$\gamma\delta$ T Cells Predict Outcome in Zoledronate-Treated Breast Cancer Patients

JOANNE L. WELTON,^a SALVADOR MARTÍ,^a MOHAMMED H. MAHDI,^c CLARE BOOBIER,^c PETER J. BARRETT-LEE,^{b,c} MATTHIAS EBERL^a

^aCardiff Institute of Infection and Immunity and ^bInstitute of Cancer and Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom; ^cVelindre Cancer Centre, Cardiff, United Kingdom

Disclosures of potential conflicts of interest may be found at the end of this article.

We read with great interest the article by Valachis et al. [1], published in *The Oncologist*. In a comprehensive meta-analysis of 15 randomized clinical trials on adjuvant therapy for breast cancer patients with zoledronate, the authors identified a significant overall survival benefit with zoledronate treatment, in agreement with one smaller meta-analysis [2] but not another [3] conducted earlier. These new findings support the call for zoledronate to be considered as a new standard of care in adjuvant breast cancer therapy [4].

The dilemma remains that the biological mechanism underlying the antitumor role of zoledronate is unclear, as Valachis et al. correctly highlight [1]. The clinical benefit may in fact stem, at least in part, from the activity of zoledronate on the patient's immune system by specifically stimulating V γ 9/V δ 2 T cells, which compose 0.5%–5% of circulating T cells in healthy adults [5]. Targeted immunotherapy studies directly exploiting the potent non-major histocompatibility complex-restricted cytotoxicity of V γ 9/V δ 2 T cells have shown excellent safety profiles and promising clinical observations in a variety of hematological and solid cancers [6], including metastatic breast cancer [7].

Studies by us and others have demonstrated that zoledronate treatment induces a rapid and long-lasting conversion of the peripheral V γ 9/V δ 2 T-cell phenotype from CD27⁺CD45RA⁺ central memory T (T_{CM}) cells to CD27⁺CD45RA⁺ effector memory T (T_{EM}) cells in many, but not all, individuals [7–9]. In Figure 1, zoledronate-treated breast cancer patients with stable disease showed elevated proportions of V γ 9/V δ 2 T_{EM} cells at the time of blood sampling compared with patients with progressing disease and untreated controls (Fig. 1A). No such differences were observed with regard to patient age, time on treatment, or pretreatment clinical status. We then stratified the same patient cohort according to their follow-up disease status at 3–9 months after blood sampling. This showed successful conversion from T_{CM} to T_{EM} cells (as evident by a lower frequency of T_{CM} cells) in patients who were stable 3–9 months after blood sampling compared with patients who went on to show progressing disease (Fig. 1B).

Our analysis illustrates the diagnostic and prognostic potential of a $\gamma\delta$ T-cell-based blood test and implies a link between immune responsiveness and positive outcome with zoledronate therapy, thereby contributing to our

Figure 1. $\gamma\delta$ T-cell memory subsets in metastatic breast cancer patients in relation to clinical outcome. **(A):** Proportion of CD27⁺CD45RA⁺ effector memory T cells among circulating V γ 9/V δ 2 T cells in 10 zoledronate-treated patients in relation to clinical outcome at the time of sampling and in comparison to three untreated patients. **(B):** Proportion of CD27⁺CD45RA⁺ central memory T cells in relation to subsequent clinical outcome at 3–9 months after sampling. Patients received at least two cycles of zoledronic acid (Zometa; Novartis International, Basel, Switzerland, <http://www.novartis.com>) for >1 month and <8 months and had no other active malignancies within the previous year; no active or uncontrolled infections; no autoimmune disorders or serious allergic reactions; no use of immunosuppressives during the previous 3 months; and no prior chemotherapy or cytotoxic agents during the previous 6 months.

Abbreviations: AUROC, area under the receiver operator curve; CI, confidence interval; PD, progressing disease; SD, stable disease; T_{CM} cells, central memory T cells; T_{EM} cells, effector memory T cells.

understanding of the as yet unexplained clinical benefit of adjuvant therapy. These findings are particularly intriguing because new treatments specifically targeting bone resorption, such as denosumab, lack this potential to act on

Correspondence: Matthias Eberl, Ph.D., Cardiff Institute of Infection and Immunity, Henry Wellcome Building, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, Wales, United Kingdom. Telephone: 44-29-206-87011; Fax: 44-29-206-87018; E-Mail: eberlm@cf.ac.uk Received March 19, 2013; accepted for publication July 3, 2013. ©AlphaMed Press 1083-7159/2013/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2013-0097>

$\gamma\delta$ T cells and may not yield the direct antitumor benefit observed with zoledronate.

ACKNOWLEDGMENTS

This study was approved by the North West Wales Local Ethics Committee under reference number 08/WNo01/48 and supported by the Cardiff CR-UK Centre Development

Fund and Cancer Research UK project Grant C28524/A9497.

DISCLOSURES

Peter J. Barrett-Lee: Novartis (H). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

REFERENCES

1. Valachis A, Polyzos NP, Coleman RE et al. Adjuvant therapy with zoledronic acid in patients with breast cancer: A systematic review and meta-analysis. *The Oncologist* 2013;18:353–361.
2. Huang WW, Huang C, Liu J et al. Zoledronic acid as an adjuvant therapy in patients with breast cancer: A systematic review and meta-analysis. *PLoS One* 2012;7:e40783.
3. Yan T, Yin W, Zhou Q et al. The efficacy of zoledronic acid in breast cancer adjuvant therapy: A meta-analysis of randomised controlled trials. *Eur J Cancer* 2012;48:187–195.
4. Gnant M. Adjuvant bisphosphonates: A new standard of care? *Curr Opin Oncol* 2012;24:635–642.
5. Kunzmann V, Wilhelm M. Adjuvant zoledronic acid for breast cancer: Mechanism of action? *Lancet Oncol* 2011;12:991–992.
6. Fournié JJ, Sicard H, Poupot M et al. What lessons can be learned from $\gamma\delta$ T cell-based cancer immunotherapy trials? *Cell Mol Immunol* 2013;10:35–41.
7. Meraviglia S, Eberl M, Vermijlen D et al. In vivo manipulation of V γ 9V δ 2 T cells with zoledronate and low-dose interleukin-2 for immunotherapy of advanced breast cancer patients. *Clin Exp Immunol* 2010;161:290–297.
8. Santini D, Martini F, Fratto ME et al. In vivo effects of zoledronic acid on peripheral $\gamma\delta$ T lymphocytes in early breast cancer patients. *Cancer Immunol Immunother* 2009;58:31–38.
9. Welton JL, Morgan MP, Martí S et al. Monocytes and $\gamma\delta$ T cells control the acute-phase response to intravenous zoledronate: Insights from a phase IV safety trial. *J Bone Miner Res* 2013;28:464–471.