European Journal of Cancer (2015) 51, 1812–1821

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Hypocalcaemia in patients with metastatic bone disease treated with denosumab

Jean-Jacques Body [a,*], Henry G. Bone [b], Richard H. de Boer [c], Alison Stopeck [d], Catherine Van Poznak [e], Ronaldo Damião [f], Karim Fizazi [g], David H. Henry [h], Toni Ibrahim [i], Allan Lipton [j], Fred Saad [k], Neal Shore [l], Toshimi Takano [m], Adam J. Shaywitz [n,1], Huei Wang [n], Oswaldo L. Bracco [n,2], Ada Braun [n,3], Paul J. Kostenuik [n]

[a] *Department of Medicine, CHU Brugmann, Université Libre de Bruxelles, Brussels, Belgium*
[b] *Michigan Bone and Mineral Clinic, Detroit, MI, USA*
[c] *Department of Medical Oncology, Royal Melbourne Hospital, Melbourne, VIC, Australia*
[d] *Stony Brook Cancer Center, Stony Brook, NY, USA*
[e] *Department of Medical Oncology, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI, USA*
[f] *Department of Urology, Hospital Universitario Pedro Ernesto, Rio de Janeiro, Brazil*
[g] *Department of Medical Oncology, Institut Gustave Roussy, University of Paris Sud, Villejuif, France*
[h] *Department of Medicine, Joan Karnell Cancer Center at Pennsylvania Hospital, Philadelphia, PA, USA*
[i] *Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS-Osteoncology and Rare Tumors Center, Meldola, Italy*
[j] *Division of Oncology, Pennsylvania State University, Milton S. Hershey Medical Center, Hershey, PA, USA*
[k] *Department of Surgery, University of Montreal Hospital Center, Montreal, QC, Canada*
[l] *Department of Urology, Carolina Urologic Research Center, Myrtle Beach, SC, USA*
[m] *Department of Medical Oncology, Toranomon Hospital, Tokyo, Japan*
[n] *Department of Hematology/Oncology, Amgen Inc., Thousand Oaks, CA, USA*

Received 9 March 2015; received in revised form 13 May 2015; accepted 14 May 2015
Available online 17 June 2015

* *Corresponding author at:* Place Van Gehuchten, CHU Brugmann, Université Libre de Bruxelles (ULB), 1020 Brussels, Belgium. Tel.: +32 2 477 2600; fax: +32 2 477 2527.

E-mail addresses: jean-jacques.body@chu-brugmann.be (J.-J. Body), hgbone.md@att.net (H.G. Bone), richard.deboer@wh.org.au (R.H. de Boer), Alison.Stopeck@stonybrookmedicine.edu (A. Stopeck), cvanpoz@med.umich.edu (C. Van Poznak), damiao@email.com (R. Damião), Karim.FIZAZI@igr.fr (K. Fizazi), dhhenry@juno.com (D.H. Henry), toni.ibrahim@irst.emr.it (T. Ibrahim), alipton@hmc.psu.edu (A. Lipton), fredsaad@videotron.ca (F. Saad), nshore@gsuro.com (N. Shore), takano@toranomon.gr.jp (T. Takano), ashaywitz@gmail.com (A.J. Shaywitz), hueiw@amgen.com (H. Wang), ozzie.bracco@gmail.com (O.L. Bracco), adahbraun@yahoo.de (A. Braun), PKost@roadrunner.com (Paul J. Kostenuik).

[1] Current address: Department of Clinical Sciences, BioMarin Pharmaceuticals Inc., Novato, CA, USA.
[2] Current address: Clinical Safety and Risk Management, Merck Research Laboratories, North Wales, PA, USA.
[3] Current address: Pharmaceutical Group, Biothera, Eagan, MN, USA.

http://dx.doi.org/10.1016/j.ejca.2015.05.016

KEYWORDS
Denosumab
Zoledronic acid
Hypocalcaemia
Risk factors
Bone metastasis

Abstract ***Background:*** This analysis was performed to further characterise treatment-emergent hypocalcaemia in patients with bone metastases receiving denosumab.
Methods: Laboratory abnormalities and adverse events of hypocalcaemia in patients with metastatic bone disease were analysed using data from three identically designed phase 3 trials of subcutaneous denosumab 120 mg ($n = 2841$) versus intravenous zoledronic acid 4 mg ($n = 2836$).
Results: The overall incidence of laboratory events of hypocalcaemia grade $\geqslant 2$ was higher with denosumab (12.4%) than with zoledronic acid (5.3%). Hypocalcaemia events were primarily grade 2 in severity and usually occurred within the first 6 months of treatment. Patients who reported taking calcium and/or vitamin D supplements had a lower incidence of hypocalcaemia. Prostate cancer or small-cell lung cancer, reduced creatinine clearance and higher baseline bone turnover markers of urinary N-telopeptide of type I collagen (uNTx; >50 versus $\leqslant 50$ nmol/mmol) and bone-specific alkaline phosphatase (BSAP; >20.77 μg/L [median] versus $\leqslant 20.77$ μg/L) values were important risk factors for developing hypocalcaemia. The risk associated with increased baseline BSAP levels was greater among patients who had >2 bone metastases at baseline versus those with $\leqslant 2$ bone metastases at baseline.
Conclusion: Hypocalcaemia was more frequent with denosumab versus zoledronic acid, consistent with denosumab's greater antiresorptive effect. Low serum calcium levels and potential vitamin D deficiency should be corrected before initiating treatment with a potent osteoclast inhibitor, and corrected serum calcium levels should be monitored during treatment. Adequate calcium and vitamin D intake appears to substantially reduce the risk of hypocalcaemia.

1. Introduction

Many patients with malignancies develop bone metastases during disease progression [1–3]. Bone metastases disrupt the normal homeostasis between bone formation and resorption by promoting osteoclast maturation and activity and increased bone resorption [1]. The shift toward increased bone resorption may result in bone destruction and skeletal-related events (SREs), such as pathologic fracture, spinal cord compression, severe pain and the need for skeletal radiation or surgery [1].

Antiresorptive agents used to treat bone metastases inhibit osteoclastic bone resorption and reduce skeletal calcium release into circulation. Hypocalcaemia has been observed with oral and intravenous bisphosphonates and the receptor activator of nuclear factor-kappa β ligand (RANKL) inhibitor denosumab [1,4–7]. Risk factors for hypocalcaemia include osteoblastic metastases [8,9] or extensive osteoid, as in osteomalacia [10], both of which may act as a calcium sink. Low baseline serum calcium concentrations [11] and concurrent corticosteroids [11] have also been associated with hypocalcaemia on antiresorptive therapy.

A combined analysis of three phase 3 trials in patients with metastatic bone disease showed denosumab was superior to zoledronic acid (ZA) in preventing SREs [5]. The overall safety profiles of denosumab and ZA were generally similar; however, hypocalcaemia was more frequent with denosumab [5]. This retrospective analysis assessed hypocalcaemia based on laboratory abnormalities and clinical evaluations collected during these trials to further identify and characterise potential risk factors.

2. Patients and methods

2.1. Patients

This retrospective analysis included patient-level data from three identically designed, double-blind, double-dummy, international phase 3 trials comparing the efficacy and safety of denosumab versus ZA for the prevention of SREs in patients with metastatic bone disease and advanced breast cancer (NCT00321464) [12], prostate cancer (NCT00321620) [13], or other solid tumours or multiple myeloma (NCT00330759) [14]. Details of the study designs and results are published [5,12–14]. Patients provided written informed consent; the study protocols were approved by each site's ethics committee.

2.2. Study design and treatment

Patients received either subcutaneous denosumab 120 mg (plus intravenous placebo) or intravenous

infusion of ZA 4 mg (plus subcutaneous placebo) every 4 weeks. ZA dosing was adjusted for renal impairment per the label and withheld as needed for renal insufficiency. Daily calcium ($\geqslant$500 mg) and vitamin D ($\geqslant$400 IU equivalent) were strongly recommended for all patients; supplement intake was collected by patient report. Study treatment was withheld for any patient who experienced grade 3 or 4 hypocalcaemia (or any other adverse event [AE]) reported by the investigator as treatment related, and resumed when the event resolved to grade $\leqslant$1.

2.3. Study assessments

Serum calcium (albumin-corrected if albumin was <4 g/dL), measured every 4 weeks by a central laboratory, was analysed to identify hypocalcaemia. The incidence of laboratory hypocalcaemia by grade, defined by the Common Terminology Criteria for Adverse Events, version 3.0 [15], was evaluated by treatment group.

2.4. Hypocalcaemia risk factors

Potential risk factors for hypocalcaemia that were evaluated were sex and race, calcium and/or vitamin D supplementation, baseline calculated creatinine clearance, tumour type, bone lesion type, length of exposure to study drug, baseline levels of bone-specific alkaline phosphatase (BSAP) and urinary N-telopeptide of type I collagen (uNTx), and number of bone metastases at baseline. Hypocalcaemia incidence was also summarised by baseline levels of calculated creatinine clearance (ie, 30–<60 mL/min, 60–<90 mL/min and $\geqslant$90 mL/min). The first occurrence of hypocalcaemia was evaluated by tumour type, time period and timing after dose.

2.5. Statistical analysis

The duration of the first occurrence of hypocalcaemia was calculated from the date of onset of hypocalcaemia grade $\geqslant$2 to the resolution date (AE) or to a lower grade of hypocalcaemia (laboratory values). The median duration of first occurrence of hypocalcaemia based on central laboratory results was estimated using the Kaplan–Meier method. The impact of on-study calcium/vitamin D supplement use on the risk of developing hypocalcaemia for denosumab-treated patients was evaluated using a time-dependent Cox proportional hazards model.

A Cox proportional hazards model was used to assess the baseline covariate significance of disease characteristics on the risk of developing grade $\geqslant$2 hypocalcaemia in univariate and multivariate analyses. Baseline characteristics with $P < 0.05$ from the univariate analyses were included in the multivariate model. The interactions of corrected uNTx and number of bone metastases, and BSAP and number of bone metastases, were explored using Cox proportional hazards models with the interaction term and the associated baseline covariates in the models separately.

3. Results

3.1. Baseline characteristics

Across the three trials, patients were randomised to denosumab ($n = 2862$) or ZA ($n = 2861$; Table 1). In both treatment groups, baseline characteristics were generally similar between patients who did and did not develop on-study hypocalcaemia. Overall, 2841 and 2836 patients in the denosumab and ZA groups, respectively, received $\geqslant$1 dose of study drug and were included in this analysis.

3.2. Incidence of hypocalcaemia

The incidence of laboratory-detected hypocalcaemia was higher for denosumab than for ZA overall and for each grade (Fig. 1A). Similarly, the incidence of investigator-reported hypocalcaemia AEs was greater for denosumab than for ZA (Fig. 1B). The majority of hypocalcaemia events were grade 2; no fatal hypocalcaemia events occurred during the trials.

Supplementation with calcium and/or vitamin D was associated with fewer hypocalcaemia AEs for both treatment groups (Fig. 1C and D). With denosumab, the risk of developing an AE of hypocalcaemia was 40% lower among patients who reported taking supplements compared with those who did not (hazard ratio [HR], 0.60 [95% confidence interval (CI), 0.45–0.81]; $P = 0.0007$). For ZA, the risk of developing hypocalcaemia was 27% lower for those who reported taking supplements compared with those who did not (HR, 0.73 [95% CI, 0.48–1.11]; $P = 0.14$).

3.3. First occurrence of hypocalcaemia

Hypocalcaemia occurred earlier for denosumab-treated than for ZA-treated patients. The median (Q1, Q3) time to first occurrence of hypocalcaemia grade $\geqslant$2 was 3.8 (1.8, 10.1) months with denosumab and 6.5 (2.2, 12.0) months with ZA. The median (Q1, Q3) time to first occurrence of hypocalcaemia grade $\geqslant$3 was longer in both groups: 4.6 (1.8, 12.6) and 7.8 (3.7, 13.8) months, respectively. The corresponding median (Q1, Q3) times to first occurrence of an AE of hypocalcaemia were 2.8 (1.0, 7.1) and 3.5 (1.0, 9.1) months.

Of the 353 denosumab-treated patients with hypocalcaemia grade $\geqslant$2, more than half had prostate cancer (Table 2). For most denosumab-treated patients and

Table 1
Demographics and baseline characteristics of randomised patients.

Characteristic, *n* (%) or median (Q1, Q3)	Denosumab ($n = 2862$)		Zoledronic acid ($n = 2861$)	
	With hypocalcaemia[a] ($n = 353$)	Without hypocalcaemia ($n = 2509$)	With hypocalcaemia[a] ($n = 149$)	Without hypocalcaemia ($n = 2712$)
Sex				
Female	116 (32.9)	1200 (47.8)	78 (52.3)	1271 (46.9)
Male	237 (67.1)	1309 (52.2)	71 (47.7)	1441 (53.1)
Age, years	65 (57, 74)	62 (54, 71)	62 (54, 70)	63 (54, 72)
ECOG performance status				
0	147 (41.6)	1015 (40.5)	70 (47.0)	1080 (39.8)
1	164 (46.5)	1259 (50.2)	65 (43.6)	1331 (49.1)
2	42 (11.9)	230 (9.2)	14 (9.4)	290 (10.7)
Albumin-adjusted serum calcium[b]				
mg/dL	9.6 (9.3, 10.0)	9.8 (9.5, 10.1)	9.7 (9.4, 9.9)	9.8 (9.5, 10.1)
mmol/L	2.4 (2.3, 2.5)	2.5 (2.4, 2.5)	2.4 (2.4, 2.5)	2.5 (2.4, 2.5)
Serum creatinine, μmol/L	79.6 (61.9, 97.2)	70.7 (61.9, 88.4)	70.7 (61.4, 89.7)	71.6 (61.9, 88.4)
Calculated creatinine clearance >60 mL/min[c]	278 (78.8)	2068 (82.4)	120 (80.5)	2222 (81.9)
Bone turnover markers[d]				
uNTx, nmol/mmol	59.5 (29.9, 155.4)	41.9 (23.9, 75.2)	49.5 (26.4, 99.4)	42.7 (24.9, 79.5)
BSAP, μg/L	41.4 (18.6, 109.5)	19.8 (13.7, 35.4)	22.0 (14.6, 48.4)	20.5 (13.4, 39.1)
Received study drug[e]	353 (100)	2486 (99.1)	149 (100)	2689 (99.2)

BSAP, bone-specific alkaline phosphatase; ECOG, Eastern Cooperative Oncology Group; uNTx, urinary N-telopeptide of type I collagen.

[a] Defined as a laboratory grade $\geqslant 2$ event of hypocalcaemia.

[b] Calcium value was adjusted if albumin level was <4 g/dL.

[c] The Cockroft–Gault formula was used to estimate creatinine clearance. Values were missing for 19 patients treated with denosumab and 31 patients treated with zoledronic acid.

[d] Based on patients with baseline values of uNTx or BSAP.

[e] Two patients randomised to zoledronic acid received denosumab; these two patients are included in the denosumab-treated group for the subsequent analyses.

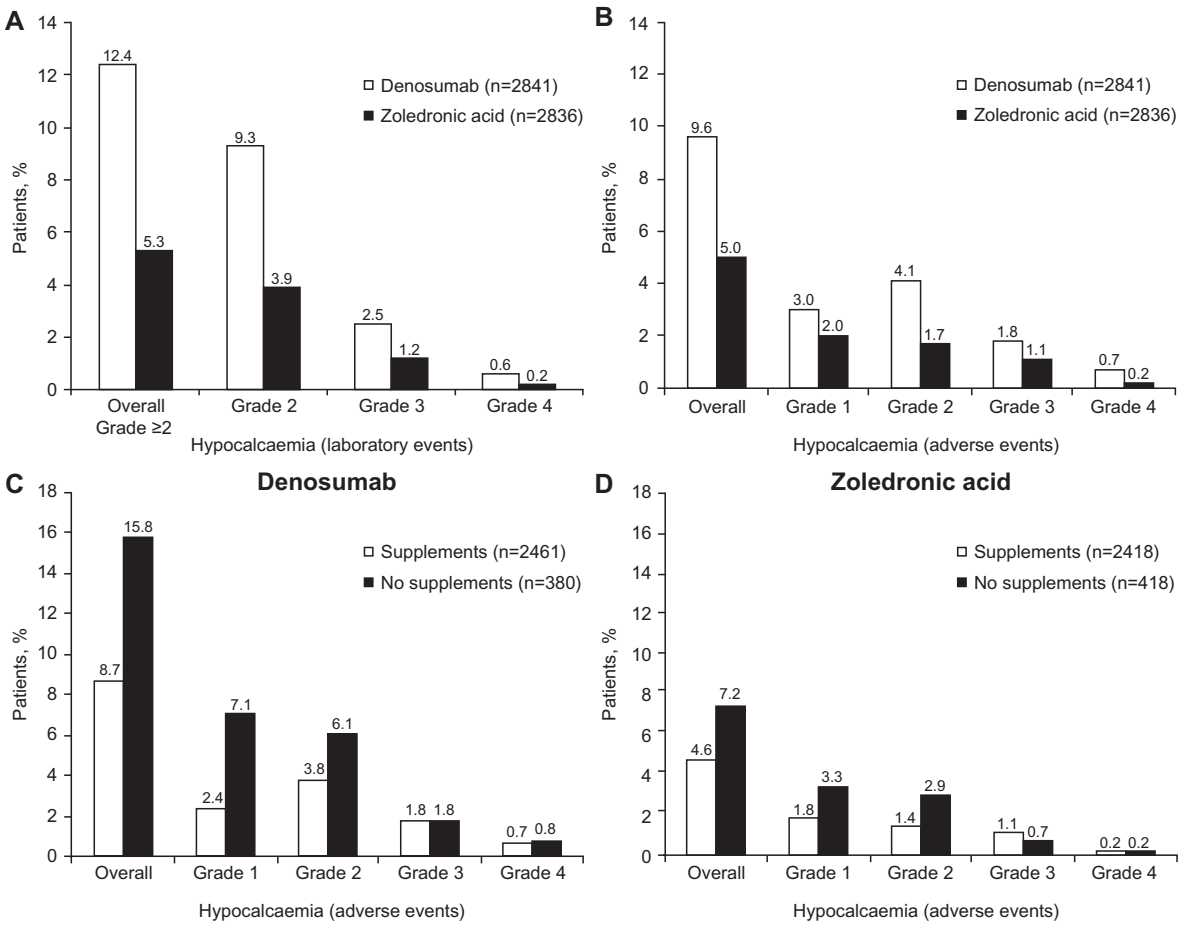

Fig. 1. Overall incidence of hypocalcaemia, including laboratory grade ⩾2 events (A), adverse events of hypocalcaemia (B), denosumab adverse events of hypocalcaemia by supplement subgroups (C), and zoledronic acid adverse events of hypocalcaemia by supplement subgroups (D). Maximum grade experienced by each patient based on the Common Terminology Criteria for Adverse Events, version 3.0. The supplements subgroup included patients who reported taking oral calcium and/or vitamin D at any time during the study, excluding those who reported supplement use only after their first hypocalcaemia event. The no-supplements subgroup comprised patients who never reported taking oral calcium and/or vitamin D during the study as well as those who reported supplement use only after their first hypocalcaemia event.

across all tumour types, the first occurrence of grade ⩾2 hypocalcaemia or a hypocalcaemia AE was ⩽6 months after initiating treatment (Table 2); 20.4% of denosumab-treated patients and 16.1% of ZA-treated patients experienced grade ⩾2 hypocalcaemia between the first and second doses (Table 3).

Hypocalcaemia generally resolved by the next scheduled study visit. Using the beginning and end dates for the event-of-interest AEs to define duration, the first occurrence of hypocalcaemia lasted approximately a median of 3 weeks. Similarly, the Kaplan–Meier estimate of the median duration for first occurrence of hypocalcaemia was 30 days for denosumab and 29 days for ZA. Of the 502 patients who experienced grade ⩾2 hypocalcaemia, 3 (0.6%) discontinued the study because of an AE of hypocalcaemia.

Most patients with hypocalcaemia experienced only one hypocalcaemic episode; 43% of denosumab-treated patients with hypocalcaemia and 32% of ZA-treated patients with hypocalcaemia had a recurrent event. Among denosumab-treated patients, recurrence rates were highest for those with prostate cancer (94/943, 10%) and lowest for those with non–small-cell lung cancer (NSCLC; 3/345, 1%).

3.4. Factors associated with hypocalcaemia

In the denosumab-treated group, more men than women experienced grade ⩾2 hypocalcaemia (15.4% versus 8.9%). The ZA-treated group did not show a difference based on sex (4.7% versus 5.8%). Neither treatment group showed differences based on race (Table 4).

For each tumour type, the incidence of hypocalcaemia was higher with denosumab than ZA (Fig. 2). Among denosumab-treated patients, those with prostate cancer or small-cell lung cancer (SCLC) had the highest incidence of hypocalcaemia. For patients receiving ZA, the incidence of hypocalcaemia was highest in those with SCLC or multiple myeloma. Those with NSCLC had the lowest incidence in both groups.

Table 2
First occurrence of hypocalcaemia by tumour type and time period in patients treated with denosumab.

Tumour type, *n* (%)	Time period			
	0–6 months	>6–12 months	>12–24 months	>24 months
Laboratory event of grade ⩾2 hypocalcaemia				
All tumour types ($n = 353$)	219 (62.0)	67 (19.0)	59 (16.7)	8 (2.3)
Breast cancer ($n = 86$)	44 (51.2)	22 (25.6)	19 (22.1)	1 (1.2)
Prostate cancer ($n = 193$)	115 (59.6)	39 (20.2)	32 (16.6)	7 (3.6)
Multiple myeloma ($n = 11$)	8 (72.7)	1 (9.1)	2 (18.2)	0 (0)
SCLC ($n = 11$)	10 (90.9)	0 (0)	1 (9.1)	0 (0)
NSCLC ($n = 18$)	15 (83.3)	3 (16.7)	0 (0)	0 (0)
Other ($n = 34$)	27 (79.4)	2 (5.9)	5 (14.7)	0 (0)
Adverse events of hypocalcaemia				
All tumour types ($n = 313$)	193 (61.7)	72 (23.0)	42 (13.4)	6 (1.9)
Breast cancer ($n = 65$)	36 (55.4)	15 (23.1)	13 (20.0)	1 (1.5)
Prostate cancer ($n = 137$)	80 (58.4)	35 (25.5)	17 (12.4)	5 (3.6)
Multiple myeloma ($n = 15$)	9 (60.0)	3 (20.0)	3 (20.0)	0 (0)
SCLC ($n = 5$)	4 (80.0)	1 (20.0)	0 (0)	0 (0)
NSCLC ($n = 36$)	28 (77.8)	5 (13.9)	3 (8.3)	0 (0)
Other ($n = 55$)	36 (65.5)	13 (23.6)	6 (10.9)	0 (0)

NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

Table 3
Timing of first occurrence of hypocalcaemia by dose.[a]

Dose timing, *n* (%)	Grade ⩾2 hypocalcaemia		Grade ⩾3 hypocalcaemia	
	Denosumab ($n = 353$)	Zoledronic acid ($n = 149$)	Denosumab ($n = 88$)	Zoledronic acid ($n = 38$)
Between doses 1 and 2	72 (20.4)	24 (16.1)	19 (21.6)	2 (5.3)
Between doses 2 and 3	55 (15.6)	14 (9.4)	6 (6.8)	3 (7.9)
Between doses 3 and 4	34 (9.6)	8 (5.4)	10 (11.4)	3 (7.9)
Between doses 4 and 5	22 (6.2)	8 (5.4)	6 (6.8)	3 (7.9)
Between doses 5 and 6	23 (6.5)	8 (5.4)	7 (8.0)	2 (5.3)
After dose 6	147 (41.6)	87 (58.4)	40 (45.5)	25 (65.8)

[a] Includes patients who received one or more doses of study drug and experienced an adverse event of hypocalcaemia.

The incidence of laboratory events of hypocalcaemia was higher in denosumab-treated patients with lower baseline values of creatinine clearance compared with those with higher values. In the denosumab-treated group, 15.5% of patients with baseline creatinine clearance 30 to <60 mL/min had a hypocalcaemia event, compared with 12.9% of those with values 60 to <90 mL/min and 11.0% of those with values ⩾90 mL/min. The corresponding percentages in the ZA-treated group were 5.8% (30–<60 mL/min), 4.4% (60–<90 mL/min) and 5.8% (⩾90 mL/min).

The denosumab-treated patients who developed hypocalcaemia had higher baseline uNTx and BSAP levels than those who did not (Table 1). Similarly, the ZA-treated patients who developed hypocalcaemia also had higher baseline uNTx levels than those who did not, although baseline BSAP levels were similar.

Covariate analyses of baseline characteristics showed several factors associated with risk of developing grade ⩾ 2 hypocalcaemia in denosumab-treated patients. In the univariate analysis, male sex, prostate cancer, SCLC, reduced creatinine clearance (30–<60 mL/min), higher baseline values of uNTx and BSAP, >2 bone metastases at baseline, interaction of baseline values of BSAP and the number of bone metastases, and osteoblastic lesions were significantly associated with an increased risk of developing hypocalcaemia (Table 4).

In the multivariate analysis, an increased risk of developing hypocalcaemia was found for patients with prostate cancer or SCLC, reduced creatinine clearance (30–<60 mL/min) and higher baseline values of uNTx and BSAP (Table 4). The risk associated with increased baseline BSAP levels was greater among patients who also had >2 bone metastases at baseline versus patients with ⩽2 bone metastases. Bone lesion type (osteolytic, osteoblastic, or mixed) at baseline did not increase the risk for developing hypocalcaemia (all $P \geqslant 0.2697$).

4. Discussion

Hypocalcaemia occurred more frequently in denosumab recipients than in ZA recipients, as measured by both laboratory values and AEs. The risk of developing hypocalcaemia AEs was 40% lower in denosumab-treated patients who reported taking calcium/vitamin D supplements. Univariate analysis identified several risk factors associated with the development

Table 4
Baseline factors and risk of developing hypocalcaemia grade ⩾2.

Baseline disease characteristic	Univariate analysis[a]			Multivariate analysis		
	Point estimate	95% CI	*P* value	Point estimate	95% CI	*P* value
Sex						
Male ($n = 1535$)	2.083	1.667–2.603	<0.0001	0.761	0.459–1.262	0.2902
Female ($n = 1306$)						
Race						
White ($n = 2404$)	1.080	0.801–1.457	0.6141			
Non-white ($n = 437$)						
Tumour type						
Breast cancer ($n = 1020$)	1.024	0.615–1.706	0.9263	0.849	0.441–1.635	0.6249
Prostate cancer ($n = 943$)	2.966	1.827–4.814	<0.0001	2.193	1.210–3.974	0.0096
Multiple myeloma ($n = 86$)	1.582	0.746–3.356	0.2319	2.082	0.908–4.771	0.0831
SCLC ($n = 61$)	4.211	1.989–8.915	0.0002	4.982	2.252–11.021	<0.0001
Other ($n = 386$)	1.546	0.873–2.738	0.1354	1.484	0.767–2.869	0.2409
NSCLC ($n = 345$)						
Baseline creatinine clearance, mL/min						
30–<60 ($n = 470$)	1.649	1.242–2.189	0.0005	1.414	1.039–1.924	0.0276
60–<90 ($n = 1080$)	1.207	0.954–1.527	0.1162	1.183	0.919–1.524	0.1918
⩾90 ($n = 1268$)						
Baseline corrected uNTx level (>50 versus ⩽50 nmol/mmol; $n = 1125$ versus 1429, respectively)	1.971	1.581–2.457	<0.0001	1.305	1.018–1.673	0.0360
Baseline BSAP level (>median versus ⩽ median; 20.77 μg/L; $n = 1319$ versus 1305, respectively)	2.702	2.134–3.419	<0.0001	1.734	1.280–2.348	0.0004
Bone metastases (>2 versus ⩽2; $n = 695$ versus 2146, respectively)	1.814	1.459–2.144	<0.0001	0.631	0.312–1.275	0.1995
Interaction between baseline BSAP level (>median versus ⩽median; 20.77 μg/L) and number of bone metastases (>2 versus ⩽ 2)	1.981	1.028–3.821	0.0412	2.419	1.154–5.071	0.0193
BSAP level (>median versus ⩽median; 20.77 μg/L) and >2 bone metastases at baseline	–	–	–	4.193	2.091–8.410	–
BSAP level (>median versus ⩽median; 20.77 μg/L) and ⩽2 bone metastases at baseline	–	–	–	1.734	1.280–2.348	–
Interaction between baseline corrected uNTx level (>50 versus ⩽ 50 nmol/mmol) and number of bone metastases (>2 versus ⩽ 2)	1.614	0.973–2.676	0.0638	–	–	–
Lesion type						
Osteolytic ($n = 380$)	1.040	0.698–1.552	0.8458	0.881	0.554–1.401	0.5922
Osteoblastic ($n = 1023$)	1.994	1.527–2.605	<0.0001	1.197	0.870–1.647	0.2697
Mixed ($n = 537$)	1.299	0.928–1.818	0.1268	0.952	0.638–1.421	0.8105
Not seen ($n = 901$)						

BSAP, bone-specific alkaline phosphatase; CI, confidence interval; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer; uNTx, urinary N-telopeptide of type I collagen.
[a] Univariate covariate analysis stratified by study. Only baseline characteristics with $P < 0.05$ from the univariate analyses were included in the multivariate model.

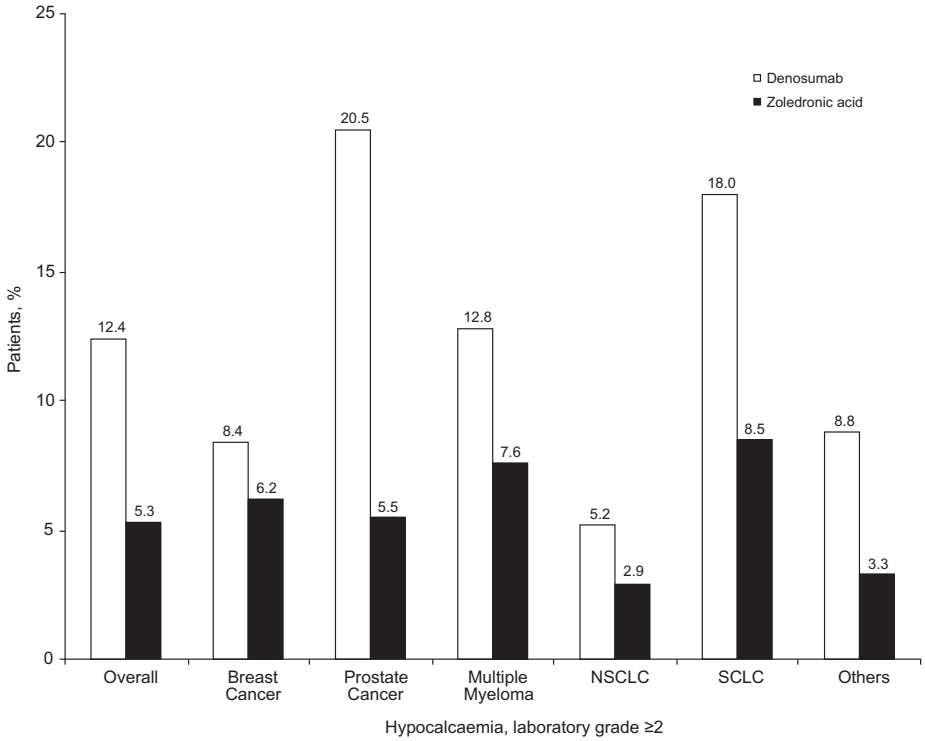

Fig. 2. Incidence of laboratory grade ⩾2 hypocalcaemia by tumour type. NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

of grade ⩾2 hypocalcaemia, including male sex, prostate cancer or SCLC, reduced creatinine clearance, higher baseline values of uNTx and BSAP, >2 bone metastases at baseline, and osteoblastic lesions. In the multivariate analysis, risk factors associated with hypocalcaemia included prostate cancer or SCLC, reduced creatinine clearance and higher baseline values of uNTx and BSAP.

Denosumab and ZA inhibit bone resorption by distinct mechanisms, and preclinical and clinical data indicate RANKL inhibitors are more potent at reducing bone turnover and destruction and the associated release of skeletal calcium than intravenous bisphosphonates [16,17]. Importantly, the identified risk factors for hypocalcaemia with denosumab were similar to those previously ascribed to potent bisphosphonates, such as vitamin D deficiency, renal insufficiency and prostate cancer [18–20]. In our analysis, calcium and/or vitamin D supplementation at any time during denosumab therapy significantly lowered the risk of AEs of hypocalcaemia.

Previous analyses of hypocalcaemia in cancer patients treated with denosumab have relied primarily on clinical AE reports, which only include symptomatic hypocalcaemia events [21,22]. Results from a meta-analysis of data from seven randomised controlled trials demonstrated a higher risk of AEs of hypocalcaemia among denosumab versus control groups [23]. Our analysis included laboratory events of hypocalcaemia using patient-level data from three identically designed trials, including non-symptomatic events.

Osteoclast inhibition rarely results in clinically important declines in blood calcium in healthy individuals [24]. Clinically meaningful hypocalcaemia during antiresorptive therapy is typically associated with inadequate vitamin D intake; insufficient calcium intake; impaired renal function; hypoparathyroidism; or extensive osteoid due to high bone turnover, osteomalacia, rapid skeletal growth, or Paget disease [25–28]. In our analysis, denosumab-treated patients who developed hypocalcaemia had higher baseline uNTx levels and median baseline BSAP levels twice as high as those who did not. Elevated BSAP levels may indicate potential calcium deposition in osteoid and undermineralised bone matrix, a phenomenon that can persist for weeks or months after osteoclast inhibition. These results suggest that patients with high bone turnover may be more susceptible to hypocalcaemia when osteoclasts are inhibited, particularly when calcium and vitamin D intake is insufficient. This observation corroborates the importance of calcium and vitamin D intake in compensating for antiresorptive-mediated reductions in bone resorption.

Hypocalcaemia incidence with denosumab was lower in patients with normal baseline creatinine clearance levels, consistent with the kidney's role in compensating for decreased skeletal calcium mobilisation. Osteoclast inhibition via denosumab typically leads to increased parathyroid hormone (PTH) release [29], and functionally impaired kidneys may be less effective at producing active vitamin D ($1,25(OH)_2D_3$; calcitriol) in response to this PTH signal. Serum calcitriol was not measured

in these studies, but impaired renal production of calcitriol could diminish intestinal calcium absorption, leading to increased risk of hypocalcaemia with antiresorptive therapy. In this analysis, treatment with ZA in patients with the lowest levels of creatinine clearance had a similar subject incidence of hypocalcaemia compared with those with normal creatinine clearance. This could be explained by the downward dose adjustment of ZA in patients with impaired renal function (in 18% of patients) [5], which may have mitigated hypocalcaemia incidence.

Our findings suggest a potentially important contribution of osteoid as a risk factor for hypocalcaemia. Hypocalcaemia was most common in patients with prostate cancer, in whom skeletal metastases often produce newly formed bone and mineralising osteoid, which can continue to absorb extracellular calcium after osteoclastic activity is inhibited. Recent preclinical data indicate that calcium absorption by osteoid plays an important role in serum calcium reductions after osteoclasts are inhibited by denosumab [30]. Furthermore, across all tumour types, the risk of hypocalcaemia was greatest during the first 6 months after initiating denosumab. Denosumab maximally decreases bone resorption markers within 2 weeks, whereas bone formation markers reach maximal reductions after 3–6 months [21,31,32]. Thus, although denosumab rapidly decreases skeletal calcium release, the newly formed undermineralised matrix may continue to absorb extracellular calcium for several weeks or months [31]. The rate of occurrence of hypocalcaemia in the current study was lower and generally stable beyond 6 months after initiating denosumab, consistent with the predicted closure and mineralisation of the remodeling space [33].

It is unlikely that hypocalcaemia in clinical practice persists for as long as suggested in our analysis [34]. Frequent measurements used in clinical practice to monitor for resolution of hypocalcaemia were not required or reported in these trials; thus, resolution may have occurred between study visits but was not detected until the next study-directed visit.

In summary, hypocalcaemia is a known risk associated with antiresorptive therapies, including denosumab 120 mg. It is important to correct hypocalcaemia, or any potential vitamin D deficiency, before initiating denosumab or another antiresorptive agent, particularly in patients exhibiting factors associated with increased risk (prostate cancer or SCLC, reduced creatinine clearance and higher baseline values of uNTx and BSAP). Hypocalcaemia associated with antiresorptive therapies may be prevented by counseling patients on the importance of adequate calcium and vitamin D intake, advising them of the pertinent symptoms of hypocalcaemia, and monitoring of serum calcium levels, particularly within the first weeks, during therapy. Hypocalcaemia occurring in this setting should be appropriately managed, including administration of intravenous calcium if required. With proactive and careful monitoring, hypocalcaemia is preventable and effectively manageable when manifested.

Conflict of interest statement

J-JB has served as a consultant for and received research funding from Amgen and has received honoraria from Amgen and Novartis. AL has received honoraria and research funding from Amgen. DHH has served as a consultant for and has received research funding and honoraria from Amgen, Ortho Biotech, and Watson. AS has served as a consultant for Amgen and has received honoraria from Amgen and GlaxoSmithKline and research funding from Amgen and Novartis. KF has served as a consultant for Amgen and Novartis and has received honoraria from Amgen. HGB has served as a consultant for and received honoraria and research funding from Amgen and Novartis. FS has served as a consultant for and received research funding and honoraria from Amgen and Novartis. CVP has received research funding from Amgen and Novartis. RdB has served as a consultant for and received honoraria from Novartis and has received research funding and other remuneration from Amgen and Novartis. NS has served as a consultant for Amgen, Astellas, Bayer, Dendreon, Janssen, and Medivation. TI has served as a consultant for and received honoraria from Amgen, Ipsen, and Novartis. TT has served as a consultant for and received honoraria from Daiichi-Sankyo. RD has no conflicts to disclose. PJK has served as a consultant for Grunenthal. HW is an employee of and owns stock or stock options in Amgen Inc. OLB, AB, AJS, and PJK are former employees of Amgen Inc.

Acknowledgements

This study was supported by Amgen Inc. Employees of Amgen Inc. were involved in the study design and the collection, analysis and interpretation of data. Michele Vivirito, Rick Davis, MS, and Amy Foreman-Wykert, PhD, were supported by Amgen Inc. and assisted the authors with manuscript drafting, editing and formatting.

References

[1] Body JJ. Denosumab for the management of bone disease in patients with solid tumors. Expert Rev Anticancer Ther 2012;12:307–22.
[2] Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat Rev 2001;27:165–76.
[3] Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. Clin Cancer Res 2006;12:6243s–9s.

[4] Jodrell DI, Iveson TJ, Smith IE. Symptomatic hypocalcaemia after treatment with high-dose aminohydroxypropylidene diphosphonate. Lancet 1987;1:622.
[5] Lipton A, Fizazi K, Stopeck AT, et al. Superiority of denosumab to zoledronic acid for prevention of skeletal-related events: a combined analysis of 3 pivotal, randomised, phase 3 trials. Eur J Cancer 2012;48:3082–92.
[6] Gartrell BA, Coleman RE, Fizazi K, et al. Toxicities following treatment with bisphosphonates and receptor activator of nuclear factor-kappaB ligand inhibitors in patients with advanced prostate cancer. Eur Urol 2014;65:278–86.
[7] Smith MR, Saad F, Oudard S, et al. Denosumab and bone metastasis-free survival in men with nonmetastatic castration-resistant prostate cancer: exploratory analyses by baseline prostate-specific antigen doubling time. J Clin Oncol 2013;31:3800–6.
[8] Gulley JL, Wu S, Arlen PM, Dahut WL. Persistent hypocalcemia induced by zoledronic acid in a patient with androgen-independent prostate cancer and extensive bone metastases. Clin Genitourin Cancer 2007;5:403–5.
[9] Champallou C, Basuyau JP, Veyret C, et al. Hypocalcemia following pamidronate administration for bone metastases of solid tumor: three clinical case reports. J Pain Symptom Manage 2003;25:185–90.
[10] Riches PL, McRorie E, Fraser WD, Determann C, van't Hof R, Ralston SH. Osteoporosis associated with neutralizing autoantibodies against osteoprotegerin. N Engl J Med 2009;361:1459–65.
[11] Hanamura M, Iwamoto T, Soga N, Sugimura Y, Okuda M. Risk factors contributing to the development of hypocalcemia after zoledronic acid administration in patients with bone metastases of solid tumor. Biol Pharm Bull 2010;33:721–4.
[12] Stopeck AT, Lipton A, Body JJ, et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol 2010;28:5132–9.
[13] Fizazi K, Carducci M, Smith M, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. Lancet 2011;377:813–22.
[14] Henry DH, Costa L, Goldwasser F, et al. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. J Clin Oncol 2011;29:1125–32.
[15] US Department of Health and Human Services. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). <http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf>; 2006 [accessed 06.06.14].
[16] Morony S, Warmington K, Adamu S, et al. The inhibition of RANKL causes greater suppression of bone resorption and hypercalcemia compared with bisphosphonates in two models of humoral hypercalcemia of malignancy. Endocrinology 2005;146:3235–43.
[17] Body JJ, Lipton A, Gralow J, et al. Effects of denosumab in patients with bone metastases with and without previous bisphosphonate exposure. J Bone Miner Res 2010;25:440–6.
[18] Liamis G, Milionis HJ, Elisaf M. A review of drug-induced hypocalcemia. J Bone Miner Metab 2009;27:635–42.
[19] Recker RR, Lewiecki EM, Miller PD, Reiffel J. Safety of bisphosphonates in the treatment of osteoporosis. Am J Med 2009;122:S22–32.
[20] Zuradelli M, Masci G, Biancofiore G, et al. High incidence of hypocalcemia and serum creatinine increase in patients with bone metastases treated with zoledronic acid. Oncologist 2009;14:548–56.
[21] Lipton A, Steger GG, Figueroa J, et al. Randomized active-controlled phase II study of denosumab efficacy and safety in patients with breast cancer-related bone metastases. J Clin Oncol 2007;25:4431–7.
[22] Fizazi K, Bosserman L, Gao G, Skacel T, Markus R. Denosumab treatment of prostate cancer with bone metastases and increased urine N-telopeptide levels after therapy with intravenous bisphosphonates: results of a randomized phase II trial. J Urol 2009;182:509–15 [discussion 15–6].
[23] Qi WX, Lin F, He AN, Tang LN, Shen Z, Yao Y. Incidence and risk of denosumab-related hypocalcemia in cancer patients: a systematic review and pooled analysis of randomized controlled studies. Curr Med Res Opin 2013;29:1067–73.
[24] Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR. The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res 2001;16:348–60.
[25] Maalouf NM, Heller HJ, Odvina CV, Kim PJ, Sakhaee K. Bisphosphonate-induced hypocalcemia: report of 3 cases and review of literature. Endocr Pract 2006;12:48–53.
[26] McCormick BB, Davis J, Burns KD. Severe hypocalcemia following denosumab injection in a hemodialysis patient. Am J Kidney Dis 2012;60:626–8.
[27] Rosen CJ, Brown S. Severe hypocalcemia after intravenous bisphosphonate therapy in occult vitamin D deficiency. N Engl J Med 2003;348:1503–4.
[28] Whitson HE, Lobaugh B, Lyles KW. Severe hypocalcemia following bisphosphonate treatment in a patient with Paget's disease of bone. Bone 2006;39:954–8.
[29] Block GA, Bone HG, Fang L, Lee E, Padhi D. A single-dose study of denosumab in patients with various degrees of renal impairment. J Bone Miner Res 2012;27:1471–9.
[30] Kostenuik PJ, Smith SY, Samadfam R, Jolette J, Zhou L, Ominsky MS. Effects of denosumab, alendronate, or denosumab following alendronate on bone turnover, calcium homeostasis, bone mass and bone strength in ovariectomized cynomolgus monkeys. J Bone Miner Res 2015;30:657–69.
[31] Frost HM. Some ABCs of skeletal pathophysiology. III: Bone balance and the delta B.BMU. Calcif Tissue Int 1989;45:131–3.
[32] Kostenuik PJ, Smith SY, Jolette J, Schroeder J, Pyrah I, Ominsky MS. Decreased bone remodeling and porosity are associated with improved bone strength in ovariectomized cynomolgus monkeys treated with denosumab, a fully human RANKL antibody. Bone 2011;49:151–61.
[33] Gallagher JC, Jerpbak CM, Jee WS, Johnson KA, DeLuca HF, Riggs BL. 1,25-Dihydroxyvitamin D3: short- and long-term effects on bone and calcium metabolism in patients with postmenopausal osteoporosis. Proc Natl Acad Sci USA 1982;79:3325–9.
[34] Body JJ, Bouillon R. Emergencies of calcium homeostasis. Rev Endocr Metab Disord 2003;4:167–75.