The value of immunotherapy for the treatment of cancer has been clearly established over the past few decades. However, the adverse effects associated with the systemic activation of the immune system have limited the use of immunotherapeutic regimens to malignancies for which standard of care approaches are poorly effective. Acute myeloid leukemia (AML) is a prime example of a neoplasm in which immunotherapy, primarily in the form of allogeneic stem cell transplantation (allo-SCT), is generally administered to patients undergoing relapse or bearing markers of aggressive disease, such as high-risk cytogenetic or molecular abnormalities. The main limitation to the use of allo-SCT is the significant rates of non-specific, off-target adverse effects. Nevertheless, the percentage of AML patients that are actually cured by allo-SCT highlights the sensitivity of AML to immunotherapy, specifically to the graft vs. leukemia (GvL) effect.

The identification of tumor-associated antigens (TAAs) that can be used to focus immune responses against neoplastic cells, including malignant stem cell populations, is therefore critical for the future success of novel immunotherapies. The ideal TAA should be (1) expressed by a wide panel of cancers and primarily (if not solely) by malignant cells, including cancer stem cells, (2) efficiently processed by the antigen presentation machinery and (3) immunogenic. So far, a few AML-associated antigens have been discovered and bear promise. These include, but are not limited to, PR1,1 Wilms’ tumor 1 (WT1),2 and receptor for hyaluronan acid–mediated motility (RHAMM).3 However, no TAAs discovered to date are universally expressed by leukemias and, given the heterogeneity of AMLs, it seems unlikely that a universal antigen expressed by all AML subtypes exists. Furthermore, tumor cells alter their antigenic features as a mechanism to escape immune responses. To address these issues, a multi-antigen approach that approximates the polyclonal immune responses stemming from allo-SCT while avoiding the off-target phenomena that underpin graft vs. host disease could be developed to improve disease outcome in AML patients treated with immunotherapy (Fig. 1).

Paul Ehrlich discovered the granules of polymorphonuclear (PMN) leukocytes in the early 1900s. PMN granules have been classified into four types, based on their content, tendency to be secreted and timing of biosynthesis. We have focused on our efforts on azurophil granules because they are synthesized early during the differentiation of myeloid cells, a stage that reflects the immature cells characterizing AML. The formation of granules and the expression of granule-associated proteases are indeed shut off as myeloid cells mature. Because the majority of AML subtypes involve myeloid cells that are not confined to an immature stage of development, the production of granule contents oftentimes continues, resulting in the overexpression of granule proteases by malignant cells. Although the overexpression of granule proteases makes them ideal leukemia-associated antigens, normal cells also express these proteins. However, normal cells do so at comparatively lower levels, and hence their presentation on MHC Class I molecules is relatively inefficient.4,5

Three factors paved the way for the pursuit of cathepsin G (CG) as a novel target for the immunotherapy of AML. First, we successfully targeted PR1, a nonameric HLA-A*0201-restricted peptide derived from the azurophil granule protease neutrophil elastase (NE) and proteinase 3 (P3). Molldrem et al. pioneered the development of PR1 for the therapy of myeloid leukemia, in particular by investigating PR1-based vaccines in AML patients and—more recently—by engineering an anti-PR1/HLA-A*0201 antibody.6 Second, the CG-derived peptide that displays the highest affinity for HLA-A*0201, CG1 (FLLPTGAEA), was shown to be a naturally processed epitope on the surface of CML blasts.7 Third, immune responses against CG, although not against the CG1 peptide, have previously been reported in malignant and autoimmune diseases.8

There are many parallels between CG, NE and P3. Like NE and P3, CG plays a role in inflammation, has bactericidal properties and is involved in leukemogenesis. We have previously shown that azurophil granules are disrupted in AML cells, allowing for the cytosolic spillage of NE

Gheath Alatrash
Section of Transplant Immunology; Department of Stem Cell Transplantation and Cellular Therapy; University of Texas MD Anderson Cancer Center; Houston, TX USA

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Cathepsin G (CG) is a serine protease normally found within the azurophil granules of neutrophils. CG is expressed during the early stages of normal myeloid differentiation and—aberrantly—by myeloid leukemia cells. We have recently identified CG-derived HLA-A*0201-binding peptides that constitute promising targets for the immunotherapy of myeloid leukemia.
and P3. Along similar lines, CG appears to be localized outside azurophil granules in AML cells, possibly facilitating processing and presentation on MHC Class I molecules. Unlike PR1, which is found in the mature forms of NE and P3, CG1 belongs in the leader sequence of CG, which is naturally cleaved during the processing of the protein. The cleavage of the leader sequence may further facilitate the presentation of CG1, which exhibits the highest predicted binding affinity for HLA-A*0201 as compared with all other HLA-A*0201-binding CG-derived peptides. A key feature that differentiates CG from NE and P3 is that the synthesis of the former is regulated by a distinct promoter. This explains the expression pattern of CG, differing from that of NE and P3, as we and others have confirmed. Furthermore, Jin et al. have demonstrated that CG is downregulated in AML patients bearing the t(8;21) translocation, further highlighting the antigenic heterogeneity of AML.

Despite the promising results obtained by targeting CG, several issues remain unresolved. Which patient populations would obtain clinical benefits from CG-targeting immunotherapeutic approaches? Are there other CG-derived peptides that would be more effective than CG1? How do CG-targeting approaches affect normal hematopoiesis in vivo? In spite of these open questions, the discovery of CG adds to our armamentarium of TAAs that can be targeted by immunotherapeutic approaches for the treatment of AML and possibly other tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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