Targeting DNA Repair Mechanisms with Spironolactone to Enhance LP-184 CNS Pharmacodynamics and Efficacy

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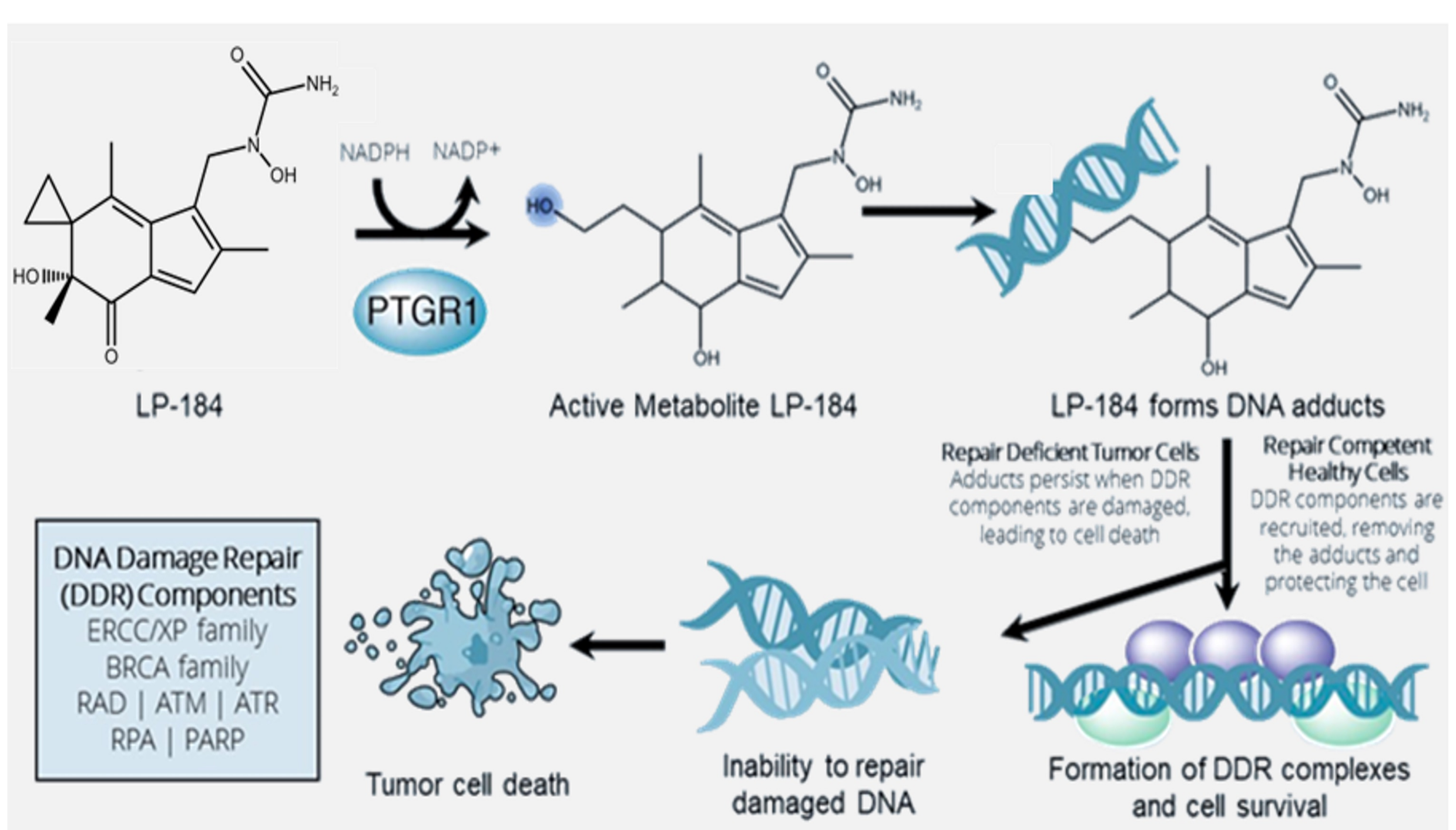
Abstract

Limited CNS bioavailability and on-target pharmacodynamics are major obstacles to developing new and effective systemic therapies for GBM and other primary brain tumors. Strategies to overcome these obstacles include blood-brain barrier disruption to enhance CNS penetration and brain penetrant drug combinations that enhance tumor cell chemosensitivity through synthetic lethality. LP-184 is a synthetic acylfulvene class small molecule alkylator that induces DNA damage and inhibits MGMT-expressing GBM cell viability with IC50 range ~20-300 nM. LP-184 improves survival in orthotopic GBM xenograft models with pre-clinical brain Cmax ~840 nM and brain tumor Cmax ~2,500 nM. Pre-clinical brain/plasma and brain tumor plasma ratios are 0.11 and 0.2, respectively. Pharmacokinetics from a first-in-human Phase 1a study (NCT05933265) shows plasma Cmax ~1000 nM at likely the RDE, predicting >IC50-range of brain and tumor concentrations based on in vitro and in vivo pre-clinical data. Transcription-coupled nucleotide excision repair (TC-NER) reverses LP-184 DNA damage and unbiased GSEA coupled with LP-184 sensitivity screens identified ERCC3, a TC-NER complex helicase, as a target for sensitizing GBM cells to LP-184. Spironolactone, an FDA-approved BBB-permeable aldosterone inhibitor, is predicted to sensitize GBM to LP-184 via multiple molecular targets. Treating GBM models including PDX-derived cells with spironolactone (25 µM) induces ERCC3 protein degradation by up to 95% and sensitizes cells to LP-184 in vitro (~5-fold IC50 reduction). Kinetic analysis of spironolactone-induced (35 mg/kg daily) ERCC3 degradation in orthotopic GBM PDX xenografts revealed ~50%, 56% and 79% degradation on treatment days -2, -1, and 0, respectively. Furthermore, spironolactone enhances orthotopic GBM PDX responses to LP-184. Machine learning approaches also associate spironolactone-induced GBM cell sensitization to LP-184 with down-regulation of the TC-NER complex endonuclease ercc1 and the DNA damage sensor cetn2. These findings provide a rationale and framework for a planned Phase 1b/2a trial to evaluate LP-184 + spironolactone therapy against recurrent GBM.

LP-184: Mechnism of Action



GBM with higher PTGR1 levels compared to normal brain sufficient to activate LP-184

PTGR1 expression level in GBM	Number of patients	Log2 (normalized RNAseq as transcripts per million + 1) range	Percentage
Low	0	0	0%
Intermediate	148	6.31 - 9.71	89.16%
High	18	9.76 to 10.61	10.84%

Normal brain (GTXe data) mean **3.67 (range 0-6.06)**

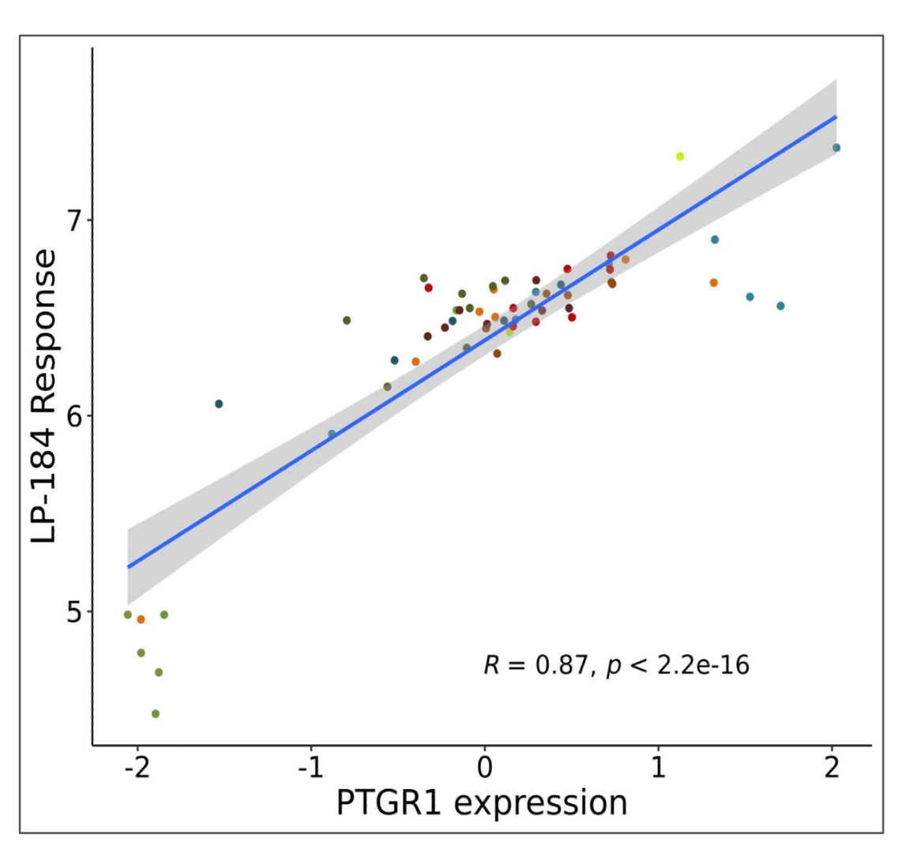
Glioblastoma (TCGA data) mean 4.98 (1.3x higher)

Alkylator Chemotherapy Comparison

	LP-184	Temozolomide	Nitrosourea (CCNU)
Molecular weight (kD)	304	194	233
DNA repair system	TC-NER & HR	MGMT	HR
Tumor/blood concentration ratio	0.2	0.2	0.5
IC_{50}	200nM	500 mM	50 mM
Bioactivation	Prodrug, conversion by intracellular PTGR1	Prodrug, spontaneous conversion by hydrolysis to MTIC	Prodrug, spontaneous conversion by hydrolysis

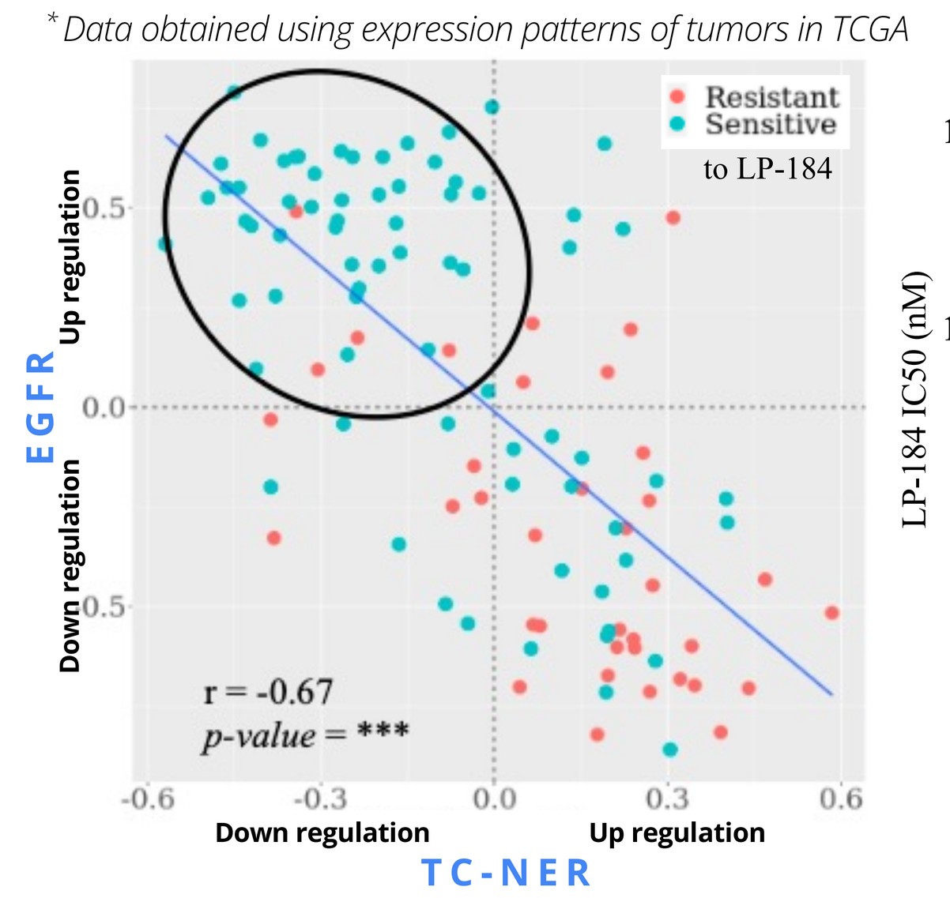
Preclinical Results

In silico comparison of LP-184 anti-tumor activity versus expression of PTGR1

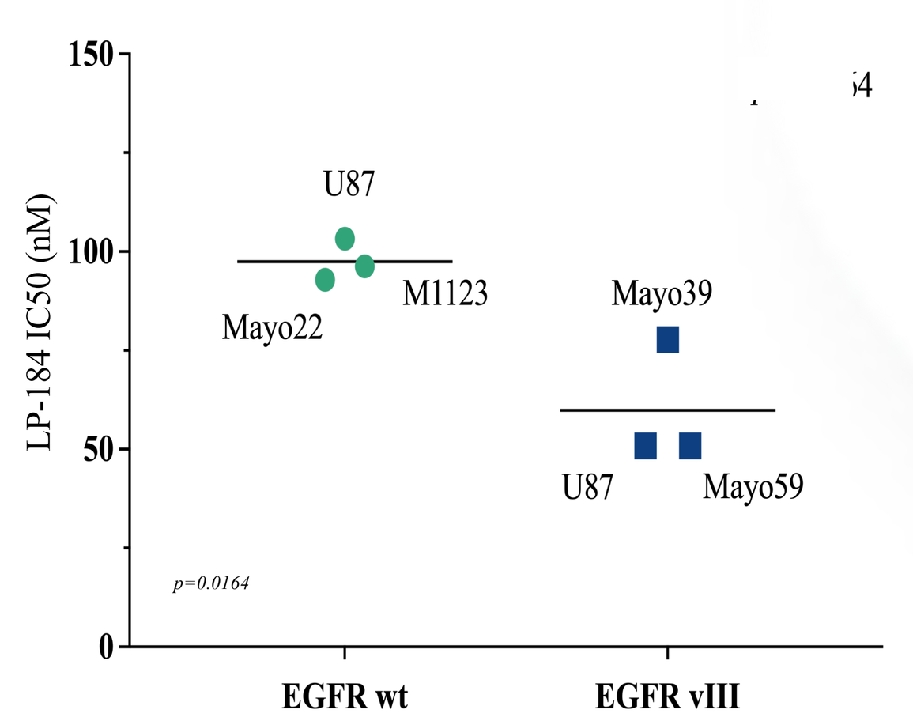


In silico LP-184 sensitivity correlates with low TC-NER & high EGFR signaling pathway

* Data obtained using expression patterns of tumors in TCGA



EGFR altered GBM cell lines show enhanced sensitivity to LP-184



LP-184 penetrates the blood brain barrier (BBB) with favorable CNS tumor bioavailability

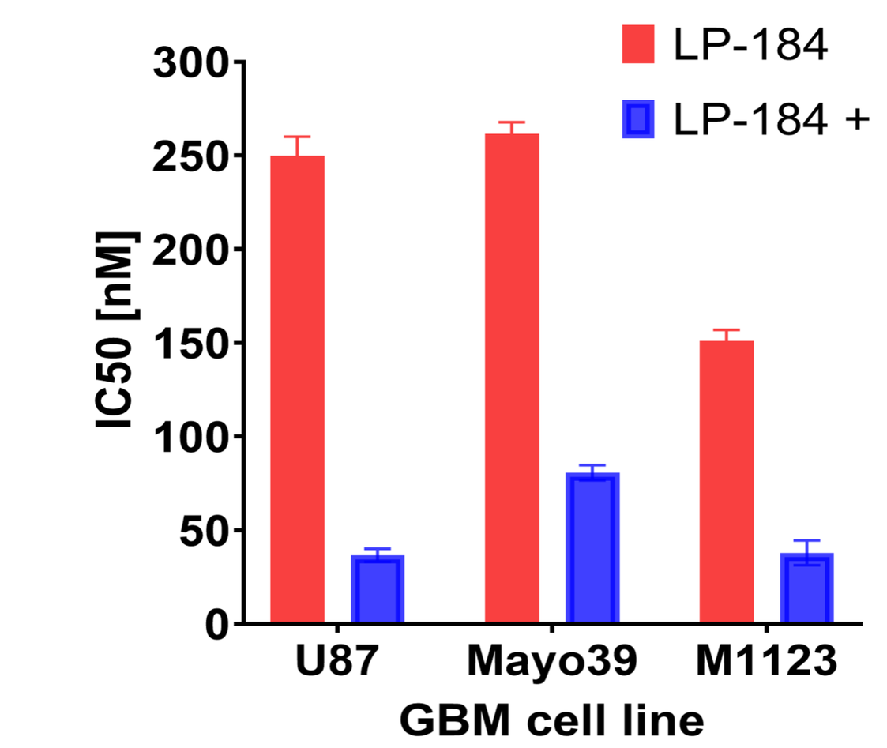
Matrix	LP-184 4 mg/kg single i.v. dose pharmacokinetic data (Mean ± SEM)						LP-184 Brain Tissue / Plasma Ratio	Historical TMZ Brain Tissue / Plasma Ratio
	C_{max} (ng/g or ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/g Or ng.h/mL)	Half-Life (h)	C_{max} (nM)	AUC (nM)		
Plasma	3438 ± 125	0.0833	1592 ± 48.7	0.236	11296 ± 410	5231 ± 160	-	-
Peritumoral Brain	279 ± 20.2	0.250	165 ± 11.6	0.383	916 ± 66	542 ± 38	**0.103**	**0.118**
Contralateral Brain	223 ± 50.6	0.250	123 ± 15.9	0.444	732 ± 166	404 ± 52	0.077 ± 0.12	-
Brain Tumor	773 ± 58.6	0.0833	319 ± 36.4	0.281	2539 ± 193	1048 ± 120	**0.200**	**0.202**

- LP-184 pharmacokinetic analyses in SCID mice bearing orthotopic GBM xenografts showed normal brain/plasma ratio 0.1 (Cmax = 916 nM) and brain tumor/plasma ratio 0.2 (Cmax = 2539 nM).
- LP-184 BBB permeability is comparable to TMZ and brain tumor Cmax achieved after a single i.v. infusion is greater than mean LP-184 IC50 (~200 nM) for sensitive GBM cell models.

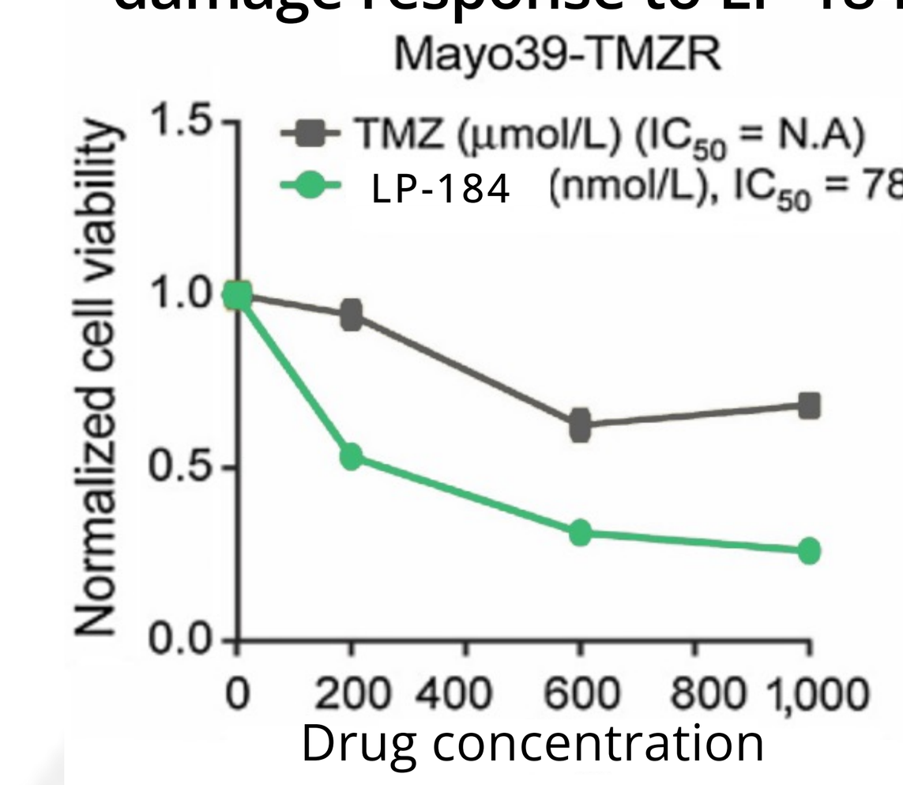
Preclinical Results

Combination with Spironolactone enhances the in vitro and in vivo anti-tumor efficacy of LP-184

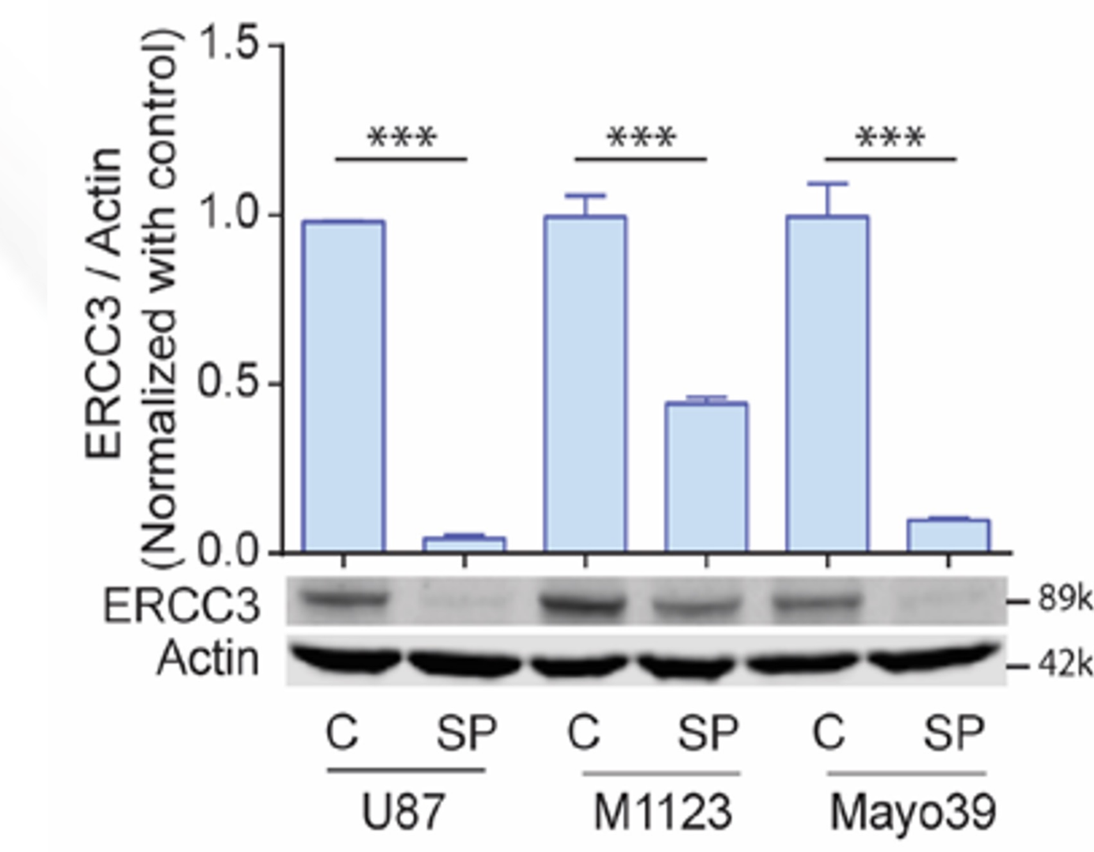
A 3-6 fold increase in LP-184 sensitivity



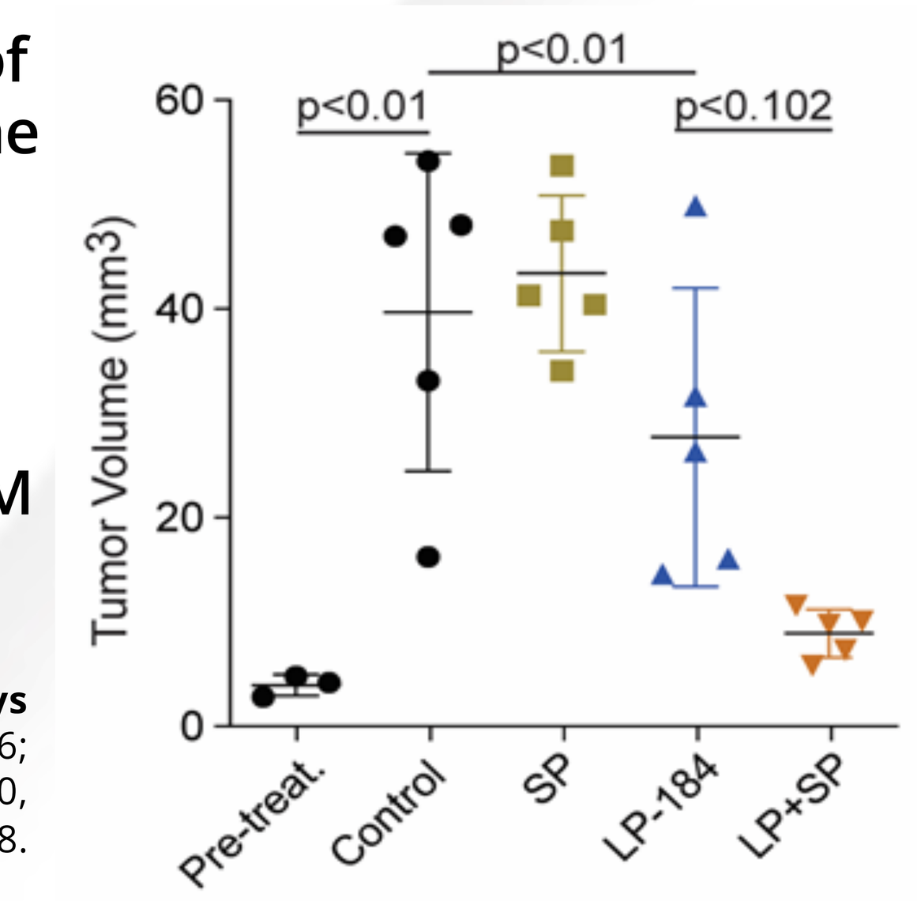
B 3 fold increase in γH2AX DNA damage response to LP-184



C 24h SP treatment depleted ERCC3 protein by up to 95%

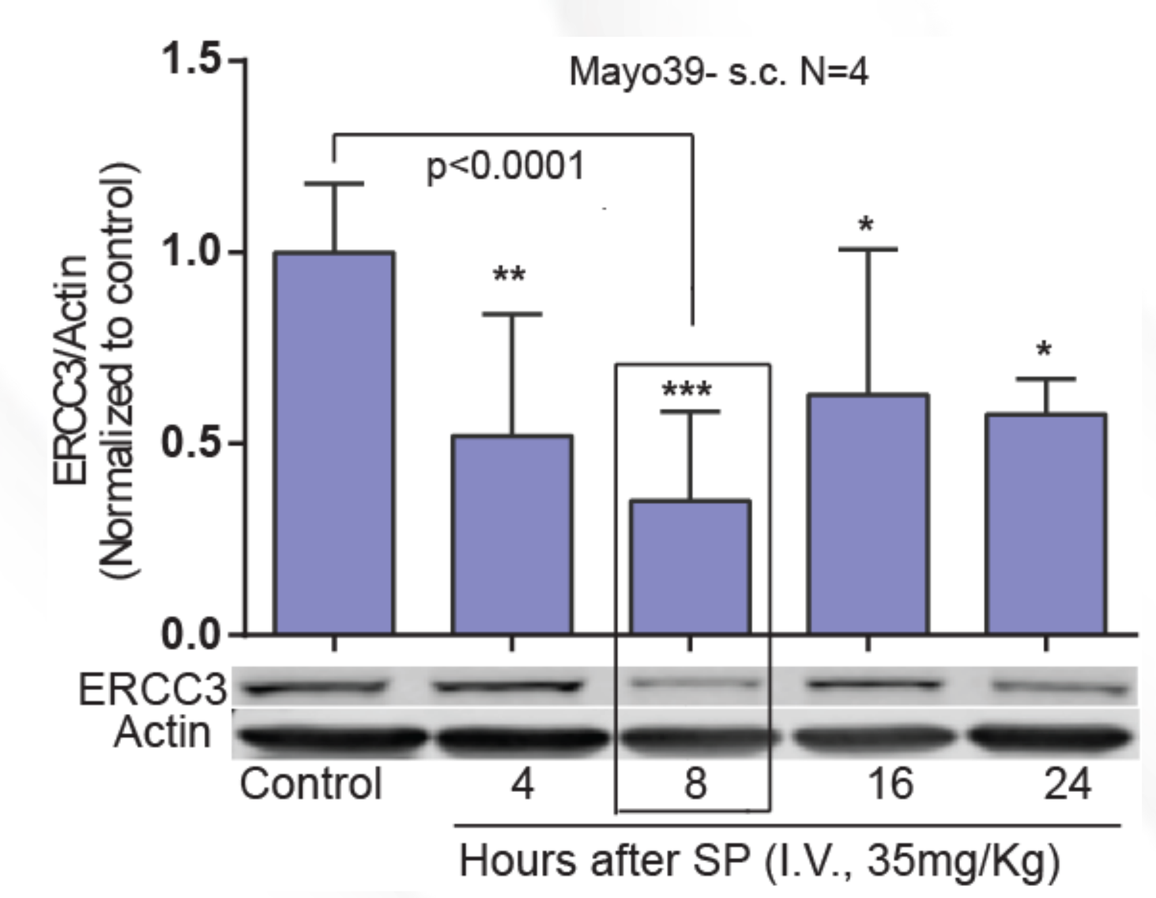


D Combination treatment of LP-184 and Spironolactone in vivo induced complete tumor regression with prolonged duration of response in orthotopic M1123 mesenchymal GBM



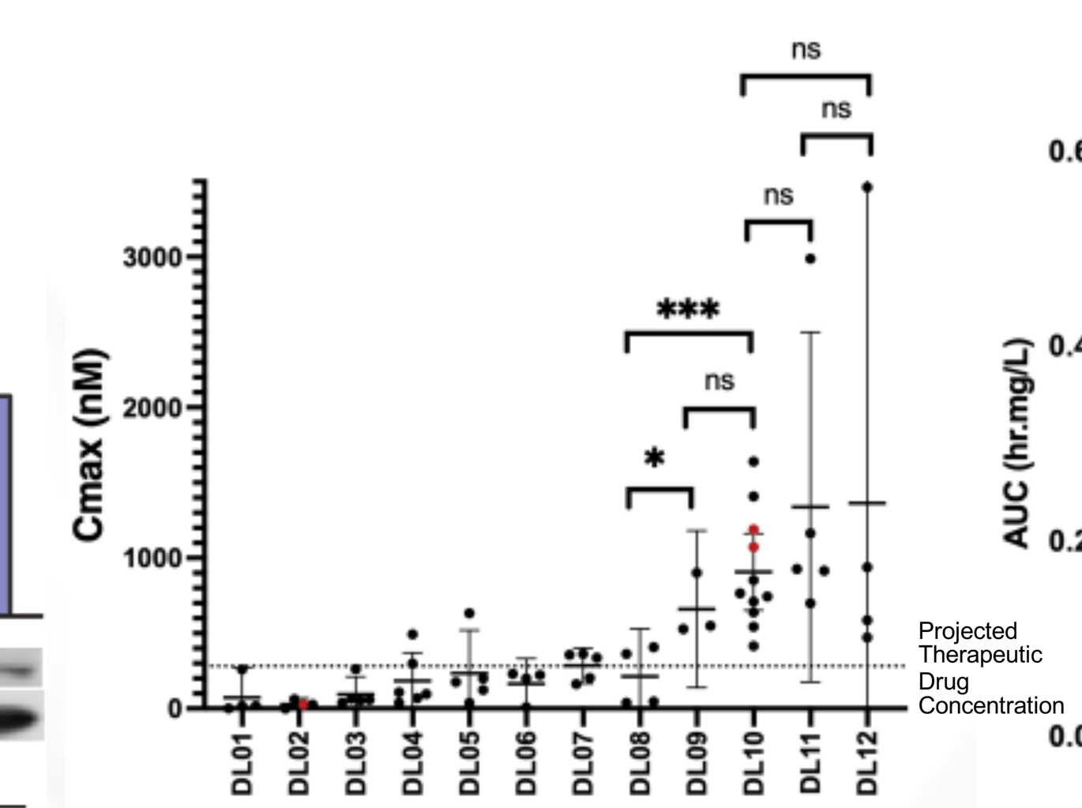
- Spironolactone treatment leads to depletion of ERCC3 protein and up to 6x increased sensitivity to LP-184 treatment
- Spironolactone monotherapy had no effect on tumor growth compared with vehicle-treated
- LP-184 alone and combined with Spironolactone induced complete or near complete tumor regression.
- Combining Spironolactone with LP-184 generated more durable responses

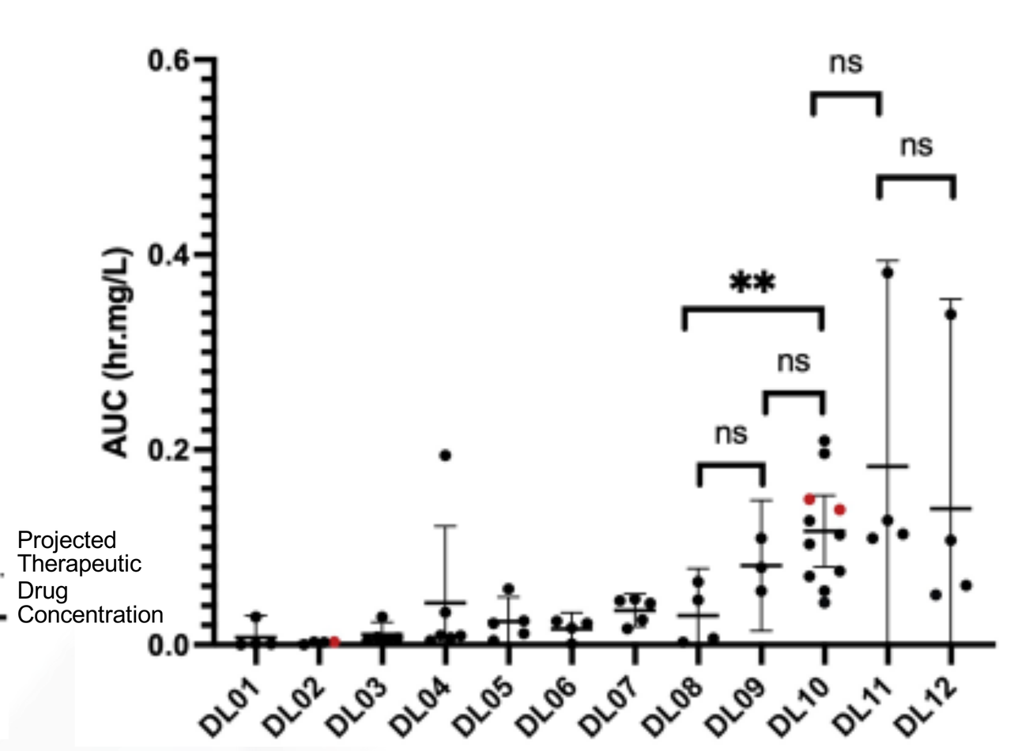
ERCC3 levels nadir 8 hours post Spironolactone administration in Mayo39 GBM xenografts

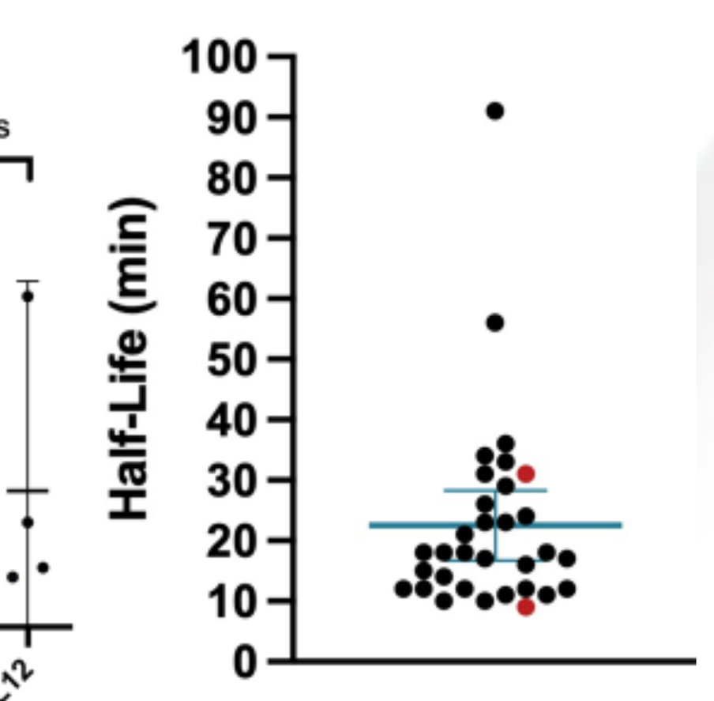


Clinical Results

LP-184 Phase 1a Study - Pharmacokinetic Cmax and AUC versus Dose Level







Conclusion

- LP-184 is a novel potent brain penetrant alkylator
- Bioactivation occurs intracellularly by the enzyme PTGR1
- Alkylation by LP-184 is repaired by NER which can be pharmacologically inhibited by predose spironolactone resulting in increased sensitivity to LP-184
- FIH PK data suggest that LP is likely to achieve sufficient clinical CNS bioavailability (200 nM) well above in vitro IC50 (~50 nM) when combined with SP

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