The unique binding mode of NTRC 0066-0, a novel inhibitor of the spindle assembly checkpoint kinase TTK (Mps1), leads to long target residence time and potent anti-tumor activity

Jos de Man, Joost C.M. Uitdehaag, Nicole Willemsen-Seegers, Jan Gerard Sterrenburg, Joeri J.P. de Wit, Jeroen A.D.M. de Roos, Martine B.W. Prinsen, Rogier C. Buijsman, Guido J.R. Zaman. Netherlands Translational Research Center B.V. (NTRC), Molenstraat 110, 5342 CC Oss, The Netherlands

An abnormal number of chromosomes, or 'aneuploidy', is a common feature of solid human tumors and a predictor of poor prognosis in breast, lung, brain and colorectal cancer. Aneuploidy is caused by malfunctioning of the Spindle Assembly Checkpoint (SAC), a surveillance mechanism that ensures the fidelity of chromosome segregation. The protein kinase TTK (commonly referred to as Mps1) is a component of the SAC. Inhibition of TTK gene expression by RNA interference and inhibition of TTK kinase activity by small molecule kinase inhibitors causes chromosome missegregation and cancer cell death.

A novel class of compounds was identified that potently inhibits TTK enzyme activity and cancer cell line proliferation [1]. Its binding mode and that of reference inhibitors was characterized by protein crystallography. Binding kinetics and target residence time were determined by surface plasmon resonance using Biacore T200. Anti-proliferative activity was measured on a broad panel of cancer cell lines [2].

The clinical candidate, NTRC 0066-0, inhibits TTK with subnanomolar potency (IC_{50}) in a kinase enzyme assay and is more than 200 times selective over 276 kinases examined, including mitotic and cell cycle dependent kinases (CDKs). X-ray structures of the TTK kinase domain in complex with NTRC 0066-0 and analogs indicate that this class of compounds induces a large conformational shift in the glycine-rich loop, invoking an inactive kinase conformation. In surface plasmon resonance experiments, NTRC 0066-0 exhibited slow dissociation kinetics, resulting in a long target residence time. Parallel surface plasmon resonance experiments with mitotic kinases confirmed the exquisite selectivity of NTRC 0066-0 for TTK over Aurora and Polo-like kinases. NTRC 0066-0 potently inhibited the proliferation of a wide variety of human cancer cell lines with potencies in the same range as marketed cytotoxic agents. The crystal structure, binding kinetics and cellular potency of NTRC 0066-0 were compared to that of other TTK inhibitors such as Mps1-IN-1, NMS-P715, MPI-0479605, Mps-BAY2b and Bay 1161909 as well as analogs from the NTRC 0066-0 series. This suggest that the unique binding mode of NTRC 0066-0 results in long target residence time which contributes to its strong anti-tumor activity. In subsequent mouse xenograft models of human cancer cell lines, NTRC 0066-0 inhibited tumor growth as a single agent after oral administration at 20 mg per kg.

NTRC 0066-0 is a novel TTK inhibitor with outstanding *in vitro* properties and potent anti-tumor activity in mouse xenograft models. Our data suggest that long target residence time corresponds with potent cellular activity for TTK inhibitors.

References

[1] Maia *et al.* (2015) *Annals of Oncology* 26, 2180-2192; [2] Uitdehaag *et al.* (2014) PLOS ONE 9(3) e92146.