Is it time to abandon RHAMM/HMMR as a candidate antigen for immunotherapy of acute myeloid leukemia?

Dolores J. Schendel

Institute of Molecular Immunology and Clinical Cooperation Group "Immune Monitoring", Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany. E-mail: schendel@helmholtz-muenchen.de

E-mail: lpagano@rm.unicatt.it doi:10.3324/haematol.2012.077099

A major dilemma in the treatment of acute myeloid leukemia (AML) is the fact that current chemotherapies lead to complete remission in the majority of patients but overall survival at five years is less than 25%.[1] Treatment failure is ascribed to the presence of small numbers of tumor cells (minimal residual disease, MRD), potentially including leukemic stem cells (LSC), that are resistant to chemotherapy and lead to subsequent relapse. Post-remission therapy of MRD using allogeneic stem cell transplantation (SCT) has lowered rates of relapse and improved survival, demonstrating that the immune system can eliminate MRD and cure AML. However, relapse still occurs after allogeneic SCT.[2] Therefore, alternative strategies are needed to mobilize more effective immune responses to combat AML relapse, such as vaccines or adoptive T-cell therapies.

A central problem in the development of immunotherapies for AML is the selection of leukemia-associated antigens (LAA) to serve as targets that allow T cells to distinguish blasts and LSC from normal hematopoietic stem cells (HSC). RHAMM/HMMR (CD168), the receptor for hyaluronic acid mediated motility, has been considered a potential LAA for T-cell targeting of AML. This was based on several observations: i) there is a large amount of literature documenting the expression of this receptor on various forms of leukemia and lymphoma, including AML.[3] Levels of mRNA expression in HSC have not been considered relevant when compared with the very high expression in leukemic blasts. To date, studies of protein expression on HSC have not been conclusive; ii) expression of RHAMM/HMMR mRNA was detected in blood samples of the majority of newly diagnosed AML patients and high expression was correlated with poor prognosis.[4,5] Therefore, patients could benefit from therapy that is not restricted to a particular AML subtype; iii) the expression of RHAMM/HMMR, as the name implies, is associated with cell migration. The importance of this characteristic for tumor cell invasion and spread may guard against selection of antigen-loss variants; iv) RHAMM-specific T cells were found in AML patients showing that this LAA can be seen by immune cells. Furthermore, the presence of these immune responses was associated with better clinical outcome in AML;[6] v) finally, RHAMM-derived peptide vaccination resulted in potent immune responses that were linked with a clinical benefit, while a negative impact on normal HSC function was not reported.[7,8]

Together these observations support the hypothesis that RHAMM/HMMR may be suitable for AML, by fulfilling several parameters that have been identified as important in the selection of candidate antigens for immunotherapy for cancer.[9] Nevertheless, two further considerations are equally important. The first is whether RHAMM/HMMR is expressed on LSC. The second is whether the low level of RHAMM/HMMR expression on normal HSC is of any relevance in the face of a very potent T-cell response.

The studies of Snauwaert *et al.* in this current issue of *Haematologica* are the first to provide clear information about the expression of RHAMM/HMMR on LSC at both mRNA and protein levels.[10] The authors applied multi-parameter flow cytometry together with mRNA analysis to analyze sorted cell populations from fresh and cultured leukemia samples obtained from blood and bone marrow of 13 AML patients. The authors concluded that expression of RHAMM/HMMR in LSC is no greater than the background levels detected in healthy HSC. These analyses were extended to mRNA and protein expression during cell cycle progression whereby substantial increases were found in rapidly proliferating progeny. Within mixed AML populations, the clear cycling cells increased RHAMM/HMMR expression while background levels were retained in a small population of supposedly quiescent LSC. Importantly, very high levels of expression were found in proliferating healthy $CD34^+$ cells *in vitro*, detected at both mRNA and protein levels. These observations were confirmed *in vivo* after reconstitution of immunodeficient mice with normal $CD34^+$ cells, which showed dramatic upregulation of RHAMM/HMMR in proliferating cells that were re-isolated two weeks after HSC transplantation.

Based on these observations, the authors conclude that RHAMM/HMMR is not a suitable target candidate for immunotherapy of AML; RHAMM/HMMR-specific T cells may well recognize AML blasts, but they will leave LSC untouched. In addition, healthy cycling $CD34^+$ cells will likely be susceptible to T-cell mediated attack, with life-threatening consequences.

In independent studies, we have directly addressed the issue of whether or not low level RHAMM/HMMR expression in uncultured $CD34^+$ cells, isolated from blood or bone marrow, allows recognition by highly potent HMMR-specific T cells.[11] Here we used dendritic cell priming to obtain allo-restricted HMMR-specific T cells that were restricted by HLA-A2. When the TCR of one high-avidity HMMR-specific T cell was transferred into activated peripheral blood lymphocytes (PBL) of normal donors, it was possible to transfer recognition of $HLA\text{-}A2^+HMMR^+$ tumor cells, opening up the possibility of using this receptor for TCR gene therapy. HMMR-specific TCR-engineered lymphocytes, when adoptively transferred into immunodeficient mice previously transplanted with AML tumor cells, could substantially retard leukemia outgrowth after transfer of very few TCR-expressing lymphocytes.

We then assessed the impact of HMMR-specific TCR-engineered lymphocytes on normal HSC *in vitro* and *in vivo*[11] (DJ Schendel *et al.*, unpublished observations, 2012). Co-cultures of human $CD34^+$ HSC from $HLA\text{-}A2^+$ donors with TCR-engineered PBL strongly reduced colony forming units of all types

when compared to co-cultures using HSC derived from HLA-A2$^-$ donors. This demonstrated that levels of HMMR expression allowed HLA-A2-restricted T-cell recognition of HSC or early progenitors. These findings were extended to reconstitution experiments in HLA-A2-transgenic mice. When HSC-containing bone marrow-derived cells of these mice were pre-cultured with TCR-engineered HMMR-specific T cells before transplantation, they failed to reconstitute hematopoiesis in irradiated mice and the animals died as rapidly as non-transplanted control animals. Therefore, RHAMM/HMMR expression on HSC or early progenitors was adequate for recognition and clearly dangerous in the face of highly potent HMMR-specific T cells.[11]

Snauwaert *et al.* also report that RHAMM/HMMR is expressed in activated lymphocytes, confirming and extending our previous observations.[12] The importance of this finding is exemplified by our study of survivin as another candidate LAA for AML. Here we also generated allo-restricted T cells bearing TCR that were restricted by HLA-A2 in their specific recognition of survivin$^+$ tumor cells. When a high-affinity survivin-specific TCR was introduced into activated PBL, it was possible to transfer survivin-specific tumor recognition, suggesting its posible use in TCR gene therapy. However, major differences were seen when this TCR was transferred into PBL of HLA-A2$^-$ *versus* HLA-A2$^+$ recipient lymphocytes. In the former case, T cells could be expanded and showed high potency killing of HLA-A2$^+$survivin$^+$ tumor cells, but transfer of the same TCR into cells of HLA-A2$^+$ donors led to high apoptosis and loss of recipient PBL. This reflected MHC-restricted fratricide that occurred due to expression of survivin in PBL, allowing HLA-A2-restricted self-recognition by the TCR-engineered cells. mRNA and protein expression does not mean that antigen processing and presentation will automatically allow sufficient epitope expression for T-cell mediated fratricide. Ongoing studies assess whether MHC-restricted fratricide also occurs after transfer of a highly potent RHAMM/HMMR-specific TCR into HLA-A2$^+$ lymphocytes. If fratricide does occur, then HMMR will likely fail to yield high avidity T cells when used as an LAA for vaccines.

So do these results lead us to the conclusion that RHAMM/HMMR should be abandoned as an LAA for immunotherapy of AML? Snauwaert *et al.*[10] argue that it should. Low RHAMM/HMMR expression in LSC may not allow effective T-cell recognition, although this still has to be determined using high avidity T cells. The levels of HMMR in normal HSC, which are comparable to LSC, were clearly sufficient to be eliminated by our highly potent HMMR-specific T cells, so LSC may also be seen. But it is precisely the recognition of normal HSC that is particularly dangerous.

On the other hand, in particular clinical settings, perhaps RHAMM/HMMR should still be considered as a potential LAA for immunotherapy in AML. The strict HLA-A2-restricted recognition of our HMMR-specific TCR indicates that it could be used in TCR gene therapy in an HLA-A2-mismatched setting of SCT. In this case, HLA-A2$^-$ donor lymphocytes would be TCR-engineered, bypassing the issue of potential MHC-restricted fratricide. After adoptive transfer into HLA-A2$^+$ AML patients, clinical benefit would be expected at two levels: residual autologous HLA-A2$^+$ HSC would be eliminated, leading to faster donor chimerism, and residual HLA-A2$^+$ leukemic cells would be targeted, potentially allowing curative treatment because of the immediate availability of potent effector T cells to rapidly eliminate MRD. Thus, due to the high medical need to control AML relapse after SCT, for the moment, HMMR should remain on the list of candidate LAAs.

Dolores J. Schendel is Director of the Institute of Molecular Immunology of the Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany. Her laboratory is developing immunotherapies for cancer using dendritic cells and adoptive T-cell therapy.

Acknowledgments: I wish to thank Stefani Spranger, Bernhard Frankenberger, Irmela Jeremias, Matthias Leisegang, Virna Marin, Barbara Mosetter, Silke Raffegerst, Wolfgang Uckert, Agnieszka Wieczorek and Susanne Wilde for their substantial contributions in deciphering and implementing T-cell mediated responses to HMMR for the clinical benefit of patients with AML. Our work is supported by the German Research Foundation (SFB-TR36) and the Helmholtz Alliance for Immunotherapy of Cancer.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

References

1. Seer Cancer Statistics Review 1975-2007. Available from: http://seer.cancer.gov/csr/ 1975-2007.
2. Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood. 2008;112(12):4371-83.
3. Greiner J, Bullinger L, Guinn BA, Döhner H, Schmitt M. Leukemia-associated antigens are critical for the proliferation of acute myeloid leukemia cells. Clin Cancer Res. 2008;14(22):7161-6.
4. Greiner J, Ringhoffer M, Taniguchi M, Schmitt A, Kirchner D, Krähn G, et al. Receptor for hyaluronan acid-mediated motility (RHAMM) is a new immunogenic leukemia-associated antigen in acute and chronic myeloid leukemia. Exp Hematol. 2002;30(9):1029-35.
5. Greiner J, Ringhoffer M, Taniguchi M, Li L, Schmitt A, Shiku H, et al. mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the development of specific immunotherapies. Int J Cancer. 2004;108(5):704-11.
6. Greiner J, Li L, Ringhoffer M, Barth TF, Giannopoulos K, Guillaume P, et al. Identification and characterization of epitopes of the receptor for hyaluronic acid-mediated motility (RHAMM/CD168) recognized by CD8+ T cells of HLA-A2-positive patients with acute myeloid leukemia. Blood. 2005;106(3):938-45.
7. Schmitt M, Schmitt A, Rojewski MT, Chen J, Giannopoulos K, Fei F, et al. RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. Blood. 2008;111(3):1357-65.
8. Greiner J, Schmitt A, Giannopoulos K, Rojewski MT, Götz M, Funk I, et al. High-dose RHAMM-R3 peptide vaccination for patients with acute myeloid leukemia, myelodysplastic syndrome and multiple myeloma. Haematologica. 2010;95(7):1191-7.
9. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: A National Cancer Institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15(17):5323-37.
10. Snauwaert S, Vanhee S, Goetgeluk G, Verstichel G, Van Caeneghem Y, Vleghe I, et al. RHAMM/HMMR (CD168) is not an ideal target antigen for immunotherapy of acute myeloid leukemia. Haematologica 2012;97(10):1539-47.
11. Spranger S, Jeremias I, Wilde S, Leisegang M, Stärck L, Mosetter B, et al. T cell receptor-transgenic lymphocytes specific for HMMR/Rhamm limit tumor outgrowth in vivo. Blood. 2012;119(15):34409.
12. Leisegang M, Wilde S, Spranger S, Milosevic S, Frankenberger B, Uckert W, et al. MHC-restricted fratricide of human lymphocytes expressing survivin-specific transgenic T cell receptors. J Clin Invest. 2010;120(11):3869-77.