WNT signalling in prostate cancer

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Abstract Genome sequencing and gene expression analyses of prostate tumours have highlighted the potential importance of genetic and epigenetic changes observed in WNT signalling pathway components in prostate tumours, particularly in the development of castration-resistant prostate cancer. WNT signalling is also important in the prostate tumour microenvironment, where WNT proteins secreted by the tumour stroma promote therapy resistance, and in prostate cancer stem or progenitor cells, where WNT-β-catenin signals promote self-renewal or expansion. Preclinical studies have demonstrated the potential of inhibitors that target WNT-receptor complexes at the membrane or that block the interaction of β-catenin with LEF1 and the androgen receptor, in preventing prostate cancer progression. Some Wnt signalling inhibitors are in Phase I trials, but they have yet to be tested in patients with prostate cancer.

Prostate cancer was the most commonly diagnosed cancer and second leading cause of cancer death in men in the USA in 2016\(^1\). Androgen deprivation therapy (ADT) is the standard of care for men with prostate cancer, owing to the essential role of the androgen receptor (AR) in the normal growth and development of the prostate gland, as well as in prostate carcinogenesis\(^2\,^3\,^4\). Unfortunately, most tumours progress to an aggressive state, known as castration-resistant prostate cancer (CRPC), despite ADT\(^5\,^6\,^7\). Several mechanisms, most of which are AR-dependent, are involved in the development of resistance. These mechanisms include the amplification and/or mutation of AR, expression of AR splice variants, increased production of androgens, and changes in the activity or expression of AR coactivators and corepressors\(^7\,^8\,^9\). However, other, often AR-independent, signals have been implicated in the acquisition of resistance, among them are those triggered by WNT family proteins\(^10\).

This Review focuses on new findings since our last review of the topic in 2012\(^11\). These data demonstrate that the field of WNT signalling research has progressed both at the level of basic research, in which noncanonical WNT
signalling is now recognized as an important contributor to prostate cancer progression and new regulators of canonical WNT signalling at the plasma membrane have been identified, and at the clinical level, where WNT signalling might soon receive approval for clinical use, as WNT signalling inhibitors are now in early stage clinical trials \(^\text{12–34}\) (Table 2).

### [H1] An overview of WNT signalling

The WNT family comprises 19 cysteine-rich, secreted lipoglycoproteins that have fundamental roles during embryonic and organ development by regulating stem cell self-renewal and cell proliferation, migration, and differentiation \(^{35–37}\). WNTs bind to transmembrane frizzled (FZD) receptors and a variety of coreceptors, including low-density lipoprotein receptor (LRP)4 to LRP6, tyrosine-protein kinase transmembrane receptor ROR1 and ROR2, and tyrosine-protein kinase RYK (RYK) \(^{38}\) to activate canonical (β-catenin-dependent) and noncanonical (β-catenin-independent) signals (Fig. 1).

A hallmark of canonical WNT signalling is the stabilization and nuclear translocation of the protein β-catenin. In the absence of WNT ligands, β-catenin is recruited and degraded by the destruction complex, whose components include axin, glycogen synthase kinase-3 (GSK-3), casein kinase 1 (CK1) and adenomatous polyposis coli protein (APC). WNT binding to FZD receptors and LRP5 and LRP6 coreceptors results in the phosphorylation of the latter by CK1 and GSK-3 and recruitment of axin and dishevelled (DVL) to the plasma membrane, thereby disrupting the destruction complex. This disruption results in the stabilization of β-catenin, which accumulates in the cytoplasm and enters the nucleus, binding to T-cell factor/lymphoid enhancer-binding factor 1 (TCF/LEF) family transcription factors and coactivators, such as the CREB-binding protein (CBP)–histone acetyltransferase p300 (p300 HAT) family and B-cell lymphoma 9 protein (BCL9) and B-cell lymphoma 9-like protein (BCL9L), and regulating the expression of WNT target genes \(^{39}\) (Fig. 1).

A novel branch of WNT signalling, WNT-dependent stabilization of proteins (WNT–STOP) \(^{40}\), was described in 2014. In this signalling cascade, the signal is initiated by WNT binding to LRP6 but the effects are transcription-independent, involving cyclin-Y, rather than β-catenin \(^{40}\). WNT–STOP signals
promote protein stabilization at mitosis. The targets are involved cell-cycle progression, endolysosomal biogenesis, DNA remodelling and the cytoskeleton, so WNT-STOP signals could be important in cancer initiation and progression\(^{41}\) (Fig. 1).

Noncanonical WNT signals are less well defined than canonical signals. Noncanonical signals are traditionally subdivided into planar cell polarity (PCP) and WNT–Ca\(^{2+}\) pathways\(^{39}\), although additional β-catenin- and TCF-independent signals also exist\(^{42}\). Noncanonical WNT signals are activated upon WNT binding to FZD receptors and tyrosine-kinase-like coreceptors, resulting in the recruitment and activation of DVL. The PCP pathway has two parallel branches involving small GTPases: Rho, which activates Rho-associated kinase (ROCK) and Rac, which is linked to c-Jun-N-terminal kinase (JNK), and signalling by activator protein 1 (AP-1) family transcription factors. Cytoskeletal and transcriptional changes associated with these small GTPases regulate cell adhesion and migration\(^{39,42-44}\). Activation of the WNT–Ca\(^{2+}\) pathway stimulates Ca\(^{2+}\) release from the endoplasmic reticulum, promoting activation of G-proteins, protein kinase C (PKC) and Ca\(^{2+}\)/calmodulin-dependent kinase type II (CaMKII). These events can also result in the activation of transcription factors, such as nuclear factor of activated T-cells (NFAT), which promotes cell growth, survival, invasion, and angiogenesis\(^{42,43,45,46}\) (Fig. 1).

In 2010, novel associations were identified between WNT and Hippo signalling, which regulates organ size, tissue homeostasis, and patterning\(^{47}\). Hippo signals are transduced by yes-associated protein (YAP), transcriptional coactivator with PDZ-binding motif (TAZ), and TEA domain family transcription factors (TEADs)\(^{48}\). YAP-TAZ signals antagonize canonical WNT signalling by binding to components of the destruction complex, such as axin\(^{49}\), and regulating nuclear translocation of β-catenin (Fig.1). In addition, a noncanonical WNT–YAP-TAZ signalling axis has been described, in which WNT binding to FZD and ROR activates G\(_{\alpha_{12}}, G_{\alpha_{13}},\) Rho, and large tumour suppressor homolog (LATS)1 and LATS2 to induce YAP-TAZ signals and TEAD-dependent transcription\(^{50}\). The importance of this alternative WNT signalling pathway in prostate cancer is yet to be examined.
The specific mechanisms by which WNT proteins stimulate these different responses are not fully elucidated, but are anticipated to involve the binding and activation of distinct WNT receptors\textsuperscript{51,52}. FZD family receptors have a role in most WNT signalling pathways. These seven-pass transmembrane proteins have an extracellular, cysteine-rich domain (CRD) that is essential and sufficient for WNT binding\textsuperscript{53–55}. The crystal structure of the mouse Fzd8 CRD bound to Xenopus Wnt-8 shows the ligand resembling a hand with the thumb and index finger grasping the receptor. The thumb’s palmitoleic acid group projects into a deep groove in the CRD (Site 1) while the finger interacts with a small depression in the CRD (Site 2)\textsuperscript{56} (Fig. 2). The lipid thumb domain of Wnt-8 interacts with residues conserved among FZD family members, accounting for the promiscuity of WNT-FZD interactions. On the other hand, some Site 2 residues are not conserved, Met149, for example, is present in 5 of 10 mammalian FZD proteins\textsuperscript{56}, suggesting that Site 2 interactions are responsible for binding specificity\textsuperscript{56}. The structure also suggested a potential binding site for WNT coreceptors: a conserved solvent-exposed patch of 10 residues on the opposite side of Wnt-8 to the FZD-binding site\textsuperscript{56,38} (Fig. 2). Additional insight into WNT-FZD interactions comes from two independent structural studies of FZD CRDs in complex with fatty acids, which show that the acyl group promotes FZD CRD dimerization and support a 1:2 stoichiometry model for WNT-FZD complexes\textsuperscript{57,58}. Moreover, palmitoylated WNT-5A and WNT-8A stabilize the FZD4-CRD-CRD interaction, suggesting that FZD receptors form signalosomes (multiprotein complexes containing membrane-localized Wnt receptors and cytosolic protein complexes containing AXIN and DVL capable of transmitting the WNT signal) upon WNT binding and that the WNT palmitoleoyl group is crucial for this interaction\textsuperscript{57}. Additional studies will be required to determine how these interactions orchestrate WNT signalosome assembly.

Apart from FZD receptors, the single-pass transmembrane WNT receptors LRP5 and LRP6 are best known for transducing β-catenin-dependent signals. These receptors form a trimeric complex with WNT and FZD that is essential for activation of the canonical pathway\textsuperscript{38,55}. By contrast, ROR1 and ROR2 have CRDs related to the FZD CRD and activate noncanonical signals\textsuperscript{38,42,59}. In addition, RYK binds WNT proteins via a WNT inhibitory factor (WIF) domain
and is implicated in canonical and noncanonical signalling\textsuperscript{38,59}, and protein-
yrosine kinase 7 (PTK7); also known as colon carcinoma kinase 4 (CCK4),
which is involved in the PCP pathway and has a less well-defined WNT binding
site than RYK, ROR1 and ROR2\textsuperscript{60}. ROR1, ROR2, RYK, and PTK7 have
intracellular tyrosine-kinase-like domains but they are most likely pseudokinases because these domains lack key residues found at the catalytic
site of active kinases and they are devoid of detectable kinase activity in \textit{in vitro}
assays. Although it remains possible that the conditions for their catalytic
activation have not been found, it is more likely that they function in association
with FZDs, other WNT receptors and allosteric activation of intracellular and
other receptor tyrosine kinases\textsuperscript{59,61,62}. By contrast, the ROR-related kinase,
muscle, skeletal receptor tyrosine-protein kinase (MuSK), which also binds
WNTs via a CRD, is catalytically active\textsuperscript{38}, as is CAM-1, the \textit{Caenorhabditis
elegans} ROR homolog, suggesting that the signalling mechanisms of this
family of kinases have diverged.

Regulation of WNT signalling by secreted WNT antagonists is more complex
than that of the receptors. These antagonists can be divided into two classes:
those that bind WNT proteins, such as secreted frizzled-related proteins (sFRPs), WIF-1 and Cerberus, and those that associate with the WNT
coreceptors LRP5 and LRP6, namely Dickkopf-related protein (DKK)-1, DKK-
2, and DKK-4\textsuperscript{63–65}. DKK-3 is a unique member of this family that does not bind
LRP5 or LRP6, but can affect WNT signalling indirectly\textsuperscript{65}. Adding further
complexity, sFRPs can also bind to FZD receptors and activate or inhibit WNT
signalling\textsuperscript{63,66}.

Results of studies published between 2013 and 2016 have revealed additional
mechanisms of regulation of WNT signalling at the cell membrane\textsuperscript{64,65,67}. The
transmembrane E3 ubiquitin ligases ZNRF3 and RNF43 inhibit WNT signalling
by targeting FZD receptors for ubiquitination and degradation. ZNRF3 and
RNF43, in turn, are regulated by R-spondins (RSPO1–RSPO4), which are
secreted proteins that enhance WNT signalling by simultaneously binding
ZNRF3 and RNF43 and leucine-rich repeat-containing G protein-coupled
receptors (LGR4–LGR6) to promote ubiquitination and membrane clearance of
ZNRF3 and RNF43, facilitating FZD receptor stabilisation\textsuperscript{67,68} (Fig. 3).
[H1] WNT signalling pathway alterations

Dysregulation of the WNT signalling pathway has been observed in several types of cancer, and some major alterations have been found in prostate cancer in the past 5 years (Table 1)\(^{10,11,35,69}\).

[H2] Canonical effectors

β-catenin is the key effector of the canonical WNT pathway. The stability of β-catenin is tightly regulated by a destruction complex of associated proteins, including axin and APC, which promotes its phosphorylation, ubiquitination, and degradation; the presence of cytoplasmic and/or nuclear β-catenin provides an indication of dysregulation. By contrast, the pool of β-catenin at the plasma membrane is protected from degradation by its interaction with cadherins. A considerable proportion of prostate tumours have increased levels of β-catenin in the cytoplasm and/or nucleus either as a result of gene mutations, or, more frequently, nongenomic changes in the expression of inhibitors and activators of WNT signalling\(^{11}\).

Activating mutations in the gene encoding β-catenin (CTNNB1) and inactivating mutations in the genes that encode proteins in the destruction complex (APC and AXIN1) increase WNT–β-catenin signalling in many types of cancer, particularly colorectal cancer, but are infrequent in prostate cancer\(^{10,11}\). However, genetic and epigenetic changes have been discovered that activate WNT–β-catenin signalling and could, therefore, contribute to prostate cancer progression. Genetic changes that activate WNT–β-catenin signalling are more frequently observed in castration-resistant prostate cancer (CRPC) than in treatment-naïve prostate cancer. Recurrent alterations in CTNNB1 were observed in 12% of CRPC samples\(^{70}\). Comparison of CRPC and high-grade, untreated, localized prostate tumours uncovered alterations in APC in 22% of lethal, castration-resistant tumours and in none of the untreated tumours\(^{71}\), and a multi-institutional study of 150 metastatic CRPC tumours revealed genomic alterations affecting APC and CTNNB1 in 18% of samples\(^{72}\). In addition, analysis of single nucleotide polymorphisms (SNPs) in WNT pathway genes identified links between gene variants of APC and reduced PSA-free survival\(^{73}\) and prostate cancer progression\(^{74}\).

A variety of cell systems and mouse models have been used to determine the
outcome of β-catenin stabilization in non-malignant prostate cells and prostate cancer cells\textsuperscript{10,11}. Studies in mice show that genetic activation of WNT–β-catenin signalling by a stabilized form of β-catenin or by Apc deletion results in high-grade prostate intraepithelial neoplasia (PIN). However, progression to invasive carcinoma requires a second event, for example, overexpression of serine protease hepsin\textsuperscript{75} or deletion of phosphatase and tensin homolog (PTEN)\textsuperscript{76}.

Association of β-catenin with binding partners in the nucleus, such as TCF/LEF family transcription factors, protects β-catenin from the destruction complex by competing with APC and axin for binding to the β-catenin armadillo repeat domain, as is the case at the plasma membrane. All four TCF/LEF family members are expressed to varying extents in the prostate and in prostate tumours, but LEF1 has been the focus of most studies, as it is upregulated in androgen-independent tumours\textsuperscript{77}. LEF1 is a WNT-β-catenin target gene and also a target gene of transcriptional regulator ERG (ERG), which is upregulated in ~50% of prostate tumours\textsuperscript{78}. A 2016 study comparing β-catenin and LEF1 levels in prostate tumour microarray samples (TMAs) showed increases in the proportion of cells coexpressing β-catenin and LEF1 in localized prostate cancer and in tumour metastases, which suggests a role for β-catenin–LEF1-mediated transcription in both malignant transformation and metastasis of prostate cancer\textsuperscript{79}.

TCF/LEF family members are the key mediators of β-catenin-dependent transcription, other transcription factors interact with β-catenin in specific cell types and tissues. The AR is a crucial partner for β-catenin in prostate cancer. Given the influence of AR in prostate cancer, the importance and consequences of this interaction have been studied in detail\textsuperscript{11,80,81}. Coexpression of AR and cytoplasmic β-catenin in patient tumour samples correlates with high primary Gleason grade (4-5), disease progression, and PSA levels in patients\textsuperscript{82}, and AR and WNT signalling pathways are enriched in patients with early-onset (diagnosed at ≤ 50 years of age) prostate cancer\textsuperscript{83}.

In mice, the overexpression of AR and stabilized β-catenin in prostate epithelial cells accelerates tumour development and invasion and reduces survival, suggesting that increased AR signalling enhances β-catenin-mediated prostate tumour initiation\textsuperscript{84}. Castration of these mice resulted in tumour regression,
implying that AR signalling is also required for tumour maintenance\textsuperscript{84}. If and how AR signalling and β-catenin-dependent signalling work together is not well understood. In this mouse study, both AR and β-catenin were recruited to regions of the myc proto-oncogene protein (c-Myc) locus containing TCF/LEF family transcription factor binding sites\textsuperscript{84}. In addition, gene expression microarray analysis showed that AR overexpression potentiated expression of some genes induced by stabilized β-catenin, including Myc, Spp1, and Egr1, which promote metastasis. However, it also reduced expression of the direct β-catenin gene targets Lef1 and Axin\textsuperscript{2}\textsuperscript{84}, consistent with previous studies in human cells showing competition between TCF/LEF family transcription factors and AR for binding to β-catenin\textsuperscript{10,11}. The interactions among AR, β-catenin, and TCF/LEF family transcription factors can be affected by the activation state of AR, with hormone deprivation or treatment with the antiandrogen enzalutamide redirecting β-catenin from AR to TCF-4, leading to activation of WNT–β-catenin signalling\textsuperscript{85}. These observations highlight the pathophysiological relevance and the complexity of the link between AR and WNT–β-catenin signalling in prostate cancer and suggest that a combination of antiandrogens and WNT inhibitors could improve the effectiveness of current treatments targeting the AR\textsuperscript{85}. Other nuclear proteins might also activate WNT–β-catenin signalling in hormone-deprived cells, such as the nuclear form of the tyrosine-protein kinase MET, nMET\textsuperscript{86}, and transcription factor SOX (SOX)-4\textsuperscript{87,88}.

[H1] WNT ligands and their receptors

The low frequency of WNT signalling pathway gene mutations in the majority of prostate tumours has encouraged the study of upstream components of the pathway. Novel molecular mechanisms have been discovered that stabilize WNT receptors at the membrane to sustain WNT signals. However, these receptors still require the presence of WNT ligands, the study of which has been hampered by their low expression and/or the lack of specific antibodies. For example, gene expression analysis indicates that WNT1 mRNA is low or undetectable in prostate cancer cell lines \textsuperscript{78,89,90}, lower in organ confined (pT2) and non-organ confined (pT3/4) tumours than in benign prostatic hyperplasia, and highly expressed in advanced prostate cancer\textsuperscript{90}, whereas
immunohistochemical analysis detected high expression of WNT-1 to be highly expressed in prostate cancer cell lines, primary tumours and metastases.

An emerging theme is the activation of noncanonical WNT signalling in advanced prostate cancer and/or in CRPC. WNT-5a is upregulated in prostate cancer and can promote prostate tumour cell invasion through FZD2 and ROR2. Moreover, Wnt5a haploinsufficiency in a mouse model of prostate cancer prevents the early onset and early lethality of prostate tumours. However, some reports contradict the protumorigenic effect of WNT-5A, correlating high WNT-5A expression with improved outcomes in localized, low-grade prostate cancer. In other cancers such as liver, breast and lung, the WNT5A-FZD2 complex has been reported to associate with the protein tyrosine kinase FYN and signal transducer and activator of transcription 3 (STAT3) to promote the epithelial–mesenchymal transition (EMT) and cell migration. One possible explanation for the discrepancy is that the tumour-suppressing role of WNT-5A results from inhibition of canonical signalling and its tumour-promoting role from activation of noncanonical signalling, consistent with association of increased expression of WNT5A and FZD2 with metastasis and biochemical recurrence. Evidence exists that supports a role for WNT-5A in the development of resistance to ADT: WNT5A mRNA is found in circulating tumour cells (CTCs) from patients with CRPC and from patients with prostate cancer whose disease progressed whilst they were undergoing treatment with the AR inhibitor enzalutamide. WNT-7B, which had been previously observed to be upregulated in prostate cancer, was shown to be an AR target gene in CRPC. WNT-7B activates a noncanonical signal involving PKC and can induce an osteoblastic response in bone. WNT-11 is also upregulated in prostate cancer, but, in contrast to WNT-7B, its expression is repressed by androgens. WNT11 mRNA expression is elevated in tumours from patients receiving ADT and cell studies show that this WNT is required for prostate cancer cell invasion and neuroendocrine-like differentiation. Many WNTs are observed to be upregulated in certain settings: mRNA expression of WNT5B, WNT6, WNT10A, and WNT16 is increased in tumours from patients after ADT, and WNT2, WNT3A, and WNT11 mRNAs are induced by ERG, an oncogene expressed in prostate cancer exhibiting TMPRSS2–ERG gene
ERG also induces expression of FZD3, FZD5, FZD7, and FZD8 mRNAs, and also FZD4, which has a role in EMT. Independent studies reported increased expression of FZD8 and its closest relative, FZD5, in advanced prostate cancer. In addition, SOX9, a critical effector of ERG, is required for increased expression of FZD7, which is also upregulated in patients after ADT. Evidence for increased expression of WNT coreceptors in prostate cancer is not as readily available as the evidence for that of the FZD family. However, ROR1 protein was shown to be expressed in 19 of 21 prostate tumours, as well as in many other types of cancer, such as lung, ovarian and colorectal, in which it is linked to increased AKT and CREB phosphorylation and increased proliferation. Together, these studies highlight a limited number of WNTs and WNT receptors as potential therapeutic targets, particularly in the setting of CRPC.

[H1] WNT signalling regulators

A common theme in prostate cancer is the loss of expression of genes that encode secreted factors that inhibit WNT signalling, usually through promoter methylation. These include genes that encode WNT binding proteins, such as WIF-1 (for which restoration of expression reduces tumour cell motility and reverses EMT) and sFRP1 (expression of which inversely correlates with β-catenin expression and is a favourable predictive and prognostic biomarker in prostate cancer). Another study showed reduced mRNA expression of SFRP1 and SFRP5 in prostate cancer but an increase in SFRP4. SFRP2 gene expression is also downregulated in prostate cancer by promoter methylation. sFRPs do not always inhibit WNT signalling; for example, sFRP2 potentiates WNT-16B signalling to promote PC3 prostate cancer cell resistance to mitoxantrone, a genotoxic agent frequently administered to prostate cancer patients as a second-line therapy.

Dickkopf proteins can be protumorigenic in some settings, despite their function as WNT inhibitors and the observation that they are frequently silenced in cancer by gene promoter methylation. In prostate cancer, high levels of DKK-1 in serum are associated with poor patient prognosis. Furthermore,
overexpression of DKK-1 promotes prostate tumour growth and the incidence of bone metastases\textsuperscript{121}, and induces osteolytic lesions\textsuperscript{122}. Some of the effects of DKK-1 have been interpreted in the context of its ability to inhibit canonical signalling, resulting in activation of noncanonical signalling. However, the discovery in 2016 of an unrelated receptor for DKK-1, cytoskeleton-associated protein 4 (CKAP4), indicates that DKK-1 can also enhance cell proliferation independently of its effects on WNT signalling\textsuperscript{123}.

Among the other proteins that regulate WNT signalling at the cell surface, mutations in the ubiquitin ligases RNF43 and ZNRF3 and gene fusions that increase expression of RSPO2 were detected at an overall frequency of 6\% in a panel of 150 metastatic CRPC tumours\textsuperscript{72}, suggesting novel therapeutic options might be a possibility for some patients with prostate cancer.

[H1] WNT and the tumour microenvironment

The development and progression of cancer involves several signals that rely on the intrinsic properties of the tumour cells. However the interaction of cancer cells with other cell types in the tumour microenvironment, both in the primary tumour and at sites of metastasis, is also important\textsuperscript{124}.

Prostate stromal cells secrete several WNT family members that can influence tumorigenesis and disease progression (Fig. 4). This secretion is exemplified by studies in mice showing that stromal Wnt-3a activates canonical Wnt signalling in the epithelium, facilitating progression of PIN lesions to adenocarcinoma and resistance to androgen deprivation\textsuperscript{125}. Stromal overexpression of high mobility group protein HMGI-C in mice was observed to promote development of multifocal PIN lesions that were accompanied by increased expression of Wnt2, Wnt4, and Wnt9a, and could be suppressed by overexpression of sFRP1 and Dkk1\textsuperscript{126}. WNT10B is among the genes upregulated in human prostate cancer stroma, and silencing it reduces the protumorigenic effects of stromal cells on LNCaP tumour growth in xenograft assays\textsuperscript{127}. WNT signals from the tumour microenvironment are also involved in the development of resistance to therapy. Therapy-induced DNA damage increases the expression of WNT-16B in prostate fibroblasts\textsuperscript{128}, in which it acts in a paracrine manner to activate canonical WNT signalling in tumour cells and
promotes therapy resistance and disease progression\textsuperscript{128}. Further studies are required to determine the importance of stromal-derived WNT-16B and of other WNT family members, such as WNT-5A\textsuperscript{129}, at different stages of prostate cancer.

**[H1] WNT signalling and cancer stem cells**

In prostate and other cancers, such as breast, pancreas, colon, lung and brain, the cancer stem (or progenitor) cell (CSC) population shares many characteristics with somatic stem cells, such as immortality and self-renewal, and is believed to be a source of tumour-initiating cells and also responsible for tumour recurrence, metastasis, and chemoresistance\textsuperscript{130,131}. WNT–β-catenin signalling was shown to promote prostate CSC renewal and expansion in 2009\textsuperscript{132}. In 2015, one study showed that shRNA-mediated silencing of the tumour suppressor gene DAB2IP (disabled homolog 2-interacting protein) in human prostate epithelial cells generated CSC with activated WNT–β-catenin signalling\textsuperscript{133}. In this model, WNT–β-catenin signals directly induced expression of the stem cell marker gene CD44, and inhibitors of WNT secretion synergized with docetaxel to inhibit tumour growth \textit{in vivo}\textsuperscript{133}. In addition, the mRNA and protein expression of ALDH1A, another CSC marker that is also a direct target of WNT–β-catenin signalling, is elevated in radioresistant prostate cancer cells\textsuperscript{134}. Inhibition of WNT–β-catenin signalling using the tankyrase inhibitor XAV939 or siRNA-mediated knockdown of β-catenin reduced ALDH1A mRNA and protein levels, reduced the population of ALDH-positive CSCs and increased cancer cell sensitivity to irradiation\textsuperscript{134}. These results provide rationale for using inhibitors of WNT–β-catenin signalling to target prostate CSCs and thereby reduce the development of tumour resistance to therapy\textsuperscript{135}.

**[H1] Targeting WNT signalling**

The variety of changes observed in WNT pathway components has the potential to provide therapeutic opportunities, particularly in advanced prostate cancer. Several drugs that target WNT signalling are in development (Fig. 5), but very few of them are being tested for the treatment of prostate cancer\textsuperscript{136}. This omission might reflect a general impression that WNT signalling has a
minor role in prostate cancer, based on the low frequency of WNT pathway mutations in primary tumours. The higher frequency of APC mutations, identification of novel mutations in CRPC, and the increasing evidence that noncanonical WNT signals are active in prostate cancer, are expected to stimulate further interest in this area of drug development.

Specific inhibitors of WNT signalling have been investigated and several FDA-approved drugs in current use affect WNT–β-catenin signalling\textsuperscript{137}, and at least one of these — aspirin — is associated with reduced prostate cancer risk\textsuperscript{138}. Specific WNT pathway inhibitors can be classified into functional groups: inhibition of WNT secretion, regulation of antagonists and agonists, targeting WNT receptor interactions, preventing DVL activation, stabilizing the destruction complex and targeting nuclear β-catenin partners.

\textbf{[H2] Inhibition of WNT secretion}

Porcupine, a membrane-bound O-acetyltransferase, is uniquely responsible for WNT palmitoylation of WNT proteins, which is essential for their secretion\textsuperscript{139}. Targeting porcupine has been used extensively to inhibit WNT signalling in different cancer types. The first reported porcupine inhibitors, Inhibitors of WNT Production (IWPs, the best known of which is IWP-2), were identified in a synthetic chemical screen\textsuperscript{140}. Several other porcupine inhibitors have since been reported. These inhibitors include WNT-C59, which inhibits growth of WNT-1-expressing mammary tumours in mice\textsuperscript{141}, LGK974\textsuperscript{142}, an orally bioavailable inhibitor that reduces tumour growth in rodent models of breast cancer, head and neck cancer\textsuperscript{142}, and of xenografts of pancreatic tumours with RNF43 mutations\textsuperscript{143}; and ETC-159, another orally bioavailable molecule, which inhibits growth of patient-derived xenografts of colorectal cancers with RSPO fusions\textsuperscript{144}. WNT974 (previously known as LGK974) entered a phase I trial in 2011 (still ongoing), for patients with tumours that have mutations in RNF43 and ZNRF3 or RSPO gene fusions\textsuperscript{12}. Preliminary clinical data suggested WNT974 has a manageable safety profile and potential for anti-tumour activity\textsuperscript{145}. An ongoing phase I/II trial is using WNT974 in combination with the RAF inhibitor LGX818 and the EGFR inhibitor Cetuximab in patients with BRAFV600-mutant KRAS wild-type metastatic colorectal cancer harboring RNF43 mutations or RSPO fusions\textsuperscript{13}. ETC-159 (longer name ETC-1922159)
has also entered a phase I trial in patients with advanced solid tumours and preliminary data indicate that it is safe and well-tolerated and dose escalation is ongoing (Table 2). The identification of RNF43 and ZNRF3 mutations and RSPO2 gene fusions in CRPC in 2015 provides hope that porcupine inhibitors might also be assessed in patients with advanced prostate cancer.

[H2] Regulation of antagonists and agonists

Many secreted WNT antagonists are epigenetically silenced in cancer, and drugs that target the epigenetic machinery can restore their expression. For example, treatment of renal cancer cells or breast cancer cells with combined therapy of the histone deacetylase (HDAC) inhibitor romidepsin and the DNA methyltransferase (DNMT) inhibitor decitabine reactivates SFRP1 expression to inhibit growth and induce apoptosis in an sFRP1-dependent manner. Application of recombinant sFRP1 proteins could also be a therapeutic option, as is the case for DKK-3, which preclinical studies have shown is an effective treatment for prostate cancer and other cancers, including breast cancer, gastric carcinoma and malignant mesothelioma. A phase I/II clinical trial started in 2013 using an adenoviral vector expressing DKK-3 in patients with localized prostate cancer have reported promising results, showing reduced metastatic tumour growth in a case study of a patient with treatment-resistant disease and anti-tumour activity and reduced biochemical recurrence-free survival in patients with high-risk localised prostate cancer (Table 2).

Another approach to targeting tumours that contain RSPO fusions is to use function-blocking antibodies; as has been done for RSPO3 in colorectal cancer. RSPO3 antibodies (OMP131R10) are now in phase I clinical trials for advanced solid tumours and metastatic colorectal cancer with results expected in 2018 (Table 2).

[H2] Targeting WNT receptor interactions

WNT ligands and their receptors are attractive targets for therapy because they are accessible to monoclonal antibodies and small molecules. WNT-5A has been targeted using antibodies and a WNT-5A-derived peptide called Foxy-5. The WNT-5A antibodies reduce metastasis of gastric tumour cells but have not been tested for their effects on prostate cancer metastasis. These antibodies do not block WNT receptor binding, but they inhibit WNT-5A-induced internalization of FZD2 and ROR2. Foxy-5 is a formylated hexapeptide that
inhibits breast cancer metastasis\textsuperscript{156,157}. The first phase I trial failed to reach a maximum tolerated dose\textsuperscript{17}, so a second trial is recruiting patients with metastatic breast, colon, or prostate cancer\textsuperscript{18} (Table 2). Given that the antibodies inhibit WNT-5A signalling and Foxy-5 activates it, and in light of the contrasting reports on the prognosis of high WNT-5A expression in prostate cancer, stratifying patients before using treatments that target WNT-5A will be important.

Antibodies are not the only tool for targeting WNT proteins: a recombinant fusion of the ligand-binding domain of FZD8 and the crystallisable fragment domain of a human IgG1 (FZD8-Fc, also known as OMP-54F28 and Ipafricept) is a decoy WNT receptor that inhibits the proliferation of CSCs and induces tumour differentiation\textsuperscript{158}. In a phase Ia clinical trial, this drug was well tolerated and demonstrated evidence of Wnt pathway modulation and potential early single-agent activity manifested by prolonged stable disease\textsuperscript{19,159}. It is now being trialled in combination with other drugs in ovarian\textsuperscript{20,160}, liver\textsuperscript{21} and pancreatic cancer\textsuperscript{22} (Table 2).

The success of porcupine inhibitors in blocking all WNTs and the possibility of redundancy among members of the WNT family in cancer has led to increased efforts to target WNT receptors, rather than WNT proteins. FZD7 is one of most frequently upregulated WNT receptors in many cancers, including intestinal cancer, hepatocellular carcinoma, gastric cancer and breast cancer, and it has been targeted using both decoy receptor and antibody approaches\textsuperscript{161}. Vantictumab (OMP-18R5) targets FZD1, FZD2, FZD5, FZD7, and FZD8. In preclinical models, it reduces CSC frequency and induces differentiation of tumorigenic cells to cell types that are less tumorigenic and/or more susceptible to conventional chemotherapy. It exhibits strong anti-tumour activity in combination with other approved therapies in patient-derived xenograft (PDX) models, including cancers of the pancreas, breast, lung, liver, ovary and colon\textsuperscript{162}. Phase Ib clinical trials of vantictumab in combination with standard-of-care chemotherapy have been carried out in HER2-negative breast cancer and advanced pancreatic cancer\textsuperscript{23–26} (Table 2). In 2017, vantictumab and ipafricept were shown to potentiate the effects of taxanes in PDX models. This involved sensitization of cancer cells to taxanes and required treatment with WNT inhibition prior to mitotic blockade with paclitaxel\textsuperscript{163}. This combination strategy
has been incorporated into ongoing clinical trials of ipafricept and vantictumab with other chemotherapeutic agents\textsuperscript{20,22,25,26}. Other FZD antibodies are patented for the treatment of cancer, for example there were plans to test OTSA101, a radiolabelled antibody against FZD10\textsuperscript{164}, in phase I trials for synovial sarcoma was found to be feasible and safe but the number of patients accrued was too small to continue the study\textsuperscript{27,165} (\textbf{Table 2}). In 2016, phage display was used to generate antibodies that recognise FZD5 and FZD8 that inhibit the growth of \textit{RNF43}-mutant pancreatic tumour cells \textit{in vivo}\textsuperscript{166}. Among the non-FZD family receptors being targeted for therapy, LRP6 and ROR1 might be relevant to prostate cancer. The antihelminthic drug niclosamide has been reported to induce apoptosis in prostate cancer by targeting LRP6\textsuperscript{167}. However, this therapy was also shown to downregulate oncogenic variants of AR and overcome enzalutamide resistance in CRPC\textsuperscript{168,169}, so whether its effects relate to LRP6 or AR, or both, is unclear. Other agents that target LRP6 exist and can affect prostate cancer cells, including salinomycin, which induces LRP6 degradation\textsuperscript{170}, and Mesd, which acts as a chaperone for LRP5 and LRP6 and inhibits prostate tumour xenograft growth\textsuperscript{171}. ROR1 expression is increased in a number of cancers, including prostate cancer\textsuperscript{113,172}. Given the importance of ROR1 in chronic lymphocytic leukaemia, a variety of approaches have been used to target it, including antibodies, chimeric antigen receptor T cells, and antibody–drug conjugates\textsuperscript{173}. Among the antibodies, UC-961 (cirmtuzumab) is recruiting patients for phase I clinical studies for chronic lymphocytic leukaemia\textsuperscript{28–30} and breast cancer\textsuperscript{31} (\textbf{Table 2}).

\textbf{[H2] Preventing DVL activation}

The DVL PDZ domain binds to the carboxyl terminal region of FZD receptors and is essential for signal transduction. Among the three DVL family members, DVL2 is upregulated in prostate tumours and silencing it inhibits prostate cancer cell proliferation and migration\textsuperscript{174}. A number of DVL inhibitors have been reported, one of which has been shown to inhibit proliferation of the prostate cancer cell line PC-3\textsuperscript{175}.

\textbf{[H2] Stabilizing the destruction complex}
Tankyrases 1 and 2 are poly (ADP-ribose) transferases (PARPs) that target axin1 and axin2 for proteasomal degradation. Their inhibitors are widely used to promote β-catenin degradation, thereby inhibit canonical WNT signalling\textsuperscript{176}. Several structurally distinct tankyrase inhibitors are now being developed for possible clinical use. Structure-based optimization of one of these, XAV939, resulted in the development of a more selective and orally bioavailable inhibitor, NVP-TNKS656, which inhibited WNT signalling in mouse mammary tumour virus (MMTV)–WNT-1 tumour-bearing mice\textsuperscript{177}. Evidence exists that chronic WNT stimulation, which increases expression of the β-catenin partners LEF1 and B-cell CLL/lymphoma 9-like protein (BCL9L), renders tumour cells resistant to tankyrase inhibition because they shield β-catenin from axin\textsuperscript{178}. This shielding might occur in prostate tumours that express high levels of LEF1\textsuperscript{79}. Finally, it should be noted that tankyrase inhibitors might have effects not directly related to WNT signalling, as tankyrases bind and regulate several other proteins, including PTEN and angiomotin (AMOT) family proteins\textsuperscript{176,179,180}.

**[H2] Targeting nuclear β-catenin partners**

Transcriptional activation by β-catenin requires interaction both with TCF/LEF family transcription factors and other coactivators, such as CBP or p300, which associate with the carboxyl terminal transactivation domain in β-catenin. Thus, the disruption of these interactions might provide new therapies. Small molecule inhibitors of β-catenin-responsive transcription have been identified that disrupt the β-catenin–TCF4 interaction, including the inhibitors of catenin responsive transcription (iCRTs)\textsuperscript{181}, BC21, which was identified by virtual docking studies using a compound library and the crystal structures of β-catenin alone and in complexes with TCF3 and TCF4\textsuperscript{182}, and LF3, a 4-thioureido-benzenesulfonamide derivative identified in a high-throughput screen of 16,000 compounds\textsuperscript{183}. In 2004, ICG-001, a first-in-class modulator of WNT signalling, was observed to inhibit binding of β-catenin to CBP\textsuperscript{184}, and a derivative, PRI-724, is in phase I clinical trials in different solid tumors\textsuperscript{32} and has been reported to have an acceptable toxicity profile\textsuperscript{185}. Results are anticipated from completed phase I trials in patients with pancreatic cancer\textsuperscript{33} and leukemia\textsuperscript{34} (Table 2). In addition, a compound named Windorphen has been identified that prevents
p300 binding to β-catenin and reduces the viability of PC3 and DU145 cells \textit{in vitro}\textsuperscript{186}. Both CBP and p300 have many other partners, so these compounds also have WNT-independent effects that can lead to induction of caspase-3 dependent apoptosis and cell-cycle arrest\textsuperscript{187}. Two further inhibitors that affect β-catenin-dependent transcription are triptonide, a small molecule identified from the traditional Chinese medicine \textit{Tripterygium wilfordii} that targets the C-terminal domain of β-catenin\textsuperscript{188}, and an orally bioavailable WNT inhibitor that acts at or downstream of TCF, identified in a cell-based screen\textsuperscript{189}. The former has been shown to promote apoptosis of cancer cells, including PC3 and DU145 prostate cancer cells\textsuperscript{188} and the later inhibited tumour growth in a colon cancer xenografts\textsuperscript{189}, suggesting they have therapeutic potential.

Given the number of studies on the interaction between β-catenin with AR, the fact that no drug screens targeting this complex have been performed is surprising. However, a group of investigators used the overlap of the interaction domains of AR and TCF-4 on β-catenin and observed that iCRT3\textsuperscript{181} inhibited both AR and WNT–β-catenin signalling and reduced prostate tumour growth \textit{in vivo}\textsuperscript{190}. The effect of iCRT3 on AR binding to target genes was accompanied by a reduction in binding of coactivator-associated arginine methyltransferase 1 (CARM1 also known as PRMT4), a cofactor for AR and β-catenin and another therapeutic target\textsuperscript{190}.

[H1] Conclusions

WNT signalling has fundamental roles in prostate carcinogenesis. Both autocrine signalling in tumour cells and paracrine signalling from the tumour microenvironment are involved in prostate tumorigenesis, metastasis, and therapy resistance. Given the possibility that drugs that specifically target WNT signalling components will reach the clinic, the question that arises is whether canonical or noncanonical WNT signals are more critical in prostate cancer. The answer might be tumour stage-dependent and context dependent. Canonical WNT signals could be more important to support prostate cancer stem (or progenitor) cell proliferation and/or survival, than noncanonical signals, particularly under conditions of androgen deprivation, whereas noncanonical WNT signals, by endowing tumour cells with migratory and/or invasive
properties, might be more important at early stages of tumour spread. Both could be critical in metastatic CRPC, in which both activating mutations in canonical WNT signalling and activated noncanonical WNT signalling have been observed. Several agents that target WNT signalling at different levels of the pathway have been developed, and some of these have reached clinical trials (Table 2). However, not many of them have been tested as possible therapies for prostate cancer. Mutations in CTNNB1 and APC are relatively infrequent in primary tumours, but they are more prevalent than originally thought in advanced tumours and CRPC, and are accompanied by increased noncanonical WNT signalling. In addition, novel WNT pathway mutations have been discovered in CRPC. Together, these new discoveries provide hope for a subset of patients who might benefit from drugs currently in clinical trials that inhibit WNT signalling, particularly those affecting WNT secretion and/or WNT receptor binding, as well as those preventing β-catenin interaction with key transcription factors, such as LEF1 and AR.

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Figure 1 | WNT signalling pathways. In the absence of WNT ligands (WNT OFF), β-catenin is recruited and degraded by the destruction complex (adenomatous polyposis coli protein (APC), axin, glycogen synthase kinase-3 (GSK-3) and casein kinase 1 (CK1)) and yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ)), which have been linked to WNT signalling, and WNT target genes are repressed. Binding of WNT ligands to frizzled (FZD) and either LRP5 or LRP6 to activate canonical signalling or ROR1, ROR2, RYK, PTK7 or MuSK to activate and noncanonical signalling (WNT ON). Canonical signals disrupt the destruction complex, stabilizing β-catenin, which enters the nucleus and binds T-cell factor/lymphoid enhancer-binding factor 1 (TCF/LEF) family transcription factors to activate gene expression. The WNT–STOP pathway is a transcription-independent pathway involving low-density lipoprotein receptor (LRP) and cyclin Y. Noncanonical signals activate small GTPases and various kinases, mobilize Ca\(^{2+}\) and activate activator protein 1 (AP-1) family and nuclear factor of activated T-cells (NFAT) transcription factors. New noncanonical signals involve activation of YAP and TAZ, the SRC family tyrosine protein kinase FYN and signal transducer and activator of transcription (STAT) family members.
Fig 1

**WNT OFF**

- WNT
- sFRP
- FZD
- LRP
- ZNRF3–RNF43
- β-cate

**WNT ON**

- Canonical WNT signalling
- Protein stabilization
- Cell growth and division

**WNT–STOP**

- YAP–TAZ
- TEAD
- FYN–STAT

**Noncanonical**

- PCP
- Rho
- Rac
- PLC
- DAG
- IP3
- JNK
- PKC
- Ca

- Actin remodelling
- Cell adhesion
- Canonical WNT signalling

**Cell migration**

Nature Reviews | Urology
Figure 2 Crystal structure of Wnt8-FZD8 CRD complex
Representative image of Xenopus wnt8 (purple) bound to FZD8-CRD (blue). The interaction is mediated through the lipid thumb (“Site 1”) and the index finger (“Site 2”) of wnt8. Sites 1 and 2 are involved in Wnt-FZD promiscuity or specificity, respectively. Patch residues are involved in wnt binding to coreceptors leading to ternary complexes. Figure adapted from Reference 38.
**Figure 3 | WNT signalling regulation by RNF43, ZNRF3 and RSPO.** RNF43 and ZNRF3 ubiquitin ligases inhibit WNT signalling by ubiquitinating frizzled (FZD) receptors, promoting their degradation. R-spondin (RSPO) binds RNF43 and/or ZNRF3 and leucine-rich repeat-containing G protein-coupled receptors (LGR5), resulting in membrane clearance of RNF43 and ZNRF3 and increased FZD stability. Mutations in RNF43 and ZNRF3 or overexpression of RSPO as a result of a RSPO gene fusion activate WNT signalling.
**Fig 2**

- **Patch residues**
- **Ternary complex**
- **Xwnt8**
- **Conserved residues**
- **WNT–FZD Promiscuity**
- **N-terminal domain**
- **Site 1**
- **Index finger**
- **Lipid thumb**
- **Site 2**
- **Nonconserved residues**
- **WNT–FZD Specificity**
- **PAM**
- **FZD8 CRD**
- **C-terminal domain**

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**Fig 3**

- **WNT off**
- **β-catenin degradation**
- **FZD degradation**
- **LRP and/or ZNRF3 and/or RNF43 degradation**
- **Mutated ZNRF3 and/or RNF43**
- **RSPO fusion**
- **β-catenin**
- **APC**
- **AXIN**
- **GSK3**
- **CK1**
- **YAP–TAZ**
- **β-catenin degradation**
- **APC**
- **β-catenin**
- **GSK3**
- **CK1**
- **YAP–TAZ**
- **β-catenin**
- **APC**
- **β-catenin**
- **GSK3**
- **CK1**
- **YAP–TAZ**

**Nature Reviews | Urology**
Figure 4 | Paracrine WNT signals from the tumour microenvironment.
Paracrine WNT signals from the tumour microenvironment can contribute to tumour progression (WNT-10B\textsuperscript{100}) and therapy resistance (SFRP2\textsuperscript{91} and WNT-16B\textsuperscript{101} or WNT-5A and bone morphogenetic protein (BMP) 6\textsuperscript{102}).
Fig 4

Normal prostate

Prostate cancer initiation

Invasive tumour

Therapy resistance

Resistant tumour

Radiation

WNT-10B (canonical)

SFRP2

WNT-5A (noncanonical)

BMP6

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**Figure 5 | Drugs that target WNT signalling.** May drugs have been developed that act on different targets at different levels in the WNT signalling pathway. The therapeutics are inhibitors of WNT secretion (red), regulators of WNT antagonist and antagonist function (blue), drugs that target WNT receptor interactions (green), drugs that prevent dishevelled (DVL) activation (pink), drugs that stabilize the destruction complex (grey), and drugs that target β-catenin partners in the nucleus (purple). NPs, nanoparticles.
Table 1 | Key changes in WNT signalling pathway components in prostate cancer

<table>
<thead>
<tr>
<th>WNT pathway component</th>
<th>Alteration</th>
<th>Disease stage</th>
<th>Effect on WNT signalling, and/or on prostate cancer cells, and on patients if known</th>
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<tbody>
<tr>
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<td>CRPC44,46</td>
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<td>Inactivating mutations SNPs</td>
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Ligands

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<th>Noncanonical: increased CRPC growth, bone metastasis; metastasis and recurrence71 Increased survival, improved outcome68-70</th>
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<td>Upregulation</td>
<td>Tumours74 CTCs from patients receiving ADT73</td>
<td>Noncanonical; induces osteoblastic response in bone</td>
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<tr>
<td>WNT11</td>
<td>Upregulation</td>
<td>ADT77, metastases76</td>
<td>Noncanonical; promotes invasion and neuroendocrine-like differentiation78</td>
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<tr>
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<td>Upregulation</td>
<td>Tumour stroma101</td>
<td>Canonical; therapy resistance101</td>
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</tbody>
</table>

Receptors

| FZD2 | Upregulation | CRPC | Recurrence71 |
| FZD4 | Upregulation | ERG positive tumours100 | Canonical and noncanonical; EMT70 |
| FZD5 | Upregulation | Tumours90 | Noncanonical |
| FZD8 | Upregulation | Tumours111 | Canonical and noncanonical |
| ROR1 | Upregulation | Tumours86 | Noncanonical; not known |

Regulators

<table>
<thead>
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<th>Downregulation</th>
<th>Tumours86,89</th>
<th>Reduced survival86</th>
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</tr>
<tr>
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<td>Downregulation</td>
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</tbody>
</table>
ZNRF3 or RNF43 | Inactivating mutations | CRPC<sup>46</sup> | Potentiation of WNT signals
---|---|---|---
RSPO2 | Upregulation (gene fusion) | CRPC<sup>46</sup> | Potentiation of WNT signals

APC, adenomatous polyposis coli protein; CRPC, castration-resistant prostate cancer; DKK, Dickkopf-related protein; FZD, frizzled; ROR, tyrosine-protein kinase transmembrane receptor; RSPO, R-spondin; SFRP, secreted frizzled-related protein

Table 2 | WNT pathway inhibitors in clinical trials

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target</th>
<th>Clinical trial</th>
<th>Phase</th>
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<td><strong>Inhibition of Wnt secretion</strong></td>
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<td>LGK974 (also known as WNT974)</td>
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<td>ETC-159 (also known as ETC-1922159)</td>
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<td>NCT02521844</td>
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<td><strong>Regulation of antagonists and agonists</strong></td>
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<td>Adenovirus expressing DKK3 (also known as REIC)</td>
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<td><strong>Targeting WNT receptor interactions</strong></td>
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CBP, CREB-binding protein; DKK, Dickkopf-related protein; FZD, frizzled; ROR, tyrosine protein kinase transmembrane receptor; RSPO, R-spondin; SFRP, secreted frizzled-related protein

Competing interests statement
The authors declare no competing interests.
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