Development of a Potent DNA Damaging Agent LP-284 for Treatment of Mantle Cell Lymphoma

J. Zhou[1], A. Kulkarni[1], C. Love[2], L. Happ[3], D. Sturtevant[1], N. Biyani[1], R.L. Peru y Colón De Portugal[1], K. Bhatia[1], S. Dave[2,3]

[1]Lantern Pharma Inc., Dallas, Texas, United States [2]Duke University, Durham, North Carolina, United States [3]Data Driven Biosciences, North Carolina, United States

INTRODUCTION

Background

- Mantle cell lymphoma (MCL) is characterized by constitutively deregulated cyclin D1 gene, impaired DNA damage response pathways, hyperactive B-cell receptor (BCR) signaling[1,2], and high incidence of MYC abnormality[3]. Targeting these deregulated pathways, such as using Bruton's tyrosine kinase inhibitors (BTKi) to block BCR signaling[2], has transformed the treatment landscape of MCL.
- DNA damaging agents are of tremendous importance in cancer treatment because defective DNA repair mechanisms in tumor cells can make them particularly vulnerable. DNA lesions at the transcribed strand of active genes can also impede expression of the oncogene-induced pathways which cancer cells are addicted to[4] by inducing degradation of RNA polymerase II or creating transcription blocks[5].
- LP-284 is a novel small molecule DNA damaging agent being developed for the treatment of MCL. The DNA lesions induced by LP-284 are repaired by transcription-coupled nucleotide excision DNA repair. We previously reported LP-284's nanomolar potency across 6 MCL cell lines, including cells resistant to BTKi, Bortezomib, and Venetoclax[6].

Aims

- To evaluate the anti-tumor effects of LP-284 in MCL subcutaneous xenograft mouse models
- To explore LP-284's mechanism of action in inducing DNA damage and blocking transcription
- To assess LP-284's toxicity and pharmacokinetics in dogs

RESULTS: LP-284's strong *in vivo* potency

LP-284 prolongs survival time of MCL xenograft mice and results in greater tumor shrinkage than Ibrutinib or Bortezomib

Fig. 1A

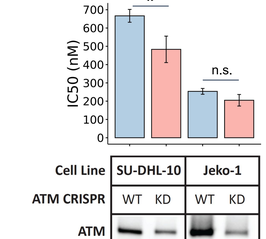

Table 1

	4 mg/kg LP-284	2 mg/kg LP-284	50 mg/kg Ibrutinib	1 mg/kg Bortezomib
Day 17 %TGI	113%	63%	8%	22%

Fig. 1B

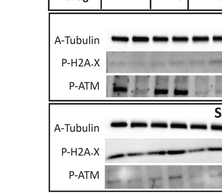

Figure 1 JeKo-1 subcutaneous xenograft models (N=8/arm) were treated with vehicle, LP-284 (i.v., 2 or 4 mg/kg, day 1, 3, 5, 7, 9, 17, 19, 21, 23, 25), Ibrutinib (p.o., 50 mg/kg, daily), or Bortezomib (i.p., 1 mg/kg, day 1, 4, 7,10). **1A)** tumor volume; **1B)** survival curves of LP-284 and vehicle arms; **Table 1)** comparison of tumor growth inhibition (TGI) at day 17

LP-284 results in near complete regression of MCL xenograft tumors that were pretreated by but resistant to Ibrutinib or Bortezomib

Fig. 2A

Fig. 2B

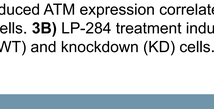

Fig. 2C

Figure 2 Ibrutinib (p.o., 50 mg/kg) or Bortezomib (i.p., 1 mg/kg) pretreated JeKo-1 subcutaneous xenograft tumors were further treated by vehicle only or 4 mg/kg LP-284 only (N=4/arm). **2A)** LP-284 resulted in near complete regression of resistant tumors with initial size of ~ 2000 mm^3 ; **2B)** representative tumor photos; **2C)** prolonged survival time in LP-284 treated mice

RESULTS: LP-284 down-regulates key MCL signaling pathways

Fig. 4A

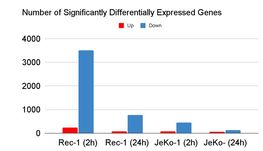

Fig. 4B GSEA: MYC target pathway

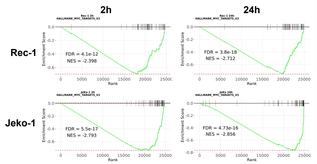

Fig. 4C GSEA: cyclin D-associated events at G1

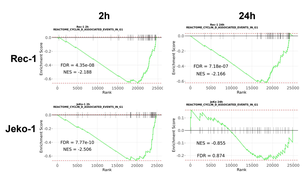

Fig. 4D GSEA: downstream signaling events of BCR

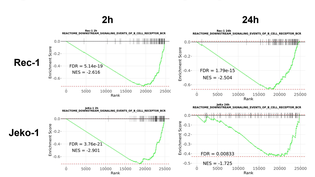

Figure 4A) Two MCL cell lines Rec-1 and JeKo-1 were treated by LP-284 at ½ IC50 or vehicle controls. Samples were collected at 2-hour and 24-hour post-treatment for RNASeq. LP-284 treatment led to more down-regulated than up-regulated genes. **4B-D)** Gene Set Enrichment Analysis (GSEA) showed that the hallmark MYC target pathway (4B), the reactome cyclin D associated events in G1 (4C), and the reactome downstream signaling events of BCR (4D) were significantly down-regulated by LP-284. FDR: false discovery rate; NES: normalized enrichment scores

RESULTS: LP-284 induces DNA damage responses regardless of ATM deficiency

Fig. 3A

Cell Line	SU-DHL-10		Jeko-1	
ATM CRISPR	WT	KD	WT	KD
ATM				
A-Tubulin				

Fig. 3B

Time	0 hours		2 hours		6 hours		24 hours	
ATM CRISPR	WT	KD	WT	KD	WT	KD	WT	KD
Drug	-	-	-	+	-	+	-	+

Figure 3A) Knocking down the ATM gene in double-hit lymphoma SU-DHL-10 cells significantly reduced LP-284's IC50 by 1.4 fold (*: p<0.05). Reduced ATM expression correlated with ~1.2 fold lower though not significant (n.s.) IC50 in JeKo-1 MCL cells. **3B)** LP-284 treatment induced phosphorylation of gamma H2AX and ATM in both ATM wild type (WT) and knockdown (KD) cells. Phosphorylation was more evident 6 hours after treatment.

RESULTS: LP-284's toxicological profiles in dogs (non-GLP)

- LP-284 was administered by i.v. once a day on days 1, 8, and 15 and at 0.3, 0.6, or 1.2 mg/kg/dose in dogs in a pilot non-GLP toxicological study. Adverse effects in body weight and clinical pathology were only observed at ≥0.6 mg/kg/dose.
- At the microscopic level, 0.3 mg/kg/dose LP-284-related hematological changes were considered non-adverse based on the small magnitude and no associated changes in clinical hematology compared with the control animals.
- At 0.3 mg/kg (close to No Observed Adverse Effect Level) in dogs, LP-284's pharmacokinetics parameters measured on dosing days 1 and 15 showed: $t_{1/2}$ = 40-50 min, C_{max} = ~ 800 nM; t_{max} = 5 minutes

DISCUSSION

Conclusions

- When compared with Ibrutinib, Bortezomib, or the vehicle control, LP-284 significantly delays tumor progression and prolongs survival of JeKo-1 MCL xenograft mouse models.
- LP-284 shrinks and delays the progression of MCL xenograft tumors that were pretreated by but resistant to Ibrutinib or Bortezomib.
- LP-284 induces DNA damage as evidenced by phosphorylation of gamma H2AX and ATM. Knocking down ATM, the core DNA repair response gene mutated in ~50% of MCL patients[7], makes cells 1.2~1.4 fold more sensitive to LP-284.
- Treating MCL cells with LP-284 resulted in downregulation of the cyclin D associated pathway, MYC target pathway, and downstream signaling of BCR, all of which contribute to MCL oncogenesis[1].
- The pilot toxicological and pharmacokinetic study results support further development of LP-284.

Projects in Progress

- IND-enabling studies (to be completed Q1, 2023)
- Validation of LP-284 as a novel agent targeting oncogene addiction
- Identification of synergistic drug combinations and optimization of dosing regimen in animal models
- Artificial intelligence facilitated biomarker discovery

REFERENCES

1. Jares P, Colomer D, Campo E. Molecular pathogenesis of mantle cell lymphoma. J Clin Invest. 2012 Oct;122(10):3416-23.
2. Merolle MI, Ahmed M, Nomie K, Wang ML. The B cell receptor signaling pathway in mantle cell lymphoma. Oncotarget. 2018 Mar 27;9(38):25332-25341.
3. Yi S, Zou D, Li C, Zhong S, Chen W, Li Z, Xiong W, Liu W, Liu E, Cui R, Ru K, Zhang P, Xu Y, An G, Lv R, Qi J, Wang J, Cheng T, Qiu L. High incidence of MYC and BCL2 abnormalities in mantle cell lymphoma, although only MYC abnormality predicts poor survival. Oncotarget. 2015 Dec 8;6(39):42362-71.
4. Pagliarini R, Shao W, Sellers WR. Oncogene addiction: pathways of therapeutic response, resistance, and road maps toward a cure. EMBO Rep. 2015 Mar;16(3):280-96.
5. Steurer B, Janssens RC, Geijer ME, Aprile-Garcia F, Geverts B, Theil AF, Hummel B, van Royen ME, Evers B, Bernards R, Houtsmuller AB, Sawarkar R, Marteijn J. DNA damage-induced transcription stress triggers the genome-wide degradation of promoter-bound Pol II. Nat Commun. 2022 Jun 24;13(1):3624.
6. Zhou J, Sturtevant D, Biyani N, Bhatia K, Dave S. MCL-319 LP-284 - A Highly Potent Small Molecule Targeting Mantle Cell Lymphoma. Clin Lymphoma Myeloma Leuk. 2022 Oct;22 Suppl 2:S396.
7. Camacho E, Hernández L, Hernández S, Tort F, Bellosillo B, Beá S, Bosch F, Montserrat E, Cardesa A, Fernández PL, Campo E. ATM gene inactivation in mantle cell lymphoma mainly occurs by truncating mutations and missense mutations involving the phosphatidylinositol-3 kinase domain and is associated with increasing numbers of chromosomal imbalances. Blood. 2002 Jan 1;99(1):238-44.