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(54) Title: HEAD AND NECK SQUAMOUS CELL CARCINOMA ASSAYS

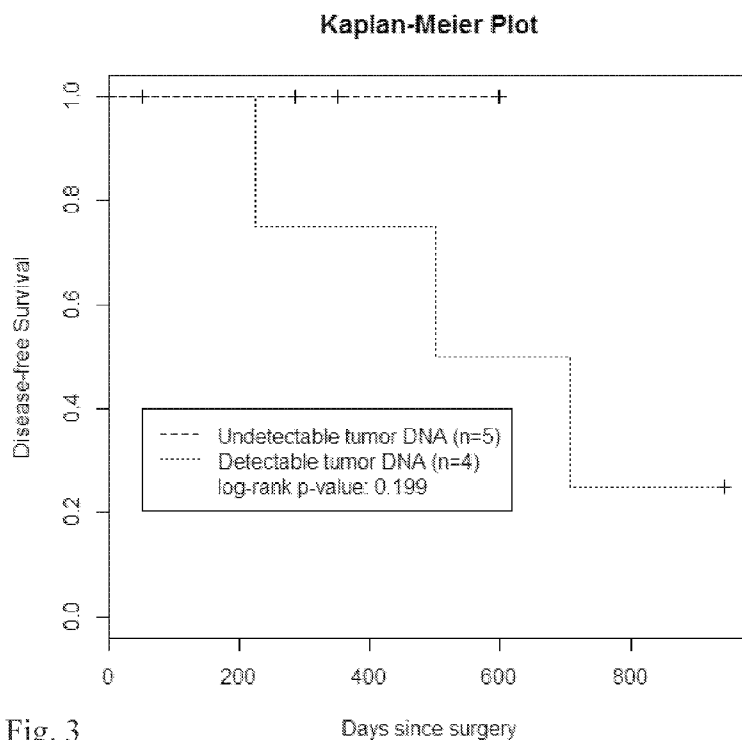


Fig. 3

(57) Abstract: We queried DNA from saliva or plasma of 93 HNSCC patients, searching for so-
matic mutations or human papillomavirus genes,
collectively referred to as tumor DNA. When both
plasma and saliva were tested, tumor DNA was de-
tected in 96% (95% CI, 84% to 99%) of 47 pa-
tients. The fractions of patients with detectable tu-
mor DNA in early- and late-stage disease were
100% (n=10) and 95% (n=37), respectively. Saliva
is preferentially enriched for tumor DNA from the
oral cavity, whereas plasma is preferentially en-
riched for tumor DNA from the other sites. Tumor
DNA in the saliva and plasma is a valuable bio-
marker for detection of HNSCC.



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HEAD AND NECK SQUAMOUS CELL CARCINOMA ASSAYS

[01] This invention was made with government support under CA 43460, CA 57345, and DE019032 awarded by NIH. The government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

[02] This invention is related to the area of cancer. In particular, it relates to assays for cancer.

BACKGROUND OF THE INVENTION

[03] Head and neck squamous cell carcinomas (HNSCC) are the 7th most common cancer worldwide, occurring in more than half a million new patients each year and >50,000 patients in the United States alone (1, 2). The incidence of certain types of HNSCC appears to be increasing, especially among young people, in part due to the increasing prevalence of human papilloma virus (HPV) (3-7). HNSCCs are associated with a poor five-year overall survival of only ~50% that has remained relatively unchanged, especially for patients with HPV-negative tumors (8). Only a few targeted therapies for this disease are available, in part because of the paucity of activating mutations in oncogenes that contribute to tumor development; most genetic alterations in HNSCCs inactivate tumor suppressor genes (9-12). There are also no available biomarkers for HNSCC to measure disease burden or response to therapy, further limiting progress in mitigating the impact of this often morbid and potentially lethal disease on human health.

[04] Though HNSCC tumors are usually classified based on histology, their biomedical properties, including demographics, risks factors, and clinical behavior, differ by anatomic site (Fig. 1) (13, 14). Anatomically, the tumors are categorized as squamous cell carcinomas (SCC) of the oral cavity (including the oral tongue), oropharynx (including the base of the tongue), larynx, and hypopharynx. Oral cavity SCC, with the exception of those of the oral tongue, is declining in incidence in the United States due to

the reduction in cigarette smoking (4). In contrast, there is an increasing incidence of oropharyngeal SCC involving the palatine and lingual (base of tongue) tonsils, particularly in younger men. These tumors are often associated with HPV. The survival of these patients is better than for those whose tumors are unassociated with HPV (6, 15). Laryngeal SCC is declining in incidence and, unlike HNSCC at other sites, is generally associated with limited regional metastasis due to anatomic barriers (16). The hypopharynx is the least common site for HNSCC and has decreasing incidence but relatively poor prognosis (17, 18).

- [05] The idea that the genetic alterations present in tumors can be used as biomarkers for cancer was proposed more than two decades ago (19-22). The advantage of genetic alterations over conventional biomarkers such as carcinoembryonic antigen (CEA) or prostate specific antigen (PSA) is that genetic changes are exquisitely specific for neoplastic cells. One challenge in exploiting genetic alterations for this purpose is that the concentration of mutant templates is often low in bodily fluids. Over the last several years, however, technological advances have made it possible to detect such mutations even when they are rare. These advances have facilitated the detection of altered DNA sequences in plasma, stool, Pap smear fluids, sputum, and urine (20, 21, 23-30). There is a continuing need in the art for more sensitive means to detect cancers.

SUMMARY OF THE INVENTION

- [06] According to one aspect of the invention a method is provided. The method comprises assaying for tumor DNA in plasma from a Head and Neck Squamous Cell Carcinoma (HNSCC) patient; and assaying for tumor DNA in saliva from a HNSCC patient.
- [07] According to another aspect of the invention another method is provided. The method comprises assaying for tumor DNA in plasma from a subject; and assaying for tumor DNA in saliva from a subject.

- [08] These and other embodiments which will be apparent to those of skill in the art upon reading the specification provide the art with

BRIEF DESCRIPTION OF THE DRAWINGS

- [09] Fig. 1. Schematic drawing showing the shedding of tumor DNA from head and neck cancers into the saliva or plasma. Tumors from various anatomic locations shed DNA fragments containing tumor-specific mutations and HPV DNA into the saliva or the circulation. The detectability of tumor DNA in the saliva varied with anatomic location of the tumor, with the highest sensitivity for oral cavity cancers. The detectability in plasma varied much less in regards to the tumor's anatomic location.
- [10] Fig. 2. (Fig. S1.) Tumor DNA is detectable in the saliva of patients before recurrence becomes clinically evident. Nine patients were followed-up for a median of 12 months after surgery. Dashed lines transition to solid lines when tumor DNA was detected after surgery. *Twenty-five months prior to surgery, patient 367 also underwent chemoradiation therapy (not shown).
- [11] Fig. 3. (Fig. S2.) Patients with undetectable tumor DNA after surgery have better disease-free survival.
- [12] Figs. 4A-4B. (Table S1.) Patient demographics. The clinical information, tumor characteristics, and presence of tumor DNA in samples collected from 93 patients enrolled in this study are listed (OC = oral cavity, OP = oropharynx, L = larynx, H = hypopharynx, and NA = not available).
- [13] Fig. 5. (Table S2.) Primer sequences used in multiplex assay for identification of driver mutations in tumors. Amplicons included commonly mutated gene regions in HNSCC. Forward primers (SEQ ID NO: 5-49) and Reverse primers (SEQ ID NO: 50-94).

- [14] Figs. 6A-6U. (Table S3). Amounts of tumor-derived DNA in saliva and plasma. The mutation identified in the tumor of each **patient** is listed, along with the fractions of mutant allele in saliva and plasma. In addition, the numbers of mutant and wild-type reads in patient samples vs. controls are listed for each mutation assayed. For patients with recurrent disease, treatments received and the interval prior to sample collection are listed. Patients in **BOLD** had both saliva and plasma available (OC = oral cavity, OP = oropharynx, H = hypopharynx, and L = larynx).

DETAILED DESCRIPTION OF THE INVENTION

- [15] The inventors have developed assays that are useful for detecting, monitoring, and predicting the course of head and neck squamous cell carcinoma (HNSCC). Some of the cancers are associated with HPV infection, and the cancer cells and body fluids contain HPV viral sequences. Some of the cancers are not associated with HPV infection but rather contain somatic mutations that are specific to the cancer. By testing both saliva and plasma of a subject, an extremely high level of sensitivity can be achieved.
- [16] HPV sequences which can be used in the assay are any that are found in HNSCC. Typically, the most prevalent are HPV-16 sequences, although HPV-18 sequences are also often found in cancers. Any HPV genetic sequence can be used, including any of genes E1, E2, E3, E4, E5, E6, E7, L1, and L2. Particularly useful genetic sequences are E7 sequences or parts of the E7 sequence.
- [17] Somatic mutations that frequently occur in HNSCC include but are not limited to TP53, NOTCH1, PI3KCA, CDKN2A, FBXW7, HRAS, and NRAS. In following a single patient one may determine a somatic mutation in a gene in a biopsy or surgical sample. That somatic mutation may be used in subsequent assays in the same or different sample types. If no somatic mutation or HPV infection has been determined for a patient, a panel of frequently-involved-in-HNSCC genes may be used including an HPV-16

sequence, TP53, PIK3CA, NOTCH1, and CDKNA2, for example. Other panels of other frequently mutated genes in HNSCC and HPV sequences may be used as well.

- [18] Samples to be assayed for HNSCC related nucleic acid sequences may be from saliva, plasma, or both saliva and plasma. The nucleic acids assayed may be in cells, extracellular, or both. Samples may be collected at multiple time points and assayed in real time, *i.e.*, close to the time of collection, or they may be held and assayed in batches. However, a plasma sample and a saliva sample should preferably be collected at the same time from a subject.
- [19] Subjects may have been diagnosed with HNSCC or not prior to performing assays. Subjects may have been subjected to surgery to remove or debulk tumor. Subjects may be at elevated risk for HNSCC due to risky behavior such as exposure to HPV, exposure to tobacco smoke, either primary or secondary, and/or heavy use of alcohol. Subjects may be male or female. Subjects may be related to a patient with HNSCC or not.
- [20] Current diagnostic methods for HNSCC make challenging the detection of early disease, assessment of response to treatment, and differentiation between the adverse effects of treatment vs. persistent or recurrent disease. These issues collectively compromise clinical decision-making and impair patient management. Though it is now abundantly clear that all cancers, including HNSCC, are the result of genetic alterations, this knowledge is just beginning to be applied to meet diagnostic challenges such as those described above (10). In this study, we show that tumor-derived DNA can be detected in the saliva of patients with HNSCC. We also show that the evaluation of plasma can complement that of saliva, together allowing detection of tumor-derived DNA in readily obtainable bodily fluids in >90% of the studied patients. Our findings enable clinical tests designed for the earlier detection of HNSCC, either for patients at high risk for the disease or patients previously treated for HNSCC who are at risk for disease recurrence. Moreover, these results enable monitoring the response to treatment.

- [21] There were several notable findings in this study. The sensitivity for detection of tumor-derived DNA in the saliva was site-dependent and most efficient for tumors in the oral cavity. Not only was tumor DNA detectable in every one of the 46 patients with cancers of the oral cavity, but the fraction of mutant DNA in the saliva was particularly high (median 0.65%, interquartile range 0.17% - 2.2%, mean 3.46%). Moreover, early stage oral cavity cancers were highly detectable; 75% of the patients were oral cavity cancer were at an early stage (Stage I or II), and all were detectable. The high fraction of tumor DNA in the saliva of patients with oral cancers makes anatomical sense and demonstrates the advantage of examining local bodily fluids for optimal sensitivity in this type of assay.
- [22] HNSCCs distal to the oral cavity (oropharynx, larynx, and hypopharynx) were still often detectable through the examination of saliva, but the frequency of their detection (47%, 70%, and 67%, respectively) and the fraction of mutant alleles (median 0.015%) were considerably lower than found in the oral cavity (0.65%). Anatomical locations likely explain this difference. Gargling may be used to increase the detectability of tumor DNA in these distal compartments.
- [23] One striking aspect of this study is the increased sensitivity demonstrated when both compartments are assayed. This increased sensitivity is possible only because of the exquisite specificity of mutant DNA as biomarker; because no false positives are expected, any number of assays can be combined, increasing sensitivity without compromising specificity. The combination of saliva and plasma allowed detection of 96% of the cancers when both fluids were available, higher than obtained with either saliva or plasma alone.
- [24] Our study enables the use of saliva and plasma to reveal the presence of HNSCCs. In each patient, we first evaluated the tumor, then used an alteration (either the presence of HPV or a somatic mutation) to query the saliva or plasma. In a clinical context, a panel of genes should be used to assess each case. Fortunately, technologies are available for finding mutations, even those present at low frequencies (25, 35). Based on the results

presented herein, as well as large studies of HNSCC genetics (9, 11, 12), a panel including HPV16 DNA sequences, *TP53*, *PIK3CA*, *NOTCH1*, and *CDKN2A* would be able to detect >95% of invasive HNSCCs. Another limitation of our study is that the number of early stage cancers beyond the oral cavity was small, in part reflecting the unfortunate fact that most of these cancers are detected only when they are late-stage. Future larger studies should be able to determine how often early stage cancers of the oropharynx, larynx, and hypopharynx can be detected using the approach described here. The fact that at least 70% of the oropharyngeal cancers in the United States are associated with HPV simplifies this task (6, 15).

[25] One important application of our results is in the diagnosis of clinically suspicious lesions. The often complex and highly specialized nature of current HNSCC diagnostic procedures can lead to delays in diagnosis and treatment, negatively impacting prognosis and survival (41-46). These delays could be prevented in many patients through the examination of saliva and plasma for tumor DNA. Such a test could potentially be incorporated into routine examinations to complement current diagnostic modalities and inform clinical decision-making. Another application of our results is in disease monitoring and surveillance. In nine patients with positive pre-treatment saliva and/or plasma, samples were collected at various times after surgery. The fact that no mutations were identified after surgery in the five patients whose tumors did not recur highlights the specificity of the mutation-based assay. It was also encouraging that we identified tumor DNA in the saliva of patients whose tumors were found to recur at the clinical level only months later, indicating that these tests can provide a clinically meaningful lead time. The results presented here indicate that the presence of tumor DNA in either saliva or plasma can be used to help manage patients who appear free of disease after definitive treatment by clinical criteria.

[26] The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLES

- [27] In this study, we determined whether genetically altered DNA could be detected in the saliva or plasma of HNSCC patients with tumors of various stages and anatomical sites. We chose these two bodily fluids for obvious reasons: plasma has been shown to harbor tumor DNA from many cancers, including HNSCC, though only a few HNSCCs, all of late stage, have been previously examined (23, 31, 32). Tumor DNA that is released from the basal side of HNSCC epithelial cells into the lymphatics or venous system should be detectable in this compartment. On the other hand, DNA that is released primarily on the apical side of HNSCC should be detectable in the saliva (23, 31, 32). The studies described below were performed to test these hypotheses

EXAMPLE 1

MATERIALS AND METHODS

- [28] Study design

- [29] This was a retrospective study with sample collection performed prospectively from 93 HNSCC patients donating saliva, 47 of whom also donating plasma. Data analysis was performed in a blinded fashion and all patient samples were de-identified.

Samples

- [30] All samples from the 93 patients in this study were collected using Institutional Review Board (IRB) approved protocols at Johns Hopkins University and MD Anderson Medical Center. None of the patients in the current study were included in the previously published study from our groups, in which the genomic landscapes of HNSCC were described (9). Saliva samples were collected before definitive treatment for primary

HNSCC (n=71, 76% of 93 patients) and before salvage treatment for recurrent HNSCC (n=22, 24% of 93 patients). In a subset of these patients (n=9), post-treatment saliva was also collected for surveillance. Most patients (95% of the 93) underwent a biopsy of the primary tumor and/or metastatic lymph node, on average 44 days prior to the first sample collection. (Fig. 6.) For the 22 patients with recurrent disease, previous treatment including an iteration of surgery, radiation, and/or chemotherapy was completed an average of 2.9 years before sample collection. (Fig. 6.)

- [31] Whole blood was collected from 47 of the 93 patients before treatment. Four to 10 mL of plasma was used for DNA purification, with the average amount of plasma being 6 mL.
- [32] Saliva was collected using two different protocols. Under the JHU protocol, patients were asked to swish 15-20 mL of 0.9% sodium chloride in their mouths for 10-15 seconds before spitting into the collection tube. Under the MD Anderson protocol, patients were asked to allow saliva to collect in the floor of the mouth for five minutes without swallowing before spitting into the collection vial. There was no significant difference in the amounts of DNA purified, the fraction of mutant DNA, or the amount of HPV sequences found with the two protocols. Saliva was frozen at -80 C until DNA purification, and the entire volume of saliva, without centrifugation of cells, was used for DNA purification. The amount of saliva used averaged 15 mL (range 10 to 20 mL).
- [33] When fresh tumor tissue from a surgical specimen of invasive SCC was available, it was immediately frozen at -80°C. When frozen tissue was not available, formalin-fixed, paraffin-embedded (FFPE) tissues were used for DNA purification. In either case (fresh-frozen or FFPE), tumors were macro-dissected to ensure neoplastic cellularity exceeding 30%. DNA was purified from the white blood cell pellet (normal DNA), saliva, plasma, and tumor using an AllPrep kit (Qiagen, cat #80204).

Tumor Mutational Profiling

- [34] A tiered approach was used to identify a somatic mutation within each tumor. Initially, the presence of HPV16 and HPV18 was assessed using the primers specific for the E7 oncogene of these variants (HPV16: TGTGACTCTACGCTTCGGTTG (SEQ ID NO: 1) and GCCCATTAACAGGTCTTCCA (SEQ ID NO: 2); HPV18: GCATGGACCTAAGGCAACAT (SEQ ID NO: 3) and GAAGGTCAACCGGAATTTTCAT (SEQ ID NO: 4)). When no HPV was present, multiplex PCR reactions containing primers amplifying regions of interest in *TP53*, *PIK3CA*, *CDKN2A*, *FBXW7*, *HRAS*, and *NRAS* were used to identify driver mutations in the tumors (Fig. 5). If a mutation was not identified in the queried regions, paired-end libraries of DNA from the tumors and white blood cell pellets of each patient were prepared and used for low-coverage whole genome sequencing or exomic sequencing as previously described (47). Massively parallel sequencing was carried out on an Illumina HiSeq instrument, either in the Goldman Sequencing Facility at Johns Hopkins Medical Institutions or at PGDx, Inc. Point mutations were identified as previously described (9, 47, 48), using the following criteria: allele fraction > 20%, >5 reads for point mutations, and >1 read for translocations. Genomic rearrangements were identified through an analysis of discordantly mapping paired-end reads. The discordantly mapping paired-end reads were grouped into 1kb bins when at least 5 distinct tag pairs (with distinct start sites) spanned the same two 1kb bins, and further annotated based on the approximate breakpoint (34). One selected mutation was confirmed through an independent PCR and sequencing reaction, and then used to query the saliva or plasma.

Mutation Detection in Saliva and Plasma

- [35] The same primers used to detect HPV16 in tumor DNA via PCR were used to detect HPV16 sequences in the DNA from saliva or plasma. Each saliva DNA or plasma DNA sample was assessed in at least three independent PCR assays, and all three assays had to be positive for the sample to be counted as positive. As an additional control for specificity, the PCR products were sequenced to ensure that they represented HPV16 sequences. To quantify the amount of HPV16 sequences present in saliva or plasma, we used digital PCR with the same primers (36). Digital PCR was also used to quantify the

amount of sequences with translocation using primers spanning the breakpoints, as previously described (49). For evaluation of point mutations in saliva or plasma, we used Safe-SeqS, a PCR-based error-reduction technology for detection of low frequency mutations in reactions each containing up to 3 ng of input DNA (23, 25). High quality sequence reads were selected based on quality scores, which were generated by the sequencing instrument to indicate the probability a base was called in error (50). The template-specific portion of the reads was matched to reference sequences. Reads from a common template molecule were then grouped based on the unique identifier sequences (UIDs) that were incorporated as molecular barcodes. Artifactual mutations introduced during the sample preparation or sequencing steps were reduced by requiring a mutation to be present in >90% of reads in each UID family ("supermutant"). Each PCR assay for each plasma or saliva sample was independently repeated at least three times, with the mutant allele fractions defined as the total number of supermutants divided by the total number of UIDs in all experiments. DNA from normal individuals was used as control, using at least five independent assays per queried mutation. Only saliva or plasma samples in which the mutant allele fractions significantly exceeded their frequencies in control DNA (P-value < 0.05) were scored as positive) (details provided in Fig. 6.).

Statistical Analysis

- [36] Sensitivity for the detection of tumor-specific mutations in the blood and saliva was calculated by tumor site, stage, and among HPV-associated tumors. Ability to detect tumor DNA in saliva and/or plasma was tested using Fisher's exact tests, and Wilcoxon rank sum tests were used to compare amounts of tumor DNA in saliva vs. plasma (51). For the comparison of mutant fractions in patients vs. control in Safe-SeqS assays, p-values were calculated using a two-sided chi-squared test of equal proportions or Fisher's Exact Test when conditions of the Chi-squared test are not met. The concordance between mutant fractions in saliva and plasma was calculated using Pearson's product-moment correlation coefficient, a standard measure of linear dependence between two variables. All statistical analyses were performed using the R statistical package version 3.1.2.

EXAMPLE 2

Mutations in primary tumors

- [37] Ninety-three patients with HNSCC were enrolled in this study. Their average age was 60 and the majority (83%) were male, as is typical of HNSCC patients (Fig. 4). Forty-six, 34, 10, and 3 samples were from the oral cavity, oropharynx, larynx, and hypopharynx, respectively. Twenty patients (22%) had early (Stage I or II) disease and the remaining 73 patients (78%) had advanced (Stage III or IV) disease.
- [38] To begin this study, we attempted to identify at least one genetic alteration in each tumor. We first searched for the presence of either HPV16 or HPV18 sequences in tumor DNA. HPV is a well-established etiologic agent for a growing subset of HNSCCs, specifically oropharyngeal SCC (6, 15). With PCR primer pairs specific for the E7 gene of the high-risk HPV types responsible for the overwhelming majority of HPV-associated HNSCC, we identified 30 patients (32%) whose tumors contained HPV16 DNA and no patients with HPV18. The preponderance of HPV16 is not surprising given prior epidemiologic studies of this tumor type (33). In the other 63 patients (all of those without HPV), we searched for somatic mutations in genes or gene regions commonly altered in HNPCC, including *TP53*, *PIK3CA*, *CDKN2A*, *FBXW7*, *HRAS*, and *NRAS*, using multiplex PCR and massively parallel sequencing (Fig. 5) (2, 11, 12). This allowed us to identify a driver mutation in 58 of the 63 samples. In the remaining 5 samples, genome-wide sequencing was performed at low coverage with the goal of identifying one driver mutation or translocation as previously described (34). Ultimately, we identified and validated one genetic alteration in each of these 63 samples (Fig. 6). The most commonly mutated gene was *TP53* (86% of 63 patients). We also searched for mutations in the tumors of 25 of the patients with HPV and found mutations in twelve of those samples (Fig. 6).

EXAMPLE 3

Mutations in saliva and plasma

- [39] Important characteristics of screening tests are that samples can be easily collected without discomfort and that the collection process is standardized. To achieve these goals, we used oral rinses, plasma, and commercially available kits to prepare DNA for conventional genotyping purposes. For saliva, we used the entire contents of the collection tube (including cells and cell debris) to prepare DNA. Of the 93 patients who donated saliva for this study prior to their surgery, 47 patients (51%) volunteered to donate plasma at the same time. DNA from plasma was purified as previously described (23). Digital PCR was used to query HPV sequences and translocations (36), whereas point mutations were assessed by Safe SeqS, a PCR-based technology for the detection of low-frequency mutations, as previously described (23, 25, 34-36).
- [40] Tumor DNA was identified in 76% (n=93) and 87% (n=47) of the saliva and plasma samples from these patients, respectively (**Table 1, Figs. 4 and 6**). In the subset of patients with both plasma and saliva samples, 96% (n=47) of patients had a tumor-specific alteration identified in at least one bodily fluid. Twenty-one of the 47 patients had HPV-positive tumors. Eighteen of the 21 patients (86%) had detectable HPV DNA in their plasma and/or saliva (**Table 1, Figs. 4 and 6**). Because HPV-16 is rarely found in oral specimens of healthy individuals, we analyzed 10 saliva or plasma samples from patients whose tumors were not HPV-positive as controls (37, 38). As expected, no HPV was detected in any of these samples, confirming the specificity of the test. In all 26 patients without HPV-positive tumors, endogenous DNA mutations, mostly in the *TP53* gene (92%), were identified in plasma or saliva. (Fig. 6.) Thus, somatic mutations and HPV sequences were both useful as biomarkers for malignancy. The sensitivity of these biomarkers for detecting cancer was greatly improved when both plasma and saliva were examined, compared to testing saliva or plasma alone. There was no significant correlation between the amounts of tumor DNA in saliva vs. plasma in the patients in

whom both sample types were available (correlation coefficient of 0.074, p-value = 0.74). However, the utility of saliva vs. plasma, and HPV vs. somatic mutations, differed considerably with respect to the site of disease, as noted below.

Table 1: Detection of Tumor Derived DNA in Saliva and Plasma. The percentages of patients whose tumors were detectable through the examination of saliva, plasma, or both are shown, grouped by tumor site, stage, and HPV status.

	Saliva - % with mutations (95% confidence intervals) (total number studied)	Plasma - % with mutations (95% confidence intervals) (total number studied)	Saliva or plasma - % with mutations (95% confidence intervals) (total number studied)*
Site			
Oral cavity	100% (92%-100%) (46)	80% (52%-96%) (15)	100% (78%-100%) (15)
Oropharynx	47% (30%-65%) (34)	91% (71%-99%) (22)	91% (71%-99%) (22)
Larynx	70% (35%-93%) (10)	86% (42%-99%) (7)	100% (59%-100%) (7)
Hypopharynx	67% (9.4%-99%) (3)	100% (29%-100%) (3)	100% (29%-100%) (3)
Stage			
Early (I and II)	100% (83%-100%) (20)	70% (35%-93%) (10)	100% (69%-100%) (10)
Late (III and IV)	70% (56%-80%) (73)	92% (78%-98%) (37)	95% (82%-99%) (37)
HPV			
Positive	40% (23%-59%) (30)	86% (64%-97%) (21)	86% (64%-97%) (21)
Total	76% (66%-85%) (93)	87% (74%-95%) (47)	96% (85%-99%) (47)

*Includes only patients from whom both saliva and plasma were available.

EXAMPLE 4

Site

[41] All (100%) of the 46 patients with oral cavity cancers harbored detectable tumor DNA in their saliva (**Table 1**). The sensitivities of detection in saliva of malignancy at sites not directly sampled by an oral rinse were lower: 47% (n = 34), 70% (n = 10), and 67% (n = 3) of patients with oropharyngeal cancers, laryngeal cancers, and hypopharyngeal cancers

had detectable tumor DNA, respectively. The detection rate of tumor DNA in plasma varied less with site, as expected: 80% (n=15), 91% (n=22), 86% (n=7), and 100% (n=3) of tumors of the oral cavity, oropharynx, larynx, and hypopharynx, respectively, had detectable tumor DNA in plasma.

- [42] It is well-known that HPV-associated tumors are most often found at specific sites, particularly the oropharynx. Twenty-nine of the 34 (85%) oropharyngeal cancers were HPV-positive. The remaining five oropharyngeal cancers were negative for HPV by PCR, were associated with tobacco use, and harbored *TP53* mutations. In striking contrast, all but one of 59 samples from the oral cavity, larynx, and hypopharynx were HPV-negative. The finding that only 1 of the 46 oral cavity cancers tested was HPV-positive is consistent with recent evidence about the low prevalence of HPV-related cancers in the oral cavity (39, 40). For the HPV-associated cancers, which represent 30 (32%) of the total HNSCCs in our study, the presence of HPV DNA in bodily fluids represents a very convenient marker: HPV was detected in 40% (n= 30) of saliva samples and 86% (n=21) of available plasma samples with a single primer pair specific for the E7 gene of HPV16 (Table 1).
- [43] Collectively, these data indicate that plasma rather than saliva is the optimal fluid for detecting tumor DNA in tumors of the oropharynx, larynx, and hypopharynx. Of the 32 patients with tumors from these sites in which both plasma and saliva were available, mutant DNA was detected in more plasma samples than saliva samples (29 vs. 18, respectively). More importantly, the amount of detectable mutant DNA alleles, expressed as a fraction of the total alleles assessed, was ~10-fold higher in the plasma compared with the saliva of these patients (median 0.146% vs. 0.015%, p=0.005, Wilcoxon rank sum test). (Fig. 6.) The higher fraction of alleles considerably simplifies the task of identifying such mutations. This pattern was not observed in the oral cavity: the fraction of patients harboring mutant DNA, as well as the mutant allele fraction, was similar in the saliva and plasma (median 0.65% vs. 0.54%, p=0.14, Wilcoxon rank sum test) (Fig. 6.)

EXAMPLE 5

Stage

- [44] The majority of HNSCC patients have advanced disease (Stage III or IV) at diagnosis (2). Accordingly, only 22% of the 93 patients in our cohort presented with early stage disease. (Fig. 4) Overall, tumor-specific DNA could be detected in the plasma or saliva of 100% (n=20) and 86% (n=73) of patients with early and advanced disease, respectively (p=0.116, Fisher's exact test). Saliva provided a more sensitive predictor of early stage disease than plasma: 100% (15 of 15 oral cavity cancers, 3 of 3 oropharyngeal cancers, and 2 of 2 laryngeal cancers) vs. 70% (5 of 7 oral cavity cancers, 2 of 2 oropharyngeal cancers, and 0 of 1 laryngeal cancers), respectively (p=0.03, Fisher's exact test; **Table 1**). Contributing to the high sensitivity in saliva was the fact that 75% (15 of 20) of the early-stage cancers in the study were from the oral cavity, which are most readily detectable in saliva and are preferentially treated with surgery, explaining their enrichment in our study. As expected, plasma provided a more sensitive predictor than saliva in patients with advanced stage disease 92% (n=37) vs. 70% (n=73), respectively (p=0.008, Fisher's exact test; **Table 1**). When segregated by nodal status, tumor-specific DNA could be detected in the plasma or saliva of 83% (n=59) and 100% (n=34) of patients with or without nodal metastasis, respectively. When both saliva and plasma were available, there was little difference between the detectability of cancers with respect to stage of disease (**Table 1**). An important caveat is that only five patients with early stage disease of non-oral cavity sites were available; though tumor DNA was detectable in all of these patients (all had detectable tumor DNA in saliva; two of the three patients with available plasma also had detectable tumor DNA in their plasma), the amount of tumor DNA was considerably lower than that of late-stage patients (median 0.007% vs 0.06%; p=0.03, Wilcoxon rank sum test).

EXAMPLE 6

HPV

- [45] Thirty patients harbored HPV type 16 DNA in their tumors when assessed by PCR and none had HPV-18. Of these thirty tumors, 29 (97%) were thought to be HPV-associated upon clinical presentation on the basis of in situ hybridization with high-risk HPV sequences or immunohistochemistry with antibodies to p16; in one case, the HPV status had not been determined in the clinic. Additionally, there were no patients who were considered to have HPV-associated tumors in the clinic and did not have HPV16 DNA identified in their tumors by PCR. This supports the specificity and sensitivity of our assays. As expected from the literature, all except one of the thirty tumors containing HPV DNA were found in the oropharynx (15, 39). And as expected, plasma from HPV-associated tumors was more informative than saliva; HPV DNA was detectable in the plasma of 86% (n=21) patients but in only 40% (n=30) of the saliva from these patients (Table 1).

EXAMPLE 7

Surveillance

- [46] Though not the primary purpose of this study, it was of interest to determine whether tumor DNA could be found in the saliva or plasma of patients after surgical removal of their tumors. "Follow-up" samples were available in nine patients in whom tumor DNA could be identified prior to therapy. Three of these patients were found to have tumor DNA in their saliva or plasma after surgery but before clinical evidence of disease recurrence (Fig. 2). For example, patient HN 399, with cancer of the oral cavity, was found to have tumor DNA in his saliva and plasma 4 months after surgery, whereas the recurrence only became evident clinically 19 months later (23.6 months after surgery). Similarly, tumor DNA was found in the saliva and plasma of patient HN 402, with cancer of the oral cavity, at 8 months after surgery, nine months before the recurrence was clinically evident. Patient HN 380 with cancer of the larynx was found to have tumor

DNA in his saliva 7 months after surgery, before any clinical or radiologic evidence of disease recurrence; the patient died of recurrent disease soon thereafter. Tumor DNA was detectable in the saliva of patient HN 367 with cancer of the oropharynx 25 months after surgery; at the time of writing (36 months after surgery), no biopsy-proven disease is yet evident but the clinical course has been complicated with suspicious imaging for locoregional and metastatic disease. No tumor DNA was detectable in the saliva and/or plasma of the other five patients in whom samples were available, all of whom have shown no clinical evidence of recurrence for a median follow-up of 12 months. (Fig. 3)

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The disclosure of each reference cited is expressly incorporated herein.

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We claim:

1. A method comprising:
 - a. assaying for tumor DNA in plasma from a Head and Neck Squamous Cell Carcinoma (HNSCC) patient; and
 - b. assaying for tumor DNA in saliva from a HNSCC patient.
2. The method of claim 1 wherein the tumor DNA is assayed using a HPV-16 nucleic acid.
3. The method of claim 1 wherein the tumor DNA is assayed using a HPV E7 nucleic acid.
4. The method of claim 1 wherein the tumor DNA is assayed using a TP53 nucleic acid.
5. The method of claim 1 wherein the tumor DNA is assayed using mutant nucleic acids selected from the group consisting of TP53, NOTCH1, PI3KCA, CDKN2A, FBXW7, HRAS, and NRAS.
6. The method of claim 1 wherein the HNSCC patient has undergone surgical removal of an HNSCC tumor
7. The method of claim 1 wherein the HNSCC is in the patient's oral cavity.
8. The method of claim 1 wherein the HNSCC is in the patient's oropharynx.
9. The method of claim 1 wherein the HNSCC is in the patient's larynx.
10. The method of claim 1 wherein the HNSCC is in the patient's hypopharynx.
11. The method of claim 1 wherein the tumor DNA is determined using a nucleic acid from each of HPV-16, TP53, PIK3CA, NOTCH1, and CDKNA2.
12. The method of claim 1 wherein step (a) and step (b) are performed on samples collected from the patient at the same time.
13. The method of claim 12 wherein step (a) and step (b) are performed on a plurality of samples collected from the patient at a plurality of times.
14. The method of claim 1 wherein the steps of assaying for tumor DNA comprise performing sequencing reactions.
15. The method of claim 14 wherein molecular barcodes are incorporated into sequencing templates prior to sequencing.

16. The method of claim 14 wherein adapter oligonucleotides are attached to sequencing templates prior to sequencing, thereby forming molecular barcodes in sequencing templates adjacent to said adapter oligonucleotides.
17. A method comprising:
 - a. assaying for tumor DNA in plasma from s subject; and
 - b. assaying for tumor DNA in saliva from a subject.
18. The method of claim 14 wherein the subject is at elevated risk for HNSCC.
19. The method of claim 18 wherein the subject is a tobacco smoker, was a tobacco smoker, or was exposed to second hand tobacco smoke.
20. The method of claim 18 wherein the subject is a heavy user of alcohol.
21. The method of claim 17 wherein step (a) and step (b) are performed on samples collected from the patient at the same time.
22. The method of claim 17 wherein the tumor DNA is determined using a nucleic acid from each of HPV-16, TP53, PIK3CA, NOTCH1, and CDKNA2.
23. The method of claim 17 wherein the steps of assaying for tumor DNA comprise performing sequencing reactions.
24. The method of claim 23 wherein molecular barcodes are incorporated into sequencing templates prior to sequencing.
25. The method of claim 23 wherein adapter oligonucleotides are attached to sequencing templates prior to sequencing, thereby forming molecular barcodes in sequencing templates adjacent to said adapter oligonucleotides.

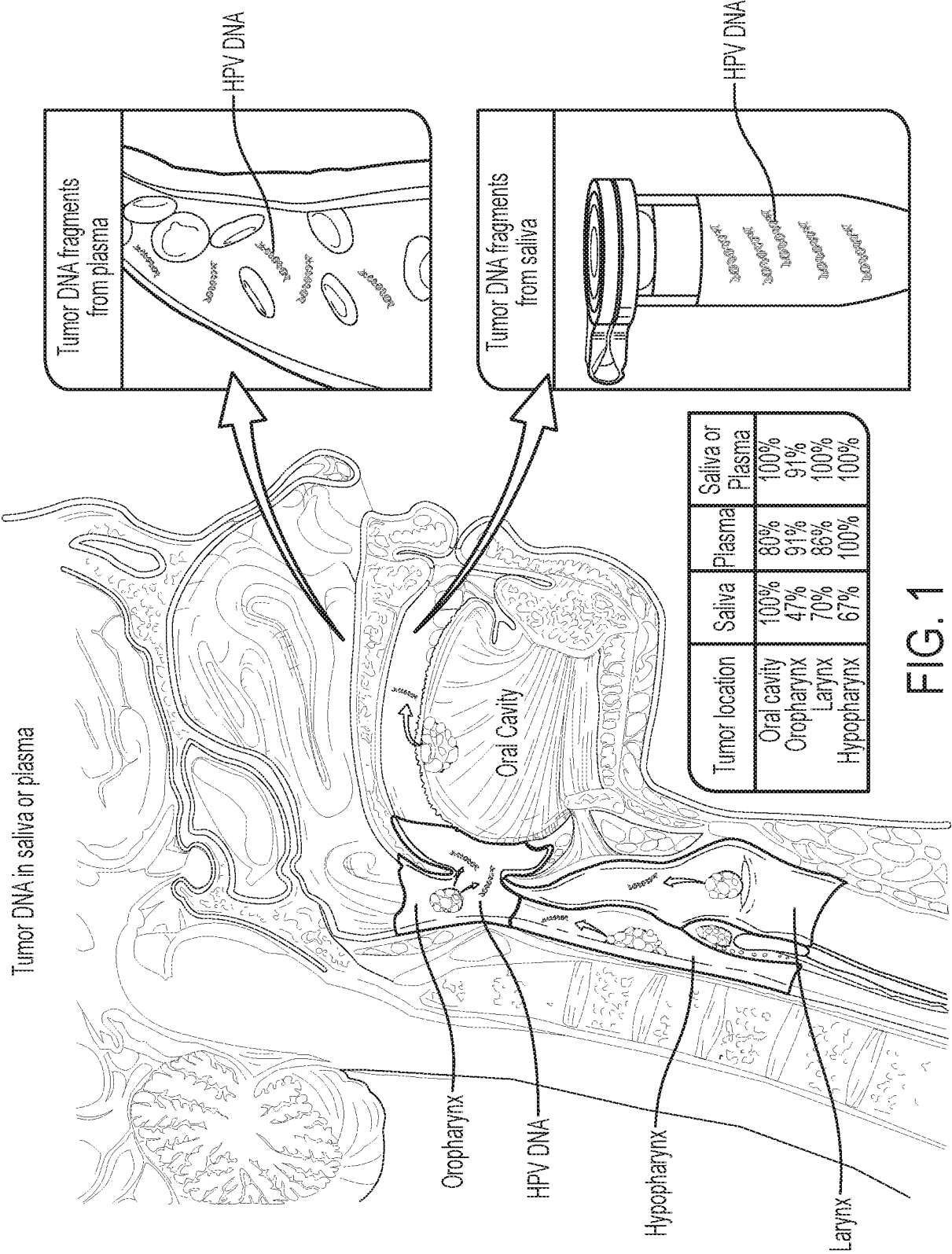


FIG. 1

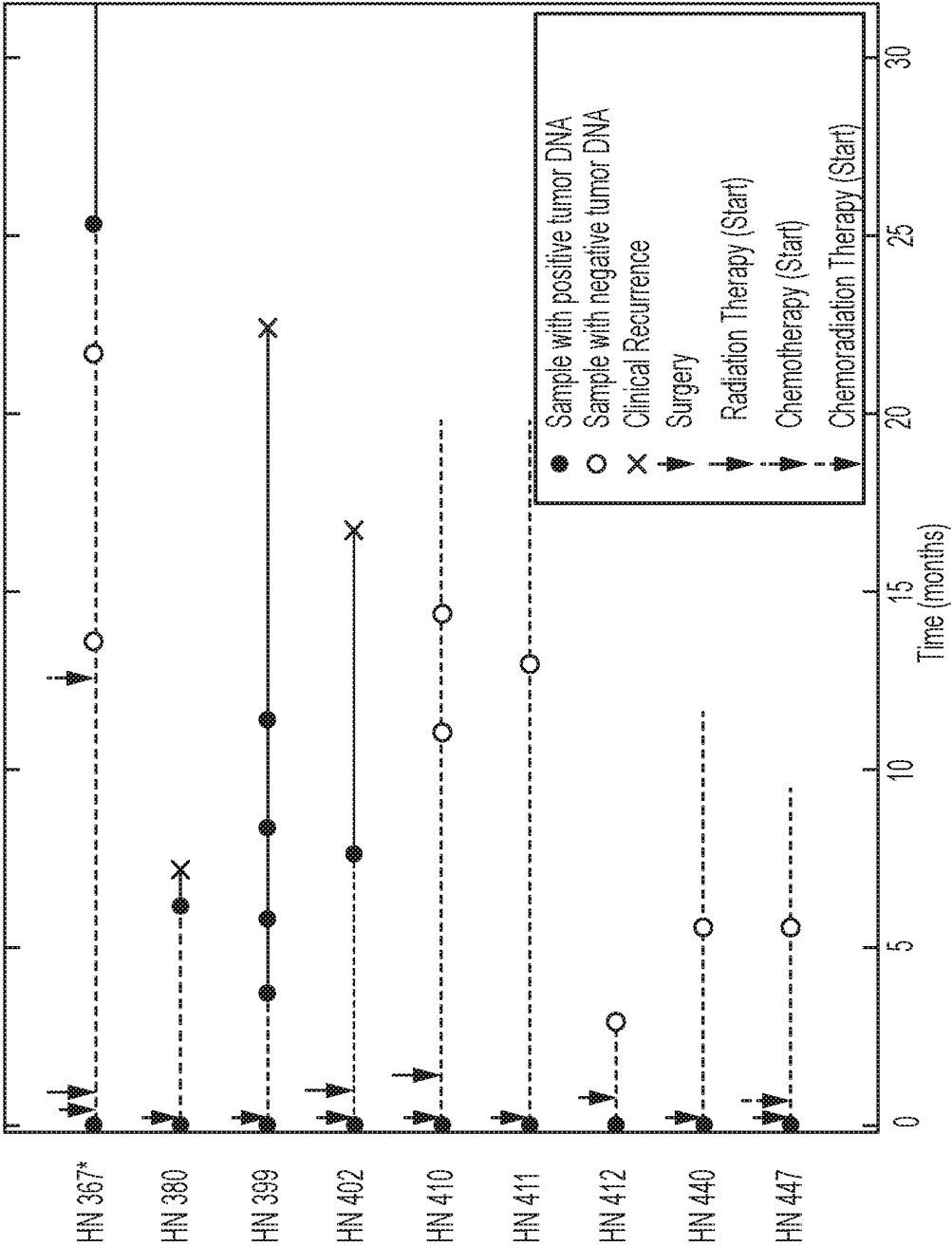


FIG. 2

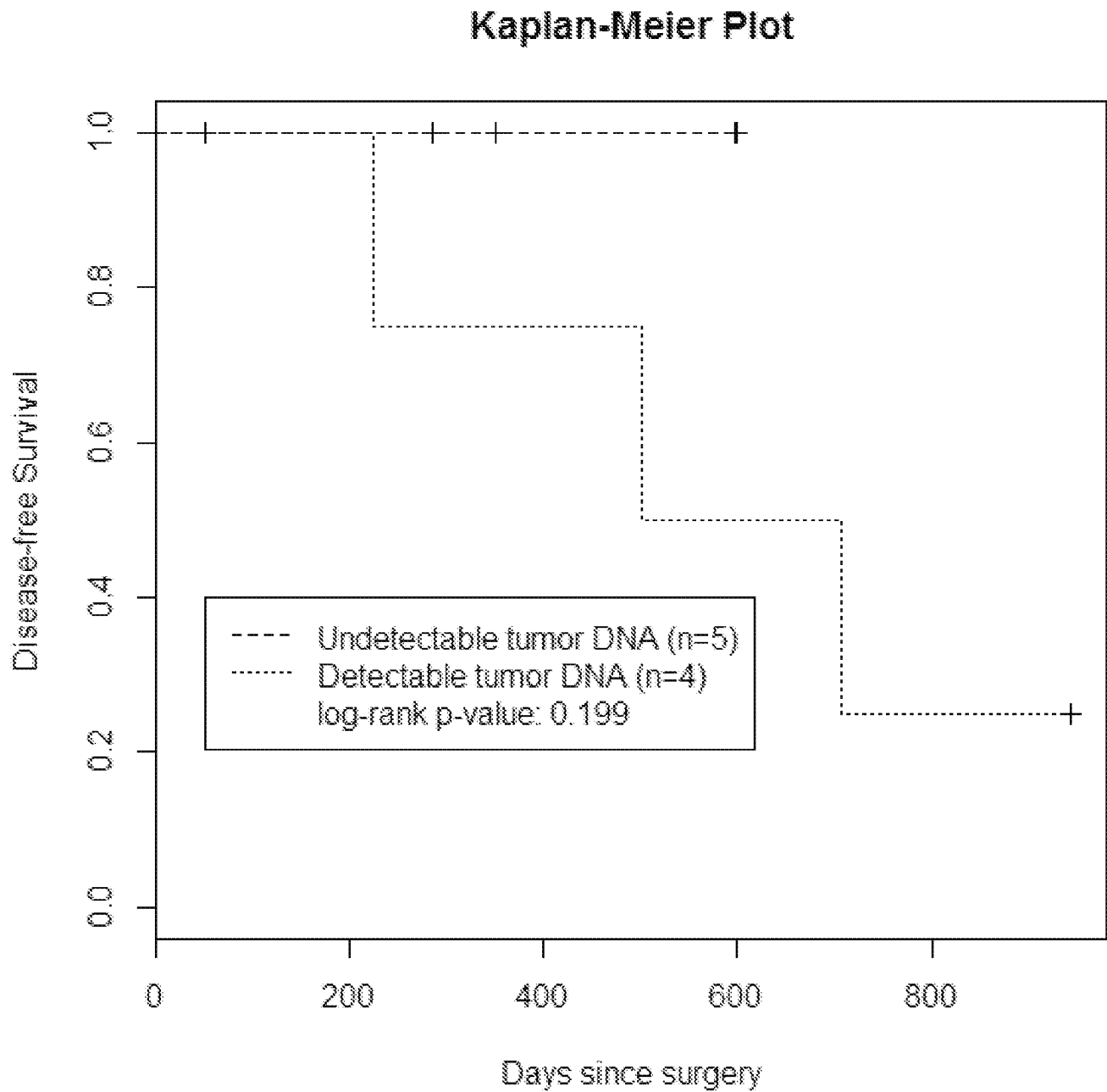


Fig. 3

Table S1: Patient Demographics

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Patient ID	Age at Diagnosis	Gender	Site	Subsite	Stage	T Classification	N Classification	Tumor HPV (by PCR)	Saliva Tumor DNA	Plasma Tumor DNA
HN 358	34	M	OC	Tongue	III	T2	N1	-	+	-
HN 359	67	M	L	Glottic	III	T3	N0	-	+	+
HN 361	68	M	OC	Tongue	I	T1	N0	-	+	+
HN 362	56	M	OP	Base of tongue	IV	T1	N2	+	-	NA
HN 363	61	F	OC	Buccal mucosa	II	T2	N0	-	+	+
HN 364	61	M	OC	Tongue	IV	T4	N2	-	+	+
HN 365	57	M	OP	Soft palate	II	T2	N0	+	+	+
HN 366	70	M	OC	Alveolar ridge	IV	T4a	N0	-	+	+
HN 367	58	M	OP	Base of tongue	IV	T2	N2	+	+	+
HN 368	50	M	OC	Tongue	I	T1	N0	-	+	+
HN 369	74	M	L	Supraglottic	II	T2	N0	-	+	NA
HN 370	54	M	OC	Buccal mucosa	IV	T2	N2	-	+	+
HN 371	77	F	OC	Tongue	I	T1	N0	-	+	NA
HN 372	61	M	OP	Base of tongue	II	T2	N0	-	+	+
HN 373	69	M	L	Supraglottic	III	T3	N0	-	-	+
HN 375	54	M	OC	Alveolar ridge	I	T1	N0	-	+	NA
HN 377	55	M	OP	Base of tongue	IV	T2	N2	+	-	+
HN 380	65	M	L	Transglottic	IV	T4a	N0	-	+	+
HN 381	45	M	OC	Tongue	II	T2	N0	-	+	+
HN 382	48	F	OC	Buccal mucosa	IV	T4a	N0	-	+	NA
HN 383	61	M	OC	Tongue	I	T1	N0	-	+	+
HN 384	68	M	OP	Base of tongue	IV	T1	N2	+	-	+
HN 385	54	F	L	Supraglottic	IV	T4a	N1	-	+	+
HN 386	75	M	OC	Lip	IV	T4	N2	-	+	NA
HN 389	59	M	OC	Hard palate	IV	T4b	N0	-	+	+
HN 390	61	M	OP	Tonsil	IV	T2	N3	+	+	+
HN 391	54	M	OP	Unknown	IV	Tx	N2	+	-	NA
HN 392	65	M	OP	Base of tongue	IV	T2	N2	+	+	+
HN 393	83	M	OP	Base of tongue	IV	T1	N2	+	-	NA
HN 394	51	M	L	Glottic	IV	T4a	N2	-	-	NA
HN 395	55	M	OP	Tonsil	IV	T4a	N2	+	+	+
HN 396	58	M	OP	Base of tongue	IV	T1	N2	+	-	+
HN 397	55	M	OP	Tonsil	IV	T2	N3	-	-	NA
HN 398	59	M	OC	Floor of mouth	III	T1	N1	-	+	NA
HN 399	77	F	OC	Floor of mouth	I	T1	N0	-	+	-
HN 400	50	M	OP	Tonsil	IV	T1	N2	+	-	+
HN 401	52	M	OP	Base of tongue	IV	T2	N2	+	+	+
HN 402	38	M	OC	Tongue	III	T1	N1	-	+	+
HN 404	65	F	OP	Tonsil	IV	T2	N2	+	-	-

FIG. 4A

HN 405	65	M	H	Post cricoid	IV	T4b	N2	-	+	+
HN 406	65	M	OP	Base of tongue	III	T3	N0	-	+	NA
HN 407	61	M	L	Transglottic	IV	T4a	N0	-	+	+
HN 408	61	M	OP	Tonsil	I	T1	N0	-	+	NA
HN 409	68	M	OC	Tongue	IV	T4	N2	-	+	NA
HN 410	58	M	OP	Tonsil	IV	T1	N2	+	+	+
HN 411	59	M	OP	Base of tongue	III	T1	N1	+	-	+
HN 412	58	M	OC	Tongue	III	T3	N0	-	+	NA
HN 413	51	M	L	Supraglottic	IV	T4a	N2	-	-	+
HN 414	59	M	OP	Base of tongue	III	T2	N1	+	+	+
HN 415	56	M	OC	Tongue	II	T2	N0	+	+	NA
HN 416	65	M	OC	Tongue	IV	T4	N2	-	+	NA
HN 417	44	M	OC	Tongue	IV	T2	N2	-	+	NA
HN 418	73	F	O	Alveolar ridge	I	T	N	-	+	AN
HN 419	55	M	OC	Tongue	VI	T23	N0	-	+	NA
HN 420	40	M	OC	Tongue	IV	T2	N2	-	+	NA
HN 421	62	M	OC	Floor of mouth	IV	T4	N2	-	+	NA
HN 423	35	F	OC	Tongue	IV	T3	N2	-	+	NA
HN 424	50	M	OC	Tongue	IV	T4a	N0	-	+	NA
HN 425	68	M	OC	Floor of mouth	IV	T3	N2	-	+	NA
HN 426	71	M	O	Alveolar ridge	I	T4	N2	-	+	AN
HN 427	53	M	OC	Floor of mouth	IV	T4	N1	-	+	AN
HN 428	58	F	OC	Tongue	II	T2	N0	-	+	AN
HN 429	61	F	OC	Tongue	IV	T2	N2	-	+	AN
HN 430	55	M	OC	Floor of mouth	II	T2	N0	-	+	AN
HN 431	63	F	OC	Tongue	II	T2	N0	-	+	AN
HN 432	71	F	OC	Tongue	II	T2	N0	-	+	AN
HN 433	61	M	O	Alveolar ridge	I	T	N	-	+	AN
HN 435	67	M	OC	Retromolar trigone	IV	T4a	N2	-	+	AN
HN 436	62	M	OC	Tongue	IV	T2	N2	-	+	AN
HN 438	56	F	OC	Tongue	I	T1	N0	-	+	NA
HN 439	65	M	H	Piriform sinus	IV	T4a	N1	-	-	+
HN 440	53	M	OP	Base of tongue	III	T1	N1	+	-	+
HN 441	80	F	OP	Tonsil	III	T1	N1	+	+	NA
HN 443	52	M	OC	Floor of mouth	IV	T4a	N2	-	+	+
HN 444	83	M	OP	Base of tongue	III	T3	N0	+	+	NA
HN 445	74	F	OC	Tongue	I	T1	N0	-	+	-
HN 447	45	M	OP	Tonsil	IV	T2	N2	+	-	+
HN 449	52	M	OP	Tonsil	IV	T3	N2	+	+	+

FIG. 4A Cont

HN 450	45	M	OP	Unknown	III	Tx	N1	+	-	+
HN 451	82	M	OC	Tongue	IV	T2	N2	-	+	+
HN 452	60	M	OP	Base of tongue	IV	T1	N2	+	-	+
HN 454	86	M	OC	Tongue	IV	T1	N3	-	+	NA
HN 456	60	M	H	Piriform sinus	IV	T4a	N0	-	+	+
HN 457	67	M	OP	Base of tongue	III	T3	N1	-	+	NA
HN 458	58	M	L	Glottic	IV	T4a	N0	-	+	NA
HN 459	54	M	OC	Tongue	IV	T3	N2	-	+	NA
HN 460	54	M	OP	Base of tongue	IV	T2	N2	+	-	NA
HN 462	54	F	OC	Tongue	IV	T2	N2	-	+	NA
HN 463	68	M	L	Glottic	I	T1a	N0	-	+	-
HN 464	47	M	OP	Base of tongue	IV	T1	N2	+	-	-
HN 469	58	M	OP	Tonsil	IV	T2	N2	+	+	+
HN 473	45	M	OP	Base of tongue	III	T1	N1	+	-	NA
HN 474	39	M	OP	Base of tongue	IV	T2	N2	+	-	NA

OC = oral cavity, OP = oropharynx, H = hypopharynx, L = larynx, and NA = not available

FIG. 4B

Table S2: Primer sequences used in multiplex assay for identification of driver mutations in tumors.

Gene	Genomic coordinates of amplified region*	Forward primer Sequence	Reverse primer sequence	Amplicon length (bp)
FBXW7	chr4:153249328-153249457	GAAGTCCCAACCATGACAAGA	CGGACACTCAAAGTGTGGAA	130
FBXW7	chr4:153247295-153247409	TTGTTTTTCTGTTTCTCCCTCTG	CTGCAACATGACCCATCAAA	115
FBXW7	chr4:153247211-153247339	TTGAGACAGGCCAGTGTTCAT	CAGTCTGGATCCACACC	129
NRAS	chr1:115258719-115258840	GATGTGGCTGCCCAATTAAC	GATTGTAGTGGCTTTTCC	122
NRAS	chr1:115256485-115256609	ACACCCACAGGATCTTACAG	CGCTGTCTCATGATTGG	125
PIK3CA	chr3:178916781-178916899	CCCCCTCCATCAACTTCTTC	GAAAAAGCCGAAGGTCAAA	119
PIK3CA	chr3:178921442-178921555	CAGACGCAATTCACAGCTA	ACATTACGTAGGTGCACAAA	114
PIK3CA	chr3:178927879-178928007	AGAGATGATTGTGAATTTTCCCTTTT	AGTTTATATTTCCCCATGCCAAT	129
PIK3CA	chr3:178951924-178952055	GCATGCCAATCTCTTCATAATC	TCCAAAGCCTCTTGTCTAGT	132
PIK3CA	chr3:178951991-178952119	TTTGATGACATTCATACATTCG	GATCCAATCCATTTTGTGTCCAG	129
PIK3CA	chr3:178936018-178936140	CAATGAATTAAGGAAATGACAAA	CTCCATTTTAGCATTACCTGTGAC	123
HRAS	chr11:534245-534371	GGCAGGAGACCCCTGTAGGAG	GTTCTGGATCAGCTGGATGG	127
HRAS	chr11:533804-533926	GATGGCAACACACACAGGA	GTGGTCATTGATGGGAGAC	123
CDKN2A	chr9:21971073-21971187	CCGAGTGGGGAGCTG	CACAGCGTGTCCAGGAAG	115
CDKN2A	chr9:21970874-21971002	ACAAATTCACATCATCAGTCCTC	AGGAGCTGGGCCATCG	129
CDKN2A	chr9:21970967-21971091	GCAGGTACCGTCCGACAT	CTTCTGGACACGCTGGT	125
CDKN2A	chr9:21971115-21971237	GGGTGGGTGAGAGTGG	TGGCTCTGACCAATTCGTCTCT	123
CDKN2A	chr9:21970997-21971138	GCTCCTCAGCCAGGTCCA	GACCCGCCACTCTCAC	142
CDKN2A	chr9:21974743-21974859	CACCTCCTCTACCCGACCC	GGGAGAGCAGGCAGC	117
CDKN2A	chr9:21974684-21974798	GGCCTCCGACCGTAACATTT	AGCCTTCGGCTGACTGG	115
CDKN2A	chr9:21974652-21974761	CTCCCGCTGCAGACCCCT	GGTCCGGTAGAGGAGGTG	110
CDKN2A	chr9:21968163-21968300	CTGTAGGACCTTCGGTGACTG	TGTGCCACACATCTTTGACC	138

FIG. 5

TP53	chr17:7579818-7579933	CCCTTCCAATGGATCCACTCAC	ACTGCCTTCCGGGTCAC	116
TP53	chr17:7579679-7579789	AGCCCCCTAGCAGACCT	CAGCCCAACCTTGTCTT	111
TP53	chr17:7579502-7579616	TGACTGCTCTTTTCAACCATC	TCATCTGGACCTGGGTCTTC	115
TP53	chr17:7579441-7579572	GCAATGGATGATTGATGCTG	CGGTGTAGGAGCTGCTGG	132
TP53	chr17:7579388-7579501	AGCTCCAGAAATGCCAGAG	TGGGAAGGACAGAAATGA	114
TP53	chr17:7579338-7579463	CTGCACAGACGCTCCTAC	CAGAAATGCAAGAAGCCAGA	126
TP53	chr17:7579270-7579396	GCAATGAAGTCTCATGGAAGC	CCCTTCCAGAAAACCTACC	127
TP53	chr17:7578475-7578593	GCCCTGACTTTCAACTCTGTCT	GGGGTGTGGAATCAACC	119
TP53	chr17:7578417-7578540	CTCGTCAATGTGCTGTGACT	CAACAAGATGTTTGGCAACTG	124
TP53	chr17:7578335-7578455	GCCATGGCCATCTACAAGC	ACCAGCCCTGTCGTCTTC	121
TP53	chr17:7578211-7578326	GTCCCAAGCCCTCTGATT	CGAAAAGTGTTCGTGTCATCCA	116
TP53	chr17:7578137-7578260	GTGGAAGGAAATTTGGGTGT	CTTAACCCCTCCTCCACAG	124
TP53	chr17:7577513-7577635	TGTGATGATGGTGAGGATGG	TCATCTTGGGCTGTGTTATC	123
TP53	chr17:7577480-7577606	TGGCTCTGACTGTACCACCATC	GTGGCAAGTGGCTCCTGA	127
TP53	chr17:7577068-7577188	TGCCCTTGTCTCTCTTTTCC	GCGGAGATTCTCTTCTCTGT	121
TP53	chr17:7576997-7577121	CGTGTTGTGCCGTGCTCG	GCTTCTTGTCTGCTGCTT	125
TP53	chr17:7576840-7576956	TTTTATCACCTTTCCTTGCCCT	CAAGACTTAGTACCTGAAGGTGAA	117
TP53	chr17:7576782-7576898	AAGAAGAAACGGCATTTTGAG	CCAGCCAAAGAAGAACAC	117
TP53	chr17:7573947-7574059	CCCTGGCTCCTTCCCAG	CTTCTCCCCCTCCTCTGTTG	113
TP53	chr17:7573881-7574007	GTTCGAGAGCTGAATGAGG	TAGGAAGGACGGGAGTAGG	127
TP53	chr17:7572929-7573043	AGTCTGAGTCAGGCCCTTCTG	ATGTCATCTCTCCTCCCTGCT	115
TP53	chr17:7572929-7573043	ATGTCATCTCTCCTCCCTGCT	AGTCTGAGTCAGGCCCTTCTG	115
TP53	chr17:7572893-7573009	GCCACTGAAGTCCAAAAG	GAGGCTGTCACTGGGGAAC	117

*Coordinates refer to the human reference genome hg19 release (Genome Reference Consortium GRCh37, Feb 2009).

FIG. 5 Cont

Table S3: Levels of Tumor-derived DNA in Saliva and Plasma

PATIENT				Site	HPV status of Tumor	Gene mutated in Tumor
Patient ID	Interval between Biopsy and Sample Collection (days)	Treatment Prior to Sample Collection for Patients with Recurrence (days prior)				
HN 358	78	No prior treatment	OC	HPV negative	TP53	
HN 359	335	RT (449)	L	HPV negative	TP53	
HN 361	29	No prior treatment	OC	HPV negative	TP53	
HN 362	66	No prior treatment	OP	HPV16 positive	PIK3CA	
HN 363	24	Surgery (151)	OC	HPV negative	TP53	
HN 364	21	Surgery, RT (324)	OC	HPV negative	TP53	
HN 365	28	CRT (244)	OP	HPV16 positive	HPV16	
HN 366	35	No prior treatment	OC	HPV negative	TP53	
HN 367	24	CRT (774)	OP	HPV16 positive	PIK3CA	
HN 368	15	No prior treatment	OC	HPV negative	TP53	
HN 369	20	RT (190)	L	HPV negative	TP53	
HN 370	34	No prior treatment	OC	HPV negative	TP53	
HN 371	40	No prior treatment	OC	HPV negative	NOTCH1	
HN 372	75	Surgery, RT (3503)	OP	HPV negative	TP53	
HN 373	No prior biopsy	CRT (161)	L	HPV negative	TP53	
HN 375		No prior treatment	OC	HPV negative	TP53	
HN 377	No prior biopsy	Surgery (709)	OP	HPV negative	TP53	
HN 380		CRT (432)	OP	HPV16 positive	HPV16	
HN 381	27	CRT (194)	L	HPV negative	TP53	
HN 382	32	No prior treatment	OC	HPV negative	TP53	
HN 383	14		OC	HPV negative	TP53	
HN 384	26		OC	HPV negative	TP53	
HN 385	49		OP	HPV16 positive	PIK3CA	
HN 386	93	No prior treatment	L	HPV negative	TP53	
HN 389	84	Surgery (1249)	OC	HPV negative	TP53	
HN 390	35	No prior treatment	OC	HPV negative	PIK3CA	
HN 391	33	No prior treatment	OP	HPV16 positive	HPV16	
HN 392	48	No prior treatment	OP	HPV16 positive	HPV16	
HN 393	9	No prior treatment	OP	HPV16 positive	FBXW7	
HN 394	28	No prior treatment	OP	HPV16 positive	HPV16	
HN 395	27	RT (337)	L	HPV negative	TP53	
HN 396	23	No prior treatment	OP	HPV16 positive	HPV16	
	9	No prior treatment	OP	HPV16 positive	PIK3CA	

Fig. 6A

HN 397	42	No prior treatment	OP	HPV negative	TP53
HN 398	23	No prior treatment	OC	HPV negative	NA
HN 399	No prior biopsy	No prior treatment	OC	HPV negative	TP53
HN 400	18	No prior treatment	OP	HPV16 positive	PIK3CA
HN 401	23	CRT (226)	OP	HPV16 positive	HPV16
HN 402	35	No prior treatment	OC	HPV negative	TP53
HN 404	36	No prior treatment	OP	HPV16 positive	NRAS
HN 405	53	No prior treatment	H	HPV negative	TP53
HN 406	21	No prior treatment	OP	HPV negative	TP53
HN 407	20	No prior treatment	L	HPV negative	TP53
HN 408	42	Surgery, RT (1783)	OP	HPV negative	TP53
HN 409	28	No prior treatment	OC	HPV negative	CDKN2A
HN 410	25	No prior treatment	OP	HPV16 positive	HPV16
HN 411	78	No prior treatment	OP	HPV16 positive	HPV16
HN 412	20	No prior treatment	OC	HPV negative	NA
HN 413	109	No prior treatment	L	HPV negative	TP53
HN 414	22	Surgery, RT (3757)	OP	HPV16 positive	PIK3CA
HN 415	69	No prior treatment	OC	HPV16 positive	HPV16
HN 416	25	No prior treatment	OC	HPV negative	NOTCH1
HN 417	58	No prior treatment	OC	HPV negative	TP53
HN 418	74	No prior treatment	OC	HPV negative	TP53
HN 419	22	No prior treatment	OC	HPV negative	TP53
HN 420	31	No prior treatment	OC	HPV negative	TP53
HN 421	68	No prior treatment	OC	HPV negative	NOTCH1
HN 423	35	No prior treatment	OC	HPV negative	TP53
HN 424	107	No prior treatment	OC	HPV negative	TP53
HN 425	36	No prior treatment	OC	HPV negative	TP53
HN 426	31	No prior treatment	OC	HPV negative	TP53
HN 427	45	No prior treatment	OC	HPV negative	CDKN2A
HN 428	55	No prior treatment	OC	HPV negative	TP53
HN 429	58	No prior treatment	OC	HPV negative	TP53
HN 430	49	No prior treatment	OC	HPV negative	TP53
HN 431	39	No prior treatment	OC	HPV negative	TP53
HN 432	58	No prior treatment	OC	HPV negative	TP53
HN 433	49	Surgery, CRT (1095)	OC	HPV negative	TP53
HN 435	77	No prior treatment	OC	HPV negative	TP53
HN 436	52	No prior treatment	OC	HPV negative	TP53
HN 438	22	No prior treatment	OC	HPV negative	TP53

Fig. 6B

Fig. 6D

TUMOR				Somatic Mutation: Patient FRACTION of MUTANT reads
Type of Mutation	Genomic Position	cDNA Change	Amino Acid Change	
SBS	7578524	406C>T	Q136*	0.541%
SBS	7577082	856G>A	E286K	0.009%
SBS	7578268	581T>G	L194R	0.245%
SBS	178936091	1633G>A	E545K	0.000%
SBS	7576851	c.993+2T>C	NA	5.443%
SBS	7576928	c.920-2A>G	NA	0.106%
NA	NA	NA	NA	Only HPV assessed
SBS	7578553	377A>G	Y126C	3.296%
SBS	178936082	1624G>A	E542K	0.003%
SBS	7578550	380C>A	S127Y	11.467%
INDEL	7577094	c.844_845insCTGTGCGCC	R282fs	0.001%
SBS	7577094	844C>T	R282W	2.390%
SBS	139411837	1442G>T	G481V	1.628%
SBS	7577120	818G>T	R273L	0.007%
SBS	7574021	1006G>T	E336*	0.000%
SBS	7577574	707A>G	Y236C	0.040%
NA	NA	NA	NA	Only HPV assessed
SBS	7577082	856G>A	E286K	0.026%
SBS	7577097	841G>A	D281N	0.282%
SBS	7577570	711G>A	M237I	6.120%
SBS	7577094	844C>T	R282W	0.190%
SBS	178936091	1633G>A	E545K	0.000%
SBS	7579358	329G>T	R110L	0.004%
SBS	7578500	430C>T	Q144*	0.168%
SBS	178952085	3140A>G	H1047R	3.497%
NA	NA	NA	NA	Only HPV assessed
NA	NA	NA	NA	Only HPV assessed
SBS	153249384	1394C>T	R465H	0.005%
NA	NA	NA	NA	Only HPV assessed
SBS	7578179	670G>T	E224*	0.000%
NA	NA	NA	NA	Only HPV assessed
SBS	178916876	263G>A	R88Q	0.000%

SBS	7578478		452C>A	P151H	0.000%
Translocation	chr11:69467879-chr11:69469070		NA	NA	43.000%
SBS	7577121		817C>T	R273C	1.850%
SBS	178936091		1633G>A	E545K	0.000%
NA	NA		NA	NA	Only HPV assessed
SBS	7577120		818G>A	R273H	0.027%
SBS	115256530		181C>A	Q61K	0.000%
SBS	7577538		743G>A	R248Q	0.033%
SBS	7574000		1027G>T	E343*	0.005%
SBS	7578406		524G>A	R175H	0.024%
SBS	7577568		713G>T	C238F	0.006%
INDEL	21971125-21971127		231delTC	T77fs	0.028%
NA	NA		NA	NA	Only HPV assessed
NA	NA		NA	NA	Only HPV assessed
Translocation	chr18:45662870-chr11:69291050		NA	NA	41.000%
SBS	7577538		743G>T	R248L	0.000%
SBS	178936082		1624G>A	E542K	0.000%
NA	NA		NA	NA	Only HPV assessed
INDEL	139396749		5359delC	L1787fs	0.060%
SBS	7578406		524G>A	R175H	0.560%
SBS	7577538		743G>A	R248Q	6.097%
SBS	7578271		578A>T	H193L	0.217%
SBS	7574003		1024C>T	R342*	7.347%
INDEL	139418317		255_256insA	Y85fs	3.469%
SBS	7577081		857A>C	E286A	2.104%
SBS	7577046		892G>T	E298*	0.653%
INDEL	7577498		c.782+1_782+2insC	NA	0.068%
SBS	21968242		c.458-1G>A	NA	1.744%
SBS	7578392		538G>T	E180*	0.559%
SBS	7577121		817C>T	R273C	1.086%
SBS	7577574		707A>G	Y236C	2.072%
SBS	7578212		637C>T	R213*	0.759%
SBS	7574017		1010G>T	R337L	1.263%
SBS	7577118		820G>T	V274F	1.430%
SBS	7578458		472C>G	R158G	0.204%
SBS	7578550		380C>A	S127Y	2.200%
SBS	7579366		321C>G	Y107*	0.880%
SBS	7577590		691A>G	T231A	0.062%

Fig. 6E

Fig. 6F

SBS	7578188	661G>T	E221*	0.000%
NA	NA	NA	NA	Only HPV assessed
NA	NA	NA	NA	Only HPV assessed
INDEL	7579590	97-2_97delAGT	S33fs	0.570%
SBS	178936082	1624G>A	E542K	0.020%
SBS	178952085	3140A>G	H1047R	0.151%
NA	NA	NA	NA	Only HPV assessed
SBS	153249384	1394C>T	R465H	0.019%
NA	NA	NA	NA	Only HPV assessed
SBS	7577094	844C>T	R282W	0.419%
SBS	153247289	1513C>G	R505G	0.000%
SBS	7578492	438G>A	W146*	0.121%
SBS	7574018	1009C>T	R337C	0.020%
SBS	7579358	329G>T	R110L	0.113%
SBS	7577121	817C>T	R273C	0.106%
SBS	7578271	578A>G	H193R	0.076%
SBS	178936091	1633G>A	E545K	0.000%
SBS	7578492	438G>A	W146*	0.073%
SBS	7578208	641A>G	H214R	0.015%
NA	NA	NA	NA	Only HPV assessed
NA	NA	NA	NA	Only HPV assessed
NA	NA	NA	NA	Only HPV assessed
NA	NA	NA	NA	Only HPV assessed

SALIVA

Somatic Mutation: Patient NUMBER of MUTANT reads	Somatic Mutation: Patient NUMBER of WILD-TYPE reads	Somatic Mutation: CONTROL FRACTION of MUTANT reads
657	120798	0.000%
19	205715	0.000%
845	343819	0.004%
0	32958	0.000%
8843	153613	0.001%
189	177609	0.001%
Only HPV assessed	Only HPV assessed	Only HPV assessed
4905	143907	0.000%
28	862561	0.000%
22073	170413	0.002%
10	1190330	0.000%
9233	377059	0.006%
9504	574434	0.000%
29	426931	0.000%
0	79917	0.000%
123	310509	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
107	414013	0.000%
911	322360	0.000%
20130	308811	0.000%
394	207299	0.006%
0	33081	0.000%
29	717919	0.000%
166	98840	0.011%
5130	141558	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
Only HPV assessed	Only HPV assessed	Only HPV assessed
8	165676	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
0	111372	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
0	248376	0.000%

Fig. 6G

0	174729	0.000%
4301	5701	0.000%
5696	302191	0.014%
0	44937	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
90	336318	0.000%
0	435825	0.000%
57	175443	0.001%
22	446306	0.000%
94	398354	0.004%
18	294486	0.001%
102	370428	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
Only HPV assessed	Only HPV assessed	Only HPV assessed
4099	5899	0.000%
0	139509	0.000%
0	118473	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
98	164341	0.000%
698	123940	0.011%
19333	297749	0.006%
302	139354	0.000%
32586	410934	0.004%
9920	276064	0.000%
3222	149904	0.000%
2935	446813	0.000%
253	374009	0.000%
842	47438	0.002%
390	69396	0.003%
2141	195085	0.009%
188	8878	0.000%
968	126514	0.012%
4979	389245	0.001%
1870	128915	0.000%
119	58051	0.000%
3592	159698	0.000%
2415	272049	0.000%
210	340200	0.001%

Fig. 6H

Fig. 6I

0	525321	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
Only HPV assessed	Only HPV assessed	Only HPV assessed
3610	629240	0.000%
97	481403	0.000%
359	237184	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
68	364936	0.002%
Only HPV assessed	Only HPV assessed	Only HPV assessed
1506	358329	0.024%
0	466506	0.000%
113	92902	0.000%
69	351123	0.003%
2360	2086288	0.000%
32	30565	0.014%
248	327670	0.000%
0	94842	0.000%
44	60139	0.000%
61	404291	0.000%
Only HPV assessed	Only HPV assessed	Only HPV assessed
Only HPV assessed	Only HPV assessed	Only HPV assessed
Only HPV assessed	Only HPV assessed	Only HPV assessed
Only HPV assessed	Only HPV assessed	Only HPV assessed

Fig. 6J

Somatic Mutation: CONTROL NUMBER of MUTANT reads	Somatic Mutation: CONTROL NUMBER of WILD-TYPE reads	Somatic Mutation: P-value (Patient vs. Control)
0	78462	3.00E-94
0	190784	7.25E-05
6	137370	5.81E-72
0	99828	NA
3	244530	0.00E+00
2	285252	7.88E-66
Only HPV assessed	Only HPV assessed	NA
0	78462	0.00E+00
0	165648	1.66E-02
4	229130	0.00E+00
0	3671899	7.73E-07
6	94302	0.00E+00
0	74781	2.77E-270
2	523939	1.40E-07
0	75699	NA
0	222409	1.49E-20
Only HPV assessed	Only HPV assessed	NA
0	304190	1.81E-18
0	94302	1.37E-59
0	73755	0.00E+00
6	94302	2.01E-37
0	99828	NA
0	297066	1.11E-03
4	36747	7.42E-13
0	132561	0.00E+00
Only HPV assessed	Only HPV assessed	NA
Only HPV assessed	Only HPV assessed	NA
0	113715	2.48E-02
Only HPV assessed	Only HPV assessed	NA
0	8730	NA
Only HPV assessed	Only HPV assessed	NA
0	75132	NA

0	36747	NA
0	9998	NA
14	101826	0.00E+00
0	99828	NA
Only HPV assessed	Only HPV assessed	NA
0	101826	3.49E-07
0	96993	NA
1	73755	6.60E-06
0	151305	1.29E-02
8	189739	2.35E-07
2	222409	5.83E-03
0	62736	5.93E-05
Only HPV assessed	Only HPV assessed	NA
Only HPV assessed	Only HPV assessed	NA
0	11051	NA
0	73755	NA
0	99828	NA
Only HPV assessed	Only HPV assessed	NA
0	12387	1.17E-02
16	140283	2.58E-162
12	215316	0.00E+00
0	243910	3.26E-116
14	323181	0.00E+00
0	52392	0.00E+00
0	94302	0.00E+00
0	94302	2.58E-136
0	213470	6.50E-33
2	103908	0.00E+00
4	140283	9.79E-169
25	274420	0.00E+00
0	177032	0.00E+00
21	182440	2.16E-288
3	323181	0.00E+00
0	101826	0.00E+00
0	36747	9.36E-18
0	78462	0.00E+00
0	250810	0.00E+00
3	215316	1.04E-28

Fig. 6K

Fig. 6L

0	8730	NA
Only HPV assessed	Only HPV assessed	NA
Only HPV assessed	Only HPV assessed	NA
0	84825	1.60E-107
0	156321	3.93E-08
0	132561	3.49E-45
Only HPV assessed	Only HPV assessed	NA
4	217514	4.92E-08
Only HPV assessed	Only HPV assessed	NA
24	101826	2.91E-83
0	247275	NA
0	36747	4.71E-11
2	75699	1.72E-03
0	297066	8.44E-75
14	101826	2.82E-13
0	137370	4.25E-24
0	99828	NA
0	36747	4.93E-07
0	354892	6.53E-13
Only HPV assessed	Only HPV assessed	NA
Only HPV assessed	Only HPV assessed	NA
Only HPV assessed	Only HPV assessed	NA
Only HPV assessed	Only HPV assessed	NA

		Somatic Mutation: Patient FRACTION of MUTANT reads	Somatic Mutation: Patient NUMBER of MUTANT reads
HPV: templates/ng DNA			
Not quantified	0.000%	0	
Not quantified	0.019%	54	
Not quantified	0.017%	21	
No HPV templates found	No plasma available	No plasma available	
Not quantified	0.796%	1999	
Not quantified	0.901%	1831	
59.8	Only HPV assessed	Only HPV assessed	
Not quantified	0.582%	674	
No HPV templates found	0.423%	379	
Not quantified	0.867%	338	
Not quantified	No plasma available	No plasma available	
Not quantified	0.498%	251	
Not quantified	No plasma available	No plasma available	
Not quantified	0.082%	63	
Not quantified	0.092%	173	
No HPV templates found	No plasma available	No plasma available	
Not quantified	Only HPV assessed	Only HPV assessed	
Not quantified	1.379%	415	
Not quantified	1.852%	854	
Not quantified	No plasma available	No plasma available	
No HPV templates found	0.031%	5	
Not quantified	0.022%	19	
Not quantified	0.089%	116	
Not quantified	No plasma available	No plasma available	
	0.022%	97	
0.6	Only HPV assessed	Only HPV assessed	
No HPV templates found	No plasma available	No plasma available	
0.3	0.638%	770	
No HPV templates found	No plasma available	No plasma available	
Not quantified	No plasma available	No plasma available	
54.1	Only HPV assessed	Only HPV assessed	
No HPV templates found	0.344%	331	

Fig. 6M

Fig. 60

Not quantified No HPV templates found	2.490% Only HPV assessed No plasma available	2300 Only HPV assessed No plasma available
0.3 Not quantified	0.066% No plasma available	27 No plasma available
7.2 Not quantified	0.000% Only HPV assessed	0 Only HPV assessed
15.6 No HPV templates found Not quantified	0.084% Only HPV assessed	48 Only HPV assessed
No HPV templates found Not quantified	0.869% No plasma available	1513 No plasma available
Not quantified	0.069% No plasma available	128 No plasma available
Not quantified	2.858% No plasma available No plasma available No plasma available No plasma available	2224 No plasma available No plasma available No plasma available No plasma available
No HPV templates found Not quantified	0.000% Only HPV assessed	0 Only HPV assessed
No HPV templates found No HPV templates found	Only HPV assessed No plasma available No plasma available	Only HPV assessed No plasma available No plasma available
1.9 No HPV templates found No HPV templates found		

PLASMA			
Somatic Mutation: Patient NUMBER of WILD-TYPE reads	Somatic Mutation: CONTROL FRACTION of MUTANT reads	Somatic Mutation: CONTROL NUMBER of MUTANT reads	Somatic Mutation: CONTROL NUMBER of MUTANT reads
69216	0.000%	0	0
282174	0.002%	3	3
120144	0.001%	3	3
No plasma available	No plasma available	No plasma available	No plasma available
251160	0.000%	0	0
203268	0.000%	0	0
Only HPV assessed	Only HPV assessed	Only HPV assessed	Only HPV assessed
115812	0.000%	0	0
89622	0.000%	0	0
39024	0.000%	0	0
No plasma available	No plasma available	No plasma available	No plasma available
50454	0.010%	10	10
No plasma available	No plasma available	No plasma available	No plasma available
76758	0.000%	0	0
187566	0.000%	0	0
No plasma available	No plasma available	No plasma available	No plasma available
Only HPV assessed	Only HPV assessed	Only HPV assessed	Only HPV assessed
30132	0.002%	3	3
46104	0.000%	0	0
No plasma available	No plasma available	No plasma available	No plasma available
16158	0.004%	5	5
87564	0.000%	0	0
130764	0.000%	0	0
No plasma available	No plasma available	No plasma available	No plasma available
440476	0.000%	0	0
Only HPV assessed	Only HPV assessed	Only HPV assessed	Only HPV assessed
No plasma available	No plasma available	No plasma available	No plasma available
120570	0.004%	5	5
No plasma available	No plasma available	No plasma available	No plasma available
No plasma available	No plasma available	No plasma available	No plasma available
Only HPV assessed	Only HPV assessed	Only HPV assessed	Only HPV assessed
96294	0.011%	26	26

Fig. 6P

Fig. 6R

92358	0.000%	0
Only HPV assessed No plasma available	Only HPV assessed No plasma available	Only HPV assessed No plasma available
41466	0.000%	0
No plasma available	No plasma available	No plasma available
119568	0.000%	0
Only HPV assessed	Only HPV assessed	Only HPV assessed
57852	0.004%	5
Only HPV assessed	Only HPV assessed	Only HPV assessed
174054	0.010%	10
187146	0.001%	2
No plasma available	No plasma available	No plasma available
77814	0.007%	10
No plasma available No plasma available No plasma available No plasma available No plasma available	No plasma available No plasma available No plasma available No plasma available No plasma available	No plasma available No plasma available No plasma available No plasma available No plasma available
44520	0.000%	0
Only HPV assessed	Only HPV assessed	Only HPV assessed
Only HPV assessed No plasma available No plasma available	Only HPV assessed No plasma available No plasma available	Only HPV assessed No plasma available No plasma available

Somatic Mutation: CONTROL NUMBER of WILD-TYPE reads	Somatic Mutation: P-value (Patient vs. Control)	HPV: templates/ng DNA
81680	NA	Not quantified
128972	4.05E-05	Not quantified
292883	1.24E-09	Not quantified
No plasma available	NA	No plasma available
143400	3.89E-249	No HPV templates found
162245	0.00E+00	No HPV templates found
Only HPV assessed	NA	63
108590	1.19E-138	Not quantified
155200	8.88E-144	45.5
108590	1.95E-204	No HPV templates found
No plasma available	NA	No plasma available
96060	6.68E-97	Not quantified
No plasma available	NA	No plasma available
118580	2.16E-22	Not quantified
148060	3.80E-31	Not quantified
No plasma available	NA	No plasma available
Only HPV assessed	NA	6386.6
128972	0.00E+00	No HPV templates found
96070	0.00E+00	No HPV templates found
No plasma available	NA	No plasma available
140730	1.87E-03	Not quantified
155200	2.64E-08	8.9
148650	4.82E-30	Not quantified
No plasma available	NA	Not quantified
166610	2.79E-09	No plasma available
Only HPV assessed	NA	Not quantified
No plasma available	NA	4
126570	1.64E-173	No plasma available
No plasma available	NA	33.1
No plasma available	NA	No plasma available
Only HPV assessed	NA	No plasma available
235469	7.38E-154	57.8
		369.2

Fig. 6S

153345	Only HPV assessed No plasma available	0.00E+00 NA	Not quantified
		3.4	No plasma available Not quantified
123775	No plasma available	2.07E-18 NA	No plasma available Not quantified
166610	Only HPV assessed	NA	11.8
126570	Only HPV assessed	6.40E-20 NA	67.4
96060		3.07E-177	7.9
227918	No plasma available	6.51E-34 NA	No HPV templates found
148050	No plasma available No plasma available No plasma available No plasma available	0.00E+00 NA NA NA NA	13.7
			No plasma available No HPV templates found No plasma available No plasma available No plasma available
			Not quantified
			No HPV templates found
153345	Only HPV assessed Only HPV assessed No plasma available No plasma available	NA NA NA NA NA NA NA	4.5
			No plasma available No plasma available

A. CLASSIFICATION OF SUBJECT MATTER**C12Q 1/68(2006.01)I**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q 1/68; G01N 33/574

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: head and neck squamous cell carcinoma, plasma, saliva, tumor DNA, HPV, TP53, NOTCH1, PI3KCAm CDKN2A, FBXW7, HRAS, NRAS**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AHN et al., "Saliva and plasma quantitative polymerase chain reaction-based detection and surveillance of human papillomavirus-related head and neck cancer" JAMA Otolaryngology-Head & Neck Surgery, Vol.140, No.9, pp.846-854 (31 July 2014) See abstract.	1-3, 6-10, 12-21, 23-25
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Further documents are listed in the continuation of Box C.



See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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International Application Division

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/037793

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	LUTZKY et al., "Biomarkers for cancers of the head and neck" Clinical Medicine Insights: Ear, Nose and Throat, Vol.1, pp.5-15 (2008) See the whole document.	1-25
PX	WANG et al., "Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck squamous cell carcinomas" Science Translational Medicine, Vol.7, Issue 293, Article No.293ra104, Internal pages 1-9 (24 June 2015) See pages 3-5.	1-25

INTERNATIONAL SEARCH REPORT

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datePatent family
member(s)Publication
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