The Bruton tyrosine kinase inhibitor zanubrutinib (BGB-3111) demonstrated synergies with other anti-lymphoma targeted agents

Inhibition of Bruton tyrosine kinase (BTK) is a well-established therapeutic approach in B-cell malignancies and two BTK inhibitors, ibrutinib and acalabrutinib, have been approved by the U.S. Food and Drug Administration for use in this setting.[1] Zanubrutinib (BGB-3111) is an investigational second-generation irreversible BTK inhibitor that has been shown to have lower off-target inhibitory activity on other kinases, including interleukin-2-inducible T-cell kinase (ITK), Janus kinase 3 (JAK3) and epidermal growth factor receptor (EGFR).[2] Zanubrutinib is under active clinical investigation for use in lymphoid neoplasms. Here, we evaluated the effects of zanubrutinib in combination with other targeted agents in human lymphoma cell lines.

Cell lines derived from activated B-cell (ABC) diffuse large B-cell lymphoma (DLBCL; n=3) or mantle cell lymphoma (MCL; n=2) were exposed for 72 h to increasing doses of zanubrutinib (provided by BeiGene) alone and in combination with increasing doses of other compounds (acquired from Selleckem), as previously described.[3] Cell line identity was assessed by short tandem repeat DNA fingerprinting (Promega GenePrint 10 System kit). Synergism was defined in the presence of a Chou-Talalay combination index between 0.3 and 0.9, additivity for a combination index between 0.9 and 1.1, and antagonism/no benefit for a combination index >1.1.[3]

As determined by the half maximal inhibitory concentration (IC_{50}), single-agent zanubrutinib showed antitumor activity in the nanomolar range in one MCL line (REC1, IC_{50} 0.9 nM) and two ABC DLBCL lines (TMD8, IC_{50} 0.4 nM; OCI-Ly-10, IC_{50} 1.5 nM), while no antitumor activity with concentrations of the drug up to 5 μM was seen in the remaining cell lines, namely the ibrutinib-resistant ABC DLBCL SU-DHL-2 and U2932 lines, and the ibrutinib-sensitive MCL cell line, Jeko1. The pattern of activity was similar to that seen with other novel BTK inhibitors (acalabrutinib and spebrutinib), which appear active in a lower number of cell lines than the first-generation BTK inhibitor, namely only in those that are very sensitive to ibrutinib (IC_{50} values lower than 5-10 nM at 72 h).[4] Since downregulation of interferon regulatory factor 4 (IRF4) only occurs in MCL cell lines that are sensitive to ibrutinib,[5,6] we exposed the dual ibrutinib-/ zanubrutinib-sensitive cell line REC1 and ibrutinib-sensitive/zanubrutinib-resistant Jeko1 cell line to ibrutinib, zanubrutinib, spebrutinib, and dimethylsulfoxide as a control. While the levels of phosphorylated BTK were downregulated in both cell lines, IRF4 protein levels were decreased only in REC1. Thus, the non-BTK mediated effect of ibrutinib could explain its antitumor activity in the group of cell lines with a discordant sensitivity between the different BTK inhibitors. This is consistent with the higher selectivity of the new inhibitors for BTK[1,2] which, based on phase I-II studies, have been associated with lower clinical toxicity than that of ibrutinib despite maintained clinical activity.[7-10] The current and planned phase III trials (*NCT02477696, NCT03053440, NCT02477696*, and *NCT02735876*) comparing first- and second-generation BTK inhibitors in different hematologic disorders should allow us to have a better idea of the contribution of the non-BTK-mediated antitumor activity to the clinical results achieved with ibrutinib.[1]

When used in combination in the ibrutinib-sensitive models, zanubrutinib achieved synergism in all of the cell lines tested with the addition of the MEK inhibitor,

Table 1. Effects of zanubrutinib-containing combinations in activated B-cell diffuse large B-cell lymphoma and mantle cell lymphoma cell lines.

Combination partner	Histology	Cell line	Median combination index	95% CI
Birabresib	ABC DLBCL	OCI-Ly-10	0.49	0.35-0.58
	ABC DLBCL	SUDHL-2	0.85	0.78-0.92
	ABC DLBCL	TMD8	1.05	0.97-1.17
	MCL	JEKO-1	0.42	0.36-0.46
	MCL	REC1	0.97	0.89-1.3
Pimasertib	ABC DLBCL	OCI-Ly-10	0.41	0.35-0.43
	ABC DLBCL	SUDHL-2	0.62	0.39-0.76
	ABC DLBCL	TMD8	0.71	0.62-0.81
	MCL	JEKO-1	0.11	0.09-0.17
	MCL	REC1	0.54	0.43-0.63
Selinexor	ABC DLBCL	OCI-Ly-10	0.53	0.41-0.73
	ABC DLBCL	SUDHL-2	>3	-
	ABC DLBCL	TMD8	0.95	0.57-1.23
	MCL	JEKO-1	0.4	0.35-0.49
	MCL	REC1	0.71	0.57-0.86
Venetoclax	ABC DLBCL	OCI-Ly-10	0.43	0.19-0.63
	ABC DLBCL	SUDHL-2	0.72	0.31-1.2
	ABC DLBCL	TMD8	0.83	0.63-1.08
	MCL	JEKO-1	0.02	0.01-0.02
	MCL	REC1	0.71	0.52-0.97

95% CI: 95% confidence interval; ABC DLBCL: activated B-cell diffuse large B-cell lymphoma; MCL: mantle cell lymphoma; 95% CI: 95% confidence interval.

pimasertib, and the BCL2 inhibitor, venetoclax. Zanubrutinib plus the BET bromodomain inhibitor, birabresib (MK8628/OTX-015), was synergistic in three and additive in two cell lines, and the XPO1 antagonist, selinexor, was beneficial in four cell lines (synergistic in three, additive in one) (Table 1). The improved antitumor activity was believed to be due to an increased cytotoxic effect as demonstrated by higher numbers of cells of the ABC DLBCL OCI-Ly-10 cell line in the subG0 fraction after 24 h of exposure to the combinations in comparison to the numbers following exposure to the single agents (Figure 2). These combination results are in addition to the synergism shown when combining zanubrutinib with the immunomodulatory drug lenalidomide in MCL cell lines,[11] and to what has been reported when combining other BTK inhibitors with targeted agents.[1] It is worth mentioning that the combinations studied were not able to overcome the primary resistance to zanubrutinib observed in the ABC DLBCL cell lines: while the addition of selinexor did not decrease the sensitivity to zanubrutinib, even the benefit of combining the BTK inhibitor with the other three targeted agents was limited.

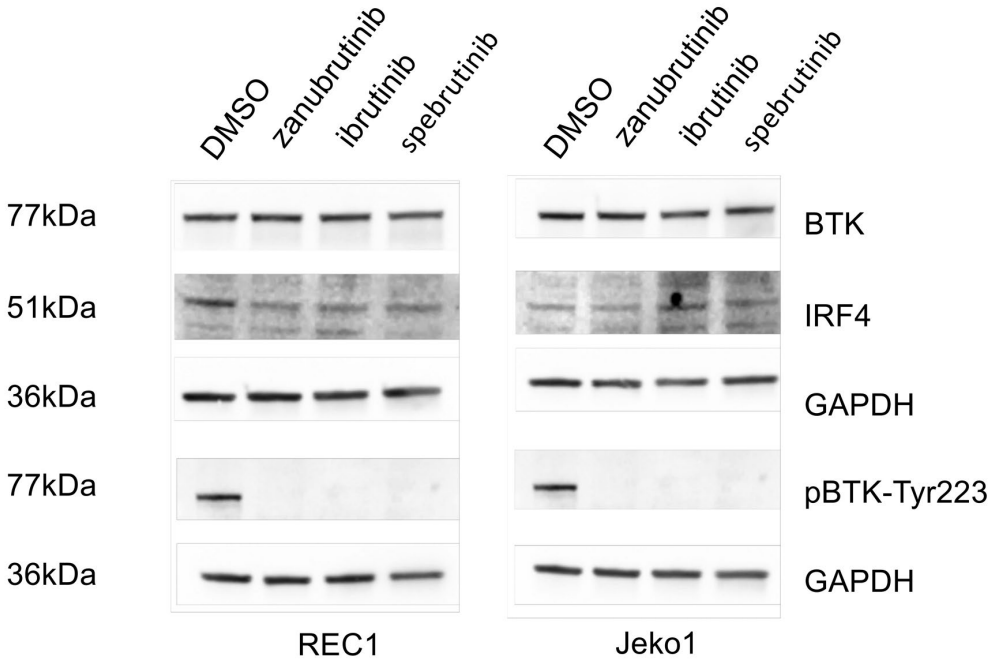

Figure 1. Assessment of IRF4 and pBTK levels in mantle cell lymphoma cell lines exposed to first- and second-generation Bruton tyrosine kinase inhibitors. The REC1 and Jeko1 cell lines were exposed to ibrutinib (500 nM), zanubrutinib (500 nM), spebrutinib (500 nM), and dimethylsulfoxide (DMSO) for 24 h. Protein extraction and western blotting were performed as previously described.[3] The figure shows representative results from experiments performed in duplicate.

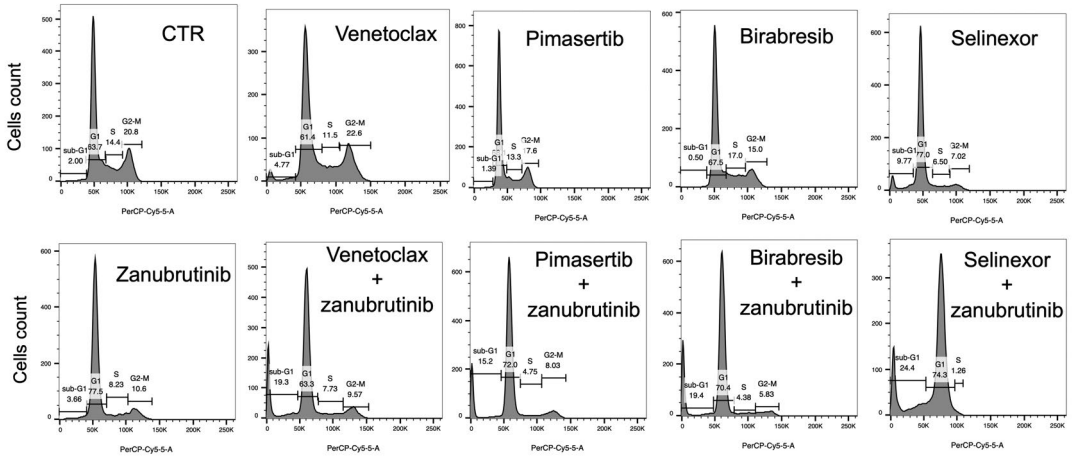

Figure 2. Cell cycle distribution after treatment with zanubrutinib-containing combinations. The activated B-cell diffuse large B-cell lymphoma OCI-Ly-10 cell line was exposed for 24 h to dimethylsulfoxide (DMSO) as a control, to zanubrutinib (50 nM), venetoclax (50 nM), pimasertib (1 μM), birabresib (100 nM) as single agents or to zanubrutinib-containing combinations (same concentrations as single agents). Cell cycle analysis was performed as previously described.[3] The figure shows representative results from experiments performed in duplicate.

In conclusion, zanubrutinib was active as a single agent *in vitro* in ABC DLBCL and MCL cell lines with high sensitivity to ibrutinib, and showed synergism when combined with birabresib, selinexor, and especially with pimasertib or venetoclax.

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