

Cancer- and Immunobiology



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Targeting the PI3K/mTOR pathway in cancer, immunity and neurodegenerative disease

The phosphoinositide 3-kinase (PI3K) – mechanistic target of rapamycin (mTOR) axis is key to cancer progression, but also regulates inflammatory, allergic and metabolic events and plays a role in neurodegenerative disease. The four class I PI3Ks are the only PI3K family members to produce $\text{PtdIns}(3,4,5)\text{P}_3$, which acts a docking site for effector proteins with phosphoinositide-binding domains. While class IA PI3Ks associate with phosphorylated growth factor receptors and their substrates, the sole class IB, PI3K γ , is activated by G protein-coupled receptors (GPCR). The PI3K γ catalytic subunit p110 γ is bound to either a p84 or p101 adapter subunit. We have demonstrated with various approaches that the p84 and p101 subunits have non-redundant functions, and that the full activation of the p84-p110 γ complex requires activated, GTP-loaded Ras proteins. This makes mast cells, expressing exclusively p84, susceptible to drugs interfering with Ras membrane attachment, while macrophages with p84 and p101 maintain GPCR signaling [Fig. 1]. This suggests that targeting of p84-p110 γ complexes provide a cell-selective path to alleviate allergic responses, without impairing host defense mechanisms (Jin *et al.*, 2020).

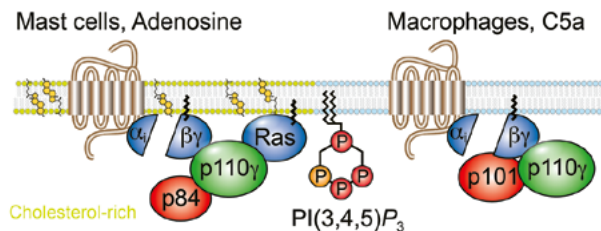


Fig. 1: Class IB PI3K γ is activated downstream of activated GPCRs by G $\beta\gamma$ subunits of trimeric G proteins. Its catalytic subunit p110 γ exists at the plasma membrane in two complexes, either bound to the adapter subunit p84 (also dubbed p87^{PIKAP}) or p101. The p84-containing complex operates in a cholesterol-rich submembrane compartment and requires activated Ras to produce phosphoinositide (3,4,5)-tris phosphate [PI(3,4,5)P $_3$]. Using isoprenylation inhibitors of Ras, we could demonstrate sensitivity in mast cell PI3K γ signaling operating via p84, and resistance on macrophages with both p101 and p84 (Jin *et al.*, 2020).

Due to numerous oncogenic inputs that activate PI3Ks in cancer, PI3Ks have been recognized as valuable drug targets. In spite of major efforts, only a handful of PI3K inhibitors (PI3Ki) have been approved up-to-date. Besides the investigation of mechanisms of PI3K isoform activation in cancer, inflammation and allergy, we have developed a number of drug-like molecules targeting the PI3K-mTOR pathway. Of these, the PQR309/bimiralisib pan-PI3Ki (Beaufils *et al.*, 2017) has been explored in phase II clinical trials in solid tumors and lymphoma. A consequence of targeting all class I PI3Ks are mechanism-based systemic feedback loops, such as drug-induced hyperglycemia and compensatory hyperinsulinemia. Especially the latter interferes with optimal drug action of PI3Kis, and might occur because of a simultaneous inhibition of PI3K α and PI3K β . Moreover, we have elucidated off-target effects of PI3K inhibitors that are chemically closely related to PQR309: while BKM120/buparlisib and PQR309 have similar potencies as PI3K inhibitors, BKM120 also interacts with tubulin. In consequence, the dominant BKM120-induced cell fate in 66-cell lines was mitotic arrest. A subsequent structural, chemical and biological analysis revealed how BKM120 binds to the colchicine-binding pocket of tubulin, an off-target interaction that is structurally prevented in PQR309. Exploration of PQR309/BKM120 target engagement also revealed a corrected orientation of BKM120 in PI3K [Fig. 2; (Bohnacker *et al.*, 2017)]. These

investigations spurred to the discovery of novel microtubule disrupting agents, and the development of dual PI3K/mTOR (PQR514, PQR530) and highly selective mTOR kinase inhibitors (mTORKi), such as PQR620 (Rageot *et al.*, 2018) and its metabolically stabler follow-up compound PQR626 (Borsari *et al.*, 2020).

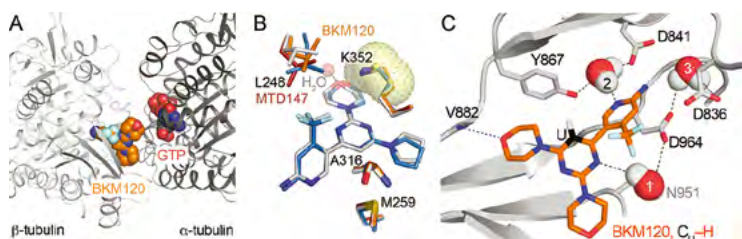


Fig. 2: BKM120 is a PI3K inhibitor that has entered >80 clinical trials. **A)** In (Bohnacker *et al.*, 2017) we showed that BKM120 interacts with microtubules and binds to the so-called colchicine-binding site on β -tubulin, explaining the subsequent depolymerization of microtubules **B)** A combination of chemical structure function relation (SAR) and X-Ray protein structure studies determined the exact rotational positioning of BKM120 and location of MTD147 in tubulin. **C)** The studies in **B)** triggered a review of a previously published BKM120/PI3K structure, which we identified as a low affinity binding mode. The correct orientation of BKM120 in PI3K shown here depicts important conserved water molecule interactions, which form an interactive water network required for efficient binding (Bohnacker *et al.*, 2017)

In contrast to pan-PI3Kis, PQR620 and PQR626 did not provoke hyperinsulinemia, and seem well tolerated. These compounds were therefore successfully used in animal models to attenuate lymphoma and ovarian cancer. As PQR620 and PQR626 are brain permeable, they were evaluated in other diseases emerging from an overactivated mTOR pathway (TORopathies). In patients with Tuberous sclerosis complex (TSC) mutated, benign hyperplastic lesions (hamartomas) are present in brain, kidney, heart, skin, lung, and liver. Morbidity is often dominated by neurological effects, including subependymal nodules (SENs >90 %), subependymal giant cell astrocytomas (SEGAs <20 %), mental impairment, and epilepsy (~90 %). PQR620 and PQR626 displayed an excellent efficiency in the attenuation of epileptic seizures in a TSC-induced (GFAP-Cre x *Tsc1^{flox/flox}*) mouse model with frequent spontaneous seizures [Fig. 3]. The elimination of seizures in the mouse TSC null model above, and an increased seizure threshold in a mouse model of chronic epilepsy (Brandt *et al.*, 2018) are a clear demonstration of the efficacy of mTORKi action in the central nervous system.

Selected Publications

- Borsari C, Keles E, Rageot D, Treyer A, Bohnacker T, Bissegger L, De Pascale M, Melone A, Sriramaratnam R, Beaufils F, Hamburger M, Hebeisen P, Löscher W, Fabbro D, Hillmann P and Wymann MP (2020). 4-(Difluoromethyl)-5-(4-((3R,5S)-3,5-dimethylmorpholino)-6-((R)-3-methylmorpholino)1,3,5-triazin-2-yl)pyridin-2-amine (PQR626), a Potent, Orally Available, and Brain-Penetrant mTOR Inhibitor for the Treatment of Neurological Disorders. *J Med Chem*, 63, 13595–13617.
- Jin JR, Gogvadze E, Xavier AR, Bohnacker T, Voelzmann J and Wymann MP (2020). PI3Ky Regulatory Protein p84 Determines Mast Cell Sensitivity to Ras Inhibition-Moving Towards Cell Specific PI3K Targeting. *Front Immunol* 11, 585070.
- Rageot D, Bohnacker T, Melone A, Langlois JB, Borsari C, Hillmann P, Sele AM, Beaufils F, Zvelebil M, Hebeisen P, Löscher W, Burke J, Fabbro D and Wymann MP (2018). Discovery and Preclinical Characterization of 5-[4,6-Bis((3-oxa-8-azabicyclo[3.2.1]oc-

tan-8-yl))-1,3,5-triazin-2-yl]-4-(difluoromethyl)pyridin-2-amine (PQR620), a Highly Potent and Selective mTORC1/2 Inhibitor for Cancer and Neurological Disorders. *J Med Chem* 61, 10084–10105.

Beaufils F, Cmiljanovic N, Cmiljanovic V, Bohnacker T, Melone A, Marone R, Jackson E, Zhang X, Sele A, Borsari C, Mestan J, Hebeisen P, Hillmann P, Giese B, Zvelebil M, Fabbro D, Williams RL, Rageot D and Wymann MP (2017). 5-(4,6-Dimorpholino-1,3,5-triazin-2-yl)-4-(trifluoromethyl)pyridin-2-amine (PQR309), a Potent, Brain-Penetrant, Orally Bioavailable, Pan-Class I PI3K/mTOR Inhibitor as Clinical Candidate in Oncology. *J Med Chem* 60, 7524–7538.

Bohnacker T, Protá AE, Beaufils F, Burke JE, Melone A, Inglis AJ, Rageot D, Sele A M, Cmiljanovic V, Cmiljanovic N, Bargsten K, Aher A, Akhmanova A, Díaz JF, Fabbro D, Zvelebil M, Williams RL, Steinmetz MO and Wymann MP (2017). Deconvolution of Buparlisib's mechanism of action defines specific PI3K and tubulin inhibitors for therapeutic intervention. *Nat Commun* 8, 14683.

Connection to Clinical Practice

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PI3K and mTOR inhibitors in clinical trials

The development of drugs targeting PI3K and mTOR kinase is an intense area in pharmaceutical industry. A spin-off company of the University of Basel, PIQUR Therapeutics, has completed phase I clinical trials with PQR309. PQR309 is a potent, brain penetrable pan-PI3K inhibitor with moderate action on mTOR kinase activity. Initial studies showed hyperglycemia, an adverse effect typical for this class of compounds, which could be attenuated by intermittent dosing schedules. Phase II studies have been carried out in lymphomas, solid tumors, and in a drug combination trial using PQR309 with eribulin (piqur.com; clinicaltrials.gov). Recently, a topical formulation of PQR309 has been developed to be tested in cutaneous T-Cell Lymphoma (CTCL) and plaque psoriasis (PPSO).

Funding by Swiss CTI and Innosuisse has promoted research at University of Basel and allowed the generation of a number of novel lead compounds targeting PI3K and mTOR with defined selectivity profiles. Besides cancer, other TORopathies were explored, including Tuberous sclerosis complex (TSC). Another collaboration with AstraZeneca (Gothenburg, Sweden) elucidated a novel class of PI3Ky inhibitors with a novel mode of action involving conformational changes of the PI3Ky catalytic subunit. These inhibitors are destined to unleash myeloid cells' anti-tumoral responses.

The currently established chemical space at UniBas provides further target specificities and opportunities, which are currently explored. Further questions aim to understand the biology of tumor drug resistance to PI3K/mTOR inhibitors, and include novel tools generated for chemical genetics.

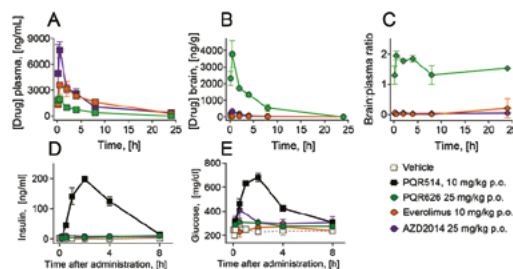


Fig. 3: **A)** PQR626 (Borsari *et al.*, 2020) displays a good pharmacology with a bit shorter half-life in rodents as compared to PQR620 (Rageot *et al.*, 2018). **B, C)** PQR626 shows a high brain:plasma distribution as compared to Everolimus and AZD2014, and is thus expected to cause less systemic side effects when targeting the CNS. **D, E)** Unlike a potent pan-PI3Ki (PQR514), mTOR inhibitors did not cause hyperinsulinemia or elevated plasma glucose levels.