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Combination of the BTK inhibitor CC-292 and bendamustine prevents leukemia development in the E?-TCL1 AT model of CLL

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia in Western countries and is characterized by a clonal expansion of mature CD5+ B cells that accumulate in peripheral blood (PB), bone marrow (BM) and secondary lymphoid tissues. CLL is classified in two molecular subtypes. Patients with a mutated IGHV (M-CLL) display an indolent CLL whereas patients with unmutated IGHV (U-CLL) display an aggressive CLL. Bendamustine is a well-tolerated agent, feasible therapy for elder and non-fit CLL patients in combination with rituximab lacking del(17p). Patients with del(17p) were shown to poorly respond to bendamustine plus rituximab and signals from the microenvironment play a key role in the chemoresistance of CLL cells to bendamustine. Therefore, nowadays, in vitro studies and ongoing clinical trials explore effects of combinations of bendamustine with new generation monoclonal antibodies and novel targeted agents.

Ibrutinib, the first-in-class Bruton's tyrosine kinase (BTK) inhibitor, was approved for the treatment of previously untreated and relapsed/refractory (R/R) CLL patients with or without del(17p). This was prompted by the unprecedented improvement in progression free survival (PFS), overall survival (OS) and high overall response rates (ORR) in corresponding clinical trials.

However, resistance to ibrutinib has emerged due to the acquisition of BTK and phospholipase C?2 (PLC?2) mutations. In addition, intolerance to ibrutinib's side effects is a common cause for discontinuation, probably due to the low specificity of the drug. So far, ibrutinib remains the only BTK inhibitor approved for the treatment of CLL and for all exposed, the availability of more specific BTK inhibitors is warranted.

Spebrutinib (CC-292) is a new orally available BTK inhibitor that covalently and irreversibly binds the ATP-binding pocket of the same Cys481 as ibrutinib but with increased specificity for BTK and no inhibition of several other kinases, such as ITK. In R/R CLL patients, it achieved high nodal and partial response rates.

OBJECTIVES We evaluated the antitumor profile of CC-292 in in vitro and in vivo CLL models, and we tested the potential efficacy of combining CC-292 with bendamustine in an adoptive transfer TCL1 mouse model (AT Eμ-TCL1).

METHODS

The in vitro effect of CC-292 was analyzed in primary cells from PB, BM and LN samples of CLL patients. We analyzed the cytotoxic effect of CC-292 in tumor cells in terms of apoptosis induction (by annexin-V/PI staining) and reduction of the intracellular ATP levels (performing the CellTiter-Glo® Luminiscent Cell Viability Assay).

The effect of CC-292 in CLL proliferation was analyzed by labeling CLL primary cells with 0.5?M CFSE and then cultured for 6 and 9 days in an enriched RPMI-1640 medium used for long-term cultures supplemented with recombinant human IL-15 to sustain survival and CpG DNA TLR-9 ligand (ODN) to induce cell proliferation. As a BTK inhibitor, we assessed the ability of CC-292 to interfere with the BCR signaling through the quantification of the activating phosphorylation of BTK (Y223) and its downstream target PLCg2 (Y759), its impact on CLL chemotaxis towards CXCL12 and tumor cell activation by measuring the surface expression of the early activation marker CD69 on CLL cells cocultured with the CLL microenvironment-mimicking HK and HS-5 cell lines. In these systems, we also tested the combination of CC-292 and bendamustine. Both the mechanism of CC-292 and the

promising in vitro results of the drug combination were validated in vivo in the AT TCL1 model. This was generated by the inoculation of leukemic cells (1 × 107) obtained from splenocytes that had been adoptively transferred before from leukemic E?-TCL1 donor mice (kindly provided by C. Croce; Ohio State University) into C57BL/6N wild-type mice. Fifteen mg/kg CC-292 were administered twice daily via oral gavage, whereas 25 mg/kg bendamustine were administered intravenously once weekly. Mice were euthanized after 11 days of treatment and single cell suspensions were obtained from the BM, inguinal LN and spleen. Hematological counts were obtained weekly in an automated hemocytometer. Tumor load and tumor proliferation were quantified in the different compartments typically affected in CLL. The composition and function of the T cell compartment in the spleen was also analyzed.

CONCLUSIONS

1. CC-292 is a selective and effective BTK inhibitor that blocks CLL chemotaxis towards the lymphoid tissues and overcomes stroma-induced tumor cell activation.
2. CC-292 modestly induces apoptosis but potently inhibits tumor proliferation.
3. CC-292 cooperates with bendamustine to reduce tumor survival, proliferation and expansion both in vitro and in the TCL1 AT mouse model of CLL.
4. Its increased selectivity, reflected by a lower interference and impact on T cells could provide CLL patients with a safer option for disease control.