in vivo *31*: 23-34 (2017)
doi:10.21873/invivo.11020

Review

Prevalence of *EGFR* Tyrosine Kinase Domain Mutations in Head and Neck Squamous Cell Carcinoma: Cohort Study and Systematic Review

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Abstract. *Background: Mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase domain (TKD) are associated with response and resistance to targeted therapy. The EGFR mutation status in patients with advanced oral and oropharyngeal squamous cell carcinoma (OOSCC) was evaluated. A systematic literature review was undertaken to summarize current evidence and estimate the overall prevalence of EGFR TKD mutations in patients with head and neck squamous cell carcinoma (HNSCC). Materials and Methods: Genomic DNA was extracted from formalin-fixed, paraffin-embedded tumor samples of 113 patients with OOSCC. Pyrosequencing was performed to investigate mutations in EGFR exons 18 to 21. Medline databases were searched for relevant studies. Studies reporting mutations in the EGFR TKD in HNSCC were eligible for inclusion in the systematic review. Results: No mutations in the EGFR TKD were observed in 113 samples of OOSCC. A total of 53 eligible studies were included in the systematic review. In total, from the review, 117 patients harboring a total of 159 EGFR TKD mutations were reported among 4122 patients with HNSCC. The overall prevalence of EGFR TKD mutations in HNSCC was 2.8%. Conclusion: Large-scale studies are warranted to provide further evidence regarding the mutation status of EGFR in patients with HNSCC.*

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Key Words: Oral and oropharyngeal cancer, *EGFR* kinase domain, mutations, pyrosequencing, review.

Head and neck squamous cell carcinoma (HNSCC) remains a challenging disease despite intensive clinical and translational research (1-3). A subset of head and neck cancer is caused by human papillomavirus (HPV) and represents a biologically distinct entity (4). In the past decades, several treatment strategies have been applied to treat HNSCC, however, survival outcomes have not substantially changed, emphasizing the need for more personalized medicine (5-8). Many efforts have, therefore, been made to identify predictive biomarkers and tailor treatment to the individual patient based on their own genetic and molecular profile.

The epidermal growth factor receptor (EGFR) is a transmembrane cell surface receptor belonging to the human epidermal growth factor receptor (HER) family of receptor tyrosine kinases. EGFR overexpression occurs in more than 90% of HNSCCs and has been correlated with poor outcome (9). Robust preclinical evidence underlines the role of EGFR in the development of HNSCC, showing that EGFR activation triggers several downstream signaling pathways that play a crucial role in cancer pathogenesis (3, 10, 11). In this context, strategies for inhibition of EGFR signaling using monoclonal antibodies and tyrosine kinase inhibitors (TKIs) have been investigated intensively in clinical trials. *De novo* or acquired resistance to EGFR-targeted therapy, however, has led to a modest survival benefit for patients with HNSCC, while up-to-date predictive biomarkers of treatment response remain elusive (8, 12, 13).

In non-small cell lung carcinoma (NSCLC), patients with activating mutations in the *EGFR* tyrosine kinase domain (TKD) are sensitive to small-molecule EGFR TKIs such as gefitinib, erlotinib, and afatinib (14-17). Given that mutations in the *EGFR* TKD may help in the selection of patients for EGFR TKIs or other targeted therapies, the *EGFR* mutation status in treatment-naïve patients with locally advanced oral and oropharyngeal squamous cell carcinoma (OOSCC) was retrospectively evaluated. In addition, a systematic literature review was undertaken to

Table I. *Patient clinical and histopathological characteristics.*

Characteristic	Total No. (%)	Pathological response, n (%) pCR	Non-pCR
Patients	113 (100)	46 (41)	67 (59)
Age (years)			
≤60	72 (64)	29 (40)	43 (60)
>60	41 (36)	17 (42)	24 (58)
Gender			
Male	83 (73)	28 (34)	55 (66)
Female	30 (27)	18 (60)	12 (40)
Smoking			
Current	94 (83)	37 (39)	57 (61)
Former or never	19 (17)	9 (47)	10 (53)
Alcohol use			
Current	83 (73)	36 (43)	47 (57)
Former or never	30 (27)	10 (33)	20 (67)
Tumor site			
Oral cavity	97 (86)	40 (41)	57 (59)
Oropharynx	16 (14)	6 (37)	10 (63)
Clinical TNM stage			
Stage III	6 (5)	4 (67)	2 (33)
Stage IV	107 (95)	42 (39)	65 (61)
HPV status			
HPV−	104 (92)	44 (42)	60 (58)
HPV+	9 (8)	2 (22)	7 (78)

pCR, Pathological complete response; HPV, human papillomavirus.

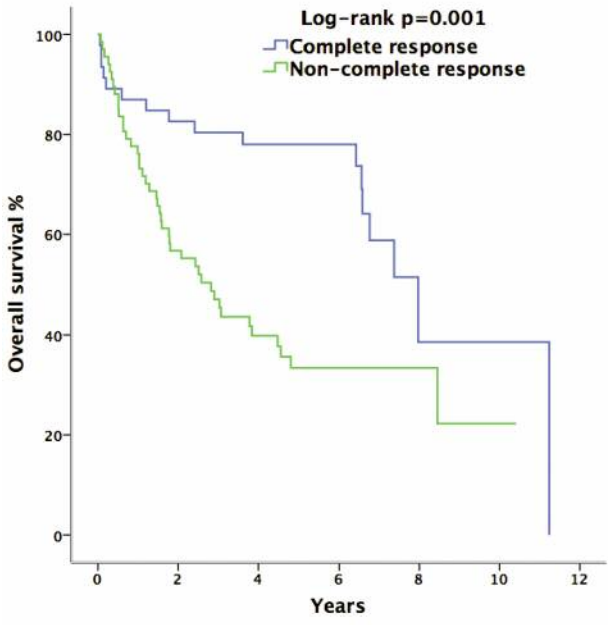


Figure 1. *Kaplan–Meier estimates of the probability of overall survival in 113 patients with oral and oropharyngeal cancer according to pathological response to neoadjuvant treatment.*

summarize current evidence regarding the *EGFR* mutation status in HNSCC. The present study aimed to estimate the overall prevalence of *EGFR* TKD mutations in patients with HNSCC and whether differences in the prevalence of *EGFR* mutations exist between patients with HNSCC across different countries and geographic regions.

Materials and Methods

Population of cohort study. This study included 113 patients diagnosed with primary locally advanced OOSCC who underwent neoadjuvant chemoradiation followed by tumor resection at the Department of Radiotherapy and Department of Cranio-Maxillofacial and Oral Surgery at the Medical University of Vienna between 2000 and 2009. All of them had: biopsy-proven OOSCC, available pretreatment biopsy tumor tissues, clinical TNM stage III or IV disease, no distant metastasis, no previous history of head and neck cancer, performance status and laboratory parameters permitting chemoradiotherapy and surgery, and had undergone complete resection (R0) of the primary tumor. The multimodal treatment comprised neoadjuvant chemotherapy with mitomycin C (15-20 mg/m^2, *i.v.* bolus injection on day 1) and 5-fluorouracil (750 mg/m^2/day, continuous infusion on days 1-5), concurrent with radiation therapy delivered over 5 weeks up to a total dose of 50 Gy (25 fractions of 2 Gy per day) followed by post-treatment radical surgery. Surgery was performed 4-8 weeks after the end of radiotherapy. Patients were followed-up regularly for further 5 years.

Patient data were obtained from the Vienna General Hospital Patient Information System (AKIM). Clinical and pathological TNM staging was based on the seventh edition of the classification Union for International Cancer Control (UICC) (18). The surgical specimens were histopathologically evaluated by means of an institutional standard protocol. Pathological complete response (pCR) was defined by the absence of residual cancer within both the primary tumor site and regional lymph nodes. A study-specific patient number was given to patients to protect their identity. The Ethics Committee of the Medical University of Vienna approved this research (approval number: 774/2008).

HPV testing. E6/E7-specific quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to determine the HPV16/18 status. Results were corroborated by p16 (cyclin-dependent kinase inhibitor 2A) immunohistochemical staining.

EGFR mutation testing. EGFR mutations were studied in DNA extracted from formalin-fixed, paraffin-embedded tissue blocks routinely archived at the Department of Pathology at the Medical University of Vienna. For each patient, one section of an appropriate tumor block was stained with hematoxylin and eosin to confirm the presence of viable carcinoma cells. DNA was extracted from tumor samples confirmed to have >50% cancer cells. From each block, 5-μm-thick sections were cut for DNA extraction performed using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. *EGFR* mutation analysis was carried out with Therascreen® Pyro Kit (Qiagen, Hilden, Germany). The *EGFR* kit enables detection and quantitation of the most common mutations in codon 719 (exon 18), exon 19 deletions, codon 768 and 790 (exon 20) and codon 858-861 (exon 21) of the *EGFR* gene.

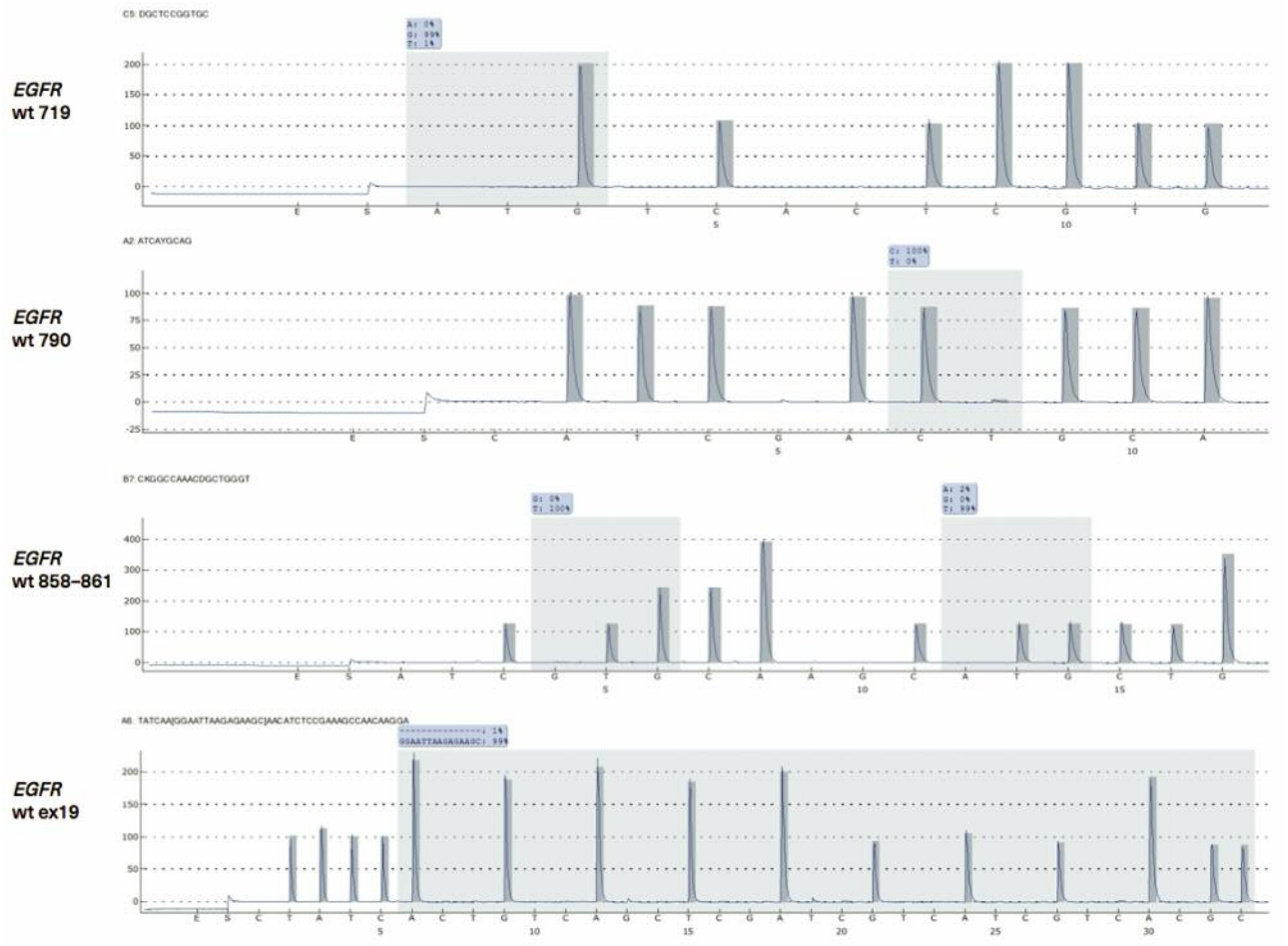


Figure 2. *Representative pyromarks of wild-type EGFR in exons 18-21.*

After the extraction of genomic DNA, *EGFR* was amplified by PCR with HotStarTaq DNA Polymerase (Qiagen) after the DNA concentration of each sample was adjusted to 2 ng/µl. The PCR conditions were 95˚C for 15 min for the initial activation of HotStarTaq DNA Poymerase, followed by a 3-step-cycling: denaturation at 95˚C for 20 s, annealing at 53˚C for 30 s and extension at 72˚C for 20 s for 42 cycles. Finally, incubation at 72˚C for 5 min was accomplished for the final extension. After amplification, the immobilization of PCR products on Streptavidin Sepharose High-Performance (GE Healthcare, Uppsala, Sweden) beads was performed using a volume of 10 µl PCR product which was added to a 24-well PCR plate containing 70 µl master mix [2 µl Streptavidin Sepharose High-Performance beads, 40 µl of PyroMark Binding Buffer (Qiagen) and 28 µl water]. The next step was the preparation of single-stranded DNA with a PyroMark Q24 Vacuum Workstation (Qiagen) and the annealing of the sequencing primer (included in the Therascreen® Pyro Kit; Qiagen) to the template. Pyrosequencing of the samples was then carried out on a PyroMark Q24 MDx system (Qiagen). The results were analyzed with PyroMark Q24 software (version 2.0.6; Qiagen). Pyrosequencing results in the initial round of sequencing were confirmed by subsequent runs of independent PCRs and pyrosequencing, as well as by Sanger sequencing.

Systematic literature review: Data sources, search strategy, selection of studies, and data extraction. This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (19). Medline databases (hosts: PubMed and OVID) from inception up to October 20, 2016 were searched for relevant studies using the key words “head and neck cancer”, “EGFR”, and “mutation”. No search restriction was applied. The complete search strategy can be found in Appendix A. In addition, manual searches were conducted on the web and by reviewing the reference lists of the retrieved articles.

Studies reporting the mutation status of the *EGFR* TKD in tumor tissues of patients with HNSCC were eligible for inclusion in the systematic review. For quantitative synthesis, only studies reporting the prevalence of *EGFR* TKD mutations in HNSCC were considered. Letters and unpublished research were not included in the present review. Case reports were considered as qualitative evidence. Two reviewers (CP and RP) independently carried out study selection and data extraction. Any disagreements between

reviewers were resolved by consensus involving a third reviewer (JE). The reviewers independently screened all records that were identified by the search strategy. Duplicate publications were excluded both electronically and manually. The selected records were pooled, retrieved as full-text publications, and assessed for eligibility. The two reviewers independently extracted data from each eligible study using a predefined data-abstraction sheet. The following data were collected: name of the first author, year of publication, study location, characteristics of study cohorts (sample size, tumor stage, tumor site), source of tumor profiled, exon location and type of *EGFR* mutations, detection methods, prognostic effect of *EGFR* mutations, and the prevalence of EGFR mutations. The PRISMA flow diagram was used to describe the study selection processes.

Statistical analysis. For the cohort study, patient characteristics were summarized using descriptive statistics. Categorical variables are described with frequencies and percentages. Patient demographic, clinical, and tumor characteristics were tested for association with pathological resonse using the chi-square test for categorical variables. Overall survival was defined as the time from surgery to death from any cause or to date of last follow-up. The Kaplan–Meier method was used for overall survival assessment and the log-rank test to compare differences in survival between groups. A two-sided *p*-value of less than 0.05 was considered statistically significant.

The systematic review was quantitatively analyzed to pool the overall prevalence of *EGFR* mutations in HNSCC. Subgroup analysis was performed to assess the prevalence of *EGFR* mutations according to geographic region. Prevalence of *EGFR* mutations was defined as the proportion of patients with *EGFR*-mutated tumors among patients who underwent the a mutation testing and was assessed as percentage with the 95% confidence interval (CI) (20). Subgroups of geographic regions (Europe, North America, Southeast Asia, and South Asia) were generated if two or more studies on a specific geographic region were present. Study location was defined based on the country where the patients were recruited in the study. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS®, version 21.0; IBM Corp., Armonk, NY, USA).

Results

Description of patient cohort and survival analysis. The clinical and histopathological characteristics of 113 study patients are presented in Table I. The median age of patients was 58 years (range=24-79 years) and most of the patients were male (73%) and current smokers (83%). The primary tumor was predominantly located in the oral cavity (86%). Pathological complete response to neoadjuvant chemoradiotherapy was observed in 46 patients (41%). Nine patients (8%) were HPV-positive. Among HPV-positive patients, two (22%) achieved a complete pathological response. Of 16 oropharyngeal tumours, two samples (13%) were positive for HPV, compared to seven HPV-positive samples (7%) out of 97 oral cavity tumors. At 2 years, the overall survival rate of the cohort was 66% and at 5 years 46%. The median follow-up time was 4.6 years by which time 56 (50%) patients had died. The overall survival of the 113 patients with OOSCC was assessed according to the pathological response status using the Kaplan–Meier method (Figure 1). The median overall survival was significantly higher in patients with tumors showing pathological complete response compared with those having non-complete tumor regression (7.9 *versus* 2.8 years respectively, log-rank p=0.001).

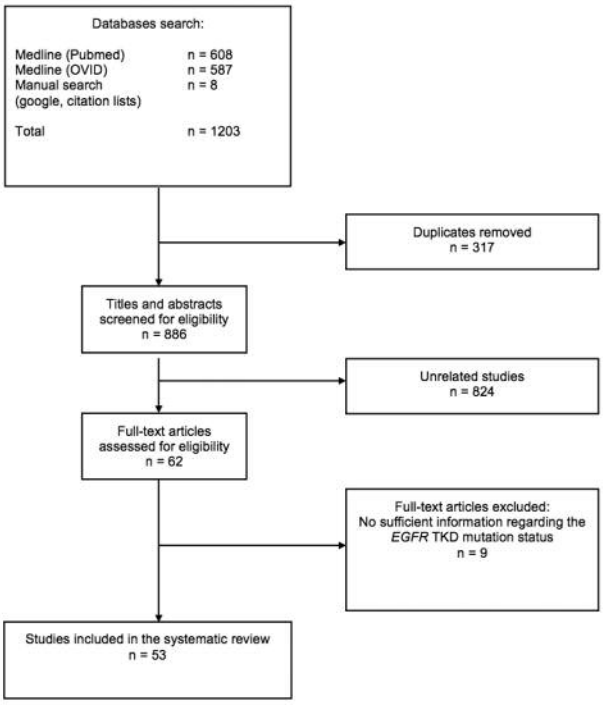
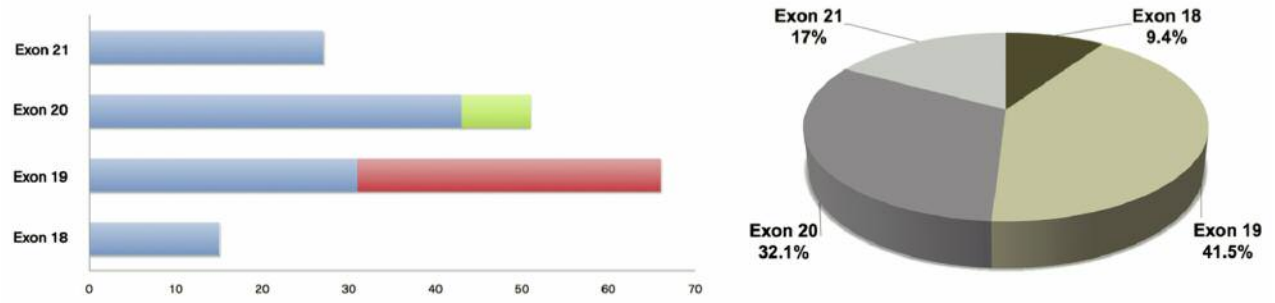


Figure 3. *Flow diagram of the study selection process*.

Mutation status of EGFR. The kinase domain of *EGFR* (exons 18-21) was analyzed in tumor samples from 113 treatment-naïve patients with primary locally advanced OOSCC. Using pyrosequencing technology, no *EGFR* mutations were detected in any of the 113 tumor tissues (Figure 2).

Characteristics of studies included in the systematic review. Overall, 53 eligible studies were included in the present systematic review. The literature search of databases and reference lists yielded 1203 records. After removing duplicates, 886 records remained to be screened. After screening of titles and abstracts, 824 studies that reported on unrelated topics were excluded. The full-text papers of the remaining 62 studies were reviewed in depth. A total of nine studies did not provide sufficient information regarding the *EGFR* TKD mutation status and were discarded, thus 53 studies were included in the qualitative review and the quantitative synthesis. The PRISMA flow diagram was used to illustrate the study selection process (Figure 3).



***EGFR* TKD mutations by exon location**

Mutation type	Exon 18 N	Exon 18 (%)	Exon 19 N	Exon 19 (%)	Exon 20 N	Exon 20 (%)	Exon 21 N	Exon 21 (%)	Total N	Total (%)
Missense	15	(9.4)	31	(19.5)	43	(27.1)	27	(17)	116	(73)
Deletion	0	(0)	35	(22)	0	(0)	0	(0)	35	(22)
Insertion	0	(0)	0	(0)	8	(5)	0	(0)	8	(5)
Total N (%)	15	(9.4)	66	(41.5)	51	(32.1)	27	(17)	159	(100)

Figure 4. *Distribution of mutations in the EGFR kinase domain categorized by exon location and mutation type.*

Table II shows the main characteristics of the studies included in the systematic review. All studies were published between 2005 and 2013 in peer-reviewed journals. Most of the studies were conducted in the USA (n=19), followed by the Republic of Korea (n=5), and India (n=4). The source for tumor DNA mutation profiling was fresh frozen tumor samples (n=26), formalin-fixed paraffin-embedded tumor tissue (n=23), or both (n=4). Three studies investigated the prognostic impact of *EGFR* mutation status on HNSCC survival using the Kaplan–Meier method/Cox proportional hazards regression model (21-23).

Overall prevalence of EGFR TKD mutations in HNSCC and distribution of mutations by exon location and type. In total, 117 patients harboring a total of 159 *EGFR* TKD mutations were reported among 4122 patients with HNSCC. The overall pooled prevalence of *EGFR* TKD mutations in HNSCC was 2.8% (95% CI=2.4-3.4%). The distribution of mutations in the *EGFR* TKD categorized by exon location and mutation type is shown in Figure 4. Among *EGFR*-mutated HNSCCs, the majority of TKD mutations were located in exon 19 (41.5%, 95% CI=33.8-49.6%), followed by exon 20 (32.1%, 95% CI=25.0-40.0%), exon 21 (17.0%, 95% CI=11.7-23.9%), and exon 18 (9.4%, 95% CI=5.6-15.3%). Of all *EGFR* mutations, missense mutations in exons 18-21 occurred in 73% (95% CI=65.2-79.6%), followed by deletions in exon 19 (22%, 95% CI=16.0-29.4%) and insertion mutations in exon 20 (5%, 95% CI=2.4-10.0%). The missense mutations T790M in exon 20 and L858R in exon 21 well-known in NSCLC occurred in 7.5% (95% CI=4.1-13.1%) and 2.5% (95% CI=0.8-6.7%) of all *EGFR* mutations, respectively.

Prevalence of EGFR TKD mutations in HNSCC by study location. The *EGFR* mutation prevalence classified by study location (countries and geographic regions) is shown in Table III. The prevalence of *EGFR*-mutated HNSCCs was estimated in four geographic regions: the highest prevalence was shown in Southeast Asia (4.9%, 95% CI=3.7-6.4%), followed by North America (2.7%, 95% CI=2.1-3.6%; all 19 studies were conducted in the USA), and Europe (1.3%, 95% CI=0.7-2.6%), whereas in South Asia (all four studies were conducted in India), no *EGFR* TKD mutations were detected. Additionally, one study from South America stated the finding of two *EGFR* mutations in 45 tumor samples (prevalence 4.4%, 95% CI=0.8-16.4%) and one study from Australia reported one *EGFR* mutation in 60 tumor samples (prevalence 1.7%, 95% CI=0.1-10.1%). When considering *EGFR* TKD mutations in individual countries, the Republic of Korea had the highest prevalence with 15.1% (95% CI=11.2-20.1%), followed by the Czech Republic (6.9%, 95% CI=1.2-24.2%), and Greece (3.3%, 95% CI=0.8-9.9%).

Discussion

In this study, pyrosequencing technology was used to identify the *EGFR* mutation status in pretreatment tumor samples of patients with locally advanced OOSCC. No *EGFR* TKD mutations were observed among 113 cases of

Table II. *Main characteristics of 53 eligible studies included in the systematic review.*

First author (Reference)	Year	Study location	Site/source of tumor profiled	Stage	No. of patients	Patients with *EGFR* mutation, n (%)	Exon location of *EGFR* mutation	Detection method	Prognostic role impact of *EGFR* mutations
Current study	2016	Austria	OC/OP – FFPE	III/IV	113	0 (0)	–	PCR, pyrosequencing, Sanger	NA
Ock *et al*. (28)	2016	Republic of Korea	H&N – FFPE	I-IV	71	19 (26.7)	18-21	Targeted NGS	NA
Feldmann *et al*. (34)	2016	USA	H&N – FFPE	NM+M	360	3 (0.8)	19, 20	Targeted NGS, Sanger	NA
Chau *et al*. (35)	2016	USA	H&N – FFPE	I-IV	213	0 (0)	–	Targeted NGS	NA
Wu *et al*. (36)	2016	USA	H&N – FFPE	I-IV	214	42 (20)	18-20	MALDI-TOF MS	NA
Huang *et al*. (37)	2016	Republic of Korea	H&N – fresh frozen	IV	18	0 (0)	–	Whole exome sequencing	NA
TCGA (25)	2015	USA	H&N – fresh frozen	I-IV	279	1 (0.4)	18	Whole exome sequencing	NA
Vettore *et al*. (38)	2015	Singapore	Tongue – fresh frozen	I-IV	78	0 (0)	–	Whole exome/ targeted NGS	NA
Kim *et al*. (39)	2015	Republic of Korea	H&N – FFPE/ Fresh frozen	III/IV	33	2 (6)	19-21	Targeted NGS	NA
Seiwert *et al*. (26)	2015	USA	H&N – fresh frozen	III-IV	120	0 (0)	–	Targeted NGS	NA
Pickering *et al*. (40)	2014	USA	Tongue – fresh frozen	I-IV	42	0 (0)	–	Whole exome sequencing	NA
Wang *et al*. (41)	2014	China	Larynx – FFPE	I-IV	132	3 (2.3)	20, 21	Multiplex PCR	NA
Tan *et al*. (42)	2014	Singapore	Tongue – fresh frozen	I-IV	66	0 (0)	–	PCR, MALDI-TOF MS	NA
McBride *et al*. (43)	2014	USA	H&N – FFPE	I-IV	64	1 (2)	20	PCR, sequencing	NA
Mehta *et al*. (44)	2014	India	OC – fresh frozen	NA	40	0 (0)	–	PCR, sequencing	NA
Boeckx *et al*. (45)	2014	Belgium	OP/Larynx – FFPE	III-IV	52	0 (0)	–	HRMA	NA
Nagalakshmi *et al*. (46)	2014	India	H&N – fresh frozen	I-IV	129	0 (0)	–	PCR, SSCP, sequencing	NA
Gaykalova *et al*. (47)	2014	USA	H&N – fresh frozen	I-IV	37	0 (0)	–	Targeted NGS	NA
Argiris *et al*.(48)	2013	USA	H&N – FFPE	IV	69	1 (1.4)	20	PCR, pyrosequencing	NA
Maiti *et al*. (49)	2013	India	H&N –fresh frozen	I-IV	148	0 (0)	–	PCR, SSCP, sequencing	NA
Lechner *et al*. (50)	2013	UK	OP – FFPE	I-IV	40	0 (0)	–	Targeted NGS	NA
ICGC (51)	2013	India	OC –fresh frozen	II-IV	50	0 (0)	–	Whole exome sequencing	NA
Pickering *et al*. (52)	2013	USA	OC –fresh frozen	II-IV	35	0 (0)	–	Whole exome sequencing	NA
Fanjul-Fernandez *et al*. (53)	2013	Spain	Larynx – fresh frozen	IV	4	0 (0)	–	Whole exome sequencing	NA
Bontognali *et al*. (54)	2013	Switzerland	H&N –fresh frozen	II,IV	6	0 (0)	–	PCR, sequencing	NA
Bahassi *et al*. (55)	2013	USA	Larynx – FFPE	IV	Case report	1 (100)	18	PCR, sequencing	Positive impact on TR
Smilek *et al*. (56)	2012	Czech Republic	H&N – FFPE	III-IV	29	2 (6.9)	19	Real-Time PCR	Negative impact on TR
Tan *et al*. (57)	2012	Singapore	H&N – FFPE	III-IV	15	2 (13.3)	18, 19	PCR, sequencing	NA
Friedland *et al*. (58)	2012	Australia	H&N – FFPE	NA	60	1 (1.6)	NA	PCR, SSCP, sequencing	NA
Szabo *et al*. (59)	2011	Hungary	H&N – FFPE	I-IV	71	0 (0)	–	Real-time PCR, HRMA	NA
Hsie *et al*. (60)	2011	Taiwan	OC – FFPE	I-IV	56	2 (3.57)	21	PCR, sequencing	NA
Morris *et al*. (61)	2011	USA	H&N – fresh frozen	I-IV	31	0 (0)	–	PCR, sequencing	NA
Agrawal *et al*. (62)	2011	USA	H&N –fresh frozen	II-IV	32	0 (0)	–	Whole exome sequencing	NA
Stransky *et al*. (27)	2011	USA	H&N –fresh frozen	P/R	74	0 (0)	–	Whole exome sequencing	NA
Murray *et al*. (22)	2010	Greece	H&N – FFPE	IV	92	3 (3.3)	19, 21	PCR, sequencing	NS impact on OS
Szymanska *et al*. (63)	2010	South America	UADT –fresh frozen	I-IV	45	2 (4.4)	19, 21	PCR, sequencing	NA

Table II. *Continued*

Table II. *Continued*

First author (Reference)	Year	Study location	Site/source of tumor profiled	Stage	No. of patients	Patients with *EGFR* mutation, n (%)	Exon location of *EGFR* mutation	Detection method	Prognostic role impact of *EGFR* mutations
Van Damme *et al*. (64)	2010	Belgium	Tonsil – FFPE	NA	24	0 (0)	–	PCR, sequencing	NA
Keller *et al*. (65)	2010	USA	OP/Larynx – fresh frozen	I-IV	60	2 (3.3)	19	PCR, sequencing	NA
Hama *et al*. (21)	2009	Japan	H&N –fresh frozen	I-IV	82	5 (6.1)	18, 20, 21	PCR, sequencing	Positive impact on DFS
Huang *et al*. (66)	2009	Taiwan	OC – fresh frozen	I-IV	172	0 (0)	–	PCR, sequencing	NA
Carlson *et al*. (67)	2009	USA	H&N –fresh frozen	NA	20	0 (0)	–	PCR, sequencing	NA
Jin *et al*. (68)	2009	China	H&N –fresh frozen	I-IV	96	0 (0)	–	PCR, sequencing	NA
Schwentner *et al*. (69)	2008	Austria	H&N – FFPE/ fresh frozen	NA	127	3 (2.4)	19, 20	PCR, sequencing	NA
Sheikh Ali *et al*. (70)	2008	Japan	H&N –fresh frozen	I-IV	91	0 (0)	–	PCR, sequencing	NA
Chiang *et al*. (71)	2008	Taiwan	OC – FFPE	I-IV	20	0 (0)	–	PCR, sequencing	NA
Na *et al*. (23)	2007	Republic of Korea	Tongue/tonsil – FFPE	I-IV	108	17 (15.7)	19-21	PCR, sequencing	NS impact on OS
Temam *et al*. (72)	2007	USA/France	H&N – FFPE/ fresh frozen	I-IV	134	0 (0)	–	PCR, sequencing	NA
Lemos-Gonzalez *et al*. (73)	2007	Spain	H&N – fresh frozen	NA	31	0 (0)	–	PCR, SSCP, sequencing	NA
Perrone *et al*. (74)	2006	Italy	OP – FFPE	I-IV	40	1 (2.5)	19	PCR, sequencing	NA
Chung *et al*. (75)	2006	USA	H&N – fresh frozen	I-IV	52	0 (0)	–	PCR, sequencing	NA
Willmore-Payne *et al*. (76)	2006	USA	H&N – FFPE	NA	24	2 (8.3)	19, 20	PCR, HRMA, sequencing	NA
Cohen *et al*. (77)	2005	USA	H&N – FFPE/ fresh frozen	NA	82	0 (0)	–	PCR, sequencing	NA
Lee *et al*. (78)	2005	Republic of Korea	H&N – FFPE	NA	41	3 (7.3)	19	PCR, SSCP, sequencing	NA

NA, Not available; NS, non-significant; EGFR, epidermal growth factor receptor; TKD, tyrosine kinase domain; OC, oral cavity; OP, oropharynx; H&N, head and neck; UADT, upper aerodigestive tract (included oral cavity, pharynx, larynx and oesophagus); FFPE, formalin-fixed paraffin-embedded; OS, overall survival; DFS, disease-free survival; TR, treatment response; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MFI, median fluorescence intensity; HRMA, high resolution melting analysis; SSCP, single-strand conformational polymorphism; NGS, next-generation sequencing.

OOSCC. The results of this cohort study showed, however, a strong association of pathological complete response to neoadjuvant treatment with improved overall survival of patients with OOSCC, thus indicating the need for discovery of predictive biomarkers.

The identification of activating mutations in the *EGFR* TKD in a subset of NSCLC and their association with substantial sensitivity to gefitinib, erlotinib or afatinib represents an important milestone in the therapy of this malignancy (14, 17, 24). Driven by the paradigm in NSCLC, several studies in HNSCC attempted to define the mutational spectrum of the *EGFR* TKD. To date, genomic data from whole exome sequencing and targeted next-generation sequencing studies have provided a comprehensive landscape of genomic alterations in HNSCC (25-27). In a recent study, Ock *et al*. using targeted next-generation sequencing identified EGFR TKD mutations in 19 out of 71 (26.7%) HNSCCs (28). The Cancer Genome Atlas data from whole exome sequencing of HNSCCs demonstrated, however, that only one out of 279 (0.4%) tumor samples from HNSCCs harbored a missense mutation in the *EGFR* TKD (25). Given this background, the present systematic review aimed to summarize current evidence regarding the *EGFR* mutation status in HNSCC. Based on the quantitative data analysis, this study demonstrated that the overall prevalence of *EGFR* TKD mutations in HNSCC is 2.8%. This study revealed that the *EGFR* mutation prevalence in patients with HNSCC varies modestly across geographic regions, with the highest prevalence shown (4.9%) in Southeast Asia and the lowest in South Asia (0%). The *EGFR* mutation prevalence within the population of Southeast Asia varies by country, from approximately 1% in Taiwan to 15% in the Republic of

Korea. In addition, this study showed that the EGFR mutation status in HNSCC has been insufficiently assessed worldwide as evident from the limited number of studies conducted in Australia (n=1) and South America (n=1), and the lack of data from several large geographic regions, particularly Africa, Central America, the Middle East, and Central Asia. Therefore, it is apparent that large-scale and multicenter studies are necessary to provide more definitive answers regarding the prevalence of *EGFR* mutations across geographic regions and countries and to assess their potential clinical value in patients with HNSCC.

In this systematic review, the overall *EGFR* mutation status in HNSCC according to exon location and mutation type was explored. The data showed that the most prevalent *EGFR* kinase domain mutations, accounting for 73% of all *EGFR* mutations in HNSCC, are missense mutations in exons 18-21. The L858R substitution, well-known in NSCLC, which comprises about 40% of all *EGFR* mutations in NSCLC and is associated with sensitivity to EGFR TKIs, was found in only 2.5% of all *EGFR*-mutated HNSCCs (29). The missense mutation T790M in exon 20, which is associated with acquired resistance to EGFR TKIs in about half of all patients with NSCLC, was found in 7.5% of all *EGFR* mutations in HNSCC (17). In-frame deletions in exon 19, which account for about 45% of all EGFR mutations in NSCLC and are linked to responsiveness to EGFR TKIs, were observed in 22% of all *EGFR*-mutated HNSCCs (24). Insertion mutations in exon 20, which occur in about 3% of all *EGFR* mutations in NSCLC and are frequently associated with resistance to EGFR TKIs, were observed in 5% of all *EGFR* mutations in HNSCC (30). Taken together, it is clear that substantial differences exist between HNSCC and NSCLC regarding the distribution of mutations within exons 18-21 of the EGFR TKD. Unlike NSCLC, *EGFR* mutations in HNSCC do not involve specific hotspots but are rather scattered throughout exons 18 to 21. Thus, mutation screening in HNSCC should not be limited to the NSCLC hotspot regions in exons 19 and 21 of *EGFR*. Moreover, given that the overall prevalence of *EGFR* TKD mutations in HNSCC is 2.8%, it is challenging to identify specific *EGFR* mutations related to response or resistance to anti-EGFR therapy or other targeted therapies (31).

The present cohort study has some weaknesses, including its retrospective nature and the relatively small sample size. Additionally, next-generation sequencing methods to compare and validate the results of the *EGFR* mutation testing by pyrosequencing were not used. However, recent studies have shown that pyrosequencing has the ability to detect *EGFR* mutations at a low ratio of mutant to wild-type alleles and thus provides high analytical sensitivity for identifying *EGFR* mutations (32, 33). The systematic review is limited in several ways. Firstly, high heterogeneity has to be assumed across the study populations given the differences in study location, tumor site, stage and interventions. Secondly, various mutation testing methods with different sensitivities in detecting *EGFR* mutations were used across studies. Thirdly, a number of studies limited their *EGFR* mutation testing to hotspot regions in exons 19 and 21, thus the true prevalence of *EGFR* mutations might in fact be under-reported. Taken together, the results of the quantitative synthesis should be interpreted cautiously.

In the emerging era of personalized medicine, the identification of clinically useful prognostic and, most importantly, predictive biomarkers to guide treatment decision in patients with cancer is urgently needed. In this study, no mutations were detected using pyrosequencing when analyzing the *EGFR* TKD mutation status in a cohort of 113 patients with advanced OOSCC. In addition, the systematic review demonstrated that *EGFR* TKD mutations are rare in HNSCC, with an overall prevalence of 2.8% and modest variation in the prevalence across countries and

Table III. *Prevalence of epidermal growth factor receptor (EGFR) kinase domain mutations in patients with head and neck cancer by country and geographic region.*

Geographic region/country	No. of studies*	Patients with *EGFR* mutation/ total patients	*EGFR* mutation prevalence, % (95% CI)
Overall	53	117/4122	2.8 (2.4-3.4)
Europe	13	9/670	1.3 (0.7-2.6)
Austria	2	3/240	1.3 (0.3-3.9)
Spain	2	0/35	0
Belgium	2	0/76	0
UK	1	0/40	0
Switzerland	1	0/6	0
Czech Republic	1	2/29	6.9 (1.2-24.2)
Hungary	1	0/71	0
Greece	1	3/92	3.3 (0.8-9.9)
France	1	0/41	0
Italy	1	1/40	2.5 (0.1-14.7)
North America	19	52/1901	2.7 (2.1-3.6)
USA	19	52/1901	2.7 (2.1-3.6)
Southeast Asia	15	53/1079	4.9 (3.7-6.4)
Republic of Korea	5	41/271	15.1 (11.2-20.1)
Taiwan	3	2/248	0.8 (0.1-3.2)
Singapore	3	2/159	1.3 (0.2-4.9)
Japan	2	5/173	2.9 (1.1-7.0)
China	2	3/228	1.3 (0.3-4.1)
South Asia	4	0/367	0
India	4	0/367	0
South America	1	2/45	4.4 (0.8-16.4)
Australia	1	1/60	1.7 (0.1-10.1)

*The study of Temam *et al.* (72) included patients from two distinct study locations: 41 patients from France and 93 from the United States, who were categorized into European and North American populations, respectively. The study of Bahassi *et al.* (55) was not considered for prevalence estimation (case report).

geographic regions. Large-scale studies are warranted to provide further up-to-date evidence regarding the mutation status of *EGFR* in patients with HNSCC and to investigate whether the *EGFR* mutation profile of individual tumors is associated with sensitivity or resistance to targeted therapy.

Conflicts of Interest

None declared.

Financial Disclosure

None.

Grant Support

This project was supported by fund of the Oesterreichische Nationalbank, Anniversary Fund, Project Number 13469.

Acknowledgements

The Author thanks Martin Filipits, Anita Brandstetter, and Ágnes Bilecz for their excellent technical support. The Author thanks Robert Pillerstorf and Johannes Engelmann for their contribution to the systematic review with regard to study selection and data extraction.

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Received November 7, 2016
Revised December 6, 2016
Accepted December 8, 2016