Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190

REVIEW

# WNT as a Driver and Dependency in Cancer

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**ABSTRACT** The WNT signaling pathway is a critical regulator of development and adult tissue homeostasis and becomes dysregulated in many cancer types. Although hyperactivation of WNT signaling is common, the type and frequency of genetic WNT pathway alterations can vary dramatically between different cancers, highlighting possible cancer-specific mechanisms for WNT-driven disease. In this review, we discuss how WNT pathway disruption contributes to tumorigenesis in different organs and how WNT affects the tumor cell and immune microenvironment. Finally, we describe recent and ongoing efforts to target oncogenic WNT signaling as a therapeutic strategy.

**Significance:** WNT signaling is a fundamental regulator of tissue homeostasis and oncogenic driver in many cancer types. In this review, we highlight recent advances in our understanding of WNT signaling in cancer, particularly the complexities of WNT activation in distinct cancer types, its role in immune evasion, and the challenge of targeting the WNT pathway as a therapeutic strategy.

## INTRODUCTION

WNT signaling is a critical molecular rheostat that guides a range of physiologic processes, including embryonic development, lineage commitment, adult stem cell homeostasis, and tissue regeneration. The first member of the WNT family was identified more than 30 years ago as the Int-1 proto-oncogene in a mouse mammary tumor virus model (1). Int-1 was later identified as the homolog of the *Drosophila melanogaster* segment polarity gene *wingless*, and thus, the term "WNT" was born from the fusion of both gene names (2). Although first described in a cancer setting, much of our fundamental understanding of WNT biology has come from studying development in model organisms such as *Drosophila, Xenopus laevis*, and the mouse. Indeed, these systems continue to be a critical resource in efforts to define all WNT signaling components, their functions, and how they serve to control normal WNT signaling. In this review, we will focus on abnormal or dysregulated WNT signaling, how it drives cancer, its role in stemness and immune evasion, and the progress and challenges of targeting the WNT pathway as a therapeutic strategy.

## β-CATENIN–DEPENDENT WNT SIGNALING

The WNT family consists of 19 secreted glycoproteins, which orchestrate cell fate specification, cell proliferation, cell migration, dorsal axis formation, asymmetric cell division, and many more functions, depending on cell and tissue context (3, 4). The downstream effects of WNTs have traditionally been separated into two sometimes overlapping categories: canonical (β-catenin–dependent) and noncanonical (β-catenin–independent). The β-catenin–dependent pathway is induced by WNT ligands binding to Frizzled (FZD) and LRP5/6 coreceptor complexes, which initiate intracellular signaling and membrane recruitment of scaffold proteins (AXIN1/2 and DVL). This induces disruption of the core destruction complex [AXIN, APC, casein kinase 1α (CK1α), and glycogen synthase kinase 3 (GSK3)], resulting in stabilization of β-catenin and its subsequent nuclear localization (ref. 3; Fig. 1). Numerous other proteins can interface with and modulate the core WNT pathway, including the tankyrase enzymes (TNKS and TNKS2) that elevate WNT signaling by targeting AXIN1/2 for degradation (5). In the nucleus, β-catenin binds to members of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family and recruit transcriptional coactivators, including p300 and/or CREB-binding proteins (CBP), to drive a WNT transcriptional program. In the absence of WNT ligands, β-catenin is tagged for degradation via sequential phosphorylation by CK1α and GSK3 on serine and threonine residues (S45/T41/S37/S33) at the N-terminus. Phosphorylated β-catenin is recognized by a ubiquitin ligase complex that includes β-transducin repeat containing E3 ubiquitin protein ligase (β-TrCP), leading to polyubiquitination and proteasomal degradation. Although not usually considered core members of the complex, Hippo pathway regulators YAP and TAZ (WWTR1) have been shown to play an important role in β-TrCP recruitment and β-catenin inactivation (6). Interestingly, YAP/TAZ downstream activity is also modulated by the TNKS enzymes through regulation of angiomotin (AMOT) proteins (7).

Before any intracellular pathway activation occurs, WNT ligands must be secreted from a WNT-producing cell to activate signaling in a WNT-responsive cell. The production of WNT ligands is tightly controlled and requires both posttranslational

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Cancer Discov 2021;11:2413–29

**doi:** 10.1158/2159-8290.CD-21-0190

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



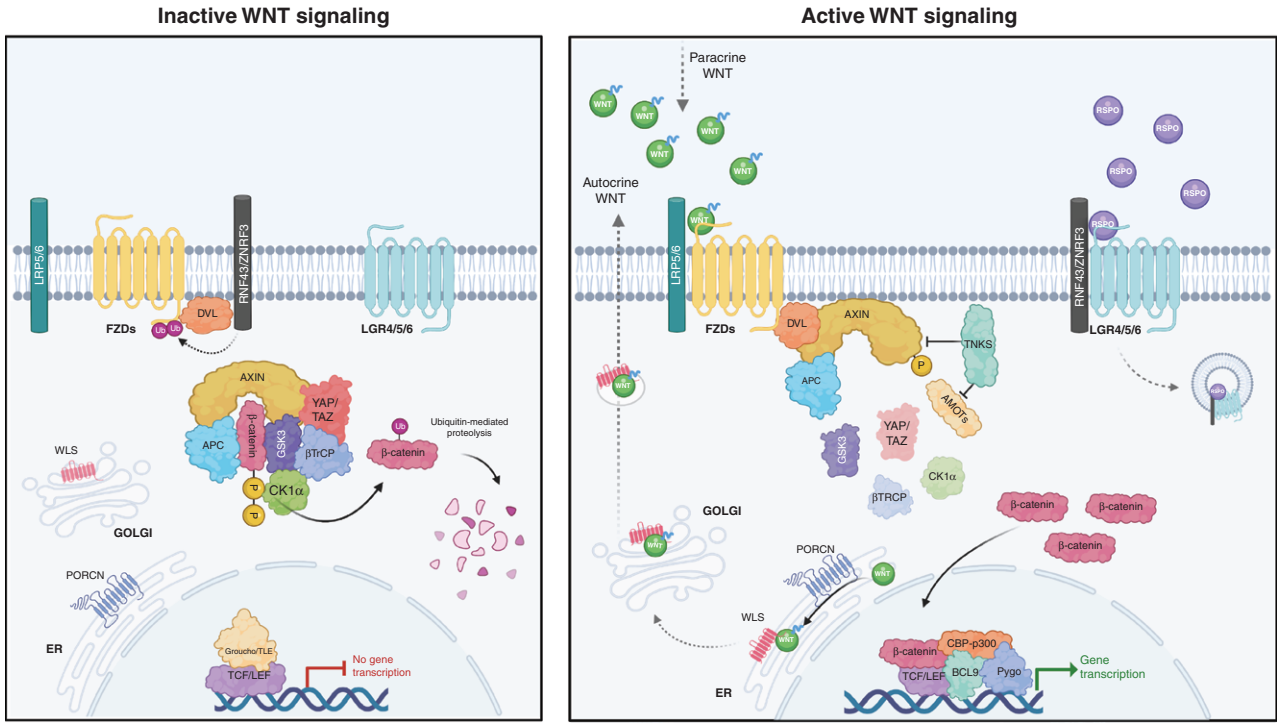


**Figure 1.** Overview of the WNT signaling pathway. In the absence of WNT ligand (inactive WNT signaling), accumulated β-catenin bound in the destruction complex by AXIN and APC is phosphorylated by CK1α and GSK3, leading to its ubiquitination and proteasomal degradation by β-TrCP/YAP/TAZ. In the presence of WNT ligands (active WNT signaling), LRP5/6 and FZD coreceptors associate, leading to activation and recruitment of AXIN1/2 and DVL to the membrane, disrupting the destruction complex. This results in stabilization and nuclear localization of β-catenin. In the nucleus, β-catenin binds to the TCF/LEF, recruiting coactivators p300 and CBP to induce WNT target gene transcription. Created with BioRender.com.

modification by a serine O-palmitoleoyltransferase (PORCN) and association with Wntless (WLS or GPR177) in the endoplasmic reticulum to be secreted into the extracellular space (8). The enzymatic processing of WNTs by PORCN is an Achilles' heel of the pathway that has been exploited to target WNT signaling pharmacologically, as will be discussed later.

In addition to the family of WNT ligands, there are a range of other extracellular modulators of WNT signaling. R-spondins 1 to 4 (RSPO1–4) are small, secreted proteins that bind to leucine-rich repeat-containing G-protein-coupled receptors (LGR4–6) to enhance WNT ligand-driven activation (9, 10). Despite their name, LGR4–6 do not actually function as G-protein-coupled receptors. Rather, RSPO-bound LGR receptors bind to and sequester the transmembrane E3 ligases RNF43 and ZNRF3, preventing them from marking WNT–FZD receptors for lysosomal degradation (11, 12). This results in accumulation of FZD receptors on the cell surface and amplification of the response to WNT ligand stimulation. The importance of the RSPO/LGR5/RNF43 module in controlling WNT pathway activation is evident across several cancer types with chromosome rearrangements driving overexpression of RSPO2 and RSPO3 in colorectal cancer, whereas inactivating mutations in RNF43 are observed in colorectal cancer, pancreatic ductal adenocarcinoma (PDAC), and endometrial cancers (13–15). Canonical WNT signaling is antagonized by several secreted proteins, including Dickkopf (DKK1) and members of the secreted frizzled-related proteins that bind and sequester WNT ligands (16, 17).

The general paradigm of WNT/β-catenin signaling has been recognized for more than a decade and is strongly supported by experimental evidence, yet the picture is far from complete. The order and kinetics of phosphorylation and protein–protein interactions at the cell membrane "signalosome" remain an area of active investigation (18–20), as do the precise interactions that govern destruction complex binding, protein degradation, and β-catenin nuclear translocation (21–24). The dynamics of protein complex interactions are beginning to be revealed through the *in vitro* reconstruction of the destruction complex, whereas newer tools such as real-time pathway reporters provide insight into the kinetics of response, particularly in complex *in vivo* tissues (25, 26). Further elucidation of how WNT signaling is tuned at each step of the process will provide an important "ground truth" for deciphering and interpreting WNT dysregulation in cancer.

## β-CATENIN–INDEPENDENT WNT SIGNALING

The β-catenin–independent pathway is comparatively more diverse and less characterized than that of the canonical WNT pathway. By definition, the β-catenin–independent pathway operates without a β-catenin–mediated transcriptional response and regulates different signaling outputs, including cell polarity and migration (27, 28). Like WNT/β-catenin signaling, the β-catenin–independent pathway is initiated by WNT ligands (e.g., WNT11 and WNT5A) binding to a panel of receptors, including FZD, ROR2, ROR1, or RYK, resulting in

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activation of downstream effectors (29). WNT ligands are often grouped into canonical and noncanonical classes, but recent evidence suggests that WNT ligands once considered "canonical" WNTs, like WNT3A, can activate β-catenin–independent signaling (30). Given this cross-talk, it is difficult to disentangle how the β-catenin–independent pathway individually contributes to cancer phenotypes, although there is evidence for both pro- and antitumorigenic roles (31). For instance, the WNT5A–ROR2 axis is a prominent ligand receptor pair in the β-catenin–independent pathway that regulates planar cell polarity and tissue patterning. This signal exerts a tumor-suppressive role in colorectal cancer while conferring invasiveness in other cancer cell types (32). Through the regulation of migration and cell polarity, β-catenin–independent signaling likely influences tumorigenic behavior, although it is not clear that this arm of the pathway acts as a primary disease driver.

## WNT SIGNALING AND TISSUE HOMEOSTASIS

WNT not only is critical for the development of many organ systems but also plays a fundamental role in the maintenance of actively self-renewing tissues and in regeneration post-injury. Sustained WNT pathway activity is essential for homeostasis of the intestine, hair follicles, and hematopoietic system (3, 33–35), whereas the induction of high WNT signaling is important for wound repair in a wide range of tissues, including skin, lung, pancreas, and liver (36–39).

The intestinal mucosa is an archetypal example of WNT dependence in both normal tissue function and wound repair, and due to its unique cellular arrangement, it provides a clear picture of how spatiotemporal WNT activity controls organ function. The small and large intestine are the most rapidly renewed tissues in adult mammals, with the average life cycle of an individual epithelial cell less than a week. In mice, up to 200 new cells are generated per intestinal crypt each day, and the entire epithelium is turned over within 3 to 5 days (40). The engine that drives this incredible flux is the LGR5-positive crypt base columnar (CBC) stem cell that resides at the base of the crypt (Fig. 2). Self-renewal of CBC cells is maintained by high levels of WNT ligand, produced and secreted by underlying stromal cells and interdigitated "niche cells," such as the Paneth cell in the small intestine (refs. 41–43; Fig. 2). The differential regulation of β-catenin by transcriptional cofactors preserves the narrow window of WNT activity to govern the fate of intestinal stem cells (44). Recently, Borrelli and colleagues (44) revealed that C-terminal coactivators of β-catenin act as a binary on/off switch for β-catenin transcription of WNT target genes, whereas N-terminal coactivators fine-tune β-catenin transcriptional output to the exact level required for proliferation and self-renewal of intestinal stem cells. Rapid proliferation in the crypt results in a continuous movement of cells in an upward motion, pushing cells out of the crypt where they begin to differentiate into all intestinal epithelial lineages and finally end their life cycle by undergoing apical extrusion or shedding at the tip of the villus (45). This cellular "conveyor belt" leading to physical separation of cells from the WNT-high crypt niche is a key factor driving intestinal differentiation. The various processes that control lineage specification during exit from the crypt are complex and have been well covered elsewhere (46).

## THE CURIOUS CASE OF WNT PATHWAY MUTATIONS IN CANCER

Oncogenic activation of the WNT pathway is observed to varying degrees across a range of cancers. Cancer-associated WNT hyperactivation can be WNT ligand–dependent or downstream of the ligand–receptor interface. Most mutations in *RNF43* or *RSPO* result in ligand-dependent WNT signaling and are sensitive to drugs that block WNT production (see below). Alterations, particularly truncating mutations in *APC* and *AXIN1*, disrupt the negative regulation of β-catenin by the destruction complex, whereas direct missense mutations or small in-frame deletions in β-catenin (*CTNNB1*) promote signaling by rendering the protein insensitive to proteosomal degradation (47). Although the outcome of each type of WNT pathway alteration is increased downstream transcriptional response, the level of pathway induction and, intriguingly, the pattern of genetic alterations that drive WNT activation in each cancer type are different (Fig. 3A and B).

In colorectal cancer, WNT hyperactivation is almost exclusively driven by truncating mutations in *APC*, whereas alterations in *CTNNB1, AXIN1/2, RNF43*, and *RSPO2/3* (not shown) combined account for less than 15% of all WNT pathway changes. In other tumor types such as gastric, lung, prostate, ovarian, and breast cancer, *APC* mutations are also common but account for only half of all WNT pathway disruptions. In contrast, hepatocellular carcinoma (HCC), melanoma, uterine carcinoma, and PDAC rarely present with *APC* alterations but instead frequently harbor β-catenin (HCC, melanoma, and uterine carcinoma) and *RNF43* (PDAC) mutations. Each of these mutations, particularly *APC* and β-catenin mutations, is a potent driver of WNT signaling, so why, then, is there such a strong bias in mutation pattern between different cancer types?

The first and most obvious possibility is that environmental carcinogens or cell-intrinsic mutational signatures unique to each tissue type led to cancer-specific genetic changes. Indeed, there is some evidence for this in HCC. Through analysis of whole-genome mutational patterns, Letouzé and colleagues (48) reported that liver cancer–specific mutational processes account for most *CTNNB1* hotspot mutations in this disease. Moreover, despite their different patterns in human cancers, in animal models, engineered mutations in *APC* and β-catenin drive very similar disease progression in the liver, lung, and colon (49–55), suggesting that functional differences may not be the dominant factor in defining cancer-selective WNT pathway alterations.

Although mutational processes are clearly important, they are likely not the whole story. By comparing observed and expected frequencies of mutations in different cancer types (which harbor distinct underlying mutational processes), Temko and colleagues (56) argue that biological selection can be dominant to mutational processes within a given cancer type. In particular, they compare *APC* and *CTNNB1* mutations in liver, uterine, and colorectal cancers and note that selection for APC mutations is observed only in colorectal cancer. Consistent with the notion that *APC* mutations are favored in colorectal cancer, germline mutations in *APC* strongly predispose patients to the development of benign and malignant tumors in the colon (57), whereas other organs are less dramatically affected.

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



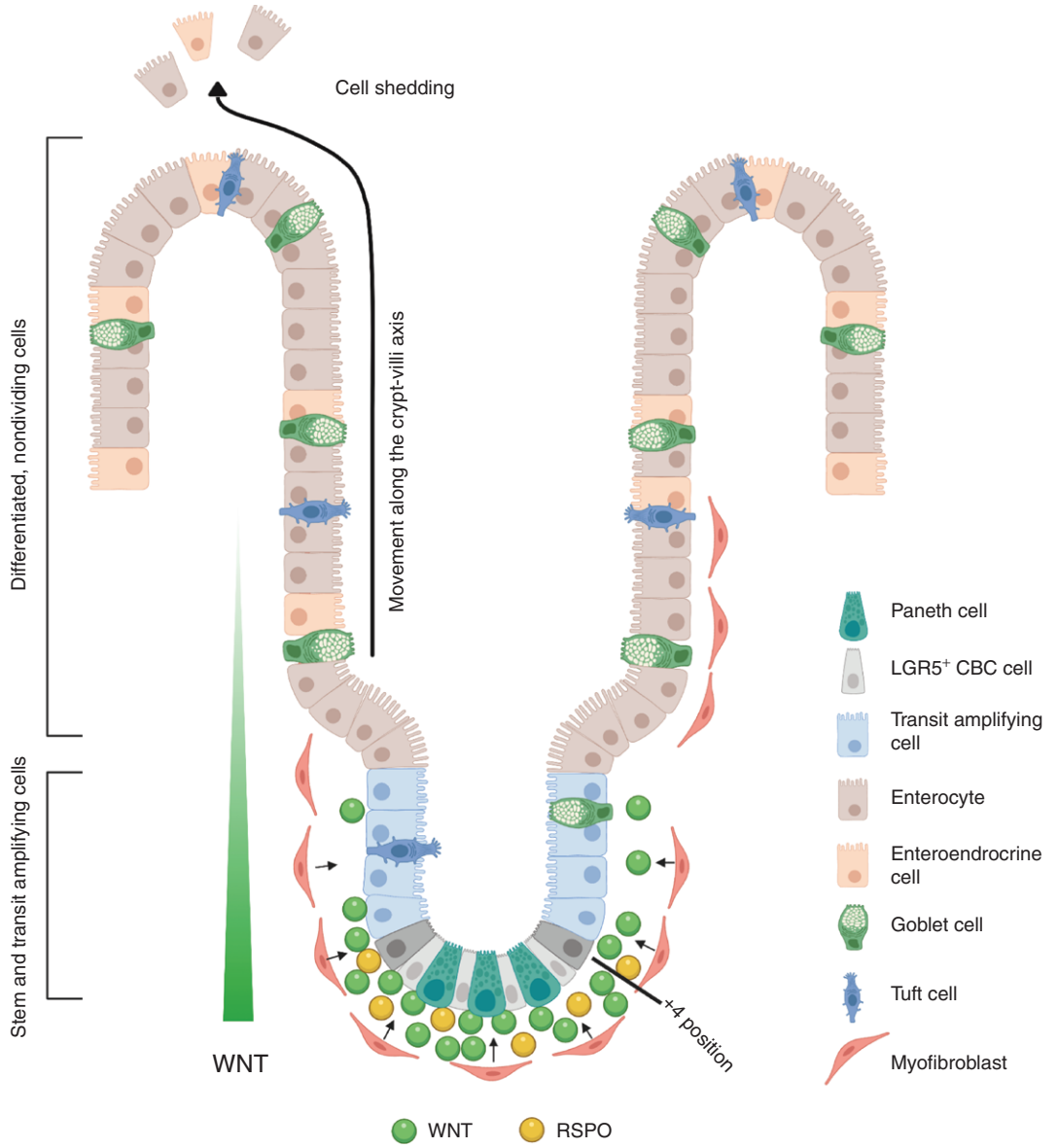


**Figure 2.** WNT signaling in the small intestine. WNT ligand produced and secreted predominantly by underlying stromal cells drives continuous proliferation in the intestinal crypt via LGR5+ crypt base columnar (CBC) cells, interdigitated with Paneth cells. Proliferation drives the upward motion of cells, through the transit-amplifying zone, where they continue to rapidly divide, and ultimately into the villus region, where committed cells differentiate into all intestinal epithelial lineages. Cells are shed from the monolayer at the tip of the villus. Created with BioRender.com.

Exactly what might drive selection for APC disruption (over β-catenin mutation) is not clear, although it is worth noting that APC is a large multidomain, multifunctional protein whose truncation or loss may cause pleiotropic effects in different cell types. APC has WNT/β-catenin–independent roles in DNA repair, apoptosis, spindle assembly, chromosome segregation, and cytoskeletal regulation through interaction with microtubules (58–60). In fact, through detailed analysis of intestinal crypts, Näthke and colleagues (61) propose that APC truncation disrupts the orientation and asymmetry of cell division and may contribute to early tumor development by delaying cell transit from the crypt base. Like APC, β-catenin is also a multifunctional protein that, in addition to acting as a WNT transcription factor, interacts with E-cadherin at the epithelial adherens junction, which is essential for cell–cell contacts and tissue remodeling (62). However, unlike APC, in most cancers *CTNNB1* mutations are present on only a single allele, with rare cases of loss of heterozygosity. Thus, it is likely the remaining wild-type β-catenin protein can support any lacking normal functions of mutant β-catenin at the membrane. To our knowledge, the impact of loss of heterozygosity has not been investigated in *CTNNB1*-mutant tumors, so this point remains speculation.

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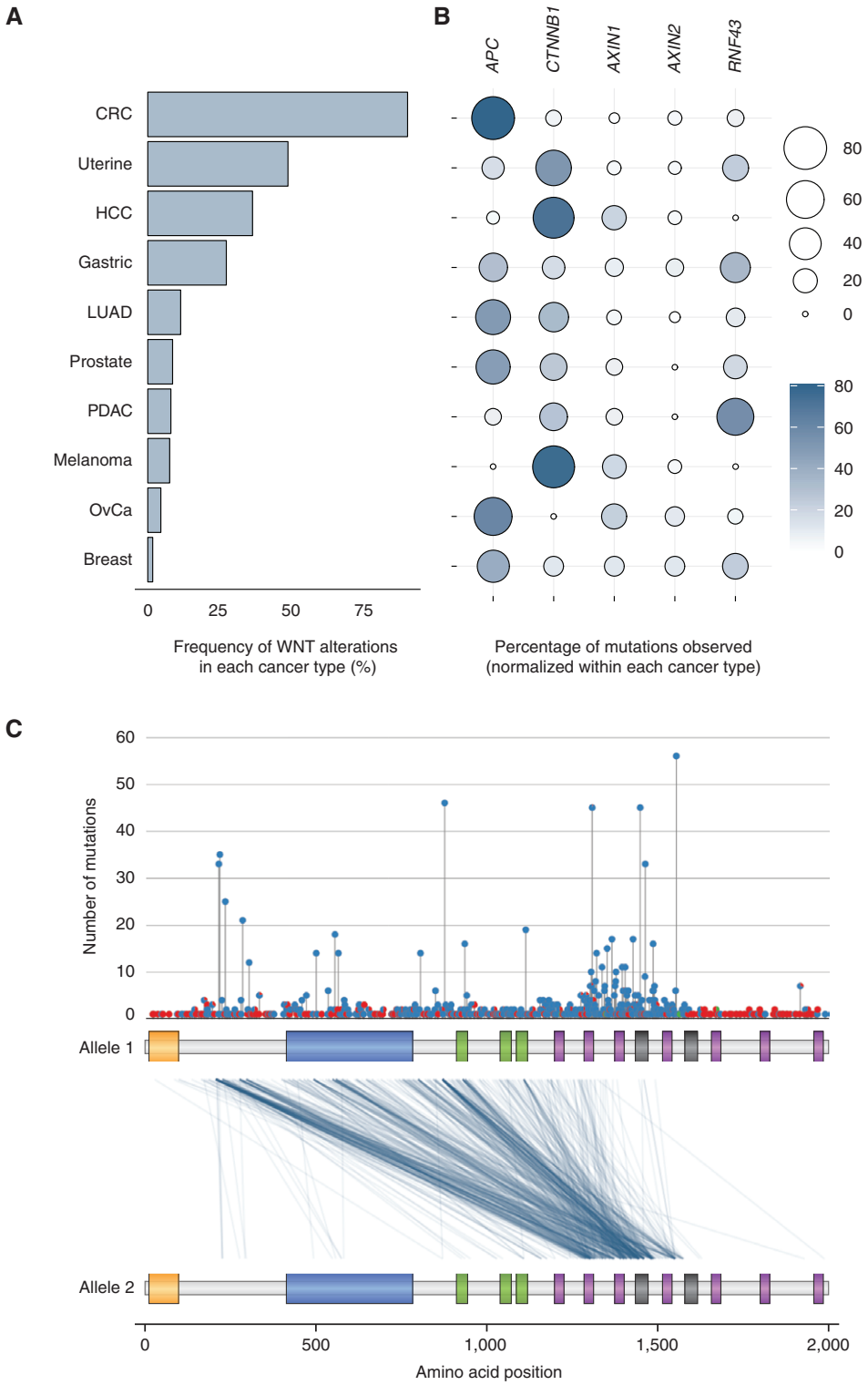


**Figure 3.** WNT pathway mutation distribution across cancer types. **A,** Bar graph representing the frequency of alterations in core WNT regulators (*APC, CTNNB1, AXIN1, AXIN2, RNF43*) across different tumor types shown as the percentage of all tumors analyzed. OvCa, ovarian cancer. **B,** Bubble plot showing the frequency of mutations in each WNT regulator, within the WNT-mutant subset of each cancer type. **C,** Lollipop plot showing number and position of truncating (blue) and missense (red) mutations in *APC* in colorectal cancers. Plot below shows the position of mutations on each of two *APC* alleles in a given tumor, relative to the N-terminal portion of the APC protein (total length is 2,843 amino acids). Many colorectal cancers show early *APC* truncations, but most cancers contain at least one allele truncated within the mutation cluster region between amino acids 1200 and 1600. All data derived from publicly available TCGA PanCan database (cbioportal.org).

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## THE "JUST RIGHT" OR "GOLDILOCKS" HYPOTHESIS

In addition to mutation signatures and non–WNT-related functions of key proteins, the relative level of WNT/β-catenin activation may have a strong impact on the selection of specific WNT alterations in cancer. The "Goldilocks" theory posits that WNT hyperactivation at an intermediate level (not too cold and not too hot) is ideal for cell transformation (63). The precise "ideal" level has been difficult to define experimentally, however, perhaps reflecting a varying set point across different tissues or cell types (64, 65). It is noteworthy that distinct *APC* mutations can produce different levels of canonical WNT pathway activation for tumorigenesis in colorectal cancer and have been associated with tumor type (microsatellite instable vs. stable), location, and response to targeted therapies (64, 66, 67). Furthermore, although many types of truncating *APC* mutations are observed in colorectal (and other) cancers, most tumor cells carry at least one allele truncated within the mutation cluster region located between amino acids 1200 and 1600 (Fig. 3C). Experimental evidence suggests that such mutations provide a hyperactivated, but not maximal, WNT response (67, 68).

Thus, although there is clear bias in the types and frequency of different WNT pathway alterations in distinct cancer types, the underlying determinants of this are unclear, and so a key facet of our understanding of WNT as a cancer driver remains elusive.

## WNT ACROSS DIFFERENT CANCER TYPES

### Colorectal Cancer

The WNT signaling pathway is the most dominant regulator of stem cell maintenance and proliferation in the gastrointestinal tract, driving complete renewal of the intestinal epithelium every 3 to 5 days (69, 70). Given this, it is not surprising that alterations in the WNT signaling pathway are a near-universal feature of colorectal cancer, with more than 90% of colorectal cancers harboring mutations in *APC, CTNNB1, RNF43, AXIN1*, and *RSPO* genes (Fig. 3; ref. 71). *APC* mutations were recognized as a likely initiating event in colorectal cancer around 30 years ago following the parallel identification of *APC* as the gene mutated in human familial adenomatous polyposis (FAP; ref. 72) and in the intestinal cancer–prone $Apc^{Min}$ (multiple intestinal neoplasias) mouse (73). Development of engineered animal models that enabled timed disruption of *Apc* or activation of *Ctnnb1* directly showed that WNT activation is sufficient to trigger hyperproliferation and block differentiation in the intestinal crypt (53–55, 74). In particular, oncogenic WNT activation within LGR5-positive stem cells drives rapid hyperplastic growth (74, 75). Similarly, WNT induction in $BMI1^+$, $LRIG1^+$, and $DCLK1^+$ stem cell compartments can also initiate tumor growth, although the kinetics of adenoma development vary and, in the case of DCLK1, require tissue injury and/or inflammation to instigate tumor growth (76–78). These data support a "bottom-up" model whereby WNT mutations in normal crypt base stem cells drive tumor growth. In contrast, Schwitalla and colleagues (79) demonstrated that coincident activation of KRAS and NFκB can induce dedifferentiation and transformation of enterocytes in a "top-down" model of tumor development. Whatever the path to tumor development, in mice and in humans, colorectal cancers show high WNT-associated stem cell–like signatures (80). In fact, lineage-tracing experiments *in vivo* in mice show that LGR5-positive cells represent about 5% to 10% of cells in adenomas and give rise to all cancer cell lineages as well as to additional LGR5-positive cells (81). Consistent with a key role for stem cell–like tumor propagation, ablation of cells expressing stem markers such as BMI1 (82) or direct elimination of LGR5-expressing cells (83, 84) can reduce intestinal tumor burden.

As demonstrated in the case of KRAS and NFκB, contribution of "non-WNT" factors can play a major role in determining the outcome of oncogenic WNT mutations in individual cells. For example, the homeobox transcription factor HOXA5, an important repressor of intestinal stem cell fate, is suppressed by WNT but can attenuate WNT-driven cancer phenotypes if overexpressed or induced by retinoids (85). Alternatively, some proteins play a more indirect role in WNT regulation, such as the histone H3K36 methyltransferase SETD2, which restricts the WNT-dependent expansion of the stem cell compartment by influencing splicing of the WNT regulator DVL (86). WNT effects can also be bolstered by synergy with other oncogenic pathways. In particular, mutations in WNT and RAS/MAPK pathway genes are frequently observed in the same tumors (71) and, in animal models, show clear cooperativity in driving the early stages of tumorigenesis (87–89). Although phenotypically WNT and KRAS are cooperative drivers, the signaling mechanics between them is complicated. APC and KRAS cooperate to drive activation of cancer stem cells and tumor growth *in vivo* (90), whereas inhibition of MEK can show potent activation of the WNT pathway with increased stem cell plasticity (91). Similarly, Kabiri and colleagues (92) propose that WNT and RAS/MAPK pathways are mutually repressive to maintain the pool of intestinal stem cells at the crypt base. Fully elucidating the molecular details of how these two key pathways intersect and in what context may help determine future treatment strategies.

However WNT is activated or augmented in colorectal cancer, it is usually a disease driver. Inhibiting WNT and/or β-catenin directly essentially eliminates LGR5-positive cells, suppresses proliferation, and drives cell differentiation (55, 67, 93, 94). In human colorectal cancer cells, overexpression of wild-type APC is sufficient to downregulate WNT signaling, induce expression of differentiation markers, and reduce tumor growth (95). Similarly, restoration of endogenous APC expression in an *in vivo* APC-silencing colorectal cancer model is sufficient to cause rapid disease regression, even in the presence of oncogenic *Kras* and *Trp53* mutations (55). In this murine example, lineage tracing revealed that tumor cells could reintegrate within the normal epithelial monolayer and produce functional differentiated epithelial cells, highlighting the key role of WNT in controlling the switch between normal and transformed behavior in colorectal cancer.

Like APC and *CTNNB1, RSPO* fusions also act as tumor initiators and cancer drivers. Recurrent chromosome rearrangements creating *EIF3E–RSPO2* and *PTPRK–RSPO3* fusions are mutually exclusive with other WNT alterations in colorectal cancer, supporting a redundant role in oncogenic WNT

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



activation (96). In clinical samples, *RSPO* fusions appear to be enriched in traditional serrated adenomas (97), although mouse models have not supported a direct link between *RSPO* fusions and this distinct adenoma subtype (98, 99). This may reflect a difference in the cell of origin, mutational processes, and/or other cooperating genetic events absent in the "RSPO-only" animal models. Creation of *RSPO* fusions in the murine intestine using inducible *in vivo* CRISPR/Cas9 (99) or via cDNA expression (98) provided the first evidence that these events are tumor-initiating, whereas multiple studies have shown that blocking WNT secretion via PORCN inhibitors or directly inhibiting RSPO itself can block tumor growth (93, 99, 100). Similar to *RSPO* fusions, inactivating mutations in *RNF43* are largely exclusive to *APC* mutations but, unlike other WNT alterations, are enriched in the microsatellite-instable (MSI-H) tumors (13). Cancer-associated changes are predominantly nonsense and frameshift truncating mutations, spread throughout the coding sequence. Loss of the *RNF43* locus or early truncations disrupt negative regulation of WNT receptor complexes and drive ligand-dependent activation of the pathway. As predicted, murine or patient-derived organoids and patient-derived xenograft (PDX) tumors with these lesions are sensitive to PORCN inhibitors (101–105). Similar sensitivity is also seen in other tumor types, including pancreatic PDX models (106).

Two other types of *RNF43* mutations reveal the complexity of how this protein controls WNT output and highlight the importance of understanding if and how specific mutations promote cancer growth. The most frequently observed *RNF43* mutation is a frameshift over a short polyguanine tract at G658–G659 (G659Vfs*41). Despite its recurrence in colorectal cancers, this alteration has only minor effects on RNF43 activity and does not confer WNT hyperactivation. The lack of functional impact in colorectal cancer and gastric cancers suggests that G659Vfs*41 is likely a passenger mutation associated with microsatellite instability (101, 107, 108). Recently, Spit and colleagues (103) described a third category of *RNF43* truncations within a 50–amino acid region (K514-Q563) in the C-terminal half of the protein. These truncations act to sequester or "trap" CK1 at the plasma membrane, depleting it from the destruction complex and promoting β-catenin accumulation. Consequently, cells with *RNF43* trapping mutations show WNT ligand-independent activation of the pathway and are insensitive to PORCN inhibition.

The dominant role of WNT signaling in colorectal cancer makes it a favorable target of therapeutic interventions. As discussed further below, a variety of approaches and drugs have been developed to target hyperactive WNT and are in early-phase clinical trials. We do not yet know the outcome of many of these studies, but it is evident from preclinical model systems that a deep understanding of recurrent WNT pathway mutations and the dependencies they create will be important for the development and deployment of effective treatments.

## Liver Cancer

Similar to colorectal cancer, the WNT signaling pathway is hyperactivated in a high proportion of HCCs, with frequent hotspot mutations in *CTNNB1* (~30%) and inactivating alterations in *AXIN1* (15%) or, less commonly, *APC* (1.6%; ref. 109). The frequency of *CTNNB1* mutations differs based on cause. Hepatitis C virus–associated tumors have a significantly higher frequency of *CTNNB1* mutations (28%) compared with hepatitis B virus–associated disease (11%; ref. 110). However, unlike colorectal cancer and gastric cancer, the role of WNT signaling as a driver or cooperating event in HCC pathogenesis is unclear. Work by Nejak-Bowen and colleagues (111) supports WNT as a cooperative event, showing that overexpression of degradation-resistant β-catenin alone is not enough to drive HCC initiation *in vivo*. Furthermore, transcriptomics suggests activation of the WNT/β-catenin pathway is restricted to middle to late stages of hepatocarcinogenesis (112). The role of β-catenin as a functional promoter of tumor progression is further supported by the observation that nuclear β-catenin is associated with late-stage HCC, even in the absence of oncogenic mutation (113). In addition to β-catenin mutation, WNT pathway activation is also associated with upregulation of WNT ligands (WNT1/3/5a/10b) or coreceptors (FZD3/6/7, LRP6) and downregulation of the antagonist of the WNT pathway (sFRP1/4/5 and DKK3/4; ref. 114). The WNT–TGFβ class of HCC is linked with a more aggressive phenotype, whereas those containing *CTNNB1* mutations tend to be less aggressive, more differentiated tumors (115).

Although activating WNT mutations may be a middle- to late-stage event in HCC, the story may be different in pediatric liver cancer. Hepatoblastoma is the most common pediatric liver cancer, most often diagnosed in young, preschool-age children (<3 years old). Given the early onset, hepatoblastomas carry relatively few genomic alterations, but *CTNNB1* mutations have been observed in up to 75% of cases (116, 117). Similarly, hepatoblastomas have been associated with APC mutation–linked FAP, suggesting WNT has a major driving role in this disease (118). What underlies the difference in WNT mutation prevalence and susceptibility to WNT stimuli in these distinct but related cancers is unclear, but it seems reasonable to assume that cell or origin/cell identity plays a key role in WNT-driven tumorigenesis in the liver.

## Lung Cancer

Lung adenocarcinoma (LUAD) is the most common lung cancer subtype and the leading cause of cancer-related death, globally. In normal lung, WNT signaling is critical for both lung development and regeneration (119–121). Likewise, LUAD is frequently associated with increased expression of WNT pathway–activating genes such as WNT ligands and FZD receptors, as well as downregulation of negative regulators of the pathway such as APC, AXIN1, and DKKs (122). Depending on the assay used to quantify, it is estimated that 35% to 70% of LUADs have active WNT signaling (123, 124), although, unlike colorectal and liver cancers, only approximately 10% of tumors carry canonical oncogenic mutations in *APC, CTNNB1*, or *RNF43*.

These genomic data imply that downstream activation of WNT is important in LUAD but that it can be mutationally acquired or ligand-dependent. Consistent with the observation of *APC* and *CTNNB1* mutations in some human LUADs, forced activation of the WNT pathway using engineered genetic alleles promotes progression of *Kras*- or *Braf*-mutant lung tumors (49, 50, 125). Moreover, recent work suggests that, like human LUAD, murine models carrying oncogenic

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



*Kras* and *Trp53* mutations, but without genetic WNT alterations, also rely on WNT ligands for tumor progression (126). Interestingly, in this setting, WNT-high LGR5-positive cancer cells reside in close proximity to PORCN$^+$ cancer cells capable of secreting WNT ligand, thus forming a cancer stem cell niche, similar to those observed in normal tissues but derived entirely of cancer cells. Indeed, inactivation of PORCN in LUAD cells (but not in the stroma) blocks tumor progression (126). Collectively, these data support the notion that cancer cell–intrinsic WNT signaling is an important component of lung cancer initiation and progression, but WNT activation may be ligand dependent or independent. In addition, the work in murine models implies that the creation of a WNT-dependent cancer cell–derived niche is a critical step in driving ligand-dependent tumor growth.

## WNT SIGNALING IN METASTASIS

Metastasis is a hallmark of late-stage cancer, which presents as a therapeutic challenge responsible for more than 90% of cancer-related mortality (127). Direct genetic evidence linking WNT signaling with metastatic progression in human cancers is scarce, although there are abundant examples in preclinical models that WNT signaling promotes stemness, proliferation, and cell motility (115, 128–138), which may contribute to metastatic phenotypes.

In cases such as colorectal and gastric cancer, WNT activating mutations are early events and so common that they are rarely associated only with metastatic behavior. Indeed, in animal models that mimic tumor progression through adenoma–adenocarcinoma metastasis, elevated WNT signaling is required for tumor cell survival at all sites (139). This is in agreement with the notion that metastasis is driven by plasticity and/or nongenetic alterations triggered by environmental cues (140). De Sousa e Melo and colleagues (83) first described a critical role for LGR5-positive cancer stem cells in the establishment of colorectal cancer–derived liver metastasis, and recently Fumagalli and colleagues (141) built on this model, revealing that highly plastic LGR5-negative stem cells drive dissemination prior to consolidation and growth through an emergent LGR5-positive stem cell population in the metastatic site. Although not directly tested, these data propose a model in which WNT does not drive the metastatic process per se but is required in both the primary tumor and metastatic site to establish and maintain tumorigenesis. Similar to colorectal cancer, once breast cancer cells form a metastatic niche, WNT ligands are recruited to reestablish signaling and maintain tumor growth (142, 143). Yet, timed suppression of WNT may also be important for tumor survival. Malladi and colleagues (144) showed that transient suppression of WNT signaling via DKK1 induces a slow cycling state, driving immune evasion and long-term survival of latent, metastatic-initiating cells. As discussed below, WNT may also contribute to metastatic spread indirectly by promoting an immunosuppressive microenvironment, allowing the establishment and growth of distant lesions.

One setting in which WNT seems to be preferentially involved in driving metastatic progression is prostate cancer. A recent comparison of localized and metastatic prostate cancers revealed that *APC* mutations are enriched specifically in metastatic tumors (145). This association was independent of androgen sensitivity, suggesting it is more strongly linked to metastatic progression. Using organoids and *in situ* genetically engineered mouse models, Leibold and colleagues (145) confirmed that activation of WNT by disruption of APC is sufficient to promote metastatic spread and that WNT suppression may be an effective strategy to target disseminated disease.

## WNT SIGNALING AND ANTITUMOR IMMUNITY

Since the introduction and success of immune checkpoint inhibitors and immune cell–based therapies for cancer treatment, understanding how developing cancers adapt to evade immune detection has become the most immediate goal in cancer research. In the effort to identify strategies to further improve immunotherapy outcomes and achieve long-term cancer remission, the WNT signaling pathway has emerged as a possible key to modulating immune cell function in cancers.

WNT signaling is a known regulator of immune cell function, notably suppressing the maturation and differentiation of T cells and dendritic cells (34). Active WNT signaling can promote increased survival of regulatory T cells, alter differentiation of CD4$^+$ cells to adopt a protumorigenic Th17 subtype, impair differentiation of CD8$^+$ effector T cells, and drive dendritic cells into a more tolerogenic regulatory state (146–148). For instance, in melanoma, WNT signaling supports an immunosuppressive microenvironment by enhancing production of IL10 and IL12, which results in impaired dendritic cell and effector T-cell function (149). Moreover, using an immune-competent murine melanoma model, Spranger and colleagues (150) highlighted a direct role for WNT activation in driving T-cell exclusion and resistance to immune checkpoint inhibitors. They showed that WNT suppressed the recruitment of BATF3$^+$ dendritic cells, resulting in a failure to prime CD8$^+$ T cells in the tumor-draining lymph node (150). Near-identical results were seen in HCC, in which induction of mutant β-catenin in *in situ*–derived MYC;p53$^{-/-}$ tumors resulted in immune evasion and resistance to anti-PD-1 treatment (151). Given the frequency of *CTNNB1* mutations in HCC, these data highlight the potential therapeutic benefit of targeting WNT in combination with approved immunotherapies.

Beyond these two clear functionally validated examples, correlative analyses across 31 cancer types show that active WNT signaling is associated with non–T-cell inflamed tumors (152). Furthermore, in colorectal cancer, in which WNT is a clear driver, Grasso and colleagues (153) identified a correlation between high WNT transcriptional signatures and low T-cell infiltration, irrespective of colorectal cancer subtype and mutational load. Whether WNT signaling explains the current poor clinical response of colorectal cancers to immunotherapies remains to be tested, but recent preclinical work with syngeneic colorectal cancer models suggests that WNT-targeted agents may enhance antitumor immunity (154, 155).

It is important to note that WNT may not be universally immunosuppressive. As mentioned, suppression of WNT via DKK1 induces latency associated with downregulation of cell surface immune sensors. This allows latent metastatic cells to persist long term by evading immune surveillance (145). Together, these data highlight key roles for WNT signaling in mediating antitumor immunity, and although most evidence

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



**Table 1. Wnt inhibitors used in the clinic**

| Compound | Cancer type | Trial identifier |
|---|---|---|
| WNT ligand or receptor targeting agents | | |
| OMP-54F28 (ipafricept) | Ovarian cancer | NCT01608867 |
| | Pancreatic cancer | NCT02092363 |
| | HCC | NCT02050178 |
| | | NCT02069145 |
| OMP-18R5/vantictumab | NSCLC | NCT01345201 |
| | Pancreatic cancer | NCT01957007 |
| | Metastatic HER2-negative breast cancer | NCT02005315 |
| | | NCT01973309 |
| WNT974 (LGK974) | Pancreatic | NCT01351103 |
| | *BRAF*-mutant and metastatic colorectal cancer | NCT02278133 |
| | Melanoma | |
| | TNBC | |
| | Head and neck squamous cell cancer | |
| | Cervical squamous cell cancer | |
| | Esophageal squamous cell cancer | |
| | Lung squamous cell cancer | |
| ETC-1922159 | Advanced solid tumors | NCT02521844 |
| RXC004 | Solid tumors | NCT03447470 |
| Compounds that promote β-catenin degradation | | |
| CWP232291 | AML | NCT03055286 |
| | CML | NCT01398462 |
| | Myelodysplastic syndrome | NCT02426723 |
| | Myelofibrosis | |
| | Multiple myeloma | |
| E7447 (2X-121) | Ovarian cancer | NCT03878849 |
| | Breast cancer | NCT03562832 |
| | Solid tumors | NCT01618136 |
| | TNBC | |
| | Melanoma | |
| Antagonists of β-catenin–mediated expression | | |
| PRI-724 | Pancreatic cancer | NCT01764477 |
| | AML | NCT01606579 |
| | CML | NCT02413853 |
| | Colorectal cancer | |
| E7386 | Solid neoplasms | NCT03833700 |
| | Colorectal cancer neoplasms | NCT03264664 |
| SM08502 | Solid tumors | NCT0335066 |

Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; NSCLC, non–small cell lung cancer; TNBC, triple-negative breast cancer.

suggests that WNT suppression would be an attractive therapeutic strategy, further understanding the impact of WNT regulation on antitumor immune function will be critical to implement such approaches safely.

## THERAPEUTIC TARGETING OF THE WNT SIGNALING PATHWAY: INHIBITORS, CLINICAL TRIALS, AND RESISTANCE

### Targeting the WNT Pathway

Given the high frequency of WNT pathway activation in cancer and its clear role in driving tumor progression and immunosuppression, there is immense interest in targeting the WNT pathway for cancer therapy. Current strategies to target WNT signaling can be grouped into three categories: ligand- or receptor-targeting agents, agents that promote degradation of β-catenin, and antagonists of β-catenin–mediated transcription. An overview of inhibitors in completed or ongoing clinical trials is presented in Table 1 and Fig. 4.

#### *WNT Ligand and Receptor Targeting Agents*

Cancers driven by *RSPO* fusions, *RNF43* mutations, and autocrine/paracrine WNT activation rely on the engagement of the WNT ligand with cell surface receptors (Fig. 1).

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



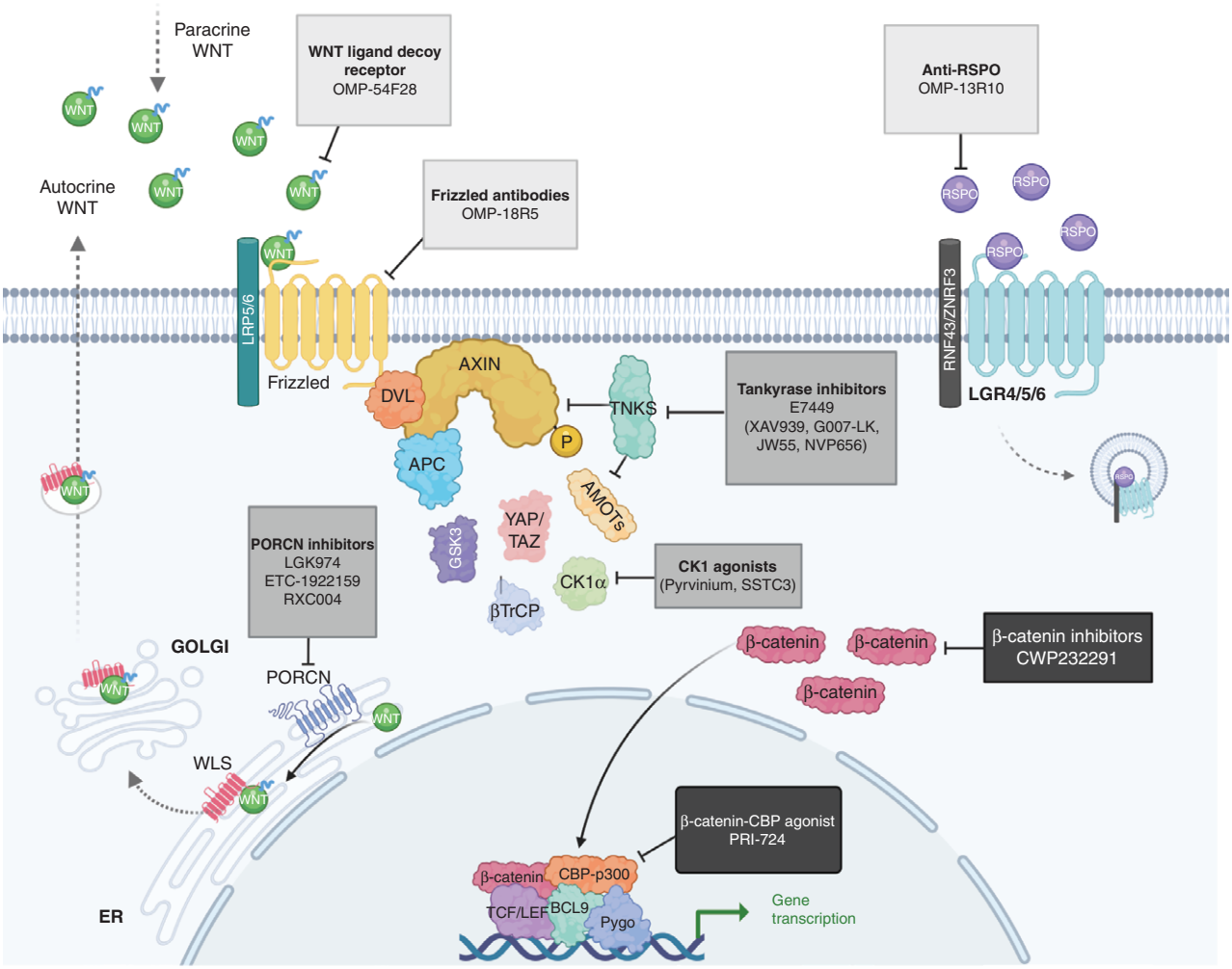


**Figure 4.** WNT pathway inhibitors in clinical trials. Schematic representation of the canonical WNT signaling pathway with inhibitors at various points along the pathway that are currently in clinical trials. Other validated inhibitors not yet in clinical trials are noted in parentheses. See also Table 1. Created with BioRender.com. ER, endoplasmic reticulum.

Multiple pharmacologic strategies have been developed to intercept this upstream WNT trigger, including the direct inhibition of WNT ligand secretion, WNT–FZD antagonists, or RSPO3 neutralizing antibodies.

WNT–FZD binding can be blocked through direct inhibition of the receptor interface or by sequestration of the WNT ligand. OMP-54F28 is a fusion protein comprising the WNT-binding domain of FZD8 fused to a human IgG1 Fc domain and, following evidence of efficacy in preclinical models, is now in phase I clinical trials for advanced HCC, ovarian cancer, and pancreatic cancer (156). Vantictumab (OMP-18R5) is a humanized mAb that recognizes the extracellular domain of multiple FZD proteins (FZD1, 2, 5, 7, 8) and has shown antitumor efficacy in solid tumors (157) and was trialed in combination with cytotoxic chemotherapies in lung, breast, and pancreatic cancers, although dose-escalation studies revealed significant bone-related toxicities (see below).

Following the identification of RSPO2 and RSPO3 fusion colorectal cancers, neutralizing RPSO antibodies were developed to directly target these oncogenic drivers. Anti-RSPO3 antibody treatment has shown promise in RSPO fusion colorectal cancer PDX models (100), and an independently developed RSPO3 antibody (OMP-131R10) completed phase I testing in 2018 (NCT02482441). Despite the clear rationale for this approach and evidence that RSPO3 is a disease driver (100, 102), to date, RSPO-focused treatments have not progressed further clinically.

LGK974 (now known as WNT974; ref. 158), ETC-1922159, and RXC004 are PORCN inhibitors that suppress WNT secretion by preventing O-linked palmitoleoylation of WNT ligands. These inhibitors have been validated thoroughly in preclinical rodent models and have shown activity in RSPO fusion (93, 102, 159), *RNF43*-mutant (93, 104), and WNT ligand–driven cancers (158). ETC-1922159 is also reported to synergize with PI3K/mTOR inhibitors in preclinical *RNF43*-mutant pancreatic cancer models *in vivo* (160). Although preliminary results suggest WNT974 has a "manageable toxicity" profile (161), WNT974, ETC-1922159, and vantictumab (anti-FZD) have shown dose-limiting toxicities related

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



to loss of bone mass (e.g., fractures). This is mostly likely due to the essential role of WNT signaling in osteoblast differentiation and regulation of bone homeostasis (3, 162–164). Recently, Madan and colleagues (165) reported that blocking the resorption of bone during PORCN inhibitor treatment can ameliorate the negative consequences of WNT blockade in the bone while maintaining on-target WNT inhibition; ETC-1922159 is currently in phase Ib testing in combination with the RANKL inhibitor denosumab to prevent bone loss (NCT02521844).

#### *Compounds That Promote β-Catenin Degradation*

For cancers that activate WNT downstream of the receptor, signaling must be blocked at or below the regulation of β-catenin. For example, CWP232291 is a peptidomimetic drug that drives degradation of β-catenin via activation of caspases and is currently in phase I clinical trials as a single agent in acute myeloid leukemia and chronic myeloid leukemia, or in combination with lenalidomide and dexamethasone in multiple myeloma.

Other strategies aim to reengage endogenous tumor suppression by hijacking known regulators of β-catenin turnover. One such approach is via the inhibition of TNKS enzymes (TNKS/TNKS2), which control the abundance of AXIN1 protein (Fig. 1) and can suppress WNT signaling, even in cells carrying truncated alleles of *APC* (see "Resistance to WNT Inhibition" below). In preclinical studies, TNKS inhibitors demonstrate robust inhibition of WNT signaling, proliferation, and synergistic effects with other targeted agents, including CDK4/6, EGFR, MEK inhibitors, or anti–PD-L1 (155, 166–169). Although PARP/TNKS inhibitors effectively suppress WNT signaling, there is considerable toxicity due to the dependence of normal epithelial and hematopoietic stem cells on WNT (170, 171). Such on-target toxicities remain an issue in targeting the WNT signaling pathway. One dual PARP/TNKS inhibitor that has not displayed overt intestinal toxicity in preclinical studies, E7449 (2X-121; ref. 172), is currently in phase II clinical trials for advanced ovarian cancer and metastatic breast cancer. Although E7449 shows TNKS inhibitor activity in cells, it is 50 to 100 times more potent against PARP1/PARP2 ($IC_{50}$ 1 nmol/L and 1.2 nmol/L, respectively) than TNKS/TNKS2 (50–100 nmol/L; ref. 172), and early clinical data show better responses in patients with *BRCA*-mutant cancers (173), a known predictive biomarker of PARP inhibitor response. Thus, it remains unclear what role TNKS inhibition plays in the biological activity of this particular drug.

An alternate approach to reengage dendritic cell–mediated β-catenin degradation is via direct activation of the kinases controlling β-catenin stability. Pyrvinium, an approved treatment for helminth (pinworm) infection, enhances CK1α activity and reduces WNT signaling, even in the context of stabilizing β-catenin mutations (174, 175). Pyrvinium is poorly bioavailable, although newer derivatives exhibit efficacy against several WNT-driven cancer cell lines and organoids and may hold more therapeutic promise (176).

#### *Antagonist of β-Catenin–Mediated Transcription*

If β-catenin abundance cannot be controlled, its function as an oncogenic transcription factor could be targeted by interfering with the engagement of essential transcriptional cofactors. PRI-724 is a β-catenin–CBP antagonist that specifically inhibits the β-catenin–CBP interaction while promoting the formation of β-catenin and p300 interactions, thus inhibiting the self-renewal capacity of stem cells *in vitro* (177). E7386 is a selective inhibitor that also targets the β-catenin–CBP interaction but was developed with improved microsomal stability, membrane permeability, and solubility of C-82, which is an active form of PRI-724 (178). Similarly, antagonists of β-catenin–responsive transcription specifically target the activity of β-catenin at the TCF transcriptional complex to inhibit WNT signaling (179). Although not directly targeting β-catenin, a small-molecule inhibitor of CDC-like kinase was recently shown to inhibit a range of WNT pathway genes, including downstream transcriptional targets (e.g., AXIN2) and direct regulators of the pathway (e.g., LRP5, CTNNB1), at least in part by modifying mRNA splicing (180). This drug is currently in phase I trials for patients with advanced solid tumors.

#### *Exploiting WNT Activation to Treat WNT-Driven Cancers*

Direct and potent inhibition of WNT signaling carries significant deleterious consequences for normal tissues, but there may be opportunities to exploit WNT dependence without blocking pathway activity. Using a genome-wide CRISPR-based screen, Hinze and colleagues (181) recently showed that activation of the WNT pathway sensitized leukemias to treatment with asparaginase by preventing GSK3α-mediated proteosome degradation and catabolic production of asparagine. The same approach provided dramatic survival benefit in mice transplanted with RSPO3-fusion intestinal tumor cells and in *APC*-mutant cancers when cotreated with selective GSK3α inhibitors (182).

## Resistance to WNT Inhibition

For most molecularly targeted therapies, the emergence of drug resistance is a major hurdle to long-term clinical efficacy. To date, the minimal clinical use of WNT targeting drugs has limited the analysis of human tumor response and resistance. However, the use of cell lines and preclinical murine models has provided some insight into potential mechanisms of tumor escape.

We and others recently showed that sensitivity to TNKS inhibition can be dictated by type of truncating *APC* mutations present in a tumor or cell line (67, 183). Surprisingly, the types of *APC* mutations that predict sensitivity or resistance to TNKS inhibition in these two studies were not the same, suggesting that there might be other factors that influence this response. Consistent with this idea, Menon and colleagues (166) suggest that activation of KRAS may be associated with TNKS inhibitor resistance. This has not been formally demonstrated, but it is worth noting that treatment of cancer cell lines and xenografts with MEK or EGFR inhibitors can resensitize cells to treatment with TNKS inhibitors (167, 169, 184, 185).

Using two human PTPRK–RSPO3 fusion cell lines, Picco and colleagues (186) showed that although RSPO-fusion colorectal cancers are sensitive to PORCN inhibitors, downstream activation of WNT signaling via disruptive mutations in *AXIN1* can lead to the emergence of drug-resistant cells. Such mutation-driven pathway reactivation is reminiscent of drug resistance in other settings, such as treatment of *EGFR*-mutant lung cancers

Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



with EGFR inhibitors. In the RSPO3 example, the VACO6 cells that developed *AXIN1* mutations are MSI-H and thus have a hypermutation phenotype. Most RSPO-fusion human tumors are microsatellite stable, so it is unclear whether this would be the dominant outcome in a clinical setting (96, 187). Nevertheless, it provides a clear example of the need to accurately profile tumor mutations and ensure there are no downstream WNT alterations present when initiating treatment with receptor- or ligand-based WNT inhibitors.

Even in the case of effective tumor-intrinsic suppression of WNT production, it is possible that niche-supporting cells in the microenvironment may supplement WNT production. Although it has not yet been demonstrated in tumors, Virshup and colleagues (188) showed that normal intestinal stem cells can bypass the toxic effect of PORCN inhibitors due to juxtaposed WNT-producing stroma that avoids PORCN inhibitions via efficient drug efflux. Similarly, Seino and colleagues (106) identified a subtype of pancreatic cancer organoids that depend on WNT production from cancer-associated stroma as well as tumor cells that produce their own WNT ligands. In both of these contexts, tumor cells remain sensitive to PORCN inhibitors, but there was a third subtype that was at least partially resistant to PORCN inhibitor treatment, although the mechanism for this was not clear.

We recently described a genotype-dependent but nongenetic mechanism of WNT inhibitor resistance (102), whereby induction of a YAP/TAZ-driven transcriptional program downstream of TGFβ signaling induced lineage reversion to a WNT-independent embryonic state (189). A similar phenomenon has been reported during tissue regeneration in the intestine (190, 191). Interestingly, in *AXIN1*-mutant HCC, transformation can occur in the absence of WNT hyperactivation, associated with oncogenic signatures of Notch and YAP/TAZ (192). Recently, Kawasaki and colleagues (193) described a second form of lineage plasticity whereby transition to a neuroendocrine-like state, termed gastroenteropancreatic neuroendocrine neoplasms (GEP–NEN), can bypass the dependence on WNT pathway activity. Reminiscent of neuroendocrine transitions observed in prostate and lung cancers (194–196), the emergence of GEP–NENs is strongly correlated with disruption of both p53 and RB1. As clinical trials of WNT inhibitors expand, it will be important to monitor lineage transitions as a possible driver of therapy failure. A mechanistic understanding of how to prevent or reverse such effects could be critical for achieving the greatest impact of WNT-targeted treatments.

## CONCLUDING REMARKS

Since its discovery more than 30 years ago, investigation of WNT pathway signaling in normal development and cancer has revealed an array of fascinating biological mechanisms. Our ever-expanding understanding of how WNT is activated and promotes cancer progression has highlighted opportunities to tackle the deadliest malignancies. We must now exploit what we have learned and explore new approaches to WNT-focused therapy, including broadening our gaze beyond the tumor cell. Defining the way in which WNT activation interacts with and is modulated by the tumor microenvironment and the immune system is a major challenge for the next decade and may usher in a new wave of efforts to drive WNT-targeted treatments into the clinic.

### Authors' Disclosures

T. Tammela reports personal fees from Gerson Lehrman Group and Guidepoint outside the submitted work. L.E. Dow reports personal fees from Mirimus Inc, grants from American Cancer Society, grants from NIH/NCI, and grants from Starr Cancer Consortium outside the submitted work. No disclosures were reported by the other author.

### Acknowledgments

We thank Alyna Katti for discussion and critical reading of the manuscript. L.E. Dow received support from the American Cancer Society under a Research Scholar Grant (131461-RSG-17-202-01-TBG) and the NCI (1R01CA222517-01A1). L.E. Dow and T. Tammela received support from the Starr Cancer Consortium (SCC I14-0023).

Received February 12, 2021; revised April 30, 2021; accepted June 11, 2021; published first September 13, 2021.

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Published OnlineFirst September 13, 2021; DOI: 10.1158/2159-8290.CD-21-0190



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# WNT as a Driver and Dependency in Cancer

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*Cancer Discov* 2021;11:2413-2429. Published OnlineFirst September 13, 2021.