838

Synergistic *in vivo* activity of the ATR inhibitor BAY 1895344 in combination with the targeted alpha therapy radium-223 dichloride in a preclinical tumor model mimicking bone metastatic castration-resistant prostate cancer (mCRPC)

Antje Margret Wengner[1], Gerhard Siemeister[1], Ulrich Luecking[1], Julien Lefranc[1], Arne Scholz[1], Mari Suominen[2], Kirstin Meyer[1], Eleni Lagkadinou[1] and Dominik Mumberg[1]

[1] Bayer AG, Pharmaceutical Division, Berlin, Germany, [2]Pharmatest Services Ltd, Turku, Finland

INTRODUCTION

- Prostate cancer is frequently associated with metastases to bone. Bone metastases are often osteoblastic, causing the formation of fragile bone, increased risk of fractures, severe bone pain, significant morbidity and poor prognosis.
- Radium-223 dichloride (Ra-223, Xofigo®), an alpha particle (α)-emitting calcium-mimetic that selectively binds to hydroxyapatite in the bone, provides targeted radiation therapy against bone metastases[1] and improves the overall survival of prostate cancer patients with bone metastases.[2]
- Xofigo® has been approved throughout the world for the treatment of castration-resistant prostate cancer with symptomatic bone metastases (mCRPC) and no known visceral metastatic disease.
- In a mouse model of osteolytic breast cancer bone metastasis, Ra-223 reduced the development of osteolytic lesions and improved survival by inducing DNA double strand breaks in tumor cells and by decreasing the number of osteoclasts.[3]
- The ataxia telangiectasia and Rad3-related (ATR) kinase plays a central role in the DNA damage response (DDR) of eukaryotic cells being activated by a broad spectrum of DNA damage, including double-strand breaks (DSB) and lesions derived from interference with DNA replication as well as increased replication stress.[4,5,6]
- ATR has the ability to affect the survival of oncogenically compromised cells through DDR activation, and thus, represents an intriguing potential therapy target in cancers characterized by increased DNA damage, deficiency in DDR, or replication stress.
- BAY 1895344 is a potent and selective oral ATR kinase inhibitor (ATRi), which exhibits high preclinical monotherapy efficacy in various cancers with DDR deficiencies.[7]
- We have previously reported that combination therapy with the ATRi BAY 1895344 and Ra-223 showed synergistic anti-tumor efficacy in the intratibial human LNCaP mCRPC xenograft model in mice.[7]
- The aim of this study was to deepen our understanding of the ATRi BAY 1895344 and Ra-223 combination treatment by assessing the effect of different doses and dosing schedules on the anti-tumor efficacy of the combination treatment, and by evaluating the mode-of-action in the intratibial LNCaP-Luc prostate cancer model mimicking mCRPC.

RESULTS

The ATRi BAY 1895344 shows synergistic anti-tumor efficacy in combination with Ra-223 in a CRPC model mimicking bone metastasis

- Combination of BAY 1895344 with Ra-223 showed synergistic anti-tumor efficacy in comparison to corresponding monotherapies in the human LNCaP model mimicking mCRPC, as demonstrated by reduced serum PSA and PINP, markers for tumor burden and abnormal bone growth, respectively (**Fig. 1**).
- No critical body weight loss (> 10%) was observed for any treatment group.

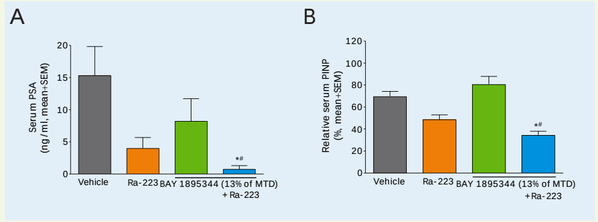

Figure 1: Serum (A) PSA and (B) PINP levels in the LNCaP mCRPC model after treatment with BAY 1895344 and/or Ra-223. Reduced PSA and PINP levels indicated inhibition the of tumor and abnormal bone growth, respectively. Intratibial LNCaP (*ATM*^{wt}, *ARID1A*^{wt}, *BRCA2*^{hmt}, *TP53*^{mut}, *MYC*^{hmt}) prostate cancer growth in the bone of male NOD/Scid mice. The mice were dosed with vehicle control, Ra-223 (300 kBq/kg, Q4W, i.v.), BAY 1895344 (20 mg/kg, QD, 2 days on/ 5 days off, p.o., 13% of MTD), or a combination of Ra-223 and BAY 1895344 for 6 weeks. BAY 1895344 single-agent MTD: 50 mg/kg, 2QD, 3 days on/ 4 days off = 300 mg/kg per week. *, P < 0.05 vs vehicle; #, P < 0.05 vs Ra-223 (one-way ANOVA on ranks followed by Dunnett's test for multiple comparisons). i.v., intravenously; mCRPC, metastatic castration-resistant prostate cancer; MTD, maximum tolerated dose; PINP, N-terminal procollagen type I; p.o., per os (orally); PSA, prostate specific antigen; Q4W, every 4 weeks; QD, once daily.

The ATRi BAY 1895344 in combination with Ra-223 inhibits pathological tumor-induced bone formation

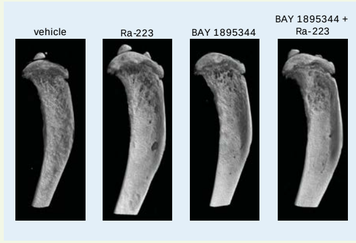

Figure 2. Representative micro computed tomography (micro-CT) images of the tumor-bearing tibia of mice inoculated intratibially with LNCaP human prostate cancer cells and treated with (A) vehicle, (B) Ra-223, (C) BAY 1895344, (D) BAY 1895344 and Ra-223. Intratibial LNCaP (*ATM*^{wt}, *ARID1A*^{wt}, *BRCA2*^{hmt}, *TP53*^{mut}, *MYC*^{hmt}) prostate tumors growing in the bone of male NOD/Scid mice. The mice were dosed with vehicle, Ra-223 (300 kBq/kg, Q4W, i.v.), BAY 1895344 (20 mg/kg, QD, 2 days on/ 5 days off, p.o.), or a combination of Ra-223 and BAY 1895344 for 6 weeks.

The ATRi BAY 1895344 in combination with Ra-223 increases DNA damage in tumor cells in the LNCaP mCRPC model

- BAY 1895344 in combination with Ra-223 showed an enhanced effect on DNA damage induction and toxicity, as indicated by an increased level of γ-H2AX foci in LNCaP tumor-bearing bone compared to respective monotherapies (**Fig. 3**).

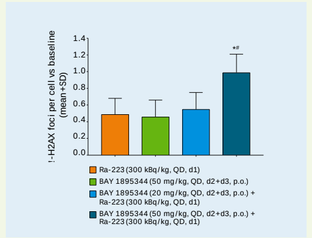

Figure 3. Induction of DNA damage in LNCaP tumor-bearing bone of NOD/Scid mice after treatment with BAY 1895344 and Ra-223 as mono or combination therapy, as determined by the number of γ-H2AX foci in tumor cells. γ-H2AX foci per cell vs baseline (average count) in intratibial LNCaP (*ATM*^{wt}, *ARID1A*^{wt}, *BRCA2*^{hmt}, *TP53*^{mut}, *MYC*^{hmt}) prostate cancer tumors grown in bone of male NOD/Scid mice. Six weeks after tumor cell inoculation, the mice were dosed with vehicle, Ra-223 (300 kBq/kg, QD, d1, i.v.), BAY 1895344 (50 mg/kg, QD, d2+d3, p.o.), or a combination of Ra 223 and BAY 1895344 (20 or 50 mg/kg). Samples were prepared 96 hours after treatment start (d5). Statistical analysis was performed using a linear fixed-effects model and the comparisons were carried out using model contrasts. The obtained p-values were adjusted for multiple comparisons. *, P < 0.05 vs vehicle; #, P < 0.05 vs Ra-223 and BAY 1895344 monotherapies. d, day; γ-H2AX, phospho-Ser139 histone protein H2AX; i.v., intravenously; p.o., per os (orally); QD, once daily.

The ATRi BAY 1895344 in combination with Ra-223 shows the highest anti-tumor efficacy when BAY 1895344 treatment starts 24 h after Ra-223

- All tested BAY 1895344 dosing schedules in combination with Ra-223 showed anti-tumor efficacy *in vivo*, as demonstrated by significant decreases in serum PSA levels (**Fig. 4A**), total bone lesion area (**Fig. 4B**), and tumor burden (BLI signal; **Fig. 4C**).
- The highest anti-tumor efficacy was achieved when BAY 1895344 treatment was started 24h after the first Ra-223 dose, with BAY 1895344 being administered once daily in weekly cycles for 2 days on/ 5 days off and Ra-223 being applied every 4 weeks.
- No critical body weight loss (> 10%) was observed for any treatment group.

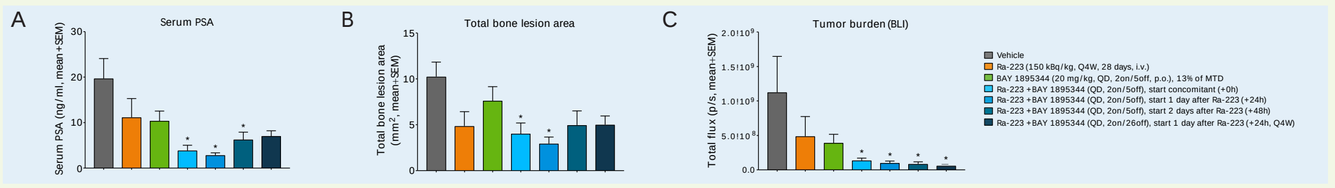

Figure 4: Anti-tumor efficacy of different dosing schedules of the ATRi BAY 1895344 in combination with Ra-223. The *in vivo* anti-tumor efficacy of different dosing schedules of BAY 1895344 in combination with Ra-223 as determined by **(A)** Serum PSA levels, **(B)** total bone lesion area (osteoblastic lesions) monitored by X-ray imaging and **(C)** tumor burden (BLI signal) in the intratibial LNCaP-Luc mCRPC model. Intratibial LNCaP-Luc (*ATM*^{wt}, *ARID1A*^{hmt}, *BRCA2*^{wt}, *TP53*^{wt}, *MYC*^{hmt}, luciferase-transfected) prostate cancer growth in the bone of male NOD/Scid mice. The mice were dosed with vehicle, Ra-223 (150 kBq/kg, Q4W, i.v.), BAY 1895344 (20 mg/kg, QD, 2 days on/ 5 days off, p.o., 13% of MTD), or a combination of Ra-223 and BAY 1895344 for 6 weeks. In combination treatments, BAY 1895344 treatment started concomitant to Ra-223 (+ 0h), 24 hours after Ra-223 (+ 24h), 48 hours after Ra-223 (+ 48h) in a 2 days on/ 5 days off weekly treatment cycle, or 24 hours after each Ra-223 dosing for 2 days on/ 26 days off (+ 24h, Q4W). *, P < 0.05 vs vehicle (one-way ANOVA on ranks followed by Dunnett's test for multiple comparisons). BLI, bioluminescent imaging; i.v., intravenously; mCRPC, metastatic castration-resistant prostate cancer; MTD, maximum tolerated dose; PSA, prostate specific antigen; p.o., per os (orally); Q4W, every 4 weeks; QD, once daily.

Varying doses of the ATRi BAY 1895344 in combination with Ra-223 show anti-tumor efficacy

- All tested doses of BAY 1895344 in combination with Ra-223 showed *in vivo* anti-tumor efficacy as demonstrated by significant decreases in serum PSA (**Fig. 5A**) or PINP (**Fig. 5B**) levels, total bone lesion area (**Fig. 5C**) and tumor burden (BLI signal, **Fig. 5D**) compared to vehicle control.
- The highest anti-tumor efficacy was achieved with 20 mg/kg BAY 1895344 administered once daily for 2 days on/ 5 days off, or with a 40 mg/kg dose administered once daily for 1 day on/ 6 days off starting 24 hours after the first Ra-223 dose and given in continued weekly cycles.
- No critical body weight loss (> 10%) was observed for any treatment group.

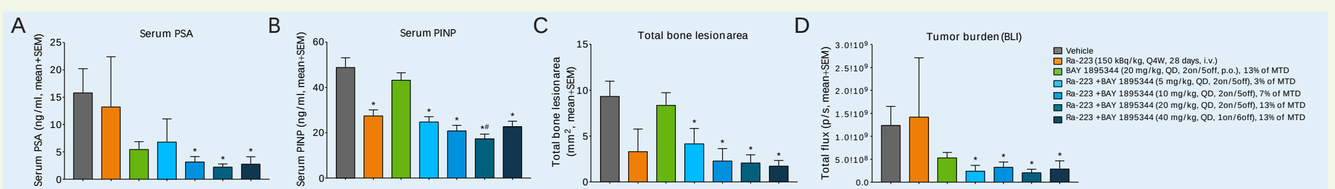

Figure 5: Anti-tumor efficacy of different doses of the ATRi BAY 1895344 in combination with Ra-223. The in vivo anti-tumor efficacy of different doses of BAY 1895344 in combination with Ra-223 as determined by **(A)** Serum PSA or **(B)** PINP levels, **(C)** total bone lesion area (osteoblastic lesions) monitored by X-ray imaging and **(D)** tumor burden (BLI signal) in the intratibial LNCaP-Luc mCRPC model. Reduced PSA and PINP levels, total bone lesion area and BLI signal indicated inhibition of tumor growth. Intratibial LNCaP-Luc (*ATM*^{wt}, *ARID1A*^{wt}, *BRCA2*^{hmt}, *TP53*^{hmt}, *MYC*^{hmt}, luciferase-transfected) prostate cancer growth in the bone of male NOD/Scid mice. The mice were dosed with vehicle, Ra-223 (150 kBq/kg, Q4W, i.v.), BAY 1895344 (20 mg/kg, QD, 2 days on/ 5 days off, p.o.), or a combination of Ra 223 and BAY 1895344 for 6 weeks. In combination treatments, BAY 1895344 was dosed once daily (QD) orally with 5, 10 or 20 mg/kg for 2 days on/ 5 days off, or with 40 mg/kg for 1 day on/ 6 days in weekly cycles; treatment started 24 hours after the first Ra-223 dosing. BAY 1895344 applied combination dose (%) related to single-agent MTD (50 mg/kg, 2QD, 3 days on/ 4 days off = 300 mg/kg per week). *, P < 0.05 vs vehicle; #, P < 0.05 vs Ra-223 (one-way ANOVA on ranks followed by Dunnett's test for multiple comparisons). BLI, bioluminescent imaging; i.v., intravenously; mCRPC, metastatic castration-resistant prostate cancer; MTD, maximum tolerated dose; PINP, N-terminal procollagen type I; p.o., per os (orally); PSA, prostate specific antigen; Q4W, every 4 weeks; QD, once daily.

CONCLUSIONS

- The ATRi BAY 1895344 shows high combination potential with DNA damage-inducing Ra-223 therapy and presents a potential new treatment option for CRPC patients with bone metastases.
- The highest anti-tumor efficacy with BAY 1895344 and Ra-223 combination therapy is achieved when BAY 1895344 treatment is initiated 24 hours after the first Ra-223 dose.
- BAY 1895344 in combination with Ra-223 shows anti-tumor efficacy with doses ranging from 3-13% of the single-agent maximum tolerated dose.
- BAY 1895344 is currently under clinical investigation in patients with advanced solid tumors and lymphomas (NCT03188965).

REFERENCES

1.Corey *et al., Prostate* 2002;**52**;20-33.
2.ALSYMPCA trial, NTC00699751, Parker *et al., NEJM* 2014;**18**;213-33.
3. Suominen *et al., JNCI* 2013;**105**;908-918.
4. Maréchal and Zou, *Cold Spring Harb Perspect Biol* 2013;**5**,a012716.
5. Karnitz and Zou, *Clin Cancer Res* 2015;**21**;4780-5.
6. Taylor and Lindsay, *Future Oncol* 2016;**12**;221-37.
7. Wengner *et al., Cancer Res* 2017;**77**;836.

ACKNOWLEDGEMENTS

Aurexel Life Sciences Ltd. (www.aurexel.com) is thanked for editorial assistance in the preparation of this poster, funded by Bayer AG.

Poster presented at the AACR Annual Meeting, April 14-18, 2018, Chicago, IL, USA