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ORIGINAL RESEARCH

The prognostic role of NK cells and their ligands in squamous cell carcinoma of the head and neck: a systematic review and meta-analysis

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ABSTRACT

Background: Despite the improvement in therapeutic interventions, 5-year survival rates in Head and Neck Squamous Cell Carcinoma (HNSCC) are limited. HNSCC is an immunogenic cancer type for which molecular stratification markers are lacking. Tumor-infiltrating lymphocytes (TILs) have shown a favorable prognostic role in different cancer types. This study focused on the prognostic role of NK cells in HNSCC.
Methods: A systematic search was conducted in Pubmed/Medline and Embase. Articles that correlated the presence of intratumoral NK cells, activating/inhibiting receptors, death receptors, or their ligands with clinicopathologic characteristics or survival were included. A meta-analysis was performed that assessed the association between CD56+ and CD57+ and overall survival (OS), disease-free survival (DFS), and progression-free survival (PFS).
Results: A pooled analysis indicated a favorable prognostic role of CD56+ and CD57+ NK cells for OS (HR 0.19 CI 0.11–0.35). NK cell markers NKp46 and Granzyme B (GrB) also have a favorable prognostic role. NK cell ligand Fas correlated with better survival and better characteristics. NK cell marker Fas-L, NK cell ligands CEACAM1, RCAS1, CD70 and TRAIL-R, and effector molecules of these ligands, FADD and FAP1, correlated to features of worse prognosis.
Conclusion: A favorable prognostic role of NK cells in HNSCC was found in this review. Some studies implied the opposite, indicating the fine balance between pro- and anti-tumor functions of NK cells. Future studies using homogeneous patient cohorts regarding tumor subsite and treatment modality, are necessary to further provide insight into the prognostic role of NK cells.

Introduction

The majority of Head and Neck Cancers (HNC) comprise squamous cell carcinomas arising from the stratified epithelium of the oral cavity (OC), nasopharynx (NP), hypopharynx (HP), oropharynx (OP) and larynx (L).[1] HNC is the sixth most common cancer worldwide with approximately 600,000 new cases per year.[2,3] The mortality due to Head and Neck Squamous Cell Carcinoma (HNSCC) is 380.000 per year, making it the seventh most common cause of cancer-related mortality worldwide.[4] In recent decades, research has focused on identifying prognostic biomarkers in HNSCC to counteract major issues: late diagnosis and locoregional recurrence.

The archetypal patient with HNSCC is of male gender between the ages of 50–70 years, with a history of tobacco and alcohol use, both identified as risk factors.[5] Over the last decades another risk factor has been identified: the human papillomavirus (HPV). The most HPV positive HNSCC occurs in the oropharynx of a younger population.[6,7]

Roughly 60% of HNSCC patients have an advanced stage disease at the time of diagnosis, due to limited awareness and knowledge of alarm symptoms, and the characteristic rapid proliferation of HNSCC.[8] Currently, treatment methods are insufficient; locoregional recurrence occurs in 15-50% of patients,[9] and for all clinical stages combined, five-year survival rates are between 56%-62%.[10,11] This calls for reliable prognostic biomarkers that enable clinicians to stratify patients in risk groups and treating them accordingly, improving personalized medicine. Cancer development is facilitated by the inability of the immune system to recognize and eliminate tumor cells. In HNSCC, an immunogenic cancer type, immune escape mechanisms are key to tumor initiation and progression. Since the host immune system plays an important role in the development of cancer, the scientific necessity arose to further employ the immune system for the treatment of HNSCC. Currently, cetuximab, an EGFR (endothelial growth factor receptor) inhibitor, is used in case a contradiction for chemotherapy exists.[12,13] The antitumor effect of cetuximab is strengthened by binding the FcY receptor on NK cells enabling antibody-dependent cellular cytotoxicity (ADCC).[14]

Immunological markers have shown to be prognostic indicators, even superior to the TNM staging system.[15] In lung, colorectal and breast cancer, studies have shown that tumor-infiltrating lymphocytes have a favorable prognostic role.[16–18]

A meta-analysis conducted by de Ruiter et al. (2017) reports findings in concordance with the latter for HNSCC.[19]

NK cells are an essential component of the innate immune system as an early line of defense against tumor cells and act by killing them. They could, therefore, function as a prognostic biomarker in HNSCC. NK cell subpopulations can be defined based on CD56 expression on the surface of the cells, in which CD56^{bright} cells express a relatively high density of CD56 and CD56^{dim} cells express the relatively low density of CD56. Classically, NK cells are divided in CD56^{bright} CD57^{−} immune regulatory cells and CD56^{dim} CD57^{+} cytotoxic cells.[20] In most studies CD56 is the archetypal phenotypic marker of NK cells.[21] Another marker, CD57, is a marker of differentiated and highly cytotoxic NK cells and is often used in studies, as it has been described as a phenotypically stable NK cell marker.[22]

NK cells are regulated by activating membrane-bound receptors that enable lysis of target cells that fail to express sufficient levels of MHC class 1 (missing self hypothesis) and by inhibitory receptors that protect cells that do express MHC class 1. These inhibitory and activating receptors are killer-cell immunoglobulin like receptors (KIRs). Important activating receptors are NKG2D, that has MICA/B as a ligand, and NpK30, NpK44, and NpK46, that have a broad variety of ligands. Upon activation, NK cells release granules containing perforin and granzymes, and produce cytokines, causing cell death. NK cells also express death receptor ligands (Fas-L, TNF-α, TRAIL), that induce cell death upon binding death receptors (Fas, TRAIL-R (DR4, DR5)) on target cells.[23] These receptors can serve as potential markers for NK cells in HNSCC. In breast and colorectal cancer, reviews report that NK cells are of positive prognostic value.[24,25]

Objectives

This study was conducted to shed light on the prognostic role of NK cells and their ligands in HNSCC by systematically reviewing the literature. The main goal was to include all studies that assessed tumor infiltration with CD56+ and/or CD57+ lymphocytes, NK cell-activating/inhibiting receptors, death receptors or their ligands as prognostic biomarkers in HNSCC.

Results

Study selection

The initial search yielded 3520 references, after the removal of 180 duplicates. After title/abstract screening, reviewers SKB and ERU selected 122 articles, which were subjected to full-text reading. Of the selected 122 articles, 9 were not available, 4 were in Chinese, 1 in German, 1 had missing data and 61 did not comply with our inclusion criteria regarding the determinant, domain and/or methods. Of the 46 articles that were eligible for inclusion, 13 assessed classical NK cell markers.[26–38] The activating markers group existed of three articles,[39–41] the inhibiting markers group of 5[42–46] and the death receptor group of 25.[47–71] Figure 1 provides an overview of study selection.

Critical appraisal

Critical appraisal for risk of bias was applied on the 13 articles of the classical markers group by use of the QUIPS criteria. All studies scored poorly in the study attrition domain, because none of the studies mentioned the patient's loss to follow-up. This domain was therefore not considered when assessing the overall risk of bias. Because assessing survival analysis by hazard ratios is crucial for conducting a meta-analysis, studies that did not mention hazard ratios were excluded. Exclusion was therefore not based on total bias score. The quality assessment is shown in Table 1. The four articles that mentioned hazard ratios[26–29] were selected for meta-analysis after critical appraisal; the forest plot is shown in figure 2.

Classical markers as a predictor for survival

A total of six studies reported on CD56+ cells. The presence of CD56+ NK cells was associated with a better OS (Wagner et al.: HR 0.32, CI 0.10–0.96, p 0.04; Stangl et al.: HR 0.27, CI 0.12–0.60, p 0.001), local PFS (Stangl et al.: HR 0.35, CI 0.17–0.74, p 0.005) and distant metastasis-free survival (DMFS) (Stangl et al.: HR 0.27, CI 0.13–0.55, p 0.004). A total of seven studies reported on CD57+ cells. High numbers of CD57+ NK cells were associated with a better OS (Fang et al.: HR 0.15 CI 0.06–0.36 $p < .001$; Taghavi et al.: HR 0.06 CI 0.01–0.26 $p < .001$).[26–30] The pooled meta-analysis showed an advantage for high CD56+/CD57+ NK cells regarding OS (pooled HR 0.19 CI 0.11–0.35) (Figure 2). Some studies mentioned no correlation between CD56+ NK cell number and OS or recurrence-free survival (RFS) or between CD57+ NK cells and OS (de Carvalho Fraga et al.: OR 1.04,CI 0.44–2.46, p 0.93) or DFS[31–35] One study reported on CD16+ cells and found no correlation between CD16 + cells and DFS.[36]

Classical markers as a predictor for clinicopathologic characteristics

Next, the correlation between classical markers and the known clinicopathologic characteristics that influence prognosis was investigated. One study found that high infiltration of CD57+ cells correlated with T3/T4 tumors and cervical metastasis (de Carvalho Fraga et al.: resp. OR 5.610, CI 1.-516–20.763, p 0.010 and OR 3.401, CI 1.162–9.951, p 0.025). These results indicate that in this study CD57+ cells correlated with features of worse prognosis.[40] Contradictory, many studies mention the correlation of high CD57+ cell number with features of better prognosis; absence of lymph node metastasis, early clinical stages.[37,38] Other studies have noted a trend toward features of better prognosis; fewer cases of nodal metastasis, advanced-stage disease, disease relapses, lower probability of local recurrence (LR) and death.[39,45] One study mentioned a higher number of CD56+ NK cells in a study group without metastatic disease.[46] Another study found no correlation between CD16+ NK cells and tumor location, TNM stage, or recurrence of the disease.[44]
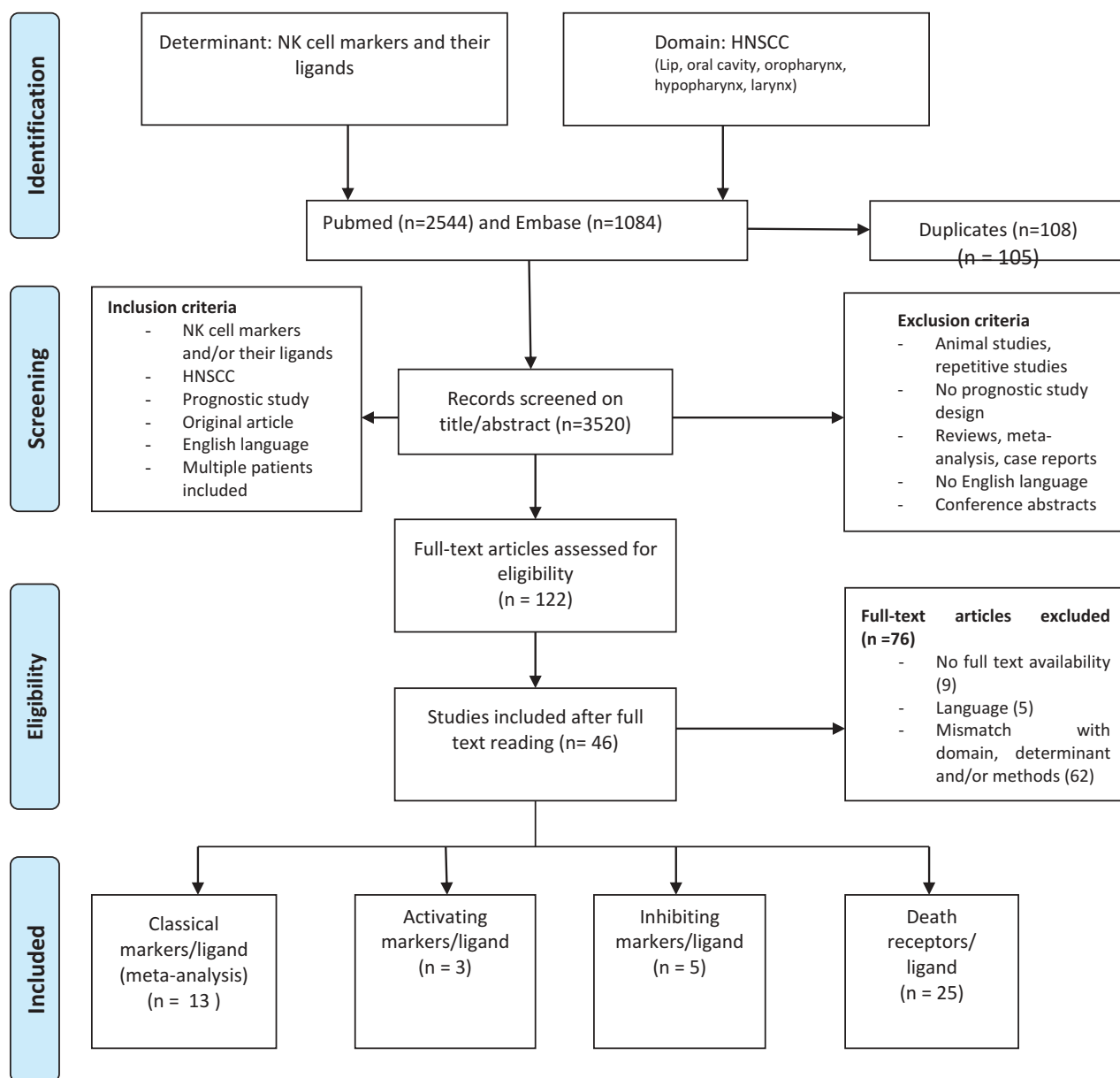
Figure 1. Flow chart of the 3520 articles initially selected, 49 were included after full-text screening. (Moher et al. 2009. prisma flow chart).[98.]

Table 1. Quality assessment of the classical marker studies. Total bias score excluded study attrition.

Study	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis and reporting	Total Bias score
Lazaris 2009	2	2	1	0	0	2	5
Zancope 2010	0	2	1	0	2	1	4
Fraga 2011	0	2	0	2	0	0	2
Sakakura 2014	0	2	0	0	2	2	4
Wagner 2015	0	2	0	0	0	0	0
Stangl 2016	0	2	0	0	0	0	0
Taghavi 2016	1	2	0	0	2	0	3
Fang 2017	1	2	0	0	1	0	2
Kartpathiou 2017	0	2	0	0	0	2	2
Maciel 2017	1	2	0	0	2	0	3
Stasikowska 2017	2	2	2	2	1	0	7
Schoenfeld 2018	0	2	0	0	2	0	2
Santos 2019	0	2	0	2	0	0	2

Supplementary Table 2 mentions the characteristics of the studies that assessed classical markers.

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Fang 2017 (CD57)	-1.8839	0.4413	31.2%	0.15 [0.06, 0.36]
Stangl 2018 (CD56)	-1.3093	0.4137	33.9%	0.27 [0.12, 0.61]
Taghavi 2016 (CD57)	-2.8542	0.7754	13.2%	0.06 [0.01, 0.26]
Wagner 2015 (CD56)	-1.152	0.567	21.8%	0.32 [0.10, 0.96]
Total (95% CI)			100.0%	0.19 [0.11, 0.35]

Heterogeneity: Tau² = 0.10; Chi² = 4.14, df = 3 (P = 0.25); I² = 27%
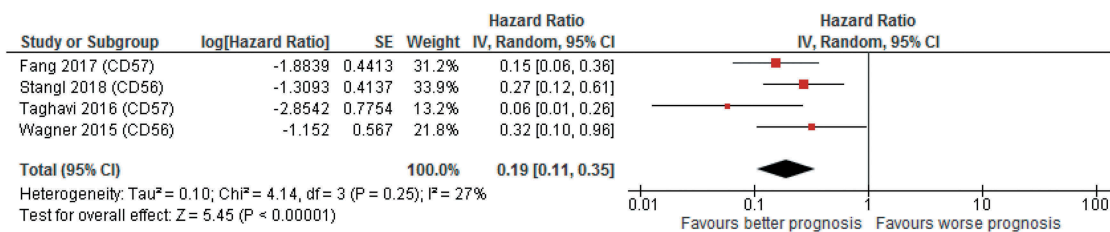Test for overall effect: Z = 5.45 (P < 0.00001)

Figure 2. Forest plot of prognostic value of CD56+/CD57+ NK cells on overall survival; high CD56/CD57 cell count correlated with better overall survival (HR 0.19, CI 0.11–0.35). (RevMan 2014).[99]

Activating markers as predictors for survival and clinicopathologic characteristics

A total of two studies reported on NKp46+ NK cells; one study mentioned that NKp46+ NK cells alone were not associated with survival and the other study reported that NKp46 + NK cells were more abundant in low-grade tumors.[39,40]

One study investigated the prognostic role of tumoral CD70 expression. Tumoral CD70 expression was higher in poorly differentiated carcinomas. There was no correlation with TNM stage. High tumor CD70 expression correlated with a trend toward lower density of TIL's.[41]

Inhibiting markers as predictors for survival and clinicopathologic characteristics

A total of four studies reported on CEACAM1. Three studies mentioned that high CEACAM1 expression correlated with worse survival and features of worse prognosis; high tumor grade, local recurrence, lymph node metastasis, distant metastasis, and high clinical stage.[42–45] One study mentioned contradictory results and found that high CEACAM1 expression correlates with better OS and DFS and features of better prognosis.[43]

RCAS1 expression in tumor cells was investigated in one study, which found that it was associated with high-grade tumors and the presence of lymph node metastasis.[46]

See Table 2 for a summary of outcomes and supplementary Table 3 for study characteristics of the activating and inhibiting markers.

Death receptors as predictors for survival and clinicopathologic characteristics

A total of four studies reported on Fas or Fas-L and four other studies reported on both markers.

Fas expression in tumor cells correlated with negative lymph nodes (de Carvalho-Neto et al.: OR 5.02 CI 1.34–18.75 p 0.017), absence of LR, lower clinical stage, better OS, better disease-specific survival (DSS) (de Carvalho-Neto et al.: HR 0.268 CI 0.083–0.862 p 0.027).[49,50,53] Contradictory, one study mentioned Fas expression associated with a higher clinical stage.[47]

Fas-L expression in the tumor is correlated with higher T stage, N stage, clinical stage, and worse DFS (de Carvalho-Neto et al.: HR 2.58 CI 1.03–6.46 p 0.044).[47,50,52,57] One study mentioned that strong Fas-L expression in lymphoid cells was associated with lymph node metastasis, low DFS, and low DSS (Peterle et al.: resp. OR 5.39 CI 1.30–22.34 p 0.02, HR 2.24, CI 1.08–4.65 p 0.03 and HR 2.49 CI 1.04–5.99 p 0.041).[51] Some studies mention no correlation of Fas or Fas-L expression with OS, DFS, DSS, or clinicopathologic parameters.[48,54–56,58]

A total of eight studies investigated the prognostic role of FADD. High FADD expression correlated with the presence of lymph node metastasis, higher tumor grade, worse DSS, worse DFS (Chien et al.: HR 1.684 CI 1.209–2345 p 0.002; Fan et al.: HR 1.003 p 0.0006), shorter DMFS (Pattje et al.: HR 2.3 CI 0.96–5.7 p 0.062) and worse OS (Chien et al.: HR 1.387 CI 1.035–1.859 p 0.029; Fan et al.: HR 1.004 p 0.000; Rasamny et al.: OR 1.72 CI 1.14–2.60 p 0.01).[59–63,66] One study found that high FADD expression showed a trend toward better local-regional control (LRC) (Schrijvers et al.: HR 3.66 CI 0.85–15.66 p 0.081), but reported no correlation with clinicopathologic parameters or OS.[64] Another study found no correlation between FADD expression and clinicopathologic characteristics or survival.[65]

Three studies reported on the prognostic role of TRAIL. The first one mentioned that high TRAIL expression correlated to worse OS.[67] The second study mentioned a positive correlation between tumor stage and TRAIL-R DR5 staining, although this correlation is on the edge of significance. TRAIL and other TRAIL-R were not associated with clinicopathologic

Table 2. The outcomes of activating and inhibiting marker studies are summarized based on high expression of the markers in question. Oral Cavity (OC), Oropharynx (OP), Hypopharynx (HP), Larynx (L), Lip (Lip), Tongue (T). Overall survival (OS), Disease-free survival (DFS), Progression-free survival (PFS), Distant metastasis-free survival (DMFS), Disease-specific survival (DSS), Local recurrence (LR), Local-regional control (LRC).

Marker (High expression)	Study	Sample Size	Subsite	Outcome
NKP46	Ikeda 2017	41	OC	No correlation with survival or characteristics
	Ladanyi 2018	50	OP, HP, L, OC	Low grade tumors
CD70	De Meulenaere 2016	95	OP, HP, L, OC	Poorly differentiated carcinomas, Lower density TIL's
CEACAM1	Shinozuka 2009	78	OC	High expression in T1 and T2 groups, Early stage disease, Better OS and DFS
	Wang 2013	74	T	High clinical stage, Lymph node metastasis
	Lucarini 2018	54	L	High tumor grade, LR, Lymph node- and distant metastasis
	Simonetti 2018	40	OC	Worse OS, Worse DSS, High tumor grade
RCAS1	Dutsch Wicherek 2009	102	OP, HP, L	High tumor grade, Lymph node metastasis

characteristics and survival.[68] The third study mentioned that in their specimens, TRAIL staining was scarce. However, they investigated TRAIL-R and found that TRAIL-R DR5 positively correlated with tumor size.[69]

One study investigated the prognostic role of Granzyme B (GrB) and found that there was a higher peritumoral density of Granzyme B in the non-metastatic study group in comparison with the metastatic study group. High peritumoral GrB correlated with better survival. GrB was higher in tumors with lower T stages, although not statistically significant.[70]

Another study investigated the prognostic role of FAP-1 and found that FAP-1 negative cases showed a better OS than FAP-1 positive cases (Nariai et al.: HR 0.317 CI 0.108–0.931 p 0.0366).[71]

See Table 3 for outcomes and supplementary table 4 for study characteristics.

Discussion

To date, specific-validated prognostic biomarkers are an unmet need to improve HNSCC treatment. NK cells are of innate origin, but also carry out the functions of the adaptive immune system. The immune system is a key component in tumor growth and control, and as such NK cells act as good candidates for possible biomarkers, due to their ability to lyse tumor cells lacking sufficient levels of MHC class 1. In this review, we investigated the prognostic role of NK cell markers and their ligands in HNSCC. The studies included in this review largely stem from the pre-immunotherapy era and should be interpreted accordingly.

Firstly, the prognostic role of the classical NK cell markers CD56+, CD57+, and CD16+ was investigated. Our meta-analysis describes that high CD56+ or CD57+ NK cell count correlated to better OS. This result can be explained by the anti-tumor response of NK cells, as they are able to kill tumor cells without prior sensitization.[72] $CD56^{dim} CD57^{+}$ NK cell subtypes are important in anti-tumor immunity and also express CD16.[13,73] CD16 is the activating FcY receptor for IgG, that enables antibody-dependent cell-mediated cytotoxicity (ADCC) when crosslinked to CD57.[74] One study investigated the prognostic role of CD16+ NK cells, and found no correlation with survival or clinicopathologic characteristics.

Important activating receptors are NKG2D, that has MICA/B as a ligand, and NKp30, NKp44, NKp46, that have a broad variety of ligands.[23] Tumor cells express NK activating receptor ligands de novo, making them susceptible to NK cell killing.[75] In this review we found that NKp46+ NK cells were more abundant in low-grade tumors, indicating a positive role of NK cells in tumor control.

One study, however, found contradictory results; high CD57+ NK cell number correlated to features of worse prognosis. This could be explained by the phenomenon of tumor escape mechanisms; escaping immune recognition by selective loss of NK activating receptor ligands, aberrant HLA types or induction of anergy in NK cells.[72,76] Absence of MHC protects against T cell activation, but enables NK cell-mediated killing of tumor cells. In this regard, a great portion of HNSCC have resorted to expressing abnormal MHC types, that shield against both T cell and NK cell activation.[77] Korrer et al. (2017) mention the upregulation of NK cell inhibitory ligand NKG2A on tumor-associated NK cells in HNSCC as an immune escape mechanism.[78]

Table 3. **The outcomes of death receptor studies are summarized based on high expression of the markers in question**. Oral Cavity (OC), Oropharynx (OP), Hypopharynx (HP), Larynx (L), Lip (Lip), Tongue (T). Overall survival (OS), Disease-free survival (DFS), Progression-free survival (PFS), Distant metastasis-free survival (DMFS), Disease-specific survival (DSS), Local recurrence (LR), Local-regional control (LRC).

Marker (High expression)	Study	Sample Size	Subsite	Outcome
Fas and Fas-L	Fuijeda 2000	58	OC, OP	No correlation with T stage, N stage, clinical stage, LR, OS, DFS
	Guler 2005	26	OC, OP	High clinical stage
	Tsuzuki 2005	58	OP	No correlation with OS
	De Carvalho-Neto 2013	60	OC	Fas: Negative lymph nodes, better DSS Fas- L: Worse DFS
Fas	Bayazit 2000	30	L	No correlation with T stage, N stage, Tumor grade, Tumor site
	Muraki 2000	46	OC	Better OS, Absence of LR, lower clinical stage.
	Jackel 2001	88	L	No correlation with OS, DSS or clinicopathologic parameters
	Asensio 2007	45	L	Better survival
Fas-L	Reichert 2002	28	OC	No correlation with T -or N stage
	Das 2011	41	OC	High clinical stage, higher T and N stage (not statistically significant)
	Fang 2013	38	OC	Lymph node metastasis
	Peterle 2015	64	OC	Fas-L expression in lymphoid cells correlated with lymph node metastasis, low DFS and low DSS
FADD	Prapinjumrune 2009	60	T	Cervical lymph node metastasis, Worse DSS
	Schrijvers 2011	92	L	Trend toward better LRC, No correlation with OS or clinicopathologic parameters
	Rasamny 2012	222	OP, OC, HP, NP	Worse OS, DSS and DFS
	Pattje 2012	177	OP, HP, L, OC	Lymph node metastasis, Shorter DMFS
	Fan 2013	200	OP, OC, L	Worse OS and DFS
	Chien 2016	339	OP, HP, OC	Lymph node metastasis, Younger age, Higher tumor grade, Worse DFS and OS
	Wachters 2017	60	L	No correlation with survival or clinicopathologic parameters
	Noorlag 2017	158	OC	Lymph node metastasis
TRAIL	Vigneswaran 2007	45	OC	High TRAIL-R DR5: higher T stage
	Carcini 2010	134	OC	Worse OS
	Erkul 2016	20	L	High TRAIL-R DR5: higher clinical stage
GrB	Costa 2010	55	OC	Better survival, Lower T stages
FAP-1	Nariai 2011	50	OC	Worse OS

The CD27-CD70 pathway enhances the cytotoxic effect of effector cells via the perforin dependent mechanism.[79] CD70 expression in the tumor should, therefore, promote NK cell killing. In this review CD70 expression correlated with features of worse prognosis, indicating the opposite. This could be explained by another hypothesis; that CD27-CD70 pathway is a mechanism of immune escape for tumor cells by inducing apoptosis in effector cells.[80]

The inhibiting ligands CEACAM1 and RCAS1 correlated with features of worse survival and prognosis. CEACAM1 is expressed on tumor cells and NK cells, and an interaction inhibits tumor cell lysis by NK cells, as it is an inhibiting receptor/ligand.[81] It also inhibits the activating NKG2D signaling, thus a high expression of CEACAM1 inhibits NK cell antitumor function. RCAS1 plays a role in immune evasion of tumor cells by inducing apoptosis in NK cells, thus correlating with features of worse prognosis in HNSCC. This is in concordance with other studies, that mention a correlation with features of worse prognosis in pancreatic ductal cell carcinoma and shorter survival in esophageal squamous cell carcinoma.[82,83]

Lastly, NK cells also express death receptor ligands (Fas-L, TNFa, TRAIL), which mediate apoptosis by binding to death receptors (Fas, TRAIL-R (DR4, DR5)) on target cells.[16] These death receptors are also expressed on T cells; the results mentioned in the following paragraph need to be interpreted as a synergistic result of T cells and NK cell function. Regarding the direct relationship between NK cells and death receptors/ligands, expression of the latter on T cells is a confounding factor.[84]

NK and T cell ligand Fas expressed on tumor cells, correlated to better survival and better clinicopathologic characteristics in HNSCC and this is in concordance with some other cancer types.[85,86] However, one study found a correlation with features of worse prognosis. Tumor cells use escape mechanisms to become resistant to Fas mediated apoptosis, for instance by downregulation of FADD, causing loss of apoptotic signaling.[87] The classic function of FADD is inducing apoptosis in cells via stimulation of different death receptors. But the FADD oriented studies included in this review found that high expression of FADD correlated to a worse prognosis. Latter finding was previously reported by researchers in data from The Cancer Genome Atlas (TCGA).[88]

It indicates a more complex and unresolved role of FADD, as it can also, for example, activate the NFkB pathway and induce cell survival.[63] Fas-L on NK and T cells mediates apoptosis when coupled to Fas on tumor cells, but one article mentioned Fas-L on NK and T cells correlated to worse survival and features of worse prognosis. Fas-L expression in tumor cells also correlated to worse survival, the hypothetical explanation for this effect is 'tumor counterattack.'[89] By expressing Fas-L, the tumor induces apoptosis in lymphoid cells. TRAIL-R expression in the tumor correlated to worse prognosis, this might be explained by the use of decoy receptors to evade apoptosis induction.[69] FAP-1 is a regulating molecule blocking Fas-mediated apoptosis, thus explaining the result that low FAP 1 correlated to a better prognosis.[90]

For some markers/ligands (GrB, NKp46, RCAS1, FAP1) the findings confirm the proposed pathophysiological mechanism as described in the literature. The other makers/ligands (CD56, CD57, CD70, CEACAM1, Fas, Fas-L, FADD, TRAIL) show bivalent results indicating more complex mechanisms underlying their function and prognostic role.

The findings of this review are bivalent, indicating a fine line between the anti- and pro-tumor function of NK cells. Previous studies identified this as an opportunity to use NK cells as a therapeutic strategy, tackling the unmet need for immunotherapy development. NK cells are good candidates; they recognize tumor cells by lack of sufficient MHC type 1 levels, subsequently activating NK cell stimulatory receptors, mediating NK cell killing. This distinctive method of NK cell killing avoids the conundrum T cell-based therapies face; dependability on presentation of specific antigens (e.g. tumor-specific antigens, tumor-associated antigens or differentiation antigens) on MHC in order to activate T cell killing. In HNSCC and various other tumor types, loss of tumoral MHC expression has been described, impairing T cell-mediated cytotoxicity.[91-93]

NK cells are known for their robust anti-tumor immunity, which is often impaired in cancers. NK cell immunotherapy, therefore, focuses on restoring the antitumor response, for example, by retargeting strategies with therapeutic antibodies or chimeric antigen receptors (CARs), by blocking KIRs with therapeutic antibodies (Lirilumab), by NK cell checkpoint inhibitors (Monalizumab: NKG2A inhibitor) or by NK cell-based adoptive immunotherapy.[87,94,95] Furthermore, TRAIL-R agonists propose as promising modulators in antitumor immunity.[96] Future research is needed to provide insight in the clinical translation of the NK cell immunotherapies.

The first general limitation of this study is heterogeneity in tumor subsite, among and within studies included in this review. In HNSCC, risk factors and pathophysiological mechanisms differ between different tumor subsites in the head and neck region, which could impact the prognostic role of NK cells among these subsites. Secondly, the studies showed heterogeneity in treatment modalities with different mechanisms of action, so the prognostic role of tumor-associated immune cells could also differ. The included studies had relatively small patients size, making it impossible to stratify patients according to treatment modality or tumor subsite. For some of the markers investigated in this review, pathophysiological mechanisms have not been clearly described yet, making interpretation of the prognostic role difficult. A limitation for the meta-analysis conducted for the classical markers was the reporting of statistical information, as only four studies provided hazard ratios. A trend in the selective reporting of p-values was observed, which might indicate reporting bias and eventually could lead to publication bias. Lastly, different NK cell subsets are known, but the majority of studies in the classical markers group only immunohistochemically stained CD56 or CD57. It would be beneficial to further characterize NK cell subsets, to provide a better understanding of the tumor microenvironment.

In conclusion, this systematic review and meta-analysis found an overall favorable prognostic role of NK cells, characterized by a different membrane and intracellular markers, in HNSCC. Some studies implied the opposite, indicating the fine balance between pro-and anti-tumor functions of NK

cells. Future studies should use homogeneous cohort regarding tumor subsite and treatment modality, and use a standardized method of reporting, in order to further provide insight in the complex balance between NK cell functions.

Methods

Search strategy

The systematic search was conducted on the 7th of March 2019 in two databases: Pubmed/Medline and EMBASE. As domain, 'head and neck squamous cell carcinoma' and synonyms of this term were used as determinant, a variety of NK cell markers and their ligands were used (Supplementary Table 1).

In- and exclusion criteria

Studies were screened based upon title and abstract. The final selection was made by full-text reading of the selected articles. Studies were eligible for inclusion if they assessed the prognostic value of NK cell markers or their ligands in patients with HNSCC (lip, OC, OP, HP, LP) by a time-to-event analysis, described as overall survival (OS), disease-free survival (DFS), progression-free survival (PFS) or by correlation to clinicopathologic characteristics. Nasopharyngeal carcinomas were excluded due to the contributing role of epstein barr virus (EBV) in the tumorigenesis. Only original articles published in English were included. Animal studies, case reports, reviews, meta-analyses or repetitive studies were excluded. Conference abstracts and studies that used techniques other than immunohistochemistry were excluded. The title/abstract screening and full-text reading were conducted by two independent researchers (ERU and SKB) and any disagreements were resolved by discussion.

Data extraction

From the references selected by full-text screening the following data were extracted: author's last name, year of publication, biomarkers, sample size, tumor subsite, tumor stage, HPV status, treatment modalities, median follow-up time, methods, scoring methods for the immunohistochemically stained samples, confounders, hazard ratios, confidence intervals, and p-values. These data were entered in a standardized form creating a synopsis of all relevant articles.

Outcome

NK cell markers and their ligands were organized in four groups: 'classical,' 'activating,' 'inhibiting,' and 'death receptors.' The classical group comprised CD56, CD57, CD16; the activating group NKp46, CD70; the inhibiting group CEACAM1, RCAS1; the death receptor group Fas, Fas-L, FADD, TRAIL, TRAL-R, FAP1. Our study focused on the classical markers as the primary outcome, strengthened by a meta-analysis. The other makers were evaluated in a narrative manner.

Critical appraisal

To assess the risk of bias of the prognostic studies included in the meta-analysis, the Quality in Prognosis studies (QUIPS) tool was used as described by Hayden et al. (2006).[97] The QUIPS tool comprises six items: study participation, attrition, prognostic factor measurement, outcome measurement, confounding and statistical analysis and reporting. For each of these items, the risk of bias was scored as low, moderate, or high. Taking into consideration our research question, we valued statistical analysis and reporting as most important when assessing the overall risk of bias, because the meta-analysis depended on the reporting of hazard ratios (HR).

Statistical analysis

In the meta-analysis, we used HR's that were defined by high NK cells vs low NK cells. If the study mentioned HR's as low NK cells vs high NK cells, we used the reciprocal. The meta-analysis was performed in review manager 5.3 by use of a random effect analysis.

Conflicts Of Interest

The authors report no conflict of interest.

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