

Review Article

Osteosarcoma Metastasis: An Unmet Clinical Need and Unique Drug Development Opportunity

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ABSTRACT

The great majority of cancer deaths result from drug-resistant recurrence that has spread from the initial tumor. Consistent with this, osteosarcoma patients will often develop metastatic disease following primary tumor resection and adjuvant chemotherapy. This latency can be explained by the presence of undetected, non-dividing disseminated tumor cells (“DTCs”) that have developed mechanisms of long-term stress adaptation and dormancy to enable their survival until re-awakened to form overt metastasis. Importantly, these mechanisms are unique and specific to the biology and the circumstances of these DTCs, namely, the conditions of the microenvironment at the secondary sites. We suggest the term “metastatic endurance” to encompass a combination of the following features of DTCs at secondary sites: stress adaptation, survival, and dormancy. To date, little progress has been made in the area of DTCs-directed therapies. Our unique perspective on metastasis biology (i.e., metastatic endurance) both arises from, and delivers, *in vitro* and *ex vivo* assays that enable efficient drug discovery and preclinical efforts that target DTCs. Pet dogs and humans share the biology of osteosarcoma metastasis and late recurrence following stress adaptation and dormancy of DTCs. Pet canine osteosarcoma drug clinical trials therefore inform human osteosarcoma trials, enabling novel potential therapies to advance with the support of a community receptive to metastasis-prevention clinical trials. In addition to the extreme importance of finding better drugs for osteosarcoma patients, this disease serves as an outstanding “model” for recurrence in other cancers and provides a unique opportunity to develop drugs that target metastatic-progression for various cancers.

Keywords: Circulating tumor cells, disseminated tumor cells, tumor dormancy, metastatic endurance, Comparative oncology

Osteosarcoma Metastasis: The Unmet Clinical Problem

Similar to other non-central nervous system solid tumors, the primary unmet therapeutic need for osteosarcoma patients is more effective therapies to prevent or treat metastatic progression. Despite successful and curative therapy of the primary tumor and adjuvant chemotherapy, long-term outcomes for osteosarcoma patients continue to be impacted by metastatic progression, most often to the lung. Most troubling is the fact that no substantive improvements in long-term outcomes have been seen in patients for over four decades [1]. Currently, approximately 35% of osteosarcoma patients who present with localized disease will progress to develop what will likely

be fatal metastatic disease. Furthermore, in patients who present with metastatic disease, over 80% will progress and succumb to metastasis [2-4].

Despite a clear benefit from adjuvant chemotherapy, it is unlikely that further intensification of chemotherapy approaches that target rapidly dividing cells will deliver further improvements to patients. It is most likely that major improvements in therapy will require a greater understanding of the biology of metastasis and an innovative translational drug development path.

Furthermore, as presented below, osteosarcoma likely shares features of metastasis biology with other cancers. Therefore, drugs that are effective in targeting certain pathways and targets related to

metastatic progression and recurrence in osteosarcoma may also be effective for other cancers. We hypothesize that new understandings and an innovative drug development path in osteosarcoma will serve to deliver much needed therapeutic leads for osteosarcoma patients, as well as for patients with other types of cancers.

The Biology of Metastasis

Metastasis is defined as the dissemination of neoplastic cells from the primary tumor to distant discontinuous secondary (or higher order) sites, where they proliferate to form a macroscopic mass [5]. Implicit in this process is the presence of a primary tumor. Metastases are not a direct extension of the primary tumor and are not dependent on the route of spread (i.e., hematogenous, lymphatic, peritoneal dissemination).

The process of metastasis occurs through the completion of a series of step-wise events that encompass the tumor cell's "journey." A tumor cell detaches from the primary tumor and invades through the basement membrane into the blood or lymphatic vessels (intravasation). While in the circulation, tumor cells must be able to resist anoikis (programmed cell death associated with loss of cellular contact), evade immune recognition, and survive physical/shear stress. Circulating tumor cells that will survive this journey must arrest (often by physical trapping in small secondary organ vessels, exit the blood vessel (extravasate) and survive cell stress at the secondary microenvironment. Those few cells that can adapt and survive stress may then colonize the secondary sites to become DTCs at these distant, secondary sites [6]. Experimental studies have shown that <0.02% of cancer cells entering the blood vessels form metastases at secondary sites [6]. Therefore, metastasis is considered to be an inefficient process. Several studies have suggested that DTCs in various cancers may have attributes of so called cancer stem cells driving the metastatic process [7-9].

We and others have found that independent of histology (including osteosarcoma) a unifying feature of successful metastatic cells is their ability to adapt to cell-stress and survive [4,10-17]. Therefore, this adaptation phenotype is linked to the ability of metastatic cells to resist more chronic stress by entering a period of dormancy.

Indeed, once the tumor cells reach their distant site, those destined to survive will launch early adaptive programs to override the new and hostile microenvironmental cues of the "foreign" tissue [18,19]. Furthermore, they will need to launch and maintain long-term adaptive programs [20]. Some of the DTCs that will survive may persist in a dormant state for many years at these secondary sites until they exit dormancy and initiate a rapid growth to form overt metastases [21,22]. This long-term survival of the DTCs while in a dormant state may account for cancer recurrence years to decades after primary tumor resection and adjuvant therapy, during which time patients are in remission [23]. Notably, DTCs that progress to form a micrometastatic mass may also be on halt from further progression to overt metastatic masses for quite some time as a consequence of an active equilibrium between proliferation and apoptosis [24]. In this steady state, there is no net increase in tumor mass. This balance was suggested to be regulated by angiogenic switch (the formation of new blood vessels) and immune surveillance [19]. Emergence of metastatic lesions at distant organs at various times after the primary tumor was removed is caused by the aforementioned steps [25]. We can envision

another possible scenario of delayed reawakening of residing DTCs or dormant micrometastases that lingered in sanctuary sites like the bone marrow [26]. We also postulate the potential for additional metastases arising from pre-existing overt metastases (before or after removal of such tumors). The steps outlined here continue, not only between the detection of the primary tumor and the development of overt metastatic tumors, but also after the detection of metastases. From a therapeutic perspective, it is therefore never too late to target DTCs as a means to inhibit metastatic progression and improve outcomes for patients.

Many of the basic tenets of this model of metastasis have been intact for over 30 years; however, a greater understanding of biologic principles associated with each step of the metastasis process has emerged from studies in osteosarcoma and other solid tumors sharing this lethal metastatic phenotype.

A New Perspective to Preventing Cancer Recurrence - Targeting the Unique Metastatic Endurance Biology of DTCs at Secondary Sites

Targeting DTCs is seen as a feasible therapeutic approach with the potential to provide substantial clinical benefits related to delaying, preventing, or reducing the frequency of cancer recurrence. A new perspective on a therapeutic approach to prevent cancer recurrence is based on targeting the critical features of DTCs, the successful metastatic tumor cells [27]. It centers around the concept that DTCs at secondary sites (which are undetected by current diagnostic procedures and may eventually transition to dividing metastatic cells and lead to the development of overt metastasis) must utilize several mechanisms to adapt to and endure their new, hostile, microenvironment in order to induce long term survival in a dormant state. Importantly, these mechanisms are unique and specific to the biology and the circumstances of the DTCs, namely, the microenvironment that acts as their 'soil'. This new perspective builds upon the ground-breaking concept by Stephen Paget, who over a century ago proposed that "metastasis will occur only when the tumor cell (the 'seed') and the microenvironment of a given organ (the 'soil') are compatible" and expands to deeper molecular and cellular levels suitable for therapeutic intervention with 21st century technology [28]. Willis and Hadfield advanced this theory and coined the term "tumor dormancy" [29], namely, a process by which tumor cells are growth-arrested in response to ectopic cues arising within their secondary sites leading to reversible mitotic arrest [22]. Consistent with this concept, it has been demonstrated in experimental models, and in humans with osteosarcoma [30], that cancer cells may colonize distant organs, where they may remain dormant for a long period of time, only emerging to proliferative metastases in specific "preferential" organs (what is known as organotropism) [31,32]. Hence, a foreign and hostile microenvironment may promote tumor dormancy of residing DTCs.

We previously demonstrated that DTCs at secondary sites have launched several stress-related adaptive processes to endure and survive the initial stress signals imposed by their ectopic microenvironment. These include stress adaptation processes such as resistance to apoptosis [33], metabolic reprogramming [34,35] endoplasmic reticulum (ER) stress response and unfolded protein response (UPR) [36].

Following adaptation to the initial stress signals, DTCs will

launch additional adaptive programs that will promote tumor dormancy to secure their long-term survival. This dormancy was shown to be mediated by impairment of DTCs engagement with the extracellular matrix (ECM) via integrins (such as beta 1 integrin) [10-12;18,37,38]. This in turn initiated under these stress conditions, ER stress signaling pathways leading to growth arrest (dormancy) and long-term survival of residing DTCs [13-15]. In essence, the ER acts as a sensor of metastatic cell stress and resultant adaptation [39] which leads to DTC survival and then potentially to dormancy. Furthermore, in addition to ER stress, failure of DTCs to engage with the ECM was shown to induce UPR [13-15]. UPR may lead to an epigenetic reprogramming and induction of ATF6alpha-Rheb-mTOR signaling pathway [14-16] culminating in adaptation of dormant DTCs *in vivo*. Autophagy, a cell survival mechanism activated in response to metabolic stress, was also shown to be critical for the survival of DTCs [17]. Tumor cells are also found in bone marrow (BM) of cancer patients long after initial treatments [40]. The BM is seen as one potential sanctuary site for tumor cells that enables long-term survival before and in addition to long-term survival at secondary sites, such as the lungs or liver. Several lines of evidence show that microenvironmental factors such as GAS6, TGFβ2 and BMP7 in the BM may be part of a non-permissive microenvironment, promoting tumor dormancy by activating adaptive stress signals in DTCs [19]. Collectively, DTCs residing at secondary sites can be either exposed directly to stress signals upon their arrival, failure to properly engage with the ECM (e.g. via beta 1 integrin) or be exposed to non-permissive microenvironmental mediators [11]. These stress conditions may initiate early stress adaptation mechanisms that will promote DTC dormancy and their long-term survival.

As a result of this realized confluence of essential cell biology mechanisms in metastasis (emerging from several labs, including work by some of the authors of this publication; Dalit Barkan and Chand Khanna, at two distinct metastasis research laboratories at the National Cancer Institute in Bethesda, MD), we suggest the term “metastatic endurance” to encompass a combination of the following features of DTCs at secondary sites and their underlying biology: stress adaptation, survival, and dormancy. In other words, stress adaptation, survival, and regulation of tumor dormancy are all intertwined biological processes executed by “lingering” DTCs at the secondary sites. As stated above, these cellular processes are distinct from the commonly studied features of cancer cells and they are fully ignored in most cancer drug development efforts (Figure 1). The hypothesis of metastatic endurance suggests targeting pathways such as beta 1 integrin, mTOR, and TGF-beta2 pathways (perhaps in combination) as valuable to improving therapeutic outcomes and preventing metastatic progression and cancer recurrence.

Several lines of evidence now point to metastatic endurance as a cohesive characterization of the metastatic phenotype across various cancers, including osteosarcoma [33-41]. It is now highly appealing to test the hypothesis that targeting metastatic endurance will yield important therapeutic advances against metastatic progression in osteosarcoma and other types of cancer.

In an additional convergence of science there is new evidence supporting the regulation of metastatic endurance at the level of epigenetic control of enhancers (so called, metastasis super-enhancer) [42,43]. These data now support the therapeutic targeting of the epigenetic regulatory apparatus to prevent metastatic progression.

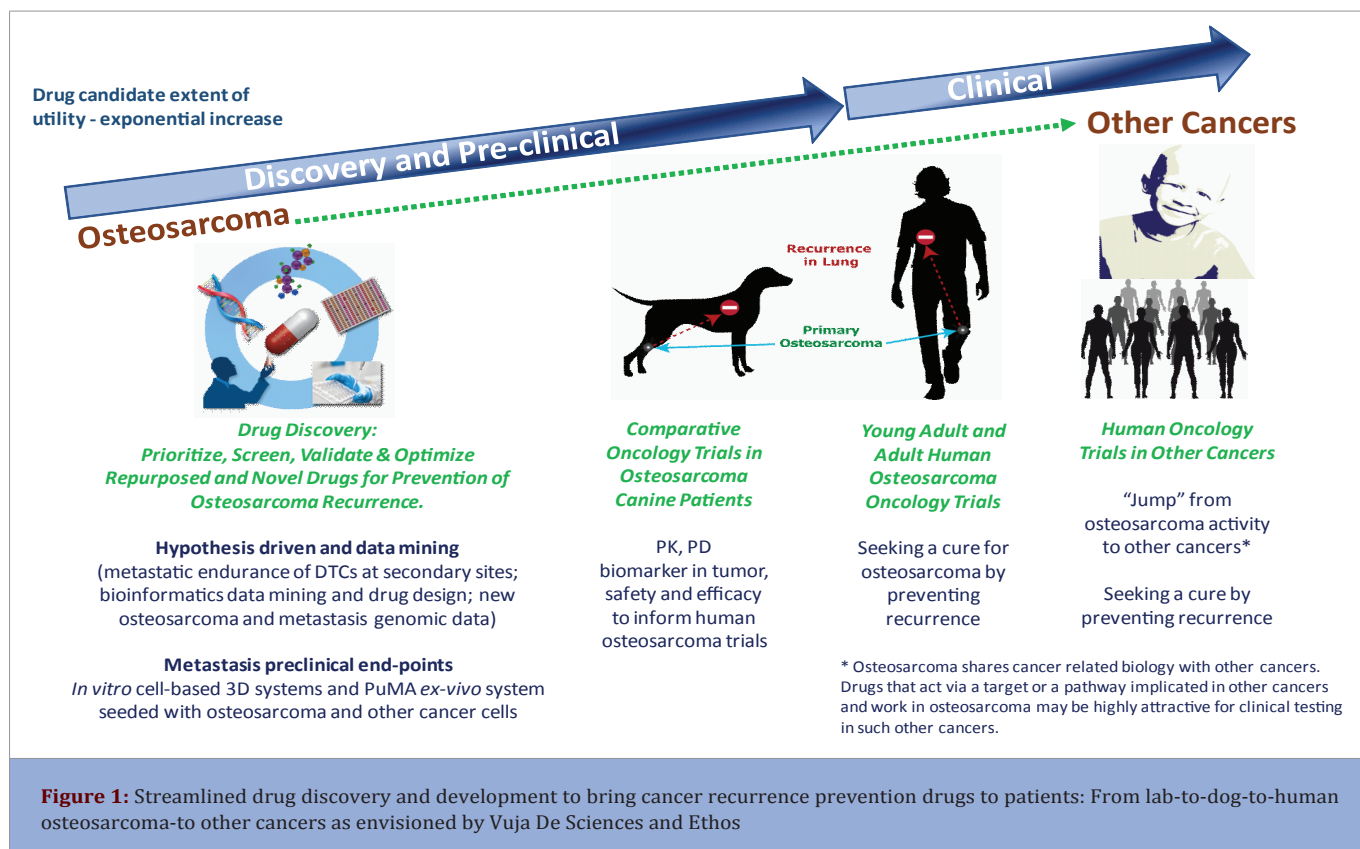
The Quagmire of Metastasis Drug Development

Despite the unequivocal and collective understanding that the primary unmet need for most cancer patients is the development of new drugs to address the problem of progression of metastasis and preventing metastatic recurrence at secondary sites, little progress has been made in this area. On the contrary, most, if not all, existing drug development efforts prioritize the development of therapeutics that will shrink an existing primary tumor in preclinical models that focus on rapidly dividing cells and resistance to apoptosis, with the expectation that overt metastatic tumors will also show a response by causing a regression of a measurable lesion. This obvious disconnect results in two distinct and significant challenges for cancer drug development. On one hand, a greater biological understanding of metastasis and cancer progression will lead to the identification of important potential therapeutic targets and pathways related to metastasis, but will not result in the identification of new drugs in conventional preclinical models. On the other hand, potential therapeutic agents that actually target critical features of the metastatic phenotype, if effective in relevant preclinical models, are in most cases, predicted to be active against metastatic progression, but are also predicted to have limited activity against measurable or primary tumors. This will result in the predicted failure of these novel therapeutics in early human clinical trials that most often require regression of a measurable lesion. Therefore, therapeutic agents that are most likely to address the primary unmet need of cancer patients are predicted to fail in a conventional cancer drug development path. Without early signals of activity in human patients, and without supportive non-clinical data, it is unlikely that these agents will advance to later stage human clinical trials where their activity is predicted to be most likely demonstrated. A new approach that better aligns unmet therapeutic needs with drug development efforts is required.

Osteosarcoma Metastasis Progression and Recurrence - A Translational Opportunity to Solve the Quagmire

Unlike other human cancers, in osteosarcoma there is now comprehensive multidisciplinary support to re-prioritize the drug development path around the essential biological mechanisms that lead to endpoints of metastatic progression. Targeting metastatic endurance is one proposed approach to achieve a clinical benefit of delayed or reduced likelihood of metastatic progression and recurrent tumors. We propose that our biological understanding of metastasis and aligned preclinical models can now deliver valuable therapeutics that may be uniquely and rapidly translated from biology through dogs with osteosarcoma to humans with osteosarcoma and potentially other solid tumors similarly associated with metastasis.

This unique opportunity to rapidly assess the therapeutic activity of agents that target metastatic progression starts with the distinctive biology of metastasis in human osteosarcoma patients that results in the high prevalence of lung metastasis as a site of metastatic recurrence. This distinctive proclivity for lung recurrence clearly defines the clinical problem for patients, treating physicians, and biologists. The reproducible pattern of lung metastasis in osteosarcoma has accelerated the design of clinical trials that can provide signals of anti-metastatic activity in human patients within the context of relatively early human clinical trials. Such trials have



been launched by the two largest cooperative groups involved in human osteosarcoma clinical trials; the Sarcoma Alliance for Research through Collaboration (SARC) and Children’s Oncology Group (COG) [3,44]. Through the COG, there is increasing evidence that recruitment to these clinical trials is feasible and often exceeds accrual expectations [45]. Rapid accrual rates and extremely high rates of clinical trial participation in osteosarcoma trials like this are further supported by an active, engaged, and sophisticated patient advocacy community. Furthermore, the development and validation of liquid biopsy technology in human osteosarcoma patients is expected to provide an additional and highly relevant circulating biomarker of disease burden that will accelerate optimization of therapeutic approaches (dose schedule regimen and combinations) [46-48].

A key to the success of the drug development process is in the ability to translate science to bed-side. The availability of rapid *in vitro* and *ex vivo* preclinical tools is a key initial step in this process (discussed here in). Suitable agents can then be tested and further developed utilizing so-called Comparative Oncology, in which dogs with naturally occurring cancer are included in clinical trials to test drugs and inform human clinical trials while potentially benefitting the canine patients. Cancer trials that include the pet dog before or in parallel with human phase I/II trials with metastasis-specific endpoints provides a key aspect to this newly proposed metastasis targeting translational approach in osteosarcoma [49]. Indeed, in osteosarcoma, nature has delivered an important translational opportunity for drug development, particularly relevant to targeting metastasis progression and prevention of cancer recurrence through the devastating problem of osteosarcoma in pet dogs. The integration of non-human clinical trials that include pet canine patients with

osteosarcoma in the identification and prioritization of therapeutic agents with the greatest potential for human benefit is an increasingly recognized solution to the quagmire of metastasis drug development. This approach has been endorsed by a variety of perspectives in the osteosarcoma drug development community, including basic scientists, cooperative groups, translational physician scientists, the NCI, NIH, the FDA, innovative biotech companies, and patient advocacy groups [50-52].

Assays of Osteosarcoma Metastasis Biology and Beyond

The biological rationale for metastatic endurance has lent itself to the development of *in vitro* and *ex vivo* assays of metastasis that can now be used to test these existing hypotheses around pathways critical for the development of metastatic endurance and to serve as a screening approach to identify novel or repurposed drugs that may interfere with this convergence in metastatic biology.

Several assay systems that are suitable and complementary have been used for studying aspects of metastatic endurance using mouse and human osteosarcoma, breast cancer, and other types of cancer cells [53-56].

A 3D reconstituted basement membrane extract (BME) model system to study tumor dormancy and metastatic outgrowth *in vitro*

This pioneering system was developed by Dalit Barkan at the National Cancer Institute to provide an *in vitro* model of tumor dormancy that enables the study of the biology of dormancy and the transition from dormancy to proliferative growth induced by

the tumor microenvironment. Several laboratories have published studies using it to study tumor dormancy [7,17,57-62]. This system mimics the *in vivo* growth characteristics of cancer cells that exhibit either dormant (D2.OR, MCF7, K7M2-AS.46) or proliferative (D2A1, MDA-MB-231, K7M2) metastatic behavior *in vitro*. Tumor cells that exhibit dormancy *in vivo* at a metastatic site (mouse and human breast cancer cells D2.OR and MCF-7, respectively, and mouse osteosarcoma cells, K7M2-AS.46) remain quiescent when cultured in the 3D-BME system under certain metabolic stress conditions. In contrast, under the same metabolic stress conditions in 2D culture, these cancer cells grow rapidly. On the other hand, cancer cells that are highly metastatic and proliferate *in vivo* (mouse and human breast cancer cells, D2A1 and MDA-MB-231, respectively, and mouse osteosarcoma cells, K7M2) readily proliferate in 3D culture after relatively short periods of quiescence [57]. In contrast, under the same conditions in 2D cell culture, these cancer cells grow rapidly with no periods of quiescence. This short, but consistent period of quiescence is important as it offers a way to study the process of exit from dormancy to a highly proliferative state. Therefore, for drug discovery and development purposes, this system with various cancer cell types can be used to identify and study compounds that act by killing DTCs and compounds that act by preventing the exit from dormancy/keeping dormant DTCs in their dormant state.

Pulmonary metastasis assay ("PuMA") to study cancer cells *ex-vivo*

This *ex vivo* system was developed at Chand Khanna's lab at the National Cancer Institute [63]. It is based on tail-vein injection of GFP-positive tumor cells followed by humane euthanasia. Then, the trachea is cannulated with an intravenous catheter and attached to a gravity perfusion apparatus. The lungs are sliced, yielding 16–20 lung slices per pair of lungs. Metastatic progression of the GFP-expressing cancer cells, from a single cell to the formation of multicellular colonies, in the mouse lung micro-environment is assessed in real time for up to 21 days. The assay was validated by prediction of the *in vivo* behavior of several mouse and human high and low-metastatic osteosarcoma, breast, and melanoma cancer *in vivo* models. PuMA can be used to test, optimize, and study the effects of compounds on DTCs.

SISgel based system for suppression and activation of the malignant phenotype

Developed at Oklahoma University in the labs of Robert Hurst and Michael Ihnat, this system utilizes SISgel, a gel-forming normal ECM material derived from Small Intestine Submucosa (SIS) [64]. Various types of mouse and human cancer cells grown on SISgel show properties of suppression and re-activation that are very similar to normal delayed metastasis and suppressed cancer cells. This technology serves to study the biology of the suppressed cancer cells and to discover and develop therapeutics to target micrometastases or suppressed cancer cells [65].

Comparative Oncology: A Path toward Better, Cost Effective Oncology Drugs

Cancer drug development is difficult and fraught with high failure rates, with fewer than 3% of cancer drugs entering human trials advancing to human approval. Therefore, it is critical that the conventional approach to oncology drug development be improved.

Many development failures occur late in drug development (so-called late attrition), thus moving disproportionate costs to patients and drug developers compared to a "fail-early model" of clinical development. Moreover, promising innovations are often "stuck" between early research, discovery, and clinical trials due to a lack of convincing data to support their potential efficacy. There is also the perception that preclinical cancer models are not "predictive" to justify the risks of human trials, to the great loss of cancer patients. These failures may be expected and are likely linked to the complex biology of cancer. In contrast to the common mouse models, metastasis is an intrinsic feature of dog solid tumors and therefore is expected to represent the true and relevant biology of metastatic progression, which when applied to well-designed non-clinical trials may substantially de-risk and inform drug development, especially in the setting of metastasis.

Comparative Oncology seeks to address the current challenges in oncology drug development through the study of naturally occurring cancers, primarily in dogs, that share the same biological complexity seen in human cancers. Given the unquestioned need and the translational and clinical infrastructure that currently exists to deliver the promise of Comparative Oncology, there is an urgency to expand the use of a comparative and parallel/integrated cancer drug development path.

There has been recognition by thought leaders and many experts that dogs with cancer provide a unique opportunity to improve the development path of new cancer drugs. There have been significant investments in the field, such as the launch of the NCI-Comparative Oncology Program, as well as similar efforts and research supported by the extramural NIH, academic research centers, comprehensive cancer centers across the US, the endorsement of the Institute of Medicine through its hosting of a strategic meeting focused on this opportunity, and by the launch of several internal programs in Comparative Oncology within the pharmaceutical and biotech industries [66-70]. Despite all of the above, Comparative Oncology is still an untapped resource.

Canine Osteosarcoma Comparative Oncology

In canines, genomic, histologic, and other biologic features of osteosarcoma are very similar to those in human osteosarcoma [71]. Naturally, unlike preclinical models in lab animals such as the mouse, the tumor and immune system are syngeneic, allowing a relevant analysis of various immune-oncology assets. Furthermore, the clinical manifestations and progression of the disease are remarkably similar between dogs and humans with osteosarcoma, including the pattern of disease metastatic spread [72]. Importantly, best care practices in canine patients and human patients are essentially identical (surgical treatment followed by chemotherapy and radiation), enabling a staged approach to clinical trials in canines with osteosarcoma that are highly comparable to human trials. This includes testing agents on top of conventional therapy or alternatively as a first-line approach to therapy. Finally, as a result of the compressed life-span of dogs compared to humans, effective drugs may be identified much more rapidly in clinical trials in dogs and used to inform human clinical development. Time to recurrence in dogs is typically four months following surgery alone, and one to two years following surgery and adjuvant chemotherapy.

Clinical trials in canine osteosarcoma patients can be designed in a phased approach, where each phase provides opportunities

to obtain important data about a drug's mechanism of action, pharmacodynamic- and pharmacokinetic-related properties, safety, administration, dosing, and ultimately its efficacy. Importantly, each phase can inform an optimal human trial or be served to "kill" a drug based on various "no go" criteria, such as no activity on the drug's target or no efficacy. This approach is also highly cost effective and supports the ability to test multiple agents ahead of a decision on which to advance toward human clinical trials. It is also possible to test novel drug combinations in canine osteosarcoma patients where economic, regulatory, and business constraints would prohibit such testing in humans.

A path in canine osteosarcoma could encompass the following steps. First, an initial phase for evaluating the pharmacodynamic/pharmacokinetic profile of the drug as a neo-adjuvant. The primary tumor can be biopsied prior and after drug exposure to assess target modulation as a function of tolerable drug exposure (PK/PD) with correlation to circulating biomarkers in the same individual and same species. Such simple studies may lead to an improved design of a human clinical trial and then rapidly integrate endpoints relevant to anti-metastatic activity.

Ethos Veterinary Health and its nonprofit incubator of scientific innovation, Ethos Discovery, proposes to facilitate this approach by bringing interested human and animal health pharma/biotech together. This approach is also being taken by Ethos along with Vuja De Sciences [73]. This approach will create synergy to leverage resources, de-risk drug development, and generate returns simultaneously within a human and animal health drug development path.

Concluding Remarks

Preventing metastatic progression and cancer recurrence is a major unmet need and can be a game-changer for patients with osteosarcoma and many types of cancers. There is an important opportunity for a streamlined cancer drug development strategy that focuses on preventing metastatic cancer progression and recurrence. Carrying out this strategy is powered by a depending understanding of the unique biology of DTCs, metastasis-specific methods and tools for drug screening, comparative oncology, and human osteosarcoma serving as an outstanding model for cancer progression in many other cancers.

The strategy (overview shown in Figure 1) involves starting with the biology of DTCs in osteosarcoma to conduct rapid and cost-effective screening of compounds in available systems *in vitro* and *ex vivo*. Then, suitable agents can be taken directly clinical trials in dogs with osteosarcoma. Based on ADMET and/or efficacy outcomes in dogs, go/no-go for human osteosarcoma drug development decisions can be made. Furthermore, dog studies can generate unprecedented data in support of human oncology trial design like never before, de-risking and enhancing the likelihood of success, and minimizing reliance on unreliable mice models. Depending on the mechanism of action of an agent, other cancers with shared biology with osteosarcoma recurrence can be pursued. Therefore, this opportunity may enable improved clinical outcomes for cancer patients beyond osteosarcoma.

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Conflict of Interest Statements

Samuel Stewart and Chand Khanna are employees of Ethos Veterinary Health. Dalit Barkan has a consulting agreement with Vuja De Sciences, Inc. and compensated with Vuja De Sciences equity. David Warshawsky is Founder and CEO of Vuja De Sciences, Inc., is employed by the company and holds stock in the company. Ethos Veterinary Health and Vuja De Sciences have a business relationship including one or more signed agreements and memorandum of understanding.

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