Abstract # 3729

MP-470, a Rad51 Suppressor and Multiple TK Inhibitor, Retards Growth of Primary Human Glioblastoma Multiforma (GBM) Cells and Synergizes Effect of Temozolomide

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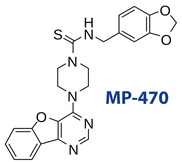
Abstract

MP-470 suppresses Rad51, essential for repair of DNA double strand breaks, and multiple tyrosine kinases including mutant c-Kit, mutant PDGFR, mutant FLT3, and c-Met. In this study, primary GBM tumor specimens from eleven patients on standard of care treatment prior to surgery were used to evaluate MP-470's efficacy as a single agent and in combination with Temozolomide. The GBM cells were tested for cell viability and placed in soft agar 96-well plates in a humidified incubator (37°C, 5% CO2). MP-470 was evaluated at 1.25, 2.5, 5, 10 and 25 μM and Temozolomide at 100, 200, 400, 800 and 1600 μM, and MCF-7 cells were used as a control. After five days of incubation anchorage-independent growth was measured by Alamar Blue assay. Susceptibility to both drugs varied across the patients, MP-470's IC_{50} ranged from 0.05 to 29.6 μM (average: 4.6 μM) and Temozolomide IC_{50} ranged from 346.5 to 1958.8 μM (average = 1058 μM). While MP-470 was effective in all 11 tumor explants, Temozolomide demonstrated activity in 10 of the 11 explants. Furthermore, MP-470 was synergistic with Temozolomide in 8 of 11 explants, at two combinations (MP-470 2.5 μM + Temozolomide 800 M and MP-470 1.25 μM + Temozolomide 400 μM). In addition, exposure sequence tests showed synergy in 10 out of 11 explants when MP-470 (1.25 μM) was followed by Temozolomide (800 μM) 24+/-2h later. In conclusion, MP-470 inhibits growth of primary human GBM explants as a single agent and is synergistic with Temozolomide in combination; the combination is more effective when MP-470 is used first followed by Temozolomide.

Introduction

- MP-470 is a novel small molecule with potent activity against several tyrosine kinase targets: mutant c-Kit, mutant PDGFR, mutant Flt3 and c-Met.
- MP-470 also sensitizes cancer cells to DNA damaging agents and XRT by suppression of Rad51 and DNA double-strand break repair.
- Resistance of Glioblastoma Multiforme (GBM) to standard DNA damaging therapies is thought to be due to increased DNA repair and inhibited apoptosis.
- MP-470 was shown to sensitize GBM cell lines to XRT by inhibiting DNA double-strand break repair (Welsh et al.)
- MP-470 is currently being studied in a Phase I clinical trial and a Phase Ib five-arm combination trial with standard-of-care anti-cancer therapies in solid tumors.



Objective

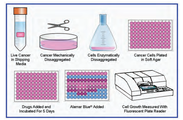
In this study, primary GBM tumor specimens from eleven patients on standard of care treatment prior to surgery were used to evaluate MP-470's efficacy as a single agent and in combination with Temozolomide.

Materials & Methods

- Viably cryopreserved glioblastoma explants were used for this study.
- Tissues used in this study (Mosaic Laboratories, Lake Forest, CA) were collected from patients presenting for routine clinical evaluation in accordance with ethics review board-approved protocols at each clinical site.

Alamar Blue Assay (Mosaic Blue Assay™)

1. Viably cryopreserved glioblastoma explants in DMSO were thawed in a manner to maintain viability.
2. Tumor cells were evaluated for viability and percent tumor prior to plating in soft agar in a 96-well plate in the presence of MP-470 (range 25-0.0390625 μM) or temozolomide (range 1600- 12.5 μM).
3. Anchorage-independent growth was measured by fluorescence using Alamar Blue (Invitrogen) on day 5. Percent inhibition was calculated by comparing the average corrected MP-470 and temozolomide results to the average corrected no drug treatment results.



Combination Index

The combination index (CI) was calculated using CalcuSyn (V2.0, Biosoft, Cambridge, United Kingdom) using the median-effect analysis derived by Chou and Talalay and the following equation:

$$CI = \frac{D^1}{D^1_x} + \frac{D^2}{D^2_x} + \frac{D^1.D^2}{D^1_x.D^2_x}$$

where D1 and D2 are the doses of drug 1 and drug 2 that have x% effect when used in combination, and Dx^1 and Dx^2 are the doses of drug 1 and drug 2 that have the same x% effect when used alone

Synergy, additivity, and antagonism are defined as

CI < 1 – Synergy
CI = 1 – Additivity
CI > 1 - Antagonism

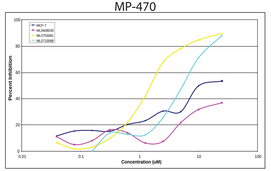
Clinical Information on Tumors

Mosaic ID	Tumor	Site	Age	Gender	Race	Prior Therapy	Comments
Titration Phase							
ML0608035	Glioblastoma	Occipital Lobe	51	F	White	Unknown	
ML0703081	Glioblastoma	Brain	44	M	White	Yes	Dexamethasone
ML0710088	Glioblastoma	Brain	59	F	White	No	
Testing Phase							
ML0610017	Glioblastoma	Brain	59	M	White	Unknown	
ML0612059	Glioblastoma	Brain	62	F	White	Unknown	
ML0701091	Glioblastoma	Brain	66	M	White	Unknown	
ML0703049	Glioblastoma	Brain	NA	F	White	Yes	Dexamethasone
ML0703063	Glioblastoma	Brain	48	M	White	Yes	Dexamethasone
ML0704021	Glioblastoma	Brain	48	F	White	Yes	Dexamethasone
ML0705060	Glioblastoma	Brain	60	M	White	Unknown	
ML0711030	Glioblastoma	Brain	35	M	White	Yes	Dexamethasone
ML0711050	Glioblastoma	Brain	43	F	White	Yes	Dexamethasone
ML0711165	Glioblastoma	Left Temporal Lobe	61	M	White	No	
ML0801114	Glioblastoma	Brain	43	F	Unknown	Yes	Dexamethasone

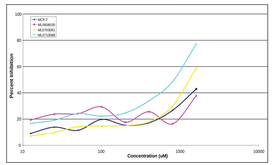
Results

Titration of MP-470 and Temozolomide in GBM Primary Cells

MP-470



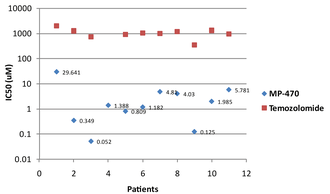
Temozolomide



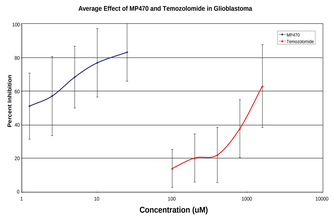
Outline of single agent and combination drug concentrations

Outline of Drug Concentrations	MP470 Concentrations	Temozolomide Concentrations
MP-470 Single Agent	25, 10, 5, 2.5, and 1.25 μM	NA
Temozolomide Single Agent	NA	1600, 800, 400, 200, and 100 μM
MP-470 + Temozolomide Combination	5, 2.5, and 1.25 μM	800, 400, and 200 μM
MP-470 (Day 0); Temozolomide (Day 1)	2.5 μM	1600, 800, 400, 200, and 100 μM
MP-470 (Day 0); Temozolomide (Day 1)	25, 10, 5, 2.5, and 1.25 μM	800 μM

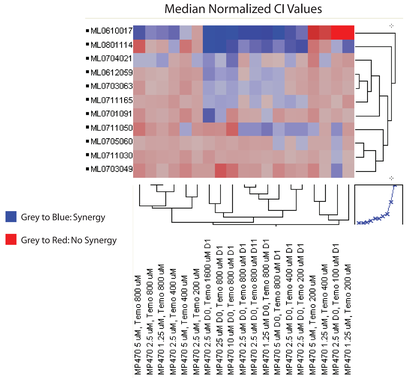
Single agent IC_{50} of MP-470 and Temozolomide in 11 GBM Primary Samples



Average effect of MP-470 and Temozolomide on 11 Glioblastoma Explants in the Almar Blue Assay.



Median Normalized CI Values



Conclusions

- MP-470 demonstrated activity in all 11 glioblastoma explants tested.
  - IC_{50} values for MP470 ranged from 0.052 μM to 29.641 μM (average = 4.56 μM).
- Temozolomide demonstrated activity in 10 out of 11 glioblastoma explants tested.
  - One specimen treated with temozolomide (ML0703049) demonstrated complete resistance to temozolomide at all 5 concentrations tested.
  - IC_{50} values for temozolomide ranged from 346.479 μM to 1958.750 μM (average = 1058.19 μM).
- Low μM MP-470 was synergistic when combined simultaneously or sequentially with Temozolomide in 10 out of 11 explants.
- A greater degree of synergy was observed when MP-470 and Temozolomide were tested sequentially compared to simultaneously.

References

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4. R. Tibes et al. A Phase-1 study of MP-470, a novel orally bioavailable small molecule with Rad51 suppression activity. Proceedings from ESMO Annual Meeting 2008.
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