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LP-184, an acylfulvene class small molecule therapeutic, is synthetically lethal in HR deficient and PARP inhibitor resistant triple negative breast cancer

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ABSTRACT

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer accounting for approximately 15% to 20% of all newly diagnosed breast cancer cases. TNBCs are defined by lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2).Due to its special molecular phenotype, it is not sensitive to endocrine therapy or targeted therapy. Therefore, chemotherapy such as anthracyclines or taxanes is the main systemic treatment, but the efficacy of conventional postoperative adjuvant chemoradiotherapy is poor. **~35% of TNBC tumors show abnormalities in the HR pathway**, making them sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) and DNA-damaging agents, although more than 40% BRCA1/2-deficient patients fail to respond to PARPi and a substantial proportion of patients acquire PARPi resistance over time [1]. TNBCs heterogeneous nature, poor prognosis and limited treatment options presents the urgent need of development of novel agents targeting tumor specific alterations. Lantern Pharma is advancing LP-184, an acylfulvene-derived prodrug that is specifically activated in tumors that overexpress the oxidoreductase enzyme Prostaglandin Reductase 1 (PTGR1), for the treatment of solid tumor indications including TNBC. LP-184 has multiple mechanisms of action (MOA). While it is synthetically lethal in tumors harboring DNA damage repair defects including Homologous Recombination (HR) deficiencies, it can also interrupt transcription. LP-184 efficacy was tested in a panel of breast cancer cell lines and patient derived TNBC xenografts models, both sensitive as well as resistant to PARP inhibitors and anthracyclines. LP-184 demonstrated **nanomolar potency in six NCI-60 breast cancer cell lines (median IC50 = 327 nM)**. Subcutaneous patient-derived TNBC xenograft mouse models were used to determine tumor volume responses to LP-184 treatment in vivo. Xenograft tumors were derived from 10 treatment-naïve HR deficient (HRD score > 50) primary TNBC patients with known BRCA1/2 loss of heterozygosity (LOH), 7 of which subsequently progressed on PARP inhibitor Olaparib. LP-184 (4mg/kg i.v., (q2d x 5 then 7 days off)x2) led to complete and durable regression in all 10 TNBC HRD-PDX models tested as compared to control ($p < 0.0001$). A **tumor growth inhibition range of 107-141%** was achieved across all the 10 models. For LP-184, T/C at the control group end day was 0% in 10/10 models, whereas across the same models for Olaparib, T/C was 0% in 2/10 models and ranged from 15 - 90% in 8/10 models. Terminal body weight change was only a transient weight loss < 4% with LP-184 treatment across all models. **LP-184 exhibited superior potency than Olaparib [2] in TNBC PDX models that carry HRD mutations including PARPi resistant models and was well tolerated in mice.** As acylfulvene-induced damage is primarily repaired by transcription-coupled nucleotide-excision repair (TC-NER) and HR pathways, response to LP-184 is influenced by tumor DNA damage repair pathway status. Recent data highlight an important role of super enhancer driven core transcription regulatory circuits in the pathogenesis of TNBCs. Our results support the superior efficacy of LP-184 in TNBCs, likely linked to the multiple MOAs, and establish LP-184 as a promising new agent for future clinical testing in TNBC patients. We finally propose that LP-184 may be broadly efficacious in solid tumors with HR and/or TC-NER pathway defects, such as pancreatic, prostate, ovarian and bladder cancers.

MATERIALS AND METHODS

Cancer cell viability assay

Breast cancer cell lines from the NCI60 panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine and inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of LP-184. After 24 h, aliquots of 100 µl of drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the final LP-184 test concentrations of 10 nM, 100 nM, 1 uM, 10 uM, 100 uM. Following drug addition, the plates were incubated for an additional 48 h at 37°C, 5 % CO2, 95 % air, and 100 % relative humidity. Sulforhodamine B assay was used to determine cell viability.

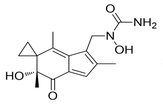
Patient-derived xenograft study

The dosing solutions of LP-184 were freshly prepared from powder material by dissolving in Ethanol and then adding sterile saline (final concentration being 5% Ethanol and 95% saline). Patient-derived TNBC models were grown as xenografts in immune-compromised athymic nude mice. Tumors of the same passage were transplanted subcutaneously onto 3-24 donor mice, passage (n-1). When these tumors reached 1080 to 1666 mm3, donor mice were sacrificed, tumors aseptically excised and dissected into fragments measuring approximately 20 mm3 and transferred in culture medium before grafting. The tumor fragment was placed in the subcutaneous tissue of the interscapular region. When tumors reached an average tumor volume of 75 - 221 mm³ animals were randomized into treatment or control groups (N=3 per group) and dosing initiated on Day 0. Tumors were measured once weekly with a digital caliper for the duration of the study (treatment + monitoring). Tumors were measured in two dimensions using calipers, and volume was calculated using the formula: Tumor Volume (mm³) = $w^2 \times l/2$, where w = width and l = length, in mm, of the tumor. Animal weights were also measured once weekly. Animal behavior was monitored daily. For each model, animals were administered intravenously QaD x 5on/7off x2 with (a) 5% ethanol and 95% saline as vehicle for the control group and (b) 4 mg/kg LP-184 in vehicle for the treatment group. Dosing occurred on days 0, 2, 4, 6, 8, 16, 18, 20, 22, 24 and animals were monitored for tumor volume and body weight until study termination on post treatment day 32. Statistical analysis was performed using GraphPad Prism version 9. Data were processed for Two-Way ANOVA using Geisser-Greenhouse correction and Sidak's post hoc analysis for group comparisons. Pictures of tumor site on the whole animal and of excised tumors were collected at study termination.

OBJECTIVES

- Evaluate *in vitro* cytotoxicity of LP-184 in a panel of breast cancer cell lines
- Determine LP-184 anti-tumor efficacy in TNBC PDX models with known HR deficiency

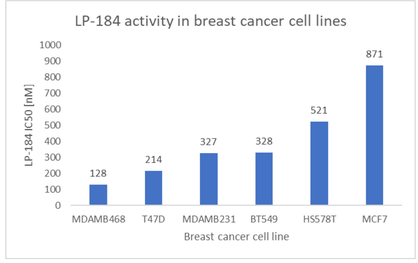
LP-184 DRUG PROFILE



- 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, prostaglandin reductase 1 (PTGR1).
- LP-184 is expected to be highly effective in treating at least **38%** of breast cancer patients based on elevated PTGR1 levels as analyzed from a TCGA dataset on 1100 clinical breast cancer samples, of which a further **16%** had deleterious DNA damage repair (DDR) mutations.

RESULTS

LP-184 shows nanomolar potency in a panel of six breast cancer cell lines



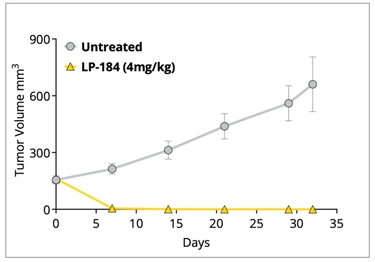
Characteristics of primary TNBC PDX models selected for evaluating *in vivo* anti-tumor efficacy of LP-184

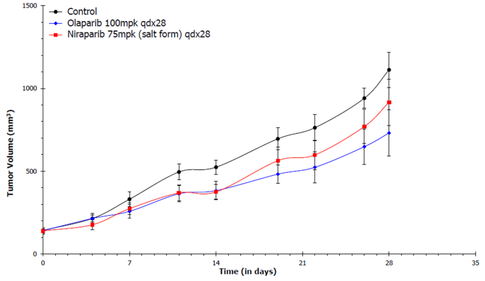
Sorting	PDX model ID	Treatment before PDX establishment	Lehman classification	RAD51 score	BRCA1/BRCA2 variants	Other relevant variants	BRCA1 LOH	BRCA2 LOH	BRCA1 promoter methylation	PTEN variants (allele frequency)	Best response to adriamycin/ cyclophosphamide	Best response to PARPi (olaparib/ niraparib)
HR Deficient and PARP resistant	HBCx-1	None	BL1	25.0	-				9.999		S	P
	HBCx-16	None	M	42.0	-		YES		12.0913	ex3-5 del (1)*	P	P
	HBCx-23	None	M	35.5	-		YES		61.6874		S	P
	HBCx-24	None	BL1	38.7					4.2948		P	P
	HBCx-28	None	BL2	36.0	BRCA1 c.212+3A>G [IVS5+3A>G]	SHLD2 loss	YES		0.9216	gene loss	S	P
	HBCx-8	RT	M	41.7	BRCA1 p.Gln81*		YES		0.3421		S	P
	HBCx-9	None	BL1	33.0	-		YES		13.8442		P	P
HR Deficient and PARPi T168	HBCx-10	None	M	1.7	BRCA2 p.Gln3036*			YES	0.542	ex3 del (1)	CR	CR
	HBCx-15	None	MSL	2.7	-	STK11 loss	YES		33.8883		CR	CR
	HBCx-T168	None	BL1	1.7	BRCA1 p.Ser1524Leufs*24		YES		0.0004		CR	CR

RESULTS

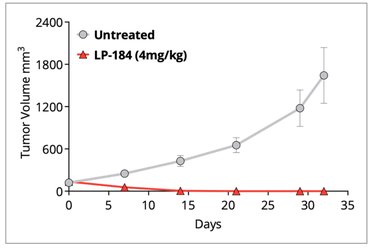
10/10 TNBC PDX models treated with single-agent LP-184 dosed at 4 mg/kg at 5 ml/kg by i.v. route, (q2d x 5 then 7 days off)x2, demonstrated complete tumor regression (-100%<ΔT/T0<-89% ; 0%<T/C%<1%).

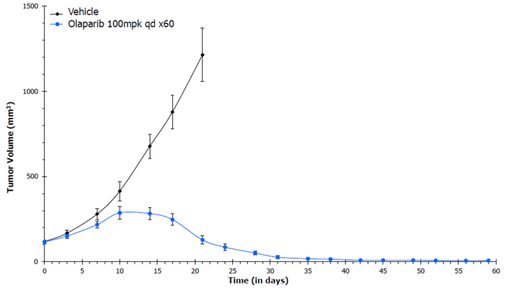
PARPi Resistant TNBC PDX Model (HBCx-1)





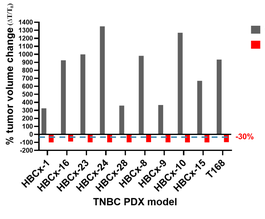
PARPi sensitive TNBC PDX Model (HBCx-10)





LP-184 is effective and tolerable in xenograft mouse models of TNBC

PDX model ID	HRD score (> 50: HRD; < 40: HRP)	Tumor growth inhibition (TGI) with LP-184 treatment (D32)	Maximum mean relative body weight change percent with 4 mg/kg LP-184
HBCx-1	50	132.2%	-2.3% (D7)
HBCx-16	57	119.6%	-4.0% (D14)
HBCx-23	69	109.9%	-2.4% (D29)
HBCx-24	55	107.3%	-8.0% (D28)
HBCx-28	63	141.4%	-9.0% (D22)
HBCx-8	65	109.9%	-1.3% (D7)
HBCx-9	55	127.6%	-3.7% (D7)
HBCx-10	57	107.9%	-2.3% (D29)
HBCx-15	68	127.1	-5.6% (D28)
T168	54	110.4%	-2.0% (D7)



Tumor response	% tumor volume change ($\Delta T/T_0$)
Tumor progression	$\Delta T/T_0 > 20\%$
Tumor stabilization	$20\% > \Delta T/T_0 > -30\%$
Tumor regression	$-30\% > \Delta T/T_0$

SUMMARY

- LP-184 demonstrated nanomolar potency in a panel of breast cancer cell lines
- Based on body weight data and clinical observations, LP-184 dosed at 4 mg/kg was well tolerated in all TNBC PDX tumor models tested.
- As monotherapy, LP-184 at 4 mg/kg demonstrated high antitumor efficacy with complete tumor regression observed in all xenografted mice across the panel of 10 TNBC PDX tumor models, irrespective of their HRD status, sensitivity to PARPi, or RAD-51-based evaluation of HRR capacity.
- LP-184 may be broadly efficacious in multiple other solid tumors with HR and/or NER pathway defects, such as pancreatic, prostate, ovarian and bladder cancers.

REFERENCES

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CONTACT

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