

Optimization of PD-1 / PD-L1 Blockade to increase NK Cells Cytotoxicity in killing Cancer Cells :A Review

Abstract

Anti-PD-1 and anti-PD-L1 are therapies that have shown success in cancer treatment. However, while treating cancer with PD-1/PD-L1 blockade is similar, the clinical response rate is still low in certain cancers at around 40%. Therefore, therapeutic strategies are needed to increase PD-L1 expression and optimize PD-1/PD-L1 blockade therapy, so as to improve satisfactory therapeutic results. In tumors, IFN γ induces the expression of PD-L1 which is a cytokine secreted by Natural Killer cells. A study showed that PM21-NK cells induced large amounts of IFN γ and transfected PM21-NK cells adaptively induced PD-L1 expression. In vitro experiments, anti-PD-L1 treatment had no direct effect on cytotoxicity or cytokine secretion by PD-1 negative PM21-NK cells in response to PD-L1+ targets. However, in vivo a significant increase in the antitumor effect of Natural Killer cells was found when combined with anti-PD-L1. PD-L1 blockade also resulted in increased persistence of Natural Killer cells in vivo and retention of their cytotoxic phenotype. These results support the use of anti-PD-L1 in combination with Natural Killer cell therapy regardless of baseline tumor PD-L1 status and suggest that Natural Killer cell therapy will likely increase the applicability of anti-PD-L1 treatment. In this review article, the author will discuss the method of optimizing PD-L1 blockade combined with Natural Killer cell therapy to increase the efficacy of treatment on cancer stem cells in the dormant phase.

Keywords: PD-1, PDL-1, Natural Killer Cell, Cancer Cells

Introduction

“Anti-PD-1 and anti-PD-L1 are therapies that have shown success in cancer treatment” [1]. “(PD-1)/programmed cell death ligand 1 (PD-L1) pathway is a programmed cell death receptor 1 pathway. Targeting this pathway has been a proven successful anticancer strategy” [2]. “In cases of head and neck squamous cell carcinoma, melanoma, lymphoma, lung cancer, colorectal cancer, liver cancer, urothelial cancer, cervical cancer, stomach cancer, kidney cancer and

breast cancer, many antibodies have been applied to the PD-1/PD-L1 pathway” [3]. “Monotherapy or combination therapy in the form of adjuvant or neo-adjuvant produces satisfactory clinical responses. Long-term remission occurs in a small proportion of cancer patients. However, while treating cancer with PD-1/PD-L1 blockade is similar, the clinical response rate is still low in certain cancers at around 40%. This is due to the lack of immune-related toxicity effects, known biomarkers, as well as innate and acquired drug resistance” [4]. “Therefore, therapeutic strategies are needed to increase PD-L1 expression and optimize PD-1/PD-L1 blockade therapy, so as to improve satisfactory therapeutic results. In tumors, IFN γ induces the expression of PD-L1 which is a cytokine secreted by Natural Killer cells. A study showed that PM21-NK cells induced large amounts of IFN γ and transfected PM21-NK cells adaptively induced PD-L1 expression. In in vitro experiments, anti-PD-L1 treatment had no direct effect on cytotoxicity or cytokine secretion by PD-1 negative PM21-NK cells in response to PD-L1+ targets. However, in vivo a significant increase in the antitumor effect of Natural Killer cells was found when combined with anti-PD-L1” [1].

Recognition of NK Cells

Natural killer (NK) cells are the main effector cells of innate immunity in eliminating various viruses, microbes and tumor cells through cytotoxic lymphocyte cell-mediated release of granzyme B, is known as NK-mediated target cell lysis. NK also produces pro-inflammatory cytokines. The ability of NK to destroy target cells depends on the levels of MHC class I molecules expressed by target cells. MSCs do not express MHC class I, so MHC does not recognized as the lysis program of NK cells, both derived from MSCs autologous and allogeneic MSCs. Active NK cells will bind ligands MSCs on NK cell surface receptors so that they can eliminate them the existence of MSC [5].

“NK cells have been divided into two main subgroups based on CD56 expression: CD56^{bright} and CD56^{dim} NK cells identified as CD3-CD56+ cells” [6]. “CD56^{bright} NK cells account for approximately 5-10% of peripheral blood NK cells and are immature NK cells that are thought to play an immunomodulatory role by producing cytokines. Meanwhile, fully mature NK cells are called CD56^{dim} NK cells which account for around 90-95% of peripheral blood NK cells. Through cell-

mediated cytotoxicity (ADCC), they kill target cells directly” [7, 8]. “CD56^{dim} NK cells are found in many lymphoid organs such as lymph nodes and tonsils. CD56^{dim} NK cells are also commonly found in peripheral blood, lungs, bone marrow and spleen. Although referred to as a distinct subtype, CD56^{bright} NK cells are also considered to be precursors of CD56^{dim} NK cells after exposure to interleukin (IL)-2 and/or IL-15 and ultimately differentiate into CD56^{dim} NK cells” [9].

NK cell recognition of “self” and “nonself” only requires precise control of NK cell function through regulation of activation and inhibition receptor and does not require rearrangement of somatic genes to produce clones that recognize different antigens (**Figure 1**) [10]. NK cell surface inhibitory receptors, especially Killer Immunoglobulin-like Receptors (KIRs), the C-type lectin receptor family CD94/NKG2, and leukocyte Ig-like receptors (LIRs/ILTs) undergo an “education” process to recognize Major Histocompatibility Complex class I molecules (MHC- I) [11], resulting in NK cells becoming inactive and building self-tolerance [12, 13]. And when NK cells recognize more activating receptors, especially DNAX accessory molecule receptor 1 (DNAM-1), Natural Cytotoxic Receptor (NCR), and natural killer group member 2 D (NKG2D) undergo lysis of target cells through a “lost self” mechanism or “induced self” [14].

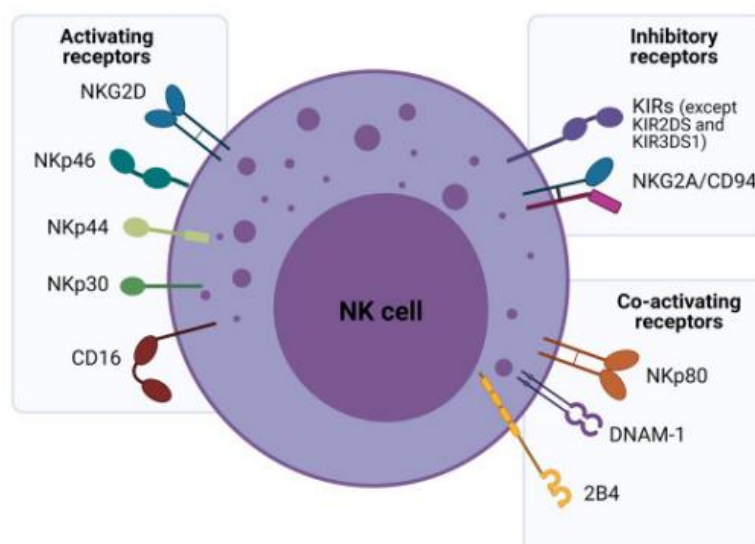


Figure 1. NK cell function through regulation of activation and inhibition receptor [10]

The Role of PD-1/PD-L1 in the Immune system and Cancer Cells

“The PD-1/PD-L1 pathway under normal circumstances negatively regulates the immune system. ITSM has the biological function of PD-1 by being phosphorylated by binding PD-L1 and subsequently activating a series of intracellular pathways and inducing immune inhibition” [3]. “PD-1 exerts its immunosuppressive effects between different T and B lymphocytes” [15].

“There are two signaling pathways involved in the immune response induced by T cells following pathogen invasion including binding of immunostimulatory APC-expressed ligands to the TCR and binding of the major histocompatibility complex (MHC) on the surface of antigen-presenting cells (APCs) to the T cell receptor (TCR)”. [30] “In effect, activating or inhibitory signals are transduced to T cells and subsequently cause T cell activation and exhaustion in regulating the immune response. TCR-mediated, PD-1/PD-L1 pathway can lead to inhibition of T cell activation. Engagement of PD-1 and PD-1 ligand in T cells results in recruitment of SHP-1/2 to the C-terminus of ITSM. Then there is dephosphorylation of TCR-associated CD-3 ζ and ZAP70 by SHP-2, so that inhibition of downstream signals occurs” [16]. “Specifically, the phosphatidylinositol 3-kinase (PI3K) pathway is suppressed, resulting in reduced expression of the cell survival gene Bcl-XL” [17]. “In addition, activation of the PI3K/AKT pathway is inhibited by TCR-induced PD-1 by activating PTEN” [18]. “In addition, PD-1 suppresses T cell proliferation by inhibiting the activation of the RAS-MEK-ERK pathway and inhibiting the activation of PKC δ , resulting in a reduction in the levels of cytokines IFN- γ and IL-2 secreted by T cells” [19, 20]. “Furthermore, by suppressing glycolysis and promoting

lipolysis and fatty acid oxidation, PD-1 signaling regulates T cell metabolism” [21]. The mechanism of the PD-1/PD-L1 pathway in the immune system and cancer is depicted in **Figure 2**.

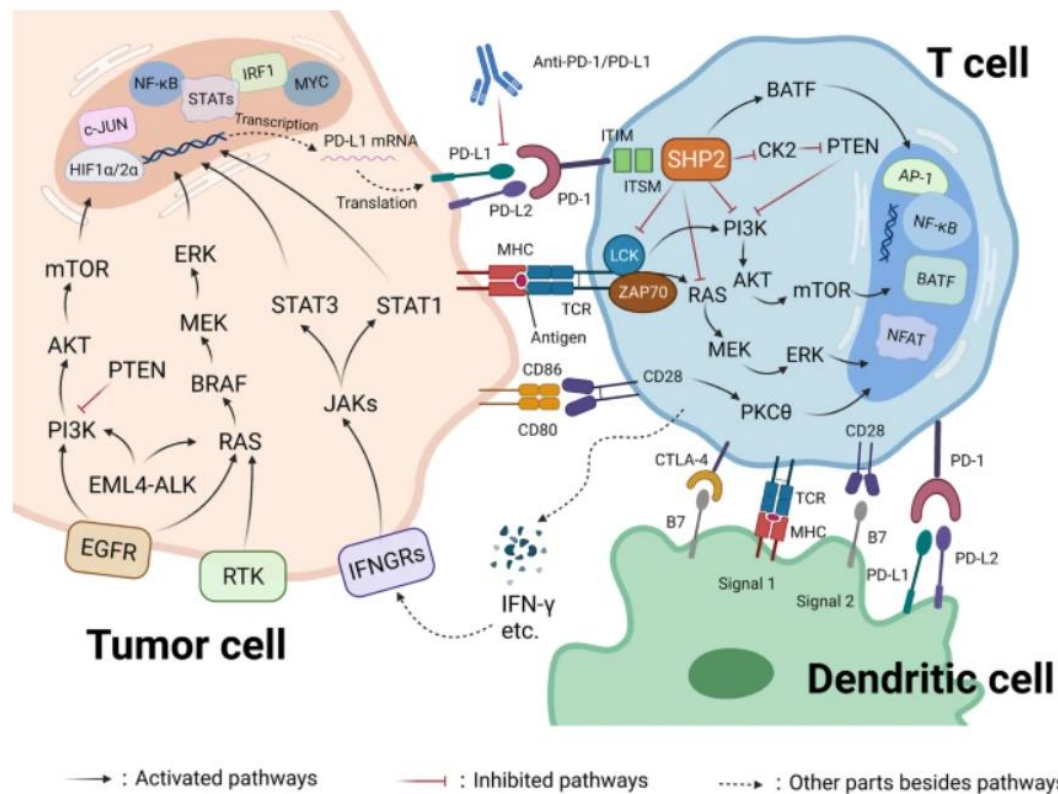


Figure 2. Mechanism of the PD-1/PD-L1 pathway in the immune system and cancer [21]

NK Cell-mediated Anticancer Mechanisms

“TRAIL is a type II transmembrane protein with homology to TNF and FasL which expressed by T cells and NK cells, but can also be found in dendritic cells, monocytes, and macrophages” [27]. “While freshly isolated NK Cells, they do not express detectable levels of TRAIL on their surface. TRAIL surface expression can be stimulated by IL-2, IL-15 or IL-12 134,135. TRAIL-mediated cytotoxicity has been shown to control NK cell-mediated cancer cells” [23]. “However, it is not yet known the mechanism by which TRAIL migrates to the lysosomal compartment and whether TRAIL is stored in the same vesicles as FasL. Surface expression TRAIL in synapses can be induced by exposure to target cells. In the case of cytokine-induced expression, this is indeed the case it is unlikely that TRAIL is concentrated in a specific subset of NK cells surface. However, unlike FasL which is soluble, TRAIL is soluble can carry out its apoptotic activity and potentially result

in the killing of cancer cells in close proximity to TRAIL secreting NK cells” [23, 26].

“TRAIL in humans binds to 4 different membrane receptors and 1 soluble receptor. TRIAL receptor subtypes that can induce apoptosis are TRAIL-R1 and TRAIL-R2. While TRAIL-R3, TRAIL-R4 and Osteoprotegerin largely induces NF- κ B signaling and functions as fodder receptors to limit TRAIL binding to the pro-apoptotic TRAIL receptor. TRAIL-R1 and TRIAL-R2 represent pro-apoptotic signaling that is very similar to CD95-mediated signaling, with activation of caspase-8 and formation of DISC” [26].

Optimization of PD-1/PD-L1 Blockade in increasing NK Cell Cytotoxicity

“PD-L1 is upregulated on the surface of various types of cancer cells by IFN- γ and TNF- α . This regulation involves several endogenous carcinogenic pathways including PI3K-AKT and AMPK. PD-L1, which is regulated through the PI3K-AKT and AMPK pathways, helps cancer cells escape immunity by negatively regulating antitumor immunity after binding to PD-1. This change in PD-L1 expression through upregulation or downregulation, results in better cancer treatment efficacy when combined with immunotherapy. Upon downregulation of PD-L1 expression, the inhibited PD-L1/PD-1 axis releases the brakes on the immune system. In contrast, upregulated PD-L1 changes the tumor from a cold state to a hot state. Therefore, the PD-L1/PD-1 axis may have greater power to inhibit the antitumor immune system” [28].

Targeting PD-L1 regulation may result in better therapeutic effects. A Multistage Sensitive Nanocomplex (MUSE) loaded with the PD-L1/CD47 dual-targeting CRISPR/Cas9 system was developed as a coactivator of T cell and macrophage-mediated antitumor immune responses. In a study on mice with melanoma, the prepared MUSE had several beneficial characteristics including, rapid response to MMP-9-rich TME, high transfection efficiency, including prolonged blood circulation, increased lysosome release, and rapid nuclear localization. With these advantages, MUSE equipped with MT-CRISPR/Cas9 shows effective elimination of PD-L1 and CD47 in tumor cells and has the ability of activation of innate and adaptive antitumor immunity, thereby significantly improving the overall survival in

mouse melanoma cancer without side effects (**Figure 3**) beyond the detected target. This study provides a new alternative for the development of future CRISPR-based anticancer treatment regimens and anticancer therapies [29].

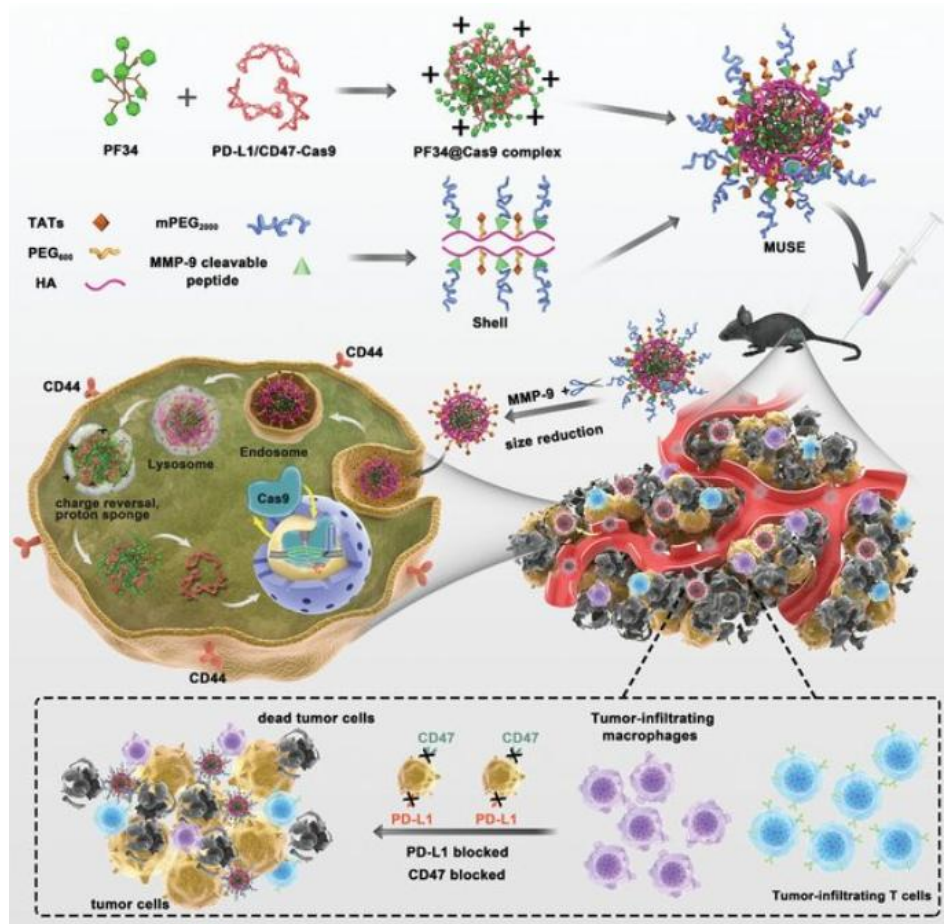


Figure 3. MUSE equipped with MT-CRISPR/Cas9 shows effective elimination of PD-L1 and CD47 in tumor cells and has the ability of activation of innate and adaptive antitumor immunity [29]

Clinical Use of Combination of PD-1/PD-L1 Blockade Agent and NK Cells

“In several cancers including renal cell carcinoma, ovarian cancer, Kaposi's sarcoma, digestive cancer and MM, PD-1 is upregulated on NK cells with varying expression levels. Functional dysregulation in NK cells occurs as a result of this upregulation. Exemplified by functionally depleted PD-1⁺ NK cells” [22]. “Several studies have shown that ligation of PD-1 on NK cells with its ligands causes death ligand programming 1 (PD-L1) on tumor cells and can deplete NK cells and strongly suppress their antitumor properties” [23]. “Combination therapy with anti-PD-1 mAbs such as nivolumab has been widely used to reconstitute NK cell function in patients with solid tumors” (**Figure 4**) [24]. “In triple negative breast

cancer cells (TNBC). The PD-L1 mAb, avelumab significantly enhanced NK cell-mediated cytotoxic effects” [23]. “PD-L1 is expressed by tumor cells to higher levels leading to a higher degree of sensitivity to avelumab-mediated ADCC. Histone deacetylase inhibitors are a combined therapeutic strategy that can result in increased NK cell tumor cell lysis and ADCC in several avelumab-mediated carcinoma cell types” [25].

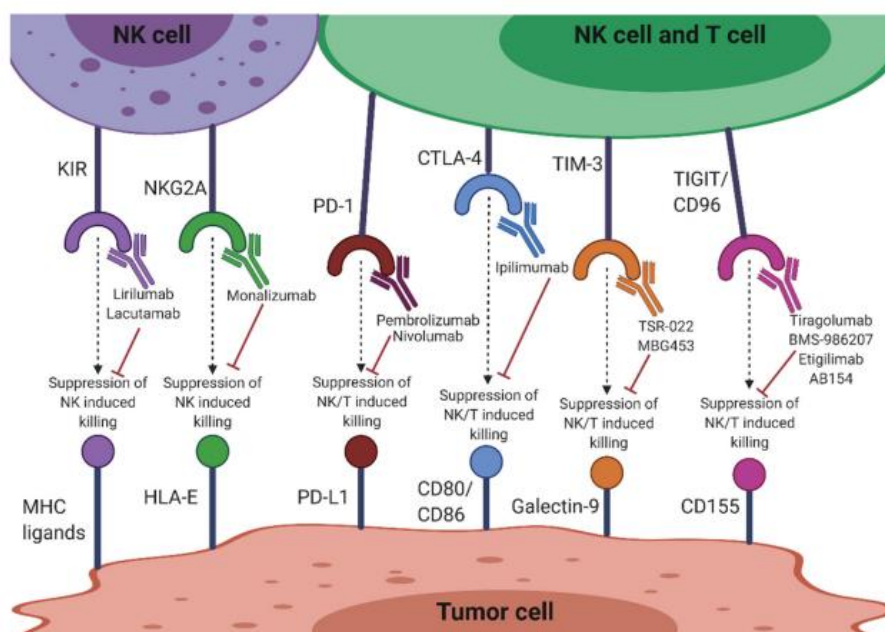


Figure 4. Targets of checkpoint inhibitors on NK cells and T cells and their respective inhibitory antibodies are being investigated for therapeutic efficacy and clinical use for cancer [24]

Therefore, targeting the PD-1/PD-L1 pathway can enhance the effect of NK-mediated tumor cell elimination in TNBC [23]. However, the role of the PD-1/PD-L1 axis in the antitumor function of NK cells appears to be TME dependent and complex. In Hodgkin's lymphoma, immune evasion through this pathway in NK cells is more prominent than in large B-cell lymphoma. Indirect emphasis by NK cells via tumor-associated macrophages express PD-L1/PD-L2 [26].

Conclusion and Future Perspectives

Innate and adaptive immunity play an important role in the immune system's response to tumors. Therefore, therapeutic strategies that are able to target both branches of immunity simultaneously have the potential to provide major therapeutic effects against cancer cells. This occurs due to blockade of the PD-

1/PD-L1 axis, which can increase the antitumor cytotoxic activity of NK cells and T cells. Tumors have a mechanism to avoid T cell recognition and tumors try to downregulate HLA I expression. However, with the optimization strategy of PD-1/PD-L1 blockade can be more efficiently recognized and killed by NK cells. The author hopes that there will be further research in vitro or in vivo on optimizing PD-1/PD-L1 blockade to increase the cytotoxic effect of NK cells in killing cancer cells.

References

1. Jeremiah L. Oyer, Sarah B. Gitto, Deborah A. Altomare, Alicja J. Copik, PD-L1 blockade enhances anti-tumor efficacy of NK cells. *Oncoimmunology*. 2018; 7(11): e1509819. Published online 2018 Aug 27. doi: 10.1080/2162402X.2018.1509819
2. Cao L, Prithviraj P, Shrestha R, Sharma R, Anaka M, Bridle KR, et al. Prognostic role of immune checkpoint regulators in cholangiocarcinoma: a pilot study. *J Clin Med*. 2021;10:2191. doi: 10.3390/jcm10102191
3. Hossain MA, Liu G, Dai B, Si Y, Yang Q, Wazir J, et al. Reinvigorating exhausted CD8(+) cytotoxic T lymphocytes in the tumor microenvironment and current strategies in cancer immunotherapy. *Med Res Rev*. 2021;41:156–201. doi: 10.1002/med.21727
4. Pitt JM, Vetizou M, Daillere R, Roberti MP, Yamazaki T, Routy B, et al. Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors. *Immunity*. 2016;44:1255–69. doi: 10.1002/adtp.201800157
5. Alex M. Abel, Chao Yang, Monica S. Thakar, Subramaniam Malarkannan. Natural Killer Cells: Development, Maturation, and Clinical Utilization. *Front Immunol*. 2018; 9: 1869. Published online 2018 Aug 13. doi: 10.3389/fimmu.2018.01869
6. Amand M, Iserentant G, Poli A, Sleiman M, Fievez V, Sanchez IP, Sauvageot N, Michel T, Aouali N, Janji B, et al. Human CD56dimCD16dim Cells As an Individualized Natural Killer Cell Subset. *Front Immunol*. 2017;8:699. doi: 10.3389/fimmu.2017.00699
7. Fortes-Andrade T, Almeida JS, Sousa LM, Santos-Rosa M, Freitas-Tavares P, Casanova JM, Rodrigues-Santos P. The Role of Natural Killer Cells in Soft Tissue Sarcoma: Prospects for Immunotherapy. *Cancers (Basel)*. 2021;13:3685. doi: 10.3390/cancers13153865
8. Bruno A, Ferlazzo G, Albini A, Noonan DM. A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis. *J Natl Cancer Inst*. 2014;106:200. doi: 10.1093/jnci/dju200

9. Romagnani C, Juelke K, Falco M, Morandi B, D'Agostino A, Costa R, Ratto G, Forte G, Carrega P, Lui G, et al. CD56^{bright}CD16⁻ killer Ig-like receptor-NK cells display longer telomeres and acquire features of CD56^{dim} NK cells upon activation. *J Immunol.* 2007;178:4947–55. doi: 10.4049/jimmunol.178.8.4947
10. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Annu Rev Immunol.* 2013;31:227–58. doi: 10.1146/annurev-immunol-020711-075005
11. Sullivan LC, Berry R, Sosnin N, Widjaja JM, Deuss FA, Balaji GR, LaGruta NL, Mirams M, Trapani JA, Rossjohn J, et al. Recognition of the Major Histocompatibility Complex (MHC) Class Ib Molecule H2–Q10 by the Natural Killer Cell Receptor Ly49C. *J Biol Chem.* 2016;291:18740–52. doi: 10.1074/jbc.M116.737130
12. Boudreau JE, Liu XR, Zhao Z, Zhang A, Shultz LD, Greiner DL, Dupont B, Hsu KC. Cell-Extrinsic MHC Class I Molecule Engagement Augments Human NK Cell Education Programmed by Cell-Intrinsic MHC Class I. *Immunity.* 2016;45:280–91. doi: 10.1016/j.immuni.2016.07.005
13. Boudreau JE, Hsu KC. Natural Killer Cell Education and the Response to Infection and Cancer Therapy: Stay Tuned. *Trends Immunol.* 2018;39:222–39. doi: 10.1016/j.it.2017.12.001
14. Rezvani K, Rouce R, Liu E, Shpall E. Engineering Natural Killer Cells for Cancer Immunotherapy. *Mol Ther.* 2017;25:1769–81. doi: 10.1016/j.ymthe.2017.06.012
15. Cai J, Wang D, Zhang G, Guo X. The role of PD-1/PD-L1 axis in treg development and function: implications for cancer immunotherapy. *Onco Targets Ther.* 2019;12:8437–45. doi: 10.2147/OTT.S221340
16. Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. *Mol Cancer.* 2019;18:10. doi: 10.1186/s12943-018-0928-4
17. Hofmeyer KA, Jeon H, Zang X. The PD-1/PD-L1 (B7–H1) pathway in chronic infection-induced cytotoxic T lymphocyte exhaustion. *J Biomed Biotechnol.* 2011;2011:1–9. doi: 10.1155/2011/451694
18. Patsoukis N, Li L, Sari D, Petkova V, Boussiotis VA. PD-1 increases PTEN phosphatase activity while decreasing PTEN protein stability by inhibiting casein kinase 2. *Mol Cell Biol.* 2013;33:3091–8. doi: 10.1128/MCB.00319-13
19. Wartewig T, Kurgys Z, Keppler S, Pechloff K, Hameister E, Ollinger R, et al. PD-1 is a haploinsufficient suppressor of T cell lymphomagenesis. *Nature.* 2017;552:121–5. doi: 10.1038/nature24649

20. Seto T, Sam D, Pan M. Mechanisms of primary and secondary resistance to immune checkpoint inhibitors in cancer. *Med Sci (Basel)*. 2019;7:14. doi: 10.3390/medsci7020014
21. Jubel JM, Barbati ZR, Burger C, Wirtz DC, Schildberg FA. The role of PD-1 in acute and chronic infection. *Front Immunol*. 2020;11:487. doi: 10.3389/fimmu.2020.00487
22. Pesce, S.; Greppi, M.; Tabellini, G.; Rampinelli, F.; Parolini, S.; Olive, D.; Moretta, L.; Moretta, A.; Marcenaro, E. Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. *J. Allergy Clin. Immunol*. 2017, 139, 335–346.e333. doi: 10.1016/j.jaci.2016.04.025
23. Julia, E.P.; Amante, A.; Pampena, M.B.; Mordoh, J.; Levy, E.M. Avelumab, an IgG1 anti-PD-L1 Immune Checkpoint Inhibitor, Triggers NK Cell-Mediated Cytotoxicity and Cytokine Production Against Triple Negative Breast Cancer Cells. *Front. Immunol*. 2018, 9, 2140. doi: 10.3389/fimmu.2018.02140
24. Pesce, S.; Greppi, M.; Grossi, F.; Del Zotto, G.; Moretta, L.; Sivori, S.; Genova, C.; Marcenaro, E. PD-1-PD-Ls Checkpoint: Insight on the Potential Role of NK Cells. *Front. Immunol*. 2019, 10, 1242. doi: 10.3389/fimmu.2019.01242
25. Hicks, K.C.; Fantini, M.; Donahue, R.N.; Schwab, A.; Knudson, K.M.; Tritsch, S.R.; Jochems, C.; Clavijo, P.E.; Allen, C.T.; Hodge, J.W.; et al. Epigenetic priming of both tumor and NK cells augments antibody-dependent cellular cytotoxicity elicited by the anti-PD-L1 antibody avelumab against multiple carcinoma cell types. *Oncoimmunology* 2018, 7, e1466018. doi: 10.1080/2162402X.2018.1466018
26. Vari, F.; Arpon, D.; Keane, C.; Hertzberg, M.S.; Talaulikar, D.; Jain, S.; Cui, Q.; Han, E.; Tobin, J.; Bird, R.; et al. Immune evasion via PD-1/PD-L1 on NK cells and monocyte/macrophages is more prominent in Hodgkin lymphoma than DLBCL. *Blood* 2018, 131, 1809–1819. doi: 10.1182/blood-2017-07-796342
27. Isabel Prager, Carsten Watzl. Mechanisms of natural killer cell-mediated cellular cytotoxicity. *J Leukoc Biol*. 2019;105:1319–1329. doi: 10.1002/JLB.MR0718-269R
28. Yousefi H, Yuan J, Keshavarz-Fathi M, Murphy JF, Rezaei N. Immunotherapy of cancers comes of age. *Expert Rev Clin Immunol*. 2017;13:1001–15. doi: 10.1080/1744666X.2017.1366315
29. Li HY, McSharry M, Bullock B, Nguyen TT, Kwak J, Poczobutt JM, et al. The tumor microenvironment regulates sensitivity of murine lung tumors to PD-1/PD-L1 antibody blockade. *Cancer Immunol Res*. 2017;5:767–77. doi: 10.1158/2326-6066.

30. Wu M, Huang Q, Xie Y, Wu X, Ma H, Zhang Y, Xia Y. Improvement of the anticancer efficacy of PD-1/PD-L1 blockade via combination therapy and PD-L1 regulation. *Journal of hematology & oncology*. 2022 Mar 12;15(1):24.

UNDER PEER REVIEW