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# Molecular target: pan-AKT in gastric cancer



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## ABSTRACT

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signalling pathway is involved in multiple cellular processes, including cell survival, proliferation, differentiation, metabolism and cytoskeletal reorganisation. The downstream effectors of this PI3K pathway are also essential for maintaining physiologic homeostasis, commonly dysregulated in most solid tumours. AKT is the key regulator in PI3K/AKT/mTOR signalling, interacting with multiple intracellular molecules. AKT activation subsequently leads to a number of potential downstream effects, and its aberrant activation results in the pathogenesis of cancer. Accordingly, as an attractive therapeutic target for cancer treatment, several AKT inhibitors are currently under development and in multiple stages of clinical trials for various types of malignancy, including gastric cancer (GC). Therefore, the authors review the significance of AKT and recent studies on AKT inhibitors in GC, focusing on the scientific background with the potential to improve treatment outcomes.

## INTRODUCTION

The prognosis for metastatic or recurrent gastric cancer (GC) remains very poor, making it the third leading cause of cancer-related death worldwide.[1] Although effective combination cytotoxic chemotherapies have been introduced and two targeted agents, trastuzumab and ramucirumab, have approved for GC treatment in first-line and second-line settings, the outcomes are still unsatisfactory.[2–5] More recently, immune checkpoint inhibitiors (ICIs) with antiprogrammed cell death protein-1 monoclonal antibodies, such as pembrolizumab and nivolumab, have led to durable and impressive responses in a minority of GC.[6 7] However, a significant proportion of patients does not respond to these therapies, plus the efficacies seem to vary depending on the tumour biology.[8] GC is well known as a complex and heterogeneous disease with various treatment outcomes.[9] Interestingly, two recent molecular classifications have provided insights on the heterogeneous nature of GC. The Cancer Genome Atlas Research Network suggested a comprehensive molecular characterisation of 295 GCs using various platforms, and proposed four distinct subtypes: Epstein-Barr virus-positive, microsatellite instability (MSI), genomically stable and tumours with chromosomal instability.[10] Another group also described four subgroups: MSI, microsatellite stable (MSS)/epithelial-to-mesenchymal transition, MSS/TP53 positive and MSS/TP53 negative.[11] Each subtype is characterised by specific gene mutations and alterations in multiple signalling pathways. Consequently, these biologic investigations have improved clinical awareness of linking tumour profiling to the selection of molecularly targeted therapies. Plus, targeting the appropriate molecules may be a promising approach for precision medicine in GC treatment.

Among these molecules, AKT, also known as protein kinase B, is a serine–threonine kinase downstream of the phosphatidylinositol 3-kinase (PI3K) signalling pathway, which controls multiple cellular processes, such as cell survival, proliferation, differentiation, metabolism and cytoskeletal reorganisation.[12 13] Functioning as a major effector protein in the PI3K pathway, AKT modulates normal cellular physiology like cell growth, motility, proliferation, metabolism and survival.[14] AKT is also one of the most hyperactivated kinases in human cancers, influencing various biological phenomena that are directly involved in tumourigenesis.[15] Frequent activation of AKT has also been reported in approximately 78% of GC.[16] Kobayashi *et al* demonstrated that increased AKT kinase activity was associated with a higher grade and poor prognosis in GC.[17] Moreover, in another molecular classification proposed by the Singapore-Duke group, mesenchymal subtype GC cell lines were found to be particularly sensitive to the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway inhibitors.[18] Therefore, cumulative evidence indicates a promising potential for targeting AKT for the effective treatment of GC. This review focuses on the role of AKT and highlights the results of recent clinical trials on AKT inhibition in GC.

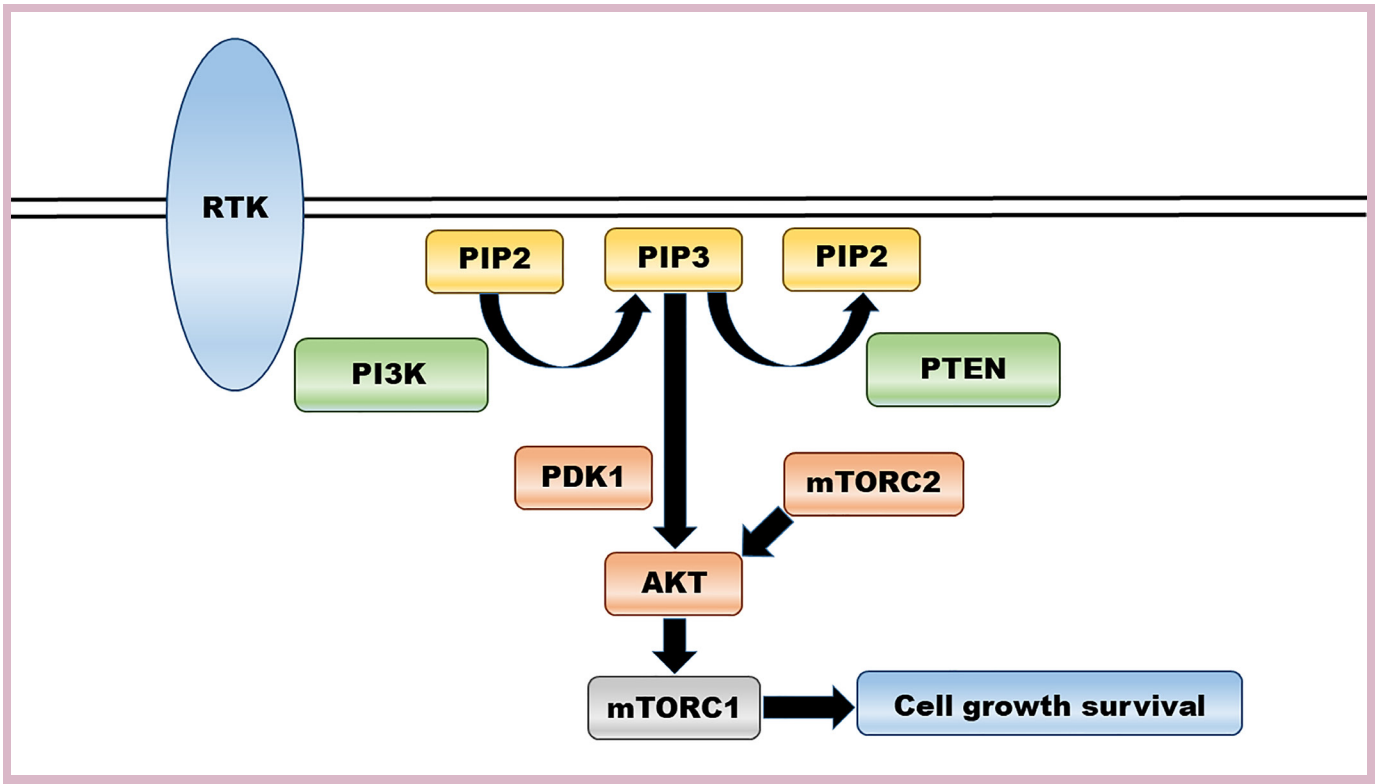


**Figure 1** Overview of PI3K/AKT/mTOR signalling pathway. AKT, protein kinase B; mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 2; PI3K, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent protein kinase 1; PIP2, phosphatidylinositol 4,5-diphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate; PTEN, phosphatase and tensin homologue; RTK, receptor tyrosine kinase.

### Overview of PI3K signalling pathway and AKT activation

The upstream target proteins of AKT are stimuli-induced receptor tyrosine kinases (RTKs) and include PI3K, mTOR complex 2 (mTORC2) and phosphoinositide-dependent protein kinase 1 (PDK1, figure 1).[14 19] The growth factor-mediated stimulation of RTKs interacts with src-homology 2 domains in PI3K, subsequently activating PI3K. This leads to the conversion of phosphatidylinositol 4,5-diphosphate (PIP2) into phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 acts as a second messenger to recruit AKT to the plasma membrane, and the binding of AKT to PIP3 induces a conformational change that results in the phosphorylation of AKT at Ser473 by mTORC2 and Thr308 by PDK1.[20] Phosphorylated AKT is an active form and promotes cell growth via the phosphorylation of mTOR complex 1. Important negative regulators of the PI3K/AKT/mTOR pathway also include the tumour suppressor gene, phosphatase and tensin homologue (PTEN), which dephosphorylate PIP3 back to PIP2. As mentioned above, AKT is an important signalling hub with numerous downstream substrates, affecting cell growth, proliferation, survival, cellular metabolism, glucose uptake and even angiogenesis.[20 21] AKT has been identified in three isoforms (AKT1, AKT2 and AKT3) transcribed from different genes and sharing an 80% amino acid homology.[22] Each member contains three conserved domains including a plekstrin homology (PH) domain in the N-terminal, central kinase domain and hydrophobic C-terminal tail (figure 2).[23] In general, AKT1 is widely expressed, the expression of AKT2 is elevated in insulin-responsive metabolic tissues and AKT3 is more highly expressed in endocrine and brain tissues.[19 20] Although still unclear how each isoform carries out a specific functional role, AKT1 promotes growth and survival, while AKT2 controls cellular invasiveness and mesenchymal characteristics.[22] In particular, AKT3 is known to play an essential role in physiologic brain development.[24]

### AKT in GC and its importance

The PI3K/AKT/mTOR pathway is the most commonly dysregulated signalling pathway in human cancer.[12] Common abnormalities and alterations in cancer include activating mutations or amplification of growth factors or RTKs, activating mutations in the p110α catalytic

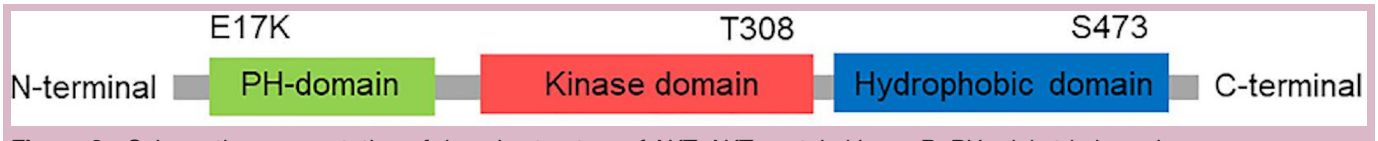


**Figure 2** Schematic representation of domain structure of AKT. AKT, protein kinase B; PH, plekstrin homology.

subunit of PI3K, loss of function and deletions in PTEN and activating mutations or amplification of AKT.[14 19 21] For instance, aberrations in the PI3K signalling pathway have been discovered in 38% of all tumour types, with PTEN loss by immunohistochemistry (IHC) occurring most frequently (30%), followed by mutations in PIK3CA (13%), PTEN (6%) and AKT1 (1%).[20 25] In GC, the PI3K/AKT/mTOR pathway has also been heavily implicated in both tumourigenesis and disease progression.[26] One early study by Staal *et al* reported a 20-fold amplification of AKT1 in one of every five GC cases in a survey of 225 human tumours.[27] It is well known that AKT2 is a frequently amplified isoform, while AKT1 is the most commonly mutated isoform in most solid tumours.[20] An AKT1 point mutation in the PH domain that substitutes an amino acid (E17K) is the most predominantly reported mutation, ranging from 0% to 4%, and confers increased activity due to pathological localisation of AKT1 in the plasma membrane.[25 28] However, AKT1 E17K mutation is very rare in GC.[29] Notably, a study of 294 cancer tissues detected AKT2 mutations in 1 of every 51 GC cases (2.0%), yet no mutations of AKT1 or AKT3.[30] In addition to E17K, E49K, L52R, C77F and Q19K mutants are known to activate AKT1, and G171R has been identified in the kinase domain of AKT3 in various types of cancer.[21 23] Meanwhile, AKT2 gene amplification has been reported in 5%–15% of pancreatic, ovarian and breast cancers.[15 31–34]

Unlike other cancers, the amplification of AKT2 has not been fully explored in GC.[28] Plus, a recent study showed a low amplification frequency of AKT1 of 0.3% in GC.[35] A study of 311 GC cases found increased AKT activity in 78%, as determined by IHC.[16] Cinti *et al* examined 50 resected GC cases and phosphorylated AKT was detected in 68% of the tumours, which correlated with the tumour aggressiveness.[36] Similarly, a link has been shown between AKT overexpression and advanced disease, suggesting a prognostic role in GC.[17 37] Therefore it is possible that AKT is activated through other mechanisms other than mutations. The importance of AKT in the PI3K pathway extends to its role in tumours with other known alterations or in the regulation of AKT signalling by microRNAs (miRNAs). A recent study reported that human epidermal growth factor receptor 2 (HER2) overexpression was significantly associated with phosphorylated AKT expression in GC tissues.[38] In this study, phosphorylated AKT expression was also correlated with poor prognosis, indicating that the PI3K/AKT pathway plays a critical role in HER2-positive GC. Another study showed that cytoplasmic AKT expression was markedly increased in PIK3CA-mutant tumours, providing a strong rationale for the clinical exploration of PI3K/AKT inhibitor combinations. Interestingly, geridonin and paclitaxel could synergistically inhibit the proliferation of GC cells via the suppression of AKT signalling.[39] Moreover, the mesenchymal subtype classified by the Singapore-Duke group is particularly sensitive to the PI3K/AKT/mTOR pathway inhibitors.[18] In addition, Lu *et al* observed that metastasis-associated lung adenocarcinoma transcript 1 competitively binds to miRNA-181a-5p, thereby upregulating AKT3 protein levels and promoting tumour growth in GC.[40] Thus, overall, these findings demonstrate the significance of AKT as a mediator of cellular proliferation and effective target for drug development in GC.

### Pan-AKT inhibitors in GC

Several promising AKT inhibitors have already been developed and are currently in various stages of clinical trials. There are two categories of AKT inhibitors.[20 21] The first type is a competitive or allosteric inhibitor of the ATP-biding site in the kinase domain, while the second type interacts with the PH domain of AKT, thereby preventing localisation of AKT in the plasma membrane. Most AKT inhibitors in clinical development inhibit all AKT isoforms referred as pan-AKT inhibitors.[21] An overview of clinical evidence of AKT inhibitors in GC is briefly summarised in table 1.

### Ipatasertib (GDC-0068)

Ipatasertib is a highly selective oral ATP-competitive pan-AKT inhibitor ($IC_{50}$=5.0–18.0 nM) which preferentially targets the phosphorylated conformation of AKT.[41 42] Ipatasertib exhibits antitumour effects against several cancer cell lines and xenograft models by inhibiting the PI3K/AKT pathway.[43 44] A first phase I study of ipatasertib demonstrated that the maximum tolerated dose (MTD) was 600 mg once daily with the two dose-limiting toxicity (DLT) at a dose level of 800 mg.[45] In terms of safety, the predominant ipatasertib-related adverse events (AEs) are diarrhoea, nausea, asthenia/fatigue and rashes. The incidence of clinically significant hyperglycaemic or rashes is relatively low with ipatasertib compared with other agents against PI3K signalling. Plus, at the MTD of 600 mg for ipatasertib, 11 (44%) of 25 patients showed the best overall response. An additional biomarker study of the same clinical samples revealed a compensatory feedback activation of extracellular-signal-regulated protein kinase and human epidermal growth factor receptor 3.[46] Accordingly, several phase II studies have evaluated the efficacy and safety for patients with triple-negative breast cancer (TNBC) and castration-resistant prostate cancer (CRPC).[47–49] LOng-Term follow-Up Study (LOTUS) is a randomised, double-blind, placebo-controlled, phase II study designed to investigate the efficacy of ipatasertib plus paclitaxel in treatment-naive locally advanced or metastatic TNBC.[50] As a result, the combination of paclitaxel/ipatasertib demonstrated an improved PFS, one of two coprimary endpoints (6.2 vs 4.9 months, HR 0.6, p=0.037). Of note, in the subset of patients with PIK3CA/AKT1/PTEN-altered tumours (n=42), the treatment benefit derived from ipatasertib was greater in patients with PIK3CA/AKT1/PTEN-altered tumours identified through next-generation sequencing (9.0 vs 4.9 months, HR 0.44, p=0.04). The ipatasertib–paclitaxel doublet in LOTUS was generally tolerated. AKT inhibition has also been studied in a neoadjuvant setting via the randomised

**Table 1** Clinical evidence of AKT inhibitors in GC

| Drug | Phase | Setting | Geographic region | Primary endpoints | Treatment arms | Patient number | RR (%) | PFS (months) | OS (months) | Common AEs | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ipatasertib | Randomised phase II | First-line | Europe, USA, Asia | PFS* | Ipatasertib/mFOLFOX6†<br>mFOLFOX6 | 71<br>82 | 52<br>56 | 6.57<br>7.52 | 12.12<br>15.67 | Diarrhoea, nausea, anorecia nausea, anorexia, neuropathy | Bang *et al*[54] |
| Capivasertib | Phase I/II biomarker-driven umbrella trial | Second-line | South Korea | RR | Capivasertib/paclitaxel | 24 | 33.3 | – | – | Hyperglycaemic, neutropenia, anaemia, diarrhoea | Lee *et al*[35] |
| Afuresertib | Phase Ib | Second-line | Asia | MTD/RP2D | Afuresertib/paclitaxel | 29 | Pending results | | | | NCT02240212 |
| Miransertib | Preclinical study | | | | AKT3 was upregulated in the majority of E-cadherin-deficient GCs, and mouse-derived gastric organoids lacking tumour suppressor gene CDH1 were sensitive to the apoptotic effects of miransertib. | | | | | | Bougen-Zhukov *et al*[82] |

*The coprimary endpoints were PFS in the intention-to-treat and biomarker-defined subgroup with PTEN low patients (<10%) as determined by IHC.
†The mFOLFOX6 regimen consisted of oxaliplatin (85 mg/m$^2$ intravenous infusion on day 1 every 14 days) coadministered with leucovorin 400 mg/m$^2$, then 5-fluorouracil 400 mg/m$^2$ administered as bolus infusion, followed by 5-fluorouracil 2400 mg/m$^2$ continuous infusion over 46 to 48 hours.
AEs, adverse events; GCs, gastric cancers; IHC, immunohistochemistry; MTD, maximum tolerated dose; OS, overall survival; PFS, progression-free survival; PTEN, phosphatase and tensin homologue; RP2D, recommended phase II dose; RR, response rate.

phase II FAIRLANE trial.[51] Although the addition of ipatasertib to neoadjuvant paclitaxel did not significantly increase the pathologic complete response (CR) rate, the overall response rate (ORR) by MRI was numerically higher with ipatasertib. Furthermore, the antitumour effect of ipatasertib was higher in the biomarker-selected patients and all the patients who showed CR had PIK3CA/AKT1/PTEN-altered tumours. Similar to these findings, a combined blockade with abiraterone and ipatasertib exhibited superior antitumour activity to abiraterone alone in patients with metastatic CRPC, especially tumours with PTEN loss.[52 53] Therefore, these evidence indicates that the identification of reliable biomarkers related with the PI3K/AKT pathway is important to facilitate precise patient selection and increase the clinical benefit to this agent. Consequently, ipatasertib continues to be actively investigated in several phase I and II clinical trials and separate phase III trials for TNBC and CRPC (table 2). In addition to these combination studies, several AKT inhibitors including ipatasertib are also being studied as a main drug in biomarker-driven trials.[41]

A recent randomised phase II trial assessed ipatasertib plus standard chemotherapy (oxaliplatin, leucovorin and 5-fluorouracil) versus chemotherapy alone in 153 patients with GC/gastro-oesophageal junction cancer as the first-line therapy.[54] Two coprimary endpoints were PFS in the intention-to-treat and biomarker-defined subgroup of patients with PTEN low (<10%) as determined by IHC. However, this trial did not meet its two endpoints for PFS and no significant difference with respect to overall survival was observed between the two groups. Plus, patients with PIK3CA mutations or amplification demonstrated no benefit with the combination study treatment. The most common AEs were nausea, diarrhoea and decreased appetite. Higher rates of severe AEs were also reported in the ipatasertib arm with common toxicity being diarrhoea, vomiting and nausea. Although the safety results were consistent with known AE profiles and there was no newly reported toxicity, the lower dose intensity of ipatasertib plus chemotherapy and higher rate of withdrawal in the experimental arm may have contributed to the lower-than-expected efficacy. There is an uncertainty regarding the best combination for ipatasertib in GC, raising the question of the interplay between ipatasertib and the chemotherapy backbone. Another consideration is that the treatment arm included more patients with poor prognostic factors, including diffuse histology, peritoneal metastasis and a higher number of metastatic sites. Ipatasertib activity may also be masked by heterogeneity of the study population, highlighting the potential need to identify specific biomarkers in order to maximise the efficacy of ipatasertib. Thus, due to low efficacy and toxicity concerns, it is unclear whether ipatasertib with conventional cytotoxic chemotherapy will be developed for GC. Notwithstanding, more recent studies are investigating the combination of ipatasertib with ICIs, such as pembrolizumab and atezolizumab, in breast and brain cancer (table 2). The outcomes of these

**Table 2** Pan-AKT inhibitors in clinical development

| Drug | Mechanism | Tumour types | Phase | Study design/combination Partner | Cliniclatrials.gov Number |
|---|---|---|---|---|---|
| Ipatasertib (GDC-0068) | ATP-competitive pan-AKT inhibitor | Breast | Ib | Trastuzumab/pertuzumab | NCT04253561 |
| | | Breast/ovary/prostate | Ib | Rucaparib | NCT03840200 |
| | | Breast | I/Ib | Carboplatin or carboplatin/paclitaxel | NCT03853707 |
| | | Solid tumours | I | Atezolizumab | NCT03673787 |
| | | Brain tumour | IIb | Pembrolizumab | NCT02430363 |
| | | Breast | Ib | Atezolizumb/paclitaxel | NCT03800836 |
| | | | | Atezolizumab/Nab-paclitaxel | |
| | | Breast | Ib | Aromatase inhibitors or fulvestrant or fulvestrant/palbociclib | NCT03959891 |
| | | Prostate | Ib/II | Abiraterone or abiraterone/apitolisib | NCT01485861 |
| | | Breast | II | Atezolizumab or atezolizumab/bevacizumab | NCT03395899 |
| | | Breast | III | Paclitaxel | NCT03337724 |
| | | Breast | III | Atezolizumab/paclitaxel | NCT04177108 |
| | | Prostate | III | Abiraterone/prednisolone | NCT03072238 |
| Capivasertib (AZD5363) | ATP-competitive pan-AKT inhibitor | Solid tumours | I | Olaparib | NCT02338622 |
| | | Solid tumours | I | Olaparib/durvalumab | NCT03772561 |
| | | Breast/ovary/endometrium | Ib | Olaparib | NCT02208375 |
| | | Prostate | I | Enzalutamide or abiraterone | NCT04087174 |
| | | Prostate | II | Enazalutamide | NCT02525068 |
| | | Breast | III | Paclitaxel | NCT03997123 |
| Afuresertib (GSK2110183) | ATP-competitive pan-AKT inhibitor | Prostate | I/II | LAE001/prednisolone | NCT04060394 |
| | | Stomach | Ib* | Paclitaxel | NCT02240212 |
| Uprosertib (GSK2141795) | ATP-competitive pan-AKT inhibitor | Multiple myeloma | II* | Trametinib | NCT01989598 |
| Miransertib (ARQ 092) | Allosteric pan-AKT and AKT1 E17K inhibitor | Ovary | Ib* | Paclitaxel or paclitaxel/carboplatin or anastrozole | NCT02476955 |

Biomarker-driven studies have been excluded from this list.
*Clinicaltrials.gov has noted no active patient enrolment in this study.

studies will hopefully provide insights on AKT inhibition combined with chemoimmunotherapy and may become an important step for future studies in GC.

### Capivasertib (AZD5363)

Capivasertib is another orally bioavailable, potent ATP-competitive pan-AKT inhibitor ($IC_{50}$=3.0–7.0 nM) that inhibits the phosphorylation of AKT substrates.[20] Several preclinical studies have demonstrated that capivasertib inhibits the growth of xenografts derived from a range of solid tumour types.[55 56] Capivasertib also significantly enhances the anticancer activity of docetaxel in GC xenograft models harbouring either PI3KCA mutation or PTEN loss.[57] In a phase I study using one continuous and two intermittent dosing schedules, the recommended phase II dose was 480 mg twice daily for 4 days followed by 3 days off, where the common AEs were diarrhoea (78%), nausea (49%) and hyperglycaemic (20%).[58] In particular, preliminary expansion data for patients with AKT1 E17K-mutant tumours showed very encouraging antitumour activity.[59] Another phase I study conducted in Japan also confirmed an intermittent dose of 480 mg to be the recommended dose, with partial response (PR) reported in two patients with AKT1 E17K mutations.[60] This data indicate that the presence of the AKT1 E17K mutation could be a potential response biomarker for this agent.[20] However, as noted before, AKT1 E17K mutations are very uncommon in GC.

This has led to several phase II studies of capivasertib in combination with paclitaxel or fulvestrant in breast cancer.[49] The phase II PAKT study investigated the addition of capivasertib to paclitaxel as the first-line therapy in 140 patients with metastatic TNBC.[61] The addition of capivasertib to first-line paclitaxel therapy for TNBC resulted in significantly longer PFS and OS. Plus, benefits were more pronounced in patients with PIK3CA/AKT1/PTEN-altered tumours. Capivasertib was also tested in a phase I/II FAKTION study of 140

postmenopausal women with metastatic oestrogen receptor (ER)-positive/HER2-negative breast cancer who had not received more than three previous lines of endocrine treatment and up to one line of chemotherapy for metastatic disease.[62 63] In this study, the primary endpoint of PFS was longer in the experimental arm (10.3 vs 4.8 months, HR 0.58, p=0.004). A further subgroup analysis also showed that the PI3K pathway alteration status did not seem to change the effect of capivasertib, In contrast to these results, a recent phase Ib/II BEECH trial investigated the efficacy of capivasertib in combination with first-line weekly paclitaxel for ER-positive/HER2-negative breast cancer show no significant PFS benefits in the overall population or PIK3CA-positive subpopulation.[64] Therefore, these results require further confirmation via ongoing phase III trial. Subsequent clinical studies and biomarker-driven trials have since validated the combination of capivasertib with other agents (table 2). For example, preliminary data demonstrated a synergistic effect of capivasertib in combination with olaparib, indicating that inhibitors of the P13K/AKT pathway potentiate a cytostatic effect of poly ADP-ribose polymerase (PARP) inhibitors in a combination therapy.[65] Recently, Lee *et al* performed a biomarker-based umbrella trial (VIKTORY; targeted agent eValuation In gastric cancer basket KORea) in GC.[35] This study classified patients with metastatic GC based on eight different biomarker groups and, among 10 associated clinical trials, included a treatment arm with capivasertib plus paclitaxel for PIK3CA mutation. It is noteworthy that the PIK3CA mutation capivasertib arm demonstrated moderate antitumour activity with an ORR of 33.3% in second-line GC, especially when compared with the low response rate (<15%) for the arm with PIK3CA wild-type capivasertib. As a result, these findings provide a strong rationale that such combinations with capivasertib and paclitaxel require further evaluation in additional phase II/III trials.

### Afuresertib (GSK2110183)

Afuresertib is an oral ATP-competitive pan-AKT inhibitor ($IC_{50}$=0.08–2.6 nM) that has been evaluated in a phase I study involving patients with haematologic malignancies.[66] On the basis of two DLTs in a 150 mg cohort, the recommended monotherapy phase II dose from this study was established at an MTD of 125 mg/day. This study showed a favourable safety profile and afuresertib appeared to be clinically active in multiple myeloma (MM). The drug-related AEs included nausea (23.3%), diarrhoea (20.5%), dyspepsia (19.2%) and fatigue (16.4%). Three patients with MM attained PR and an additional three attained minimal responses. Plus, in preclinical setting, enhanced antitumour activity was observed in MM cells when afuresertib was combined with pomalidomide plus dexamethasone. In a different phase I trial, the effect of afuresertib was examined in combination with trametinib, a mitogen-activated protein kinase (MEK) inhibitor, in 20 patients with advanced solid tumours and MM.[67] For these patient groups, an intermittent dosing schedule of a trametinib/afuresertib combination was shown to be more tolerable than continuous dosing. Thereafter, afuresertib was evaluated as a monotherapy or in combination with other anticancer agents in one phase I or two phase II studies. A phase IIa trial by Arceci *et al* demonstrated clinical activity (ORR=31%) associated with afuresertib monotherapy in patients with langerhans cell histiocytosis.[68] In a phase Ib study, a combination of afuresertib with carboplatin and paclitaxel in recurrent platinum-resistant ovarian cancer showed a favourable pharmacokinetic and toxicity profile with an MTD of afuresertib defined as 125 mg a day.[69] Chen *et al* also reported that combination therapy with ofatumumab and afuresertib was active and well-tolerated in previously treated patients with chronic lymphocytic leukaemia.[70] Based on its documented activity in other solid tumours, afuresertib in combination with paclitaxel in a second-line setting is currently testing in a phase Ib study in an unselected population (NCT02240212). Recently, many studies have revealed that the MEK/extracellular signal-regulated kinase (ERK) pathway is involved in regulating cell survival and proliferation in GC.[71] Moreover, some tumours can harbour genomic alterations in both the PI3K/AKT and MEK/ERK signalling network.[20] It is worth noting that afuresertib in combination with MEK inhibitor was shown to be beneficial for advanced solid tumours. Thus, this has significant implications for the clinical development of such combinations in GC. Plus, afuresertib is undergoing continued testing in combination with targeted therapies and chemotherapies in several phase I and II studies (table 2).

### Uprosertib (GSK2141795)

Uprosertib is an oral ATP-competitive pan-AKT kinase inhibitor ($IC_{50}$=38.0–328.0 nM) that has shown enhanced antitumour effects in combination with an MEK inhibitor in a pancreatic cancer tumour model.[72] In a phase I trial of uprosertib in patients with solid tumours, the recommended phase II dose of uprosertib for once-daily dosing was 75 mg, where the most common treatment-related AEs included diarrhoea, fatigue, vomiting and a decreased appetite.[73] In this study, PR was seen in two patients with a PIK3CA mutation and PTEN loss, respectively. Plus, a continuous dosing schedule of uprosertib in combination with trametinib was implemented in a separate phase I study.[74] Based on these results, uprosertib was evaluated in a number of clinical studies measuring the impact of a combination with trametinib. For example, a phase II clinical trial investigated the combination of uprosertib with trametinib in patients with acute myeloid leukaemia with Kirsten ras oncogene homologue (KRAS) and Neuroblastoma ras viral oncogene homologue mutations.[75] Despite the preliminary biological efficacy of this combination, no clinical benefit was observed. Exploration of the same combination in recurrent cervical cancer also revealed minimal clinical benefit.[76] When exploring a dosing of uprosertib at 50 mg and trametinib at 1.5 mg, the study closed early as the drug combination caused an unacceptable toxicity profile. This dosing combination also yielded limited clinical efficacy in recurrent endometrial cancer, with no responses at the previously recommended phase II dose.[77] Therefore, at this point in time, there are no ongoing studies, except for the treatment of MM (table 2).

### Miransertib (ARQ 092)

Miransertib is an allosteric pan-AKT and AKT1 E17K mutant inhibitor ($IC_{50}$=2.7–14.0 nM) that exhibits antitumour effects in vitro and in vivo as a monotherapy or in combination with paclitaxel.[78] In preliminary data from a phase Ib trial, the combination of miransertib and anastrozole demonstrated a manageable safety profile and preliminary efficacy in patients with PIK3CA or AKT1-mutant endometrial or ovarian cancer.[79] The most frequent AEs included a rash, hyperglycaemic and elevated liver enzyme. Durable PR was achieved in two patients with PIK3CA mutations. Meanwhile, miransertib is also being studied for the treatment of Proteus syndrome, which is a very rare mosaic overgrowth disorder caused by a somatic E17K activating mutation in the oncogene AKT1.[80] Recently, Leoni *et al* reported a case of successful treatment with miransertib of a patient with Proteus syndrome that resulted in relapsed AKT1 E17K mutant ovarian cancer, and clinical and serological remission after 22 months of treatment.[81] Despite insufficient data to support clinical cancer therapy, promising reports of antitumour activity in patients harbouring AKT1 E17K mutations support the use of biomarker-driven strategies in further clinical development of this drug. Interestingly, Bougen-Zhukov *et al* observed that AKT3 was upregulated in the majority of E-cadherin-deficient GCs, and mouse-derived gastric organoids lacking tumour suppressor gene CDH1 were sensitive to the apoptotic effects of miransertib.[82] This identification may be useful for providing new therapeutic strategies for hereditary and sporadic GC with mutations in the CDH1 gene, since germline truncation mutations in the CDH1 gene are found in 30%–50% families with hereditary diffuse GC.[83]

### Clinical perspective: resistance, toxicity and biomarkers

The PI3K/AKT/mTOR pathway comprises a complex network of crosstalk with many parallel cascades, so its inhibition induces negative feedback resulting in activation of other compensatory signalling pathways.[84] Theoretically, AKT inhibitors relieve feedback inhibition of upstream molecules and reactivate the PI3K signalling pathway by increasing the expression and phosphorylation of multiple RTKs and downstream effectors.[85] Indeed, emerging evidence supports the role of RTKs in restoring PI3K signalling following AKT inhibition.[86] Recent studies have demonstrated that other kinases can interact with AKT and stimulate cellular transformation without requiring the PI3K signalling pathway.[22] For example, in ER-positive breast cancer, a complex cycle with cross-regulatory interactions is already established between ER and the growth factor receptor network, leading to enhanced cell growth and proliferation in an AKT-independent manner.[87] Alteration of PDK1 can also activate other downstream molecules of the PI3K pathway via substrates other than AKT, such as serum-inducible and glucocorticoid-inducible protein kinase, mitogen-activated protein kinase or protein kinase C alpha.[22] In addition to PDK1, several studies have revealed AKT-independent protumouric features of mTORC2.[88 89] Moreover, the loss of PTEN and numerous miRNAs may closely contribute to the resistance to these pan-AKT inhibitors in GC.[90 91] To overcome this resistance, combination therapy regimens have been or are being tested in both preclinical and clinical settings as described in table 2. However, potential adverse effects have largely restricted the application and clinical significance of these inhibitors. While the underlying mechanisms of the PI3K/AKT/mTOR pathway inhibitor-associated toxicity are not well understood, they are likely related to a widespread role in intracellular signal transduction.[92] Similar to mTOR inhibitors, AKT inhibitors often have common toxicities, such as metabolic effects, cutaneous and mucosal effects, non-infectious pneumonitis, immunosuppression and constitutional symptoms.[84] Of note, pan-AKT inhibitors inevitably cause hyperglycaemic due to the influence of these inhibitors on glucose homeostasis.[13] In addition, the incidence and severity of AKT inhibitor-related AEs seem to be modest and better tolerated than the AEs caused by PI3K inhibitors. To decrease these toxicities, AKT isoform-specific inhibitors like MK-2206, an allosteric inhibitor with activity against AKT1 and AKT2, could be used in conjunction with a biomarker-driven approach.[13] However, concerns remain regarding the use of isoform-specific inhibitors due to a potential compensatory response that may lead to the hyperactivation of other AKT isoforms.[93]

Identification of more robust predictive biomarkers is critical to optimise treatment with these agents and avoid severe AEs and a subtherapeutic dose that often results in inadequate pathway inhibition and resistance to these agents.[13] To date, genomic alterations related with the PI3K/AKT/mTOR signalling pathway have been widely studied as potential biomarkers of response or resistance to AKT inhibitors.[94] The PIK3CA mutation status has been implemented as a predictive marker for AKT inhibitors. Plus, accumulating evidence on novel biomarkers, such as AKT mutations including AKT1 E17K, PIK3CA amplification, PTEN expression loss, KRAS mutations or the insulin level, has shown encouraging results in several studies.[94] However, the relationship between these biomarkers and the therapeutic effect of AKT inhibitors still remains unclear. Tumour heterogeneity can influence such conflicting results, and the genetic alterations vary according to various types of cancer. Thus, researchers are needed to develop more precise and standardised methods for analysing these mutations to obtain convincing data. Consequently, the challenge remains to apply the data generated through further validated clinical trials into clinical practice in order to provide precision medicine for GC in the near future.

## CONCLUSION

Multiple studies have already explored the promising potential of AKT inhibitors as monotherapies or in combination with cytotoxic and other targeted therapies for many cancers, including several validation studies for patients with GC, although recent data from a phase II study showed limited clinical activity with toxicity concerns, even in molecularly selected populations. Therefore, future research needs to focus on the detection of appropriate high-precision biomarkers with a better understanding of the complexities of the PI3K/AKT/mTOR signalling pathway, while a

biomarker-driven approach, like the VIKTORY trial, can optimise the therapeutic efficacy of AKT inhibitors in GC. Plus, the potential role of the AKT1 E17K mutation as a predictive marker needs to be explored in well-designed large-scale studies. Additional efforts are also being made to identify the best combination partners for synergistic effects and lower toxicity. This may lead to a better therapeutic index and broaden the usefulness of AKT inhibitors to overcome chemoresistance, and thereby improve treatment outcomes. In particular, it is important to assess the impact of combining ICIs with AKT inhibitors, as ICIs have become an important part of treating GC, plus the PI3K/AKT/mTOR pathway plays an essential role in the immune system. In conclusion, additional trials are needed to refine the benefits of AKT inhibitors, including specific evidence for patients with GC.

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