Targeting the DLL/Notch Signaling Pathway in Cancer: Challenges and Advances in Clinical Development

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ABSTRACT

The DLL/Notch signaling pathway plays an important role in cancer as a key driver in maintaining cancer stemness and inducing tumor angiogenesis. Many different types of DLL/Notch inhibitors have been developed and explored in clinical trials for cancer treatment, including small-molecule compounds to inhibit gamma-secretase and antibodies targeting Notch ligands or receptors. Despite promising efficacy of these inhibitors in preclinical studies, the overall clinical outcomes have been insufficient to advance to the next stage of clinical development primarily due to safety concerns or modest efficacy. To overcome the narrow therapeutic window of DLL/Notch inhibitors, diverse strategies for improving the balance between the safety and efficacy are currently being explored. Here, we review the clinical perspective and potential of DLL/Notch inhibitors as anticancer agents based on recent results from multiple clinical studies. An antibody specifically targeting Notch ligands or receptors may offer a better approach to reduce concerns about toxicity derived from broad-spectrum DLL/Notch blockers. In addition, combination therapy with an angiogenesis inhibitor targeting VEGF could be a better option for increasing anticancer efficacy. Taken together, the results of clinical trials suggest a bispecific antibody blocking the DLL/Notch and VEGF/VEGFR signaling pathways as a promising approach for effective anticancer treatment.

Introduction

The DLL/Notch signaling pathway is highly evolutionarily conserved and plays an important role in embryonic development by regulating cell proliferation and differentiation and cell-fate determination in multiple tissues and cell types (1). Notch receptors are type-1 transmembrane proteins with an extracellular domain consisting of EGF-like repeats and a negative regulatory region, including three LIN12/Notch repeats (LNR) and the juxtamembrane heterodimerization domain (**Fig. 1A**). Notch signaling is activated by the binding of a Notch ligand to a Notch receptor, which is generally mediated by cell–cell contact. The two families of Notch ligands, Delta-like ligand (DLL1, DLL3, and DLL4) and Jagged (JAG1 and JAG2), are transmembrane proteins consisting of a Delta/Serrate/LAG-2 (DSL) domain and EGF repeats (**Fig. 1A**). The DLL/Notch signaling pathway induces and regulates cell–cell communication by intercellular interaction and activation (**Fig. 1B**).

Notch receptor activation upon ligand binding involves two different cleavage processes mediated by members of the A Disintegrin and Metalloprotease (ADAM) family, specifically ADAM10 or ADAM17, within the extracellular region and gamma-secretase within the intracellular region (**Fig. 1B**). These proteolytic cleavage events generate the release of soluble Notch receptors in the extracellular space and the release of the Notch intracellular domain (NICD) in the intracellular space (**Fig. 1B**). The released NICD enters the nucleus and activates a transcriptional repressor, CBF-1/suppressor of hairless/LAG-1 (CSL; ref. 2). CSL can bind to the consensus DNA sequence in association with a SMART complex in the absence of NICD. After the released NICD interacts with CSL, the transcription of Notch target genes, such as Hairy Enhance of Split (Hes) and Hairy/Enhancer of Split related with YRPW motif (Hey) are activated in the nucleus (**Fig. 1B**; ref. 3).

As the DLL/Notch signaling pathway plays a major role in regulating specific cellular processes, dysregulation of this pathway can lead to the progression of various types of cancer and pathological disorders (1–5). Therefore, different modalities of DLL/Notch signaling pathway inhibition at each activation process have been developed and explored as anticancer agents at preclinical and clinical stages (4, 5). A class of small-molecule inhibitors, gamma-secretase inhibitors (GSIs), blocks the proteolytic cleavage of Notch receptors in the cell cytoplasm after Notch ligand binding (**Fig. 1B**). The use of GSIs in cancer treatment is based on promising preclinical data in which GSIs impaired the overall Notch signaling, resulting in significant therapeutic efficacy in preclinical models. In the case of biological agents, mAbs, antibody–drug conjugates (ADC), and bispecific antibodies specifically targeting Notch ligands or receptors were developed as investigational anticancer agents. Several antibodies targeting DLL4 or Notch receptors have been tested in multiple clinical trials based on the potent *in vitro* neutralizing activity of the corresponding DLL/Notch interactions and *in vivo* efficacy of tumor growth inhibition in preclinical xenograft models. Antibodies targeting DLL4 or Notch1, 2, or 3 are known to inhibit tumor angiogenesis and reduce cancer stemness in preclinical models. Interestingly, DLL4 antibodies disrupt the dynamic balance of tumor angiogenesis, resulting in excessive sprouting and branching and increased nonfunctional vascular density in tumors. Such effects of DLL4 blockade on tumor angiogenesis are unique and distinct from the effects of VEGF blockade in tumors. As an ADC target, DLL3 has been intensively studied in preclinical models and clinical trials of lung cancer.

The safety, tolerability, and efficacy of DLL/Notch inhibitors have been explored in multiple clinical trials (4, 5). However, most clinical programs testing DLL/Notch inhibitors were discontinued, mainly due to adverse effects, including adverse gastrointestinal

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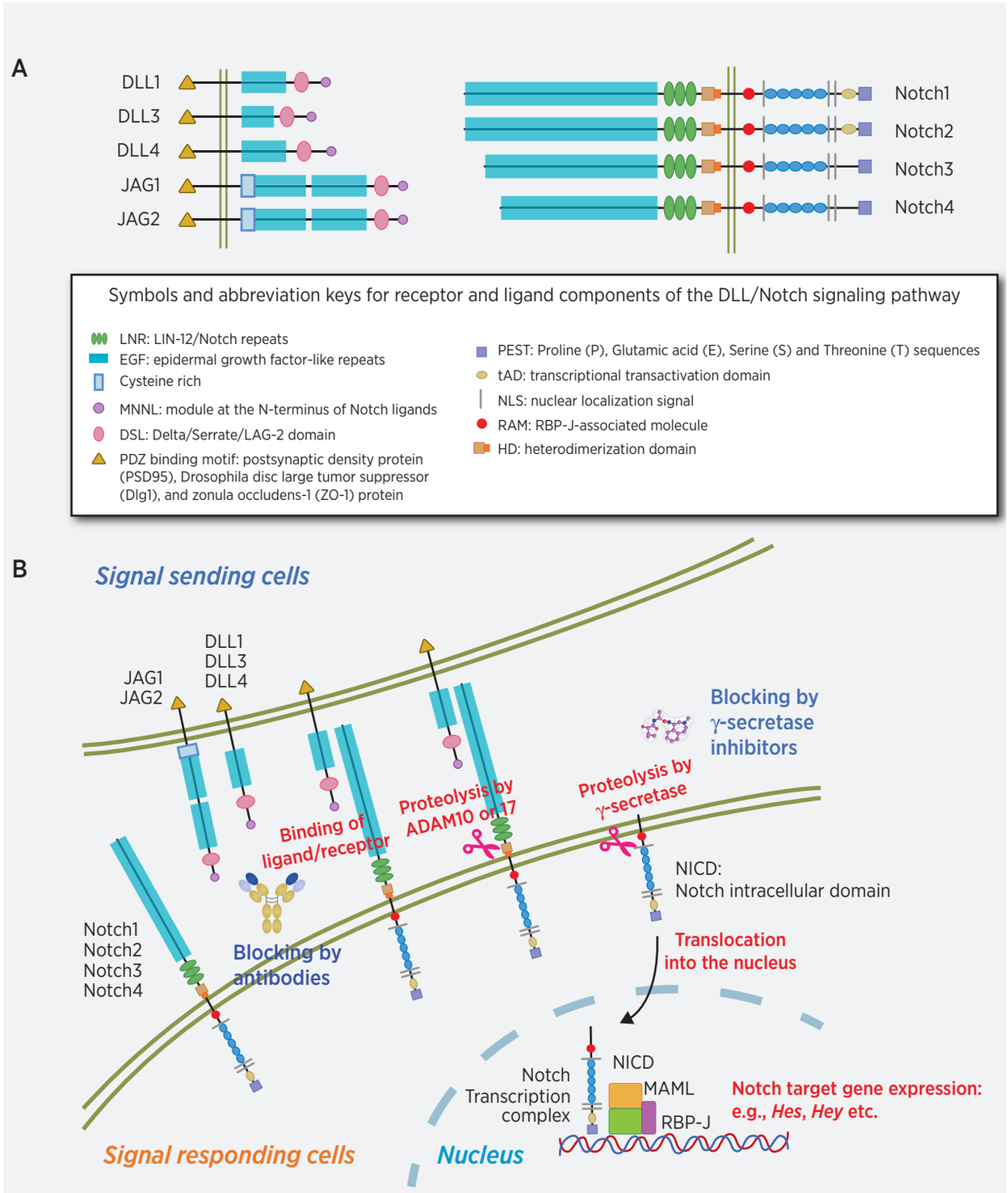


Figure 1.
Schematic diagram and structure of Notch ligands and receptors and activation of the Notch signaling pathway. **A,** Mammals have four known Notch receptors and five Notch ligands comprising two families, the Delta-like (DLL1, DLL3, DLL4: homologous to Delta in Drosophila) and Jagged (JAG1, JAG2: Homologous to Serrata in Drosophila) families, that regulate cell–cell communications by interaction and activation. **B,** The Notch signaling pathway is initiated by ligand-receptor binding, which undergoes two-step proteolytic cleavage by ADAM family proteases and gamma-secretase. The released Notch intracellular domain (NICD) translocates to the nucleus and then converts the transcription complex with DNA-binding transcription factors RBP-J (CSL), mastermind-like (MAML) protein, and other coactivator proteins to stimulate expression of Notch target genes. The Notch signaling pathway can be blocked by two major classes of Notch inhibitors. The first class is a small-molecule inhibitor of gamma-secretase inhibitors, and the second class is a mAb that specifically binds Notch ligands or receptors.

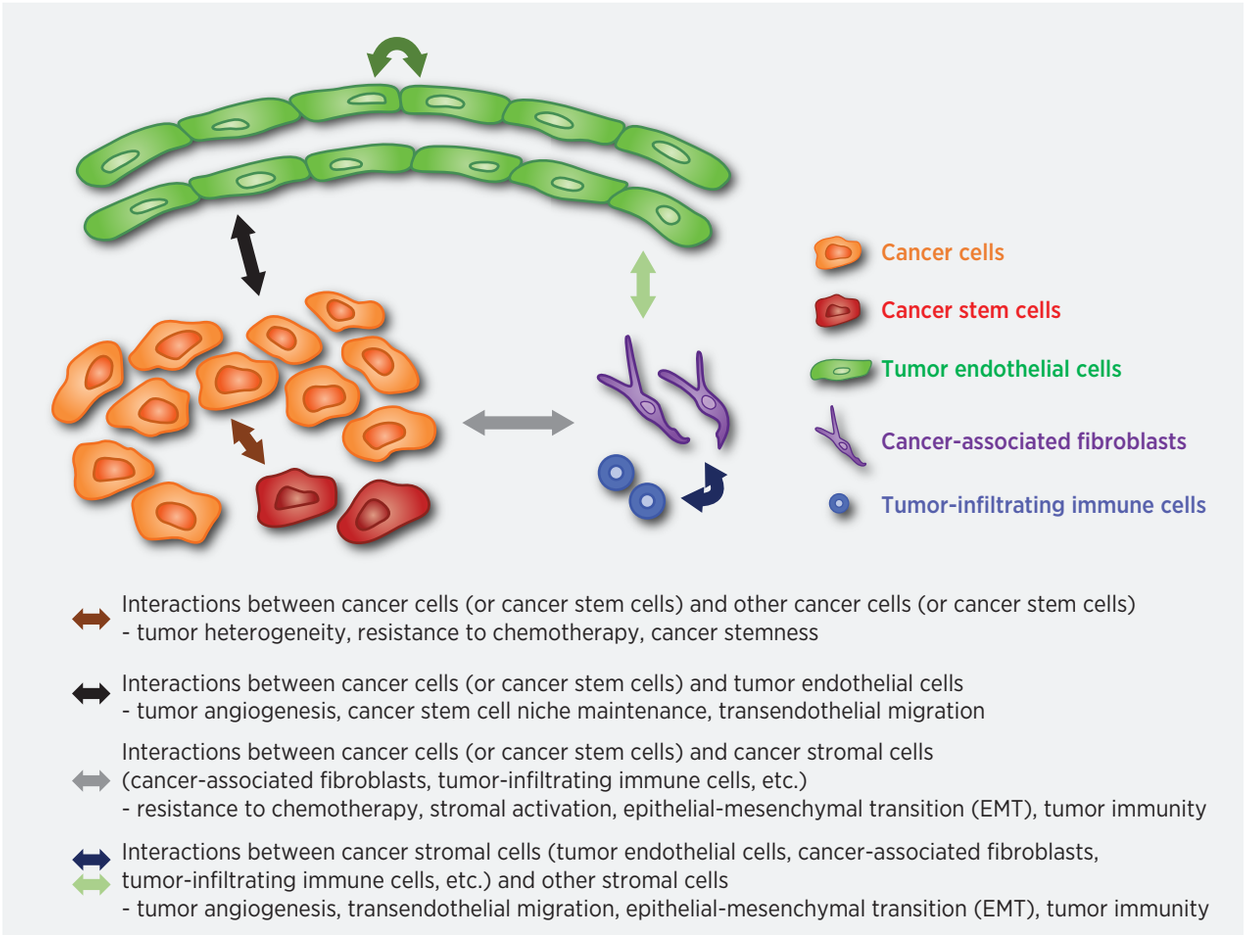


Figure 2.
Schematic diagram showing the complex networks and diverse roles of the Notch signaling pathway in cancer cells and stromal cells. Notch signaling regulates numerous complex and heterogeneous interactions between cancer cells or cancer stem cells (CSC) and their stromal cells, including tumor endothelial cells, cancer-associated fibroblast, and tumor-infiltrating immune cells. These interactions govern different aspects of tumor pathogenesis through cis- and/or trans-mediated signaling in various cancers. Interactions between cancer cells (or CSCs) with other cancer cells drive the heterogeneity of cancer, resistance to cancer therapy, and cancer stemness. Interactions between cancer cells (or CSCs) and their stromal cells coordinate tumor angiogenesis, CSC niche maintenance, transendothelial migration, resistance to chemotherapy, stromal activation, epithelial–mesenchymal transition (EMT), and tumor immunity. Interactions between cancer stromal cells and other cancer stromal cells adjust tumor angiogenesis, transendothelial migration, EMT, and tumor immunity. The Notch signaling pathway could represent oncogenic or tumor-suppressive functions in cancers dependent on the types of cancer cell or cancer stromal cell–expressing Notch ligands or receptors.

events, such as severe diarrhea, and the development of skin cancer with GSIs, or pulmonary hypertension, ventricular dysfunction, and congestive heart failure with DLL4-targeting antibodies. In this article, we review the results of relevant clinical studies of DLL/Notch-targeted therapeutics and discuss the challenges and advances in the clinical application of these investigational agents in cancer therapy.

Role of the DLL/notch signaling pathway in cancer

In pathological conditions, the DLL/Notch signaling pathway plays a pivotal role in tumor progression and angiogenesis (3–6), and both ligands and receptors have been found to be overexpressed and activated in multiple tumors, including the tumor stroma (4–8). In addition, the DLL/Notch family plays an important role in regulating the crosstalk between tumor cells and neighboring cells in the tumor stroma (4–8). Such crosstalk involves mutual *trans*-signaling interactions between tumor cells and neighboring cells, with one cell expressing a ligand and another cell expressing a receptor, as well as *cis*-signaling in which the same cell co-expresses a ligand and receptor. Crosstalk between tumor cells and tumor stroma cells through the DLL/Notch pathway could occur via multidirectional interactions between many different cell types in the tumor stroma, including tumor endothelial cells, cancer-associated fibroblasts, and tumor-infiltrating immune cells (**Fig. 2**). Therefore, the DLL/Notch signaling pathway can regulate more complex and heterogeneous interactions between tumor cells and their neighboring cells in the stroma and vice versa (7–9). These interactions have been shown to control various aspects of tumor pathogenesis, such as angiogenesis, cancer stem cell (CSC) maintenance, resistance to cancer chemotherapy, and tumor-infiltrating immune cells (refs. 8, 9; **Fig. 2**).

The DLL/Notch signaling pathway is known to drive sprouting angiogenesis in tumors via tight regulation of endothelial tip and stalk

cells (6, 8). In addition to the role of Notch signaling in tumor endothelial cells, Notch ligands or receptors expressed in cancer cells could affect the sprouting and function of adjacent tumor endothelial cells. Moreover, Notch ligands or receptors expressed in cancer cells work as an important signaling pathway to maintain CSCs in various cancers, which can reinforce cancer stemness, metastasis, heterogeneity, and resistance to conventional cancer therapies (6–9). The other aspects related to tumor progression include the tumor stromal Notch signaling of epithelial–mesenchymal transition (EMT) and tumor-infiltrating immune cells that mediates crosstalk with tumor cells or with other neighboring tumor stromal cells (8). These cell-to-cell interactions via DLL/Notch signaling are involved in remodeling the tumor stroma, as well as inducing the EMT in many tumors (5, 7, 8). The DLL/Notch signaling pathway is also known to control the functions of various immune cells in the tumor stroma such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T (Treg) cells, and T cells (8). The Notch signaling pathway can regulate tumor immune responses leading to tumor-promoting or tumor-suppressive outcome, which would be dependent on Notch activation in each type of tumor-infiltrating immune cells (7, 8).

Because of the complexity of crosstalk governed by DLL/Notch signaling in tumors and their neighboring cells, the DLL/Notch signaling pathway can also have oncogenic or tumor-suppressive functions depending on the type of cancer (refs. 5, 8, 9; **Table 1**). Generally, DLL1 binds and activates Notch receptors, leading to progression of glioma and breast cancer (10, 11). However, DLL1-mediated signaling can also result in tumor-suppressive functions in osteosarcoma, pancreatic carcinoma, and lung cancer (12–14). Signaling mediated by DLL3 plays an oncogenic role in melanoma (15, 16), bladder (17), endometrial (18), ovarian (19), pancreatic (20), and lung cancer (21). DLL4 binding also generally mediates oncogenic functions, and overexpression of DLL4 on tumor cells or tumor stromal cells, such as tumor endothelial cells, is associated with a poor clinical prognosis in many different types of cancer (22–29). Notably, DLL4 is the only Notch ligand expressed predominantly by the vascular endothelium and the only gene with haploinsufficiency that leads to vascular defects and embryonic lethality (30–32). Deletion of JAG1 from the endothelium also results in cardiovascular defects and embryonic lethality. JAG1 is overexpressed in certain types of cancer, and the overexpression is associated with a poor prognosis (33, 34). Ligand JAG2 is known to promote tumorigenicity of colon cancer and pancreatic cancer metastasis (35, 36). Upon interaction with their cognate ligands, Notch receptors regulate cell fate, behavior, and stemness, EMT, genomic stability, and metastasis of numerous cancer types (7–9, 37). Thus, the DLL/Notch signaling pathway is one of the most widely upregulated and activated pathways in multiple cancer types with a poor prognosis for patients.

DLL/notch inhibitors in clinical development for cancer treatment

On the basis of the key functions of the DLL/Notch signaling pathway in tumor pathogenesis, many different classes of DLL/Notch inhibitors have been developed and explored as anticancer agents (2–5). The first class of DLL/Notch signaling inhibitors was GSIs. These small molecules block Notch activation by preventing the cleavage function of gamma-secretase (**Fig. 1B**). GSIs were originally developed for the treatment of Alzheimer's disease due to the role of gamma-secretase in cleaving amyloid precursor protein (APP) in the central nervous system (38, 39). Several GSIs have been repurposed and developed for cancer treatment given their potent ability to block Notch receptor activation (**Table 2**; 40–47). Although efficacy data

Table 1. Summary of the functions of Notch ligands and receptors in various types of cancer.

Notch ligand or receptor	Function in different cancer	Reference
DLL1	Progression of breast and glioma cancer	Kumar *et al.* (10), Purow *et al.* (11)
	Tumor-suppressive functions in osteosarcoma, pancreatic carcinoma, and lung cancer	Pu *et al.* (12), Rajamani *et al.* (13), Biktasova *et al.* (14)
DLL3	Oncogenic functions in bladder, endometrial, lung, melanoma, ovarian, and pancreatic cancer	Ding *et al* (15), Konstantakou *et al* (16), Koshkin *et al* (17), Wang *et al* (18), Jia *et al* (19), Mullendore *et al* (20), Liu *et al* (21)
DLL4	Oncogenic functions with a poor clinical prognosis in many different types of cancer	Huang *et al* (22), Kim *et al* (23), Wang *et al* (24), Patel *et al* (25), Hu *et al* (26), Xiao *et al* (27), Jubb *et al* (28), Qiu *et al* (29)
JAG1	Overexpression with a poor prognosis	Purow *et al* (11), Huang et al (22), Xiu *et al* (33), Li *et al* (34)
JAG2	Promotion of tumorigenicity and metastasis in colon and pancreatic cancer	Vaish *et al* (35), Hu *et al* (36)
Notch1	Oncogenic functions in lung adenocarcinoma, breast cancer, cholangiocarcinoma, colorectal cancer, melanoma, and pancreatic cancer	Wang *et al* (2), Yuan *et al* (3), Meurette *et al* (8), Meisel *et al* (9), Purow *et al* (11), Mullendore *et al* (20), Liu *et al* (21), Hu *et al* (36), Aster *et al* (37)
	Tumor-suppressive functions in cutaneous squamous carcinoma, hepatocellular carcinoma, lung squamous carcinoma, prostate cancer, and small-cell lung cancer	
Notch2	Oncogenic functions in cervical, colorectal, glioblastoma, ovarian, and pancreatic cancer	Wang *et al* (2), Yuan *et al* (3), Meurette *et al* (8), Meisel *et al* (9), Jia et al (19), Mullendore *et al* (20), Liu *et al* (21), Aster *et al* (37)
	Tumor-suppressive functions in cutaneous squamous carcinoma, lung squamous carcinoma, and small-cell lung cancer	
Notch3	Oncogenic functions in non–small cell lung, ovarian, and pancreatic cancer	Wang *et al* (2), Yuan *et al* (3), Meurette *et al* (8), Meisel *et al* (9), Jia et al (19), Liu *et al* (21), Aster *et al* (37)
Notch4	Oncogenic functions in breast and pancreatic cancer	Wang *et al* (2), Yuan *et al* (3), Meurette *et al* (8), Meisel *et al* (9), Liu *et al* (21)

Table 2. Summary of gamma-secretase inhibitors (GSI) under clinical development in oncology.

GSI	Company	Clinical status	Reference
BMS-906024 (AL101)	Bristol Myers Squibb (BMS)	2	Chan *et al* (40)
BMS-986115 (AL102)	Bristol Myers Squibb (BMS)	2	Aung *et al* (41)
LY-900009	Eli Lilly	1	Pant *et al* (42)
MK-0752	Merck	2	Cook *et al* (43)
PF-03084014 (Nirogacestat)	Pfizer	3	Kummar *et al* (44), Takahashi *et al* (45)
RO4929097 (RG-4733)	Roche	2	Lee *et al* (46), Sahebjam *et al* (47)

from preclinical models have been promising, the observed efficacy for most GSIs has not translated to clinical benefit.

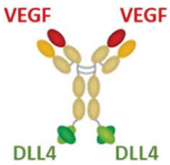
Interpreting the collective clinical data for all GSIs is complicated by the finding that GSIs appear to be functionally and pharmacologically distinct (48). The functional profiles of GSIs show differential inhibitory activity across Notch receptors Notch1–4. In addition, there are >90 gamma-secretase substrates, including APP, ERBBP4, Ephrin B2, and Cadherin (49), and all GSIs tested thus far exhibit inhibitory activity for substrates beyond the Notch family. This broad on-target activity is likely the cause of the severe adverse effects observed clinically, resulting in a limited maximum tolerated dose. Furthermore, GSIs can inhibit Notch receptor activation in normal cells and tissues, contributing to unwanted effects. Severe adverse events caused by GSIs during clinical trials were gastrointestinal toxicity, abnormal lymphoid tissues, and development of skin cancer (50, 51). Further studies investigating selective gamma-secretase–targeting agents with a better understanding of GSI structure and function, and predictive biomarkers for stratification of potentially responsive patients with cancer are essential in the development of safer and more effective GSI-based cancer therapies (51).

A strategy for more selective inhibition of the Notch signaling pathway is to discover and develop an antibody-based antagonist that can specifically bind Notch ligands or receptors. Several antibodies targeting the Notch signaling pathway have been tested and evaluated in clinical studies (**Table 3**; 52–66). Although an antibody generally has greater specificity than small molecules, most mAbs or ADCs targeting Notch ligands or receptors have demonstrated a poor balance between clinical benefits and adverse effects in clinical studies. Such adverse effects of DLL/Notch-targeting antibodies may be due to off-target effects and/or DLL/Notch signaling blockade in normal tissues. For example, gastrointestinal toxicity, such as diarrhea, nausea, and vomiting, are the most common on-target adverse events seen with DLL/Notch targeting antibodies and GSIs, which are likely caused by Notch1 inhibition in intestinal stem cells (5, 51, 61, 62). Other critical adverse events are cardiovascular toxicities, such as hypertension, acute myocardial infarction, peripheral edema, and pulmonary arterial hypertension (PAH; ref. 5). PAH, a type of high blood pressure that affects the arteries in the lungs and the heart, could be due to narrowed or dysfunctional blood vessels after DLL/Notch inhibition with DLL4-targeting antibodies (62, 67, 68). The molecular mechanism of action of DLL/Notch inhibitors that leads to PAH is not clearly understood (62, 67–69). Recent studies in preclinical mouse models have demonstrated that Notch1 deficiency in endothelial cells leads to a severe hypoxia-induced PAH associated with dysregulated contact between endothelial cells and smooth muscle cells in the pulmonary vasculature (68, 69). The results also suggest that bone morphogenetic protein (BMP) receptor-2 (BMPR2)–mediated Notch1 activation plays an important role in maintaining the normal contact between endothelial and smooth muscle cells that is necessary for endothelial cell regeneration in response to injury (68, 69). In addition, preclinical animal studies have found that a DLL4-neutralizing antibody blocks Notch1 cleavage, induces pulmonary hypertension (PH), impairs lung endothelial barrier function, and increases immune cell infiltration in vessel walls (70).

Table 3. Summary of antibody-based DLL/Notch inhibitors in clinical development in oncology.

Antibody	Target	Company	Clinical status	Reference
OMP52M51 (Brontictuzumab)	Notch1	OncoMed (Acquired by Mereo BioPharma in 2019)	1	Ferrarotto *et al* (52)
OMP-59R5 (Tarextumab)	Notch2&3	OncoMed	2	Hu *et al* (53), Smith *et al* (54)
SC16LD6.5, Rova-T (Rovalpituzumab Tesirine)	DLL3/ADC	AbbVie (Originally by Stemcentrx)	3	Morgensztern *et al* (55), Hann *et al* (56), Mansfield *et al* (57)
MEDI0639	DLL4	MedImmune	1	Falchook *et al* (58)
OMP21M18 (Demcizumab)	DLL4	OncoMed	2	Coleman *et al* (59), McKeage *et al* (60), Smith *et al* (61)
REGN421 (Enoticumab)	DLL4	Regeneron	1	Chiorean *et al* (62), Strickler *et al* (79)
AMG 757	DLL3/CD3 bispecific antibody	Amgen	2	Giffin *et al* (63), Fu *et al* (78)
ABL001 (CTX-009/ES104/ NOV1501/HD105)	DLL4/VEGF bispecific antibody	ABL Bio (Compass/Elpiscience/ Handock)	2	Lee *et al* (64)
ABT-165 (Dilpacimab)	DLL4/VEGF bispecific antibody	AbbVie	2	Gordon *et al* (65), Strickler *et al* (79)
OMP-305B83 (Navicixizumab)	DLL4/VEGF bispecific antibody	OncoMed (OncXerna Therapeutics)	3	Jimeno *et al* (66), Fu *et al* (78)

Table 4. Comparison of phase 1 study results for three bispecific antibodies targeting DLL4 and VEGF.

Antibody	OMP-305B83 (Navicixizumab)	ABT-165 (Dilpacimab)	ABL001 (CTX-009/ES104/NOV1501/HD105)
Bispecific format	Heterogeneous (asymmetric, 1+1)	DVD-IgG (symmetric tetravalent, 2+2)	IgG-scFv (symmetric tetravalent, 2+2)
			
Patient number	66 patients	55 patients	45 patients
Dosing schedule	Once every 3 weeks	Once every 2 weeks	Once every 2 weeks
Safety	1 DLT	1 DLT	No DLT
Treatment-related adverse events	Hypertension (57.6%)	Hypertension (60.0%)	Hypertension (37.8%)
	Headache (28.8%)	Headache (30.9%)	Headache (15.6%)
	Fatigue (25.8%)	Fatigue (21.8%)	Fatigue (4.4%)
	Pulmonary hypertension (18.2%)	Pulmonary hypertension (14.5%)	Pulmonary hypertension (8.9%)
	Gastrointestinal perforation (2%)	Gastrointestinal perforation (3.6%)	Gastrointestinal perforation (2.2%)
Efficacy	Partial response, $n = 4$	Partial response, $n = 6$	Partial response, $n = 3$
	Stable disease, $n = 17$	Stable disease, $n = 29$	Stable disease, $n = 21$
	Progressive disease, $n = 38$	Progressive disease, $n = 13$	Progressive disease, $n = 15$
	Not evaluable, $n = 7$	Not evaluable, $n = 7$	Not evaluable, $n = 6$
	ORR: 6.8% (4/59)	ORR: 12.5% (6/48)	ORR: 7.7% (3/39)
	DCR: 35.6% (21/59)	DCR: 72.9% (35/48)	DCR: 61.5% (24/39)
Status of further clinical study	Phase 3: Patients with ovarian cancer, fallopian tube cancer, primary peritoneal carcinoma (NCT05043402)	Phase 2: The study (NCT03368859) was discontinued after interim analysis showed lack of improved efficacy beyond bevacizumab (65).	Phase 2: Patients with biliary tract cancer (including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and ampullary carcinoma; NCT04492033)
	Combination: navicixizumab + paclitaxel		
	Navicixizumab: 3 mg/kg Q2W of a 28-day cycle (i.e., days 1 and 15)		Combination: ABL001 + paclitaxel
	Paclitaxel 80 mg/m^2 on days 1, 8, and 15 of a 28-day cycle		

Abbreviations: DCR, Disease control rate; ORR, Objective response rate.

Reduced Notch1 cleavage and activation in lung endothelial cells could be an underlying mechanism of PAH in patients with cancer administered DLL/Notch inhibitors (69, 70). Therefore, PAH is one of the most significant adverse events related to treatment of patients with cancer with DLL/Notch inhibitors. In summary, most GSIs and mAbs targeting the DLL/Notch signaling pathway were discontinued during clinical development due to safety concerns and lack of efficacy. One of the most significant adverse events from direct inhibition of Notch receptor families by GSIs or Notch targeting antibodies is gastrointestinal toxicity, such as severe diarrhea (grade 3 or 4), which has been found as a dose-limiting toxicity (DLT) during clinical trials (41–43, 54). Unlike direct blockade of Notch receptor signaling, Notch ligand inhibitors, such as DLL4 antibodies, exhibit a lower incidence of adverse gastrointestinal events, but cardiovascular side effects were reported after long-term treatment, including hypertension (grade 1–3), pulmonary hypertension (grade 1–3), and congestive heart failure (grade 3 or 4; refs. 58–62). Thus, a more careful clinical study plan should be considered and designed to reduce safety concerns by adjusting the dose and dosing schedule of DLL/Notch inhibitors.

Dual-target inhibitors of DLL4 and VEGF

To develop a more effective therapeutic agent to inhibit the DLL/Notch signaling pathway, the most important issue is how to address balancing safety and efficacy. As the effects of DLL/Notch blockade are highly dependent on the context, strength, and duration of pathway activation or inhibition, the therapeutic window associated with DLL/Notch targeting could be improved by the direct control of any of these parameters (67). Incomplete and intermittent DLL/Notch blockade by an anti-DLL4 F(ab′)2 antibody fragment demonstrated the potential to reduce dose-related liver and vascular toxicities in preclinical models while still maintaining efficacy (67). The rapid clearance of the anti-DLL4 F(ab′)2 antibody fragment allowed more flexible control over the extent and duration of DLL4 inhibition compared with the parental anti-DLL4 IgG1 antibody. However, the concept of intermittent DLL/Notch inhibition by modulating the pharmacokinetic profile to reduce toxicity was not thoroughly evaluated in clinical studies.

Another strategy to improve the therapeutic window of DLL/Notch inhibitors is to increase the specificity and potency of drug candidates. Dual targeting the DLL/Notch and VEGF/VEGFR signaling pathways

provides a promising approach given that the VEGF/VEGFR signaling pathway is the dominant force driving tumor angiogenesis, which has been clinically validated in many cancer patients (71). In addition, DLL/Notch blockade has a differentiated molecular mechanism of action for inhibiting angiogenesis in tumors compared with VEGF/VEGFR blockade (30, 31, 72). Among the multiple Notch ligands, DLL4, and antibody inhibitors of DLL4, have been widely studied. DLL4 is viewed as a promising target to augment the effects of VEGF inhibition and overcome resistance to anti-VEGF therapy (73, 74). Importantly, DLL4 upregulation in the tumor microenvironment has been shown to mediate resistance to VEGF-targeted therapies, providing additional rationale for a bispecific antibody (74). Preclinical studies have demonstrated that combination therapy with anti-DLL4 and anti-VEGF antibodies provides a synergistic effect that results in tumor growth inhibition (73, 74). On the basis of this rationale and strategy, three bispecific antibodies targeting DLL4 and VEGF, navicixizumab (OMP-305B83), dilpacimab (ABT-165), and ABL001 (CTX-009), were developed and are being evaluated for safety and efficacy in the treatment of solid tumors (**Table 4**). Navicixizumab is an asymmetric heterogeneous bispecific antibody targeting VEGF and DLL4 in each arm of the IgG antibody without binding avidity (66). Dilpacimab is a tetravalent symmetric bispecific antibody using the dual-variable domain immunoglobulin (DVD-IgG) format, in which both individual variable domains target DLL4 and VEGF in each N-terminus of IgG (75). ABL001 is also a tetravalent symmetric bispecific antibody, but it is composed of the C-terminus of the VEGF-binding IgG heavy chain conjugated with a single-chain variable fragment (scFv) targeting DLL4 (76, 77). Although the three bispecific antibodies have different formats, navicixizumab and dilpacimab maintain a sub-nanomolar range of binding affinities against both target antigens, DLL4 and VEGF, compared with each parental mAb (66, 75). As the binding moiety for DLL4 in ABL001 uses a scFv format, the binding affinity of ABL001 to DLL4 is about 10-fold weaker than that of the binding affinity of the parental DLL4-binding mAb with an IgG format (76). Nonetheless, these three bispecific antibodies have demonstrated more potent and enhanced anticancer activity than each mAb targeting DLL4 or VEGF evaluated pre-clinically in multiple human cancer xenograft models of breast, colon, gastric, glioma, ovarian, and pancreatic cancer (66, 75–77). This approach of using a bispecific antibody to simultaneously target DLL4 and VEGF has been validated as a novel and potent antiangiogenic strategy in preclinical models with affirmative *in vivo* efficacy, pharmacokinetics, and safety profiles.

Although no head-to-head comparative clinical studies have been performed, the overall results of phase 1 clinical studies have shown that all three bispecific antibodies have improved safety and efficacy profiles compared with monospecific anti-DLL4 or anti-Notch receptor antibodies and GSIs (64–66). Clinical studies of three DLL4 and VEGF dual-targeting bispecific antibodies have demonstrated a lower rate of the most common adverse events, including hypertension, headache, fatigue, pulmonary hypertension, and gastrointestinal perforation, with promising clinical outcomes in regard to anticancer activity in heavily pre-treated patients with cancer (**Table 4**). Navicixizumab was administered to 66 patients with cancer once every 3 weeks in eight dose-escalation cohorts (0.5, 1, 2.5, 3.5, 5, 7.5, 10, and 12.5 mg/kg) and an expansion cohort (7.5 mg/kg; ref. 66). The treatment-related adverse events (≥15% of patients) were hypertension (57.6%), headache (28.8%), fatigue (25.8%), and pulmonary hypertension (18.2%), with one case of DLT in a subject receiving 2.5 mg/kg (**Table 4**). In general, pulmonary hypertension was not symptomatic at low doses (≤5 mg/kg; 6 cases of grade, 1 case of grade 2), but was more severe at higher doses (4 cases of grade 2 and 1 case of grade 3; ref. 66). Dilpacimab was administered to 55 patients with advanced solid tumors once every 2 weeks in five dose-escalation cohorts (1.25, 2.5, 3.75, 5, and 7.5 mg/kg; ref. 65). Similar to navicixizumab, the most common treatment-related adverse events with dilpacimab were hypertension (60.0%), headache (30.9%), fatigue (21.8%), and pulmonary hypertension (14.5%), with one case of DLT at 2.5 mg/kg dose (**Table 4**). ABL001 was administered to 45 patients with cancer once every 2 weeks in nine dose-escalation cohorts (0.3, 1, 2.5, 5, 7.5, 10, 12.5, 15, and 17.5 mg/kg). The treatment-related adverse events with ABL001 were hypertension (37.8%), headache (15.6%), fatigue (4.4%), and pulmonary hypertension (8.9%), with no DLT, which is a better safety profile than the other bispecific antibodies (**Table 4**). Although the total number of patients with cancer was not enough to evaluate the clinical benefits of these bispecific antibodies targeting DLL4 and VEGF, the overall clinical results of monotherapy showed manageable safety profiles and preliminary signs of anticancer activity in patients. On the basis of the promising outcome of clinical phase 1 trials, further clinical studies have been performed to assess the safety and efficacy of combination treatment with DLL4/VEGF bispecific antibodies and chemotherapy drugs (**Table 4**). Recently, clinical trial results of combination treatment with chemotherapy were reported for navicixizumab plus paclitaxel, demonstrating encouraging clinical activity with manageable toxicity in bevacizumab-naïve or -treated patients with platinum-resistant ovarian cancer, but dilpacimab plus FOLFIRI was not well tolerated and did not provide a clinical benefit to patients with metastatic colorectal cancer compared with bevacizumab plus FOLFIRI (78, 79). Even though navicixizumab and dilpacimab were administered to different patients with cancer with different chemotherapies, the reason for the conflicting clinical outcomes from two bispecific antibodies should be reviewed carefully and evaluated in terms of the overall clinical design, including dose, dosing schedule, selection of cancer types with chemotherapy partner and regimen, and control group relating to treatment options (78, 79). Notably, ABL001 bispecific antibody was designed to further broaden the therapeutic window by choosing an anti-DLL4 antibody partner with modest affinity to avoid the DLL4 blockade-related adverse effects seen previously with high-affinity anti-DLL4 mAbs. In addition, ABL001 is a tetravalent, bispecific antibody with divalent binding for each target and, thus, the ability to maintain binding avidity without any steric hindrance. In summary, the most challenging issue for three DLL4 and VEGF dual-targeting bispecific antibodies is to find a well-balanced profile between safety and efficacy, which can be addressed and evaluated in clinical trials with large numbers of patients with cancer. Striking this balance is key to the successful development of a dual DLL4/VEGF antibody inhibitor for patients who have few, or have exhausted, current treatment options.

Conclusions

The DLL/Notch signaling pathway plays an important role in maintaining cancer stemness and regulating angiogenesis in solid tumors. Here, we reviewed the clinical landscape of DLL/Notch signaling inhibitors for the treatment of solid tumors. Different modalities have been tested clinically and, unlike the promising efficacy results seen in numerous preclinical studies, the clinical outcome of DLL/Notch blockade was not robust enough to warrant further clinical development due to frequent or significant observed adverse events. Improving the balance between safety and efficacy has been the most critical issue for DLL/Notch inhibitors in moving

forward with further clinical development in oncology. More studies need to be performed to determine the roles of Notch ligands and receptors in different types of cancer and stromal components. Studies to discover and develop solid predictive biomarkers for the stratification of patients with cancer who will be potentially more responsive to different types of DLL/Notch inhibitors are also important to develop better anticancer agents. In addition, the dose, dosing schedule, and combination strategy to minimize safety concerns and maximize anticancer efficacy in the clinical study design is a key driver of the clinical success of DLL/Notch inhibitors. In this regard, the bispecific antibody format to enable dual inhibition of DLL4 and VEGF, with modest DLL4 affinity, may be the best antiangiogenic strategy to successfully blunt the growth of difficult-to-treat tumors.

Authors' Disclosures

W.-K. You reports employment with ABL Bio, INC. currently working as the Head of R&D. T.J. Schuetz reports he is co-founder and CEO of Compass Therapeutics during the conduct of the study. S.H. Lee is the founder and CEO of ABL Bio, Inc. No disclosures were reported by the other author.

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