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Supplementary information

Targeting replication stress in cancer therapy

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Supplementary Table 1. Genomic alterations leading to replication stress in high-grade serous ovarian carcinoma

Mechanism of replication stress induction	Molecular alteration	Frequency in HGSOC	Comments
Loss of the G1/S checkpoint	*TP53* mutations	~ 100%	. Mutual exclusivity between *CCNE1, RB1,* and *CDKN2A* alterations . Mutual exclusivity between HRR alterations and *CCNE1* amplification - cyclin E1 overexpression which is associated with platinum and PARPi resistance . Association between HRR alterations and *RB1* loss which is related to platinum sensitivity and long-term survival . Mutual exclusivity between *KRAS* and *NF1* alterations
Premature entry into S phase	*CCNE1* amplification	20%	
	RB1 loss	11%	
	CDKN2A mRNA downregulation	32%	
DNA repair deficiencies	HRR alterations	~ 50%	
	NER alterations	~ 5%	
Oncogenic drive	*MYC* amplification	~ 40%	
	NF1 loss	~ 10%	
	KRAS amplification / mutation	11%	

HGSOC, high-grade serous ovarian carcinoma; mRNA, messenger RNA; HRR, homologous recombination repair; PARPi, PARP inhibitor

Suppelmentary table 2 Summary of pharmacokinetics and pharmacodynamics characteristics of drug candidates from early phase studies

Target	Drug names	IC50 of the target kinase	Half-life	Dosing schedule monotherapy	Dosing schedule in selected combinations	Administration
ATR	Berzosertib (M6620 / VE822)	ATR < 0.2nM	16h	240 mg/m^2 QD on day 1 q7	Carboplatin AUC5 and berzosertib 90 mg/m^2 on d 2, d9 q21 Topotecan 1.25mg/m^2 d1-5 and berzosertib 210 mg/m^2 on days 2 and 5 q21 Gemcitabine 1000mg/m^2 d1,8 and berzosertib 210mg/m^2 d2, d9	i.v.
	Ceralasertib (AZD6738)	ATR = 4nM	12h	160mg BD days 1-14 q28	Paclitaxel 80mg/m^2 d1, 8, 15 and ceralasertib 240mg BD days 1-14 q28 Olaparib 300mg BD and ceralasertib 160mg QD days 1-7 q28	p.o.
	BAY1895344	ATR = 7nM	11.5h	3 days on / 4 days off or 3 days on / 11 days off		p.o.
CHK1	Prexasertib	CHK1 = 0.9nM CHK2 = 8nM	11.5h	105mg/m^2 QD on day 1 q14	Olaparib 100mg BD d1-5, d15-19 and prexasertib 70mg/m^2 day 1 q14 Samotolosib 150mg BD d1-14 and prexasertib 105mg/m2 d1 q14d	i.v.
	SRA737	CHK1 = 1.4nM CHK2 = 1,850nM	10.5h	800mg QD continuously	Gemcitabine 250mg/m^2 d1, d8, d15 and SRA737 500mg QD on d 2-3, d8-9, d16-17 q28	p.o.
	LY2880070	CHK1 = 1.2nM	5.3h	200mg BD continuously	Gemcitabine 100mg/m^2 d1, d8, d15 and LY2880070 50mg BD d1-5, d9-13, d16-20 q21	p.o.
WEE1	Adavosertib (AZD1775 / MK-1775)	WEE1 = 5.2nM	11h	225mg BD for 2.5 days weeks 1 and 2 of q21 or 300mg QD days 1-5 and days 8-12 of q21	Carboplatin AUC5 and adavosertib 225mg BD for 2.5 days q21 Cisplatin 75mg/m^2 d1 and adavosertib: 200mg BD for 2.5 days q21 Gemcitabine 1000mg/m^2 d1, d8, d15 and adavosertib 175mg QD for 2.0 days weekly q28	p.o.
MYT1	RP-6306	MYT1 = 3.1nM	1.58h	Ongoing phase 1 dose escalation	-	p.o.

QD, once daily; BD, twice daily; AUC, area under the curve; i.v, intravenous; p.o., by mouth.

Supplementary table 3. Results of phase 1 clinical trials using ATR, CHK1, and WEE1 inhibitors

Target	Study Identifier	Agents / Schedule	Design	Patients/Accrual	Primary Endpoint	Results	Comments
ATR	NCT02487095 Thomas et al. J Clin Oncol 2018	Berzosertib / Topotecan MTD Berzosertib 210mg/m^2 d2-5 Topotecan 1.25 mg/m^2 d1-5 q21d	Phase 1	Advanced solid tumors N=21	-	Partial responses or prolonged stable disease in 3 of 5 patients with small-cell lung carcinoma	. Neutropenia G≥3 19% . Anemia G≥3 19%
	NCT02157792 Yap et al. J Clin Oncol 2020	Berzosertib / Carboplatin MTD Berzosertib 240mg/m^2 twice weekly Berzosertib 90 mg/m^2 once weekly and Carboplatin AUC 5 d1 q21d	Phase 1	Advanced solid tumors N=40	-	Monotherapy 1 partial response in a patient with ATM loss and *ARID1A* mutation Combination 1 partial response in a HGSOC germline *BRCA1* mutant patient platinum-resistant and PARPi-resistant	
	NCT02223923 Dilon et al. ESMO 2019	Ceralesrtib Ceralasertib 160mg BD d1-14 q28d	Phase 1	Advanced solid tumors N=46	-	ORR 7%	Intermittent schedule (d1-14 q28d) better tolerance than continuous schedule
	NCT02264678 Krebs et al. AAACR 2018	Ceralasertib / Olaparib / Durvalumab MTD Ceralasertib 160mg QD d1-7 Olaparib 300mg BD d1-28 Ceralasertib 80-240 mg od or bd for 1 (d22-28) or 2 (d15-28) weeks Durvalumab 1500 mg on D1 q28d	Phase 1	Advanced solid tumors +Olaparib N=45 +Durvalumab N=45	-	+Olaparib: Complete response 1 patient Partial responses in 5 patients <u>+Durvalumab:</u> Complete response 1 patient Partial responses in 2 patients	DLT thrombocytopenia and neutropenia
	NCT02630199 Kim et al. Clin Cancer Res 2021	Ceralasrtib / Paclitaxel Ceralasertib 240mg BD d1-14 Paclitaxel 80mg/m^2 d1, d8, d15 q28d	Phase 1	Advanced solid tumors N= 57 patients	-	ORR: 22.6% ORR: 33.3% among 33 patients with immunotherapy-resistant melanoma	. Neutropenia 68% . Anemia 44% . Thrombocytopenia 37%
	NCT03188965 Yap et al. Cancer Discov 2020	BAY 1895344 MTD 40 mg BID 3 days on/4 days off	Phase 1	Advanced solid tumor N=21	-	<u>ORR 19.0% (4 of 21)</u> . All 4 responders presented *ATM* loss/mutation . 1 long term responder HGSC platinum-resistant *BRCA*mut PARPi-resistant . PD-L1+ pretreatment showed upregulation of PD-L1 on treatment	. G≥3 Anemia 81.8% . G≥3 Anemia 54.5% . G≥3 Thrombocytopenia 45.5%
	NCT02264678 Yap et al. Clin Cancer Res 2021	Ceralasertib / Carboplatin MTD Ceralasertib 40mg BID d1,d2 Carboplatin AUC5 d1 q21d	Phase 1	Advanced solid tumors N=36	-	<u>. 2 partial responses in patients with absent or low ATM or SLFN11 protein expression</u>	. Anemia G≥3 39% . Thrombocytopenia G≥3 36% . Neutropenia G≥3 25%
	NCT Hong et al. J Clin Oncol 2016	Prexasertib MTD 105mg/m^2 d1 q14d	Phase 1	Advanced solid tumors N=45	-	2 partial responses in patients with squamous cell carcinomas	. Neutropenia G4 73% . Febrile neutropenia 7%
	NCT01115790 Hong et al. Clin Cancer Res 2018	Prexasertib Prexasertib 105mg/m^2 d1 q14d	Phase 1 Expansion cohort	Squamous cell carcinomas anus, head and neck, and lung N=101	ORR	<u>ORR 15%</u> for annal cancer and <u>5%</u> for head and neck	. Neutropenia G4 71% . Febrile neutropenia 12%

CHK1	NCT02124148 Hong et al. Clin Cancer Res 2021	Prexasertib / Samotolosib (PIK3CAi) MTD Prexasertib 105mg/m2 d1 q14d Samotolisib 150mg-BID	Phase 1	Solid tumors with *PIK3CA* mutations and TNBC N=53	-	<u>ORR: 15.4%</u> 13.3% tumors with *PIK3CA* mutation and 25% TNBC	. Thrombocytopenia 62.3% . Neutropenia 94.3% . Nausea 52.8%
	NCT03057145 Do et al. Clin Cancer Res 2021	Prexssertib / Olaparib MTD Prexasertib 70mg/m^2 d1 and 15 Olaparib 100mg BD, d1-5, 15-19 q28d	Phase 1	Advanced solid tumor Expansion cohort HGSOC PARPi resistant N=29	-	Partial responses in 4 of 18 patients with PARPi resistant HGSOC	. Thrombocytopenia 66% . Neutropenia 86% . Anemia (72%)
	NCT03495323 Do et al. Cancer Imm imm 2021	Prexasertib / LY3300054 (anti-PD-L1) MTD Prexasertib 70mg/m^2 d1 and 15 LY3300054 700mg d1 and 15 q28d	Phase 1	Advanced solid tumor N=17	-	Partial response in 3 patients with *CCNE1* amplified tumors	Dose limiting toxicities: 1 grade 4 neutropenia for more than 5 days, 1 febrile neutropenia, 1 AST/ALT elevation
	NCT02797977 Banerji U et al. ASCO 2019	SRA737 / Gemcitabine MTD SRA737 500mg/d Gem 250mg/m^2 d1 d8 q 21d	Phase 1	Advanced solid tumor N=81 (evaluable) 28 HGSC cohort	-	<u>ORR 11.7% (6 of 51)</u> FA pathway/*BRCA*mut ORR 25% (4 of 16)	. Nausea 50.7% . Vomiting 50.4% . Fatigue 43.2% . Diarrhea 45.3% . Anemia 33.1%
	NCT02632448 Chu et al. ASCO 2021	LY2880070 / Gemcitabine MTD LY2880070 50mg BD d1-5 every week Gemcitabine 100mg/m^2 d1,8,15 q21d	Phase 1	Advanced solid tumors N=44	-	Partial response in 1 of 12 patients with HGSOC	Most common AEs: fatigue, nausea and vomiting
WEE1	NCT01748825 Do et al. J Clin Oncol 2015	Adavosertib MTD 225mg 2qd for 2.5days w1 and w2 q21d	Phase 1	Advanced solid tumor N=25	-	2 partial responses in patients with *BRCA1/2* mutated tumors	-
	NCT00648648 Leijen et al. J Clin Oncol 2016	Adavosertib / Gemcitabine or Cisplatin or Carboplatin MTD Gemcitabine 1,000 mg/m^2 d1,d8,d15, adavosertib 175mg QD for 2.0days weekly for 3weeks q28d Cisplatin 75 mg/m^2 , adavosertib 200mg BD for 2.5days q21d Carboplatin AUC 5, adavosertib 225mg 2qd for 2.5days q21d	Phase 1	Advanced solid tumors N=202	-	<u>ORR: 17 (10%)</u> of 176 evaluable patients	Combo gem vs cis vs carbo: Anemia ≥G3: 18 vs 4 vs 20% . Neutropenia ≥G3: 36 vs 13 vs 24% . Thrombocytopenia G≥3: 31 vs 11 vs 41% . Diarrhea ≥G3: 2 vs 2 vs 15% . Vomiting: 22 vs 53 vs 44% . Fatigue: 58 vs 62 vs 59%
	NCT02508246 Mendez Clin Cancer Res 2018	Adavosertib / Cisplatin / Docetaxel MTD Adavosertib 150mg 2qd for 2.5days d1-3 Cisplatin 25 mg/m^2, docetaxel 35 mg/m^2 d1-8-15 q28d	Phase 1	Stage 3 and 4, treatment naive head and neck squamous cell carcinoma N=12	-	Partial responses in 5 of 10 evaluable patients	-
	NCT02037230 Cuneo et al. J Clin Oncol 2019	Adavosertib / Gemcitabine / Radiotherapy MTD Gemcitabine 1,000 mg/m^2 d1,8 Adavosertib 150mg d1,2,8,9 q21d	Phase 1	Locally advanced unresectable pancreatic cancer N=34	-	PFS 9.4mo OS 21.7mo	8 (24%) patients with DLT in level 1 dose
	NCT01748825 Takebe et al.	Adavosertib MTD	Phase 1	Advanced solid tumors N=42	-	ORR: 6 (14%) 4 of 10 HGSOC and 2 of 3 endometrial 2 CCNE1 overexpression	1 patient rapid progression with *WEE1* mutation and *PKMYT1* overexpression

	Clin Cancer Res 2021	Adavosertib 300mg QD d1-5, 8-12 q21d					
	NCT04158336 Tolcher et al. AACR 2021	ZN-c3 MTD 300mg QD continuously	Phase 1	Advanced solid tumors N=39	-	2 partial responses of 16 evaluable patients	

MTD, maximum tolerated dose; HGSOC, high-grade serous ovarian carcinoma; QD, once daily; BD, twice daily; AUC, area under the curve; ORR, overall response rate; PFS, progression-free survival; PARPi, PARP inhibitor

Supplementary table 4. Results of phase 2 clinical trials using ATR, CHK1, and WEE1 inhibitors

Target	Study Identifier	Agents / Schedule	Design	Patients/Accrual	Primary Endpoint	Results	Comments
ATR	NCT 02595892 Konstantinopoulos et al. Lancet Oncol 2020	Gemcitabine / Berzosertib Vs Gemcitabine Gem 1000 mg/m^2 d1 d8 q21d, berzosertib 210 mg/m^2 d2 d9 q21d	Randomized phase 2	Platinum resistant HGSOC, unlimited prior lines, up to 1 prior regimen in platinum-resistant N=70	PFS	Median PFS Gem/Berzo: 22.9 wks Gem:14.7 wks HR=0.57	. Gem/Berzosertib: Thrombocytopenia G≥3: 24% Neutropenia G≥3: 47% . In Gem alone: Thrombocytopenia G≥3: 6% Neutropenia G≥3: 39%
	NCT02567409 Pal et al. ASCO 2021	Berzosertib / Cisplatin / Gemcitabine Cisplatin 70mg/m^2 d1 Gemcitabine 1g/m^2 d1, d8 q21d Cisplatin 60mg/m^2 d1 Gemcitabine 875mg/m^2 d1, d8 plus berzosertib at 90mg/m^2 d2, d9 q21d	Randomized Phase 2	First line metastatic urothelial carcinoma N= 87	PFS	PFS: 8.0mo vs 8.0 mo ORR: 63% vs 54%	. Thrombocytopenia G≥3 39% vs 59% . Neutropenia G≥3 27% vs 37%
	NCT03780608 Kuon et al. ASCO 2021	Ceralasertib / Durvalumab Ceralasertib 240mg BD d1-14 Paclitaxel 80mg/m^2 d1, d8, d15 Durvalumab 1500mg d1 q28d	Phase 2	Metastatic melanoma after progression on anti-PD-1 or anti-PD-L1 N=30	ORR	ORR: 30% PFS: 7.1 mo	. Anemia G3 33.3% . Neutropenia G≥3 16.7% . Thrombocytopenia G≥3 23.4%
	NCT03462342 Wethington et al. ASCO 2021	Ceralesertib / Olaparib Ceralesertib 160mg QD d1-7 Olaparib 300mg BD continuously q28d	Phase 2 Single arm	Platinum-sensitive ovarian cancer progressing on PARPi N=13	ORR	ORR 46% (6 of 13 patients)	. Anemia G≥3: 1 patient (7.7%) . Neutropenia G≥3: 1 patient (7.7%) . Thrombocytopenia G≥3: 3 patients (23.1%)
CHK1	NCT 02203513 Lee JM et al. Lancet Oncol 2019	Prexasertib Prexasertib 105mg/ m^2 d1 q14d	Phase 2, Single Arm	HGSOC or HG endometrioid ovarian cancer, germline *BRCAwt* N=24 (evaluable)	ORR	ORR: 33% 8 patients, all PRs 4 of 8 PRs in tumors with CCNE1 overexpression	. Thrombocytopenia G≥3: 25% . Neutropenia G≥3: 93% . Febrile neutropenia G3: 7%
	NCT02203513 Lampert EJ et al. ASCO 2020	Prexasertib Prexasertib 105mg/m^2 d1 q14d	Phase 2 Single arm	HGSOC, *BRCA* mut N=18	-	ORR: 11% 2 patients, both plat S, one with prior PARP inhibitor treatment	. Thrombocytopenia G≥3: 14% . Neutropenia G≥3: 82% . Febrile neutropenia G3: 5%
	NCT02797964 Plummer et al. ASCO 2020	SRA737 Expansion cohort 800mg/d	Phase 1/2	Expansion cohort HGSOC enriched for *CCNE1* amp N=38	DCR	DCR 54% . No PR/CR. 2 pts maximal tumor reduction 29% and 27% . No association *CCNE1* amp	. Diarrhea 68.2% (G≥3: 19%) . Nausea 66.4% (G≥3: 2.8%) . Vomiting 51.4% (G≥3: 2.8%) . Fatigue 46.7% (G≥3: 2.8%)
	NCT02735980 Byers LA et al. Clin Lung Cancer 2021	Prexasertib Prexasertib 105mg/m2 d1 q14d	Phase 2 Parallel-cohort	Cohort 1: Platinum-sensitive Cohort 1: Platinum-resistant Small cell lung cancer	ORR	ORR 5.2% and 0% PFS 1.41 mo and 1.36mo	. Thrombocytopenia 51.8% and 50.0% . Neutropenia: 69.5% and 73.3% . Febrile neutropenia: 19.6% and 1%

WEE1	NCT 01164995 Leijen et al. J Clin Oncol 2016	Carboplatin / Adavosertib Carboplatin AUC 5 d1, adavosertib 225mg 2qd for 2.5days q21d	Phase 2, Single Arm	*TP53*-mutated ovarian cancer, refractory or resistant (<3 months) to 1st line platinum N=21 (evaluable)	ORR	<u>ORR: 43%</u> 9 patients (1 CR + 8 PR) PFS 5.3mo OS 12.6mo	. Thrombocytopenia G≥3: 48% . Neutropenia G≥3: 37%
	NCT 02151292 Lhereux et al. Lancet Oncol 2021	Gemcitabine / Adavosertib Vs Gemcitabine/Placebo Gemcitabine 1000mg/m^2 D1 D8 D15 ± adavosertib 175mg daily D1–2, D8–9, D15–16 q28d	Randomized, Phase 2	Platinum Resistant/Refractory HGSOC, unlimited prior lines N=124	PFS	<u>Median PFS</u> Gem/Adavo: 4.6 months Gem/Placebo: 3.0 months HR=0.56, ORR 21% vs 3%	Gem/Adavosertib: . Thrombocytopenia G≥3: 31% . Neutropenia G≥3: 62%
	NCT02272790 Moore K et al. ASCO 2019	Adavosertib 175–225 mg BD, several schedules + gemcitabine 800mg/m^2 d1, d8 ,d15 or paclitaxel 80mg/m^2 d1, d8, d15 or carboplatin AUC 5, d1 or pegylated liposomal doxorubicin 40 mg/m^2 d1	Non-randomized Phase 2	Platinum Resistant HGSC, ≤4 prior lines N=94	ORR	<u>ORR 31.9%</u> ORR Carboplatin/Adavosertib 66.7% (8/12) ORR *CCNE1*amp 46.1% (6/13)	. Anemia ≥G3: 33.0% . Neutropenia ≥G3: 45.7% . Thrombocytopenia G≥3: 31.9% . Diarrhea ≥G3: 10.6% . Vomiting ≥G3: 10.6%
	NCT 01164995 Oza et al. Clin Cancer Res 2020	Carbo / Taxol / Adavosertib Vs Carbo / Taxol / Placebo Paclitaxel 175mg/m^2 D1 + carboplatin AUC 5, D1 + adavosertib 225mg BD for 2.5 days q21d	Randomized Phase 2	Platinum Sensitive *TP53*-mutated ovarian cancer N=121	ePFS (by volumetric RECIST 1.1.)	<u>Median ePFS</u> Carboplatin/Taxol/Adavo: 7.9 months Carboplatin/Taxol/Placebo: 7.3 months HR=0.63	Carbo/Taxol/Adavosertib . G≥3 AEs: 78% . Anemia 53% . Diarrhea 75% . Vomiting 63% Carbo/Taxol/Placebo . G≥3 AEs:35% . Anemia 32% . Diarrhea 37% .Vomiting 27%
	NCT03668340 Liu et al. J Clin Oncol 2021	Adavosertib Adavosertib 300mg QD d1-5, 8-12 q21d	Phase 2 Single arm	HGSEC second line N=34	ORR PFS6mo	<u>ORR: 29.4%</u> <u>PFS6mo: 47.1%</u> PFS: 6.1mo	. Diarrhea 85.3%, G3 5.9% . Nausea 61.8%, G3 8.8% . Fatigue 64.7%, G3 23.5% . Anemia 67.6%, G3 23.5% . Thrombocytopenia 61.8%, G3 14.7% . Neutropenia 44.1%, G3-4 32.3%
	NCT03012477 Keenan et al. Clin Cancer Res 2020	Adavosertib / Cisplatin Cisplatin 75mg/m^2 d1 Adavosertib 200mg BD for 2.5d d1-3 q21d	Phase 2 Single arm	Metastatic TNBC first or second line N=34	ORR	<u>ORR: 26%</u> PFS: 4.9mo	. Diarrhea 35%, G3 21% . Nausea 50%, G3 6% . Anemia 29%, G3 12% . Neutropenia 29%, G3 18%
	NCT03253679 Fu et al. AACR 2021	Adavosertib Adavosertib 300mg QD d1-5, 8-12 q21d	Phase 2 Single arm	*CCNE1* amplified (CN >7) tumors N=29	ORR	<u>ORR: 26%</u> Partial response in 5 of 13 (38%) ovarian cancer patients Partial response in 1 melanoma and 1 urothelial patients	. Neutropenia G3-4 24% . Thrombocytopenia G3-4 17% . Fatigue G3-4 17% . Diarrhea G3-4 17%
	NCT03579316 Coleman et al. ASCO 2021	Adavosertib / Olaparib Adavosertib 300mg QD d1-5, 8-12 q21d	Randomized Phase 2	Platinum-sensitive ovarian cancer progressing on PARPi N=80	ORR	<u>ORR: 23% and 29%</u> PFS: 5.5mo and 6.8mo <u>*BRCA1/2* wild type ORR 31% and 39%</u>	Adavosertib + olaparib . Neutropenia G3-4 10% . Thrombocytopenia G3-4 10% . Fatigue G3-4 12% . Diarrhea G3-4 12%

		Adavosertib 150mg QD d1-3, 8-10 + Olaparib 200mg BD q21d					

HGSOC, high-grade serous ovarian carcinoma; QD, once daily; BD, twice daily; AUC, area under the curve; ORR, overall response rate; DCR, disease control rate; PR, partial response; PFS, progression-free survival; PARPi, PARP inhibitor

Supplementary Table 5. Candidate synthetic lethal associations with ATR, CHK1, and WEE1 inhibitors based on clinical and preclinical data

Molecular alteration	Causative mechanisms and frequency	Synthetic lethal partner	Mechanism of synthetic lethality
ATM loss	*ATM* missense mutations 3% of HGSOC	ATR inhibition	Cells with ATM loss rely on ATR response to orchestrate DNA repair and cell cycle checkpoint
H3K36me3 loss	Loss of SETD2, overexpression of KDM4A, or mutation of histone H3.3 *KDM4A* amplification in 6% of HGSC	WEE1 inhibition	Downregulation of *RRM2* expression leading to decrease in nucleotide pool
SWI/SNF complex dysfunction	*ARID1A* mutation in 50% of CCC and 30% EOC *SMARCA4* mutation in nearly all SCHOT	ATR inhibition	DNA decatenation leading to increased dependency on the G2/M checkpoint
Alternative telomere lengthening*	Multistep process*, ATRX* mutation is part of it but is insufficient alone.	ATR inhibition	Dependency on RPA-ATR activation of HRR to elongate the telomere

HGSOC, high-grade serous ovarian carcinoma; CCC, clear-cell carcinoma; EOC, endometrioid ovarian carcinoma; SCHOT, small-cell hypercalcemic ovarian tumor; HRR, homologous recombination repair

* Contradictory results from different studies (refs. 201 and 202)

Supplementary table 6. Candidate biomarkers of response to ATR, CHK1, and WEE1 inhibitors

Group	Molecular alteration
Genomic alterations that lead to RS	*TP53* mutations
	CCNE1 amplification
	RB1 loss
	CDKN2A mRNA downregulation
	HRR alterations
	NER alterations
	MYC amplification
	NF1 loss
Markers of cellular response to RS	pATR
	pCHK1
	pRPA
	pH2AX
	pKAP1
Synthetic lethal associations	ATM loss
	H3K36me3 loss (due to loss of SETD2, overexpression of KDM4A, or mutation of histone H3.3)
	SWI/SNF complex dysfunction (due to *ARID1A* mutation, *SMARCA4* mutations)
Markers of treatment resistance	FAM122A low
	MYT1 upregulation
	p21 upregulation
	YAP expression
Pharmacodynamic biomarkers	ATR inhibitor: ↑ pH2AX, ↑ fork instability, ↓ pCHK1, ↑ pKAP1
	CHK1 inhibitor: ↑ pH2AX, ↑ fork instability, ↑ pCHK1, ↑ pKAP1, ↑RPA
	WEE1 inhibitor: ↑ pH2AX, ↑ fork instability, ↑ pCHK1, ↑RPA, ↓pCDK1

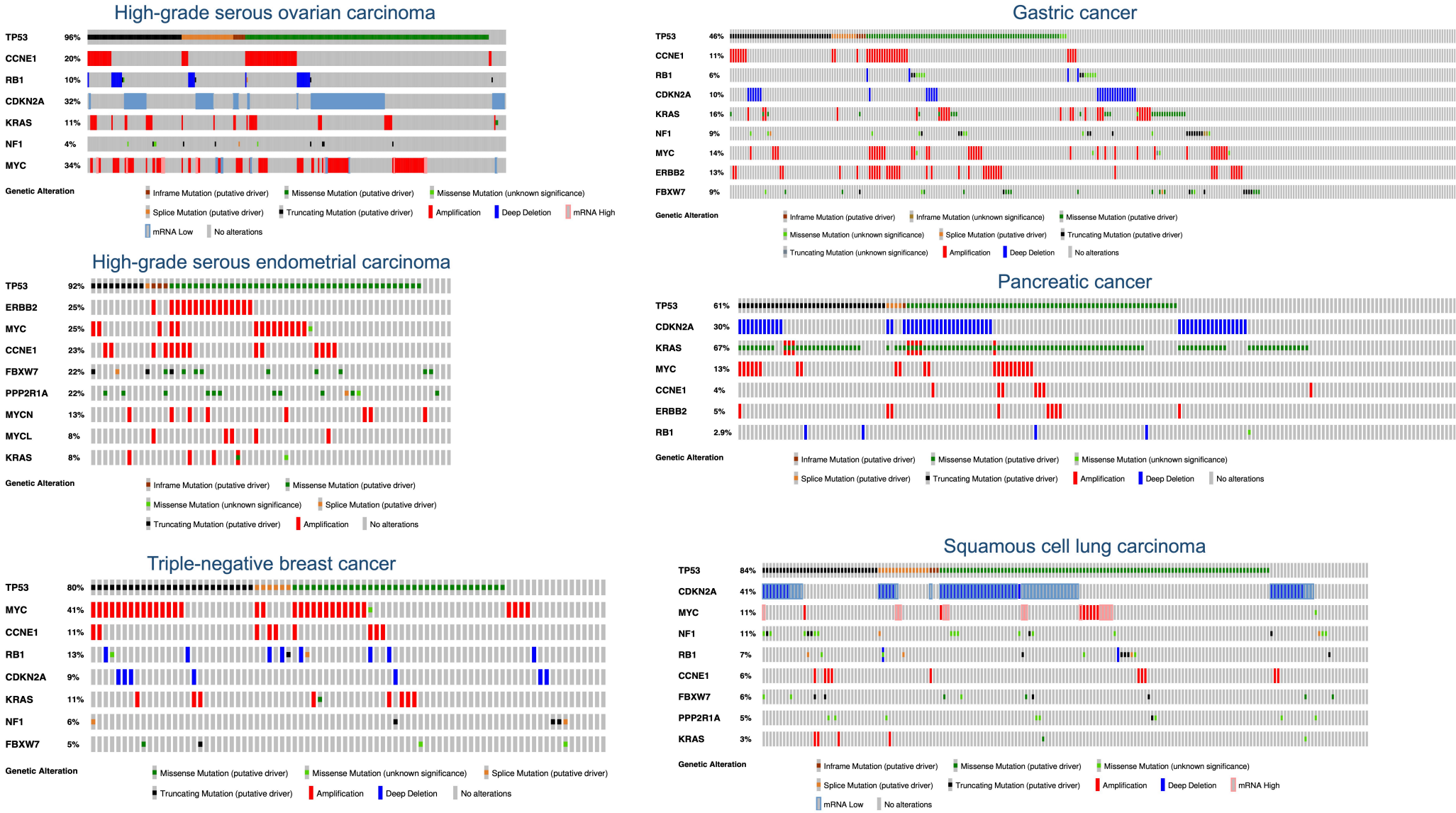
RS, replication stress; HRR, homologous recombination repair pathway; NER, nucleotide excision repair pathway

Supplementary table 7. Selected ongoing clinical trials with ATR, CHK1, and WEE1 inhibitors

Strategy	Design	Target	Agents / Schedules	Patients	Biomarkers as inclusion criteria	Status	Study Identifier
Biomarker driven monotherapy trials	Phase 2	CHK1	Prexasertib 105mg/m d1, d15 every 28 days	Advanced solid tumors exhibiting replication stress or homologous recombination deficiency	. *MYC* amplification, *CCNE1* amplification, *RB1* loss, *FBXW7* mutation . Somatic mutation in *BRCA1, BRCA2, PALB2, RAD51C, RAD51D, ATR, ATM, CHK2*, or FA pathway genes	Active, not recruiting	NCT02873975
	Phase 2	WEE1	Adavosertib once daily, d1 to 5 and d8 to 12, 21-days cycle	Advanced refractory solid tumors	. *CCNE1* amplification	Suspended	NCT03253679
Combination with chemotherapy	Phase 1	ATR	Gemcitabine 480–800 mg/m^2 d1 and d8, carboplatin AUC 4 d1, berzosertib 90–120 mg/m^2 d2 and d9, every 21 days	Platinum-sensitive ovarian cancer	. No	Recruiting	NCT02627443
	Phase 1	ATR	Gemcitabine d1 and 8, BAY 1895344 twice per day d1 to d3 and d8 to d10	. Incurable solid tumors . Expansion cohort platinum resistant ovarian cancer	. No	Not yet recruiting	NCT04616534
	Phase 1	WEE1	Gemcitabine and nab-paclitaxel d1,8 and 15; adavosertib QD d1,2,8,9,15, and 16	Unresectable pancreatic adenocarcinoma	. No	Active, not recruiting	NCT02194829
PARPi synergism	Phase 2	ATR	Ceralasertib 160mg d1 to d7, olaparib 300mg twice per day d1 to d28 every 28d	. Recurrent HGSC irrespective of platinum sensitivity	. No	Recruiting	NCT03462342 (CAPRI trial)
	Phase 2	ATR	.Ceralasertib 160mg d1 to d7, olaparib 300mg twice per day d1 to d28 every 28d .Ceralasertib 160mg d1 to d7 every 28d as single agent	. Recurrent gynecological cancers, including CCC and rare subtypes with frequent *ARID1A* loss	. No . *ARID1A* enriched	Recruiting	NCT04065269 (ATARI trial)
	Phase 2	ATR WEE1	. Olaparib alone . Olaparib and capivasertib . Olaparib and ceralasertib . Olaparib and adavosertib	. Advanced solid tumors with positive predictive biomarkers	. Mutations in HR genes or *ATM, CHK2, MRN (MRE11/ NBS1/ RAD50), APOBEC* – Olaparib and ceralasertib . Mutations in *TP53* or *KRAS* – Olaparib and adavosertib	Not yet recruiting	NCT02576444 (OLPACO trial)
	Randomized phase 2	ATR	. Ceralasertib 160mg d1 to d7, olaparib 300mg twice per day d1 to d28 every 28d . Placebo, olaparib	. HGSC relapsed after at least 6 months maintenance PARP inhibitor	. No	Recruiting	NCT04239014 (DUETTE trial)
	Randomized phase 2	ATR WEE1	. Olaparib 300 mg BD continuously . Ceralasertib 160 mg QD d1-7 + Olaparib 300 mg BD continuously q28d . Adavosertib 150 mg BD d1-3, d8-10+ Olaparib 300 mg BD continuously q21d	. Metastatic triple negative breast cancer	. No	Active, not recruiting	NCT03330847 (VIOLETTE trial)
	Phase 1	ATR	. BAY1895344 twice per day and niraparib once per day	. Advanced solid tumors and ovarian cancer . Two expansion ovarian cancer cohorts: PARPi naïve, PARPI resistant	. DDR deficiency	Recruiting	NCT04267939
	Phase 1	WEE1	. Olaparib twice per day on d1 to d5, d15 to d19, adavosertib once per day on d8 to 12 and d22 to d26	. Advanced refractory solid tumor with DDR mutations	. Mutations in *BRCA1/2, BRIP, FANCA, PALB2, ATM* . *CCNE1* amplification	Recruiting	NCT04197713 (STAR trial)
	Phase 1-2	ATR	. Ceralasertib . Ceralasertib + carboplatin . Ceralasertib + Olaparib . Ceralasertib + durvalumab	. Head and neck squamous cell carcinoma, non-small cell lung carcinoma, gastric, breast and ovarian cancer	. No	Recruiting	NCT02264678
Overcoming PARP resistance	Phase 1-2	ATR	. Olaparib and ceralasertib	. Platinum-sensitive ovarian cancer post PARPi progression	. Mutations in *BRCA1/2*	Recruiting	NCT02264678

	Phase 1	ATR	. Niraparib once daily + M4344 100–200 mg daily	. Ovarian cancer patients with disease progression while on PARPi	. No	Not yet recruiting	NCT04149145
	Phase 1	CHK1	. Olaparib intermittent schedule, and prexasertib d1 and d15 every 28 days	. Advanced solid tumors with prior PARPi treatment	. No . Expansion cohort will include only HGSC with *BRCA1/2* mutations	Active, not recruiting	NCT03057145
	Phase 2	WEE1	. Adavosertib daily d1 to d5 and d8 to d12 every 21 days . Adavosertib daily d1 to d3 and d8 d10) and olaparib twice daily d1to d21 every 21 days	. Ovarian cancer patients with disease progression while on PARPi	. No	Recruiting	NCT03579316
Immunotherapy combinations	Phase 1-2	ATR	. Berzosertib and avelumab	. Advanced solid tumors	. DDR deficient: ARID1A, ATM, ATR, ATRX, BAP1, BARD1, BRCA1/2, BRIP1, CDK12, CHEK2, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCM, MRE11A, MSH2, NBN (NBS1), PALB2, RAD51, RAD51C, RAD51D, SMARCB1, and VHL, or other related genes at the discretion of the principal investigator	Recruiting	NCT04266912
	Phase 1	ATR	. BAY1895344 and pembrolizumab	. Advanced solid tumors	. No	Recruiting	NCT04095273

QD, once daily; BD, twice daily; AUC, area under the curve; HGSC, high-grade serous ovarian carcinoma; CCC, clear cell carcinoma; HR genes, homologous recombination genes; PARPi, PARP inhibitor: DDR, DNA damage response.



Supplementary figure 1. Prevalence of various mechanisms of high replication stress in high-grade serous ovarian carcinoma, high-grade serous endometrial carcinoma, triple-negative breast cancer, gastric cancer, pancreatic cancer and squamous cell lung carcinoma from TCGA cohorts.