Clinical Trials: Targeted Therapy

Clinical Cancer Research

Phase I Trials of Anti-ENPP3 Antibody–Drug Conjugates in Advanced Refractory Renal Cell Carcinomas

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Abstract

Purpose: To determine the safety, pharmacokinetics, and recommended phase II dose of an antibody–drug conjugate (ADC) targeting ectonucleotide phosphodiesterases-pyrophosphatase 3 (ENPP3) conjugated to monomethyl auristatin F (MMAF) in subjects with advanced metastatic renal cell carcinoma (mRCC).

Patients and Methods: Two phase I studies were conducted sequentially with 2 ADCs considered equivalent, hybridoma-derived AGS-16M8F and Chinese hamster ovary–derived AGS-16C3F. AGS-16M8F was administered intravenously every 3 weeks at 5 dose levels ranging from 0.6 to 4.8 mg/kg until unacceptable toxicity or progression. The study was terminated before reaching the MTD. A second study with AGS-16C3F started with the AGS-16M8F bridging dose of 4.8 mg/kg given every 3 weeks.

Results: The AGS-16M8F study ($n = 26$) closed before reaching the MTD. The median duration of treatment was 12 weeks (1.7–83 weeks). One subject had durable partial response (PR; 83 weeks) and 1 subject had prolonged stable disease (48 weeks). In the AGS-16C3F study ($n = 34$), the protocol-defined MTD was 3.6 mg/kg, but this was not tolerated in multiple doses. Reversible keratopathy was dose limiting and required multiple dose deescalations. The 1.8 mg/kg dose was determined to be safe and was associated with clinically relevant signs of antitumor response. Three of 13 subjects at 1.8 mg/kg had durable PRs (range, 100–143 weeks). Eight subjects at 2.7 mg/kg and 1.8 mg/kg had disease control >37 weeks (37.5–141 weeks).

Conclusions: AGS-16C3F was tolerated and had durable antitumor activity at 1.8 mg/kg every 3 weeks. *Clin Cancer Res; 24(18); 4399–406. ©2018 AACR.*

Introduction

The mechanisms of resistance to chemotherapy in renal cell carcinoma (RCC) are largely unknown (1). Several hypotheses have been put forward to explain this, including increased drug efflux due to overexpression of ATP-driven pumps such as PgP (MDR1) and modified expression of tubulin isotypes affecting sensitivity to taxanes (2, 3). Antibody–drug conjugates (ADC) represent a different modality of chemotherapy in which a potent payload is delivered specifically to target-positive tumor cells, sparing normal cells to a large degree (4, 5). Importantly, due to the long half-life of ADCs, the exposure to the active component is significantly increased from hours to days when compared with traditional chemotherapy (4). This increased exposure and higher potency of the active payload for an ADC compared with traditional chemotherapy may contribute to overcoming resistance mechanisms. Furthermore, ADCs containing the noncleavable linker mcMMAF liberate the active moiety Cys-mcMMAF after processing in lysosomes (6). Cys-mcMMAF is not very membrane permeable because it is positively charged at a physiologic pH (6), facilitating accumulation in target cells and, theoretically, it is also a poor MDR1 substrate (4), reducing drug efflux. Altogether, these properties suggest that ADCs may represent a feasible treatment for RCC.

AGS-16M8F and AGS-16C3F are ADCs composed of fully human IgG_{2a} antibodies conjugated to MMAF via a noncleavable linker (7). The antibody components target ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3: CD203a), a member of the ENPP family. ENPP3 is expressed in a subset of renal tubules and on activated basophils/mast cells. Among cancers, ENPP3 is expressed by most RCCs of clear cell histology (94%) and about 60% of those with papillary histology (7). Preclinical experiments confirmed that both ADCs

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Translational Relevance

Antibody–drug conjugates (ADC) are designed to deliver a cytotoxic payload specifically to tumor cells expressing the target, while mostly sparing normal tissue. ENPP3 is a novel target specific to renal cell carcinoma (RCC) with minimal expression in normal tissue. An ADC composed of an antibody against this target conjugated to the microtubule-disrupting agent MMAF via the linker maleimidocaproyl (mc) was developed. This report describes the first-in-human experience with this ADC. ENPP3 was detected at high levels in archived tumor samples in agreement with preclinical data. Despite historical failures of cytotoxic agents in RCC, AGS-16C3F had encouraging clinical activity, with 3 PRs and tolerable toxicity. As reported with other ADCs containing mcMMAF, reversible corneal toxicity was dose limiting and independent of antibody target. These findings warrant further clinical investigation of AGS-16C3F for the treatment of RCC.

internalize and induce cytotoxicity in both *in vitro* and *in vivo* models of RCC (7).

The first product tested in humans was the cell line–derived hybridoma designated AGS-16M8F(Hyb). Although production of ADCs via hybridomas is cost and time effective for limited scale production, the method is not suitable for scaled-up production. We planned to switch to a Chinese hamster ovary (CHO) cell line system, which is suitable for later stage development, after safety and biological activities were evaluated with AGS-16M8F(Hyb). The first study was terminated before reaching the MTD when the CHO-derived product, designated AGS-16C3F(CHO), became available. A new phase I study with AGS-16C3F(CHO) was implemented from where the first study left off to continue dose escalation and to determine the AGS-16C3F(CHO) dose suitable for late-stage development.

Patients and Methods

Objectives

For both studies in subjects with metastatic RCC (mRCC), the primary objective was to evaluate the safety and pharmacokinetics of AGS-16M8F(Hyb)/AGS-16C3F(CHO), and the secondary objective was to assess the immunogenicity and effectiveness of AGS-16M8F(Hyb)/AGS-16C3F(CHO).

Study population

In both studies, eligibility criteria included a diagnosis of mRCC of all histologies, age $\geq$18 years, Eastern Cooperative Oncology Group performance status $\leq$1, adequate organ and bone marrow function; creatinine $\leq 1.5\times$ upper limit of normal (ULN) or calculated creatinine clearance > 50 mL/minute; total bilirubin $\leq 1.5\times$ ULN; AST/ALT $\leq 2.5\times$ ULN (or $\leq 5\times$ ULN if known hepatic metastases); absolute neutrophil count $\geq$1,500/μL; and platelet count $\geq$100,000/μL. Exclusion criteria common to the two studies included uncontrolled CNS metastases, significant underlying cardiac problems, investigational therapy within 4 weeks of enrollment, or thrombotic events in the prior 3 months. In addition, the AGS-16C3F(CHO) study required measurable disease as defined by RECIST version 1.1 (8), at least one prior antiangiogenic therapy for subjects with clear cell histology, and ENPP3 positivity by IHC for subjects with non–clear cell histology. Subjects who had recent cataract surgery or ocular disorders significantly affecting vision were excluded.

Study design

The AGS-16M8F(Hyb) and AGS-16C3F(CHO) studies were phase I, open-label, dose escalation clinical trials. The Institutional Review Boards at all participating institutions approved the study protocols and all subjects gave written informed consent. The studies were registered in ClinicalTrials.gov (identifier NCT01114230 and NCT01672775, respectively) and were conducted in accordance with Good Clinical Practice guidelines, as provided by the International Council on Harmonisation and principles of the Declaration of Helsinki. The AGS-16M8F(Hyb) study was conducted in 3 study sites in the United States from August 2010 to November 2012 and the AGS-16C3F(CHO) study in 9 study sites in the United States and Canada from July 2012 to February 2017.

The ADCs were given as monotherapy via intravenous infusion over 60 minutes every 3 weeks until unacceptable toxicity, progression, or investigator decision. Both studies used a 3 + 3 dose escalation design. The AGS-16C3F(CHO) study also included a dose expansion cohort for clear cell and papillary histologies after the dose escalation phase.

The AGS-16M8F(Hyb) study enrolled 26 subjects and tested 6 dose levels (0.6, 1.2, 1.8, 2.4, 3.6, and 4.8 mg/kg). The protocol was designed to explore doses up to 8 mg/kg. However, when the CHO-derived AGS-16C3F(CHO) became available, the AGS-16M8F(Hyb) study closed and a phase I study of AGS-16C3F (CHO) was opened at the highest dose (4.8 mg/kg) tested in the prior study. Toxicities at 4.8 and 3.6 mg/kg dose levels required amending the protocol to include lower dose levels. The amended protocol added planned dose levels of 2.7, 1.8, 1.2, and 0.6 mg/kg, and dose finding was done through dose deescalation.

Safety

Toxicities were graded according to the NCI Common Toxicity Criteria (NCI-CTCAE) version 4.0. A dose-limiting toxicity (DLT) in the dose-determining cohorts was defined as an adverse event (AE) occurring in cycle 1 (day 1–22) including any nonlaboratory grade 3 or higher AE. The following were also DLTs in both studies: grade 4 neutropenia lasting >5 days, grade 4 thrombocytopenia, grade 3 thrombocytopenia with bleeding, any requirement for a platelet transfusion, and grade 4 anemia unexplained by underlying disease. In the AGS-16C3F(CHO) study, DLTs also included grade 3 infusion-related reaction not resolving within 24 hours, febrile neutropenia, $\geq$grade 3 neutropenia with bacterial infection, grade 4 nonhematologic laboratory abnormalities, $\geq$grade 3 nonhematologic laboratory abnormalities with clinical consequences not resolving within 24 hours, and ALT >3× ULN with bilirubin >2× ULN.

Clinical and laboratory assessments were similar in both studies, with the exception that ophthalmology exams were required at baseline, cycle 3, and cycle 5 in the AGS-16C3F(CHO) study.

Pharmacokinetics

In both AGS-16M8F(Hyb) and AGS-16C3F studies, blood samples were collected for pharmacokinetic analysis. Pharmacokinetic analysis measured serum total antibody (serum free antibody + serum antibody drug conjugate), serum ADC, and serum Cys-mcMMAF. Pharmacokinetic parameters assessed included maximum observed plasma concentration ($C_{\max}$) after the first and fourth dose, time of maximum observed plasma concentration ($T_{\max}$) for Cys-mcMMAF only, partial area under the concentration–time curve (AUC), and terminal elimination half-life ($t_{1/2}$).

Immunogenicity

To assess immunogenicity, subject serum samples were collected prior to cycles 1 to 4 or 5 and every 12 weeks thereafter, and evaluated for anti-drug antibodies to AGS-16M8F(Hyb) or AGS-16C3F(CHO).

Antitumor effects

Objective response rate and disease control rate were assessed by RECIST 1.1 criteria. Disease evaluations were performed at baseline and every 12 weeks thereafter in the AGS-16M8F(Hyb) study and every 8 weeks in the AGS-16C3F(CHO) study.

ENPP3 expression by IHC

A mouse anti-ENPP3 mAb (M16-48(4)29.1.1.1) was generated by immunizing Balb/c mice with the ENPP3 extracellular domain (7). Sections were stained in a Bond Max IHC autostainer (Leica Biosystems). Antigen retrieval was carried out using proteinase K (Dako). M16-48(4)29.1.1.1 or MOPC21 (negative control), both at 6 μg/mL, were applied to the sections and incubated for 45 minutes at room temperature. ENPP3 was visualized using the Bond Refine Polymer Kit DC9800 (Leica Biosystems) with 3.3′-diaminobenzidine (DAB) as the chromogen. Positivity was defined as anything greater than an H-score of 0, with one exception where a sample was considered positive based on staining alone (fewer than 100 cells).

Tissue collection was optional for all subjects in the AGS-16M8F(Hyb) study; in the AGS-16C3F(CHO) study, this was required for all non–clear cell subjects and optional for clear cell subjects.

Results

Subjects and treatment

Subject characteristics in the two studies are listed in Table 1.

The AGS-16M8F(Hyb) study enrolled 26 subjects in 6 dose cohorts (6 at 0.6 mg/kg, 3 each at 1.2, 1.8, 2.4, and 3.6 mg/kg, and 8 at 4.8 mg/kg).

The AGS-16C3F(CHO) study enrolled 34 subjects. Fourteen subjects were treated in the dose-determining phase; 2 at 4.8 mg/kg, and 6 each at 3.6 and 2.7 mg/kg. Twenty subjects were treated in the dose expansion cohort, which included both 2.7 and 1.8 mg/kg dose levels. The expansion phase opened at 2.7 mg/kg and enrolled 7 subjects, but the dose was further reduced to 1.8 mg/kg due to toxicity where 3 subjects experienced an ocular AE, of which 2 discontinued treatment due to this AE. Thirteen additional subjects were enrolled in the dose expansion phase for a total of 20 subjects. The decision to reduce the dose to 1.8 mg/kg applied to all new subjects enrolled in the expansion phase and all subjects whose treatment was ongoing at that time. Five subjects' treatment was ongoing at the time at 2.7 mg/kg, 2 from dose-determining phase and 3 from the dose expansion phase. These 5 subjects received 1 to 3 doses at 2.7 mg/kg, but later continued treatment at 1.8 mg/kg (Table 2).

Safety

In the AGS-16M8F(Hyb) study, all subjects had at least one AE. The most common AEs were fatigue, thrombocytopenia, constipation, dyspnea, and nausea. There was only 1 DLT in the 0.6 mg/kg cohort, which was pulmonary embolism with dyspnea and chest pain. Dose escalation continued up to 3.6 mg/kg with 3 subjects in each cohort and without DLTs. At 4.8 mg/kg, a total of 8 subjects were enrolled. Two of the first 6 discontinued after the first dose, 1 due to progressive disease (PD) and another for metamorphosia (visual distortions). The decision was then made to enroll 2 additional subjects before closing the study and initiating the study with AGS-16C3F(CHO). There were no DLTs in the 8 subjects at 4.8 mg/kg and hence the MTD of AGS-16M8F (Hyb) was not established before study closure.

The AGS-16C3F(CHO) study started with 2 subjects at 4.8 mg/kg. Both had DLTs: grade 4 keratopathy in one (keratopathy is used as an umbrella term to include any pathology affecting the cornea, i.e., keratitis, microcystic epitheliopathy, superficial keratopathy, etc.) and posterior reversible encephalopathy in the other; the latter subject was on bevacizumab treatment until 10 weeks before the first dose of AGS-16C3F. The MTD was exceeded and the dose was deescalated. Only 1 of 6 subjects treated at 3.6 mg/kg had a DLT (grade 4 thrombocytopenia), but 4 of these subjects discontinued therapy after the second dose due to keratopathy (2 subjects) or PD (2 subjects). The dose was again reduced to 2.7 mg/kg and 3 subjects were enrolled. Although there were no DLTs in these 3 subjects, 2 subjects had delayed administration of the second dose at a reduced dose of 1.8 mg/kg, 1 subject due to grade 3 fatigue based on preexisting condition and another due to grade 2 creatinine increase. A decision was therefore made to enroll 3 additional subjects at 2.7 mg/kg. No DLTs were reported from the additional 3 subjects and the study team then decided to start the expansion phase at 2.7 mg/kg. The dose initially appeared to be tolerated, but of the first 7 subjects treated, 3 subjects experienced grade 2–4 keratopathy after 1 or 2 doses, and 2 subjects discontinued treatment due to keratopathy, which was reversible. Consequently, the dose was further reduced to 1.8 mg/kg. The expansion phase continued at the 1.8 mg/kg dose level and the 5 subjects who were still being treated at 2.7 mg/kg from both the escalation and expansion cohorts were dose-reduced. The expansion phase

Table 1. Summary of subject characteristics

	AGS-16M8F(Hyb)	AGS-16C3F(CHO)
Enrollment and histology	26 total, 19 clear, 7 nonclear	Dose determining: 14 total, 10 clear, 4 non–clear cell Dose expansion: 20 total, 15 clear, 5 papillary
Gender	19 male, 7 female	27 male, 7 female
Median age	65 (47–80)	64 (46–84)
ECOG	0 (10, 38.5%), 1 (15, 57.7%), 1 missing	0 (11, 32.4%), 1 (23, 67.6%)
Median prior therapies	3 (0–8) 24/26 (92.3% had at least 1 prior line of treatment)	3 (0–7) 33/34 (97.1%) had at least 1 prior line of treatment

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2. AGS-16C3F(CHO) study dose level deescalation

Dose level	Dose determining	Expansion	Total
4.8 mg/kg	2		2
3.6 mg/kg	6		6
2.7 mg/kg	6 (2 reduced to 1.8 mg/kg)	7 (3 reduced to 1.8 mg/kg)	13
1.8 mg/kg		13	13

enrolled another 13 subjects at 1.8 mg/kg. This dose level was tolerated in multiple doses. Of the 13 subjects enrolled at 1.8 mg/kg, 12 subjects reported changes in the eyes; ophthalmology examination revealed keratitis/keratopathy in 5 subjects (38%), and 7 subjects (54%) experienced ocular symptoms with no obvious keratopathy. Only 1 of 13 subjects discontinued treatment due to keratopathy. Ocular symptoms and keratopathy were all reversible with discontinuation of the treatment. Overall, all 34 subjects had at least one AE.

The most common (>20%) AEs from the two studies are shown in Table 3. Grade 3–4 AEs are shown in Table 4.

Ocular AEs. Ophthalmology exams were not required in the AGS-16M8F(Hyb) study but were required at baseline, week 7, and week 13 in the AGS-16C3F(CHO) study.

In the AGS-16M8F(Hyb) study, ocular AEs were reported only at the 3 highest dose levels from 8 subjects (31%); 1 subject each (33%) at 2.7 and 3.6 mg/kg, and 6 subjects (75%) at 4.8 mg/kg. The 2 subjects at 2.7 and 3.6 mg/kg only reported grade 1 dry eye. Of the 6 subjects at 4.8 mg/kg dose level with ocular AEs, 4 subjects reported grade 2 blurred vision, 3 subjects had dry eye (2 grade 2 and 1 grade 3), 1 subject had grade 1 eye pruritis, and 1 subject experienced grade 1 metamorphosia.

In the AGS-16C3F(CHO) study, 29 of 34 subjects (85%) experienced ocular signs and symptoms. Keratopathy was diagnosed in 20 of 34 subjects (59%) and 9 of 34 subjects (26%) reported ocular symptoms without objective keratopathy. Five subjects (15%) did not report any ocular symptoms and showed no keratopathy; however, most only received 1 dose (2 subjects) or 2 doses (2 subjects) before discontinuing treatment; one subject received 12 doses before discontinuing treatment.

Approximately half of the subjects reported ocular symptoms such as dry eye (17/34, 50%) and blurred vision (15/34, 44%). The most common ophthalmologic findings were corneal lesions described as microcysts. Although subjects frequently reported ocular signs and symptoms, these were asynchronous with clinical findings.

Table 3. Most common (>20%) AEs

All AEs	AGS-16M8F(Hyb) $n = 26$	AGS-16C3F(CHO) $n = 34$
Subjects with at least 1 event	26 (100%)	34 (100%)
Fatigue	12 (46.2%)	24 (70.6%)
Nausea	7 (26.9%)	19 (55.9%)
Dry eye		17 (50.0%)
Decreased appetite		15 (44.1%)
Vision blurred		15 (44.1%)
Vomiting		13 (38.2%)
Thrombocytopenia	8 (30.8%)	12 (35.3%)
Headache		11 (32.4%)
Keratitis		11 (32.4%)
Anemia		10 (29.4%)
Constipation	8 (30.8%)	10 (29.4%)
Dyspnea	7 (26.9%)	10 (29.4%)
Pyrexia		8 (23.5%)
Epistaxis		7 (20.6%)
Infusion-related reaction		7 (20.6%)
Edema peripheral		7 (20.6%)

Table 4. Grade 3–4 AEs (>1 subject)

	AGS-16M8F(Hyb) $n = 26$	AGS-16C3F(CHO) $n = 34$
Subjects with at least 1 event AEs	16 (61.5%)	25 (73.5%)
Thrombocytopenia	3 (11.5%)	6 (17.6%)
Anemia		7 (20.6%)
Keratitis		6 (17.6%)
Fatigue		4 (11.8%)
Asthenia		2 (5.9%)
Back pain		2 (5.9%)
Dry eye		2 (5.9%)
Hypertension		2 (5.9%)
Nausea		2 (5.9%)
Edema		2 (5.9%)
Vision blurred		2 (5.9%)
Vomiting		2 (5.9%)
Dyspnea	2 (7.7%)	
Hypophosphatemia	2 (7.7%)	

Grade 3 corneal events were observed in 7 of 34 subjects (21%) and were reported more frequently at higher dose levels (1/2 at 4.8 mg/kg, 3/6 at 3.6 mg/kg, 2/13 at 2.7 mg/kg, and 1/13 at 1.8 mg/kg). Of these 7 subjects, 1 subject at 2.7 mg/kg also experienced a grade 4 corneal event. Six subjects discontinued study drug due to keratopathy [1 subject (50%) at 4.8 mg/kg, 2 subjects each at 3.6 and 2.7 mg/kg (33% and 15%, respectively), and 1 subject (8%) at 1.8 mg/kg]; the best overall response for these 6 subjects was 3 PD, 2 SD, and 1 NE.

Most changes in the eyes, including corneal changes, were observed early in treatment, within the first two cycles. Overall, although the frequency of ocular AEs was consistent across all dose levels, the severity was dose dependent. Severity decreased with lower doses and at 1.8 mg/kg; the eye symptoms were better tolerated and manageable. In both studies, ocular AE management method, mitigation or intervention, was not specified. The needed interventions for the management of ocular AEs were variable, determined by treating investigators and local ophthalmologists. Interventions included, but were not limited to, lubrication with artificial tears and steroid eye drops.

Keratopathy reported in both studies was reversible after study drug cessation. Time to resolution of keratopathy, both symptoms and objective findings, varied from a few weeks to several months.

Preclinical data suggest that these ocular AEs are mediated through macropinocytosis (a regulated form of endocytosis that mediates the nonselective uptake of solute molecules, nutrients and antigens) by corneal epithelial cells, which do not express ENPP3 (9).

Thrombocytopenia. A transient decrease in platelet number was observed in the first 7 to 15 days in 24 of 25 subjects (96%; 1 subject had no data) and in 32 of 34 subjects (94%) treated with AGS-16M8F(Hyb) and AGS-16C3F(CHO), respectively. Importantly, the target for this ADC, ENPP3, is not expressed in platelets or megakaryocytes (10). The median change in platelet count from baseline was −52% (min, −6.9%; max, −85%) for AGS-16M8F(Hyb) and for AGS-16C3F(CHO), this was −51% (min, −8.4%; max, −95.8%). The decreased platelet count was mostly grade 1 and 2. In the AGS-16M8F(Hyb) study, 1 subject each at 1.2, 2.7, and 4.8 mg/kg had at least 1 grade 3 platelet count decrease during the study. In the AGS-16C3F(CHO) study, 4 subjects (1 subject each at 1.8 and 2.7 mg/kg and 2 subjects at 3.6 mg/kg) experienced grade 3 and 2 subjects (1 subject each at 2.7 and 3.6 mg/kg) had grade 4 thrombocytopenia. The grade 4 event at 3.6 mg/kg was a DLT.

In general, all subjects at all dose levels experienced an initial drop in platelet count after the first dose, but there was sufficient recovery spontaneously by day 22 to receive the next dose. The platelet count stabilized at a lower than baseline threshold during treatment and improved after cessation of treatment. Study subjects were not followed long enough to determine if full recovery of platelet count would occur as subjects continued on to another treatment or died. In the AGS-16C3F(CHO) study, grade 3 and 4 thrombocytopenia were dose dependent, and the incidence of thrombocytopenia decreased with decreasing dose level. At 1.8 mg/kg, 3 of 13 subjects (23%) reported thrombocytopenia, only one of which was grade 3.

Bone marrow biopsies performed in 2 subjects at 1.8 mg/kg did not show myelosuppression. One result showed hypercellularity with increased normal megakaryocytes and the other showed normal bone marrow. In the case that showed normal bone marrow, a peripheral blood smear was performed and this result determined that platelet clumping caused the low platelet count. No bleeding events requiring transfusion, or interventions other than nasal tamponade, were observed during thrombocytopenia.

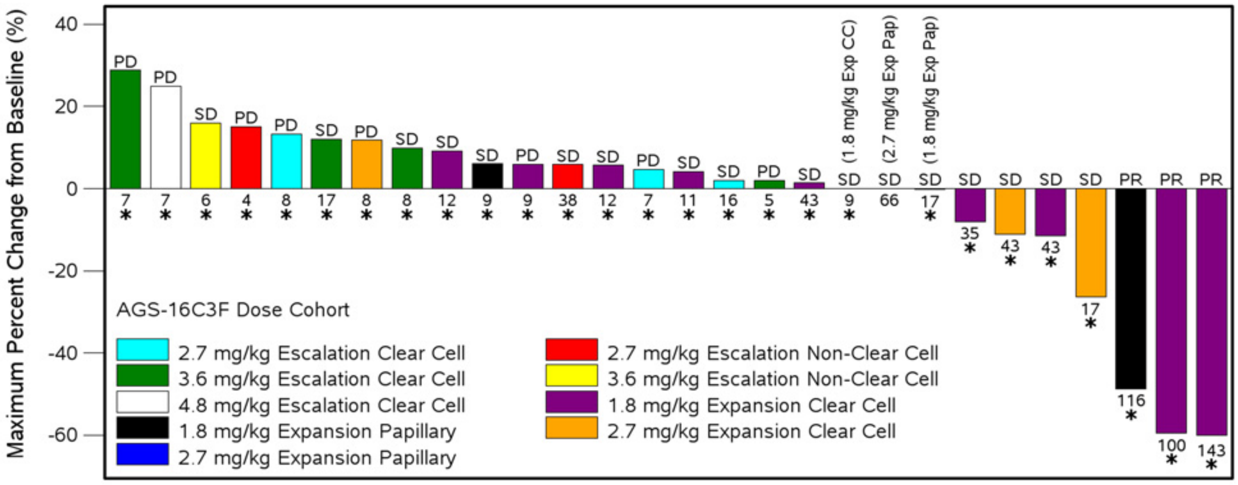
Although the mechanism of thrombocytopenia induced by AGS-16C3F(CHO) is not fully understood, preclinical data suggest that this is not a direct effect on platelets, but rather through macropinocytosis-mediated uptake by developing megakaryocytes (10).

Pharmacokinetics and immunogenicity

For every 3-week AGS-16M8F(Hyb) and AGS-16C3F(CHO) dosing, $C_{\max}$ and AUC increased linearly with increasing doses and without significant ADC accumulation (Supplementary Fig. S1; Supplementary Fig. S2; Supplementary Table S1). The AUC and $C_{\max}$ for free Cys-mcMMAF increased in line with increases in overall doses of AGS-16M8F(Hyb) and AGS-16C3F (CHO) and no accumulation was observed with repeat administration (Supplementary Fig. S3; Supplementary Fig. S4; Supplementary Table S2). The median $T_{\max}$ for peak Cys-mcMMAF concentrations from AGS-16M8F(Hyb) and AGS-16C3F(CHO) was observed at 5 hours (range, 3–8 hours) for both AGS-16M8F (Hyb) and AGS-16C3F(CHO). The total antibody concentration (TAb), comprising free antibody and ADC, was higher overall compared with ADC concentration alone, implying deconjugation of the ADC (Supplementary Fig. S5; Supplementary Fig. S6; Supplementary Table S3). Similar to the ADC pharmacokinetics, the AUC and $C_{\max}$ of the TAb and Cys-mcMMAF increased proportionally to the dose given. As summarized in Supplementary Table S4, the overall pharmacokinetic properties of both ADCs were comparable.

No subject developed immunogenicity during the study (Supplementary Table S5).

Clinical antitumor effects. In the AGS-16M8F(Hyb) study, 1 clear cell subject treated at 2.7 mg/kg had a partial response (PR) at week 23 and response was confirmed at week 29. This subject



Best overall response was determined according to RECIST:
CR=complete response, PR=partial response, SD=stable disease, PD=progressive disease
The following subjects are not represented on the graph due to having a nonevaluable best overall response:
0001-0103 (2.7 mg/kg Escalation Non-Clear Cell), 0006-0007 (3.6 mg/kg Escalation Clear Cell), 0001-0002 (4.8 Escalation Non-Clear Cell mg/kg), 0003-1005 (2.7 mg/kg Expansion Clear Cell), 0008-1004 (2.7 mg/kg Expansion Clear Cell), 0003-1102 (2.7 mg/kg Expansion Papillary)
Numbers below the bars represent number of weeks between first dose and decision made to end treatment.
Bars with an asterisk (*) indicate a subject who received prior systemic therapy.
Note: Maximum change is defined as subject's best response and calculated so that bars below 0 represent good outcomes.
Prog: csr\dev\programs\tfl\f-tumor-wtrfl-dur.sas, f14-3-2-f-tumor-wtrfl-dur.rtf (24MAY2017:16:41)

Figure 1.
Waterfall plot for AGS-16C3F(CHO): Maximum percent change from baseline in total tumor burden by best overall response and duration of treatment.

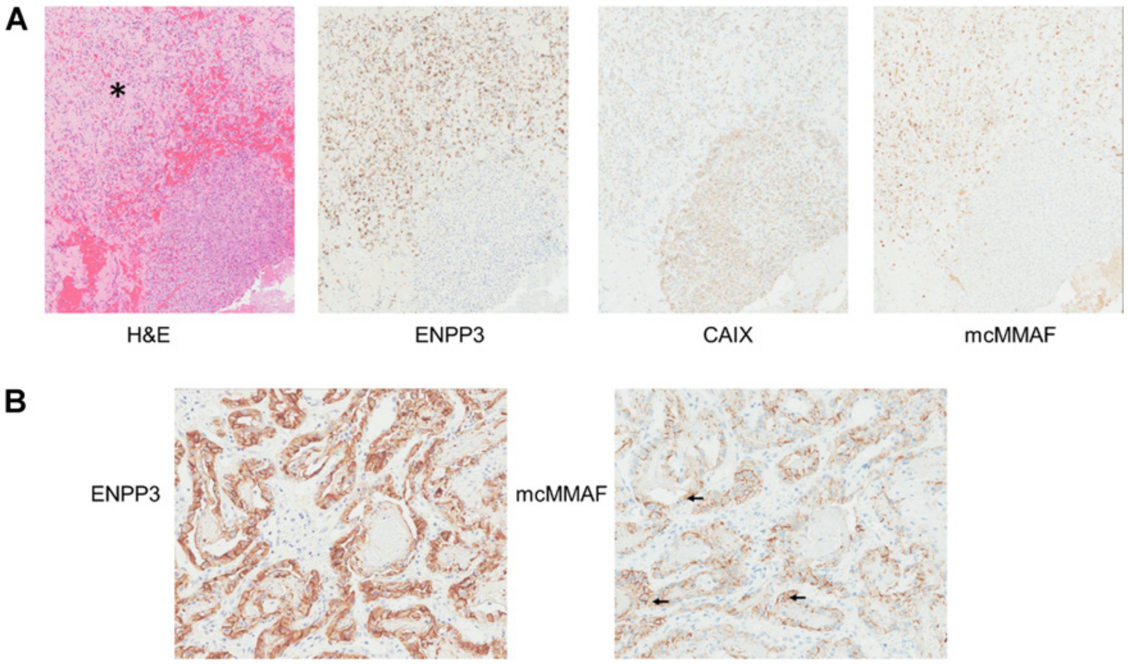


Figure 2.

Immunohistochemical characterization of tumor metastases from 2 patients. **A,** Sample of tumor from the humerus of a patient treated with AGS-16M8F(Hyb) at 2.7 mg/kg who had a PR. From left to right, hematoxylin and eosin stain showing a fibrotic area (*); ENPP3 staining shows positivity in the area of fibrosis only; CAIX staining shows tumor cell positivity throughout the section; mcMMAF showing areas of positivity where AGS-16M8F(Hyb) may be bound. **B,** Sample of tumor from the bronchus of a patient treated with AGS-16M8F(Hyb) at 0.6 mg/kg who had an SD. From left to right, ENPP3 staining of the tumor cells; mcMMAF showing areas of positivity where AGS-16M8F(Hyb) may be bound or internalized (arrows).

remained on study for 56 weeks. In addition, 1 clear cell subject treated at 0.6 mg/kg had prolonged SD (48 weeks).

In the AGS-16C3F(CHO) study, 3 subjects at 1.8 mg/kg achieved durable PR (3/13, 23%), 2 with clear cell (Fuhrman grade 2 and 3) and 1 with type II papillary histology (Fuhrman grade 3). Response was observed at week 8 (1 subject) and at week 16 (2 subjects). The 3 PR subjects were on treatment for 100, 116, and 143 weeks. The disease control rate at 1.8 mg/kg was 92% (12/13 subjects). The disease control rate for the entire study was 59% (20/34 subjects). The waterfall plot from the AGS-16C3F (CHO) study showing the maximum change from baseline in total tumor burden by best overall response and duration of treatment is shown in Fig. 1.

IHC. ENPP3 IHC staining was performed on tumor samples from 66 subjects. Only 63 were evaluable and were analyzed using the H-score system (11); 2 had an insufficient number of tumor cells and one sample was poorly processed, preventing accurate IHC staining. Of the tumors with clear cell histology, 93% (25/27) were positive, as were 73% (16/22) of the papillary carcinoma samples, although expression was somewhat lower overall in this group. Thirteen of the remaining 14 were either of unclassified (10) or chromophobe type (1) and had mixed expression with 62% (8/13) being positive (Fig. 2). The other remaining sample was a lung biopsy from a subject with RCC shown to be a primary squamous cell carcinoma rather than a renal cancer metastasis; this sample was excluded from the IHC analysis (Supplementary Table S6).

Samples of tumor metastases were collected from 2 subjects after dosing with AGS-16M8F(Hyb). One of the samples was from a subject treated at 2.7 mg/kg who had a PR. The sample from the primary tumor for this subject had an H-score of 277. The metastatic sample acquired posttreatment showed decreased ENPP3 positivity and contained 2 different areas. One area was comprised of tumor cells, as defined by CAIX staining, which were also ENPP3 positive and stained using an antibody that recognizes MMAF, demonstrating binding of AGS-16M8F(Hyb) (Fig. 2A). This area includes fibrosis suggestive of cell death. The other area of the section contained tightly packed tumor cells with no fibrosis that were positive for CAIX but negative for ENPP3 and showed no binding of AGS-16M8F(Hyb) (Fig. 2A). Lack of ENPP3 expression in these areas suggests a possible mechanism of escape from AGS-16M8F(Hyb) therapy. The second sample was from a subject who had a best overall response of SD. The sample showed high levels of ENPP3 (Fig. 2B) expression as well as surface and intracellular staining for AGS-16M8F(Hyb) after dosing with 0.6 mg/kg, the lowest dosage investigated (Fig. 2B). Altogether, these data demonstrated specific binding of AGS-16M8F(Hyb) to ENPP3-positive cells.

Discussion

The AGS-16CF phase I study results demonstrated that an ADC targeting ENPP3 can be administered safely at 1.8 mg/kg every 3 weeks and has antitumor activity in a heavily pretreated, refractory mRCC population, in both clear cell and papillary histologies. Three of the 13 subjects treated at the recommended phase II dose experienced PRs that lasted between 100 and 143 weeks (23.3 and 33.4 months). These durations of response are longer than the typical durations of response associated with second or later lines of therapy using mTOR inhibitors (9.2 months for everolimus; Choueiri, personal communication and ref. 12) or tyrosine kinase inhibitors (13 months for lenvatinib combined with everolimus; ref. 13). In addition, responses to AGS-16C3F(CHO) treatment lasted longer than the subject's response to their most recent therapy in 2 of the 3 PR cases, a finding that supports the conclusion that the responses were due to AGS-16C3F(CHO) and not to delayed effects from prior therapy (14).

We observed unexpected differences in the safety profile of AGS-16M8F(Hyb) and AGS-16C3F(CHO), 2 ADCs that contained the same antibody derived from different cell lines and carried the same payload. The two ADCs were deemed comparable based on tests of critical quality attributes that included analytic and preclinical biological characterization (7). Comparability shown by these test results allowed the AGS-16C3F(CHO) study to start with the bridging dose from the AGS-16M8F(Hyb) study.

The human pharmacokinetic properties of the 2 ADCs were comparable as well. Serum concentrations decreased multiexponentially and exposure was dose proportional. Repeated administration of AGS-16M8F(Hyb) and AGS-16C3F(CHO) over a 3-week period did not demonstrate a cumulative increase at trough levels to suggest drug accumulation over cycles. In both clinical studies, the mean terminal half-life was approximately 7 to 8 days for the intact drug and approximately 4 days for Cys-MMAF. Despite the fact that the pharmacokinetic profiles of the 2 ADCs were almost superimposable, the bridging dose of 4.8 mg/kg that was tolerable in the AGS-16M8F (Hyb) study was not tolerated in the AGS-16C3F(CHO) study, most notably for ocular AEs. As a result, the AGS-16C3F(CHO) study was modified to become a reverse dose finding study from the starting dose of 4.8 mg/kg. Multiple dose reductions were required to define the MTD and the recommended phase II dose of 1.8 mg/kg for AGS-16C3F(CHO).

The IHC analysis of the metastatic tumors of 2 patients after AGS-16C3F(CHO) treatment showed the specific targeting of this ADC to ENPP3-positive tumor cells, either membrane bound or internalized in the cytoplasm, supporting the proposed mechanism of action. Importantly, in one of the samples from a patient who experienced a PR, areas of the metastatic tumor collected after clinical progression were ENPP3 negative and AGS-16C3F(CHO) was not found in those cells. Furthermore, Cys-mcMMAF, the active metabolite, has no bystander effect due to its poor membrane permeability. Thus, disappearance of the target, ENPP3, may contribute to resistance to AGS-16C3F(CHO) therapy as it has been suggested for other ADCs (15, 16). Combination of AGS-16C3F(CHO) with other active agents may reduce the impact of the lack of bystander effect and possible loss of ENPP3. Given the small number of subjects treated in each study and 4 objective responses (PRs) in the two studies combined, response correlation to target expression, positivity or positivity strength, cannot be made. This is being investigated further in a randomized phase II study. Other potential uses of ADCs targeting ENPP3 include combination use in earlier lines of treatment of mRCC and in mastocytosis.

Although ocular signs and symptoms were commonly reported, they were asynchronous with clinical findings. Objective ocular findings were limited to the cornea and were reversible with treatment cessation. ADC-induced keratopathy has been previously described and appears to be an off-target effect (17–19).

The most objective way to grade ocular AEs is to use visual acuity of each eye, as grading according to symptoms by definition is subjective. CTCAE v4.0 has vision demarcation at 20/40 (grade 2 for vision equal to or better than 20/40, grade 3 for 20/50 to better than 20/200, and grade 4 for 20/200 and worse). However, the limitation of CTCAE ocular AE vision demarcations is the assumption that a patient has baseline vision of 20/20, which is often not the case. For example, when a patient at baseline with a vision of 20/30 experiences an ocular AE where the vision declines to 20/50, according to CTCAE v4, it would assign the AE as grade 3 simply because the vision is 20/50. The decline in two lines of visual acuity, however, should be assigned as grade 2 as is the case for a patient whose vision of 20/20 declines two lines to 20/30. Grading ocular AE by visual acuity demarcations erroneously assigns a higher grade AE, which may be reflected in this study. Future studies using the change in visual acuity (delta vision), accounting for various baseline visions to grade ocular AEs, will more accurately capture the severity of keratopathy. As such, a modified CTCAE ocular AE grading scale may be beneficial to reach consistency among participating sites.

Systemic therapies for mRCC that are in broad use include the tyrosine kinase inhibitors targeting VEGF ± MET, mTOR inhibitors, and the immune checkpoint inhibitor nivolumab. Many patients receive serial lines of therapy with tyrosine kinase inhibitors that have similar mechanisms of action, thus limiting the potential benefit of successor therapy on progression-free survival. The results of the AGS-16C3F(CHO) phase I study support further development of AGS-16C3F(CHO) or other next-generation anti-ENPP3 ADCs as potential new treatments of mRCC.

Disclosure of Potential Conflicts of Interest

R.J. Motzer is a consultant/advisory board member for Pfizer, Novartis, Merck, Genentech, Eisai, and Exelixis. T.K. Choueiri reports receiving commercial research grants from Pfizer, Bristol-Myers Squibb, and Exelixis and is a consultant/advisory board member for Pfizer, Exelixis, Novartis, Roche, Merck, Ipsen, and Bristol-Myers Squibb. D.S. Ernst reports receiving speakers bureau honoraria from Bristol-Myers Squibb and EMD Serono and is a consultant/advisory board member for Bristol-Myers Squibb, EMD Serono, Merck, and Novartis. S.K. Kim is a consultant/advisory board member for Agensys. B. Anand is an employee of NantKwest. C. Kollmannsberger is a consultant/advisory board member for Pfizer, Ipsen, Eisai, Astellas, and Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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