UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
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SÍNTESE E AVALIAÇÃO BIOLÓGICA DE INIBIDORES ENZIMÁTICOS COMO CANDIDATOS A FÁRMACOS

ISADORA SERRAGLIO FORTES

PORTO ALEGRE, 2025

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Tese de doutorado apresentada por **Isadora Serraglio Fortes** para obtenção do GRAU DE DOUTORA em Ciências Farmacêuticas.

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Co-Orientador: Prof. Dr. Rafael Roesler

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RESUMO

O “RESUMO”, que no texto completo da tese defendida ocupa a página 9, foi suprimido, uma vez que contém informações dos manuscritos em preparação para publicação em periódicos científicos.

Palavras-chave: atividade antitumoral; atividade antiviral; compostos heterocíclicos; inibidores de Akt; inibidores da M^{pro} de SARS-CoV-2.

Synthesis and biological evaluation of enzymatic inhibitors as drug candidates

ABSTRACT

O “ABSTRACT”, que no texto completo da tese defendida ocupa a página 11, foi suprimido, uma vez que contém informações dos manuscritos em preparação para publicação em periódicos científicos.

Keywords: antitumoral activity; antiviral activity; heterocyclic compounds; Akt inhibitors; SARS-CoV-2 M^{pro} inhibitors.

LISTA DE FIGURAS

A “LISTA DE FIGURAS”, que no texto completo da tese defendida ocupa a página 13, foi suprimida, uma vez que contém informações dos manuscritos em preparação para publicação em periódicos científicos.

SUMÁRIO

O “SUMÁRIO”, que no texto completo da tese defendida ocupa o intervalo de páginas 15-17, foi suprimido por conter informações dos manuscritos em preparação para publicação em periódicos científicos.

1. INTRODUÇÃO

A “INTRODUÇÃO”, cujo conteúdo, no texto completo da Tese, corresponde ao intervalo de páginas 19-30, foi suprimida por conter informações dos manuscritos em preparação para publicação em periódicos científicos.

2. OBJETIVOS

2.1 OBJETIVOS GERAIS

Síntese de compostos heterocíclicos, avaliação enzimática e celular dos compostos sintetizados na busca por novos candidatos antitumorais e antivirais.

2.2 OBJETIVOS ESPECÍFICOS

Os Objetivos Específicos, cujo conteúdo, no texto completo da Tese, corresponde a página 31, foram suprimidos por tratar-se dos objetivos presentes no manuscrito em preparação para publicação em periódicos científicos. Consta da síntese de compostos a fim de avaliar a atividade frente a enzimas e avaliar a citotoxicidade destas substâncias contra linhagens tumorais de câncer pediátrico, células não tumorais e células infectadas com vírus.

3. CAPÍTULO I: TARGETING AKT/PKB IN PEDIATRIC TUMORS: A REVIEW FROM PRECLINICAL TO CLINICAL TRIALS (Versão aceita do artigo publicado em https://doi.org/10.1016/j.phrs.2022.106403)

3.1 ABSTRACT

The serine/threonine kinase Akt is a major player in the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway, and its modulation impacts multiple cellular processes such as growth, proliferation, and survival. Several abnormalities in this pathway have been documented over the years, and these alterations were shown to have great implications in tumorigenesis and resistance to chemotherapy. Thus, multiple Akt inhibitors have been developed and tested in adult tumors, and some of them are currently undergoing phase I, II, and III clinical trials for distinct cancers that arise during adulthood. Despite that, the impact of these inhibitors is still not fully understood in pediatric tumors, and Akt-specific targeting seems to be a promising approach to treat children affected by cancers. This review summarizes recent available evidence of Akt inhibitors in pediatric cancers, from both preclinical and clinical studies. In short, we demonstrate the impact that Akt inhibition provides in tumorigenesis, and we suggest targeting the PI3K/Akt/mTOR signaling pathway, alone or in combination with other inhibitors, is a feasible tool to achieve better outcomes in pediatric tumors.

Keywords:

Pediatric cancer, PI3K/Akt/mTOR pathway, Akt inhibitor, targeted therapy

Chemical compounds studied in this article:

GSK690693 (PubChem CID 16725726); GSK2110183 (PubChem CID 92044396); GSK2141795 (PubChem CID 51042438); CCT128930 (PubChem CID 17751819); GDC-0068 (PubChem CID 24788740); AZD5363 (PubChem CID 25227436); MK-2206 (PubChem CID 24964624); Perifosine (PubChem CID 148177); Triciribine (PubChem CID 65399)

Abbreviations: **ALL**, Acute Lymphoblastic Leukemia; **AML**, Acute Myelogenous Leukemia; **AMPK**, AMP-Activated Protein Kinase; **ATP**, Adenosine Triphosphate;

BDNF, Brain-Derived Neurotrophic Factor; **B-pre ALL**, B-precursor acute lymphoblastic leukemia; **CDK2**, Cyclin-dependent *kinase* 2; **CLL**, Chronic Lymphocytic Leukemia; **CML**, Chronic Myeloid Leukemia; **CRBN**, E3 ubiquitin ligase substrate adaptor cereblon; **CSC**, Cancer Stem Cell; **DCR**, Disease Control Rate; **DLT**, Dose-Limiting Toxicities; **DNA-PK**, DNA-Dependent Protein Kinase; **EFS**, Event-Free Survival; **EGFR**, Epidermal Growth Factor Receptor; **FDA**, Food and Drug Administration; **FOXO**, Family of Forkhead Box Class O; **GPCR**, G Protein-Coupled Receptors; **GSK3**, Glycogen Synthase Kinase 3; **HER2**, Human Epidermal Growth Factor Receptor 2; **HM**, C-terminal regulatory hydrophobic motif; **HR-NB**, High-Risk Neuroblastoma; **INRC**, International Neuroblastoma Response Criteria; **JMML**, Juvenile Myelomonocytic Leukemia; **KC**, Kinase Catalytic Domain; **MAPKAP-K1**, Mitogen-activated protein kinase-activated protein kinase-1; **MEK**, Mitogen-activated protein kinase kinase; **MSK-1**, Mitogen- and stress-activated kinase 1; **MTD**, Maximum Tolerated Dose; **mTOR,** mammalian target of rapamycin; **mTORC2**, mammalian target of rapamycin complex 2; **NB**, Neuroblastoma; **p70S6K**, p70 ribosomal S6 kinase; **PDK1**, 3-Phosphoinositide-Dependent Protein Kinase 1; **PFS**, progression-free survival; **PH**, Pleckstrin Homology Domain; **PI3K**, Phosphoinositide 3-Kinase; **PIP_2**, Phosphatidylinositol 4,5-bisphosphate; **PIP_3**, Phosphatidylinositol 3,4,5-triphosphate; **PKB**, Protein Kinase B; **PPTP**, Pediatric Preclinical Testing Program; **PTEN**, Phosphatase and Tensin Homolog; **Ras-ERK** Ras-Extracellular Signal-Regulated Kinase; **RECIST**, Response Evaluation Criteria in Solid Tumors; **ROCK**, Rho-kinase 1; **RTK** - Receptor Tyrosine Kinases; **T-ALL**, T-cell acute lymphoblastic leukemia; **TPD**, targeted protein degradation; **TRKB**, Tropomysin-related Kinase B; **TSC2**, Tuberous Sclerosis Complex 2; **VHL**, von Hippel–Lindau protein.

3.2 INTRODUCTION

Pediatric tumors are one of the leading causes of death among children from 0 to 19 years old in developed countries, and 400,000 new cases are diagnosed each year worldwide [1,2]. Due to the recent advances in diagnosis and treatment, the mortality rates have had a constant decline over the last years [3,4]. However, resistance to treatment protocols is still a significant cause of death, and severe side effects of clinical intervention can lead to decreased quality of life among survivors. In fact, long-term effects of therapy have been observed in psychological, social, and physical outcomes, and higher incidence of chronic health conditions is reported in childhood cancer survivors when compared to siblings [5–7]. In the last decade, the continuous understanding of molecular factors involved in tumorigenesis and tumor progression shed light on several new targets for pediatric cancer treatment. Among them, the PI3K, protein kinase B (PKB, also known as Akt), mTOR signaling pathway (PI3K/Akt/mTOR) has been widely studied due to its major role in some of the most important cellular functions, such as proliferation, survival, growth, and protein synthesis [8]. Given its major impact on cellular function, deregulations in this pathway have been associated with cancer, diabetes, neurological and cardiovascular diseases. Thus, its modulation is a feasible way to enhance success rates of treatment against pediatric tumors.

In this review, we gather information regarding the rationale of targeting the PI3K/Akt/mTOR signaling pathway in pediatric tumors, as well as provide an overview of results concerning the efficacy of Akt inhibitors in clinical and pre-clinical studies regarding childhood malignancies, both alone and in combination with other inhibitors. Finally, we discuss the future of Akt as a target on the treatment of pediatric tumors and main challenges regarding its establishment in the clinic. To our knowledge, this is the first review to focus on the potential of Akt inhibitors in childhood cancer.

3.3 THE PI3K/AKT/MTOR SIGNALING PATHWAY

Activation of PI3K/Akt/mTOR signaling pathway initiates when G protein-coupled receptors (GPCR) or receptor tyrosine kinases (RTK) are activated via ligand binding, leading to phosphoinositide 3-kinase (PI3K) activation (Error! Reference source not f ound.). Activated PI3K triggers the conversion by its catalytic domain of phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol 3,4,5-triphosphate

(PIP_3). This reaction is regulated negatively by Phosphatase and Tensin Homolog (PTEN), which converts PIP_3 back to PIP_2 and works as a suppressor of PI3K/Akt/mTOR signaling [8,9].

Akt is a serine/threonine kinase that plays a key role on this pathway. It contains three highly conserved homologous subtypes (Akt-1/PKBα, Akt-2/PKBβ and Akt-3/PKBγ), being the first two widely expressed, and latter limited to nervous system tissues and testes [10]. Additionally, these isoforms have distinct biological relevance in cancer and other contexts [10–12], and specific isoform inhibition may have different impacts in cancer cell survival, metastasis and chemoresistance [13–15]. It has three different domains: the pleckstrin homology domain (PH) with an N-terminal fragment, kinase catalytic domain (KC), and C-terminal regulatory hydrophobic motif (HM). As other kinase enzymes, Akt can adopt different conformations due to their malleability [16]. The closed conformation is the inactive form of the enzyme, also known as ‘’PH-in’’. The PIP_3 acts as a docking site to Akt through the protein’s PH domain (opened conformation, “PH-out”) on the plasma membrane (**Error! Reference source not found.A**), and it is later activated by double phosphorylation by other kinases [17]. The 3-Phosphoinositide-Dependent Protein Kinase 1 (PDK1) phosphorylates the residue T308/T309/T305 at the KC domain in Akt-1/Akt-2/Akt-3, respectively. Subsequently, the mammalian target of rapamycin complex 2 (mTORC2) phosphorylates the S473/S474/S472 residue (Akt-1/Akt-2/Akt-3, respectively) at the HM domain for the full activation of the enzyme [17]. It is important to mention, however, that other kinases like DNA-Dependent Protein Kinase (DNA-PK) are also capable of phosphorylating Akt at S473 in stress situations [18]. After these modifications, Akt becomes active and adenosine triphosphate (ATP) molecule docks into its pocket at the KC domain (Error! Reference source not found.**A**). Thus, Akt regulates several cellular processes through transference of phosphate from ATP to its substrates, such as GSK-3, TSC2, mTOR, and the FOXO family of transcription factors [8].

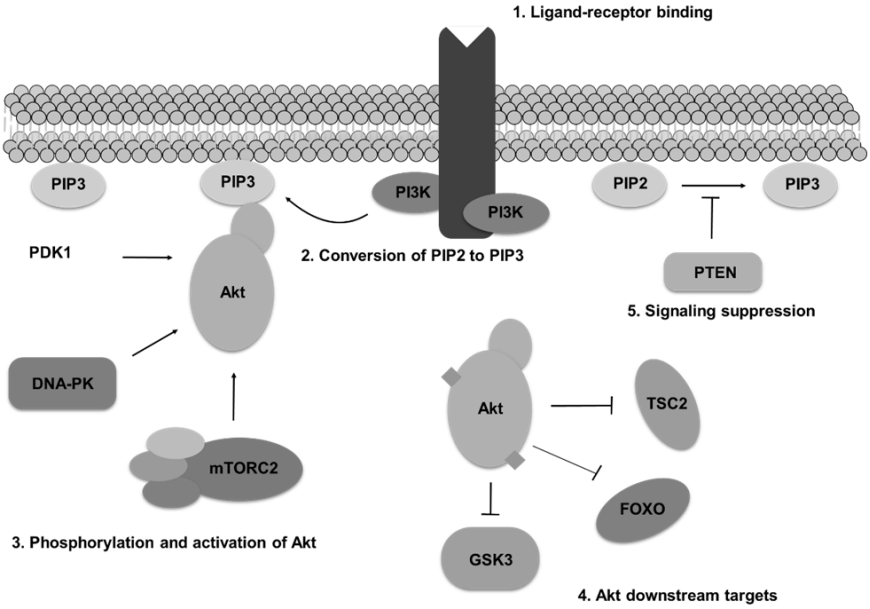


Fig. 1. PI3K/Akt/mTOR signaling pathway. Schematic review showing the activation of Akt through double phosphorylation and subsequent inhibition of proteins such as GSK3, FOXO, and TSC2. Regulating proteins such as PTEN and mTORC2 are also represented.

The PI3K/Akt/mTOR signaling pathway is long known as one of the most frequently altered pathways in human cancer [19,20]. This dysregulation is common in a wide range of tumors and is primarily a consequence of mutations or gene amplifications that affect the function of proteins related to this pathway [19–21]. Among these alterations, most occur on phosphatidylinositol 3-kinases isoforms, especially on *PIK3CA* [19]. Missense mutations on this gene, such as E542K, E545K, and H1047R, lead to constitutive activation of the protein and have been linked to tumor initiation and progression [21]. Mutations on Akt, on the other hand, are less frequent in tumors and the population in general [19,22].

In fact, the most known and common Akt mutation was reported by Carpten et al., who described a somatic E17K mutation in the PH domain of Akt-1 that leads to constitutive Akt activation [23]. Ever since, multiple variants in different domains of all three isoforms of Akt have been described and related not only to cancer but also to other pathologies such as diabetes and overgrowth syndromes [22,24]. Of note, most of the described alterations on Akt isoforms are missense or in-frame deletions/insertions, whilst rare changes are observed in the phosphorylation sites in each protein [22,25]. Many other genetic alterations on the PI3K/Akt/mTOR pathway have been

documented over the years, including the epidermal growth factor receptor variant III (EGFRvIII) deletion of exons 2–7 that leads to constitutive activation of epidermal growth factor receptors, monoallelic loss of PTEN, and other mutations on proteins such as PDK1, HER2 and other RTKs related to pathway instability and tumor maintenance [20]. Altogether, these alterations in the PI3K/Akt/mTOR pathway have instigated the development of a great number of small-molecule inhibitors as a possible new approach for cancer treatment, and multiple clinical trials are ongoing in different childhood malignancies. Importantly, some of these modifications, such as the T790M on EGFR, may confer resistance to developed inhibitors and should, therefore, be carefully considered before choosing treatment protocols. Despite the recent approval of PI3K and mTOR inhibitors to treat adult tumors [26–28], Akt inhibitors are still a little behind when it comes to development and approval, and the assessment of these molecules in pediatric tumors still needs further evaluation. Nonetheless, there are promissory results regarding pre-clinical and clinical trials encouraging the development of Akt inhibitors for cancer treatment.

Like other signaling pathways, the PI3K/Akt/mTOR is also regulated by negative and positive feedbacks to control stimulus durability, and some of its downstream targets are known to modulate the pathway's activity. In fact, as a key modulator of cell viability, the PI3K/Akt/mTOR signaling pathway also has points of cross-regulation with other major signaling pathways, such as Ras-Extracellular Signal-Regulated Kinase (Ras-ERK), NF-kB, Hippo/MST, and AMP-Activated Protein Kinase (AMPK) [8,29]. Additionally, these pathways seem to play compensatory roles, as suppression of one pathway may lead to activation of another. They also have some convergent points, in which more than one upstream effector may modulate the same downstream proteins, and consequently the same cellular processes. These complex relations are a challenge to overcome due to signaling redundancy and compensation, since they could decrease inhibitor and treatment efficacy. Understanding how alternative pathways are related to PI3K/Akt/mTOR in cancer is an important aspect to consider when planning to use Akt inhibition as an approach to treat pediatric tumors as combined therapies targeting multiple proteins from the same or different pathways might represent a feasible way to mitigate treatment resistance and to increase inhibitor effects on tumor cells [30,31].

3.4 PI3K/AKT/MTOR PATHWAY IN HEMATO-IMMUNE PEDIATRIC MALIGNANCIES

Hematologic cancers, such as lymphomas, leukemias, and myelomas, include tumors derived from the immune system and blood-forming tissues [32]. Noteworthy, leukemias are the most common malignancies in individuals from 0 to 19 years worldwide, and account for the biggest proportion of disability-adjusted life years, which includes premature mortality and healthy life lost due to disability [1]. Among tumorigenesis mechanisms, deregulation of the PI3K/Akt/mTOR pathway is a common feature throughout hematologic malignancies [33–35], even though mechanisms of upregulation may be different among specific tumor types. In acute lymphoblastic leukemia (ALL), inactivation of PTEN and mutations in the *AKT* and *PI3K* genes are the most common alterations that lead to pathway aberrant activation, although there are some significant divergences between B-ALL and T-ALL [36,37]. The opposite happens in adult multiple myeloma samples, in juvenile myelomonocytic leukemia (JMML), and in acute myelogenous leukemia (AML), in which mutations in these genes are not the most common causes of alteration of the pathway. For example, although, the PI3K/Akt/mTOR pathway is still activated in multiple myeloma, and Akt upregulation is modulated by other mechanisms such as increased secretion of growth factors and other signaling molecules [38]. Additionally, altered RAS signaling both in AML and JMML patients due to mutations in genes such as *NRAS* and *KRAS* may lead to aberrant Akt activation [39,40]. Lastly, common genetic alterations in kinases such as FLT3 are also reported to lead to increased phosphorylation in downstream signaling pathways, including the PI3K/Akt/mTOR [40]. Thus, although molecular mechanisms may be variable, PI3K/Akt/mTOR pathway aberrations have been spotted in high frequencies among hemato-immune cancers.

Over the last years, PI3K/Akt/mTOR pathway and its possible role on tumorigenesis has been extensively assessed as a new approach to treat pediatric tumors. For instance, *in vitro* studies demonstrated that induced downregulation of PI3K/Akt/mTOR pathway signaling through multiple approaches such as gene silencing, miRNA, and exposure to natural products has a great impact on cell growth and viability in a wide range of hematologic childhood tumors [41–46]; and the use of specific inhibitors has also demonstrated promising results [47–49]. Accordingly, Akt signaling has also been demonstrated to exert anti-apoptotic effects over ALL cells [50]. Furthermore, pathway

activation has been correlated to poor clinical outcomes in pediatric hematologic malignancies, as mutations in the PI3K/Akt/mTOR pathway and high expression of downstream targets such as mTOR and GSK3-β may be predictors of treatment resistance and decreased survival rates [51–54]. In a study described by Morishita et al., it was demonstrated p-Akt expression in B-precursor acute lymphoblastic leukemia (B-pre ALL) patients with poor response to chemotherapy. In addition, the overall survival and relapse-free survival decreased in patients with expression of activated Akt [51]. Accordingly, inactivation/loss of PTEN has also been suggested to have significant impact on prognosis of hemato-immune tumors, as it is known for inhibiting Akt signaling [34,55]. As reviewed by Mendes et al., different groups have reported that aberrant PTEN function is related to poorer clinical outcomes such as higher risks of relapse, although some groups could not find clinical benefits over PTEN-genotype assessment [55]. Furthermore, there is growing evidence that PI3K/Akt/mTOR signaling modulation may also increase therapeutic effect of standard protocols in hematologic tumors, and Akt inhibition could also be used to mitigate treatment failure due to treatment-resistance mechanisms [56,57]. Thus, combined therapies with conventional drugs and/or with other targeted inhibitors deserve further assessment. All this knowledge has led to the inclusion of PI3K and mTOR inhibitors in clinical trials in young patients with hematologic malignancies [58–60], and even more studies are expected to be performed in the near future.

Evidence briefly reviewed here supports that PI3K/Akt/mTOR signaling pathway is a feasible target to improve treatment success in childhood hematological cancers, as it plays major roles in cell proliferation, survival and drug resistance. However, taking into consideration heterogeneity of metabolism mechanisms between tumors, it is possible that single-target agents may not have the same efficacy in distinct diseases. Thus, combining the use of PI3K/Akt/mTOR pathway inhibitors either with targeted agents or with chemotherapy seems an appealing protocol to enhance treatment success. Furthermore, considering PI3K inhibitors have already been approved by the Food and Drug Administration (FDA) to treat adult hematological malignancies [61], targeting PI3K/Akt/mTOR pathway on pediatric hemato-immune tumors may render encouraging results.

3.5 PI3K/AKT/MTOR PATHWAY IN PEDIATRIC SOLID TUMORS

Pediatric solid tumors, altogether, account for approximately 60% of malignancies in children and adolescents aged 0 to 19 years old [62,63]. Although the distribution of these tumors may vary in different ages, most of them share many cellular and molecular features, such as the status of the PI3K/Akt/mTOR signaling pathway. Disruptions in the pathway, mainly caused by genetic alterations in key genes such as *PIK3CA* and *PTEN*, have also been spotted in a wide range of solid tumors [19], including the ones affecting children. Regarding osteosarcoma tumor, for example, a screening of 59 patients revealed genetic alterations of PI3K/Akt/mTOR pathway in 24% of patients, and it was observed almost 12% and 1.7% of patients with *PTEN* and *PIK3CA* alteration, respectively, contributing to the pathogenesis of this disease [64]. Low levels of PTEN, mutations in genes such as *PIK3CA* and *AKT3*, and mTOR activation were also observed in several central nervous system tumors like medulloblastoma, ependymoma, and glioma [65,66]. Still, these mutations do not seem to be as recurrent as in adult tumors [19]. All these alterations have been consistently suggested as one of the many molecular mechanisms related to tumor maintenance and progression, as abnormal activity of this pathway is also correlated to poor prognosis features such as metastasis and chemotherapy resistance [67,68]. Just like hematologic tumors, abnormalities in the PI3K/Akt/mTOR pathway are not uniformly distributed among pediatric solid tumors [19]. The presence of disruptive signaling of this pathway, however, has led to a deep evaluation of the PI3K/Akt/mTOR pathway's role in tumorigenesis. The increasing understanding about how this pathway supports malignancy has led to the development of several protein inhibitors over the last years and targeting Akt signaling has proven to be a feasible way to increase treatment efficacy.

For instance, multiple *in vitro* studies demonstrated the great impact of PI3K/Akt/mTOR signaling downregulation on pediatric tumor's progression, leading to cell cycle arrest, decreased cell viability, and increased apoptosis. Exposure of different malignant cells to PI3K, Akt, and mTOR inhibitors rendered significant results [69,70], which demonstrates the appealing efficacy of PI3K/Akt/mTOR inhibitors in cancer treatment. Promising results were also obtained *in vivo*, in which models demonstrated the effects of these pathway inhibitors in tumorigenesis inhibition, decreased tumor growth and

enhancement of event-free survival [71–74]. Further promising results should also be obtained in clinical trials, in which different inhibitors of the pathway are currently being evaluated in distinct tumors [60,75,76]. Interestingly, tumor cells have demonstrated increased susceptibility to chemotherapy agents after exposure to PI3K/Akt/mTOR inhibitors, and pathway targeting also seems able to sensitize resistant cells to golden standard protocols [67,77,78]. In accordance to these findings, Wei et al. demonstrated that PI3K/Akt/mTOR pathway activation was the main responsible for resistance against cisplatin in osteosarcoma cells, suggesting signaling disruption of this pathway could be an effective therapy against refractory tumors [79]. Furthermore, evaluation of co-targeting and different combinations of cytotoxic drugs have been performed by multiple groups [80–84], who have consistently suggested that patients may benefit the most from PI3K/Akt/mTOR inhibition if compounds are combined with different drugs. According to these studies, PI3K/Akt/mTOR inhibitors alone do not seem to play a great impact on tumor viability, whereas adjuvant therapies may increase the cytotoxic effect of this approach. These results corroborate the importance of assessing the impact of the PI3K/Akt/mTOR pathway in the biology of pediatric solid tumors.

Taking into consideration the results discussed above, there is a clear rationale for the investigation of PI3K/Akt/mTOR pathway inhibitors as an approach to treat pediatric tumors. Preclinical and clinical trials ongoing in a wide variety of childhood malignancies are currently assessing the efficacy of multiple small inhibitors and have been gathering promising results over the last decade. Here, we review data regarding the use of Akt enzyme inhibitors collected over the last decade.

3.6 AKT INHIBITORS

Three main classes of Akt inhibitors are investigated and entered into preclinical and clinical trials: ATP-competitive inhibitors (**Fig. 2B**), allosteric inhibitors (**Fig. 2C**), and inhibitors of PIP_3 binding (**Fig. 2D**). **Table 1** summarizes the inhibitors that were tested in pediatric tumors as well as the phase of the study.

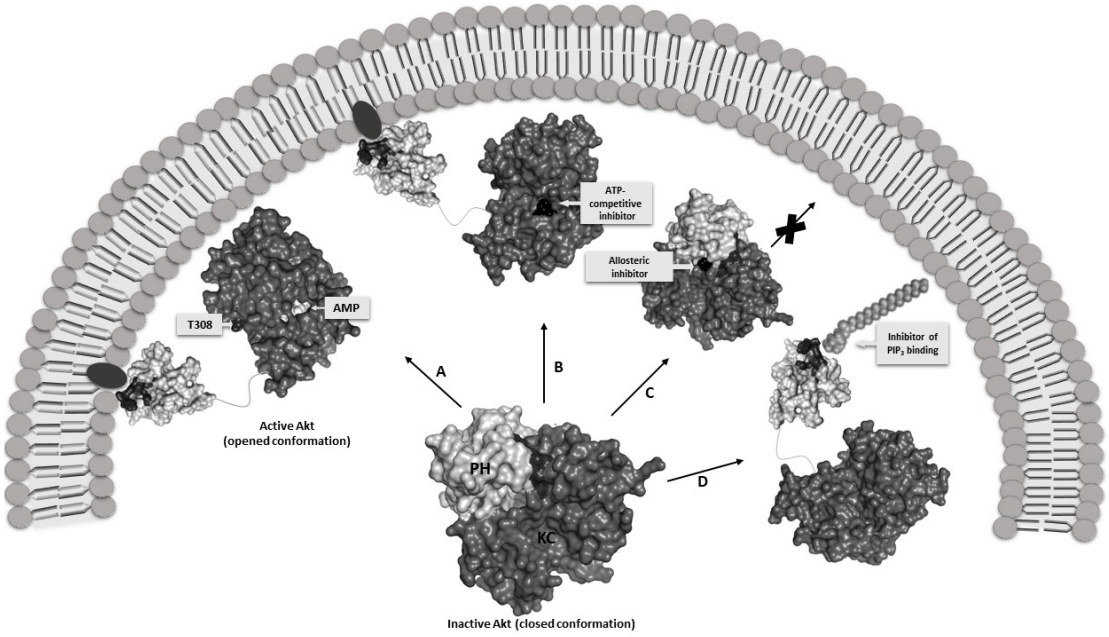


Fig. 2. Binding mode of ATP and Akt inhibitors in Akt crystal structure. **A)** When inactive Akt (closed conformation; PDB ID: 3O96) is recruited to membrane, its PH domain (PDB ID: 1UNQ) binds to PIP_3 (ellipse) at inner membrane, then Akt exposes its KC domain and is phosphorylated at T308 and S473 (this residue does not appear in this picture due to the side of the enzyme represented here) in the opened conformation. Then, ATP docks into active site. This structure was crystallized with AMP into KC domain of Akt-1 (KC domain PDB ID: 4EKK); **B)** ATP-competitive inhibitor into kinase domain of Akt-1 (KC domain PDB ID: 4GV1); **C)** Allosteric inhibitor binding into KC and PH domains of Akt-1 (PDB ID: 3O96); **D)** Inhibitor of PIP_3 binding in PH domain of Akt-1 (KC domain PDB ID: 4GV1). 3D surface structure model was generated using Open-Source Pymol 1.8.x.

3.6.1 ATP-COMPETITIVE INHIBITORS

The ATP-competitive inhibitors belong to the largest group of Akt inhibitors. These molecules occupy the same site of adenosine triphosphate (ATP) on opened PH-out conformation of Akt [85,86], preventing the enzyme to transfer the γ-phosphate of ATP to its substrates. It is important to mention, however, that Akt enzyme still can be phosphorylated at residues T308 and S473 in the presence of such inhibitors (**Fig. 2B**). Since the ATP binding pocket is highly conserved among the three isoforms of the enzyme [87], specific targeting of isoforms is still a challenge to overcome.

3.6.1.1 GSK690693

In 2005, Bamford and collaborators performed a high-throughput screening aiming to identify MSK-1 inhibitors. They found a novel inhibitor (compound **1)** bearing an amino-oxadiazole scaffold (**Fig. 3**) with IC_{50} 140 nm against MSK-1. Then, the optimization of this compound provided the azaderivative **2** (**Fig. 3**) being about 50-fold more potent than compound **1** and exhibiting activity against other kinases such as GSK3β, ROCK1, CDK2, MAPKAP-K1α, and p70S6K [88,89]. Taking into consideration the kinase inhibitory activities of these compounds, *GlaxoSmithKline* moved forward with the optimization of imidazopyridine substituents in order to obtain an inhibitor of Akt. During the structure-activity relationship studies, it was observed a mild effect in Akt activity and higher selectivity over other kinases like MSK-1 and ROCK1 when the amine side chain at C-7 position was modified. In addition, the activity against Akt-1 was improved with modifications at C-4 of the imidazopyridine with the presence of an alcohol and a dimethyl group at the terminus of alkyne. The resulting compound was **3** (**Fig. 3**), which showed an IC_{50} value of 6 nM against Akt-1 and of 1 μM regarding the inhibition of phosphorylation of GSK3β in BT474 cells. The final round of modifications at C-7 position resulted in a series of derivative which inhibited Akt-mediated phosphorylation of GSK3β in BT474 cells with IC_{50} values in submicromolar range. The compound GSK690693 was the best derivative of the imidazopyridine series since it demonstrated a higher selectivity against the Akt isoforms and great antitumoral effect [90]. Therefore, GSK690693 is a pan-Akt inhibitor with low nanomolar values of IC_{50} for Akt-1, Akt-2, Akt-3 (see **Table 1**), being at least 4-fold more selective for Akt-1 isoform than to the other two [91]. GSK690693 is an intravenous drug candidate and it was evaluated in two adult phase I clinical trials for the treatment of solid tumors and hematologic malignancies, which were later discontinued [92,93]. The sensitivity of hematologic tumors to treatment with Akt inhibitor GSK690693 was evaluated *in vitro* by Levy and colleagues, who reported a higher growth inhibition in ALL. Among the pediatric ALL lineages, J.GAMMA-1 showed the lowest value of EC_{50} (65 nM). Other hematologic tumors as non-Hodgkin lymphoma, Burkitt lymphoma, and chronic lymphocytic leukemia (CLL) cell lines were more sensitive to the treatment than chronic myeloid leukemia (CML), AML, and Hodgkin lymphoma. Of note, results regarding proliferation assay in non-tumoral $CD4^+$ peripheral T cells and mouse thymocytes treated with GSK690693 showed growth

inhibition only with EC_{50} values greater than 10 and 30 μM, respectively, suggesting a higher antiproliferative activity in tumor cells. Reduction of phosphorylation in downstream targets of Akt, such as GSK3-β and PRAS40, was also detected in a dose-dependent manner [94].

This inhibitor was also evaluated in a wide panel of pediatric tumor cell lines by the Pediatric Preclinical Testing Program (PPTP) and obtained a broad spectrum of activity. In line with results gathered by Levy and colleagues, ALL cell lines were the most sensitive to treatment, showing the lowest IC_{50} value of 6.5 nM for COG-LL-317. Regarding *in vivo* assays, however, no objective responses (tumor regression) were observed in solid nor in ALL xenografts. Interestingly, osteosarcoma xenografts showed significant stability of disease, since GSK690693 increased event-free survival (EFS) distribution in all subjects bearing this specific tumor [95]. Up to this moment, no clinical phases regarding the treatment of childhood cancers with this compound were ever developed.

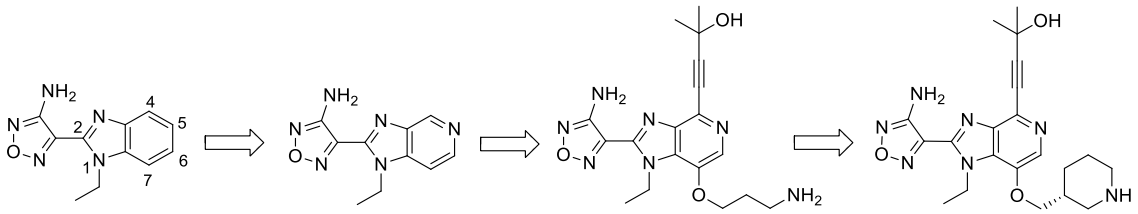


Fig. 3. Schematic representation of the discovery and optimization of the GSK690693.

6.6.1.2 GSK2110183 (Afuresertib) and GSK2141795 (Uprosertib)

In 2009, *GlaxoSmithKline* identified a pyrrolopyridinyl thiophene carboxamide series as Akt inhibitor through a screening method [96]. The structure-activity relationship studies with this series led to optimization of pyrrolopyridinyl to pyrazole group, developing the oral GSK2110183 (Afuresertib) and GSK2141795 (Uprosertib) Akt inhibitors. Due to the similar structures of these compounds, they can be discussed in the same section. The kinase selectivity profile of these inhibitors is narrower than GSK690693 [97] and the K_i values are in nanomolar range for all Akt isoforms (see **Table 1**) [98].

Afuresertib can inhibit AKT1 E17K mutant as well as Uprosertib with EC_{50} 0.2 nM, probably due to their similar binding manner into the ATP site of the enzyme [98].

Bradgen et al 2019 reported promising results after association of Afuresertib with paclitaxel and carboplatin to treat recurrent platinum-resistant ovarian cancer [99]. Afuresertib has been included in different studies as monotherapy or in combination with chemotherapy drugs in adult patients, and a single phase II study regarding Langerhans Cell Histiocytosis included a single 16-year old patient with refractory disease [100,101]. The trial demonstrated acceptable tolerability, and gastrointestinal disorders were the most common adverse effects reported by the recruited patients, along with fatigue and upper respiratory infections. Interestingly, the adolescent had similar plasma concentrations if compared to adult patients. Although it is still debated whether Langerhans Cell Histiocytosis can be considered a cancer [102], and a single adolescent was enrolled in this study, it is noteworthy that Akt inhibitors are being considered for treatment of children, and new trials on pediatric tumors with greater numbers of subjects are likely to take place in the near future.

Dumble et al 2014 tested this compound in a series of hematologic and solid pediatric tumors and demonstrated results similar to GSK2141795. Regarding hematologic cells, MOLT-4 (ALL) showed the lowest IC_{50} value (14 nM). Among solid tumors, the HEP3B cell line (hepatocellular carcinoma) was the most sensitive lineage, exhibiting an IC_{50} of 399 nM. Like GSK2141795, the effects of GSK2110183 on Akt signaling were observed by the inhibition of phosphorylation in GSK3-β, PRAS40, FOXO and Caspase 9 [98]. To our knowledge, no clinical phase studies were ever addressed regarding the use of GSK2141795 in pediatric tumors.

GSK2141795 has been included in several adult clinical trials associated with Trametinib (GSK1120212), an allosteric inhibitor of MEK1/2. Although there are preclinical studies supporting the use of PI3K/AKT and MEK inhibitors in adult cancer [98,103], subjects enrolled in clinical studies developed serious side effects and displayed limited benefits [104–107]. Therefore, further studies are important to determine whether targeting both pathways is a feasible and efficient solution in tumor treatment. To our knowledge, a single preclinical phase was performed by Dumble and collaborators 2014 evaluating the activity of GSK2141795 in a broad panel of hematologic and solid pediatric tumors. Regarding cell viability, the treatment demonstrated higher cytotoxic effects in ALL (MOLT-4 cell line) with IC_{50} 10 nM and

hepatocellular carcinoma (HEP3B cell line) with IC_{50} 154 nM among hematologic and solid tumors, respectively [98].

6.6.1.3 CCT128930

CCT128930 was developed by a partnership between the *Cancer Research UK Centre for Cancer Therapeutics* and *Astex Therapeutics* and is 30-fold more selective for Akt-2 (IC_{50} = 6 nM) than PKA (IC_{50} = 168 nM) [108]. To our knowledge, no data regarding IC_{50} values on other Akt isoforms were ever determined.

The cytotoxic effect of CCT128930 in pediatric hepatoma cell line HEP-G2 was evaluated by Wang and collaborators, who identified an IC_{50} value of 30 μM. The CCT128930 treatment in hepatoma cells also led to decreased cell size, DNA damage, impaired cell cycle progression, and induction of both apoptosis and autophagy [109].

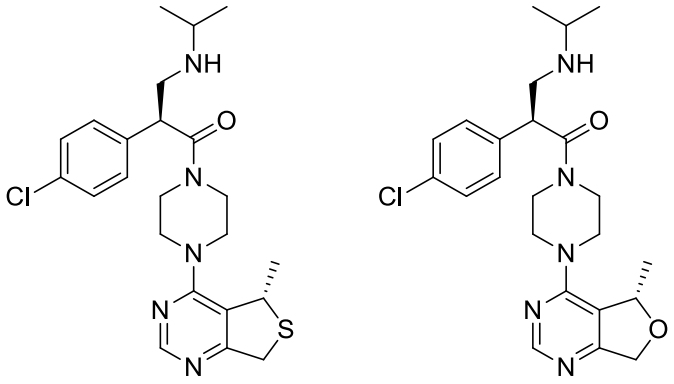
Regarding osteosarcoma, *Sun et al* 2020 measured the activity of CCT128930 in U2OS and MG-63 cells, which had cell growth inhibition in a dose-dependent manner (average inhibition of 60% at 40 μM). Further investigation about the mechanism of action demonstrated that CCT128930 induced G1-phase arrest and apoptosis in both cell lines. An analysis regarding the synergic effect between CCT128930 and VS5584 (PI3K inhibitor) also reported an enhancement in cell apoptosis and growth inhibition in these cells [110].

Combination of CCT128930 with other drugs have also been evaluated in neuroblastoma (NB) cells. Higher proliferation inhibition, as well as decreased sphere formation were obtained after association of CCT128930 (10 μM) with PD98059 (MAPK inhibitor, 100 μM) in BE(2)-C and SK-N-AS cells. It is important to mention that these results were observed in cisplatin and radiation-resistant cells (CDDP-R and Rad-R), implying this combination of drugs could be a feasible solution to treat tumors that are resistant to clinical protocols [80]. Based on *clinicaltrials.gov*, we did not identify clinical studies involving this compound.

3.6.1.4 GDC-0068 (Ipatasertib)

GDC-0068 (also known as Ipatasertib) was developed by *Array BioPharma/Genentech Inc* and demonstrated a significant inhibition of all Akt isoforms, having a similar activity against Akt-1 and Akt-3 (see **Table 1**) [111]. Previously, aiming to optimize a series of

Akt inhibitors, Bencsik and collaborators demonstrated the synthesis of compound **4** and **5** (**34** and **41** in the original publication, respectively) (**Fig. 4**), and their activity against Akt enzyme. Both of them showed similar results against Akt-1 (IC_{50} 1 nM vs 3 nm, respectively) and modest selectivity over PKA (IC_{50} 35 nM vs 52 nM, respectively) [112]. Subsequently, Blake and collaborators utilized the dihydrothieno- and dihydrofuro-pyrimidine cores of **4** and **5**, respectively, to further investigate the selective inhibiton of Akt. The substitution of dihydrocyclopentapyrimidine core with C7-(*R*)-hydroxyl combined and C5-(*R*)-methyl in saturated ring and the piperazine amide portion with isopropylamine and 4-chlorophenyl afforded GDC-0068. This compound showed 620-fold more potent to Akt-1 than PKA, and a good selectivity profile over a broad panel of 230 kinases. Since the preclinical studies demonstrated a good pharmacology profile, several clinical trials have tested Ipatasertib as targeted therapy for cancer in adults [111,113]. Currently, a single phase II clinical trial recruiting both children and adults is assessing the use of Ipatasertib on metastatic or advanced solid tumors [114]. There are other numerous clinical trials in phase III including patients with metastatic castrate-resistant prostate cancer or breast cancer, all of them using Ipatasertib with other drugs [115–119].



4 **5**

Fig. 4. Chemical structures of dihydrothieno- and dihydrofuropyrimidines as potent Akt inhibitors.

An *in vitro* assay was performed using an allosteric Akt inhibitor (MK-2206) or Ipatasertib to check the activity of these compounds in the absence of the Akt-1's PH domain, due to a genetic mutation first identified by Slotkin et al. People bearing this mutation display a loss of 96% of the PH domain from Akt-1, but the kinase domain

and phosphorylation sites (S473 and T308) are still present. Therefore, the treatment with allosteric Akt inhibitor (MK-2206) was not efficient at any concentration tested, in contrast to the use of Ipatasertib, which efficiently decreased cell growth. In this study, a 12-years-old female was identified with a neoplasm bearing this specific mutation and she was the first child patient to be treated with GDC-0068. This treatment led to a decrease in CA-125 levels, a tumor marker for ovarian cancer, although results bounced back to usual levels four months later. The patient had few adverse effects as grade 1 of diarrhea and leukopenia [120].

3.6.1.5 AZD5363 (Capivasertib)

AstraZeneca developed AZD5363 (also known as Capivasertib) compound, a potent oral Akt inhibitor with low IC_{50} values (see **Table 1**). This compound was developed based on the structure of CCT128930 and optimized analogues. Capivasertib has a pyrrolopyrimidine moiety, aiming to bind through polar interactions with residues at Akt's active site. The piperidinyl spacer, primary amine and the chlorobenzyl groups were retained to perform the same pattern of interactions as well as CCT128930 series. Capivasertib increased potency against Akt, improved selectivity against ROCK2 enzyme, and reduced hERG ion channel affinity by adding an α-substituted amide group bearing a primary alcohol [121]. Currently, Capivasertib is enrolled in multiple clinical trials. Since it showed promising results in phase II trials involving adults [122,123], new phase III studies are recruiting patients [124–127]. To our knowledge, no pediatric clinical trials involving Capivasertib were ever conducted. Then, we highlight below a preclinical phase in leukemic and medulloblastoma cells.

Lynch and collaborators investigated the sensitivity of a broad panel of cancer lineages (including pediatric lineages), to PI3K/Akt/mTOR pathway inhibitors. Among all 971 cell lines included, hematological malignancies displayed remarkable sensitivity to Akt inhibitors, as 49% of the hematological tumor cell lines evaluated were sensitive to AZD5363 exposure (sensitivity cut-off of 9 μM) [128].

Tumor cells also seem to become more sensitive to other drugs after Akt inhibition by AZD5363. Khoshamooz et al. demonstrated a synergic effect inhibiting both PI3K/Akt and Notch1 pathways, another signaling mechanism usually studied in leukemia. Co-targeting rendered a significant decrease in cell viability in all three cell lines evaluated (Jurkat, MOLT-4, and HPB-ALL) when compared to each of the inhibitors alone, even

though Jurkat and HPB-ALL cells exhibited resistance to one of the employed inhibitors. In addition, the synergic effect between inhibitors also increased the number of apoptotic cells - 90% when associated inhibitors were used, in contrast to < 80 % apoptotic cells induced by monotherapy [30]. In 2020, Patra and colleagues described similar results, as they demonstrated that dual inhibition of Akt and β-catenin had a greater impact on viability of hepatocellular carcinoma cells. Exposure to both AZD5363 and FH5363 led to cell cycle arrest and increased cell death via autophagy if compared to Akt or β-catenin inhibition alone [129]. Thus, Akt targeting through AZD5363 could be used to improve therapy effects in childhood cancer.

Interestingly, Rahmanto and colleagues demonstrated that Akt inhibition could be used to reestablish sensitivity of cisplatin-resistant medulloblastoma cells. AZD5363 exposure led to a decrease in SOX9 levels, which is a transcript factor involved in cell migration, metastasis and drug resistance. Furthermore, SOX9 levels modulation through PI3K/Akt/mTOR pathway inhibition could be suggested as a feasible tool to improve clinical outcomes in therapy-resistant patients [67].

To date, ATP-competitive Akt inhibitors have demonstrated *in vitro* cytotoxic activity in a wide range of pediatric tumors, especially in ALL cell lines. The divergence among studies, however, suggests that the efficacy of PI3K/Akt pathway inhibition is not thoroughly assessed by *in vitro* studies. Thus, the effect of these small inhibitors should be deeply characterized in xenografts and clinical trials. Of note, studies regarding the combination of ATP-competitive Akt inhibitors with other therapeutic approaches have provided promising results and suggest co-targeting may be a feasible way to overcome drug resistance and to potentiate chemotherapy efficacy. Rather than monotherapy, Akt inhibitors might as well be considered as an adjunct therapy and combination studies should also be further evaluated.

3.6.2 ALLOSTERIC INHIBITORS

Allosteric inhibitors bind between PH and KC domains, stabilizing the enzyme in the closed conformation. Therefore, the protein is not able to be recruited to the cell membrane and consequently does not proceed the signaling pathway. Thus, differently than ATP-competitive inhibitors, allosteric inhibitors impair the phosphorylation at T308 and S473 residues of the enzyme (**Fig. 2C**) [85,86].

6.6.2.1 MK-2206

Merck developed MK-2206, an oral allosteric Akt inhibitor with low values of IC_{50} (see **Table 1**), and 5-8-fold more potent to Akt-1/2 than Akt-3. It also demonstrates a high selectivity profile against the Akt enzyme compared to other protein kinases. Similar to ATP-competitive inhibitors, it has been demonstrated that MK-2206 can also inhibit the phosphorylation of downstream enzyme substrates, such as TSC2, PRAS40, and ribosomal S6 proteins [130]. Clinical trials regarding the use of MK-2206 in adult patients are still under phase I and II. To our knowledge, there is only one clinical study regarding pediatric patients treated with MK-2206 [131]. Nevertheless, we found several preclinical studies using this drug as monotherapy or in combination with other agents.

Targeting Akt with MK-2206 treatment has been demonstrated as a feasible way to decrease tumor viability in a dose-dependent manner in multiple cell lines of rhabdoid tumors, neuroblastoma, and osteosarcoma with IC_{50} lower than 20 μM [132–134]. In the PPTP, a panel of solid and hematologic pediatric tumor cell lines was exposed to MK-2206 in doses ranging from 1.0 nM to 10 μM. The lowest value of IC_{50} was 0.05 μM for the COG-LL-317 cell line and the highest value was >10 μM for SJ-GBM2 lineage. In relation to *in vivo* assays, no objective responses (tumor regression) were detected either in ALL nor in solid tumor xenografts. However, the best results were obtained in the osteosarcoma xenograft panel, since all six osteosarcoma xenografts treated by MK-2206 showed a significant increase in EFS distribution compared to the control group and three xenografts of this model had PD2 response (progressive disease with growth delay). Interestingly, the pre-clinical assays with MK-2206 showed similar behavior to GSK690693 inhibitor, since both drugs showed a prominent relative sensitivity in ALL cells and specificity for osteosarcoma xenografts with a modest antitumor activity [71].

Akutagawa and colleagues also demonstrated the effect of this inhibitor in mouse models of myeloproliferative neoplasms, as treatment was able to increase overall survival *in vivo*. Combined therapy with MAPK inhibitors suggests co-targeting of these pathways may provide improved results in animal models [73]. In fact, combined therapy with MK-2206 and other inhibitors has been assessed in multiple studies. Canté-Barrett et al. 2016 observed synergic cytotoxic effect of MEK inhibitor

(AZD6244) plus PI3K inhibitor (GDC-0941) or AZD6244 plus AKT inhibitor (MK-2206) in nearly all Ba/F3 pediatric T-cell acute lymphoblastic leukemia (T-ALL) mutant lines used, except for AKT^{E17K} and $JAK1^{T901G}$ [83]. Similar results were gathered by Neri et al., who reported a synergic cytotoxic effect in B-pre ALL cells after dual targeting of mTOR and Akt using RAD001 (Everolimus) and MK-2206, respectively. Further investigation of downstream proteins, such as 4EBP1 and GSK3-α/β, demonstrated decreased phosphorylation levels using drug association (RAD001 0.34 μM and MK-2206 1 μM) in comparison to single-agent treatment. Besides, the combinatory treatment also increased extrinsic and intrinsic apoptotic pathways, potentially induced autophagy and cell cycle arrest in G0/G1 [82]. Further investigation about the mechanism of cell death caused by MK-2206 plus rapamycin treatment was performed through MYCN-amplified NB cell lines such as NGP and SK-N-BE2. Dong et al. 2020 evaluated the changes of cells treated with this combined chemotherapy and observed the formation of autophagic vacuoles and increased levels of autophagy and necroptosis-involved proteins, both *in vitro* and *in vivo*. Assays using the NGP xenograft tumor tissues had results in agreement with *in vitro* assays. Gathered data demonstrated that the mechanism of cell death induced by this combination was MYCN-dependent [135]. Santo and co-workers 2013, reported another mechanism of cell death in a panel of NB cells, in which MK-2206 triggered apoptosis by Akt inhibition and upregulation of FOXO3a [136].

In 2012, Li and collaborators performed *in vitro* and *in vivo* studies using MK-2206 as a single agent or in combination with etoposide (DNA topoisomerase II inhibitor) or rapamycin in neuroblastoma cells. The authors observed depletion of 60% in phosphorylated Akt fraction at 0.01 μM in SK-N-AS cells and a range of 40% to 70% was detected in SY5Y, SK-N-BE2, and NGP cells at 0.1 μM when theses cells were treated by MK-2206. They also observed that the monotherapy with MK-2206 led to IC_{50} values of all eight NB cells ranging from 0.6 μM to 16.5 μM. However, the monotherapy led to the caspase-3 and 7 activation only in higher concentrations (15 μM or 20 μM), and increased ROS (reactive oxygen species) levels were observed in some lineages. Significant inhibition of tumor growth (up to 48%) was observed in different NB xenografts after treatment with MK-2206 at 200mg/kg dose; besides, there was a statistically significant increase in mice survival animals. The association of MK-2206 and etoposide led to apoptotic signaling, in contrast to etoposide alone, showing

the best result in SY5Y cells (5-fold more active for the association vs 2.2-fold for etoposide). This combination showed significant inhibition of tumor growth in all 3 xenografts tested (up to 66%) and significant increase in mice survival compared to both inhibitors used alone [81].

Other combination therapies were evaluated using MK-2206 and mTOR inhibitors. In neuroblastoma cell lines, associated treatment of MK-2206 with rapamycin significantly enhanced inhibition of cell proliferation compared to either alone in two of the evaluated cell lines. Also, treatment of mice harboring NGP tumor with MK-2206 at 200 mg/kg dose and rapamycin at 5 mg/kg, showed 76% inhibition of tumor growth and a statistically significant increase in the survival of mice compared to either compounds administered alone. Caspase-3 and 7 activities were not detected in MK-2206 plus rapamycin treatment [81]. Furthermore, the use of MK-2206 in treatment of Neurofibromatosis Type 1-related tumors has demonstrated that Akt inhibition reduces cell viability *in vitro*, but co-targeting with mTOR inhibitor, AZD8055, rendered synergistic effects *in vitro* if compared to either treatments alone. When tested in animal models, however, this same combination did not lead to any decrease in tumor growth. Further investigation suggested these results might be due to PI3K/Akt/mTOR pathway restoration after treatment-induced disruption, which has been a widely discussed point regarding Akt inhibition resistance [137].

Piovan and collaborators demonstrated that the inhibition of Akt could reverse glucocorticoid resistance in ALL. Firstly, they demonstrated that Akt-1 affects the nuclear translocation of the glucocorticoid receptor NR3C1 and impairs glucocorticoid action. The inhibition of the Akt enzyme after exposure to MK-2206 could reverse the nuclear translocation of the NR3C1 protein, thus restoring the sensitivity to glucocorticoid treatment *in vitro* and *in vivo* [56]. These findings were later corroborated by Li et al., who demonstrated that MK-2206 could reestablish or enhance prednisolone sensitivity in steroid-resistant T-ALL cells by inhibiting Akt [57]. Later, Granados and collaborators reported downregulation of Akt signaling in EGFR inhibitor-sensitive rhabdomyosarcoma (RMS) cell lines after EGFR inhibition, while the Akt activation levels continued unaltered in EGFR inhibitor-resistant cells. Treatment with MK-2206 was able to reduce cell proliferation in all sensitive and resistant cells *in vitro*, and *in vivo* assays showed tumorigenesis inhibition, enhancement of EFS, and

decreased proliferation [74]. Considering the strong impact treatment resistance has in prognosis and risk of relapse, understanding the molecular features behind this phenomenon and how to manage them is crucial to improve clinical outcomes.

Lastly, a phase I trial study enrolled 50 pediatric patients with recurrent or refractory tumors such as ependymoma, hepatocellular carcinoma, malignant glioma, and others. The study determined the maximum tolerated dose (MTD), dose-limiting toxicities (DLT), and the pharmacokinetic profile of MK-2206 administered once every other day (Schedule 1) or once weekly (Schedule 2) in 28-day cycles. It was then concluded that the recommended phase II dose is 45 mg/m^2 for the Schedule 1, different for adults which is 60 mg daily. For the second schedule, the MTD is 120 mg/m^2. In both protocols, p-Akt levels were decreased by about 6 to 8 hours after treatment. However, no objective responses were detected, having only seven patients with prolonged stabilization of disease for ≥ 3 cycles. In the first regimen, 1 of 6 patients developed grade 4 hyperglycemia and neutropenia and in the second regimen, 1 of 6 patients showed grade 3 maculopapular rash. Because of these results, the associated chemotherapy may provide an improvement of MK-2206 therapy [131].This evidence demonstrates the promising effect of MK-2206 in solid and hematological tumor cells, often presenting low values of IC_{50}. Of note, T-ALL cells showed the highest sensitivity when exposed to this compound. Also, although not consistent among different malignancies, delayed tumor growth was detected in animal models following exposure to MK-2206, as well as significant increase in EFS and survival rates in xenografts models of solid or hematological tumors. Furthermore, MK-2206 results suggested that the administration of this compound associated with other drugs have a synergistic effect, as well as it could restore the sensitivity to glucocorticoid treatment *in vitro* and *in vivo.* In conclusion, MK-2206 may be a promising therapy to overcome tumor resistance and improve clinical prognosis.

3.6.2.2 TAS-117

In 2012, TAS-117 was discovered by *Taiho Pharmaceutical Co.* as a highly potent and selective allosteric Akt inhibitor, with low nanomolar IC_{50} values for all Akt isoforms (see **Table 1**). In 2014, Mimura et al. demonstrated a relevant cytotoxic activity of this drug against multiple myeloma cells. In addition, TAS-117 decreased tumor growth *in vivo* in different xenograft models and showed a favorable pharmacokinetic profile [138,139].

Recently, a phase II study in adults with advanced solid tumors harboring PI3K/Akt mutations was completed. One patient with ovarian cancer (with *PIK3CA* E545K mutation) and two patients with breast cancer (with *PIK3CA* H1047R or Akt-1 E17K mutation) demonstrated partial and stable responses, respectively. In addition, there was not an important PFS, and it was then concluded that a combination therapy should be considered to increase antitumor activity of TAS-117 [140].
Another phase II clinical trial targeting advanced solid tumors with Akt inhibitor TAS-117 is now recruiting patients who are 12 years and older, and results are yet to be published [141].

3.6.3 INHIBITORS OF PIP_3 BINDING

This class of Akt inhibitors can interact with the phosphoinositide binding pocket of the Akt PH domain, blocking the interaction with PIP_3 at the inner membrane (**Fig. 2D** illustrates the binding of perifosine as an example). Consequently, the Akt enzyme is no longer able to be activated by PDK1, leading to signaling pathway disruption [142,143]. Although this approach may overcome the low selectivity associated with some ATP-competitive Akt inhibitors, it is important to mention that several enzymes contain a PH domain; thus, extensive assessment of selectivity should be performed to assess whether they may be susceptible to the same PIP_3 binding inhibitors [144].

3.6.3.1 PERIFOSINE (KRX-0401)

Perifosine is an oral drug that belongs to the second generation of alkylphospholipid family compounds that resulted from the substitution of the choline group of miltefosine to a piperidyl residue [145]. Its primary mechanism of action prevents Akt from binding to the cell membrane, but it is also linked to de novo synthesis of phospholipids for cell membrane, lipid metabolism and transport, induction of autophagy, shortening of telomeres in cancer cells, enhanced of ROS production, and triggering of apoptosis by the activation of cellular stress pathways [146,147]. Previous results revealed the inhibition of Akt phosphorylation in multiple myeloma cells [148], prostate carcinoma cells (PC-3), lung epithelial adenocarcinoma cells (A549) [149], and leukemia cells (U937, Jurkat and HL60) [150] after perifosine treatment.

Regarding hematologic malignancies, in 2012, Tong et al. compared the sensibility of CML and AML cells exposed to perifosine. They obtained an IC_{50} value of 4.24 μM at

48 h for pediatric lineage Kasumi-1 (AML), whereas CML cells were less sensitive to treatment even at higher doses [151]. In accordance with Levy et al. 2009, CML cells also showed decreased susceptibility to Akt inhibitor GSK690693, but the mechanism related to this phenomenon remains unclear [94,151]. The effect of perifosine in neuroblastoma cell lines and xenograft models was evaluated by Li and co-workers, who demonstrated a decrease in cell viability in increased concentrations of perifosine, reaching nearly 70% of cell death at 30 μM. Caspase-activity assays also suggested perifosine induces caspase-driven apoptosis in all analyzed cell lineages. Decreased levels of expression and p-Akt at S473 were also confirmed after treatment with perifosine. Regarding *in vivo* studies, all of the perifosine-treated mice showed: 1) a significant improvement in survival; 2) tumors either grew slowly or regressed; 3) decreased levels of p-Akt (no difference was observed in total Akt); 4) increased levels of cleaved caspase-3 in most of the tumors [72]. In 2009, Kumar et al. reported the antitumor activity of perifosine in medulloblastoma cell lines. Western blot analysis revealed inhibition of p-Akt fraction in a concentration-dependent manner and the cell viability assay demonstrated an IC_{50} value of 25 μM for both cells tested. The Akt inhibition led to increased levels of cleaved caspases-3/7/9 and cleaved PARP, and a decrease in cell cycle progression [77]. Later, Yao and collaborators studied the application of perifosine in osteosarcoma lineages. Exposure to perifosine reduced cell survival in all lines tested, especially in U2OS cells, which showed a reduction in cell viability of 25% at 1 μM and 43% at 5 μM. In accordance to Li et al 2010, they reported apoptosis activation and decreased levels of p-Akt after treatment with perifosine [78].

Regarding combined therapies, Kumar et al. demonstrated that the association of perifosine and etoposide or radiation enhanced cell death when compared to Akt inhibitor alone [77]. Similar results were published by Yao et al., who detected chemo-sensibilization to etoposide or doxorubicin when perifosine was associated with the treatment [78]. Interestingly, Li and collaborators conducted a study about the relation between Brain-Derived Neurotrophic Factor (BDNF)/TrkB signaling pathway and the development of chemo-resistance associated with the activation of the PI3K/Akt pathway in neuroblastoma. *In vitro* and *in vivo* assays showed that tumors with elevated expression of activated TrkB and Akt led to development of etoposide chemo-resistance; and previous treatment with perifosine could also restore cell sensibility to etoposide [152]. In contrast, Becher et al. 2010 did not show any significant increase

in survival when perifosine was used in combination with radiotherapy or as a single agent to treat brainstem gliomas model. One possible explanation may be due to the poor penetration of this drug through the blood brain barrier [84].

In 2016, Hua et al. demonstrated that the role of BDNF/TrkB in neuroblastoma metastasis is mediated by PI3K/Akt and MAPK pathways, as *in vitro* inhibition of PI3K (LY294002), MAPK (PD98059), Akt (perifosine), or mTOR (rapamycin) led to impairment of BDNF/TrkB-induced migration or invasion in TB3 cells. Therefore, these results suggest that the inhibition of BDNF/TrkB downstream targets may be a feasible manner to mitigate NB metastasis [68].

Papa et al. 2008, reported the highest sensibility in AMoL cells treated with perifosine, and improved antitumor effect after perifosine and etoposide cotreatment [153]. Chiarini and collaborators 2008 detected cytotoxic effect in CEM-Sensitive (IC_{50} 6.8 μM) and CEM-Resistant (characterized by overexpression of P-glycoprotein and absence of PTEN expression) (IC_{50} 19.3 μM) T-ALL cells when treated with perifosine at 24 h. P-glycoprotein carries out the efflux of several drugs to treat cancer, contributing to the development of chemoresistance. Moreover, the treatment resulted in caspase pathway activation and downregulated the expression of P-glycoprotein mRNA and protein in CEM-R cells [154].

The first phase I study ling recurrent or refractory pediatric (≤ 21 years) CNS and solid tumors treated with perifosine was concluded in 2017 (NCT00776867). The protocol treatment was performed in 5 dose levels, ranging from 25 to 125 mg/m^2/day, aiming to access safety and tolerability parameters. Almost all patients tolerated the appropriate doses and MTD could not be determined due to the saturable dose exposure at doses higher than 50 mg/m^2/day. Then, the recommended phase II dose was 50 mg/m^2/day. The majority of side effects reported was ≤ grade 2, including the reduction of leukocytes, fatigue, nausea, and hyperglycemia. Among the 23 patients treated, no objective response was observed. However, five patients showed stabilization of disease per Response Evaluation Criteria in Solid Tumors (RECIST), five had no response per International Neuroblastoma Response Criteria (INRC), and thirteen experienced disease progression per RECIST or INRC. These findings suggest that studies employing a combinatory treatment may enhance the therapeutic effects [155].

A subsequent phase I study (NCT00776867) was performed to proceed with the research of primary refractory or resistance high-risk neuroblastoma (HR-NB) patients (22 patients were ≤ 21 years; 5 patients were > 21 years). Nine of twenty-seven HR-NB patients showed progression-free survival (PFS) with an average of 54+ months from the beginning of the perifosine monotherapy. Once more, the treatment was well tolerated by all participants and no subjects had to leave the trial. These outcomes suggest that perifosine may improve the prognosis of these NB patients with a safety profile in children [156].

Matsumoto and collaborators followed the same regimen as Kushner et al. 2017 to test perifosine in nineteen Japanese patients (from 2 to 22 years) with recurrent or refractory NB in a phase I study. The most common side effects were vomiting, nausea, and diarrhea. Grade 3 or higher side effects were observed in two patients. Accordingly with modified INRC, the response rate in 18 patients was 0% and PFS was 122 days. Evaluating using RECIST, the response rate was 9% and PFS was not achieved. In both analyses, the disease control rate (DCR) was higher than 50%. The DCR contributes to a feasible introduction of perifosine monotherapy in HR-NB treatment for stabilizing disease, although combinatory therapy with perifosine may enhance the potency of the treatment [157].

Becher and collaborators 2016 described the association of perifosine with mTOR inhibitor, temsirolimus, in recurrent pediatric solid tumors in a phase I study (NCT01049841). They proposed this approach to target more than one protein from the same pathway as a tool to avoid possible compensatory mechanisms. Twenty-two patients with DIPG, high-grade glioma, medulloblastoma, ependymoma, neuroblastoma, or rhabdomyosarcoma were enrolled. Hyperglycemia, fatigue, increased AST, decreased hemoglobin, and decreased platelets were the most common side effects reported, all of them were ≤ grade 2. Grade 3 and 4 of some hematologic toxicities were observed. Twelve patients had stable disease or no response per INRC, however, there were no objective responses in this trial, suggesting that combinations with other drugs might be necessary to improve treatment success [146].

3.6.3.2 TRICIRIBINE (NSC 154020, VD-0002, VQD-002, API-2)

Triciribine is a tricyclic nucleoside, being an adenosine analog, whose synthesis was first described by Schram & Townsen in 1971 [158]. Subsequent studies investigated the effects of triciribine in the inhibition of DNA synthesis, but its exact mechanism remains unclear [159]. A screening performed by the National Cancer Institute Diversity Set detected the activity of triciribine against the Akt enzyme, and also reported a higher selectivity for Akt in contrast to other kinases, such as PI3K, PDK1, PKC, SGK1, PKA, STAT3, ERK1/2, and JNK [160]. Triciribine has been included in multiple clinical studies, but none of them involves childhood tumors.

Evangelisti at al. reported that treatment of T-ALL cells with triciribine led to inhibition of cell growth, cell cycle arrest, and triggering apoptosis by the blockage of Akt phosphorylation, leading to dephosphorylation of downstream targets of the Akt enzyme. Jurkat lineage was not sensitive to triciribine, in contrast to MOLT-4, CEM-S, and CEM-R, which IC_{50} values at 24 h were 3.5 μM, 53 μM and 18.8 μM, respectively [161]. In 2012, Huang and co-workers studied the inhibition of triciribine in Ewing sarcoma family tumors, where they established the tumorigenesis role of Akt downstream target, PRAS40 in this type of tumor. The most sensitive cell was MHH-ES-1 with an IC_{50} of 0.78 μM and the less sensitive was SK-N-MC with IC_{50} of 42.3 μM [162]. Similar results were gathered in osteosarcoma cell lines HOS and SJSA-1, when Smeester et al. observed triciribine treatment induced apoptosis and impaired cell viability and migration in both cell lines. Further assessment also demonstrated that exposure to triciribine led to decreased p-Akt and total Akt levels. Accordingly, *in vivo* studies demonstrated triciribine treatment significantly decreased tumor growth and weight in a mouse model [163].

The *in vitro* antitumor effect of triciribine was also evaluated in neuroblastoma cells and its cancer stem cell (CSC) population, which contributes to a poor prognosis of the disease. The cell viability did not decrease to less than 60% in this lineage, showing a plateau state-like at different concentrations and time periods. However, the 3D culture model showed a different profile, with decreased size and number of formed spheres in a dose-dependent manner. The cell's metastatic ability was measured by the wound healing assay, which revealed that triciribine suppressed 87% of the wound closure at 48 h in contrast to untreated cells which reached about 50%. This result suggests a

delay in disease progression and dissemination by the usage of triciribine as an adjunct therapy [164]. In line with other Akt inhibitors, a synergic effect was observed when triciribine was associated with vincristine, a standard anti-T-ALL drug [161].

Both discussed inhibitors demonstrated promising results against a wide range of tumors, triggering apoptosis and decreasing cell growth, as well as were able to induce cell cycle arrest and decrease metastasis and migration ability. As previously discussed Akt inhibitors, Perifosine and Triciribine demonstrate synergistic effect in preclinical studies if used in combination with standard protocols such as vincristine, etoposide and radiation. Furthermore, Perifosine also showed capability of reestablishing cell sensitivity to cells that had previously acquired resistance to chemotherapy. Thus, they are also suggested as a feasible way to enhance treatment efficacy. It is interesting to mention that CSC cells demonstrated higher susceptibility to Triciribine in a neuroblastoma model, which indicates encouraging results regarding tumor resistance and recurrency. Pediatric clinical trials using perifosine as monotherapy suggested a safety profile of this drug, but it is important to mention that an associated therapy is warranted to enhance the potency of treatment.

3.6.4 DUAL INHIBITORS

Another trend in cancer therapy is the design and development of dual inhibitors, which aims to inhibit two targets with a single compound. Additive and synergistic effects can be observed depending on the targets inhibited and the signaling pathways involved, consequently, enhancing efficacy and decreasing drug resistance [165]. One type of classes of compounds targeting the PI3K/AKT/mTOR pathway is the dual PI3K/mTOR inhibitors. Many of these drugs have been discovered and some of them are currently in clinical phases such as dactolisib, voxtalisib, and apitolisib.

Dactolisib (also known as BEZ235 or NVP-BEZ235, **Fig. 5**), developed by *Novartis*, is the first dual PI3K/mTOR inhibitor in clinical trials for the treatment of cancer in adult patients [166]. On the other hand, there are only preclinical studies concerning the use of dactolisib against pediatric tumors. In 2016, Hall et al. showed that dactolisib may be used as adjunct therapy with glucocorticoid treatment in T-ALL, since this dual inhibitor potentiates the cytotoxic activity of dexamethasone in *in vitro* and *in vivo* models of T-ALL was observed [167]. In 2017, Xie and collaborators demonstrated that the co-administration of dactolisib with topotecan and carboplatin in mouse model of

retinoblastoma significantly induce cell apoptosis when compared to topotecan plus carboplatin or BEZ235 treatment alone [168].

Sanofi developed the PI3K/mTOR inhibitor voxtalisib (also known as SAR245409 or XL765, **Fig. 5**), which demonstrated a safety profile and efficacy in patients with lymphoma and CLL/small lymphocytic leukemia [169]. To the best of our knowledge, no preclinical and clinical studies were carried out regarding the use of this drug in pediatric tumors.

Genentech, Inc. discovered apitolisib (GDC-0980, **Fig. 5**), another dual PI3K/mTOR inhibitor. In 2019, Al-Ghabkari et al. reported the activity of apitolisib against different pediatric leukemia cells, including KOPN8, SEM, MOLM-13, MV4;11, and TIB-202. The IC_{50} values obtained in these cell lineages ranged from 199 nM to 848 nM, and it was observed decreased levels of p-mTOR, p-Akt and p-S6. Furthermore, this study demonstrated that the cytotoxic activity of this drug is correlated with the activation of the apoptotic signaling cascade [170]. Some clinical studies with only adults are available, but the candidate showed narrow therapeutic index and the efficacy was limited by poor tolerance of patients [171,172].

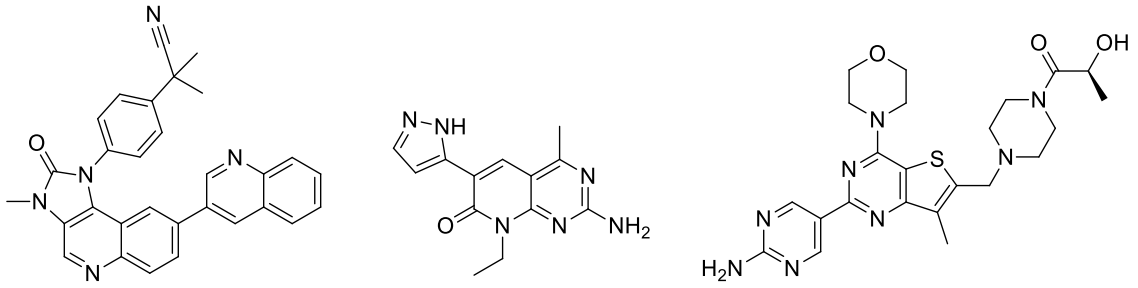


Fig. 5. Chemical structures of dual PI3K/mTOR inhibitors.

From the point of view of inhibiting Akt and other target of interest with a single compound, some drugs are in clinical trials. ONC201 (**Fig. 6**), also known as TIC10, is a potent oral drug candidate acting as an inductor of antitumoral protein named tumor necrosis factor–related apoptosis-inducing ligand (TRAIL). This drug inactivates both Akt and ERK indirectly, promoting the FOX3a translocation to the nucleus and, consequently, promoting TRAIL gene upregulation and triggering apoptosis [173,174]. Prabhu et al. demonstrated the efficacy of this drug against several cancer cell lines,

including pediatric lineages, with low micromolar range (GI_{50} of 1 to 8 μM). Further experiments with this drug proved its extended antitumoral efficacy, oral bioavailability, and safety profile [175,176]. Then, ONC201 is currently being used in several clinical trials in adults and child tumors such as glioma (NCT03416530, NCT05009992, NCT04617002, NCT02525692), and neuroendocrine tumors (NCT03034200) [177–181].

Another dual drug candidate targeting Akt are AT13148 and M2698. In recent years, AT13148 (**Fig. 6**), a potent dual Akt/ROCK inhibitor, was enrolled in a first phase in adult with solid tumors. However, no clinical response was observed, and further clinical trials are discouraged [182]. M2698 (**Fig. 6**), a dual p70S6K/Akt inhibitor was tested only in adult patients with advanced cancer. A phase I study demonstrated a well tolerability and promising results when the patients were treated with M2698 as monotherapy or combined with trastuzumab or tamoxifen [183]. It is worth mentioning that it was not found preclinical and clinical studies with AT13148 and M2698 in pediatric tumors.

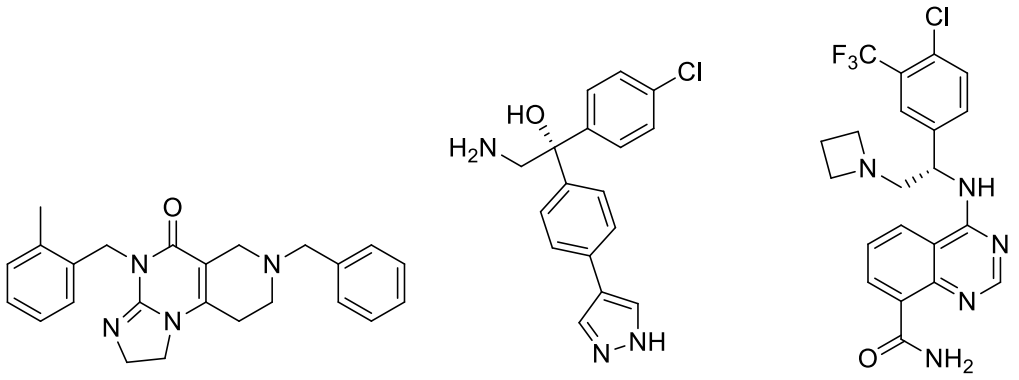


Fig. 6. Chemical structures of Akt/ERK inhibitor (ONC201), Akt/ROCK inhibitor (AT13148), and Akt/p70S6K (M2698).

The discovery of dual inhibitors in the last years was an important milestone in pharmacology. In addition, an analysis performed by Ramsay and collaborators showed that there was an increase multi-target drugs approved by FDA [165]. Observing the studies presented here, dual inhibitors have demonstrated a promising activity in most cases. A thorough investigation on simultaneous targeting should be carried out to reach maximum therapeutic effect.

3.7 FUTURE PERSPECTIVES AND CHALLENGES

Growing understanding about tumor biology allowed great improvement in cancer management, as well as the development of new chemotherapy drugs over the last decades. However, malignancies still account for almost ten million deaths annually [1], and clinical research for pediatric tumors are still scarce when compared to adult cancers. Regarding the development of new drugs and clinical protocols, multiple new PI3K/Akt/mTOR signaling pathway inhibitors have been designed to improve outcomes in oncology, since this pathway is strongly suggested as a key factor in multiple processes such as tumor progression and metastasis. A significant amount of data about PI3K/Akt/mTOR inhibitors in pediatric tumors is currently available, but still, no bibliography review was ever performed on this topic. Here, we assessed ten years of bibliography and discussed the use of Akt inhibitors in cancer patients from 0 to 19 years old, who may benefit from individualized clinical protocols using this targeted therapy.

Although they target the same kinase, distinct classes of Akt inhibitors may have different uses in the clinic, as they disrupt the PI3K/Akt/mTOR pathway in different steps of the signaling. Thus, availability of different Akt inhibitors in clinical protocols might be useful to individualized cancer therapy based on molecular and metabolic features of tumors. For instance, mutations in residues of Akt may lead to a decreasing inhibitor selectivity and/or affinity. As reported by Lazaro et al., the stability of PH-in conformation is supported by E17 interacting, through the salt bridge, with R273. In the presence of the E17K mutation, the enzyme adopts PH-out conformation and the active site is exposed, promoting the binding of ATP-competitive inhibitor preferentially. Since allosteric inhibitors require Akt closed conformation to bind, these conformational changes led by mutations may impair the docking of specific inhibitors, and consequently loss of their activity [17]. We previously mentioned a patient case comparing the activity of ATP-competitive inhibitor and allosteric inhibitor through the absence of Akt-1's PH domain, in which only the first class had therapeutic effect and could decrease tumor burden [120]. Of note, exposure to Akt ATP-competitive inhibitors has already been correlated to increased levels of phosphorylated Akt. This phenomenon of hyperphosphorylation apparently happens due to compensation mechanisms on the pathway, possibly to maintain cell homeostasis [91,94,98,184], in

which the S473 and T308 residues (as well as S474/T309 and S472/T305 in Akt-2 and Akt-3, respectively) may keep phosphorylated after binding of inhibitors into the PH-out conformation, in a time- and concentration-dependent manner. However, some studies suggested that such hyperphosphorylation occurs via Akt conformational change induced by ATP-competitive inhibitors. Then, the phosphorylated sites become unavailable to be dephosphorylated by phosphatases [86,185]. Up to this moment, no studies demonstrated allosteric inhibitors and inhibitors of PIP_3 binding could promote increased levels of p-Akt, due to the type of interaction with the enzyme [72,85,186,187]. Considering hyperphosphorylation of Akt could reverse the therapeutic effects of the enzyme's inhibition after treatment retrieval, it is worth mentioning that this topic deserves to be deeply assessed in future studies.

The interest in targeting Akt as a new cancer treatment has also increased the need for deeper knowledge about its isoforms. The development of Akt inhibitors with high degree of specificity requires a thorough examination of residues that interact with the ligand, since the Akt isoforms are highly conserved. Pan Akt inhibitors, for instance, can interact with different Akt isoforms and occasionally with other enzymes. Thus, they might have a broader range of therapeutic use, but it is crucial to bear in mind that they can also lead to higher toxicity if compared to specific inhibitors. On the other hand, although specific inhibitors render less adverse effects, their spectrum of action may be much narrower. In addition, it is known that Akt and other kinases share ATP-binding sites with >70% homology such as PKA, PKC, and Aurora A [87]. These similar features impair the development of highly specific Akt inhibitors, and ATP-competitive inhibitors may also bind multiple other kinases in higher doses. Furthermore, despite their high sequence homology, it is now known that Akt-1, 2 and 3 have differently expressed, and their functions can be quite distinct depending on the context [10–12]. In fact, recent studies have assessed differential expression and genetic alterations of Akt isoforms across multiple cancer lineages and demonstrate great heterogeneity among cancer groups [19,113]. Increasing knowledge on this topic may improve therapeutic effects and clinical outcomes in cancer treatment, as precise understanding of Akt isoform-specific signaling will lead to individualized targeting approach in the clinic. High degree of homology between isoforms, however, is still a major impairment to the design of highly isoform-specific Akt inhibitors. Also, despite recent studies, the effect on these isoforms in different tumor stages and subtypes still

needs further clarification, and their possible compensatory roles could represent a difficulty to evaluate the impact of specific inhibition of Akt isoforms.

According to preclinical and clinical results here reviewed, the association of Akt inhibitors with other protocols may maximize the potential of these compounds and improve clinical benefits. This approach is supported by the fact that the PI3K/Akt/mTOR signaling pathway is deeply connected to other important signaling mechanisms, suggesting Akt activity could be an escape gate to chemotherapy-induced cell death. Thus, the use of Akt inhibitors as adjuvant therapy to standard protocols such as etoposide and cisplatin seems to be a promising approach to improve clinical outcomes in treatment-resistant tumors. Accordingly, co-targeting of Akt and other proteins such as mTOR, MEK, and MAPK, also demonstrated better results if compared to each inhibition alone [80,81,98]. Taken together, these results suggest that Akt inhibition could rather be used as a tool to increase cytotoxic effect of standardized clinical protocols against childhood tumors, which is crucial considering the great impact of recurrency and tumor resistance in overall survival rates. Of note, treatment with Akt inhibitors was well tolerated by the majority of pediatric patients. The main adverse effects throughout several studies were grade 1 and 2 fatigue, rash, fever, vomiting and diarrhea [105,120,157]. Some grade 3 and 4 toxicities, including thrombocytopenia and neutropenia, were observed in a significant number of patients and in different studies [105,106,146,155]. Although uncommon, some dose-limiting toxicities also occurred (grade 4) hyperuricemia at dose level 4 [155], mucositis and colitis [104], and diarrhea [107].

Additionally, antibody-based therapies have been pointed out as promising and successful new approaches to target cancer cells. Relying on the expression of tumor-specific or tumor-associated antigens, these immunotherapies have revolutionized cancer treatment by targeting tumor cells and minimizing damage on the healthy tissue [188]. Several immunotherapies have already been approved by the FDA for use in oncology, many of them being alternative approaches to patients with relapsed or refractory tumors [189]. More recently, the use of antibodies has been optimized and complex therapies using bispecific antibodies and antibody-drug conjugates have been developed [190]. Following the trend of cancer immunotherapy, combined therapies of monoclonal antibodies with Akt inhibitors could also have a great impact on tumor

growth and progression. Noteworthy, Akt inhibitors such as Afuresertib, Ipatasertib, and MK-2206 have been included in different clinical trials combining Akt inhibition with monoclonal antibodies to treat advanced, relapsed, and resistant tumors in adult patients [191–194]. Although most of these studies are still recruiting, some promising results have already been published [195–197]. For example, two phase I trials have evaluated the combination of MK-2206 and trastuzumab, which targets the human epidermal growth factor receptor 2 (HER2). The first study treated 31 patients with HER2+ breast and gastroesophageal cancer. The results demonstrated significant response, or even stable disease, in patients who had cancer progression on multiple prior trastuzumab-based therapies. Further assessment indicated MK-2206's pharmacokinetics were not affected by the antibody [198]. The second study, posted in 2010, included 16 patients with HER2-oxerexpressing solid tumors. The combination presented significant clinical activity and was well tolerated [199]. Additionally, a phase II trial including 28 patients with relapsed and refractory CLL combined Afuresertib with Ofatumumab, an anti-CD20 monoclonal antibody. In general, the treatment was well-tolerated and moderate activity on tumor progression was observed [200]. Although none of these trials included children nor adolescents, gathered results support Akt-targeted therapy could also be adjuvant to antibody-based treatments, and further studies are encouraged to assess this specific approach on pediatric tumors.

Another strategy to target Akt consists of its degradation by protein degraders. This approach, named targeted protein degradation (TPD), has been used by many groups both in academic and industry fields over the last two decades. Briefly, there are two ligands connected by a linker, affording a heterobifunctional molecule. One of the moieties binds to the target of interest and the other recruits E3 ubiquitin ligase, a protein responsible for the induction of ubiquitination of the target and its subsequent proteolysis by the ubiquitin-proteasome system. Thus, the pharmacological effect of this approach can be observed for longer if compared to reversible inhibitors because the cell metabolism depends on the re-synthesis of the protein of interest. Consequently, drug administration can be done in lower doses [201]. In 2020, You et al. discovered a pan-Akt degrader, named INY-03-041, linking GDC-0068 and lenalidomide, a recruiter of the E3 ubiquitin ligase substrate adaptor cereblon (CRBN). Importantly, it was observed that the introduction of the linker did not impact the inhibitory activity against the Akt isoforms if compared to GDC-0068 alone.

Subsequently, INY-03-041 acted as a protein degrader of Akt isoforms in a dose-dependent manner, showing its maximum potency using concentrations ranging from 100 to 250 nM. It is important mention that this study demonstrated the lenalidomide moiety as responsible for the CRBN recruitment, and consequently degradation of Akt. Regarding the anti-proliferative effects, it was demonstrated that the protein degrader is 8 to 14-fold more potent if compared to GDC-0068's growth rate inhibition (GR_{50}). Besides, lower doses of INY-03-041 led to reduced levels of downstream targets such as pPRAS40, pGSK3β, and pS6 when compared to higher doses of GDC-0068. Lastly, the levels of Akt and its downstream targets were not restored after 96 hours, even in the absence of INY-03-041 treatment, reinforcing the extended cellular effects of protein degraders [202]. In 2021, Xu and colleagues described MS21 as a potent and selective Akt degrader. They synthesized this heterobifunctional ligand using the AZD5363 and a recruiter of von Hippel-Lindau (VHL) E3 ligase, connected by a linker. MS21 decreased levels of Akt and disrupt the PI3K/Akt/mTOR signaling pathway over 24 hours in tumoral cells. Of note, cells treated with AZD5363 alone were able to restore Akt downstream signaling in this period of time, and *in vivo* experiments demonstrated increased tumor growth inhibition in MS21-treated animals [203]. In subsequent research, during a structure-activity relationship study, different linkers, E3 ligase recruiters, and Akt inhibitors, including GDC-0068 and GSK690693, were discovered as a promissory Akt degraders. Two compounds, named MS98 and MS170, were selected to be further evaluated and demonstrated great bioavailability in mice [204]. Considering these promising results, Akt degraders have the potential to become a therapy against cancer, and the activity of this type of compounds against pediatric tumors also deserves to be investigated.

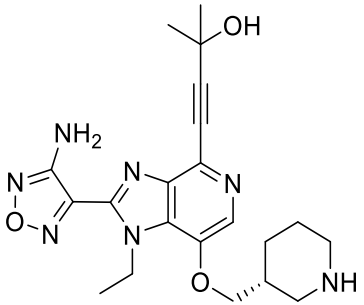
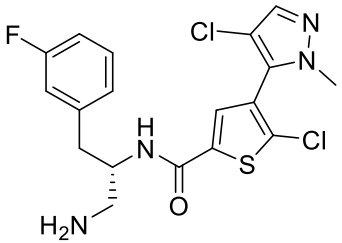
Lastly, several Akt inhibitors, such as ARQ-092, ARQ-751, BAY1125976, and RX-0201, have already been assessed in adult tumors, but their effect in pediatric malignancies is yet to be evaluated [144,205–207]. Thus, they have not been included in the present work. Noteworthy, miltefosine, also known as hexadecylphosphocholine or Impavido, is the first Akt inhibitor to be approved in the clinic in very specific cases. It prevents phosphorylation of S473 residue [208], and its intravenous administration leads to hemolytic effect. Thus, it is limited to oral and topical application, but oral administration may have nausea and vomiting as dose-limiting effects [209]. Regarding its topical application, miltefosine has been approved by several member states of the

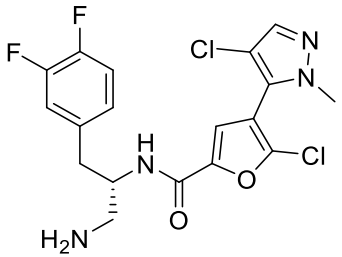
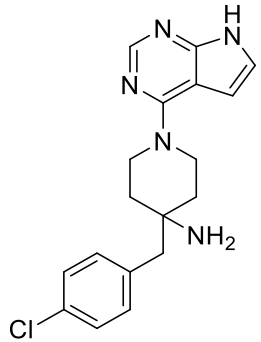
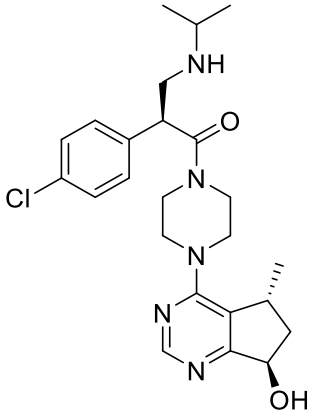
European Union for the treatment of breast cancer cutaneous metastasis [210,211]. It also demonstrated activity against *Leishmania,* and it was approved as the first oral therapy for this disease [212,213].

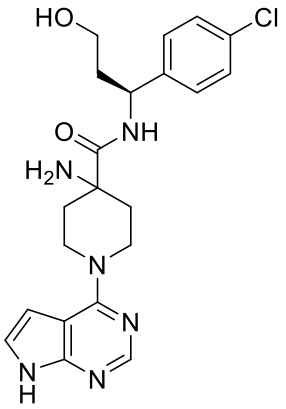
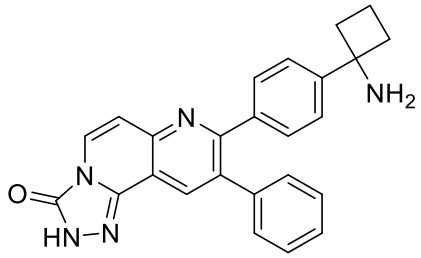
Considering gathered data up to this moment, PI3K/Akt/mTOR inhibitors show great promise in the treatment of pediatric malignancies and we strongly encourage the deep assessment of other Akt inhibitors in panels of childhood tumors. Furthermore, considering the PI3K/Akt/mTOR pathway is not disrupted in all cancers, not all patients may benefit from this targeted therapy. Of note, mutations that lead to Akt and HER2 amplification, PTEN deficiency, or PI3K dysregulation, for instance, have been correlated to increased susceptibility to Akt inhibition. Thus, genetic screening in patients could be performed to search for these specific alterations in order to delineate an individualized treatment, significantly increasing treatment success rates [1,214–216].

3.8 CONCLUSIONS

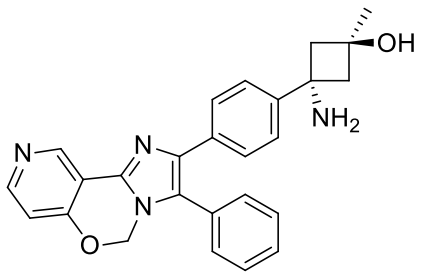
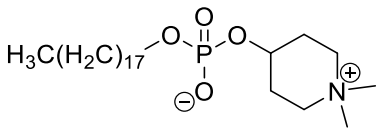
In this review, we described the three main classes of Akt inhibitors, considering their specific mechanisms of action, and their cytotoxic effect in solid and hematologic pediatric tumors. Since Akt plays a major role in tumor progression, targeting this enzyme has been considered an attractive approach to be used as an anti-cancer therapy. Taken together, the points here discussed corroborate the importance of developing different classes of Akt inhibitors as targeted therapy for pediatric tumors. Furthermore, the association of these compounds with other protein inhibitors, as well as chemotherapy drugs, has been consistently suggested as a viable approach to improve clinical outcomes in treatment-resistant tumors. Since patients may need distinct approaches of targeted therapy due to mutations in the PI3K/Akt/mTOR pathway or in Akt enzyme itself, a broad spectrum of inhibition options could improve clinical outcomes in treatment-resistant patients. We also suggest that screening of genetic mutations in Akt isoforms genes could be a feasible tool to characterize genomic abnormalities, leading to improved chance of clinical success by mitigating treatment resistance and improving individualized targeted therapy.

Class	Compound	Structure	Company	IC_{50} for Akt enzyme	Study phase	Type of tumor	References
ATP-competitive	GSK690693		GlaxoSmithKline	Akt-1 2 nmol/L, Akt-2 13 nmol/L, Akt-3 9 nmol/L [91]	Pre-clinical	ALL, AML, CLL, CML, Burkitt lymphoma, NHL, Hodgkin lymphoma	[94]
						Rhabdoid, Wilms, Ewing, alveolar rhabdomyosarcoma, medulloblastoma, ependymoma, glioblastoma, neuroblastoma, osteosarcoma, ALL	[95]
	GSK2110183 (Afuresertib)		GlaxoSmithKline	Akt-1 Ki 0.08 nM, Akt-2 2 nM, Akt-3 2.6 nM) [98]	Pre-clinical	ALL, AML, Burkitt's Lymphoma, Non-Hodgkin's Lymphoma, Head & Neck, Liver, Neuroblastoma, Sarcoma	[98]

GSK2141795 (Uprosertib)		GlaxoSmithKline	Akt-1 Ki 0.066 nM, Akt-2 1.4 nM, Akt-3 1.5 nM) [98]	Pre-clinical	ALL, AML, Burkitt's Lymphoma, Non-Hodgkin's Lymphoma, Head & Neck, Liver, Neuroblastoma, Sarcoma	[98]
CCT128930		Cancer Research UK Centre for Cancer Therapeutics, and Astex Therapeutics	Akt-2 6 nM [108], Akt-1 and Akt-3 IC_{50} values are not available	Pre-clinical	Hepatoblastoma	[109]
					Neuroblastoma	[80]
					Osteosarcoma	[110]
GDC-0068 (Ipatasertib)		Array BioPharma/Genentech Inc.	Akt-1 5 nM, Akt-2 18 nM, Akt-3 8 nM [111]	Individualized clinical trial	Epithelioid neoplasm	[120]
AZD5363 (Capivasertib)		AstraZeneca	Akt-1 3 nM, Akt-2 8 nM, Akt-3 8 nM [121]	Pre-clinical	Medulloblastoma	[67]

						Solid and hematological malignancies (large panel tested)	[128]
						T-ALL	[30]
						Hepatocellular carcinoma	[129]
Allosteric	MK-2206		Merck	Akt-1 8, Akt-2 12, and Akt-3 65 nM [130]	Pre-clinical	Rhabdoid, Wilms, Ewing, alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, medulloblastoma, glioblastoma, neuroblastoma, osteosarcoma, ALL AML, non-hodgkin lymphoma, anaplastic large-cell lymphoma	[71]
						Neuroblastoma	[81]
						Neuroblastoma	[136]
						ALL	[56]

	Osteosarcoma	[134]
	B-pre ALL	[82]
	Neuroblastoma	[132]
	T-ALL	[57]
	T-ALL	[83]
	Myeloproliferative neoplasms	[73]
	Rhabdoid tumor	[133]
	Rhabdomyosarcoma	[74]
	Neuroblastoma	[135]
	Neurofibromatosis Type 1	[137]
Phase I	Malignant glioma, Ependymoma, Non-rhabdoid soft tissue sarcoma, Rhabdomyosarcoma, Hepatocellular	[131]

						carcinoma, Osteosarcoma, Ewing sarcoma, PNET (including medulloblastoma), Neuroblastoma, Acinar cell carcinoma, Rhabdoid sarcoma, Anaplastic carcinoma, Malignant paraganglioma, Wilms tumor, Adrenal cortical carcinoma, Mixed germ cell tumor, Oligodendroglioma	
	TAS-117		Taiho Pharmaceutical Co.	Akt-1 4.8, Akt-2 1.6, and Akt-3 44 nM [138]	Phase II	Advanced solid tumors	[141]
Inhibitors of PIP_3 binding	Perifosine		Keryx Biopharmaceuticals and Aeterna Zentaris	Not available	Preclinical	AMoL	[153]
						T-ALL	[153]
						Medulloblastoma	[77]

	Brainstem glioma	[84]
	Neuroblastoma	[72]
	Neuroblastoma	[152]
	AML	[151]
	Osteosarcoma	[78]
	Neuroblastoma	[68]
Phase I	Recurrent or refractory neuroblastoma	[157]
Phase I	DIPG, high-grade glioma, medulloblastoma, ependymoma, neuroblastoma, Wilms tumor, and Ewing sarcoma	[155]
Phase I	High-risk neuroblastoma	[217]
Phase I	DIPG, high-grade glioma, medulloblastoma, ependymoma, neuroblastoma, and	[146]

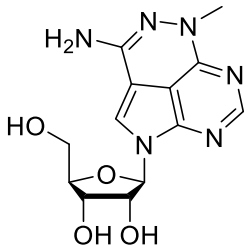
					rhabdomyosarcoma	
Triciribine		[158]	Akt-1 130 nM [218], Akt-2 and Akt-3 IC_{50} values are not available	Preclinical	T-ALL	[161]
					Ewing sarcoma	[162]
					Neuroblastoma	[164]
					Osteosarcoma	[163]

Table 1 - Akt inhibitors tested in pediatric tumors.

Declarations of interest: None

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CRediT authorship contribution statement

Conceptualization, B.T., I.F., R.R., and S.A; Writing—original draft preparation, B.T. and I.F.; Writing—review and editing, R.R. and S.A. All authors have read and agreed to the published version of the manuscript.

3.9 REFERENCES – CHAPTER I

[1] World Health Organization, CureAll framework: WHO global initiative for childhood cancer: increasing access, advancing quality, saving lives., World Heal. Organ. (2021). https://apps.who.int/iris/handle/10665/347370.

[2] World Health Organization, Global health estimates 2019: deaths by cause, age, sex, by country and by region, 2000–2019, 2020.

[3] E. Ward, C. DeSantis, A. Robbins, B. Kohler, A. Jemal, Childhood and adolescent cancer statistics, 2014, CA. Cancer J. Clin. 64 (2014) 83–103. https://doi.org/10.3322/caac.21219.

[4] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2022, CA. Cancer J. Clin. 72 (2022) 7–33. https://doi.org/10.3322/caac.21708.

[5] A.J. Galligan, Childhood Cancer Survivorship and Long-Term Outcomes, Adv. Pediatr. 64 (2017) 133–169. https://doi.org/10.1016/j.yapd.2017.03.014.

[6] L. Diller, E.J. Chow, J.G. Gurney, M.M. Hudson, N.S. Kadin-Lottick, T.I. Kawashima, W.M. Leisenring, L.R. Meacham, A.C. Mertens, D.A. Mulrooney, Chronic disease in the Childhood Cancer Survivor Study cohort: a review of published findings, J. Clin. Oncol. 27 (2009) 2339–2355. https://doi.org/10.1200/JCO.2008.21.1953.

[7] F. Erdmann, L.E. Frederiksen, A. Bonaventure, L. Mader, H. Hasle, L.L. Robison, J.F. Winther, Childhood cancer: Survival, treatment modalities, late effects and improvements over time, Cancer Epidemiol. 71 (2021) 101733. https://doi.org/10.1016/j.canep.2020.101733.

[8] B.D. Manning, A. Toker, AKT/PKB Signaling: Navigating the Network, Cell. 169 (2017) 381–405. https://doi.org/10.1016/j.cell.2017.04.001.

[9] C.-Y. Chen, J. Chen, L. He, B.L. Stiles, PTEN: Tumor Suppressor and Metabolic Regulator, Front. Endocrinol. (Lausanne). 9 (2018). https://doi.org/10.3389/fendo.2018.00338.

[10] N. Hinz, M. Jücker, Distinct functions of AKT isoforms in breast cancer: a comprehensive review, Cell Commun. Signal. 17 (2019) 154. https://doi.org/10.1186/s12964-019-0450-3.

[11] M. Sharma, C.S. Dey, Role of Akt isoforms in neuronal insulin signaling and resistance, Cell. Mol. Life Sci. 78 (2021) 7873–7898. https://doi.org/10.1007/s00018-021-03993-6.

[12] H. Yu, T. Littlewood, M. Bennett, Akt isoforms in vascular disease, Vascul. Pharmacol. 71 (2015) 57–64. https://doi.org/10.1016/j.vph.2015.03.003.

[13] S.E. Degan, I.H. Gelman, Emerging Roles for AKT Isoform Preference in Cancer

Progression Pathways, Mol. Cancer Res. 19 (2021) 1251–1257. https://doi.org/10.1158/1541-7786.MCR-20-1066.

[14] N. Roy, D. Bordoloi, J. Monisha, G. Padmavathi, J. Kotoky, R. Golla, A. Kunnumakkara, Specific Targeting of Akt Kinase Isoforms: Taking the Precise Path for Prevention and Treatment of Cancer, Curr. Drug Targets. 18 (2017) 421–435. https://doi.org/10.2174/1389450117666160307145236.

[15] B. Ma, H. Shao, X. Jiang, Z. Wang, Chuanyue, Cary, Wu, D. Whaley, A. Wells, Akt isoforms differentially provide for chemoresistance in prostate cancer, Cancer Biol. Med. 19 (2021) 635–650. https://doi.org/10.20892/j.issn.2095-3941.2020.0747.

[16] A.E. Leroux, J.O. Schulze, R.M. Biondi, AGC kinases, mechanisms of regulation and innovative drug development, Semin. Cancer Biol. 48 (2018) 1–17. https://doi.org/10.1016/j.semcancer.2017.05.011.

[17] G. Lazaro, E. Kostaras, I. Vivanco, Inhibitors in AKTion: ATP-competitive vs allosteric, Biochem. Soc. Trans. 48 (2020) 933–943. https://doi.org/10.1042/BST20190777.

[18] L. Bozulic, B.A. Hemmings, PIKKing on PKB: regulation of PKB activity by phosphorylation, Curr. Opin. Cell Biol. 21 (2009) 256–261.

[19] N. Jiang, Q. Dai, X. Su, J. Fu, X. Feng, J. Peng, Role of PI3K/AKT pathway in cancer: the framework of malignant behavior, Mol. Biol. Rep. 47 (2020) 4587–4629. https://doi.org/10.1007/s11033-020-05435-1.

[20] T.L. Yuan, L.C. Cantley, PI3K pathway alterations in cancer: variations on a theme, Oncogene. 27 (2008) 5497–5510. https://doi.org/10.1038/onc.2008.245.

[21] J. Yang, J. Nie, X. Ma, Y. Wei, Y. Peng, X. Wei, Targeting PI3K in cancer: mechanisms and advances in clinical trials, Mol. Cancer. 18 (2019) 26. https://doi.org/10.1186/s12943-019-0954-x.

[22] P. Rotwein, Variation in Akt protein kinases in human populations, Am. J. Physiol. Integr. Comp. Physiol. 313 (2017) R687–R692. https://doi.org/10.1152/ajpregu.00295.2017.

[23] J.D. Carpten, A.L. Faber, C. Horn, G.P. Donoho, S.L. Briggs, C.M. Robbins, G. Hostetter, S. Boguslawski, T.Y. Moses, S. Savage, M. Uhlik, A. Lin, J. Du, Y.-W. Qian, D.J. Zeckner, G. Tucker-Kellogg, J. Touchman, K. Patel, S. Mousses, M. Bittner, R. Schevitz, M.-H.T. Lai, K.L. Blanchard, J.E. Thomas, A transforming mutation in the pleckstrin homology domain of AKT1 in cancer, Nature. 448 (2007) 439–444. https://doi.org/10.1038/nature05933.

[24] M. OU, Z. SUN, P. ZHU, G. SUN, Y. DAI, Proteus syndrome: A case report and review of the literature, Mol. Clin. Oncol. 6 (2017) 381–383. https://doi.org/10.3892/mco.2017.1140.

[25] M. Lek, K.J. Karczewski, E. V. Minikel, K.E. Samocha, E. Banks, T. Fennell, A.H. O'Donnell-Luria, J.S. Ware, A.J. Hill, B.B. Cummings, T. Tukiainen, D.P. Birnbaum, J.A. Kosmicki, L.E. Duncan, K. Estrada, F. Zhao, J. Zou, E. Pierce-Hoffman, J. Berghout, D.N. Cooper, N. Deflaux, M. DePristo, R. Do, J. Flannick, M. Fromer, L. Gauthier, J. Goldstein, N. Gupta, D. Howrigan, A. Kiezun, M.I. Kurki, A.L. Moonshine, P. Natarajan, L. Orozco, G.M. Peloso, R. Poplin, M.A. Rivas, V. Ruano-Rubio, S.A. Rose, D.M. Ruderfer, K. Shakir, P.D. Stenson, C. Stevens, B.P. Thomas, G. Tiao, M.T. Tusie-Luna, B. Weisburd, H.H. Won, D. Yu, D.M. Altshuler, D. Ardissino, M. Boehnke, J. Danesh, S. Donnelly, R. Elosua, J.C. Florez, S.B. Gabriel, G. Getz, S.J. Glatt, C.M. Hultman, S. Kathiresan, M. Laakso, S. McCarroll, M.I. McCarthy, D. McGovern, R. McPherson, B.M. Neale, A. Palotie, S.M. Purcell, D. Saleheen, J.M. Scharf, P. Sklar, P.F. Sullivan, J. Tuomilehto, M.T. Tsuang, H.C. Watkins, J.G. Wilson, M.J. Daly, D.G. MacArthur, H.E. Abboud, G. Abecasis, C.A. Aguilar-Salinas, O. Arellano-Campos, G. Atzmon, I. Aukrust, C.L. Barr, G.I. Bell, S. Bergen, L. Bjørkhaug, J. Blangero, D.W. Bowden, C.L. Budman, N.P. Burtt, F. Centeno-Cruz, J.C. Chambers, K. Chambert, R. Clarke, R. Collins, G. Coppola, E.J. Córdova, M.L. Cortes, N.J. Cox, R. Duggirala, M. Farrall, J.C. Fernandez-Lopez, P. Fontanillas, T.M. Frayling, N.B. Freimer, C. Fuchsberger, H. García-Ortiz, A. Goel, M.J. Gómez-Vázquez, M.E. González-Villalpando, C. González-Villalpando, M.A. Grados, L. Groop, C.A. Haiman, C.L. Hanis, A.T. Hattersley, B.E. Henderson, J.C. Hopewell, A. Huerta-Chagoya, S. Islas-Andrade, S.B. Jacobs, S. Jalilzadeh, C.P. Jenkinson, J. Moran, S. Jiménez-Morale, A. Kähler, R.A. King, G. Kirov, J.S. Kooner, T. Kyriakou, J.Y. Lee, D.M. Lehman, G. Lyon, W. MacMahon, P.K. Magnusson, A. Mahajan, J. Marrugat, A. Martínez-Hernández, C.A. Mathews, G. McVean, J.B. Meigs, T. Meitinger, E. Mendoza-Caamal, J.M. Mercader, K.L. Mohlke, H. Moreno-Macías, A.P. Morris, L.A. Najmi, P.R. Njølstad, M.C. O'Donovan, M.L. Ordóñez-Sánchez, M.J. Owen, T. Park, D.L. Pauls, D. Posthuma, C. Revilla-Monsalve, L. Riba, S. Ripke, R. Rodríguez-Guillén, M. Rodríguez-Torres, P. Sandor, M. Seielstad, R. Sladek, X. Soberón, T.D. Spector, S.E. Tai, T.M. Teslovich, G. Walford, L.R. Wilkens, A.L. Williams, Analysis of protein-coding genetic variation in 60,706 humans, Nature. 536 (2016) 285–291. https://doi.org/10.1038/nature19057.

[26] T. Tian, X. Li, J. Zhang, mTOR signaling in cancer and mTOR inhibitors in solid tumor targeting therapy, Int. J. Mol. Sci. 20 (2019) 755. https://doi.org/10.3390/ijms20030755.

[27] A. Markham, Copanlisib: first global approval, Drugs. 77 (2017) 2057–2062. https://doi.org/10.1007/s40265-017-0838-6.

[28] A.K. Gopal, B.S. Kahl, S. de Vos, N.D. Wagner-Johnston, S.J. Schuster, W.J. Jurczak, I.W. Flinn, C.R. Flowers, P. Martin, A. Viardot, K.A. Blum, A.H. Goy, A.J. Davies, P.L. Zinzani, M. Dreyling, D. Johnson, L.L. Miller, L. Holes, D. Li, R.D. Dansey, W.R. Godfrey, G.A. Salles, PI3Kδ Inhibition by Idelalisib in Patients with Relapsed Indolent Lymphoma, N. Engl. J. Med. 370 (2014) 1008–1018. https://doi.org/10.1056/NEJMoa1314583.

[29] M. Ghomlaghi, A. Hart, N. Hoang, S. Shin, L.K. Nguyen, Feedback, Crosstalk and Competition: Ingredients for Emergent Non-Linear Behaviour in the

PI3K/mTOR Signalling Network, Int. J. Mol. Sci. 22 (2021) 6944. https://doi.org/10.3390/ijms22136944.

[30] H. Khoshamooz, S. Kaviani, A. Atashi, S.H.M. Hassankiadeh, Combination Effect of Notch1 and PI3K/AKT/mTOR Signaling Pathways Inhibitors on T-ALL Cell Lines, Int. J. Hematol. Stem Cell Res. 14 (2020) 99–109.

[31] A. Sathe, G. Chalaud, I. Oppolzer, K.Y. Wong, M. von Busch, S.C. Schmid, Z. Tong, M. Retz, J.E. Gschwend, W.A. Schulz, R. Nawroth, Parallel PI3K, AKT and mTOR inhibition is required to control feedback loops that limit tumor therapy, PLoS One. 13 (2018) e0190854. https://doi.org/10.1371/journal.pone.0190854.

[32] B. Faiman, M. Faiman, Living with hematologic cancer: recommendations, solutions, Cleve. Clin. J. Med. 84 (2017) 528–534. https://doi.org/10.3949/ccjm.84a.15159.

[33] A. Gutierrez, T. Sanda, R. Grebliunaite, A. Carracedo, L. Salmena, Y. Ahn, S. Dahlberg, D. Neuberg, L.A. Moreau, S.S. Winter, R. Larson, J. Zhang, A. Protopopov, L. Chin, P.P. Pandolfi, L.B. Silverman, S.P. Hunger, S.E. Sallan, A.T. Look, High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia, Blood. 114 (2009) 647–650. https://doi.org/10.1182/blood-2009-02-206722.

[34] M. Pfeifer, M. Grau, D. Lenze, S.-S. Wenzel, A. Wolf, B. Wollert-Wulf, K. Dietze, H. Nogai, B. Storek, H. Madle, PTEN loss defines a PI3K/AKT pathway-dependent germinal center subtype of diffuse large B-cell lymphoma, Proc. Natl. Acad. Sci. 110 (2013) 12420–12425. https://doi.org/10.1073/pnas.1305656110.

[35] M. Frenquelli, N. Caridi, E. Antonini, F. Storti, V. Viganò, M. Gaviraghi, M. Occhionorelli, S. Bianchessi, L. Bongiovanni, A. Spinelli, The WNT receptor ROR2 drives the interaction of multiple myeloma cells with the microenvironment through AKT activation, Leukemia. 34 (2020) 257–270. https://doi.org/10.1038/s41375-019-0486-9.

[36] V. Bardelli, S. Arniani, V. Pierini, D. Di Giacomo, T. Pierini, P. Gorello, C. Mecucci, R. La Starza, T-Cell Acute Lymphoblastic Leukemia: Biomarkers and Their Clinical Usefulness, Genes (Basel). 12 (2021) 1118. https://doi.org/10.3390/genes12081118.

[37] V. Sanchez, C. Nichols, H. Kim, E. Gang, Y.-M. Kim, Targeting PI3K Signaling in Acute Lymphoblastic Leukemia, Int. J. Mol. Sci. 20 (2019) 412. https://doi.org/10.3390/ijms20020412.

[38] V. Ramakrishnan, S. Kumar, PI3K/AKT/mTOR pathway in multiple myeloma: from basic biology to clinical promise, Leuk. Lymphoma. 59 (2018) 2524–2534. https://doi.org/10.1080/10428194.2017.1421760.

[39] C.M. Niemeyer, JMML genomics and decisions, Hematology. 2018 (2018) 307–312. https://doi.org/10.1182/asheducation-2018.1.307.

[40] S.E. Conneely, R.E. Rau, The genomics of acute myeloid leukemia in children, Cancer Metastasis Rev. 39 (2020) 189–209. https://doi.org/10.1007/s10555-020-09846-1.

[41] D. Qian, K. Chen, H. Deng, H. Rao, H. Huang, Y. Liao, X. Sun, S. Lu, Z. Yuan, D. Xie, MicroRNA-374b suppresses proliferation and promotes apoptosis in T-cell lymphoblastic lymphoma by repressing AKT1 and Wnt-16, Clin. Cancer Res. 21 (2015) 4881–4891. https://doi.org/10.1158/1078-0432.CCR-14-2947.

[42] G. Zhang, X. Gao, H. Zeng, Y. Li, X. Guo, Virosecurinine induces apoptosis in human leukemia THP-1 cells and other underlying molecular mechanisms, Oncol. Lett. 15 (2018) 849–854. https://doi.org/10.3892/ol.2017.7437.

[43] T. Yang, S. Yao, X. Zhang, Y. Guo, Andrographolide inhibits growth of human T-cell acute lymphoblastic leukemia Jurkat cells by downregulation of PI3K/AKT and upregulation of p38 MAPK pathways, Drug Des. Devel. Ther. 10 (2016) 1389–1397. https://doi.org/10.2147/DDDT.S94983.

[44] W. Pan, P. Li, Z. Guo, Q. Huang, Y. Gao, Propranolol induces regression of hemangioma cells via the down-regulation of the PI3K/Akt/eNOS/VEGF pathway, Pediatr. Blood Cancer. 62 (2015) 1414–1420. https://doi.org/10.1002/pbc.25453.

[45] P. Ehm, A. Grottke, B. Bettin, M. Jücker, Investigation of the function of the PI3-Kinase / AKT signaling pathway for leukemogenesis and therapy of acute childhood lymphoblastic leukemia (ALL), Cell. Signal. 93 (2022) 110301. https://doi.org/10.1016/j.cellsig.2022.110301.

[46] X. Liang, X. Xin, D. Qi, C. Fu, M. Ding, Silencing the PIK3CA Gene Enhances the Sensitivity of Childhood Leukemia Cells to Chemotherapy Drugs by Suppressing the Phosphorylation of Akt, Yonsei Med. J. 60 (2019) 182. https://doi.org/10.3349/ymj.2019.60.2.182.

[47] D. Barrett, V.I. Brown, S.A. Grupp, D.T. Teachey, Targeting the PI3K/AKT/mTOR Signaling Axis in Children with Hematologic Malignancies, Pediatr. Drugs. 14 (2012) 299–316. https://doi.org/10.2165/11594740-000000000-00000.

[48] Z. Ge, C. Song, Y. Ding, B.-H. Tan, D. Desai, A. Sharma, R. Gowda, F. Yue, S. Huang, V. Spiegelman, J.L. Payne, M.E. Reeves, S. Iyer, P.K. Dhanyamraju, Y. Imamura, D. Bogush, Y. Bamme, Y. Yang, M. Soliman, S. Kane, E. Dovat, J. Schramm, T. Hu, M. McGrath, Z.C. Chroneos, K.J. Payne, C. Gowda, S. Dovat, Dual targeting of MTOR as a novel therapeutic approach for high-risk B-cell acute lymphoblastic leukemia, Leukemia. 35 (2021) 1267–1278. https://doi.org/10.1038/s41375-021-01132-5.

[49] C. Evangelisti, F. Chiarini, A. Cappellini, F. Paganelli, M. Fini, S. Santi, A.M. Martelli, L.M. Neri, C. Evangelisti, Targeting Wnt/β-catenin and PI3K/Akt/mTOR pathways in T-cell acute lymphoblastic leukemia, J. Cell. Physiol. 235 (2020) 5413–5428. https://doi.org/10.1002/jcp.29429.

[50] Y. Wang, H. Hou, Z. Liang, X. Chen, X. Lian, J. Yang, Z. Zhu, H. Luo, H. Su, Q. Gong, P38 MAPK/AKT signalling is involved in IL-33-mediated anti-apoptosis in childhood acute lymphoblastic leukaemia blast cells, Ann. Med. 53 (2021) 1464–1472. https://doi.org/10.1080/07853890.2021.1970217.

[51] N. Morishita, H. Tsukahara, K. Chayama, T. Ishida, K. Washio, T. Miyamura, N. Yamashita, M. Oda, T. Morishima, Activation of Akt is associated with poor prognosis and chemotherapeutic resistance in pediatric B-precursor acute lymphoblastic leukemia, Pediatr. Blood Cancer. 59 (2012) 83–89. https://doi.org/10.1002/pbc.24034.

[52] A. Khanna, B. Bhushan, P.S. Chauhan, S. Saxena, D.K. Gupta, F. Siraj, High mTOR expression independently prognosticates poor clinical outcome to induction chemotherapy in acute lymphoblastic leukemia, Clin. Exp. Med. 18 (2018) 221–227. https://doi.org/10.1007/s10238-017-0478-x.

[53] P.P. Ruvolo, GSK-3 as a novel prognostic indicator in leukemia, Adv. Biol. Regul. 65 (2017) 26–35. https://doi.org/10.1016/j.jbior.2017.05.001.

[54] M.A. Burns, A.E. Place, K.E. Stevenson, A. Gutiérrez, S. Forrest, Y. Pikman, L.M. Vrooman, M.H. Harris, O.K. Weinberg, S.K. Hunt, J.E. O'Brien, B.L. Asselin, U.H. Athale, L.A. Clavell, P.D. Cole, L.M. Gennarini, J.M. Kahn, K.M. Kelly, C. Laverdiere, J. Leclerc, B. Michon, M.A. Schorin, M.L. Sulis, J.J.G. Welch, D.S. Neuberg, S.E. Sallan, L.B. Silverman, Identification of prognostic factors in childhood T-cell acute lymphoblastic leukemia: Results from DFCI ALL Consortium Protocols 05-001 and 11-001, Pediatr. Blood Cancer. 68 (2021) e28719. https://doi.org/10.1002/pbc.28719.

[55] R.D. Mendes, K. Canté-Barrett, R. Pieters, J.P.P. Meijerink, The relevance of PTEN-AKT in relation to NOTCH1-directed treatment strategies in T-cell acute lymphoblastic leukemia, Haematologica. 101 (2016) 1010–1017. https://doi.org/10.3324/haematol.2016.146381.

[56] E. Piovan, J. Yu, V. Tosello, D. Herranz, A. Ambesi-Impiombato, A.C. Da Silva, M. Sanchez-Martin, A. Perez-Garcia, I. Rigo, M. Castillo, Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia, Cancer Cell. 24 (2013) 766–776. https://doi.org/10.1016/j.ccr.2013.10.022.

[57] Y. Li, J.G. Buijs-Gladdines, K. Canté-Barrett, A.P. Stubbs, E.M. Vroegindeweij, W.K. Smits, R. van Marion, W.N.M. Dinjens, M. Horstmann, R.P. Kuiper, IL-7 receptor mutations and steroid resistance in pediatric T cell acute lymphoblastic leukemia: a genome sequencing study, PLoS Med. 13 (2016) e1002200. https://doi.org/10.1371/journal.pmed.1002200.

[58] ClinicalTrials.gov, Temsirolimus, Dexamethasone, Mitoxantrone Hydrochloride, Vincristine Sulfate, and Pegaspargase in Treating Young Patients With Relapsed Acute Lymphoblastic Leukemia or Non-Hodgkin Lymphoma, NIH. (2015). https://clinicaltrials.gov/ct2/show/NCT01403415 (accessed July 27, 2022).

[59] ClinicalTrials.gov, Everolimus With Multiagent Re-Induction Chemotherapy in Pediatric Patients With ALL (CRAD001NUS175T), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT01523977 (accessed July 27, 2022).

[60] ClinicalTrials.gov, Safety, Tolerability, Efficacy and Pharmacokinetics of Copanlisib in Pediatric Patients, NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT03458728 (accessed July 28, 2022).

[61] F. Janku, T.A. Yap, F. Meric-Bernstam, Targeting the PI3K pathway in cancer: are we making headway?, Nat. Rev. Clin. Oncol. 15 (2018) 273–291. https://doi.org/10.1038/nrclinonc.2018.28.

[62] W. Allen-Rhoades, S.B. Whittle, N. Rainusso, Pediatric solid tumors of infancy: An overview, Pediatr. Rev. 39 (2018) 57–67. https://doi.org/10.1542/pir.2017-0057.

[63] W. Allen-Rhoades, S.B. Whittle, N. Rainusso, Pediatric solid tumors in children and adolescents: an overview, Pediatr. Rev. 39 (2018) 444–453. https://doi.org/10.1542/pir.2017-0268.

[64] J.A. Perry, A. Kiezun, P. Tonzi, E.M. Van Allen, S.L. Carter, S.C. Baca, G.S. Cowley, A.S. Bhatt, E. Rheinbay, C.S. Pedamallu, E. Helman, A. Taylor-Weiner, A. McKenna, D.S. DeLuca, M.S. Lawrence, L. Ambrogio, C. Sougnez, A. Sivachenko, L.D. Walensky, N. Wagle, J. Mora, C. de Torres, C. Lavarino, S. Dos Santos Aguiar, J.A. Yunes, S.R. Brandalise, G.E. Mercado-Celis, J. Melendez-Zajgla, R. Cárdenas-Cardós, L. Velasco-Hidalgo, C.W.M. Roberts, L.A. Garraway, C. Rodriguez-Galindo, S.B. Gabriel, E.S. Lander, T.R. Golub, S.H. Orkin, G. Getz, K.A. Janeway, Complementary genomic approaches highlight the PI3K/mTOR pathway as a common vulnerability in osteosarcoma, Proc. Natl. Acad. Sci. 111 (2014). https://doi.org/10.1073/pnas.1419260111.

[65] K.M. Schroeder, C.M. Hoeman, O.J. Becher, Children are not just little adults: recent advances in understanding of diffuse intrinsic pontine glioma biology, Pediatr. Res. 75 (2014) 205–209. https://doi.org/10.1038/pr.2013.194.

[66] H.A. Rogers, J. Estranero, K. Gudka, R.G. Grundy, The therapeutic potential of targeting the PI3K pathway in pediatric brain tumors, Oncotarget. 8 (2017) 2083–2095. https://doi.org/10.18632/oncotarget.13781.

[67] A. Suryo Rahmanto, V. Savov, A. Brunner, S. Bolin, H. Weishaupt, A. Malyukova, G. Rosén, M. Čančer, S. Hutter, A. Sundström, D. Kawauchi, D.T. Jones, C. Spruck, M.D. Taylor, Y. Cho, S.M. Pfister, M. Kool, A. Korshunov, F.J. Swartling, O. Sangfelt, FBW7 suppression leads to SOX9 stabilization and increased malignancy in medulloblastoma, EMBO J. 35 (2016) 2192–2212. https://doi.org/10.15252/embj.201693889.

[68] Z. Hua, X. Gu, Y. Dong, F. Tan, Z. Liu, C.J. Thiele, Z. Li, PI3K and MAPK pathways mediate the BDNF/TrkB-increased metastasis in neuroblastoma, Tumor Biol. 37 (2016) 16227–16236. https://doi.org/10.1007/s13277-016-5433-z.

[69] A. Gupte, E.K. Baker, S.-S. Wan, E. Stewart, A. Loh, A.A. Shelat, C.M. Gould, A.M. Chalk, S. Taylor, K. Lackovic, Systematic screening identifies dual PI3K and mTOR inhibition as a conserved therapeutic vulnerability in osteosarcoma, Clin. Cancer Res. 21 (2015) 3216–3229. https://doi.org/10.1158/1078-0432.CCR-14-3026.

[70] T. Tao, H. Shi, M. Wang, A.R. Perez-Atayde, W.B. London, A. Gutierrez, B. Lemos, A.D. Durbin, A.T. Look, Ganglioneuromas are driven by activated AKT and can be therapeutically targeted with mTOR inhibitors, J. Exp. Med. 217 (2020) e20191871. https://doi.org/10.1084/jem.20191871.

[71] R. Gorlick, J.M. Maris, P.J. Houghton, R. Lock, H. Carol, R.T. Kurmasheva, E.A. Kolb, S.T. Keir, C.P. Reynolds, M.H. Kang, Testing of the Akt/PKB inhibitor MK-2206 by the pediatric preclinical testing program, Pediatr. Blood Cancer. 59 (2012) 518–524. https://doi.org/10.1002/pbc.23412.

[72] Z. Li, F. Tan, D.J. Liewehr, S.M. Steinberg, C.J. Thiele, In vitro and in vivo inhibition of neuroblastoma tumor cell growth by AKT inhibitor perifosine, J. Natl. Cancer Inst. 102 (2010) 758–770. https://doi.org/10.1093/jnci/djq125.

[73] J. Akutagawa, T.Q. Huang, I. Epstein, T. Chang, M. Quirindongo-Crespo, C.L. Cottonham, M. Dail, B.S. Slusher, L.S. Friedman, D. Sampath, Targeting the PI3K/Akt pathway in murine MDS/MPN driven by hyperactive Ras, Leukemia. 30 (2016) 1335–1343. https://doi.org/10.1038/leu.2016.14.

[74] V.A. Granados, U. Avirneni-Vadlamudi, P. Dalal, S.R. Scarborough, K.A. Galindo, P. Mahajan, R.L. Galindo, Selective Targeting of Myoblast Fusogenic Signaling and Differentiation-Arrest Antagonizes Rhabdomyosarcoma Cells, Cancer Res. 79 (2019) 4585–4591. https://doi.org/10.1158/0008-5472.CAN-18-2096.

[75] ClinicalTrials.gov, Ribociclib and Everolimus in Treating Children With Recurrent or Refractory Malignant Brain Tumors, NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT03387020 (accessed July 27, 2022).

[76] ClinicalTrials.gov, Targeted Therapy Directed by Genetic Testing in Treating Pediatric Patients With Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphomas, or Histiocytic Disorders (The Pediatric MATCH Screening Trial), NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT03155620 (accessed July 27, 2022).

[77] A. Kumar, H.L. Fillmore, R. Kadian, W.C. Broaddus, G.W. Tye, T.E. Van Meter, The alkylphospholipid perifosine induces apoptosis and p21-mediated cell cycle arrest in medulloblastoma, Mol. Cancer Res. 7 (2009) 1813–1821. https://doi.org/10.1158/1541-7786.MCR-09-0069.

[78] C. Yao, J. jun Wei, Z. yu Wang, H. min ding, D. Li, S. chang Yan, Y. jiang Yang, Z. ping Gu, Perifosine Induces Cell Apoptosis in Human Osteosarcoma Cells: New Implication for Osteosarcoma Therapy?, Cell Biochem. Biophys. 65 (2013) 217–227. https://doi.org/10.1007/s12013-012-9423-5.

[79] X. Wei, L. Xu, S.F. Jeddo, K. Li, X. Li, J. Li, MARK2 enhances cisplatin resistance via PI3K/AKT/NF-κB signaling pathway in osteosarcoma cells., Am. J. Transl. Res. 12 (2020) 1807–1823.

[80] K.W. Kim, J.Y. Kim, J. Qiao, R.A. Clark, C.M. Powers, H. Correa, D.H. Chung, Dual-Targeting AKT2 and ERK in cancer stem-like cells in neuroblastoma, Oncotarget. 10 (2019) 5645–5659. https://doi.org/10.18632/oncotarget.27210.

[81] Z. Li, S. Yan, N. Attayan, S. Ramalingam, C.J. Thiele, Combination of an allosteric Akt Inhibitor MK-2206 with etoposide or rapamycin enhances the antitumor growth effect in neuroblastoma, Clin. Cancer Res. 18 (2012) 3603–3615. https://doi.org/10.1158/1078-0432.CCR-11-3321.

[82] L.M. Neri, A. Cani, A.M. Martelli, C. Simioni, C. Junghanss, G. Tabellini, F. Ricci, P.L. Tazzari, P. Pagliaro, J.A. McCubrey, Targeting the PI3K/Akt/mTOR signaling pathway in B-precursor acute lymphoblastic leukemia and its therapeutic potential, Leukemia. 28 (2014) 739–748. https://doi.org/10.1038/leu.2013.226.

[83] K. Canté-Barrett, S.-H. JaP, J.G.C. Buijs-Gladdines, MEK and PI3K-AKT inhibitors synergistically block activated IL7 receptor signaling in T-cell acute lymphoblastic leukemia, Leukemia. 30 (2016) 1832–1843. https://doi.org/10.1038/leu.2016.83.

[84] O.J. Becher, D. Hambardzumyan, T.R. Walker, K. Helmy, J. Nazarian, S. Albrecht, R.L. Hiner, S. Gall, J.T. Huse, N. Jabado, Preclinical evaluation of radiation and perifosine in a genetically and histologically accurate model of brainstem glioma, Cancer Res. 70 (2010) 2548–2557. https://doi.org/10.1158/0008-5472.CAN-09-2503.

[85] K.M.Z. Savill, B.B. Lee, J. Oeh, J. Lin, E. Lin, W.-J. Chung, A. Young, W. Chen, M. Miś, K. Mesh, J. Eastham, F. Gnad, Z. Jiang, E.W. Stawiski, B. Haley, A. Daemen, X. Wang, H. Koeppen, Z. Modrusan, S.E. Martin, D. Sampath, K. Lin, Distinct resistance mechanisms arise to allosteric vs. ATP-competitive AKT inhibitors, Nat. Commun. 13 (2022) 2057. https://doi.org/10.1038/s41467-022-29655-0.

[86] K. Lin, J. Lin, W.-I. Wu, J. Ballard, B.B. Lee, S.L. Gloor, G.P.A. Vigers, T.H. Morales, L.S. Friedman, N. Skelton, B.J. Brandhuber, An ATP-Site On-Off Switch That Restricts Phosphatase Accessibility of Akt, Sci. Signal. 5 (2012). https://doi.org/10.1126/scisignal.2002618.

[87] B.R. Huck, I. Mochalkin, Recent progress towards clinically relevant ATP-competitive Akt inhibitors, Bioorganic Med. Chem. Lett. 27 (2017) 2838–2848. https://doi.org/10.1016/j.bmcl.2017.04.090.

[88] M.J. Bamford, M.J. Alberti, N. Bailey, S. Davies, D.K. Dean, A. Gaiba, S. Garland, J.D. Harling, D.K. Jung, T.A. Panchal, C.A. Parr, J.G. Steadman, A.K. Takle, J.T. Townsend, D.M. Wilson, J. Witherington, (1H-Imidazo[4,5-c]pyridin-2-yl)-1,2,5-oxadiazol-3-ylamine derivatives: A novel class of potent MSK-1-

inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 3402–3406. https://doi.org/10.1016/j.bmcl.2005.05.021.

[89] M.J. Bamford, N. Bailey, S. Davies, D.K. Dean, L. Francis, T.A. Panchal, C.A. Parr, S. Sehmi, J.G. Steadman, A.K. Takle, J.T. Townsend, D.M. Wilson, (1H-Imidazo[4,5-c]pyridin-2-yl)-1,2,5-oxadiazol-3-ylamine derivatives: Further optimisation as highly potent and selective MSK-1-inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 3407–3411. https://doi.org/10.1016/j.bmcl.2005.05.020.

[90] D.A. Heerding, N. Rhodes, J.D. Leber, T.J. Clark, R.M. Keenan, L. V. Lafrance, M. Li, I.G. Safonov, D.T. Takata, J.W. Venslavsky, D.S. Yamashita, A.E. Choudhry, R.A. Copeland, Z. Lai, M.D. Schaber, P.J. Tummino, S.L. Strum, E.R. Wood, D.R. Duckett, D. Eberwein, V.B. Knick, T.J. Lansing, R.T. McConnell, S. Zhang, E.A. Minthorn, N.O. Concha, G.L. Warren, R. Kumar, Identification of 4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3S)-3-piperidinylmethyl]oxy}-1H-imidazo[4,5-c]pyridin-4-yl)-2-methyl-3-butyn-2-ol (GSK690693), a Novel Inhibitor of AKTKinase, J. Med. Chem. 51 (2008) 5663–5679. https://doi.org/10.1021/jm8004527.

[91] N. Rhodes, D.A. Heerding, D.R. Duckett, D.J. Eberwein, V.B. Knick, T.J. Lansing, R.T. McConnell, T.M. Gilmer, S.Y. Zhang, K. Robell, J.A. Kahana, R.S. Geske, E. V. Kleymenova, A.E. Choudhry, Z. Lai, J.D. Leber, E.A. Minthorn, S.L. Strum, E.R. Wood, P.S. Huang, R.A. Copeland, R. Kumar, Characterization of an Akt kinase inhibitor with potent pharmacodynamic and antitumor activity, Cancer Res. 68 (2008) 2366–2374. https://doi.org/10.1158/0008-5472.CAN-07-5783.

[92] P. LoRusso, H. Hurwitz, E. Chiorean, R. Jewell, T. Lampkin, K. Bachman, R. Kumar, P. Lebowitz, B. Weber, AKT inhibitor GSK690693: Preliminary results from the first time in human study, in: 99th AACR Annu. Meet., American Association for Cancer Research, San Diego, CA, 2008.

[93] ClinicalTrials.gov, Open-label Study to Investigate the Safety, Tolerability, PK, and Pharmacodynamics of the AKT Inhibitor GSK690693, NIH. (2017). https://clinicaltrials.gov/ct2/show/NCT00493818?term=GSK690693&draw=2&rank=2 (accessed July 23, 2022).

[94] D.S. Levy, J.A. Kahana, R. Kumar, AKT inhibitor, GSK690693, induces growth inhibition and apoptosis in acute lymphoblastic leukemia cell lines, Blood. 113 (2009) 1723–1729. https://doi.org/10.1182/blood-2008-02-137737.

[95] H. Carol, C.L. Morton, R. Gorlick, E.A. Kolb, S.T. Keir, C.P. Reynolds, M.H. Kang, J.M. Maris, C. Billups, M.A. Smith, P.J. Houghton, R.B. Lock, Initial Testing (Stage 1) of the Akt Inhibitor GSK690693 by the Pediatric Preclinical Testing Program, Pediatr. Blood Cancer. 55 (2010) 1329–1337. https://doi.org/10.1002/pbc.22710.

[96] M.A. Seefeld, M.B. Rouse, K.C. McNulty, L. Sun, J. Wang, D.S. Yamashita, J.I. Luengo, S. Zhang, N.O.C. Elisabeth A Minthorn, D.A. Heerding, Discovery of 5-pyrrolopyridinyl-2-thiophenecarboxamides as potent AKT kinase inhibitors,

Bioorg. Med. Chem. Lett. 19 (2009) 2244–2248. https://doi.org/10.1016/j.bmcl.2009.02.094.

[97] F. Pachl, P. Plattner, B. Ruprecht, G. Médard, N. Sewald, B. Kuster, Characterization of a chemical affinity probe targeting Akt kinases, J. Proteome Res. 12 (2013) 3792–3800. https://doi.org/10.1021/pr400455j.

[98] M. Dumble, M.C. Crouthamel, S.Y. Zhang, M. Schaber, D. Levy, K. Robell, Q. Liu, D.J. Figueroa, E.A. Minthorn, M.A. Seefeld, M.B. Rouse, S.K. Rabindran, D.A. Heerding, R. Kumar, Discovery of novel AKT inhibitors with enhanced anti-tumor effects in combination with the MEK inhibitor, PLoS One. 9 (2014) e100880. https://doi.org/10.1371/journal.pone.0100880.

[99] S.P. Blagden, A.L. Hamilton, L. Mileshkin, S. Wong, A. Michael, M. Hall, J.C. Goh, A.S. Lisyanskaya, M. DeSilvio, E. Frangou, E.A. Stronach, P. Gopalakrishna, T.M. Meniawy, H. Gabra, Phase IB dose escalation and expansion study of akt inhibitor afuresertib with carboplatin and paclitaxel in recurrent platinum-resistant ovarian cancer, Clin. Cancer Res. 25 (2019) 1472–1478. https://doi.org/10.1158/1078-0432.CCR-18-2277.

[100] R.J. Arceci, C.E. Allen, I.J. Dunkel, E. Jacobsen, J. Whitlock, R. Vassallo, S.R. Morris, A. Portnoy, B.A. Reedy, D.A. Smith, R. Noble, A. Murnane, M. Cornfeld, C. Rodriguez-Galindo, M.L. Heaney, K. McClain, S. Vaiselbuh, A phase IIa study of afuresertib, an oral pan-AKT inhibitor, in patients with Langerhans cell histiocytosis, Pediatr. Blood Cancer. 64 (2017). https://doi.org/10.1002/pbc.26325.

[101] ClinicalTrials.gov, A Study to Test the Ability of and Safety of GSK2110183 in Treating Langerhans Cell Histiocytosis, NIH. (2020). https://clinicaltrials.gov/ct2/show/NCT01395004 (accessed July 23, 2022).

[102] C.E. Allen, M. Merad, K.L. McClain, Langerhans-Cell Histiocytosis, N. Engl. J. Med. 379 (2018) 856–868. https://doi.org/10.1056/NEJMra1607548.

[103] I. Sánchez-Hernández, P. Baquero, L. Calleros, A. Chiloeches, Dual inhibition of V600EBRAF and the PI3K/AKT/mtor pathway cooperates to induce apoptosis in melanoma cells through a MEK-independent mechanism, Cancer Lett. 314 (2012) 244–255. https://doi.org/10.1016/j.canlet.2011.09.037.

[104] B.K. Ragon, O. Odenike, M.R. Baer, W. Stock, G. Borthakur, K. Patel, L. Han, H. Chen, H. Ma, L. Joseph, Oral MEK 1/2 inhibitor trametinib in combination with AKT inhibitor GSK2141795 in patients with acute myeloid leukemia with RAS mutations: a phase II study, Clin. Lymphoma Myeloma Leuk. 19 (2019) 431–440. https://doi.org/10.1016/j.clml.2019.03.015.

[105] A.P. Algazi, R. Esteve-Puig, A. Nosrati, B. Hinds, A. Hobbs-Muthukumar, P. Nandoskar, S. Ortiz-Urda, P.B. Chapman, A. Daud, Dual MEK/AKT inhibition with trametinib and GSK2141795 does not yield clinical benefit in metastatic NRAS-mutant and wild-type melanoma, Pigment Cell Melanoma Res. 31 (2018) 110–114. https://doi.org/10.1111/pcmr.12644.

[106] J.F. Liu, K.P. Gray, A.A. Wright, S. Campos, P.A. Konstantinopoulos, A. Peralta, K. MacNeill, S. Morrissey, C. Whalen, D. Dillon, U.A. Matulonis, Results from a single arm, single stage phase II trial of trametinib and GSK2141795 in persistent or recurrent cervical cancer, Gynecol. Oncol. 154 (2019) 95–101. https://doi.org/10.1016/j.ygyno.2019.05.003.

[107] A.W. Tolcher, R. Kurzrock, V. Valero, R. Gonzalez, R.S. Heist, A.R. Tan, J. Means-Powell, T.L. Werner, C. Becerra, C. Wang, C. Leonowens, S. Kalyana-Sundaram, J.F. Kleha, J. Gauvin, A.M. D'Amelio, C. Ellis, N. Ibrahim, L. Yan, Phase I dose-escalation trial of the oral AKT inhibitor uprosertib in combination with the oral MEK1/MEK2 inhibitor trametinib in patients with solid tumors, Cancer Chemother. Pharmacol. 85 (2020) 673–683. https://doi.org/10.1007/s00280-020-04038-8.

[108] J.J. Caldwell, T.G. Davies, A. Donald, T. McHardy, M.G. Rowlands, G.W. Aherne, L.K. Hunter, K. Taylor, R. Ruddle, F.I. Raynaud, M. Verdonk, P. Workman, M.D. Garrett, I. Collins, Identification of 4-(4-aminopiperidin-1-yl)-7H-pyrrolo[2,3-d]pyrimidines as selective inhibitors of protein kinase B through fragment elaboration, J. Med. Chem. 51 (2008) 2147–2157. https://doi.org/10.1021/jm701437d.

[109] F.Z. Wang, Z.Y. Chang, H.R. Fei, M.F. Yang, X.Y. Yang, B.L. Sun, CCT128930 induces cell cycle arrest, DNA damage, and autophagy independent of Akt inhibition, Biochimie. 103 (2014) 118–125. https://doi.org/10.1016/j.biochi.2014.04.008.

[110] J. Sun, Y. Hou, Y. Yin, F. Wang, M. Yang, Y. Zhang, C. Fan, B. Sun, CCT128930 induces G1-phase arrest and apoptosis and synergistically enhances the anticancer efficiency of VS5584 in human osteosarcoma cells, Biomed. Pharmacother. 130 (2020) 110544. https://doi.org/10.1016/j.biopha.2020.110544.

[111] J.F. Blake, R. Xu, J.R. Bencsik, D. Xiao, N.C. Kallan, S. Schlachter, I.S. Mitchell, K.L. Spencer, A.L. Banka, E.M. Wallace, S.L. Gloor, M. Martinson, R.D. Woessner, G.P.A. Vigers, B.J. Brandhuber, J. Liang, B.S. Safina, J. Li, B. Zhang, C. Chabot, S. Do, L. Lee, J. Oeh, D. Sampath, B.B. Lee, K. Lin, B.M. Liederer, N.J. Skelton, Discovery and preclinical pharmacology of a selective ATP-competitive Akt inhibitor (GDC-0068) for the treatment of human tumors, J. Med. Chem. 55 (2012) 8110–8127. https://doi.org/10.1021/jm301024w.

[112] J.R. Bencsik, X. Dengming, J.F. Blake, N.C. Kallan, I.S. Mitchell, K.L. Spencer, R. Xu, S.L. Gloor, M. Martinson, T. Risom, R.D. Woessner, F. Dizon, W.-I. Wu, G.P.A. Vigers, B.J. Brandhuber, N.J. Skelton, W.W. Prior, L.J. Murray, Discovery of dihydrothieno- and dihydrofuropyrimidines as potent panAkt inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 7037–7041. https://doi.org/10.1016/j.bmcl.2010.09.112.

[113] H. Hua, H. Zhang, J. Chen, J. Wang, J. Liu, Y. Jiang, Targeting Akt in cancer for precision therapy, J. Hematol. Oncol. 14 (2021) 128. https://doi.org/10.1186/s13045-021-01137-8.

[114] ClinicalTrials.gov, Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study, NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04589845 (accessed July 23, 2022).

[115] ClinicalTrials.gov, A Study of Ipatasertib in Combination With Paclitaxel as a Treatment for Participants With PIK3CA/AKT1/PTEN-Altered, Locally Advanced or Metastatic, Triple-Negative Breast Cancer or Hormone Receptor-Positive, HER2-Negative Breast Cancer (IPATunity130), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT03337724 (accessed July 23, 2022).

[116] ClinicalTrials.gov, Ipatasertib Plus Abiraterone Plus Prednisone/Prednisolone, Relative to Placebo Plus Abiraterone Plus Prednisone/Prednisolone in Adult Male Patients With Metastatic Castrate-Resistant Prostate Cancer (IPATential150), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT03072238 (accessed July 23, 2022).

[117] ClinicalTrials.gov, A Study Of Ipatasertib in Combination With Atezolizumab and Paclitaxel as a Treatment for Participants With Locally Advanced or Metastatic Triple-Negative Breast Cancer., NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04177108 (accessed July 23, 2022).

[118] ClinicalTrials.gov, A Study of Ipatasertib Plus Palbociclib and Fulvestrant Versus Placebo Plus Palbociclib and Fulvestrant in Hormone Receptor Positive and HER2 Negative Locally Advanced Unresectable or Metastatic Breast Cancer (IPATunity150), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04060862 (accessed July 23, 2022).

[119] ClinicalTrials.gov, Fulvestrant and Ipatasertib for Advanced HER-2 Negative and Estrogen Receptor Positive (ER+) Breast Cancer Following Progression on First Line CDK 4/6 Inhibitor and Aromatase Inhibitor (FINER), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04650581 (accessed July 23, 2022).

[120] E.K. Slotkin, D. Diolaiti, N.N. Shukla, F.S. Dela Cruz, J.J. Clark, G. Gundem, V.D. Yellapantula, M.F. Levine, D. You, P. Ma, Patient-Driven Discovery, Therapeutic Targeting, and Post-Clinical Validation of a Novel AKT1 Fusion–Driven Cancer, Cancer Discov. 9 (2019) 605–616. https://doi.org/10.1158/2159-8290.CD-18-0953.

[121] M. Addie, P. Ballard, D. Buttar, C. Crafter, G. Currie, B.R. Davies, J. Debreczeni, H. Dry, P. Dudley, R. Greenwood, P.D. Johnson, J.G. Kettle, C. Lane, G. Lamont, A. Leach, R.W.A. Luke, J. Morris, D. Ogilvie, K. Page, M. Pass, S. Pearson, L. Ruston, Discovery of 4-amino-N-[(1S)-1-(4-chlorophenyl)-3-hydroxypropyl]-1-(7H- pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (AZD5363), an orally bioavailable, potent inhibitor of Akt kinases, J. Med. Chem. 56 (2013) 2059–2073. https://doi.org/10.1021/jm301762v.

[122] ClinicalTrials.gov, PAKT: AZD5363 in Combination With Paclitaxel in Triple-Negative Advanced or Metastatic Breast Cancer (PAKT), NIH. (2020). https://clinicaltrials.gov/ct2/show/NCT02423603 (accessed July 23, 2022).

[123] ClinicalTrials.gov, Fulvestrant +/- Akt Inhibition in Advanced Aromatase Inhibitor Resistant Breast Cancer (FAKTION), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT01992952 (accessed July 23, 2022).

[124] ClinicalTrials.gov, Capivasertib+Abiraterone as Treatment for Patients With Metastatic Hormone-sensitive Prostate Cancer and PTEN Deficiency (CAPItello-281), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04493853 (accessed July 23, 2022).

[125] ClinicalTrials.gov, Capivasertib+Fulvestrant vs Placebo+Fulvestrant as Treatment for Locally Advanced (Inoperable) or Metastatic HR+/HER2- Breast Cancer (CAPItello-291), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04305496 (accessed July 23, 2022).

[126] ClinicalTrials.gov, Capivasertib+Paclitaxel as First Line Treatment for Patients With Locally Advanced or Metastatic TNBC (CAPItello-290), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT03997123 (accessed July 23, 2022).

[127] ClinicalTrials.gov, Capivasertib + Palbociclib + Fulvestrant for HR+/HER2- Advanced Breast Cancer (CAPItello-292), NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04862663 (accessed July 23, 2022).

[128] J.T. Lynch, R. McEwen, C. Crafter, U. McDermott, M.J. Garnett, S.T. Barry, B.R. Davies, Identification of differential PI3K pathway target dependencies in T-cell acute lymphoblastic leukemia through a large cancer cell panel screen, Oncotarget. 7 (2016) 22128–22139. https://doi.org/10.18632/oncotarget.8031.

[129] T. Patra, K. Meyer, R.B. Ray, R. Ray, A combination of AZD5363 and FH5363 induces lethal autophagy in transformed hepatocytes, Cell Death Dis. 11 (2020) 540. https://doi.org/10.1038/s41419-020-02741-1.

[130] Y. Li, MK-2206: a potent oral allosteric AKT inhibitor. AACR Annual Meeting 2009: Abstract Number: DDT01-1, in: 100th AACR Annu. Meet., American Association for Cancer Research, Denver, CO, 2009. https://cancerres.aacrjournals.org/content/69/9_Supplement/DDT01-1.

[131] M. Fouladi, J.P. Perentesis, C.L. Phillips, S. Leary, J.M. Reid, R.M. McGovern, A.M. Ingle, C.H. Ahern, M.M. Ames, P. Houghton, A phase I trial of MK-2206 in children with refractory malignancies: a Children's Oncology Group study, Pediatr. Blood Cancer. 61 (2014) 1246–1251. https://doi.org/10.1002/pbc.25023.

[132] L. Qi, H. Toyoda, D. Xu, Y. Zhou, N. Sakurai, K. Amano, K. Kihira, H. Hori, E. Azuma, Y. Komada, PDK1-mTOR signaling pathway inhibitors reduce cell proliferation in MK2206 resistant neuroblastoma cells, Cancer Cell Int. 15 (2015). https://doi.org/10.1186/s12935-015-0239-4.

[133] T. Li, J. Wang, P. Liu, J. Chi, H. Yan, L. Lei, Z. Li, B. Yang, X. Wang, Insulin-like growth factor 2 axis supports the serum-independent growth of malignant rhabdoid tumor and is activated by microenvironment stress, Oncotarget. 8

(2017) 47269–47283. https://doi.org/10.18632/oncotarget.17617.

[134] M.L. Kuijjer, B.E.W.M. Van Den Akker, R. Hilhorst, M. Mommersteeg, E.P. Buddingh, M. Serra, H. Bürger, P.C.W. Hogendoorn, A.-M. Cleton-Jansen, Kinome and mRNA expression profiling of high-grade osteosarcoma cell lines implies Akt signaling as possible target for therapy, BMC Med. Genomics. 7 (2014). https://doi.org/10.1186/1755-8794-7-4.

[135] Y. Dong, W. Gong, Z. Hua, B. Chen, G. Zhao, Z. Liu, C.J. Thiele, Z. Li, Combination of Rapamycin and MK-2206 Induced Cell Death via Autophagy and Necroptosis in MYCN-Amplified Neuroblastoma Cell Lines, Front. Pharmacol. 11 (2020). https://doi.org/10.3389/fphar.2020.00031.

[136] E.E. Santo, P. Stroeken, P. V. Sluis, J. Koster, R. Versteeg, E.M. Westerhout, FOXO3a is a major target of inactivation by PI3K/AKT signaling in aggressive neuroblastoma, Cancer Res. 73 (2013) 2189–2198. https://doi.org/10.1158/0008-5472.CAN-12-3767.

[137] A. Schulte, F. Ewald, M. Spyra, D.J. Smit, W. Jiang, J. Salamon, M. Jücker, V.F. Mautner, Combined targeting of AKT and mTOR inhibits proliferation of human NF1-Associated malignant peripheral nerve sheath tumour cells in vitro but not in a xenograft mouse model in vivo, Int. J. Mol. Sci. 21 (2020) 1548. https://doi.org/10.3390/ijms21041548.

[138] N. Mimura, T. Hideshima, T. Shimomura, R. Suzuki, H. Ohguchi, O. Rizq, S. Kikuchi, Y. Yoshida, F. Cottini, J. Jakubikova, D. Cirstea, G. Gorgun, J. Minami, Y.-T. Tai, P.G. Richardson, T. Utsugi, A. Iwama, K.C. Anderson, Selective and Potent Akt Inhibition Triggers Anti-Myeloma Activities and Enhances Fatal Endoplasmic Reticulum Stress Induced by Proteasome Inhibition, Cancer Res. 74 (2014) 4458–4469. https://doi.org/10.1158/0008-5472.CAN-13-3652.

[139] T. Abe, K. Ichikawa, R. Fujita, M. Okada, K. Tanaka, N. Fujino, M. Ohkubo, K. Yonekura, T. Shimomura, T. Utsugi, A Novel Highly Potent AKT Inhibitor TAS-117 Demonstrated Synergistic Antitumor Activity in Combination with Paclitaxel Through Enhancement of Apoptosis Induction, Eur. J. Cancer. 48 (2012) 108–109. https://doi.org/10.1016/s0959-8049(12)72154-8.

[140] J.B. Lee, M. Jung, S.H. Beom, G.M. Kim, H.R. Kim, H.J. Choi, J.H. Sohn, J.B. Ahn, S.Y. Rha, H.C. Chung, Phase 2 study of TAS-117, an allosteric akt inhibitor in advanced solid tumors harboring phosphatidylinositol 3-kinase/v-akt murine thymoma viral oncogene homolog gene mutations, Invest. New Drugs. 39 (2021) 1366–1374. https://doi.org/10.1007/s10637-021-01085-7.

[141] ClinicalTrials.gov, TAS-117 in Patients With Advanced Solid Tumors Harboring Germline PTEN Mutations, NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT04770246 (accessed July 23, 2022).

[142] M.E. Mattmann, S.L. Stoops, C.W. Lindsley, Inhibition of Akt with small molecules and biologics: historical perspective and current status of the patent landscape, Expert Opin. Ther. Pat. 21 (2011) 1309–1338.

https://doi.org/10.1517/13543776.2011.587959.

[143] V. Zaremberg, S. Ganesan, M. Mahadeo, Lipids and Membrane Microdomains: The Glycerolipid and Alkylphosphocholine Class of Cancer Chemotherapeutic Drugs, in: Lipid Signal. Hum. Dis. Handb. Exp. Pharmacol. , Springer, Cham, 2019: pp. 261–288. https://doi.org/10.1007/164_2019_222.

[144] I. Hers, E.E. Vincent, J.M. Tavaré, Akt signalling in health and disease, Cell. Signal. 23 (2011) 1515–1527. https://doi.org/10.1016/j.cellsig.2011.05.004.

[145] F. Kaleağasıoğlu, D.M. Ali, M.R. Berger, Multiple Facets of Autophagy and the Emerging Role of Alkylphosphocholines as Autophagy Modulators, Front. Pharmacol. 11 (2020) 547. https://doi.org/10.3389/fphar.2020.00547.

[146] O.J. Becher, S.W. Gilheeney, Y. Khakoo, D.C. Lyden, S. Haque, K.C. De Braganca, J.M. Kolesar, J.T. Huse, S. Modak, L.H. Wexler, A phase I study of perifosine with temsirolimus for recurrent pediatric solid tumors, Pediatr. Blood Cancer. 64 (2017) e26409. https://doi.org/10.1002/pbc.26409.

[147] X. Gu, Z. Hua, Y. Dong, Y. Zhan, X. Zhang, W. Tian, Z. Liu, C.J. Thiele, Z. Li, Proteome and Acetylome Analysis Identifies Novel Pathways and Targets Regulated by Perifosine in Neuroblastoma, Sci. Reportsv. 7 (2017) 42062. https://doi.org/10.1038/srep42062.

[148] T. Hideshima, L. Catley, H. Yasui, K. Ishitsuka, N. Raje, C. Mitsiades, K. Podar, N.C. Munshi, D. Chauhan, P.G. Richardson, K.C. Anderson, Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces in vitro and in vivo cytotoxicity in human multiple myeloma cells, Blood. 107 (2006) 4053–4062. https://doi.org/10.1182/blood-2005-08-3434.

[149] G.P. Dasmahapatra, P. Didolkar, M.C. Alley, S. Ghosh, E.A. Sausville, K.K. Roy, In vitro combination treatment with perifosine and UCN-01 demonstrates synergism against prostate (PC-3) and lung (A549) epithelial adenocarcinoma cell lines, Clin. Cancer Res. 10 (2004) 5242–5252. https://doi.org/10.1158/1078-0432.CCR-03-0534.

[150] M. Rahmani, E. Reese, Y. Dai, C. Bauer, S.G. Payne, P. Dent, S. Spiegel, S. Grant, Coadministration of histone deacetylase inhibitors and perifosine synergistically induces apoptosis in human leukemia cells through Akt and ERK1/2 inactivation and the generation of ceramide and reactive oxygen species, Cancer Res. 65 (2005) 2422–2432. https://doi.org/10.1158/0008-5472.CAN-04-2440.

[151] Y. Tong, Y.Y. Liu, L.S. You, W. Bin Qian, Perifosine induces protective autophagy and upregulation of ATG5 in human chronic myelogenous leukemia cells in vitro, Acta Pharmacol. Sin. 33 (2012) 542–550. https://doi.org/10.1038/aps.2011.192.

[152] Z. Li, O. Doo-Yi, K. Nakamura, T. Carol J, Perifosine-induced inhibition of Akt attenuates BDNF/TrkB- induced chemoresistance in neuroblastoma in vivo,

Cancer. 117 (2011) 5412–5422. https://doi.org/10.1002/cncr.26133.Perifosine-induced.

[153] V. Papa, P.L. Tazzari, F. Chiarini, A. Cappellini, F. Ricci, A.M. Billi, C. Evangelisti, E. Ottaviani, G. Martinelli, N. Testoni, J.A. McCubrey, A.M. Martelli, Proapoptotic activity and chemosensitizing effect of the novel Akt inhibitor perifosine in acute myelogenous leukemia cells, Leukemia. 22 (2008) 147–160. https://doi.org/10.1038/sj.leu.2404980.

[154] F. Chiarini, M. Del Sole, S. Mongiorgi, G.C. Gaboardi, A. Cappellini, I. Mantovani, M.Y. Follo, J.A. McCubrey, A.M. Martelli, The novel Akt inhibitor, perifosine, induces caspase-dependent apoptosis and downregulates P-glycoprotein expression in multidrug-resistant human T-acute leukemia cells by a JNK-dependent mechanism, Leukemia. 22 (2008) 1106–1116. https://doi.org/10.1038/leu.2008.79.

[155] O.J. Becher, N.E. Millard, S. Modak, B.H. Kushner, S. Haque, I. Spasojevic, T.M. Trippett, S.W. Gilheeney, Y. Khakoo, D.C. Lyden, A phase I study of single-agent perifosine for recurrent or refractory pediatric CNS and solid tumors, PLoS One. 12 (2017) e0178593. https://doi.org/10.1371/journal.pone.0178593.

[156] B.H. Kushner, N. V Cheung, S. Modak, O.J. Becher, E.M. Basu, S.S. Roberts, K. Kramer, I.J. Dunkel, A phase I/Ib trial targeting the Pi3k/Akt pathway using perifosine: L ong-term progression-free survival of patients with resistant neuroblastoma, Int. J. Cancer. 140 (2017) 480–484.

[157] K. Matsumoto, H. Shichino, H. Kawamoto, Y. Kosaka, M. Chin, K. Kato, H. Mugishima, Phase I study of perifosine monotherapy in patients with recurrent or refractory neuroblastoma, Pediatr. Blood Cancer. 64 (2017) e26623. https://doi.org/10.1002/pbc.26623.

[158] K.H. Schram, L.B. Townsend, THE SYNTHESIS OF 6-AMINO-4-METII[YL-8-(g-D-RIBOFUFWOSYL)(4-H,8-H)PYRF0IO- [4,3,2-de]PYRIMIDD[4,5-c]PYRIDAFJNE, A NEW TRICYCLIC NUCLEOSIDE, Tetrahedron Lett. 49 (1971) 4757–4760.

[159] L.L. Wotring, L.B. Townsend, L.M. Jones, K.Z. Borysko, D.L. Gildersleeve, W.B. Parker, Dual Mechanisms of Inhibition of DNA Synthesis by Triciribine, Cancer Res. 50 (1990) 4891–4899.

[160] L. Yang, H.C. Dan, M. Sun, Q. Liu, X.M. Sun, R.I. Feldman, A.D. Hamilton, M. Polokoff, S. V. Nicosia, M. Herlyn, S.M. Sebti, J.Q. Cheng, Akt/protein kinase B signaling inhibitor-2, a selective small molecule inhibitor of Akt signaling with antitumor activity in cancer cells overexpressing Akt, Cancer Res. 64 (2004) 4394–4399. https://doi.org/10.1158/0008-5472.CAN-04-0343.

[161] C. Evangelisti, F. Ricci, P. Tazzari, F. Chiarini, M. Battistelli, E. Falcieri, A. Ognibene, P. Pagliaro, L. Cocco, J.A. McCubrey, A.M. Martelli, Preclinical testing of the Akt inhibitor triciribine in T-cell acute lymphoblastic leukemia, J. Cell. Physiol. 226 (2011) 822–831. https://doi.org/10.1002/jcp.22407.

[162] L. Huang, Y. Nakai, I. Kuwahara, K. Matsumoto, PRAS40 is a functionally critical target for EWS repression in Ewing sarcoma, Cancer Res. 72 (2012) 1260–1269. https://doi.org/10.1158/0008-5472.CAN-11-2254.

[163] B.A. Smeester, G.M. Draper, N.J. Slipek, A.T. Larsson, N. Stratton, E.J. Pomeroy, K.L. Becklin, K. Yamamoto, K.B. Williams, K. Laoharawee, J.J. Peterson, J.E. Abrahante, S.K. Rathe, L.J. Mills, M.R. Crosby, W.A. Hudson, E.P. Rahrmann, D.A. Largaespada, B.S. Moriarity, Implication of znf217 in accelerating tumor development and therapeutically targeting ZNF217-induced PI3K–AKT signaling for the treatment of metastatic osteosarcoma, Mol. Cancer Ther. 19 (2020) 2528–2541. https://doi.org/10.1158/1535-7163.MCT-20-0369.

[164] H.F. Bahmad, T.H. Mouhieddine, R.M. Chalhoub, S. Assi, T. Araji, F. Chamaa, M.M. Itani, A. Nokkari, F. Kobeissy, G. Daoud, W. Abou-Kheir, The Akt/mTOR pathway in cancer stem/progenitor cells is a potential therapeutic target for glioblastoma and neuroblastoma, Oncotarget. 9 (2018) 33549–33561. https://doi.org/10.18632/oncotarget.26088.

[165] R.R. Ramsay, M.R. Popovic-Nikolic, K. Nikolic, E. Uliassi, M.L. Bolognesi, A perspective on multi-target drug discovery and design for complex diseases, Clin. Transl. Med. 7 (2018). https://doi.org/10.1186/s40169-017-0181-2.

[166] F. Xu, L. Na, Y. Li, L. Chen, Roles of the PI3K/AKT/mTOR signalling pathways in neurodegenerative diseases and tumours, Cell Biosci. 10 (2020) 1–12. https://doi.org/10.1186/s13578-020-00416-0.

[167] C.P. Hall, C.P. Reynolds, M.H. Kang, Modulation of Glucocorticoid Resistance in Pediatric T-cell Acute Lymphoblastic Leukemia by Increasing BIM Expression with the PI3K/mTOR Inhibitor BEZ235, Clin. Cancer Res. 22 (2016) 621–632. https://doi.org/10.1158/1078-0432.CCR-15-0114.

[168] C. Xie, M.J. Freeman, H. Lu, X. Wang, C.L. Forster, A.L. Sarver, T.C. Hallstrom, Retinoblastoma cells activate the AKT pathway and are vulnerable to the PI3K/mTOR inhibitor NVP-BEZ235, Oncotarget. 8 (2017) 38084–38098. https://doi.org/10.18632/oncotarget.16970.

[169] J.R. Brown, M. Hamadani, J. Hayslip, A. Janssens, N. Wagner-Johnston, O. Ottmann, J. Arnason, H. Tilly, M. Millenson, F. Offner, N.Y. Gabrail, S. Ganguly, S. Ailawadhi, S. Kasar, A.P. Kater, J.K. Doorduijn, L. Gao, J.J. Lager, B. Wu, C. Egile, M.J. Kersten, Voxtalisib (XL765) in patients with relapsed or refractory non-Hodgkin lymphoma or chronic lymphocytic leukaemia: an open-label, phase 2 trial, Lancet Haematol. 5 (2018). https://doi.org/10.1016/S2352-3026(18)30030-9.

[170] A. Al-Ghabkari, M.A. Perinpanayagam, A. Narendran, Inhibition of PI3K/mTOR Pathways with GDC-0980 in Pediatric Leukemia: Impact on Abnormal FLT-3 Activity and Cooperation with Intracellular Signaling Targets, Curr. Cancer Drug Targets. 19 (2019) 828–837. https://doi.org/10.2174/1568009619666190326120833.

[171] T. Powles, M.R. Lackner, S. Oudard, B. Escudier, C. Ralph, J.E. Brown, R.E. Hawkins, D. Castellano, B.I. Rini, M.D. Staehler, A. Ravaud, W. Lin, B. O'Keeffe, Y. Wang, S. Lu, J.M. Spoerke, L.-Y. Huw, M. Byrtek, R. Zhu, J.A. Ware, R.J. Motzer, Randomized Open-Label Phase II Trial of Apitolisib (GDC-0980), a Novel Inhibitor of the PI3K/Mammalian Target of Rapamycin Pathway, Versus Everolimus in Patients With Metastatic Renal Cell Carcinoma, J. Clin. Oncol. 34 (2016) 1660–1668. https://doi.org/10.1200/JCO.2015.64.8808.

[172] V. Makker, F.O. Recio, L. Ma, U.A. Matulonis, J.O. Lauchle, H. Parmar, H.N. Gilbert, J.A. Ware, R. Zhu, S. Lu, L.-Y. Huw, Y. Wang, H. Koeppen, J.M. Spoerke, M.R. Lackner, C.A. Aghajanian, A multicenter, single-arm, open-label, phase 2 study of apitolisib (GDC-0980) for the treatment of recurrent or persistent endometrial carcinoma (MAGGIE study), Cancer. 122 (2016) 3519–3528. https://doi.org/10.1002/cncr.30286.

[173] J.E. Allen, G. Krigsfeld, P.A. Mayes, L. Patel, D.T. Dicker, A.S. Patel, N.G. Dolloff, E. Messaris, K.A. Scata, W. Wang, J.-Y. Zhou, G.S. Wu, W.S. El-Deiry, Dual Inactivation of Akt and ERK by TIC10 Signals Foxo3a Nuclear Translocation, TRAIL Gene Induction, and Potent Antitumor Effects, Sci. Transl. Med. 5 (2013). https://doi.org/10.1126/scitranslmed.3004828.

[174] C.L.B. Kline, A.P.J. Van den Heuvel, J.E. Allen, V. V. Prabhu, D.T. Dicker, W.S. El-Deiry, ONC201 kills solid tumor cells by triggering an integrated stress response dependent on ATF4 activation by specific eIF2α kinases, Sci. Signal. 9 (2016). https://doi.org/10.1126/scisignal.aac4374.

[175] V. V. Prabhu, M.K. Talekar, A.R. Lulla, C.L.B. Kline, L. Zhou, J. Hall, A.P.J. Van den Heuvel, D.T. Dicker, J. Babar, S.A. Grupp, M.J. Garnett, U. McDermott, C.H. Benes, J.J. Pu, D.F. Claxton, N. Khan, W. Oster, J.E. Allen, W.S. El-Deiry, Single agent and synergistic combinatorial efficacy of first-in-class small molecule imipridone ONC201 in hematological malignancies, Cell Cycle. 17 (2018) 468–478. https://doi.org/10.1080/15384101.2017.1403689.

[176] M.N. Stein, J.R. Bertino, H.L. Kaufman, T. Mayer, R. Moss, A. Silk, N. Chan, J. Malhotra, L. Rodriguez, J. Aisner, R.D. Aiken, B.G. Haffty, R.S. DiPaola, T. Saunders, A. Zloza, S. Damare, Y. Beckett, B. Yu, S. Najmi, C. Gabel, S. Dickerson, L. Zheng, W.S. El-Deiry, J.E. Allen, M. Stogniew, W. Oster, J.M. Mehnert, First-in-Human Clinical Trial of Oral ONC201 in Patients with Refractory Solid Tumors, Clin. Cancer Res. 23 (2017) 4163–4169. https://doi.org/10.1158/1078-0432.CCR-16-2658.

[177] ClinicalTrials.gov, ONC201 in Pediatric H3 K27M Gliomas, NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT03416530 (accessed July 28, 2022).

[178] ClinicalTrials.gov, Combination Therapy for the Treatment of Diffuse Midline Gliomas, NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT05009992?term=Combination+Therapy+for+the+Treatment+of+Diffuse+Midline+Gliomas&draw=2&rank=1 (accessed July 28, 2022).

[179] ClinicalTrials.gov, Intermediate-size Expanded Access to ONC201 for Patients With H3 K27M-mutant and/or Midline Gliomas, NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT04617002?term=Intermediate-size+Expanded+Access+to+ONC201+for+Patients+With+H3+K27M-mutant+and%2For+Midline+Gliomas&draw=2&rank=1 (accessed July 28, 2022).

[180] ClinicalTrials.gov, Oral ONC201 in Recurrent GBM, H3 K27M Glioma, and Midline Glioma, NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT02525692?term=Oral+ONC201+in+Recurrent+GBM%2C+H3+K27M+Glioma%2C+and+Midline+Glioma&draw=2&rank=1 (accessed July 28, 2022).

[181] ClinicalTrials.gov, Phase 2 Study of ONC201 in Neuroendocrine Tumors, NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT03034200?term=Phase+2+Study+of+ONC201+in+Neuroendocrine+Tumors&draw=2&rank=1 (accessed July 28, 2022).

[182] R. McLeod, R. Kumar, D. Papadatos-Pastos, J. Mateo, J.S. Brown, A.H.I. Garces, R. Ruddle, S. Decordova, S. Jueliger, R. Ferraldeschi, O. Maiques, V. Sanz-Moreno, P. Jones, S. Traub, G. Halbert, S. Mellor, K.E. Swales, F.I. Raynaud, M.D. Garrett, U. Banerji, First-in-Human Study of AT13148, a Dual ROCK-AKT Inhibitor in Patients with Solid Tumors, Clin. Cancer Res. 26 (2020) 4777–4784. https://doi.org/10.1158/1078-0432.CCR-20-0700.

[183] A.-M. Tsimberidou, J. V. Shaw, D. Juric, C. Verschraegen, A.M. Weise, J. Sarantopoulos, G. Lopes, J. Nemunaitis, M. Mita, H. Park, B. Ellers-Lenz, H. Tian, W. Xiong, R. Kaleta, R. Kurzrock, Phase 1 study of M2698, a p70S6K/AKT dual inhibitor, in patients with advanced cancer, J. Hematol. Oncol. 14 (2021). https://doi.org/10.1186/s13045-021-01132-z.

[184] E.K.H. Han, J.D. Leverson, T. McGonigal, O.J. Shah, K.W. Woods, T. Hunter, V.L. Giranda, Y. Luo, Akt inhibitor A-443654 induces rapid Akt Ser-473 phosphorylation independent of mTORC1 inhibition, Oncogene. 26 (2007) 5655–5661. https://doi.org/10.1038/sj.onc.1210343.

[185] T. Okuzumi, D. Fiedler, C. Zhang, D.C. Gray, B. Aizenstein, R. Hoffman, K.M. Shokat, Inhibitor Hijacking of Akt Activation, Nat. Chem. Biol. 5 (2009) 484–493. https://doi.org/10.1038/nchembio.183.

[186] R.S. Narayan, C.A. Fedrigo, E. Brands, R. Dik, L.J.A. Stalpers, B.G. Baumert, B.J. Slotman, B.A. Westerman, G.J. Peters, P. Sminia, The allosteric AKT inhibitor MK2206 shows a synergistic interaction with chemotherapy and radiotherapy in glioblastoma spheroid cultures, BMC Cancer. 17 (2017) 1–8. https://doi.org/10.1186/s12885-017-3193-9.

[187] S.B. Kondapaka, S.S. Singh, G.P. Dasmahapatra, E.A. Sausville, K.K. Roy, Perifosine, a novel alkylphospholipid, inhibits protein kinase B activation, Mol. Cancer Ther. 2 (2003) 1093–1103.

[188] R.S. Goydel, C. Rader, Antibody-based cancer therapy, Oncogene. 40 (2021) 3655–3664. https://doi.org/10.1038/s41388-021-01811-8.

[189] V. Bayer, An Overview of Monoclonal Antibodies, Semin. Oncol. Nurs. 35 (2019) 150927. https://doi.org/10.1016/j.soncn.2019.08.006.

[190] H. Shim, Bispecific Antibodies and Antibody–Drug Conjugates for Cancer Therapy: Technological Considerations, Biomolecules. 10 (2020) 360. https://doi.org/10.3390/biom10030360.

[191] ClinicalTrials.gov, Afuresertib +Sintilimab+Chemotherapy in Patients With Selected Solid Tumors That Resistance to Prior Anti-PD-1/PD-L1, NIH. (2022). https://clinicaltrials.gov/ct2/show/NCT05383482 (accessed July 26, 2022).

[192] ClinicalTrials.gov, Testing the Addition of an Anti-cancer Drug, Ipatasertib, to the Usual Immunotherapy Treatment (Pembrolizumab) in Patients With Recurrent or Metastatic Squamous Cell Cancer of the Head and Neck, NIH. (2021). https://clinicaltrials.gov/ct2/show/NCT05172258 (accessed July 26, 2022).

[193] ClinicalTrials.gov, A Study of Targeted Agents With Atezolizumab for Patients With Recurrent or Persistent Endometrial Cancer (EndoMAP), NIH. (2020). https://clinicaltrials.gov/ct2/show/NCT04486352 (accessed July 26, 2022).

[194] ClinicalTrials.gov, A Study of Dalotuzumab + MK-2206, Dalotuzumab + MK-0752, and Dalotuzumab + Ridaforolimus Combination Therapies in Participants With Advanced Cancer (MK-0646-027), NIH. (2018). https://clinicaltrials.gov/ct2/show/NCT01243762 (accessed July 26, 2022).

[195] C.I. Chen, S. Snitzler, O. Levina, T. (Trina) Wang, L.W. Le, H. Paul, E.N. Wei, A. Lau, M. Queau, J.B. Johnston, S. Trudel, D.A. Smith, A Phase 2 Study of Ofatumumab in Combination with a Pan-AKT Inhibitor (Afuresertib) in Previously Treated Patients with Chronic Lymphocytic Leukemia (CLL), Blood. 126 (2015) 1742–1742. https://doi.org/10.1182/blood.V126.23.1742.1742.

[196] A.J. Chien, A. Cockerill, C. Fancourt, E. Schmidt, M.M. Moasser, H.S. Rugo, M.E. Melisko, A.H. Ko, R.K. Kelley, W.M. Korn, L.J. Esserman, L. van't Veer, C. Yau, D.M. Wolf, P.N. Munster, A phase 1b study of the Akt-inhibitor MK-2206 in combination with weekly paclitaxel and trastuzumab in patients with advanced HER2-amplified solid tumor malignancies, Breast Cancer Res. Treat. 155 (2016) 521–530. https://doi.org/10.1007/s10549-016-3701-7.

[197] C. Hudis, C. Swanton, Y.Y. Janjigian, R. Lee, S. Sutherland, R. Lehman, S. Chandarlapaty, N. Hamilton, D. Gajria, J. Knowles, J. Shah, K. Shannon, E. Tetteh, D.M. Sullivan, C. Moreno, L. Yan, H.S. Han, A phase 1 study evaluating the combination of an allosteric AKT inhibitor (MK-2206) and trastuzumab in patients with HER2-positive solid tumors, Breast Cancer Res. 15 (2013) R110. https://doi.org/10.1186/bcr3577.

[198] ClinicalTrials.gov, A Study of MK-2206 in Combination With Trastuzumab and Lapatinib for the Treatment of HER2+ Solid Tumors (MK-2206-015), NIH. (2018).

https://clinicaltrials.gov/ct2/show/NCT00963547 (accessed July 27, 2022).

[199] ClinicalTrials.gov, MK-2206, Paclitaxel and Trastuzumab in Treating Patients With HER2-overexpressing Solid Tumor Malignancies, NIH. (2014). https://www.clinicaltrials.gov/ct2/show/NCT01235897 (accessed July 26, 2022).

[200] ClinicalTrials.gov, An Open-Label Phase 2 Study of Ofatumumab (Arzerra) in Combination With Oral GSK2110183 in the Treatment of Relapsed and Refractory Chronic Lymphocytic Leukemia (CLL), NIH. (2019). https://clinicaltrials.gov/ct2/show/NCT01532700 (accessed July 26, 2022).

[201] M. Békés, D.R. Langley, C.M. Crews, PROTAC targeted protein degraders: the past is prologue, Nat. Rev. Drug Discov. 21 (2022) 181–200. https://doi.org/10.1038/s41573-021-00371-6.

[202] I. You, E.C. Erickson, K.A. Donovan, N.A. Eleuteri, E.S. Fischer, N.S. Gray, A. Toker, Discovery of an AKT Degrader with Prolonged Inhibition of Downstream Signaling, Cell Chem. Biol. 27 (2020) 66–73. https://doi.org/10.1016/j.chembiol.2019.11.014.

[203] J. Xu, X. Yu, T.C. Martin, A. Bansal, K. Cheung, A. Lubin, E. Stratikopoulos, K.M. Cahuzac, L. Wang, L. Xie, R. Zhou, Y. Shen, X. Wu, S. Yao, R. Qiao, P.I. Poulikakos, X. Chen, J. Liu, J. Jin, R. Parsons, AKT Degradation Selectively Inhibits the Growth of PI3K/PTEN Pathway–Mutant Cancers with Wild-Type KRAS and BRAF by Destabilizing Aurora Kinase B, Cancer Discov. 11 (2021) 3064–3089. https://doi.org/10.1158/2159-8290.CD-20-0815.

[204] X. Yu, J. Xu, L. Xie, L. Wang, Y. Shen, K.M. Cahuzac, X. Chen, J. Liu, R.E. Parsons, J. Jin, Design, Synthesis, and Evaluation of Potent, Selective, and Bioavailable AKT Kinase Degraders, J. Med. Chem. 64 (2021) 18054–18081. https://doi.org/10.1021/acs.jmedchem.1c01476.

[205] J.S. Brown, U. Banerji, Maximising the potential of AKT inhibitors as anti-cancer treatments, Pharmacol. Ther. 172 (2017) 101–115. https://doi.org/10.1016/j.pharmthera.2016.12.001.

[206] C. Porta, C. Paglino, A. Mosca, Targeting PI3K/Akt/mTOR signaling in cancer, Front. Oncol. 4 (2014). https://doi.org/10.3389/fonc.2014.00064.

[207] G.M. Nitulescu, D. Margina, P. Juzenas, Q. Peng, O.T. Olaru, E. Saloustros, C. Fenga, D.A. Spandidos, M. Libra, A.M. Tsatsakis, Akt inhibitors in cancer treatment: The long journey from drug discovery to clinical use (Review), Int. J. Oncol. 48 (2016) 869–885. https://doi.org/10.3892/ijo.2015.3306.

[208] G.A. Ruiter, S.F. Zerp, H. Bartelink, W.J. Van Blitterswijk, M. Verheij, Anti-cancer alkyl-lysophospholipids inhibit the phosphatidylinositol 3-kinase-Akt/PKB survival pathway, Anticancer. Drugs. 14 (2003) 167–173. https://doi.org/10.1097/00001813-200302000-00011.

[209] J. Verweij, A. Planting, M. van der Burg, G. Stoter, A dose-finding study of

miltefosine (hexadecylphosphocholine) in patients with metastatic solid tumours, J. Cancer Res. Clin. Oncol. 118 (1992) 606–608. https://doi.org/10.1007/BF01211805.

[210] European Medicines Agency, EU/3/08/567, (2009). https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu308567. (accessed July 22, 2021).

[211] B.R. Leonard, J. Hardy, G. Van Tienhoven, S. Houston, P. Simmonds, M. David, J. Mansi, Randomized, double-blind, placebo-controlled, multicenter trial of 6% miltefosine solution, a topical chemotherapy in cutaneous metastases from breast cancer, J. Clin. Oncol. 19 (2001) 4150–4159. https://doi.org/10.1200/JCO.2001.19.21.4150.

[212] S. Sundar, T.K. Jha, C.P. Thakur, J. Engel, H. Sindermann, C. Fischer, K. Junge, A. Bryceson, J. Berman, Oral miltefosine for Indian visceral leishmaniasis, N. Engl. J. Med. 347 (2002) 1739–1746. https://doi.org/10.1056/NEJMoa021556.

[213] S. Sundar, P.K. Sinha, M. Rai, D.K. Verma, K. Nawin, S. Alam, J. Chakravarty, M. Vaillant, N. Verma, K. Pandey, P. Kumari, C.S. Lal, R. Arora, B. Sharma, S. Ellis, N. Strub-Wourgaft, M. Balasegaram, P. Olliaro, P. Das, F. Modabber, Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: An open-label, non-inferiority, randomised controlled trial, Lancet. 377 (2011) 477–486. https://doi.org/10.1016/S0140-6736(10)62050-8.

[214] J. Lin, D. Sampath, M.A. Nannini, B.B. Lee, M. Degtyarev, J. Oeh, H. Savage, Z. Guan, R. Hong, R. Kassees, L.B. Lee, T. Risom, S. Gross, B.M. Liederer, H. Koeppen, N.J. Skelton, J.J. Wallin, M. Belvin, E. Punnoose, L.S. Friedman, K. Lin, Targeting activated Akt with GDC-0068, a novel selective Akt inhibitor that is efficacious in multiple tumor models, Clin. Cancer Res. 19 (2013) 1760–1772. https://doi.org/10.1158/1078-0432.CCR-12-3072.

[215] T. Sangai, A. Akcakanat, H. Chen, E. Tarco, Y. Wu, K.-A. Do, T.W. Miller, C.L. Arteaga, G.B. Mills, A.M. Gonzalez-Angulo, F. Meric-Bernstam, Biomarkers of Response to Akt Inhibitor MK-2206 in Breast Cancer, Clin. Cancer Res. 18 (2012) 5816–5828. https://doi.org/10.1158/1078-0432.CCR-12-1141.

[216] B.R. Davies, H. Greenwood, P. Dudley, C. Crafter, D.H. Yu, J. Zhang, J. Li, B. Gao, Q. Ji, J. Maynard, S.A. Ricketts, D. Cross, S. Cosulich, C.C. Chresta, K. Page, J. Yates, C. Lane, R. Watson, R. Luke, D. Ogilvie, M. Pass, Preclinical pharmacology of AZD5363, an inhibitor of AKT: Pharmacodynamics, antitumor activity, and correlation of monotherapy activity with genetic background, Mol. Cancer Ther. 11 (2012) 873–887. https://doi.org/10.1158/1535-7163.MCT-11-0824-T.

[217] B.H. Kushner, N.-K. V. Cheung, S. Modak, O.J. Becher, E.M. Basu, S.S. Roberts, K. Kramer, I.J. Dunkel, A phase I/Ib trial targeting the Pi3k/Akt pathway using perifosine: Long-term progression-free survival of patients with resistant neuroblastoma, Int. J. Cancer. 140 (2017) 480–484.

https://doi.org/10.1002/ijc.30440.

[218] D.B. Gursel, Y.S. Connell-albert, R.G. Tuskan, T. Anastassiadis, J.C. Walrath, J.J. Hawes, J.C.A. Schaick, K.M. Reilly, Control of proliferation in astrocytoma cells by the receptor tyrosine kinase/PI3K/AKT signaling axis and the use of PI-103 and TCN as potential anti-astrocytoma therapies, Neuro. Oncol. 13 (2011) 610–621. https://doi.org/10.1093/neuonc/nor035.

4. CAPÍTULO II:

O texto completo do item “4. CAPÍTULO II”, presente no texto completo da tese defendida, ocupa o intervalo de páginas compreendido entre as páginas 101–133, foi suprimido por tratar-se de manuscrito em preparação para publicação em periódico científico. Consta da síntese e avaliação biológica de oxazolidinas com atividade antitumoral.

5. CAPÍTULO III:

O texto completo do item “5. CAPÍTULO III”, presente no texto completo da tese defendida, ocupa o intervalo de páginas compreendido entre as páginas 135–141, foi suprimido por tratar-se de manuscrito em preparação para publicação em periódico científico. Consta da síntese e avaliação biológica de inibidores de quinase com atividade antitumoral.

6. CAPÍTULO IV:

O texto completo do item “6. CAPÍTULO IV”, presente no texto completo da tese defendida, ocupa o intervalo de páginas compreendido entre as páginas 143–167, foi suprimido por tratar-se de manuscrito em preparação para publicação em periódico científico. Consta da síntese e avaliação biológica de inibidores de protease com atividade antiviral.

7. RESULTADOS E DISCUSSÕES GERAIS

O texto completo do item “7. RESULTADOS E DISCUSSÕES GERAIS”, presente no texto completo da tese defendida, ocupa o intervalo de páginas compreendido entre as páginas 169–173, foi suprimido por conter informações dos manuscritos em preparação para publicação em periódicos científicos. Consta da síntese e avaliação biológica de derivados heterocíclicos com atividade frente a enzimas quinase e protease.

8. CONCLUSÕES E PERSPECTIVAS

O texto completo do item “8. CONCLUSÕES E PERSPECTIVAS”, presente no texto completo da tese defendida, ocupa o intervalo de páginas compreendido entre as páginas 175–176, foi suprimido por conter informações dos manuscritos em preparação para publicação em periódicos científicos. Consta da síntese e avaliação biológica de derivados heterocíclicos com atividade frente a enzimas quinase e protease.

9. REFERÊNCIAS

O texto completo do item “9. REFERÊNCIAS”, presente no texto completo da tese defendida, ocupa o intervalo de páginas compreendido entre as páginas 177–181, foi suprimido por conter informações dos manuscritos em preparação para publicação em periódicos científicos. Consta das referências utilizadas no desenvolvimento dessa tese e dos artigos a serem publicados.

10. ANEXO A

Projeto de Doutorado Sanduíche: **Avaliação do PH122 em modelo *in vivo* e avaliação de compostos da quimoteca PHARSG em proteases de interesse para o desenvolvimento de antimicrobianos.**

O texto completo do item “10. ANEXO ”, presente no texto completo da tese defendida, ocupa o intervalo de páginas compreendido entre as páginas 183–194, foi suprimido por conter informações dos manuscritos em preparação para publicação em periódicos científicos. Consta da metodologia e dos resultados dos experimentos realizados durante o doutorado sanduíche da autora dessa tese na Universidade da Califórnia em San Diego.

11. ANEXO B - Artigos obtidos durante o período de Doutorado

11.1 Artigos como primeiro autor

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Review

Targeting Akt/PKB in pediatric tumors: A review from preclinical to clinical trials

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GSK690693 (PubChem CID 16725726)
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GSK2141795 (PubChem CID 51042438)
CCT128930 (PubChem CID17751819)
GDC-0068 (PubChem CID 24788740)
AZD5363 (PubChem CID 25227436)
MK-2206 (PubChem CID 24964624)
TAS-117 (PubChem CID 66555816)
Perifosine (PubChem CID 148177)
Triciribine (PubChem CID 65399)

ABSTRACT

The serine/threonine kinase Akt is a major player in the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway, and its modulation impacts multiple cellular processes such as growth, proliferation, and survival. Several abnormalities in this pathway have been documented over the years, and these alterations were shown to have great implications in tumorigenesis and resistance to chemotherapy. Thus, multiple Akt inhibitors have been developed and tested in adult tumors, and some of them are currently undergoing phase I, II, and III clinical trials for distinct cancers that arise during adulthood. Despite that, the impact of these inhibitors is still not fully understood in pediatric tumors, and Akt-specific targeting seems to be a promising approach to treat children affected by cancers. This review summarizes recent available evidence of Akt inhibitors in pediatric cancers, from both preclinical and clinical studies. In short, we demonstrate the impact that Akt inhibition provides in tumorigenesis, and we suggest targeting the PI3K/Akt/mTOR signaling pathway, alone or in combination with other inhibitors, is a feasible tool to achieve better outcomes in pediatric tumors.

Abbreviations: ALL, Acute Lymphoblastic Leukemia; AML, Acute Myelogenous Leukemia; AMPK, AMP-Activated Protein Kinase; ATP, Adenosine Triphosphate; BDNF, Brain-Derived Neurotrophic Factor; B-pre ALL, B-precursor acute lymphoblastic leukemia; CDK2, Cyclin-dependent kinase 2; CLL, Chronic Lymphocytic Leukemia; CML, Chronic Myeloid Leukemia; CRBN, E3 ubiquitin ligase substrate adaptor cereblon; CSC, Cancer Stem Cell; DCR, Disease Control Rate; DLT, Dose-Limiting Toxicities; DNA-PK, DNA-Dependent Protein Kinase; EFS, Event-Free Survival; EGFR, Epidermal Growth Factor Receptor; FDA, Food and Drug Administration; FOXO, Family of Forkhead Box Class O; GPCR, G Protein-Coupled Receptors; GSK3, Glycogen Synthase Kinase 3; HER2, Human Epidermal Growth Factor Receptor 2; HM, C-terminal regulatory hydrophobic motif; HR-NB, High-Risk Neuroblastoma; INRC, International Neuroblastoma Response Criteria; JMML, Juvenile Myelomonocytic Leukemia; KC, Kinase Catalytic Domain; MAPKAP-K1, Mitogen-activated protein kinase-activated protein kinase-1; MEK, Mitogen-activated protein kinase kinase; MSK-1, Mitogen- and stress-activated kinase 1; MTD, Maximum Tolerated Dose; mTOR, mammalian target of rapamycin; mTORC2, mammalian target of rapamycin complex 2; NB, Neuroblastoma; p70S6K, p70 ribosomal S6 kinase; PDK1, 3-Phosphoinositide-Dependent Protein Kinase 1; PFS, progression-free survival; PH, Pleckstrin Homology Domain; PI3K, Phosphoinositide 3-Kinase; PIP_2, Phosphatidylinositol 4,5-bisphosphate; PIP_3, Phosphatidylinositol 3,4,5-triphosphate; PKB, Protein Kinase B; PPTP, Pediatric Preclinical Testing Program; PTEN, Phosphatase and Tensin Homolog; Ras-ERK, Ras-Extracellular Signal-Regulated Kinase; RECIST, Response Evaluation Criteria in Solid Tumors; ROCK, Rho-kinase 1; RTK, Receptor Tyrosine Kinases; T-ALL, T-cell acute lymphoblastic leukemia; TPD, targeted protein degradation; TRKB, Tropomysin-related Kinase B; TSC2, Tuberous Sclerosis Complex 2; VHL, von Hippel–Lindau protein.

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Discovery of a new class based on 6-methoxypyrimidine as efficient low weight reversible inhibitors of SARS-CoV-2 main protease (M^{pro}) with antiviral activity

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Abstract: The main protease M^{pro} of SARS-CoV-2 is an important enzyme for the viral cycle and its inhibition affects replication and infection propagation. In this way, it has been reported as an excellent target for drug design. In this work, we discovered a low molecular weight non-peptide, heterocyclic inhibitor, that inactivates M^{pro}. Initially, we screened a focused chemical library of heterocyclic compounds against M^{pro} that included several cysteine protease inhibitors. A compound based on a 6-methoxypyrimidine scaffold was identified as an inhibitor of this enzyme and structure-activity relationship studies revealed all the important groups for M^{pro} inhibition. Optimization of the hit compound provided a small (306 g/mol) competitive inhibitor that rapidly inactivated M^{pro} activity with high selectivity compared to other cysteine proteases. **PH521** (**16f**) presented increased potency against SARS-CoV-2 infection of cells being 11-fold more potent than the initial hit compound.

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11.2 Artigos como co-autor

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Identification of antimycobacterial 8-hydroxyquinoline derivatives as *in vitro* enzymatic inhibitors of *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase

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ABSTRACT

The increasing prevalence of drug-resistant *Mycobacterium tuberculosis* strains stimulates the discovery of new drug candidates. Among them are 8-hydroxyquinoline (8HQ) derivatives that exhibited antimicrobial properties. Unfortunately, there is a lack of data assessing possible targets for this class mainly against *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase (*Mt*InhA), a validated target in this field. Thus, the main purpose of this study was to identify 8HQ derivatives that are active against *M. tuberculosis* and *Mt*InhA. Initially, the screening against the microorganism of a small antimicrobial library and its new derivatives that possess some structural similarity with *Mt*InhA inhibitors identified four 7-substituted-8HQ (series **5** – **5a**, **5c**, **5d** and **5i**) and four 5-substituted-8HQ active derivatives (series **7** – **7a**, **7c**, **7d** and **7j**). In general, the 7-substituted 8-HQs were more potent and, in the enzymatic assay, were able to inhibit *Mt*InhA at low micromolar range. However, the 5-substituted-8-HQs that presented antimycobacterial activity were not able to inhibit *Mt*InhA. These findings indicate the non-promiscuous nature of 8-HQ derivatives and emphasize the significance of selecting appropriate substituents to achieve *in vitro* enzyme inhibition. Finally, 7-substituted-8HQ series are promising new derivatives for structure-based drug design and further development.

1. Introduction

The *Mycobacterium tuberculosis* (Mtb) complex is responsible for most tuberculosis (TB), cases an infectious disease affecting about 10 million people worldwide [1]. TB mainly affects the lungs and can be classified as a latent infection or active disease, characterized by symptoms such as chronic cough, sustained weight loss, fever, and hemoptysis [2,3]. This disease presents risk factors such as HIV infection, undernutrition, diabetes, and alcohol abuse, and it is a significant cause of death in HIV positive patients [3]. Without of adequate treatment, TB can result in significant mortality rates [1].

TB therapy presents problems. There are about 20 drug options: being isoniazid, rifampicin, pyrazinamide, and ethambutol considered first-line drugs. However, *M. tuberculosis* presents well-known resistance

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Title:	8-Hydroxyquinoline Derivatives as Drug Candidates for the Treatment of Alzheimer's Disease
Abstract:	Alzheimer's disease (AD) is the most prevalent form of dementia among older adults worldwide. Amidst several hypotheses to explain the pathobiology of the disease are biochemical indicators such as β-amyloid (Aβ) plaques; neurofibrillary tangles, caused by hyperphosphorylated tau protein; oxidative stress; metal dyshomeostasis; low levels of acetylcholine and neuroinflammation. Considering the multifactorial nature of AD, there has been an increase in research for novel multitarget compounds, mainly utilizing molecular hybridization for drug design. In this review, we focus on the 8-hydroxyquinoline moiety, a privileged metal-binding agent with Aβ antiaggregating properties, and its derivatives aiming to have effect on multiple molecular targets. Furthermore, we discuss the most prominent structure-activity relationships found on the analyzed compounds along with the most promising strategies explored by researchers. That way, we hope to provide a comprehensive perspective on the development of anti-Alzheimer agents based on the 8-hydroxyquinoline moiety in the last decade.
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