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Provocative Questions in osteosarcoma basic and translational biology: a report from the Children's Oncology Group

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Abstract

Those diagnosed with osteosarcoma today receive the same therapy that others received over the last four decades. Extensive efforts to identify more effective or less toxic regimens have proved disappointing. As we enter a post-genomic era, now recognizing osteosarcoma not as a cancer of mutations but as one defined by p53 loss, chromosomal complexity, copy number alteration, and profound heterogeneity, emerging threads of discovery leave many hopeful that an improving understanding of biology will drive discoveries that improve clinical care. Under the organization of the Bone Tumor Biology Committee of the Children's Oncology Group, a team of clinicians and scientists sought to define the state-of-the-science and to identify questions that, if answered, have the greatest potential to drive fundamental clinical advances.

Having discussed these questions in a series of meetings, each led by invited experts, we distilled these conversations into a series of seven Provocative Questions. These include questions about the molecular events that trigger oncogenesis, the genomic and epigenomic drivers of disease, the biology of lung metastasis, research models that best predict clinical outcomes, and processes for translating findings into clinical trials.

Here, we briefly present each Provocative Question, reviewing the current scientific evidence, noting the immediate opportunities, and speculating on the impact that answered questions might have on the field. We do so with an intent to provide a framework around which investigators can build programs and collaborations to tackle the hardest problems and to establish research priorities for those developing policies and providing funding.

Precis:

Scientific advances have significantly improved our understanding of osteosarcoma but gaps in our knowledge have impaired our ability to translate new findings into new cures. Here, we present a series of questions that we believe represent the most important and most immediate unsolved problems which, if solved, might change the way we think about and treat this disease.

Keywords

osteosarcoma; pediatric oncology; translational biology; sarcoma; program development

Introduction

Osteosarcoma (OS) is an aggressive bone malignancy that primarily affects adolescents and young adults. The standard-of-care regimen used today was first introduced in the late 1970s[1] and remains largely unaltered despite numerous efforts to improve outcomes[2,3]. Patients who present with localized disease today still face 5-year overall survival rates of less than 70%; less than 20% of those who develop metastatic disease or recurrence survive beyond 3 years[4].

Recent experience suggests that transformative advances in the treatment of this disease will not likely come from intensification of antineoplastic chemotherapy[5], further focusing efforts on developing truly novel approaches. A fresh surge of scientific discovery has fueled an evolving appreciation for the complex biology of this malignancy, giving life to several ongoing clinical and basic scientific efforts that seemingly have potential to impact patient outcomes.

In an effort to create an environment that could foster collaborative thought and discussion around these opportunities, the Bone Tumor Committee of the Children's Oncology Group (COG) launched a monthly web-based conference for basic and translational scientists interested in OS research. The charge of the reformed Osteosarcoma Biology Committee was to bring together scientists committed to sharing their work, identify significant hurdles that preclude deeper understanding of OS biology, and establish new research collaborations. Participants were identified by personal referral of existing members of the COG Bone Tumor Committee, requiring only interest and a willingness to actively participate. Nearly 50 individuals were initially invited and participation continues to grow. Monthly calls routinely have more than 30 worldwide participants.

In our inaugural meeting, the group galvanized around a plan to use this forum to identify and explore key gaps in our understanding of OS biology. By methodically reviewing the most current and potentially-impactful opportunities for discovery science and clinical translation, we hoped to create a blueprint that could shape the priorities of scientists, foundations, advocacy groups, and funding agencies around common goals.

To this end, we asked members of the committee to submit one or more candidate Osteosarcoma Provocative Question that would encapsulate specific challenges to our basic, translational, and clinical understanding of the disease. We consolidated submissions into a

series of OSPQs (Box 1), then systematically reviewed the state-of-the-art and current opportunities as a group in our open meetings. We invited experts (both from within and from beyond our group) who could proficiently address each question, asking them to summarize the most current science, reserving the majority of each session for active discussion. The results of these discussions are summarized here as a series of OSPQs, refined by those discussions, and presented in a format consistent with the National Cancer Institute's Provocative Questions Initiative[6].

OSPQ1: What are the disease-initiating events in osteosarcoma and how do those events lead to genomic complexity and cellular heterogeneity?

Intent.

Despite multiple intensive efforts to genomically characterize OS, a common inciting event remains elusive. While bi-allelic loss of *TP53* appears to be a near universal requirement for the development of OS, it remains unclear whether loss of *TP53* itself is a sufficient oncogenic event. One major challenge to interpreting the results of large-scale genomic efforts is the seemingly stochastic complexity and heterogeneity encountered in OS tumors. Which of the identified alterations represent true drivers of malignancy? Which can be targeted therapeutically? Which features provide prognostic or diagnostic value?

Background.

Most OS tumors harbor inactivating *TP53* mutations or amplifications of *MDM2/4*[7,8]. The frequency of *TP53* mutations was previously underestimated until intronic rearrangements, largely in intron 1, were recognized as a frequent mechanism of somatic *TP53* loss[9,10]. The high allelic fraction of *TP53* loss suggests that mutations in *TP53* occur as a very early event in oncogenesis. Several studies, including a large meta-analysis have associated specific genetic *TP53* alterations with survival in patients with OS[11], suggesting mutation-specific phenotypes. Many tumors also bear *RB* mutations and, less commonly, mutations in *ATRX*, cell cycle proteins, and the PTEN/PI3K pathway[9].

Beyond this small set of mutations, the most common characteristic of OS is structural complexity through chromosomal rearrangements, copy number variation, kataegis, and chromothripsis, which occur broadly throughout the genome[9,12]. Widespread corruption of chromosomal structure may produce progeny with divergent patterns of copy number variation that undergo serial selection for growth advantage. Indeed, recent work has shown that a limited set of copy number patterns can group OS tumors into subtypes that may predict response to certain targeted agents[13]. These patterns, however, explain only a small number of events and the degree of cell-to-cell heterogeneity within a tumor remains unknown, though chromosomal heterogeneity could establish mechanisms for diversity that would facilitate adaptation to cellular stresses, such as chemotherapy.

Surprisingly, OS lacks common or recurrent changes in DNA repair pathway genes. Rather than accumulating this complexity over time, mathematical modeling of osteosarcomagenesis based on a cell cycle time scale suggests that the critical genetic events leading to malignancy occur abruptly within a single cell cycle[14,15]. In fact, unchecked cell

cycle progression due to *TP53* loss may be sufficient to initiate oncogenic transformation in the correct cell-of-origin and microenvironmental context.

Feasibility.

Numerous pan-cancer genomic studies have elucidated the role of single-nucleotide variants and small focal copy-number alterations in OS biology. Widespread chromosomal copy-number changes, chromothripsis, kataegis, and aneuploidy have been clearly identified as characteristic features of OS, though their role in the oncogenic process remains largely unknown. With several published and unpublished sequencing projects now completed[9,12], an effort to combine all available data into a harmonized cohort would be better powered to identify recurrent somatic events and subtypes of OS. Improving the availability of paired samples should allow more sophisticated comparison of primary, recurrent, and metastatic lesions. Emerging technologies, such as single-cell sequencing approaches, may help distinguish genomic complexity from cell-to-cell heterogeneity. Furthermore, integration of epigenomic and proteomic data may facilitate the interpretation of genomic structural variants and the elucidation of their biological effects. Finally, accurate *in vitro* and *in vivo* models, as described in other sections below, should help identify targetable dependencies engendered by osteosarcoma's unique genomic profile.

Implications of success.

A mechanistic understanding of the biological processes that drive osteosarcomagenesis and malignant progression would move the field forward in a number of substantive ways. First, a detailed grasp of the biological mechanisms that lead to malignancy in this disease would facilitate assessment of the relevance of different models of OS and may help produce models more faithful to the human disease. Second, separating the driver genetic changes from the genetic noise of passenger variants would help focus research efforts on relevant biology and may reveal specific therapeutic vulnerabilities. Indeed, if emerging ideas hold true, which hypothesize a common proximal event that sets the stage for the selection of specific patterns of copy number variation that produce growth advantage, the refinement and study of distinct subtypes could represent a paradigm shift in the way we think about treating this disease.

OSPQ2: What can epigenetic profiling tell us about osteosarcoma?

Intent.

While widespread structural variations leading to copy number gains and losses characterize the genetic landscape[9,12,23], the role of epigenetic dysregulation in osteosarcomagenesis and malignant progression remain less explored. What specific epigenetic mechanisms contribute to OS? How does the epigenetic landscape of OS compare to the putative cells of origin? Are epigenetic patterns common across the majority of osteosarcomas or heterogeneous across tumors? Are these changes heterogeneous within a single tumor site? What epigenetic changes drive progression, resistance, and metastasis? Do changes resulting from gains or losses of proteins that function within epigenetic regulatory complexes influence OS cell biology?

Background.

Increasingly, regulatory or functional changes in DNA methylation, enhancers, superenhancers and histone state have been recognized as primary drivers of pediatric cancers. While studies reporting the disease-specific epigenetic landscape of OS have not been reported, both the TARGET and Heidleberg[24] genomic efforts are generating DNA methylation data in large clinically-annotated sample sets. Preliminary reports suggest that the methylation signatures in each independent dataset do not have a clear underlying oncogenetic correlation. A few osteosarcomas have methylation patterns outside the primary clusters that coincide with genetic aberrations in epigenetic regulatory genes.

While mounting evidence suggests surprising stability of the methylome from primary to metastatic sites, focused preclinical models have recently demonstrated that OS cells possess an ability to alter enhancer profiles and patterns of transcription that enable cells to metastasize and proliferate in the lung microenvironment[25,26]. Leveraging these epigenetic patterns to identify common state-specific treatment vulnerabilities may enable the development of therapies tailored to metastatic lesions[25].

Feasibility.

Epigenetic lines of inquiry have potential to enhance our understanding of OS development, plasticity, mechanisms of metastasis, and the biology of treatment resistance. Since the underlying features that allow for numerous structural variations in OS remain unknown, it is possible that epigenetic mechanisms are involved from very early stages of osteosarcomagenesis. Methylation patterns may provide insights into the cell of origin. Targeted perturbations of specific enhancer elements may reduce metastatic potential of OS cells.

Ultimately, analyses that integrate the latest genomic, proteomic and epigenetic information will likely be the most informative. Additional insights can emerge when such analyses are enhanced with matched metastatic or relapse samples and robust clinical data. Future banking strategies for patients with OS undergoing surgical resections or biopsies should recognize the value that well-annotated samples, properly handled and preserved, sufficient for performing global analyses, might have[27].

Implications of success.

Remaining questions about OS, including mechanisms of metastasis, patient-to-patient and cell-to-cell heterogeneity, pervasive structural genetic changes, and the elusive cell of origin might be answered through a more detailed understanding of epigenetics in OS. Therapeutic avenues for targeting epigenomic dependencies are in early stages of development, but have promise for clinical translation.

OSPQ3: What can we learn from studying the inherited cancer susceptibility syndromes that increase the risk of osteosarcoma?

Intent.

Osteosarcoma is the most common childhood cancer associated with congenital loss-of-function mutations in both *TP53* (Li-Fraumeni Syndrome) and *RB1*, the two genes recurrently mutated in patients with the disease. However, increased incidence of OS is not limited to these predisposition syndromes. Indeed, germline mutations in other genes can confer even greater risk for OS. Given the poorly understood molecular origins of OS, patients carrying germline mutations that predispose to the disease present unique opportunities to learn about osteosarcomagenesis. It would be prudent to ask what might be learned from these patients and how this might help them. What subsequent molecular alterations lead to malignant progression in these patients? Why do non-TP53/non-RB1 mutations also lead to OS? Are these the same diseases? Do different types of *TP53* mutations alter the clinical phenotype of the disease?

Background.

Several cancer predisposition syndromes are associated with an increased risk of OS. The highest and most specific risk for OS occurs in patients with Rothmund-Thomson Syndrome (RTS)[28], an autosomal recessive disorder caused by germline mutations in the DNA helicase gene, *RECQL4*. (The incidence of OS in RTS patients is approximately 30%[29].) Syndromes defined by mutations in other DNA helicase genes also confer an increased risk of OS, including Bloom, Werner, and RAPADILINO syndromes[30]. OS is the most common cancer of childhood associated with Li-Fraumeni Syndrome, caused by germline *TP53* mutations, occurring with a cumulative incidence of 5–11%[31–33] in these patients. Indeed, it is estimated that approximately 10% of all patients with OS aged less than 30 years have germline *TP53* mutations[34].

Feasibility.

The major cancer susceptibility genes associated with OS are known. Animal models are available for the most common gene mutations (i.e. *TP53* and *RECQL4*). Investigators should be able to delve more deeply into the early events and networks that are important in OS development and to study how those events interact with or are influenced by other underlying germline polymorphisms. In addition, studies comparing patterns of somatic mutations and epigenomic profiles of tumors from patients with and without underlying germline cancer predisposition syndromes could be performed with existing technologies to understand the differences in tumor biology in these cohorts. Evaluation of the clinical significance of germline mutations on OS outcomes will require collaborative efforts to combine data from single institutions and cooperative groups, such as the Children's Oncology Group. Given that upfront treatment of OS in the United States is largely universal with methotrexate, doxorubicin, and cisplatin (MAP), comparisons across institutions should be feasible if a coordinated effort can be organized.

Implications of success.

The successful results of this research will define how genetic factors interact to augment the action of cancer susceptibility genes to promote OS development. Moreover, molecular investigations into the interactions of those genes and exposures may identify networks and pathways that are critical to the development of this disease. Finally, a coordinated effort to examine the outcomes of OS patients with inherited genetic cancer susceptibility may broadly inform investigators on the mechanisms that promote or inhibit clinically relevant mechanisms such as metastasis and chemosensitivity.

OSPQ4: Which research models best represent the biology of osteosarcoma?

Intent.

While researchers continue to utilize, develop, and characterize an ever-growing collection of disease models, discerning which models best represent the biology of human OS remains difficult. The translational value of scientific discoveries hinges on the answers to a series of fundamental questions that, in many cases, remain difficult to answer: How closely do cellular and animal models of OS recapitulate the biologic and ‘omic features (genomic, transcriptomic, epigenomic, etc.) of the human disease? How much (and when) does the immune and stromal environment contribute to the interpretation of results from each model? Does exposure of the model to agents with known activity in the human disease induce a similar response in the model? And, most importantly, does response in the model to novel therapies predict responses in patients with OS?

Background.

To study OS at both a basic and a preclinical level, investigators rely on a broad range of models, including established cell lines[39], patient-derived cell cultures, patient-derived xenografts[13,40–42], cell line xenografts, and genetically engineered mice[43–45]. Pet dogs that spontaneously develop OS represent a parallel patient population that presents unique opportunities for generating preclinical human data[43,46]. These models differ widely with respect to their distance from the patient, their ease and cost of use, and the fidelity with which they recapitulate different elements of the human disease. In many cases, these determinations can be difficult to make due to a lack of existing data.

One significant barrier to determining the predictability of existing models is the lack of recent clinical trials with positive results, which makes it difficult to identify predictors of efficacy. Negative clinical trials give the impression that preclinical models were not accurate in predicting response. However, others might argue that this results from setting an unreasonably low bar for calling a preclinical study “positive”.

As the number of biological leads expands and our approach to treatment becomes more sophisticated through increasingly complex precision oncology frameworks, immune- and stromal-targeting strategies, and combinatorial regimens, our need to understand which models faithfully recapitulate specific elements of OS biology becomes more urgent. From a basic science perspective, our incomplete understanding of the fundamental biology

underlying osteosarcomagenesis[44,47] and most aspects of malignant progression limits our ability to discern the value of numerous distinct models of disease.

Pet dogs frequently develop OS, which bears striking clinical, genetic, and biologic similarity to the human malignancy and almost always leads to metastatic pulmonary disease[46,48,49]. The spontaneous nature of this disease, the shared environmental context, the shorter natural history of the dog disease, and the increasing capacity of collaborative clinical trials groups within the veterinary community make this a primary early target of comparative oncology efforts[50,51]. The canine patient may become a valuable tool for vetting preclinical candidates, for answering important pharmacodynamic questions, and for the parallel evaluation of biologic responses to the perturbations induced by candidate interventions.

Feasibility.

While genomic and other high dimensional characterizations of human OS tumors and models have been completed, only very limited ‘omic comparisons of cell lines, patient-derived xenografts (PDXs), and genetically-engineered mouse models (GEMs) to primary human samples have been performed. Likewise, although comparative work in canine models suggests marked commonalities between human and canine OS[48,52], evaluation using modern techniques to compare this to the human disease still warrants attention. Such research could help scientists select models that best represent aspects of OS particularly suited to the testing of novel therapeutics, also leading to identification of biomarkers and precision implementation of clinical trials. Promising candidates in the mouse could be rapidly vetted in canine trials of new OS-targeting agents.

Using readily available models and techniques, several coordinated efforts could be considered to address this question. These might include:

- Profiling of GEMs and PDXs to reveal their genomic, epigenomic, and proteomic features. This data could be superimposed on the human data to “bin” models into appropriate subclasses (or to deem them either broadly applicable or not representative of human OS).
- Comprehensive profiling of canine tumors performed in parallel with analysis of human data to determine whether similar subtypes of OS are present or absent in dogs.
- Making the broad range of already developed OS models readily accessible to scientists, including the relevant characterization data associated with each model.
- Development of a more coordinated enterprise that integrates and aligns the work of veterinary clinical trials groups with their pediatric counterparts. Opportunities exist to coordinate the work of canine clinical trials consortia (such as the Comparative Oncology Trials Consortium and the Canine Immunotherapy Trials and Correlative Studies Group, which was created through a recent series of Moonshot grants) with that of their human counterparts (COG and other clinical

trials consortia) simply by including members of both groups in their respective planning and development meetings.

Until we have clinical results that can clarify the predictability of our models, the relative value of preclinical data remains difficult to assess. A formalized scoring system for prioritizing and valuating preclinical data exists[53], though few have utilized this system in reporting preclinical results. Improving dialogue between trialists and the research community could convey realistic expectations to the clinicians, relay important pharmacokinetic considerations, assure that optimizations to scheduling or dosing are reflected in trial design, and facilitate collection of appropriate samples for correlative study. In the short term, a retrospective analyses of the predictive performance of preclinical models relative to clinical trial outcomes could help identify aspects of experiments that are more predictive than others.

Implications of success.

As we enter an era where clinical decisions and preclinical development plans have become increasingly driven by high-level characterization of tumors and refinement of diagnostic criteria (which divide tumors into increasingly small but specific groups), the importance of understanding how our preclinical models recapitulate this biology cannot be overstated. Work done to determine which results from which preclinical models will accurately predict results in patients with OS has enormous potential to speed therapeutic development.

OSPQ5: What biological mechanisms mediate osteosarcoma lung metastasis?

Intent.

Metastatic dissemination and distant recurrence occurs primarily in the lung[54]. This natural history suggests that elucidating the mechanisms that facilitate lung metastasis might identify clinically-relevant targetable vulnerabilities. Despite the seemingly obvious potential of such strategies, we currently understand very little about the biology that drives lung colonization. What specific adaptations or intracellular signals intrinsic to certain tumor cells make OS cells survive and grow in the lung? What lung-derived signals activate survival pathways? Does lung-induced dormancy protect disseminated tumor cells from chemotherapy or mediate late metastatic relapse?

Background.

Metastatic disease is the most critical clinical factor that influences malignant progression and mortality in OS[53]. Yet we know very little about the biology underlying this metastatic process. We do know that in the early stages of metastatic lung colonization, disseminated cancer cells experience a variety of cellular stresses (e.g. redox/endoplasmic reticulum stress) that threaten their survival in the distant microenvironment[19,55,56]. Metastatic inefficiency arises from the inability of most cells to survive the stresses involved with initial arrest within a distant tissue[57–59]. Animal studies using well-characterized xenograft models of metastatic OS[60] have identified a number of molecular pathways important for metastatic

progression[18,19,61–64]. However, the clinical relevance of these potential vulnerabilities has yet to be determined in patients.

Dormancy of disseminated cells within the lung could explain the most common pattern of metastasis—late recurrence. Several groups have identified potential drivers of OS dormancy[65–67], including suggestions that tumor-lung interactions might support the dormancy state, though this remains a relatively unexplored field. Additional studies are needed to validate these findings.

Feasibility.

Since lung metastasis is a dynamic, multi-step process that involves interactions between OS cells, extracellular matrix, and lung parenchymal cells, model systems must facilitate the responses of OS cells to pharmacologic or genetic manipulation within the lung microenvironment. Several existing models are available that could help our understanding of lung-specific responses to perturbagens. For example, the *ex vivo* pulmonary metastasis assay (PuMA)[68–70] permits researchers to directly observe OS cell growth within the lung microenvironment while precisely controlling any number of experimental conditions. Many xenograft and GEM models of OS faithfully recapitulate patterns of metastasis and tumor-lung interactions observed in the human condition (discussed in OSPQ4). Intravital imaging techniques also now allow for high-resolution, longitudinal imaging of emerging metastases in context[58,71]. Single-cell whole-genome analyses offer enormous promise for furthering our mechanistic understanding of tumor-host interactions within the metastatic niche.

Implications of success.

Lung metastasis defines prognosis in patients with OS. A therapy that effectively prevents the emergence of lung metastasis could potentially save as many as 70% of the lives currently lost to the disease[53]. Therapies which render metastatic disease treatable could have even greater impact. Indeed, targeting metastatic disease has been identified as a research priority by a diverse group of experts[53], and well-vetted approaches that target prevention or treatment of metastasis would likely be prioritized for clinical evaluation.

OSPQ6. What factors limit the efficacy of immuno-oncology approaches in osteosarcoma?

Intent.

Interaction of tumor and host immune environment has long been believed to be a critical aspect of tumor survival in malignancies. Despite recent success of immune therapies in several tumor histologies and identification of some key factors that may predict response such as a high tumor mutational burden or DNA mismatch repair defects[72–74], the relationship between tumor and host immune microenvironment, their interactions and opportunities for exploitation with immune-mediated therapies remain poorly defined in OS. The key questions to answer in this context in OS include: What is known currently about the tumor immune profile? Are there differences among host immune profiles of patients with OS as compared to healthy controls and do these differences matter? Do OS primary and/or metastatic tumors have the right milieu for immune therapies to be effective? What

can we do to make immune therapies effective in these tumors and patients? What models can we use to study these questions?

Background.

OS has been considered to be immunogenic and amenable to immune therapies since the early 19th century when William Coley, considered to be the “Father of Immunotherapy”, observed responses after injecting patients with bone and soft tissue sarcomas with streptococcal-derived toxins (Coley’s toxins)[75]. In recent years, the recognition of extreme genomic complexity of OS[76,77] has led many to believe that a higher tumor mutational burden and neo-epitope antigen generation should suggest response to immune therapies such as immune checkpoint inhibition. However, while OS tumors do exhibit higher mutational burden than many pediatric cancers, this is still much lower than a majority of adult cancers[24].

Other evidence points to a potential role of the immune microenvironment in OS progression. Several studies suggest that infiltration of tumors with cytotoxic T cells as well as tumor associated macrophages predicts improved survival in patients with OS[78–80]. Further, expression of PD-1 on tumor infiltrating lymphocytes and its ligand PD-L1 on tumor cells has been shown both in primary OS tumors as well as lung metastases, suggesting that this pathway might play a role in tumor microenvironment[78,81]. When compared to healthy controls, increased expression of CTLA4 on peripheral T cells and an increased ratio of immune suppressive peripheral monocytes in patients with OS may suggest a profoundly immunosuppressive environment within tumors[79]. Preclinical studies in immunocompetent mouse models suggest that blockade of immune checkpoint pathways alone or in combination with chemotherapy (trabectedin) or radiation can reduce both primary and metastatic tumor burden in bone and soft tissue sarcomas[81–84].

Despite this preclinical evidence of immune checkpoint inhibitors activity, the response to these agents in clinical trials has been underwhelming. In SARC028 study, only one of the 22 patients with OS had an objective response to single agent pembrolizumab[85]. In the pediatric trial of nivolumab alone or in combination with ipilumumab, no objective responses were seen in patients with OS. It is clear from these results that more work needs to be done to understand the discrepancy between preclinical and clinical outcomes and to establish novel ways to improve responses to immune therapies in this disease.

Feasibility.

While initial clinical trials have been far from promising, strong preclinical data suggests an untapped potential for harnessing the immune system against OS. With an abundance of new genomic and transcriptomic data now available on OS, it is imperative that there is close collaboration between cancer immunologists and genomicists to determine which targetable immune regulatory proteins, cell therapies and combination treatments have the most potential for this disease. Our current understanding of the tumor/immune microenvironment of OS appears weak and warrants intensive biological study.

Identifying appropriate model systems for these experiments presents a particular challenge. Rodent models of disease that faithfully mimic the human immune microenvironment do not

currently exist, although efforts to develop humanized immune competent mice are ongoing[86]. Canine models and veterinary clinical trials may meet some of these needs. Efforts have been underway within Children's Oncology Group to develop clinical trials of immune checkpoint inhibitors in combination with chemoradiotherapy based on intriguing preliminary evidence suggesting that radiation might immunosensitize these tumors[83]. Other efforts to identify novel therapies that effectively modulate the immune response to OS, such as targeting IDO, B7-H3 or certain chemokines (CXCL12) are also under consideration. If designed with the proper integrated correlative biology experiments, these trials could significantly enrich our understanding of the role of the immunity in this disease.

Implications of Success.

Advances in OS therapy have stalled over many decades. Comprehensive genomic and transcriptomic profiling has thus far failed to identify easily druggable targets. Given the recent successes in other cancer types, immune therapies should be explored to determine their potential for efficacy in OS. Successful recognition of tumor and host immune environment and of barriers that prevent anti-tumor immune effectors from productively eliminating tumor cells could lead to rationally-designed immune therapy combinations in OS.

OSPQ7: Can emerging clinical trial designs compliment and accelerate the development of novel approaches to osteosarcoma?

Intent.

Most of the advances made in the treatment of OS developed empirically, during a time when few systemic options existed and little data was needed to support investment in a clinical trial. Since that time, the demand for pre-clinical data to justify the initiation of a clinical trial has grown, even as the parameters for what constitutes sufficient pre-clinical evidence remains undefined (OSPQ4). However, in a disease where definitive, prospective clinical trials often require more than 10 years from conception to initial results[87], delays in initiating trials based on good clinical intuition could result in decades of stagnation in how patients with OS are treated. Beyond identification of a potentially efficacious therapy, another opportunity cost of not having active clinical trials is reduced ability to collect and analyze biological specimens towards biomarker discovery and improved understanding of biology. Patients have been eager to test novel therapies, even when those studies are unlikely to benefit them personally[88,89]. Fortunately, newer phase II study designs facilitate trials with smaller patient cohorts, which could create more opportunities to study novel therapies for patients with recurrent disease[90]. With this, can trialists and researchers leverage smaller, leaner trial designs to not only vet treatments with strong preclinical justification, but to accelerate the development of newer ideas in a more integrated preclinical/early clinical environment? Should the go/no-go threshold be influenced by the lack of other therapeutic options for patients, such as off-label agents or competing trials? How can alternative mechanisms of efficacy such as inhibition of metastases, rather than cytotoxic effects, be tested and translated? Is it ever acceptable to test ideas directly in patients without preclinical data? Similar to questions asked for preclinical models, does

response in patients with relapsed disease predict activity in patients with newly diagnosed disease?

Background.

Accepting that additional intensification of cytotoxic agents will not improve outcomes in patients with OS[2,91], investigators have turned to an ever-lengthening list of tyrosine kinase inhibitors, immunotherapies, and targeted agents with increasingly diverse and novel mechanisms of action as potential treatments for OS. This creates a need for smaller clinical trials that can vet more potential therapies more quickly. Such efforts have long been hampered by the lack of an acceptable historical control. Fortunately, a dedicated analysis of past trials has been completed and accepted as a historical control that trialists can use to power and evaluate future clinical trials in patients with relapse OS[90]. This transition to a more relevant, easily measured efficacy threshold circumvents the problems inherent to the application of more widely used response measures inherent to OS[92]. Thus there are clear efficacy bars for phase II trials. Using these standards, trials can be conducted in under 3 years[93,94].

Other emerging lines of work could significantly affect how we conduct clinical trials in osteosarcoma. For instance, Bayesian trial designs might have particular value in rapidly evaluating multiple treatments for rare tumors like osteosarcoma[95]. Also, if OS truly represents a collection of biologically-distinct clinical entities (OSPQ1[13]), the use of biomarkers to classify individuals into smaller, biologically-related groups would dramatically affect our approach to conducting clinical trials.

Feasibility.

With no first-line therapeutic trial currently open and with a relatively small number of phase 2 trials available for relapsed patients, the number of children with osteosarcoma enrolled in NCI-sponsored clinical trials has decreased significantly compared to when frontline trials have been enrolling. This has created a situation with more patients interested in enrolling in clinical trials than there are trials available, evidenced by the rapid accrual of recent trials that have far exceeded enrollment expectations[93,94]. While numerous therapeutic strategies have some preclinical evidence supporting further investigation, very few have a level of evidence that would meet traditional standards for justifying a clinical trial. Many of these concepts invoke treatment strategies already employed in other diseases and with known safety profiles, lowering the potential risks of use in this population.

Ideally, OS research would use a more integrated approach, testing novel regimens across collections of diverse models, with laboratory experiments that educate human clinical trials[96] and canine clinical trials[48,97] and vice-versa. Each study, each experiment designed to answer important questions pertinent to that approach, all informing the ongoing development of future human studies by optimizing dose, schedule, biomarkers for inclusion/exclusion, and the thoughtful implementation of meaningful correlative studies[98].

Implications of success.

Establishing a clear threshold of preclinical data to justify a clinical trial would help those designing and planning experiments in OS. Widespread agreement concerning phase 2 efficacy thresholds should expand the number of relapse trials available by creating a transparent set of rules that facilitate study design and appropriate powering. While these represent an incredibly valuable first step, consideration should also be given to other clinical trials approaches that might also accelerate clinical trials timelines. Tighter integration of our preclinical research enterprise with our clinical trials networks could improve our ability to determine the positive or negative predictive value of preclinical data and accelerate solutions that will improve patients' chances of responding when enrolling on clinical trials.

Conclusions

While some say that history should temper the optimism of those who look for science to impact outcomes in patients with OS in the near future, recently converging efforts have matured in ways that dramatically improve our understanding of osteosarcoma's underlying biology. This acceleration of scientific discovery has the potential to drive a revolution in the care of affected patients. Our rapidly improving understanding of the genetic events that drive disease at both the genetic and epigenetic level, informed by predisposition science, coupled with studies that describe the biology of progression with increasing detail have set the stage. Growing collections of PDXs, cell lines, and banked human tissues, and an increasingly agile multi-institutional veterinary clinical trials groups should enable scientific progress with increasing efficiency. Human clinical trials consortia have begun to explore new models for evaluating candidate therapies, and immunotherapeutic strategies have become increasingly sophisticated in ways that may have promise for patients with OS. We believe that opportunities to translate scientific knowledge into clinical impact have never been greater.

However, much work remains. Our efforts highlight a number of critical gaps that could prevent us from translating our new findings into new cures for patients with OS. In organizing these gaps into a set of OSPQs, we highlight here the clear need for investment in all three phases of research: basic, translational, and clinical. Two recurring major themes ran throughout the discussions that fueled the creation of this list of OSPQs: First, while our understanding of the basic biologic mechanisms of OS genesis and progression has improved, there remain a large number of unanswered fundamental questions. Second, better characterization of preclinical models and a better understanding of their utility for predicting outcomes in patients would significantly improve our ability to validate new therapeutic candidates as they emerge. We hope that this codification of the most prominent challenges will serve to organize the efforts of government, philanthropic, and advocacy groups to facilitate meaningful science and will promote interaction within our scientific communities in ways that leverage our collective resources toward achieving a common goal —to offer our patients something far better than we can now.

We extend an open invitation to those physicians and scientists with interest in participating in our monthly calls. To initiate participation, please email the corresponding author.

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Box 1.

Osteosarcoma Provocative Questions (OSPQs)

OSPQ1: What are the disease initiating events in osteosarcoma and how do those events lead to genomic complexity and cellular heterogeneity?

OSPQ2: What can epigenetic profiling tell us about osteosarcoma?

OSPQ3: What can we learn from studying the inherited cancer susceptibility syndromes that increase the risk of osteosarcoma?

OSPQ4: Which research models best represent the biology of osteosarcoma?

OSPQ5: What biological mechanisms mediate osteosarcoma lung metastasis?

OSPQ6. What factors limit the efficacy of immuno-oncology approaches in osteosarcoma?

OSPQ7: Can emerging clinical trial designs compliment and accelerate the development of novel approaches to osteosarcoma?