Hematopoietic stem cell transplantation (HSCT) has been increasingly used as a curative treatment for acute myeloid leukemia (AML). However, relapse rates after HSCT in complete remission (CR) are reported between 30% and 70%. In addition, numerous studies suggested that secondary viral infection from a variety of viruses including Epstein-Barr virus (EBV), adenovirus (Adv), and cytomegalovirus (CMV) is among the most common causes of death post-H SCT. Currently, chimeric antigen receptor (CAR)-based T cells have been developed to treat AML in clinical studies, while virus-specific cytotoxic T cells (VST) have been proven to be able to effectively prevent or treat viral infection after HSCT. Thus it would be desirable to develop T cells with the ability of simultaneously targeting AML relapse and viral infection. In this article, we now describe the generation of VST cells that are engineered to express CAR for a specific AML cell-surface antigen CD123 (CD123-CAR-VST). Using Dendritic cells (DCs) pulsed with EBV, Adv, and CMV peptides as sources of viral antigens, we generated VST from A2 donor peripheral mononuclear cells (PBMC). VST were then transduced with retroviral vector encoding D123-CAR to generate CD123-CAR-VST. We demonstrated that CD123-CAR-VST recognized EBV, Adv, and CMV epitopes and had HLA-restricted virus-specific cytotoxic effector function against EBV target. In addition, CD123-CAR-VST retained the specificity against CD123-positive AML cell lines such as MOLM13 and THP-1 in vitro. Thus our results suggested that CD123-CAR-VST might be a valuable candidate to simultaneously prevent or treat relapse and viral infection in AML HSCT recipients.