Natural Killer T Cell – Based Cancer Immunotherapy

□□ *Commentary on Motohashi et al., p. 6079*

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In this issue of *Clinical Cancer Research*, Motohashi et al. (1) present data on the first clinical trial using adoptive transfer of invariant natural killer T (iNKT) cells in patients with advanced or recurrent non – small cell lung cancer. This study follows up on several other recently done clinical trials targeting iNKT that all aim to induce the same robust antitumor effects in humans as previously observed in murine tumor models.

iNKT constitute an evolutionary conserved T lymphocyte lineage that displays an extremely restricted T-cell antigen receptor repertoire with limited junctional diversity (Vα24-Jα18 in human and Vα14-Jα18 in mouse). iNKT can recognize certain glycolipid antigens, which may include the endogenous isoglobotrihexosylceramide and the synthetic α-galactosylceramide (α-GalCer), in the context of the monomorphic nonclassic MHC class I – like antigen-presenting molecule CD1d. One of the features that distinguish iNKT from conventional T cells is their capacity to rapidly and in some cases simultaneously produce large amounts of both T helper (Th) 1 proinflammatory (promoting cellular immune responses) and Th2-type anti-inflammatory (promoting certain antibody responses) cytokines upon triggering, thereby mediating the regulation of a variety of immune responses, including antitumor immune responses (2).

iNKT play a physiologic role in tumor immunosurveillance against carcinogen-induced tumors (3) and are required for the antitumor effects of low-dose interleukin (IL)-12 treatment (4) through their capacity for Th1 cytokine production (Fig. 1). Many studies designed to evaluate the antitumor activities of iNKT have used the model and highly specific glycolipid antigen α-GalCer (KRN7000) that was originally isolated from the marine sponge *Agelas mauritianus* in a screen for novel antitumor agents by the KIRIN pharmaceutical company (Gunma, Japan; ref. 5). Antitumor effects of α-GalCer were observed in a variety of tumor metastasis models of liver, lung, and lymph nodes, including colon carcinoma, T-cell lymphoma, sarcoma, melanoma, and lung carcinoma, suggesting broad clinical applicability (reviewed in ref. 6). On presentation of α-GalCer by CD1d-expressing antigen-presenting cells, iNKT are activated and rapidly produce large amounts of predominantly IFN-γ but also IL-4 that cumulatively result in the activation of NK cells, B cells, $CD4^+$ and $CD8^+$ T cells, and the antigen-presenting cells with which they interact. Through the production of IFN-γ, as well as through direct CD1d signaling (7), iNKT enhance dendritic cell maturation and IL-12 production. It is this cascade of events that is eventually believed to result in the development of a Th1-biased proinflammatory antitumor immune response that is pivotal for the antimetastatic activity of α-GalCer by promoting the generation of a long-lasting antitumor effector cell population and by inhibiting tumor angiogenesis (8, 9).

CD1d is constitutively expressed by dendritic cells and other antigen-presenting cells at low levels by certain other tissues and can be up-regulated in response to stress (2, 8). The possible contribution of tumor cell CD1d expression remains unclear. iNKT can mediate antitumor immunity through the activation of other immune cells, in which case tumor regression would be independent of tumor cell CD1d expression. Direct lysis of CD1d-expressing tumor cells has been reported (10), however, although some tumors might escape direct lysis by iNKT through shedding of neutral glycolipids (11) and/or their absence of costimulatory signals that could lead to altered iNKT activation and consequent suppression of antitumor responses (2, 8).

Clinical evaluation of the antitumor activity of iNKT began when i.v. administration of α-GalCer was evaluated in a phase 1 clinical trial in patients with solid tumors. Although no clinical responses were observed, this study showed that α-GalCer was well tolerated and indicated the importance of a sizeable pool of iNKT in patients treated with α-GalCer, as signs of immune activation only occurred in the smaller subset of patients with relatively normal iNKT numbers (12).

Simultaneously, studies showed quantitative and qualitative defects in the iNKT pool in various types of cancer, including colon cancer, lung cancer, breast cancer, melanoma, head and neck squamous cell carcinoma, prostate cancer, myelodysplastic syndromes, and progressive malignant melanoma, although not in glioma (reviewed in ref. 6). Murine experiments indicated that the antitumor activity of α-GalCer could be enhanced when α-GalCer was loaded onto dendritic cells (13 – 15), this has thus far been evaluated in three clinical studies. Indeed, therapy with α-GalCer-pulsed dendritic cells seemed more potent and induced inflammatory tumor responses, tumor necrosis, and decreases in tumor markers in several patients as well as expansion of iNKT over several weeks to a few months and an increase in adaptive T-cell immunity (16 – 18). Again, immunologic responses were most prominent in patients with relatively high numbers of circulating iNKT

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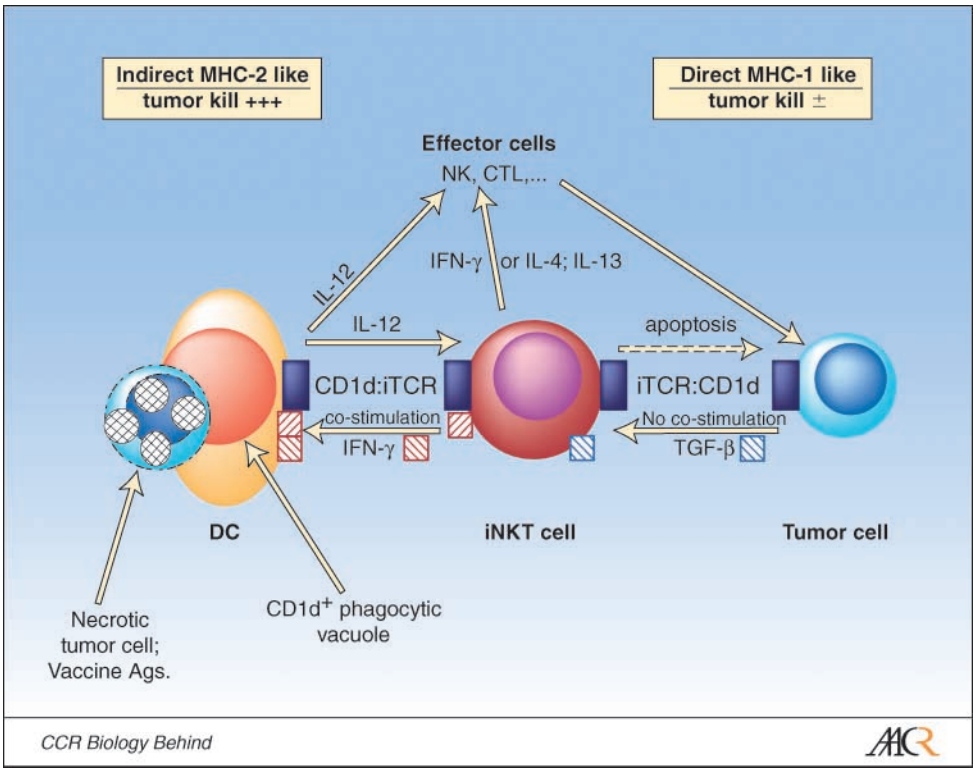

Fig. 1. Simplified model for recognition modes by iNKT. To the left, normal antigen-presenting DC enhance Th1 antitumor responses through a MHC class 2-like interaction also involving co-stimulation (red hatched to left) and DC Th1 cytokines such as IL-12 (red hatched to right). To the right, presentation by tumor cells lacking co-stimulation and secreting suppressive cytokines such as TGF-β (blue hatched to right) results in iNKT activation toward Th2 response (IL-4, IL-13) and consequent suppression of antitumor imunity. An intermediate effect might be expected if immature DC or defective intratumoral DC were the predominant presenting cells. Other variables include potential glycolipid antigens from the tumor presented directly by the tumor and/or indirectly via DC to the iNKT, the predominance of certain iNKT subsets, and the presence of other immunoregulatory cells.

(17) and *in vivo* iNKT responses were strongest using mature dendritic cells (18).

The quantitative defects in iNKT numbers that are observed in many cancer patients are accompanied by qualitative defects in the residual iNKT population, including defective IFN-γ production (19), which thereby hamper antitumor effects of the iNKT ligand α-GalCer. Using this line of thinking, beneficial clinical results may be expected from immunotherapeutic strategies directed at expansion and activation of Th1-polarized iNKT in cancer patients. This is further supported by models in which the antitumor activity of adoptively transferred iNKT was shown in murine lung cancer and melanoma (14, 15) and by accumulating evidence in man indicating that numerical defects in the size of the iNKT pool are indeed of clinical significance, as they are associated with poor prognosis in patients with neuroblastoma and colon cancer (20, 21).

In this issue of *Clinical Cancer Research*, Motohashi et al. describe the results of a clinical phase 1 study in which six patients with advanced or recurrent non–small cell lung cancer received autologous *in vitro*–expanded iNKT that were cultured from leukapheresed peripheral blood (1). Although treatment did not result in clinical responses, it was well tolerated and resulted in some interesting immunologic phenomena, although some cannot with certainty be tracked back to iNKT as the purity of the infused iNKT product was relatively low (0.3-25%), and included many classic T and NK cells. In addition, cells were transferred while still activated. This might encourage an initial response, but risks rapid clearing, as is the fate of classic activated T cells, hence the very large doses used in trials of the latter cells. As desired, the study resulted in increased numbers of circulating iNKT in several patients and was not accompanied by autoimmune phenomena, which is a concern when transferring cells that seem to regulate self-tolerance. Motohashi et al. selected patients for inclusion in their study with relatively high amounts of circulating iNKT. Although this is reasonable for toxicity analysis, one might expect that patients with more deficient iNKT populations would benefit most from administration of iNKT and that this patient group should not be excluded in future trials, which ideally would also be of larger scale allowing evaluation of clinical responses.

Clearly, the article of Motohashi et al. sets the stage for further studies that will focus on optimization of this interesting type of immunotherapy. We believe that apart from attempts to use more purified populations of iNKT, future studies should additionally focus on a comparison of the effects of different iNKT subsets, as these have been shown to be functionally distinct. $CD4^+$ iNKT produce both Th1- and Th2-type cytokines, whereas $CD4^-CD8^-$ double-negative iNKT, and especially the rarest subset, $CD8^+$ iNKT, produce Th1-type cytokines (22–24). As antitumor immune responses have traditionally been reported to benefit most from Th1-type immune responses, one would expect that these different iNKT subsets would differentially affect antitumor immune responses and this was indeed shown in recent preclinical models in which double-negative iNKT, but not $CD4^+$ iNKT, from murine liver facilitated rejection of sarcoma and melanoma (25). As iNKT from different tissues have different antitumor potential (i.e., liver > spleen > thymus; ref. 25), identification of factors responsible for these differences could be of benefit in the selection and generation of iNKT that would be expected to be most beneficial in clinical studies. In this regard, it is noteworthy that the iNKT transferred by Motohashi et al. were largely the desired double-negative iNKT. A relatively high frequency of $CD8^+$ iNKT was observed in several individuals,

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although it remains uncertain whether these cells represented true CD8αβ$^+$ or CD8αα$^+$ iNKT (i.e., activated double-negative iNKT). Other approaches that seem worthy of future clinical evaluation include the combination of iNKT transfer followed by α-GalCer treatment (ideally in the context of dendritic cells that can simultaneously be loaded with tumor-associated antigens), iNKT therapy after chemotherapy pretreatment as this seems to sensitize tumor cells to iNKT-mediated cytotoxicity (26), and direct modulation of CD1d on antigen-presenting cells (e.g., by anti-CD1d monoclonal antibodies) as this has been shown to trigger the production of the Th1-promoting cytokine IL-12 (6). Interestingly, CD4$^+$CD25$^+$ regulatory T cells, which constitute another subset of immunoregulatory T cells (reviewed in ref 1), actively down-regulate immune responses, including antitumor responses and iNKT responses (27). As this population of CD4$^+$CD25$^+$ regulatory T cells is frequently expanded in cancer patients, one can imagine that, to allow the full induction of protective immunity by iNKT, iNKT therapy might need to be combined with CD4$^+$CD25$^+$ regulatory T-cell depletion, for example, by denileukin diftitox (28).

The insight into the biology of iNKT and immunoregulatory T cells in general has expanded rapidly over recent years. iNKT therapy by itself might not be sufficient to promote long-lasting immune responses, but it has the potential to substantially stimulate the development of conventional long-lasting antitumor immune responses when combined with, for example, dendritic cell – based vaccination.

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