

A Phase I/II, Multiple-Dose, Dose-Escalation Study of Siltuximab, an Anti-Interleukin-6 Monoclonal Antibody, in Patients with Advanced Solid Tumors

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Abstract

Purpose: This phase I/II study evaluated safety, efficacy, and pharmacokinetics of escalating, multiple doses of siltuximab, a chimeric anti-interleukin (IL)-6 monoclonal antibody derived from a new Chinese hamster ovary (CHO) cell line in patients with advanced/refractory solid tumors.

Experimental Design: In the phase I dose-escalation cohorts, 20 patients with advanced/refractory solid tumors received siltuximab 2.8 or 5.5 mg/kg every 2 weeks or 11 or 15 mg/kg every 3 weeks intravenously (i.v.). In the phase I expansion ($n = 24$) and phase II cohorts ($n = 40$), patients with *Kirsten rat sarcoma-2* (*KRAS*)-mutant tumors, ovarian, pancreatic, or anti-EGF receptor (EGFR) refractory/resistant non-small cell lung cancer (NSCLC), colorectal, or H&N cancer received 15 mg/kg every 3 weeks. The phase II primary efficacy endpoint was complete response, partial response, or stable disease >6 weeks.

Results: Eighty-four patients (35 colorectal, 29 ovarian, 9 pancreatic, and 11 other) received a median of three (range, 1–45) cycles. One dose-limiting toxicity occurred at 5.5 mg/kg. Common grade ≥ 3 adverse events were hepatic function abnormalities (15%), physical health deterioration (12%), and fatigue (11%). Ten percent of patients had siltuximab-related grade ≥ 3 adverse events. Neutropenia (4%) was the only possibly related adverse event grade ≥ 3 reported in >1 patient. Serious adverse events were reported in 42%; most were related to underlying disease. The pharmacokinetic profile of CHO-derived siltuximab appears similar to the previous cell line. No objective responses occurred; 5 of 84 patients had stable disease >6 weeks. Hemoglobin increased ≥ 1.5 g/dL in 33 of 47 patients. At 11 and 15 mg/kg, completely sustained C-reactive protein suppression was observed.

Conclusions: Siltuximab monotherapy appears to be well tolerated but without clinical activity in solid tumors, including ovarian and *KRAS*-mutant cancers. The recommended phase II doses were 11 and 15 mg/kg every 3 weeks. *Clin Cancer Res*; 20(8); 2192–204. ©2014 AACR.

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Introduction

Siltuximab is a chimeric (murine–human) monoclonal antibody with high binding affinity for human interleukin (IL)-6 (1–3). IL-6 is implicated in the pathophysiology of various solid tumors. High IL-6 levels are prognostic and correlate with tumor metastasis, disease stage, and short survival in renal, prostate, breast, pancreatic, and ovarian cancers (4–9). Ovarian cancer cell lines produce IL-6 protein in the range of 10 to 10⁴ pg/10⁵ cells, which can be further increased by treatment with gonadotrophins or lysophosphatidic acid (10, 11). IL-6 may also act as an autocrine growth factor for ovarian cancer tumor cells (10).

Kirsten rat sarcoma-2 (*KRAS*) mutations are prevalent among many malignancies that respond poorly to available treatment options. More than 90% of patients with pancreatic cancer exhibit *KRAS* mutations (12). In non-small cell lung cancer (NSCLC) and colorectal cancer, *KRAS*

Translational Relevance

Interleukin (IL)-6 has been implicated in various solid tumors and may have an important role in mutant *Kirsten rat sarcoma-2 (KRAS)*-driven tumorigenesis. Siltuximab, an anti-IL-6 monoclonal antibody, has shown antitumor activity in *KRAS*-mutant tumor models and single-agent activity in patients with multicentric Castleman's disease (MCD) or renal cancer. In this phase I/II study in advanced, heavily pretreated metastatic solid tumors, multiple dose-escalating regimens of siltuximab from a new Chinese hamster ovary (CHO)-derived cell line were well tolerated and had a similar pharmacokinetic/pharmacodynamic profile to the previous Sp2/0 myeloma-derived siltuximab in renal cancer. However, no objective responses were seen irrespective of dose level or *KRAS* mutational status, suggesting that IL-6 inhibition alone is insufficient in treating advanced or refractory solid tumors. Recommended phase II doses are 11 and 15 mg/kg every 3 weeks, although no further trials are planned in solid tumors. Randomized studies of CHO-derived siltuximab are ongoing in MCD and smoldering myeloma.

mutations are present in a minority of patients but are a strong negative predictor of response to EGF receptor (EGFR)-targeted therapy (13, 14), with only a 3% response rate in patients with *KRAS*-mutant tumors compared with 27% and 35% in NSCLC and colorectal patients with wild-type *KRAS* (15).

Transformation of cells with mutant *KRAS* leads to upregulation of multiple cytokines, including IL-6 (16). Blockade of IL-6, either by small hairpin RNA or antibody, reduced *in vivo* growth and inhibited tumor angiogenesis of *KRAS*-driven tumors. Thus, IL-6 may be an important downstream effector of activated *KRAS* that can bypass the interrupted signaling induced by EGFR inhibition and affect the surrounding tumor microenvironment (16). Siltuximab has demonstrated antitumor activity in established models of *KRAS*-mutant lung and pancreatic tumors (sponsor data on file).

As a known proinflammatory cytokine, IL-6 plays a key role in the development of inflammatory anemia of cancer by stimulating the production of hepcidin, the principal regulator of iron homeostasis. Increases in hepcidin result in decreased absorption of iron, resulting in iron-reutilization restricted erythropoiesis and reduced hemoglobin levels (17). Treatment with siltuximab led to a sustained increase in hemoglobin levels in patients with renal cell carcinoma (18) and multicentric Castleman's disease (MCD; ref. 19). IL-6 is also the primary factor that drives C-reactive protein (CRP) expression (20), and an earlier pharmacokinetic/pharmacodynamic modeling study indicated that CRP was a suitable biomarker of IL-6 bioactivity (21). Suppression of CRP following siltuximab treatment was also seen in previous

clinical studies, reflecting neutralization of *in vivo* IL-6 bioactivity (21, 22).

Siltuximab derived from a murine Sp2/0 myeloma cell line was used in earlier clinical studies and had induced objective responses and provided evidence of symptom improvement as a single agent in MCD (19, 22). However, due to suboptimal product yield from the Sp2/0 cell line, a switch to a Chinese hamster ovary (CHO) cell line was made. A study in 131 healthy volunteers demonstrated pharmacokinetic comparability of a single 1.4 mg/kg i.v. dose of the new lyophilized CHO cell line-derived siltuximab against the previous Sp2/0 cell line-derived liquid i.v. formulation (article in press). However, confirmation and full characterization of the safety and pharmacokinetics of the new formulation in patients with cancer was warranted.

Herein, we report the results of a phase I/II study of CHO-derived siltuximab in patients with advanced or refractory malignant solid tumors, including ovarian and *KRAS*-mutant tumors. *KRAS* mutational status was included to assess its use as a potential selection factor for identifying patients likely to benefit from siltuximab treatment. CRP suppression following different doses was examined to identify an appropriate dosing schedule for future siltuximab studies, and the effect of siltuximab on hemoglobin was further investigated. An expeditious dose-escalation scheme was used to minimize the likelihood of exposure to subtherapeutic dosing while still enabling pharmacokinetic characterization of the anticipated dose range of 2.8 to 15 mg/kg, preserving an adequate safety margin for CHO-derived siltuximab, with the highest planned dose comparable in dose intensity to the highest Sp2/0-derived dose studied clinically and moving toward the every-3-weeks dosing schedule for future studies.

Patients and Methods

Patients

Eligible patients were ≥ 18 years old and had histologically/cytologically documented malignant solid tumors types as described below; Eastern Cooperative Oncology Group (ECOG) performance status score ≤ 2 ; and adequate bone marrow, liver, and renal function. Patients in cohorts 1 to 4 had solid tumors that progressed on/after standard therapy or for which there was no effective therapy. Patients in remaining cohorts had evaluable/measurable disease defined by Response Evaluation Criteria in Solid Tumors (RECIST) criteria v1.0, as applicable. Patients in the phase II ovarian cohort had platinum- and taxane-resistant epithelial ovarian cancer for which there was no effective therapy. Patients in the phase II *KRAS* cohort had previously received ≥ 1 line of standard chemotherapy and had known *KRAS*-mutant tumors, pancreatic cancer, or NSCLC, colorectal, or head and neck (H&N) cancer that was refractory/resistant to anti-EGFR therapy. Patients in the phase I expansion cohort 5 had to meet the same criteria as either phase II cohort. Use of prior systemic therapy or major surgery for cancer was not permitted within 4 weeks (nitrosoureas and mitomycin C not permitted within 6 weeks) before study treatment. Following a protocol amendment during expansion

cohort 5 enrollment, pretreatment with bevacizumab within 12 weeks of the start of study treatment was no longer permitted. Concomitant treatments of underlying cancer were prohibited, except for stable low-dose steroids or luteinizing hormone-releasing hormone agonists for prostate cancer. All patients provided written informed consent. This study was conducted according to the Declaration of Helsinki and was approved by the local Institutional Review Boards or ethics committees at each study site.

Study design

This was a two-part, phase I/II, open-label, dose-escalating study of single-agent siltuximab. The 5-cohort, phase I portion of the study was conducted to determine a recommended phase II dose (RP2D). Cohorts 1 to 4 each planned to enroll 1 to 6 patients sequentially to evaluate escalating siltuximab doses at 2.8 mg/kg every 2 weeks, 5.5 mg/kg every 2 weeks, 11 mg/kg every 3 weeks, and 15 mg/kg every 3 weeks in patients with all solid tumor types; each cohort included a 3-week monitoring period after dose 1 to evaluate dose-limiting toxicity (DLT) and pharmacokinetics. A study evaluation team comprising investigators, sponsor clinicians, and statisticians determined the RP2D as the highest dose at which <2 of 6 patients experienced a DLT in cohorts 2 to 4. Expansion cohort 5 evaluated the RP2D in up to 20 patients with solid tumors with unmet medical need or rationale for targeting IL-6.

The decisions to initiate the phase II ovarian cohort and the phase II *KRAS* cohort were based on the acceptability of safety data and meeting the interim analysis for preliminary efficacy for each respective patient group [i.e., ≥ 1 patients achieving tumor response or ≥ 2 of 10 patients treated at RP2D achieving tumor response or minor response defined as tumor stabilization (stable disease >6 weeks), tumor marker response, hemoglobin increase >1.0 g/dL over baseline in anemic patients, or clinical improvement]. The phase I expansion cohort 5 could continue to enroll until both phase II cohorts were opened or a determination of futility was made.

If initiated, each phase II cohort would enroll up to 20 patients to estimate tumor response. Secondary objectives of the overall study were to assess the safety profile, pharmacokinetics, immunogenicity, and pharmacodynamic effects associated with the IL-6 pathway, mechanisms of anemia, and correlation with *KRAS* mutations.

Safety

All adverse events and serious adverse events (SAEs) occurring ≤ 30 days after the last dose were reported according to the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0. SAE reporting was extended through 90 days after the last dose in phase I. DLT was defined as any grade ≥ 3 nonhematologic toxicity (except for nausea, vomiting, or diarrhea controllable with antiemetics/anti-diarrheal treatment; controllable grade 3 hypertension; or single hypersensitivity reaction per cohort), clinically relevant hematologic toxicity (e.g., grade 4 neutropenia lasting >1 week, febrile neutropenia, grade 4

thrombocytopenia lasting >1 week, or associated hemorrhage), or any other siltuximab-related toxicity that the study evaluation team considered dose-limiting.

Efficacy

Preliminary clinical efficacy was evaluated by radiologic and tumor marker assessments (e.g., CA-125) performed at screening and then every 9 weeks until progressive disease or withdrawal of consent. Major response was defined as partial response (PR) or complete response (CR) by RECIST, where applicable. Minor response was defined as stable disease confirmed >6 weeks, $\geq 50\%$ reduction in tumor marker, ≥ 1.0 g/dL increase from baseline in hemoglobin in anemic patients without transfusion/erythropoietin-stimulating agents (ESA), or clinical improvement (e.g., symptoms, performance status, and fatigue) by investigator assessment in the absence of objective progressive disease.

The primary efficacy endpoint was CR, PR, or stable disease lasting >6 weeks.

Pharmacokinetics

Serial blood samples were obtained at specified time points throughout the study. Pharmacokinetic sampling and data analysis methods are described in Supplementary Materials.

Immunogenicity

Blood samples to evaluate antibodies to siltuximab were collected at baseline and at weeks 4, 8, and 12 during follow-up and analyzed using a validated bridging immunoassay in which siltuximab-derived reagents were used to capture and detect antibodies.

Pharmacodynamics

All treated patients with appropriate postbaseline samples were evaluable for pharmacodynamic analyses. *KRAS* mutational status was evaluated in all cohorts with available archived biopsy samples by local methods and also by central testing using the DxS *KRAS* mutation kit at Genzyme Genetics. CRP was measured at baseline and several post-treatment time points using the CRP High Sensitivity Assay (23) at Covance Central Laboratory Services by immunonephelometry using the Behring Nephelometer II. The lower limit of quantification (LLOQ) for this CRP assay was 0.2 mg/L. Levels of low- and high-molecular-weight IL-6 complexes were measured at baseline using a recently developed, validated panoptic IL-6 Meso Scale Discovery-based single-plex platform with a LLOQ of 9.77 pg/mL and an upper (U)LLOQ of 10,000 pg/mL (Janssen). Bioactive Hepcidin-25 was measured using a validated, polyclonal antibody-based competitive ELISA (24) with a LLOQ of 19 ng/mL (Intrinsic LifeSciences). Pharmacodynamic sampling time points and other exploratory biomarker analyses are described in Supplementary Materials.

Statistical analyses

Descriptive statistics were used to summarize data. Sample sizes for cohorts 1 to 4 were not based on hypothesis

testing. Assuming an alternative hypothesis rate of 20%, a sample size of 20 patients was planned for expansion cohort 5 to provide 59% power and a sample size of 20 patients was planned for each phase II cohort to provide 80% power to reject a null hypothesis response rate of 5% at a fixed, one-sided level of significance of 0.1. Patients who had ≥ 1 siltuximab administration and ≥ 1 postbaseline evaluation were evaluable for efficacy response (except for hemoglobin response, for which patients must have also had baseline hemoglobin below lower limit of normal).

Results

Patient characteristics

From February 2009 to April 2011, 84 patients were enrolled and treated at 13 sites (3 France, 3 Belgium, 3 United Kingdom, 2 Spain, and 2 United States). The most common tumor types were colorectal (42%), ovarian (35%), and pancreatic (11%; Table 1). Eighteen of 23 patients in the *KRAS* cohort had sufficient samples for central laboratory testing, of which 13 tested positive locally and centrally, 3 (2 colorectal and 1 esophageal) tested positive locally but negative centrally, 1 (colorectal) who tested negative by both laboratories, and 1 (pancreatic) tested negative centrally and was not tested locally. The discrepancy between central and local *KRAS* assay methods could be due to the heterogeneity in the testing samples or different assay methods. Patients were all heavily pretreated (range, 1–15), with 49% having received 5 or more lines of prior therapy. Most patients had an ECOG performance status score of 0 (43%) or 1 (49%).

Safety

All patients were treated as assigned: cohort 1 ($n = 1$), cohort 2 ($n = 6$), cohort 3 ($n = 6$), cohort 4 ($n = 7$), expansion cohort 5 ($n = 24$), ovarian cohort ($n = 17$), and *KRAS* cohort ($n = 23$). Overall, the patients received a median of 3 (maximum 45) siltuximab doses for a median duration of 6 weeks (maximum 21 months). Seventy-five (89%) patients discontinued treatment due to progressive disease [$n = 68$ (81%)], adverse event [$n = 5$ (6%), including 1 possibly related], physician decision, or death [$n = 1$ (1%); Supplementary Fig. S1].

In the phase I part of the study, only one DLT of grade 3 jejunal perforation was reported in cohort 2 with 5.5 mg/kg every 2 weeks. This event was observed in a patient with colorectal cancer with peritoneal metastases, prior surgery (hemicolectomy), and bevacizumab use 5 weeks before first siltuximab dose. Although this event was considered unlikely to be related to siltuximab, it met the DLT criteria and led to the expansion of cohort 2 by 3 additional patients. Two other patients (in expansion cohort 5 and in *KRAS* cohort) also experienced a bowel wall event (one intestinal perforation and one pneumatosis intestinalis) in a similar context of abdominal disease, history of abdominal surgery, and recent bevacizumab use before first siltuximab dose. At the higher dose cohorts of 11 and 15 mg/kg every 3 weeks, no DLT was reported. Because no additional DLTs occurred, as recommended by the study evaluation team, the highest

dose level of 15 mg/kg every 3 weeks was further explored in the phase II portion of the study.

Almost all patients (98%) had 1 or more adverse events (Supplementary Table S1), and adverse events considered possibly related to siltuximab were reported by 44% of patients, including asthenia (8%), fatigue, thrombocytopenia (each 7%), neutropenia (6%), hepatic function abnormal, nausea, and vomiting (each 5%). In consideration of the low sample size in the first 4 cohorts, no significant differences in safety profile were observed at 11 and 15 mg/kg every 3 weeks. The most frequently reported adverse events regardless of relation to siltuximab were asthenia (27%), nausea (27%), and constipation (26%), largely consistent with symptoms of underlying disease. Adverse events reported by $\geq 10\%$ of patients overall and ≥ 2 patients in any cohort are shown in Table 2. Of 20 cases of hepatic function abnormalities, 18 were confounded by underlying liver metastases; transient, low-grade bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) elevations were observed in the remaining 2 patients.

Adverse events of grade ≥ 3 occurred in 62% of patients, but only 8 (10%) patients had grade ≥ 3 adverse events possibly related to siltuximab; of these, only neutropenia (4%) was reported in >1 patient. Most commonly reported adverse events regardless of relation to siltuximab were hepatic function abnormalities (15%), fatigue (11%), hyperbilirubinemia (7%), dyspnea (7%), general physical health deterioration (6%), abdominal pain (5%), and ascites (5%), also largely consistent with underlying disease. The maximum severity grade for fatigue was grade 2, and fatigue was transient in 4 of 6 patients who received ≥ 4 siltuximab doses. Most patients who reported grade ≥ 3 adverse events had events of no greater than grade 3 toxicity, except for hepatic function abnormalities (grade 4 in 4 patients, grade 5 in 1 patient), hyperbilirubinemia (grade 4 in 1 patient), general physical health deterioration (grade 5 in 4 patients), and dyspnea (grade 4 in 1 patient). Other grade ≥ 3 laboratory abnormalities were infrequent (neutropenia 4%, leukopenia 2%, and lymphocytopenia 4%).

Two (2%) patients had SAEs possibly related to siltuximab: 1 ovarian cohort patient had device-related infection and pulmonary embolism; and 1 *KRAS* cohort patient developed ileus, pneumatosis intestinalis, and general physical health deterioration by investigator assessment. Overall, 35 (42%) patients had SAEs regardless of relationship to siltuximab, most commonly general physical health deterioration as assessed by investigator (13%) and dyspnea (6%). All patients who reported an SAE of dyspnea had underlying lung metastases. Two patients with hemoglobin increases in the absence of transfusion or ESAs were found to have deep vein thrombosis (DVT) without apparent relationship to the hemoglobin increase. One patient had a history of DVT and continued siltuximab treatment with an increase in hemoglobin to 9.6 g/dL without recurrence of DVT. The second patient was diagnosed with DVT at the time of the first siltuximab administration (hemoglobin 8.7 g/dL) and did not experience

Table 1. Baseline demographics and disease characteristics

	Phase I					Phase II	
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Expansion cohort 5	Ovarian cohort	KRAS cohort
Patients treated	1	6	6	7	24	17	23
Female	0	4	2	6	16	17	10
Age, y	56	66 ± 13	69 ± 4	51 ± 15	60 ± 10	58 ± 10	60 ± 9
ECOG performance status score							
0	1	2	1	2	12	6	12
1	0	4	5	4	10	8	10
2	0	0	0	1	2	3	1
Tumor type							
Colorectal	0	3	2	5	8	0	17
Ovarian	0	1	1	1	9	17	0
Pancreatic	0	0	1	0	4	0	4
NSCLC	0	0	0	0	3	0	1
Esophageal	0	1	0	0	0	0	1
Other ^a	1	1	2	1	0	0	0
KRAS mutation-positive ^b	0/0	1/3	1/3	2/2	9/22	1/13	13/18
Tumor metastases							
Lung	0	4	2	5	18	4	15
Liver	0	3	6	4	17	10	20
Lung and liver	0	2	2	4	13	2	13
Bone	0	0	2	1	3	2	4
≥ 3 Sites	0	2	5	5	18	11	13
Prior therapy							
Radiotherapy	0	3	2	3	4	2	12
Systemic therapy	1	6	6	7	24	17	23
1	1	0	1	0	1	0	0
2	0	4	1	0	6	1	5
3	0	1	1	1	5	2	7
4	0	0	0	1	2	0	3
≥ 5	0	1	3	5	10	14	8
Bevacizumab	0	4	2	7	9	4	11
Serum IL-6 concentration, pg/mL	4.9	16.6 [4.9–37.8]	4.9 [4.9–15.3]	4.9 [4.9–42.3]	18.0 [4.9–785.8]	4.9 [4.9–34.1]	14.7 [4.9–490.9]

NOTE: Data presented as n, mean ± SD, or median [range].
^aOther tumor types were gastric cancer, malignant melanoma, malignant neoplasm of ampulla of Vater, thyroid cancer, ureter cancer (each n = 1).
^bPatients tested by central laboratory.

Table 2. Patients with ≥ 1 adverse events reported by $\geq 10\%$ of patients overall and ≥ 2 patients in any cohort

	Phase I						Phase II				
	Cohort 1		Cohort 2		Cohort 3		Cohort 4		Expansion cohort 5	KRAS cohort	
	2.8 mg/kg q2w	5.5 mg/kg q2w	11 mg/kg q3w	15 mg/kg q3w	15 mg/kg q3w	15 mg/kg q3w	15 mg/kg q3w	15 mg/kg q3w	15 mg/kg q3w	15 mg/kg q3w	15 mg/kg q3w
Patients treated, <i>n</i>	1	6	6	7	24	23					
Patients with ≥ 1 adverse events, %	100	100	100	100	100	91					
Asthenia	100	17	33	29	21	29					
Nausea	100	0	33	29	21	17					
Constipation	100	33	17	29	25	17					
Hepatic function abnormal	100	0	0	14	25	39					
Fatigue	0	0	33	14	33	13					
Abdominal pain	100	33	0	0	17	13					
Vomiting	0	0	33	0	13	17					
Decreased appetite	0	0	33	0	4	35					
Dyspnea	0	17	50	14	21	17					
Hyperbilirubinemia	100	0	0	0	21	22					
Diarrhea	0	33	50	14	8	0					
Headache	0	17	17	0	4	13					
Edema peripheral	0	17	33	0	4	13					
Thrombocytopenia	0	17	33	14	8	9					
Urinary tract infection	0	17	17	0	17	4					
Back pain	0	0	17	14	13	13					

Abbreviations: q2w, every 2 weeks; q3w, every 3 weeks.

any new thrombovascular events despite a subsequent hemoglobin increase to 12.2 g/dL.

Six (7%) patients required a dose delay for adverse event (one instance of reduced neutrophil count, two instances of increase in ALT/AST, and three due to other clinically significant toxicities/medical reasons). Two patients had low-grade infusion reactions: one patient had adverse events of chest pain (grade 1), nausea (grade 2), and vomiting (grade 2); another patient had infusion-site pruritus (grade 1) and infusion-site paraesthesia (grade 1). Both patients recovered without requiring further dose delays. Five patients (6%) discontinued siltuximab due to adverse events; most adverse events leading to discontinuation were considered unrelated to siltuximab, except for 1 patient with grade 4 hepatotoxicity (albumin, 43 g/dL; alkaline phosphatase, 190 U/L; ALT, 128 U/L; AST, 352 U/L; bilirubin, 34 μ mol/L; and γ -glutamyl-transferase, 1,100 U/L) and 1 patient with grade 1–2 pruritus, pollakiuria, diarrhea, upper abdominal pain, vomiting, and arthralgia. Eighteen patients died during the study: 14 due to progressive disease, 1 due to intestinal perforation, and 3 due to disease during follow-up.

Pharmacokinetics

All 84 treated patients were evaluable for pharmacokinetics. Because only 1 patient was dosed at 2.8 mg/kg, it is not possible to make definite conclusions about dose proportionality and pharmacokinetic profile at this dose level. Following doses ranging from 5.5 to 15 mg/kg, serum concentrations of siltuximab following the first dose declined in a bi-exponential manner, with a mean terminal $t_{1/2}$ ranging from approximately 15 to 20 days, and apparent dose proportionate increases in the maximum observed serum concentration (C_{max}) and area under the serum concentration versus time curve from time 0 to infinity with extrapolation of the terminal phase (AUC_{∞} ; Table 3). The mean clearance was similar and ranged from 2.97 to 4.05 mL/d/kg across the 5.5 to 15 mg/kg dose groups. On the basis of a cross-study comparison, the pharmacokinetic profile of CHO-derived siltuximab in patients with cancer appears to be similar to the pharmacokinetic profile of Sp2/0-derived siltuximab (21, 22).

Immunogenicity

Of the 40 patients with appropriate samples, none had detectable antibodies to siltuximab at baseline or any post-baseline time point.

Efficacy

The primary efficacy endpoint of CR, PR, or stable disease lasting >6 weeks was 6%, with stable disease lasting >6 weeks observed in 5 patients. One of the 5 patients with stable disease was treated at 5.5 mg/kg every 2 weeks (cohort 2, papillary thyroid cancer), and the other 4 patients were treated at 15 mg/kg every 3 weeks (2 KRAS mutation-positive colorectal cancer, 1 KRAS mutation-negative colorectal cancer, and 1 ovarian cancer). No objective response by RECIST or investigator assessment was observed. Uncon-

firmed stable disease by RECIST was observed in 13 patients [median overall survival (OS), 454 days], including 5 patients with stable disease >6 weeks (median OS, 460 days). Nine patients (11%) showed clinical improvement in symptoms or performance status by investigator assessment. Twelve (71%) of 17 evaluable patients in phase I expansion cohort 5 showed a hemoglobin response (defined as a hemoglobin increase of >1.0 g/dL over baseline at least once); therefore, protocol criteria were met to initiate both phase II cohorts.

For the phase II ovarian and KRAS cohorts, the median progression-free survival was 57 (range, 51–63) and 59 (range, 57–61) days, respectively, and the OS was 335 (range, 72–NE) and 127 (range, 85–212) days, respectively (Table 4). Of note, the longest duration on treatment was 21 months for the papillary thyroid cancer patient with stable disease in cohort 2 who had previously received 2 prior lines of therapy with sorafenib/tipifarnib and sorafenib monotherapy.

Thirty of 84 patients received subsequent therapies, including systemic therapies [$n = 28$; most commonly fluorouracil ($n = 8$) or investigational agent ($n = 6$)], radiotherapy ($n = 5$), and cancer-related surgery ($n = 4$).

Pharmacodynamics

A total of 83 patients were evaluated for serum CRP levels. At dose 1 day 8, a >50% decrease in CRP from baseline was observed in 83%, 100%, and 99% of patients treated with 5.5, 11, and 15 mg/kg siltuximab. Median CRP levels at dose 1 day 8 decreased from baseline by 82% (cohort 1; 2.8 mg/kg) to 94% in the phase I cohorts and by 92% to 93% in the phase II cohorts. The decrease in CRP levels was sustained throughout the treatment period (Fig. 1). Specifically, the median percentage decrease in cohort 3 (11 mg/kg) after dose 1 was 93% at day 8 and 98% at day 15 and remained suppressed at later time points through extended treatment and 4 weeks after the last administration. Similar CRP suppression was observed in patients dosed with 15 mg/kg every 3 weeks, with 88% to 94% median decrease by dose 1 day 8 that was sustained up to 8 weeks after the last siltuximab administration (Fig. 1). Overall, the CRP suppression observed with CHO-derived siltuximab was similar to that observed in a previous study with Sp2/0-derived siltuximab. Because of the limited clinical response and lack of relevant toxicity observed, no meaningful efficacy/safety associations were seen with the changes in CRP.

At baseline, 48 (57%) of 84 patients evaluated using the panoptict IL-6 assay (an assay that detects all forms of IL-6, i.e., high- and low-molecular-weight complexes) had measurable serum concentrations (Table 1), including 47 with elevated IL-6 (defined as ≥ 10 pg/mL). Serum concentrations of the GP80 and GP130 subunits of the IL-6R were unaffected by siltuximab and remained stable during treatment in all patients examined (data not shown). No strong or consistent changes in IL-6 network strength-related RNA expression were observed in blood samples (86 samples total, with 28 paired pre- and posttreatment) or a limited

Table 3. Siltuximab pharmacokinetic parameter estimates following the first dose^a

	Phase I					Phase II	
	Cohort 1 2.8 mg/kg q2w	Cohort 2 5.5 mg/kg q2w	Cohort 3 11 mg/kg q3w	Cohort 4 15 mg/kg q3w	Expansion cohort 5 15 mg/kg q3w	Ovarian cohort 15 mg/kg q3w	KRAS cohort 15 mg/kg q3w
Patients treated	1	6	6	7	24	17	23
AUC _{0-∞} , μg·d/mL	1	6	5	6	13	12	16
	422.1 ± NA	1,912.5 ± 793.92	3,144.9 ± 369.32	5,680.3 ± 1,725.43	5,269.2 ± 1,877.85	4,090.7 ± 1,342.20	5,449.9 ± 1,389.08
AUC _(0-t) , μg·d/mL	1	6	5	6	13	12	16
	344.8 ± NA	1,135.8 ± 242.10	2,267.2 ± 343.24	3,779.8 ± 716.64	3,102.2 ± 888.51	2,613.5 ± 768.32	3,006.0 ± 547.66
C _{max} , μg/mL	1	6	5	7	24	17	21
	56.6 ± NA	107.3 ± 23.34	218.8 ± 36.50	340.4 ± 41.88	343.0 ± 66.33	294.7 ± 78.43	322.6 ± 66.68
t _{1/2} , d	1	6	5	6	13	12	16
	12.7 ± NA	19.2 ± 8.38	16.3 ± 4.25	17.5 ± 4.98	17.4 ± 3.74	15.4 ± 4.79	20.0 ± 5.41
CL, mL/d/kg	1	6	5	6	13	12	16
	6.64 ± NA	3.28 ± 1.25	3.54 ± 0.44	2.97 ± 1.31	3.19 ± 1.09	4.05 ± 1.40	2.92 ± 0.72
V _{ss} , mL/kg	1	6	5	6	13	12	16
	105.1 ± NA	79.7 ± 21.24	77.5 ± 19.16	63.9 ± 2.37	71.3 ± 20.84	78.5 ± 21.74	75.9 ± 19.29

NOTE: Data presented as n evaluable or mean ± SD.

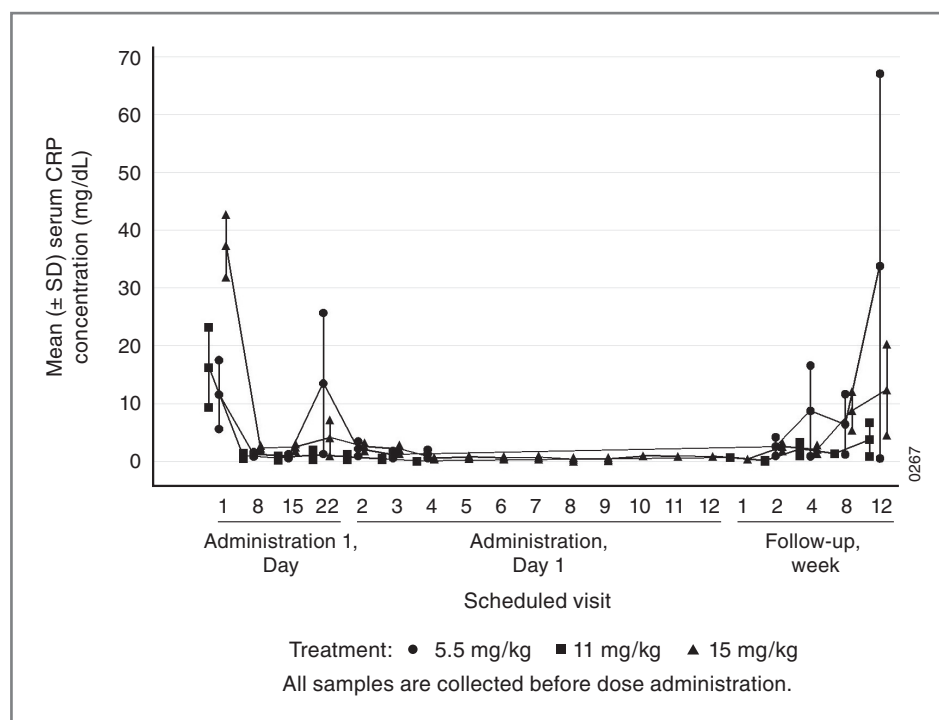
Abbreviations: CL, total systemic clearance of drug after intravenous administration; NA, not available; q2w, every 2 weeks; q3w, every 3 weeks; V_{ss}, volume of distribution at steady-state.^aPharmacokinetic sampling through day 28 for cohorts 1 to 4 and through day 21 for expansion cohort 5 and phase II cohorts.

Table 4. Efficacy

	Phase I					Phase II		
	Cohort 1 2.8 mg/kg q2w	Cohort 2 5.5 mg/kg q2w	Cohort 3 11 mg/kg q3w	Cohort 4 15 mg/kg q3w	Expansion cohort 5 15 mg/kg q3w	Ovarian cohort 15 mg/kg q3w	KRAS cohort 15 mg/kg q3w	
Patients treated	1	6	6	7	24	17	23	
Patients with CR, PR, or stable disease lasting >6 wks	0	1	0	2	0	1	1	
Stable disease >6 wks	0	1	0	2	0	1	1	
CR, PR, or stable disease lasting >6 wks rate (95% CI)	0 (NE-NE)	17 (0-64)	0 (NE-NE)	29 (4-71)	0 (NE-NE)	6 (0-29)	4 (0-22)	
Patients with stable disease								
By RECIST	0/1	3/4	3/6	2/7	2/20	1/13	2/19	
By investigator assessment	0/1	3/6	2/6	2/7	1/23	1/17	2/22	
Patients with tumor marker response	0	1	0	0	0	0	0	
CA-125	0	1	0	0	0	0	0	
Patients with hemoglobin response	0/0	1/2	3/3	1/3	12/17	7/9	9/13	
Rate	0 (NE-NE)	50 (1-99)	100 (NE-NE)	33 (1-91)	71 (44-90)	78 (40-97)	69 (39-91)	
Baseline hemoglobin, g/dL	NA	11.7 ± NA	11.0 ± 1.3	11.7 ± NA	10.6 ± 1.0	10.7 ± 0.8	10.8 ± 1.2	
Maximum increase from baseline, g/dL	NA	1.8 ± NA	2.6 ± 0.5	1.9 ± NA	2.4 ± 1.3	2.2 ± 0.9	2.0 ± 1.1	
PFS, median (95% CI)	17 (NE-NE)	131 (55-NE)	97.5 (34-194)	62 (43-381)	48 (42-60)	57 (51-63)	59 (57-61)	
OS, median (95% CI)	37 (NE-NE)	268 (207-NE)	266 (62-494)	154 (73-500)	79 (66-227)	335 (72-NE)	127 (85-212)	

NOTE: Data presented as *n*, *n*/total *n* evaluable, or % (95% CI), unless noted otherwise. Abbreviations: PFS, progression-free survival; q2w, every 2 weeks; q3w, every 3 weeks.

Figure 1. Mean (\pm SD) serum CRP concentration.



number of serial biopsy samples (19 samples total, with 12 paired pre- and posttreatment; data not shown).

The effect of IL-6 blockade on serum hepcidin levels was investigated before and following treatment with siltuximab. Seventy-five (89%) of 84 patients had detectable levels of hepcidin at baseline. Decrease in hepcidin concentrations was observed as early as 6 and 24 hours posttreatment and was generally sustained during treatment through dose 4 (Fig. 2). By dose 1 day 8, 69 (96%) of 72 patients with both baseline hepcidin above LLOQ and day 8 test results available showed 34% to 58% median decrease in hepcidin levels from baseline across all cohorts (Fig. 2). Across all cohorts, out of 33 (70%) of 47 evaluable patients who showed a hemoglobin response (defined as a hemoglobin increase of at least 1.0 g/dL over baseline), 29 patients (88%) showed hepcidin decrease at day 8 of administration 1.

Furthermore, exploratory analysis showed that among the 9 patients with elevated IL-6 (≥ 10 pg/mL) and hepcidin (≥ 65 ng/mL) at baseline, who were also anemic (i.e., hemoglobin ≤ 10 g/dL), 7 (78%) showed ≥ 1.5 g/dL hemoglobin improvement posttreatment; interestingly, 2 patients with normal IL-6 (< 10 pg/mL) but elevated hepcidin (≥ 65 ng/mL) also showed hemoglobin response (≥ 1.5 g/dL increase) at this cutoff (hemoglobin ≤ 10 g/dL). No changes in serum levels of other IL-6 pathway or anemia-associated proteins (erythropoietin, brain-derived neurotrophic factor, leptin, or bone morphogenetic protein 6) were observed in association with hemoglobin improvement in the 7 hemoglobin responders who were anemic and had elevated IL-6 and hepcidin at baseline (data not shown).

Markers associated with inflammation (IFN- γ , IL-1 β , IL-2, IL-5, IL-8, IL-10, IL-12, and TNF- α) were unaffected by

siltuximab and remained stable during treatment. Angiogenesis markers (VEGF, VEGF receptor, and basic fibroblast growth factor) also remained stable during treatment with siltuximab (data not shown).

Fresh biopsy samples for immunohistochemistry analysis were collected from a limited number of patients ($n = 13$, including 7 with pre- and posttreatment samples). Cytoplasmic, nuclear, and stromal staining of IL-6 was observed in the majority of these patients, along with staining of phosphorylated signal transducers and activators of transcription 3 (pSTAT-3), a marker associated with IL-6 signaling, in both pre- and posttreatment samples.

Association of marker expression to clinical response was not observed as no objective responses by RECIST were observed. However, 6 of 7 patients with pre- and posttreatment samples had a reduction in pSTAT-3 intensity or percentage of tumor cell staining suggestive of IL-6 neutralization and downstream signal modulation. No apparent treatment-related effects were observed in the expression of the proliferation marker Ki67 or of the apoptosis marker CC3 (data not shown).

Discussion

This phase I/II study explored multiple dose-escalating regimens of siltuximab derived from a new cell line in 84 patients with advanced, heavily pretreated, malignant solid tumors. Only 1 DLT was observed at 5.5 mg/kg every 2 weeks. Because there were no additional DLTs, dose expansion continued to the highest predefined dose level of 15 mg/kg every 3 weeks.

Although almost all (98%) treated patients reported adverse events, most events were largely driven by underlying

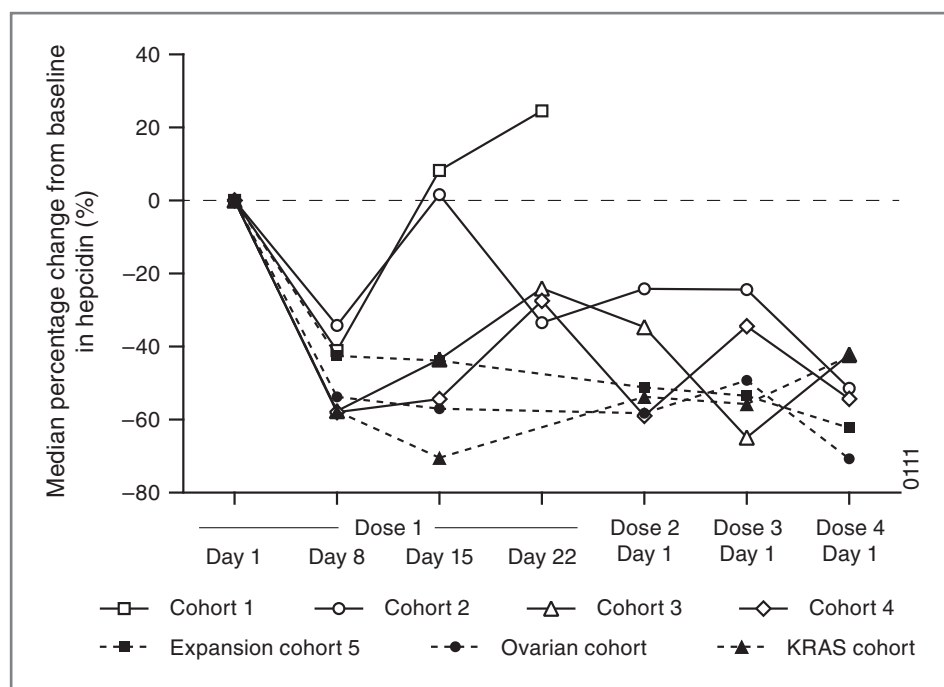


Figure 2. Median percentage change from baseline in hepcidin concentration.

metastatic disease and were consistent with the most common tumor types enrolled (e.g., ovarian, colorectal, etc.). Drug-related adverse events were mostly low grade: 29 patients (35%) had, at most, grade 1–2 adverse events and 8 patients (10%) had grade ≥ 3 adverse events. Asthenia or fatigue was reported in 39 patients, the majority of whom received less than 4 siltuximab doses, and was mostly associated with progressive disease. Hepatic dysfunction was reported by 20 (24%) patients. Eighteen of these 20 patients had confounding underlying liver metastases, and in the 2 patients without liver metastases, only transient low-grade bilirubin, AST, and ALT elevations were observed. Although a potential drug effect on liver function abnormalities cannot be excluded, a more likely explanation is disease progression in patients with underlying liver metastases. Drug-related grade ≥ 3 adverse events and SAEs were reported by only 10% and 2%, respectively. Long-term treatment up to 21 months was possible, with infrequent dose delays (13%) and discontinuations (19%) due to adverse events. Two cases of gastrointestinal perforation and a rare case of pneumatosis intestinalis, suggestive of bowel wall damage, were reported. These events were confounded by metastases, abdominal surgery, and recent bevacizumab use and were considered to be unrelated to siltuximab. With the limitation of the small sizes of the escalation cohorts, there were no apparent dose-related toxicities. There was no apparent difference in safety profile between 11 and 15 mg/kg, and no DLTs occurred at these higher dose levels.

The pharmacokinetic profile of CHO-derived siltuximab in patients with cancer seems to be similar to the pharmacokinetic profile of Sp2/0-derived siltuximab observed in previous single-agent studies (22, 25). Specifically, for the same dose and schedule, the first dose pharmacokinetic

parameter estimates of siltuximab C_{max} and AUC_{∞} are similar to the values previously reported in patients with renal cancer who received Sp2/0-derived siltuximab (21). These results are also consistent with the results of a healthy volunteer study that demonstrated pharmacokinetic comparability of CHO-derived siltuximab and Sp2/0-derived siltuximab (data on file). At the highest dose levels (11 and 15 mg/kg), complete CRP suppression suggesting adequate suppression of bioactive IL-6 was also observed, and the magnitude of the effect was similar to Sp2/0-derived siltuximab following dosing of 11 mg/kg (21, 26).

High IL-6 levels have been identified as a prognostic factor and correlated with tumor metastasis, disease stage, and short survival in renal, prostate, breast, pancreatic, and ovarian cancers (4–9). In addition, IL-6 was thought to have an important role in mutant KRAS-driven tumorigenesis (16), and we hypothesized that IL-6 inhibition may have therapeutic benefit in tumor types (13) with known KRAS mutations or poor prognosis or nonresponse to EGFR-targeted therapies (14, 15, 27, 28). However, our study was unable to detect any objective tumor response by RECIST or investigator assessment in any cohort, regardless of KRAS mutational status, despite the preclinical rationale and the observed changes in pSTAT3 during treatment. The STAT3 transcription factor is the most commonly observed member of the STAT family to be present in a constitutively activated state in many tumors (29). IL-6 binding to its receptor induces the homodimerization of the GP130 IL-6 transducer leading to phosphorylation of Janus-activated kinase 1 (JAK1). JAK1 then induces STAT3 phosphorylation and subsequent translocation to the nucleus. Reduction of p-STAT3 following siltuximab treatment was observed in the limited number of tissue samples available. This is

consistent with the hypothesis that although siltuximab is able to neutralize IL-6 and decrease downstream signaling, the highly heterogeneous nature of these tumors combined with their autocrine IL-6 status possibly contributed to the lack of clinical efficacy in this population.

With siltuximab treatment, 33 (70%) of 47 evaluable patients showed a clinically relevant increase from baseline in hemoglobin by at least 1.0 g/dL and the maximum increase from baseline was similar across cohorts. This can be considered an IL-6-related effect, as no objective response on underlying disease was seen with siltuximab treatment. Interestingly, among these patients with hemoglobin response, 88% also showed a decrease in hepcidin, a marker associated with iron regulation. This trend is consistent with the biologic rationale in the literature, in which increased IL-6 levels increases the activity of hepcidin in an inflammatory state (17). Consequently, treatment with the anti-IL-6 siltuximab reduced hepcidin levels with a general trend toward hemoglobin improvement in this study population as well as in an earlier study in B-cell non-Hodgkin's lymphoma, multiple myeloma, or MCD (22). Two patients with hemoglobin increases to a level of 9.6 and 12.2 g/dL experienced DVT in our study. There was no apparent relationship between these events and hemoglobin increase, as both patients continued to receive siltuximab with hemoglobin increase and without DVT recurrence or new thrombovascular event.

The lack of objective response seen with single-agent siltuximab in this study, the low response rate with single-agent siltuximab in a phase I/II study of renal cancer (25), and the lack of response in a small phase II study in platinum-resistant ovarian cancer (30) suggest that IL-6 inhibition alone has limited clinical benefit in advanced-stage solid tumors. It is possible that late-stage disease is not IL-6 dependent or that the effects of IL-6 are secondary to other signaling or parallel pathways, such that a multifactorial therapeutic approach is needed in advanced, refractory disease. The increase in hemoglobin associated with a decrease in hepcidin supports further investigation of the role of IL-6 in anemia of inflammation.

On the basis of the safety profile (including DLTs and adverse event profile), pharmacokinetic profile, and pharmacodynamic data (e.g., CRP) from this study, in theory both 11 and 15 mg/kg every 3 weeks could be recommended as the phase II dose of CHO-derived siltuximab. In a phase I study in hematologic malignancies, a high response rate has been seen with murine Sp2/0 myeloma

cell line–derived siltuximab monotherapy at 12 mg/kg every 3 weeks in MCD (19, 22), a disease in which systemic manifestations are primarily driven by IL-6 overproduction (31). Given the similar CRP suppression observed at 11 and 15 mg/kg every 3 weeks in this study and the high response rate observed at 12 mg/kg every 3 weeks in patients with MCD (22), a dose or equivalent dose exposure to 11 mg/kg every 3 weeks was considered adequate and appropriate for future clinical development.

Disclosure of Potential Conflicts of Interest

B. Hall has ownership interest (including patents) in Johnson & Johnson Stock. T.A. Puchalski is employed by Janssen and has ownership interest (including patents) in Johnson & Johnson. H. van de Velde is employed by Janssen Research & Development and has ownership interest (including patents) in Johnson & Johnson. R. Kurzrock has received research funding for this trial from Johnson & Johnson. No potential conflicts of interest were disclosed by the other authors.

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