Anticancer therapy

BTK degraders tackle drug resistance

Bruton's tyrosine kinase (BTK) inhibitors have revolutionized the treatment of B cell malignancies, such as chronic lymphocytic leukaemia (CLL). However, some patients develop drug resistance through acquired mutations in BTK. Montoya et al. have now described in *Science* the nature of these mutations, some of which induce a scaffolding function of BTK that enables B cell receptor (BCR) signalling. They have identified the molecular degrader NX-2127, which targets both wild-type and mutant forms of BTK and provides clinical benefit in patients with CLL.

BTK mediates B cell activation, proliferation, and signalling downstream of BCR. Covalent BTK inhibitors — such as ibrutinib, acalabrutinib, and zanubrutinib — bind cysteine 481 (C481) in BTK, preventing autophosphorylation and phosphorylation of BTK's downstream substrates. Acquired mutations in C481 impair inhibitor binding. Noncovalent inhibitors of BTK — such as pirtobrutinib and nemtabrutinib — do not require binding to C481 and can therefore overcome C481 mutant-mediated resistance. However, acquired mutations in BTK in other residues can also confer resistance to these inhibitors.

Previous findings have reported drug resistance mutations in BTK that result in loss of its kinase activity but do not affect the downstream effects, suggesting that these mutations might be neomorphic and confer BTK a scaffold function independent of its catalytic activity.

To better understand the mechanism of resistance conferred by BTK drug resistance mutations, Montoya et al. assessed the activity of full-length recombinant BTK wild-type and eight recurrent drug resistant mutants. The enzymatic activity measurements identified two different groups of mutants: kinase proficient (T474I and C481S) and kinase impaired (M437R, V416L, L528W, C481F, C481Y, and C481R). Further experiments showed that these mutations that impair BTK kinase activity still allow downstream BCR signalling — such as PLC-γ2-dependent calcium flux and nuclear factor κB (NF-κB) activation — suggesting an alternative BCR signalling mechanism in BTK kinase-impaired mutant cells.

To further investigate this potential BTK alternative mechanism, the authors carried out immunoprecipitation (IP) of labelled BTK WT or L528W overexpressed in TMD8 cells — a human BTK-dependent diffuse large B cell lymphoma cell line — followed by mass spectrometry. They found several proteins that interacted differently with BTK L528W and WT BTK in the presence or absence of BCR stimulation. Of these, hematopoietic cell kinase (HCK) and integrin-linked protein kinase (ILK) were of special interest as they are upregulated in cancer and have an important role in tumour cell proliferation. Indeed, the authors showed in a cell competition assay that BTK L528W mutant cells depended on either HCK or ILK for cell proliferation and BCR signalling compared with BTK WT cells.

Next, to target these resistant mutants that do not rely on kinase activity, the authors explored targeted protein degradation. They surveyed several high-affinity BTK binders for their ability to degrade BTK when coupled to an imide-based cereblon (CRBN) ligand. This yielded several hits that were then further optimized through structure-based drug design and medicinal chemistry until a potent, selective, and orally bioavailable BTK degrader, NX-2127, was obtained. NX-2127 can bind both BTK and the CRBN E3 ubiquitin ligase complex, inducing polyubiquitination and proteasome-dependent degradation of BTK, IKZF1, and IKZF3, both of which are lymphocyte transcription factors implicated in B cell proliferation and activation.

In vitro, NX-2127 bound all recurrent drug-resistant forms of BTK whereas ibrutinib showed impaired binding to BTK mutants compared with wild-type BTK. In TMD8 cells expressing wild-type BTK or various drug-resistant BTK mutants, NX-2127 degraded all forms of BTK and prevented a calcium response indicative of BCR signalling.

On the basis of these encouraging results, the authors initiated a first-in-human phase I trial of NX-2127 in patients with relapsed/refractory B cell malignancies, including CLL, harbouring wild-type and mutant BTK (NCT04830137). At the time of data cutoff with 23 patients, treatment with NX-2127 resulted in BTK degradation in circulating B cells at a similar rate and degree in all patients regardless of their mutational status. For patients with BTK mutations, degradation of BTK was independent of mutation type and level of enzymatic activity.

These results suggest that BTK-targeting degraders may be able to overcome the numerous resistance mechanisms that arise on treatment with BTK inhibitors, and could be applicable to other kinase inhibitor-resistant tumours.

M. Teresa Villanueva

Original article: Montoya, S. et al. Kinase-impaired BTK mutations are susceptible to clinical-stage BTK and IKZF1/3 degrader NX-2127. *Science* **383**, eadi5798 (2024)

Related article: Békés, M., Langley, D.R. & Crews, C.M. PROTAC targeted protein degraders: the past is prologue. *Nat. Rev. Drug Discov.* **21**, 181–200 (2022)