mdm2 gene amplification in estrogen receptor-positive breast cancer cells is associated with enhanced solid tumor growth and pronounced metastatic potential in humanized PDX mice and a poor outcome of luminal breast cancer disease

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PD9-07

Background

Luminal, i.e., estrogen receptor-positive (ER+) breast cancer (BC) is a heterogeneous disease in terms of tumor progression, therapy response, and relapse.[1] Additional biomarkers with a prognostic and predictive impact could facilitate advanced patient stratification and can reveal advanced therapeutic options for individual patients suffering from BC

Patients and Methods

Generation of NSG based hPDX: CD34+ hematopoietic stem cells (HSC) were isolated from the umbilical cord blood and transplanted into neonatal NOD.Cg-Prkdcscid Il2rytm1Wjl /SzJ (NSG) mice 3 hours post 1 Gy irradiation as previously described[2]. BC samples were transplanted in 7-8 weeks old humanized female NSG mice together with a s.c. 0.18 mg 17ß-estradiol pellet (Innovative Research of America). In addition, 3 previously established, patient-derived xenograft (PDX) models (PT-S2, PT-S3, and PT-S4) provided by Elisabetta Marangoni (Institute Curie, Paris, France), and 2 PDX models (PT-CTC and PT-E2) provided by Andreas Trumpp (HI-Stem, Heidelberg, Germany) were also used. Differences between tumor weight of wild type (*mdm2*^{WT}) and amplified (*mdm2*^{Amp}) hPDX models were assessed by Student`s t-test. For the functional assays *in-vitro*, ZR-75-1 BC cells were treated with the mdm2 inhibitor AMG232. Cell proliferation and apoptosis were analyzed by flow cytometry, a scratch assay was performed to analyze migration cell capacity over time, and Dunnett's multiple comparisons test was applied.

GeparTrio patient cohort for *mdm2* assessment: For this study we selected tissue specimens previously diagnosed as Luminal BC from the GeparTrio (NCT00544765) trial[3,4]. All patients within the trial received an anthracycline/taxane based neoadjuvant chemotherapy. Dual color FISH was applied on pretherapeutic TMA-samples to monitor *mdm2* gene and the cen12 region (*mdm2* score 1 corresponding to normal and score 2-3 corresponding to gain expression). Association with DFS and OS were analyzed by Cox regression models with 95% confidence interval (CI) and presented as Kaplan-Maier curves.

Primary objective: Identification and validation of biomarkers associated with successful engraftment, augmented tumor growth, and enhanced metastasis upon xenotransplantation into humanized mice.

Secondary objective: Retrospective validation and correlation of aforementioned markers with clinical outcomes of Luminal BC patients within the GeparTrio trial.

Results

Figure 1: Patient cohort included in the analysis

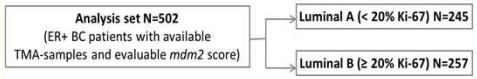
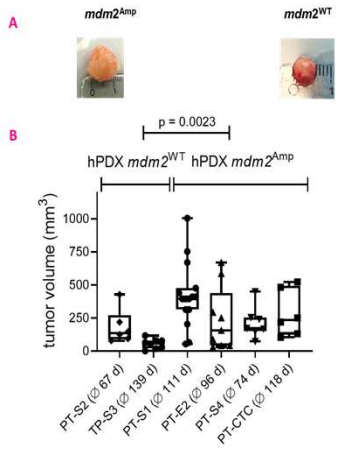


Figure 2: Representative tumor samples (A) and tumor weight (B) from *mdm2*^{Amp} and *mdm2*^{WT} hPDX models



The average days of survival of each model are indicated in brackets.

The tumor weight in all four hPDX-*mdm2*^{Amp} was significantly increased compared to hPDX-*mdm2*^{WT} (Fig. 2A & B; p = 0.0023). In addition, the hPDX-*mdm2*^{Amp} showed a significantly enhanced potential to develop lung metastasis compared to hPDX-*mdm2*^{WT} (11/14 vs 1/10, respectively, p = 0.0011; data not shown).

Figure 3: Functional assays: apoptosis (A), proliferation (B) and migration (C) on ZR-75-1 BC cells treated with the mdm2 inhibitor AMG232

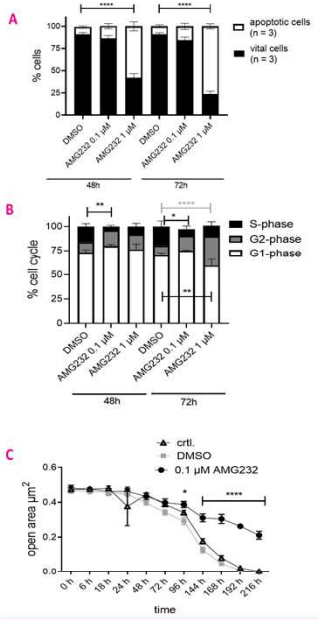
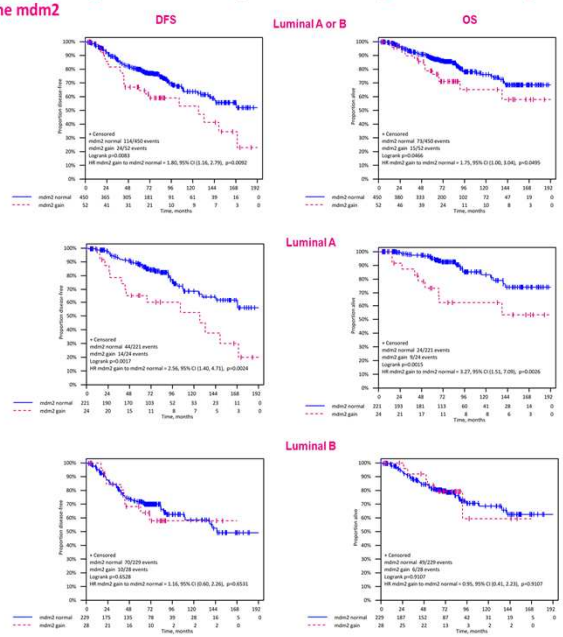


Figure 4: DFS and OS in patients with mdm2 gain versus normal expression



mdm2 targeting with 1 µM AMG232 induced a significant fraction of apoptotic cells (p < 0.0001) (Fig. 3A). In addition, AMG232 treatment caused a reduced S-phase fraction (48h: p = 0.008, 72h: p = 0.049), a reduced G1-phase after 72h (p = 0.0027) and an elevated fraction of G2 (p < 0.0001) (Fig.3B). Finally, cells exposed to *mdm2* targeting (AMG232) showed a delayed scratch overgrowth in the wound-healing assay (Fig. 3C).

Patients with *mdm2* gain showed a significantly poor DFS (HR = 1.80 [95%CI 1.16-2.79], log rank p = 0.008) and OS (HR = 1.75 [95%CI 1.00-3.05, log-rank p = 0.047) compared to those without *mdm2* alteration in the entire Luminal BC cohort. Similar results were observed in patients with Luminal-A (DFS: HR = 2.56 [95%CI 1.40-4.71], p = 0.002; OS: HR = 3.27 [95%CI 1.51-7.09], log-rank p = 0.002) but not with the Luminal-B subcohort (DFS: HR = 1.16 [95%CI 0.60-2.26], log-rank p = 0.653; OS: HR = 0.95 [95%CI 0.41-2.23], log-rank p = 0.911) (Fig. 4).

Conclusions

- An *mdm2* gene amplification propels growth and progression of ER+ BC in a preclinical humanized xenograft NSG mouse model.
- mdm2 inhibition of ER+ BC cells *in-vitro* reduces cell proliferation and migration and induces tumor cell apoptosis.
- An unfavorable impact of an *mdm2* gain on survival outcome of Luminal BC patients is mainly caused within the Luminal-A BC sub-cohort.
- Prospective studies are required to verify the suitability of *mdm2* for advanced Luminal BC stratification and therapeutic targeting of ER+ BC.

References

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Presented at: San Antonio Breast Cancer Symposium, December 7-10. 2021

This study was funded by the German Cancer Aid (Deutsche Krebshilfe, "TransLUMINAL-B" project, funding no.: 111536).

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