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

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Inhibition of Bruton's 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 (FDA) [1]. Zanubrutinib (BGB-3111) is an investigational second generation irreversible BTK inhibitor that has been shown to have a lower off-target inhibitory activity on other kinases, including ITK JAK3 and EGFR [2]. Zanubrutinib (BGB-3111) is under active clinical investigation for lymphoid neoplasms. Here, we evaluated 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 hours to increasing doses of zanubrutinib alone (provided by BeiGene) 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 (CI) between 0.3 and 0.9, additivity for CI between 0.9 and 1.1, and antagonism/no benefit for CI > 1.1 [3].

Single-agent zanubrutinib showed antitumor activity in the nanomolar range in one MCL (REC1, IC50 0.9 nM) and two ABC DLBCL (TMD8, IC50 0.4 nM; OCI-Ly-10, 1.5 nM) cell lines, while no antitumor activity with concentration of drug up to 5 µM was seen in the remaining cell lines, namely the ibrutinib-resistant ABC DLBCL SU-DHL-2 and U2932, and the ibrutinib-sensitive MCL Jeko1. The pattern of activity was similar to what has been seen with other novel BTK inhibitors (acalabrutinib or 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 (IC50 values lower than 5-10 nM at 72 hr) [4]. Since IRF4 down-regulation 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 to ibrutinib, zanubrutinib, spebrutinib, and DMSO as 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] that, based on phase I-II studies, have been associated with a lower clinical toxicity than ibrutinib despite maintained clinical activity.[7-10] The current and planned phase III trials (NCT02477696, NCT03053440, NCT02477696, NCT02735876) comparing first and second generation BTK inhibitors in different hematological 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 a synergism in all of the cell lines tested with the addition of the MEK inhibitor, pimasertib, and the BCL2 inhibitor, venetoclax. Zanubrutinib plus the BET bromodomain inhibitor, birabresib (MK8628/OTX-015), was synergistic in three and additive in two, 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 a higher number of cells in the subG0 fraction after 24 hours of exposure to the combinations versus the single agents in the ABC DLBCL OCI-Ly-10 cell line (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 studied combinations 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.

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

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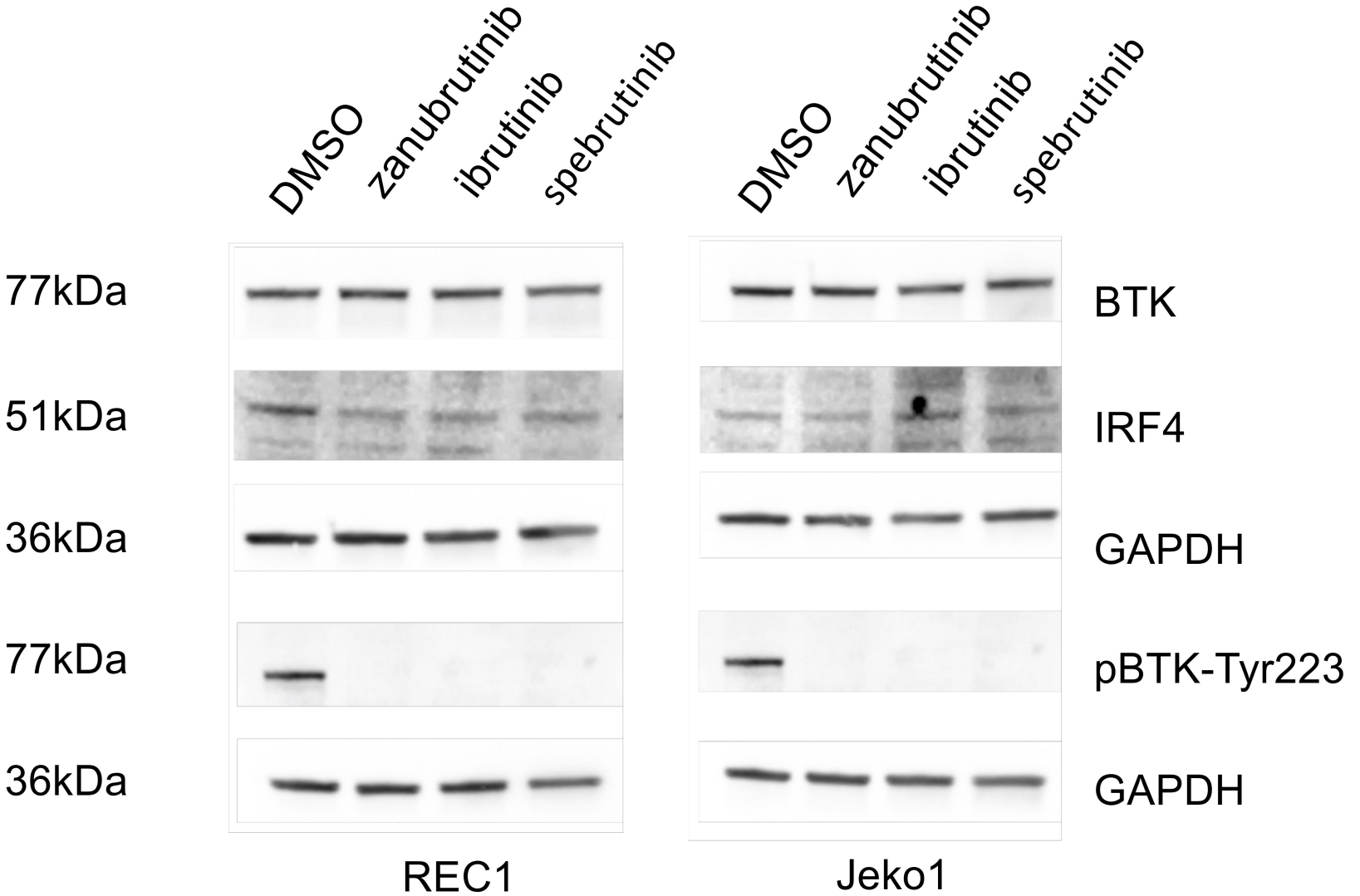
Table 1. Zanubrutinib containing combinations in ABC DLBCL and MCL cell lines.

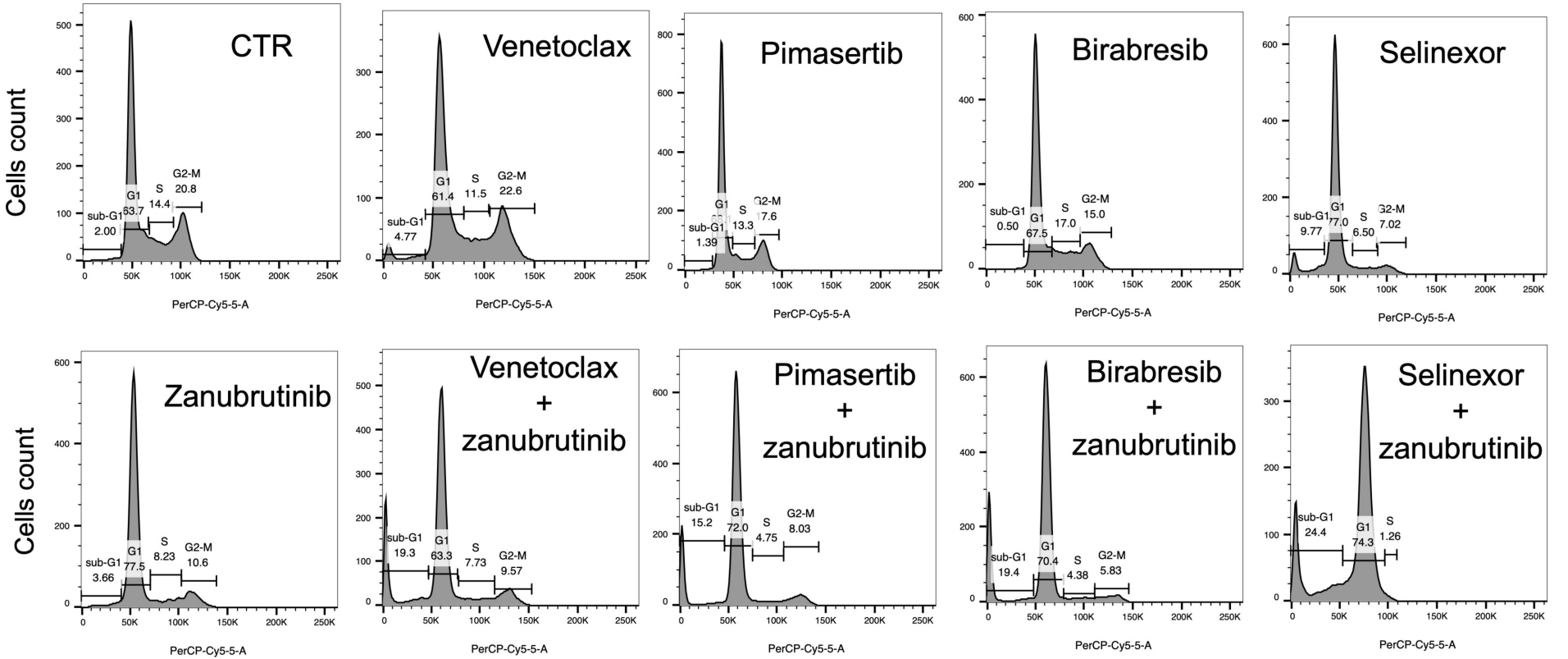
Combination Partner	Histology	Cell Line	Median Combination Index	95% C.I.
Birabresib	ABC DLBCL	OCI-Ly-10	0.49	0.35-0.58
	ABC DLBCL	SUDHL-2	0.85	0.78-0.92
	ABC DLBCL	TMD8	1.05	0.97-1.17
	MCL	JEKO-1	0.42	0.36-0.46
	MCL	REC1	0.97	0.89-1.3
Pimasertib	ABC DLBCL	OCI-Ly-10	0.41	0.35-0.43
	ABC DLBCL	SUDHL-2	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

Figure Legends

Figure 1. Assessment of IRF4 and pBTK levels in MCL cell lines exposed to 1st and 2nd generation BTK inhibitors. The REC1 and the Jeko1 cell lines were exposed to ibrutinib (500 nM), zanubrutinib (500 nM), spebrutinib (500 nM), and DMSO for 24 hours**.** The ABC DLBCL OCI-Ly-10 cell line underwent 24 hours of exposure to DMSO, as control, to zanubrutinib **(**BGB-3111; 50 nM), venetoclax (50 nM), pimasertib (1 µM), and birabresib (100 nM) as single agents or to zanubrutinib-containing combinations (same concentrations as single agents). Protein extraction and Western blot were performed as previously described.[3] The figure shows representative results from experiments performed in duplicate.

Figure 2. Cell cycle distribution after zanubrutinib-containing combinations. The ABC DLBCL OCI-Ly-10 cell line underwent 24 hours-exposure to DMSO, as 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.


DMSO
zanubrutinib
ibrutinib
spebrutinib
DMSO
zanubrutinib
ibrutinib
spebrutinib
77kDa
51kDa
36kDa
77kDa
36kDa
BTK
IRF4
GAPDH
pBTK-Tyr223
GAPDH
REC1
Jeko1


Cells count
CTR
sub-G1 2.00
G1 63.7
S 14.4
G2-M 20.8
Venetoclax
sub-G1 4.77
G1 61.4
S 11.5
G2-M 22.6
Pimasertib
sub-G1 1.39
G1
S 13.3
G2-M 17.6
Birabresib
sub-G1 0.50
G1 67.5
S 17.0
G2-M 15.0
Selinexor
sub-G1 9.77
G1 77.0
S 6.50
G2-M 7.02
Zanubrutinib
sub-G1 3.66
G1 77.5
S 8.23
G2-M 10.6
Venetoclax + zanubrutinib
sub-G1 19.3
G1 63.3
S 7.73
G2-M 9.57
Pimasertib + zanubrutinib
sub-G1 15.2
G1 72.0
S 4.75
G2-M 8.03
Birabresib + zanubrutinib
sub-G1 19.4
G1 70.4
S 4.38
G2-M 5.83
Selinexor + zanubrutinib
sub-G1 24.4
G1 74.3
S 1.26
PerCP-Cy5-5-A