IN THE SPOTLIGHT

Addressing the Controversy: Do Bisphosphonates Directly Affect Primary Tumors?

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Summary: The recent article by Junankar and colleagues focuses on demonstrating the uptake of bisphosphonates (BP) into the primary tumor in both animal models and human samples. Interestingly, the authors were able to establish tumor-associated macrophages as the cell type that takes up the BPs. These studies are an important advancement for understanding the potential benefits of using BPs as adjuvant therapy in patients with cancer. *Cancer Discov; 5(1); 14–5. ©2015 AACR.*

See related article by Junankar and colleagues, p. 35 (6).

The medical potential of bisphosphonates (BP) was first discovered by Dr. Herbert Fleisch in 1968, and they have been used clinically for diseases such as Paget disease, osteoporosis, osteogenesis imperfecta, and cancer-induced bone disease for decades (1). Despite the successful clinical use of BPs and extensive research into their mechanism of action, whether BPs induce tumor cell death directly remains a controversy. For example, several groups have shown that tumor cells are killed by BP treatment *in vitro*, but many have proposed that the concentration of BPs at the tumor does not reach significant levels to kill tumors directly and that the observed effect on tumors *in vivo* is due to indirect effects (2, 3). Adding to the controversy are recent articles by Coleman and colleagues (4, 5) that suggested no overall benefit with the addition of zoledronic acid to the standard of care, but showed a promising reduction in disease-free survival in postmenopausal women. Although these findings suggest a promising clinical approach for these women, it is unclear why the positive effects were only in this subset of patients with cancer. The authors postulate that there are several potential key differences in this population that could have led to these observations (including changes in estrogen status, age, etc.). However, these findings opened the opportunity for the use of BPs as adjuvant therapy for this population of women with bone metastatic disease.

The article by Junankar and colleagues (6) uses a unique approach to directly monitor the uptake of BPs in the primary tumor. This is the first time that a group has been able to directly address whether tumor cells *in vivo* take up BPs. To do this, the authors used intravital 2-photon *in vivo* imaging over a 48-hour time period with near-infrared–labeled BPs and GFP-labeled tumor cells to acquire movies and images. The BPs could be clearly seen moving through the bloodstream within minutes in the non–tumor-bearing fat pad. However, in the tumor-bearing fat pad, the labeled BPs leaked into the surrounding tissue, where it remained for hours. The authors go on to demonstrate that BPs bind to calcium deposits present in the primary tumor, which they also showed were present in patient samples.

Most interestingly, the authors demonstrate convincingly by flow cytometry and immunofluorescence that these BP deposits are engulfed by $CD45^+$ and $F4/80^+$ cells (6). These cells represent tumor-associated macrophages (TAM), which have been shown in numerous studies (7) to support tumor cell growth. Importantly, this group has previously demonstrated that BPs can induce apoptosis in these cells *in vitro*, suggesting that uptake of BPs within the tumors may reduce the viability of the TAMs and, thus, reduce their support of tumor growth (7, 8).

To further establish the impact of these studies, the authors examined ^{99}Tc-MDP (methylene diphosphonate) bone scans of patients. These scans rely on a radioactively labeled BP (MDP) and are frequently given to patients to examine changes in bone turnover and to assess the status of bone metastases. By searching for the presence of MDP uptake in the primary tumor, the authors were able to show that patients have similar BP uptake at the primary tumor in areas of calcification (6), which nicely demonstrated a direct clinical significance of their findings with intravital imaging in mouse models.

Although the authors were able to creatively address some of the remaining controversies in the field regarding whether BPs can affect primary tumor growth and whether tumor cells directly take up BPs *in vivo*, there are still many more questions to answer. First, what happens *in vivo* to the TAMs that have taken up the BPs? This is particularly important due to the numerous studies that focus on TAMs in the literature. If BPs do indeed cause apoptosis of the TAMs, this could explain the reduction of primary tumors seen in the adjuvant setting. Second, what varies between pre- and postmenopausal women that could explain why postmenopausal

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women seem to benefit from adjuvant BPs but not premenopausal women (4)? There are many different potential answers to this question, but perhaps there are differences between the TAMs, leaky vasculature, or changes in calcifications in pre- and postmenopausal women that alter how BPs affect the primary tumor. Third, it will be important to demonstrate that BPs do have a lasting effect on tumor metastasis and to determine if BP uptake in the primary tumor alters bone disease at later time points. This may be harder to determine, because mice treated with BPs will also have uptake in their bones, which will also likely affect metastasis to bone.

In summary, the article by Junankar and colleagues (6) directly looked at the uptake of BPs in the primary mammary fat pad tumors of live mice using real-time intravital imaging. Using this approach, the authors were able to show that the leaky vasculature caused by tumors allows BPs to enter the primary tumor where they are taken up by TAMs. This convincingly demonstrates that the tumor cells do not take up BPs *in vivo*, and that the published effects are indirect. These findings are a critical first step to better understanding how BPs can be used as an adjuvant therapy for patients with primary disease who are at high risk for developing bone metastatic disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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