

Copanlisib for treatment of B-cell malignancies: the development of a PI3K inhibitor with considerable differences to idelalisib

Günter Krause
Floyd Hassenrück
Michael Hallek

Department I of Internal Medicine,
University of Cologne, Center of
Integrated Oncology Köln Bonn,
Cologne Cluster of Excellence on
Cellular Stress Responses in Aging-
Associated Diseases (CECAD),
Cologne, Germany



Correspondence: Günter Krause
Department I of Internal Medicine,
University of Cologne, CECAD Research
Center, 26 Joseph Stelzmann Strasse,
Cologne 50931, Germany
Tel +49 221 478 84125
Fax +49 221 478 84115
Email guenter.krause@uk-koeln.de

Abstract: On the occasion of its recent approval for relapsed follicular lymphoma, we review the design and development of the pan-class I PI3K inhibitor copanlisib as a drug for the treatment of B-cell malignancies in comparison with other kinase inhibitors targeting B-cell-receptor signaling, in particular with strictly isoform- δ -selective idelalisib. In agreement with previously defined PI3K-inhibitor chemotypes, the 2,3-dihydroimidazo[1,2-*c*]quinazoline scaffold of copanlisib adopts a flat conformation in the adenine-binding pocket of the catalytic p110 subunit and further extends into a deeper-affinity pocket in contrast to idelalisib, the quinazoline moiety of which is accommodated in a newly created selectivity pocket. Copanlisib shows higher potency than other clinically developed PI3K inhibitors against all four class I isoforms, with approximately tenfold preference for p110 α and p110 δ . Owing to its potency and isoform profile, copanlisib exhibits cell-type-specific cytotoxicity against primary chronic lymphocytic leukemia cells and diffuse large B-cell lymphoma (DLBCL) cell lines at nanomolar concentrations. Moreover, copanlisib differs from idelalisib in regard to intravenous versus oral administration and weekly versus twice-daily dosing. In regard to adverse effects, intermittent intravenous treatment with copanlisib leads to fewer gastrointestinal toxicities compared with continuous oral dosing of idelalisib. In relapsed follicular lymphoma, copanlisib appears more effective and especially better tolerated than other targeted therapies. Copanlisib extends existing treatment options for this subtype of indolent non-Hodgkin lymphoma and also shows promising response rates in DLBCL, especially of the activated B-cell type.

Keywords: targeted therapy, B-cell receptor signaling, p110 isoforms, non-Hodgkin lymphoma, leukemia

Introduction

Typically for B cell malignancies as a whole, pharmacotherapy for chronic lymphocytic leukemia (CLL) started from alkylating agents and purine analogs, to evolve into the continuing era of the incorporation of monoclonal antibodies into immunochemotherapeutic regimens, until small-molecule-targeted therapies emerged in the last decade.¹ These drugs are designed specifically to inhibit signaling pathways that are crucial for the survival and interaction of neoplastic cells with the microenvironment, eg, by targeting kinases involved in B-cell receptor (BCR) signaling, such as PI3K, BTK, or antiapoptotic mitochondrial Bcl2 proteins. These molecules are targeted by idelalisib, ibrutinib, or venetoclax, which already have been approved for the treatment of CLL in North America and Europe.

PI3Ks produce the second messenger PIP₃ and determine pivotal cell functions like proliferation, survival, and metabolism, but also oncogenesis.² Expression of the PI3K

p110 δ isoform is restricted to cells of hematopoietic origin, where it is intricately involved in BCR signaling regulating cell proliferation and survival. Therefore, in malignant B cells, the PI3K pathway is constitutively activated and dependent on PI3K δ .³ Consequently the PI3K δ inhibitor idelalisib selectively induces apoptosis of CLL cells while sparing normal T or natural killer cells.⁴ Idelalisib inhibits the migration of CLL cells to CXCL12 and the secretion of chemokines by activated CLL cells.⁵ At the beginning of idelalisib treatment, transient redistribution lymphocytosis is frequently observed, as with other inhibitors of BCR signaling.^{6,7} This can be interpreted as egress of malignant B lymphocytes from protective compartments to the peripheral blood in line with these drugs exerting modest direct cytotoxicity against CLL cells while displaying predominant effects on the microenvironmental dialogue.

Here, the design and development of the pan-class I PI3K inhibitor copanlisib as a drug for the treatment of B cell malignancies are reviewed in comparison with other kinase inhibitors targeting BCR signaling, in particular idelalisib. While preclinical data are available for B-cell acute lymphoblastic leukemia (B-ALL), mantle-cell lymphoma (MCL), CLL, diffuse large B-cell lymphoma (DLBCL), and multiple myeloma (MM), copanlisib has gained approval for relapsed follicular lymphoma (FL) with ongoing clinical trials for several subtypes of indolent and aggressive B-cell non-Hodgkin lymphoma (B-NHL).

Molecular pharmacology

Design and structure–activity relationship

In an effort to develop PI3K inhibitors with potential as antiasthmatic agents, Bayer identified by high-throughput screening

the 2,3-dihydroimidazo[1,2-*c*]quinazoline scaffold as a lead that was further developed finally to yield copanlisib.⁸ This scaffold is also the basis of PIK90, which represents one of eleven chemotypes in a pharmacological map of PI3K inhibitors docked to p110 γ .⁹ Lead optimization and docking to the p110 γ structure established important features of the structure–activity relationship (Figure 1). For instance, the imidazoline N1 was postulated to bind to the hinge region of the p110 γ -binding pocket, since its substitution or oxidation diminishes inhibitor potency. Moreover, comparison of various substituents at C5 and C7 indicates that the C5 amide fits into the affinity pocket and C7 points to the sugar pocket. Similarly, C8 was found to face toward the solvent at the entrance of the binding pocket, which offered the opportunity of tuning physical, chemical, and pharmacokinetic properties of the molecule.

Inhibitor binding to pI 10, the catalytic subunit of PI3K

In docking studies on the basis of PI3K inhibitors and the X-ray structure of PI3K γ available at the time, clearly distinct binding modes were found for PI3K-inhibitor chemotypes classified as multitargeted versus isoform-selective.⁹ Representatives of these classes are PIK90 and PIK39, which were later developed into the drugs copanlisib and idelalisib, respectively. While multitargeted PI3K inhibitors adopt a flat conformation in the adenine-binding pocket of the catalytic p110 subunit and extend further into a deeper-affinity pocket, isoform-selective PI3K inhibitors extend in different dimensions, eg, as the propeller-shaped molecules PIK39 and idelalisib. For their binding to the catalytic PI3K subunit, these inhibitors exploit a conformationally mobile region

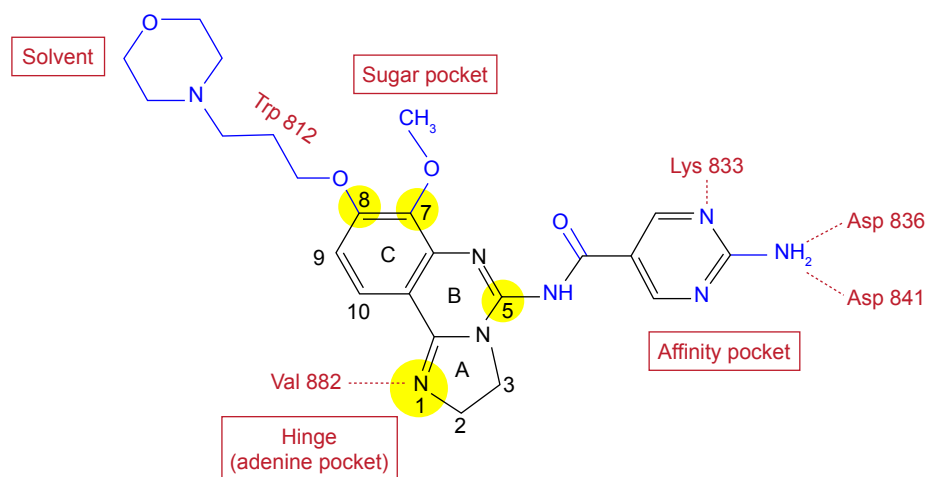


Figure 1 Molecular structure of copanlisib.

Notes: Elements of the initial lead are shown in black and subsequent modifications in blue. The structure–activity relationship of the highlighted positions of the 2,3-dihydroimidazo[1,2-*c*]quinazoline scaffold was explored extensively by numerous substitutions, as outlined in the “Design and structure–activity relationship” section. Red print indicates potential binding regions in the p110 γ structure, as well as contact residues.

including Met752, which leads to an induced fit of one of the three rotor blades of the inhibitor molecules, namely the quinazoline moiety, into a newly created selectivity pocket. Consequently, mutation of this methionine to isoleucine (M752I) affects binding of isoform-selective but not multitargeted PI3K inhibitors to p110 γ and leads to resistance to PIK39, while sensitivity to PIK90 is maintained.⁹

These proposed binding modes were confirmed by the X-ray structures of cocrystals of p110 γ and p110 δ with copanlisib and idelalisib, respectively.^{8,10} The corresponding structural information was deposited as entries 5G2N and 4XE0 in the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The structures are available for interactive viewing on the RCSB PDB website (www.rcsb.org) by means of the integrated NGL viewer.^{11,12} In the view of the binding pocket of p110 γ accommodating copanlisib (Figure 2A), the inhibitor molecule is shown in a similar orientation as in Figure 1 to facilitate comparison with the structure–activity relationship of copanlisib. Moreover, the views of copanlisib and idelalisib bound to the binding pockets of p110 γ and p110 δ , respectively, are aligned according to the methionine residues M804 or M752 and tryptophan residues W812 or W760 of p110 γ or p110 δ , respectively, to show the induced fit of idelalisib (Figure 2).

Isotype selectivity of some PI3K inhibitors in clinical development

The isotype selectivity of PI3K inhibitors was assessed by determination of the inhibitor concentrations that lead to half-maximal inhibition of PI3K activity. For this purpose,

activity assays were performed on purified recombinant PI3K isoforms in the presence of different inhibitor concentrations, mainly with detection of the reaction product PIP₃. This affords the comparison of clinically used and developed PI3K inhibitors by virtue of IC₅₀ values for individual p110 isoforms (Figure 3).^{13–20} Further PI3K inhibitors in clinical development for treatment of B-NHL, namely umbralisib (TGR1202), ACP319 (previously named AMG319), and acalisib are p110 δ -selective, but show approximately tenfold-lower potency against p110 δ activity than idelalisib.^{21–23}

Differences in the binding modes of PI3K-inhibitor chemotypes were correlated with their isoform selectivity.⁹ Copanlisib and idelalisib had been developed from PIK90 and PIK39 scaffolds respectively, which represent multitargeted and isotype-selective chemotypes of PI3K inhibitors. To qualify as isoform-selective in activity profiling, PI3K inhibitors must exhibit an approximately 30-fold difference in potency between isoforms. This threshold is reached by the isoform-selective PI3K inhibitors idelalisib, duvelisib and alpelisib, but not by the multitargeted PI3K inhibitors buparlisib, pictilisib, AZD8835, or copanlisib (Figure 3). Duvelisib has a very similar molecular structure to idelalisib, but compared to idelalisib, potency against p110 γ or p110 α is shifted slightly up or down, respectively, putting emphasis on the selectivity against p110 γ . The same high level of selectivity that idelalisib has for p110 δ recently has also been achieved for the p110 α isoform with the design of alpelisib.¹⁵ Of note, the high potency of idelalisib and duvelisib against p110 δ is surpassed only by copanlisib in the present selection and particularly among multitargeted inhibitors.

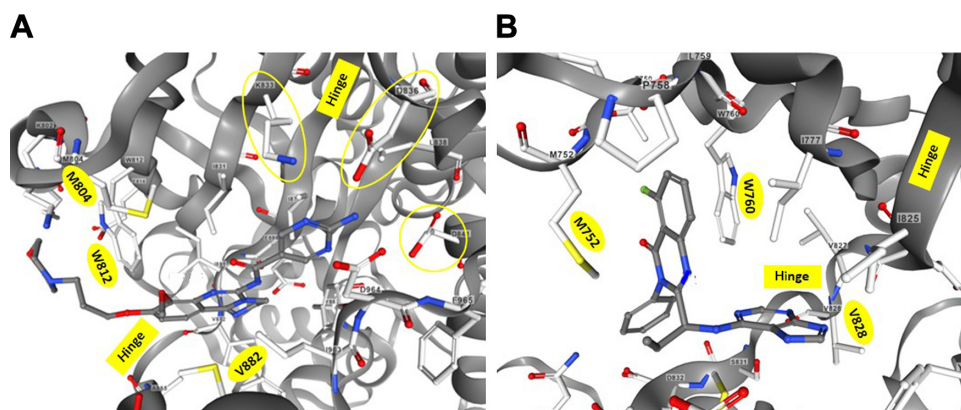


Figure 2 Inhibitor binding to p110 isoform-binding pockets.

Notes: Views of the ATP-binding clefts of p110 γ or p110 δ cocrystallized with copanlisib⁸ (A) or idelalisib¹⁰ (B) were captured from the RCSB PDB entries 5G2N and 4XE0, respectively, visualized on www.rcsb.org with the NGL viewer.^{11,12} The binding pockets opening toward the left were aligned according to the highlighted methionine residues M804 or M752 and tryptophan residues W812 or W760 of p110 γ or p110 δ , respectively, that form an induced cleft accommodating the quinazoline moiety of idelalisib (B). Instead, the flat copanlisib molecule extends into the deeper affinity pocket of p110 γ , making contact with the circled side chains of K833, D836, and D841 (A). Both inhibitors contact the highlighted valine residues of the hinge regions V882 (A) or V828 (B). The hinge regions connect the N-terminal (top) and C-terminal (bottom) lobes of p110 and roughly delimit the ATP-binding and affinity pockets in the views depicted.

Abbreviation: RCSB PDB, Research Collaboratory for Structural Bioinformatics Protein Data Bank.

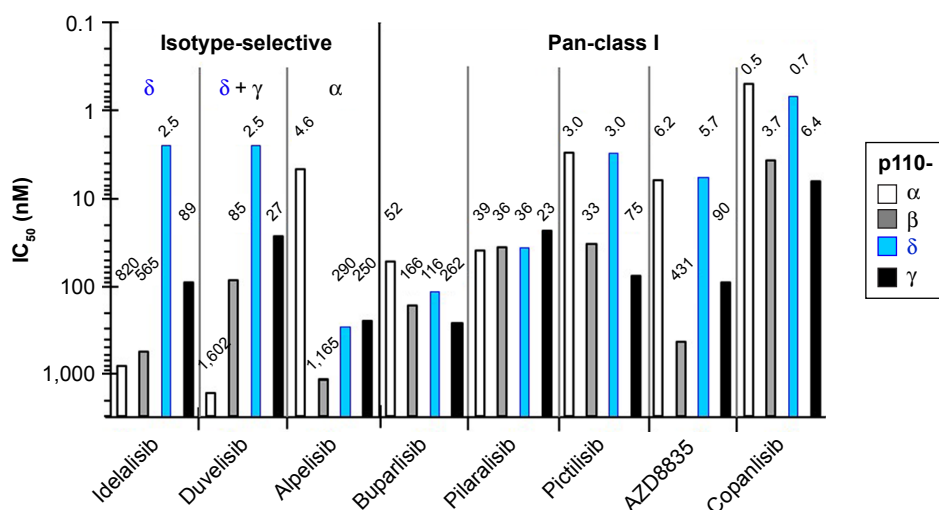


Figure 3 Isoform-selectivity profiles of PI3K inhibitors in clinical use or development.

Notes: Inhibitor potency against purified p110 catalytic subunits is represented by IC_{50} values determined in activity assays. Isoform-selectivity profiles are shown for the isoform-selective inhibitors idelalisib,¹³ duvelisib,¹⁴ and alpelisib,¹⁵ which show at least 50-fold higher potency for a certain isoform, and the pan-class I inhibitors buparlisib,¹⁶ pilaralisib,¹⁷ pictilisib,¹⁸ AZD8835,¹⁹ and copanlisib.²⁰ Figures above the columns indicate the exact individual IC_{50} (nM) values.

Abbreviation: IC_{50} , half maximal inhibitory concentration.

Copanlisib belongs to the group of multitargeted pan-class I PI3K inhibitors without relevant potency against other classes of PI3K or mTOR, for which it shows an IC_{50} of 45 nM,²⁰ and shows higher potency than other clinically developed PI3K inhibitors against all four class I isoforms, with approximately tenfold preference for p110 α and p110 δ . The selectivity of AZD8835 for p110 α and p110 δ compared to the other class I isoforms is approximately 15-fold, and thus exceeds that of other multitargeted PI3K inhibitors that predominantly target p110 α and p110 δ , namely pictilisib and copanlisib. In spite of its predominant selectivity for p110 α and p110 δ , copanlisib is the most potent inhibitor of p110 γ in the present selection and in particular a more potent p110 γ inhibitor than duvelisib. The potency of copanlisib against p110 γ also surpasses that of voxalisib, which is a similar molecule to pilaralisib, with enhanced activity against p110 γ and especially mTOR.²⁴ According to their biochemical pharmacology, copanlisib and idelalisib can be regarded as PI3K inhibitors with flat versus propeller-shaped structure, different binding modes to the active site of the catalytic p110 subunit, and comparatively higher potency versus more stringent isoform selectivity.

Impact of copanlisib on cell functions

At the next level of investigation, one needs to ask how the biochemical pharmacology of PI3K inhibitors influences the action of these substances on malignant B cells and their microenvironment. In this regard, the consequences of pan-class I inhibition with preferential targeting of p110 α and p110 δ will be compared to that of purely p110 δ selective

idelalisib. Its pan-class I selectivity profile led to copanlisib being considered also for treatment of solid tumors.^{20,25–28} Apart from cytotoxicity against tumor cells, preclinical investigation of copanlisib must also comprise that against cells of the tumor microenvironment and of the immune system. Of note, even other completely unrelated mechanisms may be involved in the cytotoxicity of PI3K inhibitors, as recently shown for buparlisib, which apart from action as an ATP-competitive PI3K inhibitor, also interferes with microtubule polymerization.²⁹ Before we specifically address the relationship of the biochemical pharmacology of copanlisib, especially its PI3K isoform-selectivity profile, with preferentially targeted B-NHL types, we discuss two issues on a more general note, namely the role of lipid kinases in the signal transduction occurring in malignant B cells and the biology of B-cell malignancies.

PI3K in BCR signaling

Class I phosphatidylinositol-3-kinases catalyze the formation of the lipid second messenger PIP_3 , which is capable of binding to the pleckstrin-homology domain of important signaling mediators, eg, Akt and BTK.² The predominantly expressed and functionally relevant catalytic PI3K subunit, namely p110 δ ,³⁰ cooperates with other kinases of BCR signaling (Figure 4). Activation of PI3K δ in mature B cells occurs chiefly via binding of the Src homology 2 (SH2) domain of the regulatory p85 subunit to a phosphotyrosine motive of the receptor CD19. Since CD19 lacks intrinsic or associated kinase activity, the required tyrosine phosphorylation is carried out by the Src family kinase Lyn, which is associated with the BCR. In addition, rather than exclusively by receptor

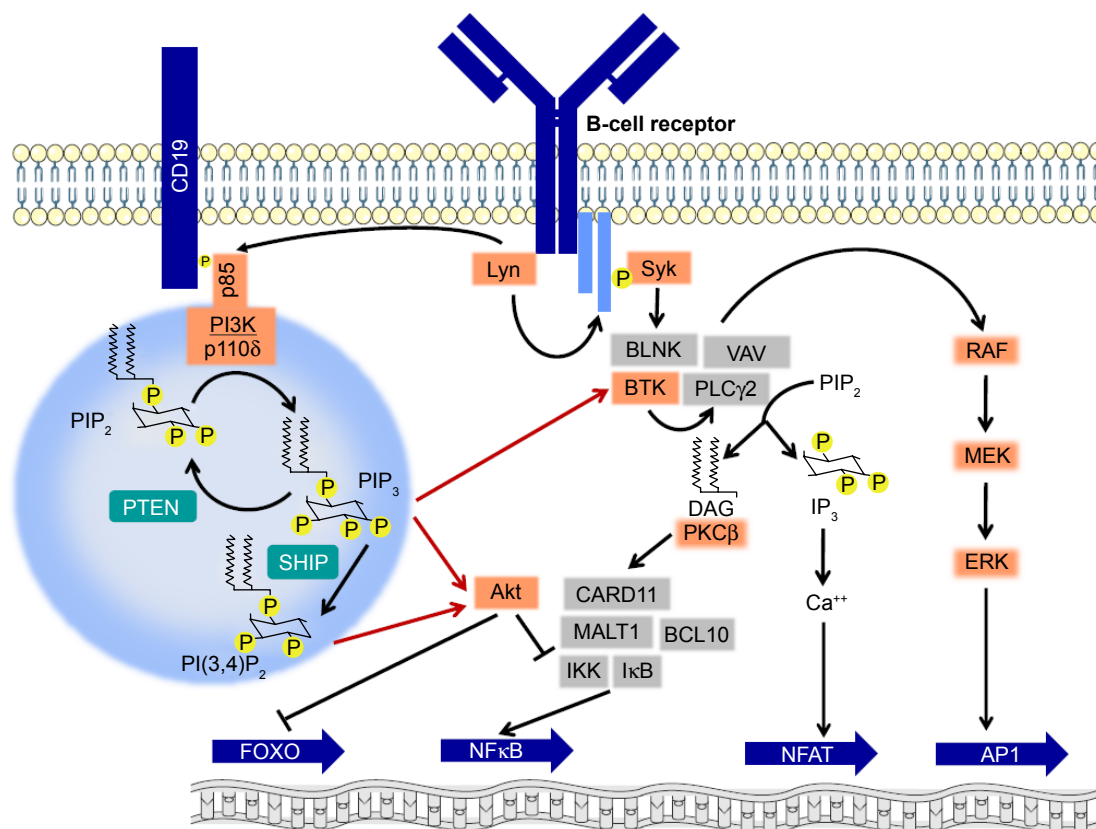


Figure 4 Involvement of PI3K δ and lipid second messengers in B-cell-receptor signaling.

Notes: Activation of PI3K δ upon stimulation of the B-cell receptor occurs via recruitment of p85 to a phosphotyrosine-docking site created by the Src-family kinase Lyn. PI3K activity leads to production of lipid second messengers and is counteracted by the phosphatases PTEN and SHIP. Red arrows indicate the activation of the kinases Akt and BTK by binding of lipid second messengers to their pleckstrin-homology domains that enables their localization at the plasma membrane. Further signaling events lead to regulation of transcription-factor activity.

Abbreviation: DAG, diacylglycerol.

tyrosine kinases, PI3K δ in B cells is commonly activated by chemokine receptors that belong to the group of G-protein-coupled receptors, which in other cell types usually activate the PI3K γ isoform. While both Akt and BTK may be recruited to the cytoplasmic membrane via their pleckstrin-homology domains and PIP₃, this may also alternatively occur by additional routes, eg, in the case of Akt via the lipid second messenger PI(4,5)P₂, which results from dephosphorylation of PIP₃ by SHIP or via the SH2 domain of BTK. PI3K inhibition, eg, by copanlisib, in contrast to inhibition of AKT alone, also blocks the ensuing RAF–MEK–ERK cascade,²⁵ which is also affected by inhibitors of BTK. A major effect of Akt activity is the phosphorylation and inactivation of the transcription factor FOXO. Other consequences of Akt activity include phosphorylation of the inhibitory IKK complex, which leads to activation of NF κ B, and of the proapoptotic Bcl2 protein BAD, which triggers its own degradation.

BCR signaling is now accepted as a common driving force of B-cell malignancies, and inhibitors have become available for kinases at several interception points in these

pathways, with those targeting p110 δ or the tyrosine kinases BTK and Syk already in or close to clinical use.³¹ While p110 δ appears indispensable for the BCR pathway stimulated by antigen, p110 α may play a role in tonic BCR signaling and inhibitor resistance. Similarly to other inhibitors of the BCR pathway, idelalisib exhibits only modest direct cytotoxicity, so that disturbance of interactions with the microenvironment, specifically inhibition of chemotaxis to CXCL12 and secretion of the chemokines CCL3 and CCL4, may offer an explanation for its clinical activity.⁵ Exactly these important functions of neoplastic B cells also depend on two other kinases involved in BCR signaling, namely BTK and Lyn, since these cell functions are rescued from inhibition by the tyrosine-kinase inhibitors ibrutinib or dasatinib, owing to expression of inhibitor-resistant kinase mutants in cell-line models.³² Interrogation of the role of the same kinases in the pathogenesis of CLL using the E μ -TCL1 mouse model revealed that loss of Lyn in leukemic cells reduces BCR signaling, including BTK phosphorylation, but does not affect leukemic cell expansion, in contrast to transplantation of

syngeneic CLL cells into Lyn- or BTK-deficient recipients, which results in strongly delayed leukemic progression and prolonged survival.³³ Therefore, the microenvironment plays a predominant role in the pathogenesis of CLL compared to neoplastic B cells, which implies that clinically effective inhibitors, such as ibrutinib and idelalisib, do not chiefly target BCR signaling in the literal sense, but different (as yet incompletely defined) signaling pathways and cell functions in accessory cells. Small-molecule inhibitors targeting the BCR pathway, especially during prolonged treatment, have the potential to promote the development of secondary lymphoma, since disruption of BCR signaling in normal and neoplastic B cells, especially by inhibition of PI3K δ , enhances the expression of AID and increases translocation frequency and genomic instability.³⁴ Whether a different balance of PI3K-isoform inhibition, as could be achieved for instance with copanlisib, could change this effect is as yet unknown.

Different types of B-cell malignancies

The definition of B-NHL by the World Health Organization comprises B-cell malignancies irrespective of their appearance as solid tumors or leukemias. Accordingly, these diseases can be arranged according to the development stage of their presumed cells of origin (Table 1).³⁵ In the course of B-cell development, pre-B cells from the bone marrow migrate to the lymph nodes and specifically germinal centers, where they experience antigen contact, V(D)J recombination and finally isotype switch before long-lived plasma cells return to the bone marrow. As reference for the preclinical and clinical data reported in this review, molecular features of B-NHL³⁶ and their incidence³⁷ are indicated. Although the malignancies listed³⁸⁻⁴⁷ are often heterogeneous within the subgroups, one can distinguish more indolent, eg, CLL, marginal-zone lymphoma, FL, and more aggressive malignancies, eg, B-ALL, MCL, Burkitt lymphoma, and DLBCL. CLL patients can be divided into prognostic subgroups with unmutated versus mutated *IGHV* genes with more aggressive and indolent course.⁴⁸ Similarly, gene-expression profiling distinguishes DLBCL of the germinal center or activated B-cell (ABC) subtypes.⁴⁹

Cytotoxicity of copanlisib against B-NHL subtypes

Given the different PI3K-isoform profiles of copanlisib and idelalisib, the sensitivity of specific B-NHL subtypes to these inhibitors could depend on the expression and functional involvement of p110 α in these entities. Genome analysis of

Table 1 Subtypes of B-NHL in the order of B-cell development

B-cell non-Hodgkin lymphoma (B-NHL) subtype	Subgroup	Reference	Cell of origin		Molecular features		Incidence	
			B-cell type	Localization	Translocation	Oncogene	Per 10 ⁵ person-years ³⁷	% of B-NHL
B-cell acute lymphoblastoid leukemia (B-ALL)		38	Immature	BM	t(9;22)	BCR-ABL	0.8	2.9
Mantle-cell lymphoma (MCL)		39	Mature	Periphery				
Chronic lymphocytic leukemia (CLL)	UM		Naive	LN or S	t(11;14)	Cyclin D1	0.5	2.0
	M		Follicular	LN or S		ZAP70	5.2	19.8
Marginal-zone lymphoma (MZL)		40	Memory	LN or S			1.0	3.7
Follicular lymphoma (FL)		41, 42	Follicular	GC	t(14;18)	BCL2	3.2	12.2
Burkitt lymphoma (BL)		43	Centrocyte	GC	t(8;14)	MYC	0.3	1.1
Diffuse large B-cell lymphoma (DLBCL)	GC	44, 45	Centroblast	GC	PTEN loss		7.1	27.3
	ABC		Plasmablast	GC	Mutant CD79, CARD11, MYD88		0.4	1.3
Waldenström macroglobulinemia (WM)		46	B cells to plasma cells	BM	MYD88 L265P		5.3	20.2
Multiple myeloma (MM)		47	Plasma cell	BM				

Notes: B-NHL subtypes listed according to development stages of presumed cells of origin; references to recent reviews of molecular features and incidence. **Abbreviations:** ABC, activated B cell; BM, bone marrow; GC, germinal center; LN, lymph node; M, mutated *IGHV* genes; MZ, marginal zone; S, spleen; UM, unmutated *IGHV* genes.

patient samples indicates amplification of PI3KCA in MCL⁵⁰ and CLL.⁵¹ In MCL, p110 δ inhibition by idelalisib blocks BCR-stimulated, but not constitutive PI3K activation, and pictilisib, which predominantly targets p110 α and p110 δ , shows significantly more cytotoxicity against MCL cell lines and primary samples than idelalisib.⁵² Accordingly, a panel of MCL cell lines exhibits high sensitivity to copanlisib.⁵³

Relatively easy access to B lymphocytes from the peripheral blood of CLL patients enables the assessment of PI3K inhibitors with different isoform selectivity on such samples. Multitargeted PI3K inhibitors, including PIK90, from the scaffold of which copanlisib was derived, show stronger inhibition of the phosphorylation of Akt and apoptosis induction in CLL cells than p110 δ -selective inhibitors.⁵⁴ From analysis of the survival of CLL cells treated with different isoform-selective PI3K inhibitors, one can conclude that in contrast to normal B cells, where p110 δ is mostly involved in signaling through the IL4 receptor and the BCR,⁵⁵ the most important PI3K isoform in the survival of CLL cells is p110 α , followed by p110 δ , whereas the viability of CLL cells is hardly affected by inhibition of p110 γ .⁵⁶ Copanlisib exhibits cytotoxicity selectively against CLL cells compared to normal B and T cells and the concentrations leading to half-maximal reduction of CLL-cell survival are more than tenfold lower for copanlisib than for idelalisib or duvelisib.⁵⁷ In addition, treatment with copanlisib also overcomes the survival advantage that CLL cells gain from stroma-cell support.

Immunohistochemistry shows higher PI3K α expression in DLBCL than in FL samples.⁵⁸ ABC-DLBCL cell lines are sensitive to 0.5 μ M idelalisib only if p110 α is knocked down by shRNA, and their proliferation and survival are inhibited by AZD8835 that predominantly targets p110 δ and p110 α .⁵⁹ Similarly, simultaneous inhibition of p110 δ and p110 α by copanlisib or a combination of idelalisib and alpelisib leads to significantly enhanced inhibition of the growth of ibrutinib-sensitive and -resistant ABC-DLBCL cell lines.⁵⁸ Since activating *CARD11* mutations seem to cause resistance against PI3K inhibition, a thorough molecular characterization is necessary for effective use of targeted therapeutics in DLBCL.⁵⁹

In agreement with isoform-expression profiles of MM samples, which show higher levels of p110 α and p110 β than normal samples, with further increases during disease progression, the survival of primary MM cells and MM cell lines is inhibited by p110 α -selective alpelisib more strongly than that of normal peripheral blood mononuclear cells.⁶⁰ Therefore, potency against p110 α may contribute to the

inhibition of constitutive and IGF1-induced Akt phosphorylation in MM cell lines by copanlisib concentrations of 100 nM or lower.⁶¹ Copanlisib inhibits cell-cycle progression and survival of primary MM cells and cell lines and the growth of subcutaneous xenografts in nude mice.

Interactions with the microenvironment

The clinical efficacy of idelalisib cannot be explained by its direct cytotoxicity against CLL cells, but by the disturbance of CLL-microenvironment interactions, such as redistribution of neoplastic B cells and production of cytokines.⁵ In the survival of CLL cells, p110 isoforms appear to be involved to different degrees, namely p110 α more than p110 δ and especially p110 γ ,⁵⁶ and pharmacological inhibition of the chemotaxis of CLL cells to CXCL12, eg, by PIK90, can also be connected with targeting p110 α .⁵⁴ In a head-to-head comparison with 1 μ M each of the clinically developed inhibitors copanlisib and duvelisib, which target p110 γ in addition to p110 δ , inhibited the chemotaxis of CLL cells to CXCL12 significantly more efficiently than idelalisib, which implies involvement of p110 γ in this process.⁵⁷ This role of p110 γ in the chemotaxis of CLL cells was substantiated by up- and downregulation of p110 γ expression and use of an exclusively p110 γ -selective inhibitor.⁶² In addition to showing increased effects on chemotaxis compared to idelalisib, the p110 δ and γ -selective PI3K inhibitor duvelisib also impacts on the BCR-mediated secretion of the chemokines CCL3 and CCL4.⁶³ According to its isoform profile and high potency, copanlisib may also be expected to affect this microenvironment interaction to a high extent. Apart from their impact on neoplastic B cells, it will be essential to understand how PI3K inhibitors influence the function of myeloid cells, T cells, and natural killer cells. For instance, selective inactivation of macrophage PI3K γ stimulates and prolongs NF κ B activation and inhibits C/EBP β activation, thus promoting an immunostimulatory transcriptional program that restores CD8⁺ T-cell activation and cytotoxicity.⁶⁴

Copanlisib in drug combinations against B-NHL

BCR-ABL-positive leukemias can be efficiently controlled by inhibitors of the tyrosine kinase Abl, unless acquired resistance arises, eg, via the T315I mutation in *ABL*. In cell lines expressing wild-type and mutant *BCR-ABL*, the cytotoxic effects of Abl inhibitors are additively enhanced by copanlisib. Remarkably, treatment with a combination of ponatinib and copanlisib abolishes tumor growth in mice after irradiation and subcutaneous or intravenous transplantation

with allografts consisting of BaF3 cells expressing *BCR-ABL* carrying the T315I-resistance mutation.⁶⁵

The occurrence of the t(9;22) translocation with concomitant deregulation of cyclin-dependent kinases in >90% of MCL cases prompted the assessment of the CDK4 inhibitor palbociclib in combination with PI3K inhibitors in the context of acquired ibrutinib resistance.⁶⁶ The prolonged early G₁ arrest induced by palbociclib sensitizes DT40 chicken-lymphoid cells to cell killing by ibrutinib if they express wild-type *BTK*, and to PI3K inhibitors, even in the presence of the *BTK*^{C481S} mutant. Owing to the role of p110 α in MCL cells,⁵² it is reasonable to assume that copanlisib can be more cytotoxic for them than idelalisib, particularly in combinations with CDK inhibitors and for overcoming ibrutinib resistance.

The irreversible BTK inhibitor ibrutinib is clinically used for treatment of CLL and MCL and acquired resistance owing to the *BTK*^{C481S} mutation is observed in both entities. By targeting a different interception point in BCR signaling, namely PI3K, duvelisib is capable of overcoming this ibrutinib resistance.⁶⁷ As a PI3K inhibitor exhibiting greater cytotoxicity against CLL cells than duvelisib,⁵⁷ copanlisib can be expected to function in a similar manner. Also in ABC-DLBCL cell lines, combinations of PI3K and BTK inhibitors lead to synergistic enhancement of cytotoxic effects and overcome ibrutinib resistance.⁶⁸

Owing to independent mechanisms of action, monoclonal antibodies are considered promising combination partners of kinase inhibitors; however, with the risk that kinase inhibition interferes with killing mechanisms of antibodies, eg, with antibody-dependent cell-mediated cytotoxicity (ADCC). As such, the irreversible BTK inhibitor ibrutinib antagonizes the ADCC of rituximab,⁶⁹ while in the presence of the PI3K δ inhibitor idelalisib, the ADCC of alemtuzumab is maintained.⁴ Survival inhibition of CLL cells by copanlisib and idelalisib is enhanced by the therapeutic anti-CD20 antibodies rituximab and obinutuzumab, while ADCC by alemtuzumab and peripheral blood mononuclear cells is not substantially impaired by idelalisib or copanlisib.⁵⁷ In a more sensitive and refined analysis of ADCC using genetically modified NK92 cells expressing CD16-176V as effector cells, copanlisib led to a considerably stronger impairment of the ADCC of anti-CD20 antibodies than idelalisib or duvelisib at the same clinically unachievable concentration of 10 μ M, but significant ADCC of obinutuzumab against Raji cells was maintained in the presence of 1 μ M copanlisib.⁷⁰ In CLL cells, PI3K inhibitors enhance the direct cytotoxicity of anti-CD37 antibodies, eg, otlertuzumab, owing to opposing

signaling by N- and C-terminal tyrosine residues of CD37.⁷¹ On primary CLL cells, the combination of the anti-CD37 antibody BI836826 with copanlisib is particularly efficacious, which can be attributed to high single agent anti-apoptotic activity of both drugs.⁷²

Emerging clinical evidence

Preclinical investigation of copanlisib in vitro was followed by in vivo studies in rat- and mouse-xenograft models.^{20,25,61} Despite an extremely short half-life in mice of only 0.7 hours, pulsatile administration of copanlisib led to much better efficacy against subcutaneous xenografts of the breast cancer cell line BT-474 than continuous treatment.²⁵ Based on preclinical evidence including xenograft models,²⁰ copanlisib was investigated in a first-in-human dose-expansion trial with patients suffering from solid and hematological tumors.⁷³ In this trial, an intermittent dosing schedule of 60 mg copanlisib on days 1, 8, and 15 of a 4-week cycle was established. In contrast, 150 mg idelalisib was administered twice daily,⁶ yielding a ≥ 35 -fold higher drug load than with copanlisib.

Adverse effects

The most common toxicities of idelalisib include elevation of liver transaminases, diarrhea, and neutropenia. These can be divided into inflammatory toxicities of presumed autoimmune origin, as well as bacterial and opportunistic infections that are possibly caused by impaired T-cell function. Concerns have emerged about the risks associated with oral PI3K δ -targeting agents, such as infections, pneumonitis, and colitis.^{74,75} Unexpected aggravation of such adverse effects in combined applications of idelalisib with rituximab and lenalidomide or with the Syk inhibitor entospletinib led to premature termination of the trials of these combinations.^{76,77} In addition safety concerns in prematurely discontinued Phase III trials of combinations containing idelalisib and rituximab with or without bendamustine were attributed to opportunistic infections, notably with *Pneumocystis jirovecii* and *Cytomegalovirus*.⁷⁸ This raises the possibility that serious adverse effects of isoform- δ -selective PI3K inhibition may be avoided by inhibitors with broader isoform selectivity or different dosing. Since data from Phase II trials with similar numbers of participating patients suffering from relapsed indolent B-NHL were available, the adverse effects of copanlisib⁷⁹ or idelalisib⁸⁰ reported in these trials were compared (Figure 5). In this comparison, hyperglycemia, an expected on-target effect of PI3K α inhibition, and hypertension were observed exclusively with copanlisib, while idelalisib led to higher incidence of gastrointestinal symptoms

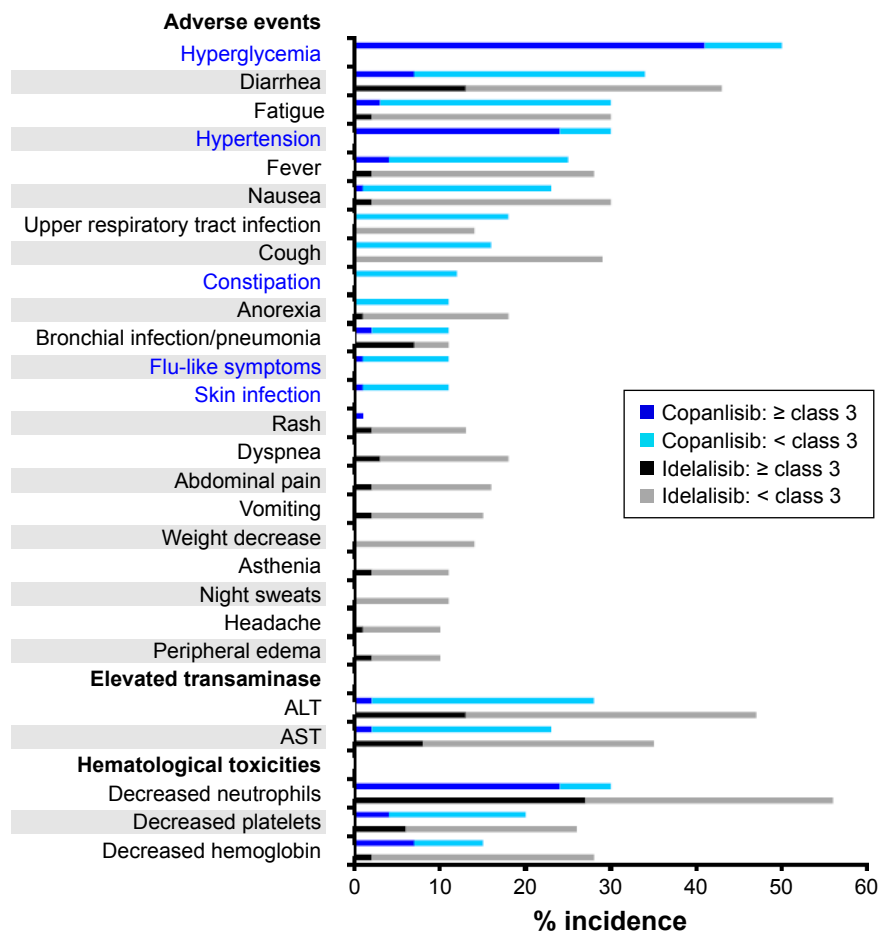


Figure 5 Different adverse effects of copanlisib and idelalisib.

Notes: The incidence of the adverse effects named among participants in trials assessing copanlisib⁷⁷ or idelalisib⁷⁸ was compared. With 141 and 125 study participants, respectively, the trials were of comparable size, and both Phase II studies comprised patients with relapsed indolent NHL. All adverse-effect categories affecting >10% of participants are listed, with those occurring exclusively with copanlisib printed in blue.

and hematological toxicities. Transient hyperglycemia is a recognized on-target effect of PI3K α inhibition that is related to insulin-receptor signaling^{9,81} and occurred also in trials of orally administered multitargeted pan class I PI3K inhibitors, namely buparlisib,⁸² pictilisib,⁸³ and pilaralisib.⁸⁴

Efficacy against different B-NHL subtypes

Although p110 α is expressed to a lesser extent than p110 δ in most subtypes of B-NHL, copanlisib might offer greater efficacy than idelalisib in situations where alternative isoforms are upregulated in response to selective PI3K δ inhibition as a resistance mechanism. The selection of B-NHL entities (Table 1) for potential clinical use of copanlisib may be guided by the molecular properties of the inhibitor and by preclinical studies, but is mainly influenced by insights with regard to adverse effects and clinical efficacy gained from pilot trials, which sometimes comprise several entities. Also for the assessment of copanlisib, a trial was conducted with

participants suffering from different subtypes of relapsed/refractory indolent or aggressive lymphomas,⁸⁵ which was continued as the CHRONOS-1 trial focusing on indolent B-NHL,⁷⁹ where a prespecified threshold of 40% overall response was exceeded, leading to accelerated approval of copanlisib for FL. Accelerated approvals allow drugs for serious conditions that fill an unmet medical need to be cleared for marketing based on a surrogate rather than measurable clinical benefit. Three placebo-controlled Phase III trials of copanlisib have been started in patients with indolent B-NHL, namely on rituximab-refractory patients (CHRONOS-2) and of copanlisib in combination with rituximab (CHRONOS-3) or chemoimmunotherapy (CHRONOS-4).⁸⁶

As measures for the efficacy of copanlisib, response rates were determined in single-armed Phase I and II trials with patients suffering from relapsed/refractory indolent or aggressive B-NHL, including FL, DLBCL, and MCL. Participating patients were heavily pretreated with chemoimmunotherapy,

usually containing rituximab. Comparisons of response rates from such single-arm trials have to be done with caution, because of differences between trials, but are the only way to estimate the clinical efficacy of copanlisib and competitor drugs before results from randomized placebo-controlled trials become available. Accordingly, overall and complete response rates obtained in a trial comprising indolent and aggressive B-NHL subtypes⁸⁵ were compared with those from trials assessing different PI3K inhibitors.⁸⁷ A similar comparison of recently published results of trials of copanlisib in relapsed indolent B-NHL, including FL, as well as more aggressive DLBCL and MCL, is presented in Table 2. In trials of indolent B-NHL and FL, the response rates observed with copanlisib⁷⁷ were superior to those with idelalisib,⁷⁸ duvelisib,⁸⁶ and buparlisib⁸⁷ to different degrees, particularly in regard to the frequency of complete responses (Table 2), but in addition to its efficacy, the more favorable side-effect profile of copanlisib compared to p110 δ -selective agents contributes to its advantages.

With chemoimmunotherapy, ABC-DLBCL has a cure rate of 40%, which is lower than that for germinal-center DLBCL. BCR-pathway inhibitors are considered for the treatment of relapsed patients, particularly of the ABC subtype. The efficacy of PI3K inhibitors for treating DLBCL observed in Phase I and II trials is compiled in Table 2. The clinical usefulness of PI3K inhibition for the treatment of DLBCL has been called into question, when in an early trial this drug was not effective in the few studied DLBCL patients, in contrast to other B-NHL subtypes.⁹² However, copanlisib

treatment of relapsed or refractory DLBCL patients resulted in encouraging responses, especially in the ABC subtype,⁹⁰ which may be explained by the requirement for simultaneous inhibition of both p110 δ and p110 α .⁵⁹ Since treatment with single-agent ibrutinib, or copanlisib, with ~40% response has only limited efficacy against ABC-DLBCLs,⁹³ combined inhibition of BTK and p110 δ and p110 α is suggested on the basis of xenograft models.⁵⁹

The percentages of ORR and CR achieved with copanlisib against MCL, similarly as against ABC-DLBCL, surpass those obtained with idelalisib to a higher extent than observed in the treatment of FL (Table 2). However, the data available so far are from single-arm Phase I trials and must be interpreted with caution, even more so for ongoing or preliminary studies with low numbers of evaluated patients.

Summarizing the properties of copanlisib and idelalisib as representatives of multitargeted versus p110 δ -selective PI3K inhibitors (Table 3) does not immediately reveal why copanlisib was first approved for the treatment of FL, rather than for an entity, where targeting p110 α in addition to p110 δ would offer an advantage, such as ABC-DLBCL or MCL. While copanlisib exhibits at least 50-fold higher cytotoxicity against malignant B cells compared to only approximately five times lower clinically achievable concentrations than idelalisib, the differences between these inhibitors in regard to disturbance of the microenvironmental dialogue are still incompletely explored. Therefore, future clinical trials may reveal a favorable pharmacological profile of copanlisib against additional types of NHL.

Table 2 Efficacy of PI3K inhibitors in single-armed clinical trials with patients suffering from different subtypes of relapsed B-NHL

Drug	Entity	Subgroups	Trial	Phase	Reference	n	% ORR	% CR
Copanlisib	i-B-NHL	SLL, FL, MZL, WM	NCT01660451 part B,	II	79	141	60	12
		FL	CHRONOS I			104	59	14
Idelalisib		SLL, FL, MZL, WM	NCT01282424	II	80	125	57	6
		FL				72	54	8
Duvelisib		SLL, FL, MZL, WM	NCT01882803,	II	88	129	47	1
		FL	DYNAMO			83	43	1
Buparlisib		FL	NCT01693614	II	89	24	25	0
Copanlisib	DLBCL	All subtypes	NCT02391116	II	90	40	25	13
		ABC-DLBCL				16	38	25
Idelalisib		All subtypes	NA	I	91	9	0	0
Buparlisib		All subtypes	NCT01693614	II	89	26	12	4
Copanlisib	MCL		NCT01660451 part A	II	85	11	64	18
Idelalisib			NCT00710528	I	92	40	40	5
Buparlisib			NCT01693614	II	89	22	23	5

Notes: Entities investigated comprised indolent B-NHL and aggressive DLBCL and MCL. For each trial, the list contains the registration number at ClinicalTrials.gov and the corresponding reference. Results are indicated separately for all i-B-NHL or DLBCL and FL or ABC-DLBCL subgroups, respectively. Apart from the numbers of study participants, the percentages of ORR and CR are indicated. SLL has similar neoplastic B cells to CLL, but affects mostly lymph nodes.

Abbreviations: ABC, activated B cell; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; i-B-NHL, indolent B-cell non-Hodgkin lymphoma; MCL, mantle-cell lymphoma; MZL, marginal-zone lymphoma; NA, not applicable; ORR, overall response rate; SLL, small lymphocytic leukemia; WM, Waldenström macroglobulinemia.

Table 3 Summary of development of copanlisib and idelalisib

Feature	Copanlisib	Idelalisib
Core scaffold	Quinazoline	Quinazoline
Molecular weight	480.53	415.42
Ligand binding to p110	Scott et al ⁸	Somoza et al ¹⁰
Molecule shape	Flat	Three-blade propeller
Extending from adenine pocket into:	Affinity pocket	Induced selectivity pocket
Contact residues	K833, D836, D841 (p110 γ)	W760, M752 (p110 δ)
Potency	Liu et al ²⁰	Lannutti et al ¹³
IC ₅₀ of p110 δ activity	0.7 nM	2.5 nM
Selectivity against p110δ		
Compared to p110 α	0.7-fold	328-fold
Compared to p110 γ	9-fold	36-fold
Cytotoxicity against malignant B cells	Göckeritz et al ⁵⁷	Göckeritz et al ⁵⁷
IC ₅₀ for survival (48 hours)		
SUDHL5 (GC-DLBCL)	~0.03 μ M	~3 μ M
JVM3 (B-PLL)	~0.4 μ M	~20 μ M
CLL samples	~0.6 μ M	~30 μ M
Clinical administration	Patnaik et al ⁷³	Brown et al ⁶
Route	Intravenous	Oral
Dosing	Intermittent	Continuous
Standard treatment scheme	60 mg once weekly (three times per 4-week cycle)	150 mg twice daily
Pharmacokinetics		
C _{max}	~0.9 μ M	~5.3 μ M
C _{trough}	~0.01 μ M	~1.2 μ M
t _{1/2}	39.2 h	8.2 h
Most frequent adverse effects	Dreyling et al ⁷⁹	Gopal et al ⁸⁰
% occurrence (\geq grade 3)	41% hyperglycemia	27% decreased neutrophils
(see Figure 5)	24% hypertension	21% elevated transaminases
	24% decreased neutrophils	13% diarrhea
Clinical efficacy		
(see Table 2)	Dreyling et al ⁷⁹	Gopal et al ⁸⁰
Follicular lymphoma	59% ORR, 14% CR	54% ORR, 8% CR

Notes: Copanlisib and idelalisib represent multi-targeted versus p110 δ -selective PI3K inhibitors, but have the clinical application for treatment of relapsed or refractory follicular lymphoma in common. This table recapitulates important properties of both drugs and permits the comparison of biochemical and cellular IC₅₀ values with clinically achieved plasma concentrations.

Abbreviations: CR, complete remission; DLBCL, diffuse large B-cell lymphoma; GC, germinal center; IC₅₀, half maximal inhibitory concentration; ORR, overall response rate; PLL, prolymphocytic leukemia; C_{max}, maximal achieved plasma concentration of drug during standard treatment; C_{trough}, lowest concentration reached by a drug before the next dose is administered; t_{1/2}, half life.

Conclusion

Copanlisib and idelalisib are PI3K inhibitors with flat versus propeller-shaped structures and belong to multitargeted versus isoform-selective PI3K inhibitors. Copanlisib shows higher potency than other clinically developed PI3K inhibitors against all four class I isoforms, with approximately tenfold preference for p110 α and p110 δ . Of note, the high potency of idelalisib and duvelisib against p110 δ is surpassed only by copanlisib among currently available multitargeted inhibitors. Simultaneous targeting of p110 δ and p110 α was most convincingly shown to be of advantage in MCL and ABC-DLBCL cells, but targeting p110 γ may be of paramount importance in interruption of the microenvironmental dialogue. Apart from targeting all four class I isoforms versus only one, copanlisib differs from idelalisib also in regard to intravenous versus oral administration and weekly versus

twice-daily dosing. These properties may contribute to fewer and less severe gastrointestinal toxicities with copanlisib compared to idelalisib. Owing to efficacy and an adverse-effect profile distinct from idelalisib, copanlisib has already expanded the drug armamentarium against relapsed FL, with promising prospects for future applications, eg, against ABC-DLBCL and potentially further B-cell malignancies.

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