Microtubule-targeting agents (MTAs) have been employed in the treatment of many cancers for decades. BAL101553 is a highly soluble product of BAL27862, a novel, small molecule, microtubule-depolymerizing agent that induces tumor cell death by activating the lysosomal cell-death checkpoint. Given heterogeneity or instability, the drug penetrates the brain and has anti-cancer activity in diverse tumor models refractory to standard MTAs or radiation therapy. Here, we report on the preclinical efficacy and tolerability of BAL101553 in xenografts from 16 GBM PDX models. 7 of 16 lines demonstrated significant (p<0.01) increase in median survival with BAL101553 versus placebo (range in median survival extension 24-47 days). The combination of BAL101553 with conventional therapies for GBM (RT and temozolomide (TMZ)) was then evaluated in select lines. In the MGMT high line, combination of RT with TMZ increased survival compared to placebo (median survival 60 days vs. 33 days, respectively, p<0.01). Extended BAL101553 monotherapy provided a short but significant extension in survival (median survival 31 days, p<0.01) while extended BAL101553 dosing during and after RT/TMZ (median survival 85 days) did not extend survival relative to RT/TMZ alone (p=NS). In contrast, in the MGMT unmethylated GBM line, combination of RT and extended BAL101553 showed increased survival (median 90 days, p<0.01) relative to either treatment alone (median survival 101553 63 days, RT 69 days or placebo 54 days). Additionally, the combination of BAL101553 with TMZ (median survival 73 days) was more effective than TMZ alone (median survival 65 days, p<0.001). Consistent with the unmethylated MGMT status, the TMZ+28 combination (median survival 60 days) was similar to RT alone (p=0.62), but the combination of extended BAL101553 with RT/TMZ (median survival 101 days, p=0.031 compared to other combination groups) was significantly more effective. To further evaluate whether BAL101553 is a true radiosensitizer, we treated mice that had previously received a combination of RT (20Gy/6x) with extended BAL101553 dosing (median survival 68 days); significantly extended survival compared to RT alone (median survival 54 days; p=0.003). Interestingly, when BAL101553 and TMZ were given to mice with established tumors, there was no increase in median survival (38 days; p=0.16). To evaluate effects on tumor progression during RT, the efficacy of an extended RT schedule (5 Gy, 6x/week) with or without 6 weeks of BAL101553 was evaluated. In this case, BAL101553 given during the RT schedule (median survival 78.10 days) extended median survival with a trend (78 days; p=0.07). Collectively, these data demonstrate that BAL101553 has broad single agent activity across a panel of GBM PDX models and suggests that the combination with RT/TMZ therapy may provide additional benefits for survival extension.

**Conclusions**

- BAL101553 inhibits GBM neurosphere growth in vitro.
- BAL101553 has single-agent activity in a flank GBM spheroid model (G105) refractory to TMZ treatment.
- In GBM orthotopic spheroids, 7 out of 16 lines (including G105) demonstrated significant increases in median survival with single-agent BAL101553 compared to placebo.
- The combination of TMZ and BAL101553 did not extend survival in a GBM MGMT unmethylated line in comparison to BAL101553 treatment alone or in a GBM MGMT methylated line in comparison to TMZ alone.
- The combination of BAL101553 with radiation or with TMZ and radiation extends survival in the GBM spheroid line in comparison to the TMZ radiation standard of care combination.
- Prolonged BAL101553 and radiation combination therapy is more effective than short-term combination therapy.