

Safety and efficacy of the antiganglioside GD3 antibody ecomeximab (KW2871) combined with high-dose interferon- α 2b in patients with metastatic melanoma

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This study evaluated the safety and clinical benefit of ecomeximab (KW2871) combined with high-dose interferon- α 2b (HDI) in patients with metastatic melanoma. We also carried out pharmacokinetic and immune monitoring studies of this combination. This was an open-label, phase 1/2 study of ecomeximab plus HDI in patients with measurable metastatic melanoma. Eligible patients received ecomeximab-HDI combination therapy: ecomeximab administered intravenously once every 2 weeks and HDI at a dose of 20 million units (MU)/m² administered intravenously for 5 consecutive days per week for the first 4 weeks (induction phase) and then at 10 MU/m² subcutaneously thrice weekly through week 14 (maintenance phase). Patients were treated with combination therapy until disease progression or limiting toxicity. Three dose-escalation cohorts (5, 10, and 20 mg/m²) of ecomeximab were planned. Thirty-six evaluable patients were enrolled including six in each of cohorts 1 and 2, and 24 in cohort 3. Median progression-free survival was 2.53 months [95% confidence interval (CI): 1.93–3.83] and it was 1.93 months (95% CI: 1.00–3.80) in cohort 3. The median overall survival was 10.28 months (95% CI: 6.93–16.77) and 7.78 months (95% CI: 6.03–13.97) in cohort 3. There was no significant difference in progression-free survival or overall survival by BRAF mutation status. The response rate was 5.6% (95% CI: 0.68–18.7), with two patients showing an objective response (one complete response and one partial response), and the clinical benefit rate was 78% (95% CI: 61–90). Stable disease as best response was observed in 26 (72%) patients including five in each of cohorts 1 and 2, and 16 in cohort 3. Treatment-emergent adverse events considered related to ecomeximab treatment occurred in four (66.7%) patients in cohort 1, five (83.3%) patients in cohort 2, and seven (29.2%) patients in cohort 3. Among TEAEs with a maximum severity of grade 3 or 4, those that

occurred only in cohort 3 were related to pain, electrolyte imbalance, blood cell decreases, and allergic reaction. Safety and efficacies considered related to ecomeximab occurred in cohort 3 and included grade 3 hypersensitivity [one (4.2%)] and grade 2 hypotension [one (4.2%)]. Regimen-limiting toxicities occurred in two (8.3%) patients in cohort 3: hypersensitivity (with hypertension, supraventricular tachycardia, bronchospasm, chills, and dyspnea) and hypotension. One patient out of 31 examined showed a low-level transient positivity for human antichimeric antibodies against ecomeximab. Pharmacokinetic measurements by enzyme-linked immunosorbent assay determined that administration of HDI does not influence serum levels of ecomeximab at 5, 10, and 20 mg/m² dose levels. Ecomeximab in combination with HDI was generally well tolerated in patients with metastatic melanoma and has shown low immunogenicity. However, the clinical activity was limited, suggesting that future development of this combination should be deprioritized and that other combinations, such as with immune checkpoint inhibitors, should be considered. *Melanoma Res* 00:000–000 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

Melanoma Research 2017, 00:000–000

Keywords: antiganglioside, interferon- α , melanoma

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Received 14 October 2016 Accepted 24 March 2017

Introduction

The incidence of melanoma in the USA continues to increase at a rate faster than any other potentially preventable malignancy. In the year 2016, 76 380 new cases of melanoma are estimated to occur, with 10 130 deaths because of melanoma [1]. Chemotherapy has never shown an overall survival (OS) benefit in metastatic melanoma, despite occasional induction of transient

disease regression [2,3]. However, immunotherapy has shown durable survival benefits, initially with high-dose interferon- α (HDI) in the adjuvant setting and high-dose interleukin-2 (IL-2) for inoperable metastatic disease [4]. More significant survival benefits have been found more recently with immune checkpoint inhibitors targeting CTLA-4 or PD-1, administered either as monotherapy or in combination [5,6]. Molecularly targeted therapy in the

form of BRAF and MEK inhibitors has also shown significant improvements in survival [7]. Nevertheless, although significant advances have been made in the management of patients with metastatic melanoma, many patients ultimately show therapeutic resistance and disease progression, necessitating the continued exploration of new therapeutic strategies.

Gangliosides are acidic glycolipids preferentially expressed on a number of neuroectodermal tumors including melanoma. The dominant gangliosides found in melanoma are GD3 and GM3 and their overexpression in this and other cancers may modulate growth factor, cell adhesion, angiogenesis, and immunosuppression [8–10]. From an immunologic standpoint, GD3 is shed from melanoma cells and has been shown to impair dendritic cell maturation and function [11]. Moreover, GD3 is more significantly expressed on the surface of T cells from patients with metastatic melanoma compared with healthy donors and GD3 blockade promotes a non-CD3 mediated, nonmajor histocompatibility complex-restricted activation of T cells [12,13]. The chimeric monoclonal antibody, ecomeximab (formerly KW2871 and KM871), targets the GD3 ganglioside antigen. Ecomeximab showed potent in-vivo antitumor effects in xenograft models [14]. Ecomeximab inhibits GD3 tumor xenografts and mediates in-vitro complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) and of GD3-expressing cell lines [14]. In-vitro synergy of ecomeximab-mediated ADCC against SK-MEL-28 GD3-positive target cells with several cytokines was observed, suggesting that combination therapy of ecomeximab with Th1-inducing cytokines may be potentially more beneficial than single antibody therapy alone [15]. A phase I study of ecomeximab was carried out in patients with metastatic melanoma [16]. At dose levels above 5 mg/m², three patients showed inflammatory responses at the tumor site and one developed a partial response (PR), whereas three had stable disease (SD) at restaging after three antibody infusions. The antibody was well tolerated, with no observed dose-limiting toxicity (DLT). In a subsequent phase I/II study, patients with stage IV melanoma were enrolled in four dosage tiers and the maximum tolerated dose was determined as 40 mg/m², which was selected for part II of the study. Two out of six patients in the 40 mg/m² cohort in part I had documented SD (7+ and 2+ months) after the first four courses of ecomeximab. Of the 14 patients evaluable for response in part II, three patients had documented SD (8+, 6+, and 2+ months). The most commonly observed adverse events were urticaria, rigors, pyrexia, and nausea.

Interferon- α (IFN- α) is a type I interferon that plays broad immunobiological roles that vary from immunoregulatory to differentiation-inducing, antiproliferative, antiangiogenic, and proapoptotic effects [17]. The immunomodulatory effects of IFN- α include supporting

a link between innate and adaptive immunity by affecting myeloid dendritic cell generation, maturation, differentiation as well as function. Type I IFNs affect cytotoxic T cells, inducing potent antitumor cell-mediated activity while also promoting natural killer cell-mediated proliferation and antitumor cytotoxicity. Enhancement of ADCC of human peripheral blood lymphocytes has been attributed to type I IFNs [17]. HDI (induction phase, 20 MU/m² intravenously, 5 days a week, for 4 weeks and a maintenance phase of 10 MU/m² subcutaneously three times a week, for 48 weeks) administered as adjuvant therapy for patients at high risk of relapse after locoregional surgery for melanoma significantly improved relapse-free survival and overall survival [18]. In the metastatic setting, however, the role of HDI has not been investigated adequately since the time of the early phase I–II trials that served as the foundation for pursuit of the adjuvant treatment regimen noted above. In advanced unresectable melanoma, most trials have used either low doses (i.e. < 20 MU/m²) or interrupted schedules (i.e. treatment for one out of every 2–4 weeks rather than daily), and have adopted more convenient nonintravenous routes of administration. Overall, irrespective of dosage, this agent has an overall response rate of about 15% (range: 6–27%), with response durations ranging from 1 to 60+ months [1].

Biologic features of ganglioside targeting and IFN- α therapy have suggested a rationale for combination treatment. Both HDI and treatment with the mouse monoclonal antibody against GD3, R24, led to increased numbers of immunohistochemistry (IHC)-detected immune cells infiltrating the tumor [8,9,11,19]. IFN- α may reverse immune dysfunction in patients with melanoma, and therefore, the ecomeximab-induced ADCC may similarly be enhanced by IFN on the basis of in-vitro and clinical data [12,13]. Ecomeximab may complement the immune-enhancing effects of IFN- α by binding to GD3 that is shed by melanoma cells. Moreover, blocking of GD3 located on the surface of T lymphocytes has been shown to enhance non-CD3 mediated, nonmajor histocompatibility complex-restricted activation of T cells [16]. HDI may theoretically increase GD3 surface expression of melanoma cells as shown *in vitro* with other inflammatory cytokines, such as tumor necrosis factor- α , IL-4, and IFN- γ , thereby increasing the target for antibody therapy [14,15]. Furthermore, animal studies testing the ecomeximab–IFN combination in mice showed equivalent enhancement of antitumor activity with IFN, followed by ecomeximab antibody and administered concurrently [20]. On the basis of these considerations, we have carried out a phase I/II study to evaluate the safety and efficacy (SAE) of the ecomeximab–IFN combination in metastatic melanoma.

Patients and methods

Patients

Eligible patients were 18 years or older and had histologically proven metastatic cutaneous, mucosal, or unknown primary melanoma that fulfilled prespecified clinical and laboratory safety criteria. The study was carried out in full conformity with the current revision of the Declaration of Helsinki, International Conference on Harmonisation Guidelines and applicable local laws and regulations. This study was approved by the Institutional Review Board and a written informed consent was obtained from all the patients.

Study design and treatment

IFN α -2b was administered at 20 MU/m² intravenously 5 consecutive days per week during weeks 1 through 4 (induction phase), and then 10 MU/m² subcutaneously three times per week until disease progression (maintenance phase). Two dose reductions of 1/3 the original dose were allowed if HDI-related DLT occurred. If a third HDI dose reduction was indicated, HDI treatment was to be stopped, but ecromeximab infusions could continue. Ecromeximab was administered intravenously on day 3, after the administration of HDI, beginning in week 1 and every 2 weeks thereafter until disease progression. Cohort 1 received ecromeximab at 5 mg/m², cohort 2 at 10 mg/m², and cohort 3 at 20 mg/m². No dose adjustments were allowed.

Toxicity and response assessments

The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events, version 3.0, were utilized for adverse event (AE) grading and reporting. AEs were attributed to HDI, ecromeximab, or the combination therapy. An AE that required reduction of the HDI dose or discontinuation of ecromeximab was considered a DLT. A regimen-limiting toxicity (RLT) was defined as an HDI-related DLT that required more than two dose reductions of HDI during the induction phase or the first 4 weeks of the maintenance phase or any ecromeximab-related or regimen-related DLT. For the purpose of tumor response assessment (RECIST), imaging studies were carried out at baseline, on days 29, 57, 85, 115, 143, 171, 227, 283, and 339, and at the end of the study. Patients who were treated beyond 49 weeks were to undergo clinical and radiologic assessments as per the standard of care.

Laboratory methods

Blood samples were tested for the development of human antichimeric antibodies (HACA) at baseline, on days 29, 115, 143, 171, 199, 227, 255, 283, 311, and 339, and at the end of the study visit. Measurement of immune responses to ecromeximab in patients' plasma was performed with a Biacore 2000 biosensor (Biacore AB, Uppsala, Sweden), enzyme-linked immunosorbent assay (ELISA) as described previously [21]. Blood

samples were drawn preceding ecromeximab infusion and 30 min after infusion through week 13 for pharmacokinetic measurements of ecromeximab antibody levels in human plasma by ELISA as described previously [22,23]. All samples were diluted two-fold first and then analyzed by a half-log serial dilution. Positive and negative controls were analyzed at the same time and the secondary antibody and substrate controls were also included. All samples were tested in triplicate. An ecromeximab standard curve was used to determine the ecromeximab concentrations of patients' sera samples.

Statistical methods

The study aimed to assess the SAE of the effects of the ecromeximab-IFN combination in metastatic melanoma. The primary endpoints were progression-free survival (PFS) and toxicity. The secondary endpoints included tumor response (RECIST), HACA, and serum ecromeximab levels. All statistical analyses were carried out using Statistical Analysis Software, version 9.2 or higher for Windows (SAS Institute Inc., Cary, North Carolina, USA). Descriptive statistics (*n*, mean, SD [24], median, minimum, and maximum) were calculated by cohort for continuous variables. Frequencies and percentages were presented by cohort for categorical and ordinal variables. Missing data were not imputed; observed cases were used in the analyses. All analyses were carried out on the safety analysis set, defined as all patients who received initial treatment with HDI and at least one infusion of ecromeximab, irrespective of duration. Baseline descriptive statistics on all evaluable patients were provided on demographic variables, laboratory parameters, toxicity, and efficacy. Survival and recurrence-free survival were estimated using the Kaplan-Meier method. The sample size chosen for cohorts 1 and 2 was based on the standard '3 + 3' dose-escalation schema in oncology trials [25]. The sample size of cohort 3 was chosen on the basis of the analysis of the primary endpoint, PFS. On the basis of phase 3 randomized clinical trials in patients with metastatic melanoma published between 2000 and 2006 [2-5], 2.5 months was estimated as a conservative (i.e. somewhat high) external standard of median PFS [2,3,26,27]. The aim of this study was to improve this PFS estimate by 70% or more to a median of 4.3 months or more for patients treated with ecromeximab combined with HDI. If the therapeutic target of 4.3 months for median PFS represented the true underlying treatment effect of ecromeximab plus HDI, then a total of 23 patients would provide 80% power to detect a statistically significant improvement ($\alpha=0.05$ one-sided test) over the 2.5-month external standard. The combination treatment would be considered safe if 20% or fewer patients experienced RLT as defined previously.

Results

Patient and disease characteristics

Thirty-seven (37) patients were enrolled in the study including one patient who never received ecromeximab. Therefore, 36 patients were considered study-eligible per protocol and were included in the safety analysis set. Cohort 1 (5 mg/m²) included six (16.7%) patients, cohort 2 (10 mg/m²) included six (16.7%) patients, and cohort 3 (20 mg/m²) included 24 (66.7%) patients. Baseline patient demographics and disease characteristics are summarized in Table 1.

Patient disposition and treatment details

RLT was observed in two (5.6%) patients in the study; both patients were in cohort 3 [two (8.3%)]. Two (5.6%) patients completed the study as planned: one (16.7%) in cohort 1 and one (4.2%) in cohort 3. The rest of the patients discontinued prematurely. The reasons for premature discontinuation were progressive disease [27 (79.4%)], unacceptable toxicity [3 (8.8%)], refusal to continue treatment or observation [two (5.9%)], and the investigator's decision to discontinue the patient [two (5.9%)]. The percentages of patients with progressive disease in each cohort were similar (78.3–83.3%). Early death, as defined by RECIST, occurred in one (2.8%) patient in the study, who was in cohort 2 [one (16.7%)]

Table 1 Patient demographics and baseline disease characteristics

	Cohort 1 (5 mg/m ²) (n = 6)	Cohort 2 (10 mg/m ²) (n = 6)	Cohort 3 (20 mg/m ²) (n = 24)	All patients (n = 36)
Age at screening (years)				
Mean (SD)	53.0 (16.54)	57.7 (15.93)	54.8 (10.73)	55.0 (12.37)
Median	55.0	61.0	53.5	54.0
Minimum, maximum	27, 70	31, 73	36, 72	27, 73
Sex [n (%)]				
Male	4 (66.7)	3 (50.0)	11 (45.8)	18 (50.0)
Female	2 (33.3)	3 (50.0)	13 (54.2)	18 (50.0)
Primary tumor [n (%)]				
Cutaneous	6 (100)	4 (66.7)	16 (66.7)	26 (72.0)
Acral	0	0	1 (4.2)	1 (2.8)
lentiginous				
Unknown	0	2 (33.3)	7 (29.2)	9 (25)
Stage at study entry [n (%)]				
IIIB	0	1 (16.7)	0	1 (2.8)
M1a	1 (16.7)	1 (16.7)	3 (12.5)	5 (13.8)
M1b	1 (16.7)	1 (16.7)	5 (20.8)	7 (19.4)
M1c	4 (66.7)	3 (50.0)	16 (66.7)	23 (63.9)
Tumor mutational status [n (%)]				
BRAF				
mutant	1 (16.7)	2 (33.3)	10 (28)	13 (36)
BRAF wild type	3 (50.0)	2 (33.3)	10 (28)	15 (42)
Unknown	2 (33.3)	2 (33.3)	4 (11)	8 (22)
Prior treatment [n (%)]				
Surgery	6 (100.0)	5 (83.3)	23 (95.8)	34 (94.4)
Radiation	0	1 (16.7)	7 (29.2)	8 (22.2)
Systemic ^a	5 (83.3)	5 (83.3)	21 (87.5)	31 (86.1)

^aSystemic (n, %): interleukin-2 (9, 25%), dacarbazine (4, 11%), carboplatin-paclitaxel (10, 28%), paclitaxel (1, 3%), ipilimumab (5, 14%), biochemotherapy (1, 3%), VEGFR2 TKI (1, 3%), cisplatin (1, 3%), temozolomide (11, 31%), tumor vaccine (6, 17%), VEGF Trap (1; 3%), adoptive TIL therapy (1, 3%).

Table 2 Treatment exposure to ecromeximab (N = 36 patients)

	Cohort 1 (5 mg/m ²) (n = 6)	Cohort 2 (10 mg/m ²) (n = 6)	Cohort 3 (20 mg/m ²) (n = 24)
Total number of ecromeximab doses received			
Mean (SD)	6.5 (1.76)	7.3 (6.80)	6.3 (7.87)
Median	7.0	5.5	4.0
Minimum, maximum	3, 8	2, 20	1, 36
Treatment duration – ecromeximab (weeks) ^a			
Mean (SD)	14.81 (7.763)	15.14 (15.375)	10.89 (16.104)
Median	12.14	9.14	6.07
Minimum, maximum	8.1, 30.1	2.1, 38.1	0.1, 72.3

^aDuration = (last dose date – first dose date + 1)/7.

and died of malignant disease. Treatment exposure is summarized in Table 2.

Efficacy

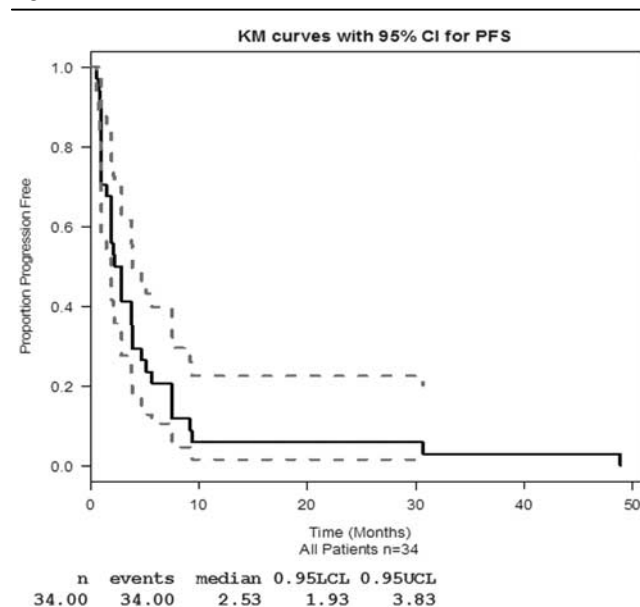
Including all evaluable patients (n = 36), the response rate was 5.6% [95% confidence interval (CI): 0.68–18.7] and the clinical benefit rate was 78% (95% CI: 61–90). A best response of complete response occurred in one (4.2%) patient in cohort 3 after 26 infusions of ecromeximab and the duration of the response was at least 27 months, after which the patient was lost to follow-up. A best response of PR occurred in one (16.7%) patient in cohort 1 after receiving two infusions of ecromeximab and the response persisted ~18 months. SD as best response was observed in 26 (72%) patients including five in each of cohorts 1 and 2, and 16 in cohort 3. For cohort 3 (n = 24), the response rate was 4.2% (95% CI: 0.11, 21) and the clinical benefit rate was 71% (95% CI: 49–87). We evaluated the response rate by BRAF mutational status that was available in 28 patients not including the two responders. The clinical benefit rate was 93% (95% CI: 64–99) and 67% (95% CI: 38–88) in BRAF mutant and wild type (WT), respectively. In cohort 3, it was 90% (95% CI: 55–99.7) and 50% (95% CI: 19–81).

The median PFS in all patients was 2.53 months (95% CI: 1.93–3.83) as shown in Fig. 1 and it was 1.93 months (95% CI: 1.00–3.80) in cohort 3. There was no significant difference in PFS between BRAF mutant and WT patients in the total population (P = 0.37) or in cohort 3 (P = 0.55). The median OS was 10.28 months (95% CI: 6.93–16.77) as shown in Fig. 2 and it was 7.78 months (95% CI: 6.03–13.97) in cohort 3. There was no significant difference in OS between BRAF mutant and WT patients in the total population (P = 0.95) or in cohort 3 (P = 0.91).

Safety

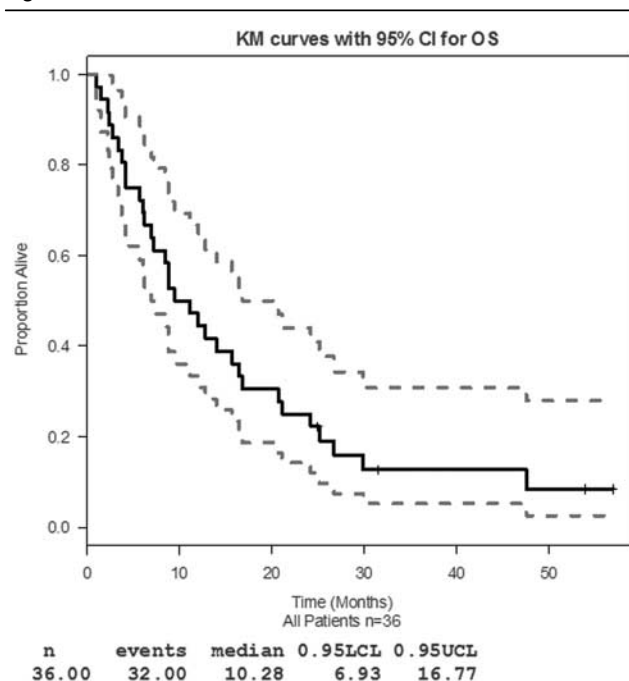
The safety analysis set included all patients (n = 36) who received initial treatment with HDI and at least one infusion of ecromeximab, irrespective of duration. All 36 patients had at least one TEAE and all had at least one

Fig. 1



Kaplan-Meier Plot of PFS with 95% CI. CI, confidence interval; PFS, progression-free survival.

Fig. 2



Kaplan-Meier Plot of OS with 95% CI. CI, confidence interval; OS, overall survival.

TEAE related to HDI treatment. TEAEs considered related to ecromeximab treatment occurred in four (66.7%) patients in cohort 1, five (83.3%) patients in cohort 2, and seven (29.2%) patients in cohort 3. Two patients, one each in cohorts 2 and 3, died of progressive

disease during the study; neither death was considered related to study drug treatment. In cohort 3, TEAEs with a maximum severity of CTCAE grade 3 or 4, and SAEs, occurred at rates similar to the rates in the other cohorts. Among TEAEs with a maximum severity of grades 3 or 4, those that occurred only in cohort 3 were related to pain, electrolyte imbalance, blood cell decreases, and allergic reaction.

SAEs considered related to ecromeximab occurred in cohort 3 and included grade 3 hypersensitivity [one (4.2%)] and grade 2 hypotension [one (4.2%)]. Both events occurred on the day of the first infusion of ecromeximab and the patients were immediately withdrawn from the study. The SAE of hypersensitivity was accompanied by symptoms of bronchospasm, dyspnea, hypertension, rigors, supraventricular tachycardia, and urticaria. SAEs that occurred in two or more patients, overall, included nausea, vomiting, hypotension, and appendicitis. The SAEs of nausea, vomiting, and hypotension occurred in cohorts 2 and 3; appendicitis occurred in cohort 1. RLTs occurred in two (8.3%) patients in cohort 3: hypersensitivity (with hypertension, supraventricular tachycardia, bronchospasm, chills, and dyspnea) and hypotension.

One patient out of 31 examined showed a low level, transient positivity for HACA against ecromeximab. Table 3 summarizes the AEs that were considered to be related to study therapy (ecromeximab and HDI).

Laboratory correlates

Biacore was used to determine ecromeximab HACA responses of patient sera in duplicate at different time points as noted earlier. Samples from 31 patients were assessed (six patients in cohort 1, 6 patients in cohort 2, and 19 patients in cohort 3). One patient out of 31 examined showed a low level, transient positivity for HACA against ecromeximab.

Pharmacokinetic measurements by ELISA determined that administration of HDI does not influence serum levels of ecromeximab at 5, 10, and 20 mg/m² dose levels. Linear relationships were observed for C_{max} and C_{min} with dose level. A total of 30 samples were assessed from five patients in cohort 1, six patients in cohort 2, and 19 in cohort 3. Data obtained from the current clinical trial were compared using an unpaired Student's *t*-test to data obtained from a previous phase I clinical trial study [22,23]. No significant differences between the two clinical studies were observed, indicating that the combination of HDI does not affect ecromeximab serum protein levels.

Discussion

This is the first human study testing ecromeximab in combination with HDI. In this study, 36 patients with metastatic melanoma were treated with the anti-ganglioside GD3 mouse/chimeric antibody ecromeximab

Table 3 Adverse events considered related to the treatment regimen (possible, probable, definite by CTCAE v.3; $\geq 10\%$ of patients) (N = 36)

	n (%)					Total
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
General disorders and administration site conditions						
Chills	28 (77.8)	6 (16.7)	0	0	0	34 (94.4)
Fatigue	6 (16.7)	9 (25.0)	18 (50.0)	0	0	33 (91.7)
Pyrexia	26 (72.2)	4 (11.1)	0	0	0	30 (83.3)
Gastrointestinal disorders						
Nausea	20 (55.6)	7 (19.4)	4 (11.1)	0	0	31 (86.1)
Vomiting	11 (30.6)	4 (11.1)	4 (11.1)	0	0	19 (52.8)
Constipation	11 (30.6)	5 (13.9)	0	0	0	16 (44.4)
Diarrhea	12 (33.3)	4 (11.1)	0	0	0	16 (44.4)
Abdominal pain	5 (13.9)	2 (5.6)	2 (5.6)	0	0	9 (25.0)
Dry mouth	5 (13.9)	0	0	0	0	5 (13.9)
Metabolism and nutrition disorders						
Anorexia	17 (47.2)	8 (22.2)	2 (5.6)	0	0	27 (75.0)
Hypoalbuminemia	8 (22.2)	8 (22.2)	0	0	0	16 (44.4)
Hyponatremia	11 (30.6)	0	2 (5.6)	0	0	13 (36.1)
Hypocalcemia	8 (22.2)	0	0	0	0	8 (22.2)
Hypokalemia	6 (16.7)	0	2 (5.6)	0	0	8 (22.2)
Laboratory investigations						
Aspartate aminotransferase increased	17 (47.2)	5 (13.9)	4 (11.1)	0	0	26 (72.2)
γ -Glutamyltransferase increased	6 (16.7)	8 (22.2)	9 (25.0)	1 (2.8)	0	24 (66.7)
Blood lactate dehydrogenase increased	16 (44.4)	4 (11.1)	3 (8.3)	0	0	23 (63.9)
Alanine aminotransferase increased	14 (38.9)	2 (5.6)	4 (11.1)	0	0	20 (55.6)
Blood creatine phosphokinase increased	11 (30.6)	3 (8.3)	1 (2.8)	0	0	15 (41.7)
Weight decreased	9 (25.0)	5 (13.9)	1 (2.8)	0	0	15 (41.7)
Blood alkaline phosphatase increased	8 (22.2)	3 (8.3)	0	0	0	11 (30.6)
Blood and lymphatic system disorders						
Leukopenia	9 (25.0)	12 (33.3)	1 (2.8)	0	0	22 (61.1)
Neutropenia	7 (19.4)	7 (19.4)	7 (19.4)	1 (2.8)	0	22 (61.1)
Lymphopenia	1 (2.8)	8 (22.2)	4 (11.1)	1 (2.8)	0	14 (38.9)
Thrombocytopenia	13 (36.1)	0	0	0	0	13 (36.1)
Anemia	2 (5.6)	2 (5.6)	2 (5.6)	0	0	6 (16.7)
Nervous system disorders						
Dysgeusia	16 (44.4)	7 (19.4)	0	0	0	23 (63.9)
Headache	13 (36.1)	4 (11.1)	1 (2.8)	0	0	18 (50.0)
Dizziness	5 (13.9)	2 (5.6)	3 (8.3)	0	0	10 (27.8)
Nystagmus	3 (8.3)	1 (2.8)	1 (2.8)	0	0	5 (13.9)
Tremor	4 (11.1)	1 (2.8)	0	0	0	5 (13.9)
Musculoskeletal and connective tissue disorders						
Myalgia	12 (33.3)	3 (8.3)	0	0	0	15 (41.7)
Pain in extremity	6 (16.7)	3 (8.3)	1 (2.8)	0	0	10 (27.8)
Back pain	3 (8.3)	2 (5.6)	1 (2.8)	1 (2.8)	0	7 (19.4)
Muscular weakness	3 (8.3)	1 (2.8)	0	0	0	4 (11.1)
Psychiatric disorders						
Anxiety	8 (22.2)	2 (5.6)	0	0	0	10 (27.8)
Depression	5 (13.9)	2 (5.6)	2 (5.6)	0	0	9 (25.0)
Insomnia	6 (16.7)	1 (2.8)	0	0	0	7 (19.4)
Confusional state	4 (11.1)	0	0	0	0	4 (11.1)
Restlessness	3 (8.3)	1 (2.8)	0	0	0	4 (11.1)
Skin and subcutaneous tissue disorders						
Pruritus	3 (8.3)	2 (5.6)	0	0	0	5 (13.9)
Rash	4 (11.1)	0	1 (2.8)	0	0	5 (13.9)
Respiratory, thoracic, and mediastinal disorders						
Cough	8 (22.2)	0	0	0	0	8 (22.2)
Dyspnea	2 (5.6)	4 (11.1)	1 (2.8)	0	0	7 (19.4)
Vascular disorders						
Hypotension	2 (5.6)	5 (13.9)	1 (2.8)	0	0	8 (22.2)
Hypertension	2 (5.6)	2 (5.6)	0	0	0	4 (11.1)
Infections and infestations						
Oral candidiasis	2 (5.6)	3 (8.3)	0	0	0	5 (13.9)

combined with HDI at ecromeximab doses of 5 mg/m² (cohort 1, six patients), 10 mg/m² (cohort 2, six patients), and 20 mg/m² (cohort 3, 24 patients).

Ecromeximab was generally well tolerated. TEAEs considered related to ecromeximab treatment occurred in four (66.7%) patients in cohort 1, five (83.3%) patients in cohort 2, and seven (29.2%) patients in cohort 3. SAEs

considered related to ecromeximab occurred in cohort 3 and included grade 3 hypersensitivity [one (4.2%)] and grade 2 hypotension [one (4.2%)] on the day of the first infusion of ecromeximab. RLTs occurred in two (8.3%) patients in cohort 3, which were hypersensitivity and hypotension. By not exceeding the threshold of 20% for RLT as specified at study design, the regimen is considered to have fulfilled the primary endpoint of safety.

In terms of efficacy, a best response of CR occurred in one (4.2%) patient in cohort 3 (90% CI: 0.21–18.29) and the duration of the response was at least 27 months. A best response of PR occurred in one (16.7%) patient in cohort 1 (90% CI: 0.85–58.18) and persisted ~18 months. SD as the best response was observed in 26 (72%) patients including five in each of cohorts 1 and 2, and 16 in cohort 3. The median PFS was 2.53 months (95% CI: 1.93–3.83), whereas the aim was to improve PFS by 70% or more to a median of 4.3 months or more. The median OS was 10.28 months (95% CI: 6.93–16.77). Therefore, the study failed to meet the primary endpoint of improvement in PFS and the overall level of clinical activity is considered modest especially compared with the significant survival benefits that have recently been shown with immune checkpoint inhibitors [5,6] and molecularly targeted antitumor therapy with BRAF and MEK inhibitors [7]. It may be worth considering testing of combination therapy of ecomeximab with immune checkpoint-targeting agents or kinase inhibitors. This is justified to counteract immune checkpoint-mediated immune suppression that is likely play a role in the limited activity observed in our study.

The search for antigens predominantly expressed on the surface of melanoma cells, with little or no normal tissue expression, has resulted in the discovery of monoclonal antibodies (mAbs) with the ability to induce immune-mediated cytotoxicity against tumor cells, such as R24 [28]. Despite the heterogeneity of GD3 expression in melanoma biopsies, response rates between 6 and 19% were observed as late as 8–10 weeks after treatment initiation. Moreover, responses were associated with high levels of ADCC, increased number of CD4⁺ cells infiltrating the tumor, elevated peripheral blood CD4⁺:CD8⁺ ratio, and lower CD56⁺/DR⁺ and CD56⁺CD16⁺ lymphocytes [8,16]. Further development of the mouse monoclonal antibody R24 was limited and a chimeric mouse/human immunoglobulin G1 anti-GD3 mAb KW2871 (ecomeximab) was developed to overcome the difficulties associated with the mouse mAb [29]. The superior safety profile and low immunogenicity of ecomeximab were shown by this study. Only one patient out of 31 examined showed a low level, transient positivity for HACA against the antibody.

In patients with advanced melanoma, peripheral blood T cells show immune dysfunction and are ‘polarized’ toward a T_{h2} (IL-4, IL-5, IL-10-producing) rather than T_{h1} (IFN- γ , IL-2-producing) response. The latter type of T cell response has been shown to be critical for effective tumor rejection [30]. Peripheral blood and tumor-infiltrating lymphocytes in patients with metastatic melanoma are dysfunctional, inappropriately stimulated (if primed by antigen-presenting cells), and prone to apoptosis when interacting with melanoma cells [31]. Their function, however, may be restored *ex vivo*, and even mediate clinically significant tumor regression,

when adoptively transferred to tumor-bearing hosts [32]. In the tumor microenvironment (TME), melanoma cells overexpress and shed gangliosides, including GD3, which may be incorporated into the cell membrane of nearby immune cells and may then result in their dysfunction and apoptosis [33]. The failure of ecomeximab mAb therapy to show significant antitumor response rates in patients with metastatic melanoma may be explained in several ways. Melanoma is heterogeneous with respect to the ganglioside expression profile that may change over the course of disease and with treatment, and has been correlated with differences in drug and radiation sensitivity [34]. In all clinical studies with anti-GD3 antibodies (i.e. R24 and KW2871), tumor progression was not related to outgrowth of GD3 negative cells, as measured by IHC in post-treatment tumor biopsies [8,16,22], suggesting that mechanisms other than mAb-mediated elimination of GD3-positive melanoma cells may also be important. The tumor cell burden is already large in patients with metastatic melanoma, and animal data suggest that anti-GD3 therapy is most effective against microscopic subclinical disease [35]. The direct effects of anti-GD3 treatment on melanoma cells have not been documented to date, although GD3 induces variable effects in cell proliferation and apoptosis. No well-powered trial has ever evaluated the therapeutic potential of the current standard HDI adjuvant regimen in metastatic melanoma. In the adjuvant setting, the HDI regimen has shown an overall 30% reduction in disease relapse and mortality rates that must be factored into consideration. Melanoma cell lines are variably sensitive to the antiproliferative effects of IFN- α , which cannot be exclusively explained by the JAK/STAT-1 signaling pathway effects, suggesting that other factors may be relevant. Such factors may include the downregulation or absence of IFN- α receptor, the expression of antagonistic proteins of IFN- α signaling, such as constitutive production of STAT3, suppressors of cytokine signaling (SOCS) proteins, and the protein inhibitor of activated STATs (PIAS-1) that have been clinically correlated with IFN- α response in other malignancies or promote immunosuppression [36,37]. Further, the tumor cell burden is significantly greater in stage IV than in stage III disease, and has been associated with T_{h2} bias that may not be easily reversed and repolarized to an effective antitumor T_{h1} phenotype. Patients with melanoma who received either HDI or the mouse monoclonal antibody against GD3, R24, showed increased numbers of IHC-detected immune cells infiltrating the tumor [8,9,11,19]. Although this may improve immunogenicity of the TME, other tolerogenic factors including immune checkpoints and suppressive factors, regulatory T cells, and myeloid-derived suppressor cells are still active in the TME, and may explain the limited clinical activity of the combination regimen. Taking these factors into account suggests that future development of this combination or of ecomeximab monotherapy should consider combinations with immune

checkpoint modulators addressing dominant tolerogenic factors within the TME.

Conclusion

Ecomeximab in combination with HDI was well tolerated in patients with metastatic melanoma and has showed low immunogenicity. However, the clinical activity of this combination is limited and future development may be more reasonable in combination with other immunotherapies, including immune checkpoint inhibitors.

Acknowledgements

The authors would like to acknowledge Mary Horak, B.S., and Ping DeBlasio, R.N. (University of Pittsburgh), and Karen Matijevich, R.N. (University of Chicago), for their roles in study coordination. For this study, KW2871 (ecromeximab) was provided by Life Science Pharmaceuticals and the study was sponsored by the Ludwig Institute for Cancer Research.

Conflicts of interest

Ahmad A. Tarhini, Stergios J. Moschos, Thomas F. Gajewski and John M. Kirkwood research grant support was provided by Ludwig Institute for Cancer Research. Ralph Venhaus is employed by Ludwig Institute for Cancer Research. For the remaining authors there are no conflicts of interest.

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