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HMPL-523, a Novel SYK Inhibitor, Showed Anti-tumor Activities *in Vitro* and *in Vivo*

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Introduction

- Spleen Tyrosine Kinase (SYK) plays a pivotal role in the regulation of B-cell receptor (BCR) signal pathway.
- Ibrutinib and Idelalisib , (targeting BTK and PI3Kδ in BCR signaling pathway, respectively), were approved for chronic lymphocytic leukemia (CLL).
- Due to the heterogeneity of B cell malignancies and relapse from current therapy, new drugs are still in great demand.
- HMPL-523 is a novel, highly potent and selective SYK inhibitor. The pre-clinical anti-tumor activity of HMPL-523 was evaluated in this study.

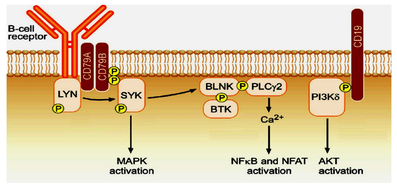


Figure 1 .BCR signaling pathway [1]

Methods

- **Cell viability assay:** Different types of cells were treated with tested compounds for 72 hours and detected by CellTiter-Glo luminescent or CCK-8 assay.
- **Apoptosis**: Cells treated with indicated drugs for 48 hours and determined by Annexin-V/PI staining or PI staining
- **Signaling pathway**: The effect of HMPL-523 on SYK signaling pathway was detected by Western blot.
- **In vivo studies**: Balb/c nude mice bearing subcutaneously implanted REC-1 cells or intravenously injected BA/F3 cells or BA/F3 $^{TEL\text{-}SYK}$ cells were used to determine the *in vivo* target inhibition and anti-tumor activity.

Results

A. HMPL-523 is a potent and selective SYK inhibitor

Kinase Inhibition	HMPL-523 IC_{50} (μM)	R406 IC_{50} (μM)
SYK*	0.025 (1×)	0.054 (1×)
FLT3*	0.063 (2.5×)	0.009 (0.2×)
KDR*	0.390 (21×)	0.061 (1.1×)
LYN*	0.921 (39×)	0.160 (3.0×)
FGFR2*	3.214 (129×)	0.057 (1.1×)
AUR A*	3.969 (159×)	0.219 (4.1×)
Other > 200 kinases**	<70% inhibition at 3 μM	N/A

*: Determined at HMP using z-lyte assay (Invitrogen) or FP (Bellbrook)

**: Determined with ^{32}P-ATP incorporation assay by Eurofins

Inhibition on p-BLNK in cell-based assay（ IC_{50}, μM)

Cell lines	HMPL-523	R406	GS-9973
REC-1, human mantle cell lymphoma	0.105	0.147	0.051
ARH-77,human plasma cell leukemia	0.173	0.824	0.228

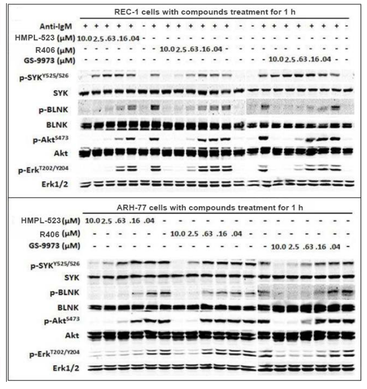


Figure 2. Inhibition on p-BLNK activation in REC-1 and ARH-77 cells

- **HMPL-523 showed higher selectivity compared to R406.**
- **HMPL-523 showed SYK and downstream signaling inhibition in REC-1 and ARH-77 cells.**
- **The activity of HMPL-523 was comparable to R406 or GS-9973 in REC-1 and ARH-77 cells.**

B. HMPL-523 inhibited cell survival and SYK signaling pathway in BA/F3$^{TEL\text{-}SYK}$

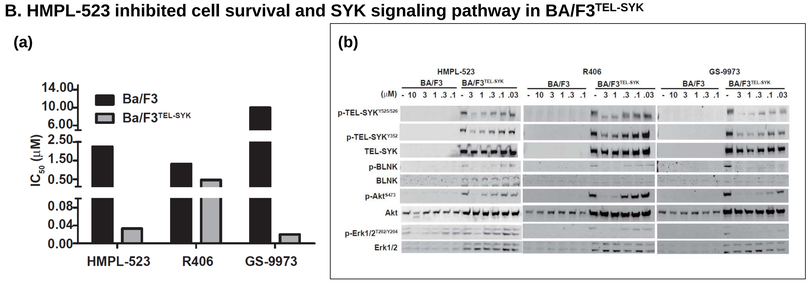


Figure 3. Effect of HMPL-523 on cell survival (a) and SYK signaling pathway (b) in BA/F3$^{TEL\text{-}SYK}$ cell which stably expressed TEL-SYK , and the parent BA/F3 cell.

- **R406 showed similar inhibition on cell survival of BA/F3 or BA/F3$^{TEL\text{-}SYK}$ indicating a poor cell selectivity. Compared with R406, HMPL-523 and GS9973 were highly selective to inhibit BA/F3$^{TEL\text{-}SYK}$.**

C. HMPL-523 inhibited cells survival in a panel of human lymphoma and leukemia cell lines and induced apoptosis in REC-1 cells

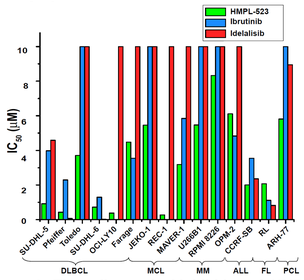


Figure 4. The cells survival inhibition of HMPL-523, ibrutinib and idelalisib in lymphoma and leukemia cell lines.

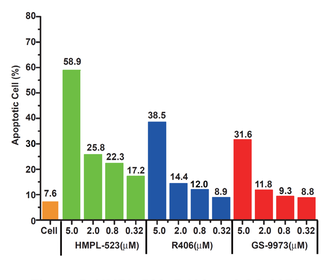
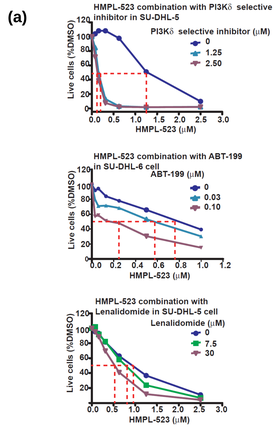


Figure 5. HMPL-523, R406 and GS-9973 increased apoptotic rate in REC-1 cells.

D. Combination of HMPL-523 with other drugs to promote cell killing in Diffuse large B-cell lymphoma (DLBCL) cells through inducing apoptosis



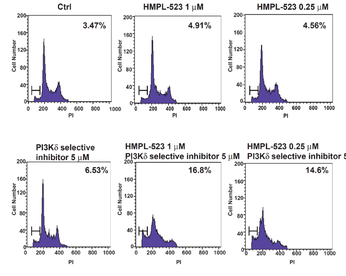
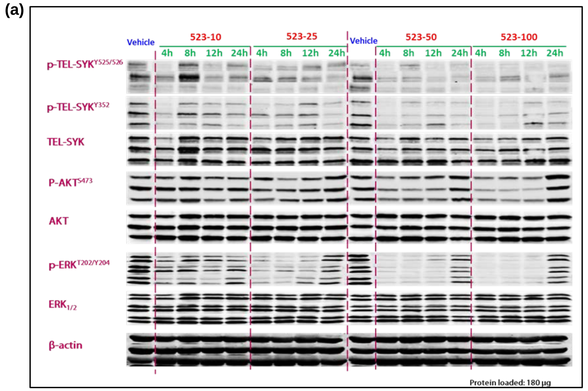


Figure 6. (a) HMPL-523 combined with other drugs to inhibit cell survival in DLBCL. (b) Co-treatment of HMPL-523 and PI3Kδ selective inhibitor caused apoptosis in SU-DHL-5 cells.

The synergistic anti-tumor effect of HMPL-523 in combination with PI3Kδ inhibitor, BCL-2 inhibitor and chemotherapy drugs in DLBCL cells may provide rationale to investigate the combination therapy in clinical trials.

E. SYK signaling inhibition and anti-tumor activity of HMPL-523 *in vivo*



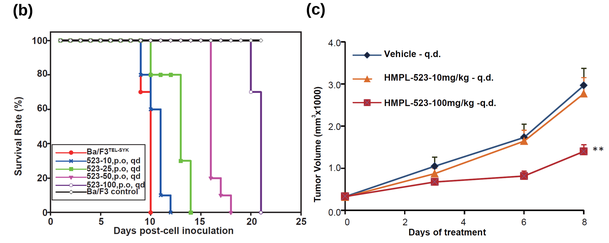


Figure 7. (a) HMPL-523 down-regulated phosphorylation of TEL-SYK^{Y352} and its downstream molecules in a dose-dependent manner in spleen of BaF3$^{TEL\text{-}SYK}$ tumor bearing mice. (b). HMPL-523 dose-dependently increased the life span of the mice bearing Ba/F3$^{TEL\text{-}SYK}$ tumors via i.v. injection (c). HMPL-523 at 100 mg/kg inhibited tumor growth in REC-1 subcutaneous xenograft model. ** p<0.01 vs. vehicle.

Summary

- **HMPL-523 is a potent and highly selective SYK inhibitor.**
- **The in vitro and in vivo anti-tumor activity of HMPL-523 is mediated by SYK signaling pathway inhibition.**
- **The synergistic anti-tumor effect of combination of HMPL-523 with other targeted therapy or chemotherapy in DLBCL cell line warrantes further investigation of combination therapy in clinical trials.**

Reference

[1] Pharmacology & Therapeutics 144 (2014) 338–348

Disclosures

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