WHITE PAPER

THE SCIENCE OF EARLIER™: IMPROVING EARLY DETECTION OF ORAL AND OROPHARYNGEAL CANCER

VIGILANTBIOSCIENCES™

Dedicated to Early Intervention. And Life.

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BACKGROUND OF ORAL CANCER

Oral squamous cell carcinoma (OSCC) is a debilitating and deadly disease where both the treatment and the disease itself can often result in disfigurement and the impairment of speech and eating function when it does not otherwise result in death. This devastating type of cancer develops from the mucosal linings of the upper aerodigestive tract (UADT) comprising the nasal cavity and paranasal sinuses, the nasopharynx, the hypopharynx, larynx, trachea, oral cavity and oropharynx with areas at highest risk for developing cancer being the floor of the mouth, the lateroventral tongue and the soft palate.[1] OSCC is the most frequent malignant tumor of the head and neck region.[2] It is also an aggressive tumor with low response to chemotherapy and basic resistance to most standard of care anticancer drugs.[3]

OSCC represents more than ninety percent of all head and neck cancers with a varied list of risk factors. For example, gender plays a role with males having this type of cancer twice as often as females.[4] Additionally, males in certain ethnic groups, such as African-Americans, have a higher incidence of oral and oropharyngeal cancers than do their Caucasian counterparts.[5] Traditionally, tobacco products, in particular smokeless tobacco products, are known to be a primary cause of this disease. Excessive alcohol use is also considered a high risk factor, especially when combined with tobacco products since the two act synergistically.[6,7] Recently, however, the human papilloma virus (HPV) infection commonly associated with sexual activity is now shown to be responsible for the rising proportion of OSCC cases independent of race, gender, tobacco use, alcohol consumption, or other risk factors.[8] Many strains of HPV infection associated with oral cancer also overlap with the same strains associated with cervical cancer. Worldwide, HPV-16 prevalence accounts for 40.6% of oropharyngeal squamous cell carcinomas, 14.9% of oral cavity squamous cell carcinomas and 13.4% of laryngeal squamous cell carcinomas.[9] HPV-18 accounts worldwide for 5.9% of oral cavity squamous cell carcinomas, 0.7% oropharyngeal squamous cell carcinomas and 1.6% of laryngeal squamous cell carcinomas.[10] It is currently unclear if HPV alone is sufficient to cause oropharyngeal cancer or if other risk factors (listed above) interact with HPV to cause these cancers.[11] In addition to the risk factors mentioned, others include the Epstein-Barr virus, gastroesophageal reflux disease (GERD), and exposure to paint fumes, plastic by-products, wood dust, asbestos and gasoline fumes.[12]

MORE THAN **90%** **OF ORAL CANCERS** ARE ORAL SQUAMOUS CELL CARCINOMA.

THE ORAL CANCER PROBLEM

Worldwide, there are more than 600,000 new cases of oral cancer each year.[13] Over 48,000 individuals in the United States will be diagnosed with oral or oropharyngeal cancer this year with an estimated 9,600 deaths resulting from this disease, equating to the killing of roughly one person per hour each day.[14] Of those 48,000 newly diagnosed individuals, only 57% will be alive in 5 years. This number has not significantly improved in decades.[15]

Survival rates 5 years from diagnosis had been stagnant for many decades at about 50%. Although the current 57% survival rate is an improvement over the last ten years, this improvement is due to the increase of HPV16 caused cancers which are more vulnerable to existing treatment modalities and can confer a significant survival advantage.[16] Therefore, it is a change in etiology and not improved early discovery or treatments that are the sole cause for improvement. Conversely, certain ethnic groups continue to have poorer outcome with survival. For example, African-American males have mortality rates that are twice as high with a five-year survival rate being only 39.5%.[17] With this understanding, the severity of this disease becomes even more glaring.

Studies also show that there is a 16-36% chance of oral cancer recurrence in addition to the probability of developing subsequent cancers elsewhere in the body due to metastasis.[18,19,20] Metastasis occurs most commonly via the bloodstream or lymphatic system. Cancer cells once detached from the tumor can have access via these routes to every portion of the body.[21] Recurrence of these cancers due to this metastasis factor is unfortunately very real, and continued follow-up care is vital to be sure that secondary cancers do not develop.

THERE ARE MORE THAN **600,000** **NEW CASES** OF ORAL CANCER EACH YEAR.

THE CURRENT STANDARD FOR ORAL CANCER SCREENING

Oral cancer screening by definition is the process by which a practitioner evaluates an asymptomatic patient to determine if he or she is likely or unlikely to have a potentially malignant or malignant lesion.[22] An important component of this screening is a thorough review of the patients' health history.

Currently, the current standard for screening is visual and tactile palpation during an extra and intra oral inspection by the healthcare professional during routine examination. This head and neck examination entails bimanual palpation of various external areas of: 1) the head and neck including the lower jaw, neck, glands and lymph nodes of this area, and 2) the oral cavity including the tongue, cheeks, floor and roof of the mouth, lips, back of the throat.[23]

During this examination, the frontline screeners traditionally look for clinical features of oral lesions that might raise suspicion of potential malignancy include sharp or distinct margins, a red component (color variation), a non-homogenous white component (surface irregularity), persistent ulceration and size larger than 1 centimeter.[24] The clinician also should view with suspicion any persistent or progressive lesion of the ventrolateral tongue or the floor of the mouth (both of which are high-risk sites for oral squamous cell carcinoma). If these types of areas are present, the current standard of oral cancer diagnosis follows which requires the histopathological examination of surgical biopsy specimens.[25]

THE ORAL CANCER CHALLENGE AND THE UNMET CLINICAL NEED

When oral cancer is identified in Stage I or Stage II, the overall five-year survival rate is over 80%. All too often, however, the manifestations of this invasive and devastating disease are found later during either stage III or stage IV periods where the five-year survival rate falls to less than 40%.[26] Unfortunately, over 60% of oral cancer patients in the United States are identified with the advanced stage of disease.[27]

THE STAGES OF ORAL CANCER[28]

The stages used to describe cancer of the lip and oral cavity

STAGE I	The cancer is less than 2 centimeters in size (about 1 inch), and has not spread to lymph nodes in the area (lymph nodes are small almond shaped structures that are found throughout the body which produce and store infection-fighting cells).
STAGE II	The cancer is more than 2 centimeters in size, but less than 4 centimeters (less than 2 inches), and has not spread to lymph nodes in the area.
STAGE III	Any of the following may be true: 1) The cancer is more than 4 centimeters in size, 2) The cancer is any size but has spread to only one lymph node on the same side of the neck as the cancer, or 3) The lymph node that contains cancer measures no more than 3 centimeters (just over one inch).
STAGE IV	Any of the following may be true: 1) The cancer has spread to tissues around the lip and oral cavity, 2) The lymph nodes in the area may or may not contain cancer, 3) The cancer is any size and has spread to more than one lymph node on the same side of the neck as the cancer, to lymph nodes on one or both sides of the neck, or to any lymph node that measures more than 6 centimeters (over 2 inches), or 4) The cancer has spread to other parts of the body.
RECURRENT	Recurrent disease means that the cancer has come back (recurred) after it has been treated. It may come back in the lip and oral cavity or in another part of the body.

Unfortunately, early-stage lesions may often be asymptomatic or mimic other conditions, whereas other lesions may not be readily evident in routine examination. Also, because malignant and benign lesions may not be clinically distinguishable, the clinician cannot predict the biological relevance of lesions on the basis of their physical features alone.[29] Our ability to identify this disease in its earliest stages with this screening modality therefore is not easy and has often eluded the medical and dental professions. The reason must be reiterated: Early oral cancers and precancerous lesions are often subtle and asymptomatic.[30] This phenomenon is often labeled as occult or hidden from plain view, and although the tissue may appear normal it often hides the truth within the cells below the surface of the mucosa.

When oral cancer lesions are easily and visually identified and biopsied, they may have likely progressed into Stage III or IV such that treatment requires aggressive surgical intervention and significant loss of the quality of life through impaired speech and eating function as well as disfigurement from the resulting surgery. Certainly by this stage, we have missed the opportunity attending to our patients at an earlier intervention when the five-year survival rates are best.

Thus, there exists a strong need to identify these occult lesions as early as possible. Efforts over the last decade or so have been made to enable medical and dental clinicians to visualize early lesions using variety of techniques through adjunctive visualization and diagnostic aids. However, we must understand these visualization modalities are not sensitive and specific (and therefore, not as accurate) for effective screening, diagnosis, or otherwise risk assessment of any type of abnormal lesion where only a definitive test can determine the biologic behavior of a lesion.[31] While there are a variety of adjunctive screening products available, it remains unclear as to whether they significantly improve early detection. To date, there have been no widely accepted early detection, screening, or risk assessment tests for oral cancer.[32] Many of the tests are very expensive and priced where payors (insurance and patients alike) are reluctant to pay for such tests particularly without demonstrated clinical utility. As a result, high costs to normal clinic practice implementing these adjuncts have served as an impediment for readily adopting these types of adjuncts into the standard of care.

PATIENTS WITH ELEVATED SOLCD44 AND PROTEIN LEVELS MAY BE **25X** MORE LIKELY TO HAVE ORAL CANCER THAN THOSE WITHOUT.

ANSWERING THE UNMET NEED

Dentistry has long been charged by groups like the US Preventive Services Task Force (USPSTF) to be at the forefront of oral cancer detection.[33] Interestingly, the USPSTF recently concluded "the current evidence is insufficient to assess the balance of benefits and harms of screening for oral cancer in asymptomatic adults." However, one would argue that it is because of the nature of the screening mechanisms available to date that led the USPSTF to this decision.

In 2004, the National Institute of Dental and Craniofacial Research (NIDCR) encouraged the research community to comprehensively decipher, catalogue, and identify human salivary proteomes (i.e. proteins).[34] This work has uncovered well over 1,100 proteins in human saliva. These proteins possess clues as to where and why they are being produced and hold the potential to help unlock underlying disease mechanisms, which are developed both locally in the oral cavity and elsewhere in the body. Saliva samples may provide the opportunity to solve the oral cancer challenge for earlier intervention.

Independent of the NIDCR initiative, however, researchers at the University of Miami (UM) had already undertaken studying key salivary protein biomarkers captured in an oral rinse. Their initial discovery lead to a landmark publication that revealed a family of proteins (CD44) expressed from OSCC and thought to promote tumorigenesis.[35] Based on this seminal work, further research was conducted over a period of 15 years. During this time, studies showed salivary solCD44 collected in 5mL of an oral rinse could effectively be used to reveal OSCC at all stages.[36] Additionally, solCD44 was shown to be elevated in a majority of OSCC cases, and with different measures of elevated total protein, solCD44 could distinguish cancer from benign disease with high accuracy using traditional lab assays and equipment and thereby supporting an effective test as an aid in diagnosis.[37] Further studies demonstrated these protein marker levels were elevated regardless of the tumor size or stage, and thereby indicating these markers to be present early in carcinogenesis,[38] and in some instances, before visual or physical symptoms develop. They also discovered that that subjects with high solCD44 and protein levels in the oral rinse ELISA assay showed varying quantifiable risk levels including certain categories revealing a 25 times more likelihood of a patient to have oral cancer than those without these elevated levels.[39]

The data culminated from these and further ongoing studies revealed that a simple yet elegant oral rinse test can be an accurate, effective, and affordable method to aid in the diagnosis of the onset of oral cancer and hold the promise to forever change the way clinicians identify and manage this horrific disease.

THE SCIENCE OF EARLIER™

In 2011, Vigilant Biosciences, Inc. ("Vigilant") licensed the intellectual property based on this research from University of Miami to commercialize this technology for use in various clinical settings. Since then, Vigilant began development of its initial, pioneering products for oral cancer using the CD44 and total protein biomarkers. The initial products under the OncAlert brand include: the OncAlert™ Oral Cancer RAPID Test (a rapid point-of-care qualitative test) kit and the OncAlert™ Oral Cancer LAB Test (a set of quantitative laboratory tests). Both products are CE Marked.

The Vigilant OncAlert™ product line will overcome the unmet market need for a simple, accurate and cost-effective early detection process. The OncAlert™ RAPID Test comprises of a lateral flow test strip with results that can be read without any capital equipment and that, together with other clinical factors, will aid the healthcare practitioner in the detection of cancer in its earliest stages in a quick (within 20 minutes) and accurate assessment.

A comparison of available test methods demonstrates the benefits of the OncAlert™ Oral Cancer product line.

DETECTION TECHNIQUE	SENSITIVITY	SPECIFICITY	REFERENCE
ONCALERT ORAL CANCER PRODUCT LINE (CE MARKED)	0.88	0.74	VIGILANT BIOSCIENCES DATA (NOT YET PUBLISHED)
STANDARD VISUAL / PHYSICAL ORAL EXAM	0.64	0.74	SANKARANARAYANAN ET AL., 2005
TOLUIDINE (DYE)	0.40	0.90	SU ET AL, 2010
CHEMILUMINESCENCE	0.00	0. 76	MEHROTRA, 2010
FLUORESCENCE	0.50	0.39	MEHROTRA, 2010
BRUSH CYTOLOGY TEST	0.52	0.29	HOLWEG-MAJERT, 2009
MRNA (IL-8, IL-1B,SAT, OAZ1)	0.45-0.79	0.72-0.77	ELASHOFF D...WONG, DT 2012

ONCALERT™ WILL OVERCOME THE UNMET MARKET NEED FOR AN **SIMPLE, ACCURATE AND COST-EFFECTIVE** TECHNOLOGY TO AID IN THE DIAGNOSIS OF ORAL CANCER.

ORAL CANCER DIAGNOSTIC AID	TUMOR-INITIATING AND STEM CELL ASSOCIATED BIOMARKER	SIMPLE TO USE	COST TO CLINIC
ONCALERT ORAL CANCER PRODUCT LINE	✓	✓	$
CHEMILUMINESCENCE			$$
ORAL HPV TEST			$$
FLUORESCENCE / REFLECTANCE LIGHT-BASED SYSTEMS			$$$$$
BRUSH CYTOLOGY			$$$
LESION VALIDATION MOLECULAR DNA TEST			$$$

NO SPECIAL TRAINING

OR STEEP LEARNING CURVE IS ASSOCIATED WITH THE ONCALERT™ ORAL CANCER TESTS.

No special training or steep learning curve is associated with the OncAlert™ tests, which will be of great benefit to the clinical offices by providing minimal disruption to the flow of the clinic.

With the OncAlert™ Oral Cancer product line, we are now on the threshold of a remarkable technology and product, which can finally provide the healthcare community with a useful tool to identify OSCC in its earliest stages and thereby aiding the clinician to provide the patient an earlier intervention in the hopes of successfully overcoming their battle with oral cancer.

ONCALERT™ ORAL CANCER PROCEDURE:

- Swish and gargle 5 ml saline in the mouth for 10 seconds.
- Spit into specimen cup.
- Insert test cassette into specimen cup.
- Wait 20 minutes for a colorimetric result indicating presence of CD44 and Total Protein with respect to pre-determined threshold levels for cutoffs in the specimen.

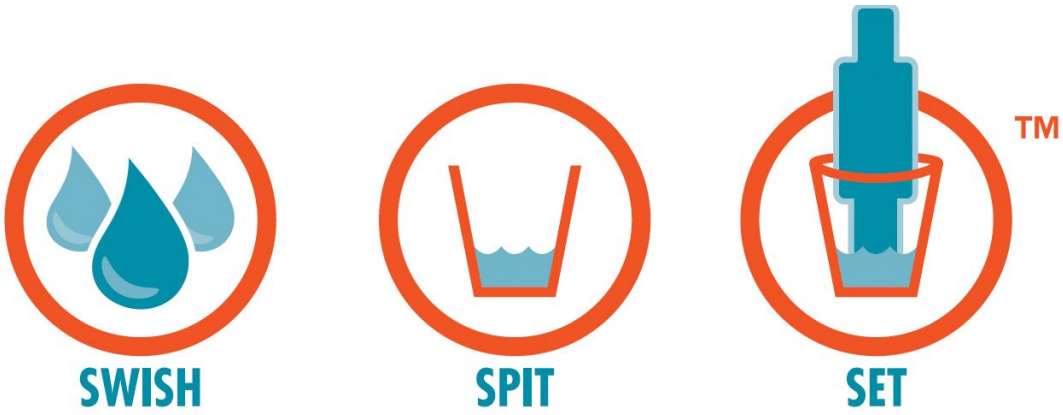
Once the patient has provided the sample, the health care provider performs an external visual and bilateral manual palpation examination and an internal and external visual and bimanual palpation head and neck examination to determine if there are any visible or palpated abnormalities, as follows:

- Lips
- Floor of Mouth
- Roof of Mouth (Hard and Soft Palate)
- Buccal Mucosa
- Tongue (dorsal and ventral surfaces and lateral borders)
- Tonsil areas
- Palpate base of tongue, floor of mouth

The observations of this examination are noted in the patient record.

Results are produced within 20 minutes. Together with the observations of the examination and the reading of the OncAlert™ Oral Cancer RAPID Test, the healthcare provider would then discuss the results with the patient during his/her examination. If the test shows a negative result, then only annual OncAlert™ testing with the OncAlert Oral Cancer RAPID Test is needed. If the test shows a positive result, the clinician will discuss this with the patient and prescribe a follow up regimen as outlined below:

WITH THE
ONCALERT™
PRODUCT LINE
WE ARE NOW ON THE THRESHOLD OF LEVERAGING THE SCIENCE OF EARLIER TO AID CLINICIANS IN THE DIAGNOSIS OF OSCC.



IF THE TEST IS POSITIVE:

- Lifestyle changes to help reduce and possibly reverse disease progression (tobacco cessation, alcohol reduction or elimination, practice good oral hygiene and improve nutrition).
- Schedule for a second OncAlert™ RAPID Test in one to three months.
  - i. If second OncAlert™ Test is negative, routine follow-up (per health care profession protocol) with repeat OncAlert™ RAPID Test annually.
  - ii. If second OncAlert™ Test is positive, refer to appropriate specialist (ENT, oral surgeon, otolaryngologist, oncologist).
    - → Specialist can perform additional tests available for further examination.
    - → Specialist orders OncAlert™ LAB test to obtain quantitative results.

Any time a lesion is identified by a clinician, a biopsy is strongly recommended. If lesion is of uncertain significance, OncAlert™ testing may be performed to guide decision-making. All patients encouraged in healthy lifestyle behaviors.

When the OncAlert™ RAPID Test is used and indicates a positive result, then the OncAlert™ LAB Test would be utilized by the clinician to aid in diagnosing and possibly monitoring an at-risk patient not diagnosed with cancer so as to provide a more detailed assessment to determine the appropriate type of intervention for the patient. The OncAlert™ products will be the first tools to simply, accurately and cost-effectively aid healthcare professionals in the early detection of cancer.

[1]Hong WK, Lippman SM, Itri LM, et al. Prevention of secondary primary tumors with isotretinoin in squamous cell carcinoma of the head and neck. N Engl J Med 1990;323: 795-801, 8257.
[2]ibid.
[3]Grimm M, Cetindis M, Lehmann M, Biegner T, Munz A, Teriete P, Kraut W and Reinert S. Association of cancer metabolism-related proteins with oral carcinogenesis – indications for chemoprevention and metabolic sensitizing of oral squamous cell carcinoma? Journal of Translational Medicine 2014, 12:208 doi:10.1186/1479-5876-12-208
[4]Surveillance, Epidemiology, and End Results (SEER) Program, National Cancer Institute. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence – SEER 17 Regs Limited-Use + Hurricane Katrina Impacted Louisiana Cases, Nov 2008 Sub (2000-2006), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2009, based on the November 2008 submission
[5]Ragin CCR, Modugno F, Gollin SM. The Epidemiology and Risk Factors of Head and Neck Cancer: a Focus on Human Papillomavirus. J DENT RES February 2007 86: 104-114
[6]Hong WK, op. cit.
[7]Head and Neck Cancer, op. cit.
[8]Fakhry C, Souza G. Discussing the diagnosis of HPV-OSCC: Common questions and answers. Oral Oncology Head and Neck Oncology, September 2013; 49 (9): 863-871.
[9]Head and Neck Cancer, op. cit.
[10]ibid.
[11]http://www.cdc.gov/std/hpv/STDFact-HPVandOropharyngealCancer.htm
[12]Head and Neck Cancer, op. cit.
[13]Mol Cancer Res April 2014 12; 571;
[14]American Cancer Society: http://www.cancer.org/cancer/oralcavityandoropharyngealcancer/detailedguide/oral-cavity-and-oropharyngeal-cancer-key-statistics
[15]http://www.oralcancerfoundation.org/facts/
[16]http://www.oralcancerfoundation.org/facts/
[17]Ragin CCR, op.cit.
[18]Hong WK, op. cit.
[19]Schwartz LH, Ozsahin M, Zhang GN, et al. Synchronous and metachronous head and neck carcinomas. Cancer 1994;74:1933-8.
[20]http://www.oralcancerfoundation.org/facts/metastasis.php
[21]http://www.cdc.gov/std/hpv/STDFact-HPVandOropharyngealCancer.htm
[22]Rethman MP, Carpenter W, Cohen EW, Epstein J, et al. Evidence-Based Clinical Recommendations Regarding Screening for Oral Squamous Cell Carcinomas. The J Am Dent Assoc 2010; 141: 509-520.
[23]http://www.sixstepscreening.org/oral-cancer/
[24]Mehrotra R, Hullmann M, Smeets R, Reichert TE, and Driemel O, "Oral cytology revisited," Journal of Oral Pathology and Medicine, vol. 38, no. 2, pp. 161–166, 2009.
[25]Ibid.
[26]Ries LAG, Young JL, Keel GE, Eisner MP, Lin YD, Horner M-J, editors. SEER Survival Monograph: Cancer Survival Among Adults: US SEER Program, 1988–2001, Patient and Tu- mor Characteristics. National Cancer Institute, SEER Program, NIH Pub. No. 07–6215, Bethesda, MD, 2007.
[27]Franzmann EJ, Reategui, Pereira LHM, Pedroso F, et al. Salivary Protein and SolCD44 Levels as a Potential Screening Tool for Early Detection of Head and Neck Squamous Cell Carcinoma. Head Neck . 2012 May ; 34(5): 687–695.
[28]http://www.oralcancerfoundation.org/discovery-diagnosis/stages-of-cancer.php
[29]Mehrotra R, op. cit.
[30]Neville BW and Day TA. Oral Cancer and Precancerous Lesions. CA: A Cancer Journal for Clinicians Volume 52, Issue 4, pages 195–215, July/August 2002
[31]Mehrotra R, op. cit.
[32]Lin H.S., Talwar H.S., Tarca A.L., Ionan A., Chatterjee M., Ye B., et al. Autoantibody approach for serum-based detection of head and neck cancer, Cancer Epidemiol Biomarkers Prev 16(2007), 2396-2405.
[33]Moyer VA; U.S. Preventive Services Task Force. Screening for oral cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2014 Jan 7;160(1):55-60. doi: 10.7326/M13-2568.
[34]Denny P, Hagen DP FK, Hardt M, et al. The proteomes of human parotid and submandibular/sublingual glands salivas collected as the ductal secretions. J Proteome Res 2008; 7: 1994-2006.
[35]Franzmann EJ, Wee DT, Civantos FJ, Goodwin WJ, Bourguignon LYW. A Novel CD44v3 Isoform is Involved in Head and Neck Squamous Cell Carcinoma Progression. Otolaryngol Head Neck Surg 2001; 24: 426-432.
[36]Franzmann EJ, Reategui EP, Carraway KL, et al. Salivary Soluble CD44: A Potential Molecular Marker for Head and Neck Cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 735-739.
[37]Franzmann EJ, Reategui EP, Pedrosos F, et al. Soluble CD44 Is a Potential Marker for the Early Detection of Head and Neck Cancer. Cancer Epidemiol Biomarkers Prev 2007; 16(7):1348–55
[38]Franzmann EJ, Reategui EP, Pereira LHM, et al. Salivary Protein and solCD44 Levels as a Potential Screening Tool for Early Detection of Head and Neck Squamous Cell Carcinoma. Published online 11 July 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/hed.21810 (Head Neck 34: 687–695, 2012)
[39]ibid.

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