

The Inhibitor of BTK (IBTK γ) plays a distinct, BTK-independent signaling role in MYD88-mutated lymphomas.

Amanda Kofides (1), Xia Liu (1), Hao Sun (1), Nicholas Tsakmaklis (1), Maria L. Guerrero (1, 2), Christopher J. Patterson (1), Shayna R. Sarosiek (1,2), Jorge J. Castillo (1, 2), Zachary R. Hunter (1, 2), Steven P. Treon (1, 2), and Shirong Liu (1, 2)

(1) Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA; (2) Department of Medicine, Harvard Medical School, Boston, MA, USA.

Introduction: The highly prevalent MYD88 L265P mutation drives malignant growth via NF- κ B activation through Bruton tyrosine kinase (BTK) in Waldenström macroglobulinemia (WM) (Yang et al., 2013), underpinning the clinical use of BTK inhibitors in WM (Treon et al., 2015). Copy number alteration studies have identified a common 6q deletion (del6q) in WM patients that affects regulators of MYD88 signaling, including IBTK. IBTK copy number loss occurs in up to half of MYD88-mutated lymphoma patients (Guerrero et al., 2018). Homozygous del6q with loss of IBTK expression has been observed in ibrutinib resistant WM patients (Jimenez et al., 2020). IBTK binds to the PH domain of BTK, thereby downregulating its activity, calcium mobilization, and NF- κ B activation (Liu et al., 2001). However, the role of IBTK in MYD88-mutated B-cell malignancies remains to be defined. We therefore investigated the functional role of IBTK in MYD88 mutated lymphomas.

Methods: IBTK isoforms were identified by whole exome sequencing. IBTK protein levels were assessed by western blot in MYD88-mutated and wild-type (WT) cell lines and in sorted CD19⁺ B cells from WM patients. Single-cell RNA sequencing with BCR sequencing was used to compare IBTK transcript levels between malignant and non-malignant B cells, and between del6q and 6q-intact cells. IBTK was overexpressed in MYD88-mutated (BCWM.1, MWCL-1, TMD8, HBL-1) and WT (OCI-Ly7, OCI-Ly19, Ramos, RPMI-8226) lines to identify interacting partners via pull-down assays and mass spectrometry phospho-proteomics. RNA-seq was performed to characterize signaling pathway changes in IBTK γ -overexpressing versus vector-control cells.

Results: Single-cell analysis of WM patients showed increased IBTK transcription in malignant versus non-tumor B cells, except in del6q cases. Of the IBTK isoforms (α , β , γ), only α and γ were expressed, with IBTK γ showing heterogeneous expression. IBTK α was consistently expressed across WM patients and MYD88-mutated lines regardless of del6q or treatment status, whereas IBTK γ was reduced in a subset of WM patients. Although both IBTK α and IBTK γ have been reported to bind and inactivate BTK (Janda et al., 2011), this had not been examined in MYD88-mutated cells. Unexpectedly, IBTK γ overexpression did not

alter BTK activation, and pull-down assays in BCWM.1 and TMD8 cells showed that IBTKy does not bind BTK; instead, BCLAF1 emerged as its dominant binding partner. HCK activity appeared to suppress this interaction: covalent (zanubrutinib) or non-covalent (pirtobrutinib) BTK inhibition did not affect IBTKy–BCLAF1 binding, while the BTK/HCK dual inhibitor KIN-8194 enhanced it. IBTKy overexpression produced two distinct signaling effects: increased inflammatory pathway activity, including elevated I κ B α phosphorylation, alongside decreased PLC γ 2 and PKC phosphorylation, the latter likely downstream of BCLAF1. Consequently, IBTKy overexpression induced apoptosis, which was enhanced by venetoclax (BCL2 inhibitor) and pacritinib (JAK2 inhibitor) but unaffected by BTK inhibition.

Conclusions: IBTKy plays a distinct, BTK-independent signaling role in MYD88-mutated malignancy, combining proinflammatory I κ B α activation with suppression of PLC γ 2/PKC signaling via BCLAF1 interaction to drive apoptosis. These findings provide novel mechanistic insights to explain BTKi resistance in del6q WM patients and support IBTKy as a potential therapeutic target.

Form record received

From International Workshop on Waldenstrom's Macroglobulinemia <pattersonkent@outlook.com>

Date Fri 31-Jul-26 5:14 PM

To Patterson, Christopher <Christopher_Patterson@DFCI.HARVARD.EDU>

External Email - Use Caution

Record saved to database with ID: 272

Form ID: 1

Form title: Abstract Submission

Form name: Abstract_Submission

Submitted at: 2026-07-31 17:13:31

Submitter IP: 170.223.207.67

User-ID: 0

Username: -

User full name: -

Submitter provider: Unknown

Submitter browser: Mozilla/5.0 (Windows NT 10.0; Win64; x64) AppleWebKit/537.36 (KHTML, like Gecko)

Chrome/150.0.0.0 Safari/537.36 Edg/150.0.0.0

Submitter operating system: win

First Name: Amanda

Last Name: Kofides

Email: amanda_kofides@dfci.harvard.edu

Registration Type: Delegate

Abstract Title: The Inhibitor of BTK (IBTKy) plays a distinct, BTK-independent signaling role in MYD88-mutated lymphomas.

Please consider me for a YIA grant: YIA Grant Consideration

Conference: IWWM13