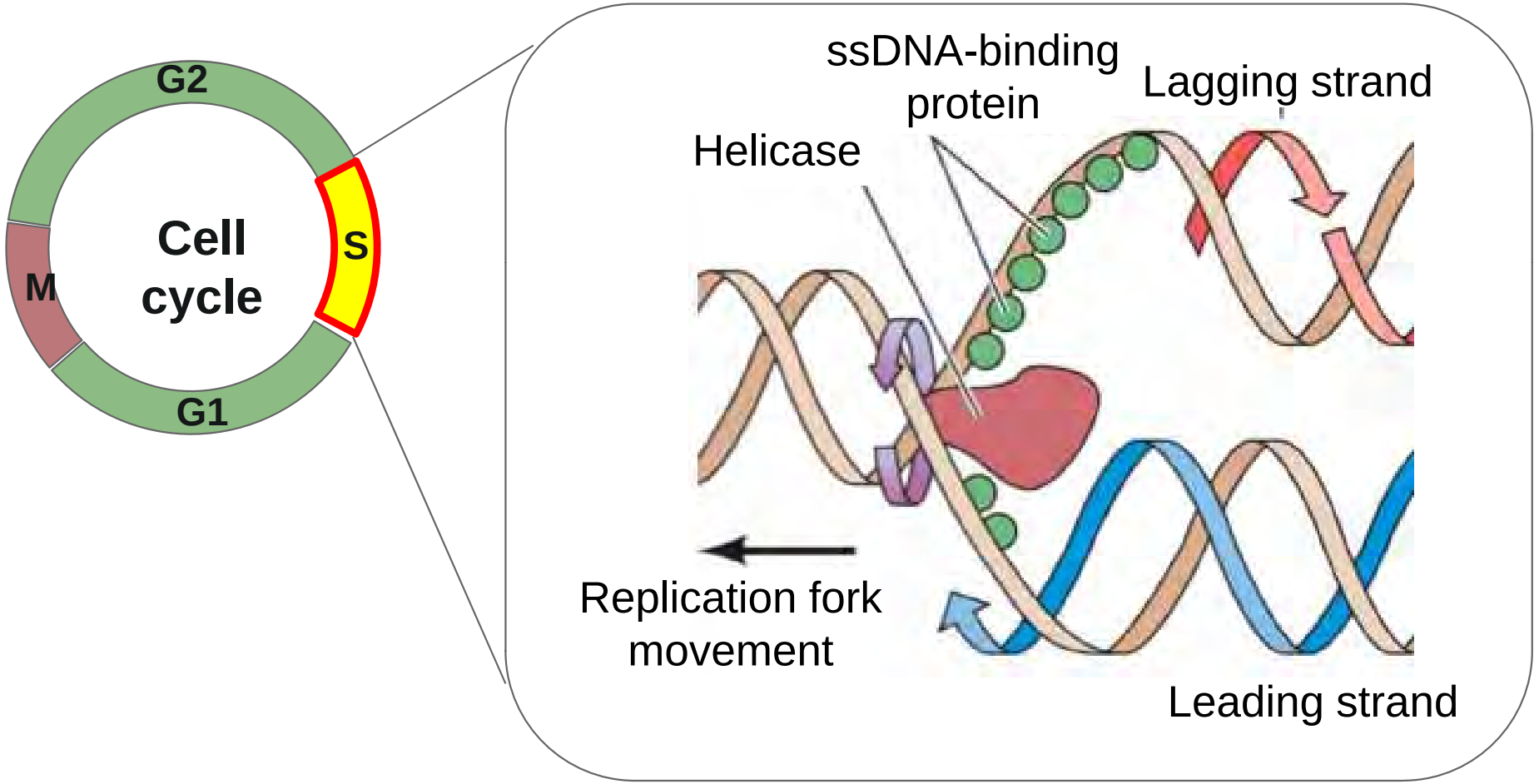
[1]Modified from New Eng. J.Med. (2006) 355, 983

[2]PNAS (2002) 99, 4592

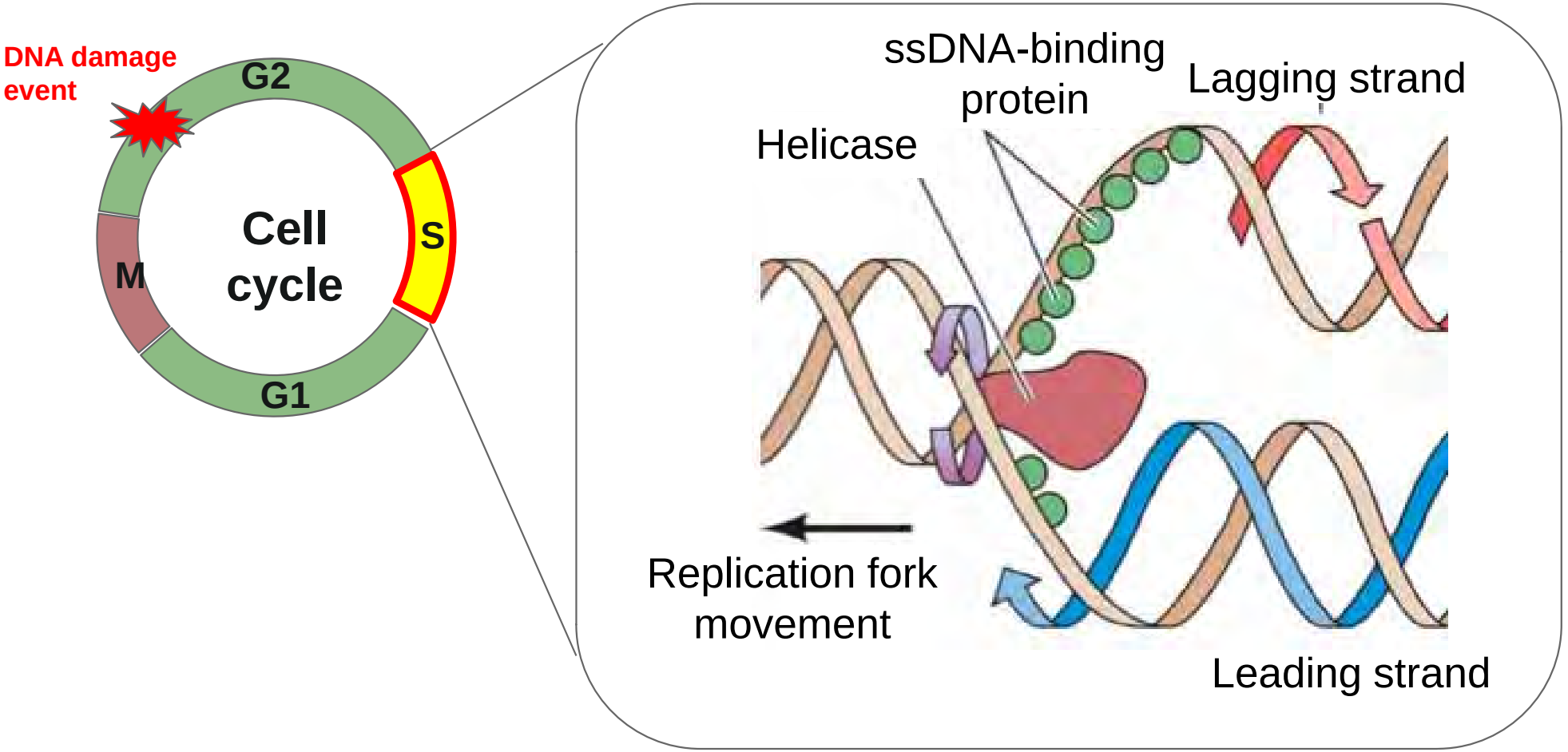
Complex surveillance and repair network in place



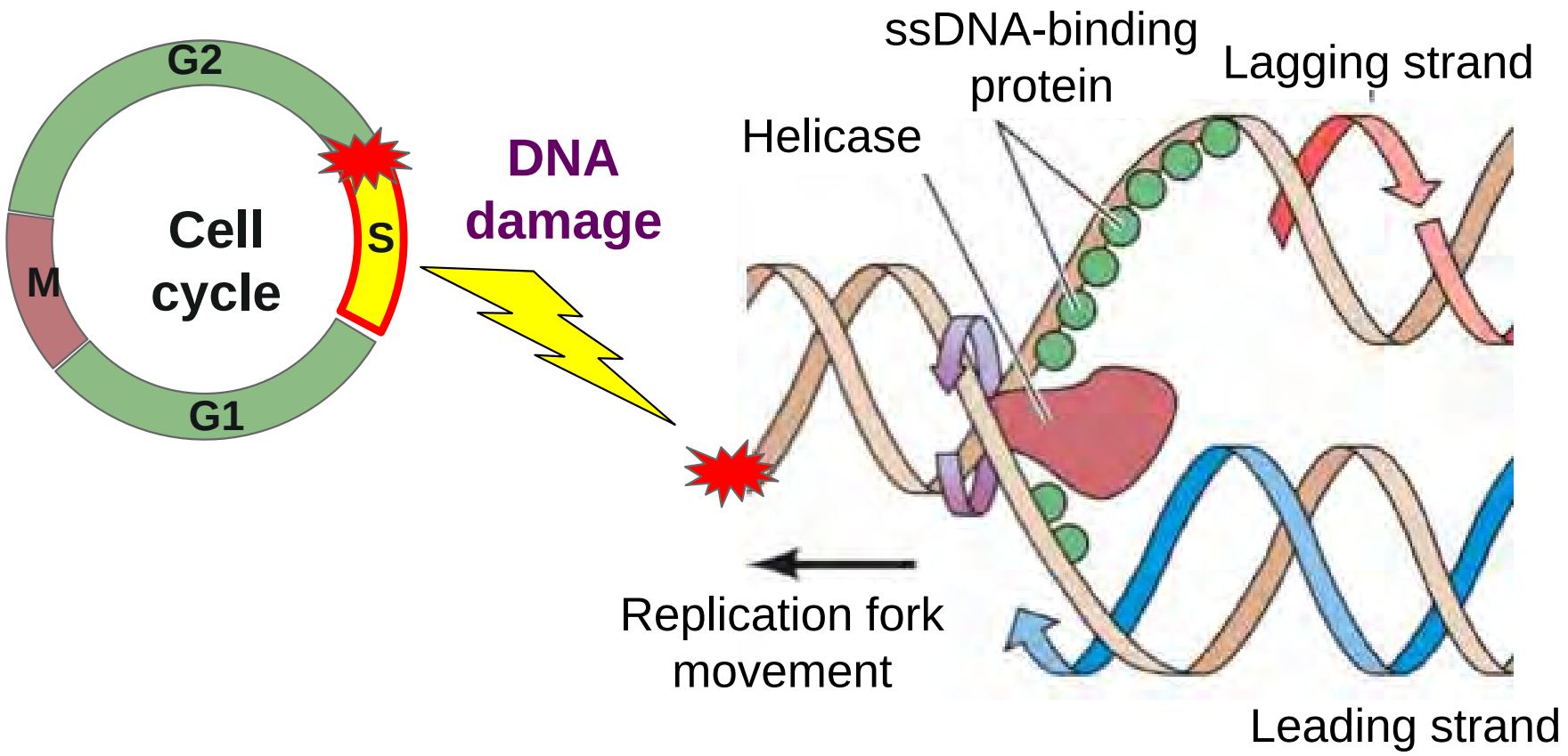
Unperturbed replication fork



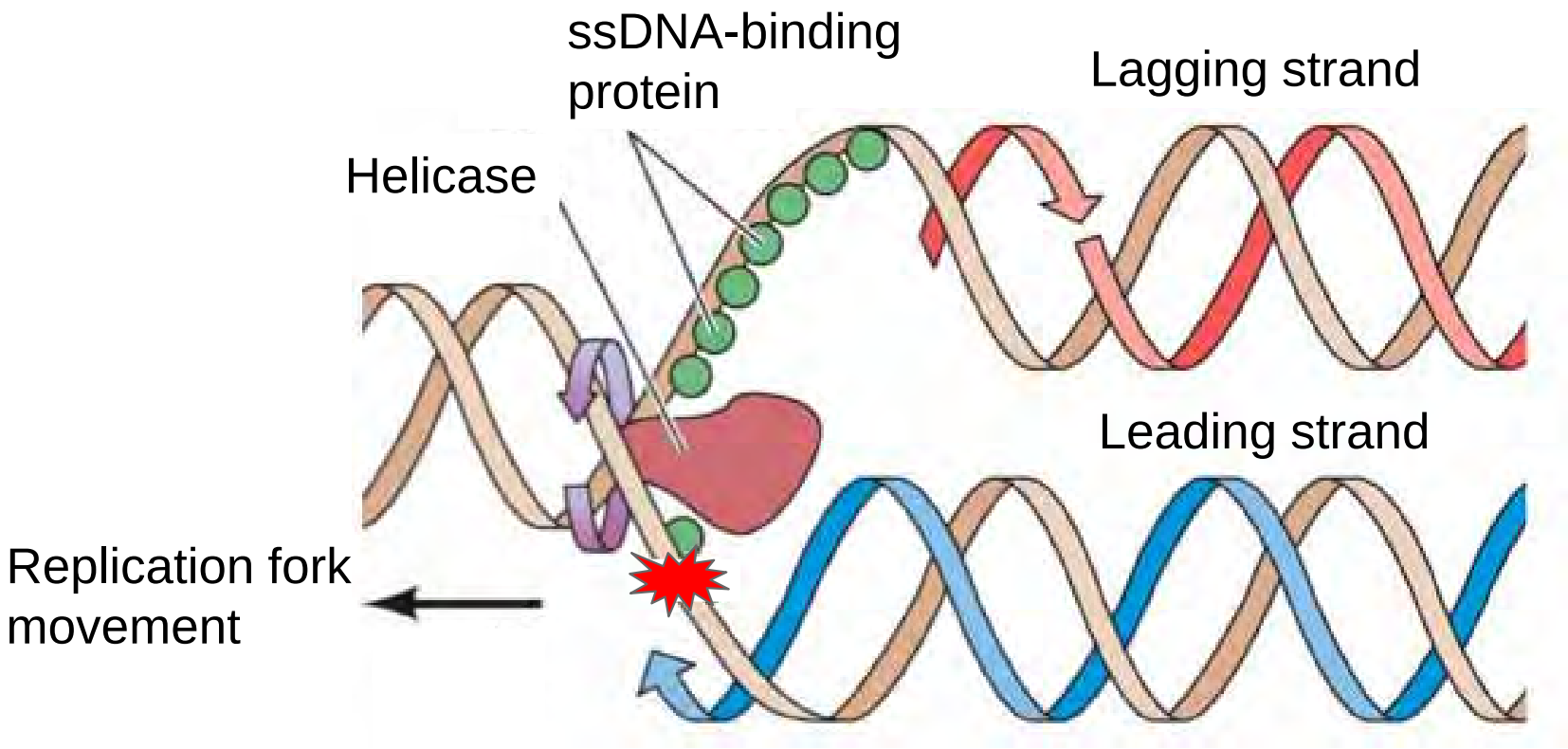
Unperturbed replication fork



Unresolved damage persists to S-phase

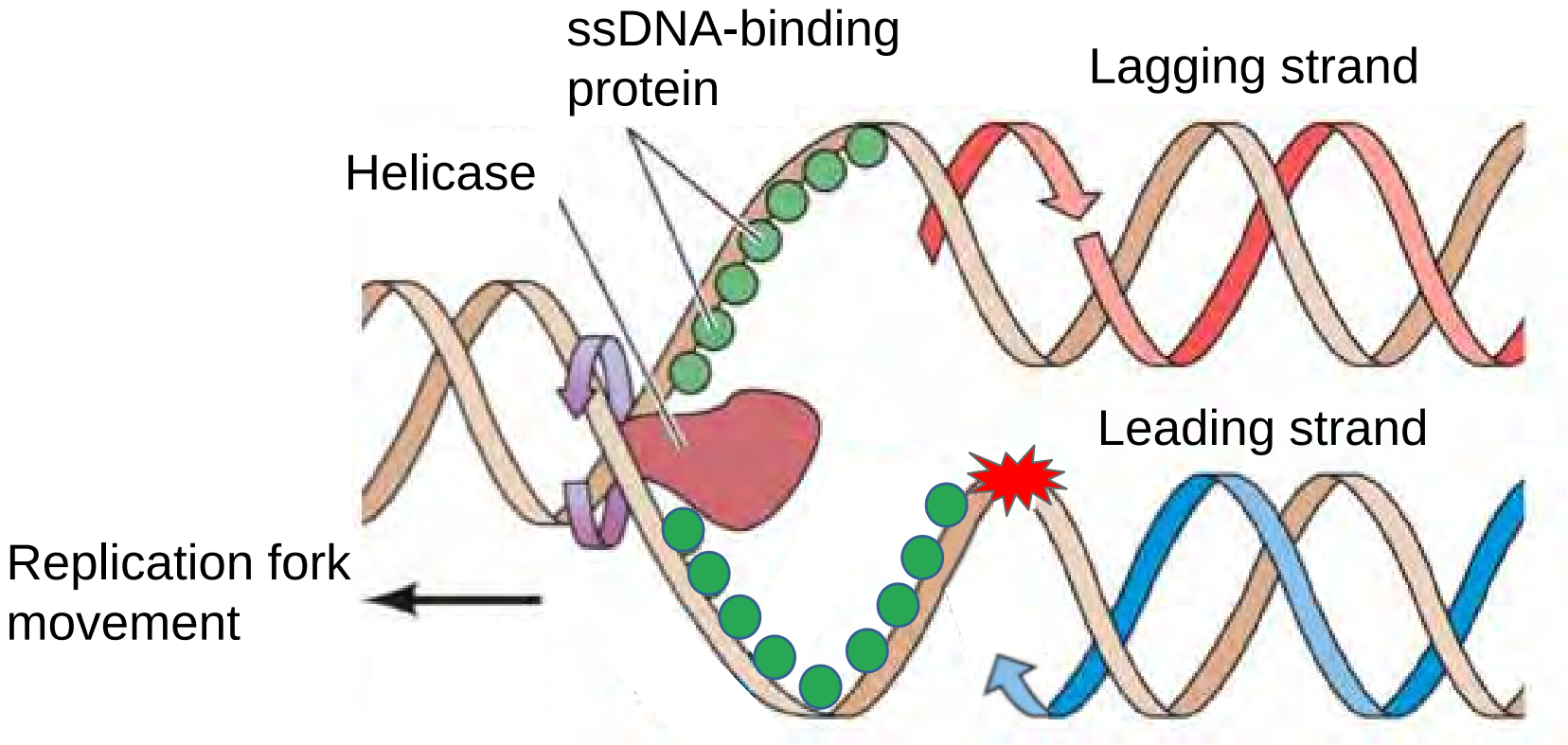


Replication fork encounters damage lesion



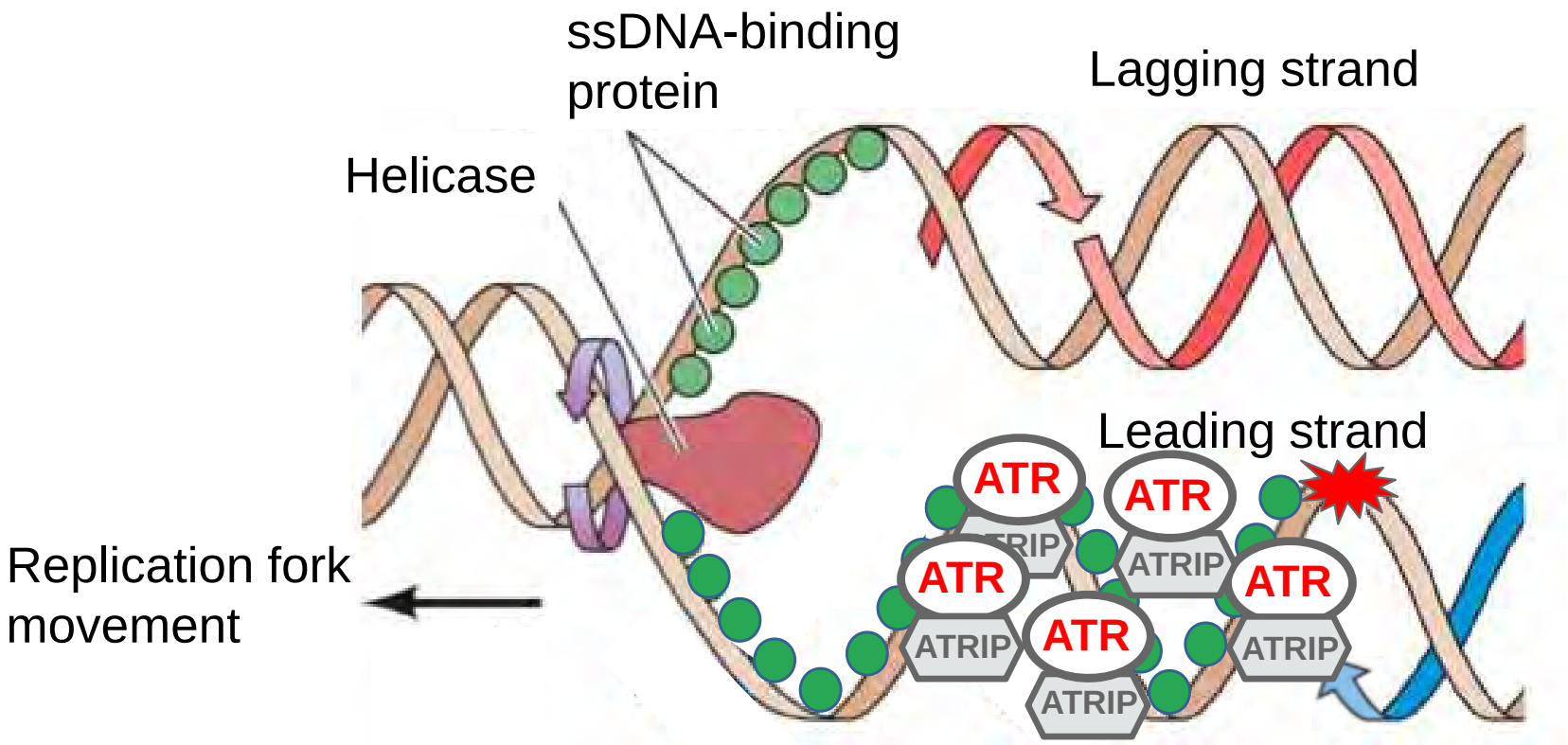
Replication fork stalls – ***Replication stress***

Uncoupled single stranded DNA is exposed



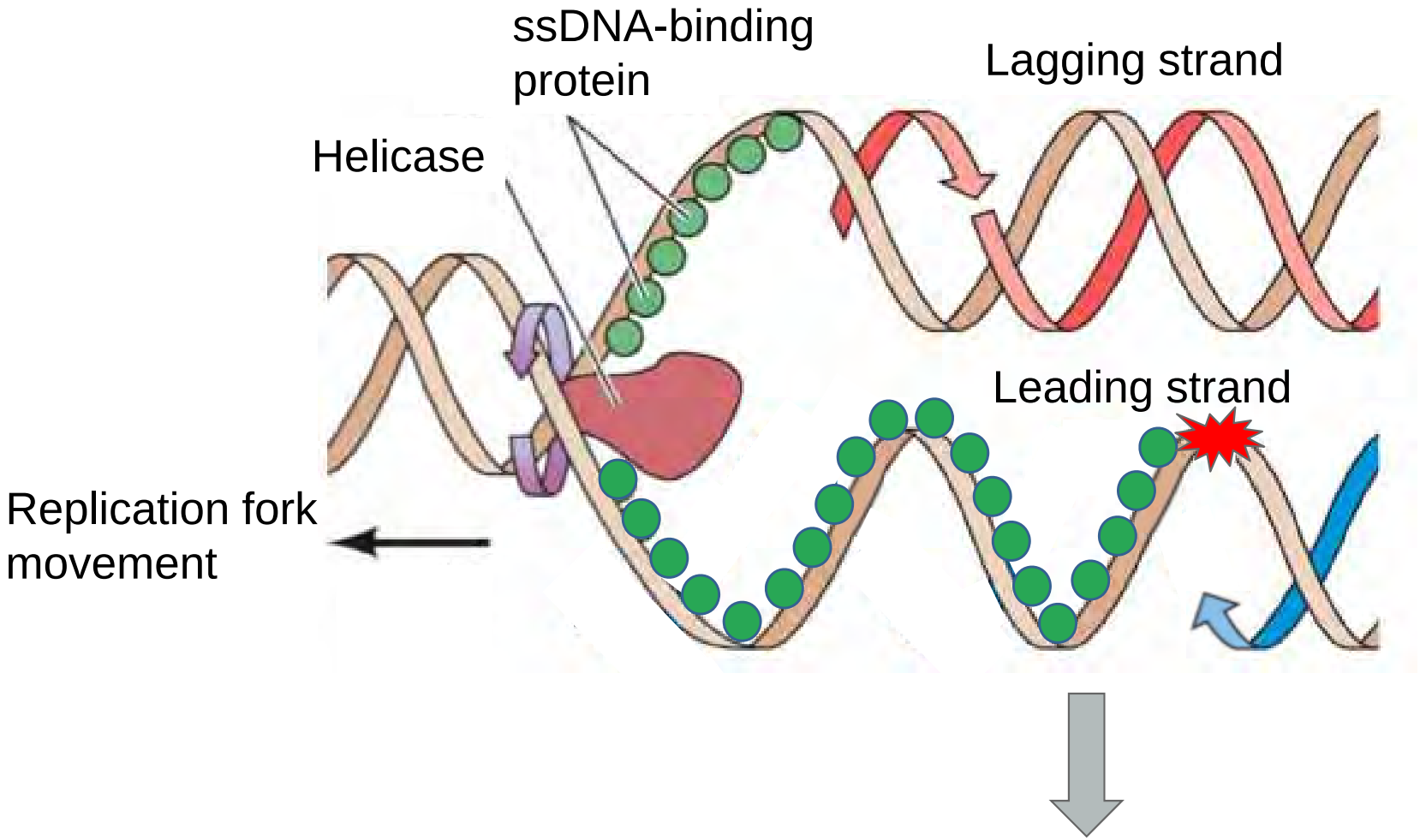
ATR kinase is recruited to stalled fork

Arrests replication, controls origin firing and directs repair



Without ATR stalled replication fork collapses

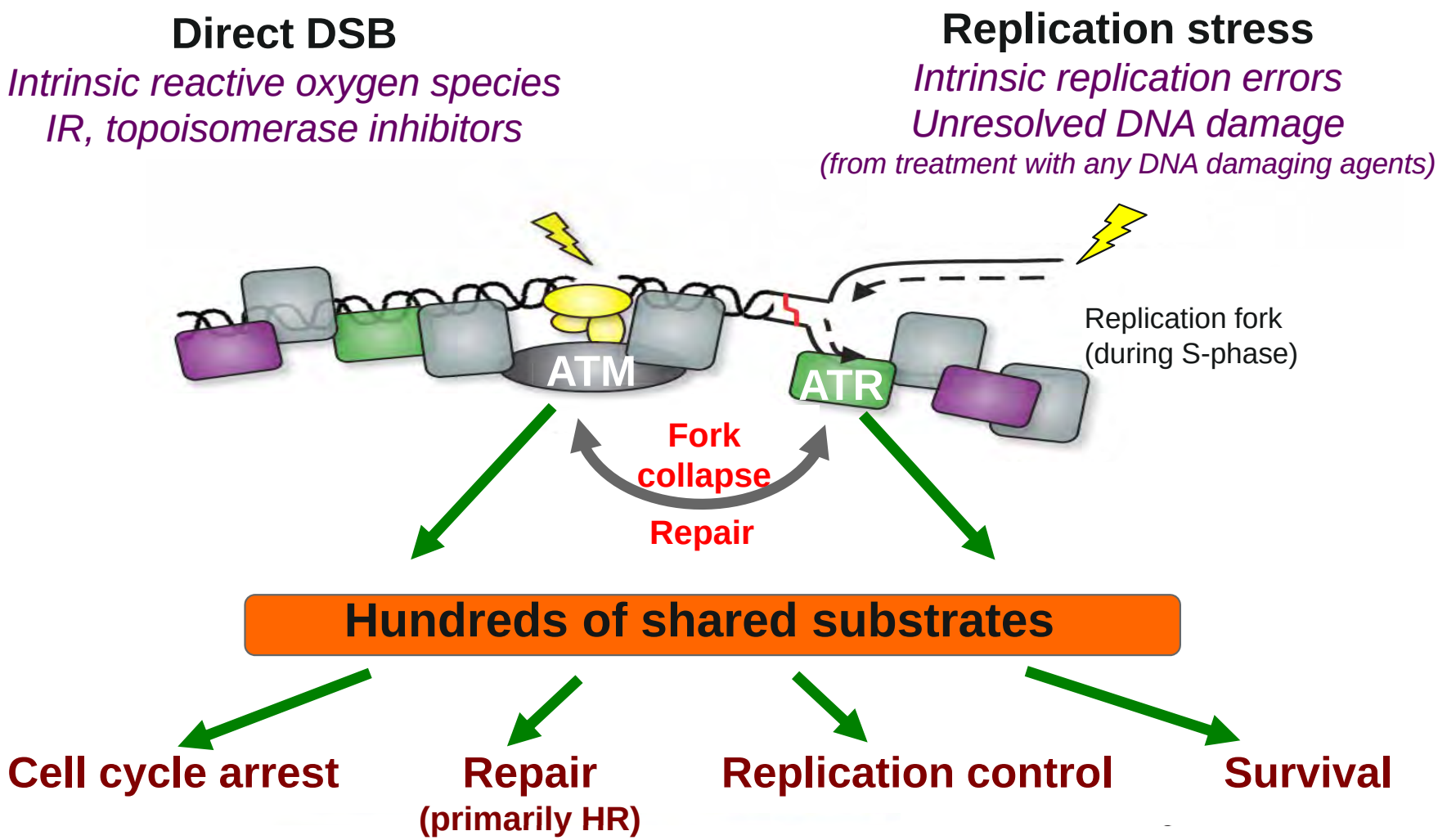
Forms a double strand break



Double Strand Break (DSB)

Extensive overlap between replication stress and DSB repair pathways

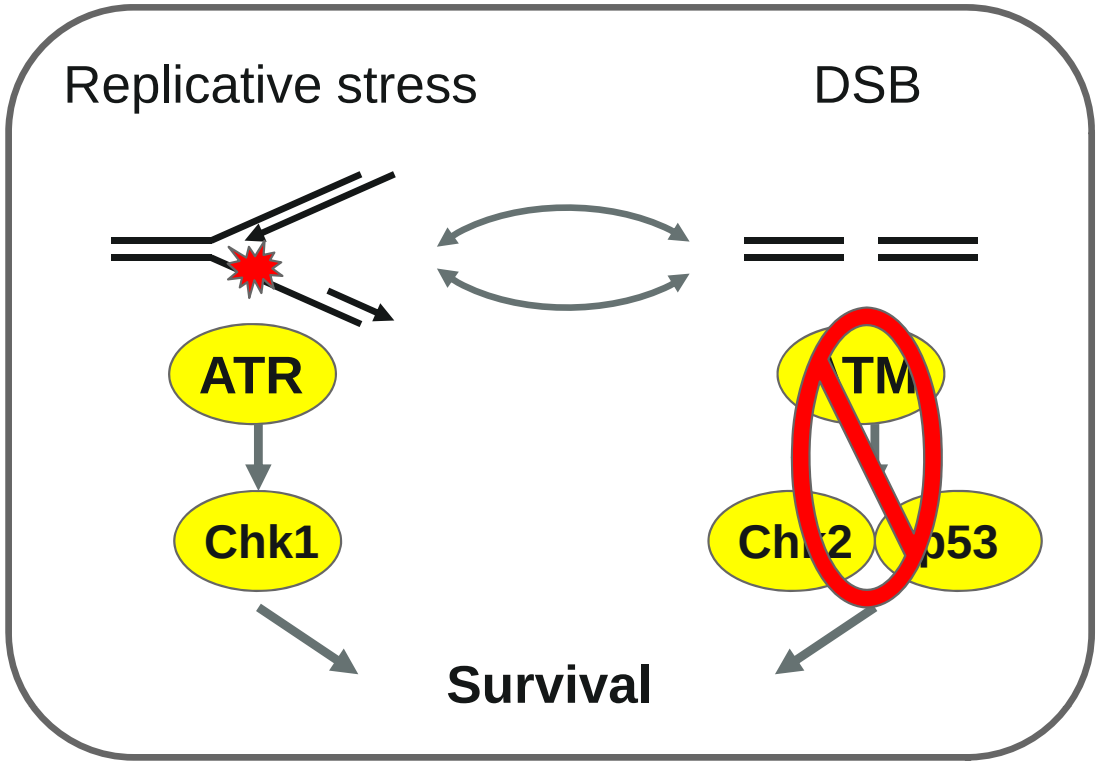
DNA damage response (DDR)



Modified from Jackson SP, Bartek J. *Nature*. 2009;461(1071):1071-1078. DOI: 10.1038/nature08467.

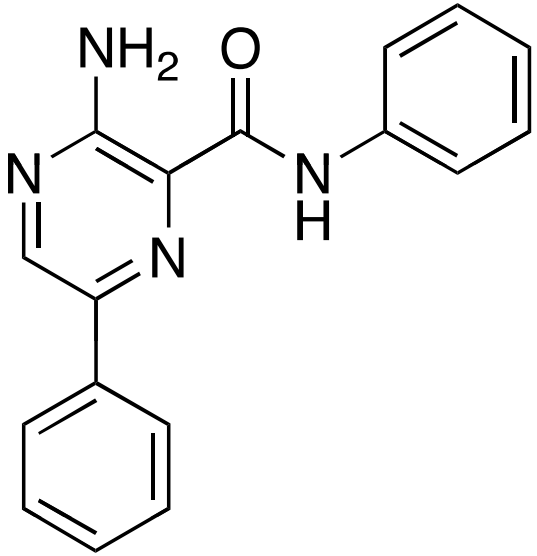
Many cancer cells appear to be reliant on ATR

- Frequent defects in overlapping repair pathways
  - p53, MRN, ATM
- High background replicative stress
  - Defects in G1 checkpoint, expression of oncogenes such as kRas, hypoxia



Discovery of ATR inhibitors

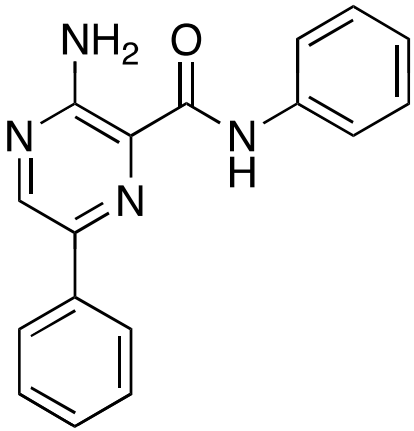
- High throughput screen of in-house library of kinase focused compounds
  - Biochemistry kinase screen using recombinant ATR

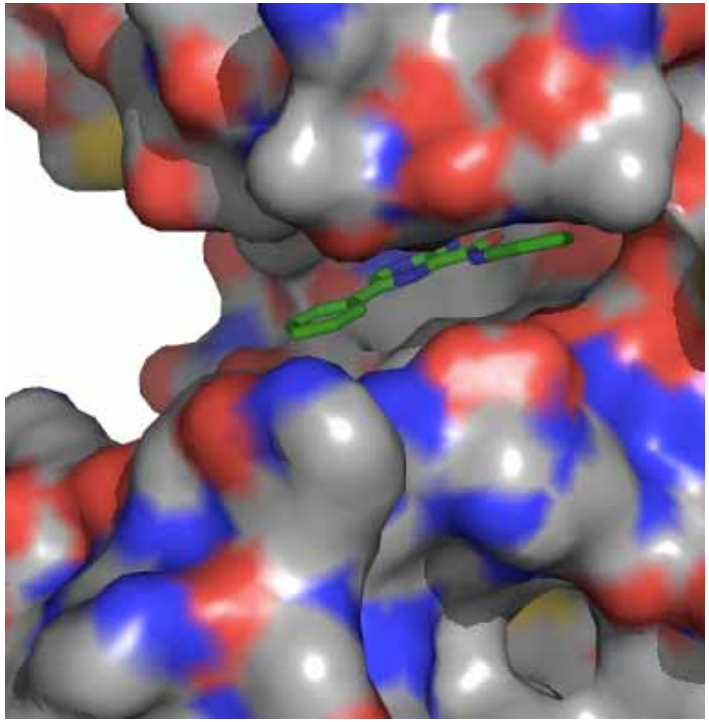


- ATP competitive mechanism of action
- Moderate ATR potency
  - Ki 0.31µM
- Encouraging selectivity
  - Ki ATM >4µM
  - Ki DNA-PK >4µM
- Poor cell activity
  - ATR biomarker IC_{50} >2.5µM

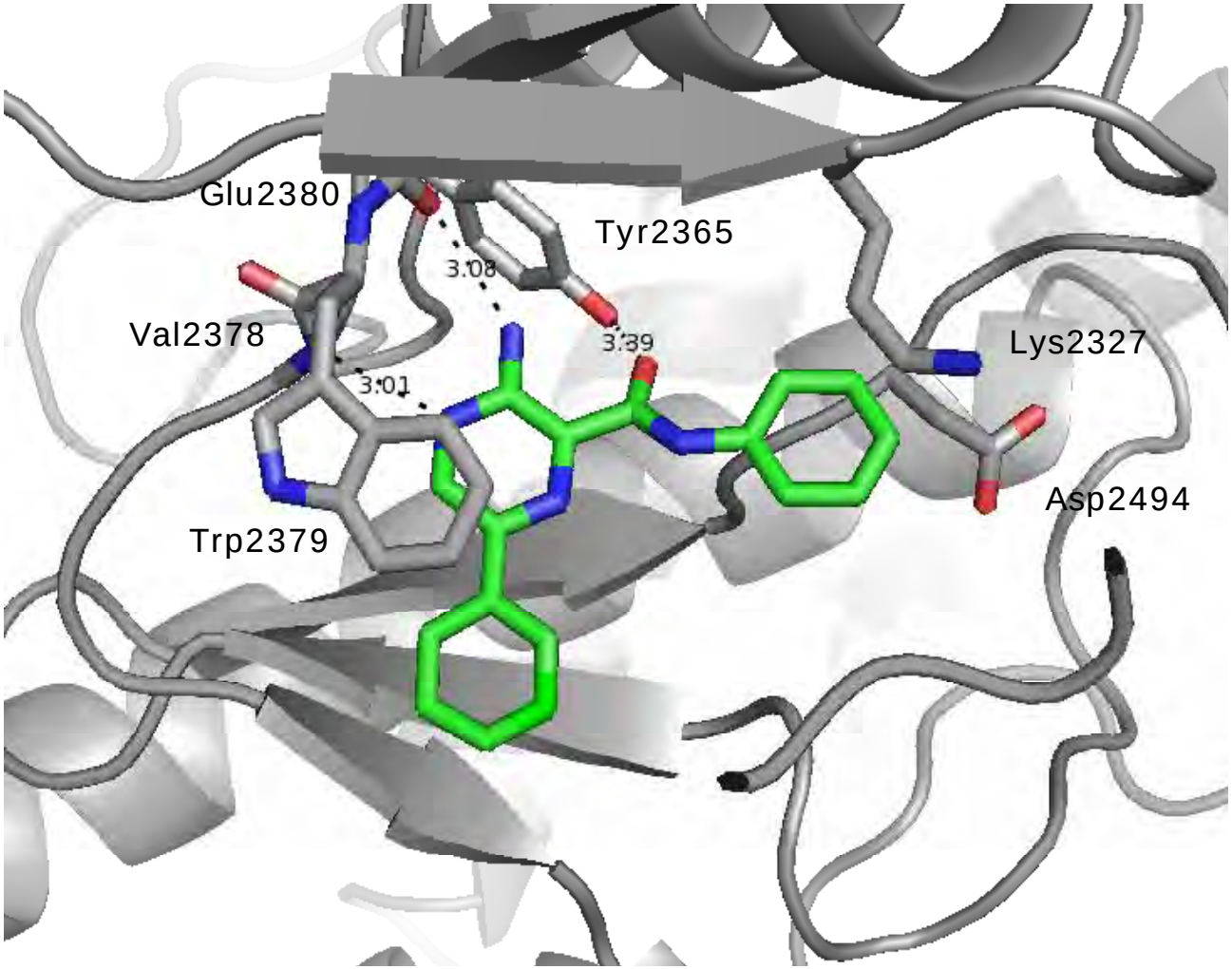
J. Med. Chem. (2011) **54**, 2320

HTS hit modeled in the ATP binding site





Homology model for ATR based on PI3K crystal structures



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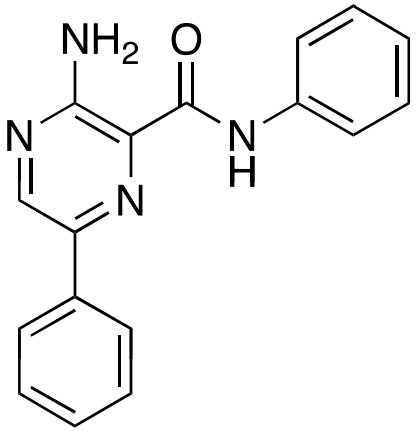
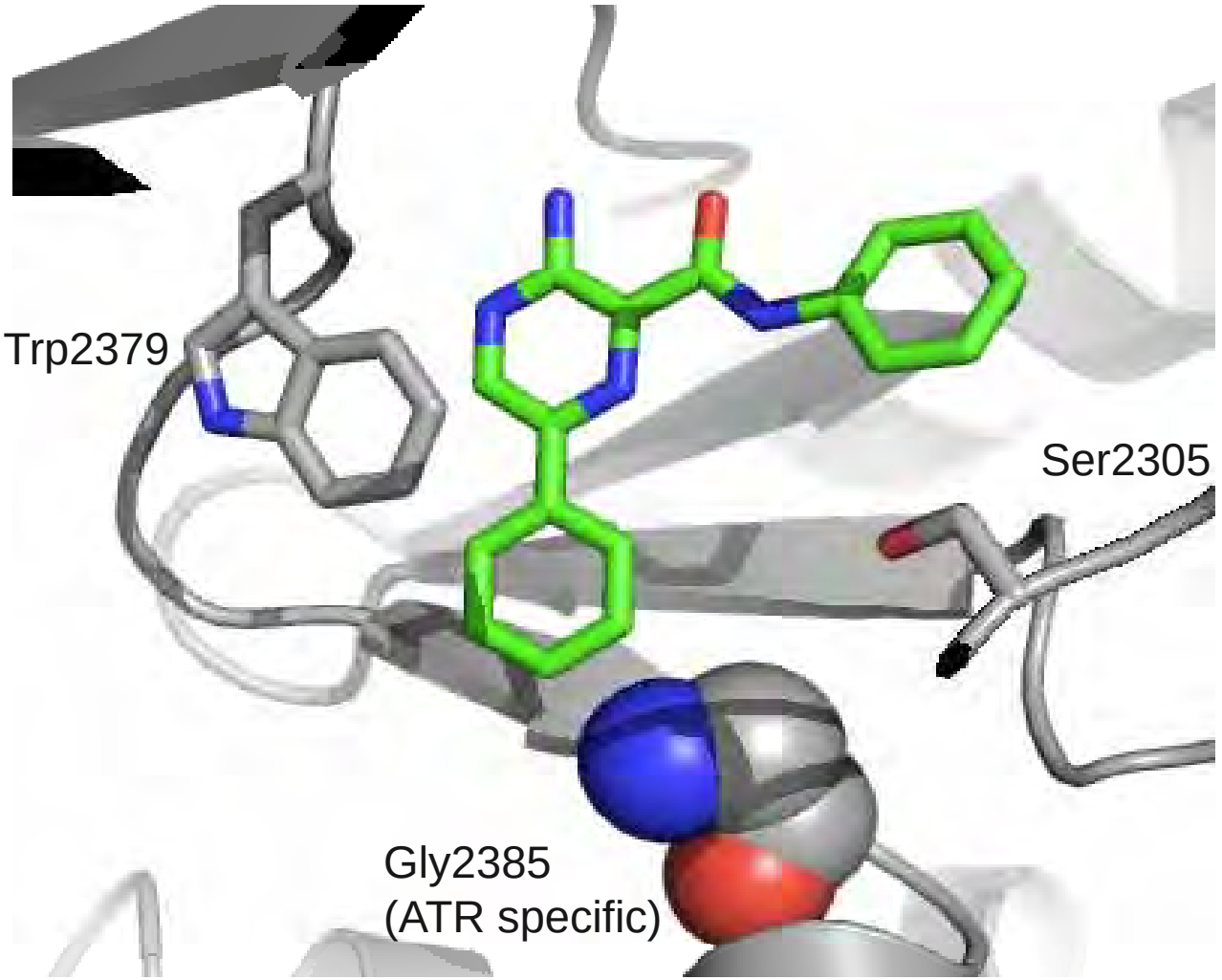
Opportunity to make beneficial interactions from the 5-phenyl group





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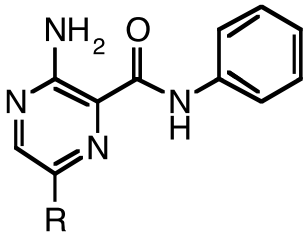
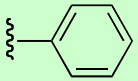
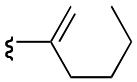
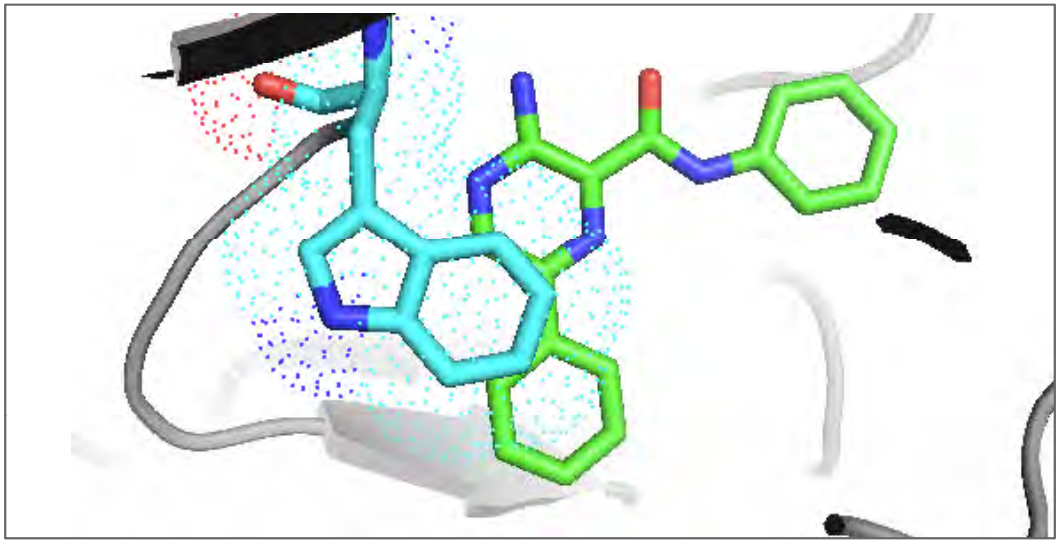
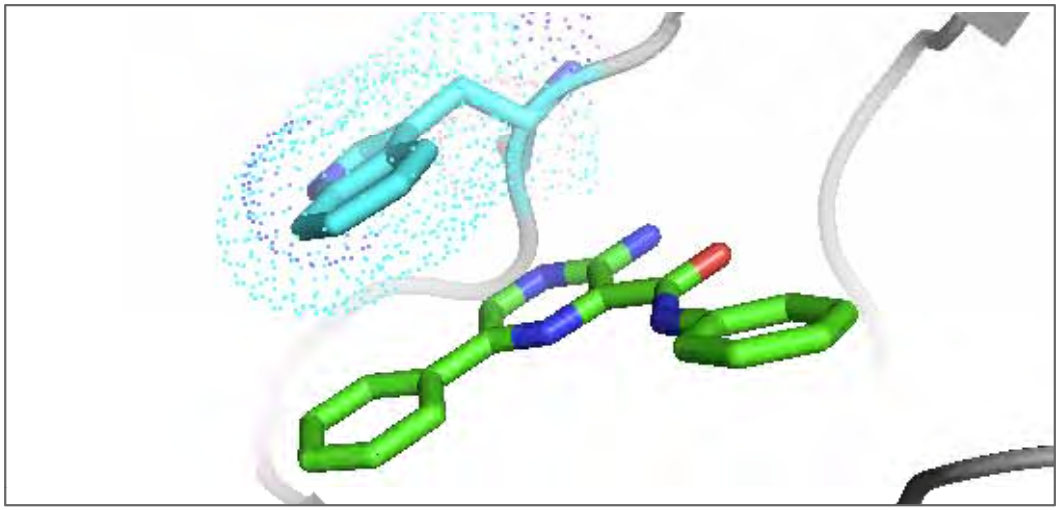
π-stack interaction with Trp2379



Compds	R	Enzyme inhibition K_i (μM)
		ATR
1		0.31
4		2.1





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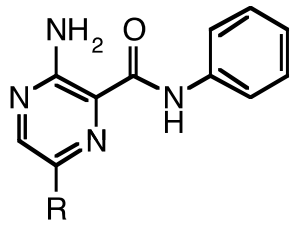
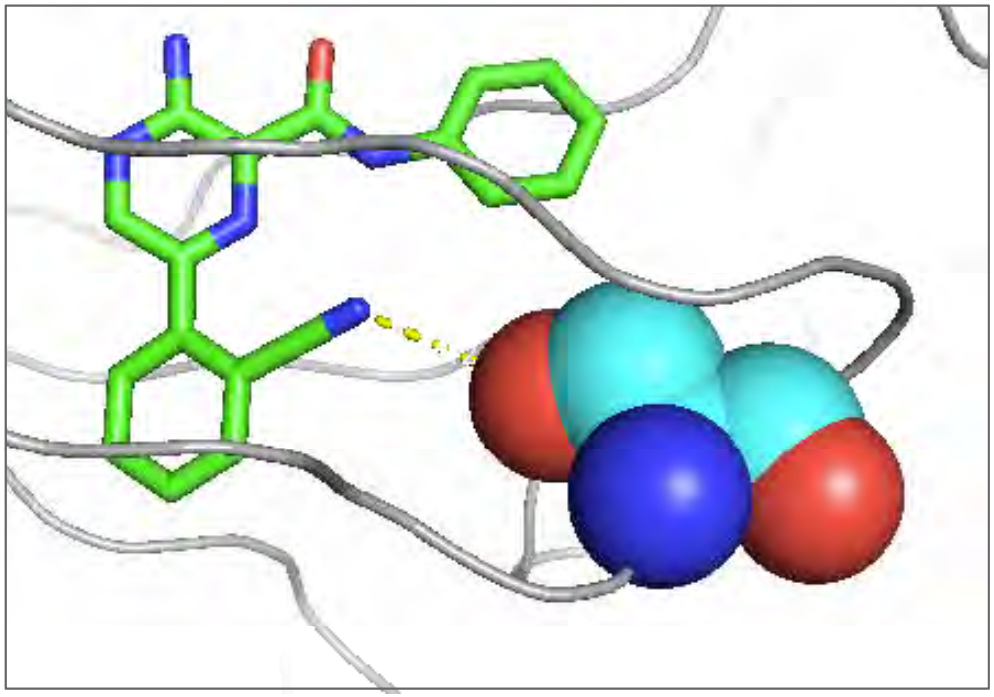
Polar interaction with Ser2305 from the *ortho*-position



Compds	R	ATR	ATM	DNAPK
1	-C_6H_5	0.31	>4	>4
5	C_6H_4 2 CN	0.012	0.32	0.72
—	C_6H_4 3 Me	0.28		



- ATM and DNA-PK have a similarly positioned polar residue
- Small meta substituents don't improve potency or selectivity

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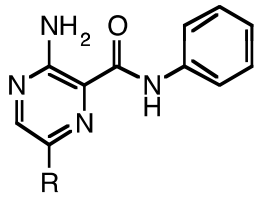
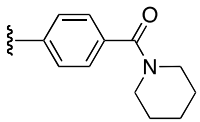
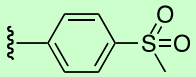
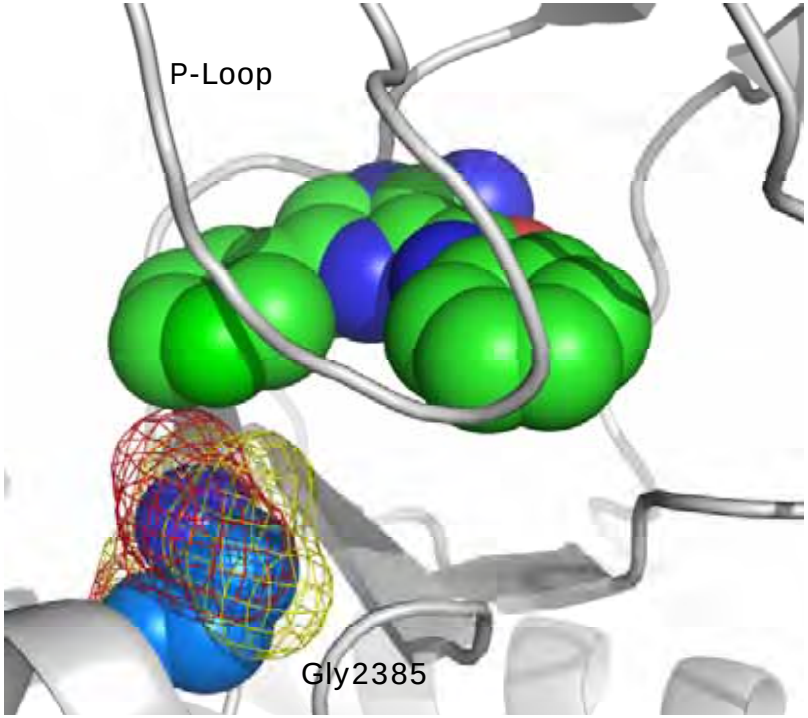
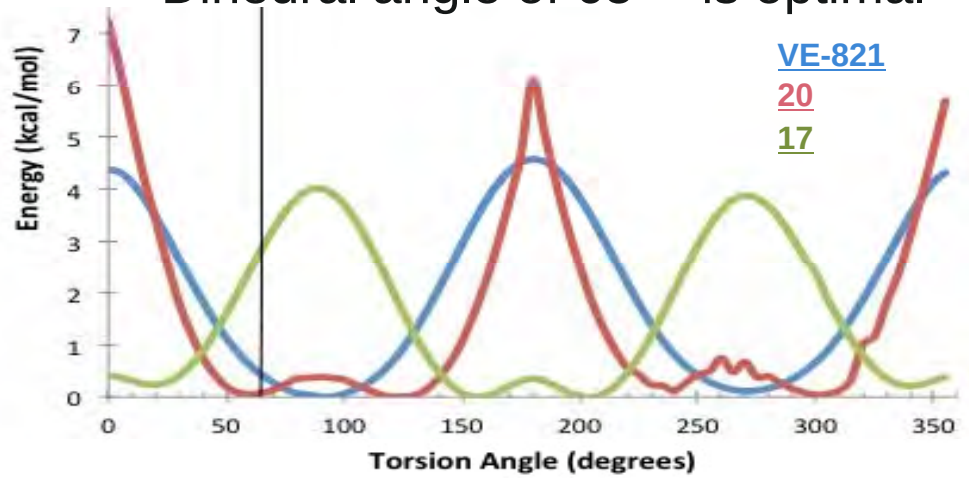
Interaction with ATR specific Gly2385 from out of plane *para*-substituents



Compds	R	Enzyme inhibition K_i (μM)		
		ATR	ATM	DNAPK
20		0.013	>4	>4
VE-821		0.013	>4	2.2



Dihedral angle of 65° is optimal



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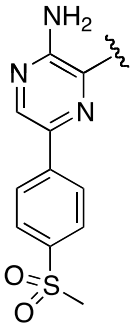
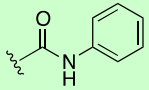
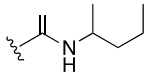
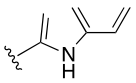
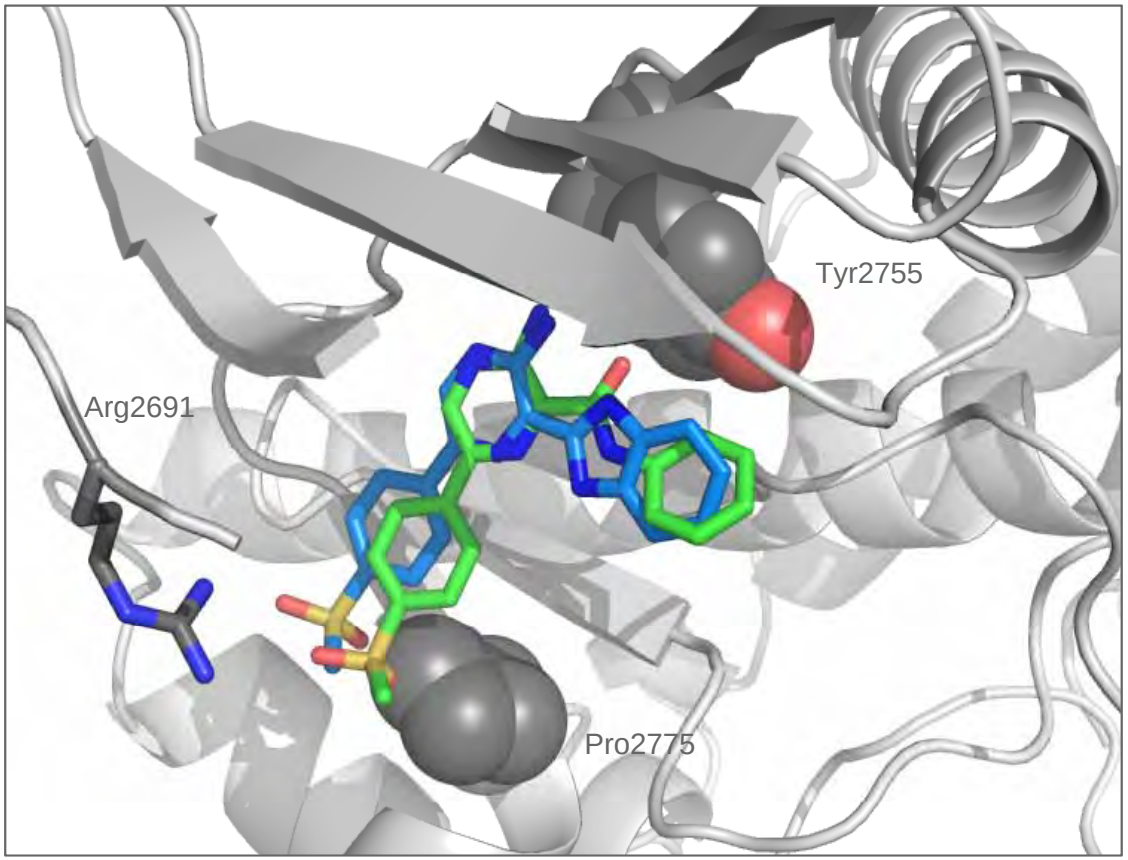
Alternatives to the anilide are tolerated but compromise selectivity



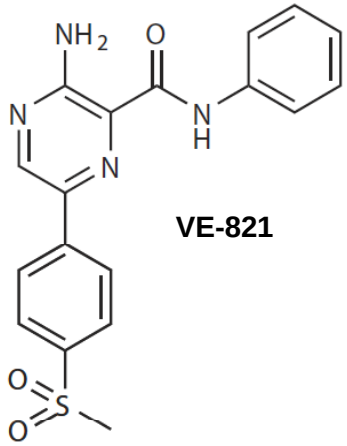
Compds	R	Enzyme inhibition K_i (μM)		
		ATR	ATM	DNAPK
VE-821		0.013	>4	2.2
25		1.8	>4	>4
				
29		0.012	0.06	1.2



- Amide functions primarily to position the aromatic group under the P-loop

J. Med. Chem. (2011) **54**, 2320

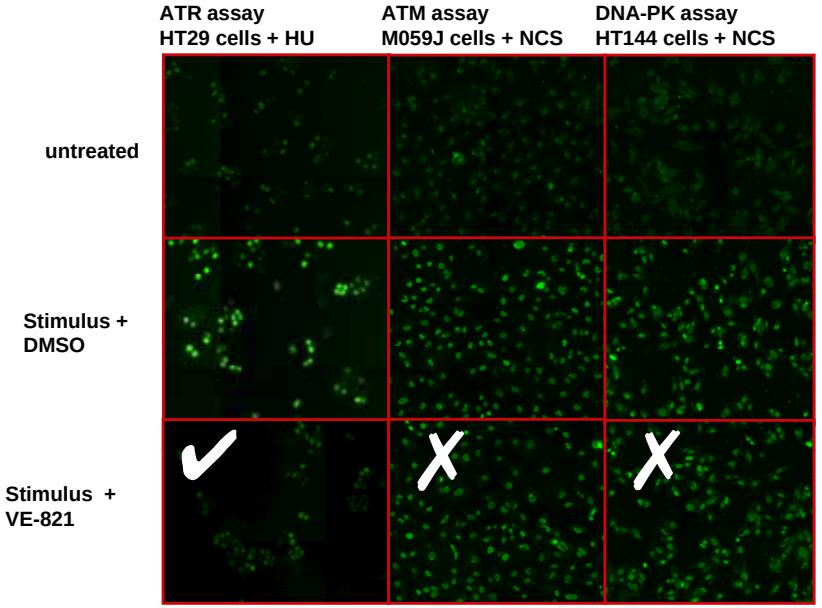
VE-821 a powerful tool to probe target biology



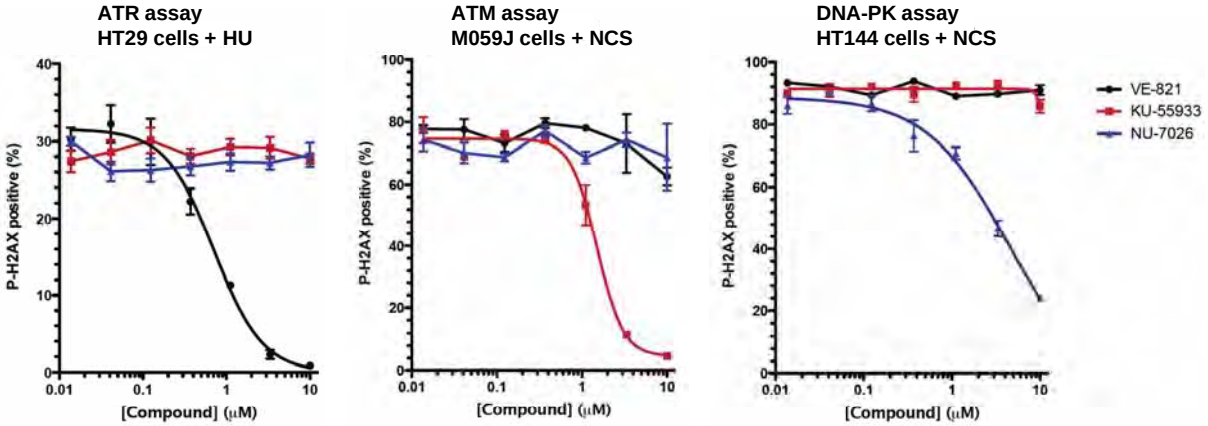
VE-821

Enzyme	Ki (μM)
ATR	0.013
ATM	16
DNA-PK	2.2
mTOR	>1
PI3Kγ	3.9

- >100-fold selectivity against 50 kinases



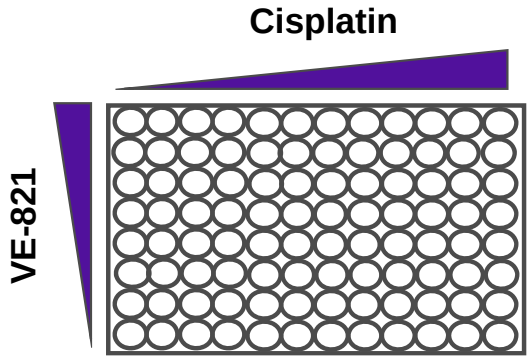
IF: P-H2AX (S139)



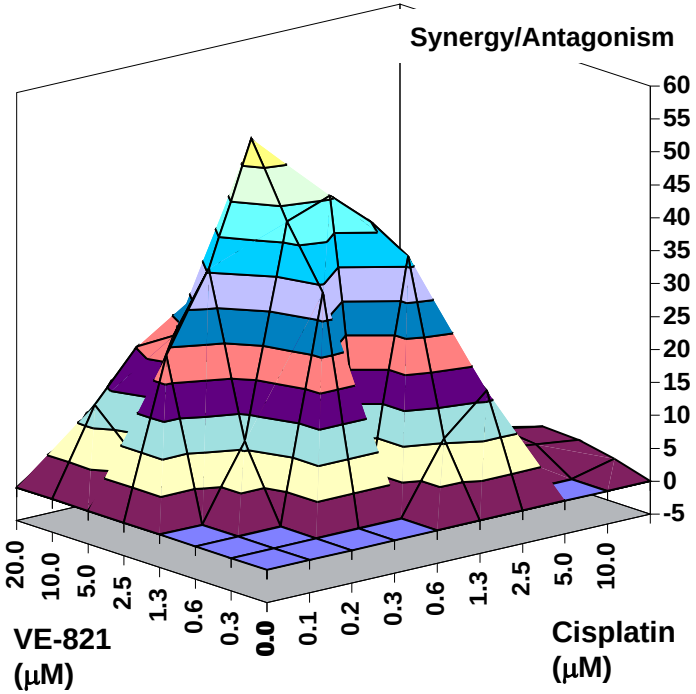
IC_{50} 0.8μM

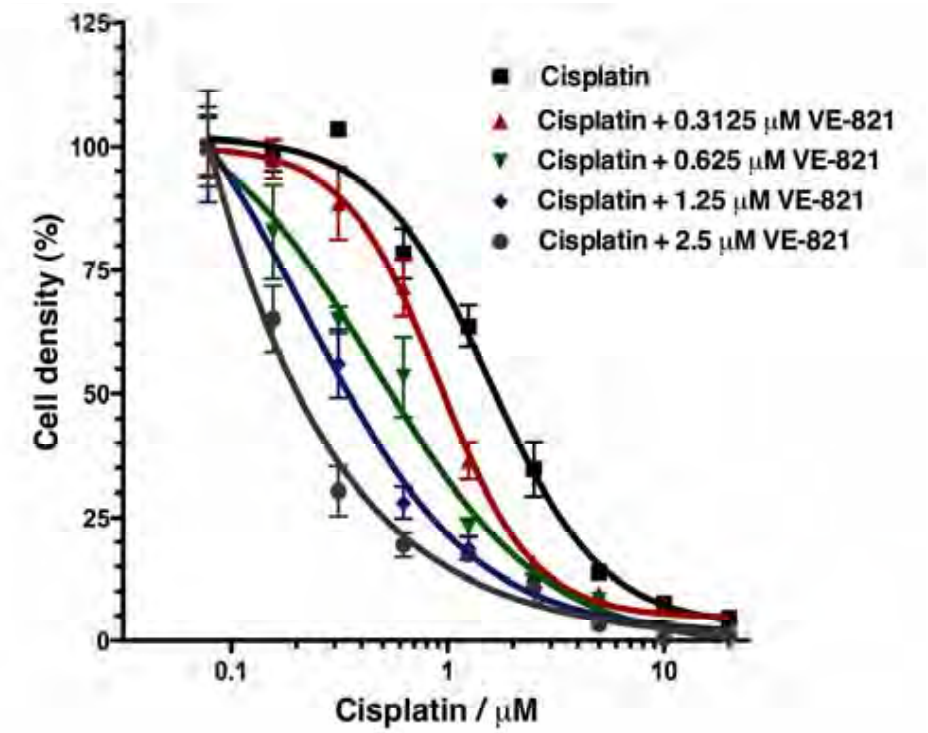
Nat. Chem. Biol. (2011) **7**, 428

ATR inhibition increases cytotoxicity of cisplatin



- Cisplatin IC50 shifts by >10-fold

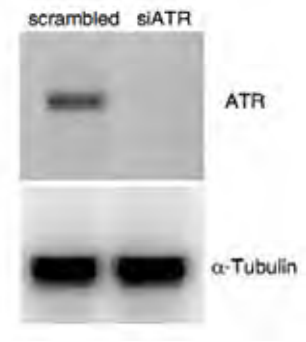


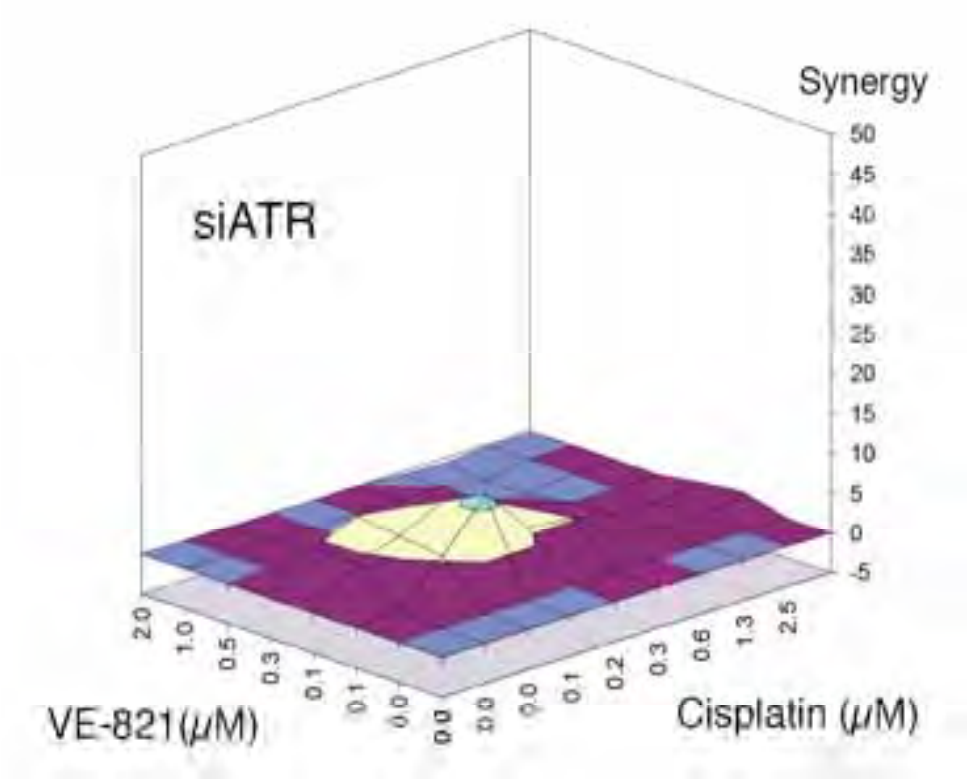


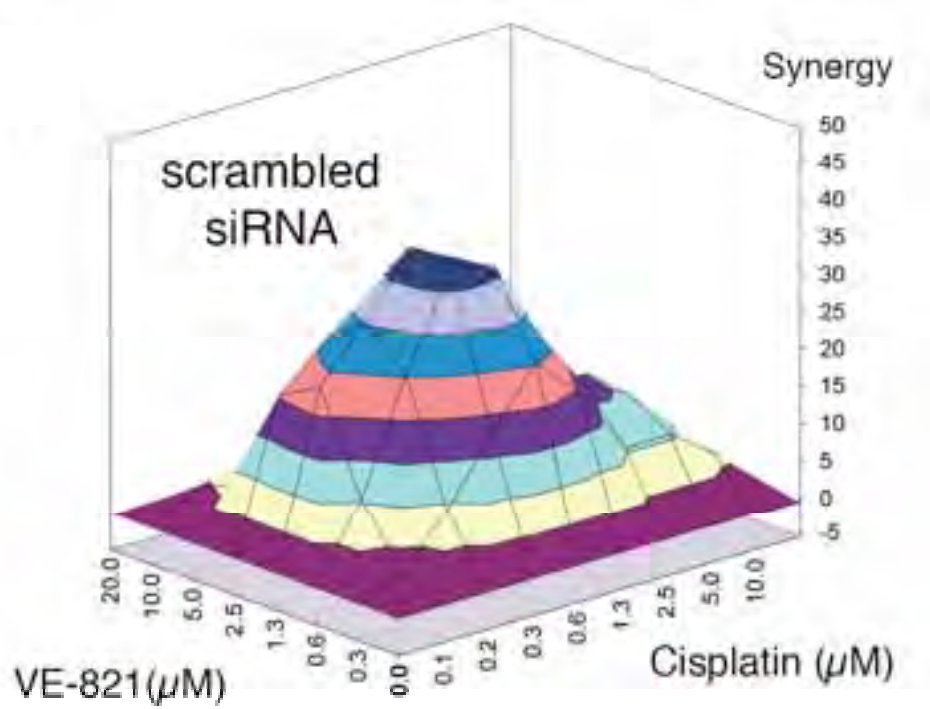
HCT116 cancer cells, MTS assay, 96h

Nat. Chem. Biol. (2011) **7**, 428

Phenotype is through inhibition of ATR

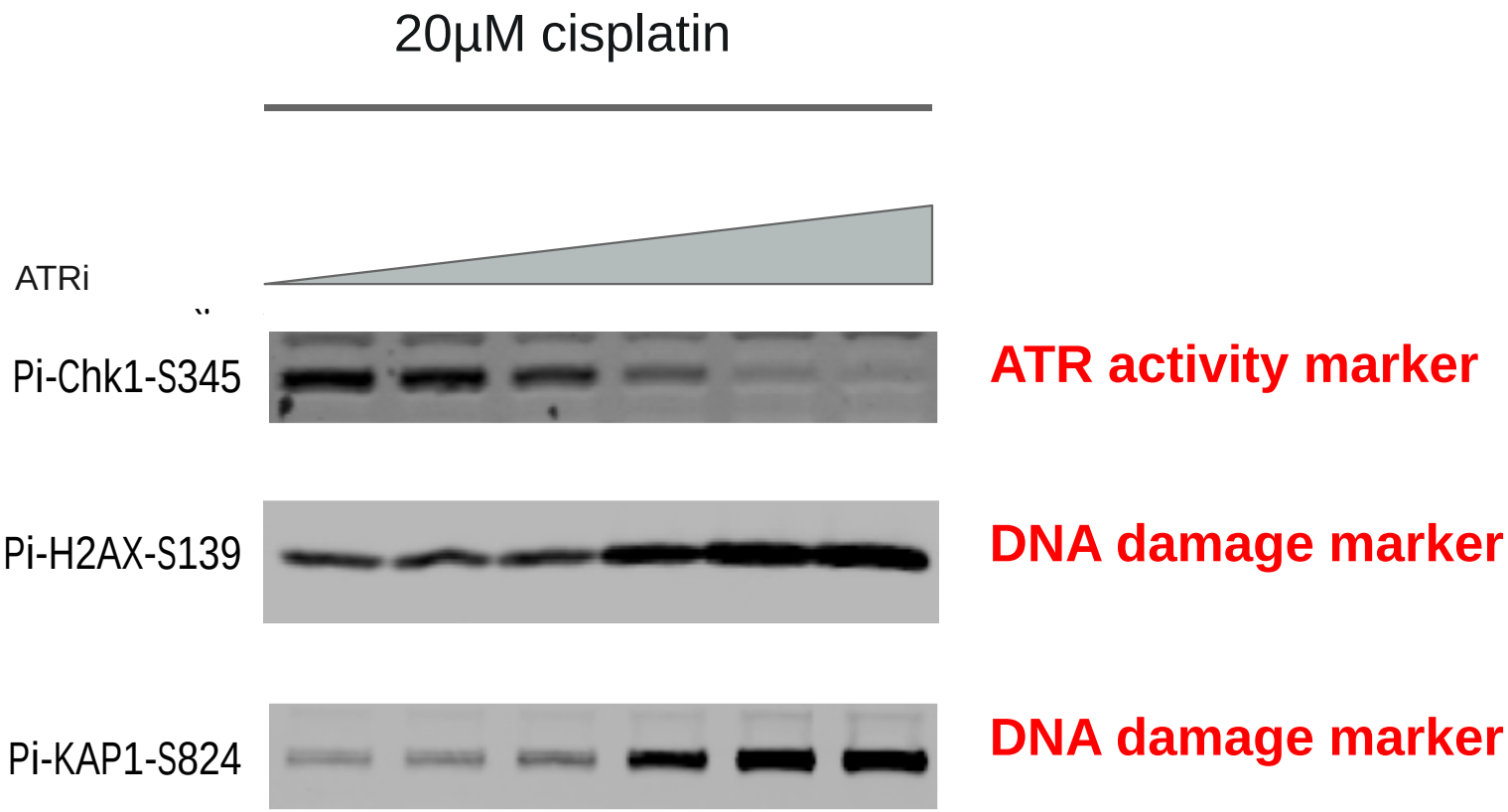






Nat. Chem. Biol. (2011) **7**, 428

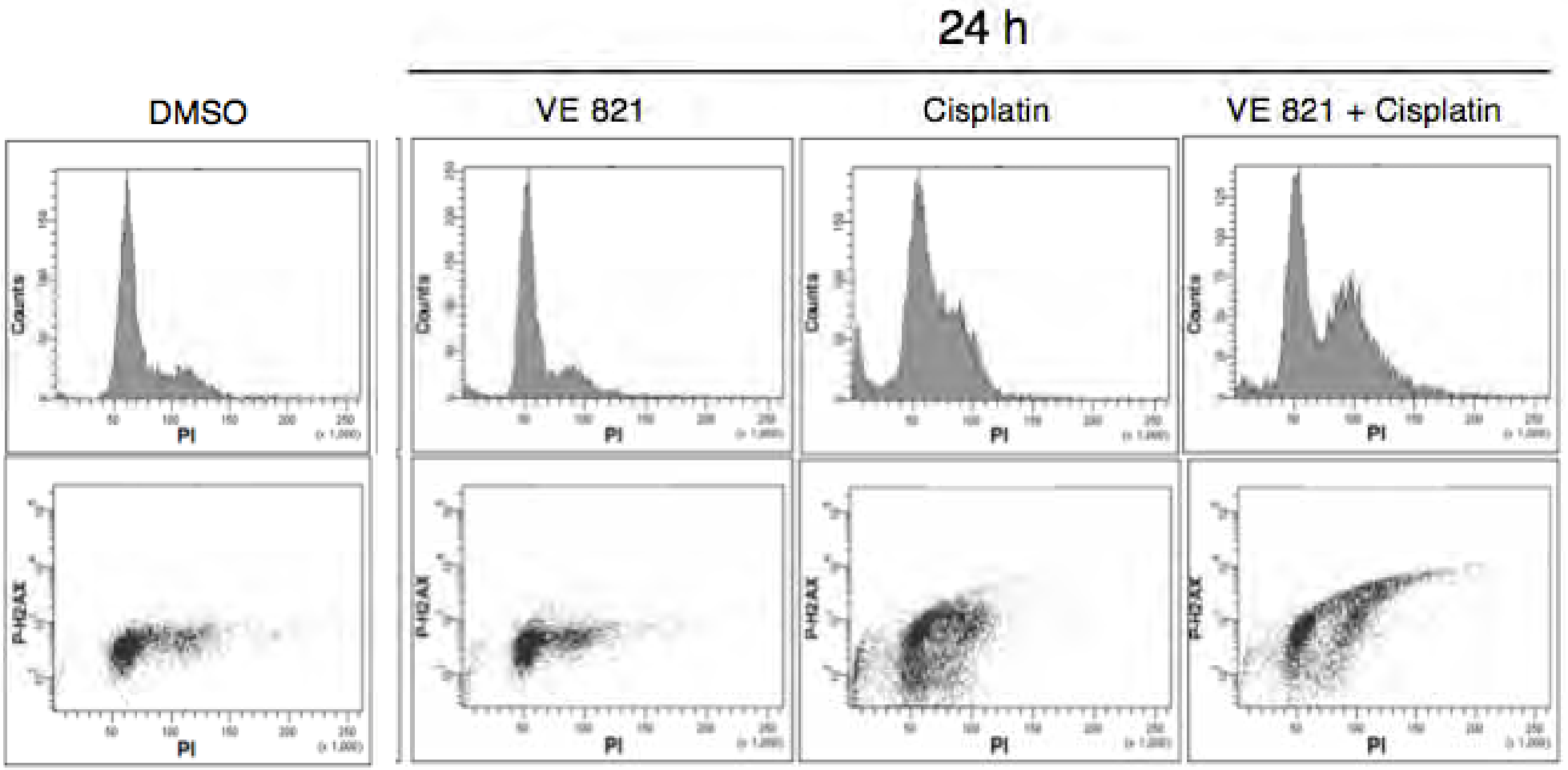
ATR inhibition enhances DNA damage



Cancer cells treated for 17h with cisplatin and ATRi

Nat. Chem. Biol. (2011) **7**, 428

Inhibition of ATR leads to S-phase checkpoint override, hyperdiploidy and elevated H2AX

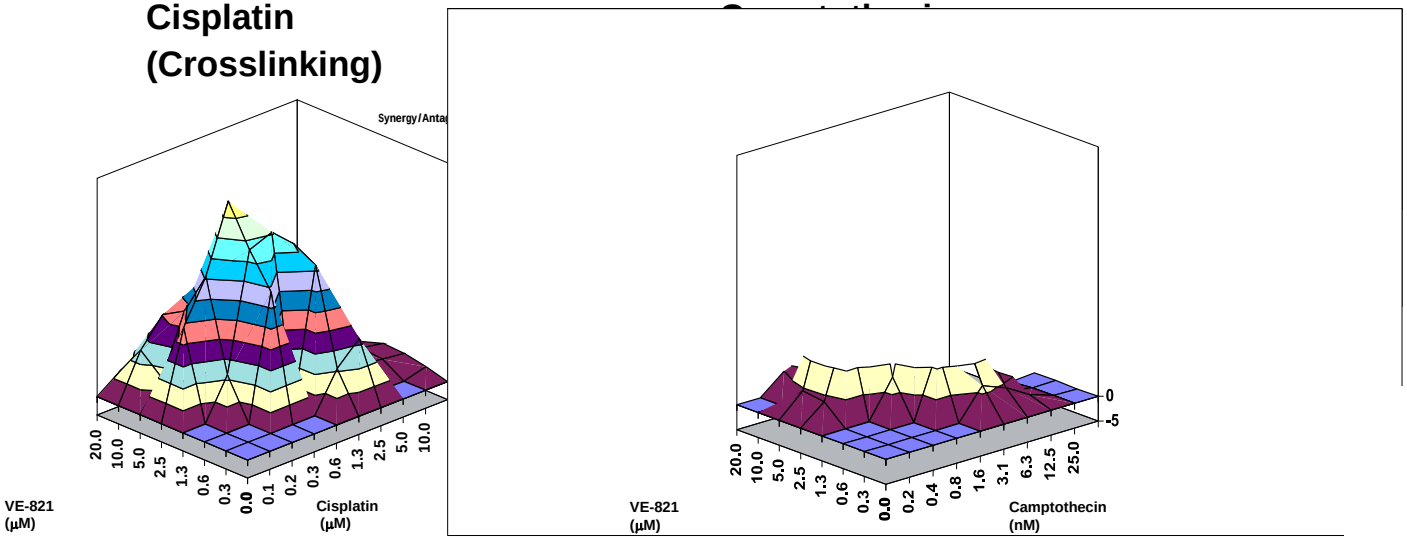


Hct116 cells treated with VE-821 / cisplatin for 24h. DNA content stained with PI and H2AX for phosphorylation at Ser139

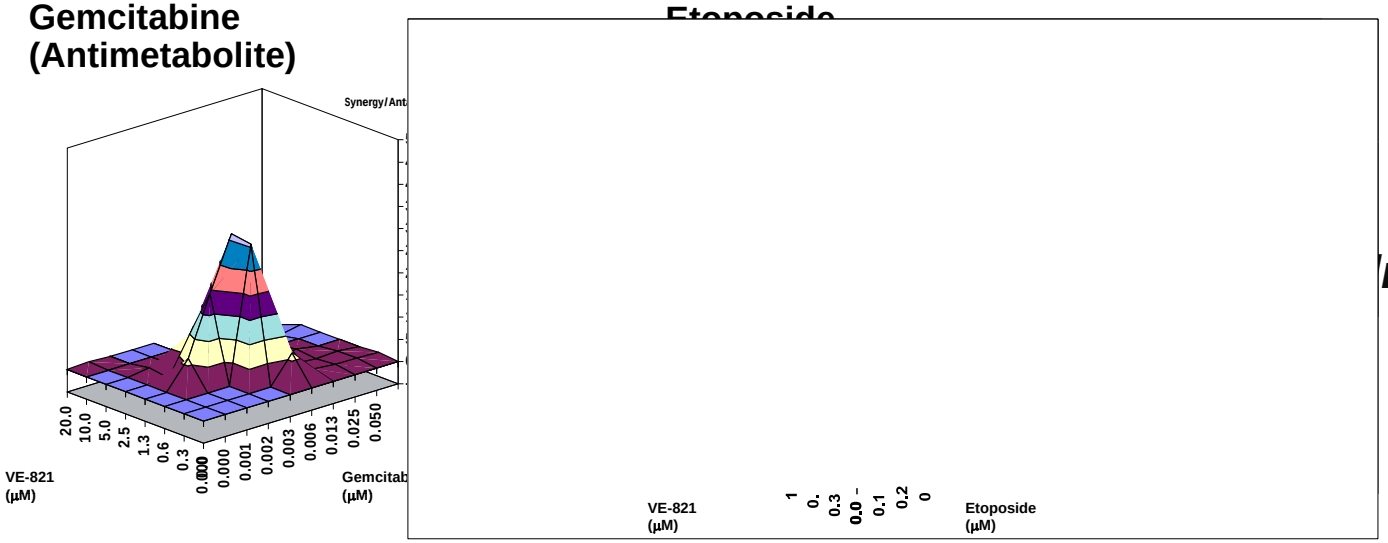
Nat. Chem. Biol. (2011) **7**, 428

ATR inhibition potentiates multiple classes of DNA damaging drug

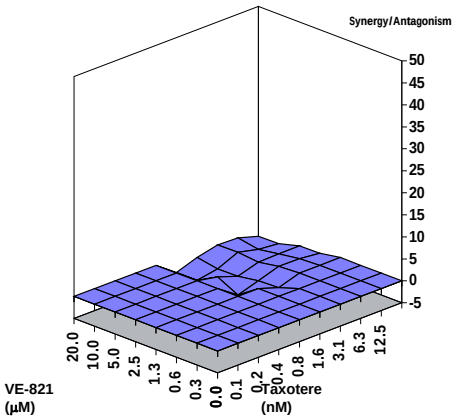
Cisplatin (Crosslinking)



Gemcitabine (Antimetabolite)



Taxotere (Antimitotic)



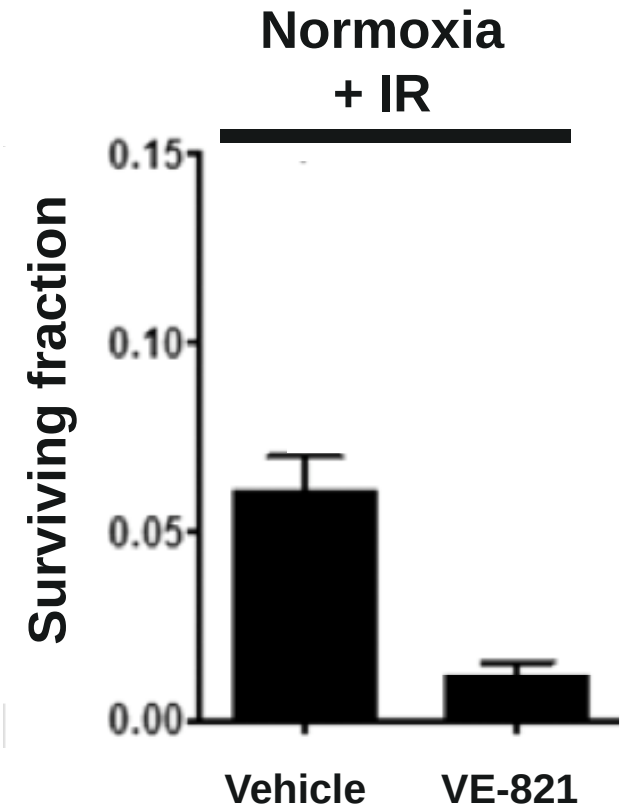
No impact with cytotoxic drugs that don't damage DNA

HCT116 cancer cells, MTS assay, 96h

Nat. Chem. Biol. (2011) **7**, 428

ATR inhibition potentiates IR

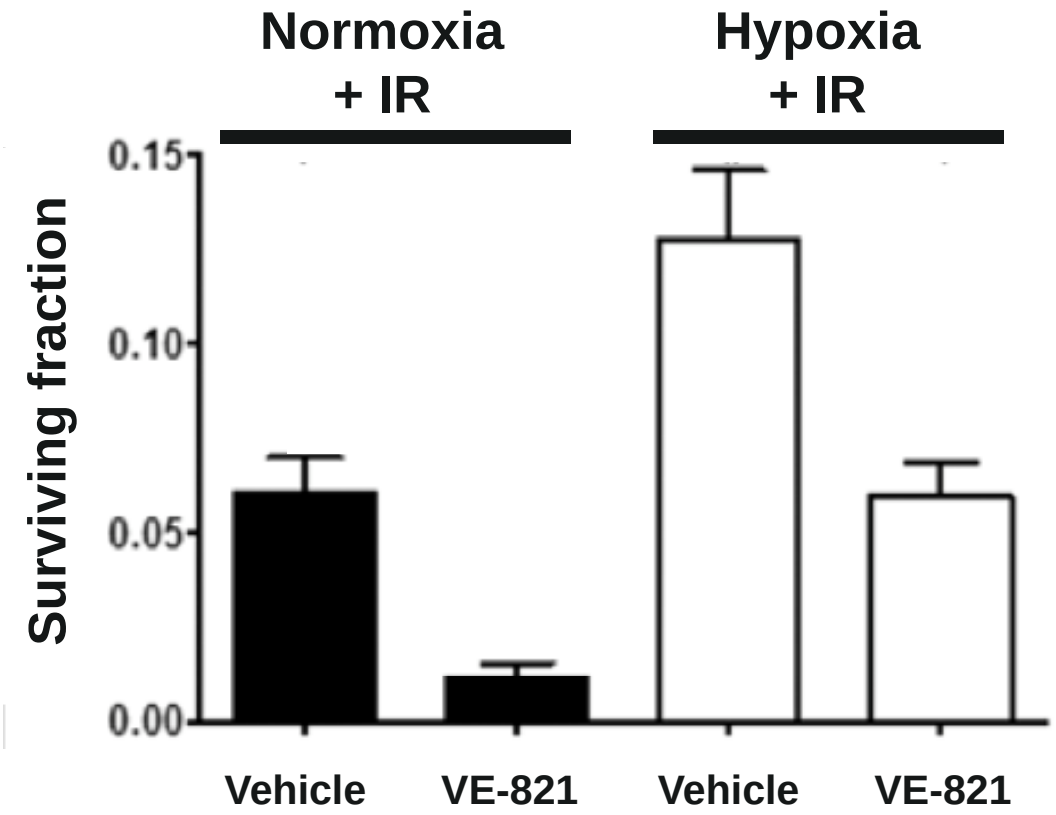
- MiaPaCa cancer cells treated with IR ± VE-821 in normoxic conditions (clonogenic assay)



Studies run at the Gray Institute Oxford University
Br J Cancer. (2012) **10**, 291

and reverses hypoxia radiation resistance

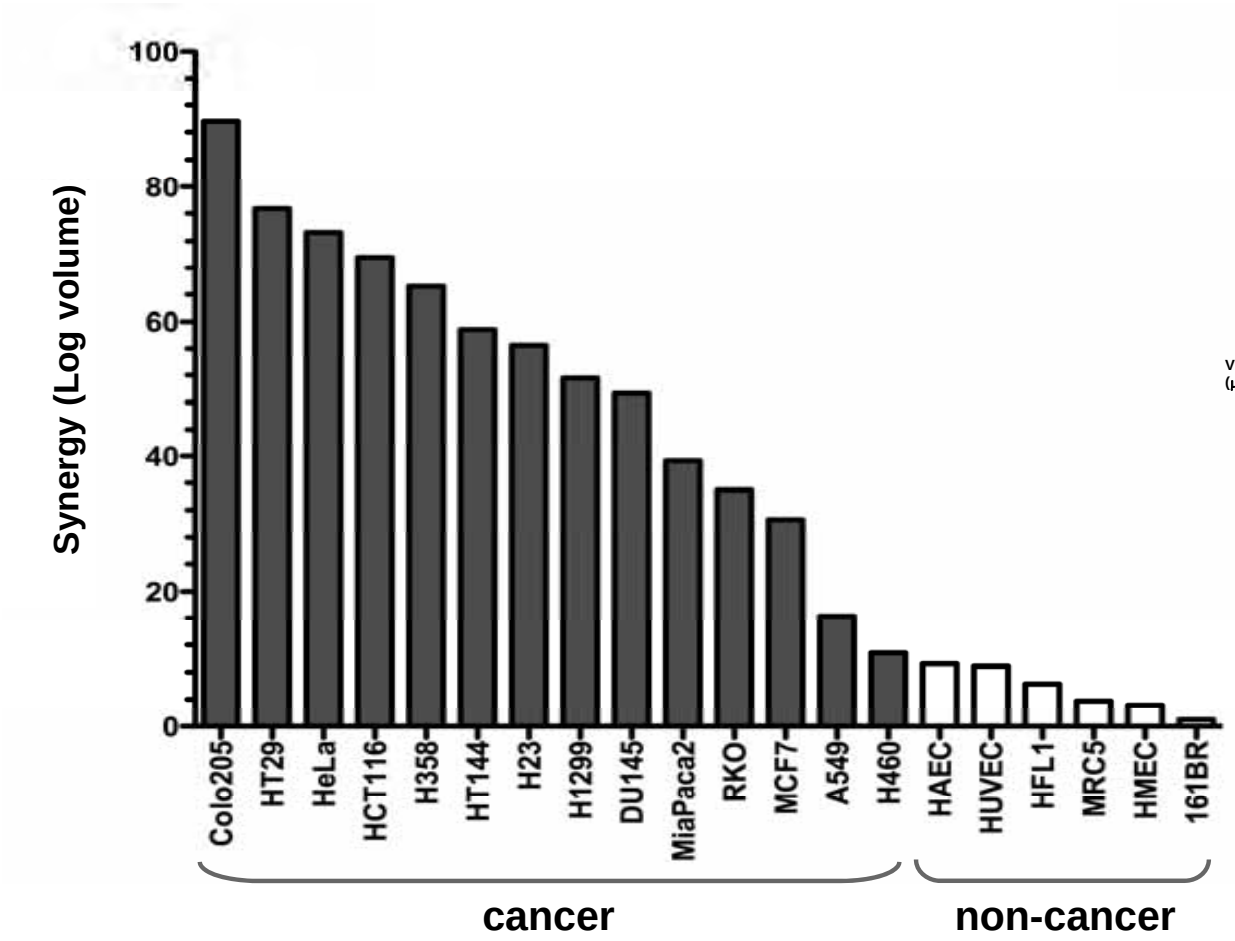
- MiaPaCa cancer cells treated with IR ± VE-821 in normoxic or hypoxic conditions (clonogenic assay)

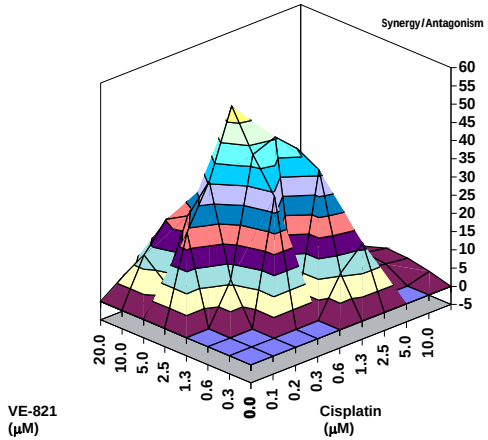


Studies run at the Gray Institute Oxford University
Br J Cancer. (2012) **10**, 291

Differential response between cancer and non-cancer cells

- Combination of VE-821 and cisplatin, volume under the synergy surface at 96h (95% confidence interval)

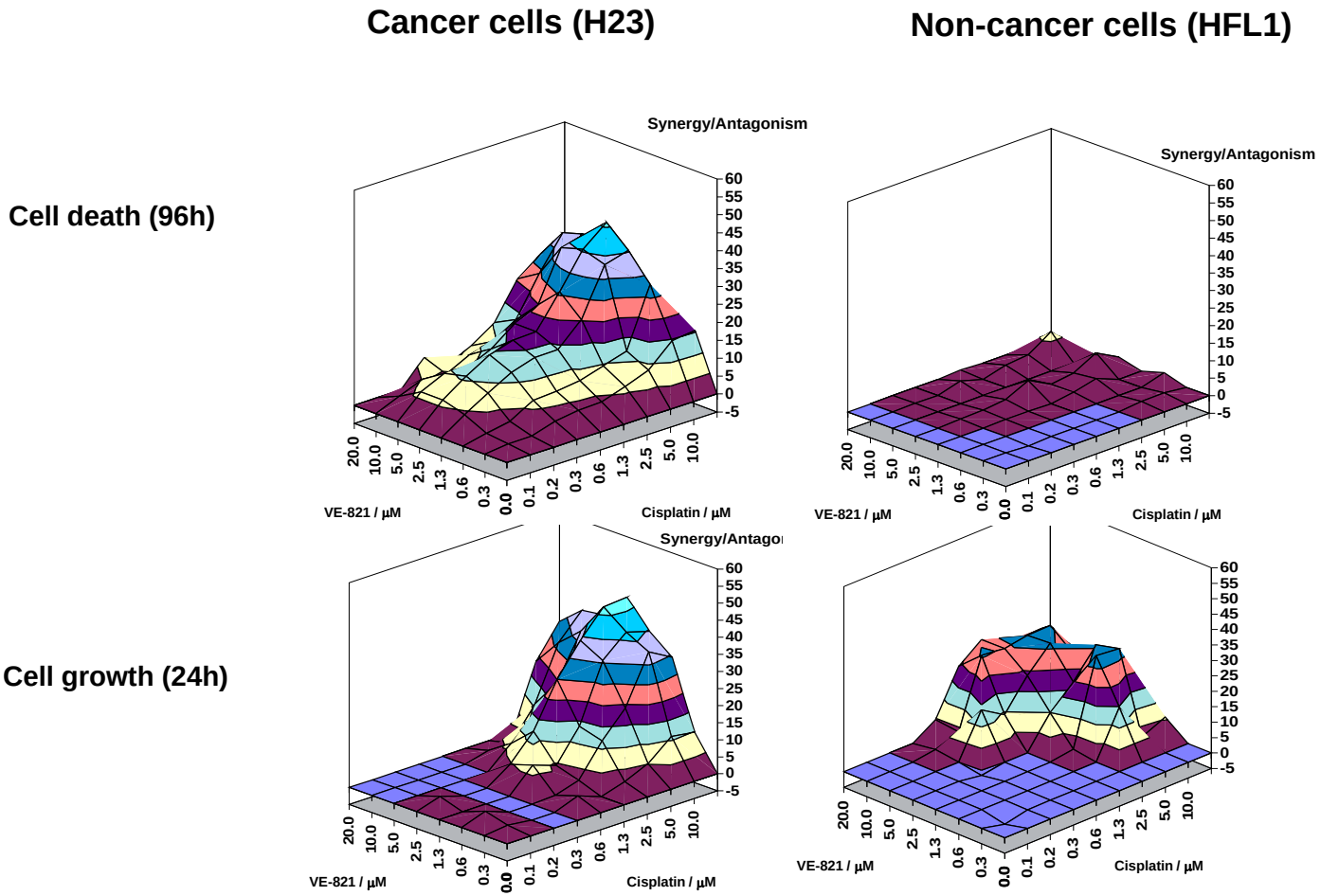




Nat. Chem. Biol. (2011) **7**, 428

Differential response between cancer and non-cancer cells

- Enhanced cytotoxicity in cancer cells but only cytostasis in non-cancer cells
  - Combination of VE-821 and cisplatin

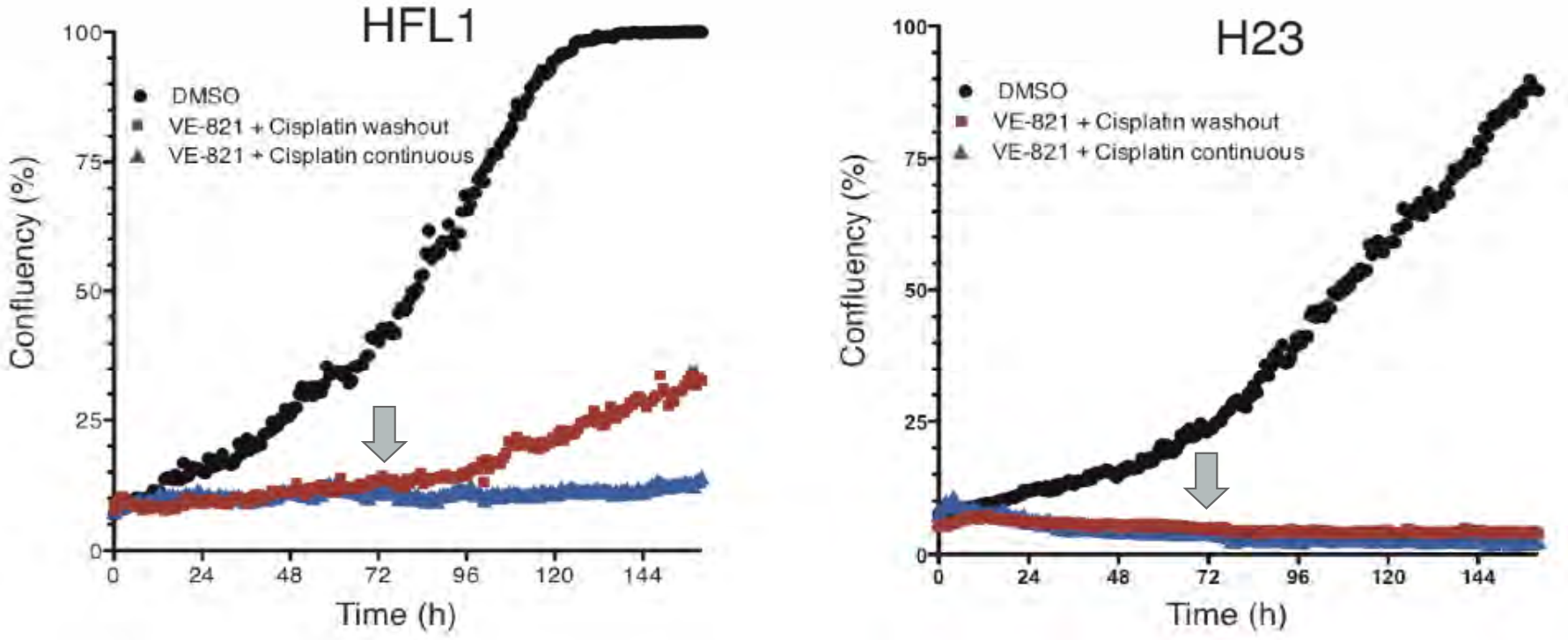


Nat. Chem. Biol. (2011) **7**, 428

Enhanced growth arrest in non-cancer cells is reversible

H23 / HFL1 cells → VE-821 + Cisplatin (72h) → VE-821 + Cisplatin (72h)

→ DMSO (72h)

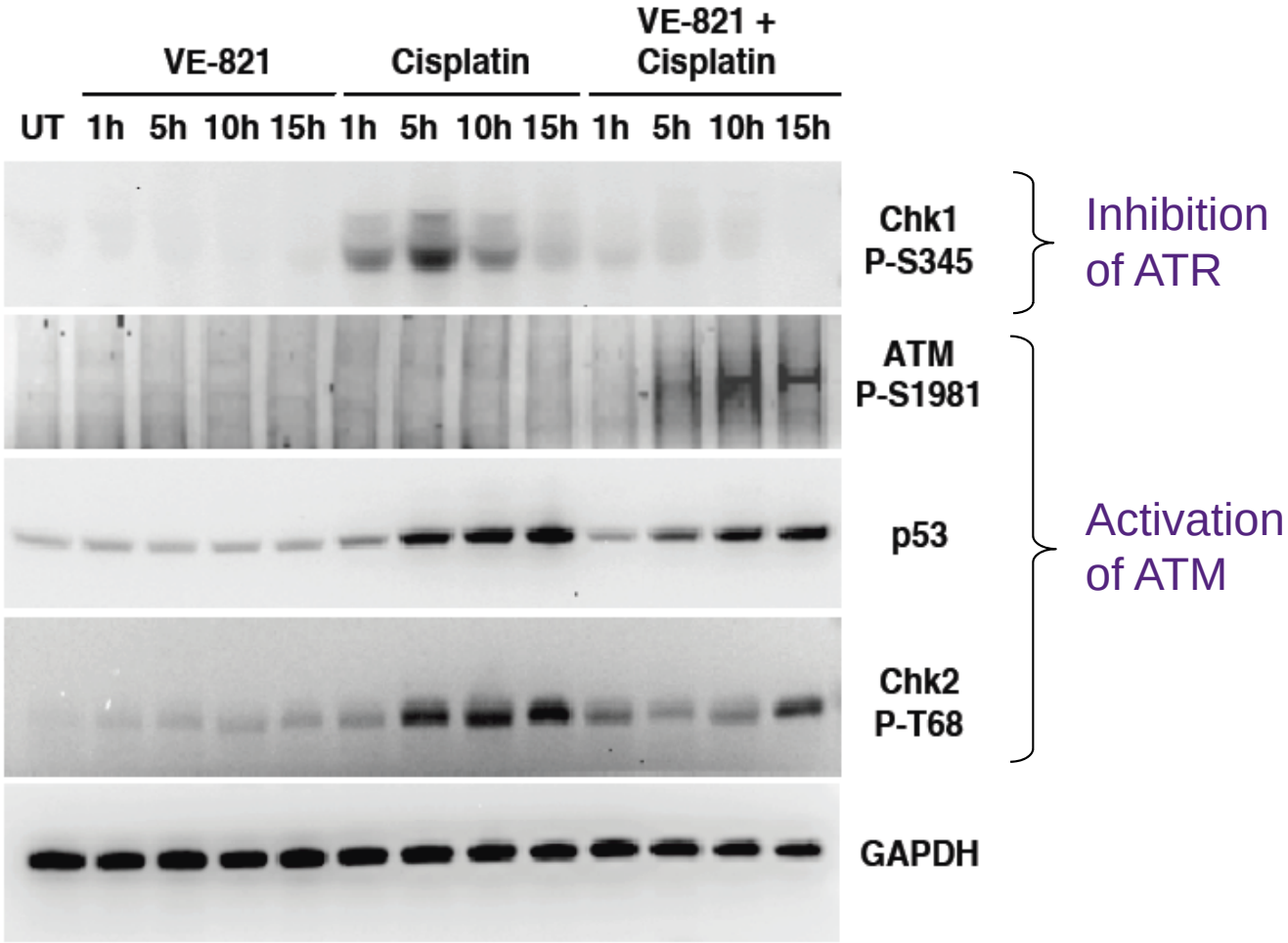


7.5μM VE-821, 1.25μM Cisplatin

Nat. Chem. Biol. (2011) **7**, 428

ATR inhibition is associated with a compensatory DDR in non-cancer cells

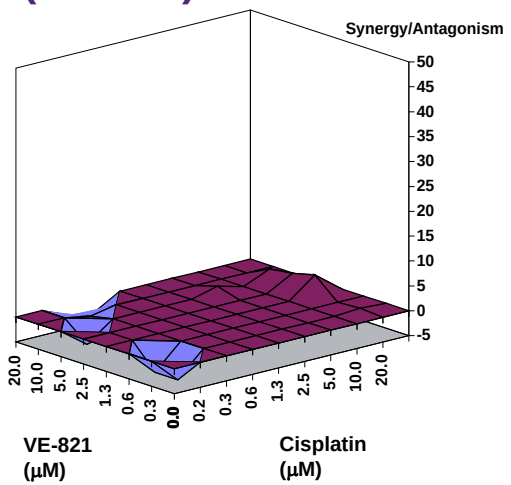
HFL1 normal fibroblasts
10μM VE-821,
100μM Cisplatin



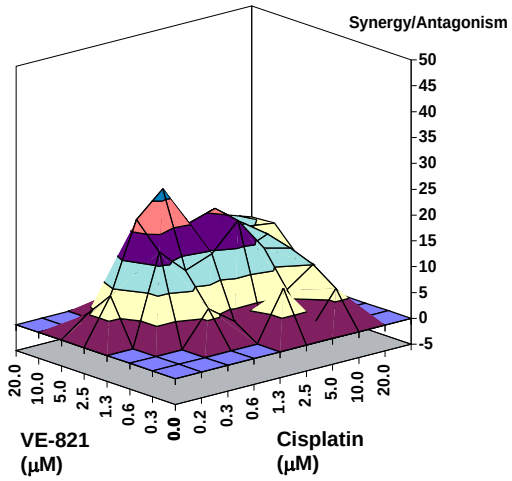
Nat. Chem. Biol. (2011) **7**, 428

Synthetic lethality with ATM pathway

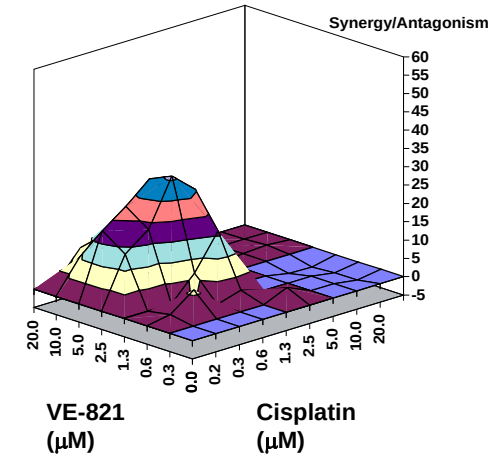
161BR (ATM wt)



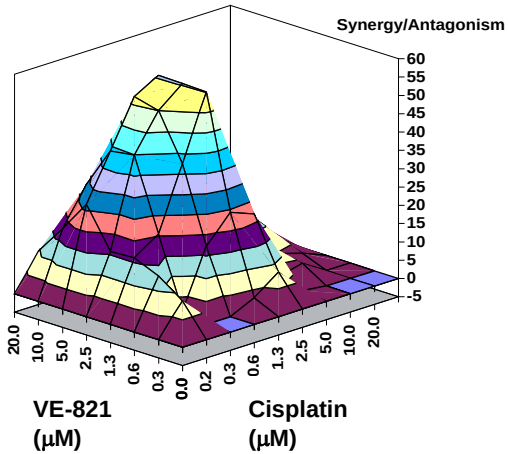
AT1BR (ATM null)



A549 (scrambled siRNA)



A549 (siRNA p53)

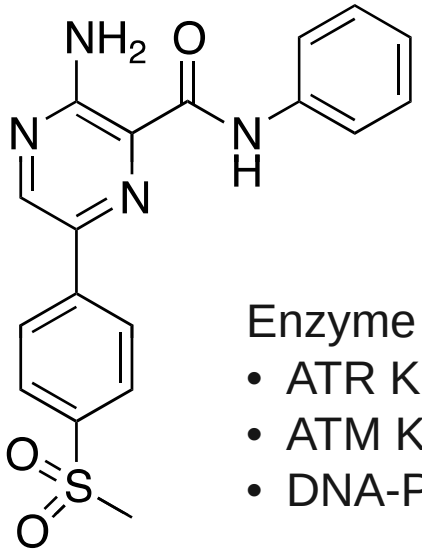


Nat. Chem. Biol. (2011) **7**, 428

From tool molecule to lead candidate

- Optimisation to improve potency and drug-like properties
  - Optimised interactions with the P-loop

VE-821

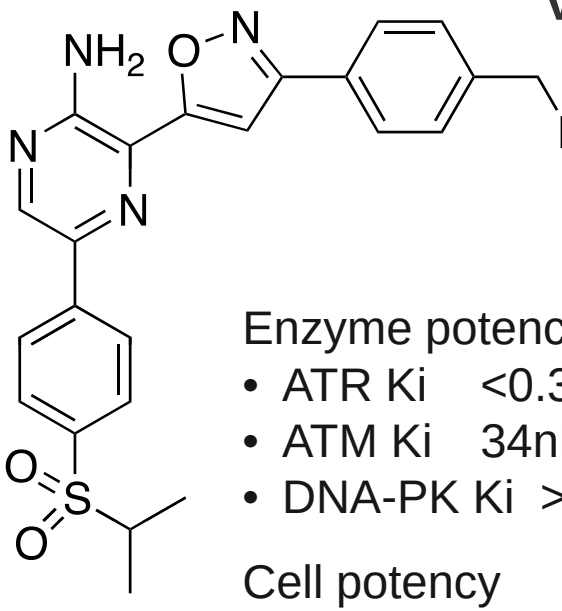


Enzyme potency and selectivity
- ATR Ki 13nM
- ATM Ki 16 000nM
- DNA-PK Ki 2200nM

Cell potency
- Biomarker IC_{50} 800nM

Solubility (pH7.4) <0.3 μM

VE-822



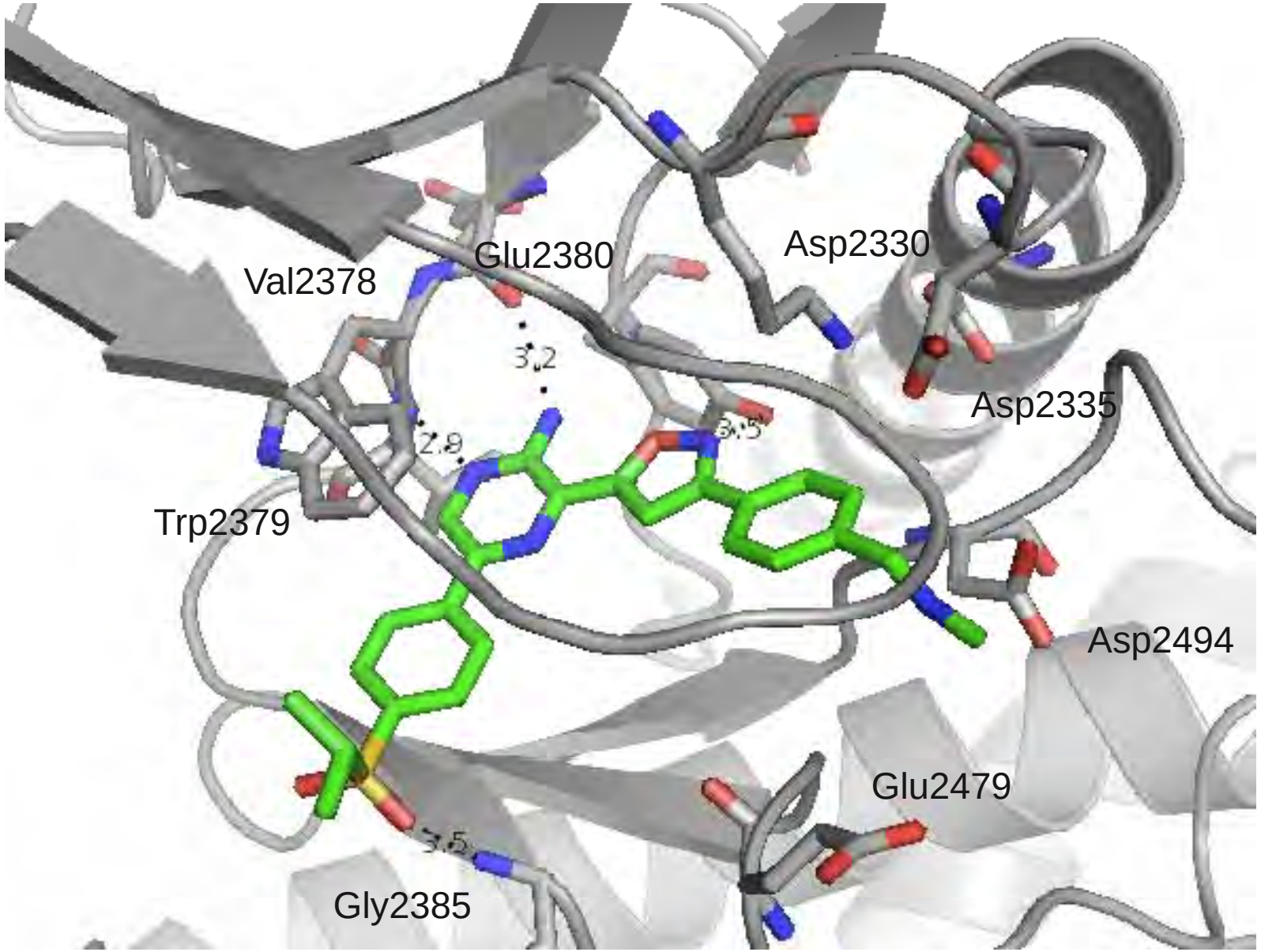
Enzyme potency and selectivity
- ATR Ki <0.3nM
- ATM Ki 34nM
- DNA-PK Ki >4000nM

Cell potency
- Biomarker IC_{50} 19nM

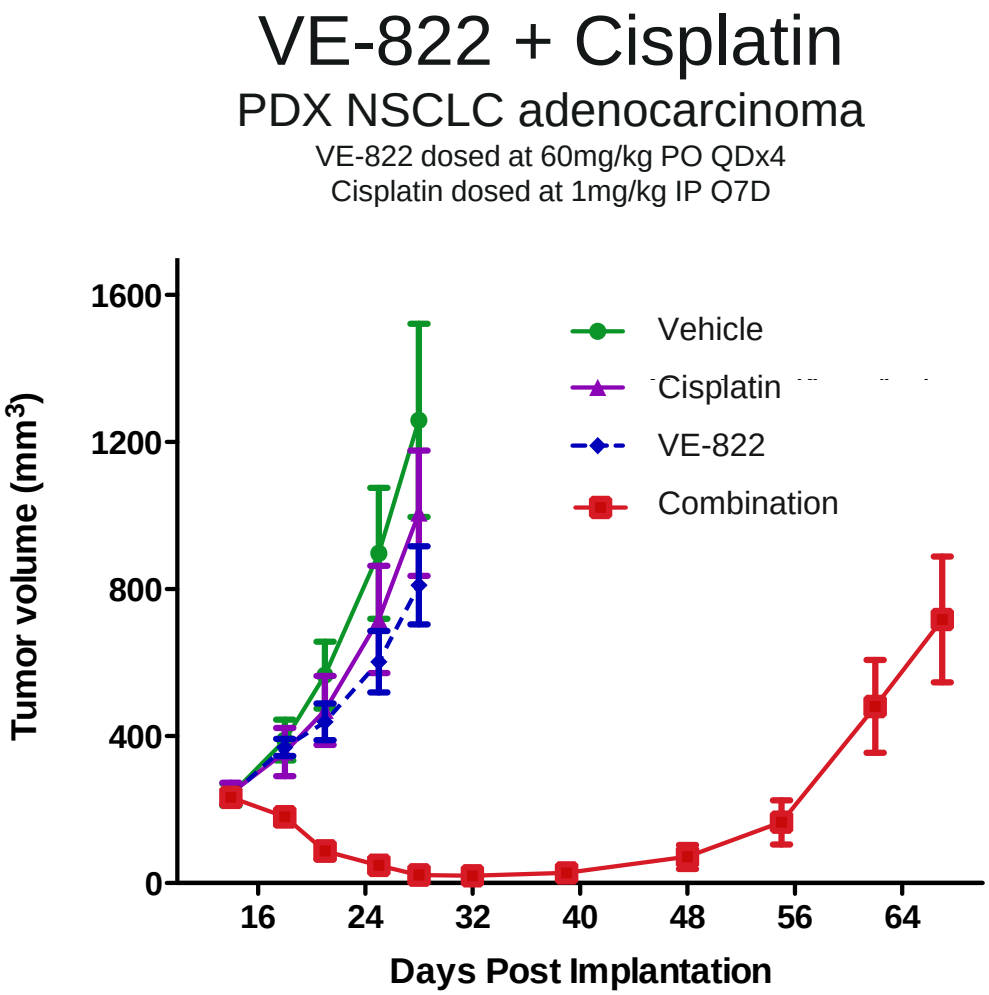
Solubility (pH7.4) 46 μM

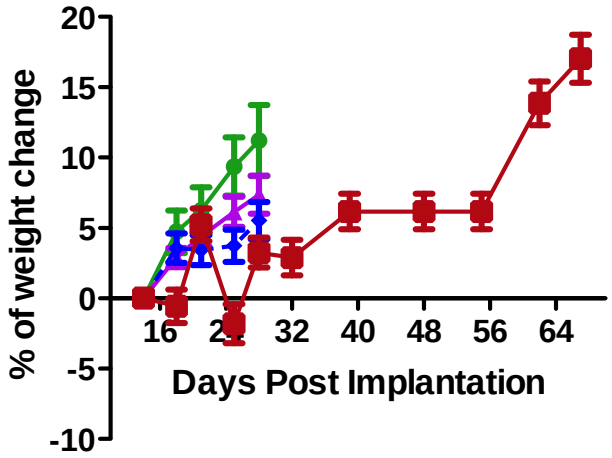
Cancer Res. (2013) **73**, LB299

VE-822 modeled in ATP binding site



VE-822 markedly improves tumor responses to cisplatin in PDX NSCLC model





Combinations well tolerated

Statistical synergy observed in 5/9 PDX NSCLC tumors

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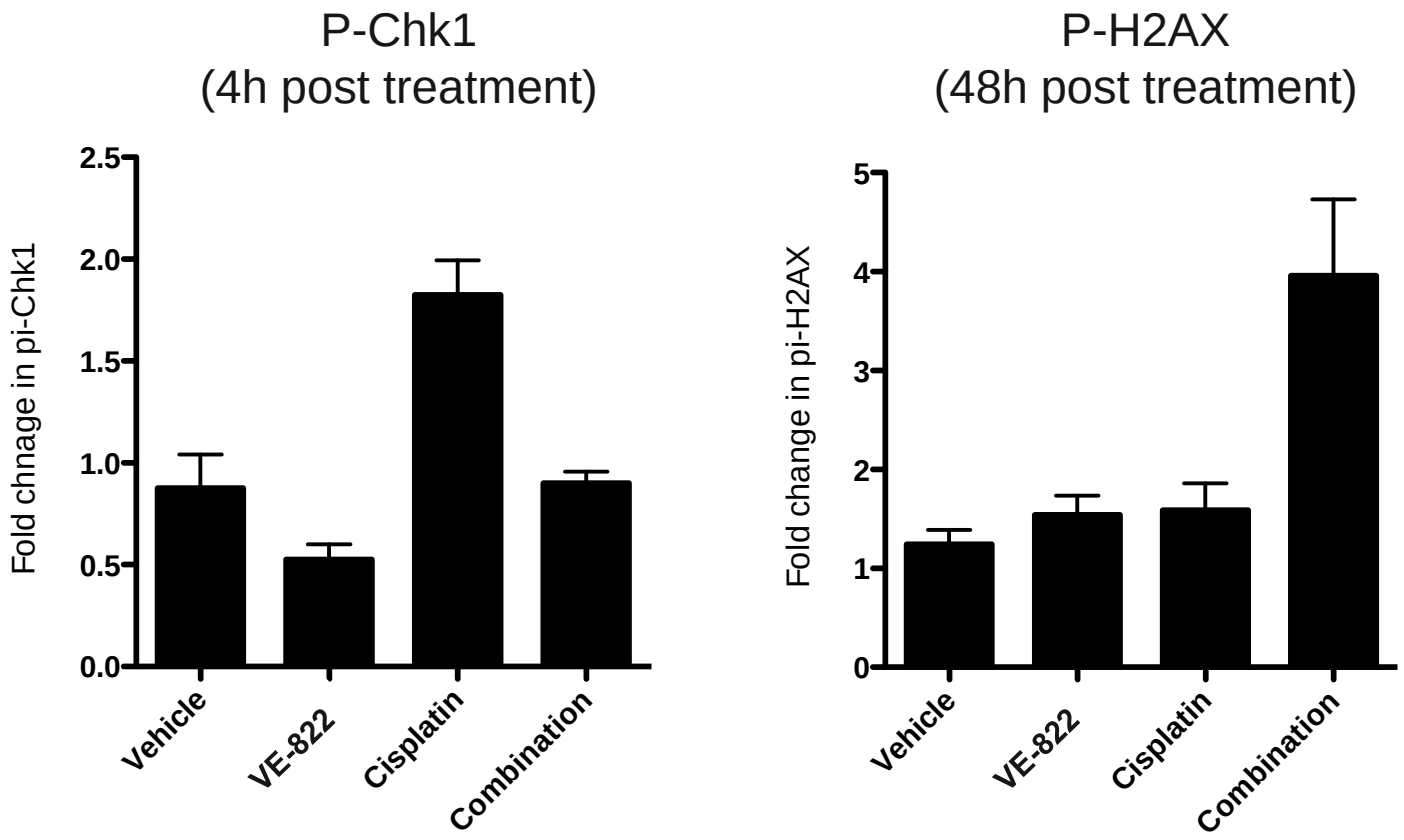
Efficacy correlates with biomarker responses

- Mice treated with single dose of VE-822 + cisplatin and tumors assessed for:
  - Inhibition of ATR by P-Chk1 at an early time point
  - Accumulation of DNA-damage by P-H2AX at late time point



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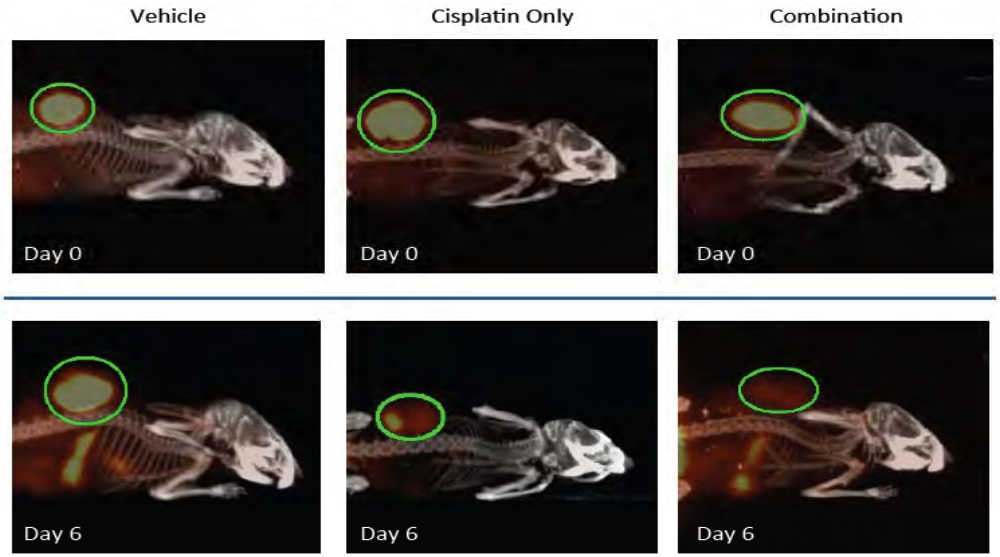
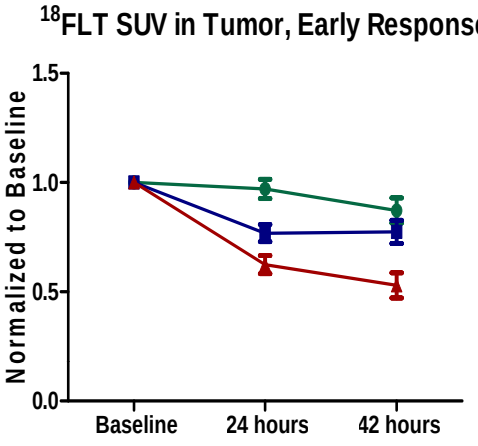
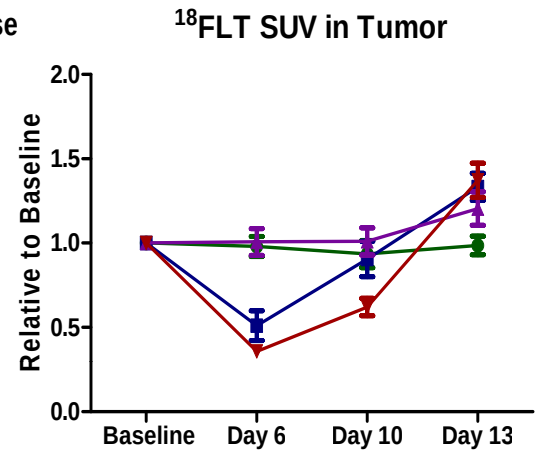
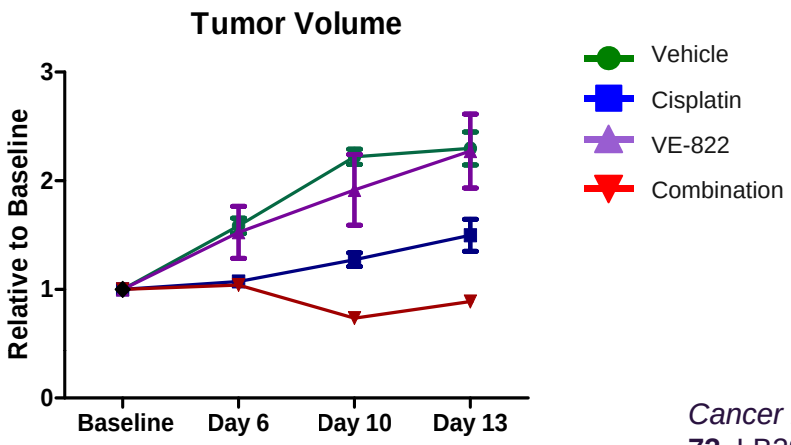
^{18}FLT-PET a potential early marker for response

- Mice treated with single dose of VE-822 and cisplatin









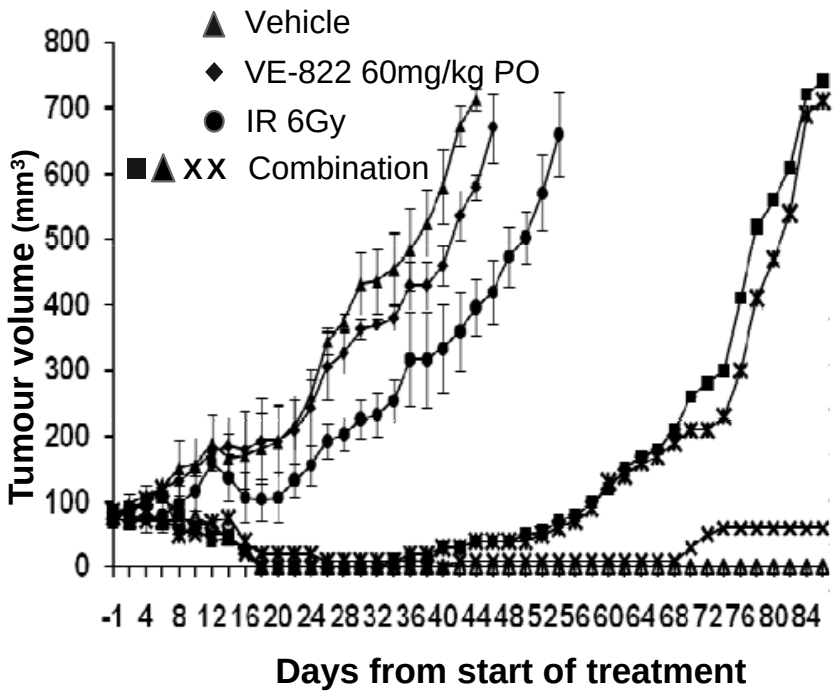
Cancer Res. (2013) **73**, LB299

VE-822 markedly improves responses to IR

MiaPaCa-2 cell line

IR dosed at 6Gy once on day 1

VE-822 dosed orally at 60mg/kg on days -1 through 3 or 5



Studies run at the Gray Institute Oxford University

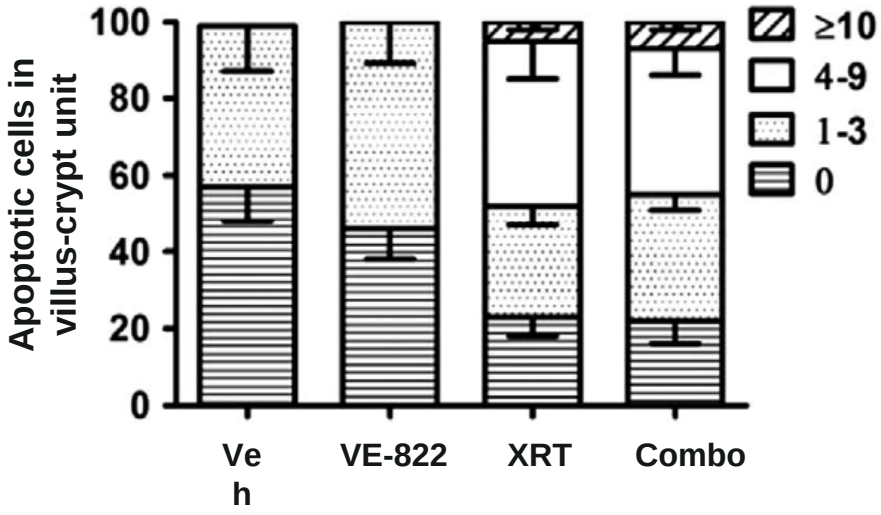
Cell Death Dis. (2012 Dec)

Combination well tolerated with no significant body weight loss

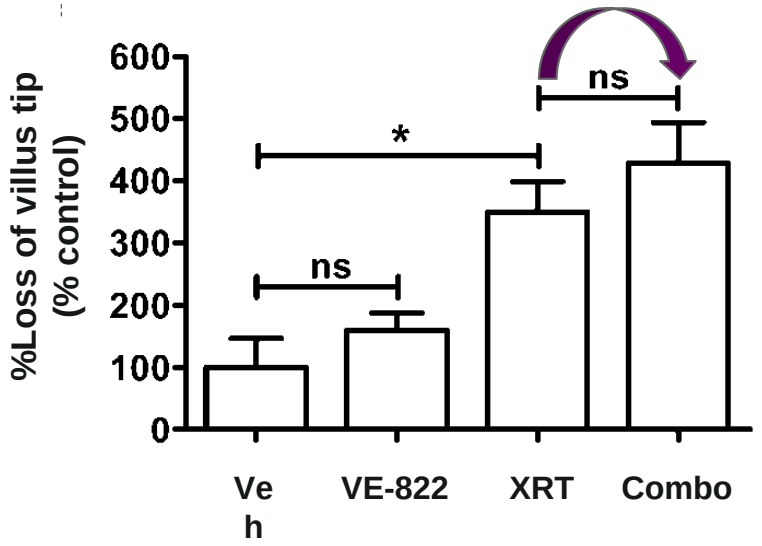
VE-822 does not enhance non-cancer cell tox

- Mice treated with single IR dose (6Gy) ± 3 doses of VE-822 (PO 60mg/kg Q2D)
  - IR beam directed through the gut
  - Animals assessed on day 5
  - Combination showed beneficial efficacy

No enhanced apoptosis



No added effect on villi length



Studies run at the Gray Institute Oxford University
Cell Death Dis. (2012 Dec)

Summary

- ATR is a key mediator for the cellular response to replication stress
  - Induced by many cancer drugs and IR
- Many cancers appear to be addicted to ATR for survival
  - Defects in alternative repair pathways
  - Expression of replication stress inducing oncogenes
  - Hypoxia
- Med-chem program led to identification of VE-822, the lead candidate
  - Potentiates the anti-tumor activity of many DNA damaging drugs and IR in mouse models of cancer at well tolerated doses
    - Normal cells tolerate inhibition of ATR with just a transient growth arrest
  - Loss of function of the compensatory ATM-p53 pathway is an important contributor to cell sensitivity

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- Project leader
  - John Pollard
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  - Yong Gu
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  - Tanya Hay
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  - Ester Hammond
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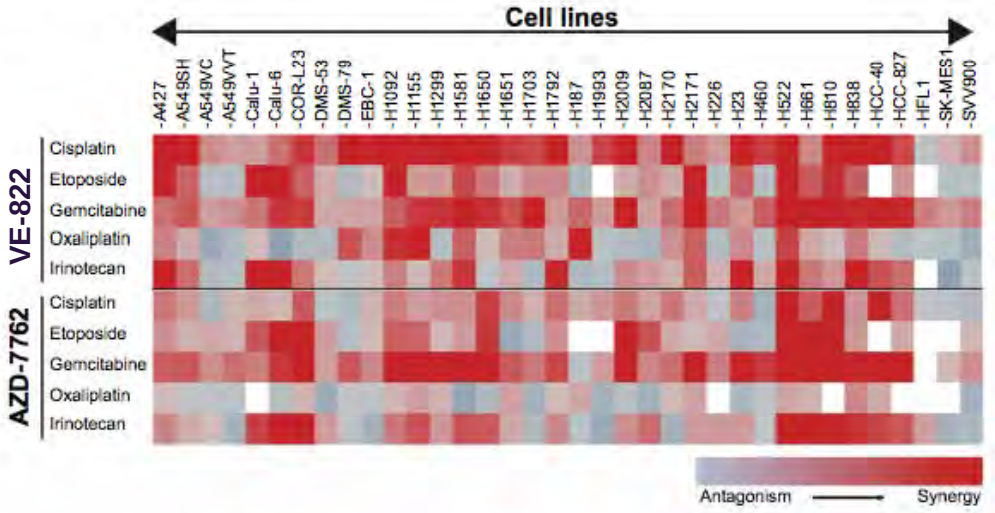
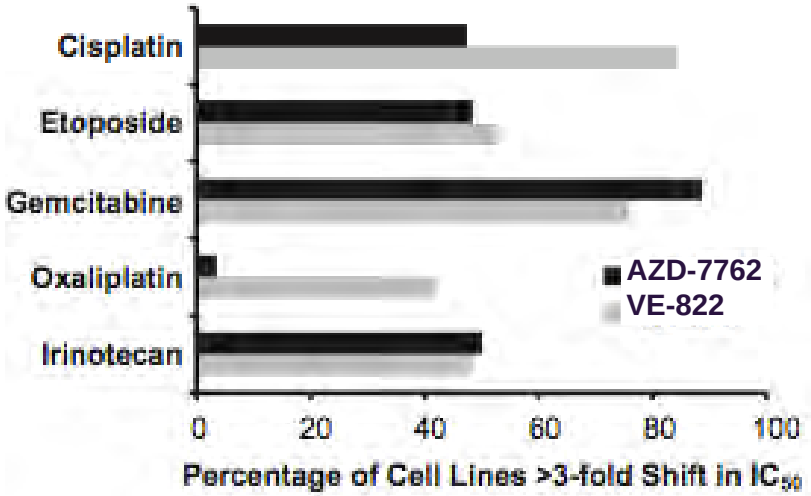
Differences in biological profile between ATR and Chk inhibition

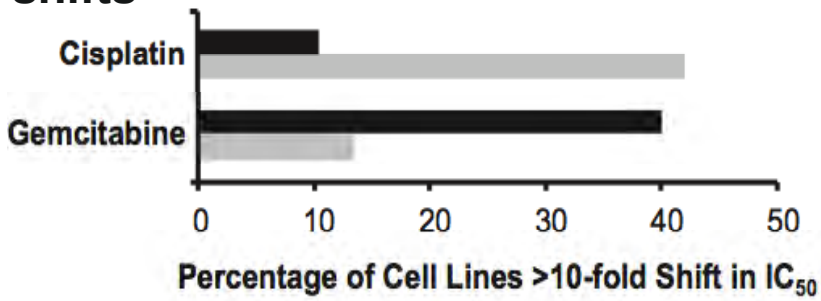


- Panel of lung lines treated with combinations of DDRi and varied DNA-damaging drugs for 96h (viability assessed by MTS)
- Data shows as maximum shifts in IC_{50} value for the DNA damaging drug on addition of DDRi

Percent of lines with >3-fold shifts



Percent of lines with >10-fold shifts

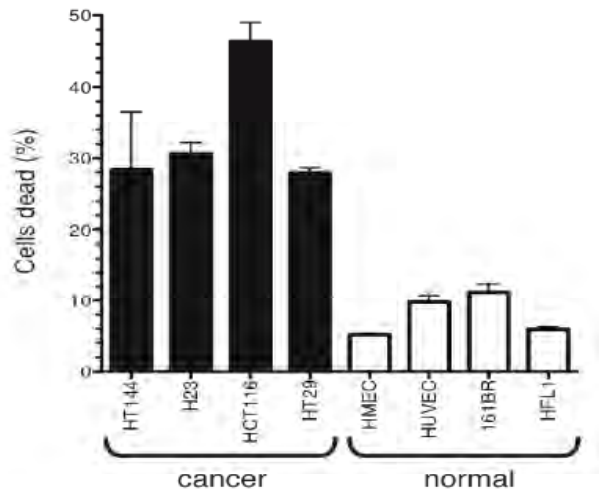


Cancer Res. (2013) **73**, LB299

Potential as a monotherapy in certain populations

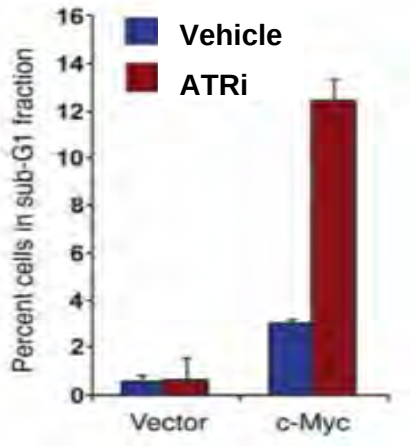
- Tumors with high replicative stress
  - E.g. oncogenic stress, hypoxia, DNA-repair defects
- Tumors with defects in compensatory DDR pathways

ATRi toxic to some cancer but not non-cancer cells



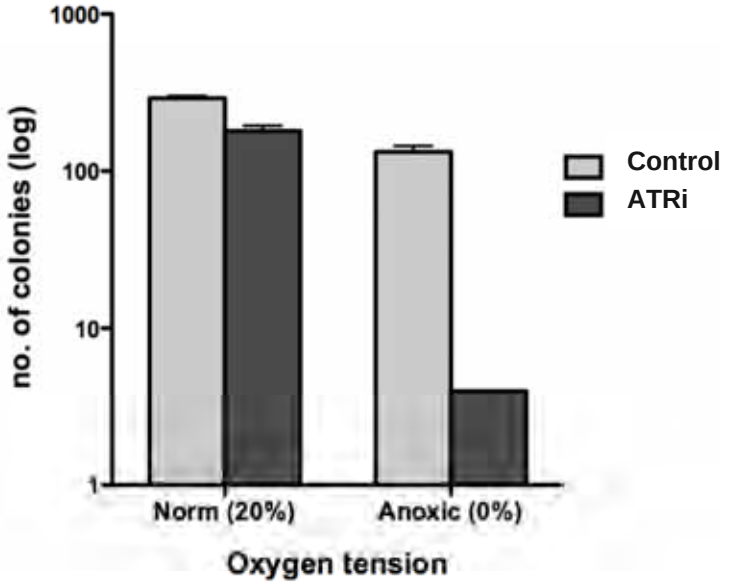
Nat. Chem. Biol. (2011) **7**, 428

Myc drives cell sensitivity to ATRi



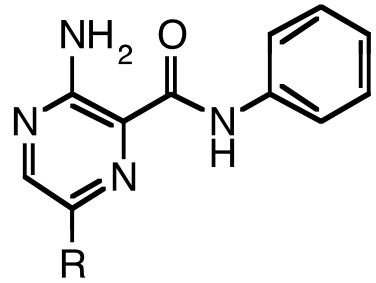
J. Clin. Inv. (2012) **122**, 241

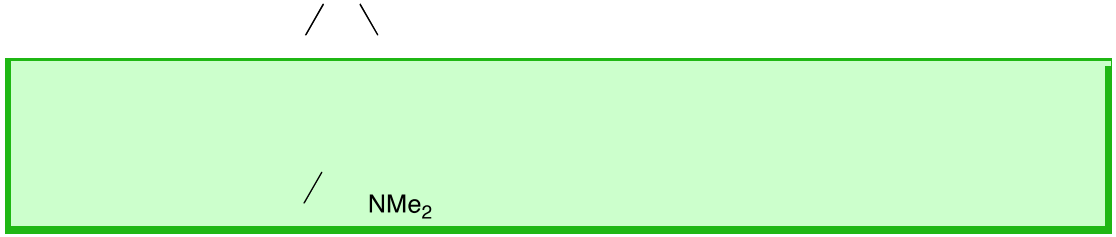
Anoxic cells highly sensitive to ATRi

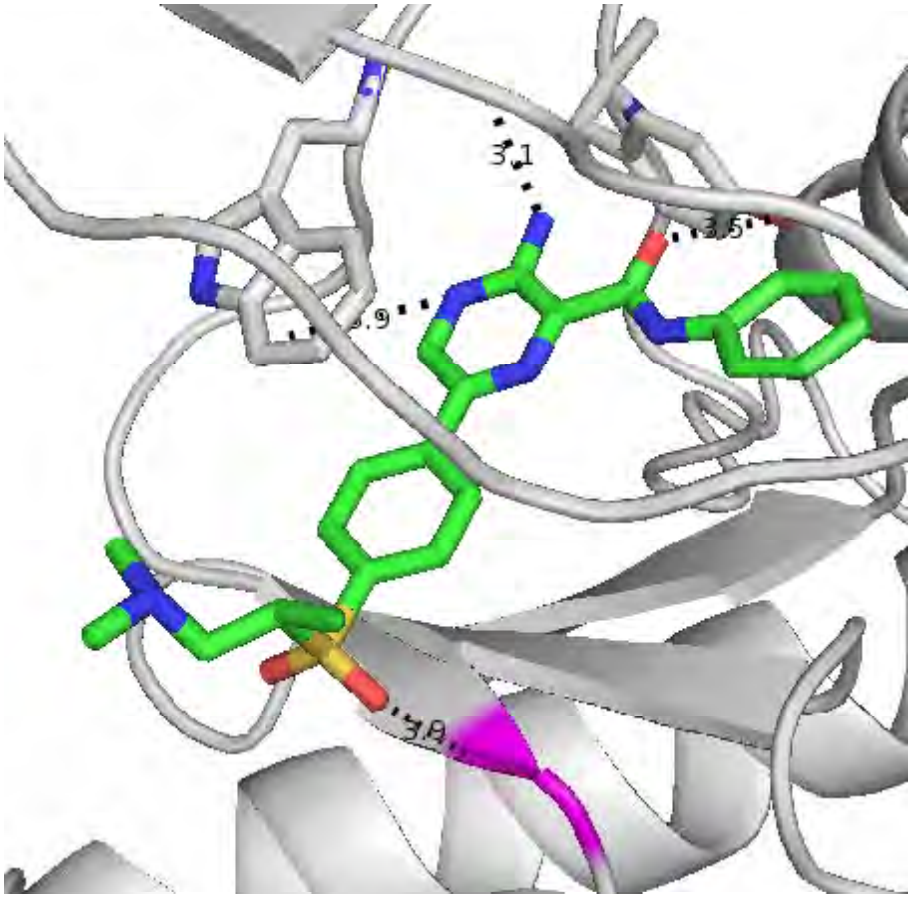


Br. J. Cancer (2012) **107**, 291

Further improvements by optimising the sulfone



Compds	R	ATR	ATM	DNAPK	IC_{50} (P-H2AX) (μM)	at pH 7.4 (μM)
						



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