OncoImmunology 2:11, e27156; November 2013; © 2013 Landes Bioscience

An allogeneic NK cell line engineered to express chimeric antigen receptors

A novel strategy of cellular immunotherapy against cancer

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In recent years, several immunotherapeutic approaches against cancer have emerged, including the adoptive transfer of T cells that have been reprogrammed to react against one or more tumor-associated antigens (TAAs). In most cases, reprogramming is achieved as T cells are engineered to express the antigen-binding domain of a TAA-specific monoclonal antibody fused to intracellular domains that are normally involved in T-cell receptor (TCR) signaling and/or co-stimulation.[1-3] In small cohorts of patients affected by hematological malignancies including both low-grade and aggressive B-cell neoplasms, significant, long-lasting clinical responses were observed upon the administration of these chimeric antigen receptor (CAR)-expressing T cells.[4,5]

To circumvent the use of autologous T cells, which requires labor-intensive steps of isolation and expansion in vitro, Klingermann's group developed an allogeneic natural killer (NK) cell line (NK-92 cells) that express CARs comprising single-chain variable fragments from murine CD19- or CD20-specific antibodies.[6] NK cells are advantageous as compared with T cells as they recognize malignant cells in a HLA-unrestricted manner and can lyse them in the absence of pre-sensitization.[7] NK cells exert cytotoxic functions by multiple mechanisms, including (1) the direct release of granzyme and perforin upon the physical interaction with target cells, (2) the so-called "antibody-dependent cell-mediated cytotoxicity" (ADCC), a process whereby NK cells kill their targets once these are opsonized by antibodies, following the binding of constant fragments (Fcs) to Fc fragment of IgG, low affinity III, receptors (FcγRIIIs), (3) the secretion of T_H1 cytokines, and (4) via the granzyme/perforin pathway upon activation by dendritic cells.[8] However, the infusion of unmodified NK cells, both as a stand-alone intervention and in combination with immunostimulatory cytokines, failed to induce any significant diseases regression in patients affected by multiple solid tumors.[9]

In a recent issue of *OncoImmunology*, Boissel et al. demonstrated that NK-92 cells engineered to express CD20-specific CARs exhibit improved cytotoxicity against primary chronic lymphocytic leukemia (CLL) cells in vitro as compared with parental NK-92 cells pulsed with various anti-CD20 monoclonal antibodies. Of note, Boissel et al. did not use the same anti-CD20 monoclonal antibodies to compare the cytotoxicity of CAR-expressing NK-92 cells to that of NK-92 cells primed for ADCC, which may have introduced a bias. Indeed, the binding affinity of monoclonal antibodies for their targets is known to affect cytotoxicity.[10] However, these results confirm and extend previous reports demonstrating the superiority of CAR-expressing NK cells over NK cells pulsed with TAA-specific antibodies at lysing cancer cells.[11] Taken together, these observations suggest that administration of monoclonal antibodies should be less effective than that of (NK or T) cells engineered to express a CAR based on the same molecule. Although no comparison of the clinical activity of these two immunotherapeutic strategies has been performed, T cells modified o express CD19-specific CARs have been shown to be clinically effective in patients that are resistant to a chimeric bispecific antibody targeting CD3 and CD19 (blinatumomab).[4] Unlike antibodies, CAR-modified cells have the potential to replicate in vivo, and the long-term persistence of these cells might underlie sustained disease control, eliminating the need for repeated infusions.[12]

The adoptive transfer of NK-92 cells expressing CD19-targeting CARs effectively eradicated human SUP-B15, but not TMD-5, leukemia cells growing in immunodeficient mice.[6] Various hypotheses can be put forward to explain this differential activity, including variations in the levels of expression of TAAs (in this case, CD19) on the surface of leukemia cells, the differential sensitivity of SUP-B15 and TMD-5 cells to apoptosis as triggered by CAR-modified NK-92 cells, and the existence of specific mechanisms developed by TMD-5 cells to escape the antineoplastic activity of NK cells. To test the hypothesis that TMD-5 cells escape the cytotoxicity of CAR-modified NK-92 cells because the CAR-bearing NK-92 cells fail to infiltrate the neoplastic bone marrow, Boissel et al. injected CAR-expressing NK cells directly into bone marrow, observing a significant antitumor effect locally but not at distant sites. Moreover, intravenously injected CAR-expressing NK-92 cells were found in the peripheral blood and spleen but not in the bone marrow, confirming the hypothesis that TMD-5 cells are insensitive to this therapeutic approach due to a homing issue. To explain such a homing bias at the molecular level, it would have been of interest to analyze the integrin and chemokine

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Submitted: 11/01/2013; Accepted: 11/01/2013
Citation: An allogeneic NK cell line engineered to express chimeric antigen receptors: A novel strategy of cellular immunotherapy against cancer. OncoImmunology 2013; 2:e27156; http://dx.doi.org/10.4161/onci.27156

receptor profile of CAR-expressing NK-92 cells. The relocalization of antigen-experienced $CD4^+$ T cells to bone marrow is dependent on integrin $\alpha2\beta1$, a collagen receptor.[13] However, since CAR-bearing NK-92 cells were able to cure SUP-B15 acute lymphoid leukemia cells (which also infiltrated the bone marrow), not only the phenotype of NK-92 cells but also features of the tumor microenvironment created by TMD-5 cells might explain their resistance to this immunotherapeutic approach.

Various strategies have been developed to correct defects in the homing of effector T or NK cells within neoplastic lesions.[14] For example, imatinib, a targeted anticancer agent, stimulates NK cells to localize next to foci of malignant cells.[15] Both the trafficking to neoplastic sites and in vivo antitumor activity of T cells modified to recognize a peptide derived from Wilms' tumor 1 (WT-1, a TAA frequently expressed by pulmonary cancers) in a HLA-A24-restricted fashion were improved when these cells were engineered to express chemokine (C-C motif) receptor 2 (CCR2), which recognizes a chemokine that is highly expressed in the lung (i.e., chemokine (C-C motif) ligand 2, CCL2).[16]

The CARs used by Boissel et al. to engineer allogeneic NK cells did not comprise the signaling domain of co-stimulatory molecules. As domains of this type have been shown to promote the persistence of adoptively transfer effector cells in vivo, they may represent a means to improve the antineoplastic activity of CAR-expressing NK cells.[4,5,12,17] Allogeneic NK cells may actually represent a weakness as compared with their autologous counterparts, because they may be rapidly rejected. However, Boissel et al. provided evidence for the expansion of allogeneic NK-92 cells in vivo.[6] In addition, allogeneic NK cells have previously been shown to be efficient for the therapy of acute myeloid leukemia.[18] The proof-of-concept for this allogeneic, CAR-based immunotherapeutic approach has been recently provide in patients affected by B-cell malignancies, as donor-derived allogeneic T cells engineered to express CD19-specific CARs were shown to induce disease regression in individuals that were insensitive to conventional donor lymphocyte infusion (DLIs) upon allogeneic hematopoietic stem cell transplantation, and were not associated with no signs of graft-vs.-host disease.[19] The good safety profile of CAR-expressing allogeneic NK cells in patients further supports the clinical development of this immunotherapeutic approach.[20]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by grants from Ligue contre le Cancer, Agence Nationale de la Recherche (ANR), Labex Immuno-Oncology, Institut National du Cancer (Carpem project), Canceropole-Region Ile de France.

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