YPN005, an oral CDK7 inhibitor, exhibits a significant antitumor activity in Myc-driven cancers.

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Background: CDK7 plays an important role in regulating cell cycle progression and gene via activation of cell cycle kinases (CDK1, CDK2, CDK4 and CDK6) and RNA polymerase II (PolII). Recent studies indicate that the inhibition of CDK7 is an attractive strategy for the treatment of cancer by down-regulation of c-Myc expression. (Wang *et al* 2018) Myc also regulates the anti-tumor immune response through CD47 and PD-L1. We have investigated the therapeutic efficacy of YPN005, a novel oral CDK7 inhibitor, in triple negative breast cancer (TNBC) and hepatocellular carcinoma (HCC).

Methods: To study the enzymatic activity of CDK7, we used luminescent kinase assay ADP-Glo^{TM} (Promega). The selectivity of YPN005 was assessed across a panel of 468 kinases (scanMAX^{sm}) by a contract research organization (DiscoverX). We evaluated antiproliferative activity of YPN005 on TNBC and HCC cells and the levels of RNA PolII phosphorylation and c-Myc expression were studied by Western Blot analyses to investigate the mechanism of action. Also, caspase 3/7 activation assay (Promega) was performed with HCC cell to confirm the induction of apoptosis. To identify a biomarker, we examined the correlation between the level of c-Myc expression and anticancer activity of YPN005 *in vitro* using HCC cells. The expression levels of PD-L1 and CD-47 mRNA by YPN005 in MDA-MB-468 cells were measured in a real-time PCR (qPCR). In addition, the effect of YPN005 on the cell surface protein expression of PD-L1 and CD47 in MDA-MB-231 cells was measured by flow cytometry. Occupancy study *in vitro* was conducted in human PBMC to measure the level of engagement of the CDK7 by YPN005. The therapeutic efficacy of YPN005 was evaluated in TNBC xenograft mouse model. YPN005 was orally administered at a dose of 10 mg/kg once a day for 3 weeks. THZ1 was intraperitoneally administered at a dose of 10 mg/kg twice a day following the same schedule. We tested *in vitro* synergistic effect of YPN005 in combination with sorafenib against HCC cell. (Supporting Information)

Results: A lead compound, YPN005-121, significantly inhibited the proliferation of TNBC and HCC cells and IC_{50}s was determined as 10-20 nM and 5-30 nM range, respectively. Inhibition of cell proliferation was accompanied by a decrease in the levels of RNA PolII phosphorylation and c-Myc expression. Western Blot analyses revealed that the sensitivity of HCC cells to a lead compound was correlated with the level of c-Myc expression. *In vivo* xenograft model of TNBC showed that oral daily administration of a lead compound for 21 days (once, and 10 mg/kg) efficiently inhibited the growth of tumor. All mice survived during the dosing period without significant changes of the hematologic profiles.

Then, lead optimization has been carried out to obtain more potent CDK7 inhibitors. YPN005-189 was selected as an optimized lead and it showed potent cell growth inhibition as well as highly potent CDK7 inhibitory activity with 4 nM. Also, Kinase panel assay is ongoing.

No. 189 showed potent cell growth inhibition in TNBC cell with 30 nM. Also, complete c-Myc depletion in TNBC cell at the 75 nM concentration was observed. With the liver cancer cell, no 189 showed 5 nM potency. Complete c-Myc depletion was observed at 100 nM concentration. Also, the activation of caspase 3/7 was observed at a highly potent concentration.

For the more, a CDK7 occupancy study was performed using human PBMC cell. At 100 nM concentration of no. 189, our results confirmed over 95% occupation of CDK7 kinase. This result is well correlated with c-Myc depletion in western blotting analysis in cancer cell. At the moment, we're preparing to carry out in vivo PK/PD study.

Then, we examined whether c-Myc depletion by our optimized lead would reduce mRNA expression of PD-L1 and CD47 in cancer cells. MDA-MB-468 was treated with 50 nM concentration of no. 189 which resulted in a significant decrease in mRNA expression levels of PD-L1 and CD47. In addition, no. 189 decreased PD-L1 expression on TNBC cell surface.

Xenograft study for no. 189 was done using a MDA-MB-468 model. We have observed tumor volume regression without any signs of toxicity during treatment periods. In addition, no. 189 showed *in vitro* synergy with sorafenib used to treat hepatocellular carcinoma.

Conclusion: We propose that oral administration of YPN005, an orally available CDK7 inhibitor, could be a potent and attractive approach to treat the Myc overexpressing cancers. YPN005-189 showed significant anti-tumor activities for Myc-driven cancer models with down regulation of immune checkpoints such as PD-L1 and CD47.

Supporting Information