An Italian Retrospective Survey on Bone Metastasis in Melanoma: Impact of Immunotherapy and Radiotherapy on Survival

Francesco Mannavola¹, Mario Mandala², Annalisa Todisco¹, Vanna Chiarion Sileni³, Marco Palla⁴, Alessandro Marco Minisini⁵, Laura Pala⁶, Francesca Morgese⁷, Lorenzo Di Guardo⁸, Luigia Stefania Stucci¹, Michele Guida⁸, Alice Indini¹, Pietro Quaglino¹⁰, Virginia Ferraresi¹¹, Riccardo Marconcini¹², Maria Chiara Tronconi¹³, Ernesto Rossi¹⁴, Olga Nigro¹⁵, Marcella Occoli¹⁶, Alessio Cortellini¹⁷, Silvia Quadri¹⁸, Giuseppe Palmieri¹⁹, Jacopo Pigozzo³, Paolo Antonio Ascierto⁶, Maria Grazia Vitale⁵, Sabino Strippoli⁶, Pier Francesco Ferrucci⁶, Rossana Berardi⁶, Giovanni Randon⁶, Pietro Cardone⁶, Giovanni Schinazzi¹⁴, Franco Silvestris¹ and Marco Tucci¹,² on behalf of the Italian Melanoma Intergroup (IMI)

¹ Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, Bari, Italy, ² Medical Oncology Unit, Department of Oncology and Hematology, Azienda Ospedaliera Papa Giovanni XXIII Hospital, Bergamo, Italy, ³ Melanoma Oncology Unit, Veneto Institute of Oncology, Scientific Institute for Research, Hospitalization and Healthcare, Padua, Italy, ⁴ Melanoma, Cancer Immunotherapy and Development Therapeutics, Istituto Nazionale Tumori IRCCS Fondazione Pascale, Naples, Italy, ⁵ Division of Melanoma, Sarcoma and Rare Tumors, European Institute of Oncology, Scientific Institute for Research, Hospitalization and Healthcare, Milan, Italy, ⁶ Oncology Clinic, Università Politecnica delle Marche, Ancona, Italy, ⁷ Melanoma Medical Oncology Unit, Department of Medical Oncology and Hematology, National Institute of Tumori, Milan, Italy, ⁸ IRCCS Giovanni Paolo II, Cancer Institute, Bari, Italy, ⁹ Department of Medical Sciences, Dermatologic Clinic, University of Turin, Turin, Italy, ¹⁰ First Division of Medical Oncology, IRCCS Regina Elena National Cancer Institute, Rome, Italy, ¹¹ Medical Oncology Department, Santa Chiara Hospital, University of Pisa, Pisa, Italy, ¹² Medical Oncology and Hematology Unit, Humanitas Cancer Center, Humanitas Clinical and Research Center, IRCCS, Rozzano, Italy, ¹³ Medical Oncology, Fondazione Policlinico Universitario ‘Agostino Gemelli’ IRCCS, Rome, Italy, ¹⁴ Medical Oncology, ASST-Sette Laghi, Ospedale di Circolo e Fondazione Macchi, Varese, Italy, ¹⁵ Medical Oncology Unit, Santa Croce and Carle Teaching Hospital, Cuneo, Italy, ¹⁶ Department of Biotechnological and Applied Clinical Sciences, San Salvatore Hospital, University of L’Aquila, L’Aquila, Italy, ¹⁷ Medical Oncology Unit, Azienda Sanitaria Locale Frosinone, Frosinone, Italy, ¹⁸ Unit of Cancer Genetics, Institute of Genetic and Biomedical Research, National Research Council, Sassari, Italy

**Background:** We performed a multicenter retrospective observational study to investigate the impact of clinical–pathological features and therapeutic strategies on both the complications and survival of patients with bone metastases (BMs) from malignant melanoma.

**Patients and Methods:** A total of 305 patients with melanoma and radiological evidence of BMs were retrospectively enrolled from 19 Italian centers. All patients received conventional treatments in accordance with each own treating physician’s practice. Both univariate and multivariate models were used to explore the impact of melanoma features, including skeletal-related events (SREs), and different treatments on both overall survival (OS) and time-to-SREs. The chi-squared test evaluated the suitability of several parameters to predict the occurrence of SREs.
INTRODUCTION

Innovative therapies have improved the survival of patients with unresectable metastatic cutaneous melanoma (CM). However, the prognosis remains poor in those harboring negative prognostic factors, such as poor performance status (PS), high tumor burden, brain metastases, and high baseline levels of lactate dehydrogenase (LDH) (1, 2). Also, the genomic landscape of melanoma influences the clinical evolution as well as innate and acquired drug resistance, thus representing an up-growing field of interest (3).

The impact of bone disease (BD) in melanoma has been scarcely investigated. Data from clinical trials indicate that the skeleton is the fourth site of metastasis after lung, liver, and brain, that occurs in about 11–18% of patients (4, 5). Bone metastases (BM) generate typical skeletal-related events (SREs), such as severe bone pain, pathological fractures, spinal cord compression, hypercalcemia, and need for radiotherapy (nRT) or surgery to the bone, and are common in breast, prostate, and lung cancers while being so far a clinical challenge in other malignancies like melanoma (6). Moreover, the potential beneficial effect of novel anti-melanoma agents on BMs and SREs is, at present, unclear (7).

Data collected from the SEER (Surveillance, Epidemiology and End Results) database and large series from individual medical centers demonstrated that BMs from CM are frequently observed in young patients and are associated with elevated LDH, while the prognosis is apparently poor and almost similar to that of patients developing brain or liver metastases (8). In addition, BMs occur in patients with CM, while they are rarely detected in those with mucosal, uveal, and acral melanoma. Furthermore, BMs are frequently revealed at diagnosis of the metastatic disease as involving single or multiple sites and frequently affect the axial skeleton.

Results: Eighty-three percent of patients had metachronous BMs. The prevalent (90%) bone metastatic site was the spine, while 45% had involvement of the appendicular skeleton. Forty-seven percent experienced at least one SRE, including palliative radiotherapy (RT) in 37% of cases. No melanoma-associated factor was predictive of the development of SREs, although patients receiving early treatment with bone-targeted agents showed 62% lower risk and delayed time of SRE occurrence. Median OS from the diagnosis of bone metastasis was 10.7 months. The multivariate analysis revealed as independent prognostic factors the number of BMs, number of metastatic organs, baseline lactate dehydrogenase levels, and treatment with targeted therapy or immunotherapy. Subgroup analyses showed the best OS (median = 16.5 months) in the subset of patients receiving both immunotherapy and palliative RT.

Conclusion: Based on our results, patients undergoing immunotherapy and palliative RT showed an OS benefit suggestive of a possible additive effect. The apparent protective role of bone targeting agent use on SREs observed in our analysis should deserve prospective evaluation.

Keywords: melanoma, bone metastases, SREs, immunotherapy, bisphosphonates, denosumab

The pathogenesis of BMs is regulated by the interplay among malignant cells, osteoclasts, osteoblasts, and immune cells. Epithelial tumor cells, indeed, produce high RANK-L (the receptor activator of nuclear factor-kB ligand) levels that engulf the bone metastatic sites, thus interfering with osteoclasts through the RANK receptor that is also expressed by cancer cells like melanoma and regulated by interferon (IFN)-γ (9). Systemic agents for the treatment of BMs include inhibitors of bone resorption and anabolic signals, namely, bone-targeting agents (BTAs), aimed at restoring the physiological bone turnover that results profoundly impaired in patients with skeletal colonization. Their use in breast, prostate, and lung cancers is currently well supported in various clinical settings (10), while their effective contribution in patients with BMs from melanoma is still debated. Recent studies reported the synergistic effect of RANK-L blockade combined with immune checkpoint inhibition in patients with BMs from melanoma, suggesting a potential clinical benefit from this strategy (11). Based on preliminary evidence (12, 13), clinical trials are currently ongoing and are aimed at exploring the overall therapeutic effect of denosumab with immunotherapy (NCT-03161756; EudraCT-2016-001925-15).

Growing interest in melanoma has also been oriented in combining an immune checkpoint inhibitor (ICI) with either systemic or local treatment to maximize the antitumor response. Ongoing clinical trials, for example, are exploring the association of targeted therapy and immunotherapy (14–16). They were supported by preclinical and translational studies showing that BRAF and MEK inhibition has immune-modulating effects that increase tumor T cell infiltration as well as tumor antigen exposition and PD-L1 expression (17, 18). Another attractive scenario is combining immunotherapy with radiotherapy (RT), the latter being often used in the case of BMs. The rationale is that tumor
irradiation induces cell death, provoking local release of tumor-derived antigens that, in turn, promote T cell cross-priming by dendritic cells and long-term immunological memory (19). This priming of the immune system by RT can synergize with immunotherapy. Consequently, trials evaluating combinations of immunotherapy and RT have progressively increased in the last years, both in melanoma and other malignancies (20).

Herein, we completed a retrospective multicentric survey in an Italian melanoma population bearing BMs to investigate the potential impact of clinical–pathological features and the therapeutic strategies on survival.

**PATIENTS AND METHODS**

**Study Population**

In this observational multicenter study, we retrospectively enrolled 305 patients with a diagnosis of melanoma and radiological evidence of BD. All patients received standard treatments in accordance with each own treating physician’s practice in 19 Italian Centers from November 1984 to March 2019. Clinical data were collected throughout the disease course and included features of melanoma, BM detection, access to systemic treatments (immunotherapy, target therapy, or chemotherapy), nRT, as well as date of first SRE and death. When available, other variables, including age, sex, melanoma primary site, histological parameters (e.g., histotype, Breslow, ulceration, number of mitoses, lymphocyte infiltrate, and nodal stage), BRAF/NRAS status, LDH levels, calcemia, time to appearance of BMs, presence of extraosseous metastases, and SRE type, were assessed. Specific information about the systemic treatments (e.g., drug sequences, objective responses, or time of disease progression) were not considered since these are out of the scope of this survey. All patients had written informed consent for clinical data collection and use for research purposes according to each own center’s rules. Ethical approval was not required for this study according to local legislation.

**Statistical Analysis**

Descriptive statistics were used for patient demographics and incidence of SREs. The chi-squared test analyzed the relationship of SRE occurrence with the clinical and pathological features of melanoma patients. Survival analyses were completed by the Kaplan–Meier method. Factors for analyses were identified of melanoma patients. Survival analyses were completed by the multivariate analysis of survival using the Cox proportional hazards regression. Patients that were lost in follow-up were not considered for survival analyses. The statistics were completed with the Medcalc software (version 12.7.0.0). A p value < 0.05 was considered significant.

**RESULTS**

**Baseline Demographic Features**

In total, 305 patients with cutaneous \((n=290)\), mucosal \((n=6)\), and uveal \((n=9)\) melanoma were enrolled in the study. The basal clinical and pathological features of the population are described in **Table 1**. Of note is that the median age at diagnosis was 56 years and 63.3% were male \((n=193)\). A BRAF mutation was documented in 59%, while NRAS mutation was present in 36% of patients. Almost 22\% \((n=23/105)\) of patients had LDH levels \(>2\) times the upper limit of the normal range (ULN) at the time of melanoma diagnosis.

The majority of patients \((97%)\) had extraosseous metastases, which included 247 patients \((81\%)\) with more than three metastatic sites and 73 patients \((24\%)\) with brain metastases. The onset of BMs and the diagnosis of primary melanoma were mostly metachronous \((83\%)\). The prevalent bone metastatic site was the spine \((90\%)\), while only 45\% of patients had involvement of the appendicular skeleton, including 10\% of them with both axial and appendicular colonization. The majority of patients \((61\%, n=183/301)\) showed less than five BMs, while 39\% \((n=118)\) harbored more lesions. Calcium levels at the time of BM diagnosis were usually normal, whereas 37\% of patients \((n=95/254)\) expressed LDH levels \(>2\) times the ULN.

Almost half of the patients received BTAs, including bisphosphonates \((40.8\%, n=119/292)\) or denosumab \((6.8\%, n=20)\). With regard to systemic treatments, 33\% of patients \((n=96/291)\) received only targeted therapy, 39\% \((n=114)\) only ICIs, and 20.6\% \((n=60)\) both ICIs and targeted agents, whereas a minority \((7\%, n=21)\) underwent only chemotherapy (CHT). Data are summarized in **Table 2**.

**Bone Disease and Development of SREs**

As shown in **Supplementary Figure S1A**, 47\% \((n=137/291)\) of patients developed at least one SRE, which included nRT in 37\% \((n=109)\), fractures in 12\% \((n=35)\), spinal cord injury in 7\% \((n=21)\), and surgery in 3\% \((n=8)\), while hypercalcemia occurred in a single case \((<1\%)\). Thirty-four patients \((12\%)\) experienced two or more SREs.

The next set of analyses (**Table 3**) explored the features of melanoma patients who experienced SREs. The median age was 59 years and 65\% were male. The majority of them showed metachronous BMs \((87\%)\) located in the axial skeleton \((56\%)\) as well as LDH levels \(\leq2\) times the ULN \((67\%)\) and normal calcemia \((89\%)\) at the time of BM detection. Notably, 66\% of patients had less than five BMs, while extraosseous metastases occurred in about 95\% of patients. Among patients who experienced at least one SRE, almost 54\% were previously treated with BTAs such as bisphosphonates.

Neither clinical parameters nor tumor-related variables correlated with SREs (data not shown). However, patients who were early treated with BTAs showed a minor SRE occurrence with respect to the untreated patients \(20\% vs. 39.6\%\);
TABLE 1 | Patient demographics and basal melanoma characteristics in the study population.

| Characteristics                        | Frequency | Percentage |
|----------------------------------------|-----------|------------|
| Age at melanoma diagnosis (n = 305)    | Median, 56 (range = 18–86) years |            |
| Sex (n = 305)                          | Male      | 193        | 63.3       |
|                                        | Female    | 112        | 36.7       |
| Primary site (n = 305)                 | Limbs     | 82         | 26.9       |
|                                        | Head and neck | 39     | 12.8       |
|                                        | Trunk     | 134        | 43.9       |
|                                        | Occult    | 35         | 11.5       |
|                                        | Mucosa    | 6          | 2.0        |
|                                        | Uvea      | 9          | 3.0        |
| Histology (n = 239)                    | SSM       | 88         | 36.8       |
|                                        | Nodular   | 95         | 39.7       |
|                                        | Acral     | 9          | 3.8        |
|                                        | Lentigo Maligna | 1   | 0.4        |
|                                        | Mucosal   | 6          | 2.5        |
|                                        | Uveal     | 9          | 3.8        |
|                                        | Other     | 31         | 13.0       |
| Breslow depth (n = 239)                | ≤1 mm     | 28         | 11.7       |
|                                        | >1–2 mm   | 48         | 20.1       |
|                                        | >2–4 mm   | 73         | 30.5       |
|                                        | >4 mm     | 90         | 37.7       |
| Ulceration (n = 239)                   | Present   | 138        | 57.7       |
|                                        | Absent    | 101        | 42.3       |
| Number of mitosis (n = 201)            | ≤1        | 46         | 22.9       |
|                                        | >1        | 155        | 77.1       |
| Tumor-infiltrating lymphocytes (n = 169)| brisk    | 37         | 21.9       |
|                                        | Absent or not brisk | 132   | 78.1       |
| Lymph node mts (n = 263)               | NO        | 133        | 50.6       |
|                                        | N+        | 130        | 49.4       |
| BRAF status (n = 285)                  | Mutated   | 168        | 58.9       |
|                                        | Wild type | 117        | 41.1       |
| NRAS status (n = 59)                   | Mutated   | 21         | 35.6       |
|                                        | Wild type | 38         | 64.4       |
| LDH levels (n = 105)                   | ≤2 × ULN  | 82         | 78.1       |
|                                        | >2 × ULN  | 23         | 21.9       |

LDH, lactate dehydrogenase; ULN, upper limit of normal; SSM, superficial spreading melanoma; mts, metastases.

odds ratio (OR) = 0.38, 95% confidence interval (CI) = 0.2–0.72, p = 0.003] (Supplementary Figure S1B). Moreover, the univariate analysis (Table 4) investigated those factors putatively associated with longer time to SRE development and revealed a correlation with the axial localization of BMs [hazard ratio (HR) = 0.61, 95% CI = 0.33–1.13, p = 0.05] and with previous use of BTAs (HR = 0.41, 95% CI = 0.26–0.66, p = 0.001). However, only the use of BTAs before the development of SREs was confirmed as an independent prognostic factor (p = 0.003).

Factors Associated With Overall Survival
At the time of data lock, 182 patients were deceased and 108 were still alive, while the other 15 resulted lost in follow-up. The median OS was 10.7 months (Supplementary Figure S2). Table 5 describes data from both univariate and multivariate analyses of the factors potentially correlated with prognosis. The univariate analysis revealed a worse prognosis in males (p = 0.03) and in patients with melanoma of the trunk (p = 0.05) as well as a number of five or more BMs (p < 0.0001), high LDH levels at metastatic BD diagnosis (p < 0.0001), and evidence of three or more metastatic sites (p = 0.0027),
TABLE 3 | Patient demographics and BM characteristics in the population who experienced SREs.

| Characteristics                             | Frequency | Percentage |
|---------------------------------------------|-----------|------------|
| Age at BM diagnosis (n = 137)               |           |            |
| Median, 59 years                            |           |            |
| Sex (n = 137)                               |           |            |
| Male                                        | 89        | 65.0       |
| Female                                      | 48        | 35.0       |
| BRAF status (n = 131)                       |           |            |
| Mutated                                     | 71        | 54.2       |
| Wild type                                   | 60        | 45.8       |
| NRAS status (n = 27)                        |           |            |
| Mutated                                     | 11        | 40.7       |
| Wild type                                   | 16        | 59.3       |
| LDH levels at BM diagnosis (n = 109)        |           |            |
| ≤2 × ULN                                    | 73        | 67.0       |
| >2 × ULN                                    | 36        | 33.0       |
| Calcaemia at BM diagnosis (n = 104)         |           |            |
| ≤ ULN                                       | 93        | 89.4       |
| > ULN                                       | 11        | 10.6       |
| BM and melanoma diagnosis (n = 137)         |           |            |
| Synchronous                                 | 18        | 13.1       |
| Metachronous                                | 119       | 86.9       |
| SRE and BM diagnosis (n = 129)              |           |            |
| Synchronous                                 | 38        | 29.5       |
| Metachronous                                | 91        | 70.5       |
| Localization of BM (n = 133)                |           |            |
| Axial                                       | 74        | 55.6       |
| Appendicular                                | 17        | 12.8       |
| Both                                        | 42        | 31.6       |
| Number of BM (n = 134)                      |           |            |
| <5                                          | 89        | 66.4       |
| ≥5                                          | 45        | 33.6       |
| Presence of extraosseous mts (n = 137)      |           |            |
| No                                          | 7         | 5.1        |
| Yes                                         | 130       | 94.9       |
| Use of BTA (n = 137)                        |           |            |
| No                                          | 63        | 46         |
| Bisphosphonates                             | 67        | 48.9       |
| Denosumab                                   | 7         | 5.1        |

BM, bone metastases; BTA, bone-targeting agents; LDH, lactate dehydrogenase; mts, metastases; SRE, skeletal-related events; ULN, upper limit of normal.

Impact of Melanoma-Dedicated Treatments on Overall Survival

The next set of analyses explored the role of systemic treatments and RT on OS. As shown in Figure 1A, patients receiving CHT alone underwent worsened survival as compared to those treated with new agents, including ICIs and/or targeted drugs (HR = 4.15, CI = 1.75–9.90, p < 0.0001). In detail, the median OS (mOS) were 16.5 (95% CI = 10.0–23.3), 13.0 (95% CI = 9.2–16.6), 9.0 (95% CI = 7.3–11.5) and 4.0 months (95% CI = 2.2–5.4) in patients comprehensively treated with (i) ICIs only, (ii) ICIs and targeted therapy, (iii) targeted therapy only, or (iv) CHT only, respectively.

Further investigations were dedicated to the BRAF-mutated population (Figure 1B). The median OS in patients who received at least one ICI (14.2 months, 95% CI = 9.7–18.0) resulted increased with respect to those treated with targeted therapy alone (8.8 months, 95% CI = 7.0–11.5), although the differences were not statistically significant (HR = 0.75, 95% CI = 0.51–1.10, p = 0.15).

Regarding bone-specific treatments (Supplementary Figure S4), patients who received BTAs showed only a modest benefit in terms of mOS as compared to the untreated ones (11 vs. 9 months), but not statistically significant differences were found (HR = 0.80, 95% CI = 0.60–1.06, p = 0.13). On the other hand, mOS was quite similar between patients who underwent RT (Figure 2A) vs. those who were never treated (10.4 vs. 9.2 months). We also verified (Figure 2B) whether or not different combinations of RT with new therapies interfered with survival. To this purpose, we divided the study population into four groups based on the treatment received: (A) ICIs and RT (n = 73); (B) ICIs without RT (n = 94); (C) targeted therapy only without ICIs (n = 64); and (D) targeted therapy plus RT and never ICIs (n = 30). We found that patients in group A achieved the best mOS (16 months, 95% CI = 10.4–20.7) with respect to either group B (13 months; HR = 0.78, 95% CI = 0.52–1.17, p = 0.23), group C (11 months; HR = 0.68, 95% CI = 7.0–14.5, p = 0.08), or group D (8.1 months; HR = 0.5, 95% CI = 0.29–0.86, p = 0.013).

DISCUSSION

The development of BMs frequently occurs in cancer with a negative impact on the quality of life and survival. Appropriate algorithms for the management of BD mainly derive from extensive prospective trials with patients harboring tumors that frequently metastasize to the skeleton, such as breast, prostate, lung, and kidney cancers (21). Otherwise, a definite evidence-based strategy for the treatment of BMs from other malignancies endowed with lower osteotropic propensity does not exist, while apparently innovative drugs such as cilengitide failed to provide satisfactory results to be applied for routine clinical practice (22, 23). Particularly, the impact of BD in melanoma has been poorly investigated, as well as the potential therapeutic strategies such as either RT or BTA use. Moreover, another unanswered question from recent registrative clinical trials in melanoma concerns the possible impact of ICIs or targeted agents in patients with BM.
The present study was aimed at exploring retrospectively the characteristics of melanoma patients bearing BD. As a result of the available literature to the topic, ours appears as a large retrospective study investigating the features of BMs in melanoma. The definition of the effective incidence of BMs in melanoma, however, is out of the scope of the study and, therefore, was not investigated.

The baseline demographic data in our melanoma population demonstrated that BMs occurred primarily in males with prevalent involvement of the axial skeleton. Almost all patients (97%) showed extrasosseous metastases. Among them, 83% developed metachronous bone and visceral metastases, while in 13% of them BMs were discovered in consequence of a SRE occurrence. The frequency of patients harboring a BRAF mutation (59%) was almost in line with that observed in the general melanoma population. On the other hand, the relative higher frequency observed for mutated NRAS (35.6%) should not be considered as a major propensity of these patients in developing BMs since the NRAS mutational status was available only in less than 20% of cases.

Other studies describing a modest incidence of bone involvement at diagnosis of metastatic melanoma probably reflect the fact that the BD was not properly suspected and investigated at diagnosis. However, one-fourth (23.9%) of melanoma patients from our series showed a brain involvement which is instead reported in about 40–60% of the general melanoma population (24). This apparent difference from our data may probably imply specific molecular mechanisms which critically drive the osteotropism of melanoma cells like those affecting the SDF1/CXCR7/CXCR4 pathway, as recently proven (25, 26).

The median survival from the onset of BM was 10.7 months, but our analysis revealed that patients receiving innovative therapies including ICIs and/or targeted agents showed a better prognosis (9.0–16.5 months) than those undergoing CHT (4.0 months). The patients analyzed in this study (n = 290) apparently showed a lower OS with respect to those enrolled in recent phase 3 clinical trials with new agents resulting in 5-year survival rates higher than 50%. However, it is noteworthy that our survey refers to the time from BM diagnosis because the information relative to the time of a comprehensive diagnosis of metastatic disease was not available. The lack of a control group without BMs also restrains the possibility of exploring the real impact of the BD in the general melanoma prognosis. Moreover, our real-world experience includes patients with clinical features that are generally excluded from clinical trials, including a suboptimal PS or brain involvement, thus reducing the possibility of a direct comparison between various case studies.

The data from large clinical trials with recent 5-year survival updates and pooled analyses defined major negative prognostic factors in metastatic melanoma that included elevated LDH levels, poor PS, and three or more metastatic sites. Despite the lack of information regarding the PS of our population, our multivariate model was in line with these results, while revealing for the first time that an elevated number of BM (five or more skeletal lesions) significantly harms the survival. Additionally, the mutational status of melanoma patients bearing

### TABLE 4 | Univariate and multivariate analyses of factors associated with time to SRE in patients with BM from melanoma.

| Factors | Effect tested | Univariate analysis | Multivariate analysis |
|---------|---------------|---------------------|----------------------|
|         |               | HR 95% CI p         | HR 95% CI p          |
| Demographics |               |                     |                      |
| Age (years) | ≤55 vs. >55   | 1.22 0.86–1.74 0.25 |                      |
| Sex       | Male vs. Female | 1.13 0.80–1.61 0.48 |                      |
| Baseline melanoma characteristics |               |                     |                      |
| Histology | SSM vs. Nodular | 0.96 0.61–1.50 0.85a |                      |
|          | Mucosal vs. Nodular | 0.95 0.23–3.94 0.91 |                      |
|          | Acral vs. Nodular | 1.42 0.47–4.34 0.25 |                      |
|          | Uveal vs. Nodular | 0.50 0.17–1.43 0.57 |                      |
|          | Others vs. Nodular | 0.81 0.34–1.93 0.64 |                      |
| BRAF genotype | V600 vs. Wild type | 0.74 0.52–1.07 0.10 |                      |
| NRAS genotype | Mutated vs. Wild type | 1.37 0.65–2.90 0.33 |                      |
| Characteristics present at BM diagnosis |               |                     |                      |
| Time of diagnosis | Synchronous vs. Metachronous | 0.75 0.47–1.20 0.27 |                      |
| Localization of BM | Axial vs. Extra-axial | 0.61 0.33–1.13 0.05 |                      |
| Number of BM | ≤5 vs. >5 | 0.98 0.68–1.41 0.73 |                      |
| Calkaemia | ≤ULN vs. >ULN | 0.70 0.28–1.72 0.35 |                      |
| LDH levels | ≤2 × ULN vs. >2 × ULN | 1.00 0.66–1.52 1.00 |                      |
| Treatment and SRE | Use of BTA before SRE | Yes vs. No | 0.41 0.26–0.66 0.001 |                      |
|          | Systemic treatment | Targeted/ICIs vs. CHT | 1.12 0.51–2.44 0.78 |                      |

BM, bone metastases; BTA, bone-targeting agents; CHT, chemotherapy; ICIs, immune checkpoint inhibitors; LDH, lactate dehydrogenase; SSM, superficial spreading melanoma; SRE, skeletal-related events; ULN, upper limit of normal. *Logrank test. Bold values are indicate to p < 0.05.
BM did not apparently influence OS, although a positive trend was seen in BRAF-mutated patients treated with immunotherapy (14.2 months) as compared to those treated with targeted therapy alone (8.8 months). These results, however, are conditioned by an *a priori* selection bias since the clinician therapeutic decisions were driven by individual prognostic factors; therefore, they should not lead to considering a superiority of immunotherapy in this setting.

SREs are major complications of BMs that restrain the quality of life in cancer patients. The 2-year cumulative incidence of SREs in breast, prostate, and lung tumors ranges from 41 to 54%, and more than half of patients develop a SRE at cancer diagnosis or thereafter. In the majority of these tumors, SREs dramatically impact on cancer-specific survival (27, 28). Similarly to previous studies (29–31), we observed that at least half of patients with BMs developed a SRE, whose nRT predominantly occurred in 37% of the studied population, while 12% of these patients experienced more than a single SRE. Moreover, in the majority of patients (85%), SREs followed the diagnosis of BMs, with about 50% of patients experiencing one SRE within 1 year from diagnosis.

No melanoma-associated factors were predictive for SRE development in our cohort, although we observed that those receiving primary treatment with BTAs showed a 62% reduced risk of experiencing SREs and delayed time to their occurrence. This apparent protective effect of BTAs in reducing the risk of SREs sounds very impressive if compared to other osteotropic tumors whose relative risk reduction ranges from 15 to 30% (32, 33). However, it is almost difficult to speculate on the possible more potent effect of BTAs in the treatment of melanoma BMs due to the retrospective nature of our study. Finally, a possible effect of BTAs on OS was not demonstrated in our melanoma population. Other studies investigated the efficacy of combining immunotherapy with anti-RANK-L monoclonal antibodies in this setting of patients, providing encouraging though weak results that undoubtedly require further investigation (11). Based on our data, the use of BTAs should be thus suggested at the time of BM detection since it reduces the incidence and delays the development of SREs.

In a different fashion from other tumors, SREs had no impact on the survival of our melanoma patients bearing BMs, including the nRT that was the most recurrent complication. The modest

---

**TABLE 5** | Univariate and multivariate analyses of baseline factors associated with OS in patients with BM from melanoma.

| Factors                                      | Effect tested | Univariate analysis |      | Multivariate analysis |      |
|----------------------------------------------|---------------|---------------------|------|-----------------------|------|
|                                              |               | HR                  | 95% CI | p         | HR                  | 95% CI | p     |
| Demographics                                 |               |                     |       |           |                     |       |       |
| Age (years) <55 vs. >55                      |               | 0.75                | 0.56–1.01 | 0.07    | 0.43                | 0.97–2.10 | 0.08  |
| Sex Male vs. Female                          |               | 1.41                | 1.05–1.89 | **0.03** | 1.43                | 0.97–2.10 | 0.08  |
| Baseline melanoma characteristics            |               |                     |       |           |                     |       |       |
| Histology SSM vs. Nodular                    |               | 0.61                | 0.42–0.89 |       | 1.43                | 0.97–2.10 | 0.08  |
| Mucosal vs. Nodular                          |               | 1.62                | 0.45–5.87 |       | 1.43                | 0.97–2.10 | 0.08  |
| Acral vs. Nodular                            |               | 0.56                | 0.23–1.34 | **0.07** | 1.43                | 0.97–2.10 | 0.08  |
| Uveal vs. Nodular                            |               | 0.96                | 0.38–2.39 |       | 1.43                | 0.97–2.10 | 0.08  |
| Others vs. Nodular                           |               | 0.79                | 0.34–1.85 |       | 1.43                | 0.97–2.10 | 0.08  |
| Melanoma site                                |               |                     |       |           |                     |       |       |
| Limbs vs. Trunk                              |               | 0.68                | 0.47–0.97 |       | 0.43                | 0.97–2.10 | 0.08  |
| Others vs. Trunk                             |               | 1.09                | 0.70–1.71 | **0.05** | 1.08                | 0.69–1.69 | 0.73  |
| Occult vs. Trunk                             |               | 0.65                | 0.41–1.03 |       | 0.43                | 0.97–2.10 | 0.08  |
| BRAF genotype                                |               |                     |       |           |                     |       |       |
| V600 vs. Wild type                           |               | 1.21                | 0.89–1.64 |       | 1.21                | 0.89–1.64 | 0.22  |
| NRAS genotype                                |               | 0.87                | 0.48–1.55 |       | 0.87                | 0.48–1.55 | 0.65  |
| Characteristics present at BM diagnosis      |               |                     |       |           |                     |       |       |
| Time of diagnosis                            |               | 1.08                | 0.73–1.59 |       | 1.08                | 0.73–1.59 | 0.68  |
| Localization of BM                           |               | 1.20                | 0.76–1.89 |       | 1.20                | 0.76–1.89 | 0.46  |
| Number of BM <5 vs. ≥5                       |               | 0.47                | 0.34–0.64 | **<0.0001** | 0.56                | 0.39–0.79 | **0.0013** |
| Calkaemia <ULN vs. >ULN                      |               | 0.54                | 0.23–1.26 |       | 0.54                | 0.23–1.26 | 0.06  |
| LDH levels ≤2 × ULN vs. >2 × ULN              |               | 0.42                | 0.30–0.60 | **<0.0001** | 0.49                | 0.34–0.71 | **0.0001** |
| Characteristics of metastatic disease        |               |                     |       |           |                     |       |       |
| Number of metastatic organs <3 vs. ≥3        |               | 0.55                | 0.40–0.77 | **0.0027** | 0.58                | 0.34–0.99 | **0.047** |
| Presence of visceral mts Yes with Brain vs. Yes w/o Brain | | 1.79 | 1.23–2.62 | **0.0002** | 1.33 | 0.88–2.01 | 0.18 |
| No vs. Yes w/o Brain                         |               | 0.42                | 0.21–0.87 |       | 0.42                | 0.21–0.87 | 0.06  |
| Treatment and SRE                            |               |                     |       |           |                     |       |       |
| Use of BTA Yes vs. No                        |               | 0.80                | 0.60–1.06 |       | 0.80                | 0.60–1.06 | 0.13  |
| SRE occurrence                               |               | 0.85                | 0.64–1.14 |       | 0.85                | 0.64–1.14 | 0.28  |
| Systemic treatment                           |               | 0.24                | 0.10–0.57 | **<0.0001** | 0.32                | 0.17–0.58 | **0.0002** |

BM, bone metastases; BTA, bone targeting agents; CHT, chemotherapy; ICIs, immune checkpoint inhibitors; LDH, lactate dehydrogenase; mts, metastases; SSM, superficial spreading melanoma; SRE, skeletal-related events; ULN, upper limit of normal; w/o, without. *Logrank test. Bold values are indicate to *p* < 0.05.*
The effect of RT on prognosis was previously demonstrated since the majority of patients showed lower survival and required further treatments to stabilize the skeletal complications (34). However, relevant data from our study concerns the protective effect in terms of survival for patients receiving RT and immunotherapy, independently of targeted agents. Therefore, it is conceivable...
that the OS benefit observed in these patients could have balanced the worse prognosis of those combining RT and targeted therapy without ICIs.

Benefits derived from the combination of RT and immunotherapy have been widely described as abscopal effect in melanoma (35) as well as in other malignancies (36) and reflects...
the regression of non-irradiated metastatic lesions at a distance from the primary site of irradiation. The putative synergic effect of RT and immunotherapy gained by our population is also suggested by the similar effect of immunotherapy vs. targeted therapy in the absence of RT, although both a defect of enrollment and radiological imaging as well as the retrospective nature of the analysis are probably limiting factors in our study. However, this combined effect also results from restoration of the immune cell activity in their systemic anticancer effect. In addition, it has been demonstrated that T cells may protect against bone loss (37), while IFN-γ counterbalances the osteoclastogenesis by interfering with RANK signaling (38). Finally, osteoblasts are also influenced by local T-helper 2 cells through parathormone (PTH) production (39), and a role of plasmacytoid dendritic cells has also been described (40).

CONCLUSION

In conclusion, we identified the number of BM as a novel prognostic factor in metastatic melanoma and observed that both reduced risk and delay in SRE development occur in patients early treated with BTAs. Despite the SREs not impacting on the survival of melanoma patients with BM, those receiving immunotherapy and requiring palliative RT obtained a major extent of benefit in terms of OS. Further prospective studies are thus needed to understand the effective role of immunotherapy in melanoma patients with BD. However, our preliminary observation suggests that palliative RT on symptomatic BMs may potentially reinforce the immune response and T cell activity in patients treated with ICIs, while the complementary activity of BTAs requires further investigation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

REFERENCES

1. Schadendorf D, Long GV, Stroakivski D, Karaszewska B, Hauschild A, Levcenko E, et al. Three-year pooled analysis of factors associated with clinical outcomes across dabrafenib and trametinib combination therapy phase 3 randomised trials. Eur J Cancer. (2017) 82:45–55. doi: 10.1016/j.ejca.2017.05.033
2. Petrelli F, Ardito R, Merelli B, Lonati V, Cabiddu M, Seghezzi S, et al. Prognostic and predictive role of elevated lactate dehydrogenase in patients with melanoma treated with immunotherapy and BRAF inhibitors: a systematic review and meta-analysis. Melanoma Res. (2018) 29:1–12. doi: 10.1097/cmr.0000000000000520
3. Cancer Genome Atlas Network. Genomic classification of cutaneous melanoma. Cell. (2015) 161:1681–96.
4. Selby HM, Sherman RS, Pack GT. A roentgen study of bone metastases from melanoma. Radiology. (1956) 67:224–8. doi: 10.1148/67.2.224
5. Tas F. Metastatic behavior in melanoma: timing, pattern, survival, and influencing factors. J Oncol. (2012) 2012:647684.
6. Zekri J, Marples M, Taylor D, Kandukurki K, McParland L, Brown JE. Complications of bone metastases from malignant melanoma. J Bone Oncol. (2017) 8:13–7. doi: 10.1016/j.bon.2017.08.003
7. Smyth MJ, Yagita H, McArthur GA. Combination anti-CTLA-4 and anti-RANKL in metastatic melanoma. J Clin Oncol. (2014) 34:e104–6. doi: 10.1200/jco.2013.51.3572
8. Abdel-Rahman O. Clinical correlates and prognostic value of different metastatic sites in patients with malignant melanoma of the skin: a SEER database analysis. J Dermatolog Treat. (2017) 29:176–81. doi: 10.1080/09546634.2017.1360987
9. Chu GC-Y, Chung LWK. RANK-mediated signaling network and cancer metastasis. Cancer Metastasis Rev. (2014) 33:497–509. doi: 10.1007/s10555-013-9488-7
10. D’Onoro S, Coleman R, Brown JE, Silvestris F. Metastatic bone disease: pathogenesis and therapeutic options: up-date on bone metastasis management. J Bone Oncol. (2018) 15:4.
11. Angela Y, Haferkamp S, Weischaup C, Ugggel S, Becker JC, Oberndorfer F, et al. Combination of denosumab and immune checkpoint inhibition: experience in

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MT, MM, VS, MP, AM, LP, FMo, LD, MG, AI, PQ, VF, RM, MCT, ER, ON, MO, AC, SQ, GP, PA, MV, SS, PF, RB, GR, PC, and GS contributed to the provision of study materials or patients. AT did the data collection. FMa contributed to the statistical analysis. FMa and MT did the data interpretation and manuscript writing. All authors are accountable for all aspects of the work, gave final approval of the manuscript, and contributed to revising the manuscript critically.

FUNDING

This work was funded by the “Precision Medicine” project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc. 2020.01652/full#supplementary-material

FIGURE S1 | (A) Percentage of patients experiencing different skeletal related events (SREs) in the study population. (B) Incidence of SREs according to the use of bone-targeted agents (BTAs). nRT, need for radiotherapy; **p < 0.01.

FIGURE S2 | Kaplan–Meier overall survival estimate in the study population (n = 290). mOS, median overall survival.

FIGURE S3 | Overall survival by number of bone metastases (BM). mOS, median overall survival; LDH, lactate dehydrogenase.

FIGURE S4 | Overall survival according to the use of bone-targeted agents (BTAs) mOS, median overall survival.
29 patients with metastatic melanoma and bone metastases. Cancer Immunol Immunother. (2019) 68:1187–94. doi: 10.1007/s00262-019-02353-5

12. Ahern E, Harjunpää H, Barkauskas D, Allen S, Takeda K, Yagita H, et al. Co-administration of RANKL and CTLA4 antibodies enhances lymphocyte-mediated antitumor immunity in mice. Clin Cancer Res. (2017) 23:5789–801. doi: 10.1158/1078-0432.CCR-17-0666

13. Ahern E, Smyth MJ, Dougall WC, Teng MWL. Roles of the RANKL-RANK axis in antitumour immunity—implications for therapy. Nat Rev Clin Oncol. (2018) 15:676–93. doi: 10.1038/s41571-018-0095-y

14. Sullivan RJ, Hamid O, Gonzalez R, Infante JR, Patel MR, Hodi FS, et al. Atezolizumab plus pembrozumab and vemurafenib in BRAF-mutated melanoma patients. Nat Med. (2019) 25:929–35. doi: 10.1038/s41591-019-0474-7

15. Ascierto PA, Ferrucci PF, Fischer R, Del Vecchio M, Atkinson V, Schmidt H, et al. Dabrafenib, trametinib and pembrozumab or placebo in BRAF-mutant melanoma. Nat Med. (2019) 25:941–6.

16. Long GV, Lebbe C, Atkinson V, Mandalà M, Nathan PD, Arance A, et al. The anti–PD-1 antibody spartalizumab (S) in combination with dabrafenib (D) and trametinib (T) in previously untreated patients (pts) with advanced BRAF V600-mutant melanoma: updated efficacy and safety from parts 1 and 2 of COMBI-I. JCO. (2020) 38:57. doi: 10.1200/jco.2020.38.5_suppl.57

17. Wilmutt JS, Long GV, Howle JR, Haydu LE, Sharma RN, Thompson JF, et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. Clin Cancer Res. (2012) 18:1386–94. doi: 10.1158/1078-0432.ccr-11-2479

18. Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res. (2013) 19:1225–31. doi: 10.1158/1078-0432.ccr-12-1630

19. Grassberger C, Ellsworth SG, Wilks MQ, Keane FK, Loeffler JS. Assessing the incidence and prognosis of skeletal-related events (SREs) and factors affecting SRE-free survival for nonsmall cell lung cancer patients with bone metastases. Tumour Biol. (2015) 37:1131–40. doi: 10.1007/s13277-015-3907-z

20. Barnes M, Tiwana MS, Kiraly A, Hutchinson M, Olson RA. Incidence of distal bone metastases in patients treated for palliative radiotherapy and associations with primary tumour types. J Bone Oncol. (2015) 4:107–9. doi: 10.1016/j.jbo.2015.10.002

21. Kawai AT, Martinez D, Saltus CW, Vassilev ZP, Soriano-Gabarrr M, Kaye JA. Incidence of skeletal-related events in patients with castration-resistant prostate cancer: an observational retrospective cohort study in the US. Prostate Cancer. (2019) 2019:5971613.

22. Bäuerle T, Komljenovic D, Merz M, Berger MR, Goodman SL, Semmler L. Does cilengitide deserve another chance? Lancet Oncol. (2016) 21:508–13. doi: 10.1016/j.lancot.2015.0377

23. Tucci M, Stucci S, Silvestris F. Does cilengitide deserve another chance? Nat Rev Drug Discov. (2015) 14:202–7. doi: 10.1038/nrd4429

24. Zhang D, Wang Z, Shang D, Yu J, Yuan S. Incidence and prognosis of bone metastases as imaged noninvasively using VCT, MRI and DCE-MRI in a longitudinal in vivo study. Int J Cancer. (2017) 128:2453–62. doi: 10.1002/ijc.30842

25. Tucci M, Mannavola F, Passarelli A, Silvestris F. Cancer treatment-induced bone metastases in patients treated for palliative radiotherapy and associations with primary tumour types. Tumour Biol. (2015) 36:1131–40. doi: 10.1007/s13277-015-3907-z

26. Ngwa W, Irabor OC, Schoenfeld JD, Hesser J, Demaria S, Formenti SC. Using immunotherapy to boost the abscopal effect. Nat Rev Cancer. (2018) 18:313–22. doi: 10.1038/nrc.2018.6

27. Stottle T, Forrster R, Schlampf I, Wolf R, Serras AF, Mayer A, et al. Stability, prognostic factors and survival of spinal bone metastases in malignant melanoma patients after palliative radiotherapy. Tumori. (2015) 101:156–61. doi: 10.3030/j.2500382

28. Ulas A, Bilici A, Durmali A, Tokluoglu S, Akinci S, Silay K, et al. Risk factors for skeletal-related events (SREs) and factors affecting SRE-free survival for non-small cell lung cancer patients with bone metastases. Tumour Biol. (2015) 37:1131–40. doi: 10.1007/s13277-015-3907-z

29. Mannavola E, Mandala, Todisco, Sileni, Palla, Minisini, Pala, Morgese, Di Guardo, Stucci, Guida, Inubini, Quaglino, Ferresi, Marcomini, Tucci. Copyright © 2020 Mannavola, Mandala, Todisco, Sileni, Palla, Minisini, Pala, Morgese, Di Guardo, Stucci, Guida, Inubini, Quaglino, Ferresi, Marcomini, Tucci. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.}