



## OPEN Changes in scanographic bone mineral density in melanoma patients treated with immunotherapy: a new irAE from real-life data

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**Background** The use of immune checkpoint inhibitors (ICIs) is increasingly important in melanoma management. While ICIs are associated with immune-related adverse events (irAEs), little is known about skeleton irAEs and they are felt to be poorly described.

**Methods** We conducted a real-life, retrospective cohort study to monitor bone mineral density (BMD) evolution over 2 years using routine Computed Tomography (CT) in melanoma patients treated with ICIs and to identify associated factors. This single-center study included 165 patients (mean age: 65 years; 44.2% women) treated with ICIs between 2014 and 2023. BMD was measured at baseline (T0), 1 year (T1), and 2 years (T2) on L1 vertebrae. Vertebral fractures were assessed on sagittal slices. Paired t-tests compared BMD values at the different time points, and we analyzed risk factors for BMD changes with regression models.

**Results** BMD significantly decreased over 2 years (mean difference: 14.02 Hounsfield Units (HU), 95% CI 10.31–17.74,  $p < 0.001$ ), with bone loss rates of 5.15% and 11.91% at 1 and 2 years, respectively. Male sex ( $\beta$  8.25,  $p = 0.002$ ) and younger age ( $\beta$  -0.34,  $p = 0.001$ ) were linked to greater BMD decline at 1 year. Disease progression or partial response correlated with greater reductions at 2 years.

**Multivariable analysis confirmed male sex as an independent risk factor for BMD loss.**

**Conclusion** ICIs are associated with significant BMD loss, particularly in men. These findings emphasize the importance of osteoporosis prevention and routine BMD monitoring during ICI therapy.

**Keywords** Osteoporosis, Vertebral fracture, Bone mineral density, Melanoma, Immunotherapy, Immune-related adverse events, Immune-checkpoint inhibitors, ROI

Melanoma represents 3% of all cancers<sup>1</sup> and was an experimental model for testing the benefits of Immune checkpoint inhibitors (ICIs), which are now widely used in organ cancers. ICIs, including anti-programmed cell death-1 (PD-1), its ligand (PD-L1) and anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4), enable restoration of the immune system in the tumor environment<sup>2</sup>, has revolutionized the management of melanoma patients, establishing them as the gold standard treatment in melanoma management<sup>3</sup>. The pathophysiological mechanism consists in restoring immunity by lymphocyte engagement with antigen-presenting cells and tumor cells<sup>4</sup>, thereby limiting cancer cells from escaping immune detection and enabling their destruction<sup>5</sup>. Engagement of PD-1 by PD-L1 leads to inhibition of lymphocyte proliferation and T-cell receptor-mediated cytokine secretion<sup>6</sup>.

By increasing cytotoxic T lymphocyte activity, immune checkpoint inhibitors (ICIs) cause toxicity know as Immune Related Adverse Events (irAEs) affecting various organs through distinct pathophysiological mechanisms<sup>7</sup>. IrAEs typically arise within 40 days but can persist or emerge long after treatment cessation, with late-onset cases being less documented due to limited follow-up<sup>8</sup>. As melanoma survival improves with ICIs, the

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number of treated patients increases, along with the incidence of delayed toxicities, including bone effects. The incidence of all-grade adverse events is approximately 58% with CTLA-4 inhibitors<sup>9</sup> and around 35% with PD-1 inhibition<sup>10,11</sup>. Mostly grade 1–2 per the Common Terminology Criteria for Adverse Events (CTCAE). However, around 10% are severe (grade 3–4) (13,14). A review by Lemiale et al.<sup>12</sup> report that 80% of patients on anti-PD-1 will experience irAEs.

Rheumatological irAEs mainly concern arthritis, PPR like and myositis. However, it is widely accepted that the pro-inflammatory state engendered by immune system activation promotes bone resorption<sup>13,14</sup>. Vertebral fractures are the most frequently reported bone irAEs under anti PD-1/PD-L1 and anti CTLA-4 therapy in the literature, raising the hypothesis of a possible bone mineral density-lowering effect of ICIs<sup>15–17</sup>. However, not all studies use objective criteria such as imaging to distinguish osteoporotic from malignant fractures.

Given the socio-economic impact of osteoporosis, there is growing interest in opportunistic screening using bone mineral density (BMD) measurement by CT, i.e., no change in the paramedical monitoring of melanoma established by the guidelines<sup>18</sup>. Several studies have demonstrated the correlation between Hounsfield unit and T-score, thus reinforcing its usefulness in clinical practice<sup>19–23</sup>, as well as the absence of volumetric BMD or microarchitectural alterations in healthy vertebrae, even in the presence of bone metastases<sup>24,25</sup>.

The primary objective of this study is to measure the scanographic evolution of BMD in melanoma patients treated with ICI at 1 and 2 years. We also aimed to describe the number and characteristics of incident vertebral fractures and factors associated with evolution. Our hypothesis is that a decrease in scanographic bone mineral density in patients undergoing immunotherapy may represent a potential adverse effect for which prevention would be essential.

## Material and methods

### Patients' recruitment

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by CLERS, the Local Research Ethics Committee for Health (ethics approval number: ID 4977). Analyses performed are available requesting corresponding author.

We performed a retrospective cohort analysis on patients with resected stage III-IV melanoma who received adjuvant treatment over periods ranging from October 2014 to December 2023 from the dermatology department's day care hospital of Caen University Hospital. Selection of cases is summarized in Fig. 1, according ESMO-GROW guidelines<sup>26</sup> and characteristics of patients are given in Table 1.

Patients who met the following criteria were included in the study: (1) Male and female aged 18 years and older, (2) Histologically diagnosed melanoma stage III-IV, (3) Treatment with ICIs for the first time with CT follow-up before and after ICI initiation. All included patients had a minimum follow-up of 1 year. Patients who met any of the following criteria were excluded from the study: (1) History of fragility bone disease or osteoporotic fracture, (2) Absence of initial imaging, (3) Identification of an osteoporotic fracture on the baseline CT without a documented history of osteoporosis, (4) Prior lumbar surgery, presence of lumbar hardware, or heterogeneous vertebral bone structure (5) Presence of lumbar spine bone metastases, either at baseline or emerging during follow-up of the 526 patients initially identified in the database, 165 were included in this study after applying the specified inclusion and exclusion criteria.

### Clinical data

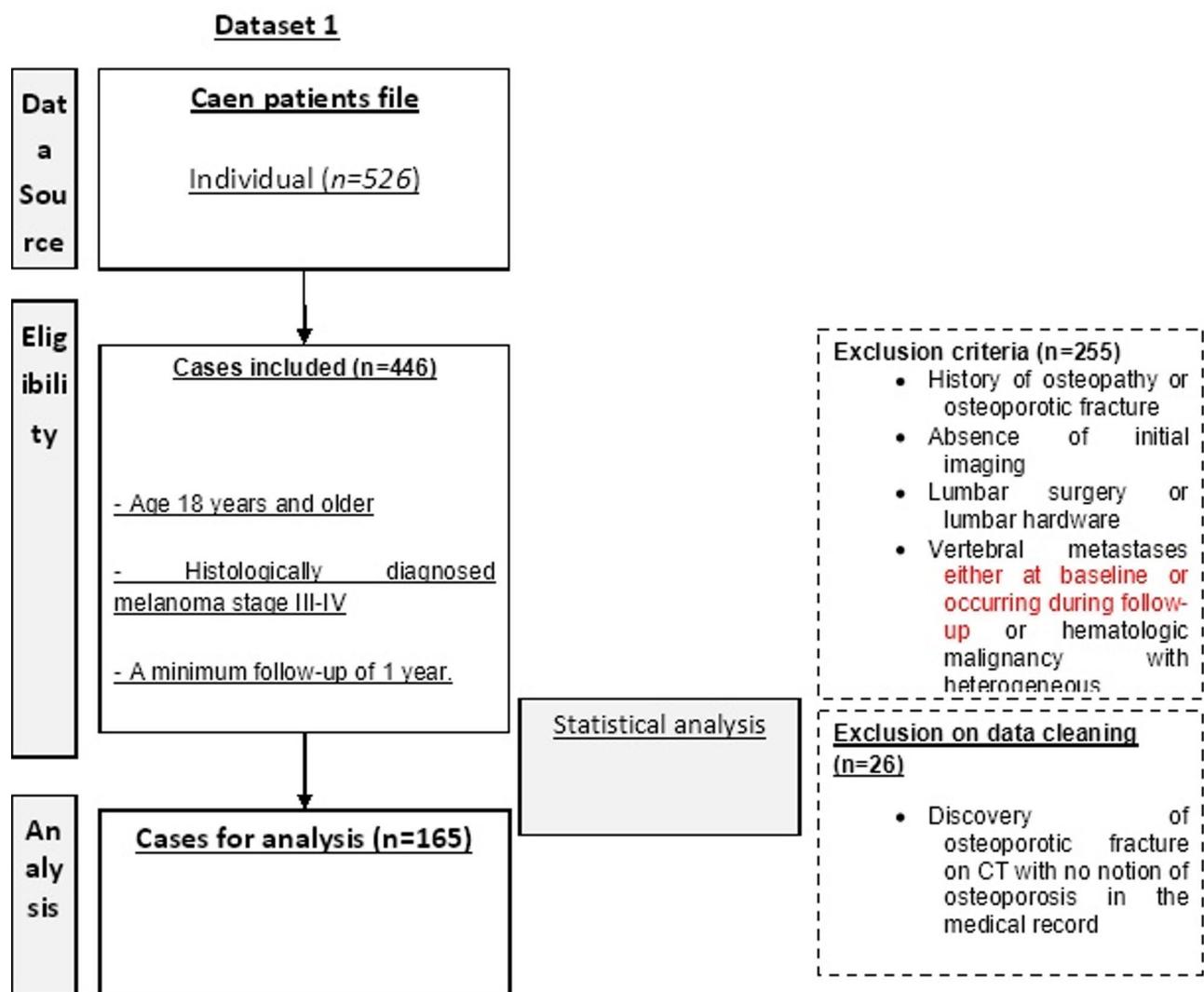
We gathered all data of melanomas and characteristics of patients i.e., demographic data (sex, age), data on primary melanoma (Breslow, date of diagnosis, stage, *BRAF* status), treatment regimens and outcomes (RESIST 1.1 criteria (Response Evaluation Criteria in Solid Tumors) defining a BOR (Best Overall Response) and classifying patients as partial response (PR), progression disease (PD), stable disease (SD) or complete response (CR)).

Risk factors for osteoporosis were collected i.e., body mass index (BMI), diabetes, chronic renal failure (GFR < 60 ml/min), smoking and alcohol consumption, prior use of chemotherapy, use of drugs such proton pump inhibitors (PPIs). Corticosteroid prescriptions (> 7.5 mg for more than 3 months) were also reviewed and were exclusively related to the management of immune-related adverse events (irAEs) induced by immunotherapy. No other indication for corticosteroid use was reported in our cohort. Finally, the use of treatments interfering with bone metabolism, such as zoledronic acid, was recorded and was solely related to the presence of bone metastases.

### Imaging protocol

BMD measurements were independently performed by two rheumatologists, each blinded to both patient clinical data and the other's assessments, following prior training by a radiologist specialized in musculoskeletal imaging. Measurements were taken at the lumbar spine of L1 vertebra on the last CT prior to the initiation of immune checkpoint inhibitors (ICIs) (T0), followed by new measurements at 1-year (T1) and 2-year (T2) intervals.

CT images were analyzed on a standard radiology picture archiving and communication system workstation. An Elliptical Region of Interest (ROI), referring to a predefined area selected for quantitative analysis, of approximately 300 mm<sup>2</sup> were manually put in the central and anterior portion of the L1 vertebral body on axial slices avoiding the cortical bone, venous plexuses, and any heterogeneities. Details for the measurement and interpretation of BMD are consistent with literature<sup>19</sup>. The final record included the HU values, calculated as the mean of both measurements.



**Fig. 1.** Flowchart, according to ESMO-GROW flowchart for real-world evidence studies in oncology.

### Incident fractures

Incident vertebral fractures were assessed on sagittal slices using the semi-quantitative Genant method, which evaluates fracture morphology and severity, graded from 0 to 3<sup>27</sup>. Only moderate (grade 2: 25–40% height loss) and severe (grade 3: >40% height loss) fractures were included in the analysis.

### Threshold of 110 UH

A threshold of 110 HU was used to define osteoporosis<sup>19</sup>. Patients with BMD > 110 HU were divided into two groups: those whose BMD remained above the threshold and those who developed osteoporosis (BMD < 110 HU), to allow for a comparison of their characteristics. A total of 47 patients were excluded from analysis as their BMD was already below 110 HU at baseline.

### Statistical analysis

Inter-operator variability in BMD measurements was assessed using the Intraclass Correlation Coefficient (ICC) and represented in scatter and Bland–Altman plots (Supplementary Fig. 1). ICC values were reported between 0 (no concordance) and 1 (perfect concordance).

Qualitative data are shown as counts and percentages, and quantitative data as mean  $\pm$  standard deviation. Scanographic BMD change over time was assessed using Paired Student's t-tests. Linear regression, with both bivariate and multivariable analysis, explored associations between melanoma characteristics, osteoporosis risk factors and BMD variation in Hounsfield units (HU) between T0-T1 and T0-T2 (positive values indicate density loss). The multivariate model included immunotherapy type and variables with  $p < 0.1$  associated with BMD variation. Model selection (one per immunotherapy) was driven with a bidirectional stepwise selection of variables based on Akaike Information Criterion (AIC). A logistic regression model was performed to compare patients whose BMD remained above 110 HU ( $n=42$ ) with those whose BMD decreased below this threshold ( $n=21$ ). Following the primary statistical analysis, a post-hoc analysis was conducted on patients lost to follow-

|                        |                   | N = 165      |
|------------------------|-------------------|--------------|
| Age (mean ± SD)        |                   | 65 (12.5)    |
| Sex (%)                | Female            | 73 (44.2)    |
| BMI (mean ± SD)        |                   | 26.90 (4.74) |
| Type (%)               | SSM               | 90 (64.3)    |
|                        | MN                | 36 (25.7)    |
|                        | ALM               | 8 (5.7)      |
|                        | MLM               | 4 (2.9)      |
|                        | LMM               | 2 (1.4)      |
| BRAF (%) *             |                   | 54 (33.5)    |
| Breslow (mean ± SD) ** |                   | 4.58 (6.89)  |
| Stage J0 (%)           | 3                 | 26 (15.8)    |
|                        | 4                 | 139 (84.2)   |
| Smoking status (%) *** | 0                 | 96 (63.6)    |
|                        | 1                 | 55 (36.4)    |
| Alcohol use (%) ****   | Yes               | 17 (11.7)    |
| Diabetes (%)           |                   | 23 (13.9)    |
| Glucocorticoid use (%) |                   | 46 (27.9)    |
| CKD (%)                |                   | 16 (9.7)     |
| Hyperthyroidism (%)    |                   | 6 (3.6)      |
| PPIs (%)               |                   | 26 (15.8)    |
| Chemotherapy (%)       |                   | 19 (11.5)    |
| Ipilimumab (%)         |                   | 30 (18.2)    |
| Nivolumab (%)          |                   | 146 (88.5)   |
| Pembrolizumab (%)      |                   | 59 (35.8)    |
| Zometa (%) +           |                   | 8 (4.)       |
| BOR at 1 year          | Complete Response | 60 (36.4)    |
|                        | Partial Response  | 28 (16.9)    |
|                        | Stability         | 29 (17.6)    |
|                        | Progression       | 46 (27.9)    |
| BOR at 2 years         | Complete Response | 55 (50.0)    |
|                        | Partial Response  | 15 (13.6)    |
|                        | Stability         | 13 (11.8)    |
|                        | Progression       | 30 (27.3)    |

**Table 1.** Patient characteristics. \*4 \*\*29, \*\*\*14, \*\*\*\*20 missing data, + 1 missing data. BMI: Body Mass Index, BRAF: BRAF V600E mutation, Breslow Index: Tumor thickness in millimeters, CKD: Chronic kidney disease, PPIs: Proton pump inhibitors, BOR: Best Overall Response.

up at T2. Student's t-test for quantitative variables and Chi-square test for qualitative variables were used to ensure there was no bias related to loss to follow-up (Supplementary Table 1). A significance level of  $p < 0.05$  was considered for all tests. Statistical analyses were performed using R version 4.3.0 Statistical Software (<https://www.programmingr.com/examples/neat-tricks/r-citation/>).

## Results

### Inter-operator variability

Inter-operator agreement for BMD measurements was excellent at any time of analysis: ICC at baseline was 0.995 (95% CI, 0.993–0.996), ICC at 1 year was 0.994 (95% CI, 0.992–0.996), and ICC at 2 years was 0.993 (95% CI, 0.991–0.996). Reproducibility was consistently excellent across all BMD measurements, as demonstrated by the Bland–Altman plots (Supplementary Methods).

There were no significant differences between patients who completed follow-up at 2 years and those lost to follow-up in terms of age, sex, baseline BMD (T0), or BMD variation between T0 and T1. For further details see Supplementary Methods.

### Patient characteristics

Demographic data and clinical characteristics of patients at baseline are summarized in Table 1. The mean age of the study population was 65 years ( $\pm 12.5$ ); 92 men (55.8%). At 2 years, 22 patients had died and 33 were lost to follow-up. The most common melanoma subtype was SSM ( $n = 90$ , 64.3%), followed by NM ( $n = 36$ , 25.7%). Most patients received PD-1 inhibitors.

### Evolution of bone mineral density

A significant decrease in scanographic BMD was observed in patients between T0 and T1, with a mean reduction of 6 HU (95% CI, 3.35–8.72;  $p < 0.001$ ). A further significant decline in BMD was observed between T1 and T2, with a mean decrease of 6.58 HU (95% CI, 3.80–9.36;  $p < 0.001$ ). Overall, from baseline to 2 years, the mean BMD decrease was 14.08 HU (95% CI, 10.40–17.76;  $p < 0.001$ ) (Fig. 2). Thus, BMD loss during the first year was estimated at 5.15%, increasing to 11.91% at the 2-year follow-up.

### Factors associated with BMD decrease from baseline to 1 year

BMD reduction between T0 and T1 was more pronounced in men ( $\beta = 8.87$ ; 95% CI, 3.62–14.11;  $p = 0.002$ ). Younger age was also associated with greater BMD loss ( $\beta = -0.3$ ; 95% CI, -0.5 to -0.09;  $p = 0.01$ ) (Table 2).

### Factors associated with BMD decrease from baseline to 2 Years

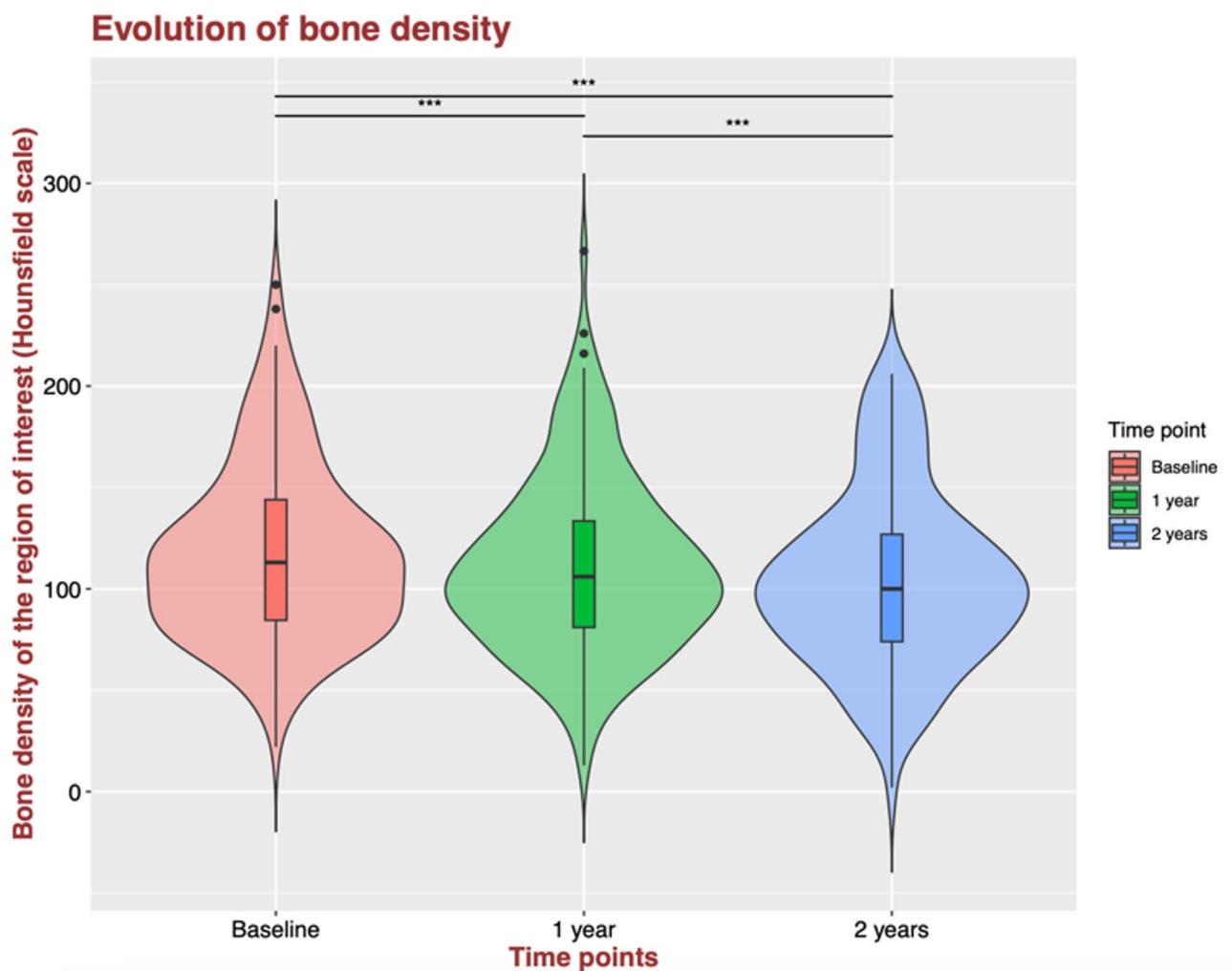
Reduction in BMD from T0 to T2 was more pronounced in men ( $\beta = 10.03$ ; 95% CI, 2.79–17.28;  $p = 0.001$ ) (Table 3). Compared to patients with a complete response, those with disease progression or partial response exhibited greater BMD decreases, with reductions of 11.20 HU (95% CI, 2.59–19.80;  $p = 0.01$ ) and 15.05 HU (95% CI, 4.06–26.03;  $p = 0.01$ ), respectively.

### Multivariable analysis

The reduction in BMD from T0 to T2 was more pronounced in men, regardless of the immunotherapy used (supplementary Tables 2–4).

### Vertebral fractures

Six patients developed vertebral fractures within 2 years of starting treatment, including two within the first year (supplementary Table 5). The age at fracture ranged from 61 to 89 years old, with half of the patients being male. The melanoma subtype was frequently SSM, wild *BRAF*, and stage 4. All patients received nivolumab, and the majority (67%) were also treated with pembrolizumab. Two patients had a history of chronic kidney disease.



**Fig. 2.** Evolution of Scanographic BMD Between T0, T1 Year, and T2 Years After Initiation of ICIs.

|  |                   | B     | 95% CI          | p. value     |
|--|-------------------|-------|-----------------|--------------|
| Sex  | Male              | 8.87  | [3.62 ; 14.11]  | <b>0.001</b> |
| Age  |                   | -0.3  | [-0.50 ; -0.09] | <b>0.005</b> |
| Histologic subtype                               | SSM               |       |                 |              |
|  | MN                | -2.16 | [-8.97 ; 4.65]  | 0.53         |
|  | Other             | -4.04 | [-10.66 ; 2.58] | 0.23         |
| BRAF status                                      | Wild              |       |                 |              |
|  | Mutated           | 3.47  | [-2.21 ; 9.14]  | 0.23         |
| Breslow (mm)                                     |                   | 0.29  | [-0.10 ; 0.68]  | 0.14         |
| AJCCv8 stage                                     | 3                 |       |                 |              |
|  | 4                 | 3.79  | [-3.58 ; 11.16] | 0.31         |
| <i>Treatment regimen</i>                         |                   |       |                 |              |
| Ipilimumab / Nivolumab combination               |                   | 4.72  | [-2.23 ; 11.66] | 0.18         |
|  | Cycles            | 1.78  | [-2.75 ; 6.32]  | 0.43         |
| Nivolumab  |                   | 2.1   | [-6.33 ; 10.53] | 0.62         |
|  | Cycles            | -0.16 | [-0.54 ; 0.22]  | 0.41         |
| Pembrolizumab                                    |                   | 1.48  | [-4.13 ; 7.10]  | 0.60         |
|  | Cycles            | -0.17 | [-1.25 ; 0.90]  | 0.75         |
| <i>Confounding factors in BMD ICI initiation</i> |                   |       |                 |              |
| BMI  |                   | -0.28 | [-0.85 ; 0.29]  | 0.34         |
| Smoking status                                   |                   | 4.77  | [-0.89 ; 10.43] | 0.10         |
| Alcohol  |                   | 4.49  | [-4.24 ; 13.22] | 0.31         |
| Diabetes   |                   | -0.75 | [-8.53 ; 7.02]  | 0.85         |
| Glucocorticoid use                               |                   | 5.18  | [-0.78 ; 11.13] | 0.09         |
| CKD  |                   | -2.4  | [-11.49 ; 6.70] | 0.60         |
| Hyperthyroidism                                  |                   | -6.96 | [-21.30 ; 7.39] | 0.34         |
| PPIs   |                   | -2.79 | [-10.16 ; 4.59] | 0.46         |
| Chemotherapy                                     |                   | -1.59 | [-10.02 ; 6.84] | 0.71         |
| Zometa   |                   | -5.61 | [-15.82 ; 4.60] | 0.28         |
| <i>Response to ICI</i>                           |                   |       |                 |              |
| BOR T1   | Complete response |       |                 |              |
|  | Progression       | -1.46 | [-8.2 ; 5.27]   | 0.67         |
|  | Partial Response  | 3.54  | [-4.33 ; 11.4]  | 0.38         |
|  | Stability         | 0.06  | [-7.71 ; 7.84]  | 0.99         |

**Table 2.** Factors Associated with BMD Variation Between T0 and T1: Bivariate Linear Regression (N = 165). AJCCv8: American Joint Committee on Cancer eighth edition, BRAF: BRAF V600E mutation, Breslow Index: Tumor thickness in millimeters, BMI: Body Mass Index, CKD: Chronic kidney disease, PPIs: Proton pump inhibitors, BOR: Best Overall Response.

Corticosteroid use was reported in 67% cases. At the time of fracture, three patients had progressive disease, one had a partial response, and two presented complete response. Finally, patients were predominantly overweight or obese, and 4 patients showing an increase in BMI in the months preceding the fracture.

### Comparison between patients with BMD above versus below the 110 HU threshold

A total of 21 patients exhibited a decrease in BMD falling below the 110 HU threshold. An association was observed between PR and scanographic osteoporosis within 2 years of immunotherapy, with an odds ratio (OR) of 6.33 (95% CI, 1.48–31.78;  $p = 0.02$ ) (supplementary Table 6). A positive association was also found between age and the likelihood of developing scanographic osteoporosis, with an OR of 1.06 (95% CI, 1.01–1.12;  $p = 0.02$ ). No significant differences were identified for other characteristics ( $p > 0.05$ ).

### Discussion

Our study underscores that scanographic BMD decreases within the 2 years following ICI initiation, especially in men and young patients, in case of progressive disease or partial response.

The greater bone loss in younger patients and male subjects could be explained by a regression-to-the-mean effect. Furthermore, our results suggest that progressive disease or partial response may be associated with a higher risk of bone mass loss. This may be attributed to a higher cumulative ICI dose in these patients compared to those with a complete response, who are typically eligible for treatment discontinuation after 6–12 months of sustained response, thereby receiving fewer cycles<sup>28</sup>. The prolonged exposure in non-responders may contribute to a persistent pro-inflammatory environment that promotes osteoclastogenesis<sup>29</sup>. However, future prospective

|  |                   | B     | 95% CI           | p. value     |
|--|-------------------|-------|------------------|--------------|
| Sex  | Male              | 10.03 | [2.79 ; 17.28]   | <b>0.001</b> |
| Age  |                   | -0.15 | [-0.43 ; 0.14]   | 0.31         |
| Histologic subtype                               | SSM               |       |                  |              |
|  | MN                | -0.82 | [-10.4 ; 8.77]   | 0.87         |
|  | Other             | -9.44 | [-18.56 ; -0.32] | <b>0.04</b>  |
| BRAF status                                      | Wild              |       |                  |              |
|  | Mutated           | 3.63  | [-4.07 ; 11.34]  | 0.35         |
| Breslow (mm)                                     |                   | 0.45  | [-0.06 ; 0.95]   | 0.08         |
| AJCCv8 stage                                     | 3                 |       |                  |              |
|  | 4                 | 1.73  | [-8.49 ; 11.95]  | 0.74         |
| Treatment regimen                                |                   |       |                  |              |
| Ipilimumab / Nivolumab combination               |                   | -3.65 | [-13.61 ; 6.32]  | 0.47         |
|  | Cycles            | 1.47  | [-2.07 ; 5.01]   | 0.41         |
| Nivolumab  |                   | 9.13  | [-1.82 ; 20.09]  | 0.10         |
|  | Cycles            | 0.02  | [-0.25 ; 0.28]   | 0.89         |
| Pembrolizumab                                    |                   | -1.70 | [-9.31 ; 5.90]   | 0.66         |
|  | Cycles            | -0.33 | [-0.91 ; 0.24]   | 0.25         |
| <i>Confounding factors in BMD ICI initiation</i> |                   |       |                  |              |
| BMI  |                   | 0.10  | [-0.64 ; 0.85]   | 0.79         |
| Smoking status                                   |                   | 1.63  | [-6.34 ; 9.60]   | 0.69         |
| Alcohol  |                   | 5.74  | [-5.91 ; 17.40]  | 0.33         |
| Diabetes   |                   | -4.27 | [-14.46 ; 5.93]  | 0.41         |
| Glucocorticoid use                               |                   | 5.28  | [-2.48 ; 13.04]  | 0.18         |
| CKD  |                   | 6.99  | [-7.18 ; 21.17]  | 0.33         |
| Hyperthyroidism                                  |                   | -1.79 | [-24.49 ; 20.90] | 0.88         |
| PPIs   |                   | -7.85 | [-18.51 ; 2.82]  | 0.15         |
| Chemotherapy                                     |                   | 6.83  | [-5.42 ; 19.09]  | 0.27         |
| Zometa   |                   | 2.03  | [-14.24 ; 18.31] | 0.80         |
| <i>Response to ICI</i>                           |                   |       |                  |              |
| BOR T1   | Complete response |       |                  |              |
|  | Progression       | -0.09 | [-10.07 ; 9.89]  | 0.99         |
|  | Partial Response  | -4.06 | [-14.30 ; 6.18]  | 0.43         |
|  | Stability         | -3.56 | [-13.80 ; 6.68]  | 0.49         |
| BOR T2   | Complete response |       |                  |              |
|  | Progression       | 11.20 | [2.59 ; 19.80]   | <b>0.01</b>  |
|  | Partial Response  | 15.05 | [4.06 ; 26.03]   | <b>0.01</b>  |
|  | Stability         | 3.13  | [-8.48 ; 14.75]  | 0.59         |

**Table 3.** Factors Associated with BMD Variation Between T0 and T2: Bivariate Linear Regression (N = 165). AJCCv8: American Joint Committee on Cancer eighth edition, BRAF: BRAF V600E mutation, Breslow Index: Tumor thickness in millimeters, BMI: Body Mass Index, CKD: Chronic kidney disease, PPIs: Proton pump inhibitors, BOR: Best Overall Response,

studies will be necessary to determine whether bone loss is specifically related to cumulative exposure, peak dosing, dosing intervals, or overall treatment burden.

Six cases of incident osteoporotic vertebral fractures were diagnosed within 2 years, with most patients being treated with PD1i and being overweight or obese. Firstly, these findings align with the literature, indicating a higher fracture prevalence with PD1i than CTLA-4i, likely due to the broader use of PD1i<sup>16</sup>. Secondly, these results suggest a potential link between obesity and the occurrence of fractures, possibly mediated by heightened sensitivity to ICIs. Indeed, obesity is classically associated with increased sensitivity to PD1i therapies in the literature, attributed to heightened PD-1 expression on T cells<sup>30</sup>. However, further studies are required to elucidate this relationship.

Further, these findings suggest a loss of bone mass that appears and persists after the discontinuation of immunotherapy. If confirmed, it may be necessary to implement earlier screening and intervention protocols for cancer survivors treated with ICI therapy, since post-treatment osteoporosis is emerging as a significant source of morbidity, to minimize bone loss and lower the probability of fractures.

Although several studies have demonstrated a strong correlation between DXA and scanographic BMD measurements<sup>19,31</sup>, with some even reporting greater sensitivity of CT in detecting trabecular bone loss<sup>32,33</sup>, CT imaging offers additional advantages by allowing separate assessment of cortical and trabecular bone. Given that

trabecular bone -especially in the vertebrae- is the earliest site affected in postmenopausal osteoporosis, this capability enables earlier detection of bone loss and provides a sensitive tool for monitoring treatment response. The absence of a calibration phantom may lead to a lack of standardization and calibration. However, this risk was partially mitigated because all patients were evaluated on the same machine during follow-up, and the reproducibility of measurements was excellent. Additionally, an important limitation of this study is the absence of a control group, as nearly all patients received immunotherapy in accordance with current clinical guidelines. In addition, no patients underwent baseline DXA measurement, as this was a retrospective study based on pre-existing imaging data. This precluded the possibility of direct comparison between CT-derived and DXA-derived BMD and limits the generalizability of our findings. Another limitation is the absence of data collection on peripheral fractures, along with the exclusion of mild fractures based on the Genant classification, which may have contributed to an underestimation of the actual number of fractures occurring during the follow-up period. Further studies are needed to assess the specific risks associated with monotherapy compared to combination regimens and additional risk factors such as family history of fractures, prior falls, and biological markers need to be considered.

One of the key strengths of this "real-world" study lies in its improved external validity and its ability to detect late-onset effects, i.e., those occurring beyond three months after the initiation of ICI<sup>34,35</sup>. Restricting the sample to melanoma, among the main tumor types for which ICIs are indicated, allowed us to study a homogeneous cohort with a low risk of secondary bone metastases. Unlike previous studies investigating fracture occurrence under immunotherapy<sup>36,37</sup>, our study excluded patients with a prior history of osteoporotic fractures or those receiving anti-osteoporotic treatments, thereby limiting major confounding factors. The use of CT imaging avoids overestimation of BMD values and consequently underestimation of osteoporosis prevalence compared to DXA<sup>38</sup>. In the context of skeletal events under ICIs, Moseley et al. reported cases of vertebral fractures occurring without densitometric osteoporosis<sup>15</sup> making opportunistic CT screening an approach to consider in prevention of osteoporosis without additional cost or radiation exposure.

## Conclusions

Bone mineral density decreases within 2 years of ICI initiation and may represent a newly emerging late-onset irAE of ICI therapy. These results highlight the need for broad preventive measures, including for individuals without traditional risk factors. Further studies are needed to clarify the direct causal impact of ICI treatment on bone biology, bone mineral density, and fracture risk, to enable appropriate monitoring and public health interventions.

## Data availability

**The datasets generated during and/or analysed during the current study are available in the research center of Caen, on request on Soukayna Baddi or Jean-Matthieu L'Orphelin.**

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## Author contributions

Conceptualization; SB, JMLO; Methodology: SB, JMLO, ACR; Formal analysis and investigation: SB, AR, DS- Writing—original draft preparation: SB, JMLO; Writing—review and editing: All authors; Supervision: JMLO

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## Declarations

## Competing interests

Jean-Matthieu L'Orphelin has received speaker and consultant honoraria from BMS, Novartis, MSD and Pierre Fabre Oncology.

## Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by CLERS, the Local Research Ethics Committee for Health (Ethics Approval Number: ID 4977). Analyses performed are available requesting corresponding author.

## Consent to participate

As a retrospective study, all patient files were checked for non-objection.

## Consent to publish

Each participant provided non opposition.

## Additional information

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