Diffusion-weighted Magnetic Resonance Imaging Characterization of Anti-tumor Activity of MLN2704 in a Bone Metastatic Cancer Model

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Introduction

Magnetic resonance imaging was used to characterize the effects of therapeutic treatment of MLN2704 on a murine model of osteoblastic prostate cancer metastasis. MLN2704, which is currently in Phase 1/2 development, is a novel therapeutic designed to deliver the maytansanoid chemotherapeutic agent DM1 directly to prostate cancer cells through the targeting of MLN591, a monoclonal antibody vehicle that binds specifically to the prostate-specific membrane antigen (PSMA). The animal model is based on the 22Rv1 prostate cancer cell line, an androgen-independent derivative of the CWR22 prostate cancer xenograft, which was engineered to express firefly luciferase and implanted directly into the proximal bone cavity of the tibia. MRI, performed on *ex vivo* samples 10 weeks after implantation, demonstrated the efficacy of MLN2704 treatment via significant reduction in tumor volume.

Methods

Sixteen CB17 *scid* mice were anesthetized with a ketamine / xylazine cocktail, and a 10 mm incision was made in the lower leg. Following separation of muscle and exposure of the upper shaft of tibia, a 3 µl suspension containing 1.5×10^5 22Rv1 Luc 1.17 cells was injected into the evacuated bone cavity. After injection, the hole was sealed with bone wax, the incision was sutured, and the animals were returned to housing. The animals were randomized into 2 groups, each with 8 animals for placebo or MLN2704 treatment. Animals received either PBS (placebo) or 5 doses of 240µg/kg (DM1 equivalents) MLN2704 delivered on a q3d schedule (dosing based on previous efficacy studies using bioluminescent imaging). 10 weeks after inoculation, the animals were euthanized by CO_2 and submitted for imaging.

MRI was performed on a Bruker DRX 8.5T vertical bore magnet using a 25 mm volume coil (courtesy of D. Burstein, CBMRR, Boston, MA). Diffusion-weighted spin-echo (DW-SE) imaging was performed with 256 × 128 data matrix, 25 mm × 18 mm FOV, and 12 – 18 1-mm-thick slices. Other DW-SE parameters: TR / TE = 1500 / 32.7 ms; Δ / δ = 15.0 / 6.0 ms; b-value = 659 s/mm^2 (g=140 mT/m); NA = 2. Tumor volume was determined by tracing the hyperintensity using Analyze (AnalyzeDirect Inc., Lenexa, KS, USA).

Results

MR images of MLN2704- and PBS-treated tumor-bearing animals (Figure 1) demonstrate the significant reduction in tumor volume as a result of MLN2704 treatment. A normal mouse leg is included in Figure 1 for reference. The arrow indicates the tumor whereas F, G, and T, indicate the femur, gastrocnemius, and tibia, respectively. Figure 2 shows the group tumor volume data. MLN2704 treatment resulted in a 91% reduction in tumor volume.

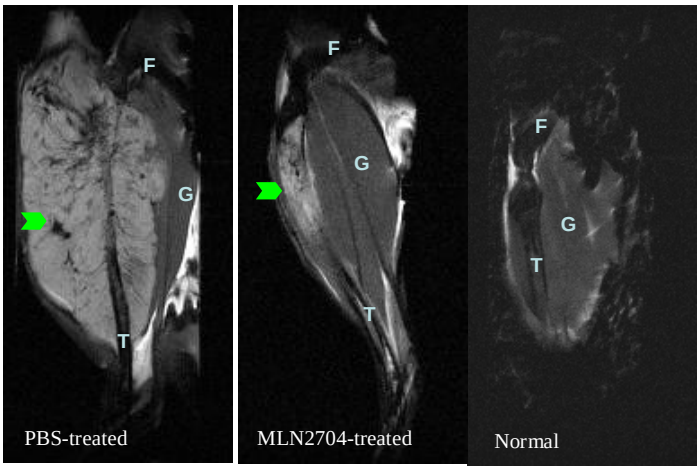

Figure 1. Comparison of PBS- and MLN2704-treated osteoblastic prostate tumors (denoted by the arrows). For comparison, a normal image is also shown (right).

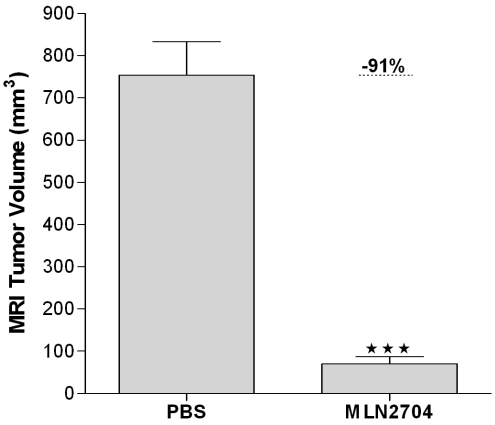

Figure 2. Tumor volumes measured from MR images. MLN2704-treated animals experienced a 91% reduction ($p<0.001$) in tumor volume as compared to PBS-treated animals.

Discussion

In man, metastatic androgen-independent prostate cancer frequently manifests metastases to bone. We have developed a murine xenograft model of bone metastases for prostate cancer. In this model, the intratibial injection of 22Rv1 Luc 1.17 cells resulted in intra- and extraosseus tumor growth and development of osteoblastic lesions. Treatment of tumor bearing mice with MLN2704 resulted in the dramatic reduction (>90%) of tumor volume with the preservation of bone architecture as compared to placebo-treated mice. Application of diffusion-weighted MR imaging illustrates a key capability for analyzing and interrogating *in vivo* tumor volumes and their modulation under therapy. The translational opportunities provided by such interrogation are enormous for clinical trials in oncology. The pharmaceutical development of MLN2704 was positively impacted by these imaging studies by further illustrating the potent anti-tumor activity of MLN2704 against the PSMA-positive 22Rv1 intraosseus tumor. These results suggest that MLN2704 may have activity against osseous metastases in prostate cancer patients.