Dendritic cells (DCs) are powerful antigen-presenting cells (APCs) that activate innate lymphocytes and elicit antigen-specific T-cell responses. DC-based immunotherapy, for instance by means of autologous DCs loaded with tumor-specific antigens ex vivo, has been used in cancer patients with some success. Unfortunately, ex vivo DC-based immunotherapy requires a large number of DCs to be generated from individual patients, and the quality of DCs largely depends on the patient’s clinical conditions. More recently, strategies for the in vivo targeting of DCs have been developed based on chimeric proteins in which a selected tumor-associated antigen is fused to an antibody specific for C-type lectin receptors (CLRs).2

Activated iNKT cells have the ability to license DCs in vivo.5 Mature DCs not only express increased co-stimulatory (i.e., CD40, CD80 and CD86) and MHC Class II molecules but also produce pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin (IL)-12, and chemokines, including CCL17 and CCL21, hence recruiting both T and iNKT cells. Finally, mature DCs acquire the capacity to induce antigen-specific T-cell responses.

Many studies have demonstrated that the co-administration of soluble or cell-associated antigens plus α-GalCer leads to the generation of antigen-specific T<sub>H1</sub> CD4<sup>+</sup> T-cell responses and cytotoxic T lymphocytes (CTLs).3,7 The timing of antigen delivery to DCs is crucial, as DCs exhibit reduced antigen uptake after maturation.8 Based on these observations, we sought to design “artificial adjuvant vector cells” (aAVCs) that would be loaded with α-GalCer and transfected with an appropriate tumor antigen-coding mRNA,9 combining the adjuvant effects of iNKT-cell activation with antigen delivery to DCs in vivo.6,9 (Fig. 1).

We first compared immune responses in mice administered with CD1d-expressing allogeneic vector cells vs. syngeneic DCs.10 Mice receiving aAVCs exhibited stronger antigen-specific T-cell responses than mice treated with DCs transfected with antigen-coding mRNA or iNKT ligand-loaded DCs transfected with antigen-coding mRNA. Interestingly, the magnitude of CD1d expression on aAVCs correlated with the strength of T-cell response.7 By means of this system, we demonstrated that α-GalCer-loaded tumor cells as well as α-GalCer-loaded allogeneic fibroblasts transfected with tumor antigen-coding mRNAs efficiently generate antigen-specific CTLs in murine models.6,9 (Fig. 1).

Next, we evaluated how efficiently human DCs cross-present tumor...
Our approach of combining antigen-expressing cells with α-GalCer closely reproduces the conditions that manifest during immune responses in vivo, leading to broad and effective adaptive immunity. We have shown that aAVCs safely initiate antigen-specific immune responses by activating both the innate and adaptive arms of the immune system. Our results support the development of aAVCs as immunotherapeutic tools against cancer.

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

References
1. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature 2007; 449:419-26; PMID:17898760; http://dx.doi.org/10.1038/nature06175
2. Geijtenbeek TB, Gringhuis SI. Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol 2009; 9:465-79; PMID:19521399; http://dx.doi.org/10.1038/nri2569
3. Fujii S, Shimizu K, Hemmi H, Steinman RM. Innate Vα14(+) natural killer T cells mature dendritic cells, leading to strong adaptive immunity. Immunol Rev 2007; 220:183-98; PMID:17979847; http://dx.doi.org/10.1111/j.1600-065X.2007.00561.x
4. Fujii S, Shimizu K, Kronenberg M, Steinman RM. Prolonged IFN-gamma-producing NKT response induced with α-galactosylceramide-loaded DCs. Nat Immunol 2002; 3:867-74; PMID:12154358; http://dx.doi.org/10.1038/nri827
5. Motohashi S, Nagato K, Kunii N, Yamamoto H, Yamasaki K, Okita K, et al. A phase I-II study of α-galactosylceramide-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer. J Immunol 2009; 182:2492-501; PMID:19201905; http://dx.doi.org/10.4049/jimmunol.0800126
6. Shimizu K, Goto A, Fukui M, Taniguchi M, Fujii S. Tumor cells loaded with α-galactosylceramide induce innate NKT and NK cell-dependent resistance to tumor implantation in mice. J Immunol 2007; 178:2853-61; PMID:17312129

Figure 1. Efficacy of artificial adjuvant vector cells for the induction of innate and adaptive immunity. Artificial adjuvant vector cells (aAVCs) are loaded with α-galactosylceramide (α-GalCer) and engineered to express tumor-specific antigens. When mice are immunized with aAVCs, natural killer (NK) and invariant NKT (iNKT) cells kill aAVCs, leading to the uptake of aAVC debris (including tumor-associated antigens) by dendritic cells (DCs) in situ. Alongside, DCs mature in response to CD40-CD40L signaling and cytokines secreted by activated iNKT cells, hence becoming able to stimulate both CD4+ T and CD8+ T cells in an antigen-specific manner.
7. Fujii S. Exploiting dendritic cells and natural killer T cells in immunotherapy against malignancies. Trends Immunol 2008; 29:242-9; PMID:18372215; http://dx.doi.org/10.1016/j.it.2008.02.002

8. Hermans IF, Silk JD, Gileadi U, Salio M, Mathew B, Ritter G, et al. NKT cells enhance CD4+ and CD8+ T cell responses to soluble antigen in vivo through direct interaction with dendritic cells. J Immunol 2003; 171:5140-7; PMID:14607913

9. Fujii S, Goro A, Shimizu K. Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT-cell ligand confer antitumor immunity. Blood 2009; 113:4262-72; PMID:19164596; http://dx.doi.org/10.1182/blood-2008-08-176446

10. Shimizu K, Mituno T, Shinga J, Asakura M, Kakimi K, Ishii Y, et al. Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust in situ immune responses by dendritic cells. Cancer Res 2013; 73:62-73; PMID:23108144; http://dx.doi.org/10.1158/0008-5472.CAN-12-0759