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Developing Understanding of the Roles of CD1d-restricted T cell Subsets in Cancer: Reversing Tumor-induced Defects

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Summary

Invariant natural killer T-cells ('iNKT') are the best-known CD1d-restricted T-cells, with recently-defined roles in controlling adaptive immunity. CD1d-restricted T-cells can rapidly produce large amounts of Th1 and/or Th2//Treg/Th17-type cytokines, thereby regulating immunity. iNKT can stimulate potent anti-tumor immune responses via production of Th1 cytokines, direct cytotoxicity, and activation of effectors. However, Th2//Treg-type iNKT can inhibit anti-tumor activity. Furthermore, iNKT are decreased and/or reversibly functionally impaired in many advanced cancers. In some cases, CD1d-restricted T-cell cancer defects can be traced to $CD1d^+$ tumor interactions, since hematopoietic, prostate, and some other tumors can express CD1d. Ligand and IL-12 can reverse iNKT defects and therapeutic opportunities exist in correcting such defects alone and in combination. Early stage clinical trials have shown potential for reconstitution of iNKT IFN-gamma responses and evidence of activity in a subset of patients, with rational new approaches to capitalize on this progress ongoing, as will be discussed here.

Keywords

cytokines; tumor immunity; CD1; CD1d-reactive T cells; iNKT; NKT

1.1. CD1d-restricted T Cell Populations-I: Invariant natural killer T cells

Natural killer T cells (NKT) are a population of innate-like T cells with unique activation properties and effector functions related to NK cells. The best characterized population of NKT cells, termed invariant NKT (iNKT), was initially identified by a restricted T cell receptor repertoire. iNKT express a canonical, invariant T cell antigen receptor comprised of Vα14 and Jα18 in mice and rats and Vα24-Jα18 in humans and non-human primates, with preferred (although not essential or invariable) Vβs, in both cases. Unlike classical T cells, which recognize peptides presented by highly polymorphic MHC molecules, iNKT cells recognize (glyco-)lipids via MHC-like, non-polymorphic CD1d molecules (1–10). The basis of iNKT regulatory function is their rapid secretion of multiple cytokines and chemokines accompanied by CD1dspecific cytotoxicity following TCR triggering (1–7). iNKT rapidly secrete large amounts of different cytokines after activation and thereby regulate immune

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responses. These products include both regulatory factors (e.g. IL-4, IL-10, IL-13) as well as pro-inflammatory agents such as IL-2, IL-17, TNFa, CCL3 (MIP1a), and IFNγ, reflecting their capacity to suppress or stimulate immune responses (1–7). iNKT were shown to contribute to immune surveillance in early stage tumors and in chemically-induced cancers (3- 5;11–13). The originally-defined prototypic high-affinity iNKT glycolipid ligand, alpha-galactosylceramide (αGalCer) was identified in a screen for anti-cancer agents. αGalCer and iNKT have subsequently been shown to have anti-cancer activity in animal models and anti-tumor potential in patients (3-5;11,12), as well as to have anti-pathogenic activity (14–18). Although exogenous CD1d ligands similar to αGalCer have been identified from pathogenic as well as non-pathogenic microorganisms (14,16,19), the identity of physiological endogenous ligands that can also mediate CD1d-dependent T cell activation remains supposed, but so far identification has been limited to ligands for subsets (14,16,19–22). Furthermore, unike T cells, iNKT can also be activated by cytokine combinations (21).

The main CD1d-expressing cell types have been identified as dendritic cells (DCs), macrophages, B and murine T cells (1–4;16,23–27). To date, physiological functions of CD1d in anti-tumor activity (11–13), tolerance induction (5) and host defense (14–18) have been best established in the case of DCs. The interaction between iNKT and APC (antigen presenting cells: monocytes, macrophages, DCs) appears to be of central importance in regulating immune responses. Monocytes or DCs loaded with αGalCer can activate iNKT *in vitro* and *in vivo*, with subsequent iNKT stimulation of DC maturation (1–10;19–22;28–31). The interactions between iNKT and DCs appear to share many features with those between conventional $CD4^+$ T cells and DCs, although priming is not required and there are other key differences. DCs pulsed with a specific Ag (in this case αGalCer) stimulate iNKT through TCR ligation and this can be enhanced by B7 (CD80/86) ligation of CD28 on the iNKT (20–22; 28–31). iNKT activation and production of IFNγ are markedly enhanced by DCs producing IL-12, with increased expression of IL-12 receptor on activated iNKT (1–7; 28–31). CD40L expression by activated iNKT can in turn activate DCs through ligation of CD40, with iNKT IFNγ further stimulating DC IL-12 (1–7; 28–31). These interactions provide a mechanism by which iNKT markedly amplify IL-12 production by DCs, and are consistent with the requirement by iNKT, in some anti-tumor responses, for low-dose exogenous and/or physiological endogenous IL-12 (1–7;11–13; 28–31). In several systems, iNKT are the primary responders to low dose IL-12 rather than NK cells (11–13;32–36). Finally, it should be noted that the profile of chemokine receptors expressed by peripheral blood iNKT indicates that they primarily traffic to peripheral tissues, consistent with their biological function being to interact with immature DC in tissues and stimulate their maturation (37,38).

1.2. CD1d-restricted T Cell Populations-II: relation of iNKT to other CD1d-reactive T cells

Human and rodent iNKT have many common features and closely resemble one another in activity and in general properties. However, iNKT frequencies are lower in humans than in mice (39). This is true both in the periphery and in the organs including liver. In fact, human liver is dominated by non-invariant CD1d-restricted T cells (40–42), many of which do not even express NK markers (41), although “tip-of-the iceberg” iNKT behave similarly (43). Furthermore, both human and murine bone marrow tend to be dominated by the “non-invariant” (‘Type 2’) CD1d-restricted NKT cells (1–7;44).

Human iNKT proportions decline with age (45,46), whereas in mice they rise (47), possibly due to declines in other lymphocyte populations. This, together with minimal surface expression of CD1d by healthy human hepatocytes (23,41) unlilke mouse (24,26), may explain why αGalcer is lethal in older mice, causing a Con-A-like acute hepatitis (48), but

has minimal effects in most adult humans. Indeed, cytokine responses to αGalcer administration are only detected in patients with higher levels of iNKT (49). Furthermore, men generally have less iNKT than women, and levels are also typically lower in those with chronic diseases of many types, including multiple auto-immune diseases and cancers 39,45,46). Therefore, despite overall conservation to the level of species cross-reactivity of iNKT with CD1d (50), there are important CD1d-restricted T cell species differences.

Finally, while all the CD1d-restricted T cells described above are αβ T cells, there is no reason in principle that γδ T cells could not also be CD1d-restricted, as have been described for other CD1 molecules (16). Indeed, such cells have been described and are not always protective. CD1d is up-regulated in murine model coxsackie virus infection and its recognition specifically by $V\gamma4^+$ T cells is associated with the autoimmune viral myocarditis *sequelae* of otherwise successful anti-picornaviral responses (51).

2.1. Principle of CD1d-restricted T cell anti-tumor activities

CD1d-restricted T cell populations physiologic role in tumor immunosurveillance is mediated at least partly through APC maturation and IL-12 induction and via both NK and $CD8^+$ T cells (11–13;21,27– 30;39). In addition, immunity against many tumor models is observed with therapeutic activation of iNKT by selective agonist α-galactosylceramide (αGalCer) presented by $CD1d^+$ APC (11–13). Sequential production of IFNγ, initiated by iNKT and subsequently produced by NK cells, is pivotal for the antimetastatic activity of αGalCer and other agents affecting CD1d-restricted T cells, such as IL-12 and cytokine combinations such as IL-12 and IL-18 (11–13;21). Direct CD1d-restricted T cell cytotoxicity may contribute via classic granule-mediated as well as TNF family mechanisms, since some tumors express CD1d (see below). Despite normal IL-4 production and activation marker up-regulation, iNKT in tumor bearing mice have defective IFNγ responses (11–13;52–54). These are reminiscent of cancer patient iNKT defects in activation by αGalCer or IL-12 (55–57). Indeed, the presence of Th1-type IFNγ-producing CD1d-restricted T cell populations is a positive prognostic indicator in a number of cancers described (see below).

2.2. CD1d-restricted T Cell Populations in Cancer Patients and CD1d Expression in Cancer

Quantitative defects in the iNKT pool are found in various types of cancer including melanoma, colon, lung, breast, head and neck squamous cell carcinoma (HNSCC), prostate cancer, myelodysplastic syndromes and progressive multiple myeloma (13;45,46;55–58). However, these differences are not absolute, there is overlap between groups, and these defects are not unique to cancers, being associated with many inflammatory conditions (59).

Importantly, these statistically significant quantitative defects are frequently accompanied in certain advanced cancers by profound but reversible defects in the capacity of iNKT to both proliferate (55–57,60) and produce IFNγ *in vitro* and *ex vivo* (55–57). Interestingly, both the proliferative defect and the Th1 response block are reversible *in vitro*, and unlike committed memory T cells, iNKT defects can therefore potentially be corrected in patients. IL-2 reverses the iNKT proliferative defect (55,60), similarly to how it well-known overcome some forms of T cell anergy. Similarly, IL-12 can reverse the block in IFNγ production in response to αGalCer *in vitro* (55), reflecting the *in vivo* block we subsequently identified in a tumor model (54). Importantly, conventional T cell responses from the same patients were normal, indicating that these defects are iNKT-specific (55–57,60). Not all cancers exhibit iNKT defects and relative iNKT cell Th2 biases are not found in all (especially early) stages or cancers and other clear exceptions have been reported (58,61).

Certain tumor types are known to express CD1d and thus can be directly recognized by iNKT (42,61–66). These include some of the very tumors where selective functional defects of iNKT have been identified: hematopoietic malignancies, prostate cancer, and certain but not all neurological tumors (54–57;61). The functional consequences of CD1d on tumor cells are not well understood, but increasing evidence suggests this may impact iNKT. For example, early and intermediate stage myeloma cells express CD1d, which can be targeted for killing, but this is lost on advanced myeloma and most myeloma cell lines (67–69). Other mostly solid human and model tumors have generally been believed to be CD1d-negative (B16 melanoma cell lines represent a good example of this), reflecting limited CD1d expression outside normal hematopoietic cell types (23–27). However, there is evidence for at least some CD1d expression on various solid tumor cell lines (70–73). Furthermore, very low levels of CD1d are sufficient for presentation to iNKT, just as for MHC-peptide complexes and other T cells. The presence of CD1d on some tumors makes them sensitive to iNKT cytotoxicity (61,63,74,75) as well as potentially direct mechanisms of apoptosis/cell suicide, as seen in $CD1d^+$ myelomas upon CD1d cross-linking (69). Therefore, CD1d expression may be a common demoninator in advanced cancers with failure of Th1 iNKT driven by tumor acting as non-professional APCs. Of course, other factors may contribute, including CD1d expression by stroma and infiltrating immune cells in various tumors and stages, as well as in response to some therapies. Indeed, another mechanism of targeting tumors by iNKT can involve them killing tumor-associated macrophage-type cells (76).

Most human cancer studies utilize blood iNKT and therefore investigate systemic effects. However, some studies have been performed using human iNKT associated with tumors. Results show that tissue and tumor-infiltrating lymphocyte (TIL) iNKT and other CD1d-reactive ‘NKT’-type cells are distinct and can be enriched relative to in matched blood (42,77–79). In liver cancer, iNKT cytokine responses can be Th1-biased (42,77), like such cells in healthy liver and other liver diseases (40–42), and unlike the equivalent Th0 cells in mouse (1–7). Interestingly, TIL can be enriched for $CD4^+$ iNKT, which at least from healthy donors, produce higher levels of regulatory/Th2 cytokines *ex vivo* (1–7;36;78). Human iNKT have shown the ability to kill tumor cells *ex vivo*. In many cancer patients, these cells are depleted from tissues, and in some cases replaced by Type 2 NKT (42,77).

There is also the remarkable finding that iNKT and other CD1d-reactive peripheral blood T cell populations producing IFNγ *in vitro* are strongly and selectively associated with improved prognosis in patients with glioma, colon cancer HNSCC, and hematological cancers (55,58,80,81). This latter specific feature of CD1d-restricted T cell Th1 responses could be of diagnostic value.

2.3. Role of iNKT in Prostate Tumor Immunity: an example of the relation of human and model studies

We first described reversible numerical and functional iNKT defects in patients with advanced prostate cancer (55). Similarly, a functional iNKT defect was found in TRAMP mice stimulated with glycolipid αGalCer *in vivo* (Figure 1; 54). Furthermore, iNKT deficiency exacerbates sensitivity to the TRAMP prostate cancer model (53). iNKT can be found in established TRAMP tumors in the TIL compartment. We characterized the interaction of tumor cells with iNKT cells from TRAMP mice *ex vivo* compared to iNKT cells from healthy mice. Systemic iNKT in both normal and TRAMP mice constitutively express low levels of CD69. However, high levels of CD69 as well as IL-12RB1 are expressed by TIL iNKT, suggesting iNKT are hyper-activated within tumors (54).

TRAMP tumor cell lines, human CaP lines and primary prostate epithelium as well as primary TRAMP tumors express CD1d (54). Moreover, CD1d on the TRAMP-C2 cell line

is functional. TRAMPC2 pulsed with αGalCer stimulate IL-2 secretion by iNKT hybridomas (54). Therefore, we tested whether tumor cells could aberrantly activate iNKT. TRAMP tumor cells induce expression of activation markers CD25, PD-1 and IL-12RB1 on primary iNKT (54). TRAMP tumor cells modestly activate primary iNKT *ex vivo* even without exogenous ligand. αGalCer+ TRAMP tumor cells induce iNKT IL-4, but not IFNγ (54). IL-4 production occurs independently of co-stimulatory molecules presented by $CD1d^+$ DC (30), whereas iNKT IFNγ is enhanced by IL-12 produced by DC (28-30). However, despite iNKT up-regulation of IL-12R, IL-12 alone was not sufficient to stimulate their IFNγ production. Only both IL-12 and the high-affinity ligand αGalCer together could induce IFNγ production by iNKT (54), thus showing that tumor cells can reversibly inhibit iNKT. This defect can be overcome by provision of strong TCR signals (such as provided by the high affinity ligand αGalCer) in combination with Th1 adjuvant IL-12.

The mechanism(s) by which iNKT in the presence of tumors acquire a Th2 like profile is not fully understood, but appears to be related to a specific defect in signaling to IFNγ production (54). As mentioned, DCs play a fundamental role in controlling iNKT effector functions, and iNKT can control DC maturation. This positive feedback loop provides a further potential *in vivo* contributing mechanism to help explain the observed qualitative iNKT defects. As discussed above, some other tumors express CD1d (62–73) as well as human and mouse prostate epithelial cells (54). Therefore, one may speculate that $CD1d^+$tumors present endogenous glycolipids to iNKT, hence there is some cytokine production even in the absence of αGalCer (54), as has been shown in other systems (82,83), leading to iNKT activation that is distinct from normal DC-induced activation. Finally, provision of exogenous high affinity ligand-loaded tumor cells has been shown to break tumor tolerance in some systems even in the absence of IL-12 (84,85).

2.4. Further Examples of NKT-related therapy

2.4.1. CD1d mAb bypasses iNKT defects in models

CD1d mAb have widely been used to block CD1d-reactive T cell activity in vivo (1–7). However, an unexpected effect of this approach has been identified. Direct CD1d mAb administration induces a potent Th1 and type 1 interferon response both *in vitro* but also *in vivo*, through maturation of dendritic type and other $CD1d^+$ antigen presenting cells, such as monocytes (86,87). As mentioned above, CD1d cross-linking of myeloma cell CD1d leads to apoptosis, which has been suggested as another element of a multi-pronged attack in $CD1d^+$ tumors (69).

As described for several tumor cell line models, anti-CD1d mAb can also delay or actually prevent tumor growth (88). In models, this approach is synergistic with other approaches, both conventional and immuno-therapeutic (89). Therefore, this may be a future alternative means to bypass defects in NKT cells of cancer patients (Figure 2).

2.4.2. Further NKT-related therapeutic Approaches

Several further direct approaches to exploit the anti-tumor activity of CD1d-restricted T cell populations have been described. For example, direct transfer of iNKT cells is therapeutically active against a range of model tumors (11–13;34).

Unlike most classical T cells, IL-12 and IL-18 combined will also activate NKT independently of the TCR (21) and this combination has been shown to enhance anti-tumor activity (11,90). NKT activity is also synergistic with NK cells in mice treated with IL-12 and IL-18 (11,21,90).

Finally in this regard, iNKT have also been shown to have powerful adjuvant-like activity for T and B cell immune responses to model antigens, a range of pathogens, and against tumors (91-98). In particular, as also mentioned above, αGalCer presented on $CD1d^+$ tumor cells or carried to DC on some CD1d-negative tumors (84,85), or presented by dendritic cells and other APC, augments anti-tumor activity in numerous models (96-99). Therefore, NKT have the potential synergise with other immuno- and conventional therapies. This is discussed further below for models and in the context of clinical trials.

2.5. Optimizing Cancer Vaccines through iNKT cells

While some potentially tumor reactive T cell clones may be lost (by central or peripheral mechanisms) in tumor-bearing individuals, other clones appear to remain naïve or tolerized and can potentially be activated by tumor vaccines. Multiple tumor vaccines have been shown to require iNKT presence for optimal activity, including both GM-CSF-transduced ('GVax') (100) and some types of CpG based vaccines (101–105). It is likely that other vaccine types are purely (but still usefully) synergistic with NKT-related therapies. Such synergy has been described, as above, for CD1d mAbs with other immunotherapies in various models (89). The efficacy of GM-CSF tumor vaccine is largely impaired in iNKT cell deficient as well as CD1d KO mice (100), which supports a critical role for iNKT in the requisite maturation of DCs for effective antigen presentation in this system. However, as in direct anti-tumor responses (11,12), CD1d-restricted T cells are not protective for all cancer vaccines, with CD1/noninvariant CD1d-restricted T cells apparently having deleterious effect on anti-tumor immunity (106).

3.1. iNKT-related clinical trials

Cumulatively, *in vivo* results from tumor models (Figure 3) and *in vitro* patient results, along with results in various other diseases have led to a range of current and planned clinical trials exploiting the NKT system in different ways (107). Initial phase clinical trials attempting to induce antitumor immunity through activation of iNKT have revolved around the ligand, αGalCer. Further details are provided below, as described in detail in other reviews in this issue of *Clin. Immunol*. (108–113).

In the first iNKT trial, in which patients with solid tumors received intravenous αGalCer, signs of immune activation only occurred in the fraction of patients with relatively normal iNKT numbers (49,109). Despite overall relatively specific effects, αGalCer has been reported to have an off-iNKT target effect of inducing type-1 IFN (114), which could also affect anti-tumor activity *in vivo*.

As murine experiments indicated that anti-tumor acitivity of αGalCer is enhanced by loading onto DC (11,12;99), similar approaches have been evaluated in clinical trials for solid and hematological malignancies. αGalCer-pulsed monocyte-derived 'DC' produced more potent immune activity, including inflammatory tumor responses, tumor necrosis and decreases in tumor markers in some patients, as well as expansion of iNKT up to a few months and an increase in adaptive T cell immunity (11-13;112,113,115-117). Local targeting of αGalCer-pulsed APC has been shown to be well-tolerated in HNSCC patients (118).

iNKT adoptive transfer has also been effective in mouse tumor models (11–13;34,119). Based on these observations and those that the size of the IFNγ-producing NKT-type cell pool also appears to correlate with survival (15, 55,58,80,81), a study evaluated feasibility of adoptive transfer of autologous iNKT-enriched populations in cancer patients, and reported that the treatment was well-tolerated and could be accompanied by clinical responses (120). Combining iNKT-enriched product with αGalCer-pulsed APC has been associated with evidence of some clinical responses as well as increased cytokine responses in HNSCC

(121). As purity of initial enriched iNKT products has been variable and mostly modest, future studies aim to use more homogeneous populations of iNKT to allow evaluation of clinical responses.

Current immunotherapeutic approaches in cancer may also be expected to be substantially more potent when combined synergistically with iNKT restoration/activation. For examples, iNKT can activate other T cells and limitations on the latter are being relieved in current trials involving anti-CTLA4 and anti PD-1 mAbs. It is likely that direct positive iNKT activation will be synergistic with approaches to inihibit conventional T cell inhibitory pathways.

Further trials have had impacts on various NKT-type populations. In pioneering model studies by several groups, bone marrow NKT-type cells (which unlike mouse liver, but like human bone marrow are dominated by *non*-invariant NKT; 4,20,44;122-125), were shown to contribute to graft-versus-tumor (GvT) activity, but also be capable of suppressing graft-versus-host disease (GvHD) (123–125). This potential double benefit was exploited in several regimens in which the non-invariant ('Type 2') NKT were enriched and activated *in vivo*. NKT in general are resistant cells, which can also recover faster than other T cells from potent stimulations and/or insults, such as pharmacological doses of IL-12, anti-CD3 mAb, steroids, etc. (1–7;21,22,90). Dr. S. Strober's group found that a clinical protocol-derived treatment with total lymphoid organ irradiation and anti-thymocyte globulin (TLI + ATG) led to rapid restoration of NKT populations ahead of conventional T cells (123–125). These types of treatment could also enhance bone marrow transplant (BMT) efficacy in multiple models (123–126). However, a note of caution was raised by the finding that iNKT DC activation and IL-12 production downstream of αGalCer could exacerbate GvHD (127). Therefore, a clinical non-invariant NKT enhancing protocol combining TLI + ATG with BMT was tested and found to have a strong protective effect against acute GvHD (128,129). In parallel, non-myeloablative BMT and kidney transplantation without immunosuppression in patients with renal failure due to myeloma led to transient mixed chimerism and tumor control without GvHD or kidney rejection (130,131). Donor leukocyte infusions (DLI) can enhance BMT anti-tumor effects and most recently recipient leukocyte infusions (RLI) have been shown to have similar activity. In particular, iNKT were the protective population in models of the RLI approach (131).

Based on the large amounts of model data, others trials, and our preclinical data summarized above, a phase I clinical trial of autologous iNKT in patients with advanced cancer was initiated (PI. Dr. S. Balk; DF/HCC #06-432), and has now completed 8 melanoma patient treatments. The approach is feasible and well-tolerated, with only local/grade 1-2 toxicity. The trial is conducted through the Dana Farber/Harvard Cancer Center, in collaboration with Drs. S. Hodi and G. Dranoff, who have extensive expertise in the conduct of melanoma clinical trials. The methods for iNKT purification and expansion are similar to those we have published previously (55,133), with modifications for clinical grade use. Dr. J. Ritz (DFCI) supervises the iNKT cell culture. Patients undergo leukapheresis yielding ~10^{10} PBMC. Residual iNKT (~ 0.01% iNKT, ~ 10^6 cells) are purified from the leukapheresis product using GLP-grade biotinylated mouse anti-human mAb (6B11) against the human invariant Vα24Jα18 TCR chain (55,133) and GMP grade antibiotin micro-beads for purification on a magnetic column, as described (133). This procedure yields populations highly enriched in iNKT (~ 50% pure), with little or no losses. The bulk of purified iNKT are then expanded *in vitro* with CD3 mAb and IL-2 (133) for therapeutic use of up to > 100 million total iNKT/infusion at 3 bi-weekly infusions. The first 3 patients received iNKT alone. Since there was no grade 3 or greater toxicity, subsequent patients additionally receive GM-CSF subcutaneously for 10 days with second and third iNKT administrations, to mobilize and activate dendritic cells. Patients are restaged as appropriate 3 – 4 weeks after

final iNKT infusion and followed thereafter. Complete formal clinical and immunological results will be reported at conclusion of the trial. In summary, these data confirm feasibility of such a clinical trial, show isolation and expansion of iNKT from patients with advanced melanoma, and infusion safety.

4.1. Conclusions

In conclusion, although iNKT cells and other CD1d-reactive T cells appear to frequently suffer attrition in advanced cancers and some other diseases, this can be reversible and mechanistic insights reveal rational approaches to restore NKT cell physiological protective activities (Figure 3) for cancer (as above), as well as for other therapeutic settings such as sickle cell disease (134). At least some 'Type 2' non-invariant CD1d-reactive T cells and even iNKT cells can inhibit anti-tumor responses, including those of other iNKT in tumor models (135-137), so CD1d-reactive T cell-related therapeutic approaches need to avoid augmenting undesirable responses alongside protective ones. Although highly specific for iNKT, based on lack of activity in Jα18 KO mice (lacking only the TCR J region used by iNKT and a small number of other T cells; 1–7), there is a report of direct type-1 IFN inducing activity of αGalCer on human liver cells (114), which could contribute either positively or negatively where αGalCer is used therapeutically. A spectrum of αGalCer analogues are now in preclinical development (e.g. 111,138), some of which will be deployed in phase 1 trials imminently. Although, as expected, toxicity has been minimal, the therapeutic potential of NKT-based approaches has not yet been fully realised in early clincal trials. However, a number of recent immunological and clinical observations suggest both that progress is being made and that various means to improve on NKT-related therapies will become available in the near future (108–113). Indeed, a wide-ranging series of approaches involving (or even bypassing) NKT cell populations are at various stages of late-stage preclinical development.

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Highlights

- There are 2 major CD1d-restricted T cell populations
- Both CD1d-restricted T cell subsets can contribute to anti-tumor immunity
- Both CD1d-restricted T cell subsets can “lose” against progressive tumors
- Both CD1d-restricted T cell subsets defects can be reversed
- Improved CD1d-restricted T cell therapies are nearing clinical trials

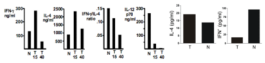

Fig. 1. αGalCer stimulated NKT-dependent cytokine production is decreased in prostate cancer model *in vivo* and *in vitro*

Left: 15 or 40 wk. old mice with prostate tumors (T) or normal controls (N) were stimulated with αGalCer *in vivo* and serum cytokines analyzed 90 min. later. **Right:** Splenocytes of 40 weeks old tumor-bearing mice or normal controls were stimulated *in vitro* with 100 pg/ml αGalCer and culture supernatants tested for IL-4 and IFNγ 24 hr. later. Cytokine levels of unstimulated mice splenocytes were below detection limit.

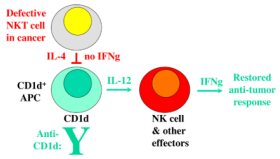

Fig. 2. Bypassing iNKT cell defects with CD1d cross-linking

iNKT defects in cancer shown in ***red***. CD1d mAb can stimulate CD1d+ APC maturation and IL-12 production (86,87). This can lead to activation of downstream Th1-type effectors such as NK cells, resulting in anti-tumor (***green***) as well as anti-viral effects (87-89). Blocking of Type 2 non- invariant CD1d-reactive T cells with Th2 bias may also contribute to net gain in anti-tumor activity. FInally, a direct pro-apoptotic effect of CD1d mAb on CD1d+ tumor cells has also been described (69), potentially augmenting this effect.

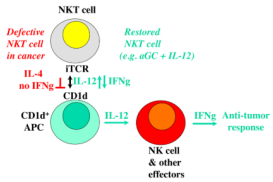

Fig. 3. Simplified model for iNKT involvement in anti-tumor responses

iNKT cell defects in cancer shown in ***red***. iNKT defects can be corrected (***green***). NKT cells can contribute to anti-tumor responses through several mechanisms, but the dominant protective pathway described to date depends on mutual stimulation of iNKT cells interacting with CD1d+ APC, such as some myeloid dendritic cells in humans, most APC in rodents.

Table

Examples of Published and Ongoing iNKT-Targeted Clinical Trials

Published iNKT Trial	**Indication**	**Ref.**
αGalcer i.v.	Advanced Cancer	49, 109
αGalcer-loaded monocyte-derived DC.	Advanced Cancer	115
αGalcer-loaded monocyte-derived matured DC.	Multiple Myeloma	116, 113
αGalcer-loaded monocyte-derived APC.	Non-Small Cell Lung Cancer	117, 112
αGalcer-loaded monocyte-derived DC.	Head & Neck Squamous Cell Cancer	118, 112
αGalcer-expanded iNKT-enriched T cells.	Non-Small Cell Lung Cancer	120, 112
αGalcer-loaded APC & expanded iNKT.	Head & Neck Squamous Cell Cancer	121, 112
Ongoing Clinical Trial	**Indication Status**	**(*04/2011*)/Ref.**
Anti-iNKTCR mAb purified & expanded iNKT.	Stage IV Melanoma	ongoing; n = 8
iNKT-expressed adenosine receptor agonist.	Sickle Cell Disease	ongoing; 134
αGalcer-loaded matured DC + lenalidomide.	Multiple Myeloma	ongoing; 113