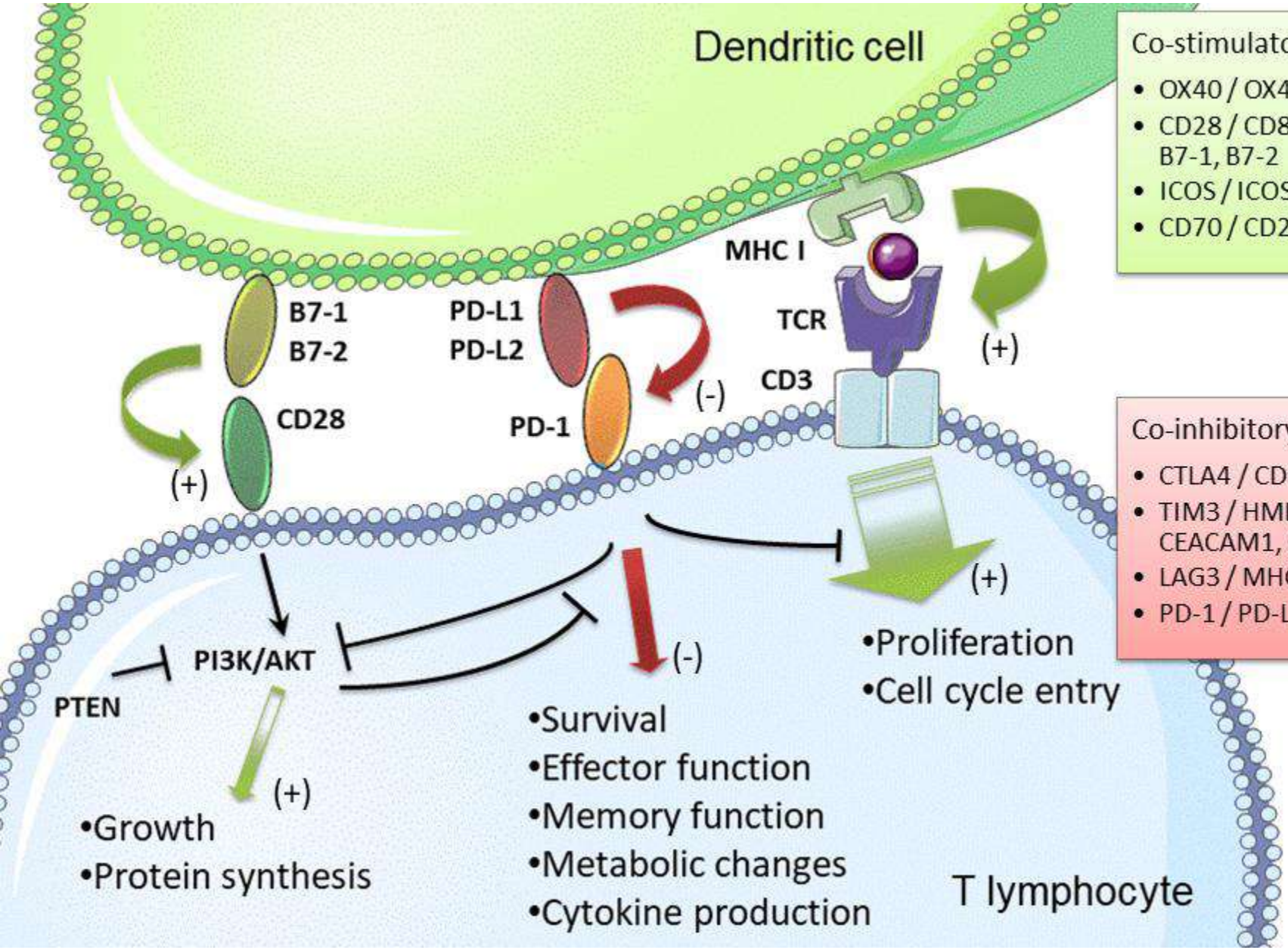
Pathologist input in the therapeutic decision

Pr Cécile Badoual
Service d'anatomopathologie,
Hôpital Européen G Pompidou, Paris
INSERM U970



S Outh-Gaer, Cancer Rev Treatment 2018

Contents lists available at ScienceDirect

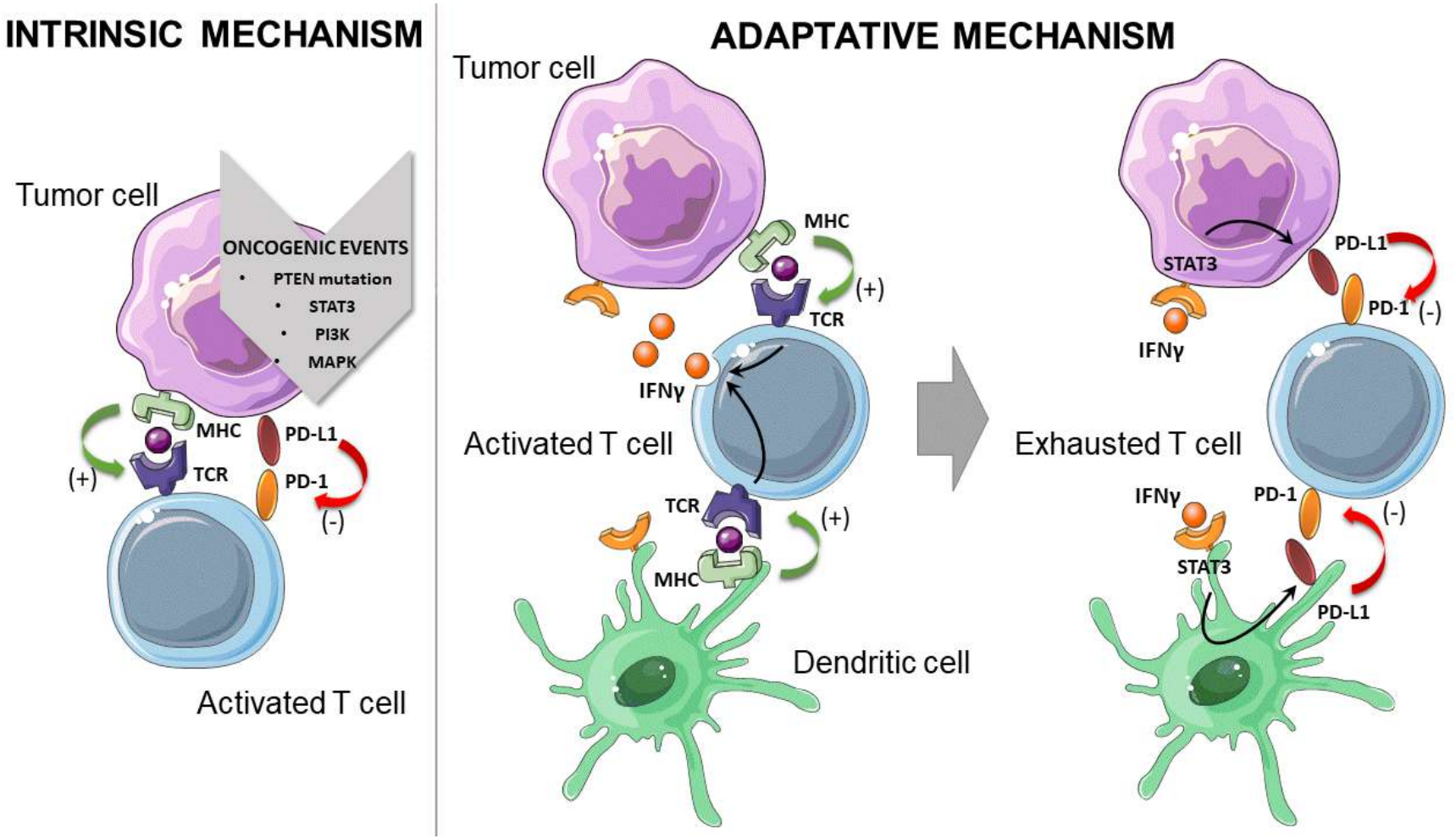
Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Anti-Tumour Treatment

Immunotherapy in head and neck cancers: A new challenge for immunologists, pathologists and clinicians

Sophie Outh-Gauer[a,b,c], Marie Alt[d], Christophe Le Tourneau[d,e], Jérémy Augustin[a], Chloé Broudin[a], Cassandre Gasne[b,c], Thomas Denize[a], Haitham Mirghani[f], Elizabeth Fabre[g], Madeleine Ménard[f], Florian Scotte[h], Eric Tartour[b,c,i], Cécile Badoual[a,b,c,*]



Tabacco/Alcohol

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 28: 1934–1941, 2017
doi:10.1093/annonc/mdx210
Published online 28 April 2017

ORIGINAL ARTICLE

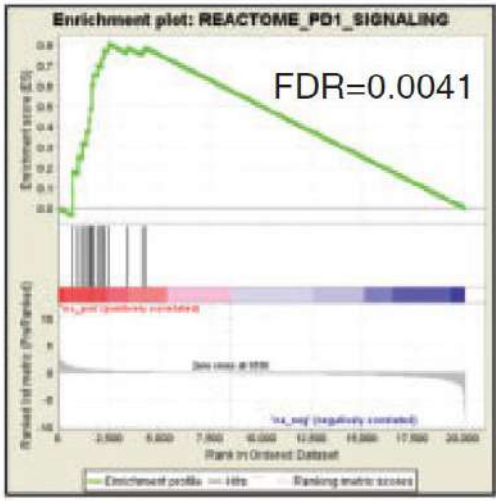
The immune microenvironment of HPV-negative oral squamous cell carcinoma from never-smokers and never-drinkers patients suggests higher clinical benefit of IDO1 and PD1/PD-L1 blockade

J.-P. Foy[1,2,3], C. Bertolus[3], M.-C. Michallet[1], S. Deneuve[4], R. Incitti[5], N. Bendriss-Vermare[1], M.-A. Albaret[1,5], S. Ortiz-Cuaran[1,5], E. Thomas[5], A. Colombe[2], C. Py[6], N. Gadot[2], J.-P. Michot[6], J. Fayette[7], A. Viari[5], B. Van den Eynde[8], P. Goudot[3], M. Devouassoux-Shisheboran[9], A. Puisieux[1], C. Caux[1], P. Zrounba[4], S. Lantuejoul[2,6] & P. Saintigny[1,2,7*]

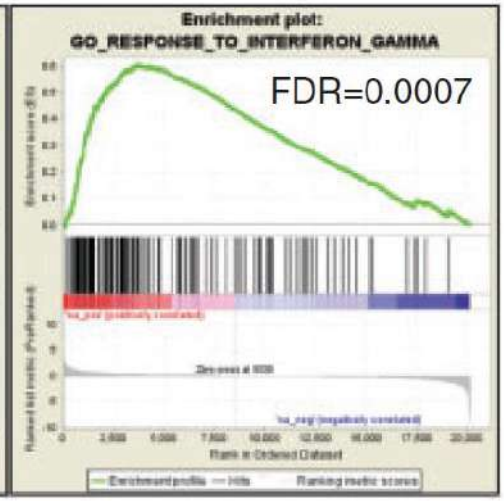
Differences between NSND /SD OSCC: immune microenvironment

Gene set enrichment analysis (GSEA) of the TGCA cohort

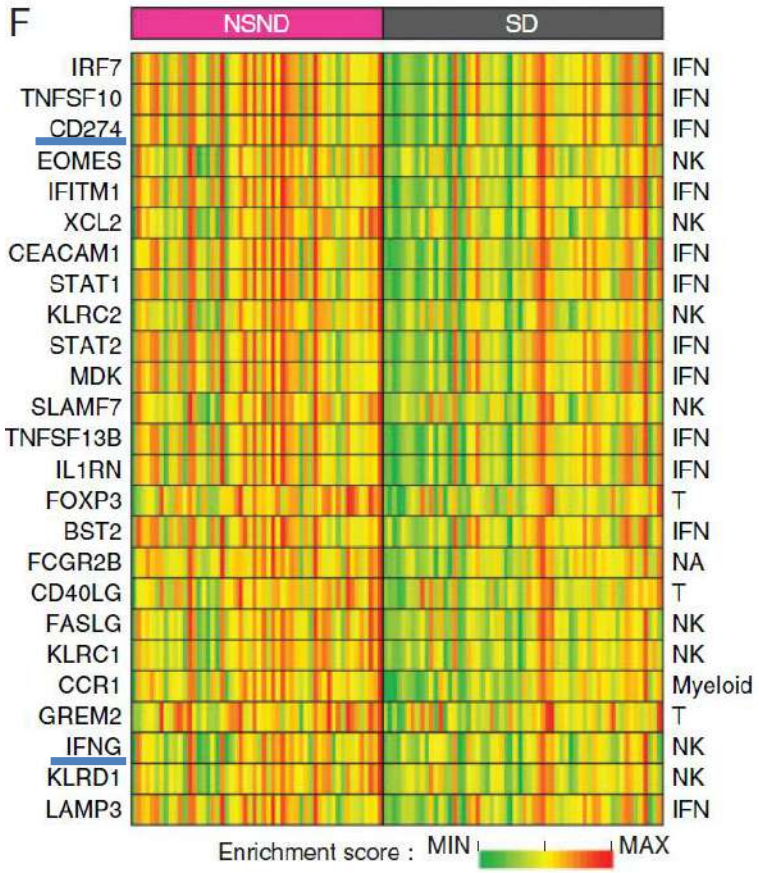
PD1



IFN-γ

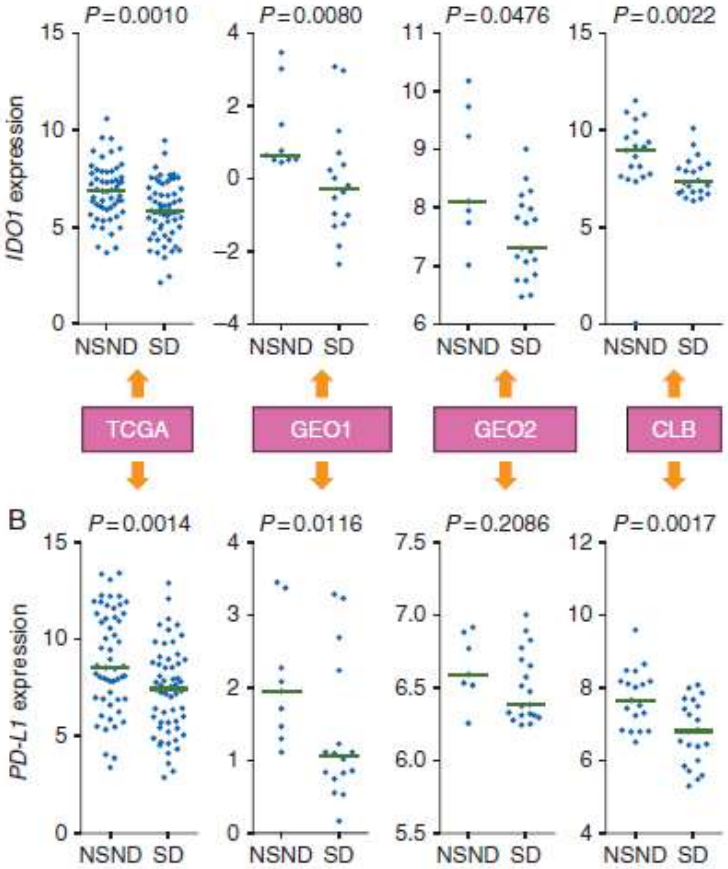


NSND OSCC
=> Largely enriched in INF-γ and PD-1 immunoe pathway



25 differential immune modulations between never-smokers and nerver-drinkers/smokers-drinkers patients

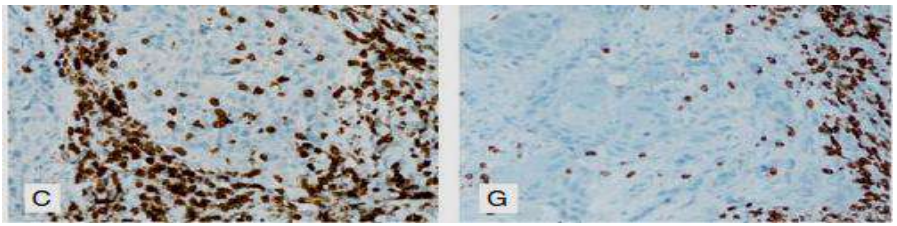
Gene expression profile in the four cohorts



IDO1 and PD-L1 overexpressed in NSND

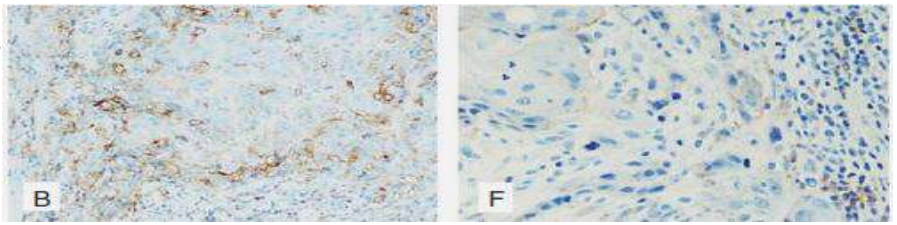
Immunohistochemestry of the CLB cohorts

CD3



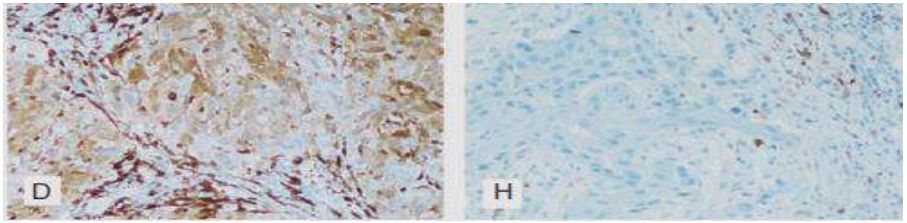
High score for tumors from NSND for CD3 with a location at the interface between tumor and stroma and within the tumor (NS)

PD-L1



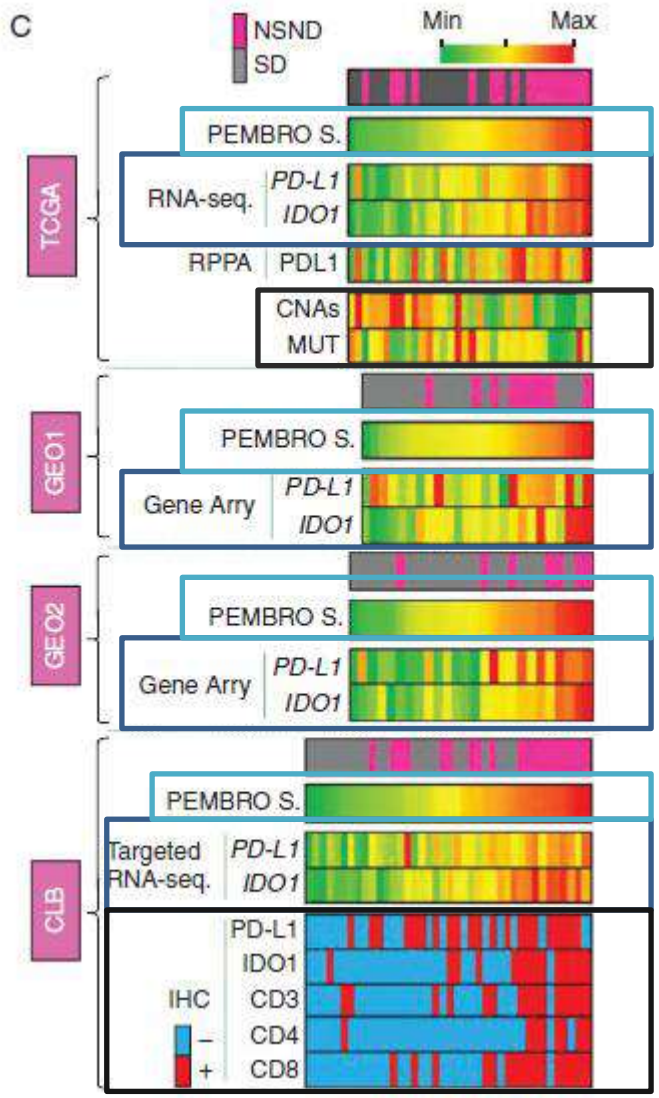
Significant overexpression of PD-L1 in tumors cells and immune cells mostly adjacent to tumors cells

IDO1



Significant overexpression of IDO1 in tumors cells and dendritic cells

NSND OSCC : IDO1 and PD-L1 overexpression, increase of des CD8 tumor infiltration, haut score de reponse au pembrolizumab



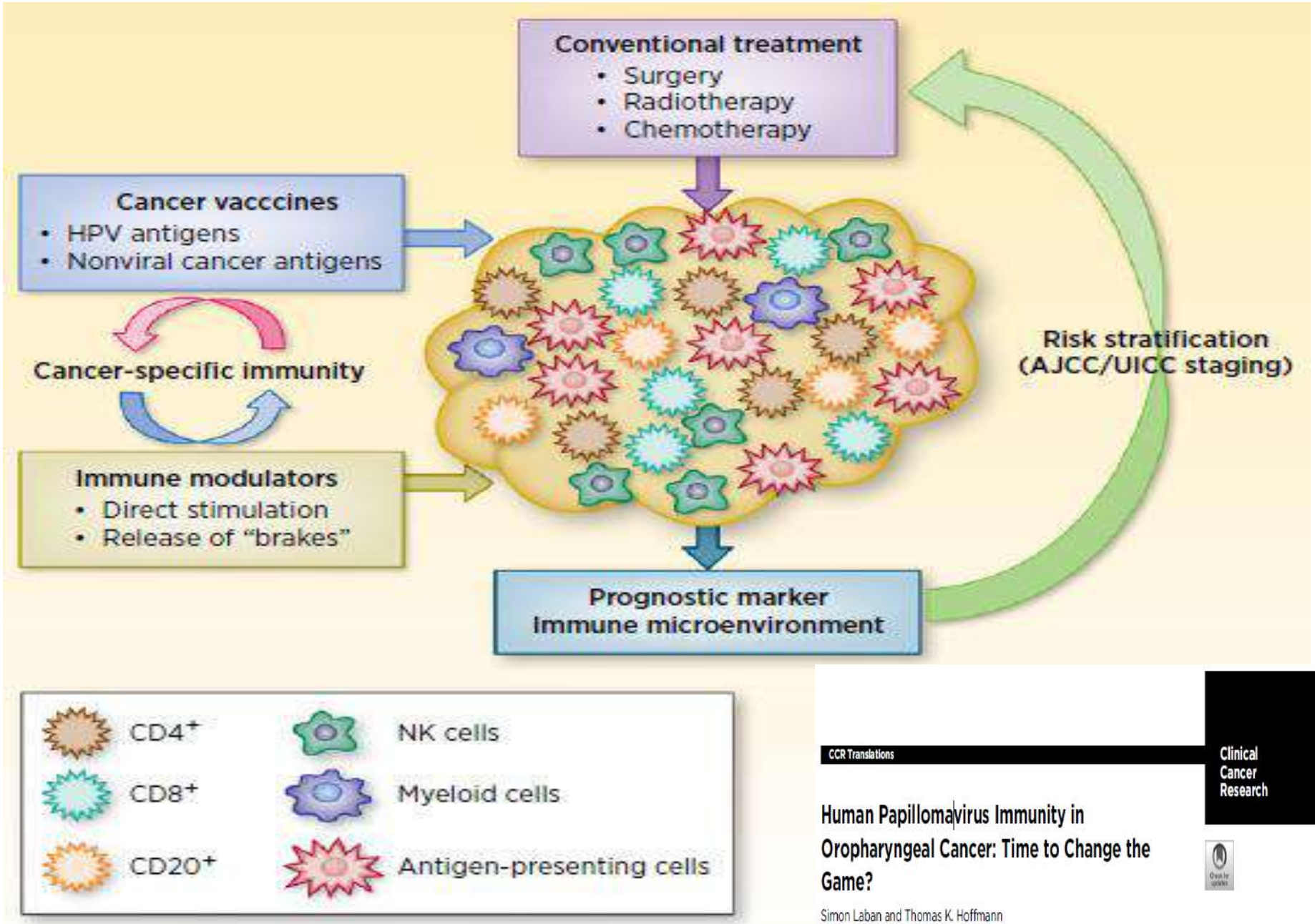
PD-L1 and IDO1 overexpression for OSCC NSND

Less mutations
Less copy number alterations

Correlation with Pembro score
=> Efficacy of pembrolizumab

Results in correlation with the immunohistochemical expression of PD-L1 and IDO1

Papillomavirus



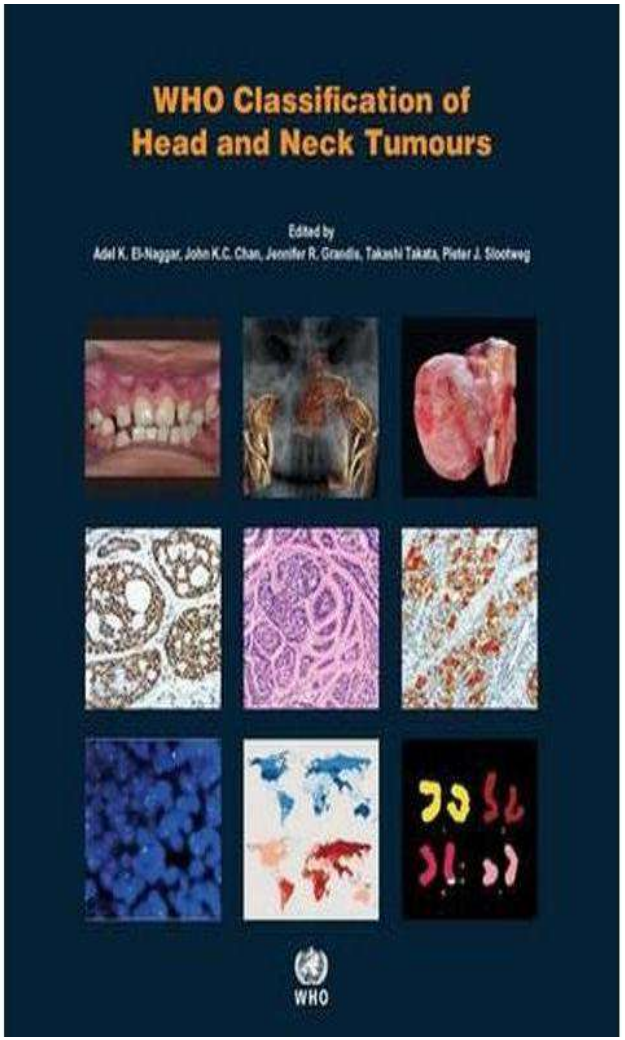
CCR Translations

Clinical Cancer Research

Human Papillomavirus Immunity in Oropharyngeal Cancer: Time to Change the Game?

Simon Laban and Thomas K. Hoffmann

Mutational profil variations due to HPV infection



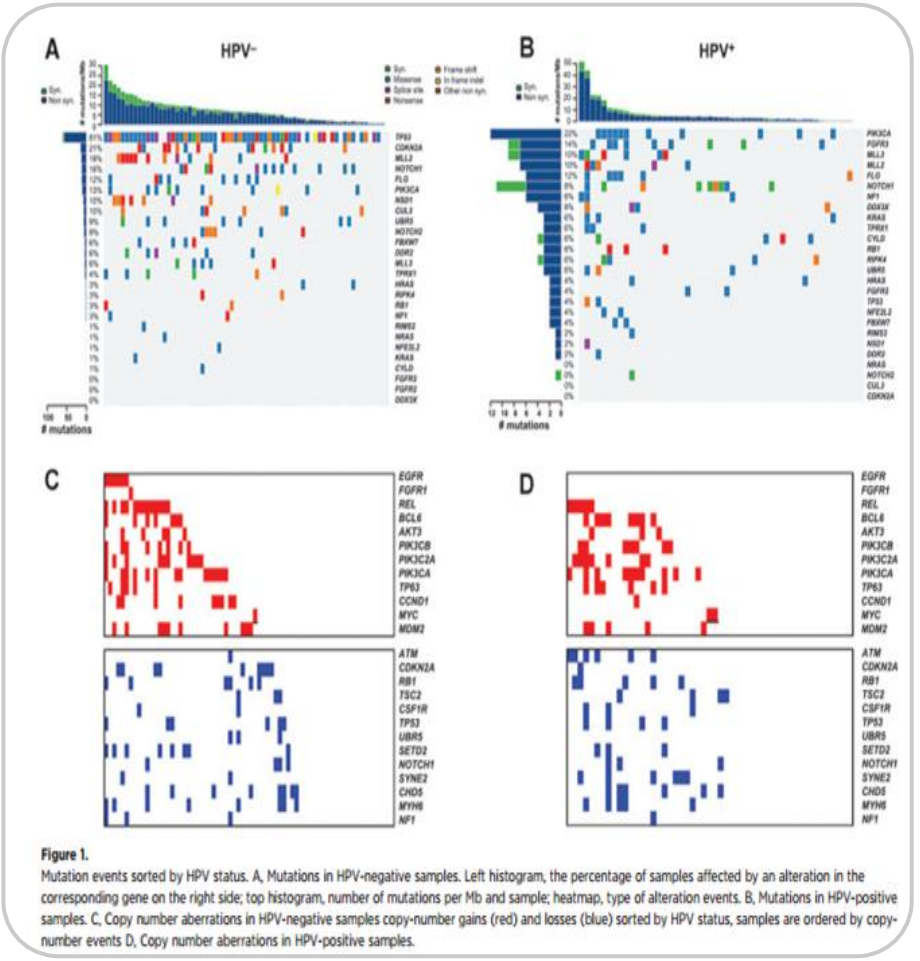
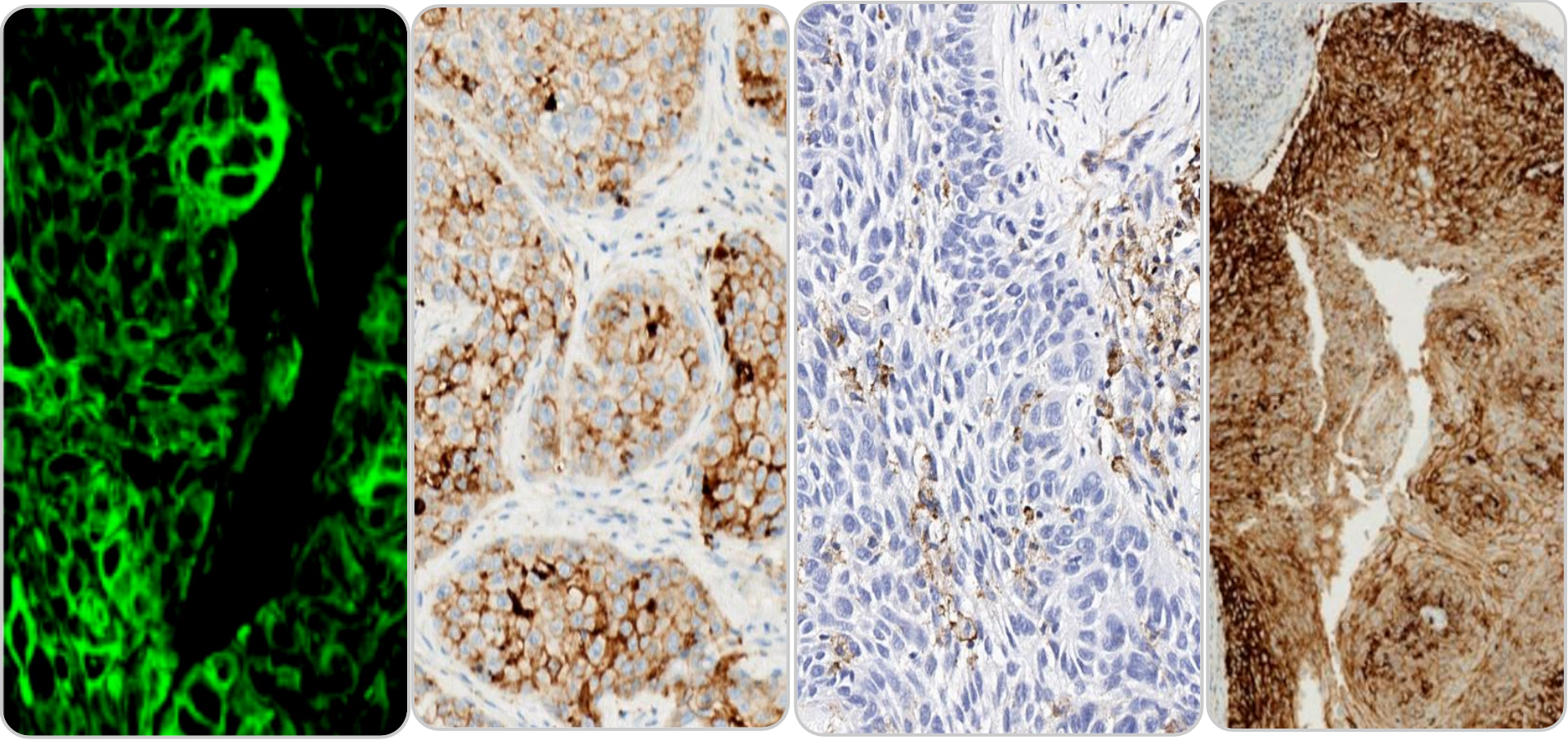


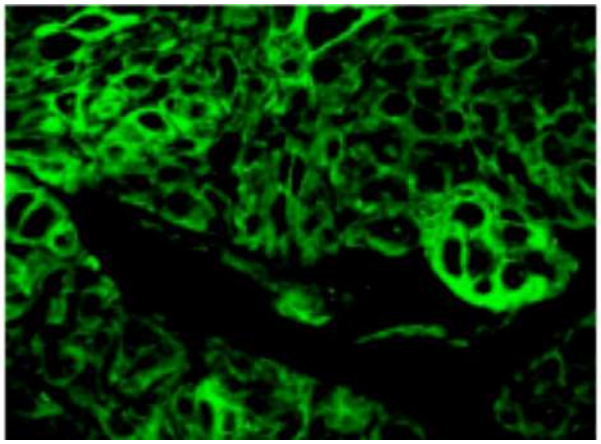
Figure 1.
Mutation events sorted by HPV status. A, Mutations in HPV-negative samples. Left histogram, the percentage of samples affected by an alteration in the corresponding gene on the right side; top histogram, number of mutations per Mb and sample; heatmap, type of alteration events. B, Mutations in HPV-positive samples. C, Copy number aberrations in HPV-negative samples copy-number gains (red) and losses (blue) sorted by HPV status, samples are ordered by copy-number events D, Copy number aberrations in HPV-positive samples.

Seiwert T, Clin Cancer Res, 2015

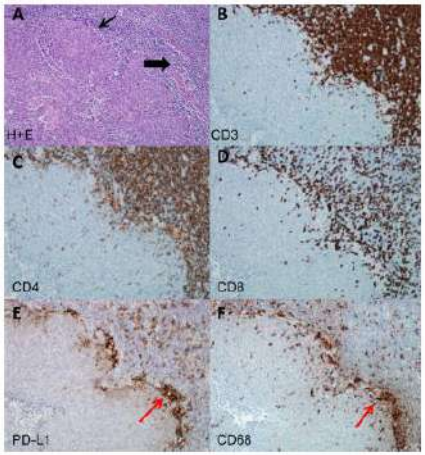
Anti-PD-L1 staining



in situ PDL1 expression in the litterature



Badoual et al. Cancer Res 2013



Lyford-Pike et al. Cancer Res 2013

PD-L1 expression in SCCHN.

Study	Primary site	Sample size	Percent of tumors with PD-L1 expression	Percent of tumors with PD-L1 expression in HPV positive cases	Percent of tumors with PD-L1 expression in HPV negative cases
Strome et al. [46]	OC, HP, L, PNS	24	66	NA	NA
Ukpo et al. [47]	OP	181	46.4	48.2	34.1
Lyford-pike et al. [45]	OP	27	59	70	29
Badoual et al. [41]	OC, OP, HP	64	51.5	62.5	40
Cho et al. [50]	OC	45	87	NA	NA
Zhang et al. [48]	NP	59	67.8	NA	NA
Hsu et al. [49]	NP	25	100	NA	NA

OC – oral cavity; OP – oropharynx; NP – nasopharynx; HP – hypopharynx; PNS – paranasal sinus; L – larynx.
NA – non-applicable.

Strome oral oncology 2014

Different cohort, studies …and various results

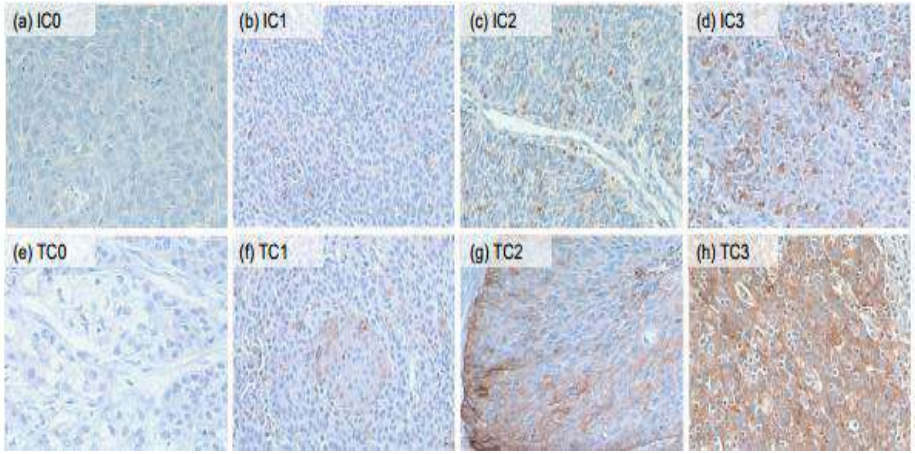
www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

PD-L1 expression on immune cells, but not on tumor cells, is a favorable prognostic factor for head and neck cancer patients

Received: 08 June 2016
Accepted: 24 October 2016



Published OnlineFirst January 3, 2013; DOI: 10.1158/0008-5472.CAN-12-2384

Microenvironment and Immunology

Cancer Research

Evidence for a Role of the PD-1:PD-L1 Pathway in Immune Resistance of HPV-Associated Head and Neck Squamous Cell Carcinoma

Sofia Lyford-Pike[1], Shiwen Peng[2], Geoffrey D. Young[1,3], Janis M. Taube[2,4], William H. Westra[1,2], Belinda Akpeng[1], Tullia C. Bruno[5], Jeremy D. Richmon[1], Hao Wang[6], Justin A. Bishop[2], Lieping Chen[9], Charles G. Drake[5,7], Suzanne L. Topalian[3,5], Drew M. Pardoll[5,8], and Sara I. Pai[1,5]

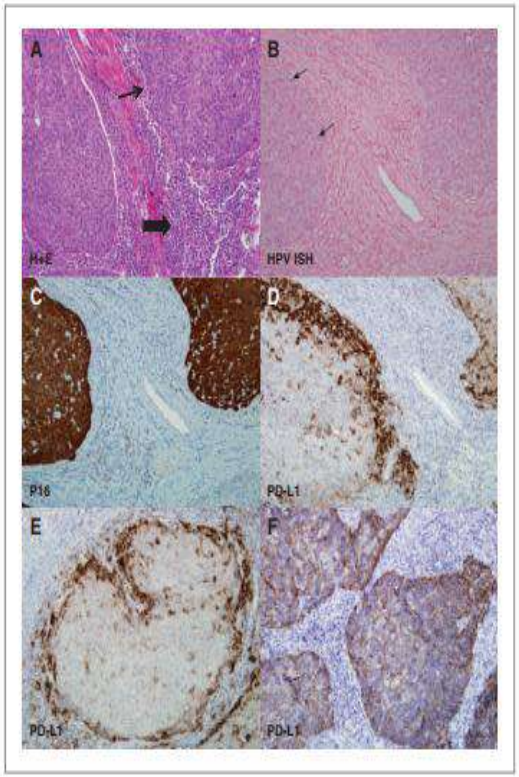


Figure 3. High levels of PD-L1 expression present in the tumor microenvironment of HPV+HNSCC. A, hematoxylin and eosin stain of HPV+HNSCC shows the prototypic tumor nests (thin arrow) surrounded by a dense inflammatory stroma (thick arrow). Serial sections evaluated for HPV ISH (arrows mark areas of blue, intranuclear staining; B); p16 IHC (C); and PD-L1 IHC (D–F). Two patterns of PD-L1 staining were observed: peripheral tumoral staining (D and E) and diffuse intratumoral staining (F). Magnification, ×400.

Different cohort, studies …and various results

Microenvironment and Immunology

Cancer Research

PD-1–Expressing Tumor-Infiltrating T Cells Are a Favorable Prognostic Biomarker in HPV-Associated Head and Neck Cancer

Cécile Badoual[1,2,3], Stéphane Hans[4], Nathalie Merillon[1,2], Cordélia Van Ryswick[1,2], Patrice Ravel[11], Nadine Benhamouda[1,2,5], Emeline Levionnois[1,2,5], Mevyn Nizard[1,2], Ali Si-Mohamed[6], Nicolas Besnier[1,2], Alain Gey[1,2,5], Rinat Rotem-Yehudar[12], Hélène Pere[1,2,6], Thi Tran[1,2], Coralie L. Guerin[1,2], Anne Chauvat[1,2], Estelle Dransart[8], Cécile Alanio[5], Sebastien Albert[13], Beatrix Barry[13], Federico Sandoval[1,2], Françoise Quintin-Colonna[1,2,14], Patrick Bruneval[1,2,3], Wolf H. Fridman[5], Francois M. Lemoine[9,10], Stephane Oudard[1,2,7], Ludger Johannes[8], Daniel Olive[15,16], Daniel Brasnu[4], and Eric Tartour[1,2,5]

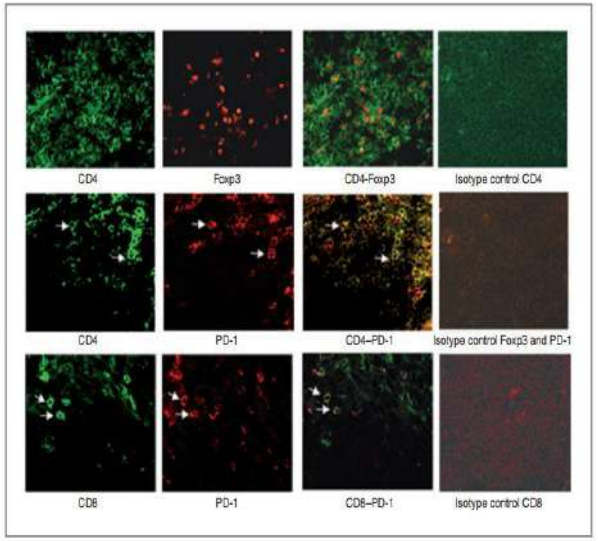


Figure 2. PD-1$^+$ and Foxp3$^+$ T cells infiltrate HPV-associated head and neck cancer. Tissue derived from biopsies of HPV-positive head and neck cancers were stained with antibodies to human CD4, CD8, Foxp3, and PD-1. Left and second column from the left, results of simple immunofluorescence acquisition with each antibody. Third column from the left, double immunofluorescence staining. Isotype control antibodies were also included in each experiment (right). Arrows indicate colocalization between the markers recognized by the specific antibodies. (Original magnification, ×400).

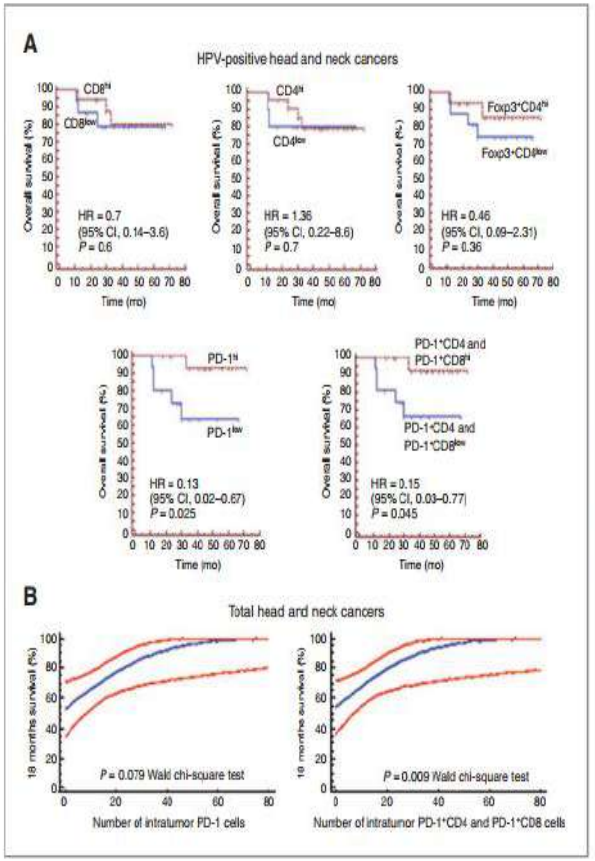


Figure 3. PD-1–positive infiltrating T cells positively correlate with survival in both HPV-positive head and neck cancer and in the overall head and neck cancer population. A, tissues derived from biopsies of HPV-positive head and neck cancers were stained with antibodies to human CD8, CD4, PD-1 by simple immunofluorescence analysis. Double immunofluorescence stainings for CD4 and PD-1, CD8 and PD-1, CD4 and Foxp3 were also conducted. For overall survival analysis, high and low levels of these various populations were defined using the median of tumor infiltration for these cells as cut-off values. B, PD-1$^+$ cells and total number of PD-1$^+$CD4$^+$ and PD-1$^+$CD8$^+$ cells were measured in biopsies derived from all head and neck cancer patients, regardless of their HPV status. The relationship between the total number of these cells selected as a quantitative variable and the 18-month survival is shown. The blue line corresponds to this relationship, whereas the red line represents the upper or lower limits of the 95% CI.

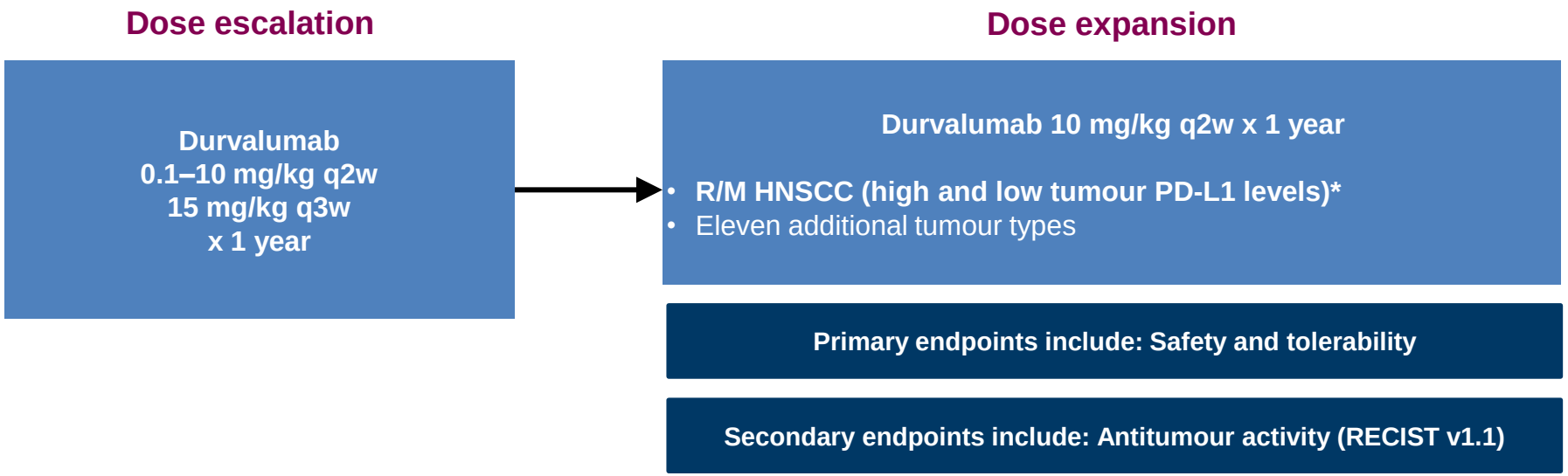
Immunotherapies blocking PD1/PDL1 pathway are efficient to treat HNSCC

- **Durable and objective answer for some patients :** 20-25%
- **Hyperprogression** 29% of R/M HNSCC treated by anti-PD-L1/PD-1 and correlation with a decreased survival (*Saada-Bouzid and al E, Ann Oncol 2017)*

It is necessary to find other biomarkers to identify

- Responders
- Hyperprogressors

Study 1108 is a Phase 1 study of durvalumab in patients with advanced solid tumours, including HNSCC



- Standard 3+3 dose-escalation phase followed by an expansion phase
- After one year of treatment, patients enter follow-up
- Treatment beyond progressive disease was permitted in the absence of clinical deterioration and if the investigator considered that the patient continued to receive benefit
- Upon progressive disease during the follow-up period, retreatment was offered for up to an additional 12 months

*High tumour PD-L1 levels: ≥25% tumour cell score; Low tumour PD-L1 levels: <25% tumour cell score; PD-L1 expression assessed using Ventana PD-L1 (SP263) assay
PD-L1, programmed cell death ligand-1; q#w, every # weeks;
RECIST, Response Evaluation Criteria in Solid Tumours; R/M, recurrent or metastatic

1. Segal NH, JCO 2015;33 Suppl Abs 3011 - ASCO 2015 (Poster 3011)

Study 1108 HNSCC : Antitumour response was higher in patients with HPV-negative R/M HNSCC

Tumour response by HPV status

	Durvalumab 10 mg/kg	
	HPV+	HPV−
RECIST response (ORR), n/N (%) 95% CI	1/25 (**4**) 0.1–20.4	4/25 (**16**) 4.5–36.1
DCR 12 weeks, n/N (%) 95% CI	6/25 (24) 9.4–45.1	6/25 (24) 9.4–45.1

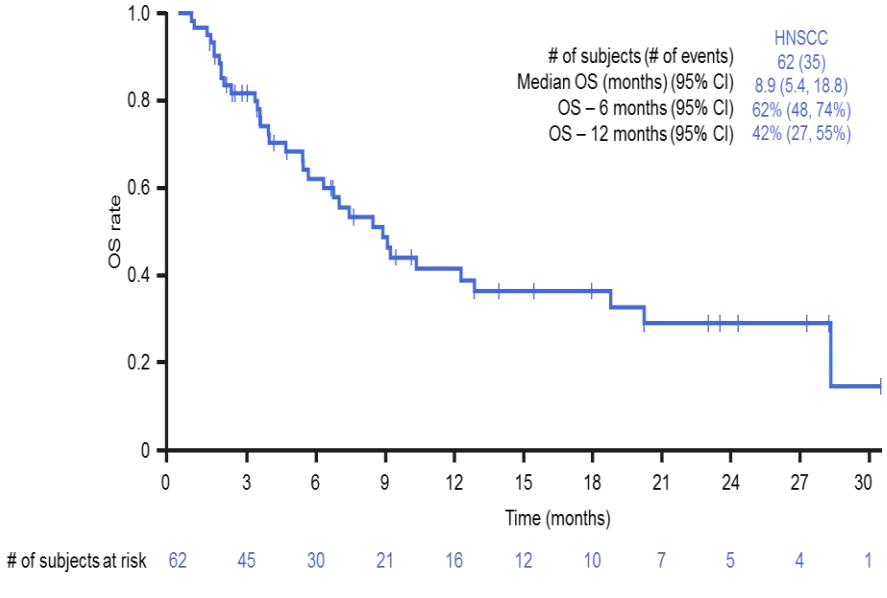
Data cutoff: 29 April, 2016. Response evaluable patients in as-treated population, with ≥12 weeks follow up, with measurable disease at baseline and ≥1 on-study scan (includes those discontinued due to disease progression or death prior to first on-study scan).

DCR (complete response [CR] + partial response [PR] + stable disease [SD] ≥12 weeks) and ORR (confirmed CR and PR) are based on RECIST v1.1.
HPV status was collected at baseline from patient records; HPV status is unknown for 2 of the 7 responding patients.
DCR, disease control rate;
ORR, objective response rate;
DCR, disease control rate;
HPV, human papillomavirus;
ORR, objective response rate;
RECIST, Response Evaluation Criteria in Solid tumours;
R/M, recurrent or metastatic

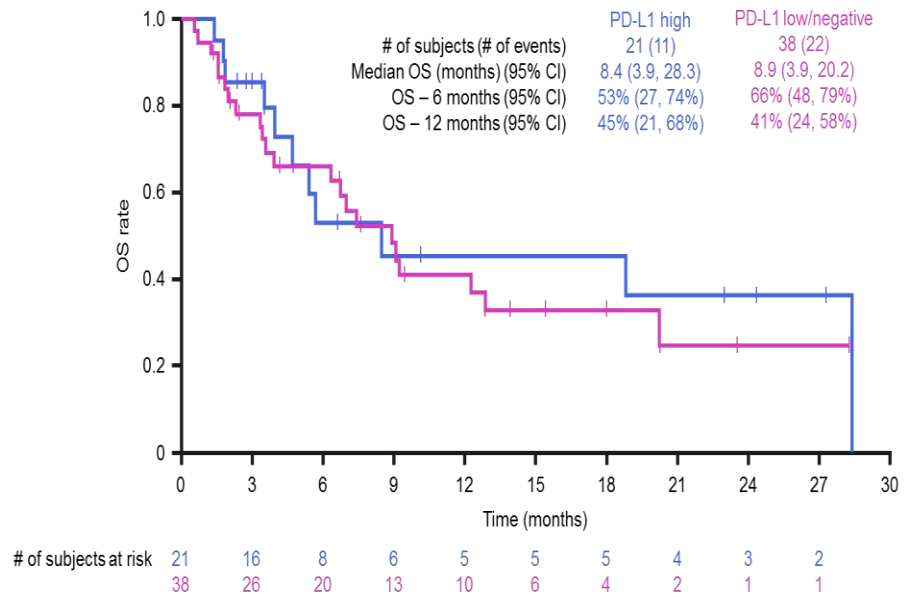
1. Segal NH, et al. Platform presentation at ESMO 2016 (949O)

Study 1108 HNSCC : OS in heavily pre-treated population: no clear difference in OS by PD-L1 expression

- OS rate in all treated HNSCC patients was 62% and 42% at 6 and 12 months, respectively



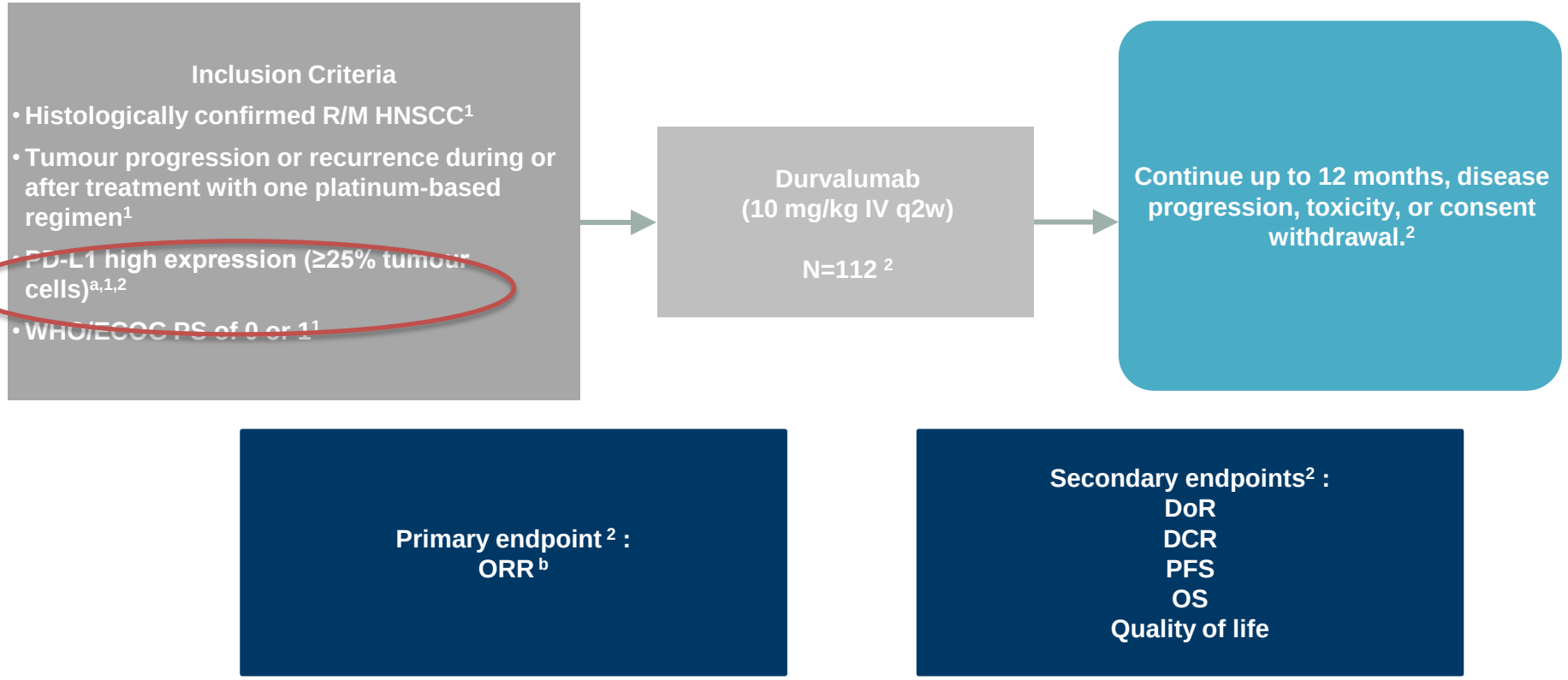
- OS rates were 53% and 66% at 6 months, and 45% and 41% at 12 months for patients with PD-L1 high and PD-L1 low/negative HNSCC, respectively



Data cutoff: 29 April, 2016. In as-treated population: all patients who received 10 mg/kg q2W durvalumab. PD-L1^{high}: ≥25% tumour cell score; PD-L1^{low}: <25% tumour cell score; PD-L1 expression assessed using Ventana PD-L1 (SP263) assay. PD-L1, programmed cell death ligand-1; R/M, recurrent or metastatic

1. Segal NH, et al. Platform presentation at ESMO 2016 (949O)

HAWK: Phase 2, global, single-arm study of durvalumab monotherapy in patients with PD-L1 high R/M HNSCC [1,2]



[a]Assessed using Ventana PD-L1 [SP263] immunohistochemical assay; [b]Using blinded independent central review assessments, according to RECIST v1.1.
DCR = disease control rate; DoR = duration of response; ECOG = Eastern Cooperative Oncology Group; HNSCC = head and neck squamous cell carcinoma; IV = intravenous; OS = overall survival; PD-L1 = programmed cell death ligand-1; PFS = progression-free survival; PS = performance status; q2w = every 2 weeks; RECIST = Response Evaluation Criteria In Solid Tumors; R/M = recurrent or metastatic; WHO = World Health Organisation.

1. US National Institutes of Health. NCT02207530. Accessed August 14, 2017.
2. Zandberg DP, HAWK Presented at: European Society of Medical Oncology. September 8-12, 2017; Madrid, Spain. Abs 1042O.

Exploratory analysis for ORR and OS by HPV status*

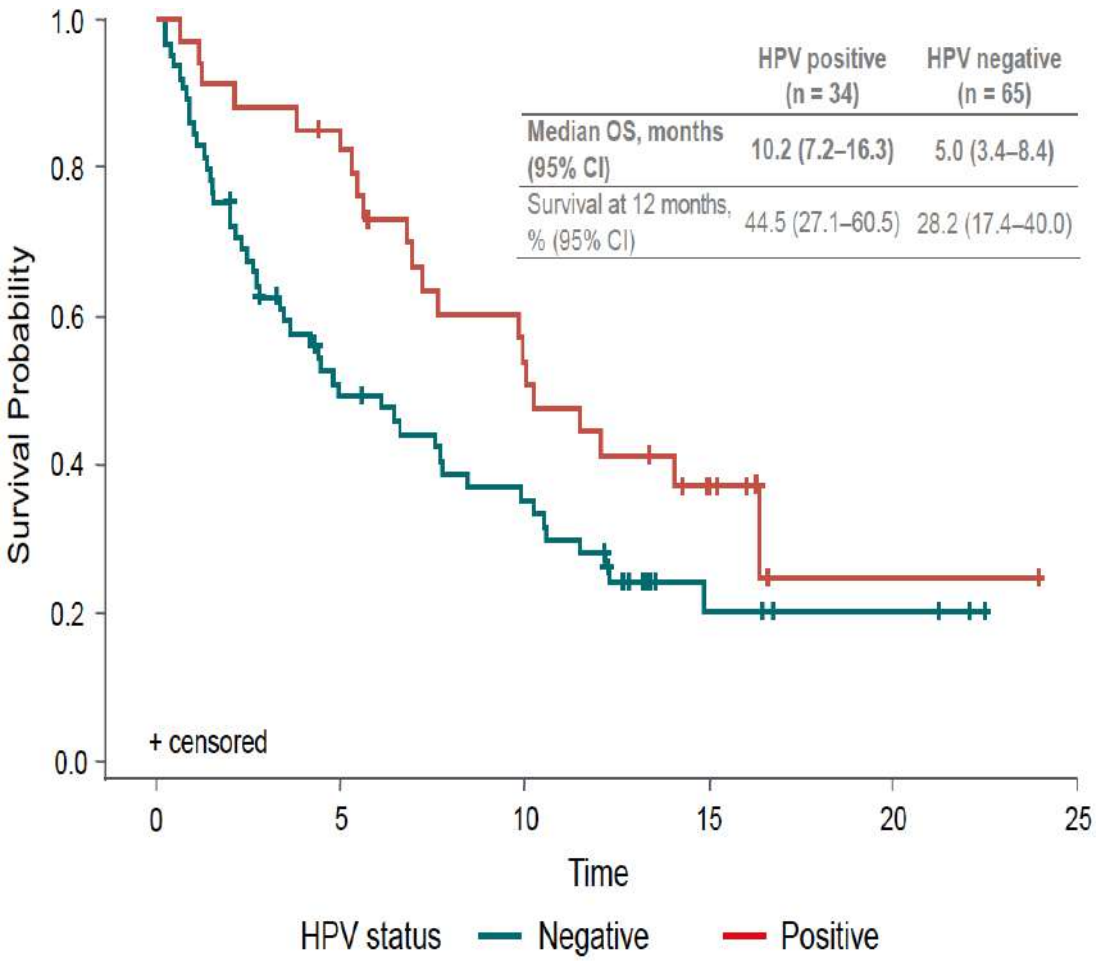
ORR by HPV Status[a]

	n	ORR, n (%)
HPV positive	**34**	10 (**29.4**)
Oropharynx	20	6 (30.0)
Oral cavity	7	1 (14.3)
Larynx	5	2 (40.0)
Hypopharynx	2	1 (50.0)
HPV negative	**65**	7 (**10.8**)
Oral cavity	33	3 (9,1)
Oropharynx	17	2 (11.8)
Hypopharynx	6	1 (16.7)
Larynx	8	1 (12.5)
Other	1	0

*Fu

[a]HPV status available on 99 patients, 13 patients had unknown HPV status.

OS by HPV Status[a]



1. Zandberg DP. ESMO 2017 Abs 1042O

PD-L1 Immuno staining : interpretation

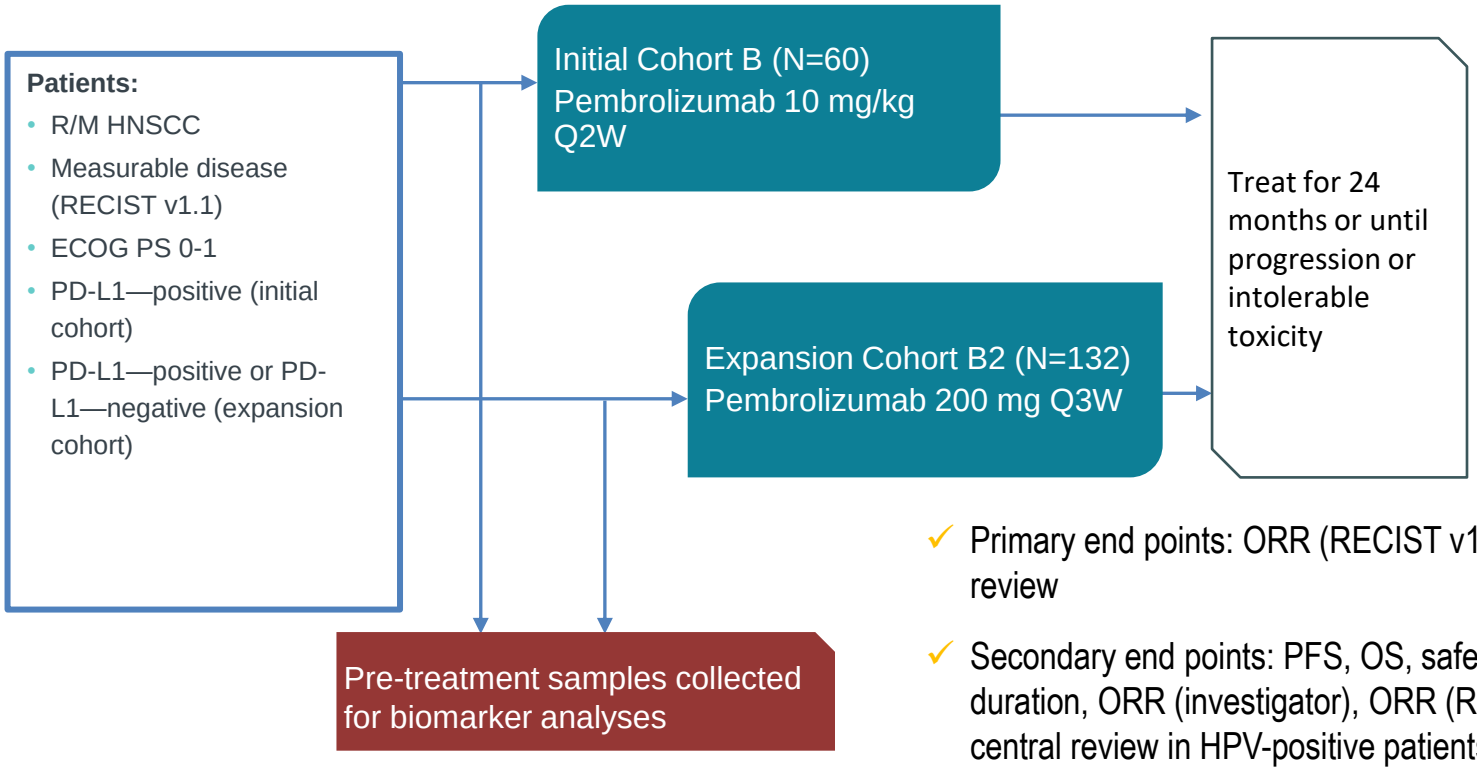
- TC : PD-L1 expressing tumor cells /total number of tumor cells in %
- IC : Comptage des cellules immunitaires / nb de cellules immunitaires stroma en %
- TPS (tumor proportion score): TC
- CPS (combined positive score) :The percentage of PD-L1 expressing tumor and infiltrating immune cells relative to the total number of tumor cells.
- MEL Score: Membrane staining in tumor and tumor-associated immune cells (intercalated mononuclear inflammatory cells only; excludes stromal cells)
- In some patient cohorts, PD-L1 staining of CPS improved the ability to predict response to therapy as compared to tumor cells alone (TPS)[1,2,3]

1. Muro et al Lancet Oncol 2016; 17: 717–26. 2. Chow et al Journal of Clinical Oncology 34 (32) Nov 2016. 3. Bellmunt et al NEJM Feb 2017.

Biomarkers and Patient Enrichment in H&N Cancers

A Phase 1b Study of Pembrolizumab in patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy

KEYNOTE-012



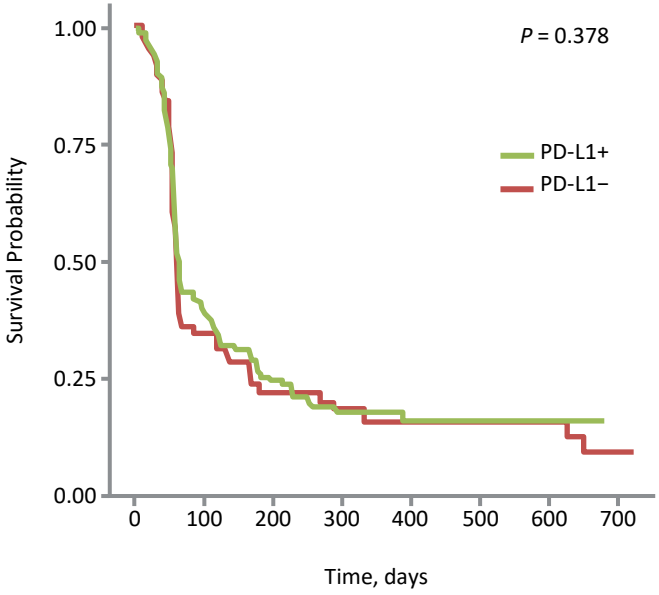
- ✓ Primary end points: ORR (RECIST v1.1) by central review
- ✓ Secondary end points: PFS, OS, safety, response duration, ORR (investigator), ORR (RECIST v1.1) by central review in HPV-positive patients, biomarkers and genomics
- ✓ Exploratory end points: PK profile

Chow LQ et al. J Clin Oncol. 2016;34(15;suppl): abstract 6010.

PD-L1 biomarker data suggested that patient selection may be enhanced by incorporation of inflammatory cells

KEYNOTE-012 Progression-Free Survival

TPS (tumor cells)

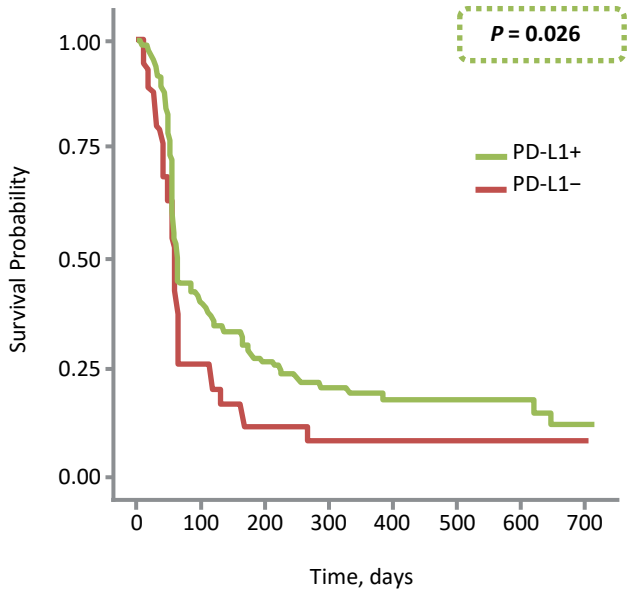


TPS<1	65	22	14	8	5	5	5	2
TPS≥1	123	48	30	21	3	3	3	0

Median (95% CI)

- PD-L1–positive, 63 days (58–98)
- PD-L1–negative, 62 days (59–67)

CPS (tumor and inflammatory cells)



CPS<1	36	9	4	3	2	2	2	1
CPS≥1	152	61	40	26	6	6	6	1

Median (95% CI)

- PD-L1–positive, 64 days (59–98)
- PD-L1–negative, 60 days (51–66)

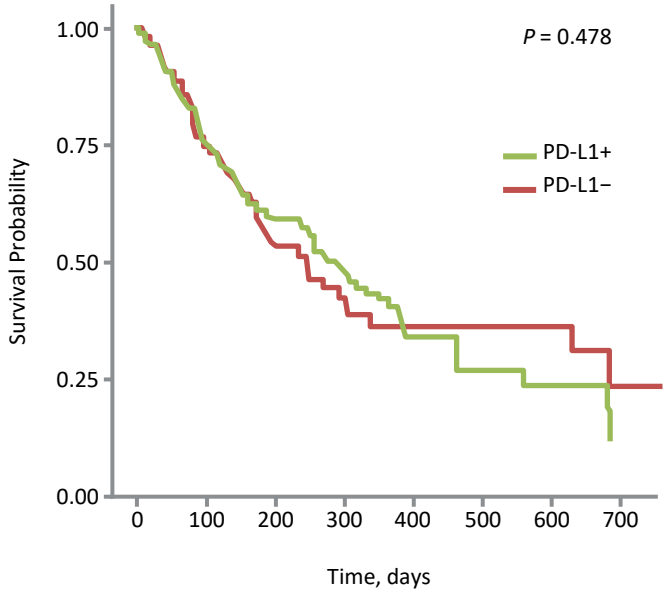
CI = confidence interval; CPS = combined positive score; ORR = overall response rate; TPS = tumor proportion score

Chow LQ et al. J Clin Oncol. 2016;34(15;suppl): abstract 6010.

PD-L1 biomarker data suggested that patient selection may be enhanced by incorporation of inflammatory cells

KEYNOTE-012 Overall Survival

TPS (tumor cells)

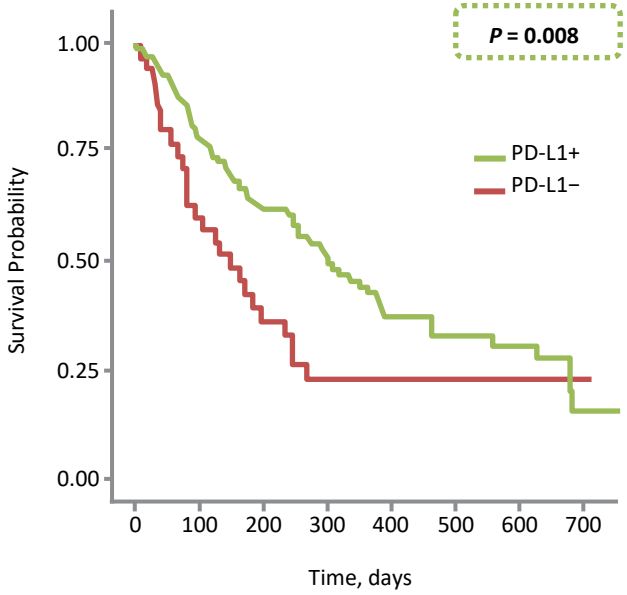


TPS<1	65	46	31	21	9	9	8	2
TPS≥1	123	88	69	53	16	8	6	0

Median (95% CI)

- PD-L1–positive, 290 days (241–377)
- PD-L1–negative, 246 days (174–626)

CPS (tumor and inflammatory cells)



CPS<1	36	21	11	7	3	3	2	1
CPS≥1	152	113	89	67	22	14	12	1

Median (95% CI)

- PD-L1–positive, 303 days (259–385)
- PD-L1–negative, 151 days (84–247)

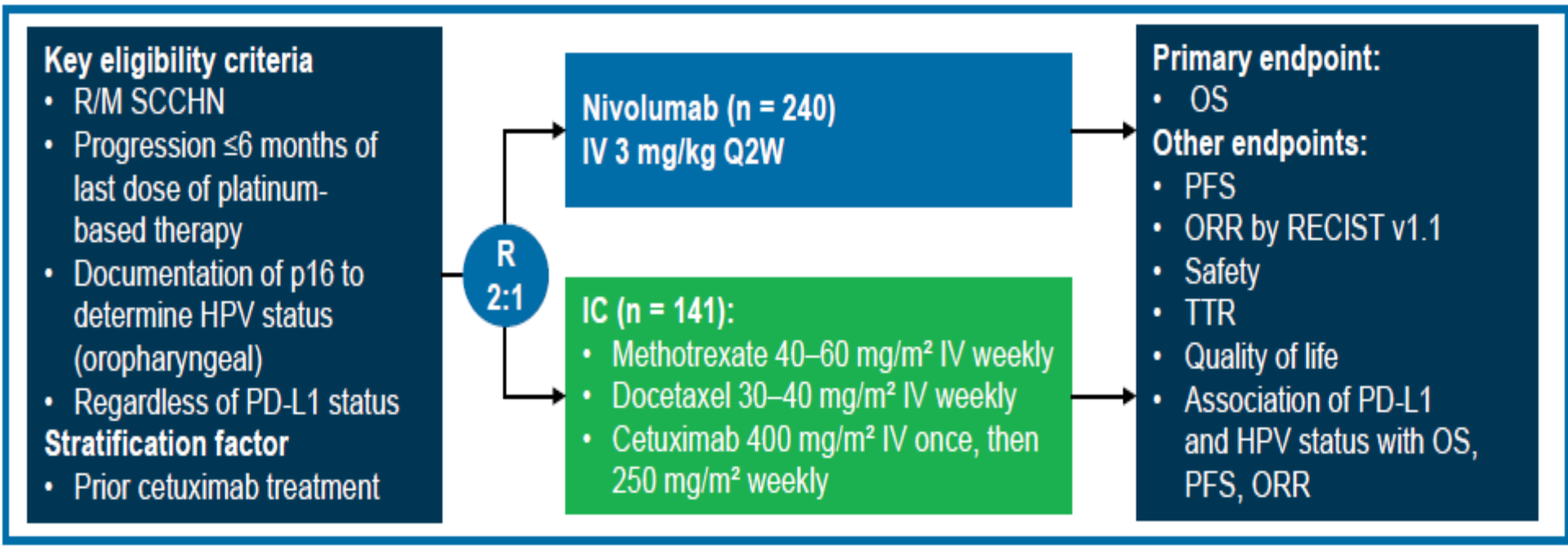
CI = confidence interval; CPS = combined positive score; ORR = overall response rate; TPS = tumor proportion score

Chow LQ et al. J Clin Oncol. 2016;34(15;suppl): abstract 6010.

Phase 3 CheckMate 141 Study Design

Nivolumab in R/M SCCHN After Platinum Therapy

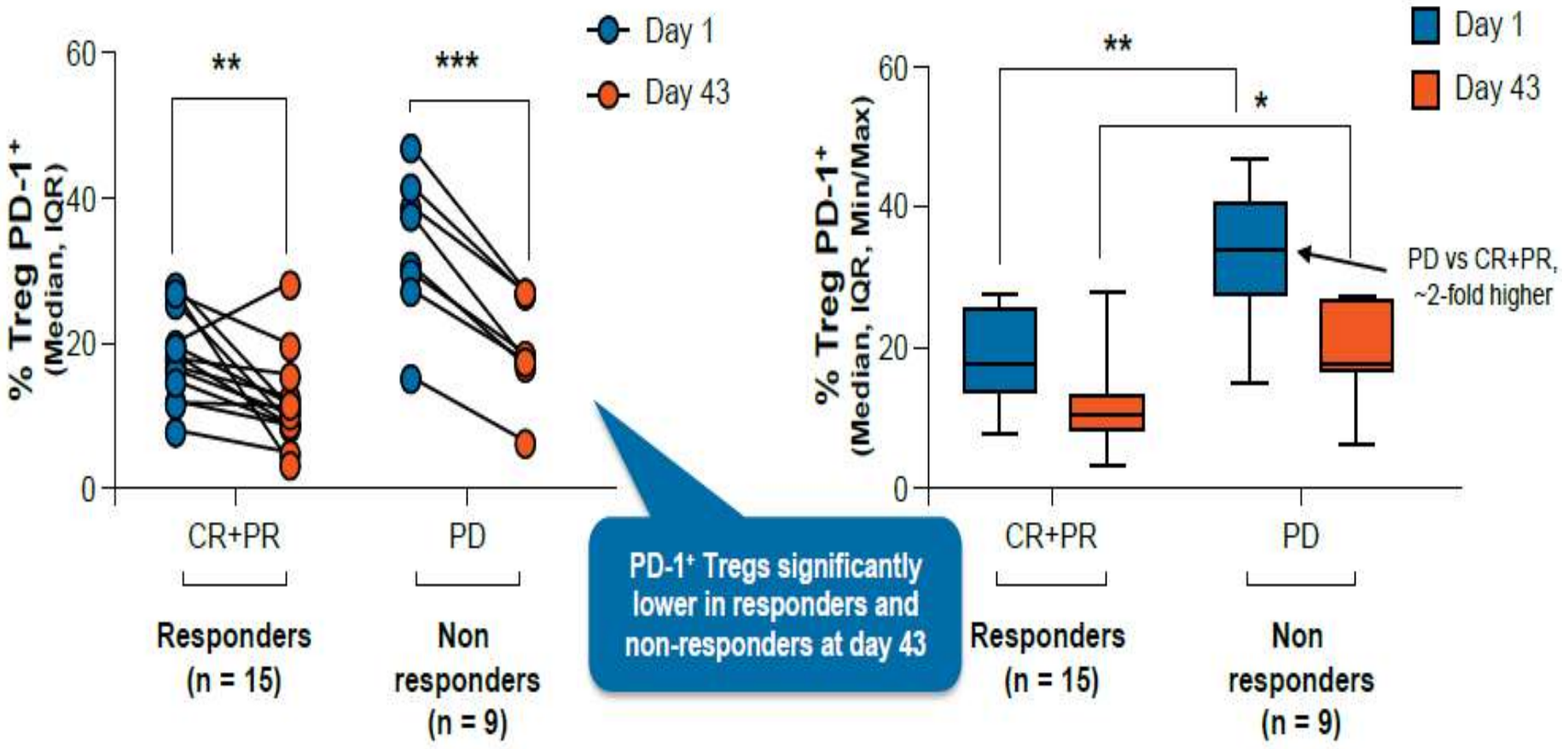
- Nivolumab is an anti-programmed death-1 (PD-1) monoclonal antibody
- In CheckMate 141 (NCT02105636), a global randomized phase 3 study, nivolumab demonstrated a statistically significant improvement in overall survival, with better tolerability and a quality of life benefit compared with investigator's choice therapy (IC) in patients with squamous cell carcinoma of the head and neck (SCCHN)



Ferris RL et al. *NEJM*. 2016;375:1856–1867

Frequency of PD-1^{+} Tregs in Nivolumab Responders vs Non-responders at Baseline and Day 43

Defined as $CD4^{+}CD25^{hi}CD127^{lo}FoxP3^{+}$



*P<0.05; **P<0.01; ***P<0.001

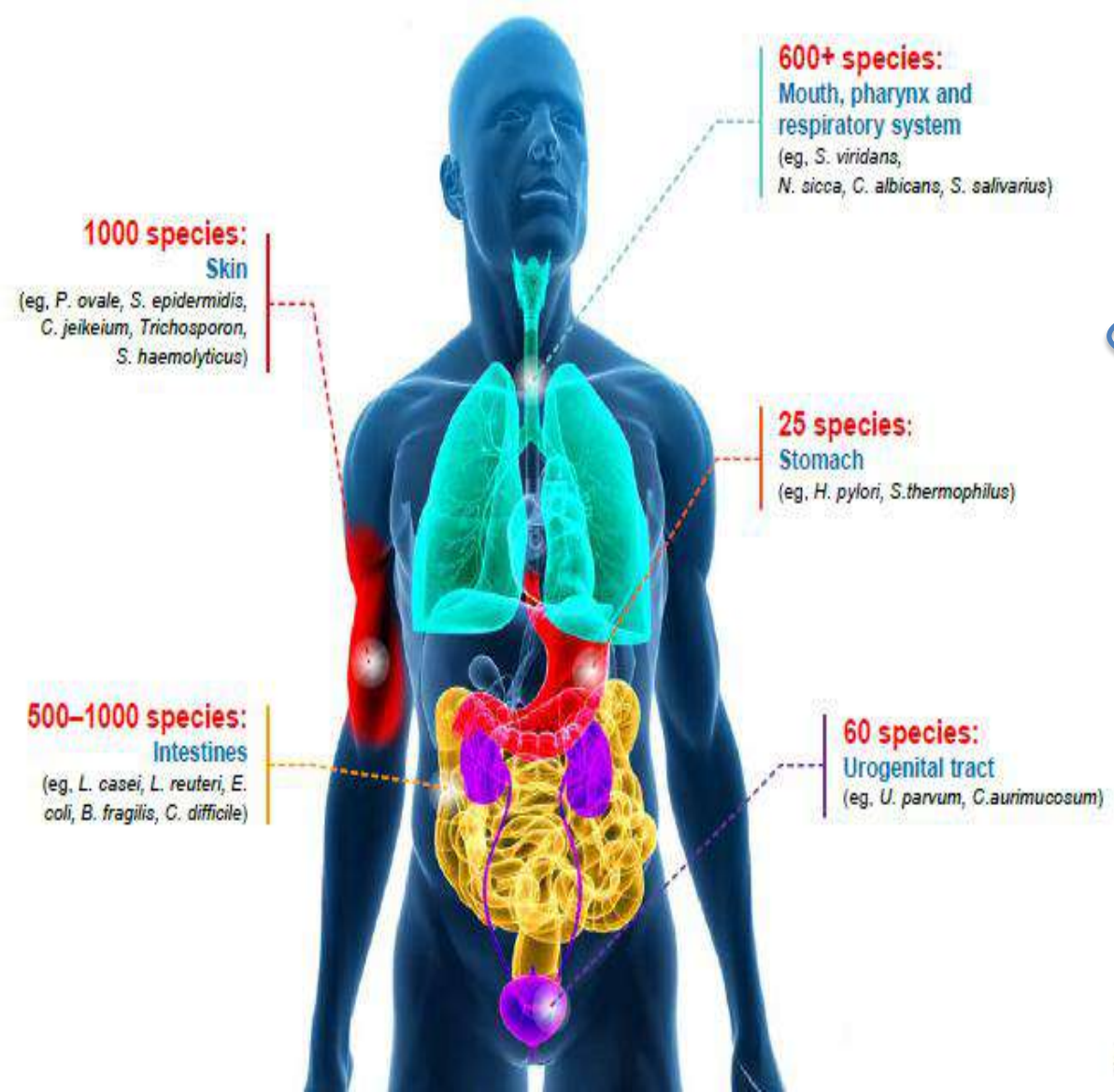
AACR American Association for Cancer Research
MEETING 2017 WASHINGTON, DC

Evaluation of Oral Microbiome Profiling as a Response Biomarker in Squamous Cell Carcinoma of the Head and Neck: Analyses from CheckMate 141

Robert L. Ferris,[1,2] George Blumenschein, Jr.,[3] Kevin Harrington,[4] Jerome Fayette,[5] Joel Guigay,[6] A. Dimitrios Colevas,[7] Lisa Licitra,[8] Everett Vokes,[9] Maura Gillison,[10] Caroline Even,[11] Cheryl Ho,[12] Makoto Tahara,[13] Robert Haddad,[14] Mark Lynch,[15] Manish Monga,[15] Somnath Bandyopadhyay,[15] Omar Jabado,[15] Henry Kao[15]

[1]University of Pittsburgh Medical Center, Pittsburgh, PA, USA; [2]University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA; [3]Department of Thoracic/Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA; [4]Institute of Cancer Research, Royal Marsden National Institute for Health Research Biomedical Research Centre, London, UK; [5]Centre Léon Bérard, Lyon, France; [6]Centre Antoine Lacassagne, FHU Oncoage, Nice, France; [7]Stanford Cancer Institute, Stanford, CA, USA; [8]Fondazione IRCCS Istituto Nazionale dei Tumori and University of Milan, Milan, Italy; [9]University of Chicago, Chicago, IL, USA; [10]Ohio State University, Columbus, OH, USA; [11]Institut Gustave Roussy, Villejuif, France; [12]BC Cancer Agency, Vancouver, BC, Canada; [13]National Cancer Center Hospital East, Kashiwa, Japan; [14]Dana–Farber Cancer Institute, Boston, MA, USA; [15]Bristol-Myers Squibb, Princeton, NJ, US

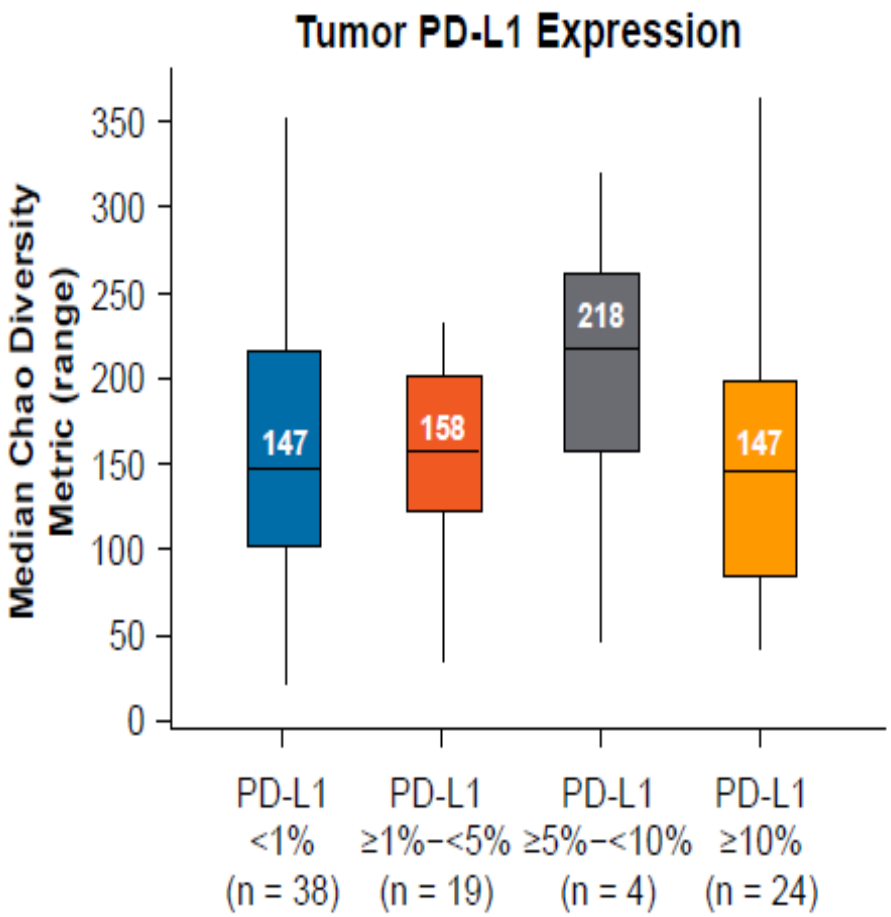
Association of the Microbiome with Immunotherapy



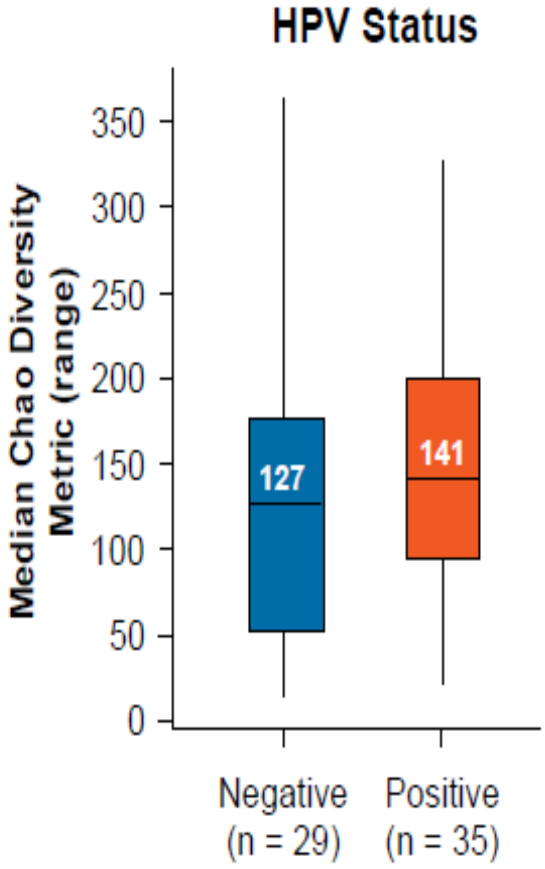
- Gut microbiota modulate effects of checkpoint immunotherapy in mouse melanoma models[1,2]
- Gut, but not oral, microbiome diversity significantly different between responders and non-responders in melanoma patients treated with anti-PD-1 therapy[3]
- **No prior evaluations of the oral microbiome in an oral tumor type**

[1]Sivan A et al. *Science*. 2015;350:1084–108
[2]Vétizou M et al. *Science*. 2015;350:1079–108
[3]Gopalakrishnan V et al. *J Clin Oncol*. 2017:35(suppl 7S):abstract

Association Between Baseline Alpha Microbial Diversity and Tumor PD-L1 Expression or HPV Status



No difference in group means (ANOVA)



No difference in group means (ANOVA)

SCC…for the moment

- No need to realise PD-L1 testing for giving immunotherapy
- PD-L1 testing needs to be enhanced and standardized
- HPV role need to be identified
- Role and implication of other biomarkers IDO, PD-L2, PD1..
- Role of immune cells CD4, CD8, macrophages…

Tumor and Immune Biomarkers Being Evaluated to Predict Better Outcomes to Immuno-Oncology Therapy

Tumor Antigens

- Biomarkers indicative of hypermutation & neo-antigens may predict response to IO treatment

Examples:

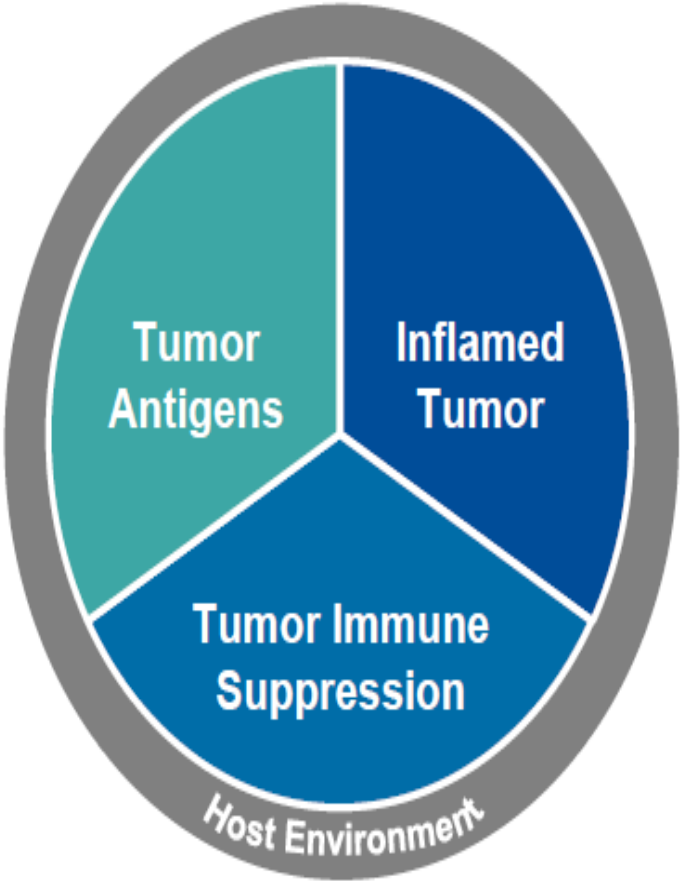
– ***TMB, MSI-High, Neo-Antigens***

Inflamed Tumor Microenvironment

- Biomarkers (intra- or peri-tumoral) indicative of an inflamed phenotype may predict response to IO treatment

Examples:

– ***PD-L1, Inflammatory Signatures***



Tumor Immune Suppression

- Biomarkers that identify tumor immune system evasion beyond PD-1/CTLA-4 to inform new IO targets and rational combinations

Examples:

– ***Tregs, MDSCs, IDO, LAG-3***

Host Environment

- Biomarkers which characterize the host environment, beyond tumor microenvironment, may predict response to IO treatment

Examples:

– ***Microbiome, Germline Genetics***

IDO = indoleamine-2,3 dioxygenase; LAG-3 = lymphocyte activation gene-3; MDSCs = myeloid-derived suppressor cells; MSI-High = microsatellite instability high; TMB = tumor mutational burden. Adapted from Blank C.U. et al., *Science* 2016;352:658–660.