## W21: Dual PAK4-NAMPT Inhibition Impacts Growth and Survival, and Increases Sensitivity to DNA-Damaging Agents in Waldenstrom Macroglobulinemia.

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p21-activated kinase 4 (PAK4) plays a significant biological and functional role in a number of malignancies, including multiple myeloma (MM). We here characterized PAK4 expression and role in Waldestrom Macroglobulinemia (WM) cells, as well effect of dual PAK4-NAMPT inhibitor (KPT-9274) against WM cell growth and viability.

We have analyzed mRNA and protein expression levels of PAK4 in WM cells, and used loss-of-function approach to investigate its contribution to WM cell viability. We observed high-level expression and functional role of PAK4 in WM, as demonstrated by analysis of cell viability and apoptosis after shRNA-mediated knockdown.

We have further tested the *in vitro* and *in vivo* effect of KPT-9274 against WM cell growth and viability. KPT-9274 is an orally administered small molecule, currently in clinical trial for the treatment of patients with advanced solid malignancies or non-Hodgkin's lymphoma (NCT02702492). KPT-9274 allosterically binds to, destabilizes and causes the degradation of PAK4. In addition to which, we have also reported that KPT-9274 depletes the synthesis of nicotinamide adenine dinucleotide (NAD) by blocking the activity of nicotinamide phosphoribosyl transferase (NAMPT), the rate-limiting enzyme in the NAD biosynthesis salvage pathway.

We observed a significant activity of KPT-9274 against WM cell growth and viability. The growth inhibitory effect was associated with decreased PAK4 expression and NAMPT activity, as well as induction of apoptotic cell death, caspases activation and PARP cleavage. A significant effect of KPT-9274 was also observed in primary tumor cells from both newly diagnosed as well as relapsed WM patients, including primary cells from ibrutinib resistant patients.

At the molecular level, we identified the DNA damage and repair pathway to be significantly impacted by KPT-9274. We observed that apart from inducing DNA damage, assessed by γH2AX expression, dual PAK4-NAMPT inhibition specifically decreased Rad51 and the double strand break repair by the homologous recombination pathway. As a result, combination of

KPT-9274 with the DNA alkylating agents bendamustine or melphalan provided a synergistic inhibition of cell viability in WM cell lines and primary patient tumor cells *in vitro* and *in vivo*.

These results support the translation of KPT-9274 into the clinic as monotherapy or in combination with alkylating agents for treatment of WM patients.