The IRAK4 inhibitor emavusertib (CA-4948) synergizes with second generation BTK inhibitors acalabrutinib and zanubrutinib in MYD88-L265P mutated lymphoma cell lines

Francesca Guidetti [1], Alberto J. Arribas [1], Eleonora Cannas [1], Emanuele Zucca [1,2], Anastasios Stathis [2], Reinhard Von Roemeling [3], Francesco Bertoni [1,2]

[1] Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, Switzerland; [2] Oncology Institute of Southern Switzerland, EOC, Bellinzona, Switzerland; *[3] Curis, Inc., Lexington, MA, USA*

BACKGROUND

IRAK4 is a protein kinase downstream the Toll-like receptor (TLR), activated through the adaptor protein MYD88, crucial for the signaling cascade that leads to NF-κB-mediated cytokine and survival factor expression. Somatic mutations in the gene coding for the adaptor protein MYD88 are common in lymphoid tumors. In particular, the MYD88-L265P mutations, known to sustain NF-κB activation, is specifically observed in a fraction of the activated B cell (ABC) diffuse large B-cell lymphomas (DLBCL), in the vast majority of lymphoplasmacytic lymphoma (LPL), and in rare marginal zone lymphomas (MZLs). So far, no success has been achieved to directly inhibit MYD88. Emavusertib (CA-4948) is an IRAK4 inhibitor, which has shown preclinical activity in ABC DLBCL and MZL models, especially in cases bearing the MYD88-L265P (Boohet et al, ASH 2018; Von Roemeling et al, Clin Cancer Res 2023; Guidetti et al, J Clin Med 2023). We and others have shown synergism when combining emavusertib with the first generation BTK inhibitor ibrutinib (Boohet et al, ASH 2018; Guidetti et al, J Clin Med 2023). Clinical trials are on-going exploring emavusertib as single agent or in combination in patients with hematological cancers or solid tumors (Joffe et al, ASCO 2022; Iqbal et al, Neuro-Oncology's Annual Scientific Meeting 2022). Since ibrutinib is associated with possible toxicities that can outbalance its clinical efficacy, here, we have tested the combination of emavusertib with acalabrutinib and zanubrutinib, two 2nd generation BTK inhibitors that have shown improved safety profile in clinical trials.

MATERIALS AND METHODS

Cell lines were exposed to five increasing concentrations of each compound as single agent or in combination for 72 h, followed by MTT assay. Emavusertib was provided by Curis. Zanubrutinib and acalabrutinib were purchased from Selleckchem (Houston, TX, USA). The beneficial effect of the combinations compared to the single agents was considered both as synergism according to the Chou-Talalay combination index (Chou. Cancer Res 2010) and as potency and efficacy according to the MuSyC algorithm (Meyer et al. Cell Syst 2019), according to the following thresholds.

Parameter	Synergism	Additivity	No Benefit/antagonism
Chou-Talalay combination index	<0.9	0.9-1.1	>1.1
Efficacy by MuSyC algorithm	>1	0-1	<0
Potency by MuSyC algorithm	>0.5	0-0.5	<0

The median values across the 5x5 concentrations used in the combination experiments were used for summary purposes.

RESULTS

Cell lines bearing the MYD88-L265P mutation and derived from ABC DLBCL (OCI-Ly-10, TMD8, HBL1) or MZL/LPL (Karpas-1718) were exposed to emavusertib, acalabrutinib or zanubrutinib as single agents or in combination. Synergism according to the Chou-Talalay index was observed when we combined emavusertib with both acalabrutinib and zanubrutinib in all four cell lines, and, based on the MuSyC algorithm, the synergistic activity appeared mainly due to an improved efficacy (i.e., maximal effect) rather than improved potency (i.e., minimal active dose) of the BTK inhibitors (Fig 1). Fig 2 shows, as examples, the effect in the OCI-Ly-10 cell line.

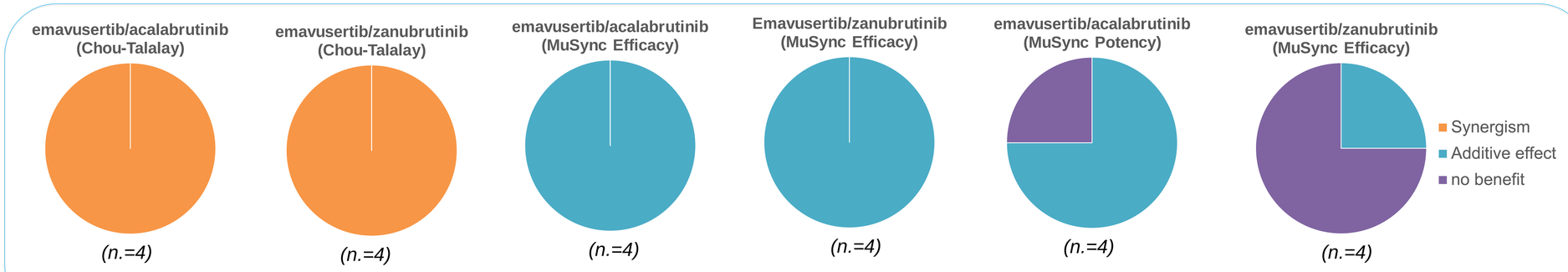

Figure 1: Pie-charts summarizing combination experiments with emavusertib plus the 2nd generation BTK inhibitors acalabrutinib and zanubrutinib in four lymphoma cell lines.

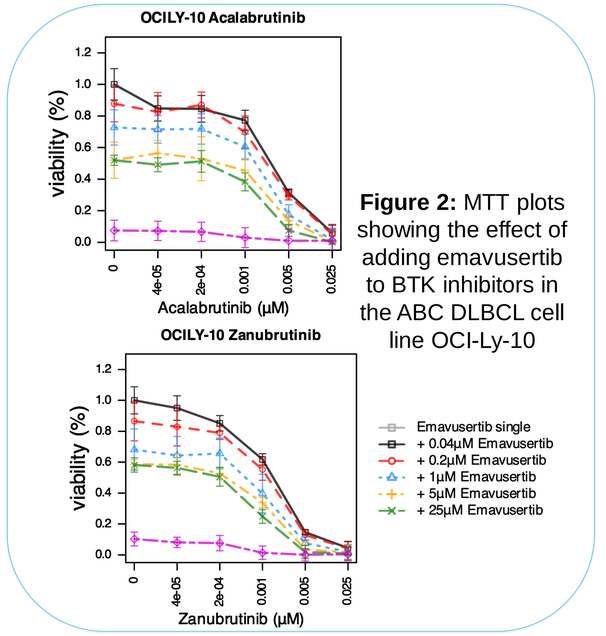

Figure 2: MTT plots showing the effect of adding emavusertib to BTK inhibitors in the ABC DLBCL cell line OCI-Ly-10

CONCLUSION

In MYD88-L265P mutated lymphoma cell lines, synergism is observed combining the IRAK4 inhibitor emavusertib with the 2nd generation BTK inhibitors acalabrutinib and zanubrutinib. Our data sustain further clinical exploration of the combination of IRAK4 and BTK inhibitors for lymphoma patients.

CONTACT

francesco.bertoni@ior.usi.ch