

*Review*

# Skeletal Metastases of Unknown Primary: Biological Landscape and Clinical Overview

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**Abstract:** Skeletal metastases of unknown primary (SMUP) represent a clinical challenge dealing with patients diagnosed with bone metastases. The management have improved significantly in the past years, however fraught with lack of evidences, approach to these patients held out hope for more systematic and tailored treatment—and some patients can achieve impressive gains. Nevertheless, in real-life practice the outlook at the beginning of the take in charge of SMUP is decidedly more somber. An incomplete translational relevance of pathological and clinical data on the mortality and morbidity rate has had unsatisfactory consequences for SMUP patients and their physicians. We examined several approaches to confront the available evidences and highlighted three key points that emerge. The characterization of the SMUP biologic profile is essential to drive clinical decisions, integrating genetic and molecular profile into a multi-step diagnostic work-up. Nonetheless, pragmatic investigation plan and therapy of SMUP cannot follow a single template; it must be adapted to different pathophysiological dynamics and coordinated with efforts of a systematic algorithm and high-quality data derived from statistically powered clinical trials within. This review argues that greater efforts are required to face the unmet need dealing with SMUP patients in oncology. Finally, we provide an original functional network analysis, identifying novel therapeutic targets.

**Keywords:** skeletal metastases of unknown primary; SMUP; bone metastases; unknown primary tumor; bisphosphonates; bone markers; tumor microenvironment

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## 1. Introduction

Skeletal metastases of unknown primary (SMUP) represent enigmatic rare metastatic tumor entity without anatomic primary sites identified. Cancer of Unknown Primary (CUP) accounts for 2% of all cancers characterized by an aggressive clinical outcome and poor response to chemotherapy. [1-2] Although almost all tumors can metastasize to the skeleton during their natural history, epithelial cancers are characterized by a particular propensity for this type of dissemination. However, modern diagnostic methods, including molecular investigations are not always sufficient to identify the primary site of the neoplasm in order to guide a targeted treatment. Multiple involved body regions are identified in more than 50% of individuals with CUP. [3] Skeleton is the third most common site of metastatic cancer after lung and liver, often representing the onset of unknown primary tumor in 8-23% of patients with poor prognostic clinical features. [4-8]. Lung cancer is the most frequently identified primary tumor (25-67%) across all literature data. The other most frequent primary malignancies are multiple myeloma, prostate cancer, lymphoma, kidney, gastrointestinal tumor and breast cancer. However, the primary site of bone metastases remains often unidentified despite diagnostic investigations and autopsy examination (Table 1).

Literature data reported that the spine is the most common site of skeletal metastasis of unknown primary (SMUP), followed by the pelvis and extremities bones. [5] Takagi et al. reported a large retrospective analysis of 286 SMUP showing that 2/3 of multiple bone lesions without differences in localization, while solitary bone metastases occur in 32.5% site. Twenty-seven percent of patients presented triple or more area of localization. The number of bone metastatic sites is related to prognosis of patients with 39 months for solitary bone metastasis and 16 months for multiple sites (7 months for triple or more area). [9]

In the above-mentioned study, the primitive site after the diagnostic investigations was diagnosed in almost 89% according to other literature data. [9] In the leftover 11% of cases the primary site remained unknown. The median overall-survival (OS) of confirmed SMUP was 11 months compared to 20 months of the overall population, highlighting that bone metastases from unknown tumor represents a poor prognostic features. [9] Of note, Hemminki et al. correlated a site-specific survival from 9,306 CUP patients with the metastatic site. The study deemed a shorter median OS of 203 patients with bone metastases (3 months). [8] Clinical evidences are seldom available and the presented data are often obtained from case series or retrospective study with insufficient characteristics homogeneity. Nonetheless, a comprehensive overview of published data is summarized in Table 1. Additional unfavorable prognostic factors for CUP comprise male gender and adenocarcinoma histotype.

**Table 1.** Bone metastases of unknown primary data overview and corresponding identified primaries and survival implications.

Authors	SMUP at diagnosis	Identified Primary Cancer	Number and Site of Primary Cancer identified	Confirmed SMUP	mOS confirmed SMUP (months)
Simon and Karluk [10]	n=12	n=6 (50%)	Kidney (3), lung (2), others (1)	n=6 (50%)	NA
Simon and Bartucci [11]	n=46	n=20 (44%)	Lung (7), kidney (6), breast = prostate (2), ovarian /thyroid/liver (1)	n=26 (56%)	NA
Nottebaert et al. [12]	n=51	n=33 (65%)	Lung (17), others (16)	n=18 (35%)	11.1
Shih et al. [13]	n=52	n=28	Lung (9), liver (8), kidney (5), prostate (3),	n=24	11

		(54%)	thyroid (2), rectum (1)	(46%)	
<b>Rougraff et al. [14]</b>	n=40	n=34 (85%)	Lung (23), kidney (4), breast/colon/liver/ bladder (1), others (3)	n=6 (15%)	NA
<b>Jacobsen et al. [15]</b>	n=29	n=24 (83%) (2 patients postmortem)	Lung (11), prostate (3), breast/lymphomas (2), kidney/ovary/pancreas/stomach/small intestine carcinoid/retroperitoneal rhabdomyosarcoma (1)	n=5 (17%)	12
<b>Katagiri et al. [16]</b>	n=64	n=59 (92%)	Lung (23), prostate (11), breast/liver (5), others (15)	n=5 (8%)	5
<b>Vandecandelaere et al. [17]</b>	n=129	n=84 (65%)	Lung (36), prostate (17), kidney (15), breast (9), stomach (2), bladder/colon/testis/pancreas/liver (1)	n=45 (35%)	6
<b>Destombe et al. [18]</b>	n=152	n=94 (88%)	Lung (37), prostate (26), breast (20), urinary system (11)	n=13 (12%)	NA
<b>Iizuka et al. [19]</b>	n=27	n=26 n= (96%)	Myeloma (7), lymphoma (3), lung (6), prostate (4), kidney/thyroid/liver/pancreas/stomach/oesophagus (1)	n=1 (4%)	NA
<b>Hemminki et al. [8]</b>	n=501	n=256 (60%)	Lung (128), urinary (29), prostate (16), breast (14), colon (12), pancreas/gastrointestinal (10), liver (9), biliary system (4), stomach (3), mediastinum (2), others (19)	n=203 (40%)	3
<b>Takagi et al. [9]</b>	n=286	n=254 (89%)	Lung (72), myeloma (41), prostate (26), lymphoma (23), kidney (18), liver (12), breast (12), gastric (10), pancreatic (10), thyroid (9), bile duct/colon (6), esophageal (3), others (6)	n=32 (11%)	11

SMUP, skeletal metastasis of unknown primary

## 2. Cancer cell homing to the bone marrow: bridging the gap between the malignancy and the neighborhood

The biological mechanisms underlying the tumors of unknown origin are still poorly understood. The identification of common etiological factors for a heterogeneous group of neoplasia, including different histotypes represents a clinical challenge. There are two main theories to explain the biology of UPT. Remarkably, most of the primary cancers in autopsy series measured less than 1 cm. The burned-out theory hypothesized that the cancer cells involute irrespective from the metastasis development by a complex interplay between the tumor microenvironment and the molecular cancer features. Alternatively, it has been proposed the existence of a peculiar progenitor cells that might be the cell of origin of the metastatic site. [20] These “rest cells” may undergo an incomplete

migration to the designated tissue. Moreover, some histotypes such as germ cell, gonadal, thymic and lymphomas can physiologically arise anywhere in the different body compartment. Next, the elucidation of spatial and clonal heterogeneity shed more light into the genomic landscape. These genetic lesions can determine specific gene expression pattern. Intriguingly, tumor-initiating cells have the potential to trans-differentiate into multiple phenotypes. [21-24] Notwithstanding, there are few studies and limited cases report. The observation of an increased incidence of metastatic primitive adenocarcinoma of unknown origin in homozygous twins affected by primary immunodeficiency linked to the X chromosome led to postulate the presence of genetic abnormalities characteristic of UPT and their role in the process of metastasis [25-26]. The complete or partial loss of the short arm of chromosome 1, for example, has been found in several cases of neoplasia of unknown primitiveness and seems to correlate with a particular capacity of the tumor to metastasize at a very early stage of its natural history, when it is not clinically detectable. A study published in 2010 based on the analysis of the Swedish Family Cancer Database support that cancer of unknown primary (CUP) may have a genetic basis.[27] The analysis showed that 2.8% of occult primary cases were familial (i.e., a parent and offspring were both diagnosed with occult primary cancer). In addition, CUP was associated with the occurrence of kidney, colorectal cancers in families, suggesting that these types of tumors are often the primary sites of the disease. [27] The incidence and the clinical relevance of the presence of overexpression of p53, bcl-2, c-myc, ras and HER-2 in neoplasm of occult primitive site is not determined and the data reported in the literature are not comprehensive. [28-32]

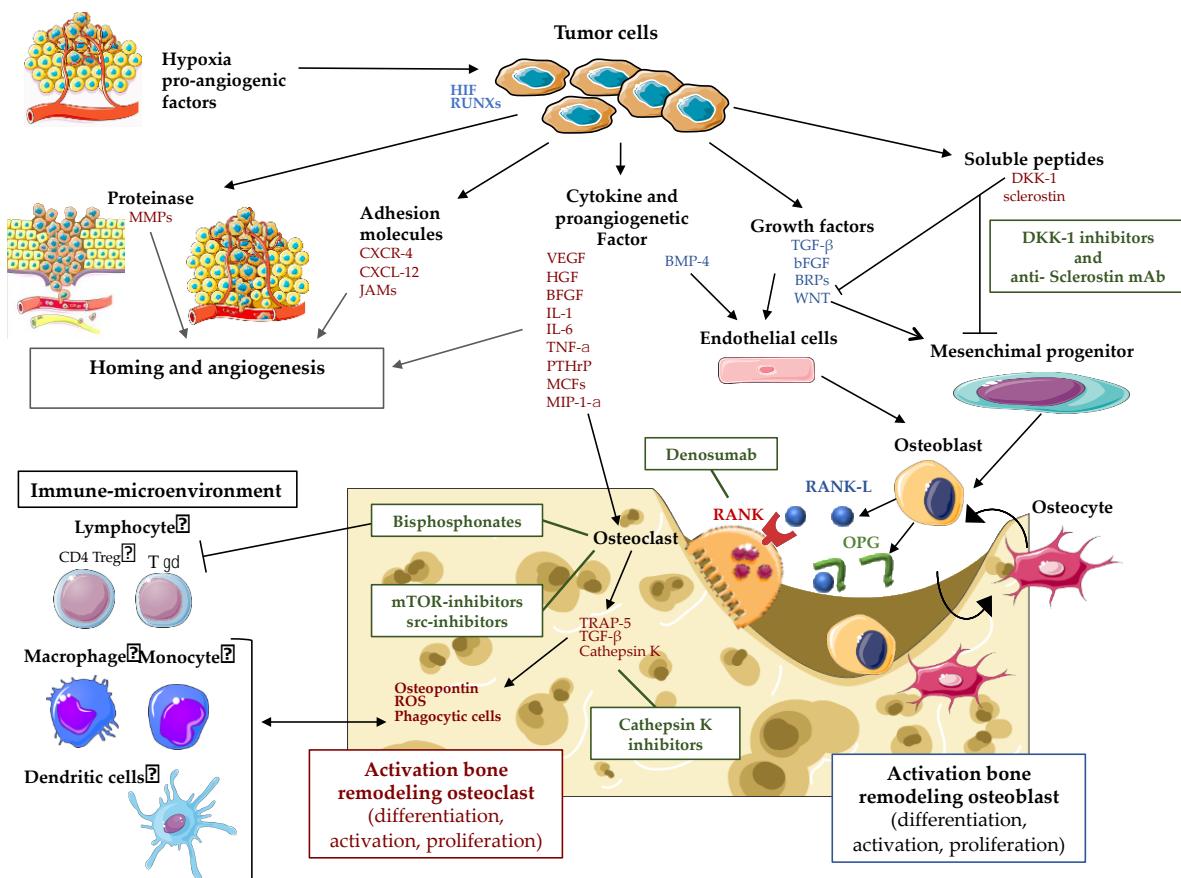
Different approaches have been proposed in order to resolve the biologic complexity underlying the pathophysiologic step to bone dissemination.

The first of the steps in the cancer-spread process to the bone is the homing to the marrow microenvironment throughout the bloodstream of the tumor cells, via the neo-angiogenesis process, through permissive bone marrow endothelial cells. [33,34]. Remarkably, a prone microenvironment is involved in the cancer vicious cycle, educating and hijacking the tumor niche to be colonized, throughout a neoplastic permissive milieu [35,36]. Ancillary to cancer intrinsic mechanisms, such peri-neoplastic infiltrate actively primes drug-resistance mechanisms both in solid and haematologic neoplasms expressing an osteotropic phenotype. [37,38] Therefore, a number of molecular actors have been considered to drive the neoplastic cells to the bone milieu, including karyotypic non-random abnormalities (i.e. t(11;22), t(15;19) [39], i12p, t(X;18), del11p, and plasma membrane protrusions and cytoskeleton system [40-41], adhesion molecule system, such as CXCL12/CXCR4 [42], junctional adhesion molecules in osteotropic tumour [43-45], focal adhesion kinases [46-48] and vascular and immune-microenvironment interactions. [49-52]

Based on the gained knowledge, mesenchymal cell numbers, their immune modulatory effects and their interactions with matrix molecules play a pivotal role in establishing a critical dependency between cancer cells and the tumor niche. Breast and prostate cancer cells home to the bone marrow, where they presumably hijack the hematopoietic stem cell niche [53], and develop metastatic lesions, are well known tumors that educate the metastatic milieu in order to make it prone to guide the dissemination process. [35] Nonetheless, the premetastatic niche remain elusive in the context of tumor of unknown origin. Mesenchymal stromal cells (MSCs) showed to be permissive when decreased in number pharmacologically in cancer cell homing and growth of bone metastases,

enhancing cancer cell homing to the bone marrow in mice. [54] In the complex relationship between the tumor cells the organ specific vasculature [55] and its microenvironment, an insufficient oxygen supply (hypoxia) is a prominent feature in various pathological processes, including tumor development and metastasis. [56,57] The central mediators during hypoxia are hypoxia inducible factors (HIF) whereas their downstream effects are tightly regulated by oxygen-dependent HIF prolyl hydroxylases (PHDs). PHD2 plays a central role during different stages of tumor development, whilst this oxygen sensor is also essential during bone mineralization, and normalization of the endothelial barrier in the bone (marrow) after stress. [58] Moreover, the perivascular niche directly influences the equilibrium between dormant tumor cells, their retention and cancer reactivation. [35] In more details, the cancer dissemination to the bone shares common steps comprising various stages. They include cell proliferation at the site of the primitive outbreak, penetration into blood and lymphatic vessels, anchoring an anatomical cell away from the primitive outbreak to the basal membrane, and the parenchima infiltration of the new anatomical site and proliferation. The process is actively supported by neoangiogenesis *via* cytokines such as "vascular endothelial growth factor" (VEFG), "basic fibroblast growth factor" (bFGF), and "transforming growth factor-alpha" (TGF- $\alpha$ ). The neo-vessels are structurally fragile and different from normal ones in that the basal membrane and extracellular matrix are easily destroyed by proteases produced by cancer cells, such as metalprotease (MMP), cathepsine D and plasminogen activator, resulting in extravascular passage of cancer cells. Cancer cells also modify adhesive capabilities and their mobility through their own factors, "hepatocyte growth factor/stromal factor" (HGF/SF) and "insulin growth factor-II" (IGF-II), or by matrix proteins such as vitronectin, fibronectin, laminin, type IV collagen, as well as host-secreted factors such as "insulin growth" (IGF-I), interleukin 6 (IL-6) and histamine. Finally, the location of bone-level cancer cells takes place in response to chemotactic stimuli by the cellular component activated by type I collagen, osteocalcin or cytokines such as "transforming growth factor-beta" (TGF- $\beta$ ) and "platelet-derived growth factor" (PDGF). The high affinity for bone by cancer cells is due both to high vascularization, and because the bone microenvironment frees up factors that promote their survival and proliferation. Cancer cells, reached at bone marrow level crossing the wall of sinusoids, invade the matrix and, once reached the endostal surface, stimulate osteoclastogenic activity and proliferate with the formation of metastases with prevailing osteolytic or osteoblastic development. These extremes cover the spectrum of neoplastic bone remodeling (Figure 1). [35,59]

The knowledge of the underlying mechanism of bone dissemination already prompted an extensive clinical investigation. Nonetheless, skeletal metastases remain a poor prognostic event with huge morbidity and mortality impact. [7,8]



**Figure 1.** Bone metastasis physiopathology: implication of cancer cell-microenvironment interactions and therapeutic target. HIF, hypoxia-inducible factors; RUNXs, Runt-related transcription factors; MMPs, matrix metallopeptidases; CXCR-4, chemokine receptor 4; CXCL2, chemokine ligand 12; JAMs, junctional adhesion molecules; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; BFGF, basic fibroblast growth factor; IL, interleukins; PTHrP, parathyroid hormone-related protein; MCFs, macrophage chemotactic factors; MIP-1- $\alpha$ , macrophage inflammatory proteins 1-alpha; BMP-4, bone morphogenetic protein 4; TGF- $\beta$ , transforming growth factor beta; bFGF, basic fibroblast growth factor; BRPs, bone resorptive proteins; WNT, Wingless-related integration site; DKK-1, Dickkopf WNT signaling pathway inhibitor 1; ; RANK, receptor activator of nuclear factor-kappaB; RANKL, receptor activator of nuclear factor-kappaB ligand; OPG, osteoprotegerin; TRAP-5, tartrate resistant acid phosphatase 5; mTOR, mammalian target of rapamycin.

### 3. Clinical Management for Skeletal Metastasis of Unknown Primary: diagnostic work-up and therapeutic approach

Skeletal metastases represent a clinical challenge approaching the diagnostic work-up for patients suffering from CUP. Clinical judgment and approaches borrowed from CUP represent reasonable pragmatic alternative and a valid paradigm to design statistically powered clinical study. Indeed, minimal basic work-up for SMUP overlaps with overall CUP having to include medical history, physical examination, basal blood and biochemical analysis (including bone metabolism), and CT

scans of thorax, abdomen and pelvis. Integrative investigation must be selected based on clinical and radiological indication such as endoscopy and serum assessment of prostate-specific antigen (PSA),  $\alpha$ -fetoprotein (AFP),  $\beta$ -human chorionic gonadotropin ( $\beta$ -HCG), chromogranin to exclude "treatable" or susceptible hormone therapy can drive a site-specific treatment. However, the tumor biopsy remains a pivotal point in the SMUP diagnostic process providing tissue suitable for light-microscopic and immunohistochemical examination and molecular characterization. [60,61] Further consequential detailed practical pathological primary and specific markers are summarized in Figure 2. Additional molecular investigation such as gene-expression profiling (GEP) assay hold the promise to deeper characterize the underlying malignancies able to guide a tailored therapy and to identify the tissue of origin in patients with occult primary cancers. [20,62-64] ICH and GEP offer a similar range of accuracy in tumor classification (approximately 75%). [65] Nonetheless, the quality of evidences available is not robust enough to allow stringent recommendation and selected classifier assays. Approaching the differential diagnosis of suspected adenocarcinoma PSA and mammography are two effective screening procedures for men and women, respectively. Breast MRI and ultrasound can efficiently complete non-diagnostic screening procedures. When painful lesions or bone scan positivity elicit further investigations, additional stepwise bone scanning is recommended, including, X-ray, MRI or contrast-enhanced CT. These procedure hold the potential to progressively clarify weigh-bearing imaging area etiology. [65]

Literature data show that the minimal basic work-up allows to identify the primary tumor in about half of the cases of SMUP. In particular, Takagi et al. reported that CT scan discovered 30% of SMUP. Bone biopsy and origin examination identify another 19% and 14% of primary site, respectively. Bone marrow puncture and PET add only 1% of diagnosis per each. [9] Despite the improvement of diagnostic methods, the primary site is not always identified.

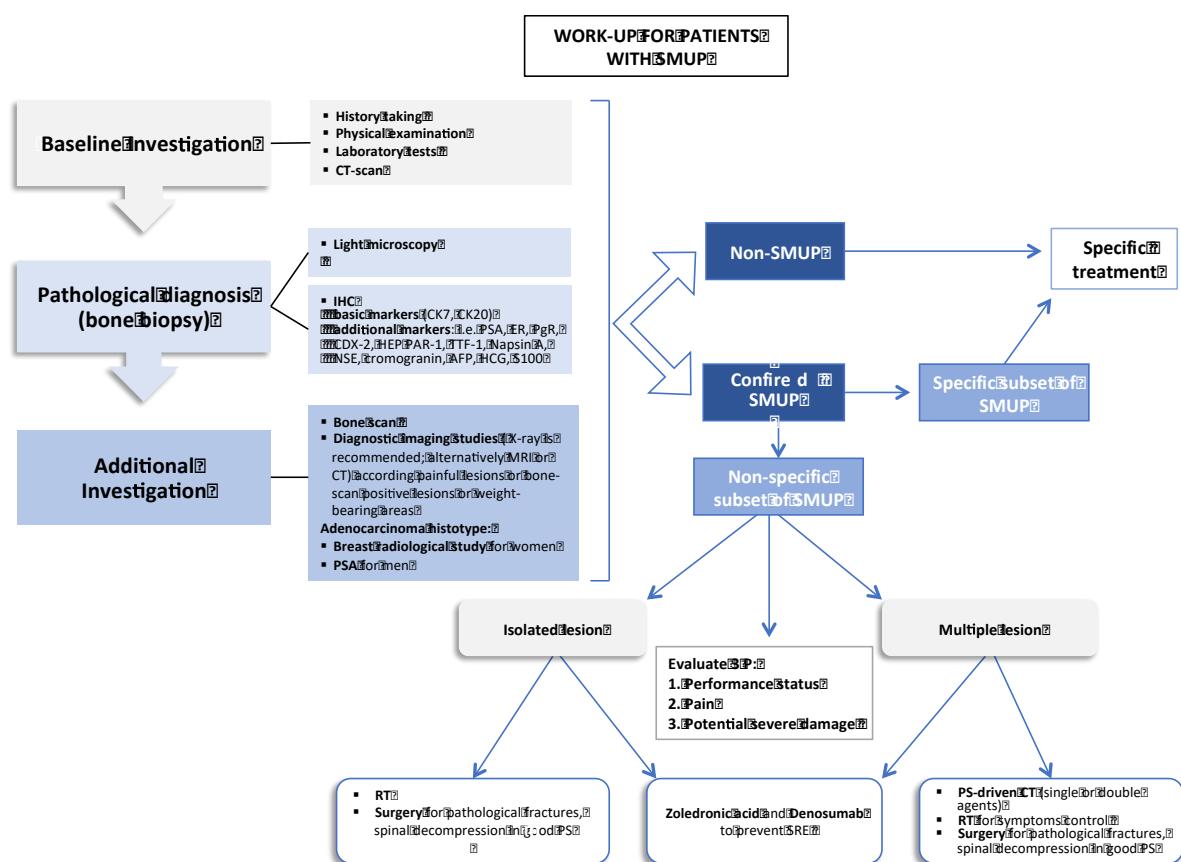
An unmet medical need of real life clinical practice of identifying the cancer primary deeply impact the choice of therapy. Therefore, in order to face this challenging task, gene-expression signatures investigation along with pathological characterization has been employed with the goal of gain an enhanced clinical survival. [20,60] In frame of this thinking, a site-specific therapy integrated with a GEP guided treatment, showed to improve the clinical outcome. [61,66]

Ensues exclusion of a non-CUP and a specific subset of CUP deserving site-oriented therapy. Next, risk-driven therapy represents the cornerstone of the clinical-judgment directed treatment approach. Therefore, CUP patient stratification into prognostically favorable and unfavorable allows differential clinical management of poorly differentiated cancer of the midline, papillary peritoneal cavity cancer of adenoma subtypes in female, women with adenocarcinoma with isolated involvement of axillary lymph nodes, and cancer of squamous type involving neck nodes. [67-69] Nonetheless, more than 80% CUP are deemed to be prognostically unfavorable, showing uncertain chemotherapy response. [70]. In the last years, prognostic scores able to homogeneously categorize CUP patients have been explored, including performance status (PS) evaluation and serum biomarkers sharing common features with known primary bone metastatic cancers (i.e. LDH and albumin), [70-74]. Bone metastasis *per se* represent a prognostic factors for survival of CUP [7,8]. The actual impact of those parameters showed to detect significant differences in terms of clinical outcome in SMUP with low PS and biomarker (with about 1 year of life expectancy) compared to poor prognosis features, such as PS>2 and elevated biomarkers level. The latter group of subjects could profit the most from palliative approach, given a mOS of 4 months. [2] An additional independent prognostic factor is the number of bone lesions that can also imply a differential therapy management constituted by integrated modality of radiotherapy and surgery when either single bone lesion or painful clinical scenario are present. Remarkably, risk fracture implies an

additional fundamental criterion to guide a multistep integrated management. Conversely, cytoreduction along with PS stratification, can guarantee a more effective treatment in bone diffuse involvement (Figure 2).

Given the poor effectiveness of chemotherapeutic agents and the results derived from metanalysis, cytoreduction should be undertaken only in symptomatic disseminated disease, when PS allow the aforementioned approaches. In all cases the goal of treatment should be represented by an improvement in the quality of life and symptoms control with the maximal minimization of the toxicity profile. In the absence of standardized high-quality evidences, the regimen of choice should be based on the histopathological data. In more details, platinum-, taxan-, gemcitabine- and irinotecan- based regimen constitute the backbone of combination therapy with proven efficacy. [75-77]

Consistent and high quality clinical evidence constitute an unmet clinical need, due to the lack of prospective controlled randomized trials designed to gain pragmatic insights from both clinical study and real-life practice.



**Figure 2.** Stepwise- clinical management of the patient with skeletal-metastasis of unknown primary (SMUP) suitable to individualized approaches.

#### 4. Bisphosphonates and bone disease modifying agents

A milestone in the clinical management of skeletal related events (SRE) in cancer, suitable for SMUP too is represented by bisphosphonates and SRE directed therapies. Bisphosphonates are effective in increasing survival and reducing skeletal complications including hypercalcemia, delaying the time

of appearance of skeletal complications and reducing bone pain in patients with metastases, particularly intravenous ones. [78-82]

Denosumab is a viable subcutaneous alternative and a preferred option where it is possible that it has shown moderate greater efficacy than Zoledronic acid in reducing skeletal events (excluding hypercalcemia). [83,84]

Several molecules with direct or indirect effects on the bone metastases progression of solid tumors are currently investigated in several clinical trials. Some of these molecules are able to act directly on the bone resorption process, targeting specific bone cells such as osteoclasts, osteoblasts, osteocytes or molecular pathways that regulate the function of these cells. In this group besides denosumab inhibitors of the endothelin 1 receptor (also expressed by osteoblasts), the inhibitors of cathepsin K (produced by osteoclasts, but also by metastatic tumor cells to the bone), drugs that interfere with the wnt/dkk1 pathway (which, among other things, regulates the function of osteoblasts and immune-cells), src inhibitors (non-receptor tyrosine kinases downstream of the rank receptor and which helps regulate the resorbing function of osteoclasts, but also the function of destruction of the bone matrix by the tumor cells are the main target addressed to date. [59,85-89]

The optimal treatment duration has not been established. Clinical trials with zoledronate and ibandronate have shown a benefit for a treatment period of at least 2 years. [90-93]

Moreover, irrespective from the appearance of a skeletal event, the continuation of Zoledronic acid therapy has been demonstrated to hold a statistically significant reduction in the occurrence of subsequent events. [94]

Prolonged treatment with prolonged Zoledronic Acid lasting more than two years, besides being associated with a low SRE rate, is characterized by a good safety profile. [95]

Winters J.P. et al presented a retrospective analysis showing that the risk of SRE on a cohort of 92 patients with bone metastases from solid tumors and with Multiple Myeloma, treated with Pamidronate or Zoledronate for over 2 years (average duration of 36 months), was greater in the first two years of treatment. [96] The toxicity profile was acceptable for both drugs, regardless of the duration of treatment.

The therapy duration is extremely heterogeneous, varying in a range from 12 weeks (in a first phase of study of denosumab) [97], to 96 weeks (for bisphosphonates) [90, 98-100] up to about 34 months in phase III studies on denosumab in breast and prostate cancer [101,102]. These studies have not provided compelling evidences regarding the optimal duration of treatment without elucidating the comparison between continuous or interrupted therapy.

Patients suffering from breast or prostate cancer, enrolled in phase III studies on denosumab, have been proposed to participate in a subsequent study on long-term treatment. Patients chose whether to continue with a further two years of treatment with denosumab or proceed with two years of follow up. A total of 948 patients accepted the continuation of therapy reaching a maximum duration of treatment of about 5 years in patients with breast cancer and about 5.6 years in patients with prostate cancer. The results of the study showed a good tolerability in the treatment with denosumab both after prolonged exposure and after switching to denosumab after previous Zoledronic acid therapy. [103]

In consideration of the aforementioned evidence, in the absence of specific data and a statistically powered clinical studies able to identify an optimal treatment period, the currently recommended duration for bone target therapy is at least 2 years suspending treatment in case of deterioration of

Performance Status. Continuation of treatment beyond the two-year limit is however recommended (especially in the case of denosumab therapy), taking into account the risks of developing skeletal events, tolerability and general clinical conditions of the patient. [104-107]

## 5. Role of bone turnover markers in diagnosis and therapy response with inhibitors of bone resorption evaluation

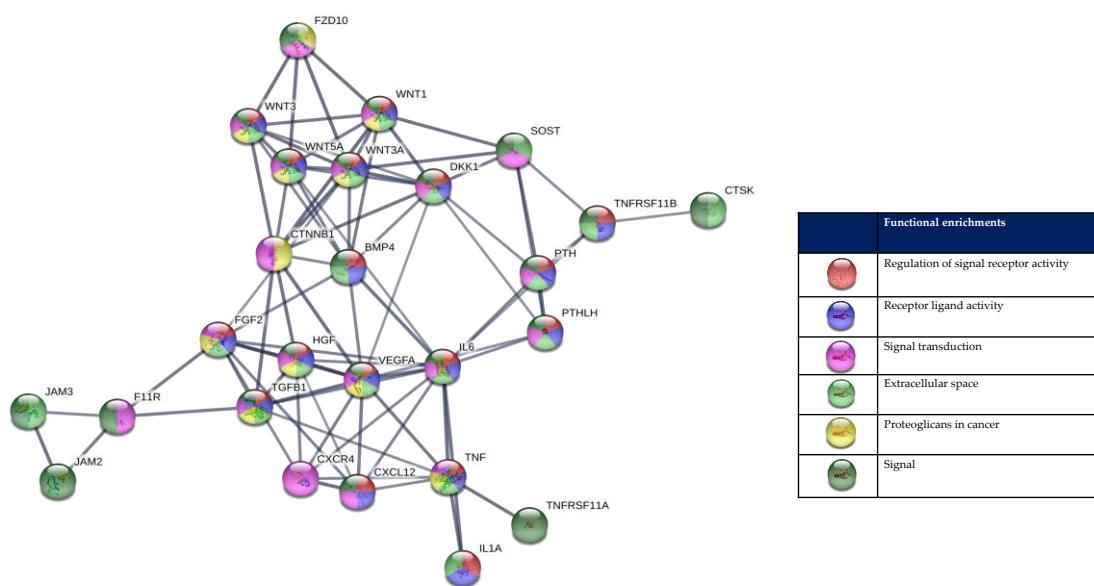
As markers of bone turnover, it is advisable to dose "bone turnover markers", defined as degradation products of type I collagen specific to bone tissue, circulating after osteoclastic digestion or pro-collagen type 1 cleavage products, expression of the neoformative synthesis activity of osteoblasts. Alkaline phosphatase (bone isoenzyme) is an 'enzyme, expression of osteoblastic activity, such as osteocalcin. PTH, calcemia, vitamin D are not strictly markers of osteoblastic and osteoclastic cellular activity. The recent important role reserved for osteocytes has led to the emergence of new potential makers such as DKK1 and sclerostin, cathepsin K and TRAP -5, very promising but still reserved for research activities. The bone turnover markers nowadays considered gold standard are P1NP (pro-type collagen) expression of neoformation activity and CTX (terminal p-type peptide of type 1 collagen) for osteoclastic resorbing activity. [108] Their clinical use in metabolic diseases of the skeleton, primarily postmenopausal osteoporosis, still finds some limitations and is not recommended by the guidelines. [109]

A potential use of bone turnover markers is to be able to drive diagnostic process investigation of SMUP. Numerous studies have shown, in a cross-sectional manner, the association between high levels of CTX and NTX and the presence of bone metastases. However, a specific marker with sufficient discriminated power was not highlighted. It should be considered that in general patients with bone metastases have a very high bone turnover for many reasons (D hypovitaminosis, age, hormonal therapy, etc.). Therefore, the specificity and accuracy of the biomarkers can be affected by non-neoplastic sources different from the metastatic site. Even in combination with diagnostic methods their sensitivity is not high, and their clinical use is not indicated. [110-111]

A potential clinical use of bone turnover markers is the prediction of skeletal complications of bone metastases (SRE or SSE) since markers level can be correlated to the extent and activity of the metastases themselves. From meta-analysis and post-hoc analysis of RCT with zoledronic acid, some markers such as NTX and ALP bone have been shown to be able to predict not only SRE but also the progression of metastatic bone disease and survival. This resulted both with the basal values (in the absence of therapy) and based on the response (after 3 months) to the treatment with zoledronic and denosumab. [112] This data finds several confirmations in the literature. For example, the levels of bone ALP and also of P1NP correlate with the progression of metastatic disease at bone level and with survival in urologic cancer, such as prostate cancer. [113] To confirm this, ALP and NTX were associated with survival in patients with bone metastases due to prostate cancer in the hormone-resistant phase. [114] Similar data have also been reported in other neoplastic histotypes and in tumor of unknown origin. [115,116]

Another potential use of bone turnover marker could be to monitor the effectiveness of zoledronic therapy. The reduction of bone turnover markers during therapy with resorption inhibitors can be used as a surrogate to evaluate efficacy on pain, and on the risk of SRE. A failure to normalize bone turnover during therapy with bone resorption inhibitors could indicate an optimal sub-effect of the same therapy. In the studies of Fizazi et coll. Studied women with breast cancer who did not

normalize bone turnover with zoledronic acid were randomized to receive denosumab or continue with zoledronic acid. In the passage to denosumab a rapid and complete normalization of the NTX values was obtained with an advantage in terms of reduction of SRE. [117] Despite clinical trial in patient with unknown origin are scanty, in the patient with SMUP, the baseline value of the bone metabolism markers and their decrease during treatment might be used with caution as a predictor of SRE, bone progression and survival. The integration of the current knowledge of the bone metastasis physiopathology along with the systematic analysis of the known molecular interaction can reasonably drive future clinical studies aimed to theragnostic target identification (Figure 3). [118-119]



**Figure 3.** Protein–protein interaction network generated with STRING v11.0 (<http://string-db.org>) database of proteins identified by literature data supervised proteomic analysis. Sector colors represent the predicted functional associations. Analysis with showed that out of the 26 molecules included in the dataset supervised functional enrichment network, 17 proteins were involved in (GO: 0010469, red sectors), 17 proteins were linked to receptor ligand activity (GO: 0048018, blue sectors), 19 proteins were related to extracellular space (GO: 0005615; light green sectors), 11 proteins were related to proteoglycans in cancer (hsa05205; yellow sectors), 19 proteins were enriched in the response to signal transduction (HAS-162582: 0006950, purple sectors) and 21 proteins were comprised in signal UniProt Keywors (KW-0879; dark green).

## 6. Conclusions

SMUP represents a diagnostic and therapeutic unmet clinical need, given the scanty evidence-based indication available and the tight underlying relationship between the challenge of diagnostic workup and the treatment choice. Besides surgical and radiotherapeutic approach, the systemic option available for patients diagnosed with SMUP is represented by bone modifying agents and chemotherapy. Nonetheless, nowadays it is mandatory to encourage patient enrollment in clinical trials involving the characterization of the genetic and molecular profile of SMUPs and to integrate standard chemotherapy associated with SRE therapy with molecular target agents.

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