Preclinical evaluation of IQS019, a novel BCR kinase inhibitor, in *in vitro* and *in vivo* models of non-Hodgkin lymphoma

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INTRODUCTION

B-cell receptor (BCR) pathway regulates multiple cellular processes which are critical for maintenance and survival of B cells, including proliferation, differentiation and cell migration. Antigen engagement to BCR extracellular domain leads to phosphorylation and activation of immunoreceptor tyrosine-based activation motifs located in the cytoplasmic portion and other proteins downstream BCR. Upon antigen binding, within the BCR signalosome the Lck/Yes novel tyrosine kinase (Lyn) recruits and phosphorylates the spleen tyrosine kinase (Syk), triggering a proliferation and survival cascade signaling that involves the phosphorylation and activation of Bruton agammaglobulinemia tyrosine kinase (Btk). Chronic BCR activation can occur in the absence of antigen engagement, in several B-cell malignancies, including diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL) (1).

Despite the promising results obtained with the first BCR kinase inhibitors in the clinical, including the Syk-targeting drug fostamatinib or the Btk inhibitor ibrutinib, the design of new compounds is warranted to improve treatment efficacy. In this context, we recently described the synthesis of a new family of 4-aminopyrido[2,3-d]pyrimidines with antitumoral activity against a panel of B lymphoid cell lines. Compound 19 (formally IQS019) was identified as the most effective and specific molecule in terms of antiproliferative activity, with growth inhibitory 50 (GI_{50}) doses in the micromolar range. Docking studies and biochemical assays further showed that the compound inhibited the activity of the BCR kinases Syk, Lyn, and Btk with higher efficacy than the reference kinase inhibitors (2).

References

1. Young RM and Staudt LM. Nat Rev Drug Discov 2013;12:229-43.
2. Puig de la Bellacasa R, *et al*. Eur J Med

AIMS

To evaluate the antitumor activity of the novel BCR kinase inhibitor IQS019 in preclinical models of B lymphoid neoplasms (CLL, MCL, FL, DLBCL).

RESULTS

Result 1 **Inhibitory activity of IQS019 against B cell-, T cell-, and leucocyte receptor-related kinases.** The kinase inhibition profile of IQS019 (10 µM) was evaluated at Proqinase using a Kinase 400-Profiler Panel ↔ 60% of the human kinome.

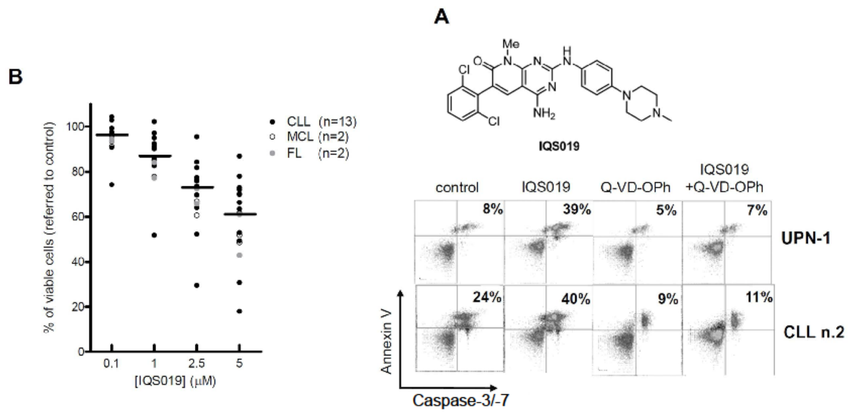
	Kinase	Residual kinase activity upon IQS019 exposure
10/61 kinases	LYN *	< 1 %
	BLK	< 1 %
	LCK	< 1 %
	SRC (GST-HIS-tag)	1 %
	FRK	1 %
	CSK	1 %
	HCK	1 %
	FYN	2 %
	BTK *	6 %
	SYK aa1-635 *	10 %

* validated by docking studies

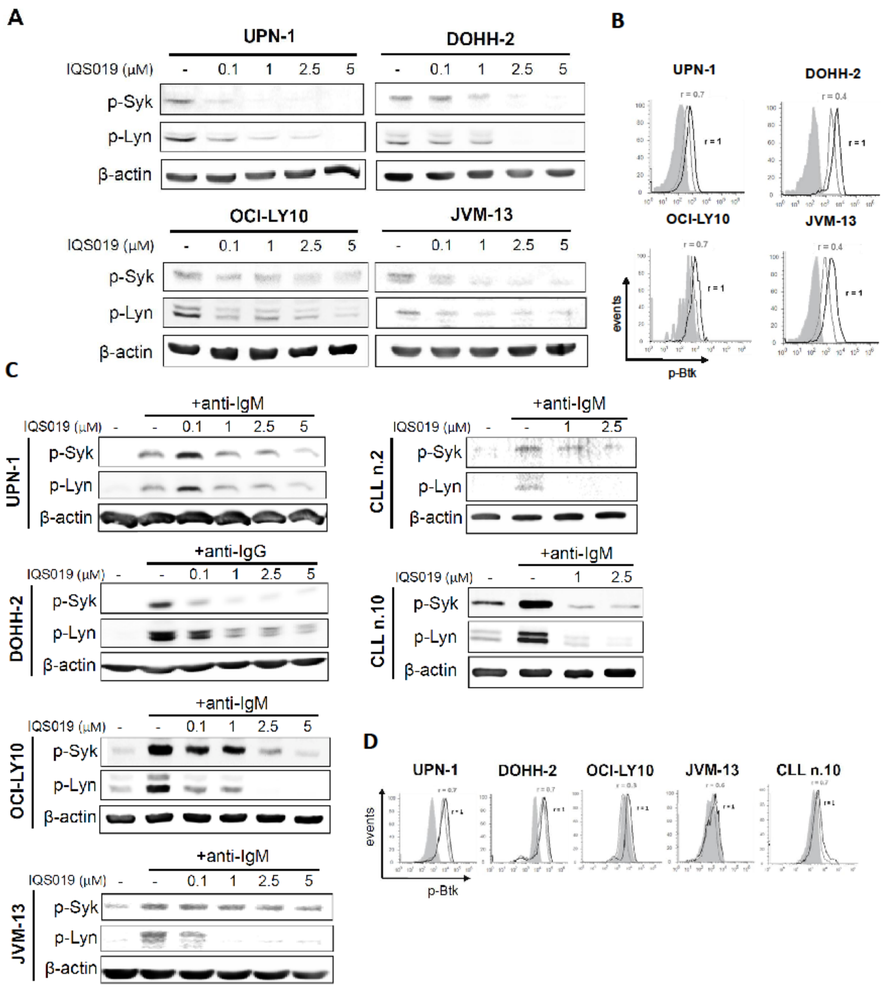
Result 2 **IQS019 decreases cell proliferation and induces a dose-dependent cell death in distinct subtypes of B lymphoid neoplasms.** (A) Chemical structure of IQS019. (B) B-NHL primary cells were incubated with IQS019 for 24h and cell viability was determined by MTT assay. (C) Cells were exposed for 24 hours to 5 µM IQS019, in the presence of absence of the pan-caspase inhibitor Q-VD-Oph. Apoptosis was determined by simultaneous cytofluorimetric detection of Annexin-V and caspase-3/7 activity.

Table 1.- Sensitivity of B lymphoid cell lines to IQS019

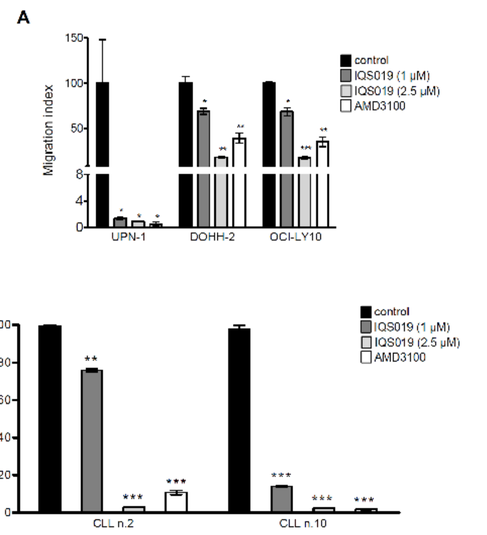
Cell lines	B lymphoid subtypes	*TP53* status[1]	IQS019 cytotoxic effect (referred to untreated cells) 1 µM, 48 hours	5 µM, 48 hours
DOHH-2	FL	wt	26%	100%
WSU-NHL	FL	del/mut	6%	80%
WSU-FSCCL	FL	wt	26%	77%
SC-1	FL	del/mut	4%	43%
JEKO-1	MCL	del/mut	21%	75%
MAVER-1	MCL	del/mut	15%	70%
UPN-1	MCL	del/mut	14%	66%
HBL-2	MCL	del/mut	12%	64%
MINO	MCL	del/mut	19%	64%
GRANTA-519	MCL	wt	26%	63%
Z-138	MCL	wt	18%	62%
JVM-2	MCL	wt	16%	58%
REC-1	MCL	wt	12%	48%
MEC-2	CLL	del/mut	12%	51%
JVM-13	CLL	wt	7%	46%
MEC-1	CLL	wt	9%	33%
SUDHL-16	GCB-DLBCL	del/mut	15%	47%
OCI-LY8	GCB-DLBCL	del/mut	2%	29%
SUDHL-8	GCB-DLBCL	del/mut	15%	32%
OCI-LY10	ABC-DLBCL	wt	3%	47%
U-2932	ABC-DLBCL	del/mut	15%	51%



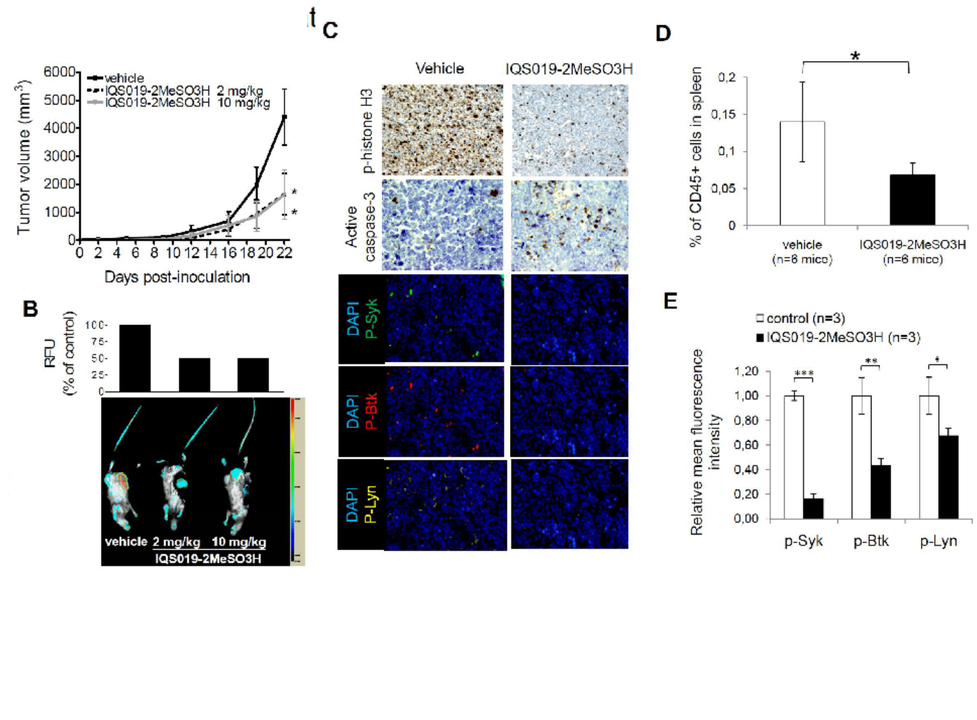
Result 3 **IQS019 impairs constitutive phosphorylation of Syk, Lyn and Btk tyrosine kinases overcomes anti-Ig-mediated BCR activation in malignant B cells.** Western Blot (A-C) and flow cytometry (B-D) detection of phospho(p)-Syk, p-Lyn and p-Btk in B-NHL cell lines and primary samples treated for 6h with increasing concentrations of IQS019, with or without anti-Ig-mediated BCR ligation. Indicated are the relative median fluorescence intensity (r) values observed after treatment with 5 µM IQS019 (grey curves), and referred to control untreated cells (black curves). Grey filled histograms: isotype control.



Result 4 **IQS019 impedes B cell chemotaxis.** Representative, CXCR4-expressing B lymphoid cell lines (A) and CLL primary samples (B) were exposed for 1.5 hours to 1 or 2.5 µM IQS019, with or without Ig-mediated BCR stimulation, followed by cytofluorimetric recounting of cells migrated towards recombinant CXCL12 in a 4 hour-Transwell assay.



Result 5 **IQS019 impairs tumor growth and homing of B lymphoid cells to spleen *in vivo*.** (A) SCID mice were inoculated with UPN-1 cells and began treatment at day 10 post-inoculation. IQS019-2MeSO3H was administrated 5 days a week for 2 weeks. (B) Lower panel, intratumoral glucose uptake was evaluated in representative mice injected intravenously with an IR800-labeled 2-deoxy glucose probe 24 hour prior sacrifice, and visualized with an Odyssey infra-red scanner (Li-Cor). Upper panel, relative fluorescence quantification by means of the Image Studio software. (C) Immunostaining of consecutive sections from representative UPN-1-derived tumors (magnification 200X). (D) Mice were injected intravenously with DOHH-2 cells and, after one week, received a 2 mg/kg dose of IQS019-2MeSO3H or vehicle, daily, for up to 14 days. Mice were then sacrificed and human (CD45+) malignant B cells were recounted from spleen. (B) CD45+/CD20+ human B cells isolated from n=3 representative animals were labeled with anti-p-Syk, anti-p-Lyn, or anti-p-Btk antibody and fluorescence was recorded on a cytometer. Shown are the relative r values among control



CONCLUSIONS

- IQS019 efficiently prevents the activating phosphorylation of the apical BCR kinases Syk, Lyn and Btk in CLL, MCL, FL and DLBCL cells.
- Inhibition of BCR signaling upon IQS019 treatment leads to reduced cell proliferation, blockade of CXCL12-dependent migration, and increased caspase-dependent apoptosis.
- In xenotransplant mouse models of MCL and FL, treatment with IQS019 results in a remarkable decrease in BCR kinase phosphorylation and mitotic index, leading to reduced tumor burden and tumor cell infiltration into the spleen.
- These results warrant further investigation and clinical development for this novel, pleiotropic BCR kinase inhibitor, in mature B lymphoid malignancies.

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