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

MASUKO KATOH[1] and MASARU KATOH[2]

[1]M & M PrecMed, Tokyo 113-0033; [2]Department of Omics Network, National Cancer Center, Tokyo 104-0045, Japan

Received September 16, 2019; Accepted November 20, 2019

DOI: 10.3892/ijmm.2019.4418

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-κB 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 γ-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

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

Key words: angiogenesis, cancer-associated fibroblasts, computer-aided diagnostics, deep learning, immune evasion, myeloid-derived suppressor cells, natural language processing, neural network, regulatory T cells, text mining, *HES1, HEY1, REST, TCF7*

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). β-1,3-N-Acetylglucosaminyltransferase lunatic fringe and β-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 γ-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-κB (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-instable (MSI) or DNA polymerase-ε 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).

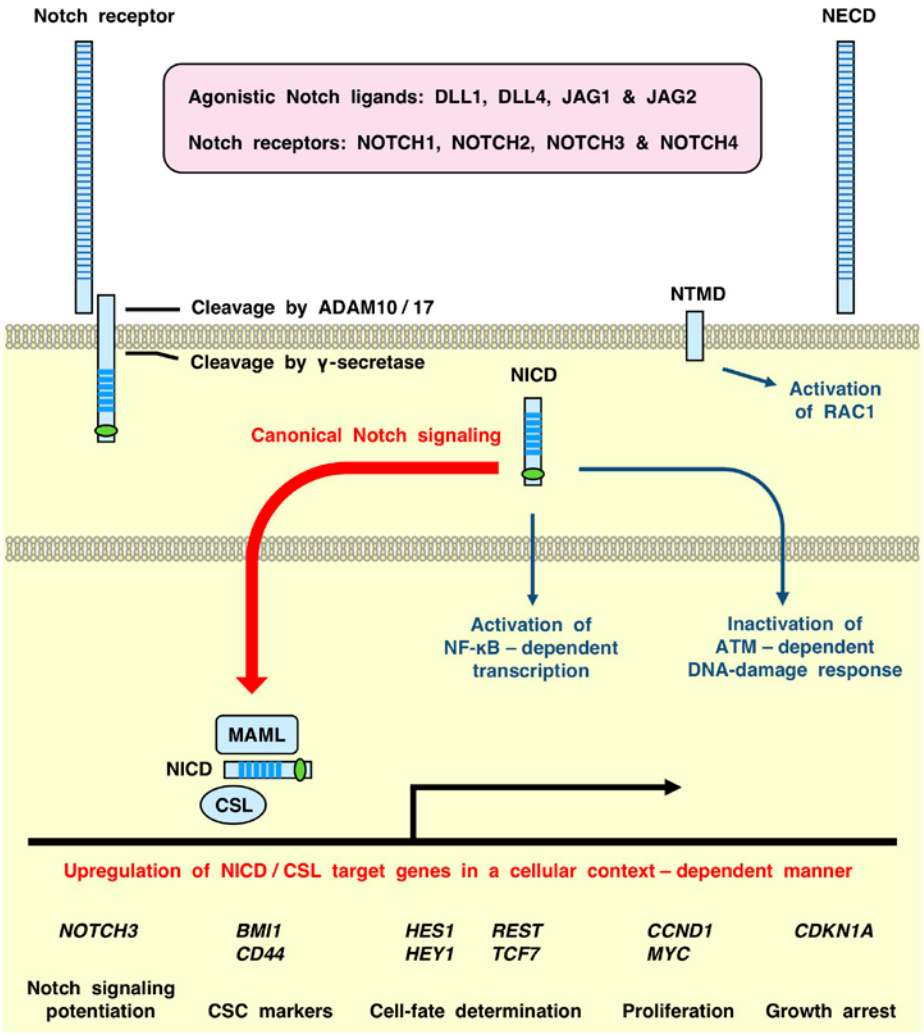

Figure 1. Overview of canonical and non-canonical Notch signaling cascades. DLL/JAG agonistic ligands trigger proteolytic cleavage of Notch receptors to generate the NECD, NTMD and NICD. Canonical Notch signaling cascades: NICD/CSL-dependent transcriptional activation of target genes, such as *BMI1, 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-κB 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 IκB kinase complex and ATM to activate NF-κB-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-κB-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

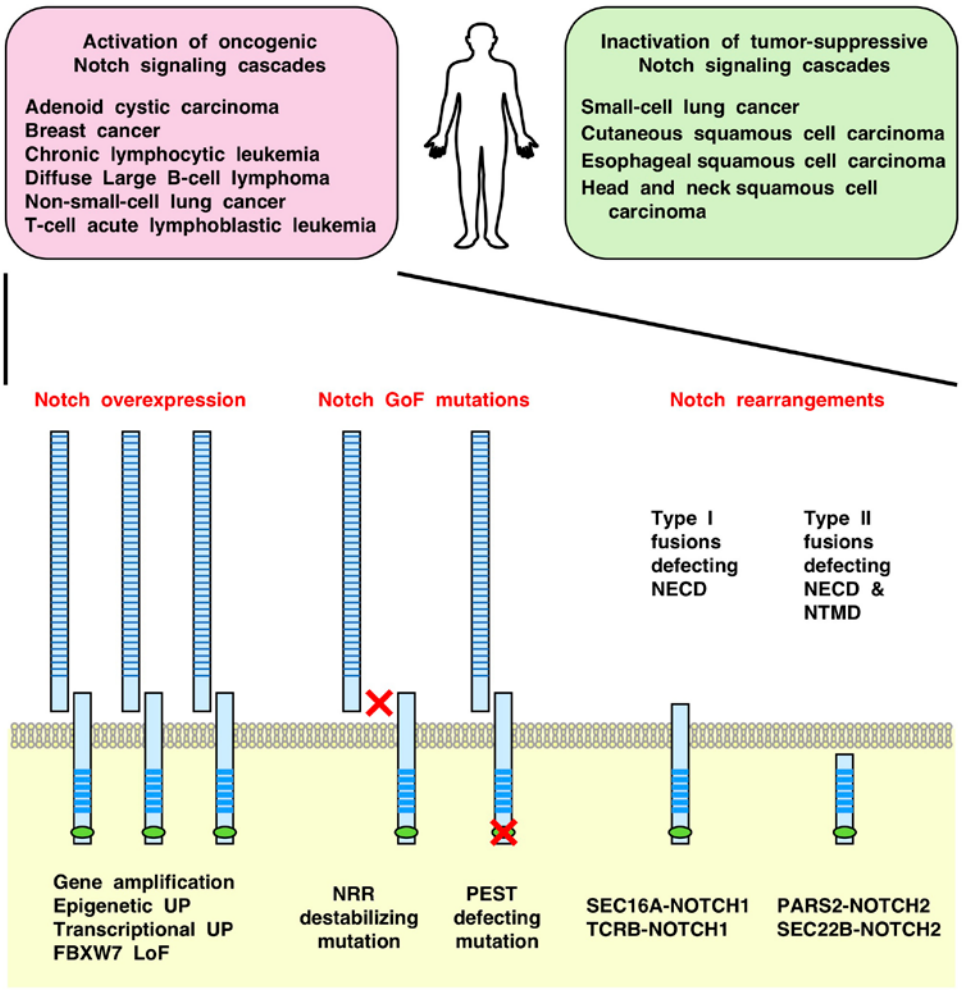

Figure 2. Genetic alterations in the Notch signaling components in human cancers. Notch signaling cascades are aberrantly activated in solid tumors and hematological malignancies owing to overexpression of Notch receptors and GoF mutations or fusions in the *NOTCH* family genes. By contrast, Notch signaling 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 β 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

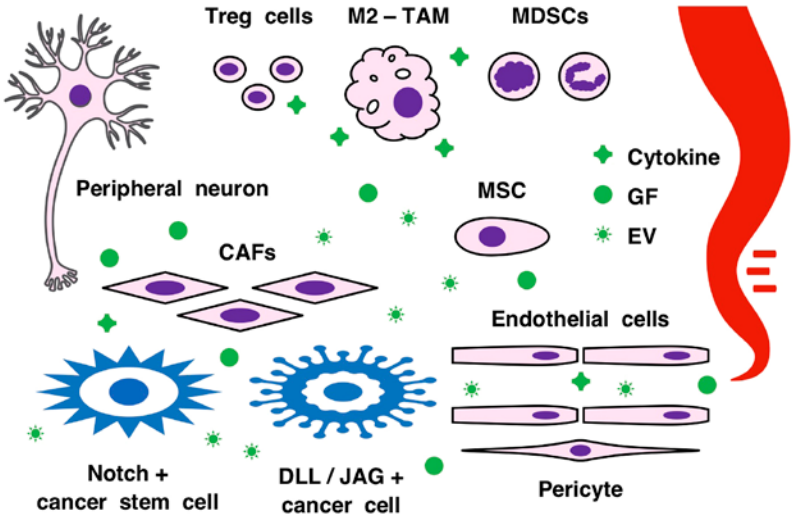

Figure 3. Notch signaling network in the tumor microenvironment. CSCs, differentiated cancer cells, CAFs, endothelial cells, MSCs, pericytes, peripheral neurons and immune cells, such as TAMs, MDSCs and regulatory T (Treg) cells, constitute the tumor microenvironment. Cancerous and non-cancerous cells 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 β-catenin/LEF1 signaling via *NRARP* upregulation (47,115), but repress WNT/β-catenin signaling through *OLFM4* upregulation (49,116). Notch and WNT signals converge on *BMI1* and *TCF7* to maintain slow-cycling cancer stem cells, partially through BMI1-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-1α, and is modulated by the crosstalk with the FGF, Hedgehog, transforming growth factor (TGF)-β, VEGF and WNT signaling cascades (102,124,125,144-147). Notch1 signaling elicited immune evasion through TGF-β 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 γ-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).

γ-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 γ-secretase complexes, is a next-generation γ-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 γ-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 γ-secretase inhibitors. Prescription to strong responders is another rational strategy to enhance the clinical benefits of γ-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 γ-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-α (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		Inhibition of S3 cleavage	Preclinical study (PSEN1-sublass GSI inhibitor for T-ALL)	(152)
	Nirogacestat	PF-03084014	Inhibition of S3 cleavage	Phase III (registration no. NCT03785964; desmoid tumors; recruiting)	(153,154)
	RO4929097		Inhibition of S3 cleavage	Phase II (Multiple trials failed, insufficient or terminated)	(155,156)
mAb	Demcizumab	OMP-21M18	Anti-DLL4 mAb	Phase II (registration no. NCT02259582; w/Chemo; NSCLC; completed)	(160)
	Enoticumab	REGN421	Anti-DLL4 mAb	Phase I (registration no. NCT00871559; solid tumors; completed)	(161)
	MEDI0639		Anti-DLL4 mAb	Phase I (registration no. NCT01577745; solid tumors; completed)	(162)
	Brontictuzumab	OMP-52M51	Anti-NOTCH1 mAb	Phase I (registration no. NCT01778439; solid tumors; completed)	(163)
	Tarextumab	OMP-59R5	Anti-NOTCH2/3 mAb	Phase II (registration no. NCT01647828; w/Chemo; Panc; completed)	(164,165)
	15D11		Anti-JAG1 mAb	Preclinical study	(220)
ADC	Rovalpituzumab tesirine	Rova-T, SC16LD6.5	Anti-DLL3 ADC	Phase III (registration no. NCT03033511; SCLC; recruiting); Phase III (registration no. NCT03061812; DLL3-high SCLC; active NR)	(170-172)
	PF-06650808		Anti-NOTCH3 ADC	Phase I (registration no. NCT02129205; solid tumors; terminated)	(173)
bsAb	AMG 757		Anti-DLL3/CD3 bsAb	Phase I (registration no. NCT03319940; SCLC; recruiting)	(174)
	ABT-165		Anti-DLL4/VEGF bsAb	Phase II (registration no. NCT03368859; w/Chemo; CRC; recruiting)	(175)
	Navicixizumab	OMP-305B83	Anti-DLL4/VEGF bsAb	Phase I (registration no. NCT02298387; solid tumors; completed)	(176)
	CT16		Anti-NOTCH2/3/EGFR bsAb	Preclinical study	(177)
	PTG12		Anti-NOTCH2/3/EGFR bsAb	Preclinical study	(178)
CAR-Ts	AMG 119		DLL3-binding CAR-Ts	Phase I (registration no. NCT03392064; SCLC; active NR)	(179)
Others	ZLDI-8		ADAM17 inhibitor	Preclinical study	(221)
	CB-103		NICD PPI inhibitor	Phase I/II (registration no. NCT03422679; cancer; recruiting)	(222)
	SAHM1		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, γ-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	Depatuxizumab 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 deruxtecan	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; ALK, ALK tyrosine kinase receptor; AXL, tyrosine-protein kinase receptor UFO; 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 receptor 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)
GPNMB	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)
LRRC15	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	Ladiratuzumab 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-α; GFRA1, GDNF family receptor α1; GPNMB, transmembrane glycoprotein NMB; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; LRRC15, 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.

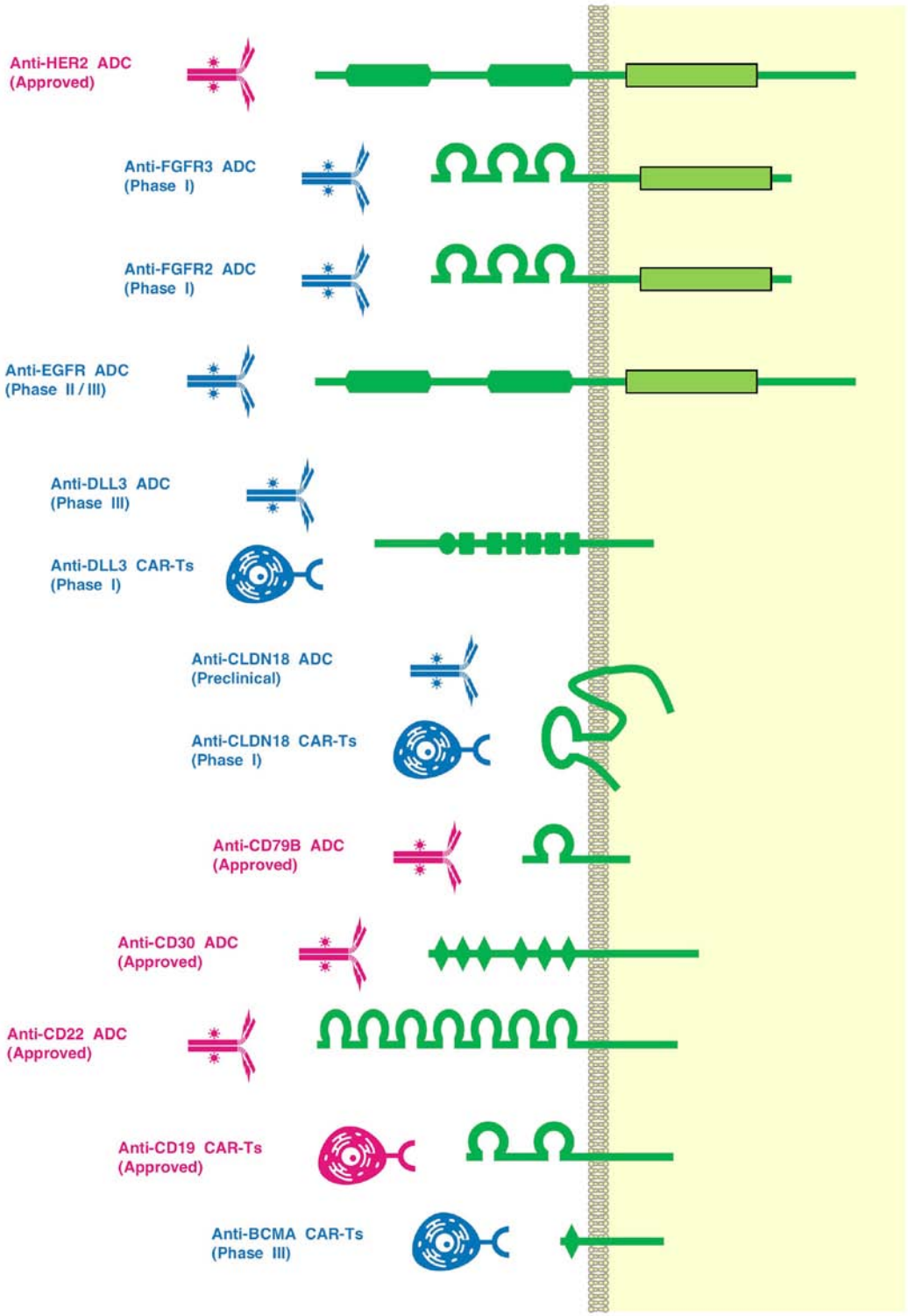

Figure 4. ADCs and CAR-Ts. ADCs or CAR-Ts targeting BCMA, CD19, CD22, CD30, CD79B, CLDN18, DLL3, EGFR, FGFR2, FGFR3, HER2 and other transmembrane or GPI-anchored proteins have been developed as investigational drugs. Anti-CD19 CAR-Ts (axicabtagene ciloleucel and tisagenlecleucel), an anti-CD22 ADC (inotuzumab 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

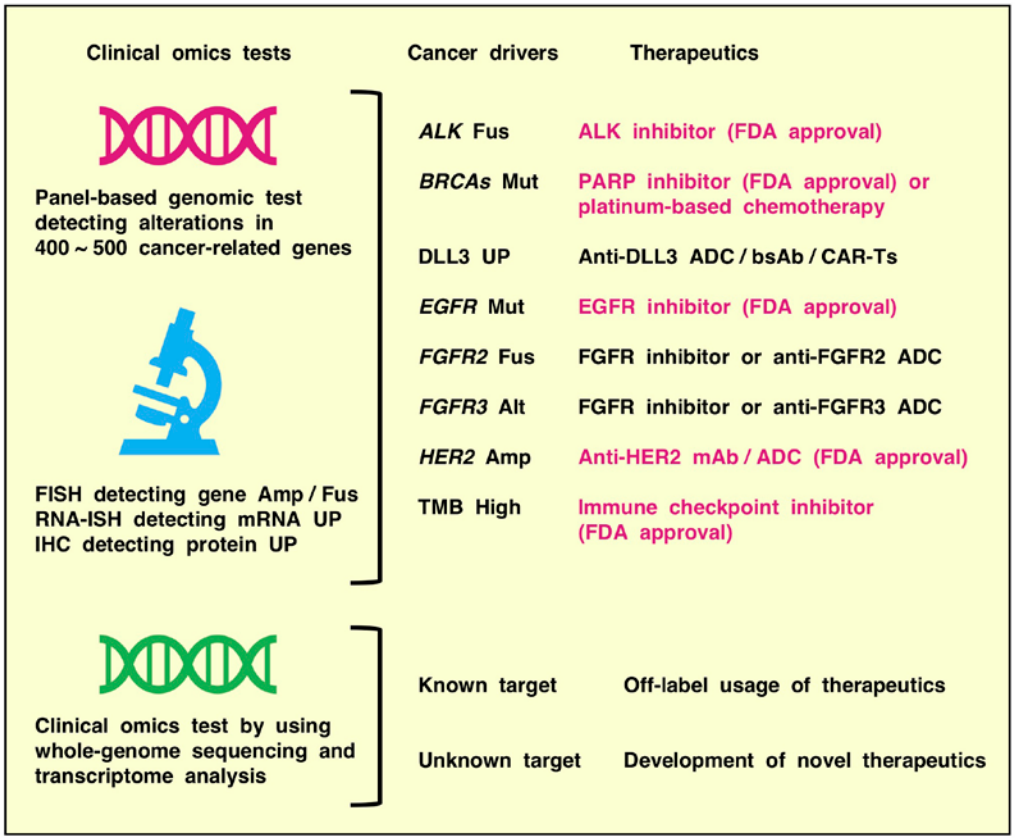

Figure 5. Clinical omics tests for precision medicine. Panel-based genomic tests detecting mutations and other alterations in 400~500 cancer-related genes, FISH detecting gene Amp or Fus, RNA-ISH detecting mRNA upregulation and IHC detecting protein UP are utilized to match drugs to cancer patients in 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 γ-secretase

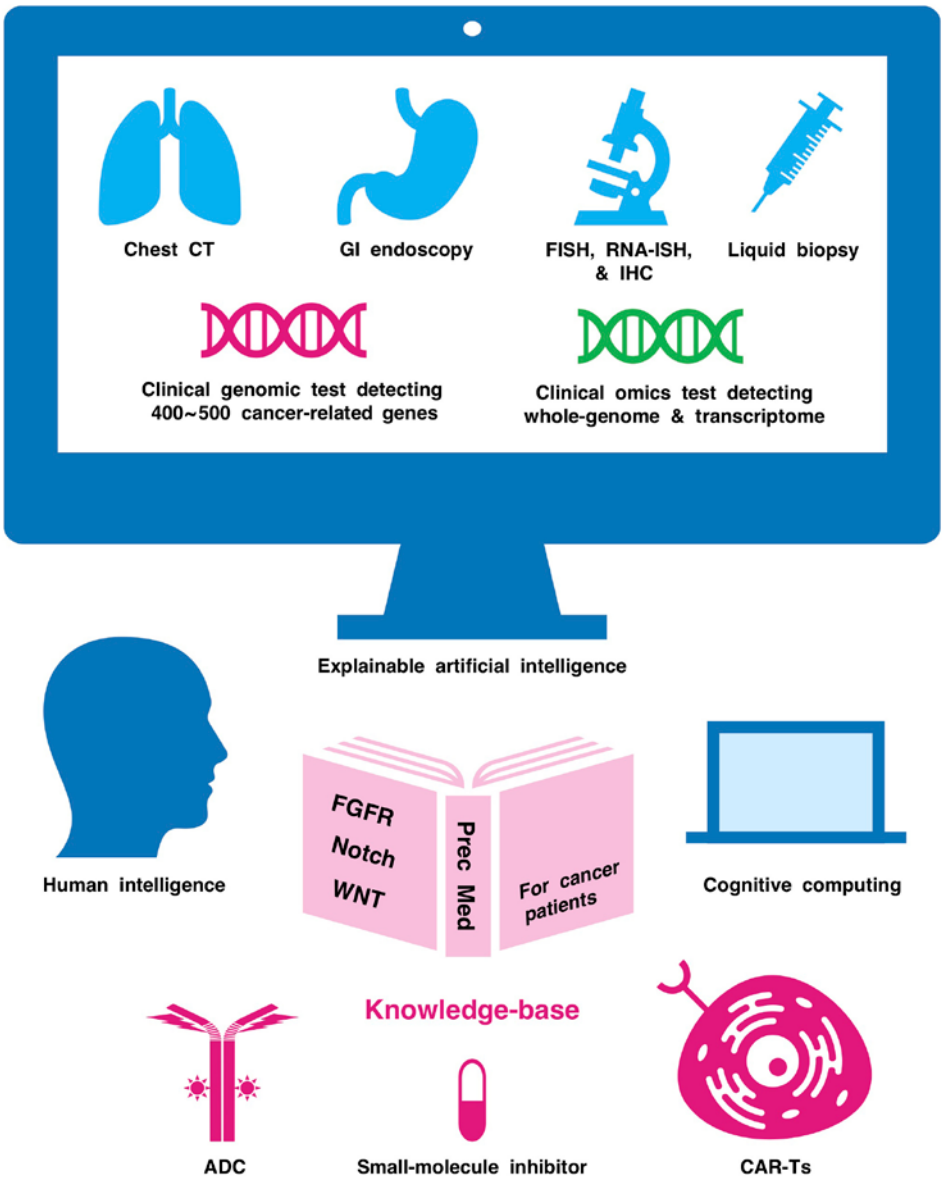

Figure 6. Human intelligence, cognitive computing and explainable artificial intelligence for omics-based precision medicine. Artificial intelligence is applied for precision medicine with chest CT, GI endoscopy and other omics-based tests, including panel-based genomic tests, FISH, RNA-ISH, IHC and 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 γ-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.

Acknowledgements

Not applicable.

Funding

This study was supported in part by a grant-in-aid from Masaru Katoh's Fund for the Knowledge-Base Project.

Availability of data and materials

Not applicable.

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.

References

1. Guruharsha KG, Kankel MW and Artavanis-Tsakonas S: The Notch signalling system: Recent insights into the complexity of a conserved pathway. Nat Rev Genet 13: 654-666, 2012.
2. Bray SJ: Notch signalling in context. Nat Rev Mol Cell Biol 17: 722-735, 2016.
3. Meurette O and Mehlen P: Notch signaling in the tumor microenvironment. Cancer Cell 34: 536-548, 2018.
4. Siebel C and Lendahl U: Notch signaling in development, tissue homeostasis, and disease. Physiol Rev 97: 1235-1294, 2017.
5. Wimmer RA, Leopoldi A, Aichinger M, Wick N, Hantusch B, Novatchkova M, Taubenschmid J, Hämmerle M, Esk C, Bagley JA, *et al*: Human blood vessel organoids as a model of diabetic vasculopathy. Nature 565: 505-510, 2019.
6. Ranganathan P, Weaver KL and Capobianco AJ: Notch signalling in solid tumours: A little bit of everything but not all the time. Nat Rev Cancer 11: 338-351, 2011.
7. Ntziachristos P, Lim JS, Sage J and Aifantis I: From fly wings to targeted cancer therapies: A centennial for notch signaling. Cancer Cell 25: 318-334, 2014.
8. Aster JC, Pear WS and Blacklow SC: The varied roles of Notch in cancer. Annu Rev Pathol 12: 245-275, 2017.
9. Nowell CS and Radtke F: Notch as a tumour suppressor. Nat Rev Cancer 17: 145-159, 2017.
10. Espinoza I and Miele L: Notch inhibitors for cancer treatment. Pharmacol Ther 139: 95-110, 2013.
11. Takebe N, Miele L, Harris PJ, Jeong W, Bando H, Kahn M, Yang SX and Ivy SP: Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: Clinical update. Nat Rev Clin Oncol 12: 445-464, 2015.
12. Owen DH, Giffin MJ, Bailis JM, Smit MD, Carbone DP and He K: DLL3: An emerging target in small cell lung cancer. J Hematol Oncol 12: 61, 2019.
13. D'Souza B, Miyamoto A and Weinmaster G: The many facets of Notch ligands. Oncogene 27: 5148-5167, 2008.
14. Kangsamaksin T, Murtomaki A, Kofler NM, Cuervo H, Chaudhri RA, Tattersall IW, Rosenstiel PE, Shawber C and Kitajewski J: NOTCH decoys that selectively block DLL/NOTCH or JAG/NOTCH disrupt angiogenesis by unique mechanisms to inhibit tumor growth. Cancer Discov 5: 182-197, 2015.
15. Kakuda S and Haltiwanger RS: Deciphering the Fringe-mediated Notch code: Identification of activating and inhibiting sites allowing discrimination between ligands. Dev Cell 40: 193-201, 2017.
16. Nandagopal N, Santat LA, LeBon L, Sprinzak D, Bronner ME and Elowitz MB: Dynamic ligand discrimination in the Notch signaling pathway. Cell 172: 869-880.e19, 2018.
17. Sjöqvist M and Andersson ER: Do as I say, Not(ch) as I do: Lateral control of cell fate. Dev Biol 447: 58-70, 2019.
18. Lambrecht BN, Vanderkerken M and Hammad H: The emerging role of ADAM metalloproteinases in immunity. Nat Rev Immunol 18: 745-758, 2018.
19. Yang G, Zhou R, Zhou Q, Guo X, Yan C, Ke M, Lei J and Shi Y: Structural basis of Notch recognition by human γ-secretase. Nature 565: 192-197, 2019.
20. Kopan R and Ilagan MX: The canonical Notch signaling pathway: Unfolding the activation mechanism. Cell 137: 216-233, 2009.
21. Vilimas T, Mascarenhas J, Palomero T, Mandal M, Buonamici S, Meng F, Thompson B, Spaulding C, Macaroun S, Alegre ML, *et al*: Targeting the NF-kappaB signaling pathway in Notch1-induced T-cell leukemia. Nat Med 13: 70-77, 2007.
22. Vermezovic J, Adamowicz M, Santarpia L, Rustighi A, Forcato M, Lucano C, Massimiliano L, Costanzo V, Bicciato S, Del Sal G and d'Adda di Fagagna F: Notch is a direct negative regulator of the DNA-damage response. Nat Struct Mol Biol 22: 417-424, 2015.
23. Polacheck WJ, Kutys ML, Yang J, Eyckmans J, Wu Y, Vasavada H, Hirschi KK and Chen CS: A non-canonical Notch complex regulates adherens junctions and vascular barrier function. Nature 552: 258-262, 2017.
24. O'Neil J, Grim J, Strack P, Rao S, Tibbitts D, Winter C, Hardwick J, Welcker M, Meijerink JP, Pieters R, *et al*: FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to γ-secretase inhibitors. J Exp Med 204: 1813-1824, 2007.
25. Wang Z, Liu P, Inuzuka H and Wei W: Roles of F-box proteins in cancer. Nat Rev Cancer 14: 233-247, 2014.
26. Shan H, Li X, Xiao X, Dai Y, Huang J, Song J, Liu M, Yang L, Lei H, Tong Y, *et al*: USP7 deubiquitinates and stabilizes NOTCH1 in T-cell acute lymphoblastic leukemia. Signal Transduct Target Ther 3: 29, 2018.
27. LaFoya B, Munroe JA, Pu X and Albig AR: Src kinase phosphorylates Notch1 to inhibit MAML binding. Sci Rep 8: 15515, 2018.
28. Ramakrishnan G, Davaakhuu G, Chung WC, Zhu H, Rana A, Filipovic A, Green AR, Atfi A, Pannuti A, Miele L and Tzivion G: AKT and 14-3-3 regulate Notch4 nuclear localization. Sci Rep 5: 8782, 2015.
29. Sun Y, Klauzinska M, Lake RJ, Lee JM, Santopietro S, Raafat A, Salomon D, Callahan R and Artavanis-Tsakonas S: Trp53 regulates Notch 4 signaling through Mdm2. J Cell Sci 124: 1067-1076, 2011.
30. McGill MA and McGlade CJ: Mammalian Numb proteins promote Notch1 receptor ubiquitination and degradation of the Notch1 intracellular domain. J Biol Chem 278: 23196-23203, 2003.
31. Pettersson S, Sczaniecka M, McLaren L, Russell F, Gladstone K, Hupp T and Wallace M: Non-degradative ubiquitination of the Notch1 receptor by the E3 ligase MDM2 activates the Notch signalling pathway. Biochem J 450: 523-536, 2013.
32. Bhardwaj A, Yang Y, Ueberheide B and Smith S: Whole proteome analysis of human tankyrase knockout cells reveals targets of tankyrase-mediated degradation. Nat Commun 8: 2214, 2017.
33. Schaller MA, Logue H, Mukherjee S, Lindell DM, Coelho AL, Lincoln P, Carson WF IV, Ito T, Cavassani KA, Chensue SW, *et al*: Delta-like 4 differentially regulates murine CD4 T cell expansion via BMI1. PLoS One 5: e12172, 2010.
34. López-Arribillaga E, Rodilla V, Pellegrinet L, Guiu J, Iglesias M, Roman AC, Gutarra S, González S, Muñoz-Cánoves P, Fernández-Salguero P, *et al*: Bmi1 regulates murine intestinal stem cell proliferation and self-renewal downstream of Notch. Development 142: 41-50, 2015.
35. Ronchini C and Capobianco AJ: Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): Implication for cell cycle disruption in transformation by Notch(ic). Mol Cell Biol 21: 5925-5934, 2001.
36. Tanis KQ, Podtelezhnikov AA, Blackman SC, Hing J, Railkar RA, Lunceford J, Klappenbach JA, Wei B, Harman A, Camargo LM, *et al*: An accessible pharmacodynamic transcriptional biomarker for Notch target engagement. Clin Pharmacol Ther 99: 370-380, 2016.
37. García-Peydró M, Fuentes P, Mosquera M, García-León MJ, Alcain J, Rodríguez A, García de Miguel P, Menéndez P, Weijer K, Spits H, *et al*: The NOTCH1/CD44 axis drives pathogenesis in a T cell acute lymphoblastic leukemia model. J Clin Invest 128: 2802-2818, 2018.

38. Rangarajan A, Talora C, Okuyama R, Nicolas M, Mammucari C, Oh H, Aster JC, Krishna S, Metzger D, Chambon P, *et al*: Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. EMBO J 20: 3427-3436, 2001.
39. Procopio MG, Laszlo C, Al Labban D, Kim DE, Bordignon P, Jo SH, Goruppi S, Menietti E, Ostano P, Ala U, *et al*: Combined CSL and p53 downregulation promotes cancer-associated fibroblast activation. Nat Cell Biol 17: 1193-1204, 2015.
40. Jarriault S, Le Bail O, Hirsinger E, Pourquié O, Logeat F, Strong CF, Brou C, Seidah NG and Isra l A: Delta-1 activation of Notch-1 signaling results in HES-1 transactivation. Mol Cell Biol 18: 7423-7431, 1998.
41. Lim JS, Ibaseta A, Fischer MM, Cancilla B, O'Young G, Cristea S, Luca VC, Yang D, Jahchan NS, Hamard C, *et al*: Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer. Nature 545: 360-364, 2017.
42. Stoeck A, Lejnine S, Truong A, Pan L, Wang H, Zang C, Yuan J, Ware C, MacLean J, Garrett-Engele PW, *et al*: Discovery of biomarkers predictive of GSI response in triple-negative breast cancer and adenoid cystic carcinoma. Cancer Discov 4: 1154-1167, 2014.
43. Maier MM and Gessler M: Comparative analysis of the human and mouse Hey1 promoter: Hey genes are new Notch target genes. Biochem Biophys Res Commun 275: 652-660, 2000.
44. Weng AP, Millholland JM, Yashiro-Ohtani Y, Arcangeli ML, Lau A, Wai C, Del Bianco C, Rodriguez CG, Sai H, Tobias J, *et al*: c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. Genes Dev 20: 2096-2109, 2006.
45. Gekas C, D'Altri T, Aligué R, González J, Espinosa L and Bigas A: β-Catenin is required for T-cell leukemia initiation and MYC transcription downstream of Notch1. Leukemia 30: 2002-2010, 2016.
46. Tottone L, Zhdanovskaya N, Carmona Pestaña Á, Zampieri M, Simeoni F, Lazzari S, Ruocco V, Pelullo M, Caiafa P, Felli MP, *et al*: Histone modifications drive aberrant Notch3 expression/activity and growth in T-ALL. Front Oncol 9: 198, 2019.
47. Pirot P, van Grunsven LA, Marine JC, Huylebroeck D and Bellefroid EJ: Direct regulation of the Nrarp gene promoter by the Notch signaling pathway. Biochem Biophys Res Commun 322: 526-534, 2004.
48. Wakabayashi N, Skoko JJ, Chartoumpekis DV, Kimura S, Slocum SL, Noda K, Palliyaguru DL, Fujimuro M, Boley PA, Tanaka Y, *et al*: Notch-Nrf2 axis: Regulation of Nrf2 gene expression and cytoprotection by Notch signaling. Mol Cell Biol 34: 653-663, 2014.
49. VanDussen KL, Carulli AJ, Keeley TM, Patel SR, Puthoff BJ, Magness ST, Tran IT, Maillard I, Siebel C, Kolterud Å, *et al*: Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. Development 139: 488-497, 2012.
50. Weber BN, Chi AW, Chavez A, Yashiro-Ohtani Y, Yang Q, Shestova O and Bhandoola A: A critical role for TCF-1 in T-lineage specification and differentiation. Nature 476: 63-68, 2011.
51. Germar K, Dose M, Konstantinou T, Zhang J, Wang H, Lobry C, Arnett KL, Blacklow SC, Aifantis I, Aster JC and Gounari F: T-cell factor 1 is a gatekeeper for T-cell specification in response to Notch signaling. Proc Natl Acad Sci USA 108: 20060-20065, 2011.
52. Bray SJ and Gomez-Lamarca M: Notch after cleavage. Curr Opin Cell Biol 51: 103-109, 2018.
53. Chen B, Jiang L, Zhong ML, Li JF, Li BS, Peng LJ, Dai YT, Cui BW, Yan TQ, Zhang WN, *et al*: Identification of fusion genes and characterization of transcriptome features in T-cell acute lymphoblastic leukemia. Proc Natl Acad Sci USA 115: 373-378, 2018.
54. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, Dimitriadoy S, Liu DL, Kantheti HS, Saghafinia S, *et al*: Oncogenic signaling pathways in The Cancer Genome Atlas. Cell 173: 321-337.e10, 2018.
55. Weng AP, Ferrando AA, Lee W, Morris JP IV, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT and Aster JC: Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306: 269-271, 2004.
56. Palomero T, Barnes KC, Real PJ, Glade Bender JL, Sulis ML, Murty VV, Colovai AI, Balbin M and Ferrando AA: CUTLL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to gamma-secretase inhibitors. Leukemia 20: 1279-1287, 2006.
57. De Bie J, Demeyer S, Alberti-Servera L, Geerdens E, Segers H, Broux M, De Keersmaecker K, Michaux L, Vandenberghe P, Voet T, *et al*: Single-cell sequencing reveals the origin and the order of mutation acquisition in T-cell acute lymphoblastic leukemia. Leukemia 32: 1358-1369, 2018.
58. Puente XS, Pinyol M, Quesada V, Conde L, Ordóñez GR, Villamor N, Escaramis G, Jares P, Beà S, González-Díaz M, *et al*: Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature 475: 101-105, 2011.
59. Fabbri G and Dalla-Favera R: The molecular pathogenesis of chronic lymphocytic leukaemia. Nat Rev Cancer 16: 145-162, 2016.
60. Karube K, Enjuanes A, Dlouhy I, Jares P, Martin-Garcia D, Nadeu F, Ordóñez GR, Rovira J, Clot G, Royo C, *et al*: Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets. Leukemia 32: 675-684, 2018.
61. González-Rincón J, Méndez M, Gómez S, García JF, Martín P, Bellas C, Pedrosa L, Rodríguez-Pinilla SM, Camacho FI, Quero C, *et al*: Unraveling transformation of follicular lymphoma to diffuse large B-cell lymphoma. PLoS One 14: e0212813, 2019.
62. Kridel R, Meissner B, Rogic S, Boyle M, Telenius A, Woolcock B, Gunawardana J, Jenkins C, Cochrane C, Ben-Neriah S, *et al*: Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. Blood 119: 1963-1971, 2012.
63. Robinson DR, Kalyana-Sundaram S, Wu YM, Shankar S, Cao X, Ateeq B, Asangani IA, Iyer M, Maher CA, Grasso CS, *et al*: Functionally recurrent rearrangements of the MAST kinase and Notch gene families in breast cancer. Nat Med 17: 1646-1651, 2011.
64. Wang K, Zhang Q, Li D, Ching K, Zhang C, Zheng X, Ozeck M, Shi S, Li X, Wang H, *et al*: PEST domain mutations in Notch receptors comprise an oncogenic driver segment in triple-negative breast cancer sensitive to a γ-secretase inhibitor. Clin Cancer Res 21: 1487-1496, 2015.
65. Robinson DR, Wu YM, Lonigro RJ, Vats P, Cobain E, Everett J, Cao X, Rabban E, Kumar-Sinha C, Raymond V, *et al*: Integrative clinical genomics of metastatic cancer. Nature 548: 297-303, 2017.
66. Westhoff B, Colaluca IN, D'Ario G, Donzelli M, Tosoni D, Volorio S, Pelosi G, Spaggiari L, Mazzarol G, Viale G, *et al*: Alterations of the Notch pathway in lung cancer. Proc Natl Acad Sci USA 106: 22293-22298, 2009.
67. Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, Proby CM, Leigh IM, Collisson EA, Gordon PB, Jakkula L, *et al*: Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. Proc Natl Acad Sci USA 108: 17761-17766, 2011.
68. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J, *et al*: Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 333: 1154-1157, 2011.
69. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, *et al*: The mutational landscape of head and neck squamous cell carcinoma. Science 333: 1157-1160, 2011.
70. Song Y, Li L, Ou Y, Gao Z, Li E, Li X, Zhang W, Wang J, Xu L, Zhou Y, *et al*: Identification of genomic alterations in oesophageal squamous cell cancer. Nature 509: 91-95, 2014.
71. Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ, Cagan A, Murai K, Mahbubani K, Stratton MR, *et al*: Somatic mutant clones colonize the human esophagus with age. Science 362: 911-917, 2018.
72. George J, Lim JS, Jang SJ, Cun Y, Ozretić L, Kong G, Leenders F, Lu X, Fernández-Cuesta L, Bosco G, *et al*: Comprehensive genomic profiles of small cell lung cancer. Nature 524: 47-53, 2015.
73. Ali SA, Justilien V, Jamieson L, Murray NR and Fields AP: Protein kinase Cι drives a NOTCH3-dependent stem-like phenotype in mutant KRAS lung adenocarcinoma. Cancer Cell 29: 367-378, 2016.
74. Bhagat TD, Zou Y, Huang S, Park J, Palmer MB, Hu C, Li W, Shenoy N, Giricz O, Choudhary G, *et al*: Notch pathway is activated via genetic and epigenetic alterations and is a therapeutic target in clear cell renal cancer. J Biol Chem 292: 837-846, 2017.
75. van Groningen T, Akogul N, Westerhout EM, Chan A, Hasselt NE, Zwijnenburg DA, Broekmans M, Stroeken P, Haneveld F, Hooijer GKJ, *et al*: A NOTCH feed-forward loop drives reprogramming from adrenergic to mesenchymal state in neuroblastoma. Nat Commun 10: 1530, 2019.

76. Ban J, Bennani-Baiti IM, Kauer M, Schaefer KL, Poremba C, Jug G, Schwentner R, Smrzka O, Muehlbacher K, Aryee DN and Kovar H: EWS-FLI1 suppresses NOTCH-activated p53 in Ewing's sarcoma. Cancer Res 68: 7100-7109, 2008.
77. Augert A, Eastwood E, Ibrahim AH, Wu N, Grunblatt E, Basom R, Liggitt D, Eaton KD, Martins R, Poirier JT, *et al*: Targeting NOTCH activation in small cell lung cancer through LSD1 inhibition. Sci Signal 12: pii: eaau2922, 2019.
78. Song Y, Zhang Y, Jiang H, Zhu Y, Liu L, Feng W, Yang L, Wang Y and Li M: Activation of Notch3 promotes pulmonary arterial smooth muscle cells proliferation via Hes1/p27Kip1 signaling pathway. FEBS Open Bio 5: 656-660, 2015.
79. Maraver A, Fernández-Marcos PJ, Herranz D, Muñoz-Martin M, Gomez-Lopez G, Cañamero M, Mulero F, Megías D, Sanchez-Carbayo M, Shen J, *et al*: Therapeutic effect of γ-secretase inhibition in KrasG12V-driven non-small cell lung carcinoma by derepression of DUSP1 and inhibition of ERK. Cancer Cell 22: 222-234, 2012.
80. Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, Caparros E, Buteau J, Brown K, Perkins SL, *et al*: Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med 13: 1203-1210, 2007.
81. Zhou X, Smith AJ, Waterhouse A, Blin G, Malaguti M, Lin CY, Osorno R, Chambers I and Lowell S: Hes1 desynchronizes differentiation of pluripotent cells by modulating STAT3 activity. Stem Cells 31: 1511-1122, 2013.
82. Weng MT, Tsao PN, Lin HL, Tung CC, Change MC, Chang YT, Wong JM and Wei SC: Hes1 increases the invasion ability of colorectal cancer cells via the STAT3-MMP14 pathway. PLoS One 10: e0144322, 2015.
83. Jin S, Mutvei AP, Chivukula IV, Andersson ER, Ramsköld D, Sandberg R, Lee KL, Kronqvist P, Mamaeva V, Ostling P, *et al*: Non-canonical Notch signaling activates IL-6/JAK/STAT signaling in breast tumor cells and is controlled by p53 and IKKα/IKKβ. Oncogene 32: 4892-4902, 2013.
84. Schreck KC, Taylor P, Marchionni L, Gopalakrishnan V, Bar EE, Gaiano N and Eberhart CG: The Notch target Hes1 directly modulates Gli1 expression and Hedgehog signaling: A potential mechanism of therapeutic resistance. Clin Cancer Res 16: 6060-6070, 2010.
85. Bonyadi Rad E, Hammerlindl H, Wels C, Popper U, Ravindran Menon D, Breiteneder H, Kitzwoegerer M, Hafner C, Herlyn M, Bergler H and Schaider H: Notch4 signaling induces a mesenchymal-epithelial-like transition in melanoma cells to suppress malignant behaviors. Cancer Res 76: 1690-1697, 2016.
86. Wang D, Xu J, Liu B, He X, Zhou L, Hu X, Qiao F, Zhang A, Xu X, Zhang H, *et al*: IL6 blockade potentiates the anti-tumor effects of γ-secretase inhibitors in Notch3-expressing breast cancer. Cell Death Differ 25: 330-339, 2018.
87. Hartman BH, Reh TA and Bermingham-McDonogh O: Notch signaling specifies prosensory domains via lateral induction in the developing mammalian inner ear. Proc Natl Acad Sci USA 107: 15792-15797, 2010.
88. Petrovic J, Formosa-Jordan P, Luna-Escalante JC, Abelló G, Ibañes M, Neves J and Giraldez F: Ligand-dependent Notch signaling strength orchestrates lateral induction and lateral inhibition in the developing inner ear. Development 141: 2313-2324, 2014.
89. Sun W, Gaykalova DA, Ochs MF, Mambo E, Arnaoutakis D, Liu Y, Loyo M, Agrawal N, Howard J, Li R, *et al*: Activation of the NOTCH pathway in head and neck cancer. Cancer Res 74: 1091-1104, 2014.
90. Turley SJ, Cremasco V and Astarita JL: Immunological hallmarks of stromal cells in the tumour microenvironment. Nat Rev Immunol 15: 669-682, 2015.
91. Valkenburg KC, de Groot AE and Pienta KJ: Targeting the tumour stroma to improve cancer therapy. Nat Rev Clin Oncol 15: 366-381, 2018.
92. Östman A and Corvigno S: Microvascular mural cells in cancer. Trends Cancer 4: 838-848, 2018.
93. Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, Bassez A, Decaluwé H, Pircher A, Van den Eynde K, *et al*: Phenotype molding of stromal cells in the lung tumor microenvironment. Nat Med 24: 1277-1289, 2018.
94. Bartoschek M, Oskolkov N, Bocci M, Lövrot J, Larsson C, Sommarin M, Madsen CD, Lindgren D, Pekar G, Karlsson G, *et al*: Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. Nat Commun 9: 5150, 2018.
95. Kalluri R: The biology and function of fibroblasts in cancer. Nat Rev Cancer 16: 582-598, 2016.
96. Altorki NK, Markowitz GJ, Gao D, Port JL, Saxena A, Stiles B, McGraw T and Mittal V: The lung microenvironment: An important regulator of tumour growth and metastasis. Nat Rev Cancer 19: 9-31, 2019.
97. Wang Z and Zöller M: Exosomes, metastases, and the miracle of cancer stem cell markers. Cancer Metastasis Rev 38: 259-295, 2019.
98. Katoh M: Canonical and non-canonical WNT signaling in cancer stem cells and their niches: Cellular heterogeneity, omics reprogramming, targeted therapy and tumor plasticity (Review). Int J Oncol 51: 1357-1369, 2017.
99. Dotto GP: Multifocal epithelial tumors and field cancerization: Stroma as a primary determinant. J Clin Invest 124: 1446-1453, 2014.
100. Shibue T and Weinberg RA: EMT, CSCs, and drug resistance: The mechanistic link and clinical implications. Nat Rev Clin Oncol 14: 611-629, 2017.
101. Schreiber RD, Old LJ and Smyth MJ: Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science 331: 1565-1570, 2011.
102. Fukumura D, Kloepper J, Amoozgar Z, Duda DG and Jain RK: Enhancing cancer immunotherapy using antiangiogenics: Opportunities and challenges. Nat Rev Clin Oncol 15: 325-340, 2018.
103. Robbins J, Blondel BJ, Gallahan D and Callahan R: Mouse mammary tumor gene int-3: A member of the Notch gene family transforms mammary epithelial cells. J Virol 66: 2594-2599, 1992.
104. Peters G, Lee AE and Dickson C: Concerted activation of two potential proto-oncogenes in carcinomas induced by mouse mammary tumour virus. Nature 320: 628-631, 1986.
105. Shackleford GM, MacArthur CA, Kwan HC and Varmus HE: Mouse mammary tumor virus infection accelerates mammary carcinogenesis in Wnt-1 transgenic mice by insertional activation of int-2/Fgf-3 and hst/Fgf-4. Proc Natl Acad Sci USA 90: 740-744, 1993.
106. Katoh M: WNT and FGF gene clusters (review). Int J Oncol 21: 1269-1273, 2002.
107. Lowther W, Wiley K, Smith GH and Callahan R: A new common integration site, Int7, for the mouse mammary tumor virus in mouse mammary tumors identifies a gene whose product has furin-like and thrombospondin-like sequences. J Virol 79: 10093-10096, 2005.
108. Theodorou V, Kimm MA, Boer M, Wessels L, Theelen W, Jonkers J and Hilkens J: MMTV insertional mutagenesis identifies genes, gene families and pathways involved in mammary cancer. Nat Genet 39: 759-769, 2007.
109. Zhan T, Rindtorff N and Boutros M: Wnt signaling in cancer. Oncogene 36: 1461-1473, 2017.
110. Morgan RG, Mortensson E and Williams AC: Targeting LGR5 in colorectal cancer: Therapeutic gold or too plastic? Br J Cancer 118: 1410-1418, 2018.
111. Estrach S, Ambler CA, Lo Celso C, Hozumi K and Watt FM: Jagged 1 is a beta-catenin target gene required for ectopic hair follicle formation in adult epidermis. Development 133: 4427-4438, 2006.
112. Ma L, Wang Y, Hui Y, Du Y, Chen Z, Feng H, Zhang S, Li N, Song J, Fang Y, *et al*: WNT/NOTCH pathway is essential for the maintenance and expansion of human MGE progenitors. Stem Cell Reports 12: 934-949, 2019.
113. Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, Ridgway RA, Seo SS, Spee B, Van Rooijen N, *et al*: Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Nat Med 18: 572-579, 2012.
114. Högström J, Heino S, Kallio P, Lähde M, Leppänen VM, Balboa D, Wiener Z and Alitalo K: Transcription factor PROX1 suppresses Notch pathway activation via the nucleosome remodeling and deacetylase complex in colorectal cancer stem-like cells. Cancer Res 78: 5820-5832, 2018.
115. Phng LK, Potente M, Leslie JD, Babbage J, Nyqvist D, Lobov I, Ondr JK, Rao S, Lang RA, Thurston G and Gerhardt H: Nrarp coordinates endothelial Notch and Wnt signaling to control vessel density in angiogenesis. Dev Cell 16: 70-82, 2009.
116. Liu W, Li H, Hong SH, Piszczek GP, Chen W and Rodgers GP: Olfactomedin 4 deletion induces colon adenocarcinoma in $Apc^{Min/+}$ mice. Oncogene 35: 5237-5247, 2016.
117. Giancotti FG: Mechanisms governing metastatic dormancy and reactivation. Cell 155: 750-764, 2013.

118. Katoh M: Multi-layered prevention and treatment of chronic inflammation, organ fibrosis and cancer associated with canonical WNT/β-catenin signaling activation (Review). Int J Mol Med 42: 713-725, 2018.
119. Dimri GP, Martinez JL, Jacobs JJ, Keblusek P, Itahana K, Van Lohuizen M, Campisi J, Wazer DE and Band V: The Bmi-1 oncogene induces telomerase activity and immortalizes human mammary epithelial cells. Cancer Res 62: 4736-4745, 2002.
120. De Jaime-Soguero A, Aulicino F, Ertaylan G, Griego A, Cerrato A, Tallam A, Del Sol A, Cosma MP and Lluis F: Wnt/Tcf1 pathway restricts embryonic stem cell cycle through activation of the Ink4/Arf locus. PLoS Genet 13: e1006682, 2017.
121. Srinivasan T, Walters J, Bu P, Than EB, Tung KL, Chen KY, Panarelli N, Milsom J, Augenlicht L, Lipkin SM and Shen X: NOTCH signaling regulates asymmetric cell fate of fast- and slow-cycling colon cancer-initiating cells. Cancer Res 76: 3411-3421, 2016.
122. Germann M, Xu H, Malaterre J, Sampurno S, Huyghe M, Cheasley D, Fre S and Ramsay RG: Tripartite interactions between Wnt signaling, Notch and Myb for stem/progenitor cell functions during intestinal tumorigenesis. Stem Cell Res 13: 355-366, 2014.
123. Mourao L, Jacquemin G, Huyghe M, Nawrocki WJ, Menssouri N, Servant N and Fre S: Lineage tracing of Notch1-expressing cells in intestinal tumours reveals a distinct population of cancer stem cells. Sci Rep 9: 888, 2019.
124. Jain RK: Normalizing tumor microenvironment to treat cancer: Bench to bedside to biomarkers. J Clin Oncol 31: 2205-2218, 2013.
125. De Palma M, Biziato D and Petrova TV: Microenvironmental regulation of tumour angiogenesis. Nat Rev Cancer 17: 457-474, 2017.
126. Yunus M, Jansson PJ, Kovacevic Z, Kalinowski DS and Richardson DR: Tumor-induced neoangiogenesis and receptor tyrosine kinases-Mechanisms and strategies for acquired resistance. Biochim Biophys Acta Gen Subj 1863: 1217-1225, 2019.
127. Potente M, Gerhardt H and Carmeliet P: Basic and therapeutic aspects of angiogenesis. Cell 146: 873-887, 2011.
128. Goel HL and Mercurio AM: VEGF targets the tumour cell. Nat Rev Cancer 13: 871-882, 2013.
129. Bridges E, Oon CE and Harris A: Notch regulation of tumor angiogenesis. Future Oncol 7: 569-588, 2011.
130. Simons M, Gordon E and Claesson-Welsh L: Mechanisms and regulation of endothelial VEGF receptor signalling. Nat Rev Mol Cell Biol 17: 611-625, 2016.
131. Wieland E, Rodriguez-Vita J, Liebler SS, Mogler C, Moll I, Herberich SE, Espinet E, Herpel E, Menuchin A, Chang-Claude J, *et al*: Endothelial Notch1 activity facilitates metastasis. Cancer Cell 31: 355-367, 2017.
132. Wohlfeil SA, Häfele V, Dietsch B, Schledzewski K, Winkler M, Zierow J, Leibing T, Mohammadi MM, Heineke J, Sticht C, *et al*: Hepatic endothelial Notch activation protects against liver metastasis by regulating endothelial-tumor cell adhesion independent of angiocrine signaling. Cancer Res 79: 598-610, 2019.
133. Radtke F, MacDonald HR and Tacchini-Cottier F: Regulation of innate and adaptive immunity by Notch. Nat Rev Immunol 13: 427-437, 2013.
134. Lobry C, Oh P, Mansour MR, Look AT and Aifantis I: Notch signaling: Switching an oncogene to a tumor suppressor. Blood 123: 2451-2459, 2014.
135. Amsen D, Helbig C and Backer RA: Notch in T cell differentiation: all things considered. Trends Immunol 36: 802-814, 2015.
136. Charbonnier LM, Wang S, Georgiev P, Sefik E and Chatila TA: Control of peripheral tolerance by regulatory T cell-intrinsic Notch signaling. Nat Immunol 16: 1162-1173, 2015.
137. Wang YC, He F, Feng F, Liu XW, Dong GY, Qin HY, Hu XB, Zheng MH, Liang L, Feng L, *et al*: Notch signaling determines the M1 versus M2 polarization of macrophages in antitumor immune responses. Cancer Res 70: 4840-4849, 2010.
138. Liu H, Wang J, Zhang M, Xuan Q, Wang Z, Lian X and Zhang Q: Jagged1 promotes aromatase inhibitor resistance by modulating tumor-associated macrophage differentiation in breast cancer patients. Breast Cancer Res Treat 166: 95-107, 2017.
139. Rashedi I, Gómez-Aristizábal A, Wang XH, Viswanathan S and Keating A: TLR3 or TLR4 activation enhances mesenchymal stromal cell-mediated Treg induction via Notch signaling. Stem Cells 35: 265-275, 2017.
140. Cahill EF, Tobin LM, Carty F, Mahon BP and English K: Jagged-1 is required for the expansion of CD4+ CD25+ FoxP3+ regulatory T cells and tolerogenic dendritic cells by murine mesenchymal stromal cells. Stem Cell Res Ther 6: 19, 2015.
141. Kared H, Adle-Biassette H, Foïs E, Masson A, Bach JF, Chatenoud L, Schneider E and Zavala F: Jagged2-expressing hematopoietic progenitors promote regulatory T cell expansion in the periphery through Notch signaling. Immunity 25: 823-834, 2006.
142. Ting HA, de Almeida Nagata D, Rasky AJ, Malinczak CA, Maillard IP, Schaller MA and Lukacs NW: Notch ligand Delta-like 4 induces epigenetic regulation of Treg cell differentiation and function in viral infection. Mucosal Immunol 11: 1524-1536, 2018.
143. Zhang L, Yu X, Zheng L, Zhang Y, Li Y, Fang Q, Gao R, Kang B, Zhang Q, Huang JY, *et al*: Lineage tracking reveals dynamic relationships of T cells in colorectal cancer. Nature 564: 268-272, 2018.
144. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, Ruas JL, Poellinger L, Lendahl U and Bondesson M: Hypoxia requires Notch signaling to maintain the undifferentiated cell state. Dev Cell 9: 617-628, 2005.
145. Katoh M: FGFR inhibitors: Effects on cancer cells, tumor microenvironment and whole-body homeostasis (Review). Int J Mol Med 38: 3-15, 2016.
146. Grazioli P, Felli MP, Screpanti I and Campese AF: The mazy case of Notch and immunoregulatory cells. J Leukoc Biol 102: 361-368, 2017.
147. Katoh M: Genomic testing, tumor microenvironment and targeted therapy of Hedgehog-related human cancers. Clin Sci (Lond) 133: 953-970, 2019.
148. Yang Z, Qi Y, Lai N, Zhang J, Chen Z, Liu M, Zhang W, Luo R and Kang S: Notch1 signaling in melanoma cells promoted tumor-induced immunosuppression via upregulation of TGF-β1. J Exp Clin Cancer Res 37: 1, 2018.
149. Mao L, Zhao ZL, Yu GT, Wu L, Deng WW, Li YC, Liu JF, Bu LL, Liu B, Kulkarni AB, *et al*: γ-Secretase inhibitor reduces immunosuppressive cells and enhances tumour immunity in head and neck squamous cell carcinoma. Int J Cancer 142: 999-1009, 2018.
150. El-Khoueiry AB, Desai J, Iyer SP, Gadgeel SM, Ramalingam SS, Horn L, LoRusso P, Bajaj G, Kollia G, Qi Z, *et al*: A phase I study of AL101, a pan-NOTCH inhibitor, in patients (pts) with locally advanced or metastatic solid tumors. J Clin Oncol 36 (15 Suppl): S2515, 2018.
151. Massard C, Azaro A, Soria JC, Lassen U, Le Tourneau C, Sarker D, Smith C, Ohnmacht U, Oakley G, Patel BKR, *et al*: First-in-human study of LY3039478, an oral Notch signaling inhibitor in advanced or metastatic cancer. Ann Oncol 29: 1911-1917, 2018.
152. Habets RA, de Bock CE, Serneels L, Lodewijckx I, Verbeke D, Nittner D, Narlawar R, Demeyer S, Dooley J, Liston A, *et al*: Safe targeting of T cell acute lymphoblastic leukemia by pathology-specific NOTCH inhibition. Sci Transl Med 11: pii: eaau6246, 2019.
153. Messersmith WA, Shapiro GI, Cleary JM, Jimeno A, Dasari A, Huang B, Shaik MN, Cesari R, Zheng X, Reynolds JM, *et al*: A phase I, dose-finding study in patients with advanced solid malignancies of the oral γ-secretase inhibitor PF-03084014. Clin Cancer Res 21: 60-67, 2015.
154. Kummar S, O'Sullivan Coyne G, Do KT, Turkbey B, Meltzer PS, Polley E, Choyke PL, Meehan R, Vilimas R, Horneffer Y, *et al*: Clinical activity of the γ-secretase inhibitor PF-03084014 in adults with desmoid tumors (aggressive fibromatosis). J Clin Oncol 35: 1561-1569, 2017.
155. Tolcher AW, Messersmith WA, Mikulski SM, Papadopoulos KP, Kwak EL, Gibbon DG, Patnaik A, Falchook GS, Dasari A, Shapiro GI, *et al*: Phase I study of RO4929097, a gamma secretase inhibitor of Notch signaling, in patients with refractory metastatic or locally advanced solid tumors. J Clin Oncol 30: 2348-2353, 2012.
156. Strosberg JR, Yeatman T, Weber J, Coppola D, Schell MJ, Han G, Almhanna K, Kim R, Valone T, Jump H and Sullivan D: A phase II study of RO4929097 in metastatic colorectal cancer. Eur J Cancer 48: 997-1003, 2012.
157. Jordan NV, Bardia A, Wittner BS, Benes C, Ligorio M, Zheng Y, Yu M, Sundaresan TK, Licausi JA, Desai R, *et al*: HER2 expression identifies dynamic functional states within circulating breast cancer cells. Nature 537: 102-106, 2016.
158. Diluvio G, Del Gaudio F, Giuli MV, Franciosa G, Giuliani E, Palermo R, Besharat ZM, Pignataro MG, Vacca A, d'Amati G, *et al*: NOTCH3 inactivation increases triple negative breast cancer sensitivity to gefitinib by promoting EGFR tyrosine dephosphorylation and its intracellular arrest. Oncogenesis 7: 42, 2018.

159. Yao J, Qian C, Shu T, Zhang X, Zhao Z and Liang Y: Combination treatment of PD98059 and DAPT in gastric cancer through induction of apoptosis and downregulation of WNT/β-catenin. Cancer Biol Ther 14: 833-839, 2013.
160. Smith DC, Eisenberg PD, Manikhas G, Chugh R, Gubens MA, Stagg RJ, Kapoun AM, Xu L, Dupont J and Sikic B: A phase I dose escalation and expansion study of the anticancer stem cell agent demcizumab (anti-DLL4) in patients with previously treated solid tumors. Clin Cancer Res 20: 6295-6303, 2014.
161. Chiorean EG, LoRusso P, Strother RM, Diamond JR, Younger A, Messersmith WA, Adriaens L, Liu L, Kao RJ, DiCioccio AT, *et al*: A phase I first-in-human study of enoticumab (REGN421), a fully human Delta-like ligand 4 (DLL4) monoclonal antibody, in patients with advanced solid tumors. Clin Cancer Res 21: 2695-2703, 2015.
162. Falchook GS, Dowlati A, Naing A, Gribbin MJ, Jenkins DW, Chang LL, Lai DW and Smith DC: Phase I study of MEDI0639 in patients with advanced solid tumors. J Clin Oncol 33 (15 Suppl): S3024, 2015.
163. Ferrarotto R, Eckhardt G, Patnaik A, LoRusso P, Faoro L, Heymach JV, Kapoun AM, Xu L and Munster P: A phase I dose-escalation and dose-expansion study of brontictuzumab in subjects with selected solid tumors. Ann Oncol 29: 1561-1568, 2018.
164. Yen WC, Fischer MM, Axelrod F, Bond C, Cain J, Cancilla B, Henner WR, Meisner R, Sato A, Shah J, *et al*: Targeting Notch signaling with a Notch2/Notch3 antagonist (tarextumab) inhibits tumor growth and decreases tumor-initiating cell frequency. Clin Cancer Res 21: 2084-2095, 2015.
165. Smith DC, Chugh R, Patnaik A, Papadopoulos KP, Wang M, Kapoun AM, Xu L, Dupont J, Stagg RJ and Tolcher A: A phase 1 dose escalation and expansion study of Tarextumab (OMP-59R5) in patients with solid tumors. Invest New Drugs 37: 722-730, 2019.
166. Beck A, Goetsch L, Dumontet C and Corvaïa N: Strategies and challenges for the next generation of antibody-drug conjugates. Nat Rev Drug Discov 16: 315-337, 2017.
167. Lambert JM and Berkenblit A: Antibody-Drug conjugates for cancer treatment. Annu Rev Med 69: 191-207, 2018.
168. Carter PJ and Lazar GA: Next generation antibody drugs: Pursuit of the 'high-hanging fruit'. Nat Rev Drug Discov 17: 197-223, 2018.
169. June CH, O'Connor RS, Kawalekar OU, Ghassemi S and Milone MC: CAR T cell immunotherapy for human cancer. Science 359: 1361-1365, 2018.
170. Saunders LR, Bankovich AJ, Anderson WC, Aujay MA, Bheddah S, Black K, Desai R, Escarpe PA, Hampl J, Laysang A, *et al*: A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. Sci Transl Med 7: 302ra136, 2015.
171. Rudin CM, Pietanza MC, Bauer TM, Ready N, Morgensztern D, Glisson BS, Byers LA, Johnson ML, Burris HA III, Robert F, *et al*: Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: A first-in-human, first-in-class, open-label, phase 1 study. Lancet Oncol 18: 42-51, 2017.
172. Carbone DP, Morgensztern D, Le Moulec S, Santana-Davila R, Ready N, Hann CL, Glisson BS, Dowlati A, Rudin CM, Lally S, *et al*: Efficacy and safety of rovalpituzumab tesirine in patients With DLL3-expressing, ≥ 3rd line small cell lung cancer: Results from the phase 2 TRINITY study. J Clin Oncol 36 (15 Suppl): S8507, 2018.
173. Rosen LS, Wesolowski R, Baffa R, Liao KH, Hua SY, Gibson BL, Pirie-Shepherd S and Tolcher AW: A phase I, dose-escalation study of PF-06650808, an anti-Notch3 antibody-drug conjugate, in patients with breast cancer and other advanced solid tumors. Invest New Drugs, Mar 18, 2019 (Epub ahead of print).
174. Smit MAD, Borghaei H, TOwonikoko TK, Hummel HD, Johnson ML, Champiat S, Salgia R, Udagawa H, Boyer MJ and Govindan R: Phase 1 study of AMG 757, a half-life extended bispecific T cell engager (BiTE) antibody construct targeting DLL3, in patients with small cell lung cancer (SCLC). J Clin Oncol 37 (15 Suppl): TPS8577, 2019.
175. Li Y, Hickson JA, Ambrosi DJ, Haasch DL, Foster-Duke KD, Eaton LJ, DiGiammarino EL, Panchal SC, Jiang F, Mudd SR, *et al*: ABT-165, a dual variable domain immunoglobulin (DVD-Ig) targeting DLL4 and VEGF, demonstrates superior efficacy and favorable safety profiles in preclinical models. Mol Cancer Ther 17: 1039-1050, 2018.
176. Jimeno A, Moore KN, Gordon M, Chugh R, Diamond JR, Aljumaily R, Mendelson D, Kapoun AM, Xu L, Stagg R and Smith DC: A first-in-human phase 1a study of the bispecific anti-DLL4/anti-VEGF antibody navicixizumab (OMP-305B83) in patients with previously treated solid tumors. Invest New Drugs 37: 461-472, 2019.
177. Hu S, Fu W, Li T, Yuan Q, Wang F, Lv G, Lv Y, Fan X, Shen Y, Lin F, *et al*: Antagonism of EGFR and Notch limits resistance to EGFR inhibitors and radiation by decreasing tumor-initiating cell frequency. Sci Transl Med 9: pii: eaag0339, 2017.
178. Fu W, Lei C, Yu Y, Liu S, Li T, Lin F, Fan X, Shen Y, Ding M, Tang Y, *et al*: EGFR/Notch antagonists enhance the response to inhibitors of the PI3K-Akt pathway by decreasing tumor-initiating cell frequency. Clin Cancer Res 25: 2835-2847, 2019.
179. Byers LA, Chiappori A and Smit MAD: Phase 1 study of AMG 119, a chimeric antigen receptor (CAR) T cell therapy targeting DLL3, in patients with relapsed/refractory small cell lung cancer (SCLC). J Clin Oncol 37 (15 Suppl): TPS8576, 2019.
180. Puca L, Gavyert K, Sailer V, Conteduca V, Dardenne E, Sigouros M, Isse K, Kearney M, Vosoughi A, Fernandez L, *et al*: Delta-like protein 3 expression and therapeutic targeting in neuroendocrine prostate cancer. Sci Transl Med 11: pii: eaav0891, 2019.
181. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, Lin Y, Braunschweig I, Hill BT, Timmerman JM, *et al*: Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): A single-arm, multicentre, phase 1-2 trial. Lancet Oncol 20: 31-42, 2019.
182. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, Jäger U, Jaglowski S, Andreadis C, Westin JR, *et al*: Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 380: 45-56, 2019.
183. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, Gökbuget N, O'Brien S, Wang K, Wang T, *et al*: Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med 375: 740-753, 2016.
184. Horwitz S, O'Connor OA, Pro B, Illidge T, Fanale M, Advani R, Bartlett NL, Christensen JH, Morschhauser F, Domingo-Domenech E, *et al*: Brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma (ECHELON-2): A global, double-blind, randomised, phase 3 trial. Lancet 393: 229-240, 2019.
185. Tilly H, Morschhauser F, Bartlett NL, Mehta A, Salles G, Haioun C, Munoz J, Chen AI, Kolibaba K, Lu D, *et al*: Polatuzumab vedotin in combination with immunochemotherapy in patients with previously untreated diffuse large B-cell lymphoma: An open-label, non-randomised, phase 1b-2 study. Lancet Oncol 20: 998-1010, 2019.
186. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh DY, Diéras V, Guardino E, *et al*: Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 367: 1783-1791, 2012.
187. Doi T, Shitara K, Naito Y, Shimomura A, Fujiwara Y, Yonemori K, Shimizu C, Shimoi T, Kuboki Y, Matsubara N, *et al*: Safety, pharmacokinetics, and antitumour activity of trastuzumab deruxtecan (DS-8201), a HER2-targeting antibody-drug conjugate, in patients with advanced breast and gastric or gastro-oesophageal tumours: A phase 1 dose-escalation study. Lancet Oncol 18: 1512-1522, 2017.
188. Banerji U, van Herpen CML, Saura C, Thistlethwaite F, Lord S, Moreno V, Macpherson IR, Boni V, Rolfo C, de Vries EGE, *et al*: Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: A phase 1 dose-escalation and dose-expansion study. Lancet Oncol 20: 1124-1135, 2019.
189. Lassman AB, van den Bent MJ, Gan HK, Reardon DA, Kumthekar P, Butowski N, Lwin Z, Mikkelsen T, Nabors LB, Papadopoulos KP, *et al*: Safety and efficacy of depatuxizumab mafodotin + temozolomide in patients with EGFR-amplified, recurrent glioblastoma: Results from an international phase I multicenter trial. Neuro Oncol 21: 106-114, 2019.
190. Moore KN, Martin LP, O'Malley DM, Matulonis UA, Konner JA, Perez RP, Bauer TM, Ruiz-Soto R and Birrer MJ: Safety and activity of mirvetuximab soravtansine (IMGN853), a folate receptor alpha-targeting antibody-drug conjugate, in platinum-resistant ovarian, fallopian tube, or primary peritoneal cancer: A phase I expansion study. J Clin Oncol 35: 1112-1118, 2017.

191. Challita-Eid PM, Satpayev D, Yang P, An Z, Morrison K, Shostak Y, Raitano A, Nadell R, Liu W, Lortie DR, *et al*: Enfortumab vedotin antibody-drug conjugate targeting Nectin-4 is a highly potent therapeutic agent in multiple preclinical cancer models. Cancer Res 76: 3003-3013, 2016.
192. Bardia A, Mayer IA, Vahdat LT, Tolaney SM, Isakoff SJ, Diamond JR, O'Shaughnessy J, Moroose RL, Santin AD, Abramson VG, *et al*: Sacituzumab govitecan-hziy in refractory metastatic triple-negative breast cancer. N Engl J Med 380: 741-751, 2019.
193. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, Liedtke M, Rosenblatt J, Maus MV, Turka A, *et al*: Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med 380: 1726-1737, 2019.
194. Merlino G, Fiascarelli A, Bigioni M, Bressan A, Carrisi C, Bellarosa D, Salerno M, Bugianesi R, Manno R, Bernadó Morales C, *et al*: MEN1309/OBT076, a first-in-class antibody-drug conjugate targeting CD205 in solid tumors. Mol Cancer Ther 18: 1533-1543, 2019.
195. Zhan X, Wang B, Li Z, Li J, Wang H, Chen L, Jiang H, Wu M, Xiao J, Peng X, *et al*: Phase I trial of Claudin 18.2-specific chimeric antigen receptor T cells for advanced gastric and pancreatic adenocarcinoma. J Clin Oncol 37 (15 Suppl): S2509, 2019.
196. García-Alonso S, Ocaña A and Pandiella A: Resistance to antibody-drug conjugates. Cancer Res 78: 2159-2165, 2018.
197. Shah NN and Fry TJ: Mechanisms of resistance to CAR T cell therapy. Nat Rev Clin Oncol 16: 372-385, 2019.
198. Singal G, Miller PG, Agarwala V, Li G, Kaushik G, Backenroth D, Gossai A, Frampton GM, Torres AZ, Lehnert EM, *et al*: Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. JAMA 321: 1391-1399, 2019.
199. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, *et al*: Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 51: 202-206, 2019.
200. Rubinstein JC, Nicolson NG and Ahuja N: Next-generation sequencing in the management of gastric and esophageal cancers. Surg Clin North Am 99: 511-527, 2019.
201. Hirsch FR, Suda K, Wiens J and Bunn PA Jr: New and emerging targeted treatments in advanced non-small-cell lung cancer. Lancet 388: 1012-1024, 2016.
202. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, Plodkowski A, Long N, Sauter JL, Rekhtman N, *et al*: Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. J Clin Oncol 36: 633-641, 2018.
203. Offin M, Rizvi H, Tenet M, Ni A, Sanchez-Vega F, Li BT, Drilon A, Kris MG, Rudin CM, Schultz N, *et al*: Tumor mutation burden and efficacy of EGFR-tyrosine kinase inhibitors in patients with EGFR-mutant lung cancers. Clin Cancer Res 25: 1063-1069, 2019.
204. Buchhalter I, Rempel E, Endris V, Allgäuer M, Neumann O, Volckmar AL, Kirchner M, Leichsenring J, Lier A, von Winterfeld M, *et al*: Size matters: Dissecting key parameters for panel-based tumor mutational burden analysis. Int J Cancer 144: 848-858, 2019.
205. Schneider G: Automating drug discovery. Nat Rev Drug Discov 17: 97-113, 2018.
206. Katoh M: Fibroblast growth factor receptors as treatment targets in clinical oncology. Nat Rev Clin Oncol 16: 105-122, 2019.
207. Somashekhar SP, Sepúlveda MJ, Puglielli S, Norden AD, Shortliffe EH, Rohit Kumar C, Rauthan A, Arun Kumar N, Patil P, Rhee K, *et al*: Watson for oncology and breast cancer treatment recommendations: Agreement with an expert multidisciplinary tumor board. Ann Oncol 29: 418-423, 2018.
208. Paley S and Karp PD: The MultiOmics explainer: Explaining omics results in the context of a pathway/genome database. BMC Bioinformatics 20: 399, 2019.
209. Cheng JZ, Ni D, Chou YH, Qin J, Tiu CM, Chang YC, Huang CS, Shen D and Chen CM: Computer-aided diagnosis with deep learning architecture: Applications to breast lesions in US images and pulmonary nodules in CT scans. Sci Rep 6: 24454, 2016.
210. Esteva A, Kuprel B, Novoa RA, Ko J, Swetter SM, Blau HM and Thrun S: Dermatologist-level classification of skin cancer with deep neural networks. Nature 542: 115-118, 2017.
211. Ardila D, Kiraly AP, Bharadwaj S, Choi B, Reicher JJ, Peng L, Tse D, Etemadi M, Ye W, Corrado G, *et al*: End-to-end lung cancer screening with three-dimensional deep learning on low-dose chest computed tomography. Nat Med 25: 954-961, 2019.
212. Dascalu A and David EO: Skin cancer detection by deep learning and sound analysis algorithms: A prospective clinical study of an elementary dermoscope. EBioMedicine 43: 107-113, 2019.
213. Mori Y, Kudo SE, Misawa M, Saito Y, Ikematsu H, Hotta K, Ohtsuka K, Urushibara F, Kataoka S, Ogawa Y, *et al*: Real-time use of artificial intelligence in identification of diminutive polyps during colonoscopy: A prospective study. Ann Intern Med 169: 357-366, 2018.
214. Aboutalib SS, Mohamed AA, Berg WA, Zuley ML, Sumkin JH and Wu S: Deep learning to distinguish recalled but benign mammography images in breast cancer screening. Clin Cancer Res 24: 5902-5909, 2018.
215. Coudray N, Ocampo PS, Sakellaropoulos T, Narula N, Snuderl M, Fenyö D, Moreira AL, Razavian N and Tsirigos A: Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. Nat Med 24: 1559-1567, 2018.
216. Kather JN, Pearson AT, Halama N, Jäger D, Krause J, Loosen SH, Marx A, Boor P, Tacke F, Neumann UP, *et al*: Deep learning can predict microsatellite instability directly from histology in gastrointestinal cancer. Nat Med 25: 1054-1056, 2019.
217. Campanella G, Hanna MG, Geneslaw L, Miraflor A, Werneck Krauss Silva V, Busam KJ, Brogi E, Reuter VE, Klimstra DS and Fuchs TJ: Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. Nat Med 25: 1301-1309, 2019.
218. Hekler A, Utikal JS, Enk AH, Solass W, Schmitt M, Klode J, Schadendorf D, Sondermann W, Franklin C, Bestvater F, *et al*: Deep learning outperformed 11 pathologists in the classification of histopathological melanoma images. Eur J Cancer 118: 91-96, 2019.
219. Topol EJ: High-performance medicine: The convergence of human and artificial intelligence. Nat Med 25: 44-56, 2019.
220. Zheng H, Bae Y, Kasimir-Bauer S, Tang R, Chen J, Ren G, Yuan M, Esposito M, Li W, Wei Y, *et al*: Therapeutic antibody targeting tumor- and osteoblastic niche-derived Jagged1 sensitizes bone metastasis to chemotherapy. Cancer Cell 32: 731-747. e6, 2017.
221. Li DD, Zhao CH, Ding HW, Wu Q, Ren TS, Wang J, Chen CQ and Zhao QC: A novel inhibitor of ADAM17 sensitizes colorectal cancer cells to 5-Fluorouracil by reversing Notch and epithelial-mesenchymal transition in vitro and in vivo. Cell Prolif 51: e12480, 2018.
222. Weber D, Lehal R, Frismantas V, Bourquin J, Bauer M, Murone M and Radtke F: 411P-Pharmacological activity of CB-103-an oral pan-NOTCH inhibitor with a novel mode of action. Ann Oncol 28 (Suppl 5): v122-v141, 2017.
223. Moellering RE, Cornejo M, Davis TN, Del Bianco C, Aster JC, Blacklow SC, Kung AL, Gilliland DG, Verdine GL and Bradner JE: Direct inhibition of the NOTCH transcription factor complex. Nature 462: 182-188, 2009.
224. Sano R, Krytska K, Larmour CE, Raman P, Martinez D, Ligon GF, Lillquist JS, Cucchi U, Orsini P, Rizzi S, *et al*: An antibody-drug conjugate directed to the ALK receptor demonstrates efficacy in preclinical models of neuroblastoma. Sci Transl Med 11: pii: eaau9732, 2019.
225. Boshuizen J, Koopman LA, Krijgsman O, Shahrabi A, van den Heuvel EG, Ligtenberg MA, Vredevoogd DW, Kemper K, Kuilman T, Song JY, *et al*: Cooperative targeting of melanoma heterogeneity with an AXL antibody-drug conjugate and BRAF/MEK inhibitors. Nat Med 24: 203-212, 2018.
226. Tao Y, Wang R, Lai Q, Wu M, Wang Y, Jiang X, Zeng L, Zhou S, Li Z, Yang T, *et al*: Targeting of DDR1 with antibody-drug conjugates has antitumor effects in a mouse model of colon carcinoma. Mol Oncol 13: 1855-1873, 2019.
227. Sommer A, Kopitz C, Schatz CA, Nising CF, Mahlert C, Lerchen HG, Stelte-Ludwig B, Hammer S, Greven S, Schuhmacher J, *et al*: Preclinical efficacy of the auristatin-based antibody-drug conjugate BAY 1187982 for the treatment of FGFR2-positive solid tumors. Cancer Res 76: 6331-6339, 2016.
228. Surguladze D, Pennello A, Ren X, Mack T, Rigby A, Balderes P, Navarro E, Amaladas N, Eastman S, Topper M, *et al*: LY3076226, a novel anti-FGFR3 antibody drug conjugate exhibits potent and durable anti-tumor activity in tumor models harboring FGFR3 mutations or fusions. Cancer Res 79 (13 Suppl): S4835, 2019.

229. Rudra-Ganguly N, Challita-Eid PM, Lowe C, Mattie M, Moon SJ, Mendelsohn BA, Leavitt M, Virata C, AVerlinsky A, Capo L, *et al*: AGS62P1, a novel site-specific antibody drug conjugate targeting FLT3 exhibits potent anti-tumor activity regardless of FLT3 kinase activation status. Cancer Res 76 (14 Suppl): S574, 2016.
230. Avilés P, Domínguez JM, Guillén MJ, Muñoz-Alonso MJ, Mateo C, Rodriguez-Acebes R, Molina-Guijarro JM, Francesch A, Martínez-Leal JF, Munt S, *et al*: MI130004, a novel antibody-drug conjugate combining trastuzumab with a molecule of marine origin, shows outstanding in iivo activity against HER2-expressing tumors. Mol Cancer Ther 17: 786-794, 2018.
231. Koganemaru S, Kuboki Y, Koga Y, Kojima T, Yamauchi M, Maeda N, Kagari T, Hirotani K, Yasunaga M, Matsumura Y and Doi T: U3-1402, a novel HER3-targeting antibody-drug conjugate, for the treatment of colorectal cancer. Mol Cancer Ther 18: 2043-2050, 2019.
232. Abrams T, Connor A, Fanton C, Cohen SB, Huber T, Miller K, Hong EE, Niu X, Kline J, Ison-Dugenny M, *et al*: Preclinical antitumor activity of a novel anti-c-KIT antibody-drug conjugate against mutant and wild-type c-KIT-positive solid tumors. Clin Cancer Res 24: 4297-4308, 2018.
233. Strickler JH, Weekes CD, Nemunaitis J, Ramanathan RK, Heist RS, Morgensztern D, Angevin E, Bauer TM, Yue H, Motwani M, *et al*: First-in-human phase I, dose-escalation and -expansion study of telisotuzumab vedotin, an antibody-drug conjugate targeting c-Met, in patients with advanced solid tumors. J Clin Oncol 36: 3298-3306, 2018.
234. Sachdev JC, Maitland ML, Sharma M, Moreno V, Boni V, Kummar S, Stringer-Reasor EM, Forero-Torres A, Lakhani NJ, Gibson B, *et al*: PF-06647020 (PF-7020), an antibody-drug conjugate (ADC) targeting protein tyrosine kinase 7 (PTK7), in patients (pts) with advanced solid tumors: Results of a phase I dose escalation and expansion study. J Clin Oncol 36 (15-Suppl): S5565, 2018.
235. Nguyen M, Miyakawa S, Kato J, Mori T, Arai T, Armanini M, Gelmon K, Yerushalmi R, Leung S, Gao D, *et al*: Preclinical efficacy and safety assessment of an antibody-drug conjugate targeting the c-RET proto-oncogene for breast carcinoma. Clin Cancer Res 21: 5552-5562, 2015.
236. Yao HP, Feng L, Suthe SR, Chen LH, Weng TH, Hu CY, Jun ES, Wu ZG, Wang WL, Kim SC, *et al*: Therapeutic efficacy, pharmacokinetic profiles, and toxicological activities of humanized antibody-drug conjugate Zt/g4-MMAE targeting RON receptor tyrosine kinase for cancer therapy. J Immunother Cancer 7: 75, 2019.
237. Berger C, Sommermeyer D, Hudecek M, Berger M, Balakrishnan A, Paszkiewicz PJ, Kosasih PL, Rader C and Riddell SR: Safety of targeting ROR1 in primates with chimeric antigen receptor-modified T cells. Cancer Immunol Res 3: 206-216, 2015.
238. Trudel S, Lendvai N, Popat R, Voorhees PM, Reeves B, Libby EN, Richardson PG, Hoos A, Gupta I, Bragulat V, *et al*: Antibody-drug conjugate, GSK2857916, in relapsed/refractory multiple myeloma: An update on safety and efficacy from dose expansion phase I study. Blood Cancer J 9: 37, 2019.
239. Godwin CD, Gale RP and Walter RB: Gemtuzumab ozogamicin in acute myeloid leukemia. Leukemia 31: 1855-1868, 2017.
240. Socinski MA, Kaye FJ, Spigel DR, Kudrik FJ, Ponce S, Ellis PM, Majem M, Lorigan P, Gandhi L, Gutierrez ME, *et al*: Phase 1/2 study of the CD56-targeting antibody-drug conjugate lorvotuzumab mertansine (IMGN901) in combination with carboplatin/etoposide in small-cell lung cancer patients with extensive-stage disease. Clin Lung Cancer 18: 68-76.e2, 2017.
241. de Bono JS, Concin N, Hong DS, Thistlethwaite FC, Machiels JP, Arkenau HT, Plummer R, Jones RH, Nielsen D, Windfeld K, *et al*: Tisotumab vedotin in patients with advanced or metastatic solid tumours (InnovaTV 201): A first-in-human, multicentre, phase 1-2 trial. Lancet Oncol 20: 383-393, 2019.
242. Dotan E, Cohen SJ, Starodub AN, Lieu CH, Messersmith WA, Simpson PS, Guarino MJ, Marshall JL, Goldberg RM, Hecht JR, *et al*: Phase I/II trial of labetuzumab govitecan (anti-CEACAM5/SN-38 antibody-drug conjugate) in patients with refractory or relapsing metastatic colorectal cancer. J Clin Oncol 35: 3338-3346, 2017.
243. Zhu G, Foletti D, Liu X, Ding S, Melton Witt J, Hasa-Moreno A, Rickert M, Holz C, Aschenbrenner L, Yang AH, *et al*: Targeting CLDN18.2 by CD3 bispecific and ADC modalities for the treatments of gastric and pancreatic cancer. Sci Rep 9: 8420, 2019.
244. Bhakta S, Crocker LM, Chen Y, Hazen M, Schutten MM, Li D, Kuijl C, Ohri R, Zhong F, Poon KA, *et al*: An anti-GDNF family receptor alpha 1 (GFRA1) antibody-drug conjugate for the treatment of hormone receptor-positive breast cancer. Mol Cancer Ther 17: 638-649, 2018.
245. Ott PA, Pavlick AC, Johnson DB, Hart LL, Infante JR, Luke JJ, Lutzky J, Rothschild NE, Spitler LE, Cowey CL, *et al*: A phase 2 study of glembatumumab vedotin, an antibody-drug conjugate targeting glycoprotein NMB, in patients with advanced melanoma. Cancer 125: 1113-1123, 2019.
246. Gong X, Azhdarinia A, Ghosh SC, Xiong W, An Z, Liu Q and Carmon KS: LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence. Mol Cancer Ther 15: 1580-1590, 2016.
247. Purcell JW, Tanlimco SG, Hickson J, Fox M, Sho M, Durkin L, Uziel T, Powers R, Foster K, McGonigal T, *et al*: LRRC15 is a novel mesenchymal protein and stromal target for antibody-drug conjugates. Cancer Res 78: 4059-4072, 2018.
248. Willuda J, Linden L, Lerchen HG, Kopitz C, Stelte-Ludwig B, Pena C, Lange C, Golfier S, Kneip C, Carrigan PE, *et al*: Preclinical antitumor efficacy of BAY 1129980-a novel auristatin-based anti-C4.4A (LYPD3) antibody-drug conjugate for the treatment of non-small cell lung cancer. Mol Cancer Ther 16: 893-904, 2017.
249. Golfier S, Kopitz C, Kahnert A, Heisler I, Schatz CA, Stelte-Ludwig B, Mayer-Bartschmid A, Unterschemmann K, Bruder S, Linden L, *et al*: Anetumab ravtansine: A novel mesothelin-targeting antibody-drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. Mol Cancer Ther 13: 1537-1548, 2014.
250. Banerjee S, Oza AM, Birrer MJ, Hamilton EP, Hasan J, Leary A, Moore KN, Mackowiak-Matejczyk B, Pikiel J, Ray-Coquard I, *et al*: Anti-NaPi2b antibody-drug conjugate lifastuzumab vedotin (DNIB0600A) compared with pegylated liposomal doxorubicin in patients with platinum-resistant ovarian cancer in a randomized, open-label, phase II study. Ann Oncol 29: 917-923, 2018.
251. Sussman D, Smith LM, Anderson ME, Duniho S, Hunter JH, Kostner H, Miyamoto JB, Nesterova A, Westendorf L, Van Epps HA, *et al*: SGN-LIV1A: A novel antibody-drug conjugate targeting LIV-1 for the treatment of metastatic breast cancer. Mol Cancer Ther 13: 2991-3000, 2014.