High CXCR4 expression in adenoid cystic carcinoma of the head and neck is associated with increased risk of locoregional recurrence

Thomas J W Klein Nulent ,[1,2] Robert J J van Es,[1,2] Matthijs H Valstar,[3,4] Ludwig E Smeele,[3,4] Laura A Smit,[5] Raquel Klein Gunnewiek,[6] Nicolaas P A Zuithoff,[7] Bart de Keizer,[8] Remco de Bree,[1] Stefan M Willems[6]

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jclinpath-2019-206273).

For numbered affiliations see end of article.

Correspondence to
Dr Thomas J W Klein Nulent, Department of Head and Neck Surgical Oncology, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands; t.j.w.kleinnulent@umcutrecht.nl

Received 14 October 2019
Revised 30 November 2019
Accepted 16 December 2019
Published Online First 16 January 2020

ABSTRACT

Aim Treatment options for head and neck adenoid cystic carcinoma (AdCC) are limited in advanced disease. Chemokine receptor type 4 (CXCR4) is present in various tumour types, including AdCC. Upregulation is associated with tumour recurrence and metastasis. New CXCR4-specific diagnostic and therapeutic target agents have recently been available. This study aimed to analyse CXCR4 expression in a cohort of primary head and neck AdCC.

Methods After histopathological revision, tumour tissues of 73 consecutive patients with AdCC over 1990–2016 were sampled on a tissue microarray. Slides were immunohistochemically stained for CXCR4 and semiquantitatively scored. Associations between protein expression and cliniopathological parameters were tested. HRs were calculated using a Cox proportional hazard model.

Results Sixty-six tumours could be analysed. CXCR4 expression was present in 81% of the tumours with a median of 29% (IQR 1–70) positive cells. Expression was univariately correlated to perineural growth (Spearman ρ .26, p=0.04) and bone invasion (Spearman ρ .32, p=0.01), but not with tumour grade.
CXCR4 expression in the primary tumour was significantly higher in tumours that recurred as compared with those that did not recur (median 60%, IQR 33–72 vs 12%, IQR 1–70, Kruskal-Wallis p=0.01). After dichotomisation, >25% of CXCR4 expressions proved an independent prognosticator for a reduced recurrence-free survival (RFS) (HR 7.2, 95% CI 1.5 to 72.4, p=0.04).

Conclusion CXCR4 is expressed in the majority of primary AdCCs and independently correlated to worse RFS, suggesting CXCR4 as a target for imaging and therapy purposes in patients with advanced AdCC.

INTRODUCTION

Adenoid cystic carcinoma (AdCC) is an uncommon malignant epithelial tumour of the secretory glands in the head and neck region. It comprises approximately 10% of all salivary gland neoplasms and 20%–35% of all salivary gland malignancies, and is the most common malignancy of the minor salivary glands. The major and minor salivary glands are equally affected, although reports are contradictory.[1–5] It arises in all age groups, with a peak incidence in the fifth and sixth decades, and women are slightly more affected.[1–3 5] AdCC originates from ductal (luminal) and basal/myoepithelial (abluminal) cells arranged in a glandular (cribriform), tubular or solid growth pattern.[6] Diagnosis can be enhanced by detecting fusion of the cellular *Myeloblastosis Gene (MYB)* to the transcription factor gene *NFIB*, which is present in the majority of AdCC cases.[1]

After surgery, adjuvant radiotherapy is usually indicated due to involved tumour margins and its typical perineural spread. A tendency for locoregional recurrence and late onset of indolent, slowly growing multiple distant metastases is reflected by local control rates of 58% and poor disease-specific survival of 54% after 10 years.[1 2 7 8]

Other negative prognosticators are advanced tumour stage, solid growth pattern, involvement of the skull base and perineural spread.[1–3] Local recurrences are difficult to cure due to previous surgical procedures and exceeded radiotherapy limits. There is only limited evidence on the efficacy of systemic chemotherapy or immunotherapy, and there is a need for alternative strategies.[9]

Chemokines (chemotactic cytokines) could have that potential. They play an important role in the immune system by chemotaxis of leucocytes during inflammatory response. Furthermore, chemokines perform a variety of functions, such as apoptosis and mitogenic and angiogenic activities and are therewith involved in embryogenesis, hematopoiesis, as well as tumour growth and metastasis.[10 11] Chemokines typically act as ligands to the G protein-coupled seven-transmembrane receptor domain. The chemokine receptor type 4 (CXCR4) is such a transmembrane receptor. The CXCR4 gene is localised on chromosome two and was originally called leucocyte-expressed seven-transmembrane domain receptor (fusin). It was renamed CXCR4 when the homeostatic chemokine 'stromal cell-derived factor 1 alpha', also known as CXC-chemokine ligand 12 (CXCL12), was identified as its natural ligand.[10 12 13] During embryonic development, CXCR4 is expressed on progenitor cells, and in the 1990s, it was discovered to serve as a coentry receptor for HIV.[14] CXCL12 is expressed in different tissues and organs, including skin, lymph nodes, lung, intestine, liver, stromal cells and endothelial cells.[11 15 16]

Besides trafficking and homeostasis of immune cells and homing and retention of hematopoietic stem cells within the bone marrow, the CXCR4/CXCL12 receptor–ligand interaction plays a

Check for updates

To cite: Klein Nulent TJW, van Es RJJ, Valstar MH, *et al*. *J Clin Pathol* 2020;**73**:476–482.

prominent role in tumourigenesis. CXCR4 overexpression is present in more than 20 human tumour types, including AdCC.[15–17] In addition, increased CXCR4 expression is in most tumours directly associated with an increased risk of local recurrence and distant metastases by promoting angiogenesis and migration of tumour cells, preferentially into metastatic sites that highly express CXCL12.[13] In the present study, we aimed to analyse CXCR4 expression in a large cohort of primary AdCC of the head and neck and the association with the the above-mentioned prognosticators and outcome, that is, locoregional recurrence, distant metastases and survival.

PATIENTS AND METHODS

Patient selection

All consecutive patients diagnosed with AdCC in the University Medical Center Utrecht and Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital between 1990 and 2016 were retrospectively reviewed. Patients were selected if they had a histology-proven primary AdCC in the head and neck region and if their primary tumour tissue cores were incorporated in a previously fabricated tissue microarray (TMA). Patients with previous salivary gland disease and/or radiotherapy to the head or neck were excluded.

CXCR4 immunohistochemistry and expression analysis

Representative TMA paraffin sections 4 µm thick were immunohistochemically stained using fully automated protocols on the Benchmark XT (Ventana Medical Systems, Tucson, Arizona, USA). For the primary antibody, a mouse antihuman CXCR4 monoclonal antibody (LEAF Purified Mouse IgG2a, κ Isotype Ctrl, Biolegend) of the IgD2a isotype was used (dilution 1/800). The tissue sections were deparaffinised with ethanol and xylene, followed by pretreatment with protease 1 (8 min) and subsequent primary antibody incubation for 32 min. Antigen antibody reactions were visualised using Ventana OptiViewTM Universal DAB Detection Kit. Finally, the slides were counterstained with haematoxylin, dehydrated and mounted.

Semiquantitative scoring of the primary AdCC tumour samples was done in a blinded fashion by a dedicated head and neck pathologist (SW) and two researchers (TKN and RKG). Discrepant cases were discussed to reach consensus. The percentage of CXCR4-positive tumour cells per tumour core was scored in increments of 5%. Total tumour CXCR4 expression of the arrayed cores was defined by the mean percentage of positive tumour cells out of the three cores. A core was considered inadequate when it contained <5% tumour tissue. Patients with less than two adeqate cores were excluded to minimise tumour heterogeneity.[18]

Clinical parameters and tumour characteristics

The following clinical parameters were retrieved from the medical files: patient's gender, age at diagnosis, tumour site, treatment regimen, (time to) recurrence or metastasis, vital status (cause of death) and date of last follow-up until 1 January 2018. Recurrence-free survival (RFS) was defined as the interval from the operation to the detection of a locoregional disease recurrence. Two dedicated head and neck pathologists (SW and LS) re-examined all H&E-stained slides for the following parameters: type and diameter of the tumour, pathological T and N stages, histopathological growth pattern and associated grade (according to the differentiation of Perzin *et al*[19]), surgical resection margins and the presence of perineural, vascular and bone invasion. When Fluorescence In Situ Hybridization (FISH) had been applied to detect the *MYB-NFIB* fusion, the tumour was defined translocated when a break-apart signal was seen in >10% of the tumour cells of at least two arrayed cores.

Statistical analysis

Consistency of CXCR4 expression within the tumour was analysed using the intraclass correlation coefficient (ICC), as earlier described.[20] Associations between CXCR4 expression and tumour characteristics were tested by calculating Spearman rank correlation coefficient. The independent samples Kruskal-Wallis (KW) test was used to compare CXCR4 expression median between the primary tumours that did and did not recur or metastasise. CXCR4 expression was dichotomised by plotting receiver operating characteristic (ROC) curves. Differences in baseline characteristics of the groups divided by dichotomisation were compared using Pearson χ^2 or Fisher's exact test with appropriate Bonferroni correction; KW was used in case of a continuous dependent parameter. Statistical analyses were performed using SPSS Statistics V.22.0 for Windows. Both univariate and multivariate survival analyses were carried out to calculate HRs with 95% CI. A Cox proportional hazard regression model was performed with SAS V.9.4. Firth's correction was applied to reduce bias of maximum likelihood estimation and, if needed, to deal with the occurrence of monotone likelihood in small-sample studies.[21] Discriminative ability of the model was assessed by computing Harrell's C-statistic.[22] A two-tailed p value of <0.05 was considered statistically significant for all analyses.

RESULTS

Patients, clinical parameters and tumour characteristics

Within the defined study period of 27 years, in total, 122 patients were diagnosed with AdCC of the head and neck. Seventy-three of them were previously randomly incorporated in a TMA. CXCR4 expression could be analysed of 66 patients: one patient was excluded because of inadequate cores; six patients were excluded because they only had one available core. Close or positive resection margins were merged because only three tumours had close margins and the treatment regimen for these two groups is equal. Cohort characteristics are summarised in table 1.

CXCR4 immunohistochemistry and expression analysis

CXCR4 expression in primary AdCC ranged from 0% to 100% (median 29%, IQR 1–70). Fourteen patients (19%) were CXCR4-negative and seven tumours (9%) showed 100% expression. In general, CXCR4 staining intensity of the matched cores per tumour was homogenous with limited spatial variability, which is reflected by a high single-core ICC of 0.89 (p<0.01). Different percentages of immunohistochemical staining are visualised in figure 1. Spearman ρ correlation coefficients and CXCR4 medians for clinical parameters and pathological characteristics of the primary tumour were calculated and listed in table 1. A significant correlation was found between high CXCR4 expression and perineural growth (Spearman ρ 0.26, p=0.04) and bone invasion (Spearman ρ 0.32, p=0.01).

Follow-up and survival analysis

Median follow-up (from diagnosis until 1 January 2018) was 55 months (IQR 32–98). Thirteen patients (20%) developed a locoregional recurrence, at a median of 42 months (IQR 21–95) after diagnosis. Distant metastases occurred in 18 patients (27%), at a median of 31 months (IQR 13–49). Metastatic sites were the

Table 1 Cohort characteristics

	n (%)	Median CXCR4 (%)	Associations (Spearman ρ)	CXCR4 0%–25%	CXCR4 >25%	χ^2 CXCR4 ≤25% vs >25%
Patients	66	29		32 (48%)	34 (52%)	
Gender						
Male	24 (36%)	34	−0.10, p=0.43	11 (46%)	13 (54%)	p=0.75
Female	42 (64%)	27		21 (50%)	21 (50%)	
Age at diagnosis (years)						
Median (IQR)	59 (44–71)		−0.01, p=0.92	58 (42–71)	61 (49–71)	p=0.78
Range	20–89			20–89	29–83	
Site and subsite						
Major salivary gland	36 (55%)	17	0.21, p=0.10	21 (58%)	15 (42%)	p=0.08
Parotid gland	15	12		8	7	p=0.67
Submandibular gland	20	22		12	8	p=0.22
Sublingual gland	1	0		1	0	p=0.30
Minor salivary and seromucous gland	30 (45%)	58	0.21, p=0.10	11 (37%)	19 (63%)	p=0.08
Oral cavity (lip/buccal mucosa/hard palate gingival)	11	83		2	9	p=0.03*
Oropharynx (soft palate/base of tongue)	6	19		3	3	p=0.94
Nasal cavity/nasopharynx/maxillary sinus	7	85		2	5	p=0.26
Larynx/trachea	3	0		3	0	p=0.07
Lacrimal gland	2	72		0	2	p=0.16
External auditory canal	1	0		1	0	p=0.30
Tumour						
pT stage (TNM seventh ed.)						
pT1	19	21	0.14, p=0.26	11	8	p=0.33
pT2	24	39		9	15	p=0.18
pT3	3	12		3	0	p=0.07
pT4a	14	49		6	8	p=0.64
pT4b	6	30		3	3	p=0.94
Nodal status						
pN0	59 (89%)	23	0.07, p=0.59	30 (51%)	29 (49%)	p=0.27
pN+	7 (11%)	60		2 (29%)	5 (71%)	
Distant metastasis						
cM0	65 (98%)	30				
cM1	1 (2%)	8				
Resection margins						
Clear (>5 mm)	13 (20%)	38	−0.02, p=0.89	5 (38%)	8 (62%)	p=0.54
Close or positive (<5 mm)	53 (80%)	23		27 (51%)	26 (49%)	
Perineural growth†						
Present	47 (71%)	38	0.26, p=0.04	19 (40%)	28 (60%)	p=0.02
Absent	18 (27%)	3		13 (72%)	5 (28%)	
Vasoinvasive growth						
Present	10 (15%)	49	0.15, p=0.23	4 (40%)	6 (60%)	p=0.53
Absent	55 (83%)	23		28 (51%)	27 (49%)	
Bone invasion†						
Present	16 (24%)	74	0.32, p=0.01	4 (25%)	12 (75%)	p=0.03
Absent	50 (76%)	20		28 (56%)	22 (44%)	
Growth pattern (Perzin grade 19)						
Tubular (grade 1)	30 (46%)	35	−0.02, p=0.89	14 (47%)	16 (53%)	p=0.79
Cribriform, <30% solid (grade 2)	26 (39%)	10		15 (58%)	11 (42%)	p=0.23
Solid (grade 3)	10 (15%)	38		3 (30%)	7 (70%)	p=0.20
MYB-NFIB fusion						
Present	28 (42%)	11	−0.09, p=0.53	17 (61%)	11 (39%)	p=0.33
Absent	20 (30%)	27		10 (51%)	10 (49%)	
Treatment						
Adjuvant radiotherapy						
Yes	63 (96%)	23	−0.14, p=0.27	32 (51%)	31 (49%)	p=0.09
No	3 (4%)	53		0	3 (100%)	

*Statistically not significant due to multiple comparisons.
†Association is considered statistically significant.
CXCR4, chemokine receptor type 4.

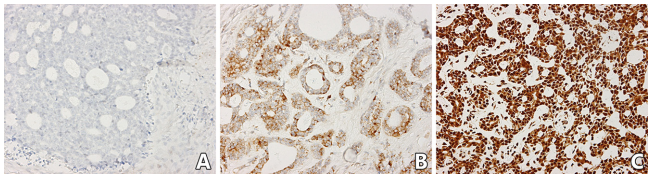

Figure 1 Immunohistochemical chemokine receptor type 4 expression in adenoid cystic carcinoma. Magnification: ×200. (A) Negative; (B) 30% positive; (C) 100% positive.

lungs in 17 patients and isolated liver metastasis in 1 patient. Besides the lungs, bone metastases were found in four patients and liver metastases in three patients. Cohort and subgroup follow-up and survival data are summarised in tables 2 and 3.

CXCR4 expression in the primary tumour was significantly higher in tumours that recurred compared with those that did not recur (median 60%, IQR 33–72 vs 12%, IQR 1–70, KW p=0.01). There was no difference in expression between primary tumours that did or did not metastasise to (the different) distant sites. Given the higher CXCR4 expression in tumours that recurred, dichotomisation was carried out by plotting an ROC curve (online supplementary figure) for CXCR4 expression by RFS. The cut-off was defined at 25% (area under the curve 0.73), the dichotomised population characteristics (0%–25% and >25%) were added to tables 1 and 2. The >25% expression group was dominated by perineural growth and bone invading tumours (Pearson χ^2 p=0.02 and p=0.03, respectively). The difference in oral cavity tumour subsite did not reach statistical significance after Bonferroni correction for multiple comparisons.

Univariate RFS analyses were carried out for relevant prognosticators using the log-rank test as shown in table 4. The multivariate Cox proportional hazard model showed a significant relation between >25% CXCR4 expression and worse RFS (HR 7.2, 95% CI 1.5 to 72.4, p=0.04). Postoperative radiotherapy extended RFS significantly (HR 25.1, 95% CI 1.9 to 339.9, p=0.03). The model incorporated growth pattern, bone invasion, perineural growth and resection margins, which were no individual predictors of RFS. Harrell's C-statistic of the predictive Cox proportional hazard model was 0.80, and that without CXCR4 expression was 0.73. Multivariate RFS survival graph is plotted in figure 2A. CXCR4 expression was in multivariate analysis no prognosticator for overall survival (OS), disease-specific survival (DSS) and metastatic-free survival (MFS). Although statistical significance is not reached, the univariate MFS survival graph (figure 2B) shows a clear divergent pattern starting 100 months after diagnosis in favour of the 0%–25% CXCR4-expressing tumours. When other prognosticators are added to this model, the possible long-term relation between high CXCR4 expression and distant metastasis can, however, no longer be demonstrated.

Table 2 Survival data

Survival by disease recurrence	n	DOD (%)	Median months
Locoregional recurrence only	6	2 (33%)	140
Distant metastases only	11	6 (55%)	51
Locoregional recurrence and distant metastases	7	6 (86%)	62

Cohort survival	N affected	Median months	% survival
Overall survival			
5 years	16	34	76
10 years	18	37	73
Disease-specific survival			
5 years	10	38	85
10 years	12	51	82
Locoregional recurrence-free survival			
5 years	9	35	86
10 years	11	37	83
Metastatic-free survival			
5 years	14	20	79
10 years	17	28	74

DOD, died of disease.

DISCUSSION

The present study demonstrates CXCR4 expression in 81% of primary AdCC samples with a median of 29% (IQR 1–70) positive cells. Interpretation of the used TMA was reliable with a high single-core ICC of 0.89 (p<0.01), indicating that one single-core is sufficiently reliable to determine the CXCR4 expression status of the whole tumour.

CXCR4 expression of the primary tumour is significantly higher in tumours that recur (KW p=0.01) and is significantly associated with perineural spread and bone invasion. Tumour expression >25% is independently correlated with reduced RFS (HR 7.2, 95% CI 1.5 to 72.4, p=0.04).

The reported high expression by immunohistochemistry and present intracellular localisation of CXCR4 in primary AdCC corresponds to the results of other smaller studies on this rare topic.[16 17 23]

A solid growth pattern is associated with a worse OS and DSS (data not shown) and is in accordance with the literature.[2 3] Various studies, however, report differently regarding an increased risk of locoregional recurrence in case of a more solid growth pattern (Perzin grade 2 or 3), which is not observed in the present results.[1 2 24] A (linear) correlation between increased CXCR4 expression and AdCC growth pattern as reported by Zushi *et al* in a small series was disputed by Phattarataratip and Dhanuthai and and also not confirmed in this study.[16 23] Interestingly, only one out of the present 14 CXCR4-negative tumours was classified Perzin grade 3 (solid growth pattern) and none of these 14 patients developed a locoregional recurrence during a median follow-up of 55 months. An increased metastatic potential by increase of CXCR4 expression could additionally not be confirmed by our results, although primary tumours that metastasised showed a higher (but statistically not significant) median CXCR4 expression (49% vs 23%). The metastatic spread of tumours is thought to be the result of a process critically regulated by chemokines and their receptors. CXCR4 has been shown to play an essential role in the metastatic spread of tumour cells to distant organs, as cells migrate along the gradient of the CXCR4-ligand CXCL12. This has also been confirmed in AdCC.[13 15 17] Moreover, an important mechanism that alters the metastatic behaviour of tumour cells in vivo is hypoxia, which enhances angiogenesis and upregulation of CXCR4 via the hypoxia-inducible factor-1α (HIF-1α).[13 15] Opposite AdCC inhibitory effects by downregulation of HIF-1α in AdCC have recently been described.[25]

The typical AdCC distant metastasic sites (lungs, liver and bone) show peak levels of CXCL12 expression and as a consequence one could have expected a significant correlation between high expression and metastatic spread.[11] In this study, distant metastases were identified median after 31 months and were equally distributed between the high and low expression groups (table 2). However, figure 2B depicts a (nonsignificant)

Table 3 Follow-up

	n (%)	Primary tumour Median CXCR4 (%)	CXCR4 0%–25%	CXCR4 >25%	χ^2 CXCR4 ≤25% vs >25%
Patients	66		32	34	
Follow-up (median months, IQR)	55, 32–98		57, 42–83	53, 31–137	p=0.63
Locoregional recurrence					
Yes	13 (20)	60%*	1 (3%)	12 (35%)	p<0.01
No	53 (80)	12%*	31 (97%)	22 (65%)	
Distant metastasis					
Yes	18 (27)	49%	7 (22%)	11 (32%)	p=0.33
No	48 (73)	23%	25 (78%)	23 (68%)	

*Differences are considered statistically significant (Kruskal-Wallis p=0.01).
CXCR4, chemokine receptor type 4.

divergent pattern starting 100 months after diagnosis indicating a worsened long-term MFS for the >25% CXCR4-expressing tumours. Significance might not have been reached due to the relatively small study population and short median follow-up time of 55 months, which are possibly insufficient to evaluate the (very) late onset of clinically irrelevant small and indolent distant AdCC metastases.[3]

In contrast to the frequent hematogenous dissemination, AdCC lymph node metastases are less common despite the high CXCL12 expression of lymph node stromal cells. One explanatory theory states that CXCL12 is involved in homing of memory T-cells via the bloodstream, and not via the lymphatic veins.[17] Studies on different (adeno)carcinomas, however, did report an increased risk of both nodal metastases and local disease recurrence in case of high primary tumour CXCR4 expression.[26–28] This is not reflected by the present results as eventually only in 20% of patients' lymph nodes were involved: seven at diagnosis and six more cases during follow-up, of which five also developed distant metastases. It was subsequently argued that high tumour CXCR4 expression is associated with worse biological parameters and agressive behaviour, resulting in an increased risk of disease progression, eventually leading to a worsened survival.[28]

Table 4 RFS analysis

	Locoregional recurrences/n (%)	RFS Univariate Log rank	RFS Multivariate HR (95% CI)
CXCR4			
0%–25%	1/32 (3%)	7.9 (p=<0.01)	7.2 (1.5 to 72.4) (p=0.04)
>25%	12/22 (55%)		
Adjuvant radiotherapy			
Yes	12/63 (19%)	19.0 (p=<0.01)	25.1 (1.9 to 339.9) (p=0.03)
No	1/3 (33%)		
Resection margins			
Clear (>5 mm)	1/13 (8%)	0.05 (p=0.49)	2.0 (0.4 to 20.6) (p=0.49)
Close or positive (<5 mm)	12/53 (23%)		
Perineural growth			
Present	11/47 (23%)	1.8 (p=0.18)	1.0 (0.2 to 6.3) (p=0.97)
Absent	2/18 (11%)		
Bone invasion			
Present	4/16 (25%)	0.03 (p=0.59)	0.7 (0.2 to 2.7) (p=0.68)
Absent	9/50 (18%)		
Growth pattern			
Tubular	5/30 (17%)	3.2 (p=0.20)	2.6 (0.5 to 12.1) (p=0.26)
Cribriform	5/26 (19%)		
Solid	3/10 (30%)		

CXCR4, chemokine receptor type 4; RFS, recurrence-free survival.

In a study on colon carcinoma, it was specifically hypothesised that by tumour alteration to a migratory phenotype, CXCL12-producing normal intestine epithelial cells would become at high risk of recurrence. Normal salivary gland cells, however, do not or at most weakly express CXCR4, but interestingly, CXCL12 was found to be expressed in areas of inflamed reactive salivary gland ductal epithelial cells and surrounding vessels in Sjogren's syndrome.[16 29 30] Inflammation around the AdCC tumour border with possible associated upregulation of CXCL12 may therefore play a role in the development of a recurrence in high CXCR4-expressing AdCCs.

CXCR4 is correlated to perineural growth and bone invasion, Spearman ρ .26 (p=0.04) and 0.32 (p=0.01), respectively. The latter could possibly be explained by involvement of the CXCL12/CXCR4 axis in mediating osteolysis by the tumour as CXCL12 has been recognised to stimulate migration of osteoclast precursors and upregulation of several pro-osteoclastic genes.[31] Both perineural growth and bone invasion are not associated with locoregional recurrence as shown in multivariate analysis and in accordance with the literature.[2] Perineural invasion is not correlated to involved resection margins and postoperative radiotherapy, which are mutually strongly correlated (Spearman ρ .37, p=0.01). Remarkably also, other patient and tumour characteristics as pT stage, tumour site and adjuvant radiotherapy did not differ significantly between free, close and positive resection margins (data not shown). Merging the patients with close and positive margins was therefore considered reliable.

A deep locoregional recurrence is a relevant problem in the management of AdCC in the case of functional irresectability or exceeding radiation limits. Conventional treatment options are no longer applicable and the results of (combinations of) chemotherapeutic or new targeted agents are limited.[9] Remarkably, cisplatin, a chemotherapeutic agent frequently used in head and neck malignancies, induces chemotherapy resistance itself by directly providing survival signals to tumour cells and indirectly by upregulation of CXCR4, which again promotes so-called 'prosurvival' pathways. In addition, CXCL12 reduces apoptosis induced by cisplatin in AdCC cells.[17] The current study presents CXCR4 as a new independent prognosticator for AdCC and may advocate comprehensive and aggressive treatment combined with stringent follow-up in CXCR4-positive tumours. Furthermore, CXCR4 may act as specific target for diagnostic and therapeutic purposes in AdCC. Antagonist drug AMD3100 and multiple other anti-CXCR4 peptides, antibodies and low-molecular-weight agents have been investigated and proven to inhibit or delay cancer progression in various tumour sites, and furthermore improved sensitisation of tumour cells to conventional chemotherapy

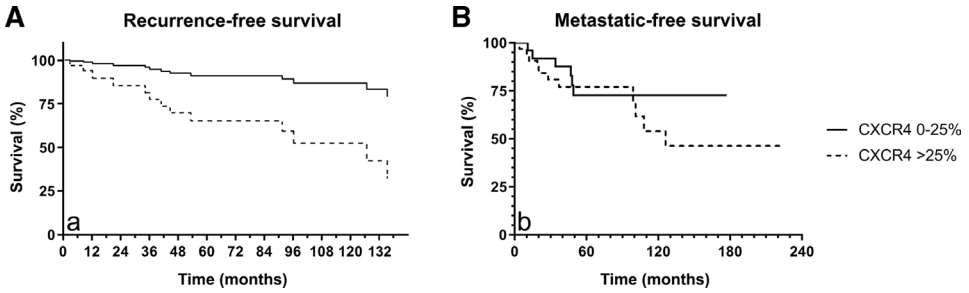

Figure 2 (A) Multivariate Cox-regression survival graph showing a worsened recurrence-free survival regarding high (>25%) versus low (0%–25%) CXCR4 expression (HR 7.2, 95% CI 1.5 to 72.4, p=0.04). (B) Univariate survival graph showing a nonsignificant diverging pattern indicating a worsened long-term metastatic-free survival in high (>25%) CXCR4-expressing tumours. CXCR4, chemokine receptor type 4.

for combined treatment.[13 15 28] Radiolabelled CXCR4 ligands have now been developed for positron emission tomography (PET)-specific imaging, which also makes these targets suitable for potential radionuclide treatment in the future.[32 33] Further research is necessary to delineate whether CXCR4 expression in AdCC is preserved in recurrent and distant tissues, and whether primary, recurrent and/or distant AdCC tumour sites are depicted on CXCR4-targeted PET/CT.

CONCLUSION

CXCR4 expression is present on 81% of primary head and neck AdCC samples in a retrospective cohort of 66 cases. High primary tumour CXCR4 expression of >25% is independently associated with worse RFS. Based on the expression levels provided by the present study, CXCR4 is a potential target for targeted imaging and possibly radionuclide therapy in AdCC.

Take home messages

- Chemokine receptor type 4 (CXCR4) is expressed in 81% of primary adenoid cystic carcinoma.
- Expression in tumours that recur was higher as compared with those that did not.
- >25% CXCR4 expression indicates reduced recurrence-free survival.
- CXCR4 may act as a target for imaging and radionuclide therapy.

Author affiliations
[1]Head and Neck Surgical Oncology, University Medical Center Utrecht, Utrecht, The Netherlands
[2]Oral and Maxillofacial Surgery, University Medical Center Utrecht, Utrecht, The Netherlands
[3]Head and Neck Oncology and Surgery, Netherlands Cancer Institute/Antoni van Leeuwenhoek, Amsterdam, The Netherlands
[4]Oral and Maxillofacial Surgery, Amsterdam UMC Cancer Center and Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, The Netherlands
[5]Pathology, Netherlands Cancer Institute/Antoni van Leeuwenhoek, Amsterdam, The Netherlands
[6]Pathology, University Medical Center Utrecht, Utrecht, The Netherlands
[7]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands
[8]Radiology and Nuclear Medicine, University Medical Center Utrecht, Utrecht, The Netherlands

Correction notice This article has been corrected since it apeared Online First. Author Stefan Willems has been corrected to Stefan M Willems. Table 1 layout has been adjusted slightly.

Handling editor Dhirendra Govender.

Contributors All authors included on this paper fulfil the criteria of authorship. There is no one else who fulfils the criteria who has been excluded as an author.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The research protocol was approved by the University Medical Center Utrecht Medical Research Ethics Committee and Biobank Research Ethics Committee (protocol numbers 16–564 and 17–073, respectively).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iD
Thomas J W Klein Nulent http://orcid.org/0000-0003-3597-4334

REFERENCES

1 Coca-Pelaz A, Rodrigo JP, Bradley PJ, *et al*. Adenoid cystic carcinoma of the head and neck – an update. *Oral Oncol* 2015;51:652–61.
2 Jang S, Patel PN, Kimple RJ, *et al*. Clinical outcomes and prognostic factors of adenoid cystic carcinoma of the head and neck. *Anticancer Res* 2017;37:3045–52.
3 Spiro RH, Huvos AG, Strong EW. Adenoid cystic carcinoma of salivary origin. A clinicopathologic study of 242 cases. *Am J Surg* 1974;128:512–20.
4 Bradley PJ. Adenoid cystic carcinoma of the head and neck: a review. *Curr Opin Otolaryngol Head Neck Surg* 2004;12:127–32.
5 Bjørndal K, Krogdahl A, Therkildsen MH, *et al*. Salivary gland carcinoma in Denmark 1990–2005: a national study of incidence, site and histology. Results of the Danish head and neck cancer Group (DAHANCA). *Oral Oncol* 2011;47:677–82.
6 Hellquist H, Skalova A. Adenoid Cystic Carcinoma. In: *Histopathology of the salivary glands*. Springer Berlin Heidelberg, 2014: 221–60.
7 Huang M, Ma D, Sun K, *et al*. Factors influencing survival rate in adenoid cystic carcinoma of the salivary glands. *Int J Oral Maxillofac Surg* 1997;26:435–9.
8 van Weert S, Reinhard R, Bloemena E, *et al*. Differences in patterns of survival in metastatic adenoid cystic carcinoma of the head and neck. *Head Neck* 2017;39:456–63.
9 Alfieri S, Granata R, Bergamini C, *et al*. Systemic therapy in metastatic salivary gland carcinomas: a pathology-driven paradigm? *Oral Oncol* 2017;66:58–63.
10 Zlotnik A, Yoshie O. The chemokine superfamily revisited. *Immunity* 2012;36:705–16.
11 Wang Z, Sun J, Feng Y, *et al*. Oncogenic roles and drug target of CXCR4/CXCL12 axis in lung cancer and cancer stem cell. *Tumor Biol* 2016;37:8515–28.
12 Oberlin E, Amara A, Bachelerie F, *et al*. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 1996;382:833–5.
13 Vandercappellen J, Van Damme J, Struyf S. The role of CXC chemokines and their receptors in cancer. *Cancer Lett* 2008;267:226–44.
14 Feng Y, Broder CC, Kennedy PE, *et al*. Hiv-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 1996;272:872–7.
15 Domanska UM, Kruizinga RC, Nagengast WB, *et al*. A review on CXCR4/CXCL12 axis in oncology: no place to hide. *Eur J Cancer* 2013;49:219–30.
16 Zushi Y, Noguchi K, Hashitani S, *et al*. Relations among expression of CXCR4, histological patterns, and metastatic potential in adenoid cystic carcinoma of the head and neck. *Int J Oncol* 2008;33:1133–9.
17 Muller A, Sonkoly E, Eulert C, *et al*. Chemokine receptors in head and neck cancer: association with metastatic spread and regulation during chemotherapy. *Int. J. Cancer* 2006;118:2147–57.
18 Goethals L, Perneel C, Debucquoy A, *et al*. A new approach to the validation of tissue microarrays. *J Pathol* 2006;208:607–14.

19 Perzin KH, Gullane P, Clairmont AC. Adenoid cystic carcinomas arising in salivary glands.A correlation of histologic features and clinical course. *Cancer* 1978;42:265–82.
20 Noorlag R, van Es RJJ, de Bree R, *et al*. Cytokeratin 19 expression in early oral squamous cell carcinoma and their metastasis: inadequate biomarker for one-step nucleic acid amplification implementation in sentinel lymph node biopsy procedure. *Head Neck* 2017;39:1864–8.
21 Heinze G, Dunkler D. Avoiding infinite estimates of time-dependent effects in small-sample survival studies. *Stat Med* 2008;27:6455–69.
22 Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;15:361–87.
23 Phattarataratip E, Dhanuthai K. Expression of C-X-C motif chemokine receptors 4 and 7 in salivary gland neoplasms. *Arch Oral Biol* 2017;83:136–44.
24 Zhang C-Y, Xia R-H, Han J, *et al*. Adenoid cystic carcinoma of the head and neck: clinicopathologic analysis of 218 cases in a Chinese population. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013;115:368–75.
25 Xiao C, Pan Y, Zeng X, *et al*. Downregulation of hypoxia-inducible factor-1α inhibits growth, invasion, and angiogenesis of human salivary adenoid cystic carcinoma cells under hypoxia. *Oncol Rep* 2018;40:1675–83.
26 Kato M, Kitayama J, Kazama S, *et al*. Expression pattern of CXC chemokine receptor-4 is correlated with lymph node metastasis in human invasive ductal carcinoma. *Breast Cancer Res* 2003;5.
27 Yoshitake N, Fukui H, Yamagishi H, *et al*. Expression of SDF-1α and nuclear CXCR4 predicts lymph node metastasis in colorectal cancer. *Br J Cancer* 2008;98:1682–9.
28 Chatterjee S, Behnam Azad B, Nimmagadda S. The intricate role of CXCR4 in cancer. *Adv Cancer Res* 2014;124:31–82.
29 Yopp AC, Shia J, Butte JM, *et al*. Cxcr4 expression predicts patient outcome and recurrence patterns after hepatic resection for colorectal liver metastases. *Ann Surg Oncol* 2012;19:339–46.
30 Barone F, Bombardieri M, Rosado MM, *et al*. CXCL13, CCL21, and CXCL12 Expression in Salivary Glands of Patients with Sjögren's Syndrome and MALT Lymphoma: Association with Reactive and Malignant Areas of Lymphoid Organization. *J Immunol* 2008;180:5130–40.
31 Coniglio SJ. Role of tumor-derived chemokines in osteolytic bone metastasis. *Front Endocrinol* 2018;9.
32 Gourni E, Demmer O, Schottelius M, *et al*. PET of CXCR4 expression by a 68Ga-labeled highly specific targeted contrast agent. *J Nucl Med* 2011;52:1803–10.
33 Schottelius M, Osl T, Poschenrieder A, *et al*. [^{177}Lu]pentixather: comprehensive preclinical characterization of a First CXCR4-directed endoradiotherapeutic agent. *Theranostics* 2017;7:2350–62.