cancers

MDPI

Review

Pharmacologic Induction of BRCAness in *BRCA*-Proficient Cancers: Expanding PARP Inhibitor Use

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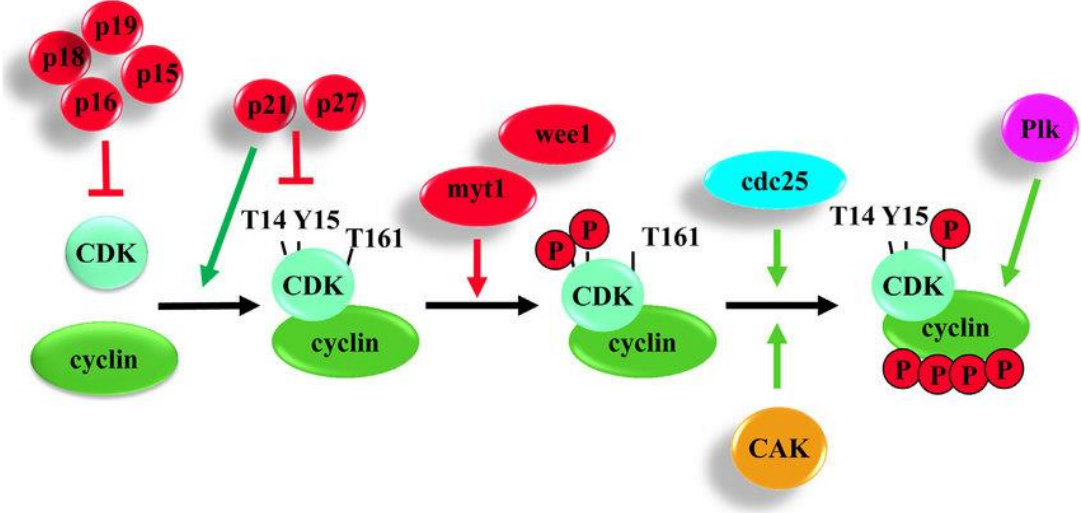
Omidreza Firuzi

Journal club presentation

17-11-1401

Cyclin-Dependent Kinases

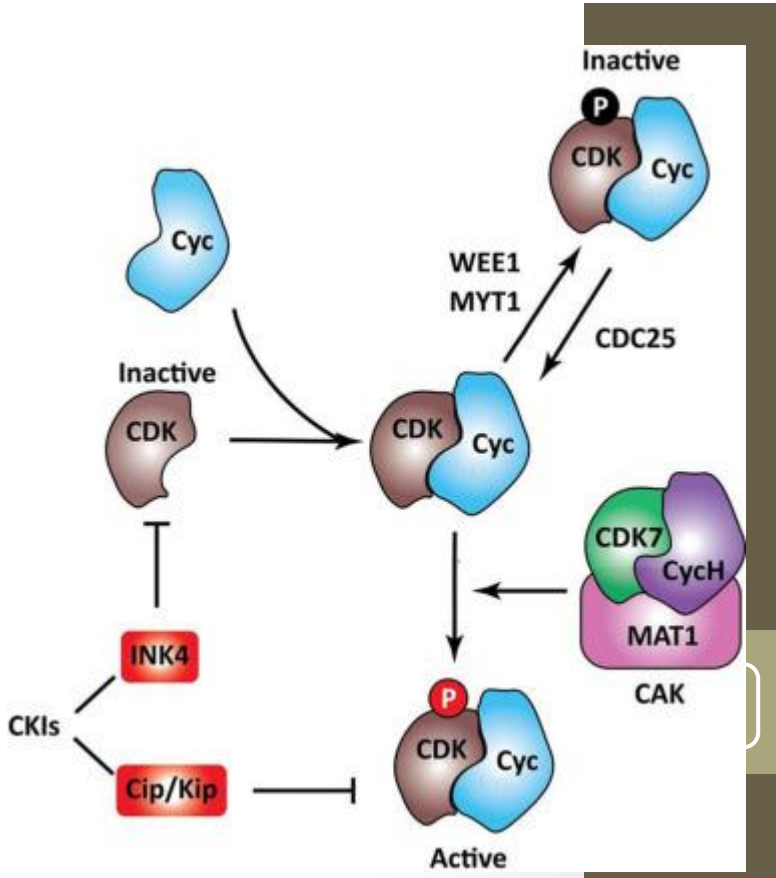


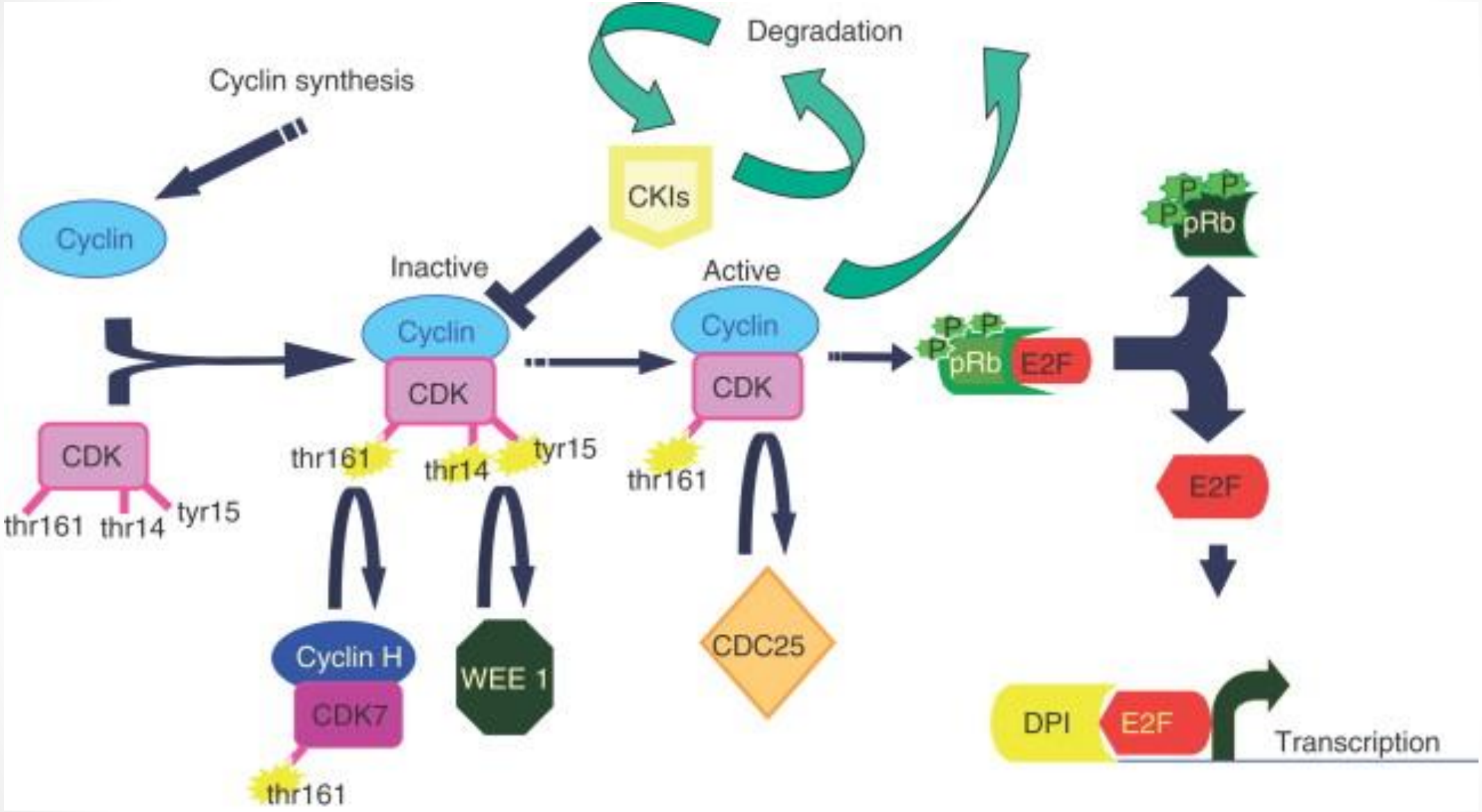
- CDKs are a family of **proline-directed Ser/Thr kinases.**

- CDKs have also an important role in the **modulation of transcription.**

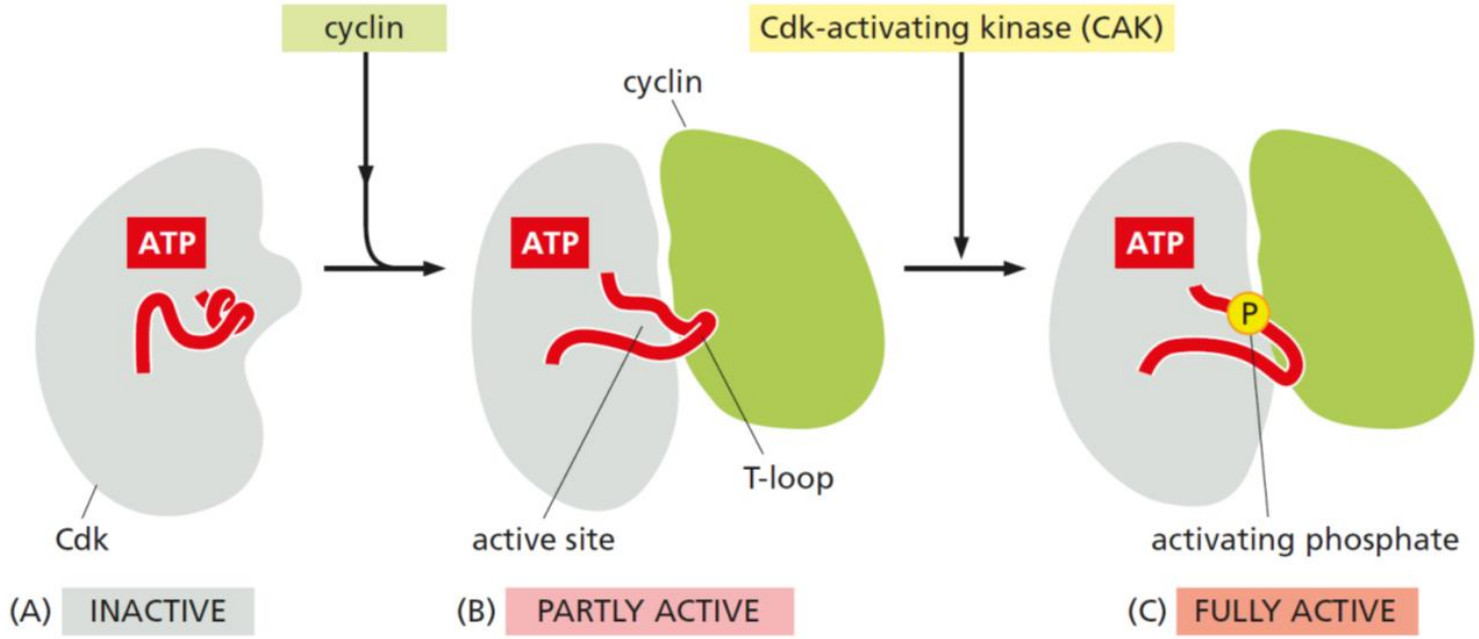
CDK activity is promoted by conformational changes induced by

- **Binding to cyclin subunits**
- **Removal of phosphorylation of Thr14/Tyr15 (WEE1** phosphorylates and **Cdc25 family phosphatases remove phosphorylation)**
- **Phosphorylation on Thr161 (CDK7)**



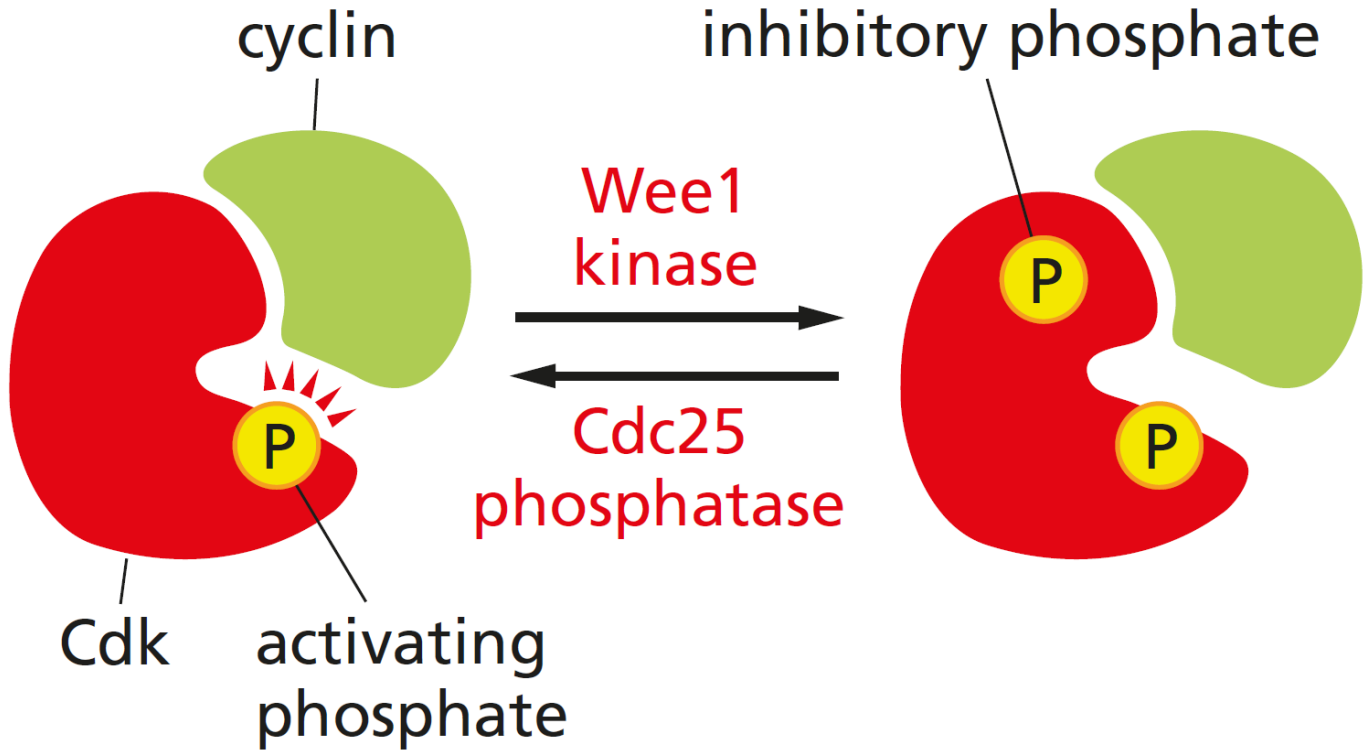

Degradation
Cyclin synthesis
CKIs
Cyclin
Inactive
Active
Cyclin
CDK
thr161
thr14
tyr15
CDK
thr161
thr14
tyr15
Cyclin
CDK
thr161
pRb
pRb
E2F
E2F
Cyclin H
CDK7
thr161
WEE 1
CDC25
DPI
E2F
Transcription

Cdk activation



- (A) Inactive state, without cyclin bound, the active site is blocked by a region of the protein called the T-loop (red).
- (B) The binding of cyclin causes the T-loop to move out of the active site, resulting in partial activation of the Cdk2.
- (C) Phosphorylation of Cdk2 (by CAK) at a threonine161 in the T-loop further activates the enzyme by changing the shape of the T-loop, improving the ability of the enzyme to bind its protein substrates.

The regulation of Cdk activity by phosphorylation



- The active cyclin–Cdk complex is turned off when the kinase Wee1 phosphorylates two closely spaced sites above the active site (For simplicity, only one inhibitory phosphate is shown).
- Removal of these phosphates by the phosphatase Cdc25 activates the cyclin-Cdk complex.

CDK roles in BRCA-ness

- CDK1 is required for the efficient recruitment of BRCA1 to DNA damage sites.

CDK1 depletion or inhibition with small molecules:

- Abrogates S phase checkpoint arrest [89]
- Reduces BRCA1 recruitment [90]
- Impairs RAD51 loading [91]

CDK1 inhibition sensitizes to PARPi, both in vitro [92] and in BRCA-wild type xenograft models [90].

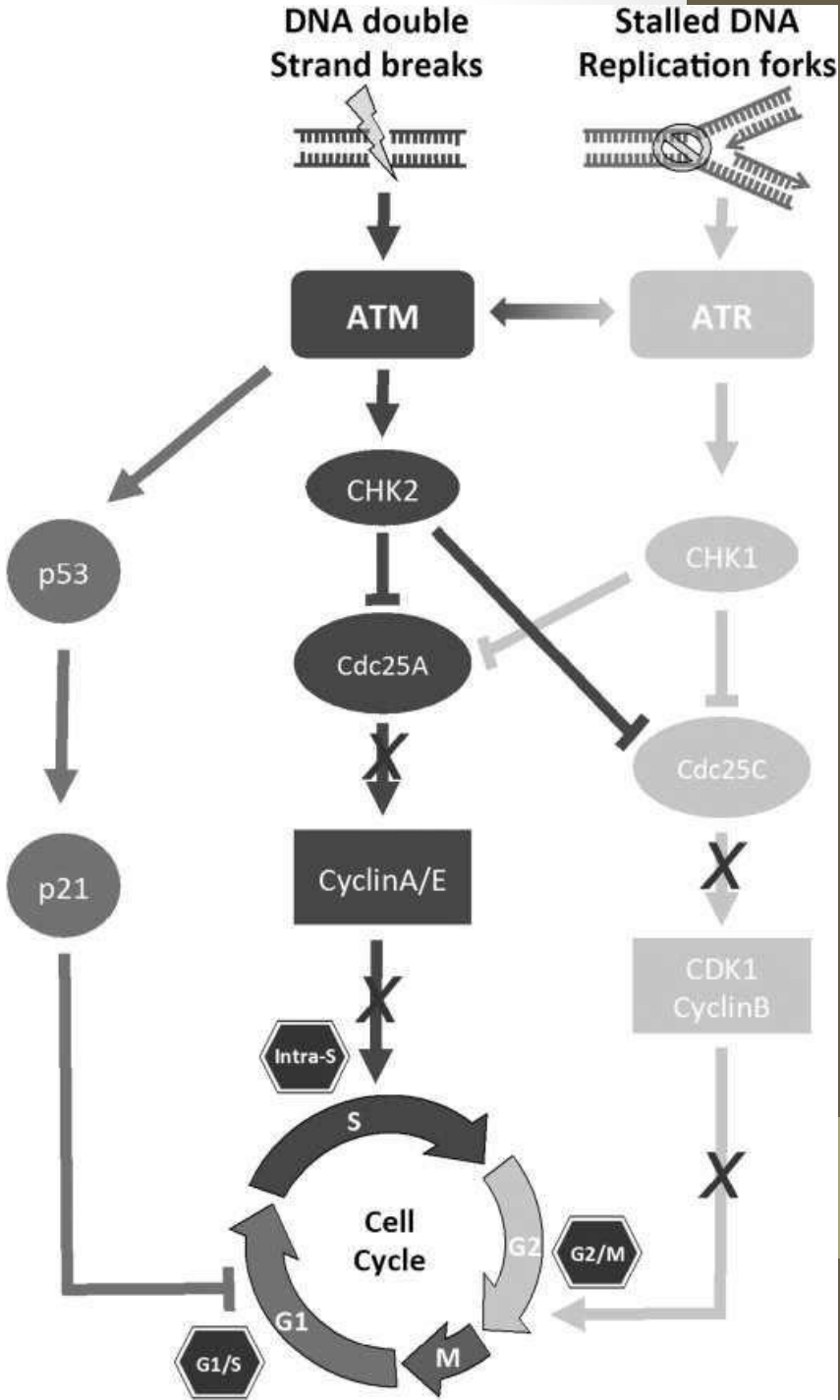
Cell cycle Checkpoints and the DNA Damage Response

- Cell cycle progression is directed by the activity of cyclin-dependent kinases (CDKs), which phosphorylate targets (transcription factors and regulatory elements such as retinoblastoma (Rb), etc)

Cell cycle arrest occurs in response to DNA damage, initiated by :

- Activation of ataxia-telangiectasia mutated (**ATM; by DSBs**)
- Activation of ataxia-telangiectasia and Rad3-related **(ATR; by single strand breaks and stalled replication forks**)
- Leading to the phosphorylation of **checkpoint kinases (CHK) 2 and 1**.

- Activated CHK1 and CHK2 antagonize the function of the Cdc25 phosphatase family, thus delaying cell cycle progression so that DNA repair can occur.



ATR Inhibitors

- ATR plays an important role in DNA damage sensing and the cellular response, particularly during S phase.

- A member of the **phosphatidylinositol-3-kinase-like kinase (PIKK) family**
- Phosphorylates **Ser/Thr-Glu** motifs on **hundreds of target proteins** across multiple processes involved in the DDR (in addition to activating CHK1 and inhibiting Cdc25, mediating cell cycle arrest).

ATR inhibition:

- Attenuates G2/M arrest following DNA damage
- Limits RAD51 focus formation
- Sensitizes to both DNA-damaging cytotoxic agents and PARPi [104].

ATR has been ascribed a role in the development of PARPi resistance in BRCA1-mutated cells, which can become 'rewired' to reverse the BRCAness phenotype.

- In BRCA1-mutant cells, the absence of BRCA1 recruitment to DSB ends is associated with the impaired recruitment of PALB2-BRCA2, which is required to load RAD51 onto resected single-stranded DNA ends to enact repair.
- ATR can bypass this block to HR by promoting PALB2-BRCA2 recruitment and subsequent RAD51 loading, reversing BRCA1 mutation-associated HR deficiency [105].
- ATR-mediated RAD51 recruitment plays an important role in the stabilization of stalled replication forks in the absence of BRCA1 [105,106].
- These ATR-dependent pathways of **reactivated HR** and **fork protection** produce **PARPi resistance** that can be abrogated by ATR knockdown or small molecule inhibitors (ATRi) [108].
- Several clinical trials combining the ATRi AZD6738 (ceralasertib, AstraZeneca, Cambridge, UK) and olaparib are underway in PARPi-naïve and PARPi-treated cancers (Table 2).

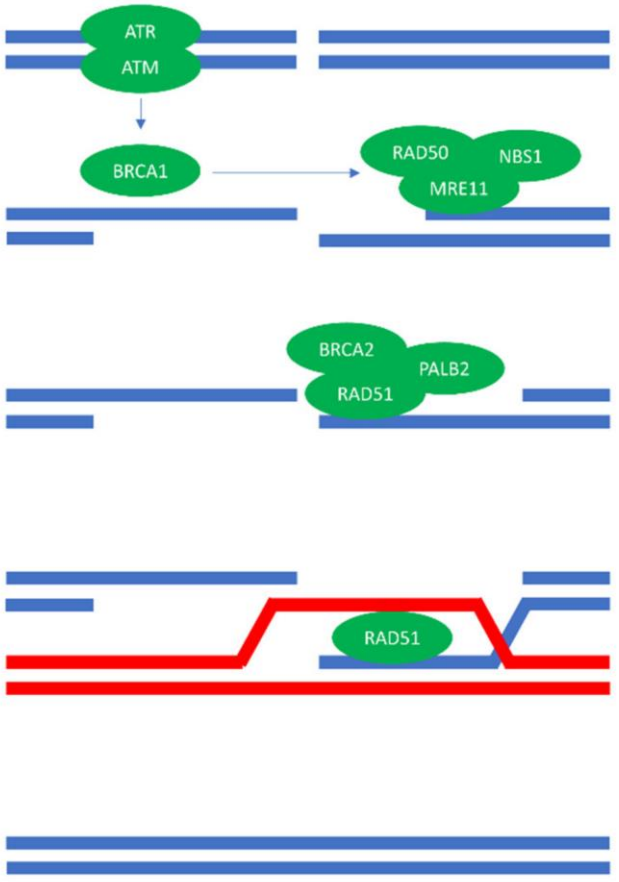


Table 2. Clinical trials evaluating cell cycle inhibitors in combination with PARPi.

ClinicalTrials.gov Identifier [86] (accessed 20 May 2022)	Phase	Status	Cell Cycle Inhibitor	PARPi	Other Drugs	Cancer
ATR inhibitor						
NCT02264678	I/Ib	Recruiting	AZD6738	Olaparib	Carboplatin, durvalumab	Advanced solid tumors
NCT02576444	II	Active, not recruiting	AZD6738	Olaparib		Advanced solid tumors with *ATM*, *CHK2*, *MRN* mutation
NCT02723864	I	Recruiting	VX-970	Veliparib	Cisplatin	Advanced refractory solid tumors
NCT02937818	II	Active, not recruiting	AZD6738, AZD1775	Olaparib	Carboplatin	Platinum-refractory small cell lung cancer (SCLC)
NCT03182634	II	Recruiting	AZD6738	Olaparib		TNBC
NCT03330847	II	Recruiting	AZD6738, AZD1775	Olaparib		2nd/3rd line TNBC
NCT03428607	II	Active, not recruiting	AZD6738	Olaparib		Relapsed SCLC
NCT03462342	II	Recruiting	AZD6738	Olaparib		Recurrent ovarian cancer, platinum-sensitive or -resistant
NCT03682289	II	Recruiting	AZD6738	Olaparib		Metastatic renal cell, urothelial, pancreatic
NCT03787680	II	Recruiting	AZD6738	Olaparib		Metastatic castration-resistant prostate
NCT03878095	II	Recruiting	AZD6738	Olaparib		*IDH*-mutant solid tumors
NCT04065269	II	Recruiting	AZD6738	Olaparib		Relapsed *ARID1A*(-) or (+) gynecological cancers
NCT04239014	II	Not yet recruiting	AZD6738	Olaparib		Platinum-sensitive relapsed epithelial ovarian with previous PARPi
NCT04298021	II	Not yet recruiting	AZD6738	Olaparib	Durvalumab	Advanced cholangiocarcinoma
NCT04417062	II	Not yet recruiting	AZD6738	Olaparib		Recurrent osteosarcoma
CHK1 inhibitor						
NCT03057145	I	Active, notrecruiting	Prexasertib	Olaparib		Advanced solid tumors
WEE1 inhibitor						
NCT02511795	Ib	Completed	AZD1775	Olaparib		Refractory solid tumors
NCT02576444	II	Not yet recruiting	AZD1775	Olaparib		Advanced solid tumors with *p53/KRAS* mutation
NCT03579316	II	Recruiting	AZD1775	Olaparib		Recurrent ovarian, peritoneal, fallopian tube
NCT04197713	I	Not yet recruiting	AZD1775	Olaparib		Advanced solid tumors with previous PARPi

CHK1 Inhibitors

- CHK1 is activated by ATR

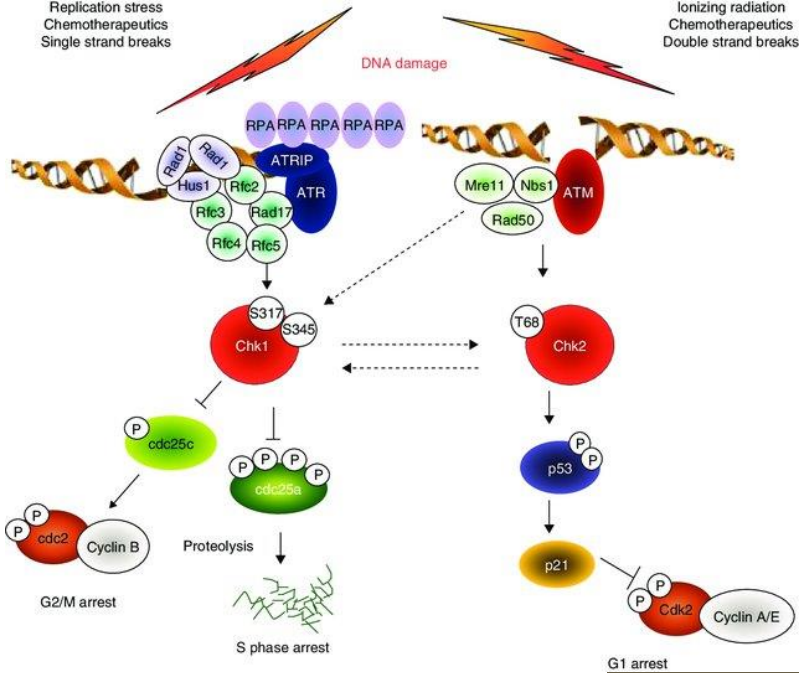
- CHK1 signaling plays an important role in the cell cycle signaling pathway in response to replication stress.

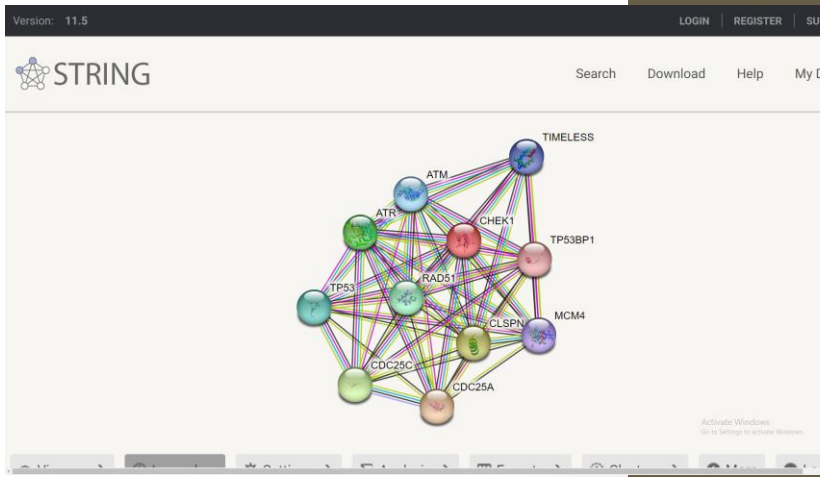
Akin to ATRi treatment, CHK1 inhibitors (CHK1i):

- **induce unrepaired DSB DNA damage** while releasing cells from cell cycle arrest into early mitosis [109].

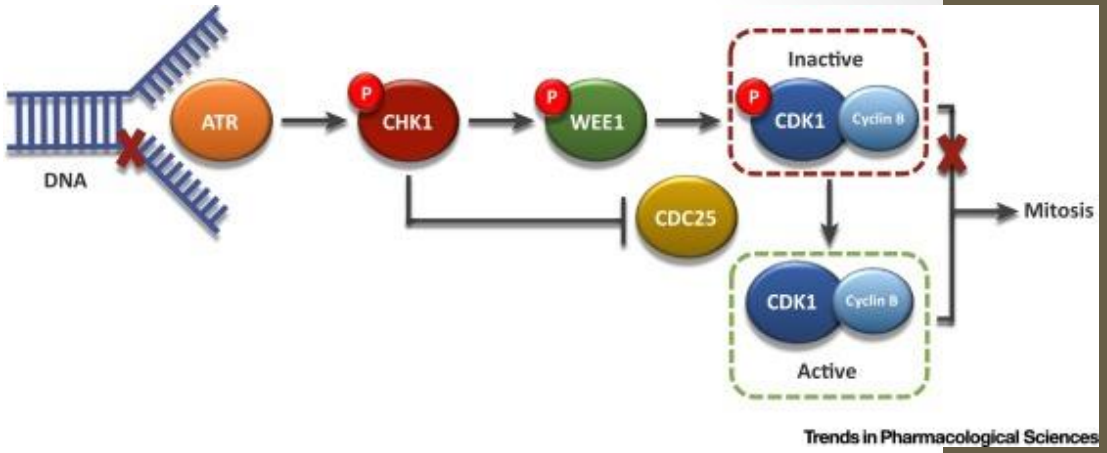
- Furthermore, **CHK1 directly phosphorylates RAD51 at Thr309, stimulating recruitment after DNA damage** [110] and suppressing proteasomal degradation [111,112].

- However, despite inducing early mitotic entry, CHK1 inhibition does not produce the marked accumulation of DNA damage and robust PARPi sensitization observed with ATRi [108,113,114].

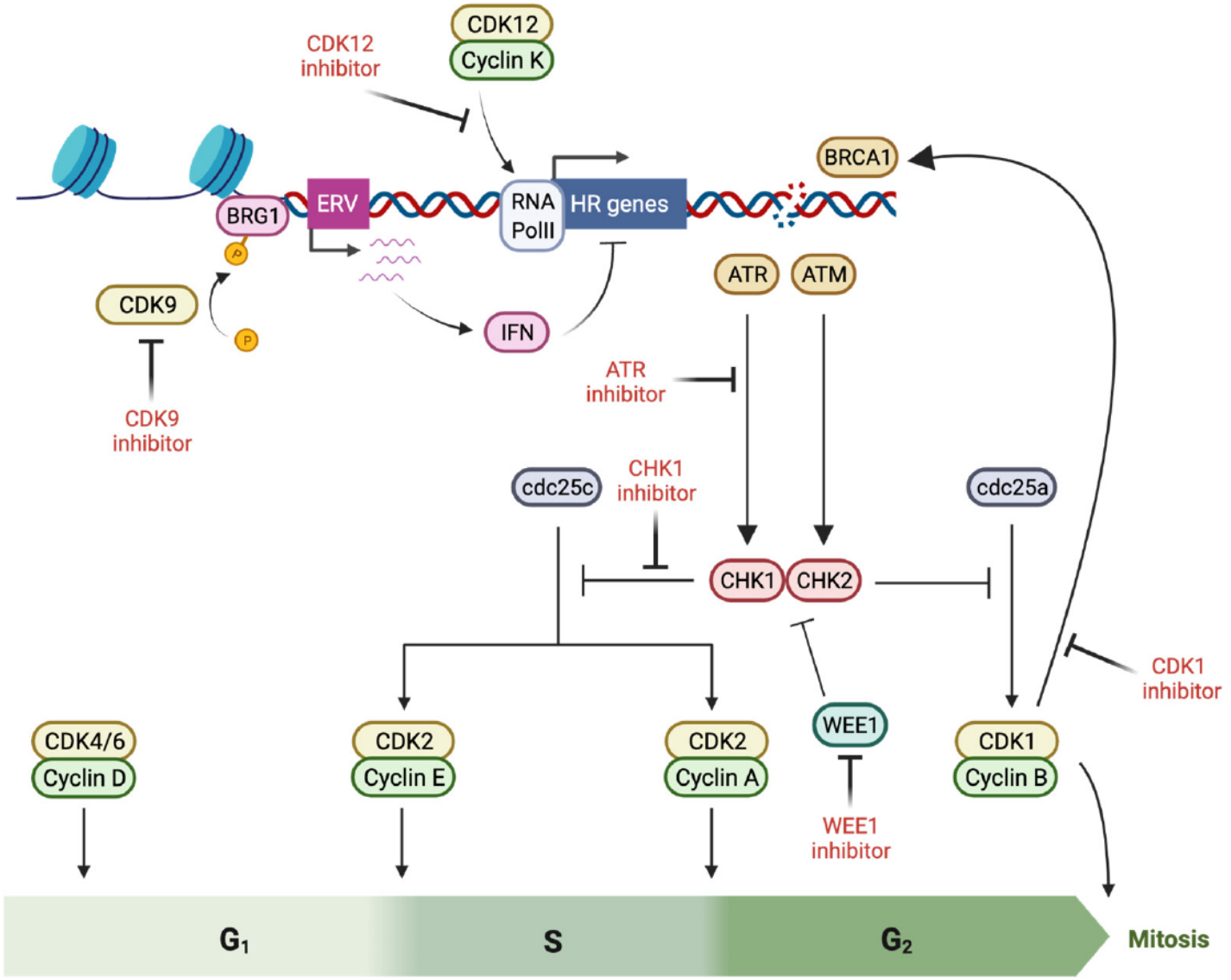




WEE1 Inhibitors



- WEE1 is activated following ATR and CHK1 phosphorylation during the DDR [116].
- The inhibition of WEE1 results in the forced activation of CDK1, **leading to the phosphorylation of BRCA2 that limits HR** [**117,118**].
- Similar to ATR, WEE1 has also been implicated in **replication fork protection.**
- Inhibition of WEE1 slows replication fork progression, limits HR, activates DDR, and forces mitotic entry, particularly in response to genotoxic agents [121].
- Accordingly, the WEE1 inhibitor AZD1775 sensitizes gastric [122], non-small cell lung cancer (NSCLC) [123], and pancreatic cancer [124] to olaparib, particularly when combined with genotoxic stress via ionizing radiation, and a small number of clinical trials are currently underway (Table 2).



- **Figure 2.** Induction of BRCAness by pharmacological targeting of cell cycle checkpoint proteins.

Tyrosine Kinase Signaling Pathways

FMS-Like Tyrosine Kinase 3 (FLT3)

- FLT3 is a receptor tyrosine kinase (RTK) that plays a role in hematopoietic cell differentiation, proliferation, and survival .

- Approximately 30% of acute myeloid leukemias (AML) carry poor prognosis FLT3 mutations, most commonly **internal tandem duplications (ITD)** that constitutively activate kinase activity.

Multitargeted tyrosine kinase inhibitors (TKIs):

- **Sunitinib and sorafenib** (limited antileukemic activity in clinical trials)
- **Gilteritinib and quizartinib** (greater FLT3 specificity and potency)

- Remissions associated with FLT3 inhibition are usually short-lived, commonly due to the persistence of therapy-refractory **leukemia stem cells** (LSCs), despite clearance of the bulk of **leukemia progenitor cells** (LPCs). Therefore, new approaches are required to enhance therapeutic **targeting of LSCs.**

- AML cells generate an elevated level of **reactive oxygen species** (ROS), leading to increased **DNA damage burden** [129].

- In certain subsets of AML, the expression of key HR proteins (including RAD51, ATM, BRCA1, and BRCA2) is suppressed, producing sensitivity to PARP inhibition [131].
- In contrast, DSB repair is proficient in FLT3-ITD high leukemic cells [132,133], rendering resistance to PARPi [131].

Treating FLT3-ITD high cells with quizartinib:

- Suppresses downstream signaling by Janus kinase 2 (JAK2) and PI3K to produce a rapid **downregulation of DSB repair proteins** (including BRCA1, BRCA2, PALB2, and RAD51)
- Sensitizes to PARP inhibition in preclinical and in vivo models [134].

- These effects may be mediated in part by the effects of PARP1 inhibition outside of DNA repair, such as STAT5 protein stabilization through PARylation [135].

c-MET

Chakraborty et al. *Oncogenesis* (2019)8:7
https://doi.org/10.1038/s41389-018-0116-9

Oncogenesis

ARTICLE Open Access

Activation of c-Met in cancer cells mediates growth-promoting signals against oxidative stress through Nrf2-HO-1

Samik Chakraborty[1,2], Murugabaskar Balan[1,2], Evelyn Flynn[1,2], David Zurakowski[2,3], Toni K. Choueiri[2,4] and Soumitro Pal[1,2]

- c-MET activation is a common event in numerous cancers, driving **proliferation, differentiation, and survival** signaling pathways.

Several small molecules and antibodies targeting the HGF-MET pathway:

- Ligand inhibitors (blocking **pro-HGF cleavage to the active form** or preventing ligand-receptor binding)
- MET receptor inhibitors (competitively antagonizing receptor binding or inhibiting MET tyrosine kinase activity).

- c-MET becomes activated following oxidative stress, inducing an antiapoptotic cytoprotective response (Nrf2) that includes the phosphorylation of PARP1 at Tyr907 located within the catalytic domain [**Chakraborty et al, 2019**].

- Phospho-Tyr907: enhances **PARylation activity**, but also **reduces PARPi binding**, and may be a predictive marker of PARPi resistance [Du et al 2016].

Inhibition of c-MET using **crizotinib** [Du et al 2016] or **HS-10,241** [Han etal, 2019]:

- Abolishes Tyr907 phosphorylation
- **Sensitizes to PARPi** in in vitro and xenograft models of TNBC, NSCLC, and high-grade serous ovarian cancer (HGSOC), independent of BRCA status.

- Elevated **c-MET expression** in BRCA-mutant TNBC cell lines **correlates to PARPi resistance** that can be reversed by c-MET inhibition. These results highlight the potential of a therapeutic strategy to **combine PARPi with c-MET inhibitors in PARPi-resistant cancers.**

nature medicine

Blocking c-Met–mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors

Yi Du[1], Hirohito Yamaguchi[1], Yongkun Wei[1], Jennifer L Hsu[1], Hung-Ling Wang[2], Yi-Hsin Hsu[1], Wan-Chi Lin[1], Wen-Hsuan Yu[1,3], Paul G Leonard[4,5], Gilbert R Lee IV[4,5], Mei-Kuang Chen[1,3], Katsuya Nakai[1], Ming-Chuan Hsu[1], Chun-Te Chen[1], Ye Sun[1], Yun Wu[6], Wei-Chao Chang[2,7], Wen-Chien Huang[8], Chien-Liang Liu[8], Yuan-Ching Chang[8], Chung-Hsuan Chen[7], Morag Park[9], Philip Jones[5], Gabriel N Hortobagyi[10] & Mien-Chie Hung[1–3,11]

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Original Article

Synergism of PARP inhibitor fluzoparib (HS10160) and MET inhibitor HS10241 in breast and ovarian cancer cells

Ye Han[1,3], Mei-Kuang Chen[3,5], Hung-Ling Wang[6,7], Jennifer L Hsu[3], Chia-Wei Li[3], Yu-Yi Chu[3], Chun-Xiao Liu[3], Lei Nie[3], Li-Chuan Chan[3,5], Clinton Yam[3,4,5], Shao-Chun Wang[6,7], Gui-Jin He[1], Gabriel N Hortobagyi[4], Xiao-Dong Tan[2], Mien-Chie Hung[3,5,6,7]

EGFR

- Binds to a variety of ligands including epidermal growth factor (**EGF**) and transforming growth factor (**TGF**).

- Upon ligand binding, inactive EGFR monomers **dimerize** to an active form, either as **homodimers** or as **heterodimers** with other members of the ErbB receptor family such as HER2.

- TKIs with specific activity against EGFR (**erlotinib**, **gefitinib**, and the EGFR/HER2-targeting **lapatinib**) and monoclonal antibodies that prevent EGFR-ligand binding (**cetuximab** and **panitumumab**) have been approved for use in a variety of EGFR expressing malignancies, including NSCLC, head and neck squamous cell carcinoma (HNSCC), and colorectal cancer.

EGFR inhibition:

Physical interaction between EGFR and **components of the classical NHEJ pathway** of DSB repair (particularly **DNA-PKCS**) [142]

Reduced levels of DNA-PK [143,144] and/or subcellular relocalization away from the nucleus [145]

Reduced NHEJ repair capacity and sensitization to radiation [146]

EGFR inhibition:

Transient downregulation of mismatch repair (MLH1, MSH2, and MSH6) and HR (BRCA2, RAD51) genes in cetuximab-sensitive colorectal cancer cell lines [147].

Hung lab have built upon their work combining MET inhibitors with PARPi:

- **EGFR cooperates with MET** in subsets of hepatocellular cancers [Dong et al 2019] and TNBCs [Chu et al, 2020] to phosphorylate **PARP1 Tyr907** in response to DNA damage, demonstrating that **dual EGFR/MET inhibition** is required in this group to block phosphorylation and **sensitize resistant cells to PARPi**.

This may further broaden the therapeutic potential of MET inhibition to overcome PARPi resistance in certain cancers.

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EGFR and c-MET cooperate to enhance resistance to PARP inhibitors in hepatocellular carcinoma

Qiongzhu Dong[1,2,*], **Yi Du**[1,*], **Hui Li**[1,3], **Chunxiao Liu**[1], **Yongkun Wei**[1], **Mei-Kuang Chen**[1,4], **Xixi Zhao**[1], **Yu-Yi Chu**[1], **Yufan Qiu**[1], **Lunxiu Qin**[2], **Hirohito Yamaguchi**[1], **Mien-Chie Hung**[1,4,5,6]

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Original Article

Blocking c-Met and EGFR reverses acquired resistance of PARP inhibitors in triple-negative breast cancer

Yu-Yi Chu[1*], Clinton Yam[1,2,6§*], Mei-Kuang Chen[1,6*], Li-Chuan Chan[1*], Min Xiao[1,7], Yong-Kun Wei[1], Hirohito Yamaguchi[1,8], Pei-Chih Lee[1,9], Ye Han[1,10], Lei Nie[1], Xian Sun[1,11], Stacy L Moulder[2], Kenneth R Hess[3], Bin Wang[4], Jennifer L Hsu[1], Gabriel N Hortobagyi[2], Jennifer Litton[2], Jeffrey T Chang[5], Mien-Chie Hung[9,1,12]

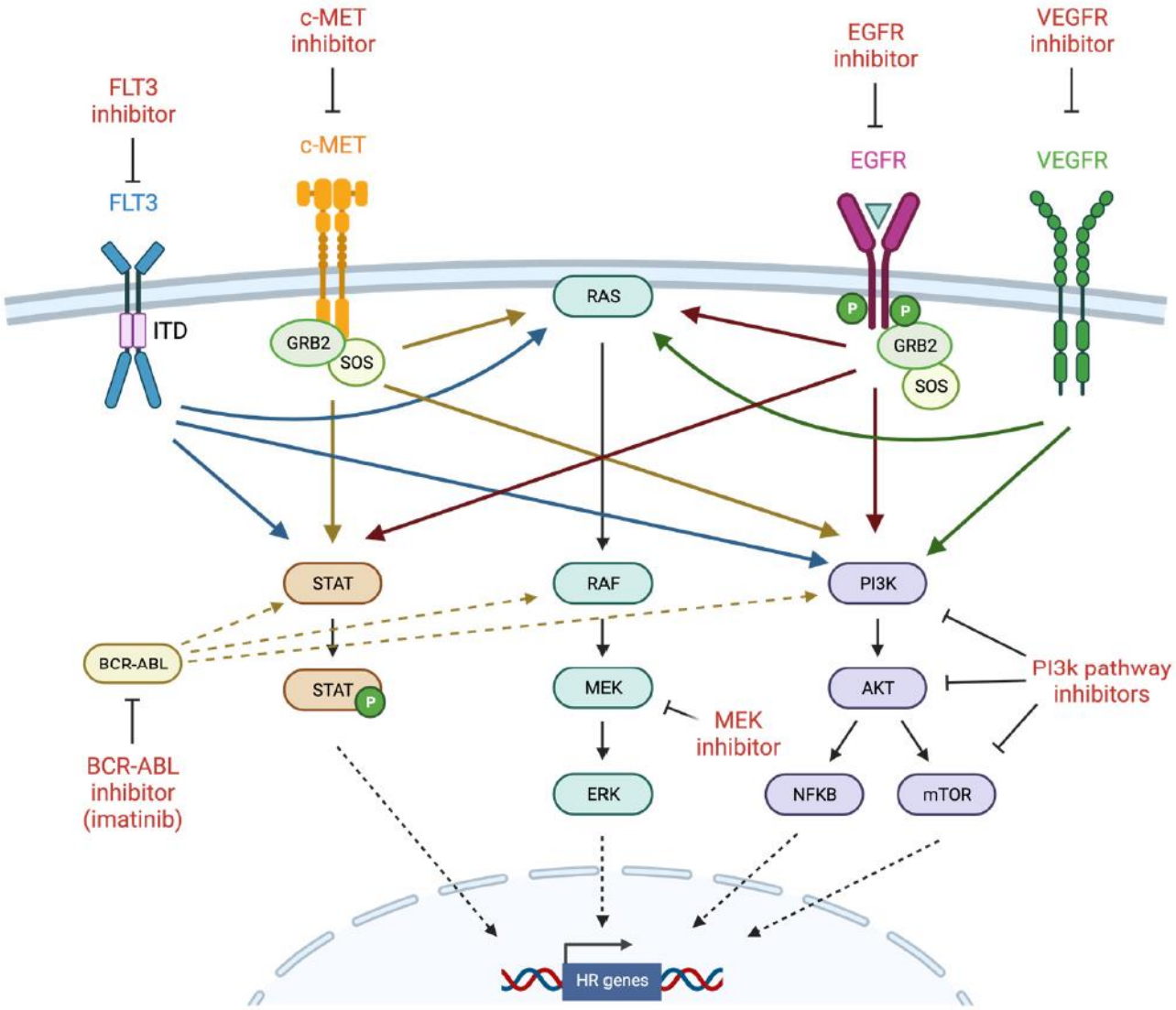


Fig 3. Induction of BRCAness by tyrosine kinase inhibitors (created with Biorender.com, accessed May 2022).

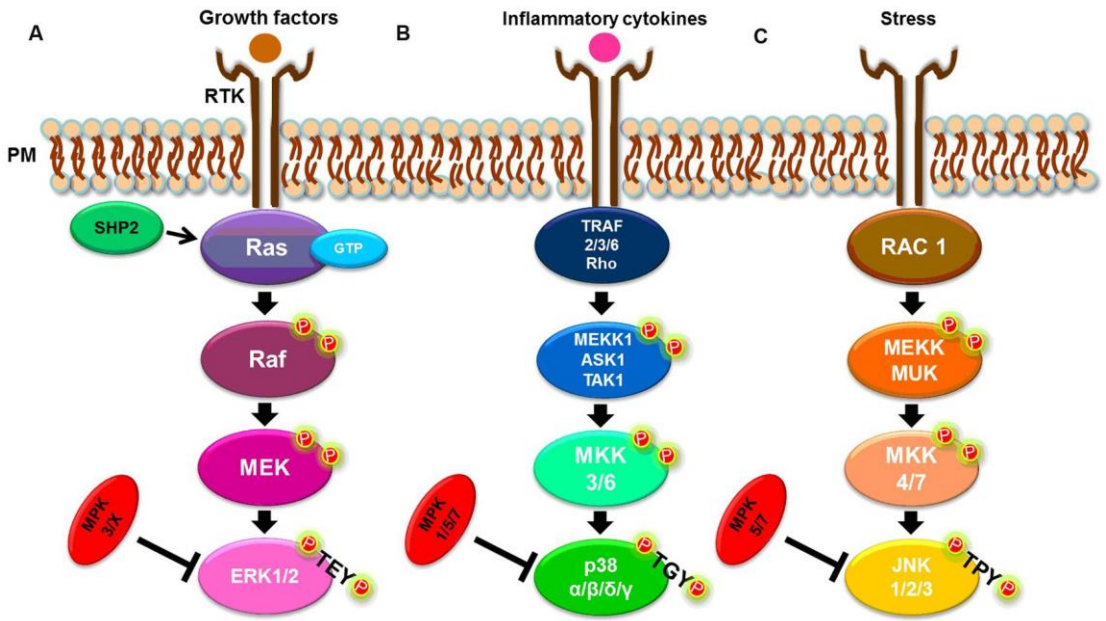
VEGFR

- Vascular endothelial growth factor receptors (VEGFRs) are tyrosine kinase receptors that play critical roles in signal transduction during **vasculogenesis and angiogenesis**.
- The abnormal expression of **VEGFR ligands (VEGFs)** by tumor-associated **macrophages** contributes to tumor **neoangiogenesis**.
- An important consequence of VEGFR inhibition is **tumor hypoxia** [151], leading to **HR defects** via the **downregulation of BRCA1, BRCA2, and RAD51** [152,153].
- In preclinical models, VEGFR inhibition is reported to sensitize to PARPi.
- A phase 2 clinical trial in platinum-sensitive HGSOC: VEGFRi/PARPi combination prolongs progression-free survival over single agent treatment [154].
- Several early-stage clinical trials combining the VEGFR inhibitor **cediranib with olaparib** are currently underway (Table 3).

Table 3. Clinical trials evaluating TKI in combination with PARPi.

ClinicalTrials.gov Identifier [86] (accessed 20 May 2022)	Phase	Status	TKI	PARPi	Other Drugs	Cancer
Pan-TKI						
NCT01116648	I/II	Recruiting	Cabozantinib	Niraparib		Advanced urothelial
EGFR inhibitor						
NCT03891615	I	Recruiting	Osimertinib	Niraparib		*EGFR*-mutant advanced lung
VEGFR inhibitor						
NCT01116648	I/II	Active, not recruiting	Cediranib	Olaparib		Recurrent ovarian, fallopian tube, peritoneal, or triple negative breast cancer
NCT02340611	II	Completed	Cediranib	Olaparib		Recurrent ovarian with prior PARPi response
NCT02345265	II	Active, not recruiting	Cediranib	Olaparib		Recurrent ovarian, fallopian tube, or peritoneal
NCT02484404	I/II	Recruiting	Cediranib	Olaparib	Durvalumab	Advanced solid tumors
NCT02498613	II	Recruiting	Cediranib	Olaparib		Advanced solid tumors
NCT02502266	II/III	Recruiting	Cediranib	Olaparib		Recurrent platinum-resistant ovarian, fallopian tube, or peritoneal
NCT02681237	II	Active, not recruiting	Cediranib	Olaparib		Recurrent ovarian with prior PARPi response
NCT02893917	II	Active, not recruiting	Cediranib	Olaparib		Metastatic castration-resistant prostate
NCT02899728	II	Terminated	Cediranib	Olaparib	Platinum, etoposide	Extensive stage small cell lung
NCT02974621	II	Recruiting	Cediranib	Olaparib		Recurrent glioblastoma
NCT03278717	III	Recruiting	Cediranib	Olaparib		Recurrent ovarian with prior platinum response
NCT03660826	II	Suspended	Cediranib	Olaparib		Metastatic endometrial
MEK inhibitor						
NCT03162627	I/II	Recruiting	Selumetinib	Olaparib		Advanced solid tumors
PI3K pathway inhibitors						
NCT02208375	Ib/II	Active, not recruiting	AZD5363 (AKT) or AZD2014 (mTOR)	Olaparib		Recurrent endometrial, ovarian, peritoneal, fallopian tube, or TNBC
NCT02511795	Ib	Completed	AZD1775 (PI3K)	Olaparib		Refractory solid tumors
NCT02576444	II	Active, not recruiting	AZD5363 (AKT)	Olaparib		Advanced solid tumors with *PTEN/PI3KCA/AKT/ARID1A* mutation
NCT03579316	II	Recruiting	AZD1775 (PI3K)	Olaparib		Recurrent ovarian, peritoneal, or fallopian tube
NCT04197713	I	Active, not recruiting	AZD1775 (PI3K)	Olaparib		Advanced solid tumors with prior PARPi response

MAPK Pathway



- The mitogen-activated protein kinase (MAPK) signaling pathway regulates a diverse range of cellular processes, including proliferation, differentiation, and survival.
- Downstream of several RTKs.
- Phosphorylates transcription factors (such as **c-MYC, c-Jun, and c-Fos**), cell cycle proteins (such as **CDK4/6** for S-phase entry), apoptotic factors (inactivating pro-apoptotic proteins such as **Bad, Bim**, and caspase 9) [155].

- Sun et al. demonstrated that transient **PARPi treatment induces Ras/MAPK activation**, producing a downregulation of the pro-apoptotic targets that induced PARPi resistance, and furthermore, recapitulated **the PARPi resistance** observed in Ras-mutant cell lines.

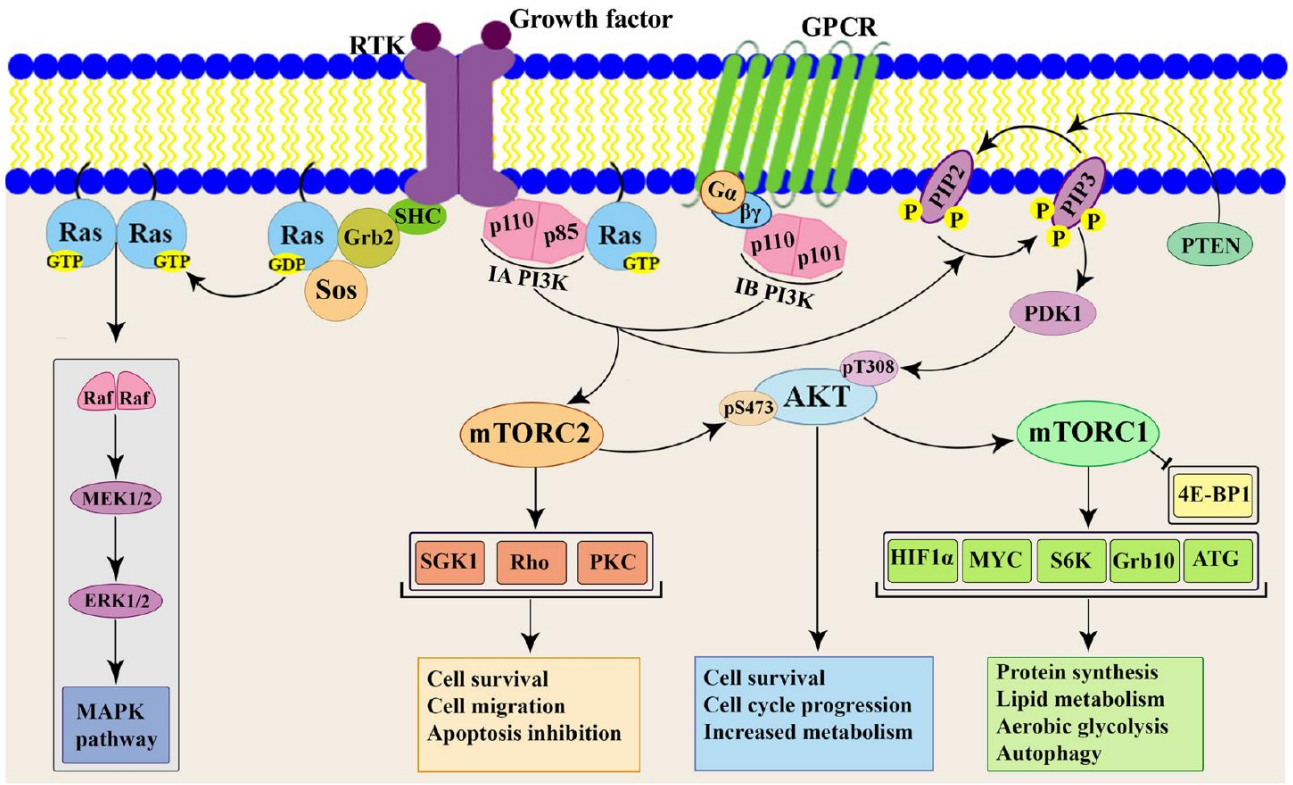
MEKi effect:

- Phosphorylation patterns of **multiple DNA repair proteins were altered**
- Altered **expression levels of DSB** repair proteins that reversed an enhanced level of DSBR observed in KRAS-mutant cells.
- Sensitized KRAS mutant cells to talazoparib, compounded by increased PARP1 expression that enhanced the accumulation of cytotoxic PARP-trapping lesions [157].

- These results were subsequently confirmed by a second group [158], suggesting a **combinatorial role for PARPi and MEKi** in the treatment of PARPi-resistant and/or KRAS-mutant tumors that is now being tested in a phase I/II trial (Table 3).

- Interestingly, PARPi synergism is not recapitulated by BRAF inhibition, likely because other RAF homologs bypass the effects of therapeutic inhibition [157].

PI3K Pathway



- The phosphoinositide 3-kinase pathway is a major effector of receptor tyrosine kinase activation, transducing signals via phospholipid generation to protein kinase B (also known as AKT), mammalian target of rapamycin (mTOR), and other downstream targets.

Mutations in cancer:

- Loss of function mutations in the negative regulator phosphatase and tensin homolog (PTEN)
- Activating mutations of other components of the PI3K pathway

PI3K has a role in **promoting the DDR** [160]:

- **Regulating the binding of the NBS1 damage sensor to DNA** [161]
- Control of **RAD51 recruitment to DSBs** [162]

The downstream effector **mTOR** also modulates the DDR:

- Maintains HR and NHEJ [163,164]
- Stimulates FANCD2 expression [165,166]

The PI3K pathway also exerts **transcriptional control** over repair gene expression:

- BRCA1/2, RAD51 [167,168]
- PRKDC (DNA-PKCS) and ATM [169].

Several studies have considered the potential for PI3K pathway inhibitors to induce DSB repair defects

In both in vitro and in vivo models of **BRCA-proficient TNBC, BRCA1/2 downregulation** induced by the following, impairs HR and sensitizes to PARPi:

- PI3K inhibitor (PI3Ki) BKM120 [167]
- mTOR inhibitors everolimus or KU0063794 [170]
- Dual PI3K/mTOR inhibitor GDC-0980 [171]

Similar results have been observed in PTEN-mutant, PI3K-activated **endometrial cancer** [172], and in PI3K-wildtype [173,174] or mutant [175] **ovarian cancer**.

- Phase I/II clinical trials examining PARPi in combination with inhibitors of PI3K, AKT, or mTOR are underway (Table 3).

Other Targets: BCR-ABL

- c-ABL tyrosine kinase is constitutively activated in most chronic myeloid leukemias (CML) following translocation adjacent to the BCR gene, **forming the BCR-ABL 'Philadelphia chromosome'**.
- Activated c-ABL interacts with multiple proliferative and survival pathways, including MAPK, PI3K, and JAK/STAT.
- c-ABL **phosphorylates RAD51** at Tyr315, enhancing complex formation with RAD52 [177,178]
- In the presence of BCR-ABL, RAD51 expression is significantly enhanced, mediated via JAK/STAT signaling [180].
- Imatinib is a multi-kinase inhibitor that possesses selectivity for BCR-ABL, along with c-kit and PDGFR, and is FDA-approved in hematological malignancies and gastrointestinal stromal tumors.

Imatinib:

- **Reduces RAD51 nuclear expression** and chromatin binding, and **inhibits HR-mediated repair** [181]
- Sensitizes to PARPi in **ovarian cancer** [174].

NAMPT Inhibition

- Nicotinamide phosphoribosyl transferase (NAMPT) is a rate-limiting enzyme required for the **generation of the PARP substrate -NAD+.**

- Small molecule inhibition of NAMPT suppresses -NAD+ synthesis, preventing PARP1 PARylation activity.

Synthetic lethality between an experimental NAMPT inhibitor and olaparib has been observed in different tumor models independent of BRCA status:

- Synergistic NAD+ depletion
- Reduction in PARylation
- Increase in DNA damage
- Induction of apoptosis [182,183].

- While this combination **does not induce a BRCAness phenotype**, it may offer an opportunity to further optimize therapeutic strategies by maximizing PARP inactivation.

Pharmacological Ascorbate

- **High doses of vitamin C** (ascorbate) have been evaluated as an anticancer therapy in a range of malignancies.

- Cytotoxicity is mediated in part through **DNA damage accumulation** resulting from the **generation of hydrogen peroxide**, which **activates PARP1** and subsequently depletes the **PARP1 substrate nicotinamide adenine dinucleotide (NAD+)** leading to ATP depletion and cell death [184].

- Although PARPi treatment prevents NAD+/ATP depletion, cell death still ensues secondary to DSB accumulation linked to the ascorbate induced **downregulation of BRCA1, BRCA2, and RAD51 [185].**

- Additionally, low doses of vitamin C, particularly in the context of vitamin C deficiency, may synergistically enhance the effects of DNMTi in hematological malignancy [186].

Conclusions

- PARP inhibitor sensitivity in BRCA-mutated breast and ovarian cancers is the prototypical example of synthetic lethality but represents only a small number of total cancer diagnoses.
- To expand PARPi utility into a wider setting, research has primarily focused on the identification of other genetic and epigenetic determinants of BRCAness.
- BRCAness—and hence PARPi sensitivity—may also be pharmaceutically induced.
- With the exploration of the additional roles of PARP in the regulation of gene expression and protein translation, this may increase the targets for the induction of BRCAness.