APG-2449, a Novel Focal Adhesion Kinase (FAK) Inhibitor, Exhibits Antileukemic Activity and Enhances Lisaftoclax (APG-2575)-Induced Apoptosis in Acute Myeloid Leukemia (AML)

Zhou Yu,[1] Zhiyan Liang,[2] Xinyi Yao,[1] Yanqiu Ji,[1] Dajun Yang,[1-3] and Yifan Zhai[1,2,4*]

[1]Ascentage Pharma (Suzhou) Co., Ltd., Suzhou, China; [2]Ascentage Pharma Group Inc., Rockville, Maryland; [3]Department of Experimental Research, State Key Laboratory of Oncology in South China Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China; [4]Guangzhou Healthquest Pharma Co., Ltd., Guangzhou, China.
*Corresponding author: yzhai@ascentage.com

INTRODUCTION

- High FAK expression is associated with increased blast proliferation and poor prognosis in AML.[1]
- Preclinical and clinical studies have demonstrated that selective targeting of BCL-2 has broad antitumor activity against hematologic malignancies, including AML, myelodysplastic syndrome, and chronic lymphocytic leukemia.[2]
- In AML, resistance to apoptosis induced by BCL-2 inhibition is an urgent unmet medical need. It is partially mediated by preexisting and therapy-induced upregulation of MCL-1 and BCL-xL.[3]
- Considering the cross-talk between FAK and MCL-1/BCL-xL pathways, we hypothesize that FAK inhibition could downregulate expression of MCL-1 and BCL-xL, and thus enhance activity of a BCL-2 inhibitor (BCL-2i) in both sensitive and insensitive AML cells (**Fig. 1**).
- Lisaftoclax (APG-2575) and APG-2449 are novel BCL-2 and ALK/ROS1/FAK inhibitors with well tolerable safety profiles and potent clinical activity in patients with hematologic and solid malignancies, respectively.[4,5] Lisaftoclax and APG-2449 are under study as investigational drugs and not yet approved in the United States.

AIM

- The aim of this study was to evaluate whether APG-2449 can enhance lisaftoclax antitumor activity in preclinical models of AML.

METHODS

- AML cells were treated with APG-2449 alone or in combination with lisaftoclax, and cell viability was measured by Cell Titer-Glo® luminescent cell viability assays.
- Cellular apoptosis was evaluated by annexin V/propidium iodide staining and flow cytometry.
- Protein expression was measured by western blot.
- The antitumor activity of APG-2449 and lisaftoclax alone or in combination were assessed in AML cell-derived xenografts (CDX) in mice.

RESULTS

- As a single agent, APG-2449 can inhibit proliferation of BCL-2i-sensitive and insensitive AML cell lines (**Table 1**).
- APG-2449 combined with lisaftoclax synergistically reduced cell viability and enhanced apoptosis in AML cell lines (**Fig. 2**).
- APG-2449 likely antagonized lisaftoclax induced upregulation of MCL-1 and BCL-xL and enhanced the antiproliferative activity of lisaftoclax.
- APG-2449, when used alone or combined with lisaftoclax, decreased activation of FAK and its downstream effectors (**Fig. 3A**). Silencing FAK with siRNA enhanced the antiproliferative effect of lisaftoclax in AML cells (**Fig. 3B**).
- APG-2449 synergistically enhanced the antitumor effect of lisaftoclax in both MV4-11 and OCI-AML-3 AML CDX xenograft models (**Fig. 4**).

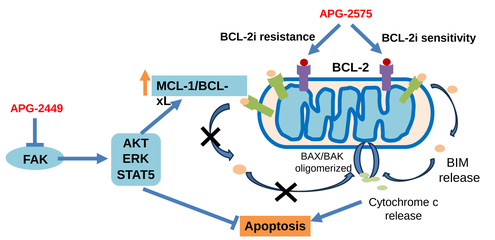


Fig. 1. Potential mechanism of the synergy between APG-2449 and lisaftoclax (APG-2575) in AML

Table1. Antiproliferation effect of APG-2449 in AML cell lines

	Cell line	IC_{50} (μM)，Mean ± SD，n = 2
BCL-2i sensitive	MV4-11	1.25±0.22
	ML-2	0.88±0.61
	MOLM-13	1.29±0.35
	HL-60	1.23±0.20
BCL-2i resistant	OCI-AML-3	6.73±2.77

CONCLUSIONS

- Our data suggest that FAK inhibition by APG-2449 augments the antileukemic activity of lisaftoclax in AML. This synergistic effect can be partially attributed to suppression of MCL-1/BCL-xL by FAK downstream pathways. This novel combination showed synergistic antileukemic activity in both BCL-2 inhibitor-sensitive and -insensitive AML cells *in vitro* and in xenograft models.
- Overall, these promising results provide a novel approach to the clinical development of lisaftoclax for treatment of patients with AML.

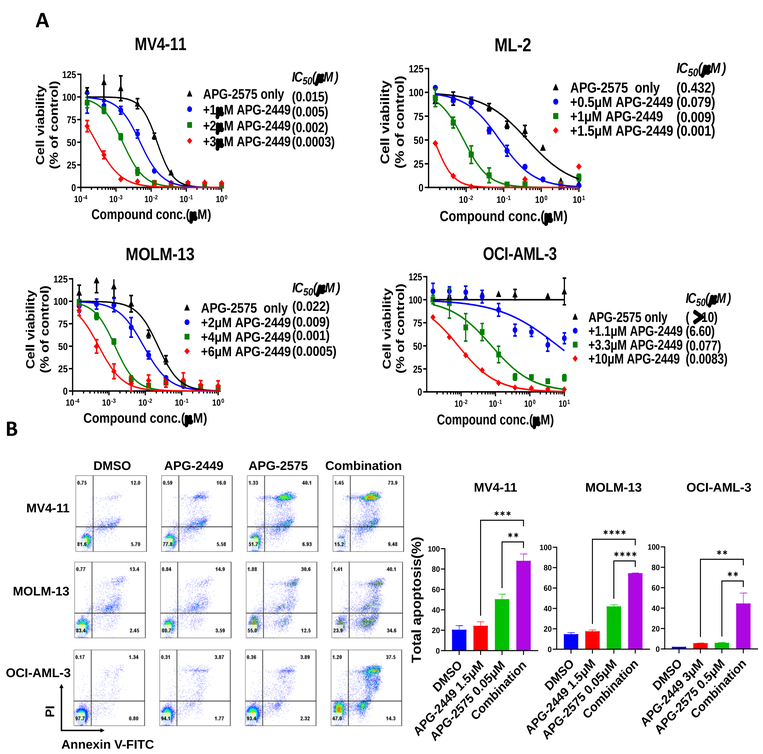


Fig. 2. APG-2449 and lisaftoclax (APG-2575) have synergistic effects in both BCL-2i-sensitive and -resistant AML cell lines. (A) AML cells were treated with indicated concentrations of APG-2449 and APG-2575 for 72 hours. Cell Titer Glo was used to evaluate cell viability. **(B)** AML cells were treated with APG-2449 alone or combined with APG-2575. Induced cell apoptosis was evaluated by flow cytometry with Annexin V-FITC and PI Staining. Values are presented as mean ± SD (n = 2 replicates), using one-way ANOVA test with Tukey's method for multiple comparison.

REFERENCES

1. Akhade VS, et al. *Leukemia* 2023;37:776-87
2. Konopleva M, Letai A. *Blood* 2018;132:1007-12.
3. Zhang Q, et al. *Sig Transduct Target Ther* 2022;7:51
4. Deng J, et al. *Clin Cancer Res* 2022;28:5455-68.
5. Fang DD, et al. *BMC Cancer* 2022;22:752.

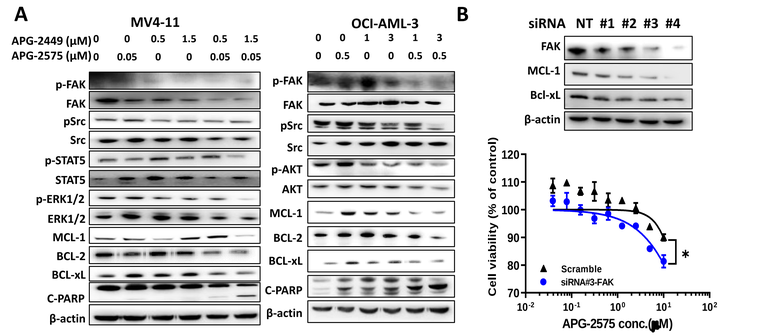
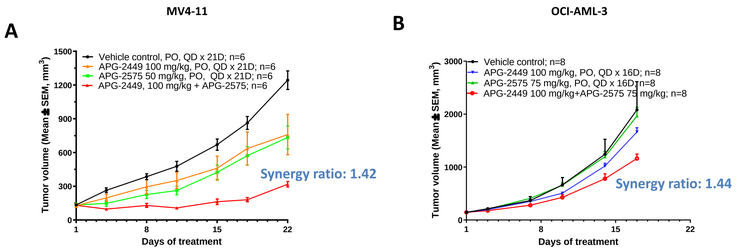


Fig. 3. APG-2449 antagonized lisaftoclax (APG-2575)-induced upregulation of MCL-1 and BCL-xL. (A) FAK and downstream pathway expression were evaluated by western blot analysis. **(B)** siRNA-mediated FAK knock-down suppressed MCL-1 and BCL-xL expression and sensitized the antiproliferation of APG-2575 in OCI-AML-3. Values are presented as mean ± SD (n = 3 replicates), *$p < 0.05$, vs. Scramble.



Group	RTV@Day 22	T/C@Day 22
Vehicle control	9.7±1.2	-
APG-2449 100 mg/kg	6.5±1.6	67
APG-2575 50 mg/kg	5.5±0.7	56
Combination	1.89±0.26$^{****\#\#\#\$\$\$\$}$	26

Group	RTV@Day 17	T/C@Day 17
Vehicle control	14.01±3.47	-
APG-2449 100 mg/kg	11.71±0.87	83.61
APG-2575 75 mg/kg	13.73±1.25	98.02
Combination	8.00±0.46$^{****\#\#\#\$\$\$\$}$	57.1

Fig. 4. APG-2449 synergistically enhanced the antitumor effect of lisaftoclax (APG-2575) *in vivo*. (A-B) MV4-11 and OCI-AML-3 human AML tumor-bearing mice were treated with APG-2449, lisaftoclax, both agents, or vehicle. Relative tumor volume changes are shown in tables, mean ± SD (n = 6 or n = 8). $^{****}p < 0.0001$, vs. vehicle; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, vs. APG-2449 100 mg/kg; $^{\$\$\$\$}p < 0.0001$ vs. APG-2575 50 mg/kg or APG-2575 75 mg/kg. Synergy ratio = $[T/C_{(APG-2449)}]*[T/C_{(APG-2575)}]/[T/C_{(combination)}]$. Synergy: Ratio > 1, synergistic; Ratio = 1, additive; Ratio < 1, antagonistic.

ACKNOWLEDGMENTS

This study was sponsored Ascentage Pharma Group Corp Ltd. (Hong Kong). Ashutosh K. Pathak, MD, PhD, MBA, FRCP (Edin.), Stephen W. Gutkin, Ndiya Ogba, PhD, and Paul O. Fletcher, PhD, with Ascentage Pharma Group Inc., provided editorial support.