Clinical Cancer Research

Results from an Integrated Safety Analysis of Urelumab, an Agonist Anti-CD137 Monoclonal Antibody

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Abstract

Purpose: Urelumab is an agonist antibody to CD137 with potential application as an immuno-oncology therapeutic. Data were analyzed to assess safety, tolerability, and pharmacodynamic activity of urelumab, including the dose selected for ongoing development in patients with advanced solid tumors and lymphoma.

Experimental Design: A total of 346 patients with advanced cancers who had progressed after standard treatment received at least one dose of urelumab in one of three dose–escalation, monotherapy studies. Urelumab was administered at doses ranging from 0.1 to 15 mg/kg. Safety analyses included treatment-related and serious adverse events (AEs), as well as treatment-related AEs leading to discontinuation and death, with a focus on liver function test abnormalities and hepatic AEs.

Results: Urelumab doses between 1 and 15 mg/kg given every 3 weeks resulted in a higher frequency of treatment-related AEs than 0.1 or 0.3 mg/kg every 3 weeks. Dose was the single most important factor contributing to transaminitis development, which was more frequent and severe at doses $\geq$1 mg/kg. At the MTD of 0.1 mg/kg every 3 weeks, urelumab was relatively well tolerated, with fatigue (16%) and nausea (13%) being the most common treatment-related AEs, and was associated with immunologic and pharmacodynamic activity demonstrated by the induction of IFN-inducible genes and cytokines.

Conclusions: Integrated evaluation of urelumab safety data showed significant transaminitis was strongly associated with doses of $\geq$1 mg/kg. However, urelumab 0.1 mg/kg every 3 weeks was demonstrated to be safe, with pharmacodynamic activity supporting continued clinical evaluation of this dose as monotherapy and in combination with other immuno-oncology agents. *Clin Cancer Res; 23(8); 1929–36. ©2016 AACR.*

Introduction

Agents designed to block the inhibitory immune checkpoints cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1) are approved for advanced melanoma (alone and in combination), and anti-PD-1 is approved for non–small cell lung cancer and renal cell carcinoma (1). Both anti-CTLA-4 and anti-PD-1 can elicit durable clinical responses and, in some patients, result in long-term remissions with no clinical evidence of cancer (2–4). To expand the clinical benefit of this approach, agents designed to target other immunoregulatory pathways are under evaluation, including antagonists of inhibitory checkpoints, such as LAG-3 and TIM-3, but also agonist antibodies against costimulatory molecules on immune cells, such as CD137 (4-1BB), CD40, GITR, and OX-40 (5–7).

CD137 is a costimulatory member of the TNF receptor superfamily that is expressed on a variety of immune cells following activation, including T cells, dendritic cells, and natural killer cells. Signaling via CD137 can lead to cytokine induction, prevention of activation-induced cell death, and upregulation of cytotoxic T-cell activity; CD137 may also reduce the infiltration of regulatory T cells into tumors (8, 9). Melero and colleagues were the first to report that agonist mAbs to CD137 eradicated large, established tumors in mice. The long-lasting antitumor activity seen in their model was primarily

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Translational Relevance

Immune checkpoint inhibitors are approved or in advanced development for several types of cancer. To expand the clinical benefit of this approach, agents designed to target other immunoregulatory receptors are under clinical evaluation, including agonist antibodies against costimulatory molecules, such as CD137. This article reports safety, tolerability, and pharmacodynamic activity data from three studies evaluating urelumab, an agonist anti-CD137 mAb, and includes information on the hepatotoxicity observed. The data showed that urelumab dose was the single most important factor contributing to the development of severe transaminitis, with significant transaminitis strongly associated with doses $\geq$1 mg/kg. In addition, urelumab 0.1 mg/kg every 3 weeks was demonstrated to be safe, with pharmacodynamic activity supporting the continued clinical evaluation of this dose as monotherapy and in combination with other immuno-oncology agents.

mediated by $CD8^+$ T cells and associated with memory responses (10).

Two mAbs to CD137 have been developed and are undergoing clinical evaluation. Urelumab (BMS-663513, clone 10C7; Bristol-Myers Squibb) is an agonist, non-ligand–blocking, fully human mAb, engineered as an IgG4 to reduce binding to Fc receptors with a hinge mutation (S228P) to improve stability (11, 12). PF-05082566 (Pfizer) is a fully human IgG2 mAb (13). In preclinical studies, urelumab enhanced IFNγ production, T-cell survival, and the cytolytic activity of antigen-specific T cells (11, 12, 14), as expected for an antibody with costimulatory activity. Urelumab entered clinical development in 2005 and was evaluated as a monotherapy in two studies, CA186-001 (NCT00309023) and CA186-006 (NCT00612664). In December 2008, enrolment was stopped for all urelumab studies following the occurrence of two hepatotoxicity-related deaths. Subsequent detailed analysis of the clinical safety data demonstrated that urelumab dose was the single most important factor contributing to the development of severe transaminitis. The urelumab clinical development program was restarted with study CA186-011 (NCT01471210) in February 2012 to evaluate monotherapy doses <1 mg/kg (15). Subsequently, three urelumab-based combination studies were opened from March 2013 onward.

Here, we report integrated safety and preliminary pharmacodynamic data from urelumab monotherapy studies CA186-001 (NCT00309023), CA186-006 (NCT00612664), and CA186-011 (NCT01471210).

Patients and Methods

Patients and study design

Aggregated data from patients participating in three urelumab studies were included in this analysis. For all studies, patients provided written, informed consent, protocols were approved by the relevant Institutional Review Boards and independent ethics committees, and conduct was in accordance with International Conference on Harmonization guidelines on Good Clinical Practice and the principles of the Declaration of Helsinki.

The study CA186-001 (-001) was an open-label, ascending, multidose, phase I/II study of urelumab in patients with metastatic or locally advanced solid malignancies who had disease progression on standard therapy or refused or were unable to receive standard treatment. Patients were assigned to one of six sequential cohorts to receive urelumab 0.3, 1, 3, 6, 10, or 15 mg/kg every 3 weeks. The primary study objective was to assess safety and tolerability of urelumab, and secondary objectives included assessing pharmacokinetics, relationship between dose and biologic effect, and antitumor activity (11).

The study CA186-006 (-006) was a randomized, multidose, open-label, parallel four-arm, phase II study in patients with stage III/IV melanoma who had received one line of prior systemic treatment with any regimen (nonexperimental or experimental) and relapsed, progressed, or did not tolerate that regimen. Patients were randomized to receive urelumab at 0.1, 1, or 5 mg/kg every 3 weeks or 1 mg/kg every 6 weeks. The primary objective was to determine the 6-month progression-free survival rate in each arm. Secondary objectives included assessment of the tumor response rate, safety profile, and pharmacokinetics and pharmacodynamic parameters.

The study CA186-011 (-011) is an ongoing phase I study that included dose escalation (part 1) using a 6 + 9 design, cohort expansion (part 2), and tumor-specific cohort expansion (part 3). In part 1, successive cohorts of patients with advanced and/or metastatic solid tumors were treated as follows: cohort 1 with 0.1 mg/kg every 3 weeks and cohort 2 with 0.3 mg/kg every 3 weeks. In part 2, both cohorts (1 + 2) were expanded to 20 patients with advanced solid tumors. In part 3, additional tumor-specific cohorts with B-cell non-Hodgkin lymphoma, colorectal cancer, and head and neck cancer (10 patients each) were enrolled at the highest tolerated dose of 0.3 mg/kg every 3 weeks. The primary objective was to evaluate safety and define dose-limiting toxicity and MTD of urelumab; secondary objectives included assessment of preliminary antitumor activity, pharmacokinetics, and immunogenicity.

Assessments

In all studies, safety was evaluated using the NCI Common Terminology Criteria for Adverse Events (v3.0 in -001 and -006, and v4 in -011). Adverse events (AEs) were mapped to preferred term and body system using the Medical Dictionary for Regulatory Activities. Safety assessments were based on medical review of AE reports, vital sign measurements, physical examinations, and clinical laboratory tests.

Serum samples were collected to characterize urelumab pharmacokinetics and immunogenicity after multiple doses. The noncompartmental analysis and population pharmacokinetics analysis were used to estimate pharmacokinetics parameters and derive an individual estimate of the average concentration at steady state ($C_{avg_{ss}}$) in a total of 333 patients for exposure–response analysis (see Supplementary Methods and Supplementary Tables S1 and S2). Blood samples for pharmacodynamic assessments were collected at prespecified time points from consenting patients ($n = 32$).

Data analysis

All patients treated in studies -001 and -006 were included in the analysis, as well as patients treated up to a data cutoff of September 26, 2014, from study -011. A total of 346 patients received at least one dose of urelumab and were evaluable for safety. An integrated safety analysis determined the incidence of all treatment-related AEs, including serious AEs (SAEs), and

Table 1. Patient demographics

	Study -001 $n = 115$[a]	Study -006 $n = 159$[b,c]	Study -011 $n = 73$
Median age, years	61.0	59.0	60.0
<65 years, %	65	65.4	67.1
≥65 years, %	35	34.6	32.9
Sex, %			
Male	53	59	55
Female	47	41	45
ECOG PS, %			
0	51	77[c]	40
1	44	20	59
2	0	2	0
Race, %			
White	97	99	93
Other	3	1	7
Tumor type, %			
Melanoma	48	100	8
Kidney	23	0	4
Ovary	24	0	1
Pancreas	0	0	1
Prostate	1	0	0
NSCLC	0	0	15
Head and neck	0	0	21
Colorectal	0	0	22
NHL	0	0	11
Other	3	0	16

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; NHL, non-Hodgkin lymphoma; NSCLC, non–small cell lung cancer.
[a]Performance status data not available for five patients in study -001.
[b]Data are for all randomized patients; one patient was randomized but not treated.
[c]Performance status data not available for one patient in study -006.

treatment-related AEs leading to discontinuation and death. A detailed analysis of liver function test (LFT) results [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (Tbili)] was performed considering toxicity grade, dose level, and cycle for each study. Possible alternative causes for transaminitis were also evaluated.

Results

Patient characteristics

Between 2005 and 2008, 115 patients were enrolled and treated in -001; 159 patients were enrolled into -006, 158 of whom received urelumab, and between 2011 and 2014, 73 patients were enrolled and treated in -011. Baseline demographics for all enrolled patients ($N = 347$) are shown (Table 1).

Overview of treatment-related AEs

Across all studies, urelumab doses between 1 and 15 mg/kg resulted in a higher frequency of treatment-related AEs than doses of 0.1 or 0.3 mg/kg (Table 2). At doses ≥1 mg/kg, the most frequent treatment-related AEs were increased AST (27%), increased ALT (27%), and fatigue (24%), although there was no evidence of dose dependency. At 0.3 mg/kg, increased AST and fatigue were the most frequent treatment-related AEs (both 14%), and at 0.1 mg/kg, fatigue (16%) and nausea (13%) were the most common treatment-related AEs. Grade 3 or higher treatment-related AEs were more frequent at urelumab doses of 1 to 15 mg/kg than at the 0.1 and 0.3 mg/kg doses (Table 2).

There was a similar pattern for treatment-related SAEs, with the majority occurring at doses ≥1 mg/kg and the most common being increased ALT, increased AST, and fatigue (Table 2). At 0.3 mg/kg, there were seven treatment-related SAEs (two increased ALT, two increased AST, one abnormal LFT, one acute hepatitis, and one enteritis). There were two treatment-related SAEs at 0.1 mg/kg: increased ALT and erysipelas (Table 2).

The rate of patient discontinuation due to a treatment-related AE was higher with urelumab ≥1 mg/kg compared with 0.1 and 0.3 mg/kg. At ≥1 mg/kg, 37 of 229 patients (16%) discontinued due to a treatment-related AE; at 0.3 mg/kg, 5 of 56 patients (9%) discontinued, and at 0.1 mg/kg, 7 of 61 patients (11%) discontinued.

Two deaths occurred at the higher dose range (1 and 5 mg/kg, respectively). Both deaths were attributed to drug-related

Table 2. Investigator-reported treatment-related AEs[a] and SAEs[b]

Event	Urelumab 0.1 mg/kg ($n = 61$)[c]	Urelumab 0.3 mg/kg ($n = 56$)[c]	Urelumab ≥1 mg/kg ($n = 229$)[c]
Any grade ≤4 AEs[a], % (grade 3–4, %)[d]			
AST increased	8.2	14.3 (3.6)	27.1 (13.5)
ALT increased	6.6 (1.6)	10.7 (3.6)	26.6 (16.6)
Fatigue	16.4	14.3	24.0
Rash	4.9	7.1	19.7
Nausea	13.1	3.6	13.5
Pruritus	4.9	5.4	13.1
Decreased appetite	8.2	3.6	12.2
Pyrexia	4.9	1.8	12.2
Diarrhea	3.3	3.6	12.2
Asthenia	8.2	0	7.9
Headache	1.6	1.8	7.0
Neutropenia	4.9 (3.3)	0	6.1 (2.6)
Vomiting	3.3	0	5.2
Any grade ≤4 SAEs[a], % (grade 3–4, %)[d]			
ALT increased	1.6	3.6 (3.6)	5.2 (5.2)
AST increased		3.6 (3.6)	4.8 (4.4)
Neutropenia			3.1 (3.1)
Thrombocytopenia			2.2 (1.3)
Febrile neutropenia	1.6 (1.6)[e]		1.7 (1.7)
Cellulitis			1.3 (0.9)
Anemia			0.9 (0.4)
Leukopenia			0.9 (0.9)
Fatigue			0.9 (0.4)
Nausea			0.4 (0.4)
Decreased appetite			0.4 (0.4)
Pyrexia			0.4 (0.4)
Increased blood bilirubin			0.4 (0.4)
Abnormal LFT		1.8 (1.8)	0.4 (0.4)
Abdominal pain			0.4 (0.4)
Hepatic failure			0.4 (0.4)
Hyperbilirubinemia			0.4 (0.4)
Anaphylactic shock			0.4 (0.4)
Decreased neutrophil count			0.4 (0.4)
Acute hepatitis		1.8 (1.8)	
Erysipelas	1.6		
Enteritis		1.8	
Grade 5 SAEs[a]			
Malignant neoplasm progression			0.4
Autoimmune hepatitis			0.4

[a]Data shown are for treatment-related AEs occurring in ≥5% patients in any dose group; all grade 3, 4, and 5 SAEs and grade 1 or 2 SAEs reported more than once are shown. Please note that the classification of AEs as either treatment-related or serious (both of which are presented in this table) is different, per the criteria defined in the protocols.
[b]Data are from the 346 patients who received at least one dose of urelumab.
[c]Patients received urelumab every 3 weeks, except 40 patients from study -006 who received 1 mg/kg every 6 weeks.
[d]Absence of parentheses indicates no grade 3–4 AEs or SAEs were reported.
[e]Febrile neutropenia was deemed to be unrelated to urelumab after the data cut-off date for this report.

Table 3. Treatment-related abnormal LFT laboratory values by dose[a]

	Urelumab dose		
	0.1 mg/kg ($n = 61$)[b]	0.3 mg/kg ($n = 56$)[b]	≥1 mg/kg ($n = 229$)[b]
ALT, *n* (%)			
Total grade 1–4	19 (31)	28 (50)	135 (59)
Grade 1	18 (30)	21 (38)	63 (28)
Grade 2	1 (2)	3 (5)	28 (12)
Grade 3	0	3 (5)	27 (12)
Grade 4	0	1 (2)	17 (7)
AST, *n* (%)			
Total grade 1–4	23 (38)	29 (52)	136 (59)
Grade 1	22 (36)	21 (38)	79 (34)
Grade 2	0	4 (7)	18 (8)
Grade 3	1 (2)	3 (5)	29 (13)
Grade 4	0	1 (2)	10 (4)
Total bilirubin, *n* (%)			
Total grade 1–4	10 (16)	3 (5)	40 (18)
Grade 1	10 (16)	1 (2)	22 (10)
Grade 2	0	1 (2)	9 (4)
Grade 3	0	1 (2)	5 (2)
Grade 4	0	0	4 (2)

[a]Data are from the 346 patients who received at least one dose of urelumab.
[b]Patients received urelumab every 3 weeks, except 40 patients from study -006 who received 1 mg/kg every 6 weeks.

hepatotoxicity, and the patients were enrolled in study -006 in the 1 mg/kg (every 3 weeks) and 5 mg/kg (every 3 weeks) arms, respectively. Neither patient was taking concomitant medications with known potential for hepatotoxicity, and both received immunosuppressant medication for management. Postmortem examination was conducted in only one of the two patients and was noncontributory. One patient treated with urelumab monotherapy at 6 mg/kg who developed severe transaminase elevations and hyperbilirubinemia also underwent a liver biopsy; histopathology examination was consistent with bile duct injury consistent with drug-related liver injury, with only a moderate mixed inflammatory infiltrate containing lymphocytes and other inflammatory cells, including neutrophils.

Of note, the patient in the 1 mg/kg (every 3 weeks) arm had previously been treated with an mAb to CTLA-4 and had developed one immune-related AE, panhypopituitarism, while on prior treatment with CTLA-4 blockade.

Transaminitis

Transaminitis was more frequent at urelumab doses ≥1 mg/kg (Table 3). Exposure–response analyses demonstrated a correlation between the occurrence and severity of transaminitis with exposure [average observed concentration (C_{avg})] and suggested that the risk of developing clinically relevant transaminitis may be substantially increased at exposures (higher C_{avg}) achieved with urelumab doses ≥1 mg/kg every 3 weeks (Fig. 1).

Correlative analyses of data from studies -001 and -006 ($n = 270$) were performed to assess whether certain clinical features (liver function abnormalities at baseline, baseline liver metastases, and acetaminophen or statin use on study) could predict which patients were more likely to experience transaminitis with urelumab across the dose range evaluated. There was no clear association between AST or ALT abnormality (grade ≥1) at baseline and increased AST or ALT of grade ≥3, nor was there any association between baseline liver metastases or acetaminophen or statin use while on study and increased AST or ALT of grade ≥2 (data not shown).

Rechallenge after transaminase elevation. Twenty-five patients who developed increased AST, ALT, and/or Tbili of grade ≥2 were rechallenged with urelumab. Patients were rechallenged with the same dose of urelumab, except one patient who received a lower dose. Of these, 15 patients (60%) did not have recurrence of grade ≥2 LFT AE. This group included one patient at 0.1 mg/kg and two patients at 0.3 mg/kg; the remaining 12 patients were receiving urelumab at ≥1 mg/kg, including one patient at 10 mg/kg. Ten of the patients who were rechallenged had recurrence of grade ≥2 LFT AE; five of these patients (1 at 0.3 mg/kg and the remainder at 1 mg/kg) continued urelumab treatment, and five discontinued treatment for reasons other than toxicity (all at ≥1 mg/kg). Two patients with grade 3 or 4 ALT or AST elevations were retreated with the original treatment dose of 1 mg/kg after laboratory values returned to below the upper limit of normal. Both patients tolerated retreatment, with ALT and AST fluctuating between normal and grade 2. No patient discontinued study drug due to an AE upon rechallenge.

Management of transaminitis. In study -011, patients with grade 2 transaminitis AEs were managed by withholding urelumab treatment until the AEs were grade ≤1. Patients with grade 3 or higher AEs received treatment with systemic corticosteroids and discontinued urelumab. All of the three patients in -011 with grade 3 increased AST/ALT who received corticosteroids responded to treatment and were not rechallenged with urelumab.

Cytopenias

Laboratory evaluations showed grade 1 to 4 reductions in absolute neutrophil, platelet, and leukocyte counts across the dose range evaluated (Table 4). Most AEs were grade 1 or 2 in severity; the 0.1 mg/kg dose was associated with the lowest frequency of these AEs. Leukopenia and neutropenia events were not associated with infections, nor were there any episodes of bleeding related to thrombocytopenia (see prior section). Febrile neutropenia was not reported in any patient who received <1 mg/kg (four events reported at doses ≥1 mg/kg; Table 2).

Pharmacokinetics

The observed serum concentration:time profile across the dose range evaluated is shown in Supplementary Fig. S1. The pharmacokinetics of single-agent urelumab was studied over the range of 0.1 to 15 mg/kg administered as multiple doses every 3 weeks and one cohort at 1 mg/kg every 6 weeks. Urelumab exposure increased dose proportionally across the studied dose range. The half-life of urelumab was estimated from population pharmacokinetics to be approximately 18 days, with the minimum concentration (C_{min}) at steady state being greater than the pharmacologically active exposure level based on the *in vitro* human T cell–binding affinity of 0.1 μg/mL (data not shown).

Pharmacodynamic activity

Treatment with urelumab 0.1 mg/kg induced a range of IFN-induced cytokines (Fig. 2) and IFN response genes (Supplementary Fig. S2). The level of several IFN-induced cytokines was increased in serum at 1 week after urelumab administration (day 8 samples were not collected; day 29 was 1 week after the second dose); similarly, the expression of several IFN response genes was increased in whole-blood samples at approximately 3 and/or 7 days following urelumab administration and appeared to return to pretreatment levels by day 22.

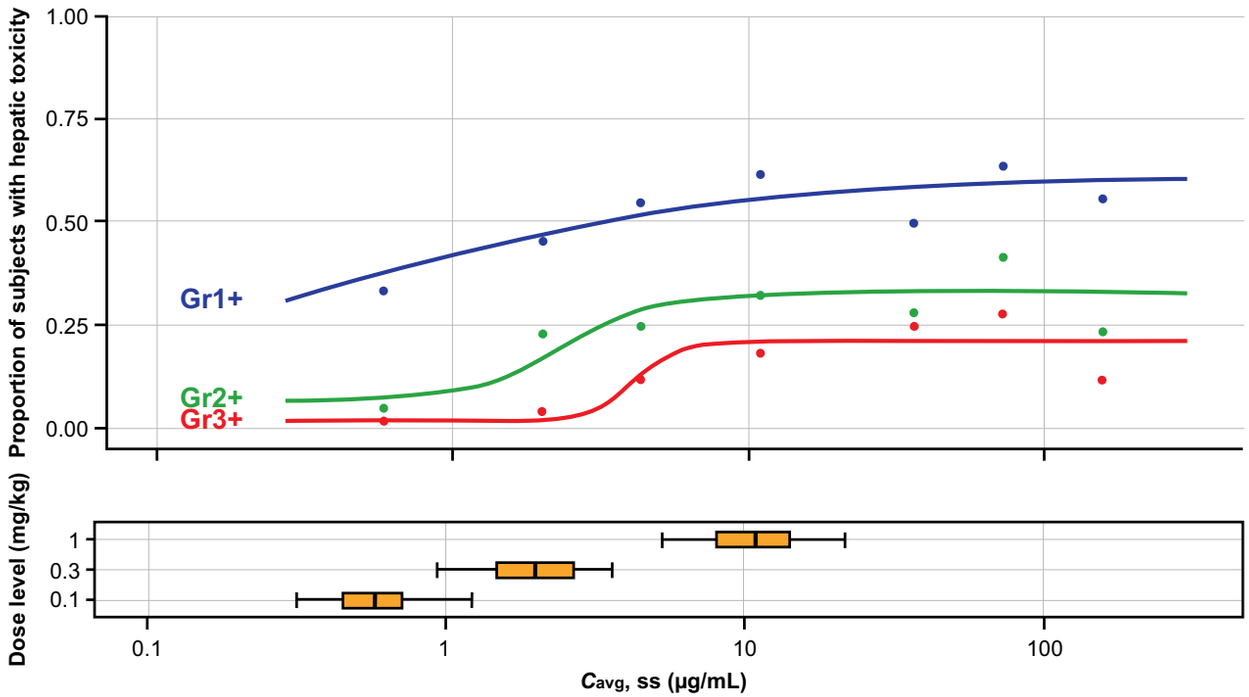

Figure 1.

Exposure–response analysis of urelumab transaminitis. The solid line represents model-estimated proportion of patients with LFT elevations at grade 1, 2, or 3 shown as blue, green, and orange lines, respectively. The observed proportion of patients with LFT elevations at grade 1, 2, or 3 and above is represented by solid points at the median C_{avg} for each tested dose level. Horizontal box-and-whisker plots show C_{avg} distribution at 0.1, 0.3, and 1 mg/kg. C_{avg}, average concentration; Gr, grade; ss, steady state. $n = 333$.

Discussion

The costimulatory receptor CD137 is a promising target for cancer immunotherapy (8, 9). This integrated safety analysis is one of the first reports of an agonist mAb targeting CD137. Overall safety data from evaluation of urelumab at doses of 0.1 to 15 mg/kg across three studies support 0.1 mg/kg every 3 weeks as the MTD. Experience with urelumab at 0.1 mg/kg every 3 weeks in 61 patients with advanced malignancies demonstrated acceptable safety and tolerability and pharmacodynamic activity consistent with immune activation. The significant transaminitis that led to the suspension of the clinical program was strongly associated with exposure to urelumab at doses of 1 mg/kg and above, suggesting a reasonable separation between the potential efficacious dose and the doses associated with toxicity.

Doses ≥1 mg/kg were associated with a high incidence of moderate-to-severe hepatic toxicity (ranging from asymptomatic increases in laboratory measures of LFTs to hepatic failure). Outcomes from analysis of LFTs and hepatic AEs showed that transaminitis was strongly associated with exposure to urelumab ≥1 mg/kg. In contrast, doses <1 mg/kg every 3 weeks were associated with the lowest probability of severe transaminitis and generally a low frequency of other AEs, predominantly grade 1 or 2 AEs. Accordingly, the clinical program was restarted to investigate 0.1 and 0.3 mg/kg every-3-week doses and included a clinical safety plan to monitor and manage transaminitis. Concomitant transaminitis (grade 4 increased AST and ALT) and hyperbilirubinemia (grade 3 increased Tbili) were reported in a single patient receiving 0.3 mg/kg in study -011; this dose was deemed to have exceeded the MTD, and further investigation of 0.3 mg/kg was stopped. Although hepatotoxicity in this patient resolved following treatment with immunosuppressive therapy per protocol-specified guidelines, further investigation of the 0.3 mg/kg dose was stopped, and the

Table 4. Treatment-related abnormal hematology laboratory values by dose[a]

	Urelumab dose		
	0.1 mg/kg ($n = 61$)[b]	**0.3 mg/kg ($n = 56$)[b]**	**≥1 mg/kg ($n = 229$)[b]**
Absolute neutropenia, *n* (%)			
Total grade 1–4	14 (23)	19 (34)	64 (28)
Grade 1	6 (10)	9 (16)	28 (12)
Grade 2	3 (5)	3 (5)	13 (6)
Grade 3	2 (3)	3 (5)	7 (3)
Grade 4	3 (5)	4 (7)	16 (7)
Thrombocytopenia, *n* (%)			
Total grade 1–4	12 (20)	25 (45)	63 (28)
Grade 1	7 (11)	24 (43)	46 (20)
Grade 2	3 (5)	0	7 (3)
Grade 3	1 (2)	0	7 (3)
Grade 4	0	1 (2)	3 (1)
Leukopenia, *n* (%)			
Total grade 1–4	10 (33)	19 (34)	84 (37)
Grade 1	13 (21)	8 (14)	50 (22)
Grade 2	2 (3)	5 (9)	15 (7)
Grade 3	4 (7)	4 (7)	14 (6)
Grade 4	1 (2)	2 (4)	5 (2)

[a]Data from the 344 patients; data not available from two patients who received urelumab ≥1 mg/kg.

[b]Patients received urelumab every 3 weeks, except 40 patients from study -006 who received 1 mg/kg every 6 weeks.

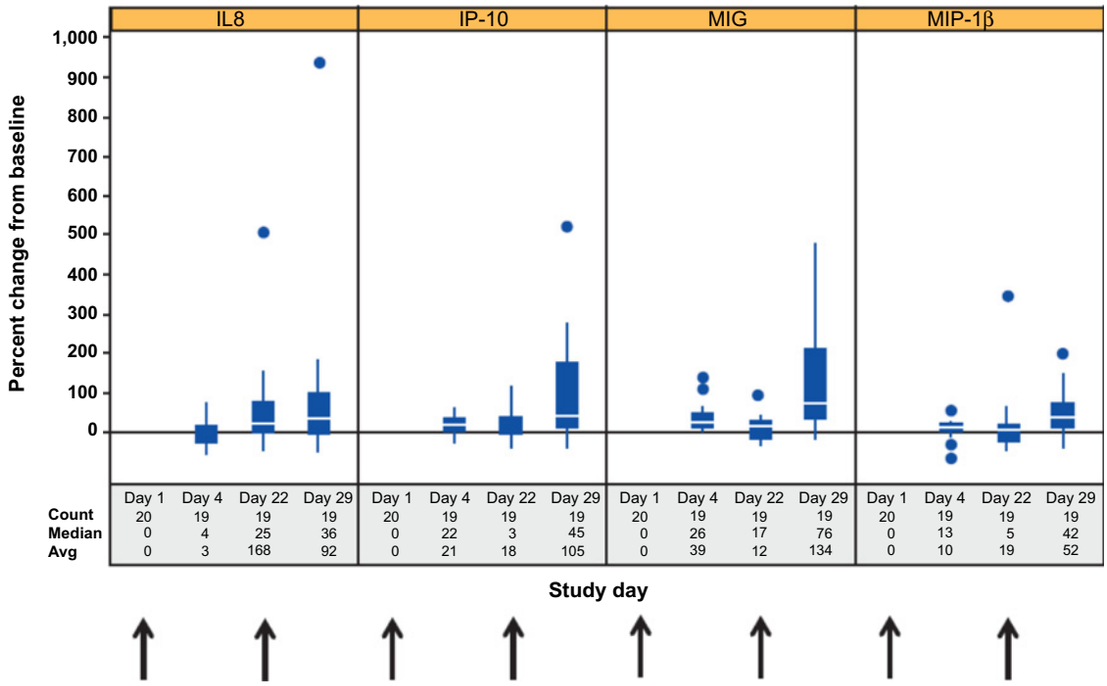

Figure 2.

Induction of a range of IFN-induced cytokines at 0.1 mg/kg urelumab. IFN-induced cytokines, including IL8, IFNγ-inducible protein 10 (IP-10), monokine induced by IFNγ (MIG or CXCL9), and macrophage-inflammatory protein-1β (MIP-1β) were measured in the serum of 19 patients from study -011 who received urelumab 0.1 mg/kg. Percentage change from baseline for each cytokine was plotted. Arrows, treatment days. Samples collected on treatment days were collected predose. Count, number of samples tested per time point; median, the quantity lying at the midpoint of a frequency distribution of observed values or quantities; Avg (abbreviation for average), the result obtained by adding together several quantities and then dividing this total by the number of quantities. One outlier data point for IL8 day 22 had a 2,220% change from baseline and is off the scale of this graph but is incorporated into the summary statistics table shown below the box plots.

urelumab dose was reduced to 0.1 mg/kg every 3 weeks in all patients.

No risk factor predisposing patients to developing transaminitis was identified in the integrated safety analysis of the completed studies -001 and -006 ($n = 169$). Although the evaluation of data from study -011 is ongoing, preliminary evaluation is similarly consistent with no identifiable risk factor for transaminitis.

Most of the patients who had their treatment withheld because of grade 2 transaminitis were able to resume treatment at the same dose with no recurrence of these AEs. Experience gained managing transaminitis in studies -001, -006, and -011, and in managing transaminitis induced by other immune checkpoint inhibitors, contributed to the development of the management strategies included in current urelumab study protocols. In general, patients receiving urelumab require close monitoring of LFTs. For grade 2 LFT AEs, urelumab dosing was delayed, alternative causes were investigated, and LFTs were monitored at increased frequency; for grade 3 or 4 AEs or grade 2 events that were worsening or persisting for more than 5 to 7 days, urelumab was discontinued, LFTs were monitored at increased frequency, and corticosteroids were administered.

Observations in murine models provide a possible explanation for liver-related AEs associated with agonist CD137 mAbs. Liver lymphocyte infiltration was reported in several studies of mice treated with a mouse anti-CD137 mAb (16–18). Although preliminary data from preclinical toxicity models suggest hepatic infiltration by lymphocytes (following cross-linking of CD137 receptor) as being possibly involved in producing liver toxicity, additional preclinical studies are ongoing to further address the mechanism of action of urelumab-related AEs (Bristol-Myers Squibb, unpublished data).

Aside from transaminitis, urelumab was not associated with the immune-mediated AEs commonly reported with CTLA-4 and PD-1/PD-L1 immune checkpoint inhibitors that affect the gastrointestinal, skin, pulmonary, and endocrine systems (19). Neutropenia, which is uncommon with CTLA-4 and PD-1 immune checkpoint inhibitors (19), was observed with urelumab. Drug-related febrile neutropenia was not noted at the urelumab MTD.

Although the mechanism of urelumab-related neutropenia is a matter of ongoing investigation, data from a murine model of listeria showed that CD137-mediated stimulation of neutrophils in early infection caused rapid production of inflammatory cytokines/chemokines and subsequent infiltration of neutrophils and monocytes crucial for eliminating the infecting bacteria (20).

The clinical evaluation of urelumab is ongoing at 0.1 mg/kg every 3 weeks as monotherapy and in combination with targeted mAbs, cetuximab, rituximab, or elotuzumab (NCT01775631, NCT02110082, and NCT02252263). This approach is based on preclinical data that showed enhancement of antibody-dependent cellular cytotoxicity when an agonist CD137 mAb was given after a targeted mAb (21–23). A study is also in progress to

evaluate urelumab in combination with nivolumab, an anti-PD-1 immune checkpoint inhibitor, in patients with advanced solid tumors and B-cell non-Hodgkin lymphoma (NCT02253992). Preclinical data suggest that combined targeting of costimulatory and inhibitory immune checkpoint pathways may enhance antitumor immune responses and prolong survival and could reduce the incidence of immune-mediated AEs, including liver inflammation (16, 24–26).

In summary, urelumab at 0.1 mg/kg every 3 weeks is well tolerated with evidence of immunologic activity. Clinical studies evaluating urelumab as monotherapy and in combination with other immuno-oncology agents are ongoing.

Disclosure of Potential Conflicts of Interest

N.H. Segal reports receiving commercial research grants from Bristol-Myers Squibb, Genentech/Roche, MedImmune/Astra Zeneca, Merck, and Pfizer; and is a consultant/advisory board member for Bristol-Myers Squibb, Genentech/Roche, MedImmune/Astra Zeneca, and Pfizer. T.F. Logan is a consultant/advisory board member for Prometheus. F.S. Hodi reports receiving commercial research grants from Bristol-Myers Squibb, and is a consultant/advisory board member for Bristol-Myers Squibb, EMD Serono, Genentech, Merck, and Novartis. D. McDermott is a consultant/advisory board member for Array BioPharm, Bristol-Myers Squibb, Eisai, Exelixis, Genentech BioOncology, Merck, Novartis, Pfizer, and Prometheus. I. Melero reports receiving commercial research grants from Pfizer, and is a consultant/advisory board member for AstraZeneca, Bioncotech, Boehringer Ingelheim, Bristol-Myers Squibb, Lilly, Merck Serono. O. Hamid reports receiving speakers bureau honoraria from Amgen, Bristol-Myers Squibb, Genentech, and Novartis; and is a consultant/advisory board member for Amgen, Bristol-Myers Squibb, Merck, Novartis, and Roche. H. Schmidt reports receiving speakers bureau honoraria from Bristol-Myers Squibb, and is a consultant/advisory board member for Bristol-Myers Squibb, GlaxoSmithKline, MSD, and Roche. C. Robert is a consultant/advisory board member for Amgen, Bristol-Myers Squibb, MSD, Novartis, and Roche. P.A. Ascierto reports receiving commercial research grants from Array, Bristol-Myers Squibb, Roche-Genentech, and Ventana; and is a consultant/advisory board member for Amgen, Array, Bristol-Myers Squibb, MSD, Novartis, Roche-Genentech, and Ventana. M. Maio is a consultant/advisory board member for Bristol-Myers Squibb. W.J. Urba reports receiving commercial research grants from Bristol-Myers Squibb and MedImmune; and is a consultant/advisory board member for Green peptide, and MedImmune/AstraZeneca. M. Jure-Kunkel holds ownership interest (including patents) in Bristol-Myers Squibb. M. Sznol is a consultant/advisory board member for Astra-Zeneca, Bristol-Myers Squibb, Genentech-Roche, Janssen, Kyowa-kirin, Lilly, Lion Biotechnologies, Merck, Nectar, Novartis Pfizer, and Symphogen. R. Levy reports receiving commercial research grants from Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The authors take full responsibility for the content of this publication and confirm that it reflects their viewpoint and medical expertise.

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References

1. U.S. Food and Drug Administration. FDA approved drug products. Available from: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.
2. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443–54.
3. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol 2015;33:1889–94.
4. Larkin J, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 2015;373:23–34.
5. Antonia SJ, Larkin J, Ascierto PA. Immuno-oncology combinations: a review of clinical experience and future prospects. Clin Cancer Res 2014;20:6258–68.
6. Shin DS, Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol 2015;33:23–35.
7. Moran AE, Kovacsovics-Bankowski M, Weinberg AD. The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy. Curr Opin Immunol 2013;25:230–7.
8. Vinay DS, Kwon BS. 4-1BB (CD137), an inducible costimulatory receptor, as a specific target for cancer therapy. BMB Rep 2014;47:122–9.
9. Yonezawa A, Dutt S, Chester C, Kim J, Kohrt HE. Boosting cancer immunotherapy with anti-CD137 antibody therapy. Clin Cancer Res 2015;21:3113–20.
10. Melero I, Shuford WW, Newby SA, Aruffo A, Ledbetter JA, Hellström KE, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med 1997;3:682–5.
11. Sznol M, Hodi FS, Margolin K, McDermott D, Ernstoff M, Kirkwood J, et al. Phase I/II study of BMS-663513, a fully-human anti-CD137 agonist monoclonal antibody, in patients with advanced cancer. J Clin Oncol 2008;26:15s (suppl; abstr 3007).
12. Jure-Kunkel MN, Calarota S, Girit E, Abraham R, Balimane P, Price K, et al. Functional characterization of fully human anti-CD137 antibodies. In: Proceedings of the 97th Annual Meeting of the American Association for Cancer Research; 2006 Apr 1–5; Washington, DC. Philadelphia, PA: AACR; 2006. Abstract nr 4759.
13. Fisher TS, Kamperschroer C, Oliphant T, Love VA, Lira PD, Doyonnas R, et al. Targeting of 4-1BB by monoclonal antibody PF-05082566 enhances T-cell function and promotes anti-tumor activity. Cancer Immunol Immunother 2012;61:1721–33.
14. Chacon JA, Wu RC, Sukhumalchandra P, Molldrem JJ, Sarnaik A, Pilon-Thomas S, et al. Co-stimulation through 4-1BB/CD137 improves the

expansion and function of CD8(+) melanoma tumor-infiltrating lymphocytes for adoptive T-cell therapy. PLoS One 2013;8:e60031.
15. Melero I, Gangadhar TC, Kohrt HE, Segel NH, Logan T, Urba WJ, et al. A phase I study of the safety, tolerability, pharmacokinetics, and immunoregulatory activity of urelumab (BMS-663513) in subjects with advanced and/or metastatic solid tumors and relapsed/refractory B-cell non-Hodgkin's lymphoma (B-NHL). J Clin Oncol 31, 2013; (suppl; abstr TPS3107).
16. Kocak E, Lute K, Chang X, May KF Jr, Exten KR, Zhang H, et al. Combination therapy with anti-CTL antigen-4 and anti-4-1BB antibodies enhances cancer immunity and reduces autoimmunity. Cancer Res 2006;66:7276–84.
17. Niu L, Strahotin S, Hewes B, Zhang B, Zhang Y, Archer D, et al. Cytokine-mediated disruption of lymphocyte trafficking, hemopoiesis, and induction of lymphopenia, anemia, and thrombocytopenia in anti-CD137-treated mice. J Immunol 2007;178:4194–213.
18. Dubrot J, Milheiro F, Alfaro C, Palazón A, Martinez-Forero I, Perez-Gracia JL, et al. Treatment with anti-CD137 mAbs causes intense accumulations of liver T cells without selective antitumor immunotherapeutic effects in this organ. Cancer Immunol Immunother 2010;59:1223–33.
19. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol 2015;33:1974–82.
20. Lee SC, Ju SA, Sung BH, Heo SK, Cho HR, Lee EA, et al. Stimulation of the molecule 4-1BB enhances host defense against *Listeria monocytogenes* infection in mice by inducing rapid infiltration and activation of neutrophils and monocytes. Infect Immun 2009;77:2168–76.
21. Houot R, Kohrt HE, Marabelle A, Levy R. Targeting immune effector cells to promote antibody-induced cytotoxicity in cancer immunotherapy. Trends Immunol 2011;32:510–6.
22. Kohrt HE, Houot R, Goldstein MJ, Weiskopf K, Alizadeh AA, Brody J, et al. CD137 stimulation enhances the antilymphoma activity of anti-CD20 antibodies. Blood 2011;117:2423–32.
23. Kohrt HE, Colevas AD, Houot R, Weiskopf K, Goldstein MJ, Lund P, et al. Targeting CD137 enhances the efficacy of cetuximab. J Clin Invest 2014;124:2668–82.
24. Dai M, Wei H, Yip YY, Feng Q, He K, Popov V, et al. Long-lasting complete regression of established mouse tumors by counteracting Th2 inflammation. J Immunother 2013;36:248–57.
25. Wei H, Zhao L, Li W, Fan K, Qian W, Hou S, et al. Combinatorial PD-1 blockade and CD137 activation has therapeutic efficacy in murine cancer models and synergizes with cisplatin. PLoS One 2013;8:e84927.
26. Mueller AMS, Hebb J, Idit Sagiv-Barfi I, Marabelle A, Houot R, Rajapaksa A, et al. Sequential tumor and dual immune targeted immunotherapy: antilymphoma activity of rituximab with 4-1bb stimulation and PD-1 blockade. J Immunother Cancer 2014;2(Suppl 3):P106.