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**CCR-14-3195-R**

**SUPPLEMENTARY FIGURE LEGENDS 1-6**

**Supplementary Figure 1.** *Combination therapy with macitentan and TMZ leads to regression of established LN-229 glioblastomas.* Representative bioluminescent images from mice that were treated with vehicle (control), macitentan, TMZ, or combination therapy with macitentan plus TMZ. The timeline above the images shows the therapy schedule and indicates when a given image was obtained. The color scale located beside each image reflects the detected photon counts emerging from active luciferase within the animal, which may be used as an indicator of tumor burden. The photon count for each representative mouse is shown at the right of each image.

**Supplementary Figure 2.** *Combination therapy with macitentan plus TMZ eradicates experimental glioblastomas.* (A) Representative gross images of brains collected from mice bearing LN-229 glioblastomas and treated with vehicle, TMZ, macitentan, or combined TMZ plus macitentan therapy. (B) Upper panel shows representative histologic images of normal brain and a three-week-old LN-229 glioblastoma. Lower panel shows histologic images of LN-229 glioblastomas that were harvested from mice that were treated for 21 days with either vehicle, TMZ, macitentan, or TMZ plus macitentan. Note: Necrotic zone in GBM of mice treated with TMZ and macitentan. (C) Representative histologic images of brains harvested from mice that were implanted with either LN-229, LN-229Res, or D54Res glioma cells. Mice were treated with either vehicle, TMZ, macitentan, or TMZ plus macitentan therapy until they were euthanized due to disease or the conclusion of the study (TMZ plus macitentan ).

**Supplementary Figure 3.** *Combination therapy with macitentan and TMZ produces durable responses in mice with established LN-229 glioblastomas.* (A) Kaplan-Meier plot of mice bearing orthotopically implanted LN-229 glioblastomas that were treated with vehicle (control) (*n*=8), macitentan (*n*=8), TMZ (*n*=8), or macitentan plus TMZ (*n*=16). Treatment was stopped on day 98 when only mice treated with macitentan plus TMZ were alive. These mice did not exhibit evidence of a brain mass and were subjected to continued monitoring for tumor recurrence. (B) *Recurrent tumors may respond to additional cycles of macitentan plus TMZ therapy.* Recurrent disease was detected in two mice that had completed a course of combination therapy with macitentan plus TMZ. One mouse (upper panel) responded to additional cycles of macitentan plus TMZ and was declared disease-free on day 258. The second mouse (lower panel) progressed on combination therapy and died on day 167. (C) *Long-term treatment of recurrent LN-229 glioblastoma with macitentan plus TMZ is well tolerated and produces stable disease.* Recurrent disease was detected in a mouse 97 days after completing a course of macitentan plus TMZ therapy. The mouse was immediately restarted on the combination regimen and was determined to have stable disease when it was euthanized on day 302.

**Supplementary Figure 4.** *Macitentan suppresses activation of AKT and MAPK signaling pathways on glioma cells and tumor-associated endothelial cells in TMZ-resistant D54Res glioblastomas.* (A) Representative immunofluorescent images of ETAR, ETBR, pAKT, and pMAPK (each depicted in green) and endothelial cells (shown in red) in *D54Res* tumors that were treated with vehicle (control), TMZ, macitentan, or macitentanplus TMZ for a period of three weeks. (B) Macitentan down-regulates anti-apoptotic protein expression in D54Res glioblastomas and sensitizes glioma cells and tumor-associated endothelial cells to TMZ.Representative immunofluorescent images of anti-apoptotic proteins Bcl2L1, Gsta5, and Twist1 (depicted in green) and tumor-associated endothelial cells (shown in red) in TMZ-resistant D54Res glioblastomas that were treated with vehicle (control), TMZ, macitentan, or macitentan plus TMZ for a period of three weeks. Sections from D54Res glioblastomas were also labeled with a proliferation marker (Ki67) and an apoptotic marker (TUNEL), both of which are depicted in green. At least 5 photomicrographs were collected from each tumor. Scale bar=50 μm. *n*=3 mice/group.

**Supplementary Figure 5.** *MGMT promoter methylation in orthotopically implanted LN-229, LN-229Res, and D54Res glioblastomas.* Mice harboring established LN-229, LN-229Res, or D54Res glioblastomas were treated for a period of four weeks. Macitentan was administered daily at a dose of 10 mg/kg, while TMZ (7.5 mg/kg) was administered daily on a one-week-on two-weeks-off schedule. Pooled DNA samples from each of the treatment groups were processed for assessment of *MGMT* promoter methylation by methylation-specific PCR. The amplification products were resolved by electrophoresis and the corresponding ethidium bromide-stained gel is shown. Mr, relative molecular weight markers; U, unmethylated; M, methylated; M/U ratio, ratio of methylated to unmethylated *MGMT* in sample.

**Supplementary Figure 6.** *Effects of treatment on glioblastoma-associated blood vessels.* (A) Representative image from tumor vascular permeability studies. Vascular permeability was assessed in established LN-229 glioblastomas that were treated with vehicle or 10 mg/kg macitentan for a period of three weeks. Following the conclusion of treatment, the mice were injected with NaFl (green), which was allowed to circulate for 10 minutes. Blood vessels were labeled with an antibody directed against CD31 (red). Scale bar=50 μm. *n*=10. (B) Representative MR images of small (upper panel) and large (lower panel) LN-229 glioblastomas from mice that were treated daily with vehicle or 10 mg/kg macitentan for period of three weeks. Images were obtained weekly once the treatment started. T2 and then T1 pre-contrast images were collected and then the mice were administered a bolus injection of 0.2 mmol/kg Gd-DTPA contrast agent. Three minutes later, T1 post-enhancement images were obtained. *n*=8. (C) Graph represents pericyte coverage of blood vessels in normal (non-tumor-bearing) mouse brain tissue and tumor-associated vessels of mice harboring LN-229 glioblastomas that were treated with vehicle, TMZ, macitentan, or TMZ plus macitentan for three weeks. A minimum of 3 samples from each group were analyzed, and a minimum of 4 regions from each sample were evaluated. Data are expressed as mean ± s.e.m. (D) Graph represents the MVD in brain tissue from non-tumor-bearing (normal) mice and in LN-229 glioblastomas from mice that were treated with vehicle, TMZ, macitentan, or TMZ plus macitentan. A minimum of 3 samples from each group were analyzed and at least 4 regions from each sample were evaluated. Data are expressed as mean ± s.e.m. \**P*<0.05, \*\**P*<0.0001. Statistical analysis was performed using the Student’s *t*-test. C, control; T, TMZ; M, macitentan; T+M, TMZ plus macitentan.