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The discovery of tarloxotinib bromide*: A first-in-class hypoxia-activated tyrosine kinase inhibitor

We have been interested for many years in imparting increased tumour-selectivity to tyrosine kinase inhibitors (TKI) to improve their therapeutic index. One approach involves exploiting the presence of hypoxia in tumours as a unique physiological target capable of supporting reductive metabolism of hypoxia-activated prodrugs (HAP). We have previously reported the synthesis of SN29966, a prototype HAP of a known irreversible inhibitor of the epidermal growth factor receptor (EGFR). SN29966, bears a 4-nitroimidazole bioreductive "trigger" that fragments following one-electron reduction under hypoxia to release the irreversible TKI (Mol Cancer Ther., 2009; 8(12 Sup), C46; Tetrahedron, 2013, 69, 9130).

During lead optimisation of SN29966 we synthesised structural variations around the 4-nitroimidazole trigger class including electron-donating and electron-withdrawing substituents at the *N*-1 and *C*-2 positions of the imidazole ring system. In addition, we investigated heterocyclic trigger variations, such as substituted 2-nitroimidazoles and 2-nitropyrroles. The HAP analogues synthesised were studied, relative to SN29966, with respect to aqueous solubility and stability. Their one-electron reduction potential, prodrug fragmentation rate and hypoxia-dependent anti-proliferative activity in A431 epidermoid carcinoma, SKOV3 ovarian carcinoma and H1975 non-small cell lung cancer (NSCLC) cells was measured. Preferred derivatives were advanced to plasma pharmacokinetic studies and the biodistribution of each HAP to tumour, liver and skin was assessed along with their efficacy in NIH-III mice bearing subcutaneous H1975 xenografts. These studies identified the 1-methyl-4-nitroimidazole trigger, linked to the TKI through the imidazole 5-position, as possessing optimal properties. This preferred trigger was then conjugated to a series of novel substituted 4-anilinopyrido[3,4-*d*]pyrimidine irreversible EGFR/HER2 inhibitors, resulting in a pre-lead series of nitromethylaryl quaternary ammonium salt (NMQ) prodrugs that were evaluated *in vitro* and *in vivo*. This identified tarloxotinib bromide (TH-4000; "tarloxotinib") as an optimised hypoxia-activated irreversible EGFR/HER2 inhibitor for clinical evaluation.

A first-in-man Phase 1 dose-escalation trial of tarloxotinib administered as a once weekly one hour intravenous infusion has been completed, demonstrating a maximum tolerated dose of 150 mg/m^2/week (NCT01631279). Threshold Pharmaceuticals in collaboration with the Academic Thoracic Oncology Medical Investigators Consortium (ATOMIC) has now initiated Phase 2 clinical evaluation of tarloxotinib for the treatment of patients with mutant EGFR NSCLC who have been previously treated with an EGFR TKI and are progressing on treatment, but have not acquired the T790M resistance mutation (NCT02454842). A second Phase 2 trial of tarloxotinib in patients with advanced squamous cell carcinoma of the head and neck (SCCHN) or skin (SCCS) is also ongoing (NCT02449681).

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