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Targeting the Wnt/beta-catenin Pathway in Cancer: Update on Effectors and Inhibitors

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Abstract

The Wnt/beta-catenin pathway is a family of proteins that is implicated in many vital cellular functions like stem cell regeneration and organogenesis. Several intra-cellular signal transduction pathways are induced by Wnt, notably the Wnt/beta-catenin dependent pathway or canonical pathway and the non-canonical or beta-catenin-independent pathway, the latter includes the Wnt/Ca²⁺ and Planar Cell Polarity pathway (PCP). Wnt activation occurs at the intestinal crypt floor, and is critical to optimal maintenance of stem cells. Colorectal cancers show evidence of Wnt signaling pathway activation and this is associated with loss of function of the tumor regulator APC. Wnt activation has been observed in breast, lung, and hematopoietic malignancies and contributes to tumor recurrence. The Wnt pathway cross talks with the Notch and Sonic Hedgehog pathways, which has implications for therapeutic interventions in cancers. There are significant challenges in targeting the Wnt pathway, including finding agents that are efficacious without damaging the system of normal somatic stem cell function in cellular repair and tissue homeostasis. Here, we comprehensively review the Wnt pathway and its interactions with the Notch and Sonic Hedgehog pathways. We present the state of the field in effectors and inhibitors of Wnt signaling, including updates on clinical trials in various cancers with inhibitors of Wnt, Notch, and Sonic Hedgehog.

Graphical abstract

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Keywords

Wnt; beta-catenin; colorectal cancers; gamma secretase; Hedgehog; Targeted therapy

Introduction

The Wnt family is a group of proteins implicated in many cellular functions: organ formation, stem cell renewal, and cell survival [1]. In humans, the Wnt family consists of cysteine rich glycoproteins that act as ligands for as many as fifteen receptors and co-receptors [2]. Extracellular Wnt can trigger varied intra-cellular signal transduction pathways, like the Wnt/beta-catenin dependent or canonical pathway and the beta-catenin-independent or non-canonical pathway (Figure 1,2b). Examples of the beta-catenin-independent pathway include the Wnt/Ca²⁺ pathway as well as the Planar Cell Polarity pathway (PCP) [3]. The beta-catenin-dependent signaling pathway is triggered by the binding of Wnt ligand to the LRP-5/6 receptors (low-density lipoprotein receptor) and Frizzled receptors. This in turn activates Dishevelled (DVL), causing recruitment of the complex (Axin, GSK-3 beta, CK1, APC) to the receptor. [4–6]. The Wnt – Frizzled-Axin - LRP-5/6 complex sequesters cytosolic GSK-3 beta rendering it incapable of phosphorylating beta-catenin. There is accumulation of un-phosphorylated beta-catenin in the cytosol which migrates to the nucleus, interacting there with T cell-specific factor (TCF)/lymphoid enhancer-binding factor (LEF) and co-activators, like Pygopus (Pygo) and Bcl-9, to turn on the Wnt target genes such as c-Myc, cyclin D1 and Cdkn1a [6].

Without Wnt, the beta-catenin in the cytosol undergoes phosphorylation by GSK-3 beta and CK1 and subsequent sequestration in the beta-catenin destruction complex, (APC, GSK-3 beta, CK1, Axin). This phosphorylated complex allows for the E3 ubiquitin ligase called beta-TrCP to attach to the beta-catenin at a binding site, that enhances its ubiquitination leading to subsequent proteasomal degradation [7–8] (Figure 2a). One of the Non-canonical Wnt pathways includes the PCP or Planar cell polarity pathway. This can be initiated by Wnt interaction with Frizzled receptors, with co-receptors RYK and ROR which control the activity of small GTPases such as RhoA that play a role in regulation of the remodeling of the cytoskeleton [8] (Figure 2b). Wnt interaction with Frizzled leads to Dvl activation [8]. myosin and the Rho-associated kinase (ROCK) are activated by Rho GTPase, altering the mechanism of actin and cytoskeleton rearrangement. There is in tandem activation of Rac GTPase and activated Rac then stimulates JNK activity (c-Jun N-terminal kinase) [9].

In the Wnt/Ca²⁺ pathway, activated by Wnt 5A, the frizzled FZD2 cleaves guanine nucleotide binding protein (G-protein), into protein beta/gamma subunits G-protein alpha-t2 causing Ca²⁺ to be released into the cytosol promoting differentiation in the neuronal system. Calcium activates CaMK II and Calmodulin, enhancing phosphorylation of Tcf/Lef (T-cell factor and lymphoid enhancer factor) thus suppressing the canonical Wnt pathway. The mechanisms by which Wnt5a can also interact via the canonical pathway are not completely mapped out though it is speculated that the LRP5 co-receptor is activated along with FZD4 and FZD5 receptors [10–14].

Axin and APC- Negative regulators of Wnt

Axin serves as a scaffold protein recruiting GSK3 β and CKI α (caspase kinase alpha) along with APC to form a complex with beta-catenin resulting in beta-catenin phosphorylation, ultimately causing its degradation. Axin also plays a key role in Wnt signaling initiation. PPPSP motifs on the cytoplasmic tail of LRP6 are phosphorylated upon Wnt activation. This in turn causes recruitment of Axin complexes to the membrane destabilizing beta-catenin complex in the cytoplasm. Axin is post translationally modified by phosphorylation/dephosphorylation. Without Wnt, Axin is phosphorylated, increasing its binding affinity with beta-catenin, leading to stabilization of Axin. When Wnt stimulation is present, Axin is dephosphorylated, resulting in less binding with beta-catenin and consequently Axin degradation [15–18].

APC supplies the framework for a destruction complex together with GSK3 β and Axin that promotes phosphorylation and subsequent proteasomal degradation of beta-catenin. In addition, APC enhances export of beta-catenin from the nucleus, which reduces the amount of nuclear beta-catenin for interaction with TCF. Furthermore, APC can bind to beta-catenin, thereby blocking the beta-catenin interaction with TCF/LEF [19–20].

RNF43 and RSPO signaling modulation of Wnt

RNF43 (Ring finger protein 43) and the homolog ZNRF3 are transmembrane E3 ligases that dispose of the surface Wnt receptors and promote FZD receptor turnover. R-spondins or RSPO's are a group of proteins that together bind to the extracellular domains of LGR4/5 and RNF43/ZNRF3, resulting in increased cell surface FZD receptors as this binding causes ubiquitination and clearance of RNF43/ZNRF3[21,22].

Wnt and Notch signaling pathway cross- talk

It is thought that the Wnt-beta catenin pathways and Notch pathways interact for Drosophila wing development [23]. Importantly, the Notch target gene Hes1, which encodes a strong basic helix–loop–helix (bHLH) transcriptional repressor, is regulated by beta-catenin-mediated Wnt signaling [24]. There is some evidence that direct interaction between beta-catenin and TCF activates Notch in colorectal cancer cells through regulation of Jagged1 expression. Beta-catenin interaction with Notch-1 leads to decreased Notch-1 ubiquitination, causing Hes-1 expression to increase, which is associated with tumorigenesis. The serine/threonine kinase GSK3 β , is an important node in Wnt and Notch signaling crosstalk. It

mediates the phosphorylation of serine and threonine residues of the Notch intracellular domain (NICD-1), which in turn causes it to localize in the nucleus increasing its transcriptional activity and making it more stable [23].

Loss of Notch-1 leads to activation of beta-catenin and increases the transcriptional activity of a beta-catenin-responsive reporter construct, suggesting that Notch dampens beta-catenin-mediated responses to Wnt [24]. The non-canonical Wnt/Ca²⁺ pathway also interacts with Notch signaling. In the non-canonical Wnt/Ca²⁺ pathway, activation of CamKII by Wnt5a, induces the phosphorylation of the RBP-J-interacting corepressor SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) on serine 1407, resulting in increased promoter activity of a Notch-responsive gene [25] (Figure 2b).

Wnt- Sonic Hedgehog pathway cross-talk

The Sonic hedgehog (SHH) pathway plays a vital role in embryogenesis. It has a critical role in the development of neural structures. Activated, the Sonic hedgehog (SHH) attaches to Patch receptors leading to the increased activity of Smoothened receptors (Smo). This in turn causes the transcription of glioma-associated oncogenes homologs (Gli1/2/3) [26]. There is evidence that SHH-mediated tumorigenesis can be inhibited by Wnt. Both GSK3 β and CK1 α phosphorylate Gli3 that leads to Gli3 ubiquitination. Phosphorylated Gli3 is recognized by β -TrCP (Beta-Transducin Repeat-Containing Protein) and leads to the degradation of C-terminal peptides generating Gli3R, which subsequently inhibits Gli1 activity [27]. Sfrp-1 (secreted Frizzled-related protein-1), a suppressor of the Fused kinase, is a negative regulator of both Wnt/beta-catenin and Hedgehog/Gli signaling pathways. Two protein kinases GSK3 β and CK1 α negatively regulate both beta-catenin and Gli1 [28]. Studies have shown an inhibition of SMO could reduce protein levels of active beta-catenin [26] (Figure 3). Inhibitors of these effectors of the Wnt- beta catenin pathway are summarized in Table1.

Wnt in Cancers

Wnt pathway is upregulated in both MSI (microsatellite instable) and MSS (microsatellite stable) colorectal cancers [66]. Normally activated at the bottom of the intestinal crypts, Wnt is critical to cell repair and maintenance of stem cell functions. The primary mechanism of Wnt pathway activation is the loss of function of APC which functions as a negative regulator. Wnt/beta-catenin signaling is activated by truncated APC protein that negates destruction complex-mediated beta-catenin ubiquitination [66].

RNF43 mutations lead to loss of function; they prevent removal of Wnt receptor in the intestinal crypt, thereby causing Wnt signaling activation [21]. They are found in over 18% of colorectal and endometrial cancers [67]. RSPO translocations are noted in 4–18% of patients with gastric, ovarian, and endometrial cancer and about 9% of colorectal cancers. Both RNF43 and R-spondin fusion are mutually exclusive with APC mutations; these alterations may predict for response to inhibitors of Wnt [68].

More than half of breast cancers have activation of Wnt and that is associated with lower overall survival [69]. In a transgenic mouse model inhibition of beta-catenin-dependent

signaling in ErbB2-derived cells impaired tumor initiation and metastasis additionally, treatment of ERBB2-overexpressing tumor cells with a selective beta-catenin/CBP inhibitor significantly decreased proliferation and ErbB2 expression [70]. There is an increase in active Wnt signaling in breast cancer stem cells which was confirmed by increased expression of activated beta-catenin protein, both downstream targets AXIN2 and LEF1, and decreased expression of DKK1 protein [69–70].

In non-small cell lung cancer, there is evidence that lung cancer “stemness” is maintained by targeting the negative regulators of Wnt signaling for degradation, thereby increasing beta-catenin mediated Wnt activity [71]. Wnt ligands and receptors were shown to be expressed in the hematopoietic stem cells (HSC) and are found in the bone marrow microenvironment [72]. CML (chronic myeloid leukemia) patients in blast crisis have shown evidence of Wnt activation [73]. Wnt signaling plays a critical role in chemo-resistance in ovarian cancer and is involved in the maintenance and propagation of ovarian cancer stem cells [74]. Desmoid tumors are uncommon malignancies characterized by Wnt/ β -catenin activation as the critical step in desmoid tumor formation with tumors almost always showing mutations of the beta-catenin gene or APC [75].

Wnt-signaling pathway inhibitors in clinical trials for cancer (Table 2)

Inhibitors of the Wnt-Receptor Complex

Porcupine inhibitors (Figure 2a)—Porcupine (PORCN) is a membrane-bound O-acyltransferase (MBOAT) important for the secretion of Wnt ligands because it supplies the palmitoyl group to Wnt proteins, a crucial step for Wnt ligand secretion [76].

The Porcupine-selective inhibitor LGK974 blocks Wnt signaling and tumor growth in vivo [40]. Head and neck squamous cell carcinoma (HNSCC) cell lines carrying NOTCH1 mutations that are inactivating are particularly sensitive to inhibition by LGK974 [77]. Porcupine is a Wnt pathway target that is amenable to inhibition while sparing Wnt-dependent tissues. There are ongoing Phase 1/2 trials with LGK974 in metastatic colorectal and head and neck cancers with the characteristic mutations like Rnf43/Znrf3 [78], but results are not yet reported (Table 2).

ETC-159 is a small molecule PORCN inhibitor with efficacy in preclinical models of RSPO-translocated colorectal cancer [79]. This molecule has been in Phase I trials since July 2015, with ten patients in the original cohort (9 Colorectal cancers and 1 renal) [80]. So far, there are no responses. One patient at 2 mg and 1 at 4 mg remained in the study in stable disease for 6 cycles (Table 2.).

Antibodies against Wnt family proteins—Specific Wnt ligands or receptors found to be over expressed in many tumors can also be targeted with agent’s specific to these receptors. Monoclonal antibodies developed against Wnt-1 and Wnt-2 have evidence for Wnt inhibition leading to tumor suppression in melanoma, sarcoma, colorectal cancers, non-small cell lung carcinoma, and mesothelioma [80–81].

OMP-18R5 (Vantictumab) manufactured by OncoMed Pharmaceuticals/Bayer is a monoclonal antibody purported to target five of the ten FZD receptors. Safety and efficacy in non-small cell lung cancer, pancreatic and breast cancer are being evaluated alone or combined with chemotherapy [41]. A novel recombinant fusion protein, OMP-54F28 binds Wnt ligands and blocks Wnt signaling through its domain of an extracellular part of human Frizzled 8 receptor (fused to a human IgG1 Fc fragment) [42]. There is concern for Wnt inhibition in bone and five patients had a doubling of a bone turnover marker β -C-terminal telopeptide returning to baseline levels after a single dose of zoledronic acid. There was stable disease for a period of over six months in two patients with desmoid tumors. 4 of 4 patients at 20 mg/kg with 1 on-study tumor assessment continue study with stable disease [82]. Three Phase 1b studies are currently in progress in hepatocellular cancer with sorafenib, pancreatic cancer in combined therapy with nab-paclitaxel and gemcitabine, and in combination with paclitaxel and carboplatin in ovarian cancer [83] (Table 2.).

The first-in-class recombinant fusion protein Ipafricept (OMP-54F28) blocks Wnt signaling through binding of Wnt ligands. In patient-derived ovarian cancer xenografts, this compound has shown activity to decrease the frequency of stem cells, suppress tumor formation and promote differentiation. Interestingly, pretreatment with OMP-54F28 two to three days prior to chemotherapy shows evidence of synergy with taxanes. The first 7 of the 17 patients were dosed in 2 cohorts of q3w IPA/Carboplatin/Paclitaxel (doses of 5 & 10 mg/kg) and the next 10 patients in 2 cohorts of q3w IPA followed by Carboplatin/Paclitaxel (doses of 2 & 4 mg/kg). 6/17 (35%) had complete response (CR), 3 (18%) stable disease and 8 (47%) partial responses (PR). 82% of patients achieved a partial or complete response with main adverse events being grade 3 neutropenia in 3/17, one grade 3 hypophosphatemia and zoledronic acid was used prophylactically in post-menopausal patients [43,84].

B-Catenin-Destruction Complex Inhibitors

Tankyrase inhibitors—Tankyrase belongs to the Poly (ADP-ribose) polymerases (PARPs) family. There are two isoforms of Tankyrase, Tankyrase 1 (PARP5a) and Tankyrase 2 (PARP5b) associated with the Wnt/beta-catenin signaling. Both these tankyrase isoforms increase the degradation of axin by the ubiquitin-proteasome pathway [85]. Tankyrase inhibitor, XAV939 and IWR-1 regulate Axin by inhibiting Tankyrase 1 and Tankyrase 2 [29,30]. Mouse tumor xenografts and patient-derived sphere cultures of patients with colorectal cancer were incubated with a Tankyrase inhibitor, NVP-TNKS656 in addition to AKT and PI3K inhibitors. A high nuclear beta-catenin level predicted for apoptosis with NVP-TNKS656 in combination with PI3K and AKT inhibitors suggesting the tankyrase inhibitor could overcome resistance to these inhibitors. High FOXO3A (Forkhead box O3) activity was associated with sensitivity to NVP-TNKS656 treatment. Thirteen of forty patients had high nuclear beta-catenin content and had progressed on prior PI3K/AKT/mTOR inhibition [85]. Concerns of gastrointestinal toxicity have arisen in analysis of these inhibitors and further studies are needed [86]. There are currently no ongoing trials with Tankyrase inhibitors.

Disheveled inhibitors—Through the PDZ domain, disheveled (DVL) binds to the carboxyl terminal end of the FZD receptors, the common protein-interaction domain.

NSC668036, FJ9, and 3289–8625 are some agents that block the FZD and DVL-PDZ interaction leading then to inhibition of the signal transduction pathway [34,87].

TCF/beta-catenin Transcription Complex Inhibitors—There is great variation in Wnt signaling pathway mutations and there is a quest to find agents that can target the downstream effectors. Eight compounds were identified by high-throughput ELISA screening. PFK115-584 and CGP049090 are examples of these and can perturb the beta-catenin/TCF complex in a dose-dependent manner [88]. A major disadvantage is the non-selective nature of the inhibition of beta-catenin/TCF interaction.

Wnt co-activator antagonist—PRI-724 is a first-in-class small molecule antagonist that inhibits the interaction between beta-catenin and its transcriptional coactivator CBP (CREB-binding protein) [47]. Preclinical studies of PRI-724 in pancreatic cancer suggest this agent can promote differentiation of chemotherapy-insensitive cancer stem cells and tumor-initiating cells, inhibit stroma formation, and decrease metastatic potential. Patients had progression following 1st-line treatment with FOLFIRINOX or FOLFOX chemotherapy. A 3+3 dose cohort escalation was done with gemcitabine (1000 mg/m² on d1, 8, and 15 of 28 d cycle) and increasing doses of PRI-724 administered as a continuous infusion x7 days every other week. 20 patients were enrolled across 3 dose cohorts (doses of PRI-724 at 320, 640 and 905 mg/m²/d). 7/20 had Grade 3/4 adverse events. There was stable disease in 8 pts (40%) and 2 minor responses. 5 of 8 pts (62.5%) with elevated baseline CA19-9 levels showed a marker decline of 30%. Median PFS was 2 months (range, 0.7 to 7.7) [49].

Wnt5a Mimetics—Primary breast carcinomas with low level of Wnt 5a have been found to have a lower disease-free survival suggesting a tumor suppressor role for Wnt 5a. This has been borne out in hematopoietic, prostate, thyroid and colon cancers [89]. Conversely, in melanomas and gastric cancers Wnt 5a expression is associated with increased invasion and metastases [90]. Foxy-5 is a formulated hexapeptide that can mimic the properties of the Wnt5a molecule to impair cancer cell migration in vitro. A phase 1 study of Foxy-5 in patients with metastatic colon, breast, and prostate cancer shows no dose limiting toxicity and a phase 1b trial is ongoing [91].

Gamma Secretase Inhibitors (GSI's)—There is growing evidence for cross talk between the Notch and Wnt pathways and in a recent study CD44+ CSCs (gastric cancer stem cells) showed high expression of *HES-1* [92]. Treatment with Gamma secretase inhibitors (GSI) induced apoptosis with demonstrated evidence of inhibition of tumor sphere formation of CD44+ CSCs. Notch1 was thought to be the intermediary in the crosstalk between Wnt-beta-catenin and Notch and these cells [23,24]. Clinical trials with GSI's have included RO-4929097 with exploratory phase 1 data in pancreatic and colorectal cancer though since discontinued by manufacturer. A more promising molecule is MK-0752 in combination with ridaforolimus (MK-8669) being investigated in a phase 1 trial of patients with advanced and refractory solid tumors [56,57,93].

There is an ongoing Phase I/II study in patients with locally advanced or metastatic breast cancer of MK-0752 with docetaxel (Table 2). PF-03084014 (Pfizer Inc., Groton, CT, USA) is a selective or Notch-sparing GSI or GS (gamma secretase) modulator [54]. PF-03084014 a

small molecule GSI reduced tumor cell migration and mammosphere formation in vitro, reduced tumor cell self-renewal ability in vivo, and decreased mRNA expression of Notch target genes *HES-1*, *HES-4*, *Notch-1*, and *HEY-2* in HCC (hepatocellular cancer) xenograft tumors [94]. A Phase 1 trial in triple negative breast cancer in combination with docetaxel had a partial response rate of 4/25 (16%) and 9/25 (36%) with stable disease [54]. Gastrointestinal toxicity is a dose-limiting side-effect with Gamma secretase inhibitors. [53–58]. Of interest, gamma secretase showed activity in desmoid tumors (5/9) had an objective response [95].

Hedgehog Inhibitors—sFRP-1, a main target gene of the sonic hedgehog pathway, is involved in cross-talk between the hedgehog pathway and the Wnt pathway. Vismodegib is an FDA approved SMO inhibitor that binds directly to SMO; it is currently in use in advanced basal cell cancers. There are also ongoing phase 1/11 trials in cancers like pancreatic gastric, and prostate. Erismodegib (sonidegib) is another FDA-approved, orally bioavailable SMO antagonist used in advanced BCC and there are several ongoing Phase 1/11 trials in other malignancies [62–65]. GLI transcription factors are the terminal effectors of the Shh-SMO signaling pathway and agents called GANTs (GLI antagonists), have shown activity in cell lines and xenografts and arsenic trioxide, a FDA approved drug for acute promyelocytic leukemia, has shown activity as an inhibitor of these transcription factors [96].

Modulating Wnt in the clinical setting—Wnt-targeting therapies are varied and clinical experience nascent. Optimal use of these agents in the future will depend on matching the Wnt inhibitor with responsive alterations. As an example, Porcupine inhibitors act by blocking the secretion of Wnt ligands and may impact tumors carrying alterations e.g., RNF43 and LKB1, acting at the receptor level, in this case, the FZD receptors [78]. In contrast, APC truncating mutations are resistant to Porcupine inhibitors. since loss of APC may activate the pathway independent of Wnt ligands [79]. Tankyrase inhibitors target APC-mutated tumors, which constitute 80% of colorectal cancers, by stabilizing Axin, but gastrointestinal toxicity may limit dose [86].

Wnt inhibitors may work to eradicate the tumor resistant stem cell and thus may overcome resistance to conventional therapy including cytotoxic agents. Such an approach is currently being tested clinically for chronic myeloid leukemia with PRI-724 in combination with the kinase inhibitor Dasatinib [97].

Sorafenib (Tyrosine kinase inhibitor) and refametinib (an MEK inhibitor) inactivate beta-catenin signaling [98]. Since activating mutations in the Wnt/beta-catenin pathway is seen in many patients with HCC [99], combination of sorafenib and refametinib may represent an alternative treatment for beta-catenin-dependent HCC [98]. In desmoid tumors, a phase 1 trial of PF-03084014, an oral Notch inhibitor showed that 5 of 9 patients had a partial response [95,100]. At first glance, the mechanism of action is not apparent because Notch is upstream of beta catenin. However, beta-catenin can directly express *HES-1* and activate cell proliferation through it, and *HES-1* is induced by Notch and decreased by Notch inhibitors [99]. Molecules such as Sulindac inhibit Wnt signaling, likely by blocking the PDZ domain

of the Disheveled protein and have activity in APC-mutant colorectal cancers, reducing nuclear beta-catenin accumulation [51].

Challenges to inhibiting the Wnt pathway—For the last 30 years targeting the Wnt signaling pathway has been an exciting target for inhibition. There is aberrant Wnt signaling in many cancers but thus far, no drugs have been approved to target this pathway, though there are recent clinical trials in a many hematologic and solid malignancies. One area of concern is the Wnt-beta catenin pathway's role in maintenance of stem cells and regeneration of tissues and organs [72]. There is legitimate concern that inhibition of the Wnt pathway may affect the normal Wnt dependent stem cell population, especially in areas of fast turnover like hair follicles and the gastro-intestinal tract. The preliminary experience with Tankyrase inhibitors suggests dose limiting gastrointestinal toxicity and this may limit use.

The Wnt pathway regulates many aspects of bone formation and agonists have been studied for promoting bone growth. An undesirable side effect of Wnt inhibition is increase in markers of bone turnover though in early trials this effect seems mitigated by single doses of a bisphosphonate [84].

Additionally, an elucidation of the considerable cross talk between the cell signaling pathways will be crucial to designing an efficacious therapeutic approach. The focus of future trials should be using combination therapy with agents that affect these multiple pathways in solid and hematologic malignancies.

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References

1. Croce JC, McClay DR. Evolution of the Wnt pathways. *Wnt Signaling*. 2009;3–18.
2. Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature*. 2003 May 22; 423(6938):448–52. [PubMed: 12717451]
3. MacDonald BT, Tamai K, He X. Wnt/ β -catenin signaling: components, mechanisms, and diseases. *Developmental cell*. 2009 Jul 21; 17(1):9–26. [PubMed: 19619488]
4. Wu D, Pan W. GSK3: a multifaceted kinase in Wnt signaling. *Trends in biochemical sciences*. 2010 Mar 31; 35(3):161–8. [PubMed: 19884009]
5. Bili J, Huang YL, Davidson G, Zimmermann T, Cruciat CM, Bienz M, et al. Wnt induces LRP6 signalosomes and promotes disheveled-dependent LRP6 phosphorylation. *Science*. 2007 Jun 15; 316(5831):1619–22. [PubMed: 17569865]
6. Gordon MD, Nusse R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. *Journal of Biological Chemistry*. 2006 Aug 11; 281(32):22429–33. [PubMed: 16793760]

7. Spiegelman VS, Slaga TJ, Pagano M, Minamoto T, Ronai ZE, Fuchs SY. Wnt/ β -catenin signaling induces the expression and activity of β TrCP ubiquitin ligase receptor. *Molecular cell*. 2000 May 31; 5(5):877–82. [PubMed: 10882123]
8. Kohn AD, Moon RT. Wnt and calcium signaling: β -catenin-independent pathways. *Cell calcium*. 2005 Oct 31; 38(3):439–46. [PubMed: 16099039]
9. Topol L, Jiang X, Choi H, Garrett-Beal L, Carolan PJ, Yang Y. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent β -catenin degradation. *The Journal of cell biology*. 2003 Sep 1; 162(5):899–908. 10. [PubMed: 12952940]
10. Okamoto M, Udagawa N, Uehara S, Maeda K, Yamashita T, Nakamichi Y, et al. Noncanonical Wnt5a enhances Wnt/ β -catenin signaling during osteoblastogenesis. *Scientific reports*. 2014 Mar. 4:27. 4493.
11. Holmen SL, Salic A, Zylstra CR, Kirschner MW, Williams BO. A novel set of Wnt-Frizzled fusion proteins identifies receptor components that activate β -catenin-dependent signaling. *Journal of Biological Chemistry*. 2002 Sep 20; 277(38):34727–35. [PubMed: 12121999]
12. Jiang X, Charlat O, Zamponi R, Yang Y, Cong F. Dishevelled promotes Wnt receptor degradation through recruitment of ZNRF3/RNF43 E3 ubiquitin ligases. *Molecular cell*. 2015 May 7; 58(3): 522–33. [PubMed: 25891077]
13. MacDonald BT, He X. Frizzled and LRP5/6 Receptors for Wnt/ β -Catenin Signaling. *Cold Spring Harbor Perspectives in Biology*. 2012; 4(12):a007880. [PubMed: 23209147]
14. Jernigan KK, Cselenyi CS, Thorne CA, Hanson AJ, Tahinci E, Hajicek N, et al. 2010 G β y activates GSK3 to promote LRP6-mediated β -catenin transcriptional activity. *Science signaling*. 2010 May 11.3(121):ra37. [PubMed: 20460648]
15. Kim SE, Huang H, Zhao M, Zhang X, Zhang A, Semonov MV, et al. Wnt stabilization of β -catenin reveals principles for morphogen receptor-scaffold assemblies. *Science*. 2013 May 17; 340(6134): 867–70. [PubMed: 23579495]
16. Hart MJ, de los Santos R, Albert IN, Rubinfeld B, Polakis P. Downregulation of β -catenin by human Axin and its association with the APC tumor suppressor, β -catenin and GSK3 β . *Current Biology*. 1998 May 7; 8(10):573–81. [PubMed: 9601641]
17. Zeng X, Huang H, Tamai K, Zhang X, Harada Y, Yokota C, et al. Initiation of Wnt signaling: control of Wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions. *Development*. 2008 Jan 15; 135(2):367–75. 18. [PubMed: 18077588]
18. Schneider PN, Slusarski DC, Houston DW. Differential role of Axin RGS domain function in Wnt signaling during anteroposterior patterning and maternal axis formation. *PloS one*. 2012 Sep 5.7(9):e44096. [PubMed: 22957046]
19. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. *Cell*. 2000 Oct 13; 103(2):311–20. [PubMed: 11057903]
20. Aoki K, Taketo MM. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. *Journal of cell science*. 2007 Oct 1; 120(19):3327–35. [PubMed: 17881494]
21. Loregger A, Grandl M, Mejías-Luque R, Allgäuer M, Degenhart K, Haselmann V, et al. The E3 ligase RNF43 inhibits Wnt signaling downstream of mutated β -catenin by sequestering TCF4 to the nuclear membrane. *Sci Signal*. 2015; 8:393.
22. Zebisch M, Xu Y, Krastev C, MacDonald BT, Chen M, Gilbert RJ, He X, Jones EY. Structural and molecular basis of ZNRF3/RNF43 transmembrane ubiquitin ligase inhibition by the Wnt agonist R-spondin. *Nature communications*. 2013 Nov.14:4.
23. Collu GM, Hidalgo-Sastre A, Brennan K. Wnt–Notch signalling crosstalk in development and disease. *Cellular and molecular life sciences*. 2014 Sep 1; 71(18):3553–67. [PubMed: 24942883]
24. Borggreffe T, Lauth M, Zwijsen A, Huylebroeck D, Oswald F, Giaimo BD. The Notch intracellular domain integrates signals from Wnt, Hedgehog, TGF β /BMP and hypoxia pathways. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2016 Feb 29; 1863(2):303–13. [PubMed: 26592459]
25. Ann EJ, Kim HY, Seo MS, Mo JS, Kim MY, Yoon JH, et al. Wnt5a controls Notch1 signaling through CaMKII-mediated degradation of the SMRT corepressor protein. *Journal of Biological Chemistry*. 2012 Oct 26; 287(44):36814–29. 26. [PubMed: 22888005]

26. Song L, Li ZY, Liu WP, Zhao MR. Crosstalk between Wnt/ β -catenin and Hedgehog/Gli signaling pathways in colon cancer and implications for therapy. *Cancer biology & therapy*. 2015 Jan 2; 16(1):1–7. [PubMed: 25692617]
27. Wang B, Li Y. Evidence for the direct involvement of beta TrCP in Gli3 protein processing. *Proc Natl Acad Sci U S A*. 2006; 103:33–8. [PubMed: 16371461]
28. Meng X, Poon R, Zhang X, Cheah A, Ding Q, Hui CC, et al. Suppressor of fused negatively regulates beta-catenin signaling. *J Biol Chem*. 2001; 276:40113–9. [PubMed: 11477086]
29. Riffell JL, Lord CJ, Ashworth A. Tankyrase-targeted therapeutics: expanding opportunities in the PARP family. *Nature reviews Drug discovery*. 2012 Dec 1; 11(12):923–36. [PubMed: 23197039]
30. Tian XH, Hou WJ, Fang Y, Fan J, Tong H, Bai SL, et al. XAV939, a tankyrase 1 inhibitor, promotes cell apoptosis in neuroblastoma cell lines by inhibiting Wnt/ β -catenin signaling pathway. *Journal of Experimental & Clinical Cancer Research*. 2013 Dec 5.32(1):1. [PubMed: 23289442]
31. Ma L, Wang X, Jia T, Wei W, Chua MS, So S. Tankyrase inhibitors attenuate WNT/ β -catenin signaling and inhibit growth of hepatocellular carcinoma cells. *Oncotarget*. 2015 Sep 22.6(28):25390. [PubMed: 26246473]
32. Wessel Stratford E, Daffinrud J, Munthe E, Castro R, Waaler J, Krauss S, et al. The tankyrase-specific inhibitor JW74 affects cell cycle progression and induces apoptosis and differentiation in osteosarcoma cell lines. *Cancer medicine*. 2014 Feb 1; 3(1):36–46. [PubMed: 24403055]
33. Mo ML, Li MR, Chen Z, Liu XW, Sheng Q, Zhou HM. Inhibition of the Wnt palmitoyl transferase porcupine suppresses cell growth and downregulates the Wnt/ β -catenin pathway in gastric cancer. *Oncology letters*. 2013 May 1; 5(5):1719–23. [PubMed: 23761839]
34. Fujii N, You L, Xu Z, Uematsu K, Shan J, He B, et al. An Antagonist of Dishevelled Protein-Protein Interaction Suppresses β -Catenin-Dependent Tumor Cell Growth. *Cancer research*. 2007 Jan 15; 67(2):573–9. [PubMed: 17234765]
35. Shan J, Zhang X, Bao J, Cassell R, Zheng JJ. Synthesis of potent dishevelled PDZ domain inhibitors guided by virtual screening and NMR studies. *Chemical biology & drug design*. 2012 Apr 1; 79(4):376–83. [PubMed: 22172211]
36. Gandhirajan RK, Staib PA, Minke K, Gehrke I, Plickert G, Schlösser A, et al. Small molecule inhibitors of Wnt/ β -catenin/lef-1 signaling induces apoptosis in chronic lymphocytic leukemia cells in vitro and in vivo. *Neoplasia*. 2010 Apr 30; 12(4):326–IN6. [PubMed: 20360943]
37. Arensman MD, Telesca D, Lay AR, Kershaw KM, Wu N, Donahue TR, et al. The CREB-binding protein inhibitor ICG-001 suppresses pancreatic cancer growth. *Molecular cancer therapeutics*. 2014 Oct 1; 13(10):2303–14. [PubMed: 25082960]
38. Hallett RM, Kondratyev MK, Giacomelli AO, Nixon AM, Girgis-Gabardo A, Ilieva D, et al. Small molecule antagonists of the Wnt/beta-catenin signaling pathway target breast tumor-initiating cells in a Her2/Neu mouse model of breast cancer. *PloS one*. 2012 Mar 28.7(3):e33976. [PubMed: 22470504]
39. Masuda M, Sawa M, Yamada T. Therapeutic targets in the Wnt signaling pathway: Feasibility of targeting TNIK in colorectal cancer. *Pharmacology & therapeutics*. 2015 Dec.156:31. 1–9.
40. Liu J, Pan S, Hsieh MH, Ng N, Sun F, Wang, et al. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. *Proceedings of the National Academy of Sciences*. 2013 Dec 10; 110(50):20224–9. 41.
41. Smith DC, Rosen LS, Chugh R, Goldman JW, Xu L, Kapoun A, et al. First-in-human evaluation of the human monoclonal antibody vantictumab (OMP-18R5; anti-Frizzled) targeting the WNT pathway in a phase I study for patients with advanced solid tumors. *J Clin Oncol*. 2013 May 31.31(15 Suppl)
42. Le PN, McDermott JD, Jimeno A. Targeting the Wnt pathway in human cancers: Therapeutic targeting with a focus on OMP-54F28. *Pharmacology & therapeutics*. 2015 Feb.146:28. 1.
43. Fischer MM, Cancilla B, Yeung VP, Cattaruzza F, Chartier C, Murriel CL, et al. WNT antagonists exhibit unique combinatorial antitumor activity with taxanes by potentiating mitotic cell death. *Science Advances*. 2017 Jun 1.3(6):e1700090. [PubMed: 28691093]
44. Canesin G, Evans-Axelsson S, Hellsten R, Krzyzanowska A, Prasad CP, Bjartell A, et al. Treatment with the WNT5A-mimicking peptide Foxy-5 effectively reduces the metastatic spread

- of WNT5A-low prostate cancer cells in an orthotopic mouse model. *PloS one*. 2017 Sep 8.12(9):e0184418. [PubMed: 28886116]
45. Fukukawa C, Hanaoka H, Nagayama S, Tsunoda T, Toguchida J, Endo K, et al. Radioimmunotherapy of human synovial sarcoma using a monoclonal antibody against FZD10. *Cancer science*. 2008 Feb 1; 99(2):432–40. [PubMed: 18271942]
 46. Cortes JE, Faderl S, Pagel J, Jung CW, Yoon SS, Koh Y, et al. Phase 1 study of CWP232291 in relapsed/refractory acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). *InASCO Annual Meeting Proceedings*. 2015 May 20.33(15_suppl):7044.
 47. Lenz HJ, Kahn M. Safely targeting cancer stem cells via selective catenin coactivator antagonism. *Cancer science*. 2014 Sep 1; 105(9):1087–92. 48. [PubMed: 24975284]
 48. Pozzi S, Fulciniti M, Yan H, Vallet S, Eda H, Patel K, et al. In vivo and in vitro effects of a novel anti-Dkk1 neutralizing antibody in multiple myeloma. *Bone*. 2013; 53:487–496. [PubMed: 23333523]
 49. Ko AH, Chiorean EG, Kwak EL, Lenz HJ, Nadler PI, Wood DL, et al. Final results of a phase Ib dose-escalation study of PRI-724a CBP/beta-catenin modulator, plus gemcitabine (GEM) in patients with advanced pancreatic adenocarcinoma (APC) as second-line therapy after FOLFIRINOX or FOLFOX.
 50. Arend RC, Londoño-Joshi AI, Samant RS, Li Y, Conner M, Hidalgo B, et al. Inhibition of Wnt/ β -catenin pathway by niclosamide: A therapeutic target for ovarian cancer. *Gynecologic oncology*. 2014 Jul 31; 134(1):112–20. [PubMed: 24736023]
 51. Tai WP, Hu PJ, Wu J, Lin XC. The inhibition of Wnt/ β -catenin signaling pathway in human colon cancer cells by sulindac. *Tumori*. 2014; 100(1):97–101. [PubMed: 24675499]
 52. Li B, Flaveny CA, Giambelli C, Fei DL, Han L, Hang BI, et al. Repurposing the FDA-approved pinworm drug pyrrvinium as a novel chemotherapeutic agent for intestinal polyposis. *PloS one*. 2014 Jul 8.9(7):e101969. [PubMed: 25003333]
 53. Krop I, Demuth T, Guthrie T, Wen PY, Mason WP, Chinnaiyan P, et al. Phase I pharmacologic and pharmacodynamic study of the gamma secretase (Notch) inhibitor MK-0752 in adult patients with advanced solid tumors. *Journal of Clinical Oncology*. 2012 Apr 30; 30(19):2307–13. [PubMed: 22547604]
 54. Schott AF, Landis MD, Dontu G, Griffith KA, Layman RM, Krop I, et al. Preclinical and clinical studies of gamma secretase inhibitors with docetaxel on human breast tumors. *Clinical Cancer Research*. 2013 Mar 15; 19(6):1512–24. [PubMed: 23340294]
 55. Yao J, Qian C. Inhibition of Notch3 enhances sensitivity to gemcitabine in pancreatic cancer through an inactivation of PI3K/Akt-dependent pathway. *Medical Oncology*. 2010 Sep 1; 27(3):1017–22. [PubMed: 19816816]
 56. Tolcher AW, Messersmith WA, Mikulski SM, Papadopoulos KP, Kwak EL, Gibbon DG, et al. Phase I study of RO4929097, a gamma secretase inhibitor of Notch signaling, in patients with refractory metastatic or locally advanced solid tumors. *Journal of clinical oncology*. 2012 Apr 23; 30(19):2348–53. [PubMed: 22529266]
 57. De Jesus-Acosta A, Laheru D, Maitra A, Arcaroli J, Rudek MA, Dasari A, et al. A phase II study of the gamma secretase inhibitor RO4929097 in patients with previously treated metastatic pancreatic adenocarcinoma. *Investigational new drugs*. 2014 Aug 1; 32(4):739–45. [PubMed: 24668033]
 58. Papayannidis C, DeAngelo DJ, Stock W, Huang B, Shaik MN, Cesari R, et al. A Phase 1 study of the novel gamma-secretase inhibitor PF-03084014 in patients with T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma. *Blood cancer journal*. 2015 Sep 1.5(9):e350. [PubMed: 26407235]
 59. Zweidler-McKay PA, DeAngelo DJ, Douer D, Dombret H, Ottmann OG, Vey N, Thomas DA, et al. The safety and activity of BMS-906024, a gamma secretase inhibitor (GSI) with anti-notch activity, in patients with relapsed T-cell acute lymphoblastic leukemia (T-ALL): initial results of a phase 1 trial. *Blood*. 2014 Dec 6.124(21):968.
 60. Jenkins DW, Ross S, Veldman-Jones M, Foltz IN, Clavette BC, Manchulenko K, et al. MEDI0639: a novel therapeutic antibody targeting DLL4 modulates endothelial cell function and angiogenesis in vivo. *Molecular cancer therapeutics*. 2012 Jun.7:61.

61. Patnaik A, LoRusso P, Munster P, Tolcher AW, Davis SL, Heymach J, et al. Safety and early evidence of activity of a first-in-human phase I study of the novel cancer stem cell (CSC) targeting antibody OMP-52M51 (anti-Notch1) administered intravenously to patients with certain advanced solid tumors. *European Journal of Cancer*. 2014 Nov;50:1. 7. [PubMed: 24183379]
62. Catenacci DV, Junttila MR, Karrison T, Bahary N, Horiba MN, Nattam SR, et al. Randomized phase Ib/II study of gemcitabine plus placebo or vismodegib, a Hedgehog pathway inhibitor, in patients with metastatic pancreatic cancer. *Journal of Clinical Oncology*. 2015 Nov 2; 33(36): 4284–92. [PubMed: 26527777]
63. Steg AD, Katre AA, Bevis KS, Ziebarth A, Dobbin ZC, Shah MM, et al. Smoothed antagonists reverse taxane resistance in ovarian cancer. *Molecular cancer therapeutics*. 2012 Jul 1; 11(7):1587–97. [PubMed: 22553355]
64. Aberger F, Hutterer E, Sternberg C, del Burgo PJ, Hartmann TN. Acute myeloid leukemia—strategies and challenges for targeting oncogenic Hedgehog/GLI signaling. *Cell Communication and Signaling*. 2017 Jan 25.15(1):8. [PubMed: 28122581]
65. Campbell VT, Nadesan PP, Wang Y, Whetstone H, McGovern K, Read M, et al. Abstract LB-380: Direct targeting of the Hedgehog pathway in primary chondrosarcoma xenografts with the Smoothed inhibitor IPI-926.
66. Vermeulen L, Felipe De, Sousa EM, Van Der Heijden M, Cameron K, De Jong JH, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nature cell biology*. 2010 May 1; 12(5):468–76. 67. [PubMed: 20418870]
67. Giannakis M, Hodis E, Mu XJ, Yamauchi M, Rosenbluh J, Cibulskis K, et al. RNF43 is frequently mutated in colorectal and endometrial cancers. *Nature genetics*. 2014 Dec 1; 46(12):1264–6. [PubMed: 25344691]
68. Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB, et al. Recurrent R-spondin fusions in colon cancer. *Nature*. 2012 Aug 30; 488(7413):660–4. [PubMed: 22895193]
69. Khrantsov AI, Khrantsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/ β -catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. *The American journal of pathology*. 2010 Jun 30; 176(6):2911–20. [PubMed: 20395444]
70. Schade B, Lesurf R, Sanguin-Gendreau V, Bui T, Deblois G, O'Toole SA, et al. B-Catenin signaling is a critical event in ErbB2-mediated mammary tumor progression. *Cancer Res*. 2013; 73(14):4474–87. [PubMed: 23720052]
71. Teng Y, Wang X, Wang Y, Ma D. Wnt/ β -catenin signaling regulates cancer stem cells in lung cancer A549 cells. *Biochemical and biophysical research communications*. 2010 Feb 12; 392(3): 373–9. [PubMed: 20074550]
72. Staal FJ, Sen JM. The canonical Wnt signaling pathway plays an important role in lymphopoiesis and hematopoiesis. *Eur J Immunol*. 2008; 38:1788–1794. [PubMed: 18581335]
73. Peterson LF, Turbiak AJ, Giannola DM, Donato N, Showalter HH, Fearon ER, et al. Wnt-Pathway Directed Compound Targets Blast Crisis and Chronic Phase CML Leukemia Stem Progenitors. *Blood*. 2009 Nov 20.114(22):2168. [PubMed: 19589924]
74. Nagaraj AB, Joseph P, Kovalenko O, Singh S, Armstrong A, Redline R, Resnick K, Zanolini K, Waggoner S, DiFeo A. Critical role of Wnt/ β -catenin signaling in driving epithelial ovarian cancer platinum resistance. *Oncotarget*. 2015 Sep 15.6(27):23720. [PubMed: 26125441]
75. Huss S, Nehles J, Binot E, Wardelmann E, Mittler J, Kleine MA, Knstlinger H, Hartmann W, Hohenberger P, Merkelbach-Bruse S, Buettner R. β -Catenin (CTNNB1) mutations and clinicopathological features of mesenteric desmoid-type fibromatosis. *Histopathology*. 2013 Jan 1; 62(2):294–304. [PubMed: 23020601]
76. Wang X, Moon J, Dodge ME, Pan X, Zhang L, Hanson JM, et al. The development of highly potent inhibitors for porcupine. *Journal of medicinal chemistry*. 2013 Mar 19; 56(6):2700–4. [PubMed: 23477365]
77. Giefing M, Wierzbicka M, Szyfter K, Brenner JC, Braakhuis BJ, Brakenhoff RH, Takes RP, et al. Moving towards personalised therapy in head and neck squamous cell carcinoma through analysis of next generation sequencing data. *European journal of cancer*. 2016 Mar.55:31. 147–57.

78. Koo BK, van Es JH, van den Born M, Clevers H. Porcupine inhibitor suppresses paracrine Wnt-driven growth of Rnf43; Znf3-mutant neoplasia. *Proceedings of the National Academy of Sciences*. 2015 Jun 16; 112(24):7548–50.
79. Teneggi V, Ng M, Tan DS, Subbiah V, Weekes C, Diermayr V, et al. 1520 A phase 1, first-in-human dose escalation study of ETC-159 in advanced or metastatic solid tumours. *Annals of Oncology*. 2016 Dec 1.27(suppl_9)
80. He B, You L, Uematsu K, Xu Z, Lee AY, Matsangou M, et al. A monoclonal antibody against Wnt-1 induces apoptosis in human cancer cells. *Neoplasia*. 2004 Jan 1; 6(1):7–14. [PubMed: 15068666]
81. Mikami I, You L, He B, Xu Z, Batra S, Lee AY, et al. Efficacy of Wnt-1 monoclonal antibody in sarcoma cells. *BMC cancer*. 2005 May 24.5(1):53. [PubMed: 15913453]
82. Jimeno A, Gordon MS, Chugh R, Messersmith WA, Mendelson DS, Dupont J, et al. A first-in-human phase 1 study of anticancer stem cell agent OMP-54F28 (FZD8-Fc), decoy receptor for WNT ligands, in patients with advanced solid tumors.
83. Yeung P, Beviglia L, Cancilla B, Dee-Hoskins C, Evans J, Fischer MM, et al. Wnt pathway antagonist OMP-54F28 (FZD8-Fc) inhibits tumor growth and reduces tumor-initiating cell frequency in patient-derived hepatocellular carcinoma and ovarian cancer xenograft models. *AACR Annual Meeting*. 2014 Apr.:5–9.
84. Weekes C, Berlin J, Lenz HJ, O'Neil B, Messersmith W, Cohen S, et al. Phase 1b study of WNT inhibitor ipafricept (IPA, decoy receptor for WNT ligands) with nab-paclitaxel (Nab-P) and gemcitabine (G) in patients (pts) with previously untreated stage IV pancreatic cancer (PC). *Annals of Oncology*. 2016 Oct 1.27(suppl 6):367PD. [PubMed: 26924595]
85. Arqués O, Chicote I, Puig I, Tenbaum SP, Argilés G, Dienstmann R, et al. Tankyrase inhibition blocks Wnt/ β -catenin pathway and reverts resistance to PI3K and AKT inhibitors in the treatment of colorectal cancer. *Clinical Cancer Research*. 2016 Feb 1; 22(3):644–56. [PubMed: 26224873]
86. Zhong Y, Katavolos P, Nguyen T, Lau T, Boggs J, Sambrone A, et al. Tankyrase inhibition causes reversible intestinal toxicity in mice with a therapeutic index < 1. *Toxicologic pathology*. 2016 Feb; 44(2):267–78. [PubMed: 26692561]
87. Grandy D, Shan J, Zhang X, Rao S, Akunuru S, Li H, Zhang Y, Alpatov I, Zhang XA, Lang RA, Shi DL. Discovery and characterization of a small molecule inhibitor of the PDZ domain of dishevelled. *Journal of Biological Chemistry*. 2009 Jun 12; 284(24):16256–63. [PubMed: 19383605]
88. Lepourcelet M, Chen YN, France DS, Wang H, Crews P, Petersen F, et al. Small-molecule antagonists of the oncogenic Tcf/ β -catenin protein complex. *Cancer cell*. 2004 Jan 31; 5(1):91–102. 89. [PubMed: 14749129]
89. Säfholm A, Tuomela J, Rosenkvist J, Dejmek J, Härkönen P, Andersson T. The Wnt-5a–Derived Hexapeptide Foxy-5 Inhibits Breast Cancer Metastasis In vivo by Targeting Cell Motility. *Clinical Cancer Research*. 2008 Oct 15; 14(20):6556–63. [PubMed: 18927296]
90. Kurayoshi M, Oue N, Yamamoto H, Kishida M, Inoue A, Asahara T, Yasui W, Kikuchi A. Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. *Cancer research*. 2006 Nov 1; 66(21):10439–48. [PubMed: 17079465]
91. Soerensen PG, Andersson T, Buhl U, Moelvadgaard T, Jensen PB, Brunner N, et al. Phase I dose-escalating study to evaluate the safety, tolerability, and pharmacokinetic and pharmacodynamic profiles of Foxy-5 in patients with metastatic breast, colorectal, or prostate cancer.
92. Barat S, Chen X, Cuong Bui K, Bozko P, Götze J, Christgen M, et al. Gamma-Secretase Inhibitor IX (GSI) Impairs Concomitant Activation of Notch and Wnt-Beta-Catenin Pathways in CD44+ Gastric Cancer Stem Cells. *Stem cells translational medicine*. 2017 Mar 1; 6(3):819–29. [PubMed: 28186678]
93. Piha-Paul SA, Munster PN, Hollebecque A, Argiles G, Dajani O, Cheng JD, et al. Results of a phase 1 trial combining ridaforolimus and MK-0752 in patients with advanced solid tumours. *European Journal of Cancer*. 2015 Sep 30; 51(14):1865–73. [PubMed: 26199039]
94. Olsauskas-Kuprys R, Zlobin A, Osipo C. Gamma secretase inhibitors of Notch signaling. *Onco Targets Ther*. 2013 Jul.6:1. 943–55. [PubMed: 23319867]

95. Gounder MM. Notch inhibition in desmoids: “Sure it works in practice, but does it work in theory?”. *Cancer*. 2015 Nov 15; 121(22):3933–7. [PubMed: 26348868]
96. Infante P, Alfonsi R, Botta B, Mori M, Di Marcotullio L. Targeting GLI factors to inhibit the Hedgehog pathway. *Trends in pharmacological sciences*. 2015 Aug 31; 36(8):547–58. [PubMed: 26072120]
97. Zhou H, Mak PY, Mu H, Mak DH, Kouji H, Konopleva M, et al. Combination of Tyrosine Kinase Inhibitor with β -Catenin/CBP Modulator C82 Reverses TKI Resistance, Eradicates Quiescent CML Stem/Progenitors Cells, and Overcomes MSC-Associated Microenvironmental Protection. *Blood*. 2014 Dec 6.124(21):401.
98. White BD, Chien AJ, Dawson DW. Dysregulation of Wnt/ β -catenin signaling in gastrointestinal cancers. *Gastroenterology*. 2012 Feb 29; 142(2):219–32. [PubMed: 22155636]
99. Hung HT, Ong R, Puehler F, Scholz A, Politz O, Zopf D, et al. Sorafenib/Refametinib potently inhibits Wnt/ β -catenin in vitro and patient-derived xenograft models of human hepatocellular carcinoma.
100. Messersmith WA, Shapiro GI, Cleary JM, Jimeno A, Dasari A, Huang B, et al. A phase I, dose-finding study in patients with advanced solid malignancies of the oral γ -secretase inhibitor PF-03084014. *Clinical Cancer Research*. 2015 Jan 1; 21(1):60–67. [PubMed: 25231399]

Highlights

- Wnt is a family of proteins involved in stem cell renewal and organogenesis.
- Wnt pathway activation occurs in many cancers and contributes to tumor recurrence.
- Wnt has significant cross talk with Notch and Sonic Hedgehog pathways.
- Update on clinical trials of agents targeting Wnt, Notch, and Sonic Hedgehog

Fig 1.

The Wnt pathway can be classified as canonical and non-canonical. In the canonical pathway, Wnt signaling when it is on inhibits the degradation of β -catenin, which can regulate transcription of many genes. Wnt signaling is activated by binding of Wnt proteins to surface receptors composed of the seven transmembrane frizzled proteins and the LRP5/6. Upon binding, the cytoplasmic protein disheveled (Dvl) is activated. Activation of Dvl induces the dissociation of GSK-3 β from Axin and leads to the inhibition of GSK-3 β . Next, the phosphorylation and degradation of β -catenin is inhibited because of the inactivation of the “destruction complex”. Subsequently, stabilized β -catenin translocates into the nucleus leading to transcription of target genes like C-Myc and Cyclin D1. RNF43 (Ring finger protein 43) / ZNRF3 promote FZD receptor turnover. R-spondins or RSPO's bind to RNF43/ ZNRF3 causing their ubiquitination and clearance, resulting in increased cell surface FZD receptors.

Fig 2a.

Canonical Wnt Pathway and Inhibitors of the Wnt/beta-Catenin Signaling Pathway
 schematic representation of the Canonical Wnt Pathway and pharmacologic inhibitors of the Wnt/beta-catenin signaling pathway.

Fig 2b.

Non-Canonical Wnt Pathway and the Notch and Sonic Hedgehog Pathway-The two major non-canonical pathways are Wnt/calcium and Planar Cell Polarity (PCP) pathways. In the Wnt/calcium pathway, Wnt binding to Frizzled activates Dvl, causing calcium release from the endoplasmic reticulum, activating calcium-binding proteins including protein kinase C (PKC) and calmodulin-dependent kinase II (CamKII). Signal transduction through Ca²⁺ activates the nuclear factor of activated T cells (NFAT). The Wnt/PCP pathway is mediated by the GTPases RhoA and Ras, which through the RhoA-Rho-associated kinase (ROCK) axis or JNK, can exert effects on the cytoskeleton. A schematic of the Notch and Sonic Hedgehog pathway is shown.

Fig 3.

Cross-Talk between the Wnt, Notch and Sonic Hedgehog Pathways- Beta-catenin can drive activation of Notch signaling by increasing expression of the *JAG1* gene, which encodes the Notch ligand Jagged1. Sfrp-1 may be a negative regulator for both SHH and Wnt/beta-catenin pathway.

Table 1Examples of drugs/agents that inhibit Wnt/ β -Catenin Signaling

Compound	Target/Receptor	Manufacturer/Type of agent/Other targets	Preclinical vs clinical trial (phase) vs FDA approved*	Ref
Preclinical Agents				
Tankyrases inhibitors				
IWR1	Tankyrases1, 2 inhibitors	Tocris Bioscience/Small Molecule	Preclinical	29
XAV939	Tankyrases1, 2 inhibitors	Novartis/Small Molecule	Preclinical	30
NVP- TNKS656	Tankyrases1, 2 inhibitors	AbMole Bioscience/Small Molecule/ Parp Inhibitor	Preclinical	31
JW74	Tankyrases1, 2 inhibitors	Tocris Bioscience/Small Molecule	Preclinical	32
Porcupine inhibitors				
IWP-2	Porcupine inhibitors	Tocris Bioscience/Small Molecule	Preclinical	33
Disheveled inhibitors				
NSC668036	Disheveled ((Dvl)	Tocris Bioscience/Small Molecule	Preclinical	34
J01-017a	Binds to PDZ-Dvl	Small Molecule	Preclinical	35
TCF/β-catenin Inhibitors				
PKF115-584	β -catenin/LEF-1 inhibitor	Novartis/Small molecule	Preclinical	36
ICG-001	CREB binding protein / CBP	Enzo Life Sciences/Small molecule	Preclinical	37
PKF118-310	Selective inhibitor β -catenin/TCF	EMD-Millipore/Small Molecule	Preclinical	38
NCB-0846	TNIK inhibitor	Carna Biosciences/Small molecule	Preclinical	39
Clinical Trial Phase I/Ia/Ib				
LGK974	Porcupine inhibitors	Novartis/Pyridines; Small molecules	Phase I (NCT01351103) in Melanoma, breast cancer and pancreatic CA	40
Wnt antibodies				
OMP-18R5 (Vantictumab)	Frizzled receptor	Onco Med Pharmaceuticals/Cellgene Fzd8-Fc fusion protein	Open –label Phase Ib dose escalation study in solid tumors(NCT01345201).Phase I in breast cancer(NCT01973309), non-small cell lung cancer(NCT01957007) and pancreatic cancer(NCT02005315)	41
OMP- 54 F28 (Ipafricept)	Fzd8-Fc fusion protein	Onco Med Pharmaceuticals/Bayer Acts as Decoy receptor	Phase I trial in solid tumors (NCT01608867) Phase Ib trial in hepatocellular carcinoma(NCT02069145), ovarian cancer(NCT02092363), and pancreatic cancer(NCT02050178)	42,43
Foxy-5	FZD ₅ Peptide	WntResearch AB	Phase I in metastatic breast, colorectal, and prostate cancer(NCT02020291)	44
OTSA 101	FZD ₁₀ mAb	Centre Léon Bérard, OncoTherapy Science/Monoclonal antibody	Phase I in synovial sarcoma(NCT01469975)	45
Beta-catenin inhibitors				
CWP232291	Induces β -catenin degradation	JW Pharmaceuticals/ Small molecule	Phase I in Acute Myeloid Leukemia(NCT01398462)	46
PRI-724	B-catenin/CBP	Prism/Eisai pharmaceuticals/ Small molecule	Phase Ia in solid tumors(NCT01302405), Phase Ib in colorectal(NCT0132405) and pancreatic cancer(NCT01764477)	47
Clinical Trial Phase I/II				

Compound	Target/Receptor	Manufacturer/Type of agent/Other targets	Preclinical vs clinical trial (phase) vs FDA approved*	Ref
DKN-01	DKK, dickkopf-related protein	Leap Therapeutics	Phase I/II in Multiple Myeloma(NCT01457417)	48
PRI-724	Beta-catenin/CBP	Prism/Eisai pharmaceuticals/ Small molecule	Phase I/II in Myeloid leukemia(NCT01764477)	49
FDA approved (off label with preclinical data only)				
Niclosamide	Downregulates Dvl-2	Taj Pharma/Anti-helminthic	FDA approved	50
Sulindac	PDZ domain of Dishevelled(Dvl)	Merck & Co., Inc./Non-steroidal anti-inflammatory drug	FDA approved	51
Pyrrvinium	CK1 α	U.S. Pharmacopeia/Anti-helminthic	FDA approved	52
Examples of Inhibitors of Notch Signaling				
MK0752	Gamma Secretase Inhibitor (GSI)	Merck/Small Molecule	Phase 1 advanced solid tumor(NCT00106145)	53
MK0752 + do cetaxel	GSI	Merck/Small Molecule	Phase I/II; breast cancer (NCT00645333)	54
MK0752 + Ge mcitabine	GSI	Merck/Small Molecule	Phase I/II; pancreatic cancer (NCT01098344)	55
RO4929097	GSI	Roche/Small molecule	Phase II, colorectal cancer (NCT01116687)	56
RO4929097	GSI	Roche/Small molecule	Phase II; pancreatic cancer (NCT01232829)	57
PF-03084014	GSI	Pfizer/Small molecule	Phase 1 in Acute T cell lymphoblastic leukaemia/lymphoma and solid tumors(NCT00878189)	58
BMS-906024	Pan Notch Inhibitor	Bristol-Myers Squibb/Small molecule	Phase 1 in Acute T cell lymphoblastic leukaemia/lymphoma and solid tumors(NCT01653470, NCT01292655, NCT01363817)	59
MEDI0639	DLL4-specific Notch antibody	MedImmune	Phase 1 advanced solid tumors (NCT01577745)	60
OMP-52M51	Notch 1-specific antibody	GlaxoSmithKline/OncoMed Pharmaceuticals	Phase 1 solid tumors and lymphoid malignancies (NCT01703572, NCT01778439)	61
Examples of Inhibitors of Hedgehog signaling				
Vismodegib	SMO inhibitor	Roche/Genentech	Advanced pancreatic cancer, prostate cancer, gastric cancers(NCT01195415, NCT01088815, NCT00878163)	62
Erismodegib/Sonidigib	SMO inhibitor	Novartis	Recurrent ovarian cancer, triple negative breast cancer, advanced solid tumors (NCT02195973, NCT02027376, NCT00961896)	63
Glasdegib	SMO inhibitor	Pfizer	Myelofibrosis (NCT02226172)	64
Saridegib(IPI-926)	SMO inhibitor	Infinity	Pancreatic adenocarcinoma (NCT01383538)	65

Table 2

Wnt inhibitors in clinical trials for cancers
Wnt- Receptor Complex Inhibitors

Compound	Mechanism of Action	Phase of Trial	Manufacturer	Diseases	Concomitant Therapy	Response/Adverse effects	Trial Identifier
LGK974	Porcupine inhibitor	1/2	Novartis	Metastatic CRC with Wnt pathway mutations, Head and neck Squamous cell cancers with Notch mutations	Biological: PDR001	No clinical data	NCT02278133 NCT02649530
ETC 159	Porcupine Inhibitors	1	D3-Institute experimental therapeutics	Refractory solid tumors, 10 patients (9 CRC, 1 Renal)	Oral single agent	2 stable disease (SD).	NCT02521844
OMP18RS(V antictumab)	Anti Fzd7 antibody	1	Bayer, OncoMed	Non-small cell lung cancer, Pancreatic cancer, Metastatic breast cancer	With Nab- Paclitaxel and Gemcitabine in Pancreatic Ca. With taxanes in Her2 neg MBC and NSCLA	19 pts pancreatic cancer, 8 partial response (PR), 4 SD Prolonged SD in 3 NET's	NCT01957007 NCT02005315 NCT01973309
OMP-54F28 (Ipafricept)	Fzd-Fc Decoy receptor	1	Bayer, OncoMed	Hepatocellular carcinoma, ovarian cancer, pancreatic cancer	In ovarian cancer given 2 days before carboplatin and taxol chemotherapy. Trials with Nab-paclitaxel in pancreatic cancer and sorafenib (HCC)	Ovarian cancer 17 patients, 6 (35%) complete response (CR), 8 (47%) (PR) and 3 (18%) SD Pancreatic cancer – 14, 4 PR and 7 SD.	NCT02092363 NCT02050178
OMP131R10	Anti-R- spondin 3 antibody	1	Oncomed/Cel gene	RSPO3 positive metastatic Colorectal cancer	FOLFIRI (FOL = Leucovorin Calcium (Folinic Acid)F = Fluorouracil IRI = Irinotecan Hydrochloride)	No clinical data yet	NCT02482441
OTSA 101	Yttrium90 radiolabeled anti Fzd10 antibody	1	Oncotherapy Science	Synovial Sarcoma	Single agent	1 fatal thrombocytopenia	NCT01469975
PRI-724	TCF-CBP interaction	1/2	Prism Biolab	Acute and chronic myeloid leukemia, Colorectal cancers,	Gemcitabine in Pancreatic cancer,	8 pts (40%), 2 minor responses, 5 of 8 pts	NCT01764477
	inhibitor			pancreatic adeno CA	Dasatinib or Cytarabine in Chronic myeloid leukemia.	(62.5%) 7 Gr3/4 AE.	NCT02413853 NCT01606579
Foxy 5	Wnt 5 a derived peptide	1	Wnt Research	Breast cancer, Colorectal cancer, Prostate cancer	Single agent	No dose limiting toxicity in phase 1 trial.	NCT02655952

Compound	Mechanism of Action	Phase of Trial	Manufacturer	Diseases	Concomitant Therapy	Response/Adverse effects	Trial Identifier
RO4929097	Gamma Secretase Inhibitors	Phase 2	Roche	Pancreatic cancer, metastatic CRC	Single agent	12 patients with pancreatic cancer, 3 SD.	<u>NCT01116687</u>
MK0752	GSI	Phase 1/2	Merck	Breast cancer, Pancreatic cancer	Docetaxel in breast cancer Gemcitabine in Pancreatic ca	Breast ca in 30 patients, 9 had PR, 8 SD, and 3 PD, RR of 45%	<u>NCT00645333</u>
PF- 03084014	GSI	Phase 1	Pfizer	Triple negative breast cancer	Docetaxel	Four (16%) of 25 PR; nine (36%) SD.	<u>NCT01876251</u>

Abbreviations
CBP (CREB- binding protein),
CRC (colorectal cancer)
DMBC (Metastatic Breast Cancer)
NET (Neuro-endocrine tumors)
NSCLCA (Non-small cell lung cancer)
PPFS (Progression free survival)
RSP03 (R-spondin 3)
ITCF (T cell factor)