**Supplementary figures for:**

**The novel ATR inhibitor BAY 1895344 is efficacious as monotherapy and combined with DNA damage-inducing or repair-compromising therapies in preclinical cancer models**

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**Supplementary Figures**

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**Figure S1. Waterfall plots of *in vitro* anti-proliferative IC50 values of BAY 1895344 in a panel of cancer cell lines.** (**A**) Cancer cell lines harboring ATM mutations as well as (**B**) lymphoma cell lines (both marked in green) showed increased ATRi sensitivity demonstrated by IC50 values of < 100 nM (summarized in Table S3).

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**Figure S2. Body weight change in human (A-B) GRANTA-519 mantle cell lymphoma (C) A2780 ovarian cancer, (D) PC-3 prostate cancer, (E) LOVO colorectal cancer and (F) REC-1 xenograft models in mice.**

The optimal dose and treatment schedule for BAY 1895344 was 50 mg/kg, p.o., 2QD 3 days on/ 4 days off, based on efficacy and tolerability in human xenograft models in mice (*n* = 10/group). AZD6738 and M6620 were used at their known maximal tolerated doses: AZD6738 (50 mg/kg, QD, p.o.), M6620 (100 mg/kg, QD, p.o.). The dosing schedule for 5‑FU was 50 mg/kg, QW, i.p.

Body weight change is defined as the percentage change of the actual mean body weight compared to initial mean body weight (at the time point of treatment start).

i.p., intraperitoneally; p.o., per os/ orally; QD, once daily; 2QD, twice daily; QW, once weekly.



**Figure S3. Body weight change following treatment with BAY 1895344 in combination with the chemotherapy drug carboplatin, external beam radiation therapy (EBRT), the PARPi olaparib, or the AR antagonist darolutamide, in xenograft tumor models in mice.**

(**A**) Body weight change in the IGROV-1 human ovarian cancer xenograft model in female nude (nu/nu) mice (*n* = 10/group) treated with BAY 1895344 (10 or 20 mg/kg, p.o., QD, 2 days on/ 5 days off) in combination with carboplatin (50 mg/kg , i.p., QW) or BAY 1895344 as a single agent at MTD (50 mg/kg, p.o., 2QD, 3 days on/ 4 days off).

(**B**) Body weight change in the human LOVO colorectal cancer xenograft model in female nude mice (*n* = 10/group) treated with BAY 1895344 (20 or 50 mg/kg, p.o., 2QD 2 days on/ 5 days off) in combination with radiation (EBRT). Radiation (5 Gy) was applied directly on the subcutaneously growing tumors on days 12 and 27 after tumor inoculation.

(**C**). Body weight change in the MDA-MB-436 human breast cancer xenograft model in female NOD/SCID mice (*n* = 10/group) treated with BAY 1895344 (20 mg/kg, p.o., 2QD, 3 days on/ 4 days off) in combination with olaparib (50 mg/kg, i.p., QD) or BAY 1895344 as single agent only at MTD (50 mg/kg, p.o., 2QD, 3 days on/ 4 days off).

(**D**) Body weight change in the 22Rv1 human prostate cancer xenograft model in male SCID mice (*n* = 10/group) treated with BAY 1895344 (20 mg/kg, p.o., 2QD, 3 days on/ 4 days off) in combination with olaparib (20 mg/kg, i.p., QD) or olaparib as single agent only at MTD (50 mg/kg, i.p., QD).

(**E**) Body weight change in the hormone-dependent human LAPC-4 human prostate cancer xenograft model in male SCID mice (*n* = 11/group) treated with BAY 1895344 (20 mg/kg, p.o., 2QD 3 days on/ 4 days off) in combination with darolutamide (100 mg/kg, p.o., QD).

(**F**) Body weight change in the hormone-dependent human LAPC-4 human prostate cancer xenograft model in male SCID mice (*n* = 10/group) treated with BAY 1895344 (20 mg/kg, p.o., 2QD 3 days on/ 4 days off) in combination with darolutamide (100 mg/kg, p.o., QD) and radiation (5 Gy Q7Dx2).

Body weight change is defined as the percentage change of the actual mean body weight compared to initial mean body weight (at the time point of treatment start).

Gy, Gray; i.p. intraperitoneally; p.o., per os/ orally; QD, once daily; 2QD, twice daily; QW, once per week.

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**Figure S4. DDR gene expression levels in LAPC-4 cells treated with BAY 1895344 in combination with the AR antagonist darolutamide.**

(**A-B**) BRCA1, (**C-D**) EXO1 and (**E-F**) MCM10 gene expression levels (relative expression to cyclophilin A) were determined by quantitative PCR after 24 or 48 h treatment. All values are shown as mean±SD of three biological replicates.

LAPC-4 cells were stimulated with 1 nM methyltrienolone (R1881), a synthetic androgen agonist and treated with 75 nM BAY 1894344, 2 µM darolutamide or a combination of both, for 24 or 48 h. Treatment effects were assessed by comparison with the respective androgen treated sample (R1881) and statistical analysis was performed using Dunnett´s multiple comparisons test. \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001