SYNERGISTIC ANTITUMOR EFFECT OF A NOVEL FIRST-IN-CLASS SMALL MOLECULE OPB-111077 THAT INHIBITS MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION IN COMBINATION WITH ALKYLATING AGENT

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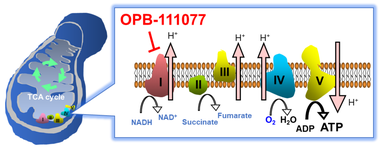
BACKGROUND

Cancer cells are known to exhibit Warburg effect, but it has been recently reported that mitochondrial oxidative phosphorylation (OXPHOS) is also important for tumor cell proliferation, and that metabolic reprogramming from glycolysis to OXPHOS is one of the resistance mechanisms to antitumor drugs. OPB-111077, synthesized by Otsuka Pharmaceutical Co., Ltd., is a novel orally available small molecule compound that inhibits mitochondrial respiratory chain complex I, suppressing energy production through OXPHOS. OPB-111077 showed antitumor effects against various human hematological and solid tumors in cultured cells and cell line-derived xenograft models. Here, we report the combination therapeutic strategy based on the cancer energy metabolism of OPB-111077. Currently, we are conducting a combination therapy of OPB-111077 with bendamustine and rituximab in Phase 1 clinical trial for relapsed/refractory DLBCL in Japan and Korea (NCT04049825), and we have reported the preliminary results from dose-escalation stage in ASH 2023 (DOI:10.1182/blood-2023-179716).



RESULTS

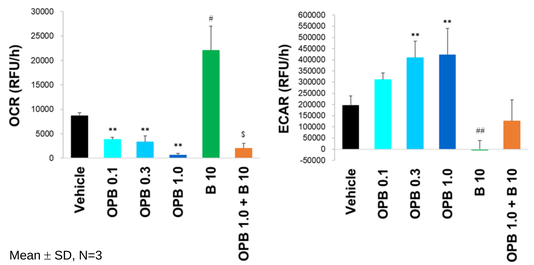
Figure 1. Antitumor effect of OPB-111077 on human blood tumor cell lines

The IC50 values of growth inhibitory effect obtained by OPB-111077 treatment for 5 days using WST-8 colorimetric method. N=3.

Cell Line	Origin		IC_{50} (nmol/L)	95% CI (nmol/L)
KU812	Leukemia	Chronic myelogenous leukemia	18.6	15.4～22.5
KG-1		Acute myelogenous leukemia	120.4	78.6～183.3
HEL 92.1.7		Erythroleukemia	525.3	367.0～757.1
PCM6	Myeloma	B-cell lymphoblastoid-like	109.9	88.5～136.4
SU-DHL-1	Lymphoma	Anaplastic large cell lymphoma	202.1	117.4～305.2
OCI-Ly7		Diffuse large B-cell lymphoma	243.3	195.0～302.8
OCI-Ly18		Diffuse large B-cell lymphoma (MYC/BCL2 double-hit)	203.4	149.2～282.4
DOHH-2		Follicular lymphoma	313.7	155.7～685.0
REC-1		Mantle cell lymphoma	255.5	149.5～486.2

Figure 3. Bendamustine reprogramming of cancer cells to OXPHOS

OCI-Ly7 cells were pretreated with vehicle or 10 μM bendamustine (B) for 24 hours then, 0.1, 0.3 or 1.0 μM OPB-111077 (OPB) were added. Oxygen consumption rate and extracellular acidification rate were measured using MitoXpress Xtra Oxygen Consumption Assay Kit and pH-Xtra Glycolysis Assay Kit, respectively. ** P < 0.005 vs vehicle group (one-tailed Williams test). # P < 0.05 and ## P < 0.01 vs vehicle group (unpaired t-test). $ P < 0.01 (testing interaction effects using two-way ANOVA with OPB-111077 and bendamustine presence or absence as factors).



Mean ± SD, N=3

Figure 2. Synergistic antitumor effect by the combination of OPB-111077 and bendamustine on CDX and PDX models

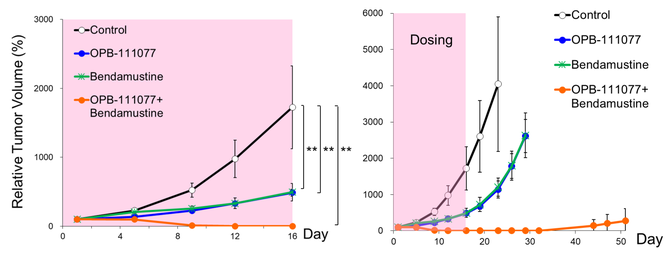
(A) OPB-111077 and bendamustine were treated for 5 days on lymphoma cell lines, and the combination index values were calculated from growth inhibitory effects obtained by CellTiter-Fluor™. The upper confidence limit of the combination index was less than 1, which is judged to be synergistic. (B) The effect of antitumor (left) and tumor growth delay (right) on OCI-Ly7 subcutaneously transplanted into SCID mice. Using a mixed effects model, a Dunnett's test was performed between the control and each OPB-111077-treated group (**p<0.01). (C) The combined antitumor effect on lymphoma PDX model.

(A)

N=3

Cell Line	Combination Index	95% CI	Effect
OCI-Ly7	0.67	0.37～0.96	Synergistic
REC-1	0.65	0.34～0.96	Synergistic

(B)



OPB-111077 30mg/kg: p.o. for 14 days, Bendamustine 15mg/kg: i.p. for 5 days, Mean ± SD, N=6

(C)

ID of PDX	Subtype	Tumor Growth Inhibition (TGI%) on Day 15		
		OPB-111077	Bendamustine	OPB-111077 Bendamustine
LY0257	DLBCL	100.0	-27.2	100.0
LY12962	DLBCL	100.0	80.9	100.0
LY12966	DLBCL	100.0	45.7	100.0
LY2264	DLBCL	80.3	-6.5	100.0
LY2266	B cell lymphoma with plasma cell differentiation	73.0	19.8	73.7
LY2298	DLBCL	92.7	35.3	88.5
LY2318	DLBCL	76.3	-2.4	100.0
LY3148	Burkitt Lymphoma	100.0	73.8	100.0
LY3463	DLBCL	100.0	-5.4	100.0
LY3604	DLBCL	99.3	44.3	99.2
LY3786	DLBCL	79.6	13.9	89.8
LY6701	DLBCL	100.0	25.3	100.0
LY6933	DLBCL	75.5	51.6	100.0
LY6934	DLBCL	100.0	91.9	100.0
Mean TGI%		91.2	31.5	96.5
Rate of Complete Response (TGI 100%)		50.0 % (7/14)	0.0 % (0/14)	71.4 % (10/14)

OPB-111077 200mg/kg: p.o. for 14 days, Bendamustine 10mg/kg: i.p. for 2 days, N=4

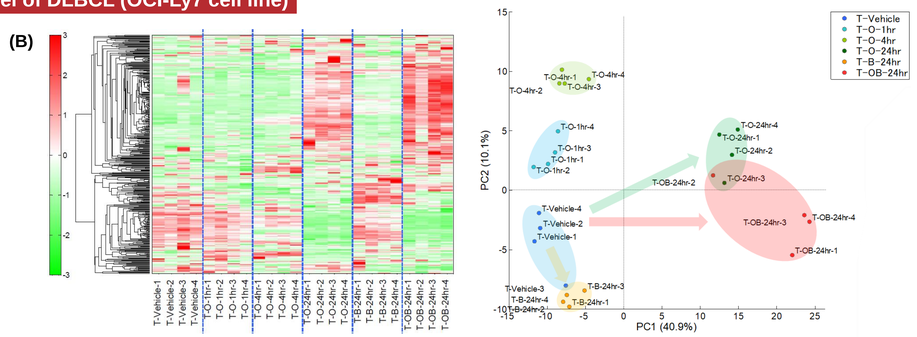
Figure 4. Metabolome analysis using a CDX model of DLBCL (OCI-Ly7 cell line)

(A) Group (B) Hierarchical clustering analysis and principal component analysis of tumor tissue. (C) Enrichment analysis was performed using the sets of metabolites extracted comparison of two groups and PCA. Metabolic pathways of TCA cycle-related, pyrimidine, fatty acid and lipid and Branched Chain Amino Acid were altered in response to OPB-111077 and bendamustine.

(A)

Group	Compound	Dose	Time after dose	n
Vehicle	Vehicle	-	24 hr	4
O-1hr	OPB-111077	100 mg/kg	1 hr	4
O-4hr	OPB-111077	100 mg/kg	4 hr	4
O-24hr	OPB-111077	100 mg/kg	24hr	4
B-24hr	Bendamustine-5days	15mg/kg	24 hr	4
OB-24hr	Benda-5days + OPB	15+100mg/kg	24 hr	4

(B)

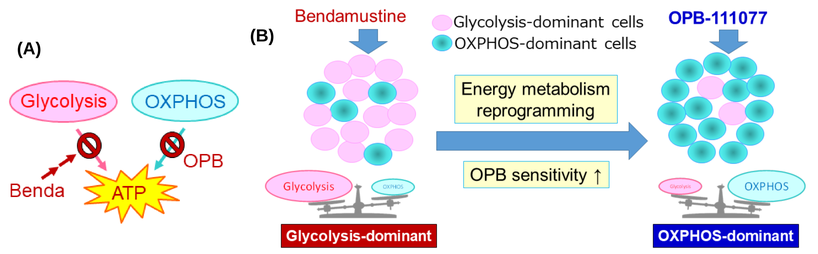


(C)

Group	OPB 1h	OPB 4hr	OPB 24hr	Benda 24hr	OPB + Benda 24hr
Enriched Pathways	Pyrimidine metabolism	• TCA • Glyoxylate and dicarboxylate metabolism • Pyruvate metabolism • Pyrimidine metabolism • Amino and nucleotide sugar metabolism	Pyrimidine metabolism	• Sphingolipid metabolism • Steroid biosynthesis	• Biosynthesis of unsaturated fatty acids • Arachidonic acid metabolism • Valine, leucine and isoleucine degradation

CONCLUSIONS

- OPB-111077, an OXPHOS inhibitor, exhibited a synergistic antitumor effect in combination with bendamustine.
- OPB-111077 inhibited OXPHOS while enhancing glycolysis, however, when combined with bendamustine, the enhanced glycolysis was suppressed (A).
- Bendamustine reprogrammed cancer cells to OXPHOS (B), suggesting that the tumor environment became more conducive to the antitumor effects of OPB-111077 when combined with bendamustine.
- We propose a novel anticancer therapeutic strategy that combines OXPHOS inhibitors with alkylating agents. OPB-111077 could be treated in a wide range of applications as a combination therapy with the alkylating agents for lymphoma.



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