

# Precision medicine for human cancers with Notch signaling dysregulation (Review)

MASUKO KATOH<sup>1</sup> and MASARU KATOH<sup>2</sup>

<sup>1</sup>M & M PrecMed, Tokyo 113-0033; <sup>2</sup>Department of Omics Network, National Cancer Center, Tokyo 104-0045, Japan

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**Abstract.** NOTCH1, NOTCH2, NOTCH3 and NOTCH4 are transmembrane receptors that transduce juxtacrine signals of the delta-like canonical Notch ligand (DLL)1, DLL3, DLL4, jagged canonical Notch ligand (JAG)1 and JAG2. Canonical Notch signaling activates the transcription of BMI1 proto-oncogene polycomb ring finger, cyclin D1, *CD44*, cyclin dependent kinase inhibitor 1A, hes family bHLH transcription factor 1, hes related family bHLH transcription factor with YRPW motif 1, *MYC*, *NOTCH3*, RE1 silencing transcription factor and transcription factor 7 in a cellular context-dependent manner, while non-canonical Notch signaling activates NF- $\kappa$ B and Rac family small GTPase 1. Notch signaling is aberrantly activated in breast cancer, non-small-cell lung cancer and hematological malignancies, such as T-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma. However, Notch signaling is inactivated in small-cell lung cancer and squamous cell carcinomas. Loss-of-function *NOTCH1* mutations are early events during esophageal tumorigenesis, whereas gain-of-function *NOTCH1* mutations are late events during T-cell leukemogenesis and B-cell lymphomagenesis. Notch signaling cascades crosstalk with fibroblast growth factor and WNT signaling cascades in the tumor microenvironment to maintain cancer stem cells and remodel the tumor microenvironment. The Notch signaling network exerts oncogenic and tumor-suppressive effects in a cancer stage- or (sub)type-dependent manner. Small-molecule  $\gamma$ -secretase inhibitors (AL101, MRK-560, nirogacestat and others) and antibody-based biologics targeting Notch ligands or receptors [ABT-165, AMG 119, rovalpituzumab tesirine (Rova-T) and others] have been developed as investigational drugs. The DLL3-targeting antibody-drug

conjugate (ADC) Rova-T, and DLL3-targeting chimeric antigen receptor-modified T cells (CAR-Ts), AMG 119, are promising anti-cancer therapeutics, as are other ADCs or CAR-Ts targeting tumor necrosis factor receptor superfamily member 17, CD19, CD22, CD30, CD79B, CD205, Claudin 18.2, fibroblast growth factor receptor (FGFR)2, FGFR3, receptor-type tyrosine-protein kinase FLT3, HER2, hepatocyte growth factor receptor, NECTIN4, inactive tyrosine-protein kinase 7, inactive tyrosine-protein kinase transmembrane receptor ROR1 and tumor-associated calcium signal transducer 2. ADCs and CAR-Ts could alter the therapeutic framework for refractory cancers, especially diffuse-type gastric cancer, ovarian cancer and pancreatic cancer with peritoneal dissemination. Phase III clinical trials of Rova-T for patients with small-cell lung cancer and a phase III clinical trial of nirogacestat for patients with desmoid tumors are ongoing. Integration of human intelligence, cognitive computing and explainable artificial intelligence is necessary to construct a Notch-related knowledge-base and optimize Notch-targeted therapy for patients with cancer.

## Contents

1. Introduction
2. Notch signaling overview
3. Notch signaling in tumor cells
4. Notch signaling in the tumor microenvironment
5. Therapeutics targeting Notch signaling cascades
6. Perspectives on Notch-targeted precision oncology

## 1. Introduction

NOTCH1, NOTCH2, NOTCH3 and NOTCH4 are cell surface receptors that transduce juxtacrine signals of delta-like canonical Notch ligand (DLL)1, DLL3, DLL4, jagged canonical Notch ligand (JAG)1 and JAG2 from adjacent cells (1-3). Germline mutations in the *NOTCH1*, *NOTCH2* and *NOTCH3* genes cause Adams-Oliver syndrome, Alagille syndrome and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, respectively (4), and DLL4-NOTCH3 signaling in human vascular organoids induces basement membrane thickening and drives vasculopathy in the diabetic microenvironment (5). By contrast, somatic alterations in the genes encoding Notch signaling

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*Correspondence to:* Dr Masaru Katoh, Department of Omics Network, National Cancer Center, 5-1-1 Tsukiji, Chuo Ward, Tokyo 104-0045, Japan  
E-mail: mkatoh-kkr@umin.ac.jp

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components drive various types of human cancer, such as breast cancer, small-cell lung cancer (SCLC) and T-cell acute lymphoblastic leukemia (T-ALL) (6-9). Notch signaling dysregulation is involved in a variety of pathologies, including cancer and non-cancerous diseases.

Small-molecule inhibitors, antagonistic monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), bispecific antibodies or biologics (bsAbs) and chimeric antigen receptor-modified T cells (CAR-Ts) targeting Notch signaling components have been developed as investigational anti-cancer drugs (10-12). The safety, tolerability and anti-tumor effects of these compounds have been studied in clinical trials; however, Notch-targeted therapeutics are not yet approved for the treatment of patients with cancer. Here, Notch signaling in the tumor microenvironment and Notch-targeted therapeutics are reviewed, and perspectives on Notch-related precision oncology are discussed with emphases on biologics, clinical sequencing and explainable artificial intelligence.

## 2. Notch signaling overview

DLL1, DLL3, DLL4, JAG1 and JAG2 are transmembrane ligands of Notch receptors (2,6,13). DLL1, DLL4, JAG1 and JAG2 are agonistic Notch ligands (Fig. 1), whereas DLL3 without the conserved N-terminal module of agonistic Notch ligands is an aberrant Notch ligand that can antagonize DLL1-Notch signaling. EGF-like repeats 1-13 in the extracellular region of NOTCH1 are involved in DLL1/4 signaling and the EGF-like repeats 10-24 of NOTCH1 are involved in JAG1/2 signaling (14).  $\beta$ -1,3-N-Acetylglucosaminyltransferase lunatic fringe and  $\beta$ -1,3-N-acetylglucosaminyltransferase manic fringe transfer N-acetylglucosamine to O-fucose on the EGF repeats in the extracellular region of Notch receptors, which enhances DLL1-NOTCH1 signaling and inhibits JAG1-NOTCH1 signaling (15). DLL1 promotes myogenesis through transient NOTCH1 activation, whereas DLL4 inhibits myogenesis through sustained NOTCH1 activation (16). The expression profile of DLL/JAG ligands and extracellular modification of Notch receptors affect receptor-ligand interactions and modulate the outputs and strength of the Notch signaling cascades (17); however, the landscape of interactions between Notch ligands and receptors, especially those of NOTCH2, NOTCH3 and NOTCH4, remain elusive.

Interactions with DLL/JAG agonistic ligands trigger sequential proteolytic cleavage of Notch receptors by disintegrin and metalloproteinase domain-containing protein (ADAM)10/17 and  $\gamma$ -secretase (2,6,18,19), which generates the following: i) Notch extracellular domain; ii) Notch transmembrane domain (NTMD); and iii) Notch intracellular domain (NICD) (Fig. 1). The NICD is then translocated to the nucleus and associates with CBF1-suppressor of hairless-LAG1 (CSL) and mastermind like proteins (MAML1, MAML2 or MAML3) to activate the transcription of target genes. NICD/CSL-dependent transcription of Notch target genes is defined as the canonical Notch signaling cascade (20), whereas CSL-independent cellular responses, such as NICD-dependent activation of NF- $\kappa$ B (21), NICD-dependent inhibition of serine-protein kinase ATM (22) and NTMD-dependent activation of Ras-related C3 botulinum toxin substrate 1 (RAC1) (23), are defined as non-canonical Notch signaling cascades (Fig. 1).

NICDs undergo posttranslational modifications such as phosphorylation, ubiquitination and PARylation. Cyclin-dependent kinase (CDK)8-dependent phosphorylation of the NOTCH1 intracellular domain (NICD1) within the intracellular proline-, glutamate-, serine- and threonine-rich region leads to F-box/WD repeat-containing protein 7 (FBXW7)-mediated ubiquitination and proteasomal degradation (24,25), whereas ubiquitin carboxyl-terminal hydrolase 7-mediated deubiquitination stabilizes NOTCH1 receptors (26). SRC-dependent phosphorylation of NICD1 within the intracellular ankyrin repeat region represses Notch signaling through blockade of the NICD1-MAML interaction and degradation of NICD1 (27). AKT-dependent phosphorylation of NICD4 at S1495, S1847, S1865 and S1917 tethers NICD4 in the cytoplasm and represses NICD4-dependent transcription (28). MDM2-dependent NICD4 ubiquitination and E3 ubiquitin-protein ligase LNX (NUMB)-dependent NICD1 ubiquitination degrade NICDs and attenuate Notch signaling (29,30), whereas MDM2-dependent NICD1 ubiquitination does not degrade NICD1 and activates Notch signaling (31). Poly [ADP-ribose] polymerase tankyrase-1 (TNKS) PARylates NOTCH1, NOTCH2 and NOTCH3, and TNKS-dependent PARylation of NOTCH2 is required for nuclear translocation of the NICD (32). Posttranslational modifications of NICDs modulate their stability and intracellular localization to fine-tune intracellular Notch signaling.

Canonical Notch signals induce the upregulation of NICD/CSL-target genes (Fig. 1), such as BMI1 proto-oncogene polycomb ring finger (*BMI1*) (33,34), cyclin D1 (*CCND1*) (35,36), *CD44* (37), *CDKN1A* (*p21*) (38,39), hes family bHLH transcription factor 1 (*HES1*) (40,41), hes family bHLH transcription factor 4 (*HES4*) (36,42), hes related family bHLH transcription factor with YRPW motif 1 (*HEY1*) (36,42,43), *MYC* (42,44,45), *NOTCH3* (42,46), Notch regulated ankyrin repeat protein (*NRARP*) (36,41,42,47), nuclear factor erythroid 2 like 2 (48), olfactomedin 4 (*OLFM4*) (49), RE1 silencing transcription factor (*REST*) (41) and transcription factor 7 (*TCF7*) (50,51). Canonical Notch target genes are upregulated in a cellular context-dependent manner through dynamic patterns of Notch signaling activation, the epigenetic status of target genes and the availability of other transcription factors (16,52).

## 3. Notch signaling in tumor cells

Notch signaling molecules are frequently altered in T-ALL (80%) (53) and microsatellite-unstable (MSI) or DNA polymerase- $\epsilon$  catalytic subunit A (POLE)-mutant subtypes of gastric and esophageal cancer (79%), colorectal cancer (70%) and uterine corpus endometrial cancer (64%) (54). Notch signaling is activated owing to gain-of-function (GoF) *NOTCH* alterations in T-ALL (55-57), chronic lymphocytic leukemia (58,59), diffuse large B cell lymphoma (60,61), mantle cell lymphoma (62), breast cancer (63-65) and non-small-cell lung cancer (NSCLC) (66) as well as loss-of-function (LoF) *FBXW7* mutations in MSI or POLE-mutant cancers and hematological malignancies (53,54) (Fig. 2). By contrast, Notch signaling is inactivated as a result of LoF *NOTCH* alterations in cutaneous squamous cell carcinoma (67), head and neck squamous cell carcinoma (HNSCC) (68,69), esophageal squamous cell carcinoma (70,71) and SCLC (72) (Fig. 2).

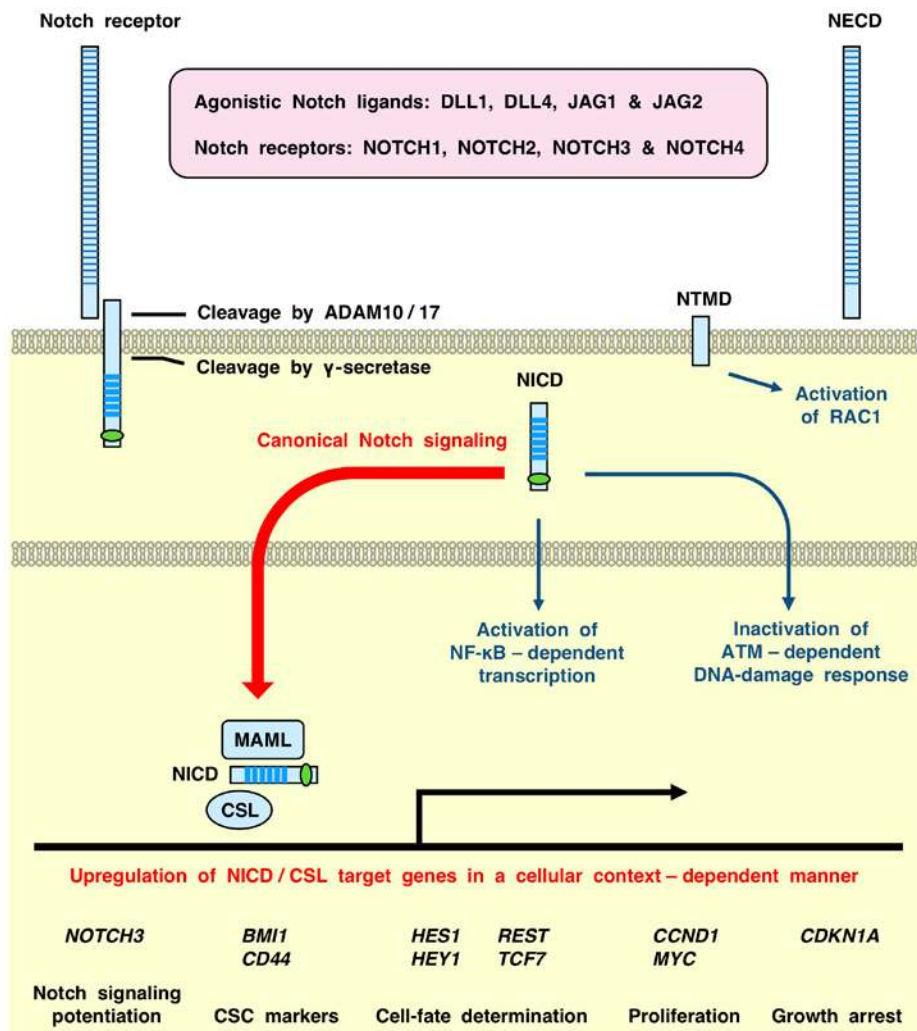


Figure 1. Overview of canonical and non-canonical Notch signaling cascades. DLL/JAG agonistic ligands trigger proteolytic cleavage of Notch receptors to generate the NECD, NTMD and NICD. Canonical Notch signaling cascades: NICD/CSL-dependent transcriptional activation of target genes, such as *BMI1*, *CCND1*, *CD44*, *HES1*, *HEY1*, *MYC*, *NOTCH3*, *REST* and *TCF7*, in a cellular context-dependent manner. Non-canonical Notch signaling cascades: CSL-independent cellular responses, such as NTMD-dependent activation of RAC1, NICD-dependent activation of NF- $\kappa$ B and NICD-dependent inhibition of ATM. DLL4-NOTCH1 signaling in endothelial cells induces NTMD-mediated assembly of cadherin-5, receptor-type tyrosine-protein phosphatase F and TRIO and F-actin-binding protein, which activates RAC1 to maintain vascular barrier function through cytoskeletal reorganization. By contrast, NOTCH1 activation in T-cell acute lymphoblastic leukemia leads to the interaction of NICD with the I $\kappa$ B kinase complex and ATM to activate NF- $\kappa$ B-dependent transcription and inhibit ATM-dependent DNA-damage response, respectively. DLL, delta-like canonical Notch ligand; JAG, jagged canonical Notch ligand; NECD, Notch extracellular domain; NTMD, Notch transmembrane domain; NICD, Notch intracellular domain; ADAM10, disintegrin and metalloproteinase domain-containing protein 10; ATM, serine-protein kinase ATM; MAML, mastermind like protein; CSL, CBF1-suppressor of hairless-LAG1; *BMI1*, BMI1 proto-oncogene polycomb ring finger; *CCND1*, cyclin D1; *HES1*, hes family bHLH transcription factor 1; *HEY1*, hes related family bHLH transcription factor with YRPW motif 1; *REST*, RE1 silencing transcription factor; *TCF7*, transcription factor 7; RAC1, Ras-related protein Rac1.

Transcriptional or epigenetic alterations also dysregulate Notch signaling in the absence of genetic alterations in the Notch signaling components (Fig. 2). Oncogenic Notch signaling is reinforced due to *NOTCH3* upregulation through ETS-related transcription factor ELF3-dependent transcription in *KRAS*-mutant lung adenocarcinoma (73); *JAG1* upregulation through CpG hypomethylation in renal cell carcinoma (74); and upregulation of *JAG1*, *MAML2*, *NOTCH1*, *NOTCH2* and *NOTCH3*, partially through increased histone H3K27 acetylation, in neuroblastoma (75). Tumor-suppressive Notch signaling is inactivated in Ewing's sarcoma due to repression of *JAG1* by RNA binding protein EWS-friend leukemia integration 1 transcription factor fusion protein (76) and repression of *NOTCH1* and *REST* through decreased H3K27 acetylation in *SCLC* (77).

Notch signaling activation promotes tumor cell proliferation or survival and *in vivo* tumorigenesis through: i) Direct upregulation of *CCND1* (35) and *MYC* (44); ii) HES1-mediated *CDKN1B* (*p27*) repression and subsequent cellular proliferation (78); iii) HES1-mediated dual specificity phosphatase 1 repression and subsequent ERK activation (79); iv) HES1-mediated phosphatase and tensin homolog repression and subsequent AKT signaling activation (80); and v) HES1-mediated STAT3 activation (81,82) and CSL-independent, NF- $\kappa$ B-dependent interleukin 6 (*IL6*) upregulation, and subsequent JAK-STAT signaling activation (83). By contrast, Notch signaling activation blocks tumor cell proliferation or survival and *in vivo* tumorigenesis through: i) Direct upregulation of *CDKN1A* (38,39); ii) HES1-mediated GLI family zinc finger 1 repression (84); iii) HEY1-mediated

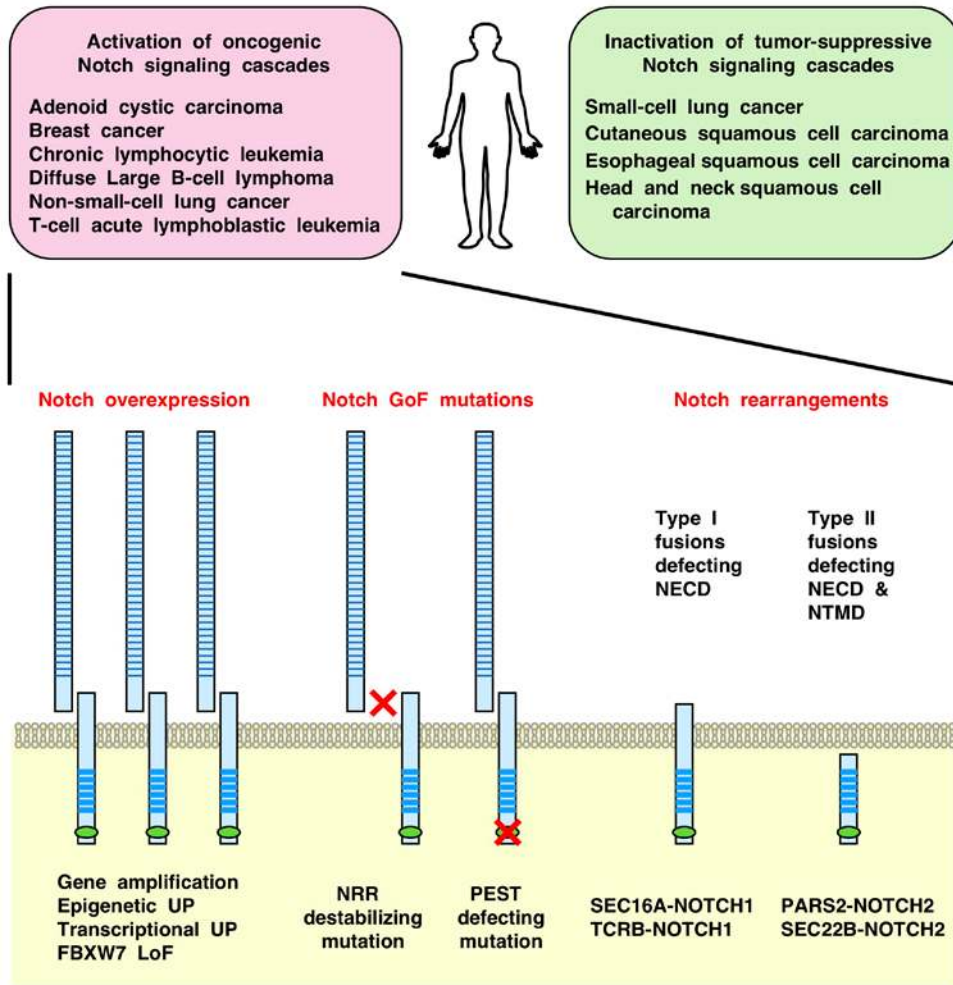


Figure 2. Genetic alterations in the Notch signaling components in human cancers. Notch signaling cascades are aberrantly activated in solid tumors and hematological malignancies owing to overexpression of Notch receptors and GoF mutations or fusions in the *NOTCH* family genes. By contrast, Notch signaling cascades are inactivated in small-cell lung cancer and squamous cell carcinomas owing to LoF mutations in the *NOTCH* family genes, especially *NOTCH1*. NECD, Notch extracellular domain; NRR, Notch negative regulatory region; NTMD, Notch transmembrane domain; PEST, proline-, glutamate-, serine- and threonine-rich domain that undergoes FBXW7-mediated ubiquitylation; UP, upregulation; GoF, gain-of-function; LoF, loss-of-function; SEC16A, protein transport protein Sec16A; TCRB, T cell receptor  $\beta$  locus; PARS2, prolyl-tRNA synthetase 2, mitochondrial; SEC22B, vesicle-trafficking protein SEC22b.

snail family transcriptional repressor 2 and twist family bHLH transcription factor 1 repression, and subsequent mesenchymal-to-epithelial transition (85); and iv) HEY1-mediated *IL6* downregulation and subsequent depletion of cancer stem cells (86). Because Notch signals drive lateral induction as well as lateral inhibition to fine-tune organ development and homeostasis (17,87,88), bifunctional cellular responses are a common feature of Notch signaling during embryogenesis, adult tissue homeostasis and tumorigenesis.

Oncogenic Notch signaling is activated in NSCLC owing to GoF *NOTCH1* mutations or ELF3-dependent *NOTCH3* upregulation (66,73), whereas tumor-suppressive Notch signaling is inactivated in SCLC owing to LoF *NOTCH1* mutations or epigenetic *NOTCH1* repression (72,77). In HNSCC, tumor-suppressive Notch signaling is inactivated owing to LoF *NOTCH1* mutations, but oncogenic Notch signaling is activated by *JAG1*, *JAG2* or *NOTCH3* upregulation (69,89). Tumor-suppressive Notch signaling is advantageous for maintaining a non-cancerous esophagus in middle-aged or elderly individuals (71), whereas oncogenic Notch signaling promotes the later stages of T-cell leukemogenesis (57) and B-cell

lymphomagenesis (61). Because Notch signals intrinsically exert both oncogenic and tumor-suppressive effects (Fig. 1), epigenetic silencing or genetic inactivation of anti-tumorigenic Notch target genes may transfer the growth advantage from LoF Notch mutants to GoF Notch mutants.

**4. Notch signaling in the tumor microenvironment**

The tumor microenvironment comprises a heterogeneous population of cancer cells, cancer-associated fibroblasts (CAFs), endothelial cells, mesenchymal stem/stromal cells (MSCs), pericytes, peripheral neurons and immune cells (90-92) (Fig. 3). Single-cell RNA sequencing (scRNAseq) revealed seven subgroups of fibroblasts, six subgroups of endothelial cells and 30 subgroups of immune cells in NSCLC (93), and four subtypes of cancer-associated fibroblasts in mouse mammary tumors (94). Cancerous and non-cancerous cells communicate via growth factors, cytokines and extracellular vesicles for paracrine signaling, and via membrane-type ligand/receptor pairs for juxtacrine signaling (3,95-97). These intercellular communications turn the anti-tumor

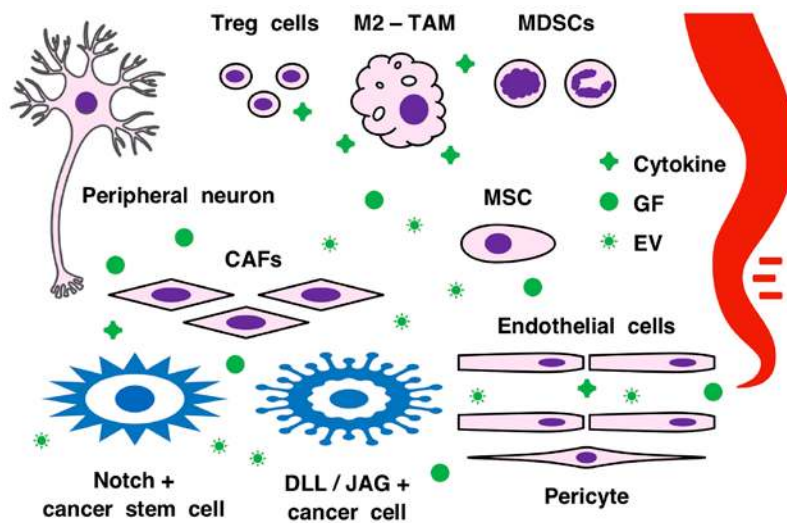


Figure 3. Notch signaling network in the tumor microenvironment. CSCs, differentiated cancer cells, CAFs, endothelial cells, MSCs, pericytes, peripheral neurons and immune cells, such as TAMs, MDSCs and regulatory T (Treg) cells, constitute the tumor microenvironment. Cancerous and non-cancerous cells communicate via Notch ligand/receptor pairs for juxtacrine signaling, as well as via cytokines, GFs and EVs for paracrine signaling. Notch signaling cascades crosstalk with FGF and WNT signaling cascades in the tumor microenvironment to support the self-renewal of CSCs and regulate angiogenesis and immunity. The Notch signaling network exerts oncogenic and tumor-suppressive functions in a cancer stage- or (sub)type-dependent manner. CAFs, cancer-associated fibroblasts; MSCs, mesenchymal stem/stromal cells; TAMs, tumor-associated macrophages; EV, extracellular vesicle; GF, growth factor, MDSC, myeloid-derived suppressor cell; CSC, cancer stem cell; DLL, delta-like canonical Notch ligand; JAG, jagged canonical Notch ligand.

microenvironment into a pro-tumor microenvironment through 'omics reprogramming' (98), which includes epigenetic changes (99), epithelial-to-mesenchymal transition (100), immunoediting (101) and vascular remodeling (102).

*Notch4* (*Int3*), fibroblast growth factor (*Fgf*) 3 (*Int2*), *Fgf4*, R-spondin (*Rspo*) 2 (*Int7*), *Rspo3*, *Wnt1* (*Int1*) and *Wnt3* (*Int4*) are proto-oncogenes that are activated by mouse mammary tumor virus (MMTV) (103-108). Notch signaling is required for the CSL-dependent expression of FGF7, FGF9, FGF10, FGF18, WNT1, WNT2 and WNT3 in dermal fibroblasts (39), while RSPO2 and RSPO3 interact with LGR4/5/6 to potentiate WNT signaling through Frizzled receptors (109,110). WNT signals enhance Notch signaling through *JAG1* and *NOTCH2* upregulation (111,112) but repress Notch signaling through *NUMB* and prospero homeobox 1 upregulation (113,114). Notch signals enhance  $\beta$ -catenin/LEF1 signaling via *NRARP* upregulation (47,115), but repress WNT/ $\beta$ -catenin signaling through *OLFM4* upregulation (49,116). Notch and WNT signals converge on *BM11* and *TCF7* to maintain slow-cycling cancer stem cells, partially through *BM11*-induced telomerase reverse transcriptase upregulation and *TCF7*-induced *CDKN2* upregulation, and on *CCND1* and *MYC* to promote tumor proliferation (34,50,51,117-121). Colorectal cancer stem cells diverge into Notch- and WNT-dependent populations, and Notch signals may not be essential for bulk tumorigenesis (122,123). Notch signaling cascades crosstalk with FGF and WNT signaling cascades to orchestrate the tumor microenvironment for the maintenance of cancer stem cells.

Tumor angiogenesis is characterized by excessive endothelial sprouting from preexisting blood vessels, which leads to overgrowth of randomly organized and leaky tumor vessels (124-126). Vascular endothelial growth factor (VEGFA) signaling through VEGF receptor 2 (VEGFR2) (KDR) and neuropilin-1 (NRP1) receptors on endothelial tip cells drives vascular sprouting and *DLL4* upregulation, and

*DLL4* signaling through Notch receptors on endothelial stalk cells restricts angiogenic sprouting and proliferation through downregulation of VEGFR2 and NRP1 (127,128). By contrast, Notch signaling induces *JAG1* upregulation to antagonize the *DLL4*-dependent 'stalk' phenotype, and promote endothelial sprouting and proliferation (129,130). NICD1-dependent Notch signaling activation in endothelial cells promotes lung metastasis (131), but that in hepatic endothelial cells represses liver metastasis (132). Thus, Notch signaling regulates tumor angiogenesis and metastasis in a context-dependent manner.

Notch signals are involved in the development and homeostasis of immune cells: *JAG1*-Notch, *DLL4*-Notch1 and *DLL1*-Notch2 signals promote the self-renewal of long-term hematopoietic stem cells, differentiation of early T-lymphocyte progenitors and differentiation of marginal zone B lymphocytes, respectively (133,134); *DLL1/4* and *JAG1/2* signals induce the differentiation of naïve T lymphocytes into Th1 and Th2 cells, respectively (135,136); *DLL1* and *JAG1* signals promote the differentiation of tumor-associated macrophages (TAMs) into M1- and M2-like phenotypes, respectively (137,138); *DLL1* or *JAG1* on MSCs and *JAG2* on hematopoietic progenitor cells induce the expansion of regulatory T (Treg) cells (139-141); and *DLL4* on dendritic cells promotes Treg differentiation (142). By contrast, Notch-related immunological reprogramming in the tumor microenvironment may be more complex; scRNAseq revealed 20 subsets of T lymphocytes, including circulating Treg cells, non-cancerous tissue-infiltrating Treg cells and cancerous tissue-infiltrating Treg cells (143). For example, Notch-mediated immune regulation in the hypoxic tumor microenvironment is potentiated by the interaction between NICD and hypoxia-induced hypoxia inducible factor-1 $\alpha$ , and is modulated by the crosstalk with the FGF, Hedgehog, transforming growth factor (TGF)- $\beta$ , VEGF and WNT signaling cascades (102,124,125,144-147). Notch1 signaling elicited immune evasion through TGF- $\beta$  upregulation and

accumulation of myeloid-derived suppressor cells (MDSCs) and Treg cells in a mouse xenograft model with B16 melanoma cells (148), and through upregulation of cytotoxic T-lymphocyte protein 4, lymphocyte activation gene 3 protein, programmed cell death protein 1 and hepatitis A virus cellular receptor 2, and accumulation of MDSCs, TAMs and Treg cells, in an engineered mouse model of HNSCC (149).

### 5. Therapeutics targeting Notch signaling cascades

Investigational drugs that target Notch signaling cascades are classified as follows: i) Small-molecule  $\gamma$ -secretase inhibitors that block the final step of ligand-induced processing of Notch receptors; ii) biologics, including mAbs, ADCs, bsAbs and CAR-Ts, that bind to the extracellular region of Notch ligands or receptors; iii) ADAM17 inhibitors that block the initial step of ligand-induced processing of Notch receptors; and iv) NICD protein-protein-interaction inhibitors that block the NICD-dependent transcription of Notch target genes (Table I).

$\gamma$ -Secretase inhibitors, such as AL101 (150), crenigacestat (151), MRK-560 (152), nirogacestat (153,154) and RO4929097 (155,156), are investigational Notch pathway inhibitors. AL101, crenigacestat, nirogacestat and RO4929097 were tolerated in phase I clinical trials with common adverse effects, such as diarrhea, fatigue, nausea and vomiting (150,151,153,155), whereas MRK-560, which selectively targets presenilin-1-containing  $\gamma$ -secretase complexes, is a next-generation  $\gamma$ -secretase inhibitor with decreased gastrointestinal toxicities (152). Multiple phase II clinical trials of RO4929097 (registration nos. NCT01116687, NCT01120275, NCT01175343 and NCT01232829) failed, had insufficient results or were terminated because of limited anti-tumor activity, partially driven by cytochrome P450 3A4-mediated drug metabolism (155,156). Combination therapy is a rational strategy to enhance the clinical benefits of  $\gamma$ -secretase inhibitors, because bypassing the activation of receptor tyrosine kinases (RTKs) (157,158) and the RAS-MEK-ERK (159), PI3K-AKT (80) and Hedgehog-GLI (84) signaling cascades elicits resistance to  $\gamma$ -secretase inhibitors. Prescription to strong responders is another rational strategy to enhance the clinical benefits of  $\gamma$ -secretase inhibitors. A phase III clinical trial of nirogacestat for desmoid tumor patients (registration no. NCT03785964) is in progress based on objective response rates (ORRs) of ~70 and ~30% in phase I (registration no. NCT00878189) and phase II (registration no. NCT01981551) clinical trials, respectively (153,154).

Antibody drugs that can selectively block Notch ligands or receptors have been predicted to be an optimal choice for cancer therapy compared with  $\gamma$ -secretase inhibitors for pan-Notch signaling blockade. Anti-DLL4 mAbs (demcizumab, enoticumab and MEDI0639) (160-162), an anti-NOTCH1 mAb (brontictuzumab) (163) and an anti-NOTCH2/3 mAb (tarextumab) (164,165) have been investigated in phase I clinical trials for the treatment of patients with cancer (Table I), and were relatively well tolerated with common adverse effects, including diarrhea, fatigue and nausea. However, because DLL4-NOTCH signaling in endothelial cells (127,128) and DLL4-NOTCH3 signaling in pericytes (5) mediate cardiovascular homeostasis, anti-DLL4 and anti-NOTCH2/3 mAbs elicit cardiovascular toxicities, such as hypertension,

acute myocardial infarction, left ventricular dysfunction and peripheral edema. The ORRs of monotherapy with anti-DLL4, anti-NOTCH1 and anti-NOTCH2/3 mAbs were <5% (160-165).

ADC, bsAb and CAR-T technologies (166-169) have been applied to enhance the benefits of therapeutic mAbs in patients with cancer. Notch-related investigational biologics include ADCs targeting DLL3 [rovalpituzumab tesirine (Rova-T)] (170-172) and NOTCH3 (PF-06650808) (173); bsAbs targeting DLL3/CD3 (AMG 757) (174), DLL4/VEGF (ABT-165 and navicixizumab) (175,176) and NOTCH2/3/EGFR (CT16 and PTG12) (177,178); and CAR-Ts targeting DLL3 (AMG 119) (179) (Table I). A phase I clinical trial of the anti-DLL4/VEGF bsAb navicixizumab in 66 patients with solid tumors (registration no. NCT02298387) showed four partial responses (PRs) in the entire cohort and three PRs among 11 patients with ovarian cancer, accompanied by adverse events such as systemic hypertension (58%) and pulmonary hypertension (18%) (176); in addition, a phase I clinical trial of the anti-NOTCH3 ADC PF-06650808 in patients with breast cancer and other solid tumors (registration no. NCT02129205) revealed a manageable safety profile and three PRs among 40 participants (173). By contrast, a phase I clinical trial of the anti-DLL3 ADC Rova-T in 74 patients with SCLC and eight patients with large-cell neuroendocrine tumors (registration no. NCT01901653) demonstrated ORRs of 17% (11/65) in the entire cohort and 38% (10/26) among DLL3-high patients, with adverse events such as thrombocytopenia and pleural effusion (171). Preliminary analysis of a phase II clinical trial of Rova-T in patients with SCLC (registration no. NCT02674568) showed an ORR of 21.6% (58/266), with manageable toxicities (172). Currently, phase III clinical trials of Rova-T for the treatment of SCLC patients (registration nos. NCT03033511 and NCT03061812) are ongoing. Regarding DLL3, phase I clinical trials of the anti-DLL3/CD3 bsAb AMG 757 (registration no. NCT03319940) and DLL3-targeting CAR-Ts AMG 119 (registration no. NCT03392064) are also in progress. Compared with DLL4 and NOTCH3, DLL3 is an ideal target for ADCs, bsAbs and CAR-Ts, because DLL3 is upregulated in SCLC and other neuroendocrine tumors, repressing Notch signaling and reciprocally upregulating REST to maintain the neuroendocrine phenotype (41,170,180).

### 6. Perspectives on Notch-targeted precision oncology

ADCs or CAR-Ts targeting RTKs (Table II) and other transmembrane or GPI-anchored proteins (Table III) are popular topics in clinical oncology. Anti-CD19 CAR-Ts (axicabtagene ciloleucel and tisagenlecleucel) (181,182), an anti-CD22 ADC (inotuzumab ozogamicin) (183), an anti-CD30 ADC (brentuximab vedotin) (184) and an anti-CD79B ADC (polatuzumab vedotin) (185) have been approved by the US Food and Drug Administration for the treatment of patients with hematological malignancies, and an anti-HER2 ADC (trastuzumab emtansine) (186) has been approved for the treatment of patients with breast cancer (Fig. 4). Trastuzumab-based ADCs with distinct linkers and payloads (trastuzumab deruxtecan and trastuzumab duocarmazine) (187,188); other ADCs targeting epidermal growth factor receptor (EGFR) (189), folate receptor- $\alpha$  (190), NECTIN4 (191) and tumor-associated calcium signal transducer 2 (192); and CAR-Ts targeting

Table I. Notch-targeted therapeutics.

Class	Drug	Alias	Mechanism of action	Stage of drug development	(Refs.)
GSI	AL101	BMS-906024	Inhibition of S3 cleavage	Phase II (registration no. NCT03691207; GoF-Notch ACC; Recruiting)	(150)
	Crenigacestat	LY3039478	Inhibition of S3 cleavage	Phase I (registration no. NCT01695005; advanced cancer; completed)	(151)
	MRK-560	PF-03084014	Inhibition of S3 cleavage	Preclinical study (PSEN1-subclass GSI inhibitor for T-ALL)	(152)
	Nirogacestat	RO4929097	Inhibition of S3 cleavage	Phase III (registration no. NCT03785964; desmoid tumors; recruiting)	(153,154)
	Demiczumab	OMP-21M18	Inhibition of S3 cleavage	Phase II (Multiple trials failed, insufficient or terminated)	(155,156)
mAb	Enoticumab	REGN421	Anti-DLL4 mAb	Phase II (registration no. NCT02259582; w/Chemo; NSCLC; completed)	(160)
	MEDI0639	OMP-52M51	Anti-DLL4 mAb	Phase I (registration no. NCT00871559; solid tumors; completed)	(161)
	Brontictuzumab	OMP-59R5	Anti-NOTCH1 mAb	Phase I (registration no. NCT01577745; solid tumors; completed)	(162)
	Tarextumab	15D11	Anti-NOTCH2/3 mAb	Phase I (registration no. NCT01778439; solid tumors; completed)	(163)
ADC	Rovalpituzumab tesirine	Rova-T, SC16LD6.5	Anti-NOTCH2/3 mAb	Phase II (registration no. NCT01647828; w/Chemo; Panc; completed)	(164,165)
	PF-06650808		Anti-JAG1 mAb	Preclinical study	(220)
bsAb	AMG 757		Anti-DLL3 ADC	Phase III (registration no. NCT03033511; SCLC; recruiting);	(170-172)
	ABT-165		Anti-NOTCH3 ADC	Phase III (registration no. NCT03061812; DLL3-high SCLC; active NR)	(173)
	Navicixizumab	OMP-305B83	Anti-DLL3/CD3 bsAb	Phase I (registration no. NCT02129205; solid tumors; terminated)	(174)
	CT16		Anti-DLL4/VEGF bsAb	Phase I (registration no. NCT03319940; SCLC; recruiting)	(175)
	PTG12		Anti-DLL4/VEGF bsAb	Phase II (registration no. NCT03368859; w/Chemo; CRC; recruiting)	(176)
CAR-Ts	AMG 119		Anti-NOTCH2/3/EGFR bsAb	Phase I (registration no. NCT02298387; solid tumors; completed)	(177)
	ZLDI-8		Anti-NOTCH2/3/EGFR bsAb	Preclinical study	(178)
Others	CB-103		DLL3-binding CAR-Ts	Preclinical study	(179)
	SAHMI		ADAM17 inhibitor	Preclinical study	(221)
			NICD PPI inhibitor	Phase I/II (registration no. NCT03422679; cancer; recruiting)	(222)
			NICD PPI inhibitor	Preclinical study	(223)

ACC, adenoid cystic carcinoma; Active NR, active, not recruiting; ADC, antibody-drug conjugate; bsAb, bispecific antibody or biologic; CAR-Ts, chimeric antigen receptor-modified T cells; CRC, colorectal cancer; GSI,  $\gamma$ -secretase inhibitor; mAb, monoclonal antibody; NICD, Notch intracellular domain; NSCLC, non-small-cell lung cancer; Panc, pancreatic cancer; PPI, protein-protein interaction; PSEN1, presenilin-1; SCLC, small-cell lung cancer; T-ALL, T-cell acute lymphoblastic leukemia; w/, with; GoF, gain-of-function; DLL, delta-like canonical Notch ligand; JAG, jagged canonical Notch ligand; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; ADAM17, disintegrin and metalloproteinase domain-containing protein.

Table II. ADCs and CAR-Ts targeting RTKs.

Target	Type	Drug name	Alias	Stage of drug development	(Refs.)
ALK	ADC	CDX-0125-TEI		Preclinical study (Rodent)	(224)
AXL	ADC	Enapotamab vedotin	AXL-107-MMAE	Phase I/II (registration no. NCT02988817; solid tumors; Recruiting)	(225)
DDR1	ADC	T4H11-DM4		Preclinical study (Rodent)	(226)
EGFR	ADC	Depatuzumab mafodotin	ABT-414	Phase II/III (registration no. NCT02573324; EGFR+ Glio; active NR)	(189)
FGFR2	ADC	BAY 1179470		Phase I (registration no. NCT02368951; solid tumors; completed in 2016)	(227)
FGFR3	ADC	LY3076226		Phase I (registration no. NCT02529553; cancer; completed in 2018)	(228)
FLT3	ADC	ASP1235	AGS62P1	Phase I (registration no. NCT02864290; AML; recruiting)	(229)
HER2	ADC	Trastuzumab emtansine	T-DM1	FDA approval (HER2+ Breast)	(186)
HER2	ADC	Trastuzumab deruxitecan	DS-8201a	Phase III (registration no. NCT03529110; HER2+ Breast; recruiting)	(187)
HER2	ADC	Trastuzumab duocarmazine	SYD985	Phase III (registration no. NCT03262935; HER2+ Breast; recruiting)	(188)
HER2	ADC	MI130004		Preclinical study (rodent; long-lasting anti-tumor effects)	(230)
HER3	ADC	U3-1402		Phase I/II (registration no. NCT02980341; HER3+ Breast; recruiting)	(231)
KIT	ADC	LOP628		Phase I (registration no. NCT02221505; KIT+ Cancers; terminated in 2015)	(232)
MET	ADC	Telisotuzumab vedotin	Teliso-V or ABBV-399	Phase II (registration no. NCT03539536; MET+ NSCLC; recruiting)	(233)
PTK7	ADC	Cofetuzumab pelidotin	PF-06647020	Phase I (registration no. NCT02222922; solid tumors; active NR)	(234)
RET	ADC	Y078-DM1		Preclinical study (rodent & primate; on-target neuropathy)	(235)
RON	ADC	H-Zt/g4-MMAE		Preclinical study (rodent & primate)	(236)
ROR1	CAR-Ts	ROR1 CAR-Ts		Preclinical study (rodent & primate)	(237)

Active NR, active, not recruiting; ADC, antibody-drug conjugate; AML, acute myeloid leukemia; Breast, breast cancer; CAR-Ts, chimeric antigen receptor-modified T cells; EGFR+, epidermal growth factor receptor-amplified; Glio, glioblastoma or gliosarcoma; NSCLC, non-small-cell lung cancer; FDA, Food and Drug Administration; RTK, receptor tyrosine kinase; AXL, ALK tyrosine kinase receptor; AXL, tyrosine-protein kinase receptor; UFG, DDR1, epithelial discoidin domain-containing receptor 1; FGFR, fibroblast growth factor receptor; FLT3, receptor-type tyrosine-protein kinase FLT3; KIT, mast/stem cell growth factor receptor Kit; PTK7, inactive tyrosine-protein kinase 7; RET, proto-oncogene tyrosine-protein kinase Ret; RON, macrophage-stimulating protein receptor; ROR1, inactive tyrosine-protein kinase transmembrane receptor ROR1.



Table III. ADCs and CAR-Ts targeting transmembrane or GPI-anchored proteins other than DLL3, NOTCH3 and RTKs.

Target	Type	Drug name	Alias	Stage of drug development	(Refs.)
BCMA	ADC	GSK2857916		Phase II (registration no. NCT03525678; multiple myeloma; active NR)	(238)
BCMA	CAR-Ts	Bb2121		Phase III (registration no. NCT03651128; multiple myeloma; recruiting)	(193)
CD19	CAR-Ts	Axicabtagene ciloleucel	KTE-C19	FDA approval (B-cell NHL)	(181)
CD19	CAR-Ts	Tisagenlecleucel	CTL019	FDA approval (B-cell ALL & NHL)	(182)
CD22	ADC	Inotuzumab ozogamicin	CMC-544	FDA approval (B-cell ALL)	(183)
CD30	ADC	Brentuximab vedotin	SGN-35	FDA approval (ALCL, HL, mycosis fungoides & PTCL)	(184)
CD33	ADC	Gemtuzumab ozogamicin	CMA-676	FDA approval (AML) and subsequent withdrawal	(239)
CD56	ADC	Lorvotuzumab mertansine	IMGN901	Phase II (registration no. NCT02452554; pediatric tumors; active NR)	(240)
CD79B	ADC	Polatuzumab vedotin	DCDS4501A	FDA approval (diffuse large B-cell lymphoma)	(185)
CD142	ADC	Tisotumab vedotin	TF-ADC	Phase I/II (registration no. NCT02001623; solid tumors; completed in 2018)	(241)
CD205	ADC	MEN1309	OBT076	Phase I (registration no. NCT03403725; solid tumors; recruiting)	(194)
CEACAM5	ADC	Labetuzumab govitecan	IMMU-130	Phase I/II (registration no. NCT01605318; colorectal cancer; completed in 2017)	(242)
CLDN18	ADC	Anti-CLDN18.2 ADC		Preclinical study (rodent)	(243)
CLDN18	CAR-Ts	CAR-CLDN18.2		Phase I (registration no. NCT03159819; Gas & Panc; recruiting)	(195)
FOLR1	ADC	Mirvetuximab soravtansine	IMGN853	Phase III (registration no. NCT02631876; ovary; active NR)	(190)
GFRA1	ADC	hu-6D3.v5-vcMMAE		Preclinical study (rodent & primate)	(244)
GPNNB	ADC	Glembatumumab vedotin	CDX-011	Phase II (registration no. NCT02302339; melanoma; terminated in 2018)	(245)
LGR5	ADC	Anti-LGR5-mc-vc-PAB-MMAE		Preclinical study (rodent)	(246)
LRRCL5	ADC	Samrotamab vedotin	ABBV-085	Phase I (registration no. NCT02565758; solid tumors; completed in 2019)	(247)
LYPD3	ADC	Lupartumab amadotin	BAY 1129980	Phase I (registration no. NCT02134197; solid tumors; completed in 2018)	(248)
MSLN	ADC	Anetumab ravtansine	BAY 94-9343	Phase II (registration no. NCT03023722; Panc; active NR)	(249)
NECTIN4	ADC	Enfortumab vedotin	ASG-22ME	Phase III (registration no. NCT03474107; urothelial cancer; recruiting)	(191)
SLC34A2	ADC	Lifastuzumab vedotin	DNIB0600A	Phase II (registration no. NCT01991210; ovarian cancer; completed in 2016)	(250)
SLC39A6	ADC	Ladiratumumab vedotin	SGN-LIV1A	Phase I/II (registration no. NCT03310957; TNBC; recruiting)	(251)
TROP2	ADC	Sacituzumab govitecan	IMMU-132	Phase III (registration no. NCT02574455; TNBC; recruiting)	(192)

Active NR, active, not recruiting; ADC, antibody-drug conjugate; ALCL, anaplastic large cell lymphoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Breast, breast cancer; CAR-Ts, chimeric antigen receptor-modified T cells; CD142, Tissue factor; Gas, gastric cancer; HL, Hodgkin lymphoma; MSLN, Mesothelin; NHL, non-Hodgkin lymphoma; Ovary, ovarian, fallopian tube or primary peritoneal cancer; Panc, pancreatic cancer; PTCL, peripheral T-cell lymphoma; RTK, receptor tyrosine kinase, SCLC, small-cell lung cancer; TNBC, triple-negative breast cancer; FDA, Food and Drug Administration; BCMA, tumor necrosis factor receptor superfamily member 17; CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; CLDN18, Claudin 18.2; FOLR1, folate receptor- $\alpha$ ; GFRA1, GDNF family receptor  $\alpha$ 1; GPNNB, transmembrane glycoprotein NMB; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; LRRCL5, leucine-rich repeat-containing protein 15; LYPD3, Ly6/PLAUR domain-containing protein 3; MSLN, mesothelin; SLC34A2, sodium-dependent phosphate transport protein 2B; SLC39A6, zinc transporter SIP6; TROP2, tumor-associated calcium signal transducer 2.

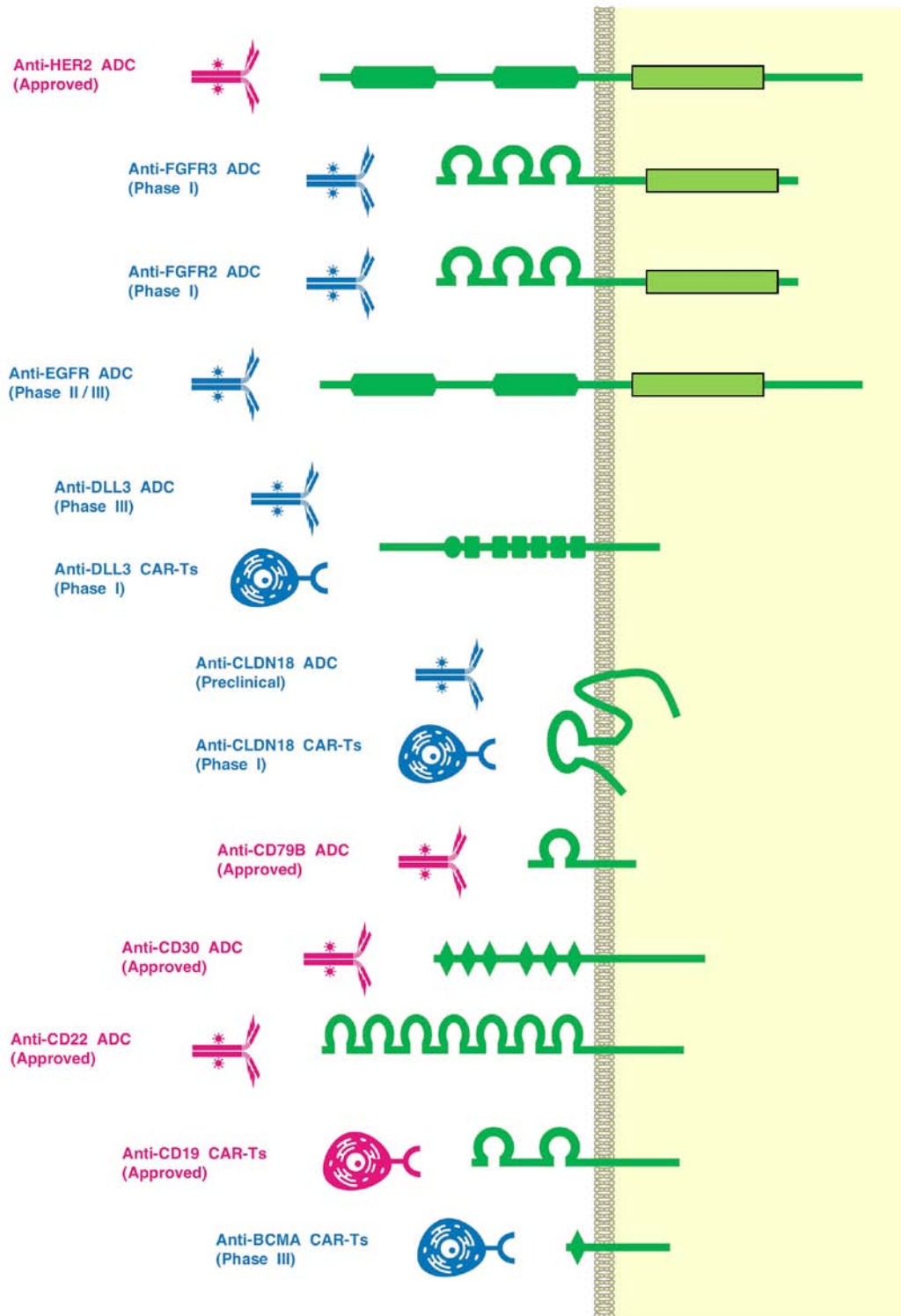


Figure 4. ADCs and CAR-Ts. ADCs or CAR-Ts targeting BCMA, CD19, CD22, CD30, CD79B, CLDN18, DLL3, EGFR, FGFR2, FGFR3, HER2 and other transmembrane or GPI-anchored proteins have been developed as investigational drugs. Anti-CD19 CAR-Ts (axicabtagene ciloleucel and tisagenlecleucel), an anti-CD22 ADC (inotuzumab ozogamicin), an anti-CD30 ADC (brentuximab vedotin), an anti-CD79B ADC (polatuzumab vedotin) and an anti-HER2 ADC (trastuzumab emtansine) have been approved by the US Food and Drug Administration for the treatment of patients with cancer. A DLL3-targeting ADC, rovalpituzumab tesirine (Rova-T), is in phase III clinical trials for the treatment of patients with small-cell lung cancer (registration nos. NCT03033511 and NCT03061812). CLDN18, Claudin 18.2; ADC, antibody-drug conjugate; CAR-Ts, chimeric antigen receptor-modified T cells; BCMA, tumor necrosis factor receptor superfamily member 17; DLL3, delta-like canonical Notch ligand 3; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor.

tumor necrosis factor receptor superfamily member 17 (193) are also in phase III clinical trials. An anti-CD205 ADC that targets mesenchymal tumor cells and CAFs (194) and

anti-Claudin-18.2 CAR-Ts that showed an ORR of 36% (4/11) in patients with gastric or pancreatic cancer (195) are cutting-edge biologics in early-stage clinical trials. ADCs and

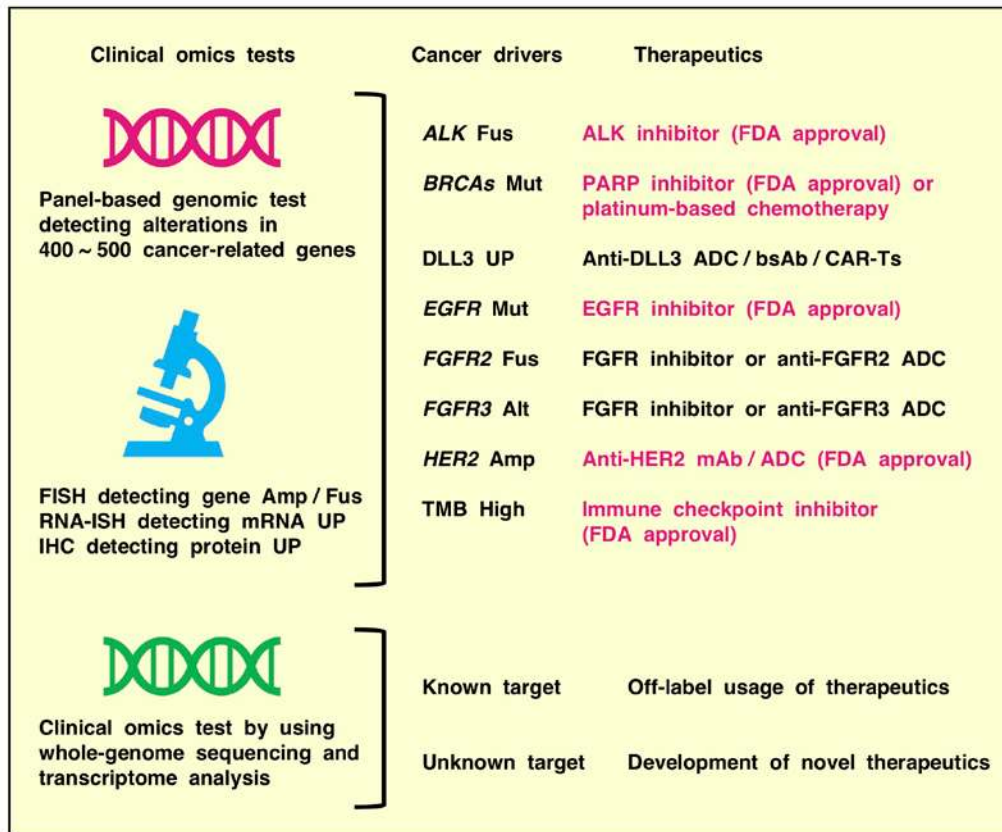


Figure 5. Clinical omics tests for precision medicine. Panel-based genomic tests detecting mutations and other alterations in 400~500 cancer-related genes, FISH detecting gene Amp or Fus, RNA-ISH detecting mRNA upregulation and IHC detecting protein UP are utilized to match drugs to cancer patients in clinical oncology. Up-to-date panel-based genomic tests are reliably applied to detect biomarkers, such as cancer drivers and the TMB. By contrast, whole-genome sequencing and transcriptome analyses is applied to explore novel therapeutic targets and biomarkers predicting therapeutic optimization in translational oncology. ADC, antibody-drug conjugate; bsAb, bispecific antibody or biologic; CAR-Ts, chimeric antigen receptor-modified T cells; mAb, monoclonal antibody; Mut, mutation; Alt, alteration; FDA, Food and Drug Administration; ALK, ALK tyrosine kinase receptor; BRCAs, BRCA DNA repair associated genes; FISH, fluorescence *in situ* hybridization; Amp, amplification; Fus, fusion; RNA-ISH, RNA *in situ* hybridization; UP, upregulation; IHC, immunohistochemistry; PARP, poly [ADP ribose] polymerase; DLL3, delta-like canonical Notch ligand 3; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; TMB, tumor mutational burden.

CAR-Ts (Tables II and III) could alter the therapeutic scheme for refractory solid tumors, especially peritoneal dissemination from diffuse-type gastric cancer, ovarian cancer and pancreatic cancer.

Repression of targeted antigens owing to the intratumoral heterogeneity and omics reprogramming of tumor cells is a common mechanism of resistance to ADCs and CAR-Ts (98,196,197). Clinical trials of ADCs in patients with solid tumors have produced disappointing results, owing to a narrow therapeutic window and unavoidable therapeutic resistance or recurrence (Tables II and III). Recruitment of new patients for the randomized phase III clinical trial of Rova-T in patients with SCLC (registration no. NCT03061812) was halted owing to shorter overall survival times in the Rova-T treatment group than in the topotecan treatment group (12). LoF *NOTCH1* mutations that decrease DLL3 dependence to suppress Notch signaling might lead to intrinsic resistance to Rova-T, whereas transdifferentiation from DLL3-high SCLC to DLL3-low SCLC or NSCLC might elicit acquired resistance to Rova-T. To enhance the clinical benefits of Rova-T in patients with SCLC, the mechanisms of resistance and biomarkers of responders should be elucidated by monitoring DLL3

expression, *NOTCH* mutations and tumor phenotypes before, during and after Rova-T therapy.

Clinical genomic tests using panel-based next-generation sequencing are utilized to match approved marketed drugs or investigational drugs to cancer patients in clinical trials in the era of precision oncology (198-200) (Fig. 5). These up-to-date genomic tests, which detect alterations in 400-500 cancer-related genes, but not out-of-date genomic tests, which detect many fewer cancer-related genes, can be reliably applied to diagnose tumor mutational burden-high cancers that predict responders to immune checkpoint inhibitors and non-responders to EGFR inhibitors (201-204). By contrast, because of their optimization for the major genetic alterations in various human cancer types, panel-based genomic tests cannot detect rare genetic alterations, promoter/enhancer mutations and epigenetic alterations that elicit aberrant activation of Notch and other oncogenic signaling pathways. Genomic tests that detect GoF mutations in the *NOTCH1*, *NOTCH2*, *NOTCH3* and *NOTCH4* genes, as well as mRNA *in situ* hybridization and immunohistochemical analyses that detect overexpression of Notch family receptors, would enhance the benefits of Notch pathway inhibitors, such as blocking mAbs and  $\gamma$ -secretase

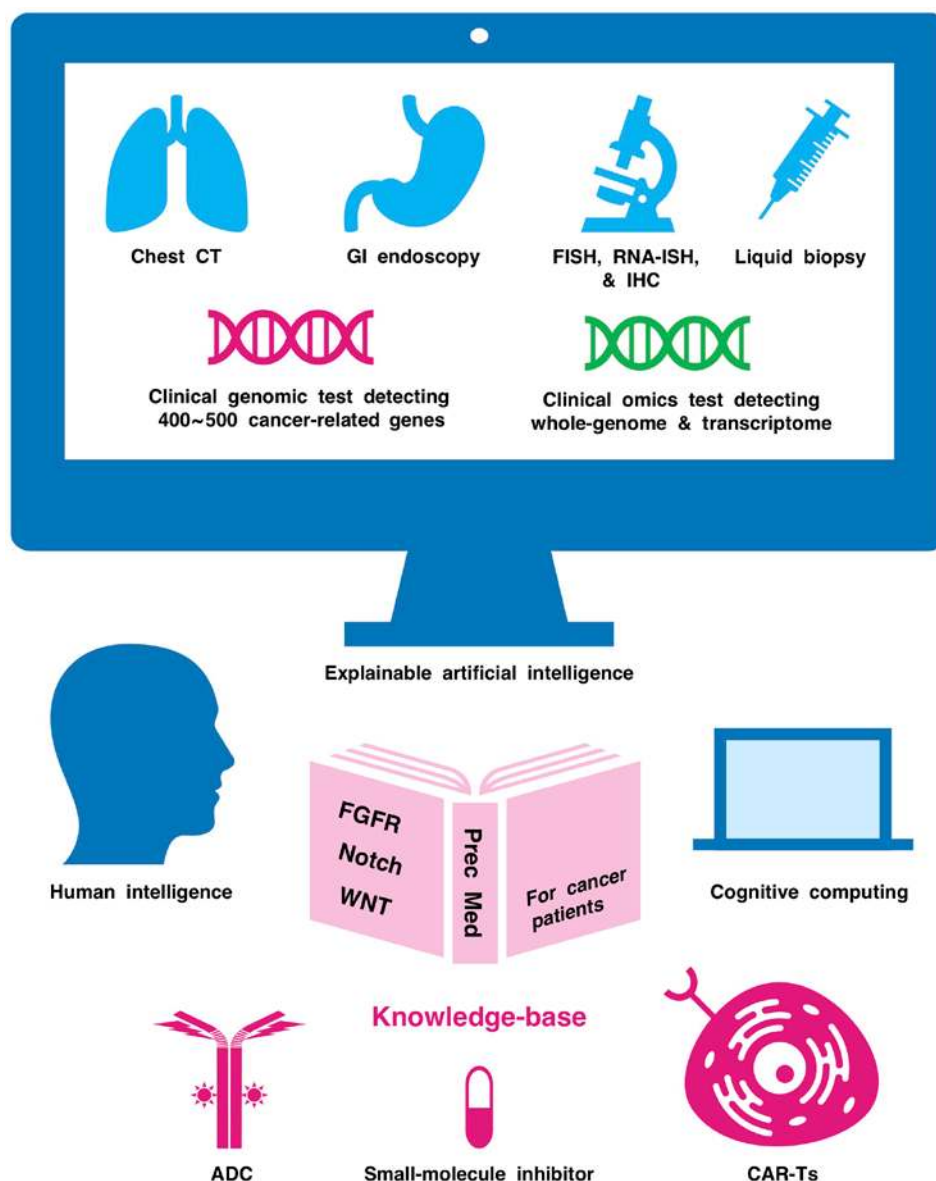


Figure 6. Human intelligence, cognitive computing and explainable artificial intelligence for omics-based precision medicine. Artificial intelligence is applied for precision medicine with chest CT, GI endoscopy and other omics-based tests, including panel-based genomic tests, FISH, RNA-ISH, IHC and liquid biopsy. Human intelligence, explainable artificial intelligence and cognitive computing should be integrated to construct a Notch-related knowledge base for the optimization of Notch-targeted therapy, such as an anti-DLL3 ADC, small-molecule  $\gamma$ -secretase inhibitors and anti-DLL3 CAR-Ts. CT, computed tomography; GI, gastrointestinal; FISH, fluorescence *in situ* hybridization; RNA-ISH, RNA *in situ* hybridization; IHC, immunohistochemistry; FGFR, fibroblast growth factor receptor; CAR-Ts, chimeric antigen receptor-modified T cells; ADC, antibody-drug conjugate; DLL3, delta-like canonical Notch ligand 3.

inhibitors, through successful positive selection of putative responders.

Whole-genome sequencing, as well as whole-exome sequencing plus transcriptome analysis, is applied for the exploration of unknown cancer drivers, and the development of novel therapeutics for known but intractable targets with the aid of human intelligence, cognitive computing and artificial intelligence in basic and translational oncology (205-208). Moreover, artificial intelligence is also applied for computer-aided diagnostic approaches (209,210), such as chest computed tomography (211), dermoscopy (212), gastrointestinal endoscopy (213), mammography (214) and histopathological diagnosis (215-218). To avoid the lack of transparency associated with black box artificial intelligence based on deep learning technologies, the development of explainable artificial

intelligence is necessary (219). Construction of a Notch-related knowledge base via human intelligence, explainable artificial intelligence, and cognitive computing based on natural language processing and text mining (Fig. 6) would promote the clinical application of Notch-targeted therapeutics in the era of omics-based precision medicine.

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**Availability of data and materials**

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**Authors' contributions**

MasukoK and MasaruK contributed to the conception of the study, performed the literature search and wrote the manuscript. MasukoK prepared the tables. MasaruK prepared the figures. All the authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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