CORR Insights**: Intratibial Injection Causes Direct Pulmonary Seeding of Osteosarcoma Cells and Is Not a Spontaneous Model of Metastasis: A Mouse Osteosarcoma Model

Daniel M. Lerman MD

Where Are We Now?

Small animal models of oncologic disease play a critical role in the elucidation of oncologic mechanisms and drug development. While imperfect, the overall algorithm of drug development follows a widely accepted course. Initially, compounds are screened in tissue culture with known cancer cell lines to assess compound efficacy in vitro. Drugs that demonstrate an effect on cancer cell lines in vitro are then introduced into small animal models. A wide range of animal models allow the assessment of antitumor efficacy, drug tolerability, pharmacokinetics and pharmacodynamics.

Drug discovery is resource intensive, requiring on average 10 years and USD 1 billion for a compound to move from basic science concept to FDA approval [12]. Despite the well accepted stepwise progression of drug development, approximately 70% of oncology drugs fail during clinical trials [1, 3]. The greatest rate of attrition occurs in Phase II and III—late and costly stages of investigation. The high rate of clinical trial failure is indicative of the complexity of the disease process and the limitations of preclinical research.

Animal models of human cancer provide essential in vivo insights into (1) pathophysiology and novel disease targets, (2) identification of therapeutic agents, and (3) their combination with established therapies [12]. In general, tumor development in animal models is more homogenous and rapid than human cancers. This provides logistical benefits for research, although it also results in limitations in terms of the degree to which the findings in animals may generalize to humans. There is a range of types of animal models, with a typical trade-off between model simplicity and generalizability to humans.

Xenograft models are created by injecting human tumor cells, or implanting primary tumor segments, into immunodeficient mice. Ectopic subcutaneous xenograft models are frequently used due to ease of implantation of tumor cells and monitoring tumor growth under the skin. These models allow for relatively cost-effective and efficient assessments of pharmacologic data, drug efficacy and toxicity. But the utility of ectopic xenografts is limited as they have been shown to be poor models for tumor histology, heterogeneity and metastatic potential [7]. The ectopic, often subcutaneous, location of tumor development fails to recreate the tumor microenvironment and the critical role that the surrounding stromal cells play in tumor development and metastasis.

Orthotopic xenografts more accurately model the tumor microenvironment. As described in the current study [10], orthotopic models are created by
the implantation of tumor cells or primary tumor fragments into an anatomically appropriate site of disease. In this case, cells from a known osteosarcoma cell line were injected into the tibia in hopes that they establish a primary tumor, develop local progression and ultimately spontaneous metastases. Compared to ectopic models, orthotopic xenograft are more resource-intensive when establishing the primary tumor and monitoring growth and/or treatment response. And, as the current study demonstrates, orthotopic models also imperfectly replicate the human disease process.

**Where Do We Need To Go?**

Regardless of the xenograft model employed, the greatest limitations are (1) the models’ reliance on an immunodeficient host animal and (2) the utilization of long-maintained cancer cell lines to simulate a de novo solid malignancy. The critical interplay between the immune system and cancer development is well established and utilized clinically [2]. Appreciation for the immune system’s integral role in oncologic management has spawned an entire field of research and drug development [4]. In osteosarcoma, the development of postoperative infection has been shown to associate with increased patient survival— alluding to the important role the immune system plays in these patients, even when primed in a crude and inadvertent manner [9].

For many cancer types there are readily available human cancer cell lines. These cell lines have been cultivated over time, consequently selecting for cells and genetic mutations that promote growth in an in vitro environment. It has been shown that the behavior of human cancer cell lines can deviate in important ways from that of in situ tumors [8]. While primary tumor fragments can be implanted into immunodeficient host animals as xenograft models, there are many obstacles to this approach—the rarity of the disease process (in sarcoma), scarcity of untreated tumor samples and the resources required for harvesting, transporting, maintaining and ultimately implanting primary tumor samples often prevents this technique from being employed on a large scale.

One strategy that eliminates the above limitations is the utilization of genetically engineered mice (GEM). Conditional disruptions of normal tumor suppressor genes and stimulation of oncogenes allows for GEM to have oncogenesis “turned on,” promoting the spontaneous development of tumors in situ. This replicates the tumor microenvironment, immune response, angiogenesis, and metastasis that is seen in human cancers [7, 12]. An osteosarcoma GEM model exists by conditional inhibition of tumor suppressor genes in osteoblast cell lineage [8]. GEM models can be limited by availability, cost of genetic engineering, and the heterogeneity of tumor development within the population. In contrast to subcutaneous ectopic xenografts, where the tumor can be easily monitored, assessing tumor growth, or response to study drugs, GEM models often require advanced imaging techniques, such as small animal MRI or PET/CT, which introduces additional limitations through cost and availability.

The high-rate of clinical trial failures has been attributed to insufficient preclinical testing and validation. The challenge of establishing a reliable, predictive preclinical model is due in large part to the inherent complexity of solid malignancies.

The genetic composition of a human cancer is dynamic both spatially and temporally. While one or more inciting genetic event(s) (such as chromosomal translocation) is responsible for the initial oncologic phenotype, once the mechanisms responsible for regulation of cellular proliferation and DNA replication are altered, genetic instability results and mutations occur in an additive manner. Therefore, the genetic composition of the cells that compromise a tumor are constantly changing, creating intratumor genetic diversity [6, 13]. Different sub-populations of malignant cells continue to accrue new mutations in unique combinations. This genetic heterogeneity results in variable cellular behavior (branch evolution) and is ultimately the substrate for the development of variable drug resistance patterns [11].

Cancer is complicated. And, just as it is critical to understand the mechanisms of human malignancies, it is important to have an appreciation for the roles and limits of any particular preclinical model. Extensive resources and energy can be spent improving an individual animal model, but invariability, each will have important limitations that ultimately preclude its utilization in isolation. If the goal is improved oncologic therapies and greater rates of therapeutic success in clinical trials, then the current structure of preclinical research should be critically evaluated.

**How Do We Get There?**

The reproducibility of preclinical data has been shown to be remarkably low. In one series of 53 “landmark” studies, the scientific findings were confirmed in only 11% [1]. This indicates a fundamental problem with the current basic science research environment. With career success dependent on publications,
In light of the tendency of the peer-review process to potentiate positive-outcome bias [5], understandably well-intentioned researchers promote their most-dramatic findings. We must realize that what we read in journals may not—indeed, probably does not—represent the full universe of findings on the subjects in question. Minimizing bias through a greater appreciation of negative and no-difference studies and investigator blinding (as in clinical trials) are ways to address the human element [1].

Improving preclinical animal modeling, will likely rely less on improvements in one particular model, and more on utilizing the spectrum of available models to initially screen new compounds in an efficient manner, and then corroborate findings in more complex, representative systems as appropriate.

References