Abstract Number: 5529

In vivo Efficacy Evaluation of ASC61, an Oral PD-L1 Inhibitor, in Two Tumor Mouse Models

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Abstract

ASC61 is a small molecule inhibitor prodrug of programmed cell death-ligand 1 (PD-L1), developed by Ascletis. ASC61 is converted to its pharmacologically active metabolite ASC61-A *in vivo* after oral dosing. *In vitro* studies have suggested that ASC61-A could induce **dimerization** and subsequent **internalization** of PD-L1 protein from the cell membrane **(Figure 1)**, interfere PD-1/PD-L1 interaction and enhance T-cell activation. Here we report the *in vivo* efficacy of ASC61 in two tumor mouse models. ASC61 was found to have comparable antitumor activities as the Food and Drug Administration (FDA) approved PD-L1 therapeutic monoclonal antibody (mAb), Atezolizumab. ASC61 has received the US IND approval. First in patient study of ASC61 is planned in Q2, 2022.

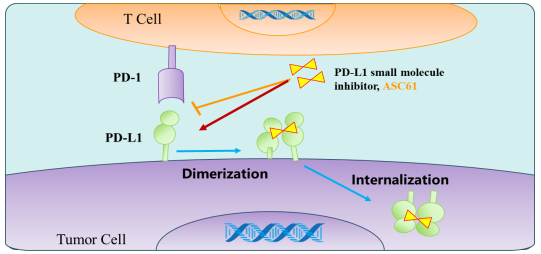

Figure 1: ASC61 inhibits the PD-1/PD-L1 interaction and promotes PD-L1 dimerization and subsequent internalization.

In vitro efficacy studies of ASC61

Regarding EC_{50}, ASC61-A demonstrated better efficiencies than competitors' compounds in *in vitro* studies.

Table 1: Efficacy validation of ASC61-A in *in vitro* studies.

Assay	EC_{50} of ASC61-A
PD-L1 dimerization	77.90 nM
PD-L1 internalization	728 nM
PD-1/PD-L1 inhibitory	0.4553 nM
PD-L1 Jurkat-NFAT reporter	0.3 nM
Hep3B-OS8-hPDL1 and T cell co-culture	2.86 nM

In vivo efficacy evaluation of ASC61 in the treatment of female BALB/c mice bearing CT-26-hPD-L1 tumors

In the syngeneic tumor mouse model, hPD-L1 expressing CT-26 cells (0.5 x 10^6) were subcutaneously inoculated at the right flank region of female BALB/c mice. When the average tumor volume reached approximately 69 mm^3, 40 mice were equally randomly assigned and treated with the predetermined regimen as shown in **Table 2**. Body weights and tumor volumes were measured regularly, and tumor growth inhibitions (TGI) of different groups were compared.

Table 2: Groups and treatment information.

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Dosing Schedule
1	8	Vehicle	--	PO	BID × 19 days
2	8	Atezolizumab	10	IP	BIW × 19 days
3	8	ASC61 (GLC01-537)	50	PO	BID × 19 days
4	8	GLC01-589	50	PO	BID × 19 days
5	8	GLC03-633	50	PO	BID × 19 days

All animals showed a gradual increase in body weight during the experiment as shown in **Figure 2A**. No significant body weight change was found between groups administered with different treatment regimens. No mouse was euthanized due to body weight loss, indicating that all treatment compounds are tolerated well in BALB/c mice.

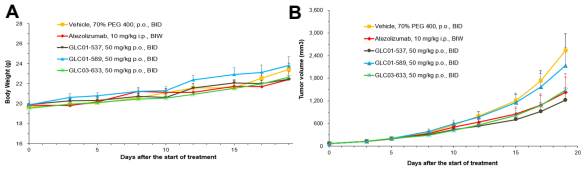

Figure 2: (A) Body weight and (B) tumor volume of female BALB/c mice bearing CT-26-hPD-L1 tumor after administering test compounds.

Mean tumor growth curves of different groups are shown in **Figure 2B**. TGI of test compounds was calculated based on tumor volume (TV) measured on Day 19 after treatment. As shown in **Table 3**, ASC61 (GLC01-537) administrated at 50 mg/kg, twice daily (BID), showed significantly inhibitory effects on the tumor growth with the best TGI value of 52.9% ($p < 0.05$), better than that of the reference drug, Atezolizumab (40.77%).

Table 3: TGI analysis on Day 19 after treatment.

Group	Tumor Size (mm^3)	T/C(%)	TGI (%)	p
Vehicle	2528±446	--	--	--
Atezolizumab	1423±406	60.27	40.77	> 0.05
ASC61 (GLC01-537)	1216±328	48.44	52.90	< 0.05
GLC01-589	2131±492	76.78	23.83	> 0.05
GLC03-633	1483±435	57.59	43.51	> 0.05

In vivo efficacy evaluation of ASC61 in the treatment of subcutaneous hPD-L1 MC38 colon cancer model in PD-1/PD-L1 dKI HuGEMM mice

In the humanized tumor mouse model, hPD-L1 expressing MC38 cells (1 x 10^6) were subcutaneously inoculated at the right flank region of female human PD-1 and PD-L1 double genes knocked-in mice, dKI HuGEMM strain. Once the mean tumor size reached approximately 78.3 mm^3, 32 mice were randomized equally into 4 groups with different treatments as shown in **Table 4** for 16 days. Body weights and tumor volumes were measured regularly, and TGI of different groups was compared.

Table 4: Groups and treatment information.

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Dosing Schedule
1	8	Vehicle	-	PO	BID × 16 days
2	8	Atezolizumab	5	IP	BIW × 16 days
3	8	ASC61	50	PO	BID × 16 days
4	8	ASC61	100	PO	BID × 16 days

ASC61 up to 100 mg/kg is well tolerated in PD-L1/PD-1 dKI mice (**Figure 3A**). ASC61 100 mg/kg, BID, produced significant antitumor efficacy with a TGI value of 63.15%, comparable with Atezolizumab 5 mg/kg treatment as shown in **Figure 3B** and **Table 5**.

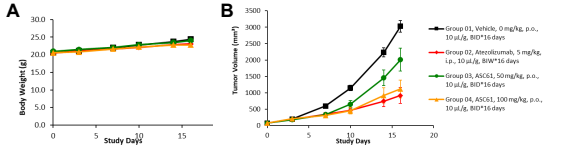

Figure 3: (A) Body weight and (B) tumor volume in subcutaneous hPD-L1 MC38 tumor model in PD-L1/PD-1 dKI mice (Mean± SEM).

Table 5: TGI analysis on Day 16 after treatment.

Group	Tumor Size (mm^3)	T/C (%)	TGI (%)	p
Vehicle	3027.54±179.16	-	-	-
Atezolizumab	919.73±244.00	30.38	69.62	<0.001
ASC61 50 mg/kg	2009.72±346.48	66.38	33.62	0.0954
ASC61 100 mg/kg	1115.61±275.17	36.85	63.15	<0.001

Median survival time (MST) was calculated by time to tumor volume reaching 3000 mm^3 or when severe tumor ulceration was observed. The increase in life-span (ILS) was calculated as follows: ILS (%) = MST of drug treated group/ MST of Vehicle group. Survival analysis showed that Atezolizumab at 5 mg/kg and ASC61 at 100 mg/kg could produce similar antitumor activities (**Table 6**).

Table 6: Survival analysis of hPD-L1 MC38 model in PD-L1/PD-1 dKI mice.

Group	MST (days)	ILS (%)	p
Vehicle	16	-	-
Atezolizumab	>23	>43.75	0.0002
ASC61 50 mg/kg	20	25.00	0.0338
ASC61 100 mg/kg	23	43.75	0.0027

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