Canonical and non-canonical WNT signaling in cancer stem cells and their niches: Cellular heterogeneity, omics reprogramming, targeted therapy and tumor plasticity (Review)

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Abstract. Cancer stem cells (CSCs), which have the potential for self-renewal, differentiation and de-differentiation, undergo epigenetic, epithelial-mesenchymal, immunological and metabolic reprogramming to adapt to the tumor microenvironment and survive host defense or therapeutic insults. Intra-tumor heterogeneity and cancer-cell plasticity give rise to therapeutic resistance and recurrence through clonal replacement and reactivation of dormant CSCs, respectively. WNT signaling cascades cross-talk with the FGF, Notch, Hedgehog and TGFβ/BMP signaling cascades and regulate expression of functional CSC markers, such as CD44, CD133 (PROM1), EPCAM and LGR5 (GPR49). Aberrant canonical and non-canonical WNT signaling in human malignancies, including breast, colorectal, gastric, lung, ovary, pancreatic, prostate and uterine cancers, leukemia and melanoma, are involved in CSC survival, bulk-tumor expansion and invasion/metastasis. WNT signaling-targeted therapeutics, such as anti-FZD1/2/5/7/8 monoclonal antibody (mAb) (vantictumab), anti-LGR5 antibody-drug conjugate (ADC) (mAb-mc-vc-PAB-MMAE), anti-PTK7 ADC (PF-06647020), anti-ROR1 mAb (cirmtuzumab), anti-RSPO3 mAb (rosmantuzumab), small-molecule porcupine inhibitors (ETC-159, WNT-C59 and WNT974), tankyrase inhibitors (AZ1366, G007-LK, NVP-TNKS656 and XAV939) and β-catenin inhibitors (BC2059, CWP232228, ICG-001 and PRI-724), are in clinical trials or preclinical studies for the treatment of patients with WNT-driven cancers. WNT signaling-targeted therapeutics are applicable for combination therapy with BCR-ABL, EGFR, FLT3, KIT or RET inhibitors to treat a subset of tyrosine kinase-driven cancers because WNT and tyrosine kinase signaling cascades converge to β-catenin for the maintenance and expansion of CSCs. WNT signaling-targeted therapeutics might also be applicable for combination therapy with immune checkpoint blockers, such as atezolizumab, avelumab, durvalumab, ipilimumab, nivolumab and pembrolizumab, to treat cancers with immune evasion, although the context-dependent effects of WNT signaling on immunity should be carefully assessed. Omics monitoring, such as genome sequencing and transcriptome tests, immunohistochemical analyses on PD-L1 (CD274), PD-1 (PDCD1), ROR1 and nuclear β-catenin and organoid-based drug screening, is necessary to determine the appropriate WNT signaling-targeted therapeutics for cancer patients.

Contents

1. Introduction

Cancer stem cells (CSCs), which show the potential for self-renewal and differentiation, have been identified in a variety of human cancers based on their tumor initiating potential *in vivo* (1-3). Clonal expansion of a minor CSC population with a drug-resistant mutation causes early recurrence, whereas reactivation of dormant CSCs into cycling CSCs owing to tumor plasticity leads to late relapse (4-6). CSCs or bulk tumor cells undergo epigenetic reprogramming (7), epithelial-mesenchymal reprogramming [epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET)] (8,9), immunological reprogramming (immuno-editing) (10,11) and metabolic reprogramming (12) to adapt to the tumor microenvironment, which is collectively defined here as 'omics reprogrammming' (Fig. 1). Since cycling CSCs that depend on aerobic glycolysis converge into quiescent mesenchymal CSCs through omics reprogramming to survive therapeutic insult for later recurrence, CSC targeting is necessary to avoid relapse

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after cancer therapy and improve the cost-effectiveness ratio of cancer precision medicine.

CD44, CD133 (PROM1), EPCAM and LGR5 (GPR49) are representative cell-surface markers of CSCs (2,13-16). LGR5, encoding an R-spondin (RSPO) receptor, is a target gene of the canonical WNT/β-catenin signaling cascade in quiescent as well as cycling stem cells, whereas CD44 and CD133 are further upregulated by WNT and RSPO signals in $LGR5^+$ cycling stem/progenitor cells (17-19). EPCAM can potentiate the canonical WNT/β-catenin signaling cascade through intra-membrane proteolysis and subsequent nuclear translocation of its intracellular C-terminal domain (20). WNT signaling cascades cross-talk with the FGF, Notch, Hedgehog and TGFβ/BMP signaling cascades to constitute the stem cell signaling network, which regulates expression of functional CSC markers (21-24).

The WNT family proteins transduce signals through the Frizzled (FZD) and LRP5/6 receptors to the WNT/β-catenin and WNT/STOP (stabilization of proteins) signaling cascades (also known as the canonical WNT signaling cascades) and through the FZD and/or ROR1/ROR2/RYK receptors to the WNT/PCP (planar cell polarity), WNT/RTK (receptor tyrosine kinase) and WNT/Ca^{2+} signaling cascades (also known as the non-canonical WNT signaling cascades) (21,25-29). The canonical WNT/β-catenin signaling cascade is involved in self-renewal of stem cells and proliferation or differentiation of progenitor cells (30-33), whereas non-canonical WNT signaling cascades are involved in maintenance of stem cells, directional cell movement or inhibition of the canonical WNT signaling cascade (34-37). Both canonical and non-canonical WNT signaling cascades play key roles in the development and evolution of CSCs.

By contrast, tumors consist of heterogeneous populations of cancer cells and non-cancerous stromal/immune cells (38,39). Intra-tumor heterogeneity of cancer cells is caused by the evolution of CSCs based on epigenetic and genetic alterations (40-42), as well as the differentiation of CSCs into bulk tumor cells (1-3), niche-like cancer supporting cells (43), endothelial-like cancer cells (44) and fibroblast-like cancer cells (45). On the other hand, intra-tumor heterogeneity of non-cancerous stromal/immune cells is orchestrated by and reciprocally orchestrates CSCs and their descendants (39,45-47). Interaction and co-evolution of CSCs and niche cells are driving forces of cancer progression. Herein, canonical and non-canonical WNT signaling in CSCs will be described, with a focus on the heterogeneity of cancer and stromal/immune cells in the tumor microenvironment; then, anti-CSC mono- and combination therapies using WNT signaling-targeted therapeutics will be reviewed with emphases on omics reprogramming and tumor plasticity.

2. Canonical WNT signaling in CSCs and their niches

Canonical WNT signaling through the FZD-LRP5/6 receptor complex leads to de-repression of β-catenin as well as STOP-target proteins, such as ATOH1, CCND1 (Cyclin D1), FOXM1, MYC (c-MYC), NRF2 (NFE2L2), PLK1, SMAD1/3/4, SNAI1 (Snail) and YAP/TAZ, from proteasomal degradation induced by GSK-3β-dependent phosphorylation and subsequent ubiquitylation (27-29,48) (Fig. 2). β-catenin stabilization and subsequent nuclear translocation leads to transcriptional activation of β-catenin-TCF/LEF target genes, such as *ATOH1, CCND1, CD44, FGF20, JAG1, LGR5, MYC* and *SNAI1*, although transcriptional outputs of the WNT/β-catenin signaling cascade are determined in a cellular context-dependent manner (e.g., epigenetic status of target genes and activities of other transcriptional regulators). ATOH1, CCND1, MYC and SNAI1 are upregulated transcriptionally and post-translationally by the β-catenin and STOP signaling cascades, respectively. Canonical WNT signals control cell fate and function through transcriptional and post-translational regulation of the omics network.

Canonical WNT signaling in CSCs is activated by WNT2B, WNT3 and other canonical WNT ligands derived from cancerous supporting cells or non-cancerous stromal cells (49-52), as well as genetic alterations in the canonical WNT/β-catenin signaling components, such as EIF3E-RSPO2 fusions, PTPRK-RSPO3 fusions, gain-of-function mutations in the *CTNNB1 (β-catenin)* gene and loss-of-function mutations in the *APC, AXIN1, AXIN2, RNF43* and *ZNRF3* genes (29,53-55). Canonical WNT signals increase the LGR5 receptor level on CSCs for the maintenance of the canonical WNT responsive state but also upregulate AXIN2, DKK1, NOTUM, RNF43 and ZNRF3 for negative feedback regulation (18-21,29). Loss-of-function mutations in the *APC, AXIN2, RNF43* and *ZNRF3* genes release CSCs from the constraints of the negative feedback regulation.

Canonical WNT signals can directly promote CSC proliferation through upregulation of CCND1, FOXM1, MYC and YAP/TAZ as described above. By contrast, canonical WNT signaling in CSCs induces expression and secretion of growth factors, such as FGFs, KIT ligand (KITLG or SCF) and VEGF (VEGFA), to fine-tune the tumor microenvironment (18,21,29). For example, MET (HGF receptor) is upregulated in human basal-like breast cancers with TP53 mutations as well as mouse basal-like breast tumors with compound gain-of-function *Ctnnb1* mutation and homozygous *Tp53* deletion (56), and combined activation of the canonical WNT/β-catenin and HGF/MET signaling cascades induces SHH upregulation in mouse mammary CSCs and subsequent activation of cancer-associate fibroblasts for the synergistic proliferation of CSCs and cancer-associate fibroblasts (57).

Together, these findings indicate that canonical WNT signaling is involved in the maintenance and expansion of CSCs through direct effects on CSCs themselves and indirect effects via CSC-stromal/immune interactions.

3. Non-canonical WNT signaling in CSCs and their niches

Non-canonical WNT signaling through FZD receptors and/or ROR1/ROR2/RYK co-receptors activates the PCP, RTK or Ca^{2+} signaling cascades (Fig. 2).

Non-canonical WNT/PCP signaling through FZD receptors and Dishevelled (DVL) adaptor proteins regulates the coordinated cellular orientation within an epithelial plane, collective cell movements during gastrulation and neurulation stages of embryogenesis and directional cell movement during invasion and metastasis of cancer cells (58-62). WNT/PCP signals are converted to actin cytoskeletal dynamics via the small G-proteins RAC and RHO (Fig. 2), and then, RAC and RHO

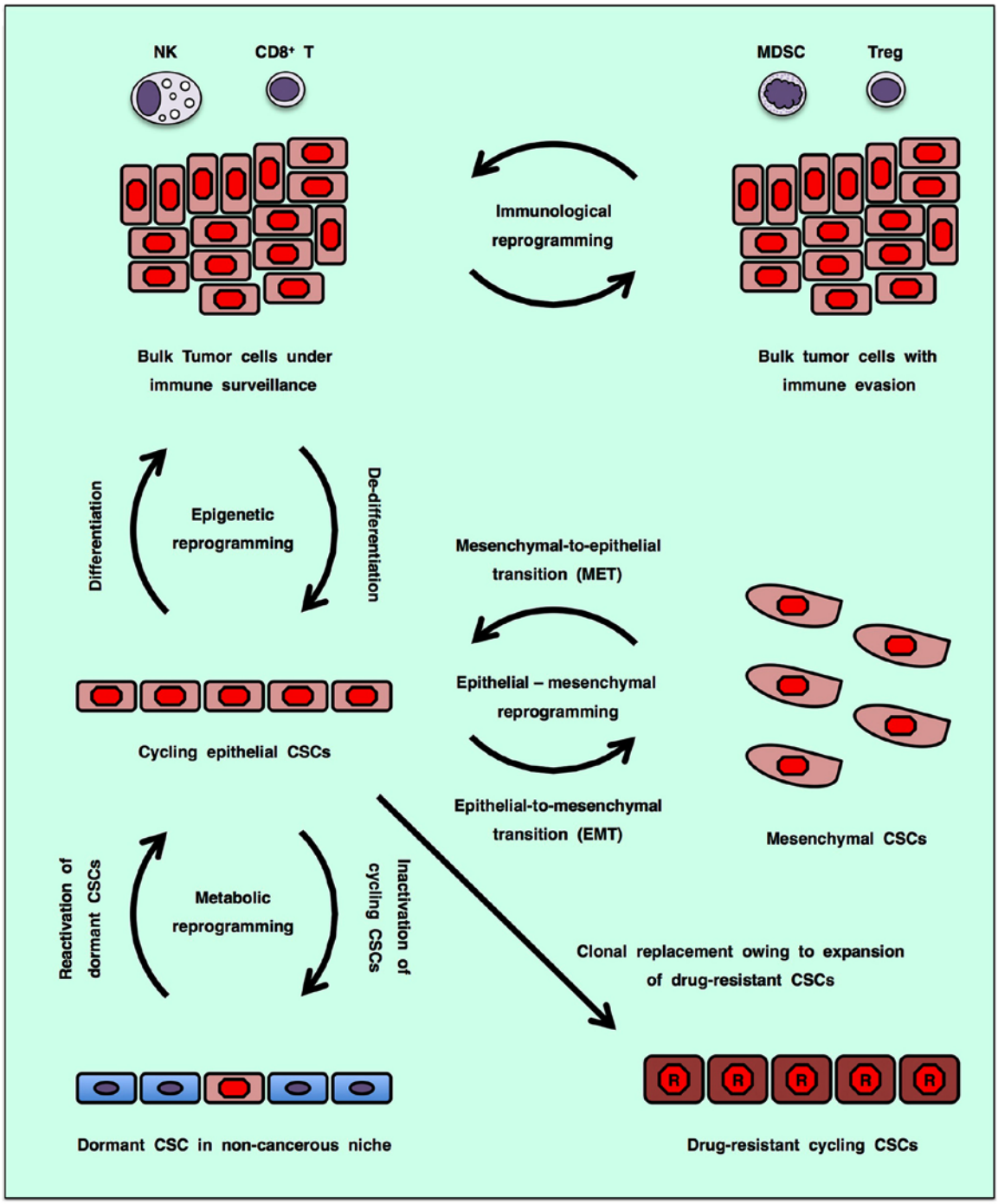

Figure 1. Therapeutic resistance owing to evolution and plasticity of cancer stem cells (CSCs). CSCs with self-renewal, differentiation and de-differentiation potentials undergo omics reprogramming, such as epigenetic reprogramming, immuno-editing (immunological reprogramming), two-way shifts between epithelial and mesenchymal states (epithelial-mesenchymal reprogramming) and two-way shifts between aerobic glycolysis and oxidative phosphorylation in the tricarboxylic acid cycle (metabolic reprogramming). Genetic or epigenetic evolution of CSCs gives rise to a repertoire of drug-resistant CSCs, which cause early recurrence through clonal expansion of drug-resistant CSCs replacing drug-sensitive bulk tumors. By contrast, the plasticity of CSCs with omics reprogramming potential gives rise to dormant CSCs to survive host defense or therapeutic insult, which cause late relapse through reactivation of dormant CSCs into cycling CSCs. CSC-targeted therapeutics are necessary to avoid drug resistance or recurrence after anticancer therapy. MDSC, myeloid-derived suppressor cell; NK, natural killer cell; Treg, regulatory T cell.

activate JNK-dependent transcription and YAP/TAZ-dependent transcription, respectively (63-66). WNT/PCP signaling regulates actin cytoskeletal dynamics, directional cell movement and JNK- or YAP/TAZ-dependent transcription.

Non-canonical WNT signaling through RTKs, such as ROR1, ROR2 and RYK, activates the PI3K-AKT signaling cascade (29,67-69). ROR1 and ROR2, with the extracellular WNT-binding FZD-like domain, are homologous to MUSK, NTRK1, NTRK2, NTRK3, DDR1 and DDR2 in their cytoplasmic tyrosine kinase domain, whereas RYK with an extracellular WNT-binding WIF domain is homologous to AXL, EGFR, ERBB2, ERBB3, ERBB4, MET, MERTK, MST1R and TYRO3 in its cytoplasmic tyrosine kinase domain (39,70-73). ROR1 and ROR2 are atypical RTKs that

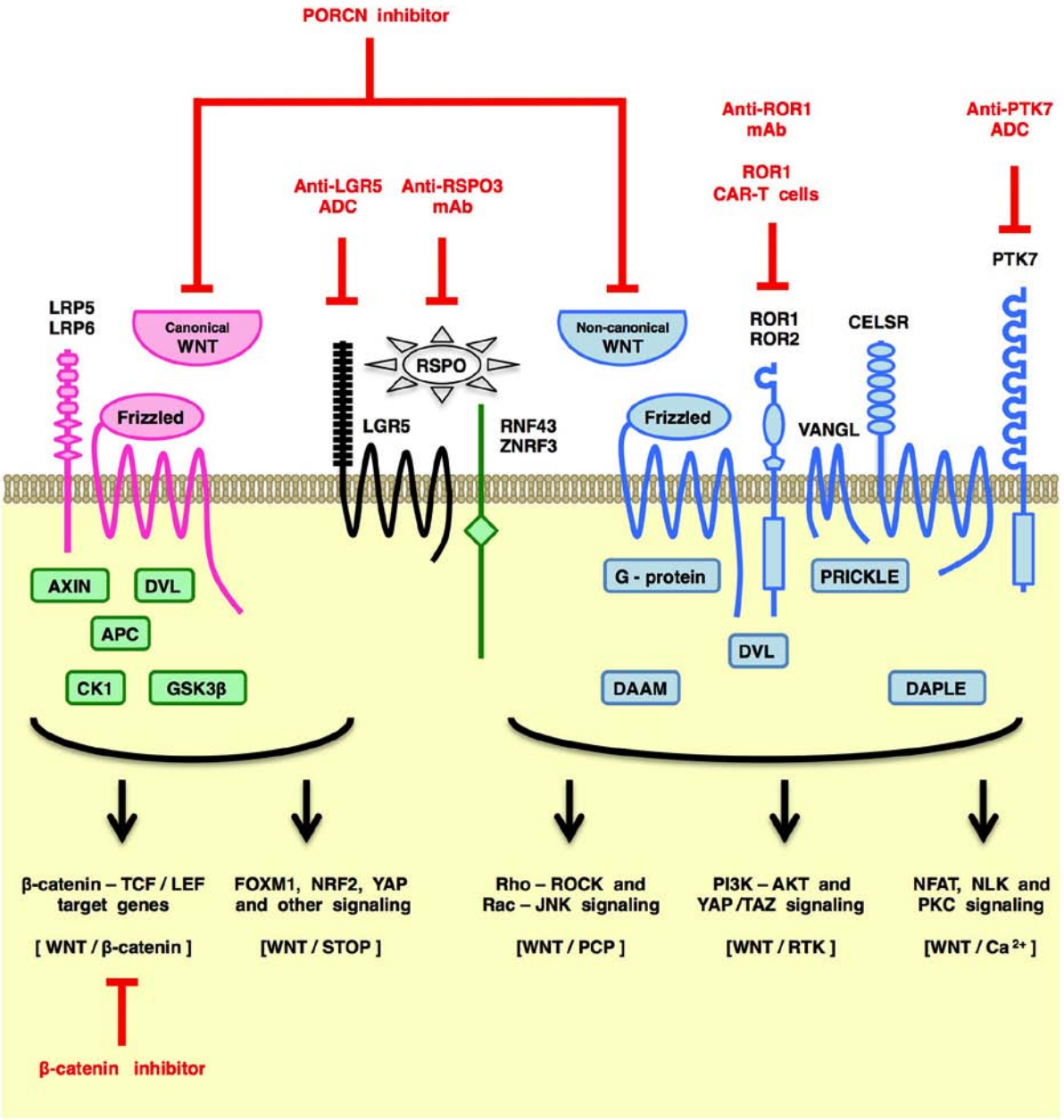

Figure 2. Overview of WNT signaling cascades and WNT signaling-targeted therapeutics. WNT signals are transduced by multiple downstream signaling cascades in a cell context-dependent manner. Canonical WNT signaling through Frizzled (FZD) and LRP5/6 receptors is transduced by the WNT/β-catenin and WNT/STOP (stabilization of proteins) signaling cascades, whereas non-canonical WNT signaling through FZD and/or ROR1/ROR2/RYK receptors is transduced by the WNT/PCP (planar cell polarity), WNT/RTK (receptor tyrosine kinase) and WNT/Ca^{2+} signaling cascades. Antibody-based drugs, such as anti-LGR5 antibody-drug conjugate (ADC), anti-RSPO3 monoclonal antibody (mAb), anti-ROR1 mAb and anti-PTK7 ADC, ROR1 chimeric antigen receptor-modified T (CAR-T) cells, porcupine (PORCN) inhibitors and β-catenin inhibitors are representative WNT signaling-targeted therapeutics in clinical trials or preclinical studies for the treatment of cancer patients.

are defective in intrinsic tyrosine kinase activity for autophosphorylation; however, ROR1 and ROR2 can be tyrosine phosphorylated by other tyrosine kinases, such as EGFR, ERBB3, MET and SRC, to activate the PI3K-AKT and YAP signaling cascades (29,74-78) (Fig. 2). WNT/RTK signaling is involved in therapeutic resistance and recurrence of human cancers in part through PI3K-AKT signaling activation.

Non-canonical WNT signals induce cytosolic Ca^{2+} elevation through Ca^{2+} release from the endoplasmic reticulum or Ca^{2+} influx from the extracellular space. WNT signaling through Frizzled receptors are involved in Ca^{2+} release from the endoplasmic reticulum via small G-protein- or SEC14L2-mediated activation of phospholipase C (PLC) and subsequent generation of inositol-1,4,5-triphosphate (IP_3) (21,79-81). WNT signaling through Polycystin 1 (PKD1) is proposed to induce Ca^{2+} influx through a TRPP2 Ca^{2+} channel (82). Ca^{2+}/Calmodulin-dependent protein kinase II (CAMK2) and Calcineurin are representative downstream effectors of the WNT/Ca^{2+} signaling cascade (Fig. 2). For example, WNT/Ca^{2+} signaling-dependent CAMK2 activation leads to phosphorylation and activation of Nemo-like kinase (NLK), which can inhibit canonical WNT/β-catenin signaling in some cells (83).

WNT-dependent CamK2 activation in cardiomyocytes gives rise to cardiac hypertrophy through phosphorylation and cytoplasmic tethering of Hdac4 and subsequent de-repression of Mef2 target genes (84). WNT/Ca^{2+} signaling-dependent Calcineurin activation leads to dephosphorylation and subsequent nuclear translocation of NFAT for the transcriptional activation of NFAT-target genes (85). By contrast, KRAS-dependent FZD8 repression in pancreatic cancer cells leads to potentiation of tumorigenesis through WNT/Ca^{2+} signaling inhibition (37). WNT/Ca^{2+} signaling to downstream effectors, such as CAMK2 and Calcineurin, is involved in a variety of cellular processes through transcriptional activation of NFAT-target genes, de-repression of MEF2-target genes and repression WNT/β-catenin-target genes in a cellular context-dependent manner.

Non-canonical WNT signaling in CSCs is activated by WNT5A, WNT11 and other non-canonical WNT ligands (58) that are secreted from cancer cells (86,87) or stromal/immune cells (88,89), as well as genetic alterations that *trans*-activate non-canonical WNT signaling cascades, such as *E2A-PBX1* fusion and MET amplification (74-76). Non-canonical WNT signaling through FZD7 activates the PI3K-AKT signaling cascade as a result of Daple (CCDC88C)-mediated dissociation of Gβγ from Gαi (90), whereas non-canonical WNT signaling through ROR1 activates PI3K-AKT signaling cascade owing to ROR1 *trans*-phosphorylation by other tyrosine kinases, such as MET and SRC (67,75). ROR1 is involved in HER3-Y1307 *trans*-phosphorylation and subsequent NSUN6-dependent MST1-K59 methylation, which induces YAP/TAZ-dependent transcriptional activation through LATS1/LATS2 inhibition (78). WNT/PCP signaling can also induce Rho-mediated LATS1/LATS2 inhibition for transcriptional activation of YAP/TAZ-target genes (91,92), whereas non-canonical WNT signaling through FZD10 induces YAP/TAZ activation through Gα13 (93). Non-canonical WNT signaling promotes survival and therapeutic resistance of CSCs through PI3K-AKT signaling activation and YAP/TAZ-mediated transcriptional activation.

By contrast, invasion and metastasis are driven by canonical WNT signaling cascades and non-canonical WNT signaling cascades. For example, canonical WNT/β-catenin and WNT/STOP signaling cascades synergistically upregulate SNAI1 to repress epithelial genes, such as *CDH1 (E-cadherin)*, for the initiation of EMT of CSCs, and non-canonical WNT signals promote invasion, survival and metastasis of CSCs or circulating tumor cells (28,29,35,62,87). Together, these findings clearly indicate that canonical WNT/β-catenin signaling as well as other WNT signaling cascades are critically involved in the malignant features of CSCs.

4. Anti-CSC mono-therapy targeting WNT signaling cascades

WNT signaling cascades are hot and cutting-edge topics in the field of translational oncology and medicinal chemistry (29,94-96). Therapeutics directly targeting WNT signaling cascades are classified into i) ligand/receptor-targeted drugs binding to ligands or transmembrane proteins involved in WNT signaling, ii) porcupine (PORCN) inhibitors abrogating WNT secretion and FZD-dependent signaling, iii) tankyrase (TNKS) inhibitors repressing WNT/β-catenin and WNT-independent signaling cascades and iv) β-catenin inhibitors blocking TCF/LEF-dependent transcription (Table I).

Human/humanized monoclonal antibody (mAb) drugs, such as anti-FZD1/2/5/7/8 mAb (vantictumab/OMP-18R5) (97), anti-FZD5 mAb (IgG-2919) (52), anti-FZD10 antibody-drug conjugate (ADC) (OTSA101-DTPA-^{90}Y) (98), anti-LGR5 ADC (mAb-mc-vc-PAB-MMAE) (99), anti-PTK7 ADC (PF-06647020) (100), anti-ROR1 mAb (cirmtuzumab/UC-961) (101) and anti-RSPO3 mAb (rosmantuzumab/OMP-131R10) (102) have been developed as large-molecule cancer therapeutics. ROR1 CAR-T cells (103) and WNT-trapping FZD8-Fc chimeric protein (ipafricept/OMP-54F28) (104) are also classified as WNT ligand/receptor-targeted drugs. Among this class of therapeutics, cirmtuzumab, ipafricept, PF-06647020, rosmantuzumab and vantictumab, which showed anti-CSC effects in preclinical model experiments, are in clinical trials to treat cancer patients (Table I).

PORCN inhibitors restrain PORCN-dependent palmitoleoylation of WNT family ligands in the endoplasmic reticulum, which obstructs WNT signaling through blockade of WNT secretion as well as palmitoleoylated WNT-mediated oligomerization of FZD receptors (105-108). ETC-159 (109), IWP-2 (110), WNT-C59 (111) and WNT974 (LGK974) (112) are small-molecule PORCN inhibitors. A preclinical study of IWP-2 on organoids derived from colorectal cancer patients revealed that PORNC inhibitors are applicable for the treatment of cancers with *RNF43* mutations but not APC mutations (52). By contrast, preclinical studies of WNT974 indicated that PORNC inhibitors repress the survival and tumor initiating potential of CSCs (43,112). ETC-159 and WNT974 are in clinical trials for the treatment of cancer patients (Table I).

TNKS inhibitors repress TNKS-dependent poly-ADP-ribosylation and subsequent degradation of negative regulators of oncogenic signaling cascades, such as AXIN family proteins, AMOT family proteins, PTEN and TERF1 (TRF1), which results in inhibition of WNT/β-catenin signaling, repression of YAP-dependent transcription, suppression of PI3K signaling and telomere shortening, respectively (113-116). AZ1366 (117), G007-LK (118), JW55 (119), NVP-TNKS656 (120) and XAV939 (121) are representative TNKS inhibitors that abrogate WNT/β-catenin signaling and tumorigenesis in preclinical mouse model experiments. TNKS inhibitors show synergistic antitumor effects with other therapeutics, such as an AKT inhibitor (API2), EGFR inhibitors (gefitinib and erlotinib), a MEK inhibitor (AZD6244), a PI3K inhibitor (BKM120) and irinotecan (117,118,120,122-124). TNKS inhibitors are promising candidates for CSC-targeted therapeutics; however, because of diverse on-target effects, TNKS inhibitors stalled in their preclinical stage.

β-catenin inhibitors block TCF/LEF-dependent transcription through inhibition of protein-protein interactions (PPI) between β-catenin and other transcriptional regulators (29,125), promotion of β-catenin degradation (126) or inhibition of β-catenin kinases, such as TNIK (127-129). BC2059 (130), CGP049090 (131), CWP232228 (132), ICG-001 (133), LF3 (134), PKF115-584 (135), PRI-724 (136) and SAH-BCL9 (137) are small-molecule β-catenin PPI inhibitors. MSAB is a small-molecule compound that binds to β-catenin and promotes proteasomal degradation of β-catenin (126). KY-05009 (128),

Table I. WNT signaling inhibitors and anti-CSC effects.

Category	Drug	Preclinical Anti-CSC TX	(Refs.)	Drug development stage	Details of clinical trials for cancer patients
Ligand/ receptor-targeted drug	Anti-FZD1/2/ 5/7/8 mAb (Vantictumab, OMP-18R5)	Breast CSC Panc CSC	(97)	P1 (NCT01345201) P1 (NCT01957007) P1 (NCT01973309) P1 (NCT02005315)	Solid tumors, Mono Solid tumors. Combo Breast, Combo Panc, Combo
	Anti-FZD5 mAb (IgG-2919)		(52)	Preclinical	
	Anti-FZD10 ADC (OTSA101-DTPA-^{90}Y)		(98)	Terminated in P1	Too slow accrual
	Anti-LGR5 ADC (mAb-mc-vc-PAB-MMAE)		(99)	Preclinical	
	Anti-PTK7 ADC (PF-06647020)	Breast CSC Lung CSC Ovary CSC	(100)	P1 (NCT02222922)	Solid tumors, Mono
	Anti-ROR1 mAb (Cirmtuzumab, UC-961)	Ovary CSC	(101)	P1 (NCT02222688) P1 (NCT02776917) P1/2 (NCT03088878)	CLL, Mono Breast, Combo CLL/MCL/SLL, Combo
	Anti-RSPO3 mAb (Rosmantuzumab, OMP-131R10)	Colorectal CSC	(102)	P1 (NCT02482441)	Solid tumors, Combo
	ROR1 CAR-T cells		(103)	Preclinical	
	WNT-trapping FZD8-Fc (Ipafricept, OMP-54F28)	Panc CSC	(104)	P1 (NCT01608867) P1 (NCT02050178) P1 (NCT02069145) P1 (NCT02092363)	Solid tumors, Mono Panc. Combo Liver, Combo Ovary, Combo
PORCN inhibitor	ETC-159		(109)	P1 (NCT02521844)	Solid tumors, Mono
	IWP-2		(110)	Preclinical	
	WNT-C59		(111)	Preclinical	
	WNT974 (LGK974)	CML CSC Lung CSC	(112) (43)	P1 (NCT01351103) P1/2 (NCT02278133)	Solid tumors, Mono mCRC, Combo
TNKS inhibitor	AZ1366		(117)	Preclinical	
	G007-LK		(118)	Preclinical	
	JW55		(119)	Preclinical	
	NVP-TNKS656		(120)	Preclinical	
	XAV939		(121)	Preclinical	
β-catenin inhibitor	BC2059	AML CSC	(130)	Preclinical	
	CGP049090		(131)	Preclinical	
	CWP232228	Liver CSC	(132)	Preclinical	
	ICG-001	Ovary CSC	(133)	Preclinical	
	KY-05009		(128)	Preclinical	
	LF3		(134)	Preclinical	
	Mebendazole		(129)	P1 (NCT01729260) P1 (NCT02644291) P1/2 (NCT01837862)	Glioma, Mono Glioma, Mono Glioma, Combo
	MSAB		(126)	Preclinical	
	PF-794		(138)	Peclinical	
	PKF115-584		(135)	Preclinical	
	PRI-724		(136)	P1 (NCT01764477) P1/2 (NCT01606579)	Panc, Combo AML/CML, Combo
	SAH-BCL9		(137)	Preclinical	

PORCN, porcupine; TNKS, tankyrase; PPI, protein-protein interaction; mAb, monoclonal antibody; bsAb, bispecific antibody; ADC, antibody-drug conjugate; P1, phase I; P2, phase II; AML, acute myeloid leukemia; Breast, breast cancer; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; Liver, hepatocellular carcinoma; MCL, mantle cell lymphoma; mCRC, metastatic colorectal cancer; NPC, nasopharyngealcarcinoma; NSCLC, non-small cell lung cancer; Ovary, ovarian cancer; Panc, pancreatic cancer; SLL, small lymphocytic lymphoma; Mono, mono-therapy; Combo, combination therapy.

mebendazole (129) and PF-794 (138) are TNIK inhibitors that repress phosphorylation of TNIK substrates, such as TCF4, FMNL2, PRICKLE1, SMAD1 and SMAD2, which leads to inhibition of β-catenin-TCF/LEF-dependent transcription and a variety of cellular processes. Among the β-catenin inhibitors mentioned above, BC2059, CWP232228 and ICG-001 repress the expansion of CSCs. The β-catenin inhibitors PRI-724 and mebendazole are in phase I/II clinical trials for cancer patients (Table I), whereas other β-catenin inhibitors are still in the preclinical stage of drug development. β-catenin inhibitors are challenging therapeutics for cancer patients.

WNT signaling cascades are the major driver of various types of human cancers (29), but the development of many WNT signaling-targeted therapeutics is stuck in the preclinical stage or phase I/II stages of clinical trials (Table I) because of the complexity of WNT signaling cascades and genetic alterations in non-enzymatic signaling components. MAb-based drugs and PORCN inhibitors with the potential to target CSCs as well as bulk cancer cells are promising therapeutics for the patients with WNT signaling-driven cancers.

5. Anti-CSC combination therapy using WNT signaling-targeted drugs

Tyrosine kinase inhibitors are rational anticancer therapeutics because tyrosine kinases with intrinsic enzyme activities are aberrantly activated in cancer cells owing to genetic alterations. Tyrosine kinase inhibitors have contributed to the improved prognosis of cancer patients and are essential for genome-based precision medicine; however, unavoidable drug resistance or recurrence is a serious issue for cancer patients and health care systems (4).

Activated tyrosine kinases, such as BCR-ABL fusion kinase, EGFR-T790M mutant, FLT3 internal tandem duplication (FLT3-ITD) mutant, KIT-D814V mutant and RET, promote β-catenin phosphorylation at Y654 to release E-cadherin-bound β-catenin from the adherens junction for its stabilization and subsequent nuclear translocation (139-143). By contrast, canonical WNT signals inhibit β-catenin phosphorylation at S33, S37, T41 and S45 to release β-catenin from proteasomal degradation for its stabilization and nuclear translocation (21,25,26,29). Since canonical WNT signals and oncogenic tyrosine kinases converge to β-catenin stabilization for the maintenance and expansion of CSCs, canonical WNT signaling inhibitors can block CSC evasion of tyrosine kinase inhibitors. For example, the porcupine inhibitor WNT974 significantly reduced residual stem/progenitor cells of chronic myeloid leukemia (CML) after treatment with the BCR-ABL inhibitor nilotinib via blockade of WNT ligand secretion into the bone marrow microenvironment (112); the β-catenin inhibitors ICG-001 and PRI-724 induced synergistic effects with the BCR-ABL inhibitors imatinib and nilotinib, respectively, on CML stem/progenitor cells (136,144); and the TNKS inhibitor AZ1366 and EGFR inhibitor gefitinib showed synergistic effects on lung cancer cells *in vivo* (124). These preclinical studies indicate that combination therapies using WNT signaling-targeted therapeutics and tyrosine kinase inhibitors might be applicable for treatment of a subset of patients with tyrosine kinase-driven cancers (Fig. 3).

Immune checkpoint blockers that abrogate interactions of ligands and inhibitory receptors on $CD8^+$ T cells are promising antitumor drugs in the clinic or clinical trials (145-151). PD-L1 (CD274) is a representative ligand for inhibitory immune signaling, whereas PD-1 (PDCD1) and CTLA4 are representative receptors for inhibitory immune signaling. Anti-PD-L1 mAbs (atezolizumab, avelumab and durvalumab), anti-PD-1 mAbs (nivolumab and pembrolizumab) and an anti-CTLA4 mAb (ipilimumab) are approved for the treatment of patients with melanoma or other types of solid tumors. Immune checkpoint blockers result in significant therapeutic effects in a subset of patients; however, the lack of benefits in other patients owing to primary or acquired resistance to immune checkpoint blockers has resulted in a cost-effectiveness issue (152-156).

Canonical WNT signaling activation in melanoma induces immune evasion through CCL4 repression and immunological reprogramming into non-T cell-infiltrated melanoma (11). Since melanoma-derived WNT5A promotes β-catenin signaling activation and subsequent IDO upregulation in dendritic cells to induce immune evasion through accumulation of regulatory T (Treg) cells, combination immunotherapy using the porcupine inhibitor WNT-C59 and anti-CTLA4 mAb showed synergistic anti-melanoma effects *in vivo* (157). By contrast, *WNT5A* and *ROR2* are relatively frequently upregulated in pretreatment tumors of melanoma patients that do not respond to PD-1 immune checkpoint blockade (158), which suggests involvement of non-canonical WNT signaling in resistance to immune checkpoint blockers. Since DKK1-dependent canonical WNT signaling inhibition or putative reciprocal non-canonical WNT signaling activation in tumor microenvironment induces immune evasion through accumulation of myeloid-derived suppressor cells (MDSCs) and depletion of T cells (159), combination therapy using anti-DKK1 mAb (BHQ880 or DKN-01) (160,161) and immune checkpoint blockers might show synergistic antitumor effects *in vivo*. WNT signaling-targeted therapeutics might be applicable for combination immunotherapy for cancer patients (Fig. 3); however, context-dependent effects of WNT signaling on immunity (4) should be kept in mind.

6. Omics monitoring for WNT signaling-targeted therapy

WNT-related human cancers are classified into three major subtypes based on signaling aberrations associated with therapeutic choices (Fig. 3): APC/CTNNB1-altered cancers with WNT/β-catenin signaling activation that can be treated with β-catenin inhibitors; RNF43/ZNRF3/RSPO2/RSPO3-altered cancers with WNT/β-catenin and other WNT signaling activation that can be treated with PORCN inhibitors, anti-FZD mAb or anti-RSPO3 mAb; and ROR1-upregulated cancers with WNT/PCP and WNT/RTK signaling activation that can be treated with anti-ROR1 mAb, anti-ROR1 x CD3 bispecific antibody and ROR1 chimeric antigen receptor-modified T (CAR-T) cells (29). Genome sequencing, transcriptomic and/or immunohistochemical tests are necessary for the detection and subtyping of WNT signaling-driven cancers and subsequent determination of appropriate WNT signaling-targeted therapeutics (Fig. 3).

WNT signaling-targeted therapeutics are also applicable for combination therapies with tyrosine kinase inhibitors or

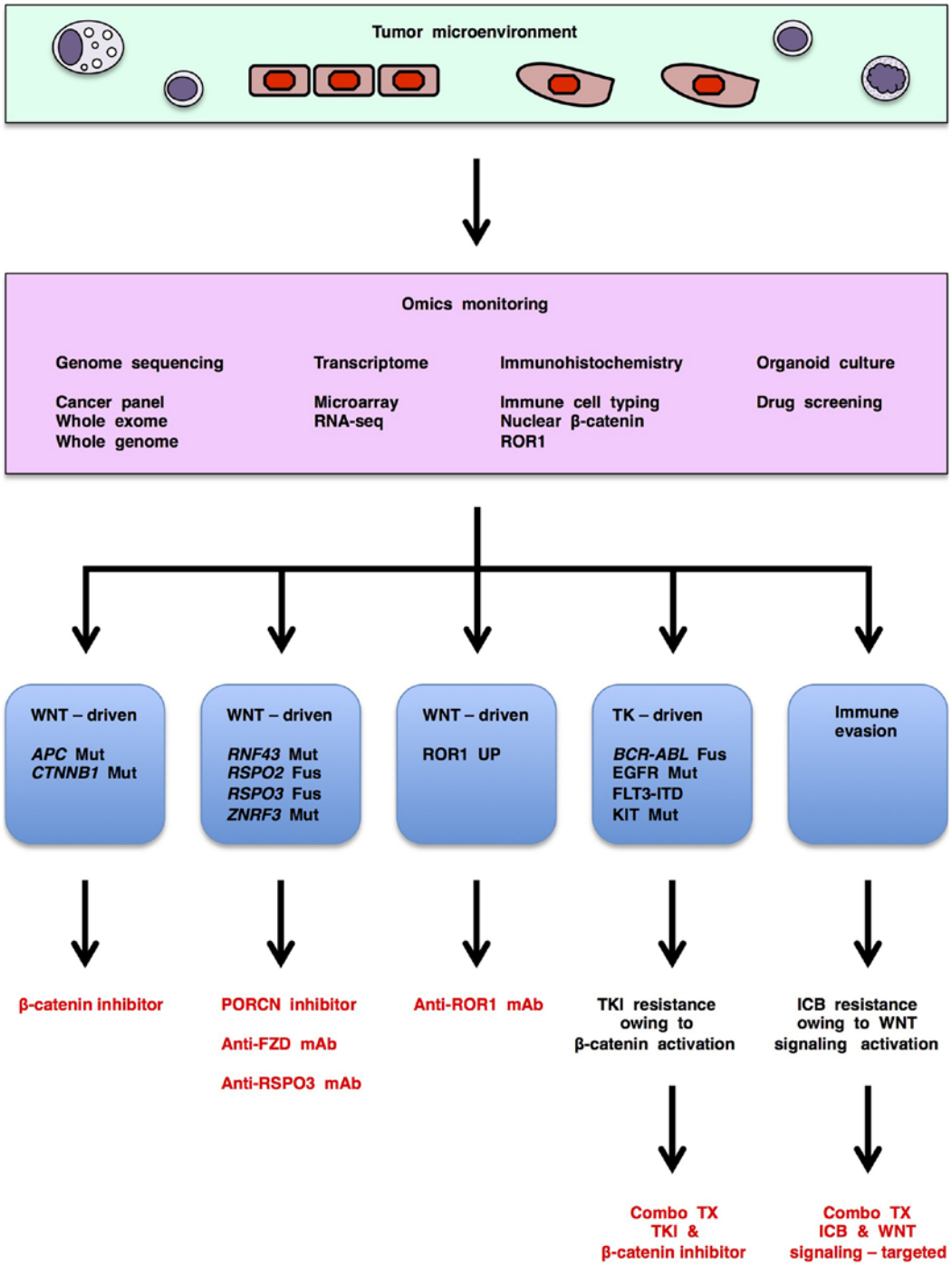

Figure 3. Investigational WNT signaling-targeted therapeutics for genome-based precision medicine. WNT signaling-targeted therapeutics are applicable for mono-therapy of WNT-related human cancers: β-catenin inhibitors for WNT-driven cancers with *APC* or *CTNNB1* alterations; porcupine (PORCN) inhibitors, anti-FZD or anti-RSPO3 monoclonal antibody (mAb) for WNT-driven cancers with *RNF43, RSPO2, RSPO3* or *ZNRF3* alterations; and anti-ROR1 mAb for WNT-driven cancers with ROR1 upregulation. By contrast, WNT signaling-targeted therapeutics are applicable for combination therapies with tyrosine kinase inhibitors (TKI) to treat a subset of tyrosine kinase (TK)-driven cancers. WNT signaling-targeted therapeutics are also applicable for combination therapies with immune checkpoint blockers (ICB) to treat cancers with immune evasion; however, because WNT signals regulate immune evasion and antitumor immunity in a context-dependent manner, monitoring of WNT signaling and immunity is mandatory to select an appropriate class of WNT signaling-targeted therapeutics for combination immunotherapy. Therefore, omics monitoring, including genome sequencing, transcriptomic, immunohistochemical and organoid-based tests, is necessary before and during selection of WNT signaling-targeted therapeutics for cancer patients. Mut, mutation; Fus, fusion.

immune checkpoint blockers as mentioned above (Fig. 3). Since resistance to tyrosine kinase inhibitors occur owing to multiple mechanisms, such as acquired drug-resistant mutations in targeted tyrosine kinases, EMT, activation of other tyrosine kinase signaling cascades to bypass targeted tyrosine kinases (4,162) and activation of WNT/β-catenin signaling cascade (Fig. 3), genomic, transcriptomic and/or immunohistochemical monitoring during tyrosine kinase inhibitor treatment is also necessary to identify a subset of patients for combination therapy with tyrosine kinase inhibitor and WNT signaling-targeted therapeutics. By contrast, because WNT signaling in the tumor microenvironment orchestrates antitumor immunity

and immune tolerance in a context-dependent manner, immune monitoring is necessary to choose the appropriate WNT signaling-targeted therapeutics for cancer patients with immune evasion (Fig. 3).

Investigational genome medicine platforms based on nucleotide sequencing of transcribed regions are applicable for determination of targeted therapeutics only in 10-24% of cancer patients (163,164). Since alterations in non-transcribed regulatory regions also drive human carcinogenesis, whole-genome sequencing rather than whole- or partial-exome sequencing is preferable to improve the precision of genome-based medicine (4,165). In addition, organoid culture is a cutting-edge technology in the fields of oncology and stem cell biology (166-168), and organoid-based tests are also used for selecting targeted therapeutics (163,166). However, because tumor-stromal/immune interactions are not recapitulated in patient-derived organoid models, immunological monitoring in the tumor microenvironment is also necessary to improve genome-based medicine.

Together, these findings indicate that 'omics monitoring', including genome sequencing, transcriptomic, immunohistochemical and organoid-based tests, before and during treatment is necessary to choose and fine-tune WNT signaling-targeted therapeutics for the treatment of cancer patients (Fig. 3).

7. Conclusion

Cancer stem cells (CSCs) are part of the tumor microenvironment and survive host defense or therapeutic insult through omics reprogramming. Aberrant WNT signaling activation in human cancers promotes CSC survival, bulk-tumor expansion and invasion/metastasis. Anti-FZD mAb, anti-ROR1 mAb, anti-RSPO3 mAb, PORCN inhibitors and β-catenin inhibitors are representative WNT signaling-targeted therapeutics in clinical trials or preclinical studies. WNT signaling-targeted therapeutics are applicable for combination therapy with tyrosine kinase inhibitors or immune checkpoint blockers. Omics monitoring is necessary for therapeutic optimization of WNT signaling-targeted therapy.

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