Persuading Natural Killer Cells to Eliminate Bad B Cells

Commentary on Altvater et al., p. 4857
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Clinical trials are underway infusing T cells genetically modified to be specific for B-cell malignancies using a chimeric antigen receptor (CAR) to redirect specificity for CD19. However, issues remain about whether the CAR can provide a fully competent application signal and whether other lymphocytes with lytic capacity can target CD19+ tumors.

In this issue of Clinical Cancer Research, Altvater and colleagues investigate whether a CD19-specific chimeric antigen receptor (CAR; or chimeric receptor, chRec) can be generated to improve the ability of natural killer (NK) cells to target the CD19 molecule on the cell surface of malignant B cells, such as acute lymphoblastic leukemia (ALL; ref. 1). NK cells are an attractive cellular platform for combining gene therapy with immune-based therapy as they have endogenous cytolytic potential. This lytic potential can be clinically harnessed by adoptively transferring haplotype-mismatched (haploidentical) NK cells that are capable of lysing acute myeloid leukemia (AML) blasts (2). The killing efficiently occurs upon mismatch between killer-cell immunoglobulin-like receptors (KIR) and their ligands found on classical human leukocyte-antigen (HLA) B and C allele groups, and has been exploited to improve the graft-versus-leukemia (GVL) effect after haploidentical hematopoietic stem-cell transplantation (HSCT), wherein engrafted alloreactive NK cells are attributed to target recipient AML blasts that lack KIR ligands (inhibiting HLA class I molecules), but present on the donor-derived NK cells (“missing ligand”; ref. 3). If engrafted haploidentical NK cells after HSCT are associated with anti-AML effect, what if haploidentical NK cells were infused without HSCT? This has been tested and shown to be effective in some patients with refractory AML when haploidentical NK cells (peripheral blood depleted ex vivo of T cells and activated with IL-2) were infused after lymphodepleting chemotherapy and administered with IL-2, to improve the survival of the infused cells (4). We are building upon this success to infuse haploidentical NK cells for patients with solid tumors, such as neuroblastoma (ClinicalTrials.gov Identifier: NCT00698009). However, the clinical experience with NK cells targeting AML has not been duplicated for adult B-cell (B-ALL).

Rather than relying on the balance between endogenous activating and inhibitory receptors on NK cells to trigger cytolyis of tumor cells, such as B-ALL blasts, investigators have genetically manipulated NK cells to express CARs to redirect specificity for CD19. As the ability of CARs to redirect specificity are evaluated in populations of lymphocytes other than T cells, it is probable that the nature of the cell to be manipulated will have to be considered to develop a CAR that is a fully functional molecule capable of activating genetically modified cells for cytosis, cytokine production and proliferation. Previously, a CD19-specific CAR was shown to activate NK cells through chimeric CD3-ζ and this signaling could be enhanced by the addition of a chimeric 4-1BB co-stimulatory endodomain (9). This work has now been expanded upon by Altvater and colleagues who generated a second-generation CAR (Fig. 1) to improve NK-cell signaling by modifying their CD19-specific CAR endodomain to include a variant of the signaling lymphocyte activation molecule (SLAM)-related receptor 2B4 (CD244), which improves CAR-dependent activation of ex vivo-propagated NK cells in response to docking with CD19. The endosomal molecule 2B4, which recognizes CD48, is endogenously expressed on NK cells, including this group’s NK cells that have been numerically expanded ex vivo on K562-derived artificial antigen presenting cells. The endogenous 2B4 co-receptor was capable of activating NK cells that express a first-generation CD19-specific CAR when targeting a CD19/“CD48” leukemia cell line. To coordinate signaling through CD3-ζ and 2B4 to target primary B-ALL blasts independent of CD48 expression, a next-generation CAR was built that signals through full-length 2B4 cytoplasmic

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Published Online First on July 28, 2009; DOI: 10.1158/1078-0432.CCR-09-0966

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domain fused to CD3-ζ (designated CD19-2B4ζ). By understanding that some of the 2B4 signaling domain’s four immunoreceptor tyrosine-based switch motifs (ITSMs) may contribute to deleterious signaling, the CD19-specific CAR was further modified to include a truncated 2B4 endodomain, shortened to just signal through the first two ITSMs, and fused to CD3-ζ (designated CD19-12B4ζ).

Just as autologous CAR+ T cells are currently being evaluated in clinical trials, preclinical data are being assembled to adoptively transfer NK cells expressing first and second generation CARs. Donor-derived NK cells may be attractive populations of cells to infuse after allogeneic HSCT to enhance the GVL-effect, for in contrast to T cells, engrafted allogeneic NK cells are not typically considered as instigators of graft-versus-host-disease (10). However, issues remain about the long-lived potential of genetically modified NK cells, and for that matter, ex vivo propagated NK cells in general, to show sustained in vivo persistence and thus survive to exert a long term antitumor effect after adoptive transfer. Nevertheless, infusions of NK cells and CAR+ NK cells are now feasible to target both AML and B-ALL, and clinical trials, perhaps concomitant with immune-modulating therapies (11), will determine whether these natural born killers can be called up in the war on cancer.

Fig. 1. Schematic showing prototypical first (black font) and second (blue font) generation CD19-specific CARs that have been expressed in A and B, T cells, and C and D, NK cells. The chimeric signaling endodomains are shown in bold type face on purple background. Not shown are “third generation” CARs, which combine CD28 and CD3-ζ endodomains with chimeric CD137 (4-1BB), as well as other costimulatory domains. The CARs are derived from the following references: A, (8), B, (12), C, (1), D, (9). TM, transmembrane.

References

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*Clin Cancer Res* Published OnlineFirst July 28, 2009.

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doi:10.1158/1078-0432.CCR-09-0966

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