Vertical inhibition of the MAPK pathway as potential treatment strategy in NRAS -mutant melanoma

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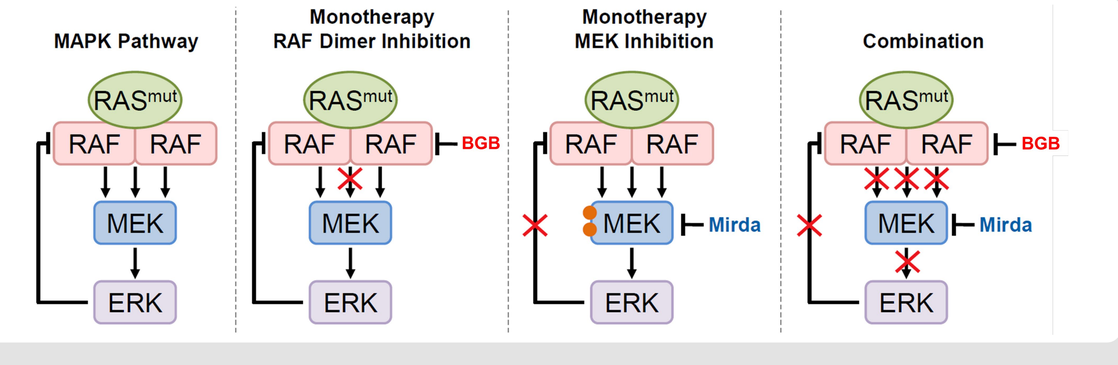
Background

- Metastatic melanoma is the most aggressive form of skin cancer. Worldwide incidences of melanoma continue to rise, with 100,000 new cases estimated in the United States during 2022, ranking melanoma as the sixth most common cancer.
- A large proportion of melanomas carry mutations in the mitogen-activated protein kinase (MAPK) signaling pathway leading to hyper-activation and proliferation of the mutated cells. Driver mutations in BRAF V600 (50%) and NRAS Q61 (20%) are the most common mutations found in melanoma.
- Newly developed FDA-approved kinase inhibitors that directly target BRAFV600E-mutated tumours have shown dramatic and long-lasting clinical efficacy, but relapses do occur.
- Treatment of tumours containing NRAS mutations have relied on inhibition of MEK, a downstream target of NRAS, as no current therapies that directly target mutant NRAS are available.
- Importantly, previous experiments using the pan-RAF dimer inhibitor Lifirafenib and the selective MEK inhibitor Mirdametinib showed striking antitumor activity after combination treatment in RAS-mutated solid tumours. Collectively, these data suggest that vertical inhibition of the MAPK in mutated melanoma tumours could result in synergistic benefits.

Therapeutic strategy



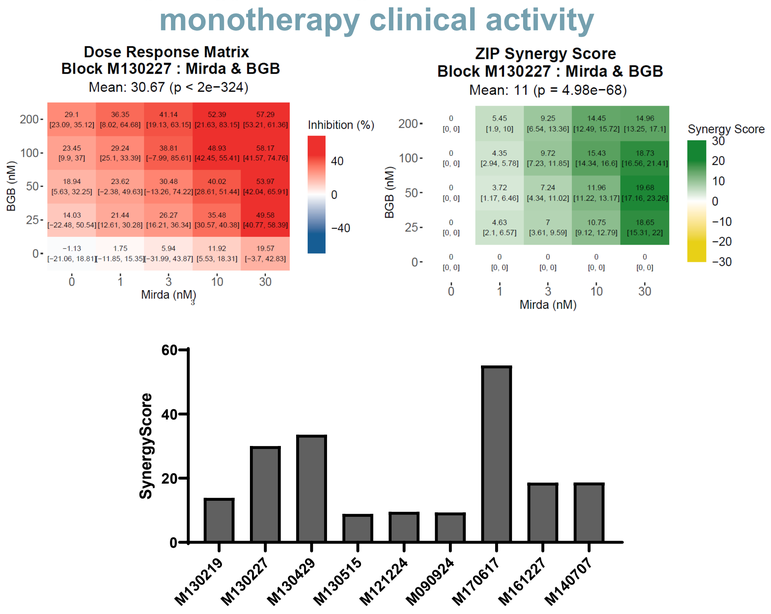
- **Mirdametinib** (PD-0325901) is an investigational, oral, potent, and selective inhibitor of MEK that has demonstrated monotherapy clinical activity in certain tumours driven by overactivation of the MAPK pathway.
- **BGB-3245** is an investigational, oral, and potent second-generation RAF dimer inhibitor. It is being investigated in an on-going clinical study (NCT04249843) in participants with advanced or refractory solid tumors harboring BRAF or NRAS mutations.

Results

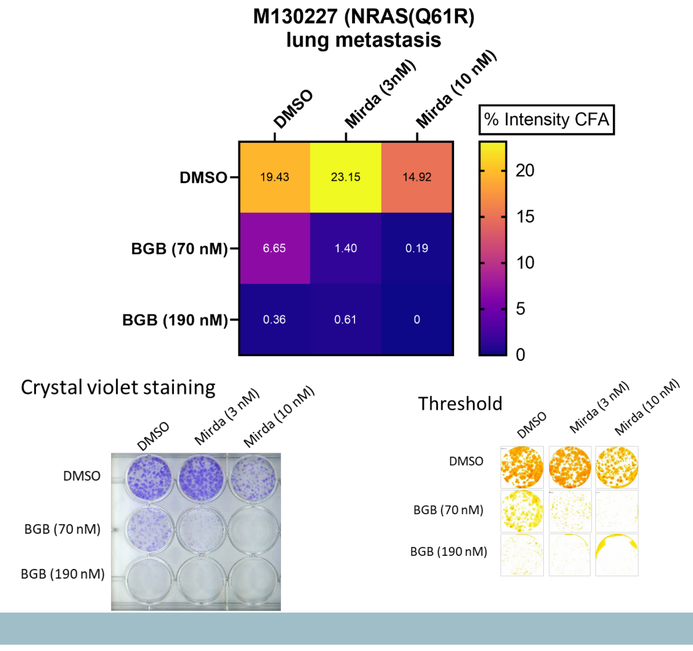
Pan-RAFi (BGB-3245) and MEKi (Mirdametinib) have inhibitory effects on primary patient-derived NRAS mutated melanoma cell lines *in vitro*

	cell culture	oncogenic mutation	MEKi IC50 (nM) Mirdametinib	panRAFi IC50 (nM) BGB-3245	IC50 (nM) Lifirafenib	IC50 (nM) Belvarafenib	IC50 (nM) Naporafenib
Bini resistant	M130219	NRAS(Q61R)	264	784	>10000	>10000	1558
	M130227	NRAS(Q61R)	360	574	1200	504	604
Bini sensitive	M130429	NRAS(Q61R)	20	279	600	533	1025
	M130515	NRAS(Q61R)	10	164	1200	240	367
BRAF/NRAS double mutated	M121224	BRAF(V600E)/ NRAS(Q61K)	26	51	440	81	191
	M150423	BRAF(V600E)/ NRAS(Q61R)	107	>10000	5000	1481	401
additional cohort 2 (other Q61x)	M140707	NRAS(Q61K)	4	168	1100	471	710
	M090924	NRAS(Q61H)	32	454	730	312	684
additional cohort 3 (previously IT treated patient)	M170617	NRAS (Q61R	14	725	9906	2274	1861
	M161227	NRAS (Q61R)	7	156	1158	173	224

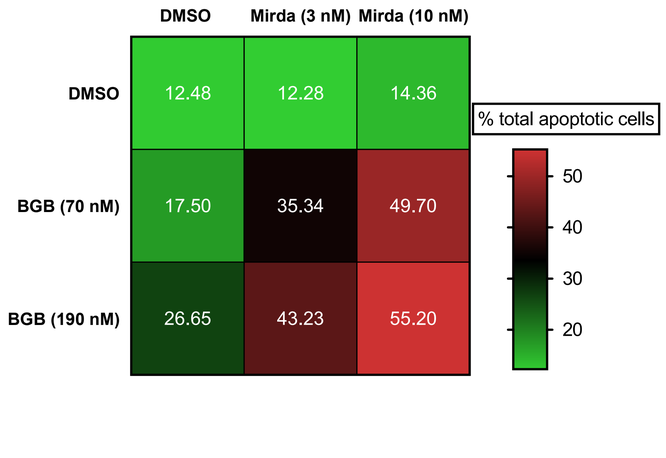
Treatment with BGB-3245 and Mirdametinib *in vitro* resulted in significant and positive synergy scoring with evidence of monotherapy clinical activity



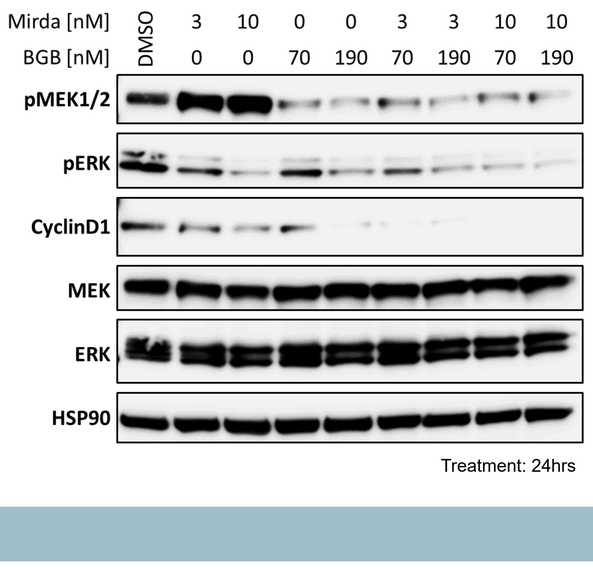
Inhibition of colony formation in long-term assays was enhanced by the combination of BGB-3245 and Mirdametinib



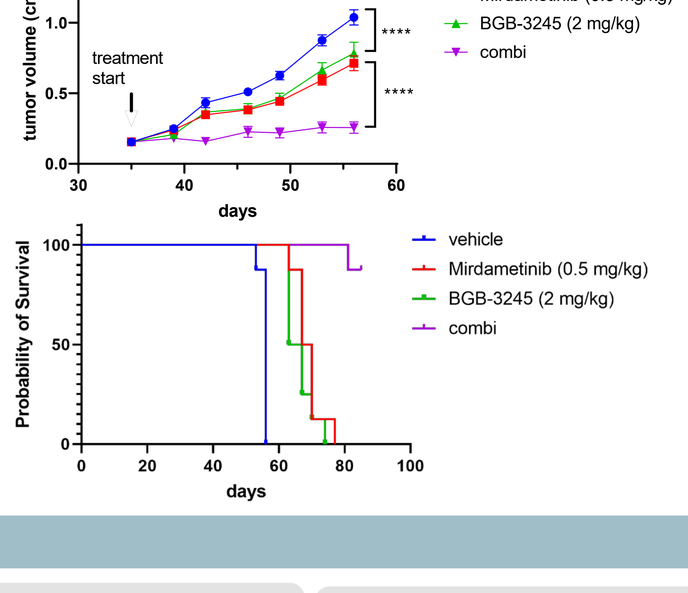
Combination of BGB-3245 and Mirdametinib induced greater frequencies of apoptosis at clinical relevant concentrations



BGB-3245 and Mirdametinib treatment resulted in dose-dependent MAPK pathway inhibition *in vitro*



In vivo combination of BGB-3245 and Mirdametinib resulted in tumour regression and survival benefit in Xenograft models



Conclusion

- This study showed significant therapeutic effects on primary patient-derived NRAS Q61 mutated melanoma cell lines, both in vitro and in vivo, using the combination of BGB-3245 and Mirdametinib at clinically relevant doses.
- Mechanistically, the combination of BGB-3245 and Mirdametinib treatment resulted in greater apoptosis of treated cell lines in vitro. This correlated with dose-dependent inhibition of the MAPK pathway proteins pMEK1/2, pERK, and Cyclin D1.
- NRAS Q61 may serve as a prospective biomarker for patient stratification in trials assessing the combination of pan-RAF and MEK inhibitors in mutant melanoma.
- Overall, these data support the clinical investigation of MAPK pathway vertical inhibition through combination treatment with BGB-3245 and Mirdametinib in patients with RAS mutations.

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