CD1d-restricted invariant natural killer T cells (iNKT) constitute an important immunoregulatory T-cell subset involved in the induction of antitumor immune responses. Here, we provide a view on the recent observation that Vγ9Vδ2-T cells, through trogocytosis of CD1d-containing membrane fragments, have the capacity to act as antigen presenting cells for iNKT.
Invariant natural killer T cells (iNKT) represent an important immunoregulatory T-cell subset. In humans, iNKT cells are mostly CD4<sup>+</sup>, or CD4<sup>-/CD8<sup>-</sup> double negative (DN). CD4<sup>+</sup> iNKT produce both T helper type 1 (Th1) cytokines, such as granulocyte macrophage colony stimulating factor (GM-CSF), interferon γ (IFNγ) and tumor necrosis factor α (TNFα) and Th2 cytokines (e.g., the interleukins IL-4 and IL13), whereas DN iNKT primarily produce Th1 cytokines. Depending on their predominant cytokine profile, iNKT can be important in autoimmunity, transplantation, anti-viral, anti-microbial, and anti-tumor immune responses. (1) Through development of long-lasting Th1-biased pro-inflammatory immune responses, iNKT were shown to play a role in various tumor-metastasis models (e.g., lymphoma, melanoma, colon carcinoma) (reviewed in ref 1 and 2). Furthermore, deficient iNKT numbers predict poor clinical outcome in various tumor types, including colon carcinoma, neuroblastoma, and head and neck squamous cell carcinoma. (3-5)

iNKT can be activated by the synthetic glycolipid α-galactosylceramide (α-GalCer) presented by CD1d-expressing antigen presenting cells (APC) and various strategies to exploit the antitumor characteristics of iNKT have been employed in immunotherapy clinical trials for advanced cancer. In a Phase I trial, intravenous (i.v.) administration of α-GalCer alone was tested and found to consistently induce the rapid depletion of circulating iNKT, with signs of immunological activation in a subset of patients with reasonable pretreatment iNKT numbers. No clinical responses were observed (reviewed in ref. 1). A second approach studied i.v. administration of immature and mature α-GalCer-pulsed monocyte derived dendritic cells (moDC) as APC. This approach appeared to be more potent as compared to i.v. α-GalCer alone, resulting in a transient immune activation (i.e., inducing changes in iNKT-, NK and T-cell numbers and stimulating higher levels of IFNγ) in patients treated with α-GalCer-pulsed immature moDC. Immune activation was further increased and prolonged in patients treated with α-GalCer-pulsed mature moDC with a >100 fold expansion of iNKT. In some of these patients these immune features correlated with signs of antitumor activity, including decreases in the presence of tumor markers and tumor necrosis (reviewed in ref. 1). Other related approaches studied i.v. or intranasal administration of α-GalCer-pulsed APC fractions, generated from peripheral blood mononuclear cells (PBMC) using GM-CSF and IL-2, either alone or in combination with intra-arterially administered autologous iNKT. These approaches also efficaciously increased iNKT and NK cell responses, as well as antitumor activity, in several patients. Of note, major side effects have not been observed in the trials performed to date (reviewed in ref. 1). As certain known
caveats restrict the utility of moDC (e.g., relatively long in vitro culture period and functional heterogeneity) or the probably even more heterogeneous and poorly defined APC fractions generated from GM-CSF and IL-2 their therapeutic predictability appears to be limited. Therefore, efforts to develop more potent platforms for iNKT-based cancer immunotherapy are required. (1,6)

Vγ9Vδ2-T cells, the predominant γδ-T-cell subset of PBMC, have been reported to exhibit characteristics of professional APCs, including antigen processing and presentation, as well as the provision of costimulatory signals. (7) Vγ9Vδ2-T are directly activated by natural (isopentenyl pyrophosphate (IPP)) and synthetic (bromohydrin pyrophosphate (BrHPP)), phosphoantigens (pAgs) presented in the context of CD277/BTN3A1. (8) Activated Vγ9Vδ2-T cells secrete a variety of chemokines and cytokines, thereby contributing to anti-inflammatory processes and cytolysis. (9) We thus reasoned that Vγ9Vδ2-T could be more suitable for clinical applications than moDCs, as they are comparatively more frequent within the peripheral blood (1–5% of PBMCs in healthy adults), mature more rapidly into professional APCs (<24 hours), home to the lymph node more efficiently and exhibit a more uniform and consistent proinflammatory status. (6,7) To address this, we set out to study the glycolipid Ag presentation capacity
of Vγ9Vδ2-T cells activated using different modes, including pAg. In contrast to prior reports for MHC Class I and MHC Class II mediated Ag presentation, α-GalCer-pulsed Vγ9Vδ2-T cells were not able to directly activate iNKT. (10) Phenotypical analyses of α-GalCer activated Vγ9Vδ2-T cells confirmed the upregulation of costimulatory markers, but showed no expression of CD1d molecules, which is required for the presentation of glycolipid Ag to iNKT. Importantly, when Vγ9Vδ2-T cells were activated using pAg-expressing cells that co-expressed relatively high levels of CD1d, we found that the so activated Vγ9Vδ2-T cells subsequently expressed CD1d and had obtained the capacity to present α-GalCer to iNKT. Additional experiments demonstrated that this acquisition of CD1d resulted from trogocytosis of CD1d-containing membrane fragments from pAg-expressing cells by Vγ9Vδ2-T. (10)

Although we found that α-GalCer loaded moDC clearly outperformed Vγ9Vδ2-T cells functioning as APCs on a per cell basis, Vγ9Vδ2-T cells have the advantage of a quicker maturation into APCs as well as that of quantitative superiority, being more numerous as compared to moDC precursors. Furthermore, culture supernatants of iNKT activated via Vγ9Vδ2-T cells as APCs were more biased toward a Th1 type cytokine profile –with a higher IFNγ/IL-4 ratio– than those iNKT cells stimulated by moDCs. However, expression of CD1d on Vγ9Vδ2-T with APC functional capacity requires trogocytosis of CD1d-containing membrane fragments from pAg-expressing donor cells, thereby causing inherent limitations in level, membrane distribution and duration of CD1d expression on Vγ9Vδ2-T APCs, thus clearly limiting their practical and widespread use. In vitro transfection of Vγ9Vδ2-T with CD1d could overcome this hurdle and thereby pave the way for future clinical assessment of the potential superiority of CD1d+ Vγ9Vδ2-T cells over moDCs with respect to iNKT-based immunotherapeutic approaches (Fig. 1A). Finally, Vγ9Vδ2-T cells are well established antitumor effector cells with the capacity to secrete proinflammatory cytokines and cytolytic molecules upon recognition of pAg expressing tumor cells. (6,7,9,10) It is therefore conceivable that Vγ9Vδ2-T combine their lytic “kiss of death” to kill pAg-expressing tumor cells with tumor-cell trogocytosis, thus acting as cytotoxic effectors and APCs in the tumor microenvironment and propagating the presentation of tumor-associated Ags to the immune system (Fig. 1B).
Acknowledgements

This work is supported by grant nr 90700309 from The Netherlands Organization for Health Research and Development (ZonMw) and grant VU 2010– 4728 from the Dutch Cancer Society (KWF).

References