Preclinical efficacy of LP-184, a tumor site activated synthetically lethal therapeutic, in glioblastoma

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Background

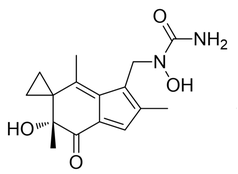
- Temozolomide, the most effective standard-of-care chemotherapy for newly diagnosed glioblastoma, is ineffective in ~70% of patients due to MGMT-driven resistance and there is no effective chemotherapy for recurrent GBM [1, 2].
- New agents with activity against TMZ-resistant and recurrent GBM are desperately needed.
- The following findings support the potential for LP-184, a novel acylfulvene-derived DNA damaging small molecule therapeutic, to fill this void, and provide the preclinical foundation for testing LP-184 in GBM patients:

(i) nanomolar activity against multiple GBM cell models including TMZ-resistant cells
(ii) favorable CNS penetration with C_{MAX} levels well above in vitro IC50s
(iii) durable regression of tumor xenografts and animal survival prolongation
(iv) synthetic lethality with Spironolactone, an FDA approved agent and clinically translatable inhibitor of nucleotide excision repair
(v) transcriptomic/pathway analyses predicting LP-184 sensitivity in clinical GBM subsets.

Objectives

- Evaluate the potency of LP-184 in an MGMT expressing GBM PDX-derived model in vitro in comparison with TMZ
- Determine the effects of LP-184 + Spironolactone combination treatment on GBM cell viability and pharmacodynamics in vitro and subcutaneous xenograft tumor responses in vivo
- Analyze gene expression and pathway biomarkers predicting LP-184 sensitivity in clinical GBM samples

LP-184 Drug Profile



- **LP-184** (hydroxyurea methylacylfulvene) is a prodrug belonging to the acylfulvene class of naturally derived small molecule therapeutics [3] and requires activation by an oxidoreductase enzyme, prostagladin reductase 1 (PTGR1).
- LP-184 is expected to be highly effective in treating **at least 11%** of GBM patients based on **elevated PTGR1** levels as analyzed from a TCGA dataset on 166 clinical GBM samples.
- The FDA has granted LP-184 an **orphan drug designation** (ODD) for the the treatment of malignant glioma.

PTGR1 Expression Profile in GBM

Fig. 1A

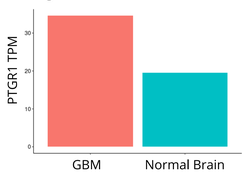


Figure 1A.
GEPIA-harmonized PTGR1 expression (TPM) analysis from GTEX normal brain and TCGA GBM highlights that PTGR1 is elevated in brain tumor tissue relative to normal brain.

Fig. 1B

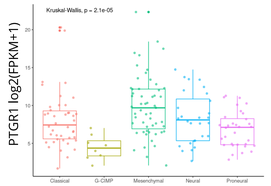


Figure 1B.
PTGR1 expression in different GBM subtypes represented in the TCGA GBM cohort, showing the highest expression in the Mesenchymal subtype.

Results

LP-184 inhibits GBM cell viability, results in durable regression of tumor xenografts and prolongs animal survival

Fig. 2

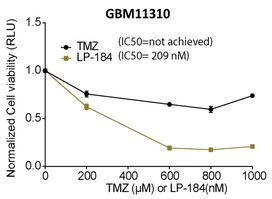


Figure 2.
LP-184 has nanomolar potency against a TMZ-resistant low passage PDX-derived GBM isolate. Cell viability in relative luminescence units (RLU). Data represents mean +/- SEM.

Fig. 3A

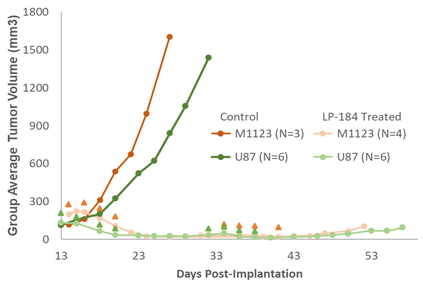


Figure 3A.
Nude mice bearing pre-established subcutaneous xenografts derived from either mesenchymal M1123 GBM neurosphere cells or U87 cells received LP-184 (4 mg/kg i.v. q.o.d x 4). Tumor regressions were observed in both M1123 and U87 models beginning as early as post-treatment day 2 with complete response or halted tumor growth in all treated animals. 107% tumor growth inhibition (TGI) was observed in both the M1123 and U87 models. 3/10 LP-184 treated U87 tumor bearing mice were entirely tumor-free from day 38 onwards until study termination. 3/4 LP-184 treated M1123 tumor bearing mice were entirely tumor-free from day 29 onwards until study termination.

Fig. 3B

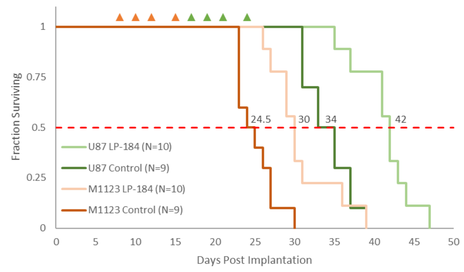


Figure 3B.
Nude mice bearing pre-established M1123 and U87 orthotopic tumor xenografts received LP-184 (4 mg/kg i.v. q.o.d X 4. A single cycle of LP-184 therapy increased survival of animals with M1123 and U87 tumor xenografts by 22% (5.5 days, $p < 0.01$) and 24% (8 days, $p < 0.001$), respectively. Median survival in days is shown at the intersection of each group with the dotted red line.

Spironolactone sensitizes GBM cells and xenografts to LP-184

Fig. 4A

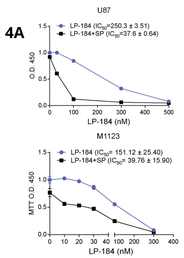


Figure 4A.
Effect of combining 25 μM spironolactone with LP-184 (72 h treatment) on viability of U87 or M1123 GBM cells. Co-treating GBM cells with LP-184 and SP decreased LP-184 IC50s 3-6 fold. Spironolactone (SP) is a blood-brain-barrier permeable agent that inhibits TC-NER by inducing ubiquitin-mediated proteolytic degradation of ERCC3 [4].

Fig. 4B

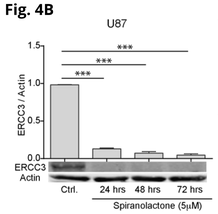


Figure 4B.
Western blot showing depletion of ERCC3 protein by up to 95% in U87 GBM cells treated with 25 μM SP over 24-72 h

Fig. 4C

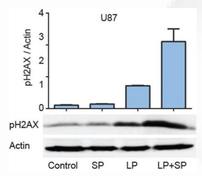


Figure 4C.
Western blot showing SP amplifying the DNA damage response (i.e. phospho-gammaH2AX induction) to LP-184 in U87 GBM cells. Co-treating GBM cells with LP-184 and SP increased the gamma-H2AX response 2-3 fold relative to LP-184 alone.

Fig. 5A

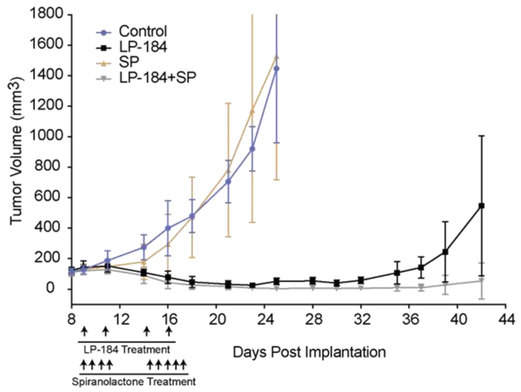


Fig. 5B

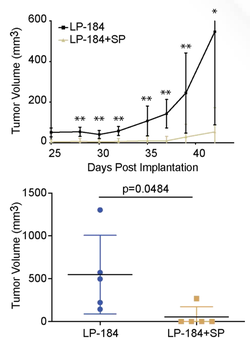


Figure 5A.
Mice with subcutaneous U87 tumor xenografts were treated with vehicle, spironolactone alone 4-5 times weekly for 2 weeks (25 mg/kg i.p.), LP-184 alone every other day for 4 doses (4 mg/kg i.v.) or LP-184 + spironolactone as indicated by arrows.

Figure 5B.
Line plots show tumor volumes vs time (N=5) and sizes of individual tumors at end of experiment on post-implantation day 42. Data represents Mean +/- SEM.

Results Cont.

Activated EGFR signaling and downregulated DNA damage repair predict LP-184 sensitivity in clinical GBM subsets

Fig. 6A

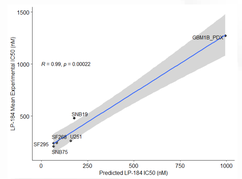


Figure 6A.
Pearson correlation plot between experimental and predicted LP-184 IC50s in GBM cell lines with full transcriptomic profiles available.

Fig. 6B

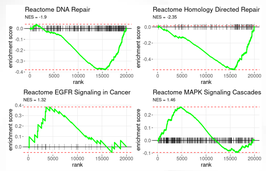


Figure 6B.
Gene Set Enrichment Analysis of Reactome Pathways based on ranked-expression derived from Pearson correlations of predicted IC50 and Gene Expression in TCGA GBM data. Subtitles indicates normalized enrichment scores.

Fig. 6C

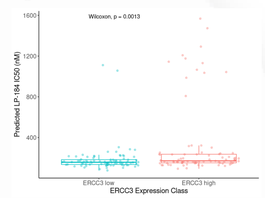


Figure 6C.
TCGA GBM clinical samples have greater predicted LP-184 sensitivity ($p = 0.0013$) in samples with lower ERCC3 expression. Clinical RNA-seq samples were divided into groups with ERCC3 expression above the mean (ERCC3 high) or below the mean (ERCC3 low).

Summary

- LP-184 is effective in TMZ-resistant preclinical GBM models and agnostic to MGMT methylation status
- ERCC3-dependent TC-NER activity was identified as a determinant of LP-184 synthetic lethality predicting that LP-184's therapeutic potential will be enhanced in patients with intrinsic or spironolactone-induced NER deficient tumors.
- LP-184 is a promising chemotherapeutic with potential clinical translation in GBM patients.
- Future directions include testing LP-184 + spironolactone combination in an intracranial xenograft model of GBM and assessing survival, tumor response and PK; and completing IND-enabling pharm/tox studies to initiate clinical trials.

References

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