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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

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Abstract

Purpose: Enoticumab (REGN421) is a fully human IgG_1 monoclonal antibody that binds human Dll4 and disrupts Notch-mediated signaling. The main objectives of this trial were to determine the safety, dose-limiting toxicities (DLT), pharmacokinetics (PK), and recommended phase II dose (RP2D) of enoticumab.

Experimental Design: Enoticumab was administered intravenously, with dose escalations from 0.25 to 4 mg/kg every 3 weeks (Q3W) and 0.75 to 3 mg/kg every 2 weeks (Q2W).

Results: Of 53 enrolled patients, 31 patients were treated Q3W and 22 patients were treated Q2W. Two DLTs occurred: grade 3 nausea (0.5 mg/kg Q3W) and grade 3 abdominal pain (1 mg/kg Q2W). An MTD was not reached on either schedule. The most frequent adverse events (AE) were fatigue, nausea, vomiting, hypertension, headache, and anorexia. Six treatment-related serious AEs were reported in 4 patients: brain natriuretic peptide (BNP) increase (0.25 mg/kg Q3W, Gr1), troponin I increase (4 mg/kg Q3W, Gr3), right ventricular dysfunction and pulmonary hypertension (1.5 mg/kg Q2W, both Gr3), and left ventricular dysfunction and pulmonary hypertension (3 mg/kg Q2W, both Gr3). Enoticumab was characterized by nonlinear, target-mediated PK, and had a terminal half-life of 8 to 9 days. With multiple Q2W or Q3W dosing, accumulation was not observed. Antitumor activity included two partial responses (non–small cell lung cancer bronchoalveolar-type with a β-catenin mutation, and ovarian cancer) and 16 patients with stable disease (3 > 6 months).

Conclusions: Enoticumab was tolerated, with RP2D of 4 mg/kg Q3W and 3 mg/kg Q2W based on PK profile and clinical activity. Responses and SD were noted in ovarian cancer and other solid tumors. *Clin Cancer Res; 21(12); 2695–703. ©2015 AACR.*

Introduction

The Delta-Notch pathway has been recognized as an important member of tumor angiogenesis and a contributor to cellular growth, differentiation, and stem cells self-renewal (1, 2). The key components of the Notch pathway are the four trans-membrane receptors (Notch 1–4) and the five membrane-bound Notch ligands: Delta-like (Dll) 1, 3, 4, and jagged (JAG) 1 and 2. Ligand binding to the receptor triggers a proteolytic cascade through γ-secretase, which generates the Notch intracellular domain (NICD) and further, transcriptional modulation of several target genes (3). The ligands can also be released from the cell surface by proteolytic cleavage and lead to transcriptional events as soluble ligands, but this has a less well-defined tumorigenic potential.

Dll4, the dominant Notch ligand, is an endothelium-specific ligand (4), critical for developmental (5, 6) and tumor angiogenesis (7). Dll4 is strongly upregulated in blood vessels of tumors compared with normal organs (8); however, it is also expressed in smaller arteries and capillaries of normal tissues. Dll4 is important for cell differentiation (9) and angiogenesis by directing the growth of tip cells from budding vessels, and generating an organized and functional vasculature (6). Tip cells are the highly polarized, filopodia-rich cells that specialize in chemotactic migration in response to growth factor gradients, in contrast with lumenized stalk cells. Dll4 expression in endothelial tip cells of the angiogenic sprouts occurs in response to hypoxia-induced VEGF and VEGFR signaling. Dll4 then activates Notch on neighboring endothelial cells, which further leads to the repression of the tip cell phenotype among the Notch receptor expressing cells (10). In turn, Notch activation on stalk endothelial cells results in downregulation of the VEGF receptor 2 (VEGFR-2), therefore

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Translational Relevance

The Delta-Notch pathway contributes to tumor angiogenesis, cell growth, differentiation, and stem cell self-renewal. Dll4, the dominant Notch ligand, is upregulated in tumor blood vessels compared with normal organs. Increased Dll4 expression has been noted in several tumor types and may be associated with worse outcomes. Preclinically, Dll4 blockade causes dysfunctional vasculature and diminished perfusion, resulting in antitumor activity, including in tumors resistant to anti-VEGF agents. Enoticumab (REGN421) is a novel IgG1 fully human anti-Dll4 monoclonal antibody. This first-in-human phase I study describes the safety, pharmacokinetics, biomarkers, and preliminary antitumor activity of enoticumab in patients with advanced solid tumors. Vascular expression of Dll4 was detected in most tumor samples, and less commonly of Notch-1 and -3 receptors, but no correlations with clinical benefit were seen. Coexpression of VEGFR-2 with Dll4 and Notch-3 suggests that combined targeting of the VEGF and Notch pathways may confer best efficacy results.

Notch provides negative feedback to reduce the activity of the VEGF/VEGFR axis, and coordinates the vascular response to VEGF during vascular sprouting morphogenesis.

Loss of Dll4 expression or Dll4 blockade leads to dramatically increased immature capillary sprouting and branching due to excessive tip cell formation and endothelial proliferation, but with reduced vessel lumen size. This overgrowth of dysfunctional vasculature results in overall diminished perfusion (7, 11). Inhibition of the Dll4 signaling also causes increased VEGFR-2 and VEGFR-3 expression (12), thus dual VEGF/VEGFR and Dll4/NOTCH inhibition is expected to have improved antitumor efficacy, and has been demonstrated in preclinical models (8).

Increased Dll4 expression has been noted in several tumor types (4, 13–15), and it may be associated with worse outcomes (13, 15–17). Preclinically, Dll4 blockade has activity against tumor xenografts such as colorectal, sarcoma, lung, breast, pancreas cancers, melanoma, and glioma, and includes tumors resistant to anti-VEGF agents (7, 11, 18).

Enoticumab (REGN421) is a novel IgG1 fully human anti-Dll4 monoclonal antibody that has been tested preclinically in humanized Dll4 mice (to allow cross-reactivity, the murine Dll4 gene was replaced by human Dll4 gene). *In vivo*, enoticumab conferred dose-dependent tumor growth inhibition of xenografts, including sarcoma, colon, and bladder tumors. Preclinical toxicology studies in cynomolgus monkeys at doses above 3 mg/kg included focal hemorrhage in the GI tract mucosa and ovaries, hepatic sinusoidal dilation, and minimal endothelial cell hypertrophy, consistent with disruption of endothelial signaling (data on file; Regeneron).

On the basis of these data and to ensure safety, the selected starting dose for this phase I study was conservatively chosen as 0.25 mg/kg administered every 3 weeks. This first-in-human phase I study reports the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD) markers, and preliminary antitumor efficacy, and recommended phase II dose for the anti-Dll4 monoclonal antibody enoticumab administered every 3 (Q3W) or every 2 weeks (Q2W) in patients with advanced solid tumors.

Patients and Methods

Patient eligibility

Eligible patients were ≥18 years, with histologically proven advanced solid malignancies, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate organ function. Exclusion criteria included brain metastases, squamous cell lung cancer, history of cardiomyopathy, left ventricular ejection fraction <50%, uncontrolled hypertension (HTN; systolic blood pressure > 150 mmHg or diastolic blood pressure > 95 mm) within 1 month before enrollment, or HTN requiring use of >2 antihypertensive medications. All patients provided written informed consent, and the study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and all applicable local regulatory requirements and laws.

Study design and treatments

This phase I, dose-escalation study (NCT00871559) was conducted at three centers in the United States. The primary objectives were to determine the safety, dose-limiting toxicities (DLT), MTD, and recommended phase II dose (RP2D) for enoticumab in two dosing schedules, Q3W and Q2W. Secondary objectives included PKs, pharmacodynamics, immunogenicity, and antitumor activity of enoticumab. Enrollment was sequential in a "3+3" design, with the Q3W schedule initiated before the Q2W schedule.

Enoticumab was administered intravenously over 30 minutes Q3W with doses of 0.25, 0.5, 1, 2, and 4 mg/kg, and Q2W with doses of 0.75, 1, 1.5, and 3 mg/kg. Dose escalation was allowed if DLT occurred in 0 of 3 or ≤1 of 6 patients in each cohort during cycle 1 (21 days for the Q3W schedule, and 28 days for the Q2W schedule).

DLTs were defined during cycle 1, as any possible treatment-related grade ≥3 toxicities. Because of preclinical toxicology findings, any grade ≥3 cardiac, gastrointestinal, or hepatic toxicities were considered DLT regardless of attribution. Additional DLTs were grade ≥2 left ventricular diastolic dysfunction, grade ≥2 cardiac ischemia, NT pro-BNP consistent with heart failure, ST segment elevation/depression ≥1 mm in 2 contiguous leads or infarction Q-wave, and any hemoglobin decline ≥2 g/dL from baseline with evidence of GI bleeding. Study treatment continued until disease progression, unacceptable toxicity (including DLT criteria above), or withdrawal of consent.

Study assessments

Safety assessments were conducted weekly throughout the study and for 60 days posttreatment. Adverse events (AE) were graded using Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. In addition to weekly BNP/NT-proBNP and troponin 1, a complete cardiac safety assessment by a cardiologist, chest X-ray, ECG, and echocardiogram, was performed every 6 to 8 weeks, depending on dosing schedule, or at any time in the presence of clinically significant cardiovascular findings. Tumor response was assessed every 8 (Q2W schedule) to 9 weeks (Q3W schedule), according to RECIST version1.0. Serum tumor markers were followed prospectively when elevated at baseline.

Pharmacokinetic analyses

Blood samples were collected on cycle 1 day 1 predose, at 0 (end of infusion), 1, 2, 3, 4, and 8 hours postdose, and on days 2, 3, 4, 8 (both schedules), and day 15 (Q3W). For cycles ≥2,

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samples were collected on day 15 (Q3W), predose and at 0 hour on days 1 and 15 (Q2W), and at the 15-, 30- and 60-day follow-up visits (both schedules). Serum was assayed for concentrations of functional enoticumab by ELISA. Because enoticumab can bind one or two molecules of target (Dll4), functional enoticumab is defined as either one or two binding sites unoccupied, and able to further bind Dll4.

Immunogenicity

Blood samples were collected for analysis of anti-enoticumab antibodies using an electrochemiluminescence bridging immunoassay (ADA): predose on cycle 1 day 1, and on day 1 of every other cycle as well as at the 15-, 30- and 60-day follow-up visits (both schedules).

Biomarker studies

Archival tumor samples were assessed for expression of Dll4, Notch 1 and 3, hypoxia-inducible factor-1 α (HIF1α), and VEGFR-2. Of specific interest was expression of Dll4 in tumor vasculature. IHC was performed using standard techniques and described in the Supplementary Materials (online only).

Results

Patients

Between June 2009 and July 2012, 53 patients (31 on Q3W schedule, and 22 on Q2W schedule) received at least one dose of enoticumab and were evaluable for safety. Forty-four of those patients were evaluable for antitumor activity. Two patients remain on treatment at time of data cut-off. Patients' baseline characteristics are summarized in Table 1.

Table 1. Characteristics of 53 enrolled patients

Characteristic	Number (%)
Age, y	
Median	56
Range	28–84
Gender	
Female	31 (58)
Male	22 (42)
ECOG performance status	
0	18 (34)
1	35 (66)
Tumor type	
Colorectal	14 (26)
Ovary	8 (15)
Pancreas	7 (13)
Sarcoma	6 (11)
Breast	6 (11)
Lung	4 (8)
Salivary gland	3 (6)
Oral (head and neck)	1 (2)
Thyroid	1 (2)
Cholangiocarcinoma	1 (2)
Hepatocellular	1 (2)
Prostate	1 (2)

Dose escalation and DLTs

In the Q3W schedule, one DLT, grade 3 nausea occurred at 0.5 mg/kg, and no further DLTs occurred up to the 4 mg/kg dose level. No MTD was reached, and 4 mg/kg represents the RP2D. In the Q2W schedule, one DLT occurred at 1 mg/kg: grade 3 abdominal pain (Table 2). Dose escalation continued until 3 mg/kg with no further DLTs and without defining an MTD. The RP2D was 3 mg/kg Q2W. Although the study was designed as a 3 + 3 dose-escalation phase I trial, cohorts at multiple dose levels were expanded despite no DLTs being observed to obtain additional clinical information in regards to laboratory abnormalities [e.g., brain natriuretic peptide (BNP) elevations] in a larger cohort of patients.

A dose-dependent increase in antitumor activity was observed for enoticumab over the range of 0.1 to 1 mg/kg in humanized SCID bearing HT1080 fibrosarcoma tumors (manuscript in preparation; Regeneron; data on file). Treatment with dose levels greater than 1 mg/kg did not further increase antitumor activity. In these studies, sustained enoticumab plasma concentrations of >2 mg/L were correlated with maximum tumor activity. Clinically, dose escalation did not continue past 4 mg/kg Q3W and 3 mg/kg Q2W, as it was demonstrated that plasma Dll4 is saturated above 1.5 mg/kg. Both 2 mg/kg Q3W and 1.5 mg/kg Q2W achieved prolonged systemic concentrations of free enoticumab above 10 mg/L. This concentration is higher than the circulating levels of enoticumab of 2 mg/L estimated to achieve maximum antitumor activity in preclinical models in humanized Dll4 SCID mice.

The median duration of exposure was 63 days overall, and the median number of cycles for each schedule and cohort are shown in Table 2.

Safety and tolerability

All patients experienced at least one treatment emergent adverse event (TEAE), and the most common were fatigue (64.2%), nausea (47.2%), vomiting (37.7%), headache (34%), HTN (32.1%), and anorexia (26.4%). The majority of events were grade 1 or 2 with only 8% of events being grade ≥3. Table 3 summarizes TEAEs, regardless of relationship to the study treatment, which occurred in >10% of the patients.

Four patients each in the Q3W and the Q2W treatment arms required at least 1 dose delay or dose reduction due to adverse

Table 2. Dose escalation and DLTs

Schedule	Dose level (mg/kg)	Patients, *n*	DLTs	DLT event	Cycles median, *n* (range)
Q3W	0.25	7	0		2 (1–4)
	0.5	6	1	Grade 3 nausea	3 (1–18)
	1	6	0		5 (2–6)
	2	6	0		3 (2–7)
	4	6	0		3 (3–4)
Q2W	0.75	6	0		3 (1–4)
	1	7[a]	1	Grade 3 abdominal pain	2 (0–9)
	1.5	3	0		4 (1–6)
	3	6	0		2 (1–9)

[a]One patient was replaced.

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Table 3. Treatment-related TEAEs in ≥10% of patients (NCI-CTCAE v3.0)

	Q3W					
Adverse event[a]	**0.25 mg/kg *N* = 7**	**0.5 mg/kg *N* = 6**	**1 mg/kg *N* = 6**	**2 mg/kg *N* = 6**	**4 mg/kg *N* = 6**	**Total *N* = 31**
Headache						7 (27%)
Grade 1	0	0	1	0	2	3 (10%)
Grade 2	1	0	2	1	0	4 (13%)
Fatigue						6 (19%)
Grade 1	0	0	2	1	1	4 (13%)
Grade 2	0	0	0	1	1	2 (7%)
Nausea						6 (19%)
Grade 1	1	0	1	2	0	4 (13%)
Grade 2	0	0	0	1	0	1 (3%)
Grade 3	0	1	0	0	0	1 (3%)
Hypertension						5 (16%)
Grade 1	0	0	1	0	0	1 (3%)
Grade 2	0	0	1	0	1	2 (7%)
Grade 3	0	0	1	1	0	2 (7%)

	Q2W				
Adverse event[a]	**0.75 mg/kg *N* = 6**	**1 mg/kg *N* = 7**	**1.5 mg/kg *N* = 3**	**3 mg/kg *N* = 6**	**Total *N* = 22**
Fatigue					10 (46%)
Grade 1	1	1	2	2	6 (27%)
Grade 2	1	1	1	1	4 (18%)
Hypertension					9 (41%)
Grade 1	0	0	0	0	0
Grade 2	2	1	2	2	7 (32%)
Grade 3	2	0	0	0	2 (9%)
Headache					7 (32%)
Grade 1	1	2	1	2	6 (27%)
Grade 2	1	0	0	0	1 (5%)
Pulmonary hypertension					3 (14%)
Grade 1	0	0	0	0	0
Grade 2	0	1	0	0	1 (5%)
Grade 3	0	0	1	1	2 (9%)

[a]Grades not listed have no events.

events or safety concerns. In the Q3W arm, reasons for dose modification or dose reduction were: ureteral stent placement ($n = 1$; unrelated; 0.5 mg/kg); grade 2 increased vaginal bleeding ($n = 1$; unrelated; 2 mg/kg); grade 1 stool for occult blood ($n = 1$; unrelated 4 mg/kg); and grade 2 increased depression (treatment-related) with grade 2 increased fatigue (unrelated; $n = 1$; 4 mg/kg). In the Q2W treatment arm, reasons for dose modification or dose reduction were: grade 2 HTN ($n = 1$, treatment-related, 0.75 mg/kg); grade 2 anemia ($n = 1$, unrelated, 0.75 mg/kg); grade 2 worsening pulmonary HTN ($n = 1$; treatment-related; 1 mg/kg); and grade 2 worsening anemia ($n = 1$ treatment-related; 3 mg/kg).

The majority of patients discontinued study treatment due to disease progression (35 patients, 66.0%). Study drug discontinuation due to AEs occurred in 10 patients (5 each in each schedule, 18.9% overall). Three of the 5 AEs in the Q3W treatment arm were cardiovascular: grade 1 elevated BNP (SAE, 0.25 mg/kg), grade 3 HTN (1 mg/kg), and grade 3 elevated troponin I (4 mg/kg); 2 other events were: grade 3 elevated AST (0.5 mg/kg) and grade 3 nausea (0.5 mg/kg). In the Q2W treatment arm, 3 of the 5 AEs that led to treatment discontinuation were cardiovascular: grade 2 worsening pulmonary HTN (1 mg/kg; treatment held for 2 weeks and resumed at 50% dose reduction once AE improved to grade 1), grade 3 pulmonary HTN with grade 3 right ventricular (RV) dysfunction (SAE, 1.5 mg/kg), and grade 3 pulmonary HTN with grade 3 left ventricular (LV) dysfunction (SAE, 3 mg/kg). The other two events were grade 3 abdominal pain (SAE, unrelated, 1 mg/kg) and grade 2 worsening anemia (3 mg/kg). All AEs leading to treatment discontinuation, except the grade 3 abdominal pain, were considered at least possibly related to enoticumab. Six patients (11.3%) discontinued treatment due to "other" reasons, including 5 patients with clinical progression.

In the Q3W schedule, the most frequent treatment-related TEAEs were headache (23%), fatigue and nausea (19% each), HTN (16%), and increased BNP, diarrhea and vomiting (10% each). In the Q2W arm, the most frequent treatment-related TEAEs were fatigue (46%), HTN (41%), headache (32%), and pulmonary HTN (14%). Most events were grade 1 or 2. All treatment-related TEAE occurring in >10% of patients are detailed in Table 4. Grade 3 treatment-related adverse events resolved or improved to grade ≤2 upon treatment discontinuation.

Six treatment-related serious AEs were reported in 4 patients: BNP increase (grade 1, 0.25 mg/kg Q3W-duration 15 days until normalization), troponin I increase (grade 3, 4 mg/kg Q3W-duration 47 days until normalization), pulmonary HTN and right ventricular dysfunction (both grade 3, 1.5 mg/kg Q2W-duration 39 days each until normalization), pulmonary HTN and left ventricular dysfunction (both grade 3, 3 mg/kg Q2W-duration 43 days pulmonary HTN until improvement to grade 2, and 91 days left ventricular dysfunction until normalization). The patient listed above at 1.5 mg/kg Q2W, also had a nonserious grade 3 right ventricular dysfunction. One

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Table 4. TEAEs in ≥10% of patients

Adverse event	Total ($N = 53$)	
	All grades, *n* (%)	Grade 3, *n* (%)
Fatigue	34 (64)	1 (2)
Nausea	25 (47)	3 (6)
Vomiting	20 (38)	0
Hypertension	19 (36)	4 (8)
Headache	18 (34)	1 (2)
Anorexia	14 (26)	0
Back pain	13 (25)	0
Diarrhea	13 (25)	0
Peripheral edema	13 (25)	0
Dyspnea	12 (23)	3 (6)
Constipation	10 (19)	0
Cough	10 (19)	0
Abdominal pain	9 (17)	3 (6)
Anemia	8 (15)	1 (2)
Insomnia	8 (15)	0
Pyrexia	7 (13)	0
Arthralgia	6 (11)	0

additional patient (1 mg/kg Q2W) experienced nonserious grade 2 pulmonary HTN (2 weeks until improvement to baseline grade 1). All three events of pulmonary HTN occurred in patients treated on the Q2W schedule, and are detailed as follows: 1 SAE of grade 3 pulmonary HTN occurred in a patient with ovarian cancer (1.5 mg/kg Q2W) on cycle 6 day 18 (159 days into the study) and resolved completely following treatment discontinuation (within 39 days); the second SAE of grade 3 pulmonary HTN in a patient with lung cancer (3 mg/kg Q2W) with history of dyspnea/hypoxia, occurred 56 days after the last dose of study drug administered on cycle 2 day 15 (overall 99 days into the study), and improved to a grade 2 AE, ongoing at the end of the study (43 days); the third nonserious AE of grade 2 pulmonary HTN in a patient with ovarian cancer and prior history of HTN and grade 1 pulmonary HTN occurred on cycle 4 day 8 (91 days into study). After a 2-week break, the latter event improved to grade 1 and the patient was retreated with enoticumab at 50% dose reduction and was able to remain on treatment for a total of eight cycles.

Five patients (9.4%) died during the study: none considered related to study treatment, 4 attributed to disease progression, and 1 patient (0.5 mg/kg Q3W) with fibrolamellar hepatocellular carcinoma due to perioperative intraabdominal hemorrhage after debulking surgery, 21 days post study-drug discontinuation.

Pharmacokinetics

Generally, the concentration–time profile of functional enoticumab was characteristic of target-mediated elimination and nonlinear PK. When functional enoticumab was above saturation, the concentration–time profile had an initial short distribution phase, followed by a bi-phasic elimination consisting of a saturating beta phase, and a terminal target-mediated elimination phase. Clearance decreased with increasing dose of enoticumab up to approximately 1.5 mg/kg. At doses greater than 1.5 mg/kg, CL, though variable, appeared dose independent. Likewise, AUC_{last} values increased in a greater than dose-proportional manner from 0.75 mg/kg to 1.5 mg/kg, and dose-proportional increases were observed above 1.5 mg/kg. Mean C_{max} increased dose proportionally, with C_{max}/Dose being dose independent across all dose levels (Fig. 1; Table 5). Consistent with dose proportionality observed with C_{max}, the volume of distribution (V_{ss}) appeared to be dose independent as well. In general, an initial short distribution phase followed by a beta elimination phase and a modest terminal target-mediated elimination phase were observed.

For the 2 or 3 week dosing intervals, a terminal half-life of 8 to 9 days was observed in the target-mediated phase. Over the first 3 to 4 dosing intervals, some increase in C_{max} occurred, but due to smaller number of patients on long-term treatment, there was no clear evidence of continued accumulation with repeated dosing.

Immunogenicity

One patient (1.5 mg/kg Q2W) who discontinued study treatment after 2 doses due to disease progression, tested positive for low titer anti-enoticumab antibodies (35 days after the initial dose). No other patients tested positive, and we cannot interpret the ADA as causative for early progression because other patients

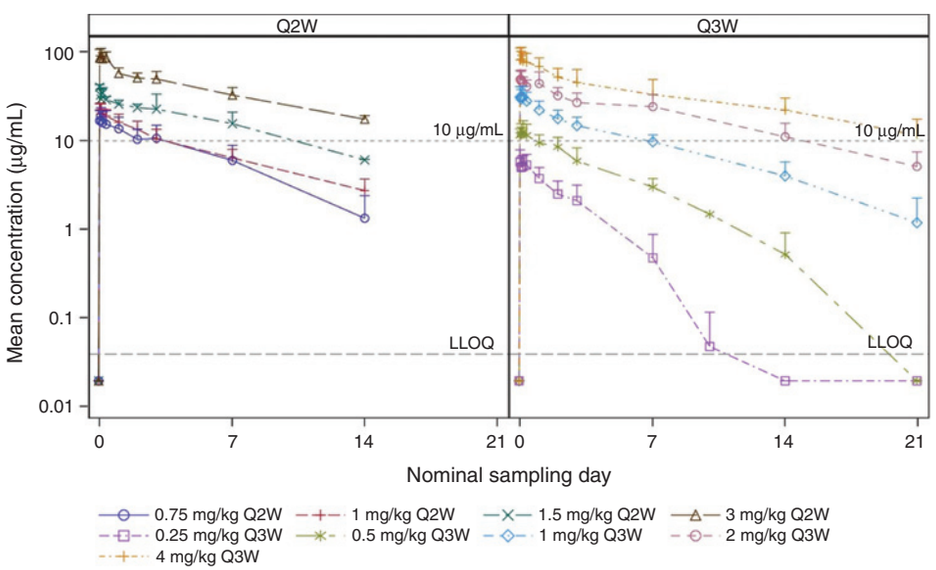

Figure 1.
Plasma concentration–time curves for enoticumab during cycle 1.

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Table 5. Summary PK parameters of functional enoticumab following the first IV infusion

Parameter/U		0.75 mg/kg Q2W *n* = 6 Mean (±SD)	1 mg/kg Q2W *n* = 6 Mean (±SD)	1.5 mg/kg Q2W *n* = 3 Mean (±SD)	3 mg/kg Q2W *n* = 6 Mean (±SD)
$t_{1/2}$	Day	4.00 (0.738)	5.13 (1.51)	6.28 (0.762)	7.74 (1.00)
CL	L/d/kg	0.00801 (0.00316)	0.00774 (0.00368)	0.00534 (0.00101)	0.00422 (0.000404)
V_{ss}	L/kg	0.0443 (0.0169)	0.0559 (0.0177)	0.0466 (0.0141)	0.0444 (0.00811)
C_{max}	mg/L	20.9 (7.27)	24.3 (4.09)	40.2 (2.63)	99.7 (16.9)
C_{max}/dose	kg/L	27.8 (9.70)	24.3 (4.09)	26.8 (1.75)	33.2 (5.62)
t_{max}	Day	0.189 (0.137)	2.40 (5.79)	0.0796 (0.0940)	0.188 (0.139)
AUC_{last}	Day/mg/L	94.3 (43.5)	118 (67.3)	233 (63.8)	522 (67.0)
AUC_{last}/dose	Day/kg/L	126 (57.9)	118 (67.3)	156 (42.5)	174 (22.3)

Parameter/U		0.25 mg/kg Q3W *n* = 7 Mean (±SD)	0.5 mg/kg Q3W *n* = 6 Mean (±SD)	1 mg/kg Q3W *n* = 6 Mean (±SD)	2 mg/kg Q3W *n* = 6 Mean (±SD)	4 mg/kg Q3W *n* = 6 Mean (±SD)
$t_{1/2}$	Day	1.73 (0.399)	3.13 (0.747)	4.30 (1.61)	6.89 (1.90)	9.25 (2.31)
CL	L/d/kg	0.0173 (0.00841)	0.00917 (0.00242)	0.00577 (0.00151)	0.00478 (0.00168)	0.00534 (0.00237)
V_{ss}	L/kg	0.0399 (0.0156)	0.0408 (0.0134)	0.0361 (0.00566)	0.0445 (0.0120)	0.0646 (0.0208)
C_{max}	mg/L	6.06 (1.83)	14.1 (4.00)	33.7 (9.58)	53.9 (13.3)	96.7 (17.7)
C_{max}/dose	kg/L	24.3 (7.30)	28.1 (8.00)	33.7 (9.58)	27.0 (6.64)	24.2 (4.43)
t_{max}	Day	0.0450 (0.0343)	0.0634 (0.0646)	0.113 (0.0682)	0.255 (0.381)	0.0799 (0.0449)
AUC_{last}	Day/mg/L	16.2 (7.70)	49.6 (18.4)	175 (42.1)	404 (118)	687 (218)
AUC_{last}/dose	Day/kg/L	65.0 (30.8)	99.2 (36.7)	175 (42.1)	202 (59.0)	172 (54.6)

Abbreviations: CL, clearance; C_{max}, maximum concentration; $t_{1/2}$, half-life; t_{max}, time to maximum concentration; V_{ss}, volume of distribution at steady state.

who were discontinued for disease progression were ADA negative.

Biomarker studies

In 39 out of 40 analyzed archival tumor samples, Dll4+ vessels were identified and in 45% of these tumor samples, all of the vessels were Dll4+. One tumor sample had no identifiable vessels and no Dll4 expression. Tumor expression of Dll4 was generally associated with $CD31^+$ tumor blood vessels (Supplementary Fig. S1). Vascular staining for VEGFR-2 was observed in 30% of tumors, and correlated with Notch-1 and Notch-3 intracellular tumor expression, as well as with Notch-3 and Dll4 vascular expression (Supplementary Table S2). Variable tumor cells or vascular expression (30%–60%) were noted for Notch-1, Notch-3, and HIF1α.

The relatively limited number of responders precluded correlative analysis with clinical response, but no significant statistical correlation was observed between the Notch biomarkers expression and time to progression (data not shown).

Antitumor activity

Among the 53 patients enrolled, 44 patients are evaluable for response (Fig. 2). Nine patients were not evaluable due to study treatment discontinuation before first follow-up tumor assessment. Partial response was confirmed in 2 patients (5%) treated at 3 mg/kg Q2W: one patient with papillary serous ovarian carcinoma heavily pretreated, and one patient with non–small cell lung cancer, bronchoalveolar type harboring a β-catenin mutation.

Sixteen patients (36%; 5 ovarian, 3 colon, 3 sarcoma, 2 breast, and 1 each with salivary gland cancer, thyroid, and cholangiocarcinoma) had stable disease as best response. Stable disease for ≥6 months was observed in 3 patients treated at: 0.5 mg/kg Q3W (374 days: KRAS wild type colon cancer with APC gene mutation and FAP syndrome), 1 mg/kg Q2W (239 days: endometrioid type ovarian carcinoma), and 1.5 mg/kg Q2W (186 days: transitional cell ovarian carcinoma). Four of 8 patients with ovarian cancer demonstrated a significant (>50%) serum CA-125 decrease, of which 2 met CA-125 response criteria (confirmed at least 28 days later).

Discussion

Several mechanisms are thought to contribute to aberrant Notch signaling, including VEGF and hypoxia. Although activating mutations in Notch 1 were described in T-cell acute and chronic lymphoblastic leukemia, an increasing number of Notch receptors' mutations have been identified in solid tumors such as colorectal, breast, lung, head and neck, and ovarian cancer (19). Because Notch may function as either tumor suppressor or oncogene in certain cancers, it is important to rationally select therapeutic targeting in solid tumors. Therapeutic Notch blockade has been reported with γ-secretase inhibitors (GSI) that affect the proteolysis of all four receptors, including those on normal intestinal cells, potentially causing gastrointestinal toxicity (20, 21). To date, clinical reports on GSI provide evidence of modest activity in gliomas (20), as well as sarcoma, melanoma, colorectal, and ovarian cancers (21).

This first-in-human phase I study reports on enoticumab, a novel anti-Dll4 agent in patients with refractory solid tumors. Although most patients experienced at least one treatment-related TEAE (72%), the majority of enoticumab-related toxicities were mild and included fatigue, headache, hypertension, and nausea. DLT, as defined by protocol, was rare (grade 3 nausea at 0.5 mg/kg Q3W and grade 3 abdominal pain at 1 mg/kg Q2W). Enoticumab appears to have a gastrointestinal toxicity profile milder than documented with GSI Notch inhibitors. Enoticumab was associated with several reversible vascular (HTN) and cardiac toxicity events, including 2 patients with grade 3 pulmonary HTN and

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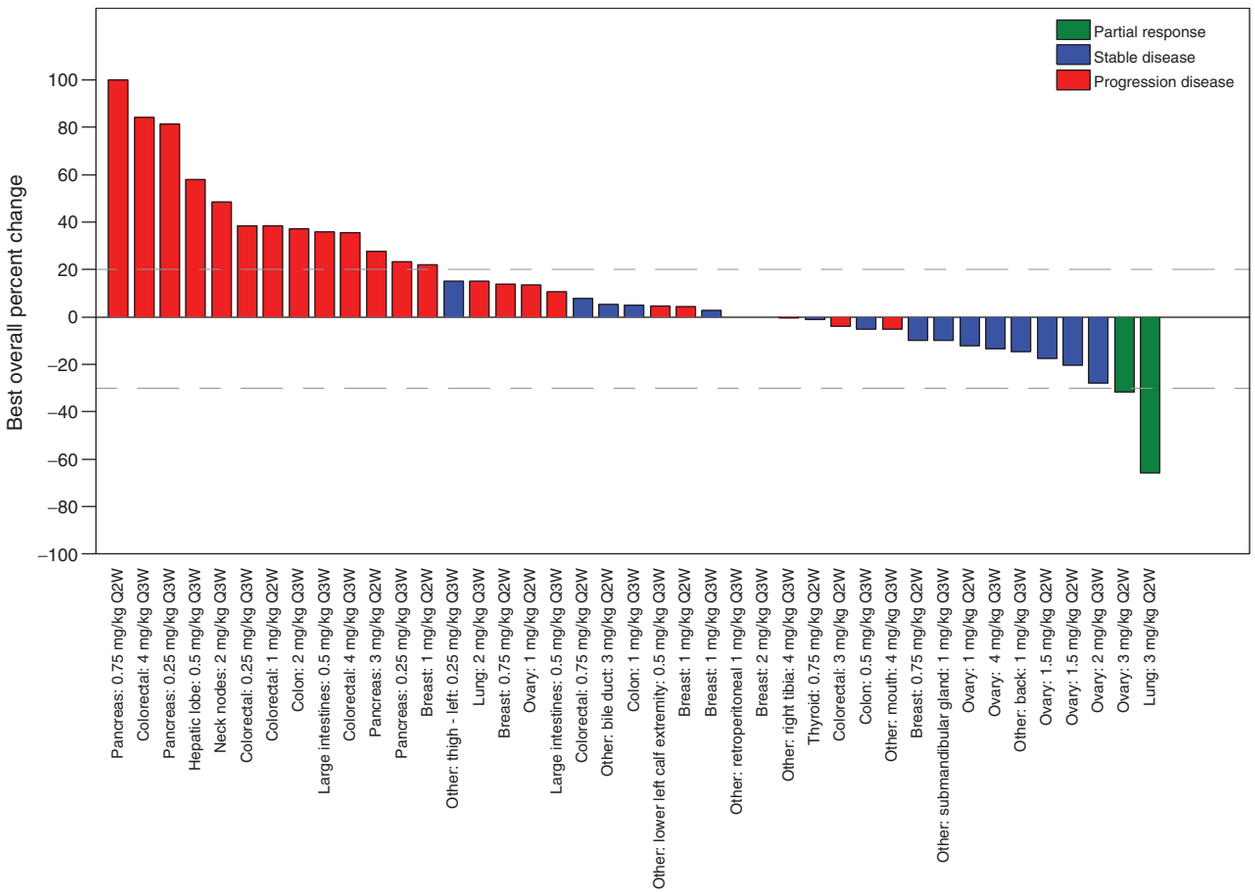

Figure 2.
Best response waterfall plot.

right or left ventricular dysfunction (same patients had pulmonary HTN with either right or left ventricular dysfunction), and 1 patient with troponin I increase. Pulmonary hypertension (13% incidence) has also been documented with the anti-Dll4 agent demcizumab (OMP-21M18), particularly after prolonged drug exposure (more than 140 days; ref. 22). In our study, we observed that grade 2 or 3 pulmonary HTN occurs after 90 days of continuous drug exposure, but one patient with underlying chronic hypoxia and NSCLC who received only 4 doses of enoticumab (3 mg/kg Q2W) developed grade 3 pulmonary HTN while off enoticumab for 56 days. It is thus important to recognize that pulmonary HTN, while reversible, seems to be a class effect with anti-Dll4 agents and predisposing factors (underlying pulmonary HTN or chronic hypoxia) may identify patients at risk. Clearly, patients undergoing prolonged anti-Dll4 treatment should undergo close cardiac monitoring with consideration for dose reductions or limiting overall exposure. Notch signaling is important for normal vascular, cardiac valvular, and myocardial development. Preclinical data suggest that low Notch activity due to mutations in Dll4 and Jagged ligands alters myocardial function (23, 24). Although not previously observed with GSIs, cardiovascular adverse events from Dll4 inhibition most likely represent mechanism-based toxicity.

It is well described that Dll4/Notch blockade results in overgrowth of a dysfunctional and chaotic vasculature with impaired lumen formation, ultimately leading to reduced tumor perfusion (7, 11). Nevertheless, Dll4 is also expressed in normal tissue capillaries and small arteries. Recently, preclinical data suggested that chronic pathway blockade with Dll4 neutralizing antibodies or loss of Notch1 may upregulate endothelium-specific genes, which can lead to sinusoidal dilation in the liver and even vascular neoplasms (25–27). We have not observed any skin lesions or cutaneous vascular neoplasms among the 53 patients in this study, many with a treatment exposure >8 weeks. Nevertheless, one patient with fibrolamellar hepatocellular carcinoma with pelvic metastases treated for 1 cycle at 0.5 mg/kg Q3W and who was off study-drug for 21 days underwent debulking metastasectomy and developed fatal perioperative bleeding. This event was thought to be due to disease, but it may have been exacerbated by the previous enoticumab exposure.

MTDs were not defined for either Q3W or Q2W schedule in this study. Nevertheless, PK analysis showed dose proportional increases in plasma concentrations, and doses of enoticumab $\geq$2 mg/kg Q3W and $\geq$1.5 mg/kg Q2W exceeded circulating concentrations of free enoticumab of 2 mg/L estimated to achieve maximum antitumor activity in preclinical models in humanized Dll4 SCID mice (manuscript in preparation, data on file; Regeneron). Enoticumab demonstrated clinical activity in several tumor types, including 2 confirmed partial responses in ovarian carcinoma and NSCLC. Prolonged stable diseases >6 months were

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seen in a colorectal adenocarcinoma patient with FAP syndrome, and 2 patients with ovarian cancer (endometrioid and transitional cell type). On the basis of PK and the antitumor activity observed, acceptable RP2Ds are 3 mg/kg Q2W and 4 mg/kg Q3W.

The cross-talk between the Notch and Wnt-β catenin pathways is known to contribute to tumorigenesis (28). Constitutive activation of Wnt/β–catenin due to mutations in β–catenin or the adenomatous polyposis coli (APC) genes may predict benefit from Notch inhibition. Although this interaction may be tissue dependent, we documented interesting sustained activity of enoticumab in tumors with β-catenin activation. One patient with bronchoalveolar NSCLC harboring a β-catenin mutation had a PR, and one heavily pretreated (including prior anti-VEGF therapy) patient with colon cancer and germline adenomatous polyposis coli (APC) gene mutation had stable disease for >12 months. The Wnt/β-catenin pathway can be activated by both germline and sporadic APC gene mutations (commonly seen in about 60% of patients with colorectal cancer). Other mechanisms for Wnt/β-catenin pathway activation have been described in colorectal cancer (KRAS/ BRAF oncogenic activation, APC promoter methylation), but our patients have not undergone on-study tumor genomic profiling. It is unclear whether germline versus sporadic APC mutations correlate with an increased benefit from anti-Dll4 therapy, but this should be further studied.

Notch is a key pathway in ovarian cancer, and Dll4 is overexpressed in up to 72% of ovarian carcinomas (15, 29). Enoticumab had activity in patients with ovarian cancer, not only serous papillary type (PR), but also in endometrioid and transitional cell tumors (SD > 6 months).

Preclinical models suggest that Notch mediates chemotherapy-resistant phenotypes, and while we did not see marked responses with single-agent enoticumab, it is possible that combining Notch/Dll4 inhibitors with select chemotherapeutic or biologic agents would enhance activity (3, 30). Notch/Dll4 is a negative regulator of the VEGF/VEGFR-2 axis, therefore Dll4 blockade is expected to activate VEGF-induced angiogenesis. In our study, grade 3 HTN was rare (8%), possibly because Dll4 is differentially expressed in tumor versus normal endothelium, and the negative feedback on VEGF/VEGFR-2 may have induced higher VEGF levels. Because productive tumor angiogenesis involves both VEGF and Notch, combined inhibition of both pathways is expected to increase efficacy.

Dll4 is a valid target for tumor inhibition, but further research needs to identify the cancer types most likely to benefit. We assessed the expression of several potential predictive biomarkers in archive tumor biopsies. Vascular expression of Dll4 was detected in most archival tumor samples, but the correlation between Dll4 levels and clinical response remains unclear and warrants further exploration. Notch1 and Notch3 expression was restricted to fewer samples suggesting involvement of different Notch receptors in Dll4-driven tumor angiogenesis. Vascular expression of VEGFR-2 correlated with vascular expression of Dll4 and Notch3, and cytoplasmic expression of Notch1 and Notch3. This finding underlies the hypothesis that both VEGF and Notch pathways contribute to tumor angiogenesis and points to potential efficacy with combined therapies. As expected, HIF1α was primarily sequestered to the nuclear region confirming high oxidative stress in the tumor microenvironment, but no correlations between HIF1α level and clinical response or other markers were observed.

In summary, enoticumab could be safely administered with manageable toxicities, including several reversible cardiovascular events. Enoticumab monotherapy demonstrated antitumor activity in molecular- and angiogenesis-relevant scenarios, and it is currently being tested in expansion cohorts in ovarian cancer.

Disclosure of Potential Conflicts of Interest

A. Harris reports receiving commercial research grants from Regeneron. No potential conflicts of interest were disclosed by the other authors.

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Clinical Cancer Research

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