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Epigenetic mechanisms dictating eradication of cancer by NK cells

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SUMMARY

Natural killer (NK) cells of the innate immune system are the first line of defense against infectious agents and cancer cells. However, only a few mechanisms that regulate eradication of tumors by NK cells have been identified. In this review, we present an account of epigenetic mechanisms that modulate the ability of NK cells to eradicate cancer cells. To date, several drugs that target epigenetic modifiers have shown clinical efficacy in cancer. Therefore, once a given epigenetic modifier is validated as a regulator of NK cell function, it can be targeted for NK cell-based cancer immunotherapies.

Epigenetics, NK cells and Cancer

The human immune system provides protection against a wide variety of pathogens and diseases, including cancer. The innate immune system comprises several cell types, including natural killer (NK) cells, which are large granular lymphocytes that represent 10 to 15% of the total circulating lymphocytes [1] but can also be tissue resident [2, 3]. NK cells play an important role in the protection against infectious pathogenic agents and serve as the first line of immunological defense against tumor initiation and progression [4, 5]. Unlike other immune cell types that are slow to attain cytotoxic activity, NK cells can readily recognize and eradicate pathogen-infected, stressed, and transformed cancer cells [4, 5].

The antitumor effects of NK cells were first shown against implanted mouse tumors in the early 1970s [6, 7]. Conversely, impaired NK cell function was later shown to increase tumor growth and metastasis [8, 9]. A recent long-term epidemiological study revealed that decreased NK cell activity is associated with higher risks of developing various cancers [10], whereas high numbers of tumor-infiltrating NK cells are associated with favorable outcomes in colorectal carcinoma, gastric cancer, and squamous cell lung cancer patients [11]. NK cells control tumor growth by recruiting conventional type 1 dendritic cells to the tumor

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CONFLICT OF INTEREST STATEMENT

Authors have no conflicts of interest to declare.

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microenvironment [12]. These observations highlight the potential use of NK cells in cancer immunotherapy.

Immunotherapies have been successful in multiple unrelated types of cancer [13], as immune suppression and immune evasion by cancer cells are known to contribute to tumor development and progression [14, 15]. Tumorigenic and metastatic cellular states are the result of a complex multistep process involving various genetic and epigenetic changes. In this review, we focus on epigenetic mechanisms in cancer and NK cells that affect NK cell-mediated recognition of rogue cancer cells and their eradication (also see, Table 1) [16–42].

Development and regulation of NK cells and mechanisms of cytotoxicity

Throughout life, NK cells develop primarily in bone marrow from hematopoietic stem cells, which differentiate first into common lymphoid progenitors and then into NK/T cell progenitors, undergoing a series of coordinated differentiation steps and acquiring different markers, receptors, and functions [43–45]. Recent studies have also demonstrated that NK cell precursors migrate to and undergo further differentiation in secondary lymphoid tissues, such as those of the uterus and liver [46–48]. We refer readers to some outstanding reviews for more details on the origin and maturation of NK cells [43, 49–52].

Although cytokines play an important role in the development and activation of NK cells [53], NK cell activities, including cytotoxicity towards target cells (e.g., cancer cells), are largely regulated by the activation or inhibition of their receptors. This is in contrast to B and T cells, primary cell types of the adoptive immune system, which have antigen specificity and a higher degree of flexibility through the arrangement of gene clusters. Nevertheless, NK cell receptors have some flexibility via the recognition of different ligands on target cells, such as the NK group 2D (NKG2D) ligands [54–56] (Figure 1A–B).

Cancer cells develop multiple strategies to evade NK cell-mediated cytotoxicity (Figure 1C). One mechanism involves the loss or shedding of NKG2D ligands, which in turn downregulates the expression of NKG2D receptor on NK cells [57, 58]. Additionally, cancer cells can also downregulate NK-activating receptors [59]. By contrast, the elevated expression of NKG2A receptors in infiltrating NK cells reduces their cytotoxic activity against renal carcinoma cells [60]. Tumor cells can also avoid eradication by NK cells by expressing the ligands for inhibitory receptors, such as non-classical human leukocyte antigens (HLA-E or HLA-G) [61, 62], and by releasing immunosuppressive cytokines, such as transforming growth factor beta (TGF- β), interleukin-10, prostaglandin, and indoleamine 2,3-dioxygenase [63–65]. These factors not only decrease NK cell activity but also inhibit NK cell maturation [66]. Additional immune inhibitory checkpoint receptors, such as programmed cell death protein 1 (PD-1), are expressed on NK cells and cancer cells expressing programmed death ligand 1 (PD-L1) inhibit the anti-cancer abilities of NK cells [67]. Similarly, the ability of NK cells to control cancer growth is modified by other immunomodulatory surface proteins (TIGIT, TIM-3, and LAG3) that affect a variety of phenotypes, including NK cell effector function and NK cell exhaustion [68–70].

Epigenetic regulation of NK cells in cancer

The effect of key immune cells (B and T cells or NK cells) against cancer cells are subject to epigenetic regulation. For example, the epigenetic state of cancer cells either makes them vulnerable to immune clearance or promotes immune evasion [71, 72]. Here, we present evidence for the role of epigenetic modulators and the mechanisms that regulate NK cell-mediated cancer cell eradication (Figure 2. Key Figure).

Epigenetic alterations are reversible and heritable changes that do not involve alteration in the DNA sequence itself. These changes are typically associated with DNA modifications, such as CpG DNA methylation or posttranslational modifications of histone proteins. However, for the purpose of this review, we use a broader definition of epigenetic alterations that includes additional non-genetic changes, such as changes in transcription factors and noncoding RNA expression/function.

Transcription factors

Transcription factors are sequence-specific DNA binding proteins that, along with other factors (e.g., epigenetic modulators, transactivators,) can either activate or repress the transcription of target genes. Several transcription factors, including nuclear factor kappa B, Cbl-C, and runt-related transcription factor 3, have been shown to modulate NK cell function and alter the eradication of tumors by NK cells [73–75]. The tumor suppressor protein p53 and the MYC oncogene are two transcription factors with a well-established relevance in both cancer and immunity [76, 77].

In half of all cancers, p53 is either mutated or deleted, and the loss of p53 has been shown to directly contribute to cancer initiation and progression [78]. A previous study showed that in cancer cells, p53 induces the expression of the NKG2D ligands ULBP1 and ULBP2 and stimulates interferon gamma (IFN- γ) production by co-cultured NK cells [79]. However, a direct role of ULBP1 and ULBP2 in mediating the production of IFN- γ was not tested in this study. In addition, p53 induces the senescence of cancer cells and NK cell-mediated clearance [80], an observation that was confirmed in a more detailed study using an Hras-transformed liver cancer model with doxycycline-inducible p53 shRNA [81]. In this model, p53 increased the secretion of multiple chemokines, particularly C-C motif chemokine ligand 2 (CCL2), which recruited NK cells to senescent tumors, rather than inducing the expression of NK cell ligands [81]. Similar to CCL2, mutations in p53 regulate the levels of other chemokines and result in the inhibition of NK cell recruitment [82]. Additional studies examining the impact of aneuploidy on p53 induction and tumor development and/or control found that DNA replication stress from complex structural aneuploidy resulted in p53 activation and senescence in cancer cells, which also secreted cytokines that promoted eradication by NK cells [83, 84].

MYC represses the expression of class 1 human leukocyte antigen in melanoma cells and potentially enhances NK cell-mediated eradication [85]. Studies using an E μ -MYC-driven mouse model of lymphoma showed that MYC is necessary for the transcription of NKG2D ligand Rae-1 [86]. Downregulation of NKG2D receptor on NK cells was observed in lymphoma-bearing mice, indicating there may be a compensatory mechanism *in vivo* by

which lymphomagenesis progresses via inactivation of NK cell-mediated cytotoxicity. The possibility that the vast number of lymphoma cells exhausted the NK cells and hampered their ability to regulate lymphoma progression was also not ruled out [86]. Additionally, a recent study using a mouse lung model of KRas^{G12D}-driven adenoma found that MYC cooperated with coactivated oncogenic Ras to cause immune suppression in part by reprogramming the stroma, which was largely driven by C-C motif chemokine ligand 9 and interleukin-23. In this model, the inactivation of MYC after tumor development reversed all the changes in the tumor stroma and caused tumor regression, which was largely dependent on returning NK cells [14]. MYC is also shown to enhance the expression of PD-L1 in cancer cells, which promotes immune evasion [82] and attenuates the ability of NK cells to clear cancer cells [67].

The signal transducer and activator of transcription (STAT) family of transcription factors also regulate NK cell function. STAT proteins play a diverse role in several biological processes and are important regulators of both the innate and the adoptive immune response [87]. For example, STAT5 plays an important role in IL-2 and IL-15-mediated signal transduction and in promoting NK cell survival, cytotoxicity, and maturation [88, 89]. STAT5 regulation of NK cell-mediated angiogenesis can act as a molecular switch to shift from tumor surveillance to tumor promotion. STAT5 normally downregulates VEGFA in NK cells, but the inhibition of STAT5 increases VEGFA production, resulting in NK cell-mediated angiogenesis and tumor growth [90]. This study suggested that STAT5 inhibitors need to be used with caution as anti-cancer agents because they may cause unexpected tumor promoting effects. Similarly, mice with a targeted mutation of STAT1 demonstrate reduced NK cell cytolytic activity in vitro and a failure to reject implanted tumor in vivo [91]. Interestingly, the inability to reject tumors in these mice was dependent upon IFN- α and IFN- γ and was not due to reduced NK cell number [91]. An analysis of mice lacking STAT3 showed normal development and normal NK cell number, but an alteration in the kinetics of IFN- γ production due to a lack of STAT3 binding to IFN- γ promoter was observed [92]. Strikingly, the loss of STAT3 in NK cells enhances tumor surveillance in various in vivo models of hematological diseases. The reduced tumor burden is partially due to increased expression of the activating ligand DNAX accessory molecule 1 as well as the lytic enzymes perforin and granzyme B [92]. Similarly, another study suggested that inhibition of STAT3 increases NK cell cytotoxicity to cancer cells by upregulating the NKG2D ligand major histocompatibility complex class I-related chain A levels by directly binding to its promoter [93]. These findings have therapeutic implications because they suggest that STAT3 inhibitors will enhance NK cell activity towards cancer cells and inhibit tumor growth in a non-autonomous manner. Overall, these studies of STAT proteins and their role in the regulation of NK cell function reveal their surprisingly diverse and non-redundant functions. A thorough evaluation of the cross-talk among different STAT transcription factor family members and their ability to regulate NK cells will require further investigation.

Chromatin regulators and DNA modifiers

Chromatin regulatory proteins are identified as “writers” “erasers,” and “readers” of histone, marks or CpG methylating or demethylating enzymes on the basis of their function [94, 95].

Chromatin regulation is important for NK cell development and function. For example, NK cell maturation requires the activity of the histone H2A deubiquitinase MYSM1 [96]. Epigenetic modulation of NK cell responses was shown in studies testing the effects of inhibitors of histone deacetylases (entinostat and givinostat) and DNA methyltransferases (5-azacytidine [Aza]) in mouse models of epithelial ovarian cancer [97]. Pretreatment of tumor epithelial cells with Aza significantly reduced ascites, alterations in the number and activation state of immune cells, including NK cells, and increase in overall survival. Treatment of ovarian cancer cells with Aza also increased IFN signaling, which was required for the tumor-suppressive effects [97]. Although the relative contribution of NK cells to these effects was not examined, there was an increase in activated NK cells in the tumor microenvironment [97]. The broad-spectrum inhibitors tested in this study have effects that include aspects beyond epigenetic regulation. Therefore, the specific epigenetic mediators responsible, including those for IFN pathway regulation, were not revealed. Another interesting study showed that *IDH1* and *IDH2* mutant gliomas escape NK cell immune surveillance via DNA methylation-based downregulation of NKG2D ligands (ULBP1 and ULBP3) [98]. These ligands were re-expressed upon cell treatment with Aza, which led to lysis of glioma cells by NK cells. These results are important because in some cancer types, including acute myelogenous leukemia, *IDH1* and *IDH2* mutations result in global DNA hypermethylation due to reduced α -ketoglutarate levels and TET2 function [98].

A recent study using a chemical screen targeting epigenetic regulators identified the histone methyltransferase enhancer of zeste homolog 2 (EZH2) as a modulator of NK cell function against hepatocellular carcinoma [99]. EZH2 inhibited the expression of ULBP1 and induced the sensitization of hepatocellular carcinoma cells to NK cells in a DNA methylation-dependent manner (via DNA methyltransferase 3A). Furthermore, NK cell differentiation is enhanced in EZH2-null or EZH2 inhibitor (UNC1999 or EPZ005687)-treated hematopoietic stem cells, which were also more effective in eradicating tumor cells [100]. Increase in methylation of lysine 27 of histone 3 in NK cells via inhibition of JMJD3/UTX demethylase was associated with an anti-inflammatory reduction in cytokine production, which confirms the importance of this histone modification in NK cell activation [101]. Similarly, the demethylating enzyme specific for trimethylated lysine 4 of histone 3, Kdm5a, is necessary for NK cell activation, and a deficiency in this enzyme reduces IFN- γ production by activated NK cells, mediated in part by SOCS1 [102].

Collectively, these studies show NK cells undergo multiple histone and DNA methylation-based modifications that affect activation and function, similar to what happens in cancer cells. Of note, some of clinically approved EZH2, IDH1, and IDH2 inhibitors are being tested in immunocompromised preclinical models of cancer, which may impact the evaluation of their anticancer efficacy. In future studies, the use of either allograft mouse tumor systems or mouse models with a humanized immune system should be encouraged, so that the impact of the immune system on the efficacy of these agents can be determined.

microRNAs

MicroRNAs (miRNAs) are small noncoding RNAs that bind to the 3'-untranslated regions of mRNAs, inducing degradation or inhibition of translation [103]. There is sufficient

evidence indicating that miRNAs regulate NK cell function [104–106]. For example, knockout of *Dicer* or *Dgcr8*, which prevents miRNA maturation [103], results in reduced survival and turnover of mouse NK cells. Survival and maturation of NK cells is also reduced in mice with lymphocyte *Dicer1* knockout [60]. *Dicer1*-deficient NK cells have enhanced activity, showed by increased degranulation and IFN- γ production in response to tumor cells and other stimuli, such as NK cell receptor ligation. The increased IFN- γ production was attributed to the reduced expression of miRNAs (miR-15a, -15b, and -16) that directly repress mouse IFN- γ .

Analysis of miRNA transcriptomes from NK cells derived from peripheral blood, cord blood, and uterine decidua revealed significant differences in miRNA profiles, suggesting different miRNAs effects on different NK cell populations [107]. For example, miR-362-5p is highly expressed in human peripheral blood NK cells and targets cyclin D1 to enhance NK cell function. miRNA-27a* targets *Prfl* and *GzmB* expression to regulate NK cell cytotoxicity [108], and miR-583 is a negative regulator of interleukin 2 receptor gamma expression and blocks NK cell differentiation [109]. Similarly, the expression of miRNA-183 is induced by TGF- β and blocks expression of DNAX-activating protein 12 kDa (DAP12). Inhibition of DAP12 creates an immunosuppressive tumor microenvironment by inhibiting NK cell function [110]. Downregulation of DAP12 is a common feature in all types of lung cancer and its expression is lower in intratumoral NK cells than in peritumoral NK cells [110]. Another TGF- β -induced miRNA, miR-27a-5p, was shown to function by inhibiting perforin and granzyme B expression and by downregulating the expression of C-X-C motif chemokine receptor 1, resulting in a limitation of NK cell migration ability [111].

Epigenetics and NK cell-based immunotherapy

Immune checkpoint therapies with antibodies against cytotoxic T lymphocyte-associated antigen 4 (e.g., ipilimumab) or programmed cell death protein 1 (e.g., pembrolizumab) or its ligand (e.g., avelumab) that engage T-cell-based eradication of cancer cells show significant clinical benefits in Hodgkin's lymphoma and melanoma [112, 113]. Similarly, success is seen with chimeric antigen receptor T-cell-based therapy [114]. By contrast, NK cell-based therapies are still in the early stages of development. NK cells from multiple myeloma and renal carcinoma patients express PD-1 on their surface and some immune checkpoint blockade therapies that primarily engage T-cells may also engage NK cells [67, 115]. However, the extent to which these therapies involve NK cell activity requires careful analysis.

New studies aim to directly enhance NK cells ability to eradicate cancer cells [13, 116]. The clinical utility of NK cell-based therapies is exemplified by the more than 180 ongoing clinical trials using these cells as of April 2018. NK cell-based immunotherapies can be largely categorized into five different types: (i) antibody-based, (ii) cytokine-based, (iii) adoptive NK cells, (iv) gene modification, and (v) small-molecule inhibitors (Figure 3, Key Figure and Table 2).

Recent studies with EZH2 inhibitors suggest that the targeting epigenetic regulators may be useful to enhancing NK cell activity [117]. EZH2 inhibitors can also enhance eradication of

hepatocellular carcinoma cells by NK cells [99]. Nevertheless, the immunotherapy effects could have arisen from the engagement of other immune cell populations. For example, EZH2 inhibition can promote T-cell-mediated clearance of melanoma cells and cooperate with anti-cytotoxic T-lymphocyte-associated antigen 4-based immunotherapy [117]. Small molecules that target epigenetic regulators have the ability to stimulate anti-cancer immune responses, further highlighting that these drugs represent new enhancers of cancer immunotherapy.

CONCLUDING REMARKS

Despite compelling evidence that NK cells play a role in preventing tumor initiation and progression, a majority of NK cell-based immunotherapies are still in the early stages of development and clinical testing. Additional understanding of the anti-cancer functions of NK cells will aid in the development of more precise and effective NK cell-based immunotherapies (see Outstanding Questions). For example, recent studies have shown important alterations in the super-enhancer functions in immune cells, including NK cells, [118, 119] and a potential future direction is to evaluate the biological impact of super-enhancers on NK cell function and develop drugs to modulate super-enhancers to stimulate NK cell-mediated target cell lysis. Additionally, understanding the epigenetic regulation of NK cell function may have a significant impact on the development of cancer prevention approaches. For example, studies have shown that NK cells are epigenetically reprogrammed and exert stronger activity as a result of exercise [120, 121]. It is still not fully understood why exercise provides cancer prevention benefits, but future studies investigating the direct role of NK cells in mediating those benefits will shed light on this aspect of NK cell function. It is worth noting that several drugs targeting various epigenetic regulators are used clinically for cancer treatment and these drugs can be used either alone or in combination with immunotherapies to enhance NK cell function. The hope is that a combination of drugs targeting epigenetic regulators and NK cell-based therapies will engage both cell intrinsic and extrinsic tumor suppressive pathways to deliver a better clinical outcome for cancer patients.

OUTSTANDING QUESTIONS

- What epigenetic regulators and states modulate NK cell-mediated eradication of cancer cells?
- Are paracrine mechanisms important in NK cell-mediated eradication of cancer cells and how are they regulated by epigenetic mechanisms?
- How do cancer cells thwart NK cells even when they have heightened stress response pathways that should render them more sensitive to NK cell-mediated eradication?
- What aspects of cancer initiation and progression are regulated by NK cells and how does NK cell function evolve with changing epigenetic landscapes in cancer cells?

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Trends Box

- NK innate immune cells inhibit tumor initiation and progression. Cancer cells develop several strategies to evade eradication by NK cells.
- Epigenetic mechanisms regulate the development and activity of NK cells, and also the ability of cancer cells to evade NK cells.
- NK cell-based immunotherapies are in early stages of clinical development. Combination of NK cell-based immunotherapy with investigational or approved epigenetic therapies will likely enhance their efficacy.

Figure 1. Function and regulation of NK cells

A. NK cell ligands on cancer cells are recognized by NK cell receptors on NK cells, which promotes NK cell-mediated eradication of cancer cells. **B.** Cancer cells develop a variety of mechanisms to evade eradication by NK cells. Immune evasion mechanisms include shedding of NK cell ligands by cancer cells, downregulation of activating receptors on NK cells, and the overexpression of inhibitory ligands by cancer cells. **C.** Cancer cells must evade NK cells, which play important roles during the multiple stages of tumor development and progression.

Figure 2, Key Figure. Epigenetic mechanisms regulate eradication of tumors by NK cells
Epigenetic regulators (transcription factors, chromatin regulators, DNA modifying enzymes, and miRNAs) regulate NK cell activity and cancer cell eradication. This can occur by cancer cells developing NK cell evasion mechanisms, for example by the downregulation of NK cell ligands (e.g., ULBP1, ULBP2, and MICA) or by the secretion of chemokines that prevent NK cell recruitment (e.g., IL-10, TGF β 1, and CCL2). Similarly, NK cells can undergo changes in the tumor microenvironment to become less effective, for example by the downregulation of activating receptors (e.g., NKG2D receptor) and the attenuation of their activity due to dampened IFN signaling.

Figure 3, Key Figure. NK cell-based cancer immunotherapies and epigenetic targets

NK cell-based immunotherapies can be largely categorized into five different types ((i) antibody-based, (ii) cytokine-based, (iii) adoptive NK cells, (iv) gene modification, and (v) small-molecule inhibitors). An improved understanding of epigenetic regulators and their roles in modulating the NK cell functions will open up new opportunities for the use of epigenetic regulator-targeting drugs to enhance NK cell-based immunotherapies. For example, small molecule inhibitors of epigenetic regulators can increase the expression of NKG2D ligands or activating receptors on cancer cells and reduce the production of immunosuppressive cytokines such as TGF β .

Table 1

Role of NK cells in cancer initiation and progression.

S. NO	Cancer type	Role of NK cells	Reference
1	Lymphoma/Leukemia	NCR1-deleted mice are shown to develop tumors faster than wild-type mice. Soluble MICA and MICB was detected in leukemia patient sera and may prevent NK cell function. CAR-NK cells expressing anti-CD19 show enhanced NK cell killing towards leukemic cells.	[16] [17] [18]
2	Neuroblastoma	CD155 levels correlate with NK cell cytotoxicity in neuroblastoma cells isolated from patients.	[19]
3	Hepatocellular carcinoma	Reduced ULBP1 and MICA/MICB expression correlate with early recurrence and reduced overall survival in HCC patients. Impairment of NK cell function leads to Hepatitis B and C virus induced HCC.	[20], [21] [22]
4	Breast cancer	IL15 ^{-/-} mice show enhanced breast cancer metastasis, in part, in a NK cell dependent manner. Mesenchymal stem cells overexpressing Sirt1 inhibits breast tumor growth via recruiting NK cells.	[23] [24]
5	Fibrosarcoma	DNAM1 receptor deficient mice show increased MCA-induced fibrosarcoma development.	[25]
6	Cervical cancer	MICA/B and ULBP1 independently predict better overall survival in cervical cancer patients.	[26]
7	Prostate cancer	NKG2D-deficient mice develop prostate tumors that expressed higher amount of NKG2D ligand compared to wild-type mice.	[27]
8	Glioma	MICA overexpression sensitizes glioma cells to NK cell-mediated eradication.	[28]
9	Colorectal Cancer	Higher expression of MICA correlates with better colorectal cancer patient survival.	[29]
10	Melanoma	Ectopic expression of NKG2D ligands in B16 cells leads potent rejection of tumor cells. Blocking of NCR and DNAM1 receptors using specific antibodies reduces NK cell killing of melanoma cells.	[30] [31]
11	Multiple myeloma	NKp30 ligand BAT3 promotes tumor lysis in a NK cell dependent manner.	[32]
12	Head and Neck cancer	Head and neck cancer patients with high NK cell activity have better disease-free survival.	[33]
13	Renal cell carcinoma	IL-21 activated NK cells inhibit the growth of renal cell carcinoma in mice.	[34]
14	Lung cancer	Gefitinib enhances NK cell-mediated clearance of EGFR mutant lung cancer cells.	[35]
15	Ovarian cancer	Depletion of ascorbic acid reduces NK cell cytotoxicity to ovarian cancer cells. IL-2 treatment significantly delays tumor growth in SCID mice engrafted with ovarian cancer.	[36] [37]
16	Endometrial cancer	HLA-E expression predicts the prognostic benefit of infiltrating NK cells in endometrial cancer	[38]
17	Pancreatic Cancer	Inhibition of NK cell checkpoint CD96 prevents relapse of pancreatic cancer.	[39]
18	Gastric Cancer	NK cells exhibit strong anti-tumor activity against gastric cancer cells.	[40]
19	Thyroid Cancer	NK cells inhibit the growth and metastasis of anaplastic thyroid cancer. NK cells eradicate anaplastic thyroid cancer in ULBP2/5/6-dependent manner and chemoattract CXCR3-positive NK cells.	[41] [42]

Table 2

Approved and investigational NK cell-based cancer immunotherapies.

S.No.	Therapy/Inhibitor/Antibody	Cancer type	Clinical Trial Stage	Clinical trial ID
1	Autologous NK cells	Advanced Kidney Cancer Metastatic melanoma Digestive cancer	Phase I/II	NCT02843607 NCT00631072 UMIN000007527
2	Allogenic NK cells	Metastatic Gastrointestinal Carcinoma High-risk AML (HINKL) Lymphoma and Solid tumors	Phase I/II	NCT02845999 NCT02229266 NCT01212341
3	NK Cell lines (Neukoplast™ (NK-92))	Hematological Malignancies	Phase I	NCT00990717 NCT00900809
4	CAR-NK cells	Relapsed/Refractory CD33+ AML Metastatic Solid Tumors Acute Lymphoblastic Leukemia	Pre-clinical/Phase I/II	NCT02944162 NCT03415100 NCT01974479
5	IL-2	Lymphoma, lung cancer and melanoma	FDA approved	
6	IL-15	Advanced Solid Tumors Acute Myelogenous Leukemia	Phase I	NCT01875601 NCT01572493 NCT01385423
7	IL-15 Superagonist (ALT-803)	Relapsed/Refractory AML	Phase II	NCT03050216
8	Anti-KIR mAB (IPH2101)	Smoldering Multiple Myeloma	Phase I	NCT01248455
9	Anti-NKG2A mAB (IPH2201/Monalizumab)	Metastatic squamous cell carcinoma Refractory Lymphoid Malignancies Hematological Malignancies Gynecological Malignancies	Phase I/II	NCT02643550 NCT02671435 NCT02921685 NCT02459301
10	mAB targeting tumor antigens (Rituximab/ Trastuzumab/Cetuximab)	Non-Hodgkin's Lymphoma Refractory Lymphoid Malignancies Breast and Gastric Cancer Advanced Solid Tumors Recurrent Non-small Cell Lung Cancer	FDA approved/Phase I/II	NCT03019640 NCT01181258 NCT02030561 NCT03319459 NCT02845856
11	Epigenetic drugs (Decitabine with Donor NK cells and Aldesleukin)	Relapsed/Refractory AML	Phase I	NCT02316964