

Bone remodeling associated with CTLA-4 inhibition: an unreported side effect

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Background Ipilimumab is a recombinant IgG1 kappa antibody against human cytotoxic T-lymphocyte antigen 4 (CTLA-4) that augments T-cell activation. It has been approved for treating unresectable or metastatic melanoma. Preclinical investigations have recently shown that CTLA-4 inhibition can cause cytokine-mediated increase in bone remodeling.

Objective To examine whether the use of ipilimumab results in increased bone remodeling that manifests as an autoimmune reaction.

Methods A retrospective case-control study of 51 patients with stage III/IV melanoma was conducted with 2 cohorts: ipilimumab cohort at the standard dose (n = 39) and chemotherapy cohort receiving an investigational chemotherapy regimen (n = 12). Outcome variables were recorded at baseline, with each treatment, and upon treatment completion. The differences in trends of bone pain and mean alkaline phosphatase (ALP) levels were assessed using a generalized linear mixed-effect model.

Results 14 of 39 patients (35.9%) in the ipilimumab cohort reported having bone pain during at least 1 of the treatment cycles, compared with 3 of 12 patients (25%) in the chemotherapy cohort, and that trend was statistically significant over time ($P = .023$). Trends of mean ALP levels between the 2 cohorts were not statistically significant ($P = .653$). There was no correlation between bone pain or mean ALP level and response to ipilimumab.

Limitations The patient-reported bone pain was dichotomized; there was a significant variability in perceived pain intensity among patients.

Conclusions Given the increasing use of CTLA-4 inhibitors in the treatment of various malignancies, physicians should be alerted to their bone remodeling effects so that they can appropriately manage the short-term side effects such as bone pain, and monitor patients for possible unknown long-term side effects. In addition, data from postmarketing studies should be evaluated for confirmation of this novel interaction.

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is an important component of the immune checkpoint pathway. CTLA-4 inhibition causes T-cell activation and proliferation, increases T-cell responsiveness, and enhances the anti-tumor immune response. CTLA-4 inhibition also results in immune-related adverse reactions such as colitis, hepatitis, and endocrinopathies. Preclinical investigations have recently shown that CTLA-4 inhibition can cause cytokine-mediated increase in bone remodeling.^{1,2(p4)} Ipilimumab, a recombinant IgG1 kappa antibody against human CTLA-4, has been approved for use in unresectable or metastatic melanoma. We hypothesize that ipilimumab results in increase in bone remodeling manifesting as an autoimmune reaction.

Methods

We conducted a retrospective case-control study of patients with stage III/IV melanoma treated at the

University of New Mexico Comprehensive Cancer Center during April 2009-July 2014. The university's Institutional Review Board approved the study.

Two cohorts were compared: an ipilimumab cohort receiving ipilimumab at 3 mg/kg every 3 weeks, and a chemotherapy cohort receiving an investigational chemotherapy regimen: carboplatin IV at an area under curve of 5 on day 1, paclitaxel IV at 175 mg/m² on day 1, and temozolomide orally at 125 mg/m² daily on days 2 to 6 every 21 days. Patients receiving at least 1 cycle of treatment were included. Those with known hepatic disease or concurrent malignancy were excluded from the study.

Serum ALP level (normal range, 38-150 international units per liter [IU/L]) and patient-reported bone pain measured by the 11-point numeric rating scale (NRS) for pain assessment were recorded before treatment initiation, on each cycle, and upon treatment completion.³ Clinical response was assessed per RECIST guidelines.⁴ Bone pain was

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dichotomized into Absent (pain intensity of 0 on the NRS, meaning no pain) or Present (pain intensity of 1-10 on the NRS, with 1 = mild pain and 10 = worst imaginable pain). Patients with a complete or partial response to the therapy were categorized as responders, and those with progressive or stable disease were categorized as nonresponders.

Descriptive statistics were generated for demographic and clinical characteristics. The primary outcome variables of interest were bone pain and mean ALP levels. Generalized linear mixed-effect models for proportion of patients with bone pain (with logit link function) and mean ALP levels (with identify link function) were used to evaluate for a difference in trends between the two cohorts over time. We used the Kenward-Roger approach to adjust for the small size of the degrees of freedom. To assess the significance of difference of the proportions of patients with bone pain and the mean ALP levels between responders and nonresponders in the ipilimumab cohort, the Fisher exact test and Wilcoxon rank-sum test were used, respectively. Statistical analyses were performed with statistical packages R (v3.1.3) and SAS (v9.4).

Results

A total of 281 patients were screened, and 51 met the inclusion criteria (39 in the ipilimumab and 12 in chemotherapy cohorts). Baseline parameters were well matched between the cohorts (Table). Of the 39 patients in the ipilimumab cohort, 14 (35.9%) had bone pain during at least one of the treatment cycles, compared with 3 of the 12 patients (25%) in the chemotherapy cohort. At baseline, 4 of 38 ipilimumab patients (10.5%; 95% confidence interval [CI], 2.9-24.8) and 2 of 12 chemotherapy patients (16.7%; 95% CI, 2.1-48.4) had bone pain. Upon treatment completion, 9 of 33 ipilimumab patients (27.3%; 95% CI, 13.3-45.5) and 0 of 12 chemotherapy patients (0%; 95% CI, 0-26.5) had bone pain. The trend of proportion of patients with bone pain over time was statistically significant between the two cohorts ($P = .023$, Figure 1). The trends of proportion of patients with bone pain were not statistically significant when stratified by the presence of bone metastasis at inclusion in the study ($P = .418$) or disease progression at treatment completion ($P = .500$).

At baseline, the mean ALP level was 89.39 IU/L (95% CI, 81.03-97.75) in the ipilimumab cohort and 114.33 IU/L (95% CI, 69.48-159.19) in the chemotherapy cohort. Upon treatment completion, the mean ALP level was 123.09 IU/L (95% C.I. 80.78-165.41) in the ipilimumab cohort and 124.24 IU/L (95% C.I. 90.88-157.62) in the chemotherapy cohort. The trend of mean ALP level over time was not statistically significant between the 2 cohorts ($P = .653$, Figure 2). There was no statistically significant difference in bone pain ($P = .236$) or mean ALP levels ($P = .196$) between responders and nonresponders in the ipilimumab cohort.

Discussion

Immune checkpoints are inhibitory pathways that are critical for maintenance of self-tolerance and regulation of appropriate immune response. CTLA-4 is present exclusively on T cells and interacts with its ligands B7.1 and B7.2. CTLA-4 competes with CD28 in binding with B7, leading to dampening of T-cell activation and function.^{5,6} Development of checkpoint inhibitors such as ipilimumab have heralded a new era of immune targeted therapies for various malignancies including malignant melanoma.

Bone remodeling involves 4 distinct but overlapping phases. The first phase involves detection of loss of bone continuity by osteocytes and activation of osteoclast precursors derived from progenitors of the monocyte-macrophage lineage. The second phase involves

TABLE Baseline characteristics of patients in the study

Characteristic	Cohort	
	Ipilimumab (n = 39)	Chemotherapy (n = 12)
Mean age, y (range)	55.8 (19-89)	62.2 (39-81)
Sex, n (%)		
Men	26 (66.7)	8 (66.7)
Women	13 (33.3)	4 (33.3)
Race, n (%) ^a		
White [Hispanic whites]	37 [0] (94.9)	10 [2] (83.3)
Others	1 (2.6)	2 (16.7)
Did not disclose	1 (2.6)	0 (0)
AJCC TNM stage, n (%)		
III	9 (23.08)	2 (16.67)
IV (%)	30 (76.92)	10 (83.33)
Documented metastasis at inclusion in the study, n (%)		
Bone	13 (33.3)	4 (33.3)
Liver	9 (23.1)	4 (33.3)
Other visceral sites	29 (74.4)	9 (75)
Clinical responses, n (%)		
For AJCC TNM stage III		
Complete response	7 (77.78)	1 (50)
Partial response	0 (0)	1 (50)
Stable disease	0 (0)	0 (0)
Progression of disease	2 (22.22)	0 (0)
For AJCC TNM stage IV		
Complete response	2 (5.13)	0 (0)
Partial response	7 (17.95)	1 (8.33)
Stable disease	6 (15.38)	2 (16.67)
Progression of disease	15 (38.46)	7 (58.33)

AJCC, American Joint Committee on Cancer; TNM, TNM staging system, where T = primary tumor, N = node involvement, M = distant metastasis

osteoclast-mediated bone resorption and concurrent recruitment of mesenchymal stem cells and osteoprogenitors. The third phase involves osteoblast differentiation and osteoid synthesis, and the fourth phase results in mineralization of osteoid and termination of bone remodeling.^{7,8}

The role of T-lymphocytes and cytokines, such as IL-1

and TNF- α , and receptor activator of NF- κ B ligand (RANK-L) in osteoclastogenesis is well studied. RANK-L is considered to be the final downstream effector of this process.⁹ T-lymphocytes have also been shown to promote osteoblast maturation and function.^{9,10} These findings suggest a significant interaction between immune system activation and bone remodeling.

The search for a reliable biomarker for immune therapy is ongoing. Although ipilimumab-associated immune-related adverse events have been suggested to predict response to therapy,¹¹ there is considerable debate on the subject. Ipilimumab's impact on bone remodeling could offer a solution.

In the current study, there was a statistically significant difference in proportion of patients with bone pain in the 2 cohorts. This was preserved with stratification based on bone metastasis at inclusion and disease progression on treatment completion making new or worsening skeletal metastasis. Furthermore, the proportion of patients with bone pain increased with each cycle for ipilimumab cohort. However, we were unable to detect an association between bone pain and response to ipilimumab.

We were not able to detect a difference in trend of mean ALP level with treatment in the two cohorts. Although it is possible that no such association exists, we believe our study was not powered to detect it. Finally, we were not able to study markers for osteoblast (bone-specific ALP) and osteoclasts (N- and C-telopeptides of type 1 collagen, deoxypyridinoline, etc) to better assess this interaction because they are not commonly clinically used.

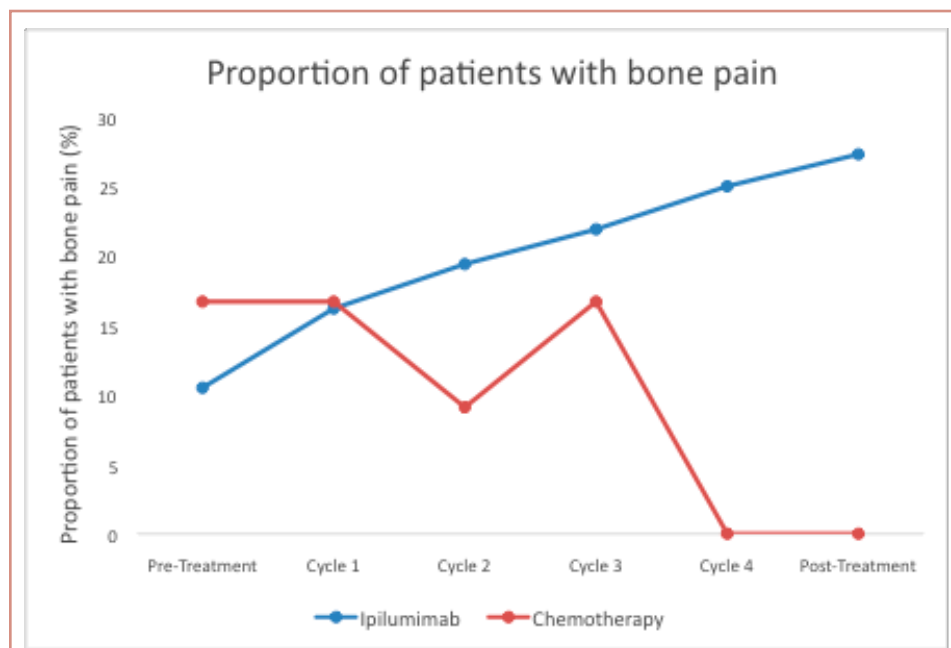


FIGURE 1 The trend of the proportion of patients with bone pain before, during, and after treatment.

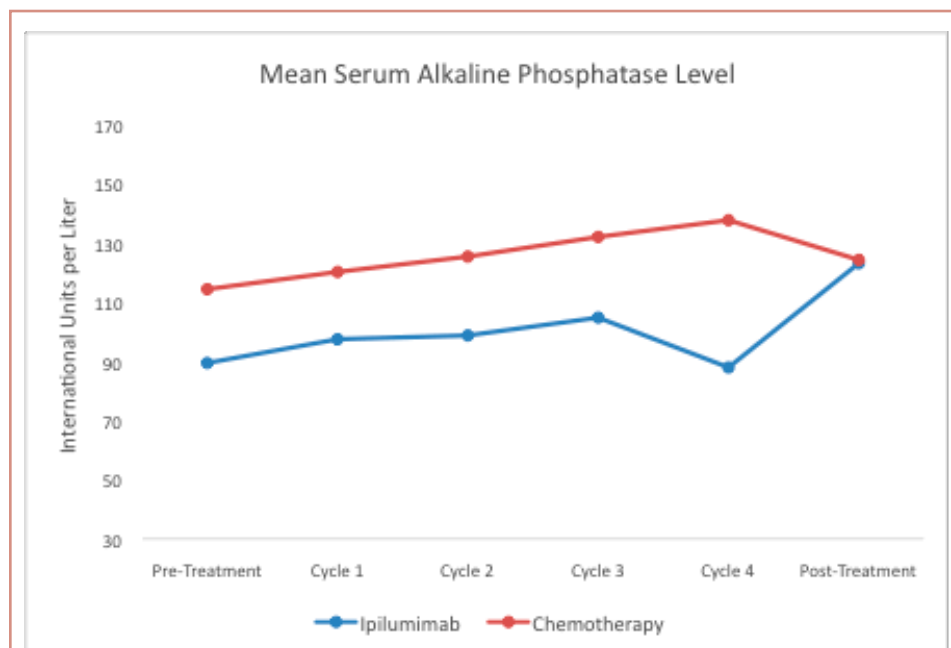


FIGURE 2 The trend of the mean serum alkaline phosphatase levels before, during, and after treatment (dotted lines denote the lower and upper limits of normal).

Regarding the limitations of our study, we chose to dichotomize the patient-reported bone pain because it is a subjective measure and there is a significant variability of the perceived pain intensity among patients. We also excluded patients with hepatitis from receiving the ipilimumab therapy and those with known hepatic disease from the study to reduce the impact of hepatic ALP on total

serum ALP levels.

In conclusion, as far as we know, this is the first clinical report suggesting a possible relationship between CTLA-4 inhibition and bone remodeling. Supported by a strong pre-clinical rationale, this side effect remains under-studied and under-recognized by clinicians. A prospective assessment of this interaction using bone specific markers is planned.

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