The novel tubulin-binding 'tumor checkpoint controller' BAL101553 has anti-cancer activity alone and in combination treatments across a panel of GBM patient-derived xenografts

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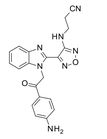
Abstract 4781

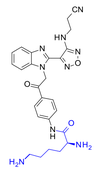
Microtubule-targeting agents (MTA) have been employed in the treatment of many cancers for decades. BAL101553 is a highly soluble prodrug of BAL27862, a novel, small molecule, microtubule-depolymerizing agent that induces tumor cell death by activating the 'spindle assembly checkpoint'. Given intravenously or orally, the drug penetrates the brain and has anti-cancer activity in diverse tumor models refractory to standard MTA or radiotherapy (RT). In this study, BAL101553 was evaluated in orthotopic xenografts from 16 GBM PDX models; 7 of 16 lines demonstrated significant ($p<0.01$) increases in median survival with BAL101553 versus placebo (range in median survival extension 24-87%). The combination of BAL101553 with conventional therapies for GBM (RT and temozolomide (TMZ)) was then evaluated in select lines. In the MGMT methylated GBM12 line, combination of RT with TMZ increased survival compared to placebo (median survival 80 days vs. 23 days, respectively; $p<0.001$). Extended BAL101553 monotherapy provided a short but significant extension in survival (median survival 31 days, $p<0.001$), while extended BAL101553 dosing during and after RT/TMZ (median survival 85 days) did not extend survival relative to RT/TMZ alone ($p=0.56$). In contrast, in the MGMT unmethylated GBM6 line, combination of RT and extended BAL101553 increased survival (median 90 days, $p<0.001$) relative to either treatment alone (median survival BAL101553 63 days; RT 69 days) or placebo (46 days). Additionally, the combination of BAL101553 with TMZ (median survival 70 days) was more effective than TMZ alone (median survival 60 days; $p=0.009$). Consistent with the unmethylated MGMT status, the TMZ/RT combination (median survival 66 days) was similar to RT alone ($p=0.62$), but the combination of extended BAL101553 with RT/TMZ (median survival 101 days; $p<0.001$ compared to other combination groups) was significantly more effective. To further evaluate whether BAL101553 is a true radiosensitizer, a second GBM6 study was performed. Also here, combination of RT (20Gy, 2wks) with extended BAL101553 dosing (median survival 66 days) significantly extended survival compared to RT alone (median survival 54 days; $p=0.002$). Interestingly, when BAL101553 dosing was limited to 2 weeks with RT, there was no increase in median survival (58 days; $p=0.16$). To evaluate effects on tumor repopulation during RT, the efficacy of an extended RT schedule (36 Gy, 6 wks) with or without 6 weeks of BAL101553 was evaluated. In this case, BAL101553 given during the RT schedule (median survival 78 days) extended median survival as compared to RT alone (61 days; $p<0.001$). Collectively, these data demonstrate that BAL101553 has broad single agent activity across a panel of GBM PDX models and suggests that combination with RT/TMZ therapy may provide additional benefits for survival extension.

Structure and Background

BAL27862 *in vitro* use

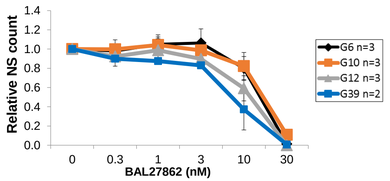


BAL101553 pro-drug- *in vivo* use



- BAL27862 is a microtubule-destabilizing drug, binds to tubulin and interferes with microtubule formation, and efficiently penetrates the brain in mouse models.
- BAL27862 binds to a different site on microtubules than currently approved microtubule-targeting agents (the colchicine site) with differing effects on microtubule dynamics.
- BAL101553 is a prodrug of BAL27862 currently in Phase I/IIa clinical studies, with oral and i.v. administration, in adult patients with advanced solid tumors.

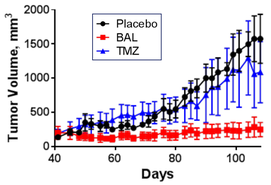
BAL27862 inhibits neurosphere formation in GBM cells



Explant cultures of GBM patient-derived xenografts (PDXs) were grown in serum-free conditions, plated in 96-well plates and treated with BAL27862 for 2-4 weeks before neurospheres (NS) were counted.

BAL101553 inhibits tumor growth *in vivo*

GBM150



Established, TMZ-refractory GBM150 flank tumors were treated with BAL - 30 mg/kg BAL101553 (daily, oral) until moribund or TMZ - 50 mg/kg TMZ on Days 1-5 every 28 days, for 3 cycles.

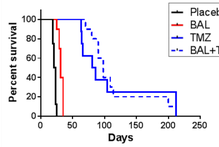
Orthotopic single agent activity

Line	MGMT promoter status	Placebo median	BAL median	Placebo mean±SD	BAL mean±SD	P value	Percent change
G6	unmethylated	48	60	47 ± 4	58 ± 10	<0.01	24%
G8	methylated	47	64	48 ± 5	64 ± 7	<0.01	36%
G10	unmethylated	35	38	35 ± 2	38 ± 5	0.04	10%
G12	methylated	23	31	22 ± 2	32 ± 3	<0.01	35%
G15	methylated	71	87	110 ± 120	89 ± 21	0.41	22%
G22TMZ	methylated	27	26	26 ± 5	27 ± 4	0.55	-4%
G26	unmethylated	51	66	52 ± 7	71 ± 17	<0.01	31%
G39	methylated	31	57	30 ± 3	55 ± 14	<0.01	87%
G59	methylated	45	56	52 ± 28	68 ± 36	0.02	23%
G84	methylated	56	73	58 ± 8	68 ± 8	<0.01	29%
G108	unmethylated	39	43	41 ± 5	49 ± 16	0.11	12%
G115	unmethylated	139	183	146 ± 40	199 ± 60	0.07	31%
G116	methylated	61	64	118 ± 117	66 ± 12	0.45	5%
G117	methylated	65	81	77 ± 22	86 ± 23	0.30	26%
G122	unmethylated	80	84	83 ± 11	90 ± 15	0.28	5%
G150	unmethylated	52	69	52 ± 6	84 ± 40	<0.01	32%

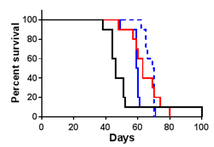
For 16 different PDX lines, mice with established orthotopic tumors were treated with placebo or 30 mg/kg BAL101553 (daily, oral) until moribund. Yellow indicates significant single agent activity at $p<0.01$. Survival medians are expressed in days.

Combination BAL101553 and TMZ treatment

GBM12
MGMT methylated



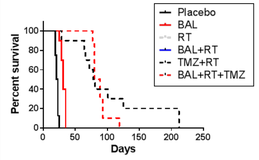
GBM6
MGMT unmethylated



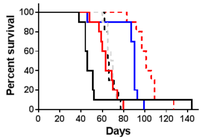
Combination therapies were studied in mice with established orthotopic tumors for GBM12 or GBM6. Starting on Day 7 (GBM12) or Day 13 (GBM6), mice were treated alone or in combination as indicated. BAL - 30 mg/kg BAL101553 (daily, oral) until moribund; TMZ - 20 mg/kg TMZ daily for 2 weeks in GBM6 and 50 mg/kg TMZ on Days 1-5 every 28 days, for 3 cycles in GBM12.

Combination BAL101553 and radiation treatment effective in select lines

GBM12
MGMT methylated



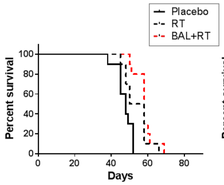
GBM6
MGMT unmethylated



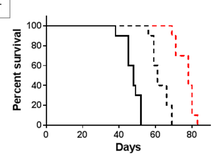
In the same experiment as above, additional arms were treated as indicated. BAL – 30 mg/kg BAL101553 (daily, oral) until moribund, RT – radiation, 20 Gy in 10 fractions over 2 weeks; TMZ - 20mg/kg TMZ daily for 2 weeks.

Concurrent BAL101553 with short vs prolonged radiation schedule

2 week schedule



6 week schedule



In a separate study, mice with established orthotopic GBM6 tumors were treated with short or protracted schedules of radiation (RT): 2 weeks - 20Gy in 10 fractions (starting on Day 14) or 6 weeks - 36Gy in 18 fractions. BAL (30mg/kg BAL101553, daily, oral) was given concurrently with RT for either 2 or 6 weeks, as appropriate.

Conclusions

- BAL27862 inhibits GBM neurosphere growth *in vitro*.
- BAL101553 has single-agent activity in a flank GBM xenograft model (G150) refractory to TMZ treatment.
- In GBM orthotopic xenografts, 7 out of 16 lines (including G150) 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 GBM6 xenograft 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.

This work was supported by funding from Basilea Pharmaceutica International Ltd and NIH.

http://www.mayo.edu/research/labs/translational-neuro-oncology