NOTCH signalling in ovarian cancer angiogenesis

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Abstract: The Notch signalling pathway is involved in the new vessel formation process by regulating tip and stalk cells, which are key cells in the sprout formation. This process is essential in both normal ovary and cancer angiogenesis and is regulated by Notch-VEGF crosstalk. Furthermore, Notch has been linked in ovary with stem cell maintenance and epithelial mesenchymal transition processes. Dysregulation of the Notch pathway is frequent in ovarian cancer (OC) and it has been associated with impaired survival and advanced stages or lymph node involvement. Notch also plays a role in chemoresistance to platinum. In this context, this pathway has emerged as an attractive target for precision medicine in OC. Two main targets of this pathway concentrate the clinical development of compounds blocking Notch: gamma secretase and Delta-like ligand 4. Most of the clinical trials including OC patients have been developed in phase I or phase Ib. Despite being in an early phase, both of these compounds, navicixizumab or demcizumab, two monoclonal antibodies targeting Dll4, showed promising efficacy data in platinum-resistant OC patients in recent studies. This review will focus on the mechanisms of the Notch pathway with special interest in angiogenesis regulation and the implication of Notch as a potential therapeutic target in OC.

Keywords: Ovarian cancer (OC); angiogenesis; Notch; Dll4; gamma-secretase

Introduction

Angiogenesis is a process by which new blood vessels will be created. The formation of new vessels is essential for tumour growth and proliferation. This process is mainly regulated by the vascular endothelial growth factor (VEGF) and its receptors (VEGFR 1-4). In ovarian cancer (OC), angiogenesis has become a crucial therapeutic target. Bevacizumab, an anti-VEGF-A, has shown activity from first line (1,2) to platinum-resistant recurrence (3), while tirosinquinase inhibitors (TKI) with anti-VEGFR activity such as pazopanib or cediranib (4) have also shown activity in OC.

Notch signalling plays a crucial role in the regulation of the vascular homeostasis. By regulating the differentiation between different phenotypes of endothelial cells, Notch promotes the sprout formation, essential for the genesis of

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new vessels.

Moreover, Notch signalling is also correlated with the acquisition and maintenance of cancer stem cell (CSC) properties in several tumours (5). The Notch pathway exerts oncogenic or tumour-suppressive effects depending on stage and subtype.

In OC Notch dysregulation is a common event. Notch 3, one of the four Notch-receptors, has been reported to be overexpressed in >20% of ovarian serous adenocarcinomas. It has been shown that deregulated expression of Notch 3 is correlated with tumour recurrence, resistance to chemotherapeutic agents, and an impaired outcome (6).

Multiple inhibitors and antagonistic monoclonal antibodies targeting Notch signalling components have been studied in preclinical and clinical trials (7). However, Notch-targeted agents have not yet been incorporated to the therapeutic armamentarium against OC.

In this review we will address the role of Notch signalling pathway in ovarian angiogenesis with particular focus on its therapeutic implications in the management of OC.

An overview of the Notch pathway

Notch is activated through cell-to-cell contact (paracrine communication) and serve as a communication mechanism with neighbouring cells. Notch pathway activation leads to cellular proliferation, differentiation, and/or apoptosis (8). As a consequence of this activation, the Notch pathway will control tissue homeostasis, playing an important role in angiogenesis regulation, and stem cell maintenance (9).

There are 4 Notch receptors (Notch 1-4) and 5 different ligands that have been identified in mammals (10). The ligands are the delta-like ligand family (Dll) 1, 3 and 4 and the serrate like Jagged1 (JAG1) and Jagged2 (JAG2) ligands (11).

Receptors are transmembrane proteins with a Notch extracellular domain (NECD) that consist of multiple EGFR-like repeats, a transmembrane domain and a large Notch intracellular domain (NICD) containing a regulation of amino acid metabolisms that interacts with the transcription factor family C promoter (CBF-1)-Su-Lag1.

Dll4/JAG ligands regulate the proteolytic cleavage of Notch receptors by disintegrin and metalloproteinase domain-containing protein (ADAM) 10/17 and gamma secretase. These cleavage events release the NECD and NICD (12,13). The expression pattern of Dll4 and JAG modifies the strength of response of the Notch cascade (14).

NICD released by gamma-secretase action, is translocated to the nucleus, where it binds to the DNA bound CBF-1/Su(H)/Lag-1 protein complex (CSL complex) recruiting the MAML1-3 co-activators. As a result, the transcription of Notch target genes is initiated (9). These genes are transcription factors of Hes (Hairy/Enhancer of split) and Hey (Hes-related) families. These genes regulate the transcription of proteins involved in cell fate determination, being the downstream effectors of the Notch signalling, and they are involved in cancer progression, chemoresistance, self-renewal promotion, and tumour initiation in various cancers (15). Hes1 locus is amplified in 2–25% in OC, followed by Hey1, Notch2 and Notch3. See *Figure 1*.

The Notch signalling cascade response can be canonical or non-canonical. The canonical signalling cascade is the NICD/CSL-dependent transcription of Notch target genes (16), whereas the non-canonical cascade refers to the CSL-independent responses such as NICD-dependent inhibition of ATM or activation of RAC1 (17,18).

The Notch pathway interacts with other signalling cascades, such as TGF-B, Wnt, EGFR and HER2 pathways, as well as the PI3K signalling cascade.

In adult tissue, Notch signalling maintains stem cells and controls tissue homeostasis. In fact, Notch signalling was first studied because of its implication in embriogenic development in mammals, where Notch has different implications in vascular and angiogenesis control such as proper cardiovascular development.

The Notch cascade is also related with cancer stemness being JAG1 the main ligand implicated. This process that regulates the ability of the cells to differentiate into different tumour cell types is crucial in cancer progression.

The Notch pathway and angiogenesis in normal ovary

The Notch pathway is related with the new vessel formation process through the differentiation of tip and stalk cells in the new sprout (19). Three types of cells are going to be involved in the formation of a new vessel: tip cells, stalk cells, and phalanx cells.

The tip cells navigate the environment via filopodia and response to vascular endothelial growth factor (VEGF). The stalk cells will form the vascular lumen, proliferate and undergo tubulogenesis. And finally phalanx cells, which are quiescent and support the sprout (20).

VEGF and Notch engage in a cross talk to balance tip and stalk cell formation. VEGF signalling through VEGF receptor 2 (VEGFR2) and neuropiline-1 (NRP1) (NRP1)
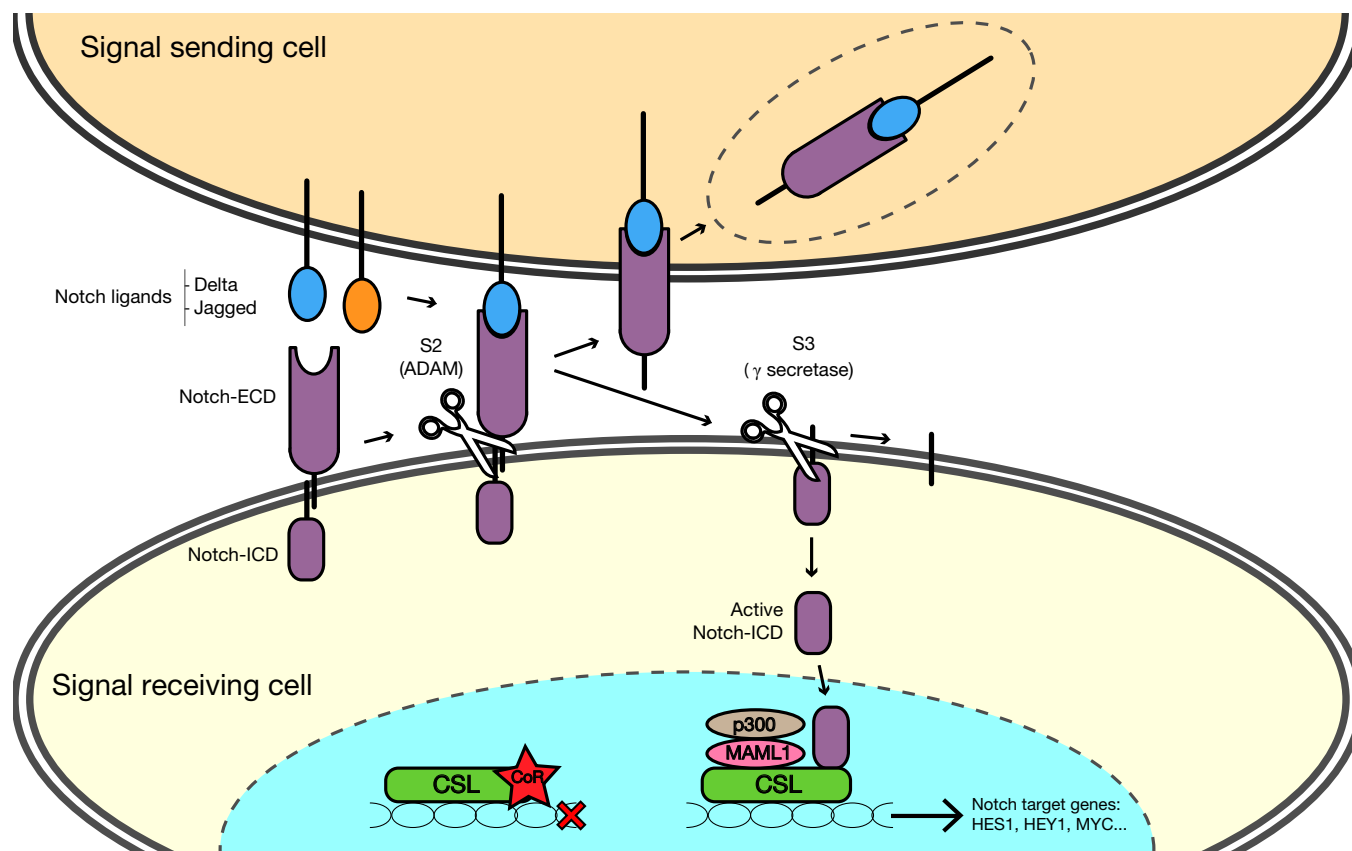
Figure 1 Notch signalling pathway. Interaction of the ligands Dll4 and Jagged with the Notch-ECD activates the secretases ADAM and gamma-secretase cleavage the two domains of Notch (ECD and ICD). Notch-ICD is released in the cytoplasm as an active form and translocates into the nucleus. In the nucleus Notch-ICD interacts with the CSL protein that in the absence of Notch-ID is inactivated by the a co-represor. Activated CSL forms a complex with proteins p300 and MAML1 that activates the transcription of Hes and Hey Notch related genes. ECD, extracellular domain. ICD, intracellular domain.

receptor on tip cells drives vascular sprouting and upregulates Dll4. Dll4 signalling through Notch receptors on endothelial stalk cells restricts angiogenic sprouting and proliferation through downregulation of VEGFR2 and NRP1 (21,22).

Notch signalling upregulates JAG1 to antagonise the Dll4-dependet stalk phenotype leading to sprout formation and proliferation

In tumour cells JAG1 has been seen to be overexpressed being involved in tumour angiogenesis (23).

Dll4 is expressed in tumour blood vessels (tip cells) and stroma. When Dll4 activates Notch 1 receptors on the adjoining endothelial stalk cell, initiates a vascular VEGF negative feedback loop, which limitates sprouting by downregulation of VEGFR-2. In this context, Dll4 regulates VEGF-mediated vascular growth by preventing

excessive branching that could potentially lead to vascular dysfunction (24).

In preclinical tumour models, inhibition of Dll4-mediated Notch signalling in tumours results in hypersprouting of nonfunctional vasculature (23).

Epithelial ovarian cells express the JAG1, Dll1 and Dll4 ligands. Dll4 is expressed in the endothelium at sites of vascular development and angiogenesis (25). Fraser *et al.* confirmed in an *in vivo* experiment with primate ovaries that Dll4 blockade increases luteal angiogenesis and microvascular density (26). Other experiments in mice also showed that Notch signalling inhibited FGF-induced angiogenesis and blocked ovarian follicle development.

In summary the Notch pathway plays an essential role in vascular homeostasis and integrity in the normal ovary.

Page 4 of 11

Perez-Fidalgo et al. Notch regulates angiogenesis and is a potential target in OC

NOTCH and ovarian cancer

The Notch pathway is frequently altered in microsatellite-instable or POLE-mutant gastric and esophageal (79%), colorectal (70%) or endometrial cancers (64%) (27). Moreover, Notch is also altered in leukemia, lymphoma, breast and lung cancers, among others (7). In OC, several studies have reported the critical roles of Notch components in cancer progression and metastasis.

The alteration of the Notch pathway genes has been reported in approximately 22% of all analysed ovarian tumours (15). A recent study found that JAG1 was overexpressed in OC cells, which stimulates the Notch pathway and promotes cell proliferation, migration, invasion, stemness, and chemoresistance (28).

VEGF, VEGFR1, Dll4, Notch1 or Notch3 in OC samples showed statistically significant higher expression than in normal ovary or than in benign ovarian tumour tissues. Dll4 expression was associated with higher expression of VEGFR1 and Notch1 with VEGFR2 and microvessel density suggesting the relevant role of Notch in ovarian angiogenesis (29).

In a recent analysis of several datasets including information from PRECOG, GENT, and CSIOVDB databases, the relevance of the Notch pathway in OC was assessed. The alteration of certain Notch-related genes has been associated with poorer prognostic factors and with an impaired survival in several studies (30). Notch-related genes were found to be differently expressed in OC and this overexpression was associated with poor overall survival (OS) and disease-free survival (DFS). High levels of Notch2, Notch3, and ADAM17 were associated with impaired OS and Notch2, Notch3, MAML1, and ADAM17 with poorer DFS.

Upregulation of Notch2, Notch3, DLL3, MAML1 and ADAM17 was associated with more advanced FIGO stages.

Notch3 expression has also been related with relapse in OC and was associated with impaired prognosis. Notch3 expression by immunohistochemistry (IHC) was significantly higher in recurrent OC than in primary OC samples from the same patients. Moreover, high Notch3 nuclear protein or mRNA levels were related with impaired OS and progression-free survival (PFS) (31,32).

Ivan *et al.* (33), studied the DNA methylation patterns and miRNAs of the Notch family and its relationship with OS. The authors analysed the clinical data from gene expression and methylation data of more than 450 patients with advanced serous OC. Higher levels of CCND1, PPARG, and RUNX1 and lower levels of their respective miRNAs were correlated with significantly poorer survival.

NOTCH and ovarian cancer angiogenesis

Angiogenesis is a key process in ovarian carcinogenesis. Wide clinical and preclinical evidence show that VEGF-mediated angiogenesis is an important pathway in cancer and an anticancer target of outmost relevance. VEGF signalling activates several downstream effectors including Notch pathway in OC (34).

Experiments in mouse models (35) showed that Dll4 silence leads to a decrease in the angiogenesis and tumour growth that was synergistic with the addition of bevacizumab.

A Chinese study (29) in 28 samples from ovarian carcinomas showed that Dll4 was positively correlated with VEGFR1 expression, one of the transmembrane receptors of VEGF, whereas Notch1 was positively correlated with VEGFR2. In this study, VEGFR2 expression in ovarian tumours was associated with an impaired prognosis with more asicites and distant metastases.

In this context, the Notch pathway is not only related with an impaired survival in OC but it is also closely related with angiogenesis biomarkers in this tumour type.

The synergistic effect of Notch targeting and anti-angiogenic treatment

As previously mentioned, the Dll4-Notch pathway and VEGF regulate each other. Dll4 in endothelial cells is upregulated by VEGF (36). In fact, Dll4 expression in tumour vessels is rapidly decreased by blocking VEGF (37). It has also been seen that Notch signalling can alter expression of three VEGF receptors (38).

Hu *et al.* confirmed that blocking both Dll4 and VEGF with siRNA and bevacizumab respectively was more effective in inhibiting angiogenesis in preclinical models than either strategy. Moreover, exposure to anti-VEGF treatment leads tumours to underexpress Dll4 (39). In this context, a double blockade of VEGF and Dll4 could potentially be synergistic improving antitumour effect in OC.

The Notch pathway implication in chemoresistance: the role of Notch3

Although OC frequently responds to chemotherapy, most patients will develop a chemoresistant recurrence. In this

context, approaches and specific biomarkers to resensitise patients to standard chemotherapies are a medical need (40).

Acquired resistance to antitumour therapies in response to repeated chemotherapy courses is a major challenge in the management of OC. However, the underlying mechanisms leading to chemoresistance in OC are poorly understood.

Notch3 pathway dysregulation is a frequent alteration in several tumours including OC (15).

Notch3 receptor has been identified as a potential oncogene and high levels of Notch3 have been found in approximately 20% of ovarian serous adenocarcinomas. Notch3 disregulation has been related with tumour recurrence, and drug resistance (31,41).

Notch3 is overexpressed in aldehyde dehydrogenase isoform 1 (ALDH1+) ovarian cells (42) supporting a link between Notch3 and CSC. Studies by McAuliffe et al. (43) showed that Notch3 played a relevant role in CSC maintenance and resistance to platinum.

Kim et al. (42) observed that higher expression levels of ALDH1 and Notch related genes, and especially Notch3, were associated with CSCs and with chemoresistant ovarian SCs and paclitaxel-resistant SKpac OC cells. In addition, Notch3 expression correlated with poor prognosis factors, such as advanced stage, lymph node, and distant metastases.

Notch3 has also been related with epithelial mesenchymal transition (EMT). In a Canadian preclinical study, the OC cell line OVCA429 was transducted with an empty vector or a retroviral vector expressing Notch3 intracellular domain or NICD3. Notch3 induced EMT in OVCA429 cells. Furthermore, OVCA429/NICD3 cell line showed higher resistance to carboplatin compared to the OVCA429 control cells (44).

Kang et al. studied the role of Notch3 specific inhibition in paclitaxel-resistant Skpac OC lines and parental SKOV3 cells. The study compared the effects on cell viability, migration, angiogenesis, cell cycle, and spheroid formation among others of either Notch3 siRNA or gamma secretase inhibitor (GSI). Notch3 silencing reversed paclitaxel-resistance re-sensitising these cells to paclitaxel therapy. Similar effects were seen after exposure of cells to GSI. Notch knockdown also depleted OC stem cells with a spheroide formation reduction and downregulation of CSC markers (45).

A recent study by Ju-Yeong et al., identified that mi-RNA-136 directly targets Notch3. Mi-RNA-136 levels were significantly lower in ovarian serous carcinoma as compared with normal control tissues. The low expression of miR-136

was associated with poorer OS in OC patients.

The artificial miR-136 overexpression leads to a decrease in cell viability, proliferation, CSC spheroid formation, and angiogenesis. Moreover, miRNA-136 expression increased apoptosis in paclitaxel-resistant SKpac cells (46).

The Notch pathway as therapeutic target: clinical development in ovarian cancer

Notch targeting with small-molecule inhibitors or antibodies is currently under development in OC. Most important targets for Notch blockade are gamma-secretase and Dll4. However, other drugs such as the natural compounds xanthohumol (47) or withaferin A (48) were assessed in the preclinical setting showing inhibition of cell growth and cell cycle arrest in OC cell lines through downregulation of Notch1 and Notch3.

Inhibiting Notch is gaining interest and recent studies have developed this strategy in the clinic as single agent or in combination with chemotherapy. See *Table 1*. In the following chapters we will focus on those studies with clinical impact in OC.

Targeting gamma-secretase

Gamma-secretase induces a proteolytic release of the NICD receptor and also participates in a wide variety of cellular functions. Targeting this secretase will avoid the cleavage and release of NICD. Gamma-secretase inhibitors (GSI) affect both ligand and receptor interactions, decreasing tumour angiogenesis, due to the cumulative effect of inhibiting Notch signalling in the tumour as well as inhibiting both JAG1 and DLL4 on the vasculature. Several GSI compounds have been developed in recent years.

A preclinical study by Chen et al. analysed the effects of MK-0752 alone or in combination with cisplatin in OC. MK-0752 alone induced cell growth inhibition, cell cycle arrest and apoptosis through downregulation of Notch1 and its downstream effectors including Hes1, XIAP, c-Myc, and MDM2. Combination of sequential cisplatin followed by MK-0752 significantly increased apoptosis and inhibited the subcutaneous xenograft growth of OC in nude mice (49).

Bocchicchio et al. studied the effect of DAPT (a gamma secretase enzyme complex inhibitor) compared to the inhibition of B-catenin and their combination. This study showed that both pathways cooperated ensuring proliferation and migration of cells. Moreover, an upregulation of the Notch pathway in the absence of Wnt

Table 1 Clinical studies of compounds targeting Notch pathway in ovarian cancer

Compound	Target	Type of drug	Phase	N (all pts)	Ovarian cancer pts	Comments
MK-0752	GSI	Inhibitor	I	103	3	Recommended doses 1,800 to 4,200 mg weekly
LY900009	GSI	Inhibitor	I	35	11	Recommended dose 30 mg 3 times a week
Crenigascestat (LY3039478)	GSI	Inhibitor	I	110	10	Well tolerated at doses with target engagement. Signs of activity
RO4929097	GSI	Inhibitor	Ib (+ gemc)	18	2	RP2D for RO 30 mg + gem 1,000. Evidence of clinical activity
			Ib (+ temsi)	17	1	RP2D for RO 20 mg + temsi 37.5 mg. Combination tolerable
			II	45	45 PROC	Insufficient activity as single agent
Enoticumab (REGN421)	Anti Dll4	Monoclonal antibody	I	53	7	RP2D 4 mg/kg three-weekly and 3 mg/kg biweekly
						1 partial response in 1 ovarian cancer patient
Navicixizumab (OMP-305B83)	Anti Dll4 and VEGF	Dual Monoclonal antibody	I	66	12	25% responses in ovarian cancer
			Ib (+ wpacl)	18	18 PROC	43% PR. 77% CBR. Median PFS 7.3 months
Demcizumab (OMP-21M18)	Anti Dll4	Monoclonal antibody	I	55	1 OGCT	Demcizumab well tolerated. Signs of activity
			Ib (+ wpacl)	19	19 PROC	21% ORR. 43% CBR

pts, patients; GSI, gamma secretase inhibitor; Dll4, delta-like ligand 4; RP2D, recommended phase 2 dose; gemc, gemcitabine; temsi, temsirolimus; wpacl, weekly paclitaxel; PROC, platinum-resistant ovarian cancer; OGCT, ovarian granulosa cell tumor; PR, parcial response; ORR, overall response rate; CBR, clinical benefit rate.

signalling suggested a crosstalk between the WNT pathway and Notch (50).

In the clinical setting different GSI have been tested in early phase trials. MK-0752 was explored a phase I trial in adult patients with advanced solid tumours. The study included 103 patients of whom 3 were OC patients. Significant inhibition of Notch signalling was observed with the 1800 to 4200 mg weekly dose levels, confirming target engagement at those doses. One patient achieved a response and 10 a stable disease (51).

LY900009 also known as crenigascestat, is an oral GSI that has been assessed in a first-in-human phase I trial. A first part of dose escalation recruited patients in cohorts of 3 patients with advanced solid tumour and a second part for dose confirmation enrolled only OC patients.

Thirty-five patients were included. Most common adverse events (AE) were diarrhea (46%), vomiting (34%), anorexia (31%), and nausea (31%). In terms of efficacy, 5 patients had stable disease (SD) as best response.

Two patients (5.7%) with leiomyosarcoma and OC received 4 courses of LY900009. The recommended MTD

schedule was 30 mg twice weekly.

Massard *et al.* studied the safety, pharmacokinetic profile, pharmacodynamic effects, and antitumour activity of crenigascestat, in patients with advanced or metastatic cancer in a phase I study. Most common adverse events were gastrointestinal. The study included 110 patients, and 10 patients with OC. This potent inhibitor of Notch signalling was well tolerated and demonstrated clinical activity in heavily pretreated patients (52).

Richter *et al.*, conducted a phase I trial of an oral GSI, RO4929097 in combination with gemcitabine. Patients with advanced solid tumours were included. This study showed promising activity and identified a potential predictive biomarker, as lower levels of Notch protein expression were related with an increased benefit (53,54).

In a phase Ib trial, RO429097 was assessed in combination with temsirolimus in refractory advanced solid tumours. The combination was tolerable with two DLTs; grade 3 rash and grade 3 mucositis in the same patient. The recommended phase 2 dose (RP2D) was established in 20 mg for RO4929097 + 37.5 mg for temsirolimus (55).

RO4929097 has been further developed in a phase II trial in OC patients. 45 women with platinum-resistant OC and treated with a maximum of 2 previous regimens were enrolled. Patients received oral RO4929097 at 20 mg 3 days on/4 days off per week in a 21-day cycle. The primary endpoint was PFS rate at the end of 4 cycles. No objective responses were observed and 15 patients (33%) had SD. The median PFS was 1.3 months (1.2–2.5 months). Treatment was well tolerated. Approximately 10% of patients discontinued treatment due to AE. In high grade serous OC, the level of NICD protein by IHC was identified as a potential biomarker for longer PFS. NICD high levels showed a trend towards longer PFS than lower levels (3.3 vs. 1.3 months, P=0.09). The drug showed insufficient activity as single agent to warrant further development in platinum-resistant OC (56).

Toxicity is a major concern in GSI treatment. A common AE is gastrointestinal toxicity and diarrhea, which is secondary to goblet cell metaplasia of small intestine attributed to the dual inhibition of Notch1 and Notch3 (57).

Targeting Dll4

Compared to GSI, Dll4 targeting leads to a massive but non productive angiogenesis instead of a reduced angiogenesis (37).

Kuhnert et al. (58), published the results of a preclinical study with REGN421 (enoticumab), an intravenous human IgG1 monoclonal antibody that binds Dll4, resulting in the inhibition of Notch pathway in OC models.

Enocitumab was administered to human tumour xenografts in immunodeficient mice engineered to express human Dll4. Exposure to enocitumab led to tumour growth inhibition and formation of nonfunctional tumour blood vessels. The antitumour effects were significantly enhanced when inhibition of VEGF with aflibercept was used simultaneously with Dll4 blockade.

Another study assessed the combination of double inhibition Dll4 and VEGF. An American group specifically assessed the role of the combination of Dll4 inhibition with the murine antibody enocitumab and the human antibody enocitumab with aflibercept or chemotherapy in orthotopic mouse models of OC. Double target of VEGF and Dll4 with enocitumab showed superior antitumour effects than either monotherapy. This study confirmed the therapeutic potential of the double blockade of VEGF and Dll4 (59).

Enocitumab was developed in the clinical setting in a phase I first-in-human trial. Enocitumab was administered with dose escalation from 0.25 to 4 mg/kg triweekly (Q3W) and 0.75 to 3 mg/kg every 2 weeks (Q2W). Of 53 patients included, 31 were treated Q3W and 22 Q2W. An MTD was not reached. Two DLTs occurred: grade 3 nausea and grade 3 abdominal pain. Antitumour activity included two partial responses (one in OC) and 16 patients with SD, of whom 3 lasted more than 6 months. Enoticumab was tolerated with a RP2D of 4 mg/kg Q3W and 3 mg/kg Q2w based on pharmacokinetics and clinical activity (60).

OMP-305B83 also knows as navicixizumab, is a bispecific anti-Dll4 and anti-VEGF dual antibody. Jimeno et al., (61) assessed at the first-in-human phase 1a study, the role of navicixizumab in previously treated solid tumours. Study objectives were the determination of MTD, safety, and efficacy, among others. Sixty-six patients were treated once every 3 weeks in 8 dose-escalation cohorts and an expansion cohort. The most commonly enrolled tumour type was OC (12) followed by colorectal (11), breast, pancreatic and endometrial cancers (4 each). MTD was not reached (only 1 DLT occurred). Most frequent treatment-related AE were hypertension (57.6%), headache (28.8%) and fatigue (25.8%). Pulmonary hypertension was mostly asymptomatic at doses ≤5 mg/kg but was more severe at higher dosis with one patient with grade 3. Regarding efficacy, 4 patients (3 OC, 1 uterine carcinosarcoma) had a partial response (PR) and 17 patients had SD. Of note, 19 patients had a reduction in the size of their target lesions including 4 responses of 12 patients with OC (25%). In this context OMP-305B83was tolerable and showed promising activity mainly in OC.

Navicixizumab had been further developed in combination with weekly paclitaxel in OC patients by Fu et al. Preliminary data of a phase Ib trial assessed this combination and were presented in ESMO 2018 (62). Eighteen patients with platinum-resistant OC treated with >2 prior therapies and/or bevacizumab were included. Paclitaxel was given on days 1, 8, and 15 and navicixizumab on days 1 and 15 of every 28-day cycle. The study had a dose escalation design testing OMP-305B83 doses of 3 or 4 mg/kg followed by an expansion cohort.

The efficacy data of navicixizumab shown in heavily pre-treated platinum resistant OC patients was encouraging and led the FDA on October 2019 to grant a fast track designation to navicixizumab for the treatment of patients with high-grade ovarian, primary peritoneal or fallopian tube cancer who have received ≥3 prior therapies and/or

Page 8 of 11

Perez-Fidalgo et al. Notch regulates angiogenesis and is a potential target in OC

prior bevacizumab.

In SGO 2020 the interim efficacy analysis of the Fu S. phase Ib study of navicixizumab in combination with paclitaxel confirmed important activity. At that time 44 patients had been included. The overall response rate (ORR) of this combination per RECIST 1.1 was 43% (n=19) including 1 complete and 18 PR. Fifteen (34%) patients had SD as best response with a clinical benefit rate (CBR) of 77%. The median time to progression was 7.3 months and the median duration of response was 5.7 months. The safety profile appeared to be manageable with hypertension being the most common AE.

Demcizumab (OMP-21M18) is an IG2 humanized monoclonal antibody that targets Dll4 leading to a potent inhibition of the Notch pathway.

A phase I trial evaluated the safety, pharmacokineics, and pharmacodynamics of demcizumab in patients with advanced malignancies. Patients were treated with 5 different dose and schedules. Fifty-five patients were treated but no more than one DLT was seen at any dose level. The MTD was not reached for either schedule. Most frequent AEs were hypertension (47%), fatigue (31%), anaemia (22%) and headache (20%). Five patients at different dose levels developed different grades of heart failure and other five were hospitalised due to bleeding episodes. Sixteen of 25 evaluable patients (64%) had stabilization of disease or response (63).

A recent phase Ib trial, the SIERRA trial, assessed the MTD or the maximum administered dose and safety of the combination of demcizumab and paclitaxel in platinum-resistant OC patients. Patients included should have received no more than 4 prior chemotherapy regimens. Following a 3+3 design, demcizumab was administered at different dose levels on days 1 and 15 in combination with weekly paclitaxel every 28 days. The doublet was administered for a maximum of 3 cycles continuing paclitaxel monotherapy until unacceptable toxicity or disease progression. The study included 19 patients. No DLTs were observed, and thus the MTD was not reached. The side effects with a frequency over 50% were diarrhea, fatigue, peripheral edema, and nausea. Pulmonary hypertension grade 2 in two patients and grade 1 in another patient was also observed. The ORR was 21% (95% CI: 6–45%) and the CBR was 43% (95% CI: 20–66%). In conclusion, the combination of demcizumab and paclitaxel showed an acceptable toxic profile and activity in a heavily pretreated platinum-resistant cohort (64).

Conclusions

The Notch pathway plays a crucial role in OC and has an impact on prognosis. Notch and VEGF are essential in OC angiogenesis and Notch has also been related with chemoresistance. Thus, Notch targeting, and mainly dual targeting of Notch and VEGF, is a promising strategy in OC. The combination of Notch inhibition with chemotherapy or antiangiogenics showed interesting activity in early phase clinical studies. Navicixizumab, a dual anti-Dll4 and anti-VEGF in combination with weekly paclitaxel showed a response rate of 43% in heavily pretreated platinum-resistant OC patients. This combination has received the first FDA fast track designation for a Notch inhibitor in OC. Although toxicity is manageable, gastrointestinal AEs and hypertension could be important. There is a need of further clinical trials in order to confirm the early signs of activity and to identify a potential biomarker of activity for GSI or Dll4 blockade.

Footnote

Roche, personal fees from amgem, personal fees from Pfizer, personal fees from Pharmamar, outside the submitted work; in addition, Dr. JAPF has a patent Predictive signature for neoadjuvant chemotherapy in breast cancer pending. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Page 10 of 11

Perez-Fidalgo et al. Notch regulates angiogenesis and is a potential target in OC

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