International Journal of
Molecular Sciences

Review

License to Kill: When iNKT Cells Are Granted the Use of Lethal Cytotoxicity

Angélica Díaz-Basabe [1,2], Francesco Strati [1] and Federica Facciotti [1,*]

1 Department of Experimental Oncology, IEO European Institute of Oncology IRCCS, 20139 Milan, Italy; angelicajulieth.diazbasabe@ieo.it (A.D.-B.); francesco.strati@ieo.it (F.S.)
2 Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, 20135 Milan, Italy
* Correspondence: federica.facciotti@ieo.it

Received: 5 May 2020; Accepted: 28 May 2020; Published: 30 May 2020

Abstract: Invariant Natural Killer T (iNKT) cells are a non-conventional, innate-like, T cell population that recognize lipid antigens presented by the cluster of differentiation (CD)1d molecule. Although iNKT cells are mostly known for mediating several immune responses due to their massive and diverse cytokine release, these cells also work as effectors in various contexts thanks to their cytotoxic potential. In this Review, we focused on iNKT cell cytotoxicity; we provide an overview of iNKT cell subsets, their activation cues, the mechanisms of iNKT cell cytotoxicity, the specific roles and outcomes of this activity in various contexts, and how iNKT killing functions are currently activated in cancer immunotherapies. Finally, we discuss the future perspectives for the better understanding and potential uses of iNKT cell killing functions in tumor immunosurveillance.

Keywords: iNKT; cytotoxicity; cancer; infections; CD1d

Invariant natural killer T (iNKT) cells are a non-conventional T cell population co-expressing natural killer (NK)-lineage receptors and a semi-invariantly re-arranged T cell receptor (TCR) [1]. The TCR of these cells is usually composed by Vα24-Jα18 (human) or Vα14-Jα18 (mouse) α chains paired with a Vβ11 (human) Vβ8, -7, or -2 (mouse) β chain [1,2]. iNKT cells recognize microbial and self-lipid antigens presented by CD1d, a non-polymorphic, MHC class I-like molecule [3]. In mice, iNKT cells are mainly located in the liver and spleen (making up of 40% and 2% of the total T cell population); in the intestine they represent approximately 1% of total lymphocytes [3,4]. Less is known about iNKT cell distribution in humans, but they appear to be less abundant [1,4]. Due to the co-expression of NK receptors and a T cell receptor, as well as various cytokine receptors, iNKT cells respond very rapidly to TCR and/or cytokine signals with immediate production of cytokines participating to innate immunity and fostering adaptive immune responses [5].Because of their developmental pathway, that includes positive selection by thymocytes, iNKT cells are functionally mature even before they exit the thymus and promptly possess an antigen-experienced phenotype [6]. iNKT cells are tissue homing and tissue resident participating and, in many cases, mediating various immune responses in peripheral organs, from tissue homeostasis to microbial infections and tumor immunosurveillance, where they can exert either a protective or a pathogenic role depending on the context and the tissue involved [4]. In this review, we focus on how iNKT cell cytotoxicity plays a crucial role from the defense and clearance of cancer cells to infectious diseases.

1. iNKT Cell Activation

The adaptive activation of iNKT cells is based on the recognition of CD1d-presented lipid antigens by the TCR (Figure 1). Exogenous antigens (Table 1) can enter the antigen-presenting cell via the mannose receptor or via endocytosis. All iNKT cells recognize and are potently activated by α-galactosylceramide (α-GalCer), a bacteria-derived glycolipid originally discovered in marine sponges [1]. Since then, several microbial antigens have been discovered both in pathogen and commensal microbes [7–14]. On the other hand, it has been demonstrated that the iNKT cell activation by self-antigens is important during thymic selection [1,15,16], infections [17–20], and autoimmune diseases [21]. Potential endogenous iNKT cell ligands include phosphatidylinositol, phosphatidylcholine, cardiolipin, sphingomyelin, lysophospholipids, gangliosides, and other glycosphingolipids, as they have been shown to bind CD1d [22].

Table 1. Microbial antigens recognized by iNKT cells (adapted from [9]).

Microorganism	Pathogenicity	Antigen
Arthrobacter	Commensal, opportunist	M-AcM-MAG
Aspergillus fumigatus and *Aspergillus niger*	Opportunists	Asperamide B
Bacteroides fragilis	Commensal, opportunist	α-GalCer (Bf)
Bacteroides vulgatus	Commensal, opportunist	α-GalCer
Borrelia burgdorferi	Pathogen	BbGL-II (1,2-di-O-acyl- 3-O-a Dgalactopyranosyl-sn-glycerol, 6)
Candida albicans	Commensal, opportunist	ChAcMan
Ehrlichia muris	Pathogen in rodents only	Not defined
Entamoeba histolytica	Opportunist	Lipopeptidophosphoglycan (EhLPPG)
Helicobacter pylori	Commensal, opportunist	Cholestoryl-a-glucosides, especially monoacyl a-CPG
Lactobacillus casei	Commensal	Glc-DAG
Leishmania donovani	Opportunist	Lipophosphoglycan (LPG)
Mycobacterium tuberculosis	Pathogen	Phosphatidylinositol mannoside PIM)
Prevotella copri	Commensal	α-GalCer
Rothia dentocariosa	Commensal, opportunist	M-AcM-MAG
Saccharopolyspora	Environmental, opportunist	M-AcM-MAG
Sphingomonas paucimobilis	Commensal, opportunist	a-glucuronosyl ceramide (GSL-1/ aGlcUCer)
Sphingomonas yanoikuyae	Environmental, commensal, opportunist	a-galacturonosyl-ceramides
Sphingomonas wittichi	No pathogenicity reported	a-galacturonosyl-ceramides
Streptococcus pneumoniae and Group B *Streptococcus*	Commensal, opportunists	SPN-Glc-DAG, SPN-Gal-Glc-DAG

Innate activation of iNKT cells, like natural killer cells, can be elicited through the balance between stimulatory and inhibitory signals via NKR (natural killer receptors) and KIR (killer-cell immunoglobulin-like receptor) [5,23]. Through the activation of some NKR, like NKG2D, iNKT cells can recognize stressed cells by the interaction with MHC-like molecules like MICA and MICB [5,24,25]. On the contrary, some NKRs and many KIRs produce inhibitory signals when they interact with classical HLA molecules [5,26]. Moreover, antigen-presenting cells, when stimulated with TLR ligands like LPS (lipopolysaccharide), secrete cytokines such as interleukins 12 and 18 that can activate iNKT cells in a CD1d-independent manner (Figure 1) [1,2,5,27,28].

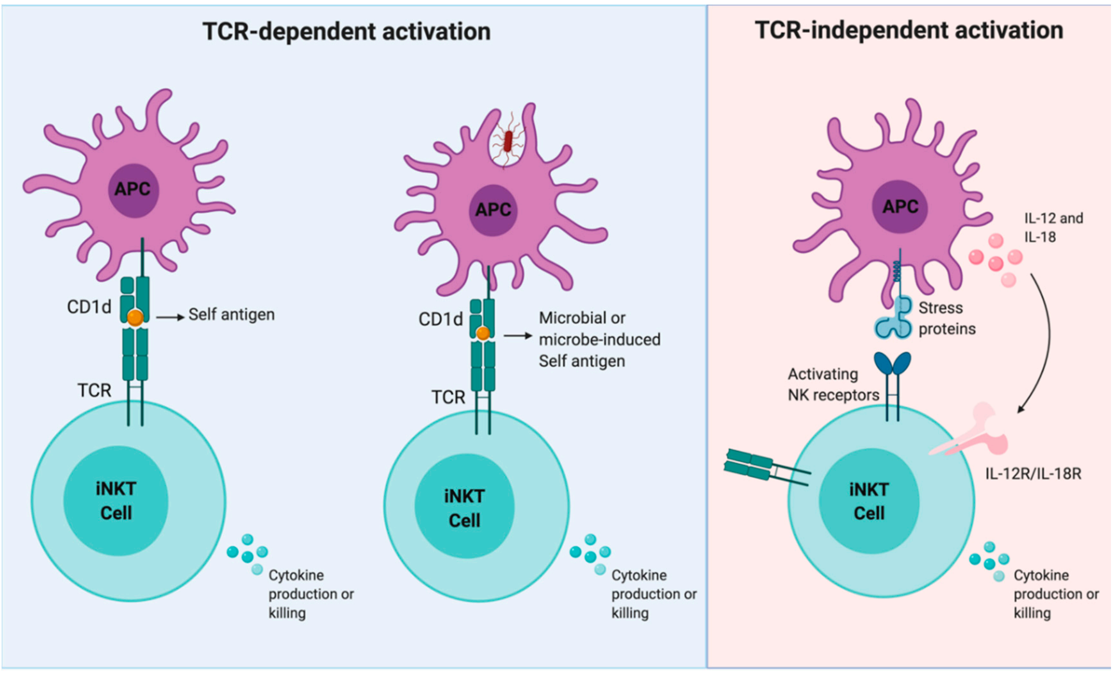

Figure 1. Mechanisms of iNKT cell activation.

2. iNKT Cell Subsets

iNKT cells are characterized by a massive and fast cytokine release shortly after activation. Human and murine iNKT cells can be classified into different functional subsets according to their cytokine secretion profile upon activation, in a way similar to the T helper classification [5,29]. NKT1 cells are similar to the T helper 1 (Th1) cells as they express the transcription factor T-bet and they secrete the typical Th1 cytokines, like IFN gamma and TNF, as well as cytotoxic molecules; NKT2 cells secrete IL-4 and IL-13; NKT17 cells are characterized by RORγt expression and IL-17A, IL-21, and IL-22 secretion [3,4]. Furthermore, an NKT10 subset has been identified in adipose tissue and intestinal polyps; these cells produce the anti-inflammatory cytokine IL-10, but, contrary to conventional Tregs, NKT10 cells do not express the transcription factor FOXP3, even if iNKT cells can express it after stimulation with TGF-β [4,29,30]. Although all subsets differentiate in the thymus, several lines of evidence have shown that further post-thymic differentiation takes place both in mice and humans [4].

After thymic development, iNKT cells can also be classified according to the expression of CD4 and CD8. In mice, iNKT cells can only be CD4+ or CD4-CD8- [2]. In humans, iNKT cells can be divided into CD4 CD8 double negative (DN), CD4 positive, or CD8 positive [2,4]. Circulating DN and CD8+ populations in humans are commonly associated with IFNγ secretion and cytotoxic activity similar to the NKT1 subset [4].

3. Immune Cell-Mediated Cytotoxicity Mechanisms

Only a few subsets of immune cells are capable of exerting cytotoxic functions. In the case of iNKT cells, this is a prerogative of the NKT1 subset, since this is the only population that expresses cytotoxic molecules [31]. In general, this NKT1 subset exert its cytotoxic activity through two main mechanisms: the death receptor pathway (or extrinsic apoptotic pathway), and the cytotoxic granule release (Figure 2) [32].

Members of the TNF family of ligands, Fas ligand (CD178, CD95L or FasL), TNF-related apoptosis ligand (TRAIL or Apo2 ligand), and TNFα are expressed by all cytotoxic populations, including iNKT cells [33–36]. Binding of these ligands to their respective receptors, Fas (CD95), death receptor 5 (DR5), and TNF receptor 1 (TNFR1), triggers the extrinsic apoptotic pathway [37]. Death ligands are usually bound to the cell membrane or packaged within cytotoxic granules [35]. Ligand-receptor interactions cause conformational changes that trigger the assembly of the DISC (death-inducing signaling complex), composed by the adaptor FAAD (Fas-associated DEATH domain protein, for Fas and DR5) or TRADD (TNFR1-associated DEATH domain protein), and pro-caspase 8. DISC formation leads to caspase 8 cleavage and activation, then caspase 8 cleaves and activates effector caspases 3 and 7, amplifying the death signal that leads to the apoptosis of the cell [37].

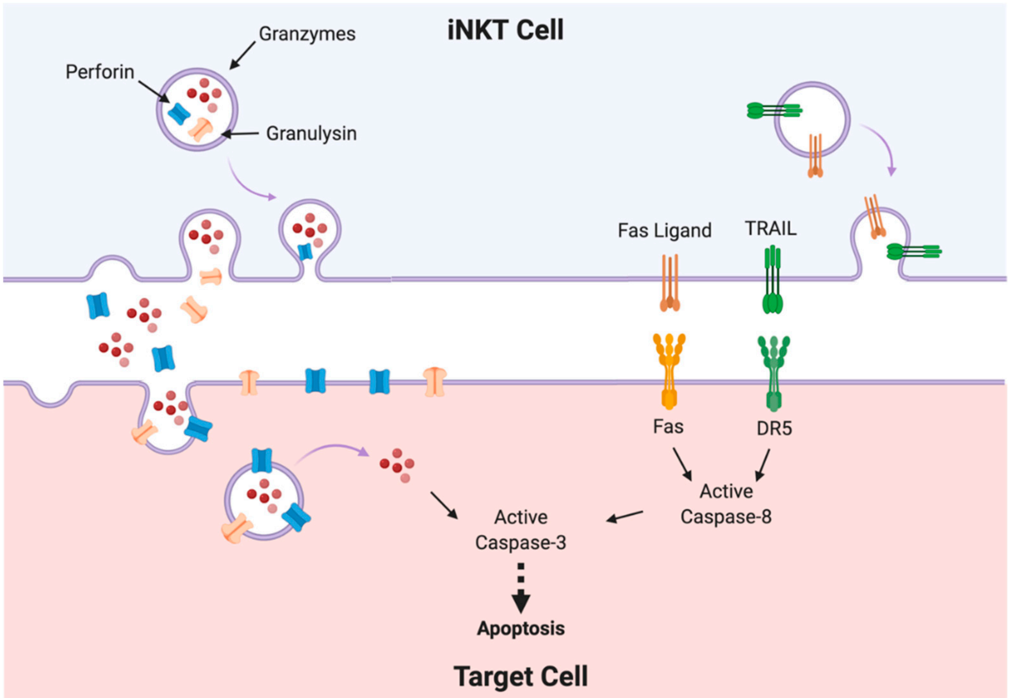

Figure 2. Cytotoxicity mechanisms used by iNKT cells.

Perforin and granzymes (especially B and A) are the major components of cytotoxic granules present in all cytotoxic populations. Cytotoxic granules can be stored in the cytosol, as for NK cells, or produced only upon activation, as for conventional T cells [35,38,39]. This mechanism, as well as the death receptor pathway, requires cell-to-cell contact in the immunological synapse. Once the contact is established, the granules polarize towards the immunological synapse, and granzymes, perforin, and other components are released towards target cells via exocytosis. Perforin forms pores in the membranes of target cells to deliver granzymes and to induce lysis [35,40–42]. Five granzymes (A, B, H, K, and M) have been identified in humans, being granzyme B the most relevant in the induction of cell death [39]. Granzyme B is the most powerful and the fastest pro-apoptotic granzyme, as very low concentrations are sufficient to induce apoptosis; its efficiency is due to the fact that it can provoke cell death either in a caspase-dependent manner through pro-caspase 3, 7 and 8 cleavage, or caspase-independent manner through BH3-only protein BID, ICAD (inhibitor of caspase-activated DNase), poly(ADP ribose) polymerase (PARP), and lamin cleavage [39,43,44]. Granzyme A, on the other hand, induces apoptosis in a slower, caspase-independent manner, and it is poorly cytotoxic in humans [45,46].

A lesser known molecule, granulysin, is also stored in the in the granules of human iNKT cells, as in other immune cytotoxic populations [47,48]. It belongs to the family of the saposin-like proteins and exists in two isoforms: the 9 kDa isoform, found in cytotoxic granules together with granzymes and perforin, is endowed of antimicrobial and cytotoxic properties; instead, the 15 kDa isoform is involved in other immune functions, such as maturation of APCs and immune cell migration [48]. In general, granulysin has been mainly correlated with direct killing of pathogens because of its potent antimicrobial activity [47–49]. More specifically, the cytotoxic activity of granulysin mainly relies on its capacity of forming pores in the membranes of pathogens and, as it has been recently discovered, tumor cells; this capacity alters the membrane permeability of the cell inducing lysis [48,49]. Furthermore, similarly to perforin, granulysin mediates its cytotoxic activity by delivering granzymes which induce apoptosis in transformed cells and substantial oxidative damage in bacteria [48,49].

4. iNKT Cell Cytotoxicity in Response to Infections

It is widely known that iNKT cells and microorganisms are in constant crosstalk. This is achieved thanks to the capacity of iNKT cells to recognize microbial and microbe-induced self-antigens, as well as being activated by innate and cytokine signals, such as IL-12 and TLRs [50–54]. Given this, iNKT cells are important in the control of homeostasis in many mucosal tissues, like the intestine and lung, but also in the response against pathogens [9,50–56]. More specifically, iNKT cells participate in the response against infectious agents mainly by mediating the activation of other immune cell types through the release of interferon (IFN) gamma [9,50–55]. However, direct killing seems to play an important role as well. Pathogen clearance by iNKT cell cytotoxicity has been found to be relevant in *Leishmania infantum*, *Mycobacterium tuberculosis, Brucella suis,* Epstein–Barr (EBV) virus, and Hepatitis B (HBV) virus infections (Table 2).

For instance, the iNKT cell role in the defense against *Leishmania* infection was established by several data [57–59], even if there is also evidence of a pathogenic role in visceral leishmaniasis [60]. The *Leishmania* species are intracellular protozoa that infect and survive inside phagocytes like neutrophils and macrophages [61]. It has been reported that iNKT cells are important in the control of *L. major* and *L. donovani* growth in vivo [57,62], and, more importantly, it has been found that they were capable of recognizing and directly eliminating *L. infantum*-infected dendritic cells due to the upregulation of CD1d [63]. Regarding the recognition of infected cells, it was previously reported that *L. donovani* synthesizes lipophosphoglycan, which was shown to activate a subset of hepatic iNKT cells when bound to CD1d [57]. The same or similar antigens could be present on other *Leishmania* species as well, but more studies must be performed on this matter.

Mycobacterium tuberculosis is particularly successful for its ability to "hide" pathogen-associated molecular patterns (PAMPs) thanks to the composition of its lipid-enriched membrane, and for invading macrophages and dendritic cells [64]. Nonetheless, several data have shown that iNKT cells are capable of arresting *M. tuberculosis* growth [11,65–67]. In one of these studies, Gansert et al. showed that infected monocyte-derived cells were targeted and eliminated by iNKT cells in a CD1d-dependent manner through granulysin expression [67]. Moreover, it was later discovered that *M. tuberculosis*-derived phosphatidylinositol mannoside (PIM) is an antigen for iNKT cells that induces IFN-γ and TNF-α secretion and lysis of CD1d-expressing HeLa cells [11], further explaining the mechanisms of iNKT cell recognition and elimination of infected cells in this context. More recently, Walker and collaborators observed that, in human immunodeficiency virus (HIV)-associated tuberculosis, iNKT cells are skewed towards a cytotoxic phenotype, as shown by the expression of CD107a, probably due to the depletion of CD4+ iNKT cells found in HIV infection [68].

Brucella species are facultative intracellular pathogens that cause fever, arthritis and osteomyelitis [69]. Bessoles and colleagues demonstrated that CD4+ iNKT cells recognized *B. suis*-infected macrophages in a CD1d-dependent manner and eliminated them via Fas ligand upregulation [69]. Despite it was demonstrated that iNKT cells targeted *B. suis*-infected macrophages through their TCR, no information about *B. suis*-derived iNKT antigens nor induction of self-antigens upon infection is available.

Viruses are probably the most popular intracellular parasites, and some lines of evidence have highlighted the role iNKT cells in viral infections. For example, some studies have reported the importance of iNKT cell-mediated immunity in Epstein-Barr virus infection [70–72]. The works from Xiao et al. and Yuling et al. showed that iNKT cell numbers, especially CD8+ iNKT cells, were significantly lower in patients with advanced EBV-associated malignancies like Hodgkin's lymphoma and nasopharyngeal carcinoma compared to healthy individuals and subjects with latent infection. Moreover, they demonstrated that CD8+ iNKT cells suppressed EBV-associated tumorigenesis in vitro and in vivo by eliminating EBV-infected cancer cells [70,71]. Furthermore, EBV provoked the downregulation of CD1d on infected B cells, event that significantly impaired iNKT cell cytotoxicity; it has been also observed that induced-CD1d expression on lymphoblastoid cell lines stimulated iNKT cell activation and killing even in the absence of α-GalCer, suggesting the role of an endogenous antigen in the recognition of EBV-infected cells [72].

Other studies have unraveled the role of other regulatory molecules in the response of iNKT cells to viruses. In this regard, it has been reported that the presence of Tim-3, a negative regulatory immune checkpoint, on iNKT cells impaired their activation and cytotoxic activity against HBV infected cells. Tim-3 blockade, both in vitro and in vivo, significantly increased iNKT cell-mediated inhibition of HBV propagation through IFN-ɣ and TNF-α production, as well as cytotoxic granule release, as reflected by the increase of CD107a expression [73].

Despite the positive role of iNKT cell cytotoxic activity in some infections, this function can also contribute to pathogenesis and disease severity in others. In particular, iNKT cells have a relevant, pathogenic role in infection-derived liver injury. For instance, some studies have shown the detrimental role of iNKT cells during Dengue virus infection, which might be in part due to the increase of Fas ligand expression, which correlates with hepatocyte cell death [74]. Besides, during Salmonella infection in mice, TLR2 signaling induced the overexpression of Fas ligand on hepatic iNKT cells, resulting in hepatocyte death and increased liver damage [75]. In another study, Chen et al. assessed the role of intestinal pathogenic bacteria, like Salmonella, on iNKT cell cytotoxicity during concanavalin A-induced hepatitis, showing that pathogenic bacteria enhanced iNKT cell cytotoxicity in the liver via iNKT-dendritic cell interactions [76].

Even if iNKT cell cytotoxicity is mainly directed towards infected cells, they are also able to directly kill cellular pathogens. For example, iNKT cells are one of the main lines of defense against Borrelia burgdorferi, etiologic agent of Lyme disease [77–79]. In fact, diacylglycerol, a lipid produced by *B. burgdoferi*, is an antigen recognized by iNKT cells [7]. Accordingly, iNKT cells represent a protective barrier against *B. burgdoferi* invasion to the joints thanks to their granzyme B-dependent bactericidal activity. This activity is limited to joint-resident iNKT cells, as neither splenic nor hepatic iNKT cells were able to eliminate *B. burgdoferi in vivo* and even in in vitro contact experiments [77]. Another example of iNKT-mediated bactericidal activity is *M. tuberculosis*. Here, as it occurred with infected cells, iNKT cells exerted their bactericidal activity through granulysin release, as it is well-known for altering mycobacterial membranes [67].

Altogether, these data demonstrate that iNKT cell cytotoxic activity can be induced by microorganisms, and this response can be both protective or contribute to infection severity.

5. iNKT Cell Cytotoxic Activity in Other Diseases

As it occurs in some infections, iNKT cell cytotoxicity can contribute to pathogenesis in other diseases (Table 2). For instance, iNKT cell pathogenic role in atherosclerosis has been validated in various murine studies [29]. Atherosclerosis is caused by the accumulation of low-density lipoproteins in the artery walls, which in turn unleashes an inflammatory response that gives rise to atherosclerotic plaques [80]. Regarding iNKT cells, one of their main proatherogenic roles is apoptosis. Indeed, Li et al. found that iNKT cells promoted atherosclerosis by inducing apoptosis in a perforin and granzyme B-dependent fashion, and these apoptotic processes further increased necrosis and inflammation [81]. Interestingly, the generation of atherosclerotic lesions by iNKT cells was dependent on cytotoxic proteins but not on cytokines, as IFN-ɣ, IL-4 and IL-21-deficient iNKT cells still augmented atherosclerosis. Nonetheless, cytotoxicity is not confined to iNKT cells in this context, as other T cell populations seem to increase their killing potential as well [80].

iNKT cells have also a detrimental role in allergic asthma [82,83]. It has been observed that iNKT cells from allergic asthma (AA) patients expressed higher levels of the activating NK receptors NKp30 and NKp46, perforin, and granzyme B, and iNKT cell cytotoxic phenotype positively correlated with disease severity, as granzyme B expression was significantly increased in patients suffering from severe to moderate AA compared to healthy individuals [82]. Moreover, it was demonstrated that CD4+ iNKT cells selectively eliminated autologous Treg cells in vitro [82], important in allergic asthma resolution as their numbers negatively correlate with IgE levels, and expression of CD39 and CD73 on Tregs has a negative correlation with Th17 cells, important in allergic inflammation [84].

Immunological hepatic injury is one of the most studied models in which iNKT cell cytotoxicity plays a pathogenic role. We have previously mentioned how pathogens induce iNKT cell-mediated liver injury by inducing Fas Ligand upregulation [76]. Furthermore, it has been observed that NK receptors, as well as TCR signaling play an important role in iNKT cell killing activity in liver injury. In particular, Kawamura and collaborators showed that NKG2A, an inhibitory NKR, inhibits iNKT cell activation and cytotoxic potential both in concanavalin A- and α-GalCer-induced hepatic injury, demonstrating that NKG2A is a major regulator in TCR-dependent iNKT cell activation [26]. Nonetheless, it was also observed that perforin-mediated killing, but not the Fas/FasL mechanism, is potentiated on iNKT cells in NKG2A-deficient mice, which indicated that not only Fas ligand upregulation is involved in iNKT cell killing of hepatocytes, but also the granzyme/perforin mechanism seems to have a relevant role.

It is now known that hypoxia plays an important role in modulating immune cell functions in various diseases, especially cancer and inflammation [85–87]. iNKT cells are not the exception, as they are sensitive to hypoxia-inducible factor activation, enhancing CD1d-dependent activation and cytokine production [88]. However, in terms of cytotoxicity, the activation of the hypoxia-inducible factor 2α (HIF-2α) on iNKT cells acts as a protective factor in renal ischemia/reperfusion injury (IRI) [89]. Specifically, it was observed that, in HIF-2α-KO iNKT cell transfer experiments, IRI was exacerbated due to a higher iNKT infiltration and Fas ligand upregulation, being the Fas/FasL pathway of particular importance in IRI pathogenesis [89]. This elucidates a new role of hypoxia on IRI, as it inhibits iNKT cell pathogenic activity by controlling Fas ligand expression and iNKT cell recruitment.

Table 2. Role of iNKT cell cytotoxicity in disease.

Disease	Role of iNKT Cell Cytotoxicity	Killing Mechanism	References
Leishmania infantum infection	Protective	Not addressed	[63]
Brucella suis infection	Protective	Fas ligand upregulation	[69]
Epstein-Barr virus infection	Protective	Infected cell killing by IFN gamma and TNF alpha production	[70–72]
Borrelia burgdoferi infection	Protective	Bacteria death by Granzyme B release	[77]
Mycobacterium tuberculosis infection	Protective	Infected cell and bacteria elimination by granulysin release	[67]
Hepatitis B virus infection	Protective	Elimination of infected cells by IFN gamma, TNF alpha production and cytotoxic granule release	[73]
Atherosclerosis	Pathogenic	Granzyme B and perforin release	[81]
Allergic asthma	Pathogenic	Increase in granzyme B and perforin. Killing of Tregs in vitro	[82]
Liver injury	Pathogenic	Hepatocyte cell death by Fas ligand upregulation, perforin and granzyme B release	[26,74–76]
Renal ischemia/reperfusion injury	Pathogenic	Fas ligand upregulation	[89]

6. iNKT Cytotoxic Activity in Antitumor Immunity

iNKT cells have been recently praised for their multiple functions during tumor immunosurveillance [5,90–93]. In particular, iNKT cells are involved in dendritic cell maturation and IL-12 production, activation of CD4+ T cells, CD8+ T cells, and B cells, and NK cell transactivation [5,27,94]. Besides, iNKT cells are able to modify the tumor microenvironment by controlling immunosuppressive populations like tumor-associated macrophages and myeloid-derived suppressor cells [5,27,95,96]. More importantly, many lines of evidence have demonstrated iNKT cell effector functions in antitumor immunity, as peripheral blood-derived human and murine iNKT cells, as well as murine hepatic and splenic iNKT cells directly kill multiple types of cancer cells both in vitro and in vivo [38,96–98].

Cytotoxic activities displayed by iNKT cells have been mostly demonstrated in blood cancers. iNKT cells are capable of targeting hematopoietic cells due to the fact that CD1d is preferentially expressed in these cells [91]. In fact, CD1d-TCR interactions are of particular importance in chronic lymphoblastic leukemia, myelomonocytic leukemias, T-cell lymphoma, and acute myeloid leukemia (AML) cell recognition [36,99–104]. Nonetheless, leukemia cells can be also targeted via NKG2D, an activating NK receptor, in a TCR-independent manner [25]. Regarding the mechanisms used by iNKT cells to eliminate tumor cells in these contexts, it has been shown that the granzyme/perforin pathway is necessary to kill cancer cells in myelomonocytic leukemia [36,101], and T-cell lymphoma [100], whereas TRAIL expression drives apoptosis in AML cells [103]. Therefore, iNKT cells do not limit the identification and elimination of blood cancer cells to a certain mechanism, as they can target malignant cells both by innate and adaptive mechanisms and kill cells both by cytotoxic molecules and death receptor pathways.

Although the iNKT cytotoxic activity has been mostly studied in hematopoietic cancers (further reviewed in [91]), iNKT cell effector activities have been also observed in solid tumors. In fact, several studies have demonstrated that human and murine iNKT cells isolated from peripheral blood, as well as murine liver, thymus, and spleen cells can directly kill colon [91,104,105], melanoma [97,98,104], lung [38,91,104,106], osteosarcoma [107], glioma [108], prostate [91], and breast cancer [91,109] cells in vitro and in vivo.

For instance, iNKT cell infiltration in colon tumors has been considered a good prognostic factor because of the observation by Tachibana et al. that infiltrating iNKT cells express an activated phenotype, characterized by CD69, Fas ligand, and granzyme B expression [110]. In other studies, researchers have assessed the potential of chemotherapeutic agents and immune adjuvants in sensitizing colon cancer cells to iNKT cell killing, obtaining promising results mainly through CD1d upregulation [91,105]. While chemotherapy induced iNKT cell cytotoxicity in vitro through the expression of TRAIL and Fas ligand [91], human colon cancer cells treated with thymosin α1 (an immune adjuvant) and α-GalCer were eliminated by peripheral blood iNKT cells through granzyme B and perforin release, and the same treatment also reduced tumor growth in NOD-SCID mice [105].

Melanoma, together with leukemias, has become one of the types of cancer in which immunotherapy has obtained more attention [111]. Although immune checkpoint blockade therapy has been proved to have success in melanoma patients [111], efforts have also been done to establish the role and therapeutic potential of iNKT cells. In particular, Kawano and collaborators [112] showed that the cytotoxic activity of iNKT cells was significantly activated by α-GalCer, and this activation inhibited liver metastases of B16 melanoma cells. However, it was also observed that the TCR was not used in the killing interactions between iNKT cells and cancer cells, indicating an NK-like mechanism of target cell recognition; moreover, cytotoxic activity was not impaired by Fas ligand blockade, suggesting the involvement of other killing mechanisms. On the other hand, Wingender et al. [97] observed that there is a positive correlation between α-GalCer-mediated protection and CD1d expression by B16 cells in mice, suggesting that TCR-CD1d interactions are still important in melanoma cell elimination in vivo.

iNKT cells and CD1d expression could be a prognostic factor in lung cancer as well. It was previously shown by Konishi et al., and confirmed by Dockry et al. that iNKT cell numbers are lower in lung cancer patients [38,106], and CD1d expression in non-small cell lung cancer NSCLC was positively correlated with overall survival [106]. Regarding iNKT cell cytotoxicity in this context, some lines of evidence have shown that peripheral blood-derived iNKT cells can eliminate lung cancer cells in vitro [38,91,104,106]. As it occurred in other types of cancer, iNKT cells could target cancer cells both in a CD1-dependent and CD1d-independent manner [38,91,104,106]. In this regard, it was also demonstrated that epigenetic induction of CD1d expression enhanced iNKT cell killing [106]. Plus, it has been also observed that degranulation and perforin release are increased and fundamental for iNKT cell cytotoxicity in vitro [38,106].

Despite most of the research in iNKT cell-mediated killing activities against tumors have focused on cancer cell elimination, some studies have addressed the role of iNKT cell cytotoxicity in the control of the tumor microenvironment, especially of CD1d-positive, myeloid-derived cells. More specifically, tumor-associated macrophages (TAMs) seem to be sensitive to iNKT cell elimination in neuroblastoma and prostate cancer models. In particular, TAMs from neuroblastoma tumors are capable of cross-presenting neuroblastoma-cell glycosphingolipids, inducing a potent cytotoxic activity by infiltrating iNKT cells against these APCs [98]. On the other hand, in the TRAMP model of oncogene-induced prostate cancer, Cortesi and colleagues [113] demonstrated a complex TAM control by iNKT cells, as they only eliminated M2-like macrophages via Fas/Fas ligand binding, but supported M1-like (antitumor) macrophages via CD40 ligand.

Hence, these results illustrate how innate and adaptive signals can unleash iNKT cell killing potential against tumors via soluble and membrane-bound cytotoxic proteins (Figure 3). We have previously mentioned that iNKT cells express NKG2D, which allows the recognition of stressed cells that express MICA, MICB, among others, and how this receptor drove iNKT cell cytotoxic activity both in a fully CD1d-independent manner or acting as TCR co-stimulator against leukemia cells [25,114–116]. Other activating innate receptors expressed by iNKT cells, such as NKp30, NKp46 and DNAM1, might also be involved in their response against tumors, as they are positively involved in natural killer and cytotoxic T cell cytotoxicity [25,82,117,118]. Cytokines can also activate iNKT cell killing activity without TCR stimulation, as it was shown for IL-18 plus IL-12 on Fas+ target cells [119]. While the innate signals received by iNKT cells can be explained by the presence of natural killer receptor-ligand interactions and cytokine activation, the lipid antigens specifically presented by tumor cells is a more intriguing topic. In this regard, it was demonstrated that iNKT cell cytotoxicity in vivo positively correlated with CD1d expression and antigen potency [97]. Some glycolipids, like gangliosides GD2, GD3, and GM2 are synthesized and presented in an altered manner in many cancers (90), and different cellular stresses commonly found in cancer cells, such as endoplasmic reticulum stress, enhance antigen-dependent iNKT activation [120,121]. However, tumor-specific antigens that activate iNKT cells are still largely unknown.

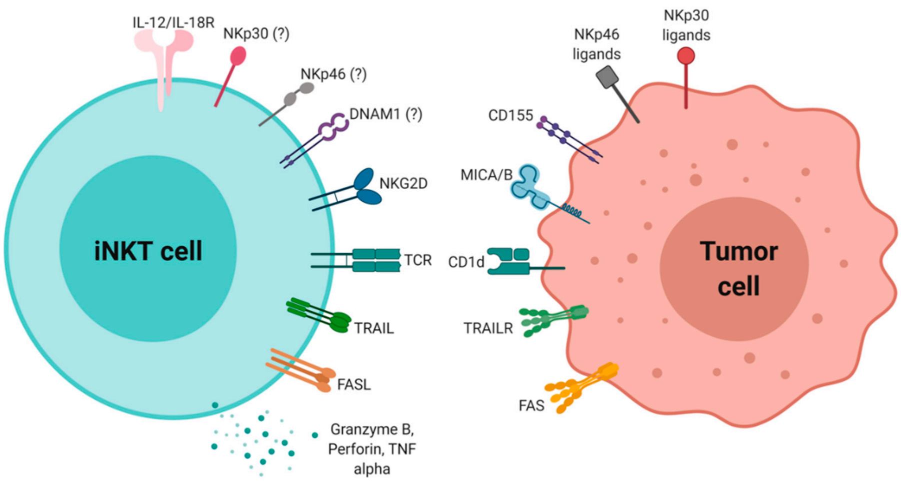

Figure 3. Activation signals and mechanisms of NKT cell cytotoxicity against tumors.

7. iNKT Cell-Based Cancer Immunotherapies

Due to the important roles of iNKT cells in cancer immunosurveillance, including the ability to kill cancer cells, iNKT cell-based immunotherapies are currently used in the fight against cancer. Most of the ongoing clinical trials on solid tumors take advantage of the strong activation given by α-GalCer through direct injection. In these, it was found an increase in cytokines typical of the NKT1 subset, such as TNF-α and IFN-γ, as well as GM-CSF [122]. However, iNKT cell anergy was increased in studies in which only α-GalCer was administered [90,94,122]. To overcome the limitations of soluble α-GalCer therapy, some trials have used α-GalCer-pulsed, autologous dendritic cell transfer as an alternative treatment, being well tolerated and inducing a positive response in some of them, measured by an increase in Th1 cytokines and an expansion in IFN-γ-producing iNKT cells [94,115,122,123]. Other efforts have been done in antigen research to identify stronger iNKT cell agonists or to modify α-GalCer. In this regard, several α-GalCer analogs were shown to elicit a strong Th1 response on iNKT cells [27,90]. Other strategies, like the modulation of metabolic pathways have also been explored for iNKT cell-based therapies. In relation to this, Fu and colleagues found that increasing iNKT cell lipid biosynthesis (mostly cholesterol synthesis) increased IFNγ production by intratumoral iNKT cells both in patients and mice [124].

More recently, adoptive cell therapies, mostly CAR-T therapy, have gained popularity especially in hematopoietic cancers, although several advances have also been done in solid tumor treatment [125–128]. iNKT cells are not the exception, as new lines of evidence have shown their potential both in preclinical and clinical studies [94,115]. CAR-T iNKT cells specific for GD2 ganglioside tested in preclinical studies for neuroblastoma and B-cell lymphoma have shown promising results, and patients have been recruited for phase I studies [27,94,115]. Moreover, studies using endogenous iNKT TCR have shown that these cells induced a strong increase in tumor cell death against various types of cancer [94].

8. Concluding Remarks

iNKT cells are an important component of innate and adaptive immunity also due to their cytotoxic functions. Although iNKT cell cytotoxicity is considered protective in responses against some infections and mostly in antitumor immunity, it also plays a negative role, enhancing pathogenesis in other immune-mediated diseases. Therefore, it is essential to decipher the signals by which iNKT cell killing functions are activated in each setting. This is particularly important in cancer research, as it is still unknown which lipid antigens and innate signals are specifically presented by tumor cells and are responsible for iNKT cell activation. This knowledge would help to understand how iNKT cell cytotoxic activity is unleashed against specific targets in order to exploit these cells in immunotherapies. Moreover, the assessment of iNKT cell triggering signals would be useful to find therapies that would arrest the iNKT cell pathogenic role in other malignancies.

Author Contributions: All authors contributed to the original draft preparation and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: This work was made possible through AIRC IG-22923 grant to FF.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

iNKT	invariant Natural Killer T cells
NKR	natural killer receptor
TCR	T cell receptor
NK	natural killer cells

References

1. Bendelac, A.; Savage, P.B.; Teyton, L. The Biology of NKT Cells. *Annu. Rev. Microbiol.* **2007**, *25*, 297–336. [CrossRef]
2. Matsuda, J.L.; Mallevaey, T.; Scott-Browne, J.; Gapin, L. CD1d-restricted iNKT cells, the "Swiss-Army knife" of the immune system. *Curr. Opin. Immunol.* **2008**, *20*, 358–368. [CrossRef]
3. Middendorp, S.; Nieuwenhuis, E.E.S. NKT cells in mucosal immunity. *Mucosal Immunol.* **2009**, *2*, 393–402. [CrossRef] [PubMed]
4. Crosby, C.M.; Kronenberg, M. specific functions of invariant natural killer T cells. *Nat. Rev. Immunol.* **2018**, *18*, 19–24. [CrossRef] [PubMed]
5. Krijgsman, D.; Hokland, M.; Kuppen, P.J.K. The role of natural killer T cells in cancer-A phenotypical and functional approach. *Front. Immunol.* **2018**, *9*, 367. [CrossRef] [PubMed]
6. Gapin, L. Development of invariant natural killer T cells. *Curr. Opin. Immunol.* **2016**, *39*, 68–74. [CrossRef]
7. Kinjo, Y.; Illarionov, P.; Vela, J.L.; Pei, B.; Girardi, E.; Li, X.; Li, Y.; Imamura, M.; Kaneko, Y.; Okawara, A.; et al. Invariant NKT cells recognize glycolipids from pathogenic Gram-positive bacteria. *Nat. Immunol.* **2012**, *12*, 966–974. [CrossRef]
8. Kinjo, Y.; Tupin, E.; Wu, D.; Fujio, M.; Garcia-navarro, R.; Benhnia, M.R.; Zajonc, D.M.; Ben-menachem, G.; Ainge, G.D.; Painter, G.F.; et al. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. 2006, 7, 978–986. *Nat. Immunol.* **2006**, *7*, 978–986. [CrossRef]
9. Hapil, F.Z.; Wingender, G. The interaction between invariant Natural Killer T cells and the mucosal microbiota. *Immunology* **2018**, *155*, 164–175. [CrossRef]
10. Shimamura, M.; Kamijo, S.; Illarionov, P. Invariant natural killer T cells stimulated with cholesteryl glycosides modulate immune responses in allergy and delayed-type hypersensitivity. *Eur. J. Immunol.* **2019**, *49*, 348–350. [CrossRef]
11. Fischer, K.; Scotet, E.; Niemeyer, M.; Koebernick, H.; Zerrahn, J.; Maillet, S.; Hurwitz, R.; Kursar, M.; Bonneville, M.; Kaufmann, S.H.E.; et al. Mycobacterial phosphatidylinositol mannoside is a natural antigen for CD1d-restricted T cells. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10685–10690. [CrossRef] [PubMed]
12. Brown, L.C.W.; Penaranda, C.; Kashyap, P.C.; Williams, B.B.; Clardy, J.; Kronenberg, M.; Sonnenburg, J.L.; Comstock, L.E.; Bluestone, J.A.; Fischbach, M.A. Production of a -Galactosylceramide by a Prominent Member of the Human Gut Microbiota. *PLoS Biol.* **2013**, *11*, e1001610.
13. Liang, S.; Webb, T.; Li, Z. Probiotics Antigens Stimulate Hepatic NKT Cells. *Hepatol. Res.* **2013**, *43*, 139–146.
14. Zajonc, D.M.; Girardi, E. Recognition of microbial glycolipids by Natural Killer T cells. *Front. Immunol.* **2015**, *6*, 1–11. [CrossRef] [PubMed]
15. Facciotti, F.; Ramanjaneyulu, G.S.; Lepore, M.; Sansano, S.; Cavallari, M.; Kistowska, M.; Forss-petter, S.; Ni, G.; Colone, A.; Singhal, A.; et al. Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus. *Nat. Immunol.* **2012**, *13*, 474. [CrossRef] [PubMed]
16. Wang, H.; Hogquist, K.A. How lipid-specific T cells become effectors: The differentiation of iNKT subsets. *Front. Immunol.* **2018**, *9*, 1450. [CrossRef]
17. De Libero, G.; Moran, A.P.; Gober, H.J.; Rossy, E.; Shamshiev, A.; Chelnokova, O.; Mazorra, Z.; Vendetti, S.; Sacchi, A.; Prendergast, M.M.; et al. Bacterial infections promote T cell recognition of self-glycolipids. *Immunity* **2005**, *22*, 763–772. [CrossRef]
18. Paget, C.; Deng, S.; Soulard, D.; Priestman, D.A.; Speca, S.; Von Gerichten, J.; Speak, A.O.; Saroha, A.; Pewzner-Jung, Y.; Futerman, A.H.; et al. TLR9-mediated dendritic cell activation uncovers mammalian ganglioside species with specific ceramide backbones that activate invariant natural killer t cells. *PLoS Biol.* **2019**, *17*, 1–26. [CrossRef]
19. Brennan, P.J.; Tatituri, R.V.V.; Brigl, M.; Kim, E.Y.; Tuli, A.; Sanderson, J.P.; Gadola, S.D.; Hsu, F.; Besra, G.S.; Brenner, M.B. Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. *Nat. Immunol.* **2011**, *12*, 1202. [CrossRef]
20. Mattner, J.; Debord, K.L.; Ismail, N.; Goff, R.D.; Cantu III, C.; Dapeng, Z.; Saint-Mezard, P.; Wang, V.; Gao, Y.; Yin, N.; et al. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* **2005**, *523*, 525–530. [CrossRef]
21. O'Keeffe, J.; Podbielska, M.; Hogan, E.L. Invariant natural killer T cells and their ligands: Focus on multiple sclerosis. *Immunology* **2015**, *145*, 468–475. [CrossRef] [PubMed]

22. Gapin, L. INKT cell autoreactivity: What is "self" and how is it recognized? *Nat. Rev. Immunol.* **2010**, *10*, 272–277. [CrossRef] [PubMed]
23. Patterson, S.; Chaidos, A.; Neville, D.C.A.; Poggi, A.; Butters, T.D.; Roberts, I.A.G.; Karadimitris, A. Human Invariant NKT Cells Display Alloreactivity Instructed by Invariant TCR-CD1d Interaction and Killer Ig Receptors. *J. Immunol.* **2008**, *181*, 3268–3276. [CrossRef] [PubMed]
24. López-Larrea, C.; Suárez-Alvarez, B.; López-Soto, A.; López-Vázquez, A.; Gonzalez, S. The NKG2D receptor: sensing stressed cells. *Trends Mol. Med.* **2008**, *14*, 179–189. [CrossRef] [PubMed]
25. Kuylenstierna, C.; Björkström, N.K.; Andersson, S.K.; Sahlström, P.; Bosnjak, L.; Paquin-Proulx, D.; Malmberg, K.J.; Ljunggren, H.G.; Moll, M.; Sandberg, J.K. NKG2D performs two functions in invariant NKT cells: Direct TCR-independent activation of NK-like cytolysis and co-stimulation of activation by CD1d. *Eur. J. Immunol.* **2011**, *41*, 1913–1923. [CrossRef] [PubMed]
26. Kawamura, T.; Takeda, K.; Kaneda, H.; Matsumoto, H.; Hayakawa, Y.; Raulet, D.H.; Ikarashi, Y.; Kronenberg, M.; Yagita, H.; Kinoshita, K.; et al. NKG2A Inhibits Invariant NKT Cell Activation in Hepatic Injury. *J. Immunol.* **2014**, *182*, 250–258. [CrossRef]
27. Bedard, M.; Salio, M.; Cerundolo, V. Harnessing the power of invariant natural killer T cells in cancer immunotherapy. *Front. Immunol.* **2017**, *8*, 1829. [CrossRef]
28. Dao, T.; Mehal, W.Z.; Crispe, I.N. IL-18 augments perforin-dependent cytotoxicity of liver NK-T cells. *J. Immunol.* **1998**, *161*, 2217–2222.
29. Getz, G.S.; Reardon, C.A. Natural killer T cells in atherosclerosis. *Nat. Rev. Cardiol.* **2017**, *14*, 304–314. [CrossRef]
30. Wang, Y.; Sedimbi, S.; Löfbom, L.; Singh, A.K.; Porcelli, S.A.; Cardell, S.L. Unique invariant natural killer T cells promote intestinal polyps by suppressing TH1 immunity and promoting regulatory T cells. *Mucosal Immunol.* **2018**, *11*, 131–143. [CrossRef]
31. Engel, I.; Seumois, G.; Chavez, L.; Samaniego-Castruita, D.; White, B.; Chawla, A.; Mock, D.; Vijayanand, P.; Kronenberg, M. Innate-like functions of natural killer T cell subsets result from highly divergent gene programs. *Nat. Immunol.* **2016**, *17*, 728–739. [CrossRef] [PubMed]
32. Igney, F.H.; Krammer, P.H. Death and anti-death: tumour resistance to apoptosis. *Nat. Rev. Cancer* **2002**, *2*, 277–288. [CrossRef] [PubMed]
33. Zhu, Y.; Huang, B.; Shi, J. Fas ligand and lytic granule differentially control cytotoxic dynamics of natural killer cell against cancer target. *Oncotarget* **2016**, *7*, 47163. [CrossRef] [PubMed]
34. Peter, M.E.; Hadji, A.; Murmann, A.E.; Brockway, S.; Putzbach, W.; Pattanayak, A.; Ceppi, P. The role of CD95 and CD95 ligand in cancer. *Cell Death Differ.* **2015**, *22*, 549–559. [CrossRef]
35. Golstein, P.; Griffiths, G.M. An early history of T cell-mediated cytotoxicity. *Nat. Rev. Immunol.* **2018**, *18*, 527–535. [CrossRef]
36. Metelitsa, L.S.; Weinberg, K.I.; Emanuel, P.D.; Seeger, R.C. Expression of CD1d by myelomonocytic leukemias provides a target for cytotoxic NKT cells. *Leukemia* **2003**, *17*, 1068–1077. [CrossRef]
37. Vucic, D.; Dixit, V.M.; Wertz, I.E. Ubiquitylation in apoptosis: A post-translational modification at the edge of life and death. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 439–452. [CrossRef]
38. Konishi, J.; Yamazaki, K.; Yokouchi, H.; Shinagawa, N.; Iwabuchi, K.; Nishimura, M. The characteristics of human NKT cells in lung cancer—CD1d independent cytotoxicity against lung cancer cells by NKT cells and decreased human NKT cell response in lung cancer patients. *Hum. Immunol.* **2004**, *65*, 1377–1388. [CrossRef]
39. Anthony, D.; Andrews, D.; Watt, S.; Trapani, J.; Smyth, M. Functional dissection of the granzyme family: Cell death and inflammation. *Immunol. Rev.* **2010**, *235*, 73–92. [CrossRef]
40. Voskoboinik, I.; Whisstock, J.C.; Trapani, J.A. Perforin and granzymes: Function, dysfunction and human pathology. *Nat. Rev. Immunol.* **2015**, *15*, 388–400. [CrossRef]
41. Thiery, J.; Keefe, D.; Boulant, S.; Boucrot, E.; Walch, M.; Martinvalet, D.; Goping, I.S.; Bleackley, R.C.; Kirchhausen, T.; Lieberman, J. Perforin pores in the endosomal membrane trigger the release of endocytosed granzyme B into the cytosol of target cells. *Nat. Immunol.* **2011**, *12*, 770–777. [CrossRef] [PubMed]
42. Metkar, S.S.; Marchioretto, M.; Antonini, V.; Lunelli, L.; Wang, B.; Gilbert, R.J.; Anderluh, G.; Roth, R.; Pooga, M.; Pardo, J.; et al. Perforin oligomers form arcs in cellular membranes: A locus for intracellular delivery of granzymes. *Cell Death Differ.* **2015**, *22*, 74–85. [CrossRef] [PubMed]
43. Afonina, I.S.; Cullen, S.P.; Martin, S.J. Cytotoxic and non-cytotoxic roles of the CTL/NK protease granzyme B. *Immunol. Rev.* **2010**, *235*, 105–116. [CrossRef]

44. Boivin, W.A.; Cooper, D.M.; Hiebert, P.R.; Granville, D.J. Intracellular versus extracellular granzyme B in immunity and disease: Challenging the dogma. *Lab. Investig.* **2009**, *89*, 1195–1220. [CrossRef]
45. Trapani, J.A.; Bird, P.I. A Renaissance in Understanding the Multiple and Diverse Functions of Granzymes? *Immunity* **2008**, *29*, 665–667. [CrossRef] [PubMed]
46. Chowdhury, D.; Lieberman, J. Death by a thousand cuts: granzyme pathways of programmed cell death. *Annu. Rev. Immunol.* **2008**, *26*, 389–420. [CrossRef] [PubMed]
47. Barry, M.; Bleackley, R.C. Cytotoxic T lymphocytes: all roads lead to death. *Nat. Rev. Immunol.* **2002**, *2*, 401–409. [CrossRef]
48. Sparrow, E.; Bodman-Smith, M.D. Granulysin: The attractive side of a natural born killer. *Immunol. Lett.* **2020**, *217*, 126–132. [CrossRef]
49. Walch, M.; Dotiwala, F.; Mulik, S.; Thiery, J.; Kirchhausen, T.; Clayberger, C.; Krensky, A.M.; Martinvalet, D.; Lieberman, J. Cytotoxic cells kill intracellular bacteria through granulysin-mediated delivery of granzymes. *Cell* **2014**, *157*, 1309–1323. [CrossRef]
50. Van Kaer, L.; Parekh, V.V.; Wu, L. The response of CD1d-restricted invariant NKT cells to microbial pathogens and their products. *Front. Immunol.* **2015**, *6*, 1–11. [CrossRef]
51. Kinjo, Y.; Takatsuka, S.; Kitano, N.; Kawakubo, S.; Abe, M.; Ueno, K.; Miyazaki, Y. Functions of CD1d-restricted invariant natural killer T cells in antimicrobial immunity and potential applications for infection control. *Front. Immunol.* **2018**, *9*, 1–8. [CrossRef] [PubMed]
52. Nagarajan, N.A.; Kronenberg, M. Invariant NKT Cells Amplify the Innate Immune Response to Lipopolysaccharide. *J. Immunol.* **2007**, *178*, 2706–2713. [CrossRef] [PubMed]
53. Brigl, M.; Tatituri, R.V.V.; Watts, G.F.M.; Bhowruth, V.; Leadbetter, E.A.; Barton, N.; Cohen, N.R.; Hsu, F.-F.; Besra, G.S.; Brenner, M.B. Innate and cytokine-driven signals, rather than microbial antigens, dominate in natural killer T cell activation during microbial infection. *J. Exp. Med.* **2011**, *208*, 1163–1177. [CrossRef] [PubMed]
54. Holzapfel, K.L.; Tyznik, A.J.; Kronenberg, M.; Hogquist, K.A. Antigen-Dependent versus -Independent Activation of Invariant NKT Cells during Infection. *J. Immunol.* **2014**, *192*, 5490–5498. [CrossRef]
55. Dowds, C.M.; Blumberg, R.S.; Zeissig, S. Control of intestinal homeostasis through crosstalk between natural killer T cells and the intestinal microbiota. *Clin. Immunol.* **2014**, *159*, 128–133. [CrossRef]
56. Zeissig, S.; Blumberg, R.S. Commensal microbial regulation of natural killer T cells at the frontiers of the mucosal immune system. *FEBS Lett.* **2014**, *588*, 4188–4194. [CrossRef]
57. Amprey, J.L.; Im, J.S.; Turco, S.J.; Murray, H.W.; Illarionov, P.A.; Besra, G.S.; Porcelli, S.A.; Späth, G.F. A subset of liver NK T cells is activated during Leishmania donovani infection by CD1d-bound lipophosphoglycan. *J. Exp. Med.* **2004**, *200*, 895–904. [CrossRef]
58. Robert-Gangneux, F.; Drogoul, A.S.; Rostan, O.; Piquet-Pellorce, C.; Cayon, J.; Lisbonne, M.; Herbelin, A.; Gascan, H.; Guiguen, C.; Samson, M.; et al. Invariant NKT cells drive hepatic cytokinic microenvironment favoring efficient granuloma formation and early control of Leishmania donovani infection. *PLoS ONE* **2012**, *7*, e33413. [CrossRef]
59. Karmakar, S.; Bhaumik, S.K.; Paul, J.; De, T. TLR4 and NKT cell synergy in immunotherapy against visceral leishmaniasis. *PLoS Pathog.* **2012**, *8*, e1002646. [CrossRef]
60. Stanley, A.C.; Zhou, Y.; Amante, F.H.; Randall, L.M.; Haque, A.; Pellicci, D.G.; Hill, G.R.; Smyth, M.J.; Godfrey, D.I.; Engwerda, C.R. Activation of invariant NKT cells exacerbates experimental visceral leishmaniasis. *PLoS Pathog.* **2008**, *4*, e1000028. [CrossRef]
61. Walker, D.M.; Oghumu, S.; Gupta, G.; Mcgwire, B.S.; Drew, M.E.; Satoskar, A.R. Mechanisms of cellular invasion by intracellular parasites Mechanisms of host cell invasion in Leishmania. *Cell. Mol. Life Sci.* **2014**, *71*, 1245–1263. [CrossRef] [PubMed]
62. Ishikawa, H.; Hisaeda, H.; Taniguchi, M.; Nakayama, T.; Sakai, T.; Maekawa, Y.; Nakano, Y.; Zhang, M.; Zhang, T.; Nishitani, M.; et al. CD4+ Valpha14 NKT cells play a crucial role in an early stage of protective immunity against infection with Leishmania major. *Int. Immunol.* **2000**, *12*, 1267–1274. [CrossRef] [PubMed]
63. Campos-martín, Y.; Colmenares, M.; López-núñez, M.; Savage, P.B.; Martínez-naves, E.; Campos-martı, Y.; Gozalbo-lo, B.; Lo, M.; Savage, P.B.; Martı, E. Immature Human Dendritic Cells Infected with Leishmania infantum Are Resistant to NK-Mediated Cytolysis but Are Efficiently Recognized by NKT Cells. *J. Immunol.* **2006**, *176*, 6172–6179. [CrossRef] [PubMed]

64. Rodriguez, D.C.; Ocampo, M.; Salazar, L.M.; Patarroyo, M.A. Quantifying intracellular Mycobacterium tuberculosis: An essential issue for in vitro assays. *Microbiologyopen* **2018**, *7*, e00588. [CrossRef] [PubMed]
65. Sada-Ovalle, I.; Chiba, A.; Gonzales, A.; Brenner, M.B.; Behar, S.M. Innate invariant NKT cells recognize Mycobacterium tuberculosis-infected macrophages, produce interferon-γ, and kill intracellular bacteria. *PLoS Pathog.* **2008**, *4*, e1000239. [CrossRef] [PubMed]
66. Rothchild, A.C.; Jayaraman, P.; Nunes-Alves, C.; Behar, S.M. iNKT Cell Production of GM-CSF Controls Mycobacterium tuberculosis. *PLoS Pathog.* **2014**, *10*, e1003805. [CrossRef]
67. Gansert, J.L.; Kiebler, V.; Engele, M.; Wittke, F.; Röllinghoff, M.; Krensky, A.M.; Porcelli, S.A.; Modlin, R.L.; Stenger, S.; Gansert, J.L.; et al. Human NKT Cells Express Granulysin and Exhibit Antimycobacterial Activity. *J. Immunol.* **2003**, *170*, 3154–3161. [CrossRef]
68. Walker, N.F.; Opondo, C.; Meintjes, G.; Jhilmeet, N.; Friedland, J.S.; Elkington, P.T.; Wilkinson, R.J.; Wilkinson, K.A. Invariant Natural Killer T-cell Dynamics in Human Immunodeficiency Virus-associated Tuberculosis. *Clin. Infect. Dis.* **2020**, *70*, 1865–1874. [CrossRef]
69. Bessoles, S.; Dudal, S.; Besra, G.S.; Sanchez, F.; Lafont, V. Human CD4+ invariant NKT cells are involved in antibacterial immunity against Brucella suis through CD1d-dependent but CD4-independent mechanisms. *Eur. J. Immunol.* **2009**, *39*, 1025–1035. [CrossRef]
70. Xiao, W.; Li, L.; Zhou, R.; Xiao, R.; Wang, Y.; Ji, X.; Wu, M.; Wang, L.; Huang, W.; Zheng, X.; et al. EBV-Induced human CD8+ NKT cells synergise CD4+ NKT cells suppressing EBV-associated tumours upon induction of Th1-bias. *Cell. Mol. Immunol.* **2009**, *6*, 367–379. [CrossRef]
71. Yuling, H.; Ruijing, X.; Li, L.; Xiang, J.; Rui, Z.; Yujuan, W.; Lijun, Z.; Chunxian, D.; Tan, X.; Xiao, W.; et al. EBV-induced human CD8+ NKT cells suppress tumorigenesis by EBV-associated malignancies. *Cancer Res.* **2009**, *69*, 7935–7944. [CrossRef] [PubMed]
72. Chung, B.K.; Tsai, K.; Allan, L.L.; Zheng, D.J.; Nie, J.C.; Biggs, C.M.; Hasan, M.R.; Kozak, F.K.; Van Den Elzen, P.; Priatel, J.J.; et al. Innate immune control of EBV-infected B cells by invariant natural killer T cells. *Blood* **2013**, *122*, 2600–2608. [CrossRef] [PubMed]
73. Xu, Y.; Wang, Z.; Du, X.; Liu, Y.; Song, X.; Wang, T.; Tan, S.; Liang, X.; Gao, L.; Ma, C. Tim-3 blockade promotes iNKT cell function to inhibit HBV replication. *J. Cell. Mol. Med.* **2018**, *22*, 3192–3201. [CrossRef] [PubMed]
74. Renneson, J.; Guabiraba, R.; Maillet, I.; Marques, R.E.; Paget, C.; Quesniaux, V.; Faveeuw, C.; Ryffel, B.; Teixeira, M.M.; Trottein, F. A Detrimental Role for Invariant Natural Killer T Cells in the Pathogenesis of Experimental Dengue Virus Infection. *Am. J. Pathol.* **2011**, *179*, 1872–1883. [CrossRef]
75. Shimizu, H.; Matsuguchi, T.; Fukuda, Y.; Nakano, I.; Hayakawa, T.; Takeuchi, O.; Akira, S.; Umemura, M.; Suda, T.; Yoshikai, Y. Toll-like receptor 2 contributes to liver injury by Salmonella infection through Fas ligand expression on NKT cells in mice. *Gastroenterology* **2002**, *123*, 1265–1277. [CrossRef]
76. Chen, J.; Wei, Y.; He, J.; Cui, G.; Zhu, Y.; Lu, C.; Ding, Y.; Xue, R.; Bai, L.; Uede, T.; et al. Natural killer T cells play a necessary role in modulating of immune-mediated liver injury by gut microbiota. *Sci. Rep.* **2014**, *4*, 7259. [CrossRef]
77. Lee, W.Y.; Sanz, M.J.; Wong, C.H.Y.; Hardy, P.O.; Salman-Dilgimen, A.; Moriarty, T.J.; Chaconas, G.; Marquesg, A.; Krawetz, R.; Mody, C.H.; et al. Invariant natural killer T cells act as an extravascular cytotoxic barrier for joint-invading Lyme Borrelia. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 13936–13941. [CrossRef]
78. Lee, W.Y.; Moriarty, T.J.; Wong, C.H.Y.; Zhou, H.; Strieter, R.M.; Van Rooijen, N.; Chaconas, G.; Kubes, P. An intravascular immune response to Borrelia burgdorferi involves Kupffer cells and iNKT cells. *Nat. Immunol.* **2010**, *11*, 295–302. [CrossRef]
79. Tupin, E.; Benhnia, M.R.E.I.; Kinjo, Y.; Patsey, R.; Lena, C.J.; Haller, M.C.; Caimano, M.J.; Imamura, M.; Wong, C.H.; Crotty, S.; et al. NKT cells prevent chronic joint inflammation after infection with Borrelia burgdorferi. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 19863–19868. [CrossRef]
80. Tabas, I.; Lichtman, A.H. Monocyte-Macrophages and T Cells in Atherosclerosis. *Immunity* **2017**, *47*, 621–634. [CrossRef]
81. Li, Y.; To, K.; Kanellakis, P.; Hosseini, H.; Deswaerte, V.; Tipping, P.; Smyth, M.J.; Toh, B.H.; Bobik, A.; Kyaw, T. CD4+ natural killer T cells potently augment aortic root atherosclerosis by perforin-and granzyme b-dependent cytotoxicity. *Circ. Res.* **2015**, *116*, 245–254. [CrossRef] [PubMed]
82. Nguyen, K.D.; Vanichsarn, C.; Nadeau, K.C. Increased cytotoxicity of CD4+ invariant NKT cells against CD4+CD25hiCD127lo/- regulatory T cells in allergic asthma. *Eur. J. Immunol.* **2008**, *38*, 2034–2045. [CrossRef] [PubMed]

83. Umetsu, D.; DeKruyff, R. Current Perspectives: focused commentary: Key cells in asthma. *J. Allergy Clin. Immunol.* **2010**, *125*, 975–979. [CrossRef] [PubMed]
84. Zissler, U.M.; Esser-Von Bieren, J.; Jakwerth, C.A.; Chaker, A.M.; Schmidt-Weber, C.B. Current and future biomarkers in allergic asthma. *Allergy Eur. J. Allergy Clin. Immunol.* **2016**, *71*, 475–494. [CrossRef]
85. Krzywinska, E.; Stockmann, C. Hypoxia, metabolism and immune cell function. *Biomedicines* **2018**, *6*, 56. [CrossRef]
86. Rundqvist, H.; Velica, P.; Barbieri, L.; Gameiro, P.; Cunha, P.P.; Gojkovic, M.; Bargiela, D.; Mijwel, S.; Ahlstedt, E.; Foskolou, I.; et al. Lactate Potentiates Differentiation and Expansion of Cytotoxic T Cells. *SSRN Electron. J.* **2019**. [CrossRef]
87. Renner, K.; Singer, K.; Koehl, G.E.; Geissler, E.K.; Peter, K.; Kreutz, M. Metabolic Hallmarks of Tumor and immune Cells in the Tumor Microenvironment. *Front. Immunol.* **2017**, *8*, 248. [CrossRef]
88. Webb, T.J.; Carey, G.B.; East, J.E.; Sun, W.; Bollino, D.R.; Kimball, A.S.; Brutkiewicz, R.R. Alterations in cellular metabolism modulate CD1d-mediated NKT-cell responses. *Pathog. Dis.* **2016**, *74*, ftw055. [CrossRef]
89. Zhang, J.; Han, C.; Dai, H.; Hou, J.; Dong, Y.; Cui, X.; Xu, L.; Zhang, M.; Xia, Q. Hypoxia-Inducible Factor-2α Limits Natural Killer T Cell Cytotoxicity in Renal Ischemia/Reperfusion Injury. *J. Am. Soc. Nephrol.* **2016**, *27*, 92–106. [CrossRef]
90. Nair, S.; Dhodapkar, M.V. Natural killer T cells in cancer immunotherapy. *Front. Immunol.* **2017**, *8*, 1178. [CrossRef]
91. Metelitsa, L.S. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans. *Clin. Immunol.* **2011**, *140*, 119–129. [CrossRef]
92. Shissler, S.C.; Lee, M.S.; Webb, T.J. Mixed signals: Co-stimulation in invariant natural killer T cell-mediated cancer immunotherapy. *Front. Immunol.* **2017**, *8*, 1447. [CrossRef] [PubMed]
93. Smyth, M.J.; Crowe, N.Y.; Hayakawa, Y.; Takeda, K.; Yagita, H.; Godfrey, D.I. NKT cells—conductors of tumor immunity ? *Curr. Opin. Immunol.* **2002**, *14*, 165–171. [CrossRef]
94. Wolf, B.J.; Choi, J.E.; Exley, M.A. Novel approaches to exploiting invariant NKT cells in cancer immunotherapy. *Front. Immunol.* **2018**, *9*, 384. [CrossRef] [PubMed]
95. Wang, Y.; Cardell, S.L. The Yin and Yang of invariant Natural Killer T Cells in Tumor immunity — Suppression of Tumor immunity in the intestine. *Front. Immunol.* **2018**, *8*, 1945. [CrossRef] [PubMed]
96. Altman, J.B.; Benavides, A.D.; Das, R.; Bassiri, H. Antitumor Responses of Invariant Natural Killer T Cells. *J. Immunol. Res.* **2015**, *2015*. [CrossRef]
97. Wingender, G.; Krebs, P.; Beutler, B.; Kronenberg, M. Antigen-specific cytotoxicity by invariant NKT cells in vivo is CD95/CD178 dependent and is correlated with antigenic potency. *J. Immunol.* **2010**, *185*, 2721–2729. [CrossRef]
98. Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Sato, H.; Kondo, E.; Harada, M.; Koseki, H.; Nakayama, T.; et al. Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated V 14 NKT cells. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5690–5693. [CrossRef]
99. Weinkove, R.; Brooks, C.R.; Carter, J.M.; Hermans, I.F.; Ronchese, F. Functional invariant natural killer T-cell and CD1d axis in chronic lymphocytic leukemia: Implications for immunotherapy. *Haematologica* **2013**, *98*, 376–384. [CrossRef]
100. Bassiri, H.; Das, R.; Guan, P.; Barrett, D.M.; Brennan, P.J.; Banerjee, P.P.; Wiener, S.J.; Orange, J.S.; Brenner, M.B.; Grupp, S.A.; et al. iNKT Cell Cytotoxic Responses Control T-Lymphoma Growth In Vitro and In Vivo. *Cancer Immunol. Res.* **2014**, *2*, 59–69. [CrossRef]
101. Nicol, A.; Nieda, M.; Koezuka, Y.; Porcelli, S.; Suzuki, K.; Tadokoro, K.; Durrant, S.; Juji, T. Human invariant Vα24+ natural killer T cells activated by a-galactosylceramide (KRN7000) have cytotoxic anti-tumour activity through mechanisms distinct from T cells and natural killer cells. *Immunology* **2000**, *99*, 229–234. [CrossRef]
102. Ghnewa, Y.G.; O'Reilly, V.P.; Vandenberghe, E.; Browne, P.V.; McElligott, A.M.; Doherty, D.G. Retinoic acid induction of CD1d expression primes chronic lymphocytic leukemia B cells for killing by CD8+ invariant natural killer T cells. *Clin. Immunol.* **2017**, *183*, 91–98. [CrossRef]
103. Nieda, M.; Nicol, A.; Koezuka, Y.; Kikuchi, A.; Lapteva, N.; Tanaka, Y.; Tokunaga, K.; Suzuki, K.; Kayagaki, N.; Yagita, H.; et al. TRAIL expression by activated human CD4+Vα24NKT cells induces in vitro and in vivo apoptosis of human acute myeloid leukemia cells. *Blood* **2001**, *97*, 2067–2074. [CrossRef] [PubMed]

104. Kawano, T.; Nakayama, T.; Kamada, N.; Kaneko, Y.; Harada, M.; Ogura, N.; Akutsu, Y.; Motohashi, S.; Iizasa, T.; Endo, H.; et al. Antitumor Cytotoxicity Mediated by Ligand-activated Human Va24 NKT Cells. *Cancer Res.* **1999**, *59*, 5102–5105. [PubMed]
105. Mattarollo, S.R.; Kenna, T.; Nieda, M.; Nicol, A.J. Chemotherapy pretreatment sensitizes solid tumor-derived cell lines to Vα24+ NKT cell-mediated cytotoxicity. *Int. J. Cancer* **2006**, *119*, 1630–1637. [CrossRef] [PubMed]
106. Ni, C.; Wu, P.; Wu, X.; Zhang, T.; Zhang, T.; Wang, Z.; Zhang, S.; Qiu, F.; Huang, J. Thymosin alpha1 enhanced cytotoxicity of iNKT cells against colon cancer via upregulating CD1d expression. *Cancer Lett.* **2015**, *356*, 579–588. [CrossRef]
107. Dockry, É.; O'Leary, S.; Gleeson, L.E.; Lyons, J.; Keane, J.; Gray, S.G.; Doherty, D.G. Epigenetic induction of CD1d expression primes lung cancer cells for killing by invariant natural killer T cells. *Oncoimmunology* **2018**, *7*, e1428156. [CrossRef]
108. Fallarini, S.; Paoletti, T.; Orsi Battaglini, N.; Lombardi, G. Invariant NKT cells increase drug-induced osteosarcoma cell death. *Br. J. Pharmacol.* **2012**, *167*, 1533–1549. [CrossRef]
109. Dhodapkar, K.M.; Cirignano, B.; Chamian, F.; Zagzag, D.; Miller, D.C.; Finlay, J.L.; Steinman, R.M. Invariant natural killer T cells are preserved in patients with glioma and exhibit antitumor lytic activity following dendritic cell-mediated expansion. *Int. J. Cancer* **2004**, *109*, 893–899. [CrossRef]
110. Hix, L.M.; Shi, Y.H.; Brutkiewicz, R.R.; Stein, P.L.; Wang, C.R.; Zhang, M. CD1d-expressing breast cancer cells modulate NKT cell-mediated antitumor immunity in a murine model of breast cancer metastasis. *PLoS ONE* **2011**, *6*, e20702. [CrossRef]
111. Tachibana, T.; Onodera, H.; Tsuruyama, T.; Mori, A.; Nagayama, S.; Hiai, H.; Imamura, M. Increased Intratumor V A 24-Positive Natural Killer T Cells: A Prognostic Factor for Primary Colorectal Carcinomas. *Clin. Cancer Res.* **2005**, *11*, 7322–7328. [CrossRef] [PubMed]
112. Drake, C.G.; Lipson, E.J.; Brahmer, J.R. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nat. Rev. Clin. Oncol.* **2014**, *11*, 24–37. [CrossRef] [PubMed]
113. Song, L.; Seeger, R.C.; Metelitsa, L.S.; Song, L.; Asgharzadeh, S.; Salo, J.; Engell, K.; Wu, H.; Sposto, R. Va24-invariant NKT cells mediate antitumor activity via killing of tumor-associated macrophages. *J. Clin. Invest.* **2009**, *119*, 1524–1536. [CrossRef] [PubMed]
114. Cortesi, F.; Delfanti, G.; Grilli, A.; Calcinotto, A.; Gorini, F.; Pucci, F.; Lucianò, R.; Grioni, M.; Recchia, A.; Benigni, F.; et al. Bimodal CD40/Fas-Dependent Crosstalk between iNKT Cells and Tumor-Associated Macrophages Impairs Prostate Cancer Progression. *Cell Rep.* **2018**, *22*, 3006–3020. [CrossRef]
115. Wensveen, F.M.; Jelenčić, V.; Polić, B. NKG2D: A master regulator of immune cell responsiveness. *Front. Immunol.* **2018**, *9*, 441. [CrossRef]
116. Fujii, S.I.; Shimizu, K. Immune Networks and Therapeutic Targeting of iNKT Cells in Cancer. *Trends Immunol.* **2019**, *40*, 984–997. [CrossRef]
117. Joshi, S.K.; Lang, M.L. Fine tuning a well-oiled machine: Influence of NK1.1 and NKG2D on NKT cell development and function. *Int. Immunopharmacol.* **2013**, *17*, 260–266. [CrossRef]
118. Barrow, A.D.; Martin, C.J.; Colonna, M. The natural cytotoxicity receptors in health and disease. *Front. Immunol.* **2019**, *10*, 909. [CrossRef]
119. Gilfillan, S.; Chan, C.J.; Cella, M.; Haynes, N.M.; Rapaport, A.S.; Boles, K.S.; Andrews, D.M.; Smyth, M.J.; Colonna, M. DNAM-1 promotes activation of cytotoxic lymphocytes by nonprofessional antigen-presenting cells and tumors. *J. Exp. Med.* **2008**, *205*, 2965–2973. [CrossRef]
120. Leite-de-Moraes, M.C.; Hameg, A.; Machavoine, F.; Koezuka, Y.; Herbelin, A.; Dy, M.; Schneider, E. A Distinct IL-18-Induced Pathway to Fully Activate NK T Lymphocytes Independently from TCR Engagement. *J. Immunol.* **1999**, *163*, 5871–5876.
121. Bedard, M.; Shrestha, D.; Priestman, D.A.; Wang, Y.; Schneider, F.; Matute, J.D.; Iyer, S.S.; Gileadi, U.; Prota, G.; Kandasamy, M.; et al. Sterile activation of invariant natural killer T cells by ER-stressed antigen-presenting cells. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 23671–23681. [CrossRef] [PubMed]
122. Govindarajan, S.; Verheugen, E.; Venken, K.; Gaublomme, D.; Maelegheer, M.; Cloots, E.; Gysens, F.; Geest, B.G.D.; Cheng, T.; Moody, D.B.; et al. ER stress in antigen-presenting cells promotes NKT cell activation through endogenous neutral lipids. *EMBO Rep.* **2020**. [CrossRef] [PubMed]
123. McEwen-Smith, R.M.; Salio, M.; Cerundolo, V. The regulatory role of invariant NKT Cells in tumor immunity. *Cancer Immunol. Res.* **2015**, *3*, 425–435. [CrossRef] [PubMed]

124. Bae, E.A.; Seo, H.; Kim, I.K.; Jeon, I.; Kang, C.Y. Roles of NKT cells in cancer immunotherapy. *Arch. Pharm. Res.* **2019**, *42*, 543–548. [CrossRef] [PubMed]
125. Fu, S.; He, K.; Tian, C.; Sun, H.; Zhu, C.; Bai, S.; Liu, J.; Wu, Q.; Xie, D.; Yue, T.; et al. Impaired lipid biosynthesis hinders anti-tumor efficacy of intratumoral iNKT cells. *Nat. Commun.* **2020**, *11*, 1–15. [CrossRef] [PubMed]
126. June, C.H.; O'Connor, R.S.; Kawalekar, O.U.; Ghassemi, S.; Milone, M.C. CAR T cell immunotherapy for human cancer. *Science* **2018**, *359*, 1361–1365. [CrossRef] [PubMed]
127. Sacchetti, B.; Botticelli, A.; Pierelli, L.; Nuti, M.; Alimandi, M. CAR-T with license to kill solid tumors in search of a winning strategy. *Int. J. Mol. Sci.* **2019**, *20*, 1903. [CrossRef]
128. Martinez, M.; Moon, E.K. CAR T cells for solid tumors: New strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front. Immunol.* **2019**, *10*, 128. [CrossRef]