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Mebendazole and Radiation in Combination Increase Survival through Anticancer Mechanisms in an Intracranial Rodent Model of Malignant Meningioma

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Abstract

Purpose—Meningiomas are a frequent tumor of the central nervous system. Although mostly benign, approximately 5% present as atypical or malignant tumors. Treatments for atypical meningiomas include gross total resection and radiotherapy, but about 33% of patients have recurrent tumors, sometimes as a higher grade. Recently, the brain penetrant anthelmintic drug, mebendazole, has shown promise as an anticancer agent in rodent models of glioblastoma and medulloblastoma.

Methods—The half maximal inhibitory concentration (IC₅₀), effect on; colony formation, cell proliferation, and caspase-3/7 markers of apoptosis of mebendazole with and without radiation was measured *in vitro*. Mice intracranially implanted with KT21MG1 human meningioma were administered mebendazole alone or in combination with radiation. Survival benefit was evaluated, while tumors were investigated by immunohistochemical staining for apoptosis, cell proliferation, and vascular density.

Results—*In vitro* experiments on meningioma cell lines showed the IC₅₀ for mebendazole in the range of 0.26 to 0.42 μ M. Mebendazole alone induced cytotoxicity, however the combination had a greater reduction in colony formation and resulted in higher levels of cleaved caspase-3. The *in vivo* study showed both, mebendazole alone and the combination, to have a survival benefit with an increase in apoptosis, and decreases in tumor cell and vascular proliferation.

Conclusion—These preclinical findings indicate that mebendazole alone or in combination with radiation can be considered for the treatment of malignant meningioma. The mechanism of action

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Author Contributions: CGS performed all the experiments assisted by TW for animal experiments. CGS and GJR created the study design. GJR originated the study hypothesis.

Conflict of Interest: Dr. Riggins is a founder of and holds equity in Benizole Therapeutics PBC. The results of the study discussed in this publication could affect the value of Benizole. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policies.

Conflict of Interest

GJR has a financial interest in Benizole Therapeutics, PBC and in mebendazole related intellectual property managed by Johns Hopkins University.

Ethical Approval

Rodent experiments were performed using a protocol approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

for this combination may include an increase in apoptosis, a reduction in proliferation and angiogenesis, or a combination of these effects.

Keywords

Meningioma; Brain; Mebendazole; Radiation; Preclinical

Introduction

Tumors of the meninges accounting for 35% of adult brain tumors [1]. Most meningiomas are benign and respond to surgical resection and/or radiation therapy [2, 3]. However, patients with the worst meningiomas, grade III malignant have poor survival with few or no treatment options [2]. For recurrent meningiomas, which are found mostly in the grade II and III, systematic chemotherapy [3, 4], α -interferon[5], somatostatin analogues[6], or VEGF inhibitors[7] have all been attempted, but with little success.

Recently, drug repurposing has been investigated for the treatment of colon cancer[8, 9] liver cancer[10], breast cancer[11], prostate cancer[12], and small cell lung cancer[13]. Mebendazole, an antiparasitic benzimidazole, has long been in worldwide use to treat helminthic infections, primarily pin, round and hook worms[14]. It is also used to treat central nervous system parasitic infections such as echinococcosis and neurocysticercosis[15, 16]. Mebendazole's mechanism depends in part on binding to alpha-tubulin to disrupt microtubule formation, leading to cytoskeletal instability[17]. Mebendazole is regarded as a relatively non-toxic drug and demonstrates antitumor activity in several types of malignancies[8, 18–20]. In glioblastoma[17] and medulloblastoma[21–23] animal models, mebendazole increased median survival. Other potential mechanisms that might contribute to survival benefit was an observed decrease in tumor angiogenesis, through the inhibition of vascular endothelial factor receptor 2 (VEGF2) [21], inhibition of the hedgehog pathway [22], and increased caspase-3 pathway activity [22].

Beyond surgery, radiation is the only routinely used therapy for the treatment of malignant meningioma, but there is much room for improvement. Patients with WHO Grade II or III meningioma treated with radiation in doses ranging from 44–66 Gy had a 5-year overall survival of 30% and 0%, respectively [24]. In a different study, patients with atypical meningioma treated with external beam radiation therapy had a 5-year progression free survival rate of 65%, but with subtotal resection, only 30% [25]. In preclinical studies of malignant meningioma the effects of radiation appear to be mixed and cell line dependent [26–28].

Here, we find that mebendazole alone and in combination with radiation decreases cell viability and induces apoptosis via the caspase-3/7 pathway in human cell lines of malignant meningioma. Furthermore, the combination increased median survival by inducing apoptosis, decreasing cell proliferation and angiogenesis in an intracranial rodent model of malignant meningioma.

Materials and Methods

Cell Lines and Cell Culture

Cell lines (KT21MG1, IOMM LEE, AC-1, SF4068, SF6717, SF1335, SF1335 + YAP) (Supplementary Table 1) were initially transfected with the hTERT vector for immortalization [29–31]. Cell lines were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/ streptomycin at 37°C with 5% CO₂.

Cell Viability, Proliferation, Survival, and Apoptosis Assays

Cell lines were plated in a 96-well plate at 2000 cells per well. Twenty-four hours after plating, the cells were treated with a range of mebendazole concentrations (0.001µm-100µm) or a single dose of radiation (12 Gy) and incubated for an additional 72 hours. Radiation dosing was achieved by the CIXDX-strahl System using the VWP-1 Software Package. Cell viability and half maximal inhibitory concentrations were calculated using the Cell Counting Kit-8 (Dojindo Molecular Technology, Japan). A Cell Proliferation ELISA Kit (Roche, Indianapolis, IN, USA) was used to measure bromodeoxyrudine (BrdUrd) incorporation according to manufacturer instructions. For clonogenic survival, KT21MG1 and IOMM LEE cells were seeded (1×10^2) and (2×10^2) into 6-well plates, respectively. After a 24 hour incubation, cells were treated with mebendazole for 72 hours. Colony formation was stained with 0.2% crystal violet and counted under a microscope. For the evaluation of apoptotic activity, KT21MG1 and IOMM LEE cells were treated with mebendazole for 72 hours with and without radiation (12 Gy) via the CIXD after 24 hours of mebendazole treatment, and evaluated by the Caspase-Glo 3/7 assay (Promega, G8091).

Immunohistochemistry and Western Blotting

Immunohistochemistry [30] was used for the detection of Ki67 (Abcam, 1:600) and CD31 (ThermoFisher, 1:50), and cleaved caspase-3 (Cell Signaling, 1:2000). For Western Blots, protein concentration was measured by a BCA [35] and 25 µg/well protein lysate was separated on a 4–12% SDS-PAGE. Primary antibodies for PARP, cleaved PARP, caspase-3, cleaved caspase-3, caspase-7 and cleaved caspase-7 were diluted 1:1000.

Animal Experiments

Six week old female athymic mice were anesthetized and using a stereotactic frame, 50,000 KT21MG1 cells expressing luciferase lentivirus[32] in 0.5µL matrigel matrix were implanted into the skull base 2mm posterior to the bregma, 2mm to the right of the bregma, and 5.8mm below the skull surface as previously described[33]. The description of the cell line can be found in Supplementary Table 1.

At day 5-post implantation of the KT21MG1 cells, mebendazole was administered in a high fat (45 kcal% fat from soybean oil and lard) mouse diet[18] (Research Diets, New Brunswick, New Jersey, USA) that was available for the mice ad libitum at a dose of 50 mg/kg/day MBZ until the end of the experiment [17]. Control animals were fed the high fat pellet diet without mebendazole. The mice were monitored daily (Supplementary Figure 1) and euthanized if neurological symptoms occurred or weight loss exceeded 15%. Survival was measured from day of implantation to euthanasia.

At day 5-post implantation the mice were treated with local radiation therapy by the Small Animal Radiation Research Platform (SARRP) [34] using a single dose of 12 Gy of radiation at a beam width of 3mm x 3mm. Using CT-Spot software (Beeley Medical, Bristol, CT) the burr hole in the skull from implantation was located. The decision to administer a single dose of 12 Gy radiation was based the successful application of this treatment in an intracranial model of NF2 mutant malignant meningioma.

Intracranial luciferase activity from tumor cells was observed by the Xenogen IVIS. Each mouse was administered D-luciferin potassium salt (Gold Biotechnology, St. Louis, MO, USA) in dPBS and analyzed in overlay mode with Living Image Software.

Statistical Analysis

Graph Pad Prism 5 Software was used for statistical analysis as previously described[30], with p-values determined by a Mantel-Cox Test. A one-way ANOVA followed by a Tukey Test compared groups ($p < 0.05$) considered statistically significant. A two-way ANOVA with a Bonferroni post-test determine significance ($p < 0.05$) by comparing treatment groups among cell lines. A two-tailed unpaired t-test compared luminescent imaging.

Results

Mebendazole and its Combination with Radiation is effective in a Malignant Meningioma Model

The KT21MG1 intracranial mouse model of malignant meningioma was used to test the *in vivo* efficacy of mebendazole alone and in combination with radiation. Five days after tumor implantation mice were administered mebendazole premixed in a high fat (HF) diet. Food consumption revealed that the mice ingested a dose of 50 mg/kg mebendazole. Our laboratory has previously shown that a dose of 12 Gy reduces xenograft tumor growth of malignant meningioma *in vivo*.

Fig. 1 represents the combined survival curve and luminescence data from two animal experiments conducted separately to show reproducibility of treatment effect. As Fig. 1A shows, the mice in the mebendazole group had a significant increase in median survival of 58% in comparison to the control (19 days vs 30 days, $p < 0.0001$), while the mice administered the combination of mebendazole and a single dose of radiation (12 Gy) had a significant increase in median survival of 105% when compared to the control (19 days vs 39 days, $p < 0.0001$). The increase in median survival in the combination group was significant to the group administered mebendazole (30 days vs 39 days, 30% increase, $p=0.0005$), and radiation alone (33.5 days vs 39 days, 16% increase, $p=0.0062$).

Luminescence images acquired twelve days after implantation (Fig. 1B and 1C) illustrated that mebendazole and radiation as single treatments significantly reduced tumor luminescence compared to control (mebendazole vs control, $p < 0.01$ and radiation vs control, $p < 0.001$). Radiation has been shown to reduce tumor growth in intracranial models of meningioma, alone and in combination with anticancer agents [28]. Tumor growth in the brain was significantly delayed by the combination of mebendazole and radiation when compared to the control (combination vs control, $p < 0.001$), or mebendazole alone

(combination vs. mebendazole, $p = 0.05$), but not to radiation alone (combination vs. radiation, $p = 0.05$) (Fig. 1C).

Mebendazole and its Combination with Radiation Decreases Cell Proliferation and Angiogenesis, and Increases Apoptosis

To evaluate the survival activity observed in the intracranial mouse model, we analyzed and compared protein expression in brain tumors from mice given mebendazole at 50mg/kg body weight, 12 Gy radiation only, and the combination of both treatments versus the untreated control. By hematoxylin and eosin staining, we confirmed tumor growth of the KT21MG1 xenograft in all four treatment groups. Histological evaluation of tumors revealed that the tumors from animals administered mebendazole or the combination were smaller in size compared to tumors from the control and radiation groups, which correlated with the decrease in bioluminescence observed during *in vivo* imaging. Overall, mebendazole alone and in combination with radiation decreased tumor size in our animal model (Fig. 2).

We evaluated the expression of cleaved caspase-3, a marker of apoptosis, since this pathway has been shown to be activated by mebendazole [22]. Immunohistochemical staining revealed low cleaved caspase-3 expression in radiation and control groups. We observed moderate expression of cleaved caspase-3 in the mebendazole only group and the highest levels of cleaved caspase-3 expression (Fig. 2) in the combination group. Immunohistochemical staining for Ki67 confirmed the combination decreased cell proliferation in tumors compared to control and single treatment groups.

Mebendazole has shown to decrease tumor angiogenesis in animal models of medulloblastoma[21] and colon cancer[18]. The CD31 protein is a useful marker for evaluation of tumor vasculature, or angiogenesis[36]. Our data illustrated that brain sections of mice treated with mebendazole alone had reduced expression of CD31 protein by immunohistochemical analysis. The expression of CD31 was further reduced in the combination group when compared to the control, or either treatment group alone, but was not significant (Fig. 2).

The Combination of Mebendazole with Radiation Decreases Survival and Cell Viability in Malignant Meningioma Cell Lines

A panel of human meningioma cell lines with varied WHO Grade and NF2 mutational status (Supplementary Table 1) were used to further investigate if mebendazole alone and in combination with radiation influence proteins involved cell proliferation and apoptosis *in vitro*. Mutations in the NF2 (neurofibromatosis Type-2 gene) have shown to increase malignancy of recurring meningiomas. Merlin, the NF2 protein product, acts as a tumor suppressor in the Hippo Pathway in cases of meningioma.

The human meningioma cell lines treated with mebendazole alone had IC_{50} values in the 0.26–0.42 μ M range (Fig. 3A). These IC_{50} values are similar to mouse glioma and human glioblastoma cells lines treated with mebendazole [17]. The effects of radiation doses (0–20 Gy) on the viability of meningioma cell lines were examined (Supplementary Figure 2). Exposure of meningioma cell lines to 8–10 Gy of radiation has previously shown to induce cell senescence and reduce cell viability[26, 37, 38]. In our study, we found the application

of 12 Gy radiation to mebendazole treated cells lowered the IC_{50s}, but only in Grade III malignant meningioma cell lines, KT21MG1 and IOMM LEE, by 22–33%, respectively.

Clonogenic activity of both cell lines, KT21MG1 and IOMM LEE, was significantly reduced with low doses of mebendazole (0.5–1 μM) in comparison to controls. Mebendazole in combination with radiation (2 Gy) further reduced colony formation compared to either agent alone (Fig. 3B and 3C).

A BrdUrd assay was used to measure cell proliferation after exposure to mebendazole with and without radiation (Fig. 4A and 4B). For both cell lines we observed a dose dependent decrease in proliferation with mebendazole alone and in combination with radiation, compared to the control.

Mebendazole Alone or in Combination with Radiation Increases Apoptosis in Human Cell Lines of Malignant Meningioma

The decrease in cell viability observed in response to mebendazole treatment may be a result of activation of apoptotic pathways [10, 22]. The activity of the caspase-3/7 pathway was measured in KT21MG1 and IOMM LEE cells treated with mebendazole alone or in combination with radiation. As illustrated in Fig. 4C, mebendazole increased caspase-3/7 activity in a dose dependent manner alone and in combination with radiation in the KT21MG1 cell line. When the effects of the combination were compared to mebendazole alone at similar doses in the KT21MG1 cell line, significance was not observed. In the IOMM LEE cell line (Fig. 4D) mebendazole increased caspase-3/7 activity over the control, but only ranging from 0.5–10 μM. A similar response in caspase-3/7 activity was observed in the IOMM LEE cell line when the combination was compared to the control. Overall, the effects of the combination were not significant when compared to mebendazole alone in the IOMM LEE cell line. At doses of 50–100 μM caspase-3/7 activity decreased drastically, potentially from drug toxicity. However, the IOMM-LEE cell line had a significant increase in caspase-3/7 activity in comparison to the KT21MG1 cell line, which needs to be further investigated.

The effect of mebendazole alone and in combination with radiation on the apoptotic process was further investigated by evaluating the expression of protein markers of apoptosis by western blot analysis (Fig. 5). The KT21MG1 treated with mebendazole ranging from 0.1–1 μM illustrated a dose dependent increase in the cleaved PARP/PARP ratio, cleaved caspase-3, and cleaved caspase-7 (Fig. 5A), while the combination of mebendazole and radiation resulted in a dose dependent increase of cleaved caspase-3, but not cleaved caspase-7. The cleaved PARP/PARP ratio remained unchanged in response to the combination in the KT21MG1 cell line (Fig. 5B). In the IOMM LEE cell line the cleaved PARP/PARP ratio, cleaved caspase-3, and cleaved caspase-7 expression increased in a dose dependent manner when treated with mebendazole (Fig. 5C). The combination increased cleaved caspase-3 and cleaved caspase-7 expression in a dose dependent manner, while the cleaved PARP/PARP ratio remained unchanged (Fig. 5D).

Discussion

Currently malignant meningiomas are treated with radiotherapy and gross total resection with limited success [39]. The five-year median survival of patients ranges from 47 to 61% [3]. Currently, there are no FDA approved chemotherapeutic regimens for malignant meningioma and chemotherapeutics offer limited benefit[3].

The anthelmintic benzimidazole class of compounds have been used for decades for the safe and effective treatment of parasitic infections of the central nervous system. Mebendazole is known to target parasitic infections by binding and disrupting alpha-tubulin in the parasite, leading to death. Efficacy of mebendazole on a broad range of cancer cell lines attribute, at least in part, to activation of cell death pathways[10, 22]. Furthermore, the anticancer effects of mebendazole have been studied in rodent models of colon cancer[18], medulloblastoma[21–23], and glioblastoma[17], and have shown promising results by impeding different pathways involved in tumor growth. In this study, we show for the first time how mebendazole administration alone or in combination effectively extends survival in preclinical models of malignant meningioma.

A dose of 50 mg/kg MBZ plus a single dose of 12 Gy radiation can impede tumor growth and significantly increase survival in a Grade III intracranial model of malignant meningioma by decreasing Ki67 and increasing cleaved caspase-3 protein expression. Mebendazole is known to reduce microvessel density and disrupt the formation of the tumor vasculature by inhibiting VEGFR2[21]. We evaluated the antiangiogenic activity of mebendazole and radiation by measuring the protein expression of CD31 in brain tumors. Combination treatment resulted in lower levels of CD31, indicative of a decrease in tumor vasculature, consistent with other brain cancer models [21].

A recent study suggested that mebendazole may sensitize brain cancer cells to radiation by altering the expression of DNA damage response proteins. When glioma cell lines were treated with mebendazole and radiation, γ H2AX1, a marker of DNA damage, was increased, while the DNA trafficking proteins Nbs1 and Chk2 were sequestered in the cytoplasm, suggesting the combination impaired DNA damage repair[40]. The combination of mebendazole and radiation seems to have a synergistic effect on inhibiting the DNA damage response. Markowitz et al. hypothesized that disrupting tubulin formation, leads to a decrease in the tubulin transport of key proteins involved in DNA repair[40]. Poruchynsky et al. demonstrated that lung and breast cancer cell lines treated with microtubule-targeting agents plus radiation increased expression of γ H2AX, an indicator of DNA damage [41]. Additional studies may be required to demonstrate how mebendazole and radiation mediate disruption of microtubule formation and DNA damage in malignant meningioma.

In summary, our study provides evidence that the combination of mebendazole and radiation can decrease cell growth and angiogenesis and increase apoptosis in preclinical models of malignant meningioma. Due to the safety and experience with mebendazole and radiation, and the lack of effective therapies for malignant meningioma grade III this should be considered for clinical trials. Additionally, atypical meningiomas grade II or those grade I

where surgical intervention is not possible, this treatment could also be considered to increase the beneficial effects of radiation therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ostrom QT, et al., CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008–2012. *Neuro Oncol*, 2015 17 Suppl 4: p. iv1–iv62. [PubMed: 26511214]
2. Bi WL, et al., Meningioma Genomics: Diagnostic, Prognostic, and Therapeutic Applications. *Front Surg*, 2016 3: p. 40. [PubMed: 27458586]
3. Kessler RA, et al., Metastatic Atypical and Anaplastic Meningioma: A Case Series and Review of the Literature. *World Neurosurg*, 2017 101: p. 47–56. [PubMed: 28143726]
4. Moazzam AA, Wagle N, and Zada G, Recent developments in chemotherapy for meningiomas: a review. *Neurosurg Focus*, 2013 35(6): p. E18. [PubMed: 24289126]
5. Chamberlain MC, IFN-alpha for recurrent surgery- and radiation-refractory high-grade meningioma: a retrospective case series. *CNS Oncol*, 2013 2(3): p. 227–35. [PubMed: 25054463]
6. Chamberlain MC, Glantz MJ, and Fadul CE, Recurrent meningioma: salvage therapy with long-acting somatostatin analogue. *Neurology*, 2007 69(10): p. 969–73. [PubMed: 17785665]
7. Lou E, et al., Bevacizumab therapy for adults with recurrent/progressive meningioma: a retrospective series. *J Neurooncol*, 2012 109(1): p. 63–70. [PubMed: 22535433]
8. Nygren P, et al., Repositioning of the anthelmintic drug mebendazole for the treatment for colon cancer. *J Cancer Res Clin Oncol*, 2013 139(12): p. 2133–40. [PubMed: 24135855]
9. Nygren P and Larsson R, Drug repositioning from bench to bedside: tumour remission by the anthelmintic drug mebendazole in refractory metastatic colon cancer. *Acta Oncol*, 2014 53(3): p. 427–8. [PubMed: 24160353]
10. Sawanyawisuth K, et al., Effect of the Antiparasitic Drug Mebendazole on Cholangiocarcinoma Growth. *Southeast Asian J Trop Med Public Health*, 2014 45(6): p. 1264–70. [PubMed: 26466412]
11. Chen HR, et al., A network based approach to drug repositioning identifies plausible candidates for breast cancer and prostate cancer. *BMC Med Genomics*, 2016 9(1): p. 51. [PubMed: 27475327]
12. Gillesen S, et al., Repurposing Metformin as Therapy for Prostate Cancer within the STAMPEDE Trial Platform. *Eur Urol*, 2016 70(6): p. 906–908. [PubMed: 27450106]
13. Jahchan NS, et al., A drug repositioning approach identifies tricyclic antidepressants as inhibitors of small cell lung cancer and other neuroendocrine tumors. *Cancer Discov*, 2013 3(12): p. 1364–77. [PubMed: 24078773]
14. McCarty TR, Turkeltaub JA, and Hotez PJ, Global progress towards eliminating gastrointestinal helminth infections. *Curr Opin Gastroenterol*, 2014 30(1): p. 18–24. [PubMed: 24241244]
15. Vutova K, et al., Effect of mebendazole on human cystic echinococcosis: the role of dosage and treatment duration. *Ann Trop Med Parasitol*, 1999 93(4): p. 357–65. [PubMed: 10656037]
16. El-On J, Benzimidazole treatment of cystic echinococcosis. *Acta Trop*, 2003 85(2): p. 243–52. [PubMed: 12606103]
17. Bai RY, et al., Antiparasitic mebendazole shows survival benefit in 2 preclinical models of glioblastoma multiforme. *Neuro Oncol*, 2011 13(9): p. 974–82. [PubMed: 21764822]

18. Williamson T, et al., Mebendazole and a non-steroidal anti-inflammatory combine to reduce tumor initiation in a colon cancer preclinical model. *Oncotarget*, 2016 7(42): p. 68571–68584. [PubMed: 27612418]
19. Doudican N, et al., Mebendazole induces apoptosis via Bcl-2 inactivation in chemoresistant melanoma cells. *Mol Cancer Res*, 2008 6(8): p. 1308–15. [PubMed: 18667591]
20. Sasaki J, et al., The anthelmintic drug mebendazole induces mitotic arrest and apoptosis by depolymerizing tubulin in non-small cell lung cancer cells. *Mol Cancer Ther*, 2002 1(13): p. 1201–9. [PubMed: 12479701]
21. Bai RY, et al., Effective treatment of diverse medulloblastoma models with mebendazole and its impact on tumor angiogenesis. *Neuro Oncol*, 2015 17(4): p. 545–54. [PubMed: 25253417]
22. Larsen AR, et al., Repurposing the anthelmintic mebendazole as a hedgehog inhibitor. *Mol Cancer Ther*, 2015 14(1): p. 3–13. [PubMed: 25376612]
23. Bai RY, et al., Brain Penetration and Efficacy of Different Mebendazole Polymorphs in a Mouse Brain Tumor Model. *Clin Cancer Res*, 2015 21(15): p. 3462–70. [PubMed: 25862759]
24. Buglione M, et al., Role of external beam radiotherapy in the treatment of relapsing meningioma. *Med Oncol*, 2014 31(3): p. 866. [PubMed: 24504843]
25. Sun SQ, et al., Management of atypical cranial meningiomas, part 2: predictors of progression and the role of adjuvant radiation after subtotal resection. *Neurosurgery*, 2014 75(4): p. 356–63; [PubMed: 24932708]
26. Ho WS, et al., LB-100, a novel Protein Phosphatase 2A (PP2A) inhibitor, sensitizes malignant meningioma cells to the therapeutic effects of radiation. *Cancer Lett*, 2018 415: p. 217–226. [PubMed: 29199006]
27. Rao Gogineni V, et al., Radiation-inducible silencing of uPA and uPAR in vitro and in vivo in meningioma. *Int J Oncol*, 2010 36(4): p. 809–16. [PubMed: 20198323]
28. Wilisch-Neumann A, et al., The integrin inhibitor cilengitide affects meningioma cell motility and invasion. *Clin Cancer Res*, 2013 19(19): p. 5402–12. [PubMed: 23948974]
29. Baia GS, et al., A genetic strategy to overcome the senescence of primary meningioma cell cultures. *J Neurooncol*, 2006 78(2): p. 113–21. [PubMed: 16554968]
30. Baia GS, et al., Yes-associated protein 1 is activated and functions as an oncogene in meningiomas. *Mol Cancer Res*, 2012 10(7): p. 904–13. [PubMed: 22618028]
31. Striedinger K, et al., The neurofibromatosis 2 tumor suppressor gene product, merlin, regulates human meningioma cell growth by signaling through YAP. *Neoplasia*, 2008 10(11): p. 1204–12. [PubMed: 18953429]
32. Hasegawa K, et al., Dual therapy of ovarian cancer using measles viruses expressing carcinoembryonic antigen and sodium iodide symporter. *Clin Cancer Res*, 2006 12(6): p. 1868–75. [PubMed: 16551872]
33. Baia GS, et al., An orthotopic skull base model of malignant meningioma. *Brain Pathol*, 2008 18(2): p. 172–9. [PubMed: 18093250]
34. Wong J, et al., High-resolution, small animal radiation research platform with x-ray tomographic guidance capabilities. *Int J Radiat Oncol Biol Phys*, 2008 71(5): p. 1591–9. [PubMed: 18640502]
35. Yamashita AS, et al., Preclinical evaluation of the combination of mTOR and proteasome inhibitors with radiotherapy in malignant peripheral nerve sheath tumors. *J Neurooncol*, 2014 118(1): p. 83–92. [PubMed: 24668609]
36. Baluk P and McDonald DM, Markers for microscopic imaging of lymphangiogenesis and angiogenesis. *Ann N Y Acad Sci*, 2008 1131: p. 1–12. [PubMed: 18519955]
37. Gravina GL, et al., The novel CXCR4 antagonist, PRX177561, reduces tumor cell proliferation and accelerates cancer stem cell differentiation in glioblastoma preclinical models. *Tumour Biol*, 2017 39(6): p. 1010428317695528. [PubMed: 28639900]
38. Lin CJ, et al., Preclinical effects of CRLX101, an investigational camptothecin-containing nanoparticle drug conjugate, on treating glioblastoma multiforme via apoptosis and antiangiogenesis. *Oncotarget*, 2016 7(27): p. 42408–42421. [PubMed: 27285755]
39. Karsy M, et al., Medical Management of Meningiomas: Current Status, Failed Treatments, and Promising Horizons. *Neurosurg Clin N Am*, 2016 27(2): p. 249–60. [PubMed: 27012389]

40. Markowitz D, et al., Microtubule-targeting agents can sensitize cancer cells to ionizing radiation by an interphase-based mechanism. *Onco Targets Ther*, 2017 10: p. 5633–5642. [PubMed: 29200877]
41. Poruchynsky MS, et al., Microtubule-targeting agents augment the toxicity of DNA-damaging agents by disrupting intracellular trafficking of DNA repair proteins. *Proc Natl Acad Sci U S A*, 2015 112(5): p. 1571–6. [PubMed: 25605897]

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A

B Day 12

C

Luminescence

Fig. 1.

Mebendazole Alone and in Combination with Radiation Increases the Median Survival of the KT21MG1 Intracranial Model of Malignant Meningioma by Decreasing Xenograft Tumor Growth. Mebendazole alone and in combination with radiation significantly extended the life of the intracranial mouse model of malignant meningioma in two combined experiments. a.) The KT21MG1 cell line was implanted intracranially into female athymic nude mice. The combination significantly increased median survival in mice compared to all groups. b.) IVIS Xenogen imaging of the KT21MG1 xenograft expressing luciferase illustrates that the combination significantly reduced the growth of the tumor in comparison to the control and mebendazole groups ($p = 0.05$) twelve days after implantation. c.) Average luminescence based on IVIS data was quantified for each treatment group

Fig. 2. Mebendazole Alone and in Combination with Radiation Decreases Xenograft Tumor Growth by Altering Pathways Involved in Apoptosis, Cell Proliferation, and Angiogenesis *in vivo*. Representative brain tissue sections were submitted to hematoxylin and eosin for each treatment group. Brain tissue sections were subjected to immunohistochemical analysis for cleaved caspase-3(10x), Ki67 (10x), and CD31 (10x) and are illustrated above for all

treatment groups. The average protein expression for each treatment group was quantified using ImageJ deconvolution.

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A

	IC ₅₀ (μM)						
	KT21MG1	IOMM LEE	SF4068	SF1335	SF1335 + YAP	AC-1	SF6717
Mebendazole	0.391±0.03	0.390±0.01	0.421±0.04	0.372±0.04	0.262±0.03	0.342±0.05	0.412±0.02
Mebendazole + Radiation	0.303±0.05	0.266±0.02	0.476±0.11	0.394±0.09	0.433±0.12	0.714±0.15	0.523±0.08

KT21MG1

B

C

Fig. 3.

The Effects of Mebendazole Alone and in Combination with Radiation on Cell Viability and Colony Formation of Meningioma Cell Lines. Meningioma cells lines were treated with different concentrations (0.001μM-100μM) of mebendazole (MBZ) alone or in combination with radiation (12 Gy) by CIXD and accessed for cell viability by CCK-8 assay. a.) The IC₅₀ values represent 72 hours of treatment with mebendazole alone and in combination with radiation. Data represents three experiments with four replicates each. b.) KT21MG1 and c.) IOMM LEE cells, both malignant meningioma cell lines, were treated with mebendazole

alone and in combination with radiation (2 Gy) to evaluate the effects of treatment on colony formation. Data is represented by three replicates. Double asterisks indicate that treatment is significant ($p < 0.05$) to DMSO treatment alone, while single asterisks indicate significance ($p < 0.05$) of a particular treatment to a particular treatment group

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	IC ₅₀ (μM)						
	KT21MG1	IOMM LEE	SF4068	SF1335	SF1335 + YAP	AC-1	SF6717
Mebendazole	0.391±0.03	0.390±0.01	0.421±0.04	0.372±0.04	0.262±0.03	0.342±0.05	0.412±0.02
Mebendazole + Radiation	0.303±0.05	0.266±0.02	0.476±0.11	0.394±0.09	0.433±0.12	0.714±0.15	0.523±0.08

Fig. 4. Mebendazole Alone and in Combination with Radiation Decreases Cell Proliferation and Increases Apoptosis in Cell Lines of Malignant Meningioma. The KT21MG1 a.) and IOMM LEE b.) cell lines were treated with different concentrations of mebendazole (0.001μM-100μM) alone for 72 hours and with a single dose of radiation(12 Gy) 24 hours after mebendazole treatment.. Proliferation activity in response to treatment was evaluated by a chemiluminescent BRDU-ELISA measuring DNA synthesis. Data represents four replicates per treatment group. The KT21MG1 c.) and IOMM LEE d.) cell lines were treated

with different concentrations of mebendazole (0.001 μ M-100 μ M) alone for 72 hours and with a single dose of radiation(12 Gy) 24 hours after mebendazole treatment. Apoptotic activity in response to treatment was evaluated by a chemiluminescent ELISA measuring caspase-3/7 pathway activity. Data represents four replicates per treatment group

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Fig. 5.

Mebendazole Alone and in Combination with Radiation Induces Apoptosis through the Caspase-3/7 Pathway in Cell Lines of Malignant Meningioma. Malignant cell lines were treated, collected, lysed, ran on an SDS-PAGE, transferred to PVDF membrane and probed for proteins markers of apoptosis. a-b.) KT21MG1 and c-d.) IOMM LEE cells were analyzed by western blot for PARP, Cleaved PARP, Caspase-3, Cleaved Caspase-3, Caspase-7, Cleaved Caspase-7, and GAPDH following treatment with mebendazole alone and in combination with radiation (12 Gy)