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ORIGINAL REPORT

Low Levels of Circulating Invariant Natural Killer T Cells Predict Poor Clinical Outcome in Patients With Head and Neck Squamous Cell Carcinoma

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ABSTRACT

Purpose
Evading antitumor immune responses is an important aspect of the pathogenesis of head and neck squamous cell carcinoma (HNSCC). Invariant CD1d-restricted natural killer T (iNKT) cells play an allegedly pivotal role in such responses via transactivation of immune effector cells. It has been reported that iNKT cells are reduced in peripheral blood of cancer patients compared with healthy controls. Here, we investigated whether the extent of this deficiency affected disease outcome in HNSCC patients.

Patients and Methods
In a prospective study, circulating iNKT cell numbers were evaluated in 47 patients before radiotherapy. Patients were stratified in three groups based on iNKT cell levels, and clinical data were obtained during a median follow-up period of 31 months.

Results
A small, compared with an intermediate or large, circulating iNKT cell fraction was significantly associated with decreased 3-year overall survival rate (39% *v* 75% and 92%, respectively), disease-specific survival rate (43% *v* 87% and 92%, respectively), and locoregional control rate (31% *v* 74% and 92%, respectively) in HNSCC patients. Cox regression revealed that the iNKT cell level, as well as clinical T stage, was an independent prognostic parameter even after correction for the confounding effect of age.

Conclusion
A severe circulating iNKT cell deficiency was related to poor clinical outcome in HNSCC patients, suggesting their critical contribution to antitumor immune responses. Furthermore, screening for iNKT cell levels may be useful for determining which patients can benefit from immunotherapeutic adjuvant therapies aimed at reconstitution of the circulating iNKT cell pool.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) represents the major histologic type among tumors of the upper aerodigestive tract, with an incidence of 500,000 new cases worldwide. Patients with early-stage disease can be cured with surgery and/or radiotherapy, and relapses are uncommon. Unfortunately, up to 75% of HNSCC patients present with locally advanced disease and require combined-modality treatment, usually consisting of surgery and radiotherapy[1,2] or chemoradiotherapy.[3] Still, the 5-year survival rate remains approximately 30% to 40%, and 60% of patients will experience a local or distant recurrence. Several therapeutic strategies may be used depending on the time and type of relapse, previous treatments, and patient's condition.

Although the initiation of HNSCC is clearly linked to environmental carcinogens (eg, tobacco and alcohol), its development can be, at least in part, attributed to the failure of the immune system to control and eradicate the cancer cells.[4,5] HNSCC are usually well infiltrated with mononuclear leukocytes, but these are generally functionally compromised. In addition to advanced conventional treatments, various immunotherapeutic approaches, including dendritic cell (DC) –based vaccines and cytokine treatments aimed at the restoration of tumor-specific responses, are currently under investigation.[6,7]

Invariant CD1d-restricted natural killer T (iNKT) cells are T lymphocytes characterized by an invariant T-cell antigen receptor-α-chain rearrangement (Vα24.Jα18 paired with Vβ11) that coexpress natural killer (NK) cell markers.[8]

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Activation of iNKT cells via antigenic recognition of the glycolipid α-galactosylceramide (αGalCer) in the context of the nonpolymorphic CD1d molecule results in a cytokine storm in which iNKT cells rapidly produce large amounts of cytokines, including interferon (IFN) -γ, granulocyte-macrophage colony-stimulating factor, interleukin-4, and interleukin-13.[9,10]

Findings from preclinical models are in support of a role for iNKT cells in promoting antitumor immunity. Treating mice with αGalCer elicited antitumor responses via the activation of host immune effector cells, resulting in the inhibition of experimental metastases (eg, lung and liver).[11-14] Furthermore, adoptive transfer of iNKT cells from transgenic mice or of αGalCer-loaded DCs inhibits development of tumors in various models, depending on activation of the innate and/or the adaptive arm of the immune system.[15-19] Also, experiments using iNKT-deficient mice revealed a necessity for IFN-γ–producing iNKT cells for efficient NK cell–dependent natural tumor immune surveillance of methylcholantrene-induced sarcomas. Adoptive transfer of iNKT cells from wild-type mice into iNKT-deficient recipients restored protection against these tumors.[20,21]

Human iNKT cells have been demonstrated to enhance T- or NK-cell function in vitro. Because they show only limited direct cytotoxicity against tumor targets, their natural role in antitumor immunity most likely lies in directing downstream effector cells.[22-26] We and others have demonstrated an overall numeric iNKT cell deficiency in peripheral blood of cancer patients compared with healthy adults.[27-29] This was unlikely the consequence of tumor growth since tumor type, tumor load, or disease stage did not correlate with circulating iNKT cell numbers. The size of the circulating iNKT cell pool was found to vary greatly between individuals, with some cancer patients having iNKT cell levels resembling those observed in age-matched healthy controls.[29] Here, we investigated whether the extent of the iNKT cell deficiency affected disease outcome in HNSCC patients treated with radiotherapy.

Table 1. Clinical Characteristics After Stratification Based on the Level of iNKT Cells/10^6 T Cells

Characteristic	iNKT/10^6 T Cells < 48 (n = 12)	48-242 (n = 23)	> 242 (n = 12)	P
Age, years				.432*
Mean	67	66	62	
Range	52-83	45-85	45-83	
Sex, No. of patients				.696†
Male	8	14	6	
Female	4	9	6	
Tumor grade, No. of patients				.841
1-2	7	13	8	
3-4	5	10	4	
Tumor status, No. of patients				.488
T1-2	9	20	11	
T3-4	3	3	1	
Nodal status, No. of patients				.339
N0	10	20	8	
N+	2	3	4	

Abbreviation: iNKT, invariant CD1d-restricted natural killer T.
*P value obtained using analysis of variance.
†P value obtained using χ^2 test.

PATIENTS AND METHODS

Patient Characteristics

The population of this prospective study consisted of a consecutive series of 47 patients who underwent primary or postoperative radiotherapy for HNSCC in the period from August 1999 to August 2002 and who were observed for a median period of 31 months (range, 5 to 55 months). All patients included in the study provided informed consent, and the institutional medical ethical committee approved the study. Five of 47 patients had a follow-up of less than 24 months while still being at risk of disease progression (ie, no evidence of disease at end of follow-up). Primary tumor sites were the oral cavity (11%), oropharynx (19%), hypopharynx (40%), larynx (13%), or other locations (17%). Pretreatment evaluation included a medical history, examination under general anesthesia with panendoscopy, and a chest radiograph in all patients. Tumor and node classification were assigned according to the staging system of the International Union Against Cancer (1997). The pretreatment characteristics are listed in Table 1. All patients were heavy smokers and drinkers.

Patients with distant metastases before radiotherapy were excluded from the analysis. Additional exclusion criteria were thyroid gland abnormalities, HIV infection, and (neo) adjuvant or concomitant chemotherapy. Patients with a history of another malignancy or prior radiation therapy were not included. Blood samples were collected 1 day before the start of radiotherapy.

Antibodies and Reagents

The following reagents were used: fluorescein isothiocyanate (FITC)-labeled antihuman CD45, FITC-labeled antihuman Vα24 (clone C15), phycoerythrin (PE) -labeled antihuman Vβ11 (clone C21; Immunotech, Marseille, France), PE-labeled antihuman CD16 (clone B7.3.1), PE-labeled antihuman CD56 (clone MY31), RPE-Cy5–labeled antihuman CD3 (clone SK7), FITC-labeled IgG1 (clone X40), PE-labeled IgG2a (clone X39), erythrocyte lysing solution (Becton Dickinson Biosciences, San Jose, CA), RPE-Cy5–labeled IgG1 (clone DAK-GO1; DAKO, Glostrup, Denmark), and FlowCount fluorospheres (Beckman-Coulter, Miami, FL).

Flow Cytometric Detection of Lymphocyte Subsets

Phenotyping of peripheral-blood lymphocytes was performed in a whole-blood analysis. Lymphocyte numbers were defined as $CD45^{high}/CD14^-$ and calculated using WBC counts and Simulset software (Becton Dickinson Biosciences). NK cells were defined as $CD16/56^+CD3^-$, and T cells were defined as $CD3^+$ lymphocytes. iNKT cells were defined by coexpression of CD3, Vα24, and Vβ11 because this combination has been demonstrated to be highly specific for αGalCer-reactive iNKT cells.[30] Flow cytometric analysis was performed on a FACSCalibur using CELLQuest software (Becton Dickinson Biosciences).

Patient Treatment Regimen

Radiotherapy was delivered using megavoltage equipment (6- to 15-MV linear accelerator). In case of primary radiotherapy, an accelerated schedule with concomitant boost technique was used. These patients were generally treated with 6 fractions a week, with a second fraction on Friday afternoon with a minimum interval of 6 hours, to a total dose of 70 Gy in 6 weeks. In case of postoperative radiotherapy, conventional fractionation schedules were used. The initial target volume, including also the elective (nodal) areas, was irradiated with a fraction dose of 2 Gy (5 times a week) to a total dose of 46 Gy. In case of negative surgical margins, the primary site was boosted using a dose per fraction of 2.5 Gy (5 times a week) to a median total dose of 56 Gy. In case of positive surgical margins, the median total dose to the primary site was 63.5 Gy. In case of lymph node metastases with extranodal spread, the median total dose to these regions was 63.5 Gy. All patients started radiotherapy on Mondays, and the overall treatment time was limited to 6 weeks.

Methodology and Statistical Analyses

Statistical analysis of the results was based on the Reporting Recommendations for Tumor Marker Prognostic Studies.[31] Data were analyzed by J.W.M and J.A.L.

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Overall survival (OS), disease-specific survival (DSS), locoregional control (LRC), and distant metastasis development (DM) were measured from the first day of radiotherapy until the time of first failure or the most recent follow-up if no relapse was detected. In the univariate analysis, OS, DSS, LRC, and DM were estimated using the Kaplan-Meier method. To test the statistical significance of different survival distributions, the log-rank test was used. Because of the relatively small cohort size, variables that were not significantly associated with survival in the univariate analysis or with less than 10 occurring events were excluded as covariates next to the iNKT cell level in further multivariate analysis. For multivariate analyses, the Cox proportional hazards model using the conditional forward stepwise procedure was applied. Other analyses performed were one-way analysis of variance, χ^2 test, and Mann-Whitney *U* test. $P < .05$ were considered significant.

RESULTS

Flow Cytometric Analysis of Lymphocyte Subsets

The distribution of peripheral-blood T-, NK-, and iNKT-cell levels within the patient population was determined by flow cytometry before radiotherapy (n = 47; Fig 1 and Table 2). All lymphocytes were significantly reduced in HNSCC patients compared with levels from age-matched healthy historical controls (n = 33). The median levels in HNSCC patients compared with age-matched controls were as follows: 1,079 T cells/μL (range, 208 to 2,920 T cells/μL) and 1,423 T cells/μL (range, 830 to 3,540 T cells/μL), respectively; 204 NK cells/μL (range, 31 to 791 NK cells/μL) and 283 NK cells/μL (range, 75 to 899 NK cells/μL), respectively; and 103 iNKT cells/mL (range, undetectable to 3,043 iNKT cells/mL) and 373 iNKT cells/mL (range, 12 to 8,137 iNKT cells/mL), respectively ($P = .0003$, .0038, and .0092, respectively; Mann-Whitney *U*). Notably, although the overall reduction of iNKT cells per million T cells in HNSCC patients compared with healthy controls did not reach statistical significance (median, 92 iNKT/10^6 T cells; range, undetectable to 12,873 iNKT/10^6 T cells in HNSCC patients; and median, 213 iNKT/10^6 T cells; range, 7 to 7,476 iNKT/10^6 T cells in controls; $P = .1075$, Mann-Whitney *U*), five of 12 patients below the 25th percentile cutoff had undetectable iNKT cells in the peripheral-blood compartment (data not shown). In contrast, all age-matched healthy individuals had detectable iNKT cells. Because T, NK, and iNKT cell numbers were not influenced by prior surgery in this cohort (data not shown), both patient groups were

Table 2. Lymphocyte Subsets in HNSCC Patients Compared With Healthy Controls

Subset	HNSCC Patients (n = 47)	Age-Matched Controls (n = 33)	*P**
Sex, No. of patients			
Male	28	19	
Female	19	14	
Age, years			.7694
Mean	65	65	
Range	45-85	44-83	
T cells/mL			.0003
Median	1,079	1,423	
Range†	544-1,415	1,115-1,952	
NK cells/mL			.0038
Median	204	283	
Range	142-264	229-392	
iNKT cells/mL			.0092
Median	103	373	
Range	40-269	52-1,805	
iNKT/10^6 T cells			.1075
Median	92	213	
Range	48-242	43-943	

NOTE. Circulating T, NK, and iNKT cell levels in all patients were determined by flow cytometry and compared with age-matched healthy individuals (historical controls).
Abbreviations: HNSCC, head and neck squamous cell carcinoma; NK, natural killer; iNKT, invariant CD1d-restricted natural killer T.
**P* values were obtained using the Mann-Whitney *U* test.
†Twenty-fifth to 75th percentile.

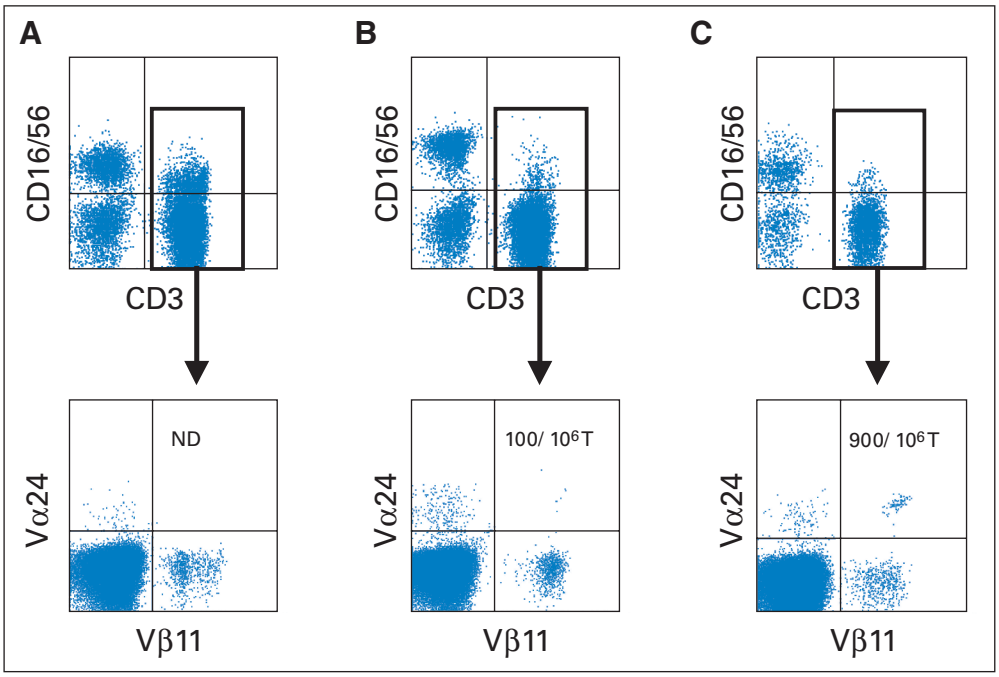

Fig 1. Flow cytometry plots of invariant CD1d-restricted natural killer T (iNKT) cells in three representative patients. The frequency of iNKT cells was identified by coexpression of the T-cell antigen receptor (TCR) -Vα24 chain and TCR-Vβ11 chain (lower panels) after gating on CD3$^+$ T cells (upper panels). Plots from individuals representative of the three patient categories are shown: (A) iNKT Low, (B) iNKT Intermediate, and (C) iNKT High (< 48, 48 to 242, and > 242 iNKT/10^6 T cells, respectively). ND, not detectable.

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grouped for further analysis. For survival analyses, patients were stratified in the following three groups for each immune parameter to be investigated: patients with low (< 25th percentile), intermediate (25th to 75th percentile), or high (> 75th percentile) numbers of iNKT, T, or NK cells.

Univariate Analyses: The Size of the iNKT but Not T or NK Cell Population Is Related to Disease Outcome

Pretreatment clinical characteristics of patients with low, intermediate, and high iNKT cell levels did not significantly differ at the start of the study and are listed in Table 1. Univariate analysis (Fig 2 and Tables 3 and 4) revealed that patients with a small circulating iNKT cell fraction before radiotherapy had a significantly decreased (disease-specific) survival period compared with patients with an intermediate or large iNKT fraction (3 year OS = 39%, 75%, and 92%, respectively; $P = .0140$, log-rank test; 3-year DSS = 43%, 87%, and 92%, respectively; $P = .0027$, log-rank test); similar results were found for LCR rate and the risk of DM (3-year LRC = 31%, 74%, and 92%, respectively; $P = .0050$, log-rank test; 3-year DM-free rate = 54%, 100%, and 92%, respectively; $P = .0102$, log-rank test). Results were comparable when patients with a small fraction of iNKT cells were

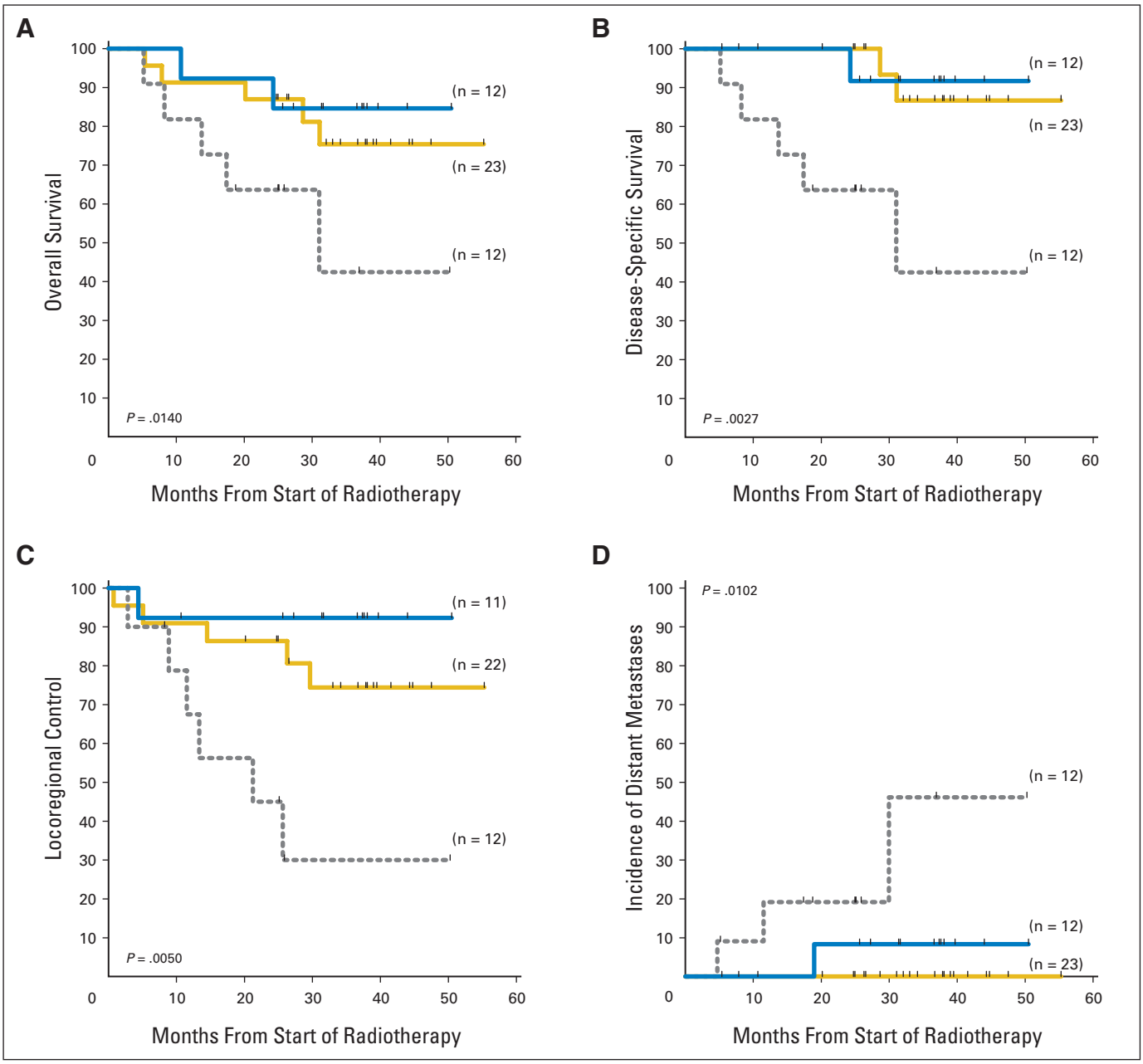

Fig 2. Kaplan-Meier analyses of (A) overall survival (OS), (B) disease-specific survival (DSS), (C) locoregional control (LRC), and (D) distant metastases (DM) in patients categorized according to the amount of iNKT/10^6 T cells. The patients were stratified according to the level of invariant CD1d-restricted natural killer T (iNKT) cells/10^6 T cells. Dashed line, < 48 iNKT/10^6 T cells; yellow solid line, 48 to 242 iNKT/10^6 T cells; blue solid line, > 242 iNKT/10^6 T cells. Plots indicate 3-year survival rates low, intermediate, and high iNKT/10^6 T cells: OS (39%, 75%, and 92%, respectively), DSS (43%, 87%, and 92%, respectively), LRC (31%, 74%, and 92%, respectively), and DM (54%, 100%, and 92%, respectively). *P* values for differences in survival distribution were obtained using log-rank statistics.

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Table 3. Log-Rank Statistics

Clinical Parameter	OS		DSS		LRC		DM	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Sex	1.91	.1699	0.46	.4996	0.09	.7673	2.11	.1463
Primary *v* postoperative RTH	1.16	.2820	1.43	.2313	0.24	.6420	0.05	.8282
T1-2 *v* T3-4	11.3	.0008*	6.31	.0120*	0.32	.5706	0.77	.3791
N0 *v* N+	0.31	.5788	1.82	.1767	0.22	.6370	2.37	.1233
Grade 1-2 *v* Grade 3-4	0.03	.8694	0.02	.8985	0.76	.3822	0.34	.5607
iNKT cells/10^6 T cells	8.53	.0140*	11.82	.0027*	10.60	.0050*	9.17	.0102*
iNKT cells/mL	18.89	.0001*	12.14	.0023*	6.45	.0398*	16.13	.0003*
T cells/mL	1.49	.4746	0.78	.6754	0.50	.7786	3.50	.1734
NK cells/mL	2.13	.3447	2.41	.2996	0.21	.9201	1.01	.6027

NOTE. Patients were stratified prior to the start of radiotherapy based on sex, treatment, disease stage, tumor grade, or iNKT, T, or NK cell levels (in case of the latter: patients with iNKT, T, or NK cell levels below the 25th percentile, between the 25th and 75th percentiles, or above the 75th percentile of the total population). The relative differences in survival distribution (χ^2), determined by log-rank statistics, are shown. Grade 1 to 2: well to moderately differentiated tumor; grade 3 to 4: poorly to very poorly differentiated tumor; T1-4: the extent of the primary tumor according to TNM classification (International Union Against Cancer 1997); N0: absence of lymph node metastases; N+: lymph node metastases detected.

Abbreviations: OS, overall survival; DSS, disease-specific survival; LRC, locoregional control; DM, distant metastasis; RTH, radiotherapy; NK, natural killer; iNKT, invariant CD1d-restricted natural killer T.

*Statistically significant.

compared with patients with an intermediate iNKT cell fraction or high iNKT cell fraction separately (OS, DSS, LRC, and DM for iNKT/10^6 T cells $<$ 48 *v* 48 to 242: P = .0337, .0035, .0103, and .0042, respectively; OS, DSS, LRC, and DM for iNKT/10^6 T cells $<$ 48 *v* $>$ 242: P = .0138, .0271, .0080, and .1177, respectively). The absolute number of iNKT cells/mL blood was also predictive of OS, DSS, LRC, and DM (P = .0001, .0023, .0398, and .0003, respectively). Clinical T stage (T1-2 *v* T3-4) was associated with (disease-specific) survival (3-year OS = 80% and 21%, respectively; P = .0008, log-rank test; 3-year DSS = 85% and 33%, respectively; P = .012, log-rank test) but not with LRC or DM. Notably, the numbers of peripheral-blood T and NK cells were not predictive of OS, DSS, LRC, or DM and, thus, were not included in further analyses (Table 4).

Multivariate Analyses: The Size of the iNKT Cell Fraction Is an Independent Prognostic Factor With Regard to OS, DSS, and LRC

Next, we established that the circulating iNKT cell level was an independent prognosticator with regard to OS, DSS, and LRC using the Cox proportional hazards model (Table 5). Namely, patients with a small circulating iNKT cell fraction ($<$ 25th percentile) before radiotherapy had a significantly decreased (disease-specific) survival period compared with patients with an intermediate or large iNKT fraction (OS: hazard ratio [HR] = 5.2 and 15, respectively; P = .015 and .019, respectively; DSS: HR = 13 and 17, respectively; P = .005 and .022, respectively); similar results were found for the risk of locoregional recurrence (LRC: HR = 4.2 and 13, respectively; P = .023 and .021, respectively) but not for the risk of developing DM (DM: P = .964 and

Table 4. Survival

Group	3-Year OS		3-Year DSS		3-Year LRC		3-Year DM-Free Survival	
	%	SE	%	SE	%	SE	%	SE
iNKT/10^6 T cells								
$<$ 48	39	19	43	20	31	17	54	24
48-242	75	10	87	9	74	11	100	—
$>$ 242	92	8	92	8	92	8	92	8
Clinical T stage								
T1-2	80	7	85	6				
T3-4	21	18	33	25				

Abbreviations: OS, overall survival; DSS, disease-specific survival; LRC, locoregional control; DM, distant metastasis; iNKT, invariant CD1d-restricted natural killer T.

Table 5. Cox Regression Analyses

Parameter	*P*	Relative Risk	95% CI
OS	.001		
iNKT/10^6 T			
$<$ 48 *v* 48-242	.015	5.2	1.4 to 19
$<$ 48 *v* $>$ 242	.019	15	1.6 to 143
Age	.004	1.1	1.0 to 1.2
DSS	.001		
iNKT/10^6 T			
$<$ 48 *v* 48-242	.005	13	2.2 to 83
$<$ 48 *v* $>$ 242	.022	17	1.5 to 200
Age	.030	1.1	1.0 to 1.2
LRC	.005		
iNKT/10^6 T			
$<$ 48 *v* 48-242	.023	4.2	1.2 to 14
$<$ 48 *v* $>$ 242	.021	13	1.5 to 111
Age	.137	NA	NA

NOTE. Patients were stratified in three groups before the start of radiotherapy: patients with iNKT cells/10^6 T cells below the 25th percentile ($<$ 48 iNKT cells/10^6 T cells), within the 25th to 75th percentiles (48 to 242 iNKT cells/10^6 T cells), and above the 75th percentile ($>$ 242 iNKT cells/10^6 T cells). The stepwise forward Cox proportional hazards model was used to investigate the predictive value of the iNKT cell level and age with regard to OS, DSS, LRC, and DM. No parameters were significantly associated with DM-free survival and, thus, are not shown. *P* values next to the clinical parameters refer to the overall significance of the regression model (Omnibus test).

Abbreviations: OS, overall survival; DSS, disease-specific survival; LRC, locoregional control; DM, distant metastasis; iNKT, invariant CD1d-restricted natural killer T; NA, not available (parameter is not significantly associated with relative risk).

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.157, respectively). Age was a significant but small confounding factor with regard to OS and DSS (HR = 1.1 in both cases; $P = .004$ and .030, respectively, Cox regression). In addition, clinical T stage (T3-4 *v* T1-2) was indicative of reduced OS and DSS (HR = 5.8 and 5.3, respectively; $P = .003$ and .024, respectively).

DISCUSSION

In this prospective study, we demonstrate for the first time that HNSCC patients with a severe numeric iNKT cell deficiency have a strikingly poor clinical outcome in response to radiotherapy. Although the levels of circulating T or NK cells were significantly reduced in HNSCC patients compared with historical age-matched healthy controls, they were not correlated to prognosis, which is in line with earlier reports.[32-34] Of note, in contrast to their levels in peripheral blood, the functional properties of tumor-infiltrating T and NK cells in HNSCC have been linked to disease outcome. In these retrospective studies, low or absent ζ-chain expression, reduced proliferation, low cytotoxicity, altered cytokine profile, and increased apoptosis of $CD8^+$ effector cells have been observed.[35] Still, immune cell dysfunction in HNSCC patients might likely also spread beyond the tumor microenvironment because similar defects have been observed in the peripheral blood of this patient group.[36-38]

Several mechanisms can be hypothesized to underlie the reduction of iNKT cells observed in cancer patients. First, the tumor might produce immunosuppressive cytokines or might shed natural glycolipids,[39] which results in overactivation and subsequent downregulation of NKT cell reactivity. Second, the tumor might indirectly cause the defective iNKT cell population (eg, by reducing the amount and/or CD1d expression of myeloid DCs because this lineage is responsible for activating iNKT cells via CD1d).[40,41] Indeed, the frequency of myeloid DCs and their HLA-DR expression was significantly lower in HNSCC patients compared with healthy controls, resulting in changes in the different T-cell subsets.[42] However, myeloid DCs reappeared in circulation 6 weeks after surgical resection,[43] whereas we have reported a retained deficiency in iNKT cell levels for up to 18 weeks after curative primary radiotherapy of HNSCC.[29] Moreover, disease stage in tumor-bearing patients did not affect circulating iNKT cell numbers. Therefore, these data indicate that either the tumor induces irreversible quantitative defects in the iNKT cell population or that a quantitative defect in the iNKT cell population (eg, based on as yet unidentified intrinsic and/or extrinsic factors) provides a risk factor for tumor development (eg, by reducing tumor immunosurveillance).[21]

To date, the clinical relevance of iNKT cells in cancer patients has received little attention. Only small-scale clinical studies suggested an important role for iNKT cells in the natural response against malignant multiple myeloma or neuroblastoma.[44,45] Dhodapkar et al[44] demonstrated that patients with progressive multiple myeloma had a marked deficiency of αGalCer-induced IFN-γ production compared with patients with nonprogressive myeloma. However, no reduced frequencies of iNKT cells were observed in either group, possibly because of the low number of patients investigated. Metelitsa et al[45] investigated the infiltration of iNKT cells using reverse transcription polymerase chain reaction in primary neuroblastomas from patients with metastatic disease. They showed that patients with tumors containing iNKT cells had a better prognosis compared with patients whose tumors did not have iNKT-cell infiltration. Tachibana et al[46] had similar results in a retrospective study in colorectal cancer patients, which demonstrated that accumulation of Vα24-positive T cells in tumors of colorectal cancer patients predicts a better prognosis. However, the prognostic value of the level of circulating iNKT cells was not addressed in these studies.

Animal studies indicate that iNKT cell–derived IFN-γ can have an important role in therapeutically induced as well as natural antitumor responses via activation of cells of both the innate and the adaptive arm of the immune system.[14,15,18,21] In line with these preclinical studies, we and others previously demonstrated that the residual circulating iNKT cells in carcinoma patients retained their ability to produce IFN-γ directly ex vivo and, thus, might still be capable to take part in antitumor responses, at least if present in appropriate numbers.[28,29] The prognostic value of circulating iNKT cell levels reported here adds to this previous notion and strengthens the hypothesis that human iNKT cells may also contribute to antitumor responses in patients. Because this would most likely involve enhancement of downstream immune effector mechanisms, future investigation of whether the size of the circulating iNKT cell pool is causally related to T- and/or NK-cell functionality in HNSCC patients is warranted.

In summary, we demonstrate here, in a prospective study, that a low level of circulating iNKT cells before radiotherapy in HNSCC patients was significantly associated with locoregional recurrence and poor (disease-specific) survival. These data strongly suggest that reconstitution of the iNKT cell pool (eg, by adoptive transfer of ex vivo expanded autologous iNKT cells) provides a promising adjuvant immunotherapeutic strategy for HNSCC. Furthermore, screening for iNKT cell levels in peripheral-blood samples provides a noninvasive, straightforward prognostic parameter and may also be useful for determining which patients can benefit from adjuvant therapies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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