LP-184, a clinical stage acylfulvene-derived tumor site activated small molecule, inhibits adult and pediatric CNS tumor cell growth

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Background

- Blood-brain barrier (BBB) permeable agents effective against recurrent, chemotherapy-resistant CNS tumors are urgently needed, particularly in **Glioblastoma multiforme (GBM) and atypical teratoid/rhabdoid tumors (ATRT)** representing extremely aggressive and lethal types of CNS malignancies [1, 2].
- LP-184 is a next-generation acylfulvene class drug candidate and has received FDA **orphan drug designations for the treatment of malignant gliomas and ATRT**.
- Lantern Pharma received **FDA clearance of the IND application for drug candidate LP-184** and plans to initiate the **first-in-human Phase 1A** clinical trial in advanced solid tumors including **CNS cancers** in Q3 2023.

Objectives

- Characterize the BBB permeability of LP-184 in vivo
- Evaluate the potency of LP-184 in GBM models compared to TMZ
- Determine the effects of LP-184 + Spironolactone combination on GBM cell viability and pharmacodynamics *in vitro* and on tumor response *in vivo*
- Test LP-184 response and anti-tumor activity across ATRT models
- Analyze the ability of LP-184 to inhibit cell viability of brain metastases in patient-derived primary lung/breast cancer models

LP-184 drug profile

- **LP-184** (hydroxyurea methylacylfulvene) is a DNA damaging agent and alkylating prodrug belonging to the **acylfulvene (AF) class** of naturally derived small molecule therapeutics [3, 4].
- LP-184 is transformed into its bioactive form by the oxidoreductase activity of the enzyme **PTGR1** (prostagladin reductase 1) [5].
- **LP-184 creates DNA adducts** at N3 of adenine base [6, 7] whereas Temozolomide (TMZ, the frontline standard of care agent for GBM) methylates O6 of guanine base [8]. The repair enzyme MGMT removes the primary TMZ-induced cytotoxic lesion, O6-methylguanine but not LP-184-induced DNA adducts.
- LP-184-induced DNA damage is likely repaired via ERCC-dependent transcription coupled nucleotide excision repair (TC-NER), suggesting potential **synergy in combination with Spironolactone** [9].
- LP-184 sensitivity is positively correlated with both MGMT expression and EGFR amplification validated by **enhanced activity in GBM models carrying unmethylated MGMT and EGFR *vIII***.

Figure 1. LP-184 structure

Results

LP-184 shows favorable CNS bioavailability and pharmacokinetics

Figure 2. (A)

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 [x]
	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 ± 0.10	0.202
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 ± 0.029	0.118

(B)

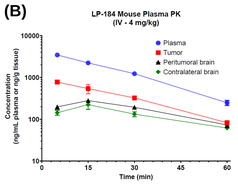


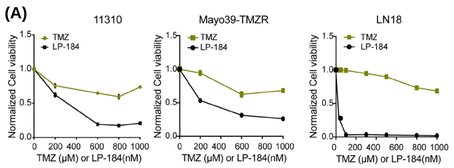
Figure 2. (A) In vivo pharmacokinetics parameters of LP-184; **(B)** LP-184 concentration-time curve in mouse plasma, brain and brain tumor tissues following a single i.v. bolus (4mg/kg).

- Pharmacokinetic analyses in SCID mice bearing orthotopic GBM xenografts showed normal brain/plasma ratio 0.1 (C_{max} = 916 nM) and brain tumor/plasma ratio 0.2 (C_{max} = 2539 nM).
- LP-184 BBB permeability is comparable to TMZ and brain C_{max} achieved (equivalent to ~800 nM) after a single i.v. infusion is greater than mean IC_{50} (~250 nM) for sensitive GBM cell models.

Results

LP-184 exhibits nanomolar potency against MGMT-expressing (*MGMT* unmethylated) and MGMT-negative TMZ-resistant GBM cells (3-day treatment) indicating >5000X increased potency over TMZ

Figure 3.



(B)

GBM model	LP-184 IC50 [nM]	TMZ IC50 [μM]
11310	209 ± 9	ND (> 1000 μM)
Mayo39-TMZR	78 ± 5.5	ND (> 1000 μM)
LN18	22 ± 3	ND (> 1000 μM)

(C)

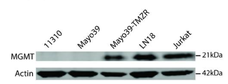
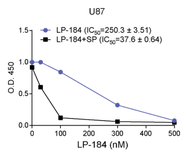


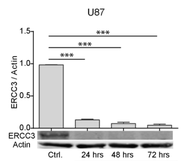
Figure 3. The patient-derived GBM xenograft line Mayo39 (*MGMT*-methylated/TMZ-sensitive) was obtained from the Mayo Clinic. TMZ-resistant Mayo39 variants (Mayo39-TMZR) were derived following prolonged culture with TMZ. The 011310 PDX line was established from a GBM resected at Johns Hopkins Hospital. LN-18 cell line, low passage Mayo39-TMZR and 011310 PDX-derived cells were maintained as monolayers in DMEM supplemented with 10% FBS. All cells were grown at 37°C in a humidified incubator with 5% CO_2. All cell lines used in the study were tested for mycoplasma and used within 20 passages of STR profiling. **(A)** LP-184 or TMZ treatments were conducted for 3 days. Dose response curves showing normalized cell viability were obtained from relative luminescence units measured in a Cell Titer-Glo 2.0 assay and **(B)** IC50s were calculated in GraphPad Prism v9. Data represent mean +/- SEM. ND = not determined; **(C)** Immunoblot showing MGMT expression [10].

Spironolactone sensitizes GBM cells and xenografts to LP-184

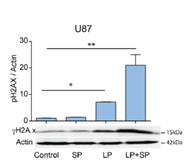
Figure 4.



(A) 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].



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



(C) Western blot showing SP amplifying the DNA damage response (i.e. phospho-γH2AX induction) to LP-184 in U87 GBM cells. Co-treating GBM cells with LP-184 and SP increased the γH2AX response ~3 fold relative to LP-184 alone.

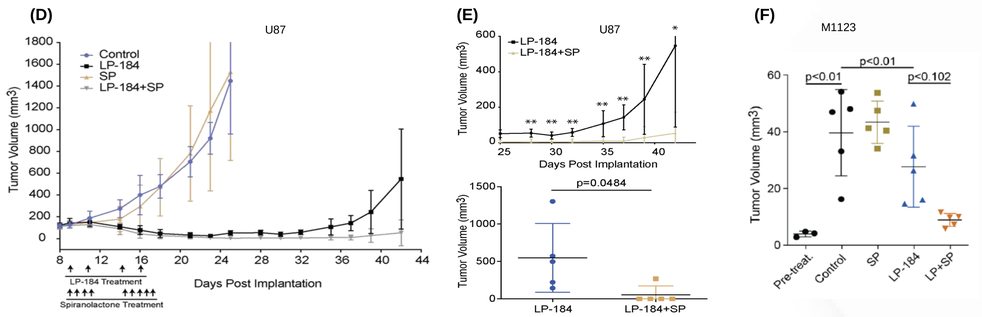


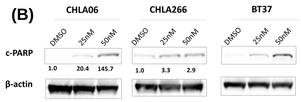
Figure 4. (D). Mice with subcutaneous U87 tumor xenografts were treated with vehicle, spironolactone alone 5 times weekly for 2 weeks (25 mg/kg i.p.), LP-184 alone every other day for 4 doses (4 mg/kg i.v.) or the combination of LP-184 + spironolactone as indicated by arrows with SP treatment initiated the day before LP-184. **(E)** Line/ scatter plots show U87 subcutaneous xenograft tumor volumes vs time (N=5 per group) and sizes of individual tumors at end of experiment on post-implantation day 42. Data represents Mean +/- SEM. **(F)** Scatter plot shows that SP (25 mg/kg) also sensitized aggressive orthotopic mesenchymal M1123 xenografts (N=5 per group) to LP-184 (4 mg/kg) in i.p. treatments [10].

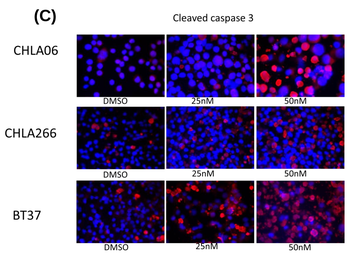
LP-184 inhibited cell growth in pediatric ATRT lines with concomitant apoptosis induction (4-day treatment)

Figure 5.

(A)

ATRT model	LP-184 IC50 [nM]
CHLA06	20 ± 2.5
CHLA266	24 ± 1.5
BT37	22 ± 3





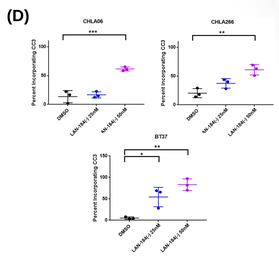


Figure 5. The ATRT cell lines CHLA06, CLHA266 and BT37 were maintained in RPMI supplemented with 10% FBS. All cells were grown at 37°C in a humidified incubator with 5% CO_2. LP-184 treatment was conducted for 4 days. **(A)** LP-184 IC50s were calculated in GraphPad Prism v9 from dose response curves obtained from relative luminescence units measured in a Cell Titer-Glo 2.0 assay. Data represent mean +/- SEM; **(B)** Western blot analysis showing PARP cleavage at 96h post LP-184 treatment as an indicator of apoptosis; **(C)** Immunofluorescence for cleaved caspase-3 at 96h post LP-184 treatment as an indicator of apoptosis and **(D)** quantification of percentage of imaged cells incorporating cleaved caspase-3.

Results

LP-184 treatment (4 mg/kg i.v.) over 2 cycles demonstrated 112% tumor growth inhibition and 2/10 tumor-free mice at study termination in ATRT subcutaneous xenografts

Figure 6.

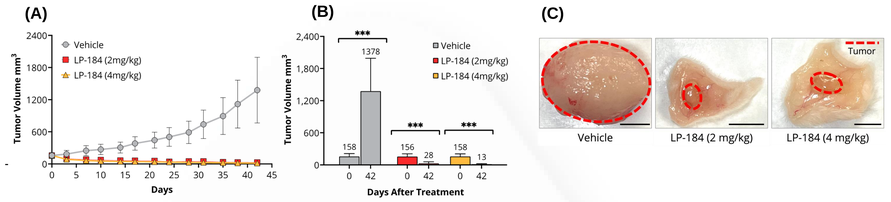


Figure 6. (A) Time-course of tumor volumes (mm^3) of CHLA-06 cell line derived xenograft (CDX) tumors treated i.v. either with vehicle, LP-184 (2 mg/kg), or LP-184 (4 mg/kg) on Days 0, 2, 4, 6, 8, 14, 16, 18, 20, 22 (2 cycles of 5 doses each). **(B)** Comparison of CHLA-06 CDX tumor volumes after treatment on day 0 and day 42 with either vehicle, LP-184 (2 mg/kg) or LP-184 (4 mg/kg). **(C)** Representative images of excised CHLA-06 CDX tumors after 42 days from mice injected with vehicle, LP- 184 (2 mg/kg), or LP-184 (4 mg/kg) [11].

LP-184 showed activity against brain metastases models from primary lung, breast, and skin cancers

Figure 7.

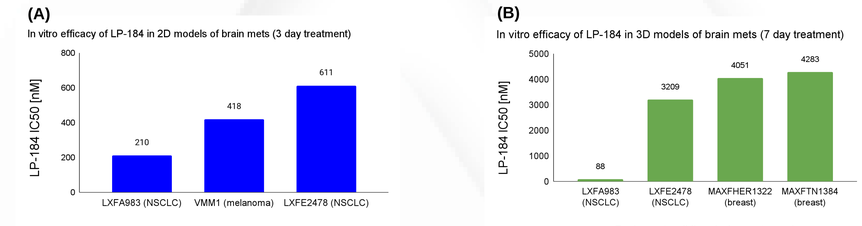


Figure 7. (A) Two models established from brain metastasis originating from primary lung cancers, LXFA983 and LXFE2478, and the VMM1 cell line representing brain metastasis from melanoma, were treated in a 2D monolayer assay *in vitro* with LP-184 across a test concentration range of 0.3 nM - 10 μM over 3 days. Viability of cells was quantified by the Cell Titer-Glo assay; **(B)** Brain metastases models originating from primary breast cancers, MAXFHER1322 and MAXFTN1384, were additionally treated in a 3D clonogenic assay *ex vivo* with LP-184 across a test concentration range of 1 nM - 10 μM over 7 days. Colony formation was assayed by staining of vital colonies with of INT and automated counting. Overall, LP-184 was found to be active across this panel of metastatic brain cancer cell lines, with IC_{50}s ranging between 88 and 4283 nM.

Summary

- LP-184 fulfills multiple requirements for clinical translation against infiltrating CNS malignancies such as GBM and ATRT.
- Pharmacokinetics in normal and tumor-bearing mice revealed effective CNS availability to tumor core (Cmax ~2500 nM), peritumoral brain (Cmax ~900 nM) and normal brain (Cmax ~730 nM) where infiltrating tumor cells reside behind a relatively intact BBB.
- 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 efficacy extends to pediatric ATRT models representing multiple molecular subsets (SHH/ TYR).
- LP-184 has the potential to treat and control brain metastases from solid tumors.

LP-184 will be further developed for CNS cancer indications under Starlight Therapeutics with the name STAR-001. Starlight Therapeutics is a clinical-stage precision oncology company focused on developing novel and effective therapies exclusively for brain and central nervous system (CNS) cancers. Starlight's programs were developed from AI, billions of oncology focused data points, and some of the world's top cancer researchers.

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