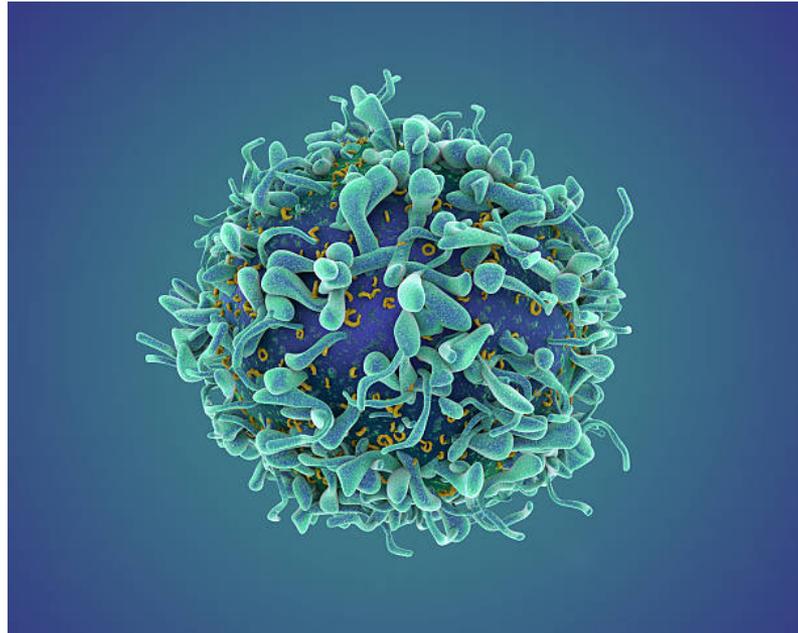


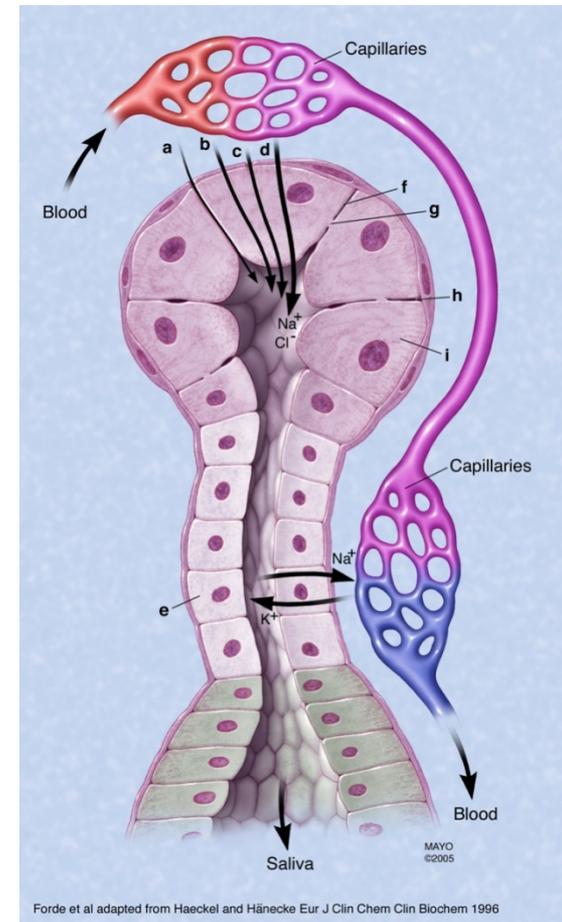
Biomarkers into saliva



Saliva used as biomarker for risk for oral cancer

Saliva composition ?

- Saliva comes from blood serum associated with oral microflora and virus :
- Hormons : cortisol, melatonin...
- Chemical : drugs, ethanol...
- Immunoglobulins : IgA
- Sugar : glucose
- Bacteria : caries, periodontal, , helicobacter pylori
- Virus : HBV, HPV
- Free mARN and cells



Saliva hormones

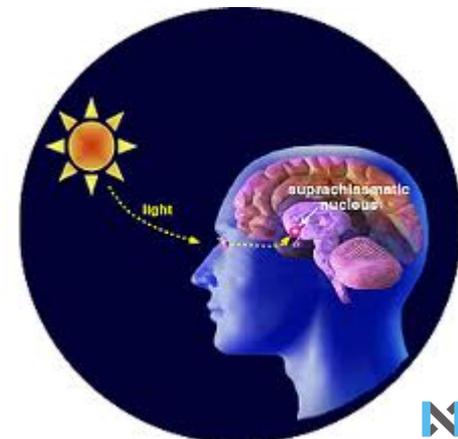
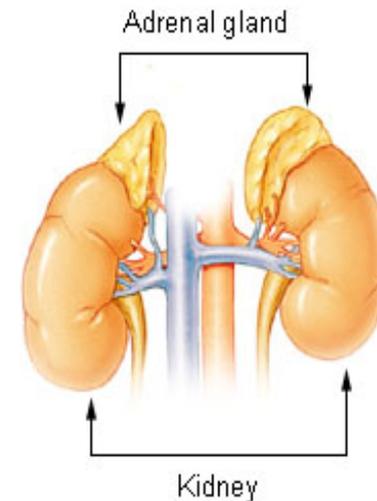
- Cortisol, melatonin, testosterone, DHEA...

2008, Restituto, et al., : cortisol dosage into saliva is equivalent to serum (Addison disease screening).

2010 Sakihara, et al., : cortisol saliva dosage is equivalent to serum and urinea (Cushing syndrome diagnostic)

2010 Bagcim, et al., : melatonin level into saliva is Equivalent to serum

Adrenal Gland



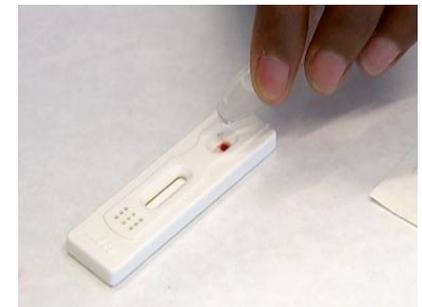
Chemical in saliva

- Alcohol, drug, organic molecules, pesticides, solvents

Ethanol, methanol, ethylene glycol are all detected into saliva & urine (Skins, et al., 2008) and exhaled breath

2011 Vindenes, et al., show that morphine, amphetamine, methamphetamine, N-desmethyldiazepam, benzodiazepines, cannabis & cocaine are very well detected into saliva

(PCP, pesticides, solvents...)



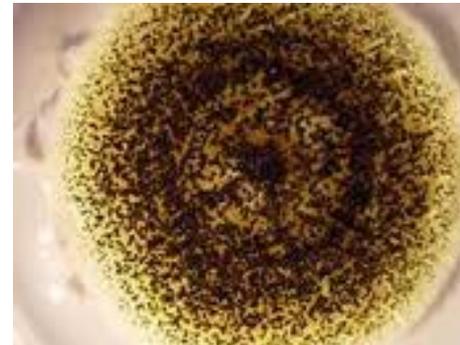
Immunoglobulins in saliva

- Allergy

IgA ratio in saliva changed for population having immuno- sensitivity to peanuts changed (2011 Peeters, et al.,)



Saliva IgA increased in presence of fungus mycotoxins (2003 Vojdani, et al.,)



Sugar in saliva and diabetic patient

- Diabetic situation

Sugar quantity into saliva increases for diabetic patients.

2009 Rao, et al., 65 proteins into saliva are specific biomarkers for type-2 diabetic.

2010 Soell, et al., overexpression of saliva chromogranin A for diabetic patients.



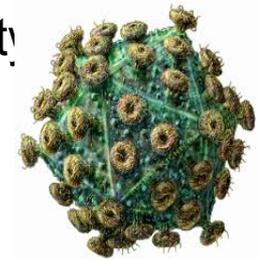
Bacteria and Virus

- Infectious diseases

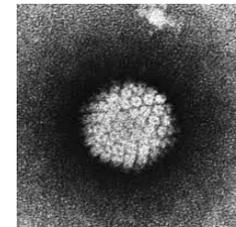
Caries bacterias (Streptococcus mutans...) cause dental plaque



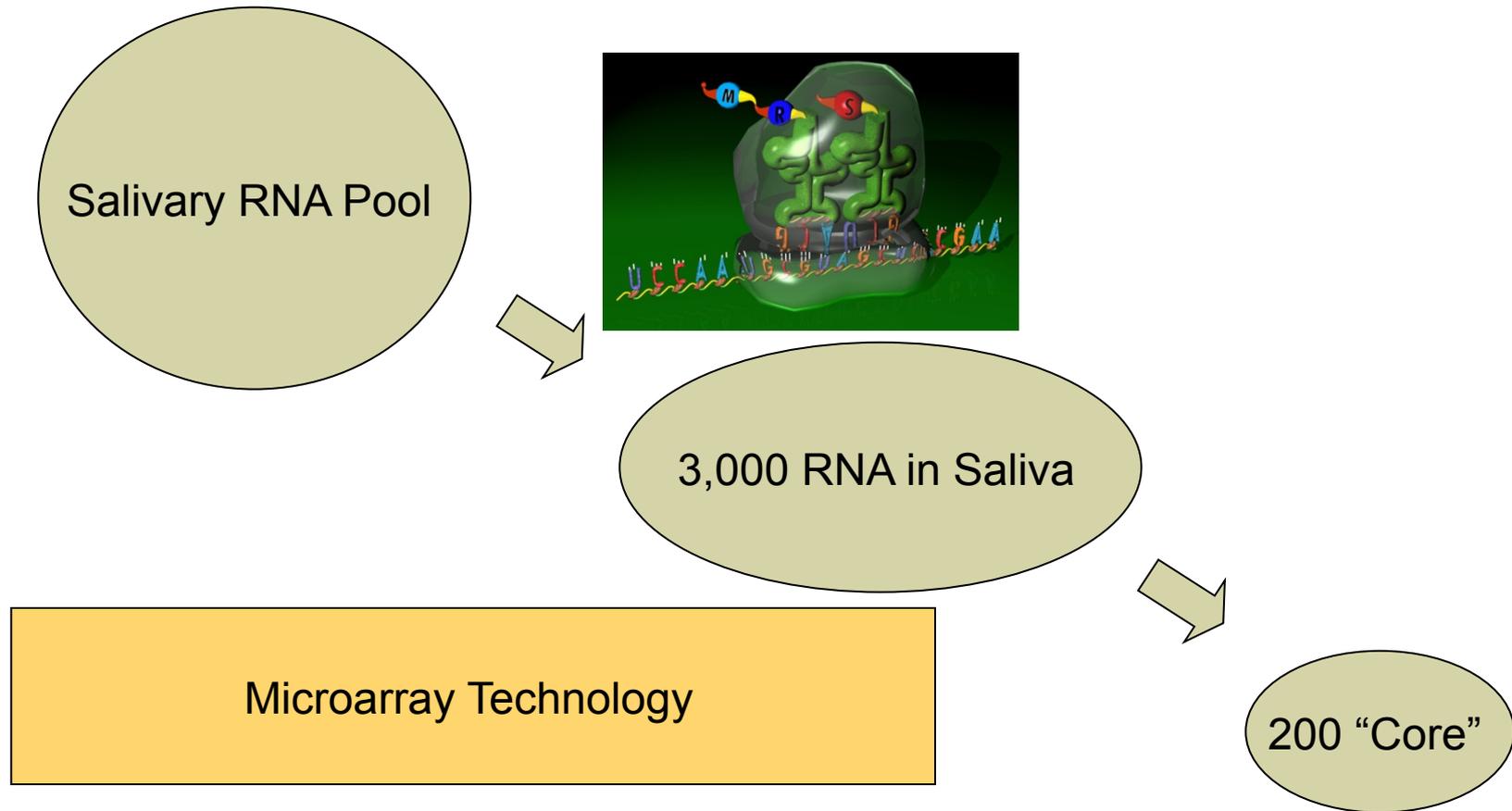
HIV : anti-HIV antibodies are present into saliva with 100% specificity (2008 by Zelin, et al., Pascoe, et al.,)



HPV is directly detected into saliva and represents a risk factor comparable to HPV in cervical cancer for Oral cancer development



Free mRNA are detected into saliva



Yang Li, et al. *Journal of Dental Research*, 83(3), 199-203, 2004

ONCORAL™

*Leveraging saliva components to
improve prognosis and quality of life of
cancer patients*

« Early diagnosis ensures better prognosis »

Dr Franck Chaubron (PhD)

CEO

Institut Clinident – Aix en Provence- France

Oral cancer

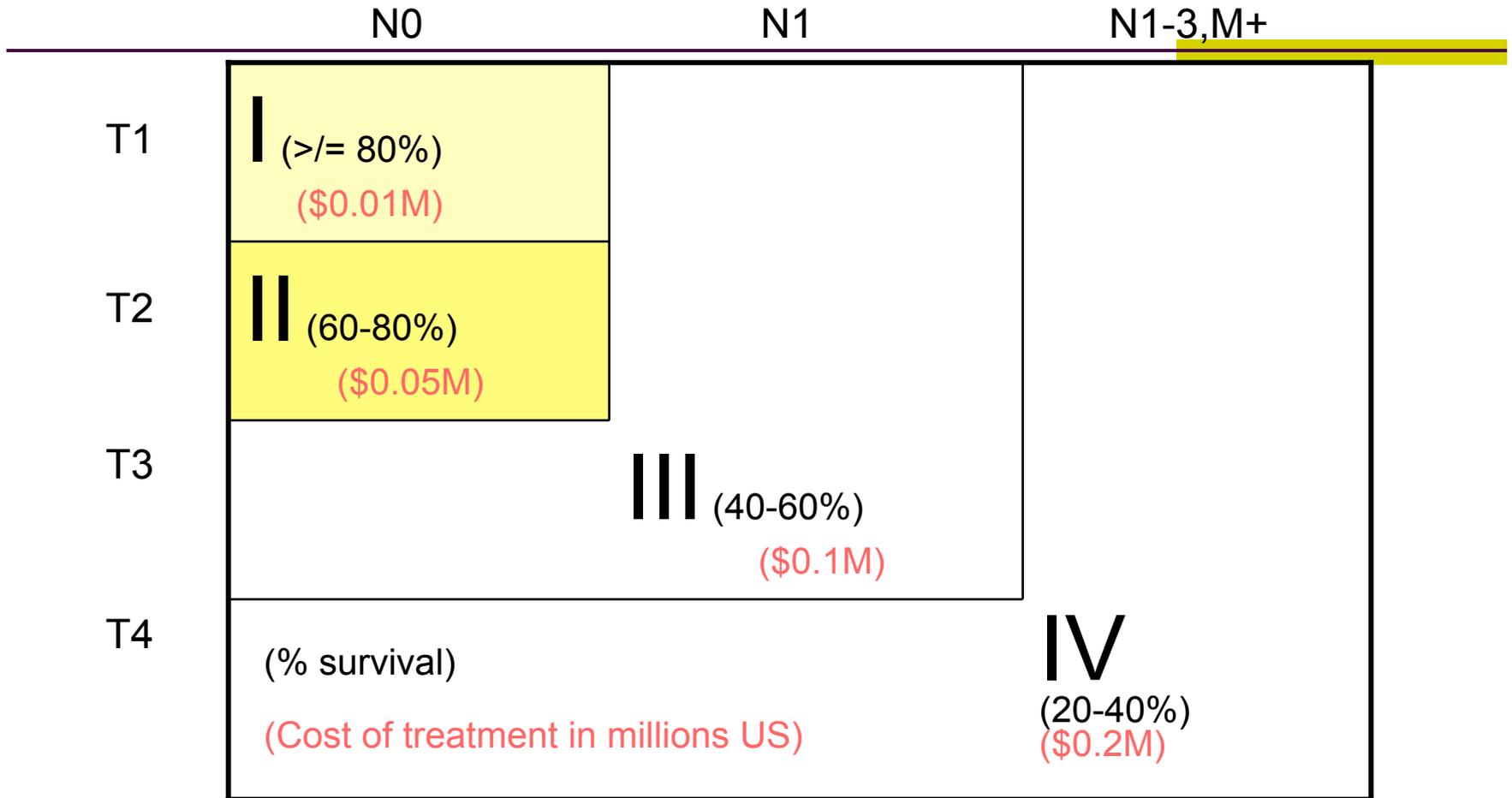
- Oral cancer is the **eighth most** common cancer worldwide and represents a significant disease burden.
- If detected at an **early stage**, survival from oral cancer is better than **90% at 5 years**, whereas **late stage disease survival is only 30%**. Therefore, there is an obvious clinical utility for novel metabolic markers that help to diagnose oral cancer at an early stage and to monitor treatment response.
- About **50 to 70% of patients** are diagnosed at an **advanced stage** depending on the countries.
- At the same time cigarette smoking declined (at least in Europe/North America), the number of oral cancer augmented. Human Papilloma Virus (HPV 16 & 18) becomes an identified cause.
- An increase of oral infected HPV patient has been observed in the past 20 years.
- 7% of the US adults are infected !

US EXPENDITURE FOR 15 MOST COMMON CANCERS

Cancer	Percent of all new cancers (2004)	Expenditures (billions)	Percent of all cancer treatment expenditures	Average Medicare payments* per individual in first year following diagnosis
Lung	12.7%	\$9.6	13.3%	\$24,700
Breast	15.9%	\$8.1	11.2%	\$11,000
Colorectal	10.7%	\$8.4	11.7%	\$24,200
Prostate	16.8%	\$8.0	11.1%	\$11,000
Lymphoma	4.6%	\$4.6	6.3%	\$21,500
Head/Neck	2.8%	\$3.2	4.4%	\$18,000
Bladder	4.4%	\$2.9	4.0%	\$12,300
Leukemia	2.4%	\$2.6	3.7%	\$18,000
Ovary	1.9%	\$2.2	3.1%	\$36,800
Kidney	2.6%	\$1.9	2.7%	\$25,300
Endometrial	2.9%	\$1.8	2.5%	\$16,200
Cervix	0.8%	\$1.7	2.4%	\$20,100
Pancreas	2.3%	\$1.5	2.1%	\$26,600
Melanoma	4.0%	\$1.5	2.0%	\$4,800
Esophagus	1.0%	\$0.8	1.1%	\$30,500
All Other	14.0%	\$13.4	18.5%	\$20,400
Total	100%	\$72.1	100%	

Source: US National Cancer Institute Report

Value proposition for early diagnostics

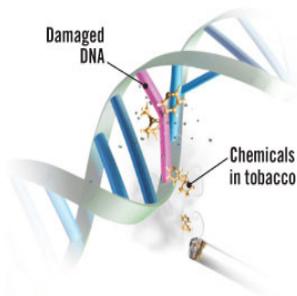


Cost treatment is reduced by 10 when detection is performed at early stage

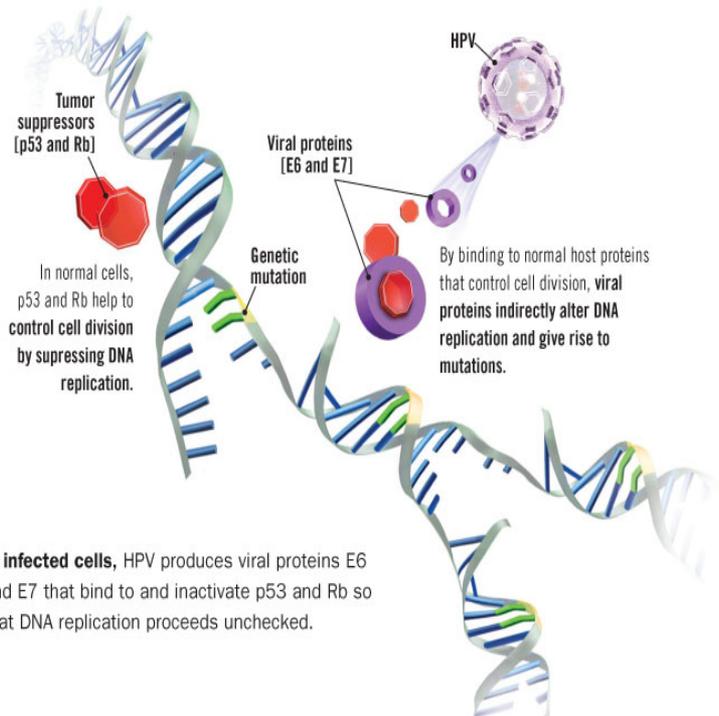
Oral cancer ethiology – from tobacco/cancer to HPV prevalence

Direct damage vs. indirect alteration >

Most oral cancers develop from direct damage to DNA caused by **tobacco-associated mutations** or indirect alteration of DNA caused by **viral proteins**.



MANY chemicals in tobacco can damage DNA directly.



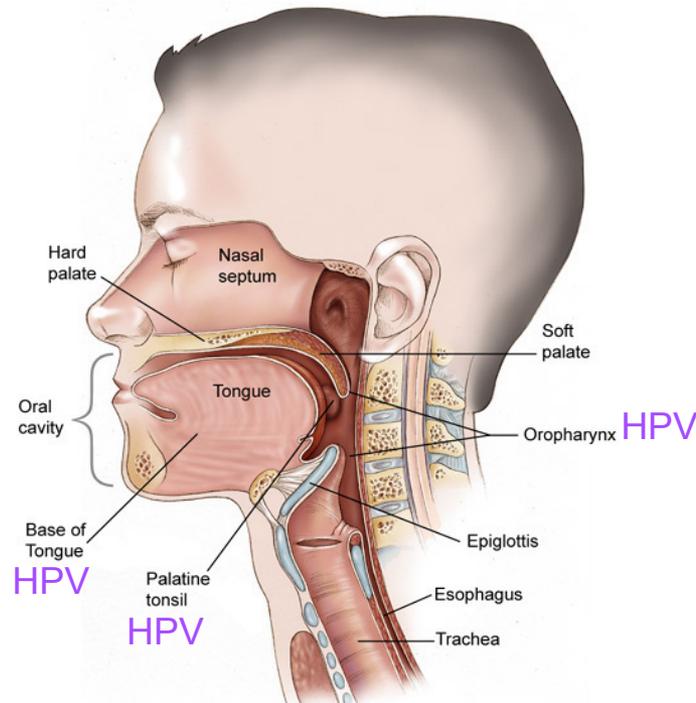
In infected cells, HPV produces viral proteins E6 and E7 that bind to and inactivate p53 and Rb so that DNA replication proceeds unchecked.

- It has now been established that the path that brings people to oral cancer contains at least two distinct etiologies; one through **tobacco and alcohol (75%)**, and another via the **HPV virus (25%)**, particularly version 16.
- In general it appears that HPV positive tumors **occur most frequently in a younger group** of individuals than tobacco related malignancies. (Tobacco oral cancers occur most frequently in the 5th through the 7th decade of life.) They also occur more in white males, and in non smokers.
- The HPV positive group is the fastest growing segment of the oral cancer population.

- HPV prevalence was identified in about **16% of tumor specimens collected between 1984 and 1989** versus about **72% of tumor specimens collected between 2000 and 2004**, a trend affecting younger populations

HPV statute and prognostics

- Using HPV infection as a measure of stratification in head and neck cancer staging



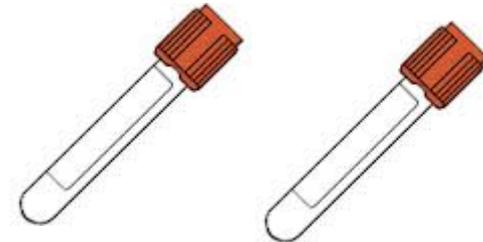
- Doctors will be able to use the results to classify these cancers as HPV positive or negative and offer treatment accordingly.
- Risk of death from HPV positive oropharyngeal cancer to be **between 50-80 per cent lower than HPV negative tumors** but patients are usually younger so may face a lifetime of treatment-related side effects.
- Classifying the HPV status of the cancer can offer eligible patients less intensive treatment with reduced side-effects. “get the most appropriate treatment for their cancer”.
- HPV-positive oropharyngeal cancers have better outcomes and fewer relapses after treatment than HPV-negative cancers.
- HPV vaccine strategy is under development in some territories (Austria) for males and females

Early analysis: new biomarkers

- HPV cancer represent 25% of oral cancer (detected into tumor cells). **Saliva HPV** is a risk factor but not a diagnosis
- 75% of oral cancer are population >45 years old associated with alcohol, tobacco... (**WHO – IARC** definition (International Agency for Research on Cancer : <http://www.iarc.fr/>))
- Developing Oncoral strategy Institut Clinident propose analysis of **specific Saliva Biomarkers for diagnosis and Saliva HPV identification for associated risk**
- **Metabolite Biomarkers** are detected into saliva
- Biomarkers are biochemical markers (**Volatile Organic Compounds – VOCs**)
- VOCs are small molecules coming from human cell metabolism (**Volatonomics**)
- VOCs are detected by **Mass Spectrometry** analysis

ONCORAL™ sampling kit

Sampling kit procedure (2 ml of saliva collected in 3 minutes)

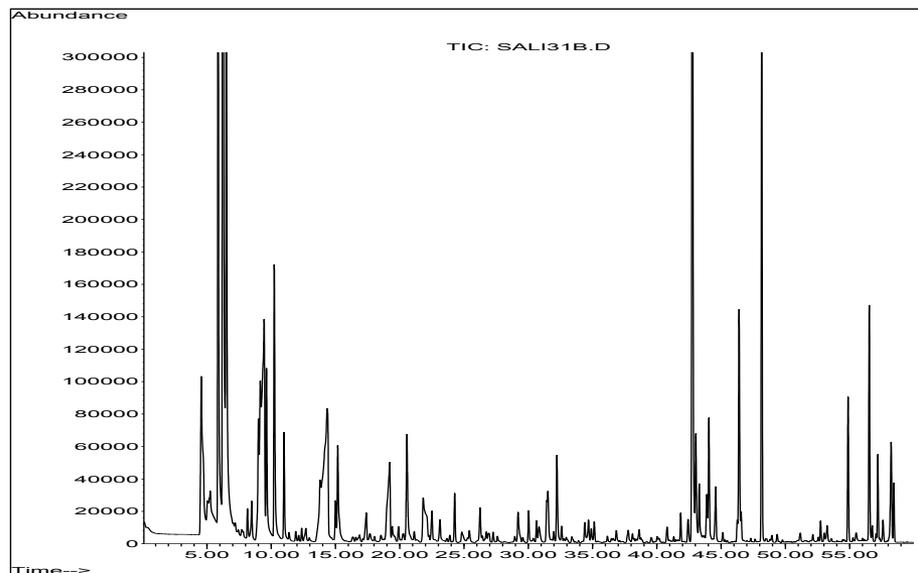


- Tartrazine
- Becher
- Vacutainer + salt (room T° stabilization)
- Safety bag



VOC analysis from Saliva

- **Saliva VOC identification** was realized with mass spectra bank Wiley 275K and with Kovats indices (KI) calculation. Relative quantification of molecules was done by SIM mode ion extraction to avoid error linked with molecular co-elutions. Peak areas corresponding to m/z of each molecule were estimated in Areas Arbitrary Unit (AAU).
- A **typical chromatogram** with the peaks representing individual compounds in saliva



VOC Results

- **107 VOCs** were identified in saliva samples to be used as Biomarkers for diagnosis
- Aromatics and esters were predominant in room-collection atmosphere, nitriles and other nitrogen compounds were supplied by the buffer solution consisting of a nitrogen molecule, 2- and 3-methylpentane and some furan compounds were known to come from fiber, septa and column. Some of these molecules may also come from tobacco. Then all analyses were performed on the **remaining 78 VOCs**.
- Among the **1767 molecular ratios calculated from the 78 VOCs**, **6 molecular ratios** were selected by a factorial discriminant analysis (FDA) to discriminate the control and the patients groups for Oral Cancer

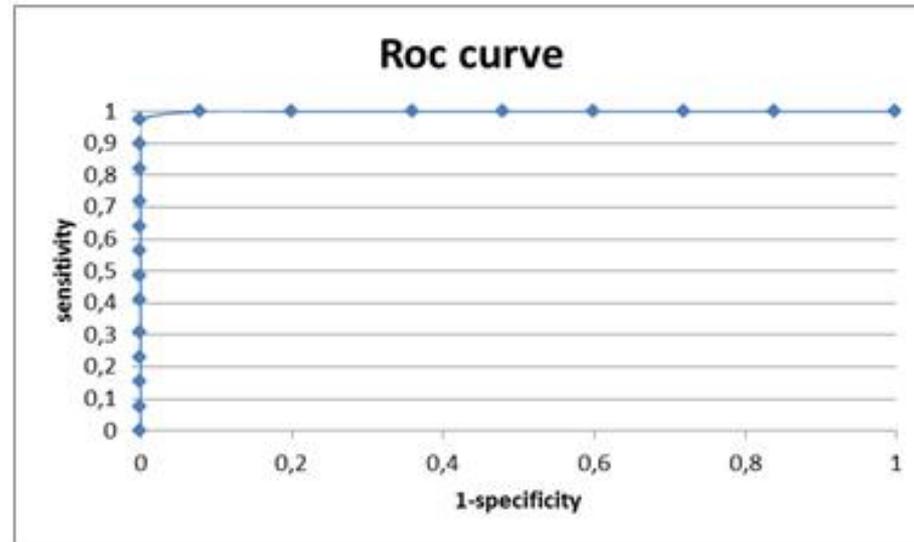
6 ratio model

The 6 molecular ratios selected by FDA with their coefficient value in the linear combination (LC) of the discrimination axis

Molecular ratio	Code	Coefficient value of the linear combination
2-butenal, 2-methyl- / methyl butanone	R1	-4,88
3-buten-2-one, 3-methyl- / 2-butenal, 2-methyl-	R2	1,21
2-pentanone, 3-methyl- / butanal	R3	-1,51
3-pentanone, 2,4-dimethyl- / methylbutanone	R4	-4,33
3-hexen-2-one, 5-methyl- / acetone	R5	70,49
Dimethylsulfide/ 1-propanol	R6	21,01
		2,51 (constant)

The classification model with the 6 molecular ratios discriminated 93.75% of the samples according to the factor "tumor". The 10 validation samples (4 controls and 6 patients) were tested in the discriminant model, and all were well classified as shown on **Erreur ! Source du renvoi introuvable.**

ROC WITH 6 RATIO MODEL



Receiver operating characteristic (ROC) curve for saliva for the diagnosis of oral cancer; (n = 64; n = 40 patients with oral cancer and n = 20 control patients); Area under the curve (AUC) is 0.999

Taking 0.6 as cutoff value for LC, the sensitivity was 92.5% and specificity was 95.8 %.

Patient monitoring

- When patients newly diagnosed with oral and oropharyngeal cancers are carefully examined, a small portion will have another cancer in a nearby area. Oncoral could be also part of this strategy to reduce that risk
- For this reason, patients with oral and oropharyngeal cancer will need to have follow-up exams for the rest of their lives and can be tested with new Biomarker strategy
- These patients also need to avoid using tobacco and alcohol, which increase the risk for these second cancers.

Thank you

