# Abstract: S154

# Title: FIRST-IN-CLASS MTHFD1 INHIBITOR ELIMINATES MALIGNANT B CELLS IN VITRO AND IN VIVO

**Abstract Type: Oral Presentation**

**Session Title: CLL biology and translational research**

**Background:**

B-cell malignancies are the most frequent hematological cancers. Despite great advance in the standard of care, there are still unmet medical needs. Dysregulated metabolism is now recognized as a crucial factor in oncogenic progression and resistance to treatment. Notably, the one-carbon (1C) cycle has emerged as a major contributor to tumor proliferation by providing building blocks for nucleotide biosynthesis (A). In a recently published work, we have highlighted the mechanism of action of a novel inhibitor of the 1C cycle. This inhibitor, called TH9619, targets the cytosolic MTHFD1 enzyme and induces the selective killing of MTHFD2-overexpressing cancer cells (Green et al, Nature Metabolism, 2023).

**Aims:**

We investigated the potential of targeting MTHFD1 as an innovative therapeutic approach for B-cell malignancies. Our study involved a comprehensive analysis of the mechanism of action, and identification of factors influencing response to treatment.

**Methods:**

The cytotoxic activity of TH9619 was evaluated in vitro on murine and human B-cell malignancies (Chronic Lymphocytic Leukemia (CLL), Richter Syndrome (RS), Mantle Cell Lymphoma (MCL), Diffuse Large B-Cell Lymphoma (DLBCL), Multiple Myeloma (MM), and Follicular Lymphoma (FL)), and in different preclinical murine models. Metabolic tracing, rescue experiments and CRISPR/Cas9 KO were carried out to elucidate the mechanism of action in sensitive and resistant cells.

**Results:**

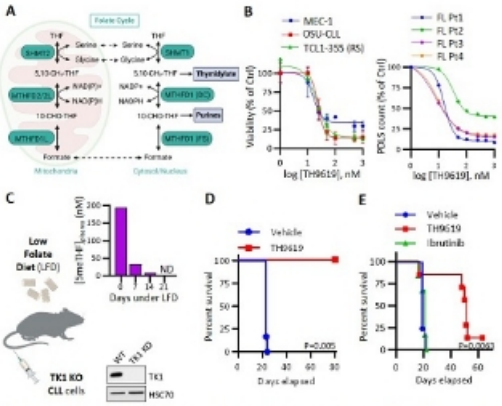
Supported by the high MTHFD2 expression reported in CLL, we evaluated TH9619 cytotoxicity in CLL and other B-cell malignancies. We demonstrated a high in vitro sensitivity to the inhibitor for CLL, MCL and DLBCL cells (IC50<50nM), while MM cells were not affected. We also showed that TH9619 is active at low nanomolar dose on CLL- and Follicular Lymphoma (FL)-patient-derived in vitro models (B). Mechanistically, we proved that TH9619 activity is mediated through a defect in thymidylate (dTMP) synthesis, leading to cell cycle arrest and apoptosis induction in sensitive cells. Indeed, TH9619 in vitro toxicity can be completely abolished by exogenous thymidine, which can be used as precursor for dTMP synthesis via the salvage pathway.

For the preclinical evaluation of TH9619, we have implemented a customized murine model, aiming to mimic human metabolite physiology. Indeed, we reported higher levels of thymidine and folate in mouse plasma compared to human, both metabolites compromising TH9619 cytotoxicity in vitro. Therefore, we developed a murine model in which (i) the use of plasma thymidine via the salvage pathway is impeded (by deleting the TK1 enzyme in CLL cells), and (ii) the plasma folate level is reduced (by feeding mice with a low folate diet) (C). Therefore, we showed that TH9619 strikingly improves mice survival in human tumor xenograft models (OSU-CLL, MEC-1) (D), and completely eradicates already established primary tumors (s.c engraftment of CLL cells). This impressive activity of TH9619 was also demonstrated in a highly aggressive RS syngeneic model, which is completely refractory to Ibrutinib treatment (E).

**Summary/Conclusion:**

We demonstrated that MTHFD1 inhibition is a valuable strategy for B-cell malignancies. We pinpoint specific metabolic features that can greatly influence the response to treatment. Our findings provide valuable insights

for the development of more effective and personalized treatments for patients.




**(A)** Major pathways of the 1C-carbon metabolism. **(B)** Cytotoxic activity of TH9619 on MEC-1, OSU-CLL, TCL1-355 (RS) cells lines, and on Patient-Derived Follicular Lymphoma Spheroids (PDLS from 4 patient (Pt) samples). **(C)** Customized murine model of CLL. Plasma concentration of 5mTHF in mouse fed with low folate diet (LFD) (upper panel). TK1 protein level after CRISPR/Cas9 KO in CLL cells (lower panel). **(D)** Kaplan-Meier survival curve of vehicle- or TH9619-treated NSG mice engrafted with TK1 KO OSU-CLL cells. **(E)** Kaplan-Meier survival curve of vehicle-, TH9619-, or Ibrutinib-treated C57BL6 mice engrafted with highly aggressive RS.

**Keywords:** Mouse model, B cell chronic lymphocytic leukemia, Inhibitor