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DISSERTATION

"Effects of Ra-223 Therapy on Bystander Breast Cancer Cells and the Immune System within the Bone Microenvironment"

by

Calvin N. Leung

M.D./Ph.D. Program

B.A. 2013, Rutgers University, New Brunswick, NJ

Thesis Advisor:

Roger W. Howell, Ph.D.
Distinguished Professor, Department of Radiology, Rutgers University

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Wednesday, August 17th, 2022
9:00 A.M.
Cancer Center G-level Conference Room, Webex

Abstract

Metastatic breast cancer often metastasizes to bone, with expected life expectancy after diagnosis only 19-25 months. Radium-223 dichloride, an alpha-emitting radiopharmaceutical current approved in the treatment of metastatic castration resistant prostate cancer (mCRPC) patients, may potentially provide benefit in bone-metastatic breast cancer patients as well. Ra-223 is a targeted radionuclide therapy for cancers located in the bone, with high bone uptake due to its calcium mimetic properties. The emitted high linear energy transfer (LET) alpha particles are extremely potent in causing damage and cell death, and allow for better normal tissue sparing compared to traditional beta particle-emitting radionuclides. However, their short traversal distance (<100 μm) makes it unlikely that direct irradiation effects alone account for the observed clinical benefits. Bystander effects from irradiated cells that cause toxic responses in unirradiated cancer cells may explain Ra-223's clinical efficacy. *In vitro*, alpha particles have demonstrated potent bystander effects, including apoptotic cell death, that may be propagated much further than the range of the alpha particles. However, their role *in vivo* remains poorly understood. Additionally, whether the use of Ra-223 dichloride clinically has been exploited to its full clinical potential remains to be determined. One potential avenue that Ra-223 dichloride may provide additional benefit to patients is through an immune response, or "abscopal effect." A successful immune response would require both the presence of immune cells in the microenvironment, as well as cytotoxic function capability. To study these questions, female Foxn athymic nude mice were injected with graded activities of Ra-223 dichloride, followed by MDA-MB-231 or MCF-7 breast cancer cell inoculation, to determine the role of the bystander effect in affecting tumor burden progression. Next, the mechanism by which the bystander effect was being propagated was explored using *ex vivo* experiments involving the use of conditioned media from tibiae of Ra-223 treated mice on breast cancer cell cultures. Finally, to determine the capacity of the immune system's ability to conduct an anti-tumor immune response after Ra-223 administration, bone marrow composition analysis was conducted on female Swiss Webster mice with fully functional immune systems. NK cell cytotoxic function was also tested in these mice to determine whether the immune cells were activated, suppressed, or unchanged via a Cr-51 release assay.

Results showed a bystander effect delaying proliferation *in vivo*, with varying sensitivity between the tested cell lines. *Ex vivo* experiments also demonstrated varying sensitivity to a secreted bystander effect on proliferation, suggesting multiple avenues in which the bystander effect is propagated in our *in vivo* model. Bone marrow composition analysis showed only transient bone marrow myelotoxicity, the main limiting factor to a Ra-223 treatment regimen. Finally, bone marrow composition analysis and NK cytotoxic function testing also suggests a time window in which the bone microenvironment may be primed for an immune response. These results, when taken together, suggests that the full range of benefits Ra-223 dichloride may offer clinically remains to be fully exploited, and more studies need to be conducted to determine how else Ra-223 may be leveraged for additional patient benefit.