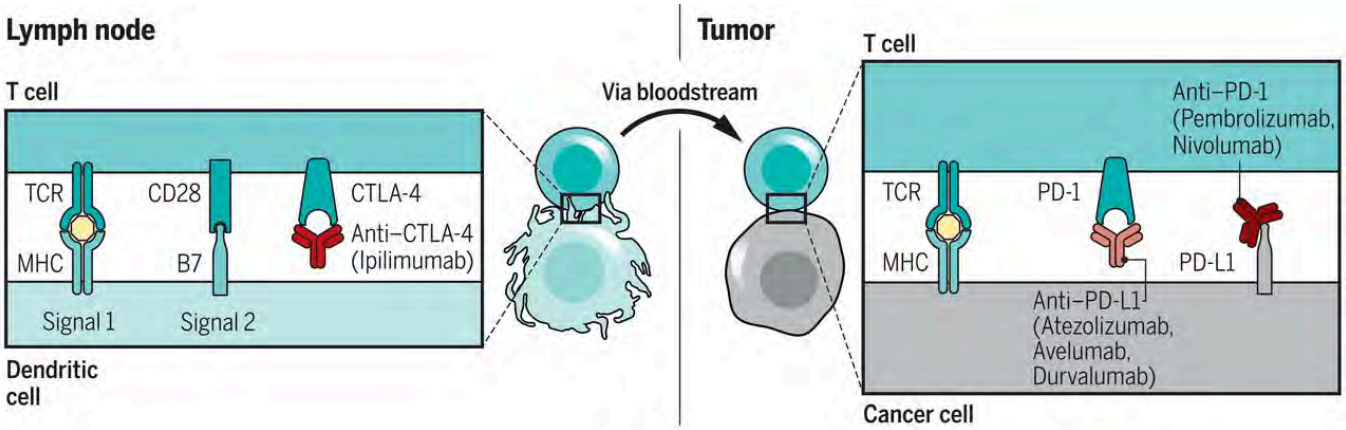
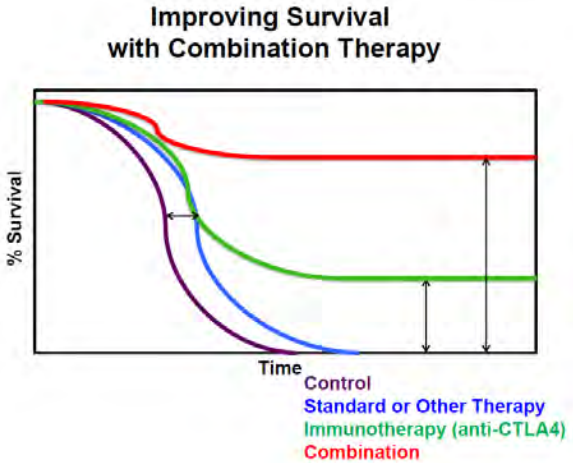
# Marqueurs prédictifs en immunothérapie : indications actuelles et perspectives

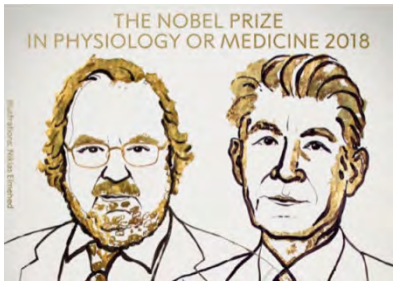
Julien Adam, MD PhD
Department of Biology and Pathology
INSERM U981 & U1186
Gustave Roussy Cancer Center
Grand Paris, France

# Immunothérapie par blocage de checkpoints immunitaires




*Ribas & Wolchok, Science 2018*



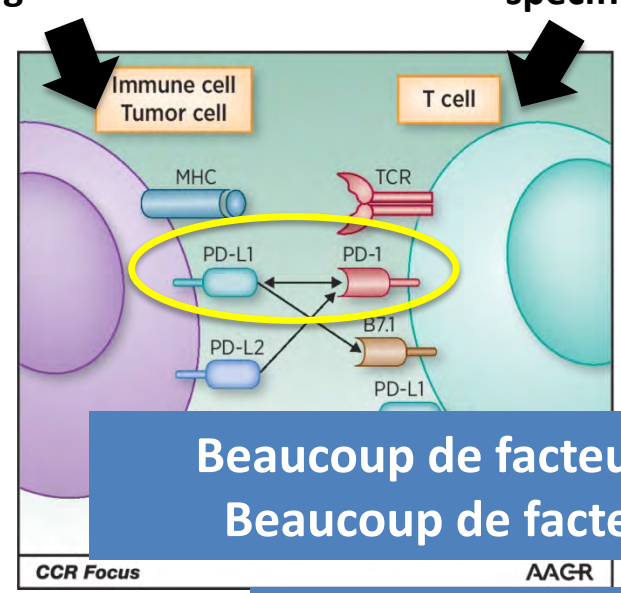



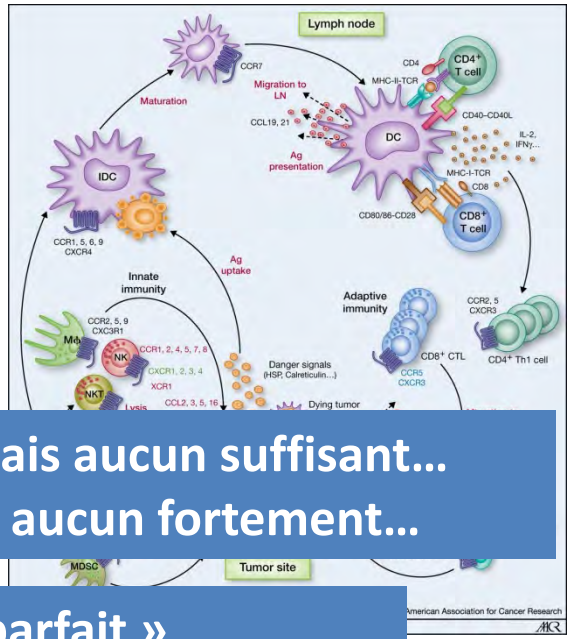
# Revenir à la biologie…

**Des cellules présentatrices d'antigènes PD-L1+**

**Des lymphocytes T spécifiques PD-1+**

**Dans un environnement complexe…**



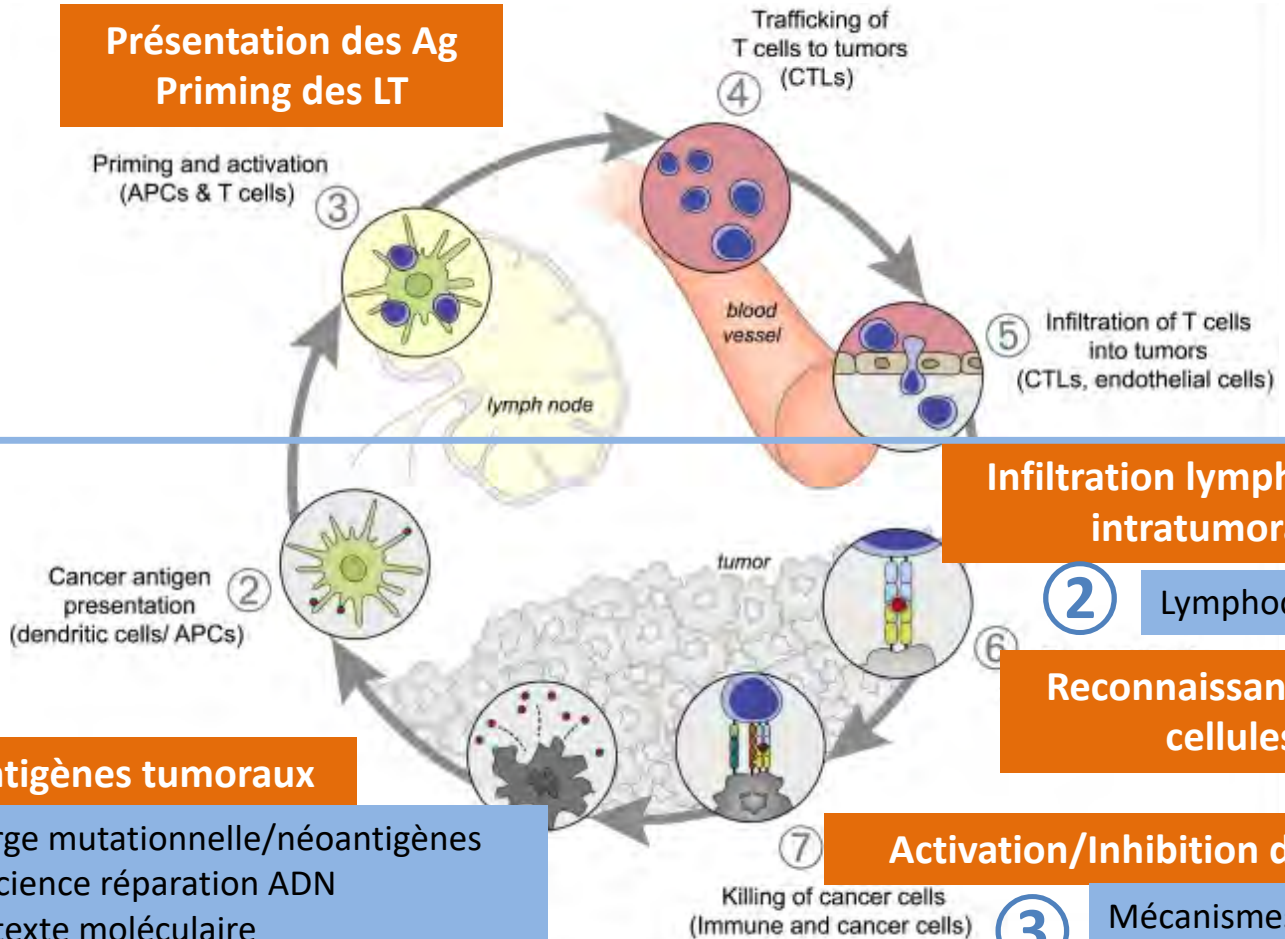




**Beaucoup de facteurs nécessaires, mais aucun suffisant…**
**Beaucoup de facteurs correlés mais aucun fortement…**

**Pas de biomarqueur « parfait »**
**Valeur prédictive positive ou négative ?**

*Soria et al. Clin Cancer Res 2015; Franciszkiewicz et al. Cancer Res, 2012*

**Présentation des Ag**
**Priming des LT**




**Infiltration lymphocytaire intratumorale**

2 Lymphocytes (in situ / ARN)

**Reconnaissance des antigènes / cellules tumorales**

**Antigènes tumoraux**

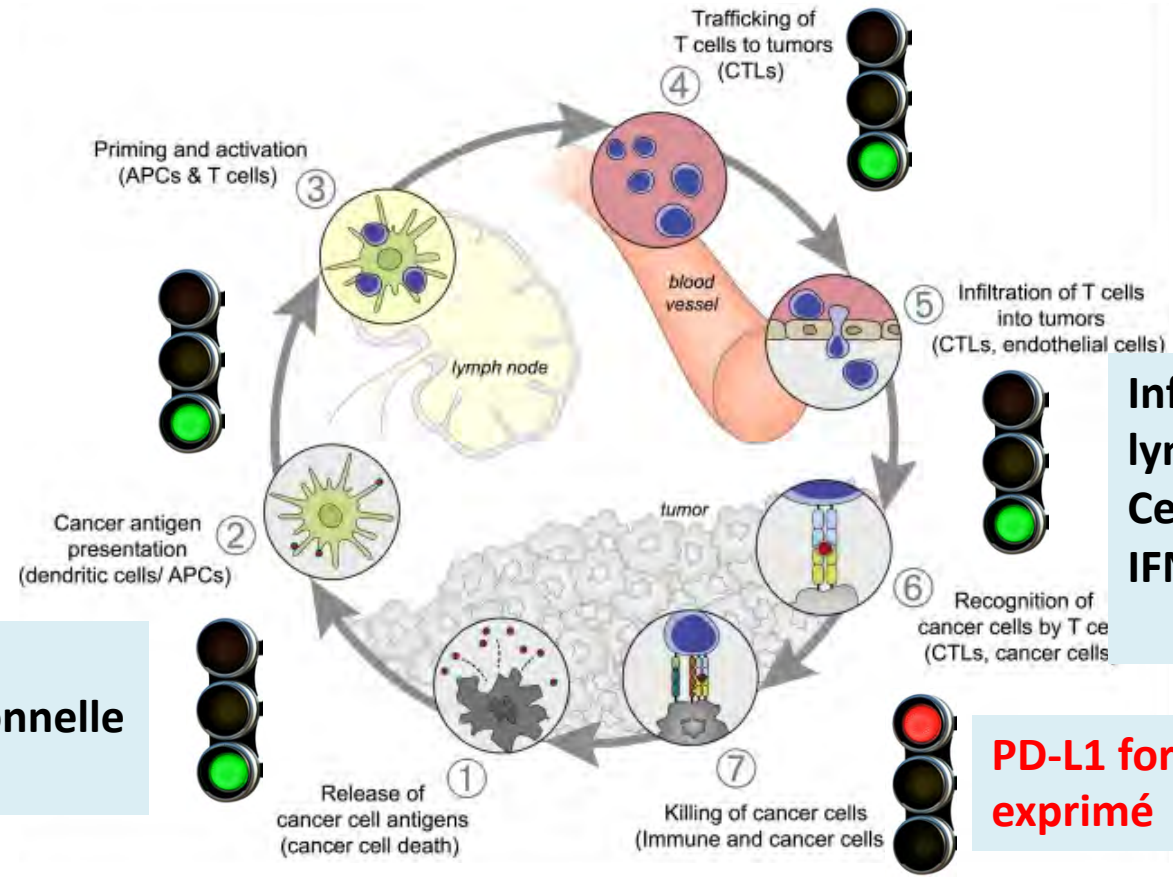
1 Charge mutationnelle/néoantigènes
Déficience réparation ADN
Contexte moléculaire

**Activation/Inhibition du LT**

3 Mécanismes d'échappement à la réponse immunitaire (dont PD-L1)

*Chen and Mellman, Immunity 2013*

# Cas de figure « idéal » pour l'efficacité des anti-PD1/L1




**Infiltration lymphocytaire élevée**
**Cellules T effectrices**
**IFN gamma**

**Charge mutationnelle élevée**

**PD-L1 fortement exprimé**

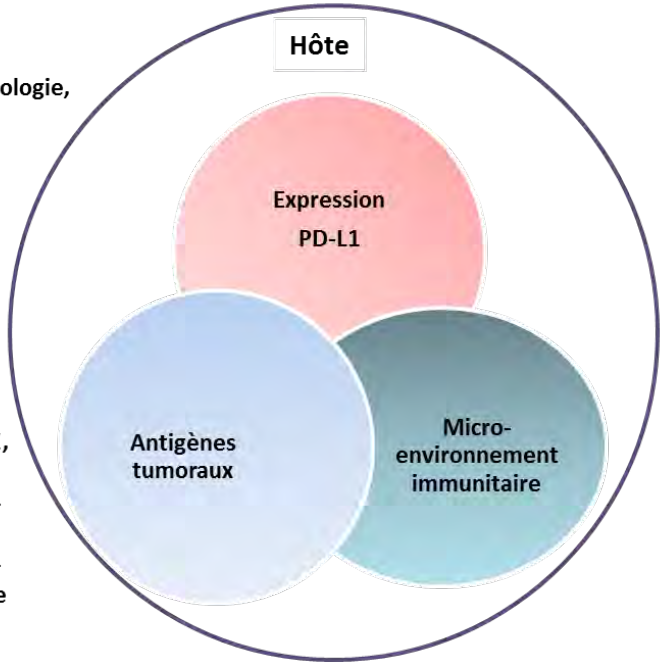
# Panorama des biomarqueurs des anti-PD-1/L1

**Caractéristiques de l'hôte**

- Cliniques : âge, sexe, PS, histologie, tabagisme
- Microbiome
- Génétique constitutionnelle

**Antigènes tumoraux**

- Mutations *EGFR*, *KRAS*, *LKB1*, p53 mutations, *ALK+*, *JAK/STAT*, amplifications PD-L1
- TMB, MSI-High/dMMR, néo-antigènes, clonalité tumorale



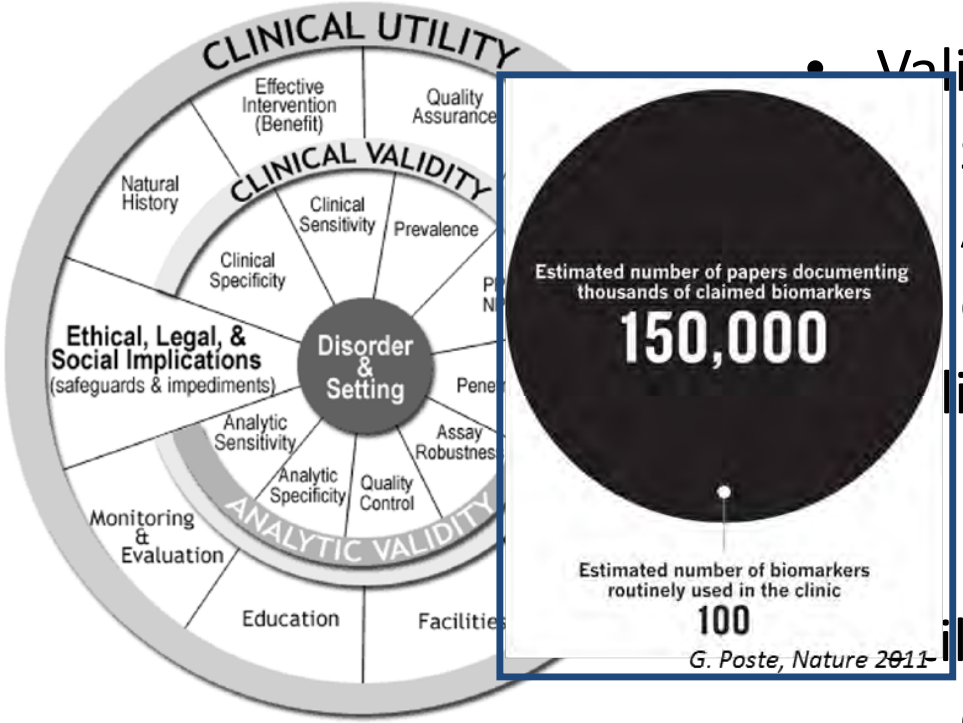

**PD-L1 expression**

- Expression protéique ou mRNA
- Cellules tumorales ou Cellules immunitaires

**Micro-environnement immunitaire**

- Tumeur « hot » ou « cold » (désert immunitaire ou immun exclu)
- CD8+T cell (infiltration et fonctionnalité)
- Voies signalisation de Interféron gamma
- Tregs, MDSCs, CD IDO+
- TIM3, LAG3, TIGIT (facteurs d'exhaustion)
- Signature transcriptomique
- Clonalité TCR

# Comment s'y retrouver ?



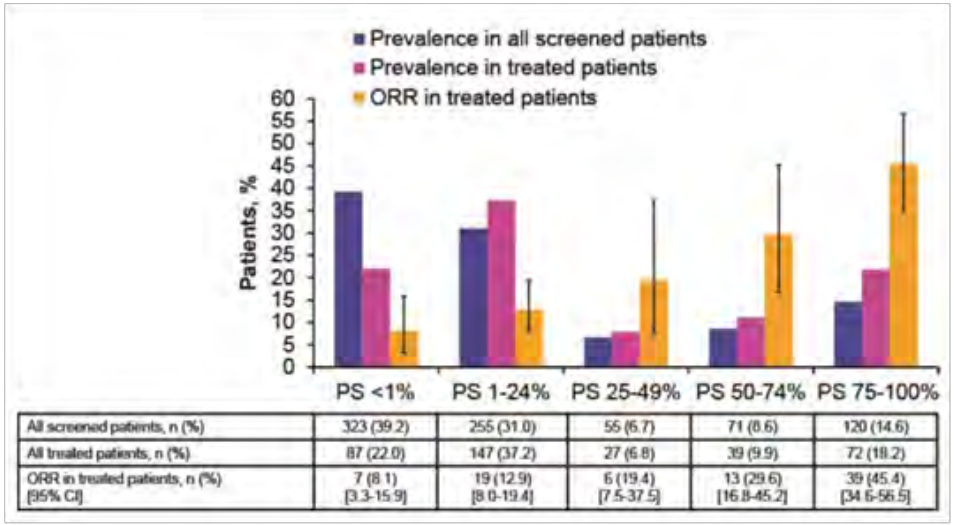




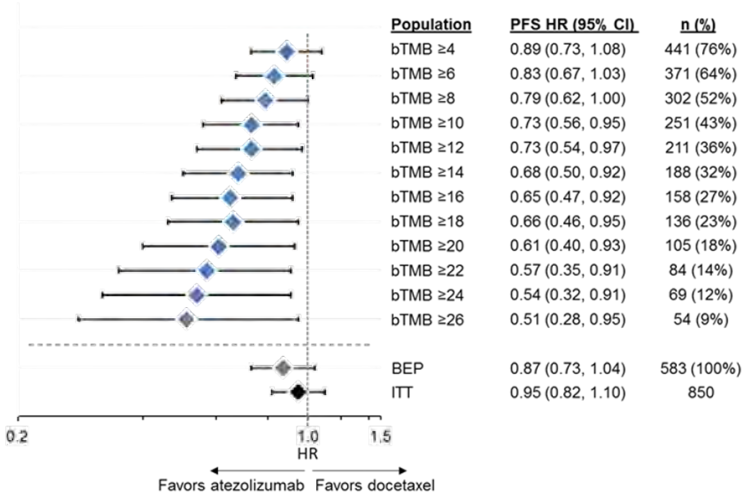
- Validation analytique
  - Standardisation, robustesse
  - Adapté aux conditions cliniques
  - Coût, délai d'analyse
- lidation clinique
  - Méthodologie adéquate
  - Pronostique versus prédictif
- ilité clinique
  - Guide la prise en charge

*Vermaelen et al. Semin Cancer Biol 2018*

# Des biomarqueurs « continus »



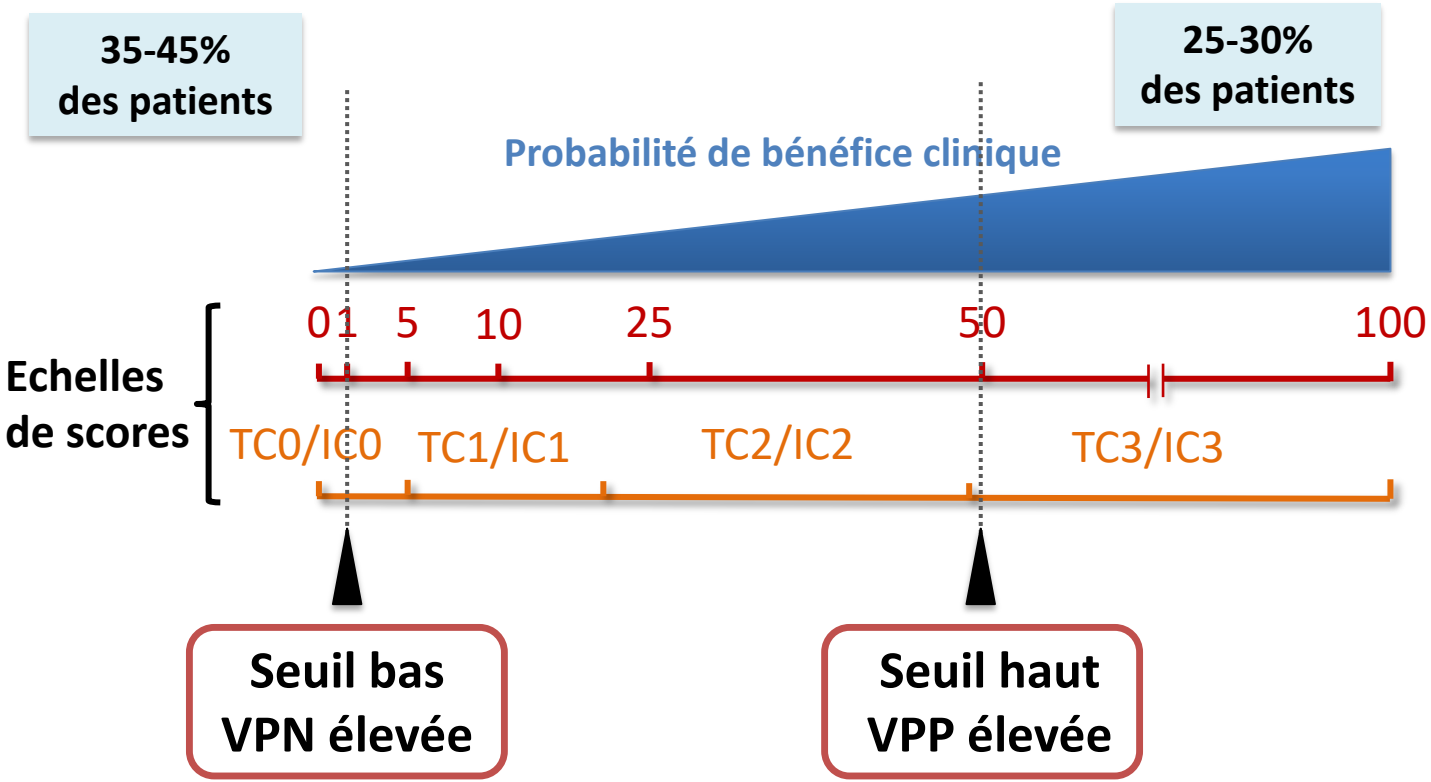

| | | | | | |
|---|---|---|---|---|---|
| All screened patients, n (%) | 323 (39.2) | 255 (31.0) | 55 (6.7) | 71 (8.6) | 120 (14.6) |
| All treated patients, n (%) | 87 (22.0) | 147 (37.2) | 27 (6.8) | 39 (9.9) | 72 (18.2) |
| ORR in treated patients, n (%) [95% CI] | 7 (8.1) [3.3-15.9] | 19 (12.9) [8.0-19.4] | 6 (19.4) [7.5-37.5] | 13 (29.6) [16.8-45.2] | 39 (45.4) [34.6-56.5] |



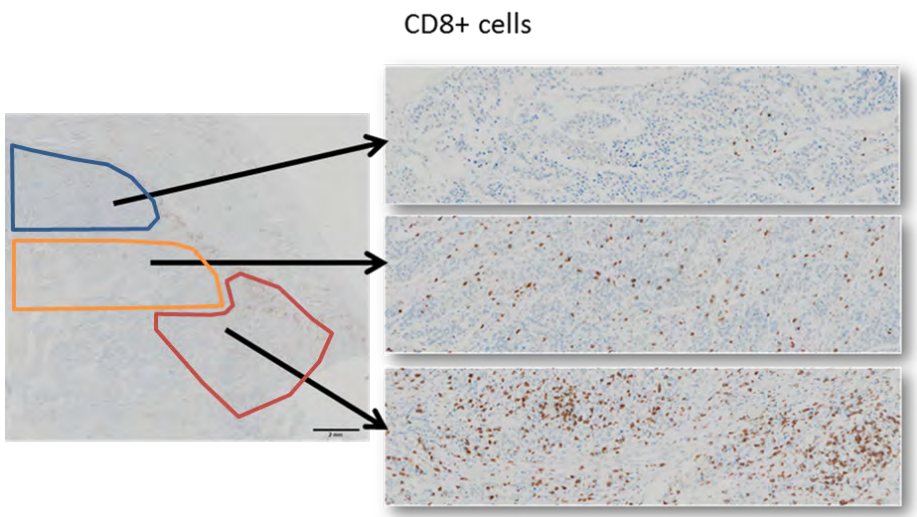

*Gandara et al., ESMO 2017*

**Comment définir les seuils (essais cliniques) ?**

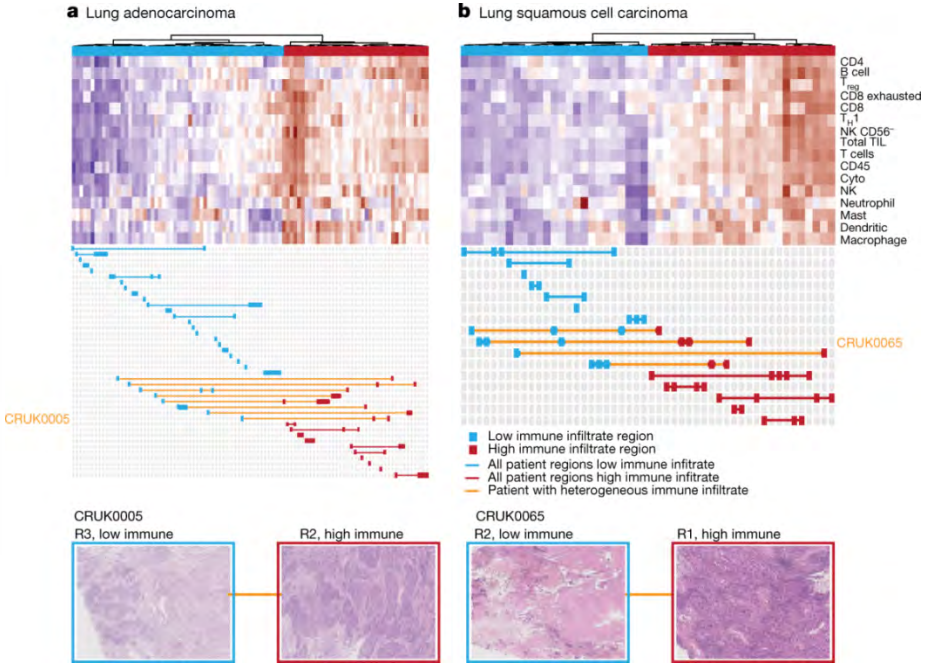
# PD-L1 : un biomarqueur continu



# Des biomarqueurs hétérogènes

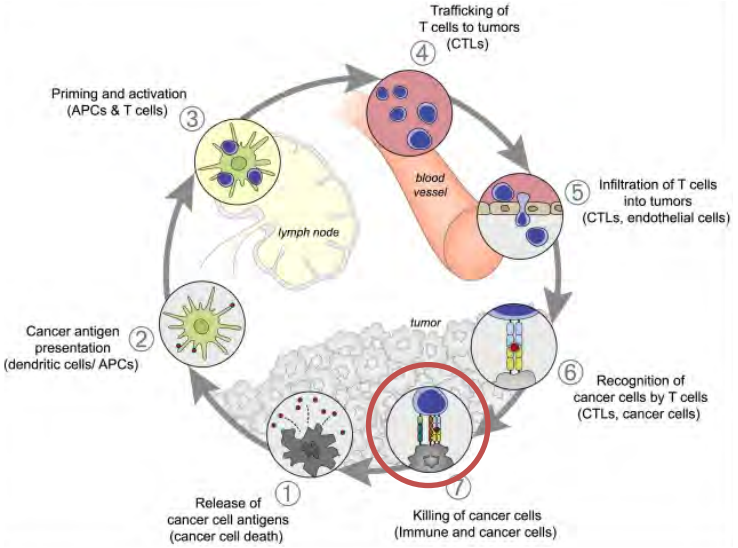



Dans l'espace
Dans le temps

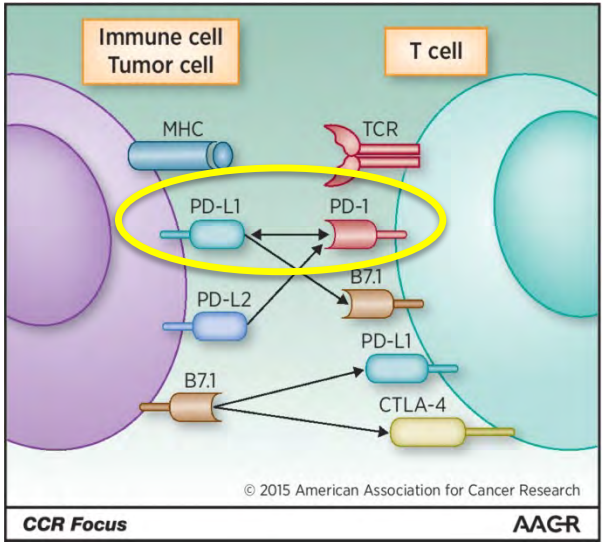


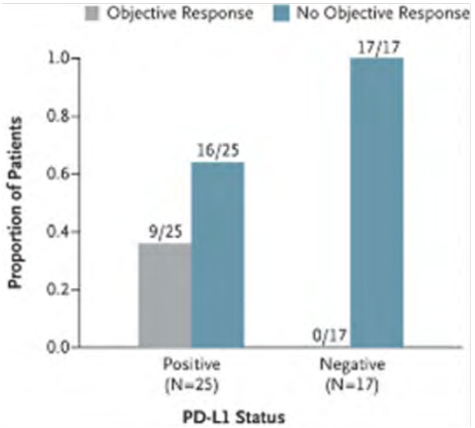

Hétérogénéité associée à des facteurs moléculaires

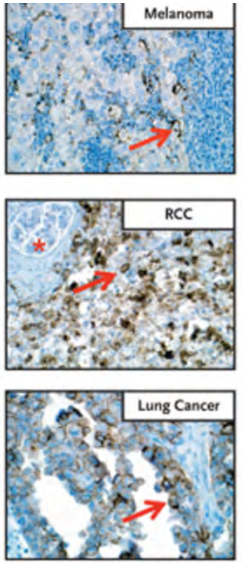
# PD-L1 comme biomarqueur dans les CPNPC



# Au début de l'histoire…




*Treatment: nivolumab, IHC clone 5H1*
*Topalian et al., NEJM, 2012*

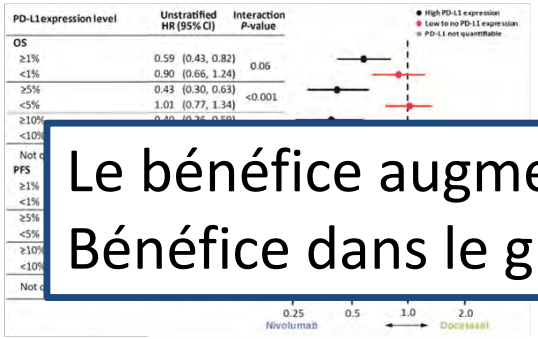
**IHC PD-L1 IHC:**

- Disponible universellement, rapide, peu cher
- Évaluée dans tous les essais cliniques

# Expression de PD-L1 et bénéfice des anti-PD1/L1
## dans les CPNPC métastatiques

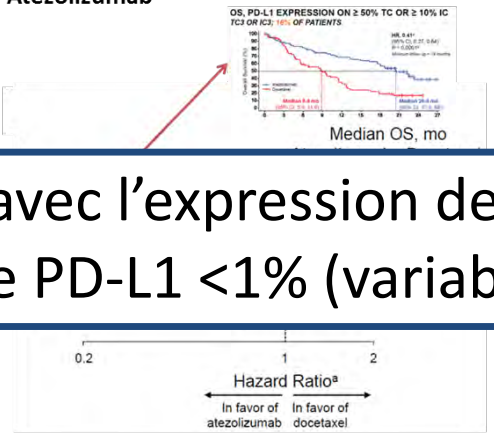
CheckMate 057

**Nivolumab** *(carcinomes non épidermoïdes)*

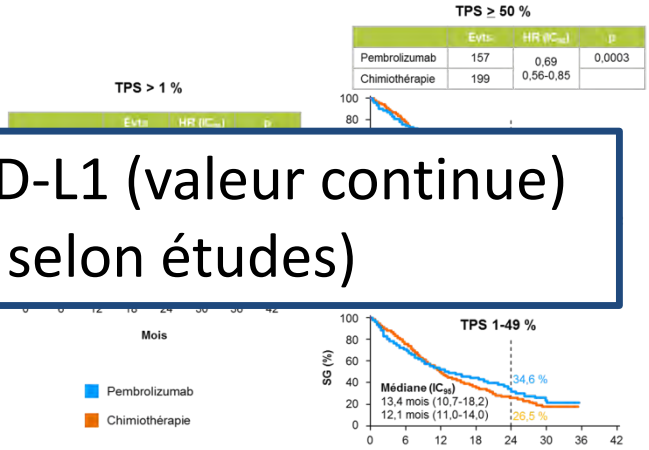



OAK

**Atezolizumab**



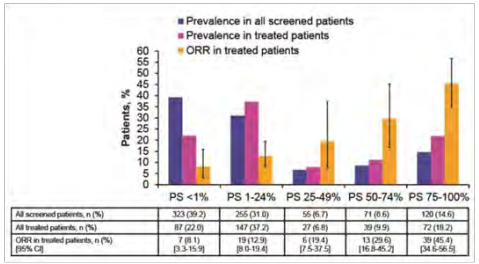

KEYNOTE-048




**Le bénéfice augmente avec l'expression de PD-L1 (valeur continue)**
**Bénéfice dans le groupe PD-L1 <1% (variable selon études)**

KEYNOTE-001

**Pembrolizumab**

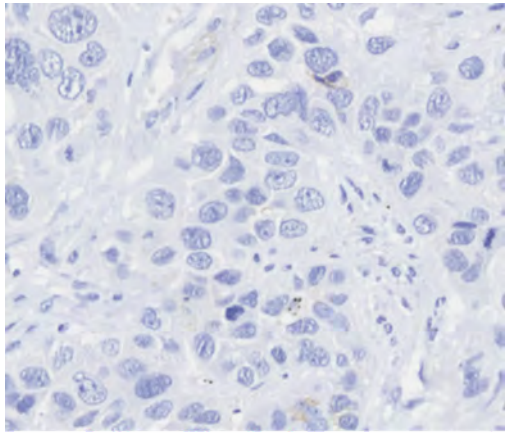



PD-L1 ≥1% HR=0.81
PD-L1 ≥20% HR=0.77
PD-L1 ≥50% HR=0.69

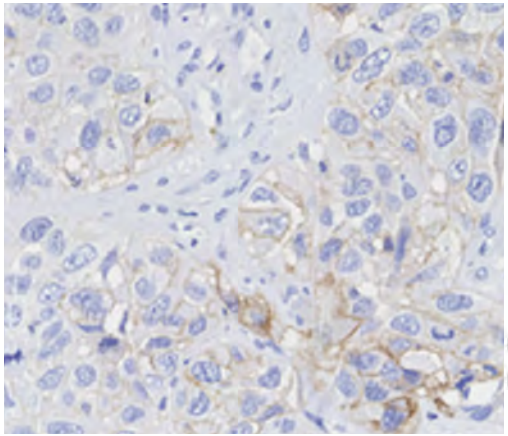
*Nivolumab: Borghaei et al., NEJM 2015*
*Pembrolizumab: Garon et al., NEJM 2015*
*Atezolizumab: Rittmeyer et al., Lancet 2016*

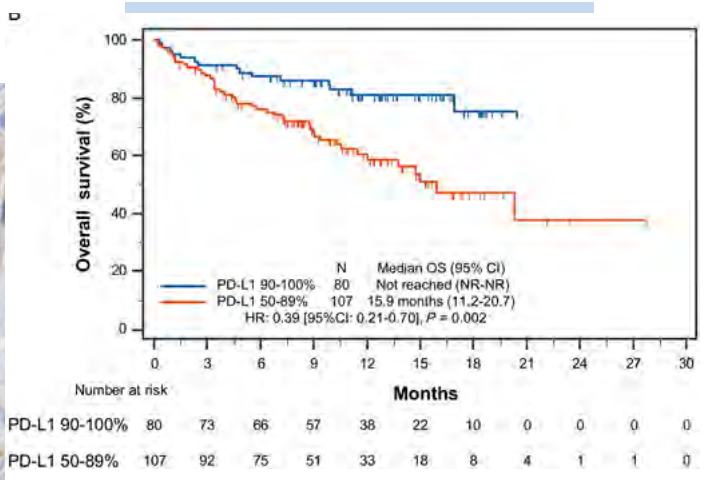
# Continuum biologique et clinique

35-45% des CPNPC



1%






*Aguilar et al. Ann Oncol 2019*

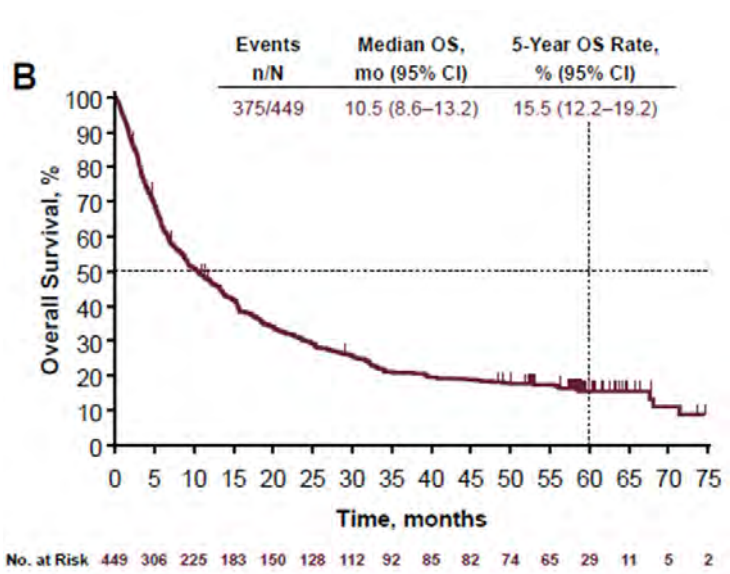
KEYNOTE 042
(pembrolizumab 1L mNSCLC)
*Mok et al. Lancet 2019*

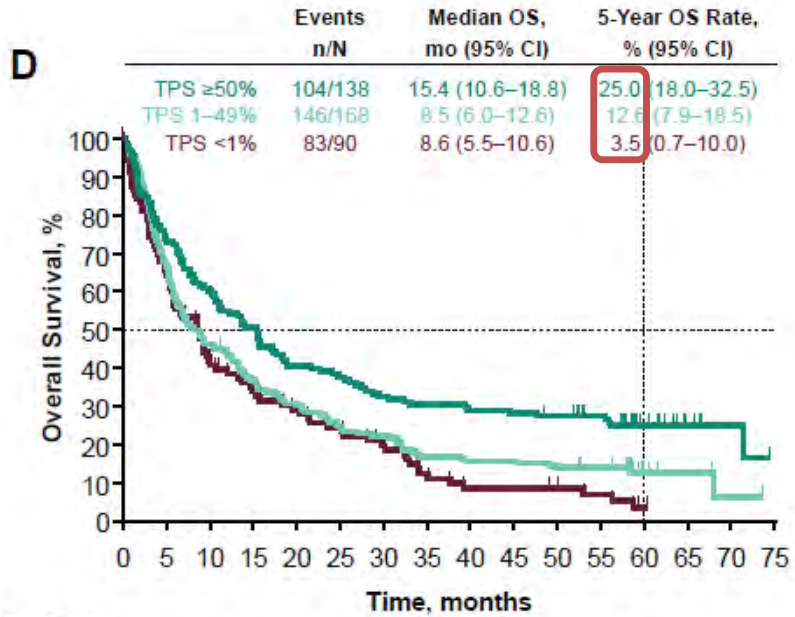
**PD-L1 ≥1%  HR=0.81**

**PD-L1 ≥20%  HR=0.77**

**PD-L1 ≥50%  HR=0.69**

# PD-L1 et bénéfice à long terme



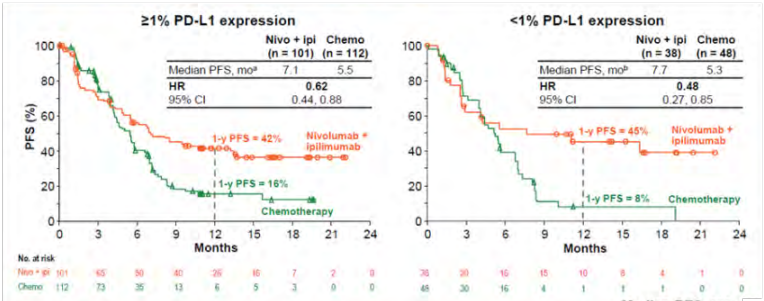




*Pembrolizumab, CPNCP métatatique après traitement par chimiothérapie à base de platine*

*Garon E et al. ASCO 2019 LBA9015*

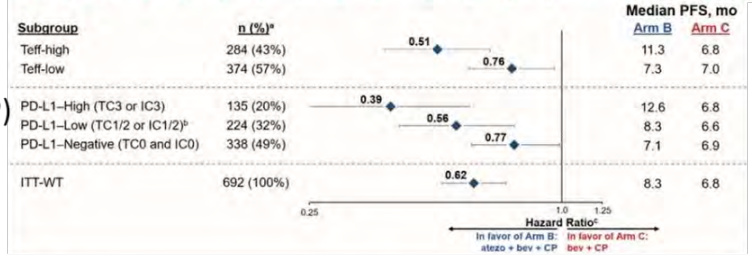
# PD-L1 et combinaisons de traitements

**CM-227** (Nivolumab+ ipilimumab)

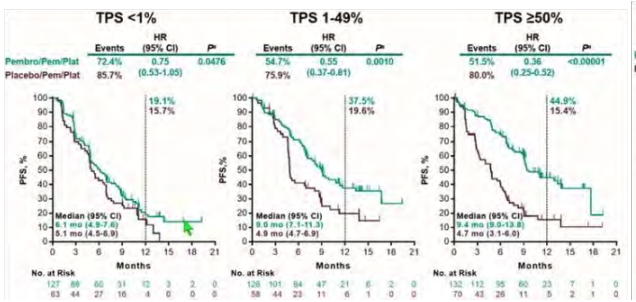


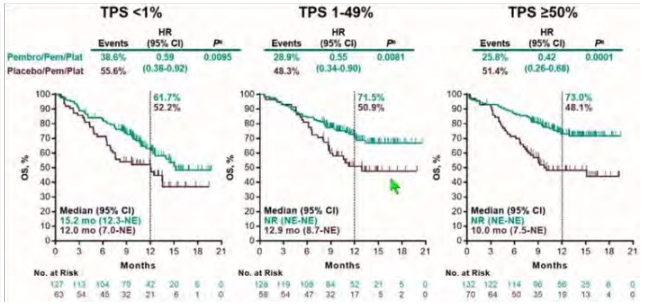

*Population: TMB ≥10 mut/Mb*

**IM-150** (Atezo+bev+CP)




**KN-189** (Pembro+CP)



# Pourquoi PD-L1 ne suffit pas ?

**L'information n'est pas exhaustive**

- L'expression par les TC peut être pertinente ou non*
- Pas de prise en compte de l'expression par les IC**

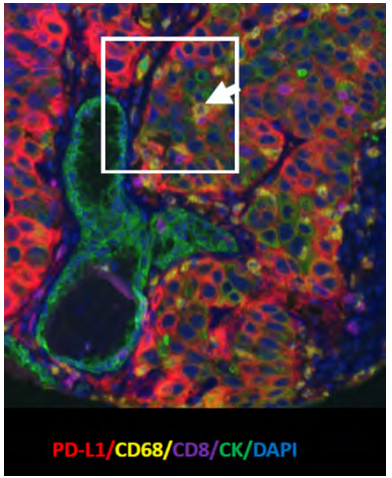
**Le prélèvement tissulaire n'est pas représentatif**

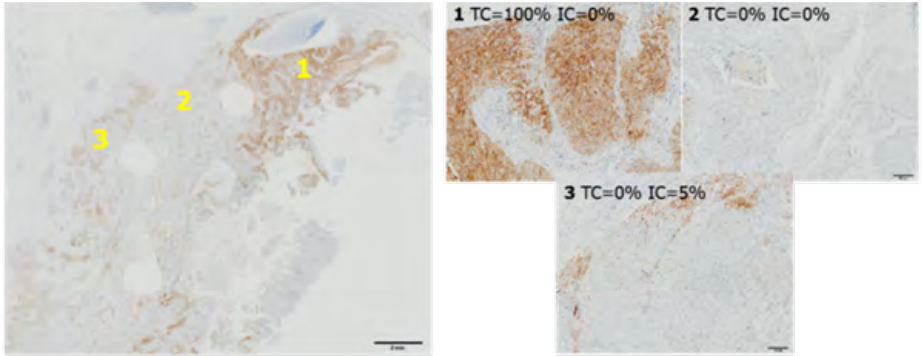
- Hétérogénéité tumorale*/**

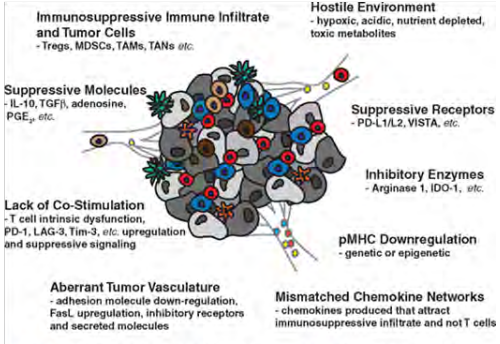
**D'autres informations sont manquantes**

- Autres voies d'immunosuppression**

** Faux positifs, ** Faux négatifs*




*D. Rimm, AACR 2019; Lanitis et al. Ann Oncol 2017*

# Implémentation pratique de PD-L1

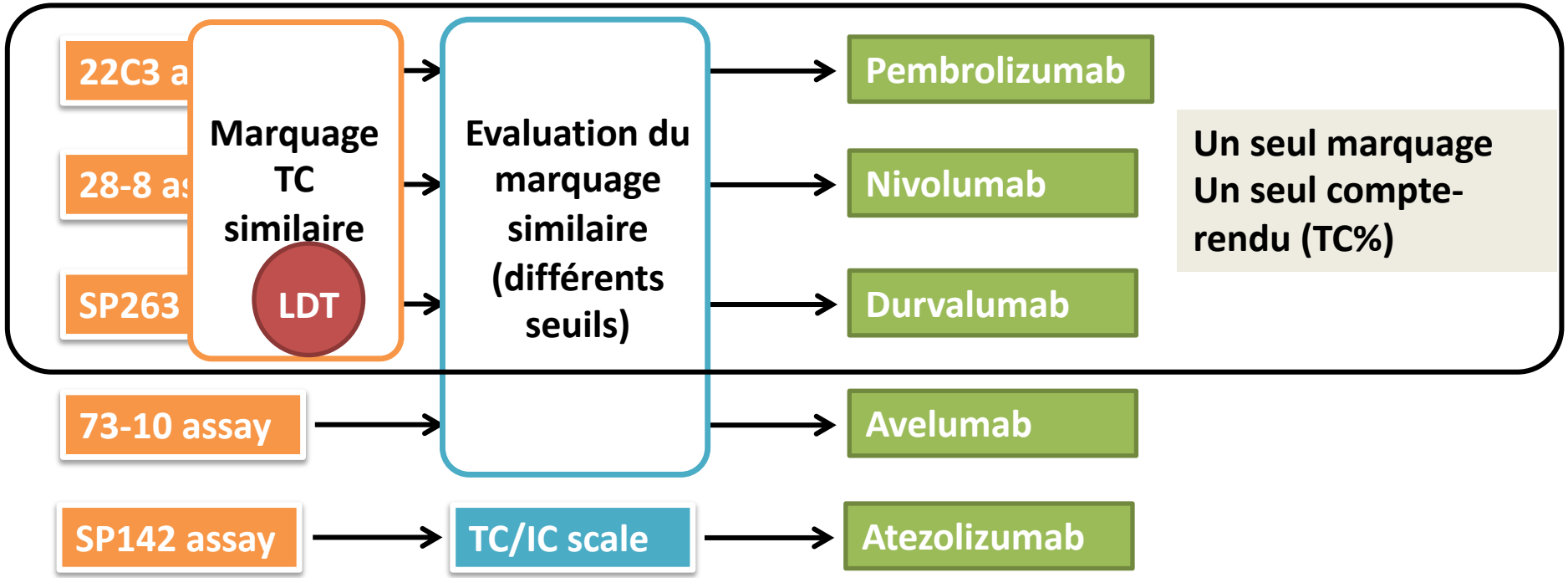
| Traitement | Clone | Epitope | Detection | Seuils de positivité |
|---|---|---|---|---|
| Nivolumab | **28-8 Dako** | EC | EnVision FLEX | TC : ≥1%, ≥5%, ≥10% |
| Pembrolizumab | **22C3 Dako** | EC | EnVision FLEX | TC : ≥1%, ≥50% |
| Durvalumab | **SP263 Ventana** | IC | OptiView | TC : ≥25% |
| Atezolizumab | **SP142 Ventana** | IC | OptiView + amplification | TC : ≥1%, ≥5%, ≥50%<br>IC : ≥1%, ≥5%, ≥10% |

**Dako** PD-L1 **28-8** pharmDx for AS Linker 48
**Dako** PD-L1 **22C3** pharmDx for AS Linker 48

**Ventana SP142** IHC Assay
**Ventana SP263** IHC assay


- **Problématiques :**
- Comparaison des tests, harmonisation et utilisation de tests maison (coût)
- Reproductibilité de l'évaluation par le pathologiste
- Anticipation et travail en réseau

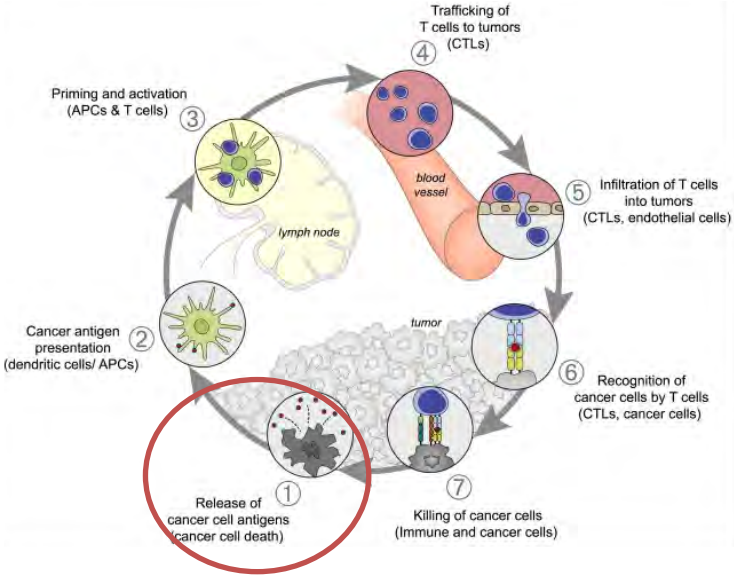
# Harmonisation du testing PD-L1 dans les CPNPC



# Peut-on faire mieux avec PD-L1 ?

- Prise en compte des cellules immunitaires : pas réaliste
- Analyse d'image : améliore la reproductibilité mais corrélation à la clinique à démontrer
- PD-L1 circulant, CTC : peu convaincant…
- ARN : corrélé/intégré à des signatures d'expression

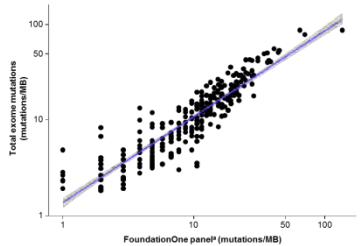
# (Néo)antigènes et charge mutationnelle



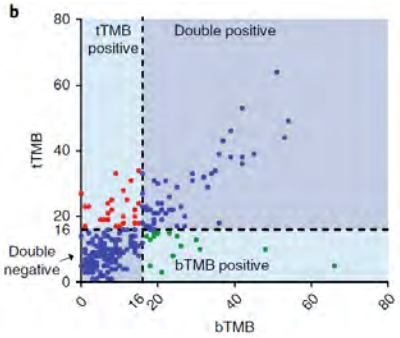
# Charge mutationnelle

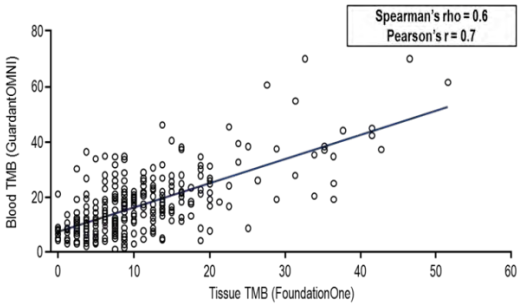
- Evalue l'immunogénicité « potentielle »
  - Pas d'évaluation directe des néoantigènes
- Hétérogénéité tumorale limitée
- WES (référence) ou panels NGS (>1 Mb)
- Différentes méthodes d'analyse bioinformatique
- ADN tissulaire ou ADN circulant

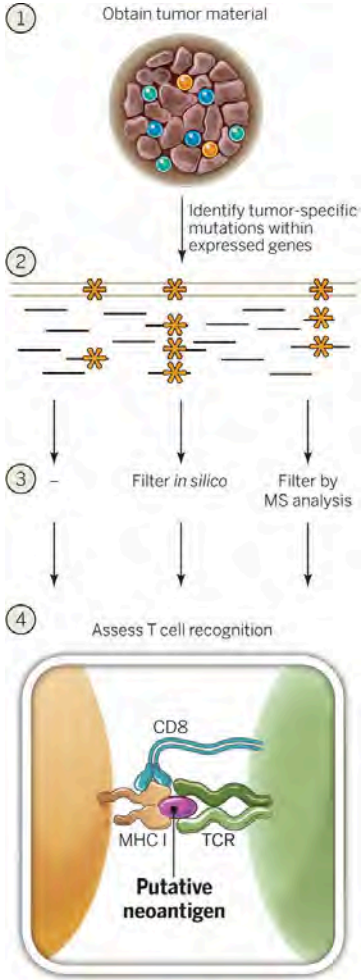
***WES vs. gene panel***




***Tissu vs. sang***

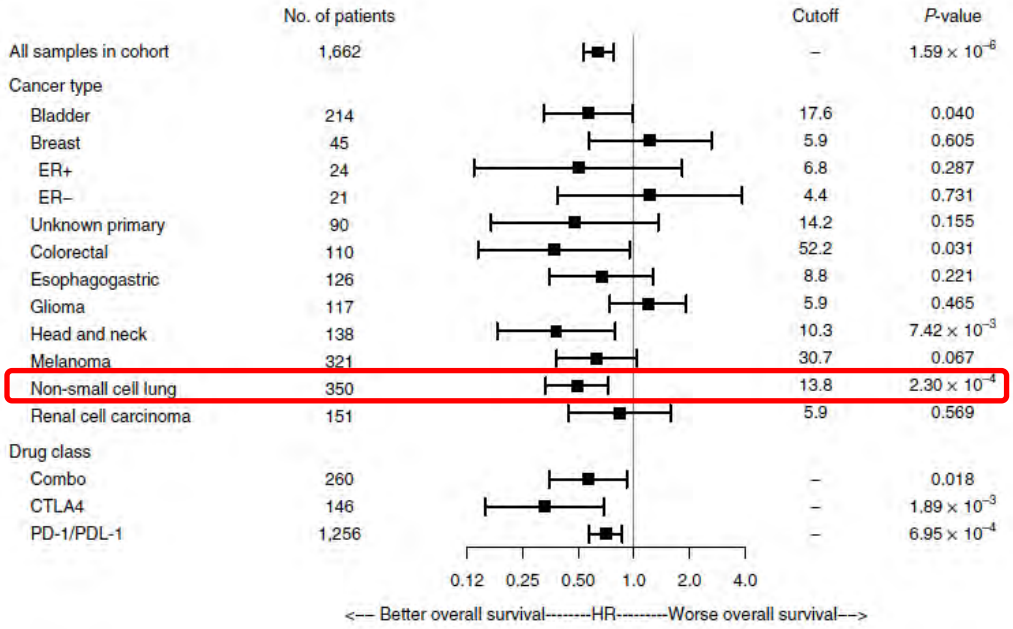



*Schumacher, Science 2015; Peters et al. AACR 2017; Gandara et al. Nat Med 2018; Peters et al.*

# Charge mutationnelle

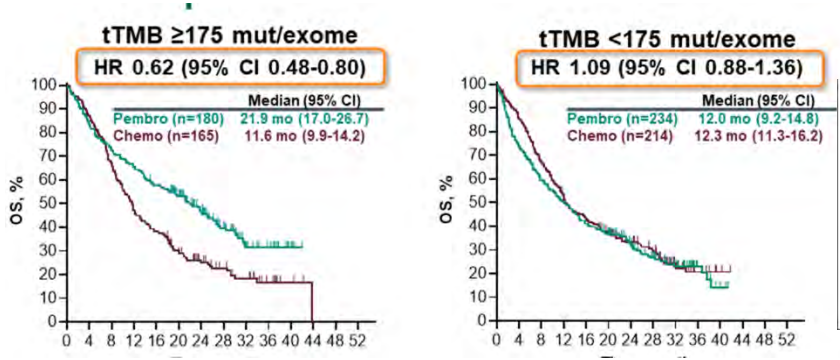



*Samstein et al. Nature Genetics, 2019*

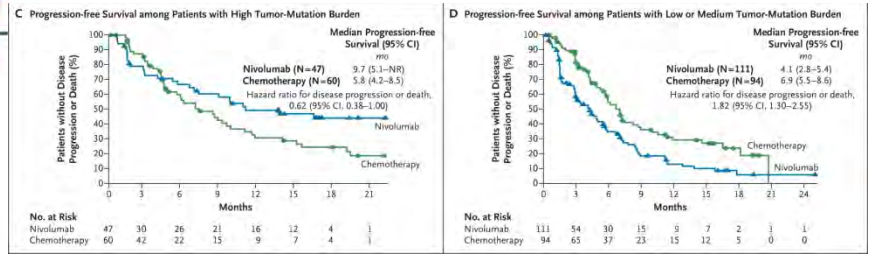
*Gandara et al. Nat Med 2018*

# Charge mutationnelle et monothérapie anti-PD(L)1

**Tissu**

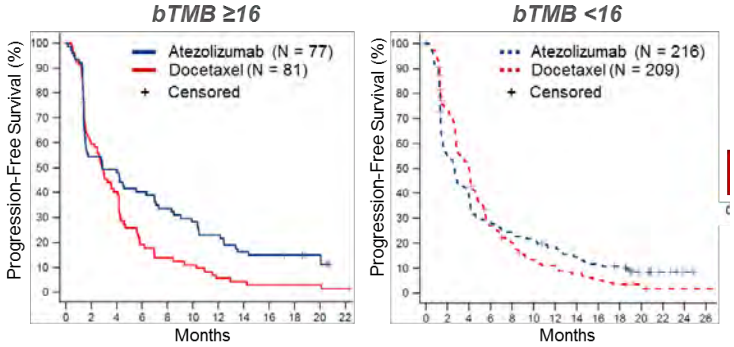



*KEYNOTE-042 (Herbst et al., ESMO 2019)*

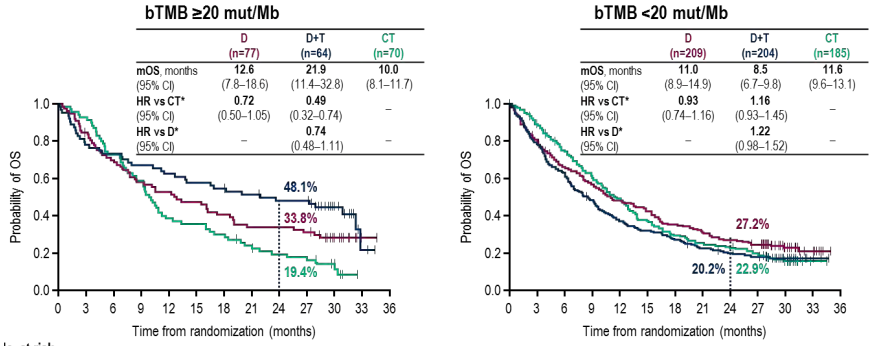



*CheckMate-026 (Carbone et al., NEJM 2017)*

**Sang**




*OAK (Gandara et al., Nat Med 2018)*




*MYSTIC (Peters et al., AACR 2019)*

# Validation prospective

## B-F1RST: Study design

**Patients with stage IIIb-IVa[a] locally advanced or metastatic NSCLC (any histology; N = 152[b])** → **Atezolizumab 1200 mg IV q3w** → **Until PD, unacceptable toxicity or loss of clinical benefit**

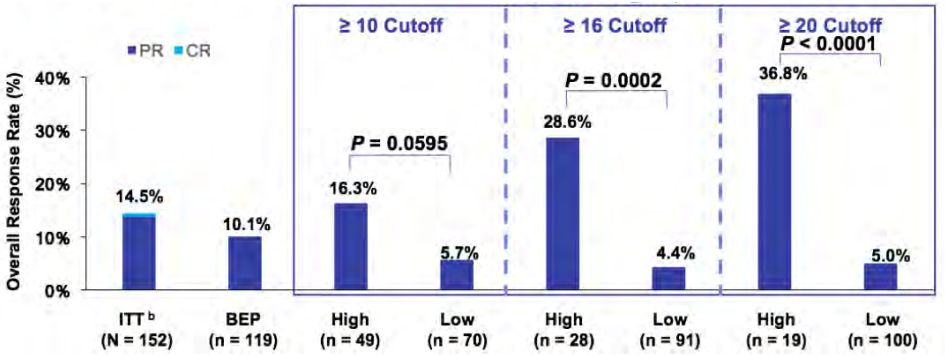
**Inclusion Criteria**
- Measurable disease per RECIST v1.1
- ECOG PS of 0 or 1
- Immunotherapy naive
- PD-L1 unselected
- Provision of blood[c]

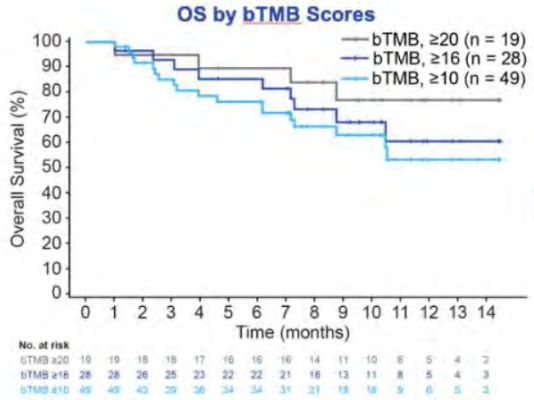
**Exclusion Criteria**
- Sensitizing *EGFR* mutations or *ALK* rearrangements
- Active brain metastases requiring treatment

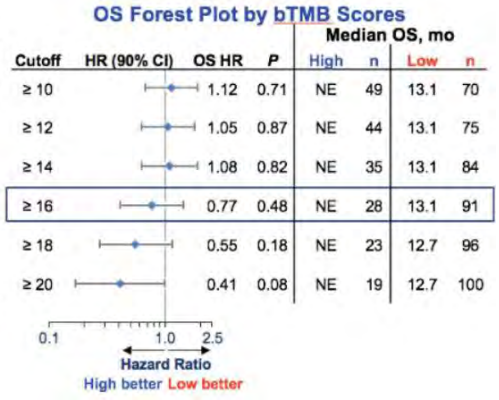
**Primary analysis**
- All enrolled patients with at least 6 months of follow-up
- Prespecified bTMB biomarker cutoff of 16

**Co-Primary Endpoints**
- Efficacy endpoint: INV-assessed ORR per RECIST v1.1
- Biomarker endpoint: INV-assessed PFS per RECIST v1.1

**Secondary Objectives**
- Safety and assessment of efficacy by INV-assessed DOR, OS



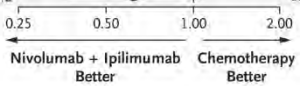




*Kim et al. ESMO 2018*

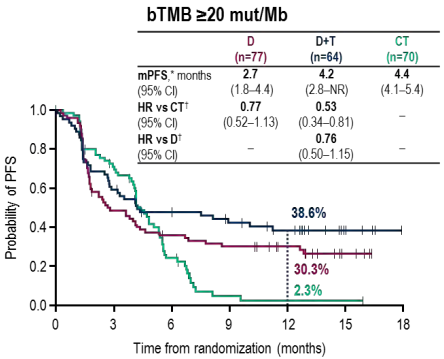
# Charge mutationnelle et combinaison anti-PD(L)1 + anti-CTLA4

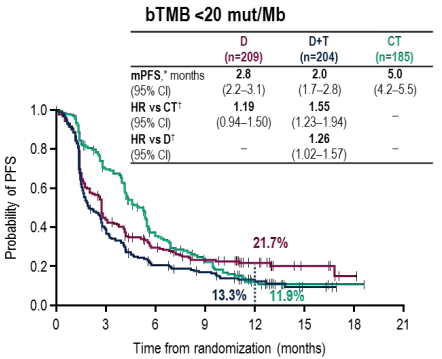
## Nivolumab + ipilimumab

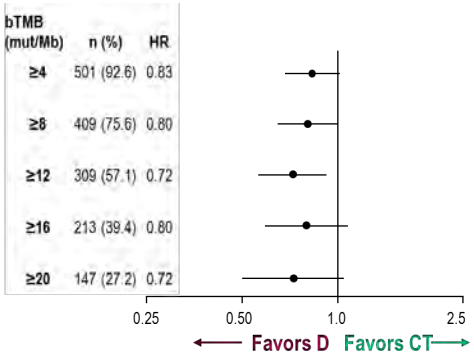
| Subgroup | No. of Patients | Median Overall Survival (months) Nivolumab + ipilimumab (N=583) | Chemotherapy (N=583) | Unstratified Hazard Ratio for Death (95% CI) |
|---|---|---|---|---|
| **Randomized Groups** | | | | |
| PD-L1 | | | | |
| All randomized | 1166 | 17.1 | 13.9 | 0.73 (0.64–0.84) |
| <1% | 373 | 17.2 | 12.2 | 0.62 (0.49–0.79) |
| ≥1% | 793 | 17.1 | 14.9 | 0.79 (0.65–0.96) |
| **Additional Exploratory Subgroup Analyses** | | | | |
| PD-L1 | | | | |
| 1–49% | 396 | 15.1 | 15.1 | 0.94 (0.75–1.18) |
| ≥50% | 397 | 21.2 | 14.0 | 0.70 (0.55–0.90) |
| Tumor mutational burden | | | | |
| Low, <10 mut/Mb | 380 | 16.2 | 12.6 | 0.75 (0.59–0.94) |
| High, ≥10 mut/Mb | 299 | 23.0 | 16.4 | 0.68 (0.51–0.91) |
| PD-L1 and tumor mutational burden (mut/Mb) combined | | | | |
| PD-L1 <1% | | | | |
| Tumor mutational burden <10 | 111 | 15.5 | 13.0 | 0.69 (0.46–1.05) |
| Tumor mutational burden ≥10 | 86 | 20.4 | 11.2 | 0.51 (0.30–0.87) |
| PD-L1 ≥1% | | | | |
| Tumor mutational burden <10 | 269 | 16.2 | 12.1 | 0.78 (0.59–1.02) |
| Tumor mutational burden ≥10 | 213 | 24.4 | 18.1 | 0.77 (0.54–1.09) |
| PD-L1 ≥50% | | | | |
| Tumor mutational burden <10 | 125 | 18.1 | 8.1 | 0.67 (0.44–1.03) |
| Tumor mutational burden ≥10 | 111 | NR | 17.2 | 0.63 (0.37–1.07) |

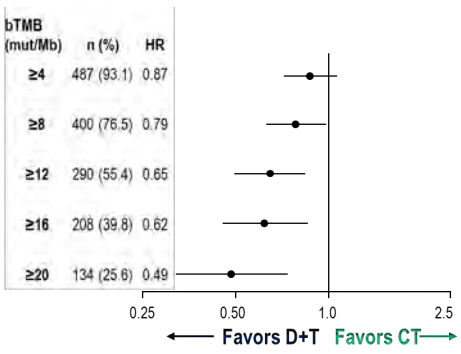



## Durvalumab + tremelimumab



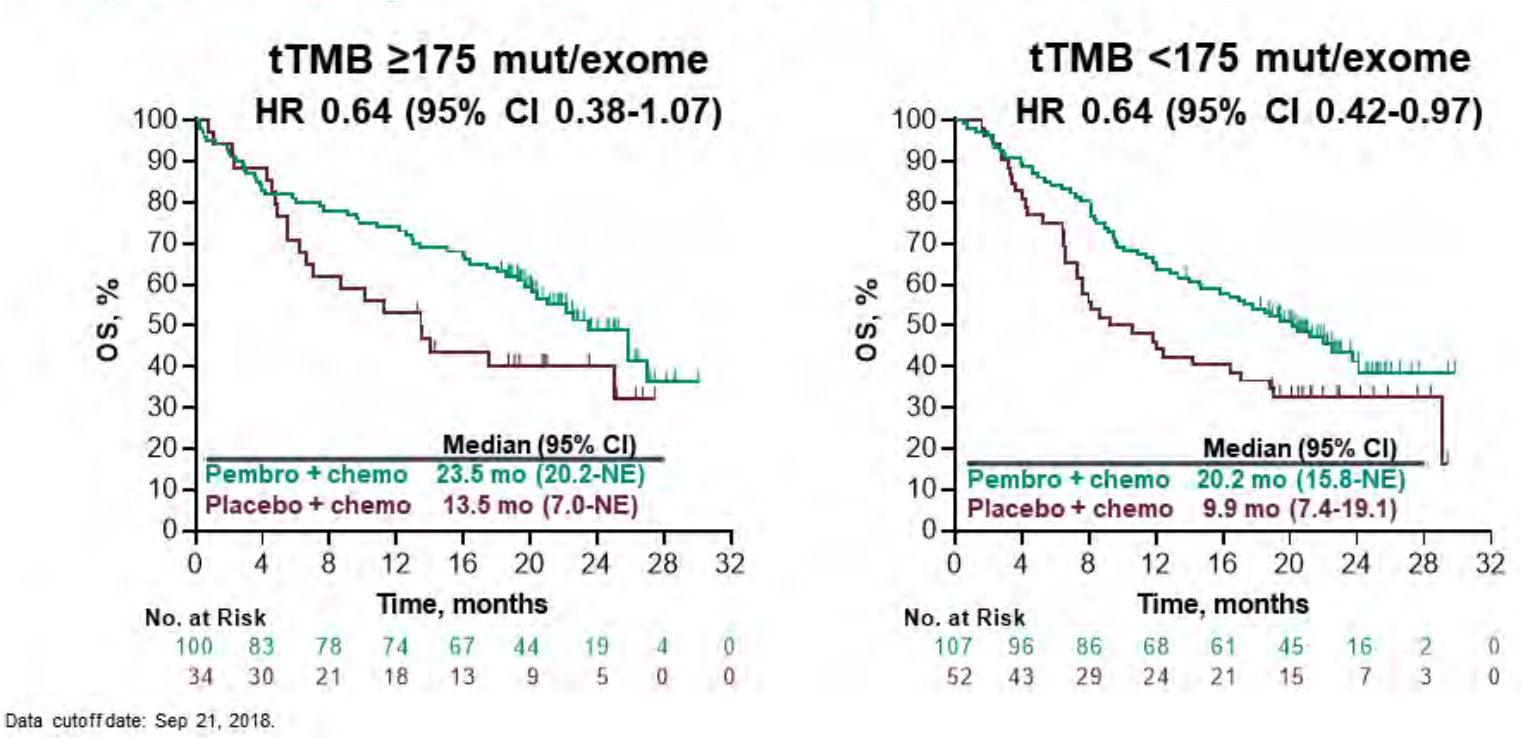




*Hellmann et al, NEJM 2019; Peters et al. AACR 2019*

# Charge mutationnelle et combinaison anti-PD1 + chimiothérapie

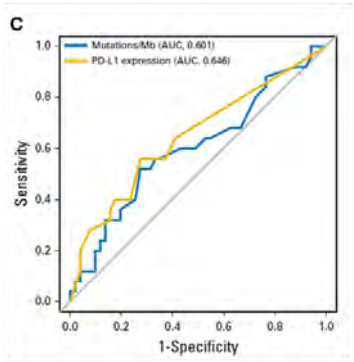


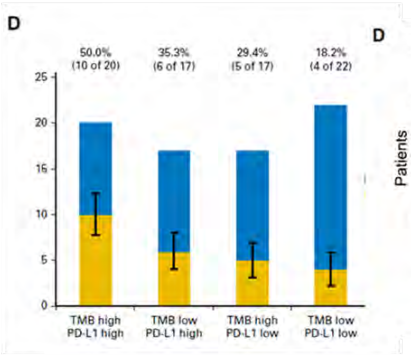

Data cutoff date: Sep 21, 2018.

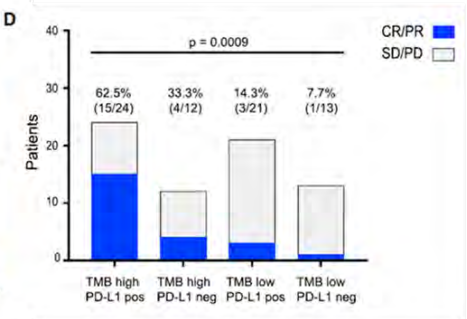
*Paz Ares et al. ESMO 2019*

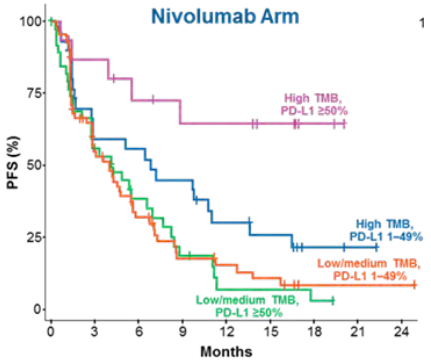
# Charge mutationnelle + …

**PD-L1**

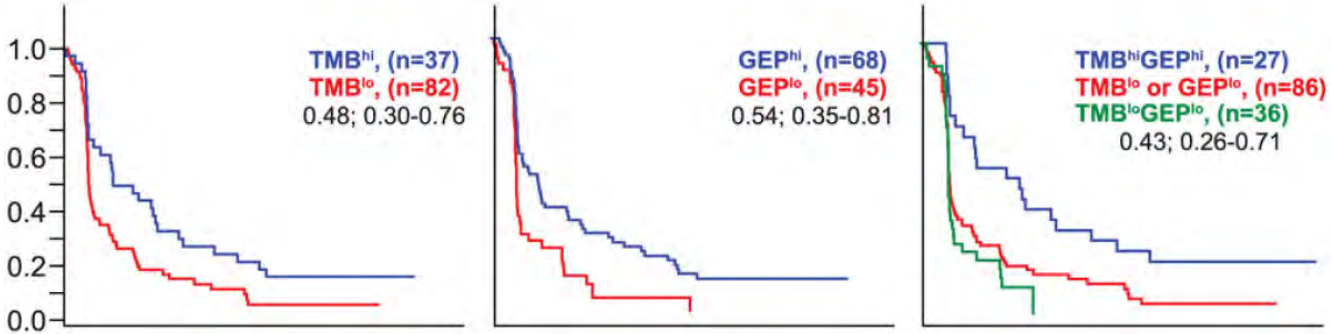


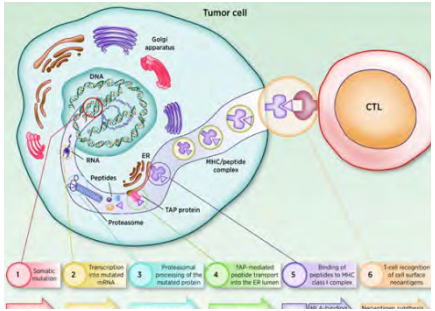

**Signature ARN**

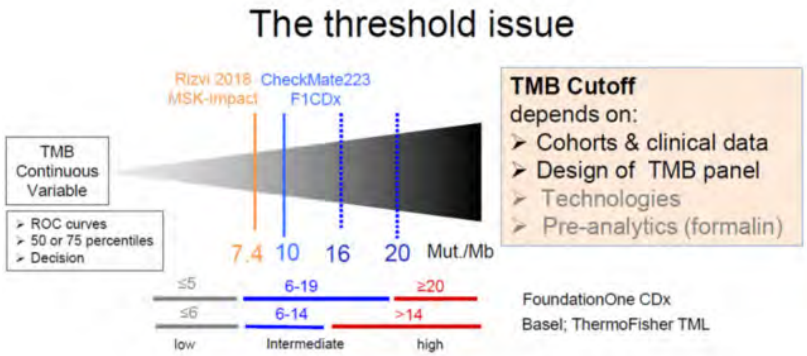



*Rizvi et al. JCO 2017; Hellmann et al. Cancer Cell 2018; Peters et al. AACR 2017; Cristescu et al. Science 2018*

# Charge mutationnelle : limitations

- Biologiques :
  - Biomarqueur indirect
  - Tous les mécanismes antigéniques ne sont pas évalués (insertions/délétions)
  - Multiples processus biologiques en « aval »
- Harmonisation de méthodes nécessaire
- Seuils multiples pour la décision clinique
- Validation clinique (analyses exploratoires principalement) et utilité actuelle ?
- Sang (ADNtc détectable ?) ou tissu ?
- Contexte : évolution des capacités de NGS



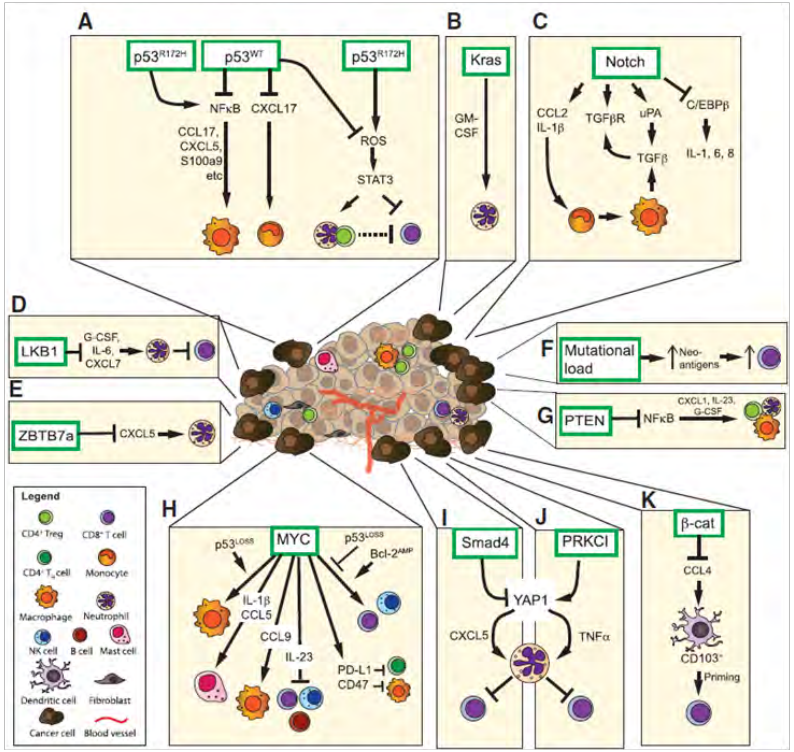




*Chabanon et al. CCR 2018*

# Caractéristiques moléculaires des tumeurs

# Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape

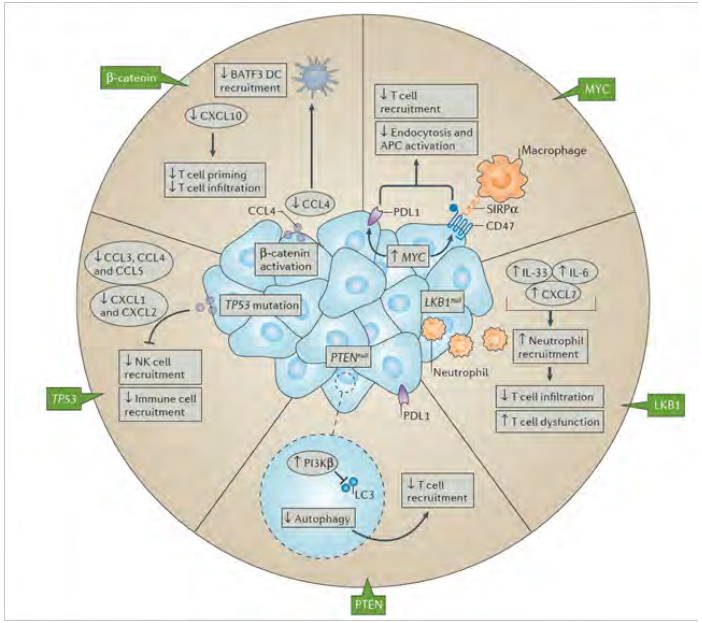
Max D. Wellenstein[1] and Karin E. de Visser[1,*]
[1]Division of Tumor Biology & Immunology, Oncode Institute, Netherlands Cancer Institute, 1066 CX Amsterdam, the Netherlands




Immunity, 2018

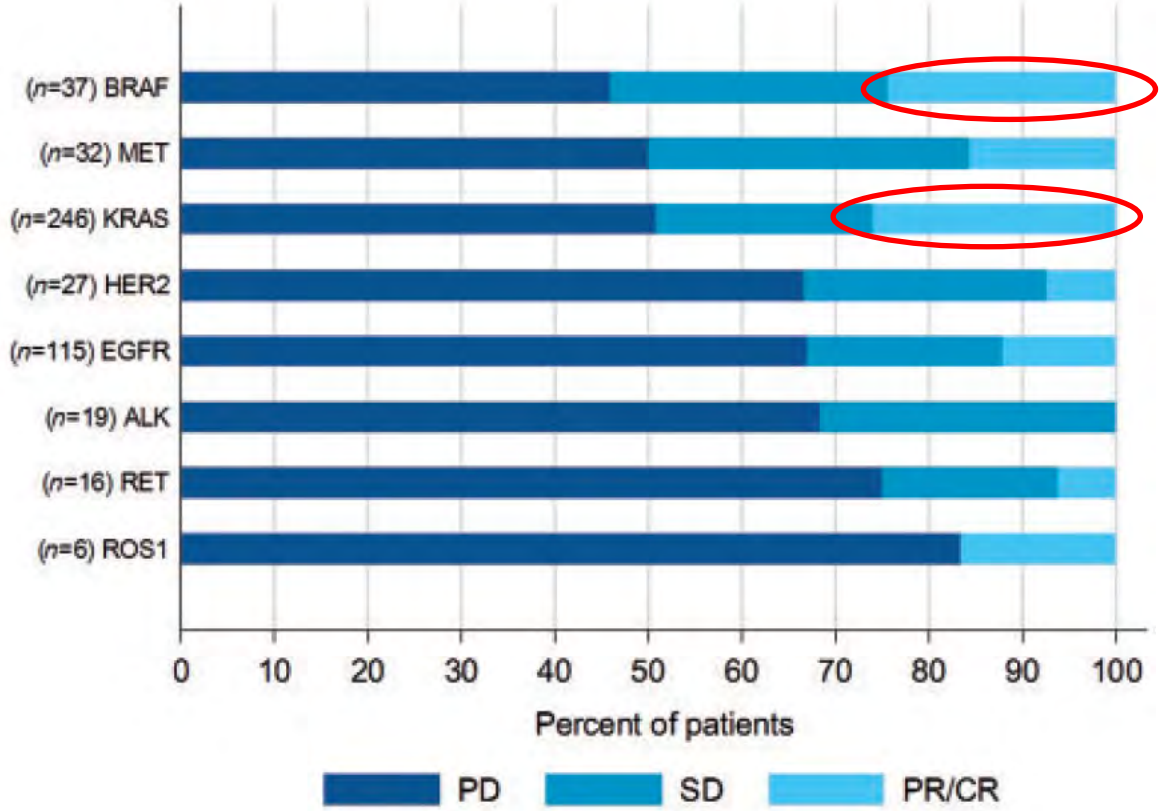
# Impact of oncogenic pathways on evasion of antitumour immune responses

*Stefani Spranger[1] and Thomas F. Gajewski[2,3]*




Nature Reviews Cancer, 2018

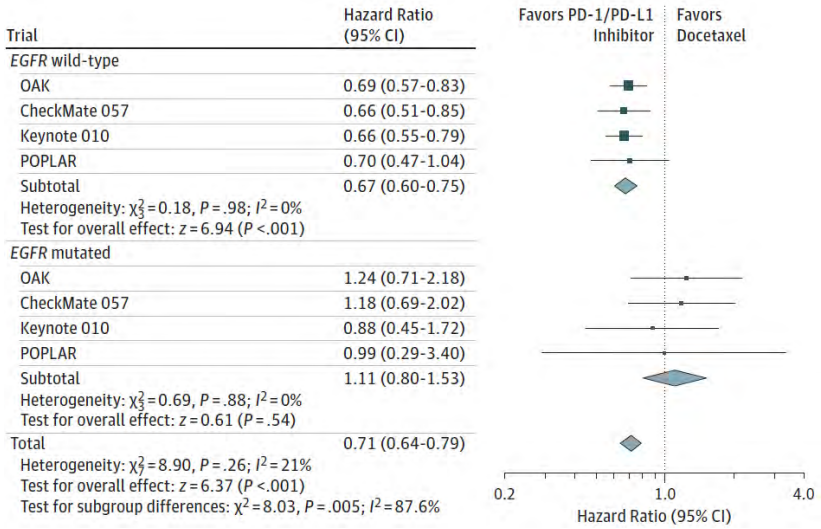
# Addictions oncogéniques et réponses aux anti-PD(L)1

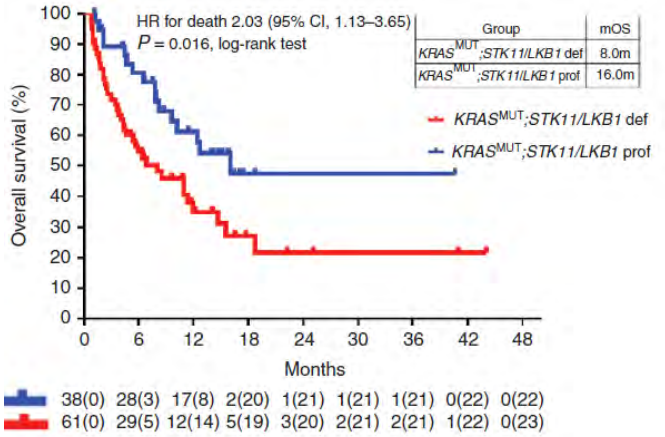



*Mazieres et al. Ann Oncol 2019*

# Altérations moléculaires et réponse aux anti-PD(L)1 (CPNPC)

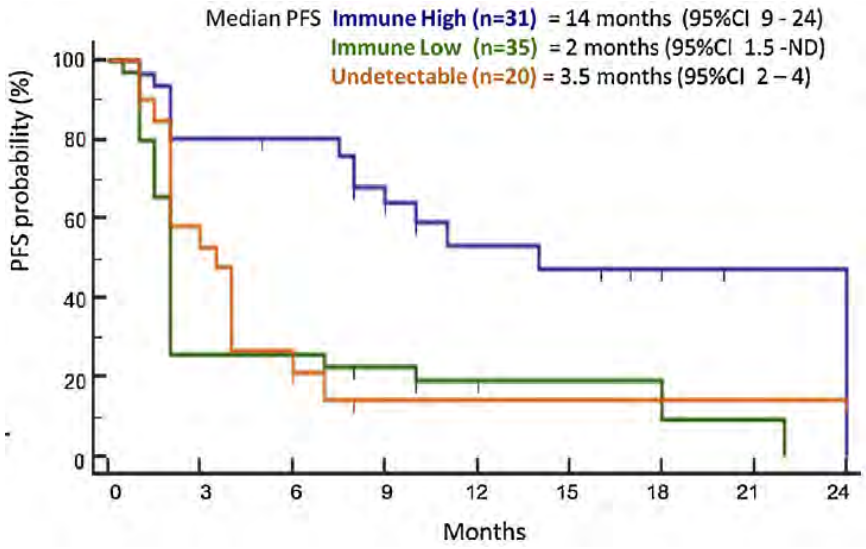
- Associés négativement : *EGFR* mut, *KRAS/STK11* comut (VPN forte)
- Associés positivement:  BRAF mut non-V600, *KRAS/TP53* comut (VPP modérée)

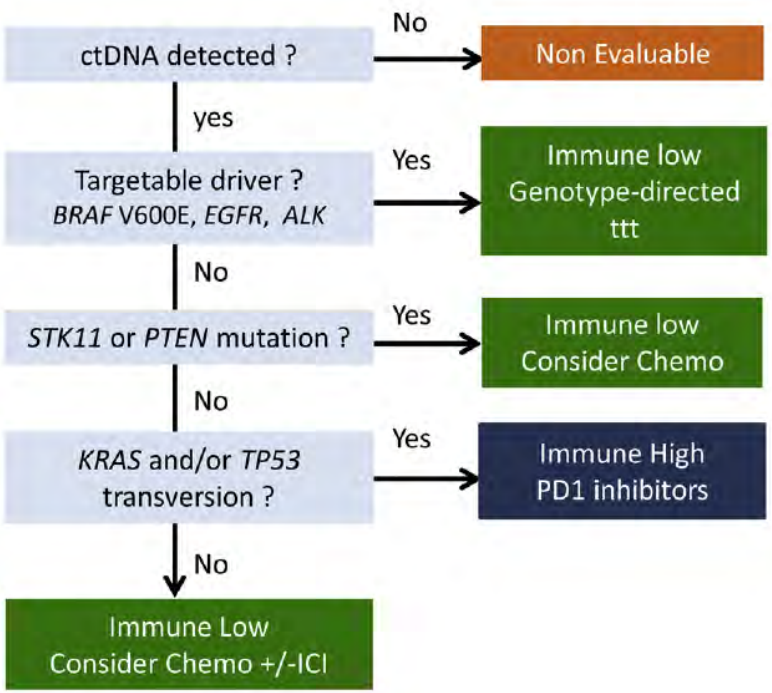



*Lee et al. JAMA Oncol 2019, Skoulidis et al. Cancer Discov 2018*

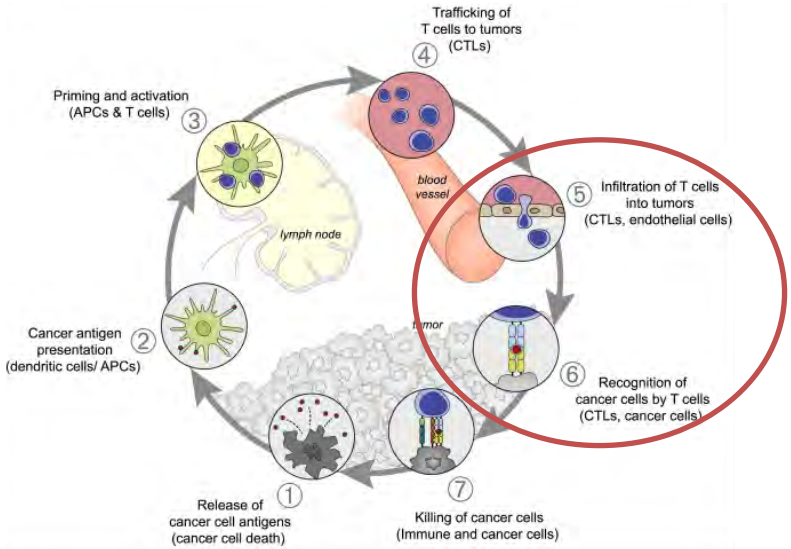
# Altérations moléculaires : ADN plasmatique



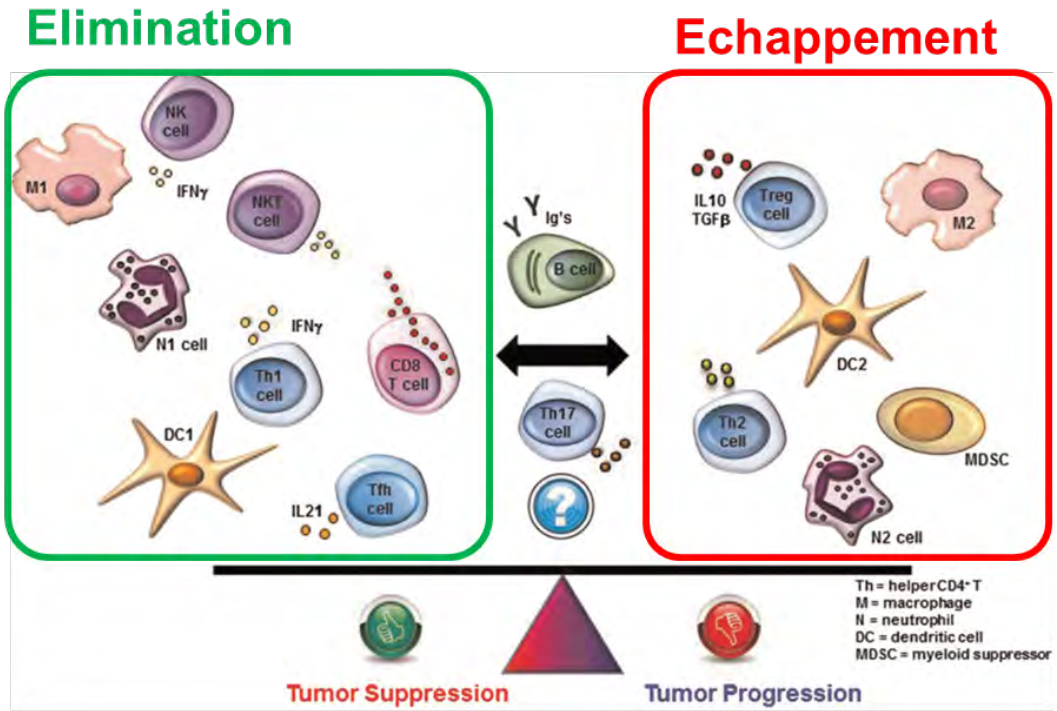



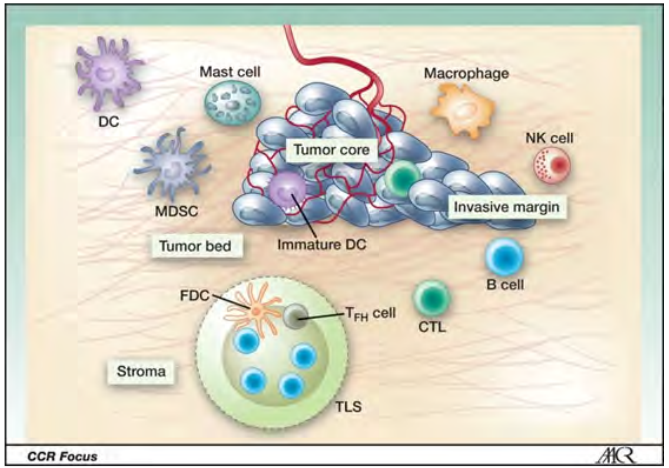

*Guibert et al. Lung Cancer 2019*

# Microenvironnement immunitaire intra-tumoral



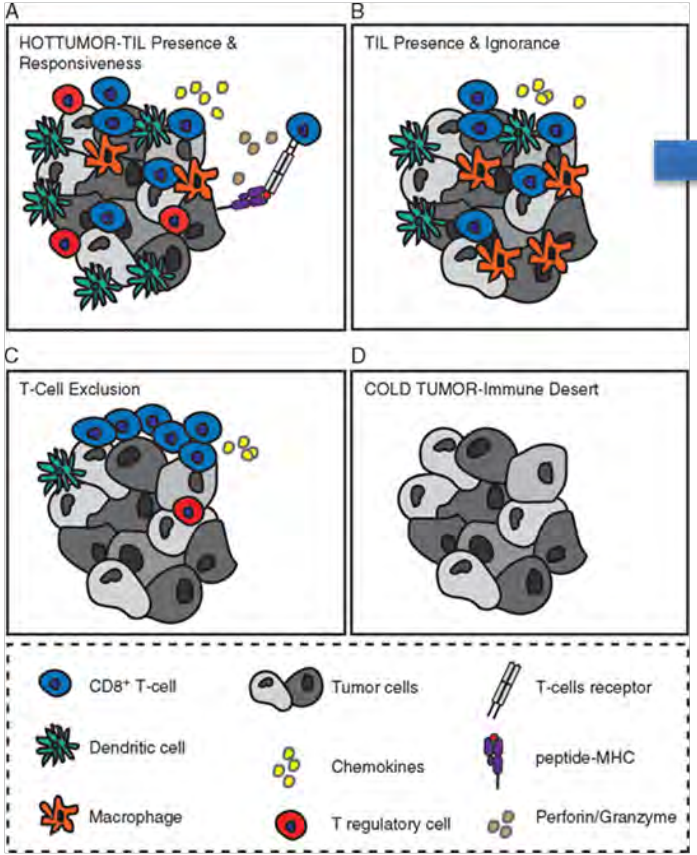
# Réponse immunitaire intra-tumorale



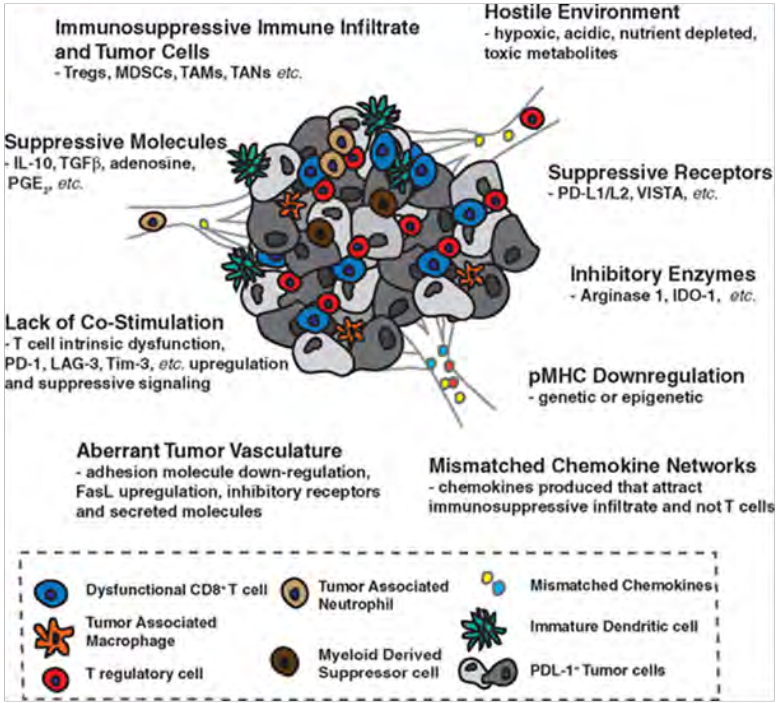




Salgado et al., Ann Oncol 2014

## Presence of T-cell inflammation




## Characterization of immune escape mechanisms




*Lanitis et al., Ann Oncol 2017*

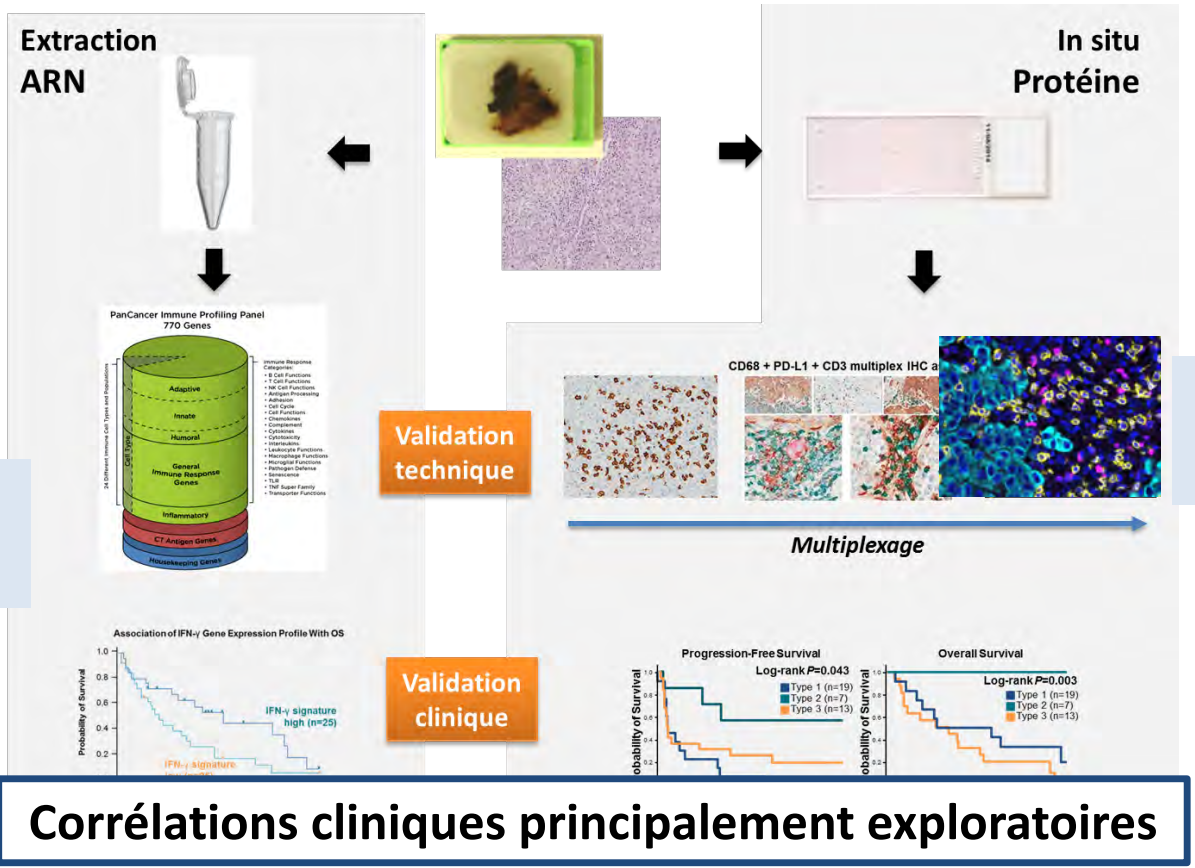
# Méthodes

## ARN

RNAseq (complet ou panel)
Nanostring

## Signatures d'expression

Th1
Cellules Teff
IFNgamma
…



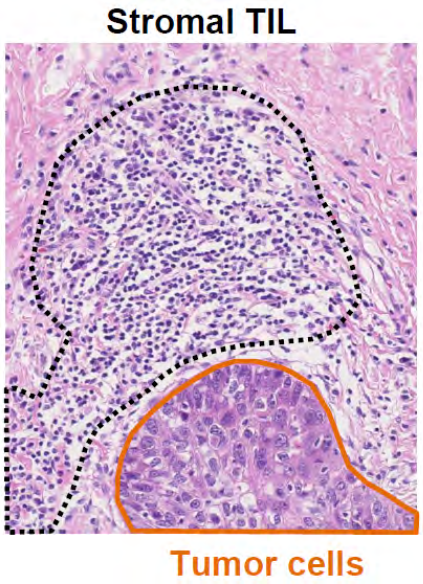

## Protéine

IHC, F-IHC
IMC

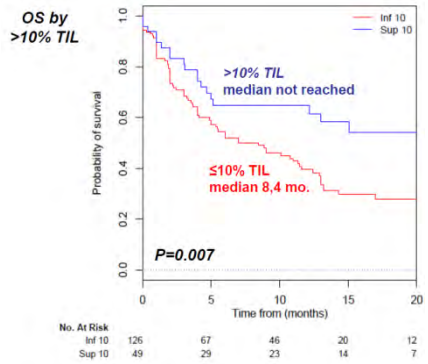
## Phénotypage de cellules

**Corrélations cliniques principalement exploratoires**

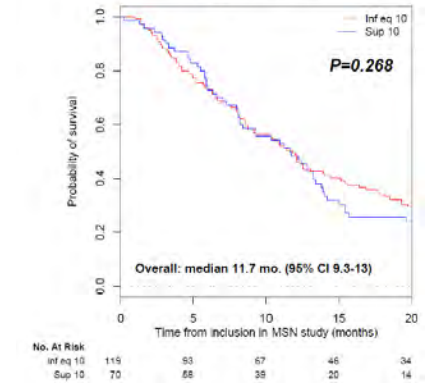
# Morphologie : TIL




***Immunotherapy***




***Chemotherapy***

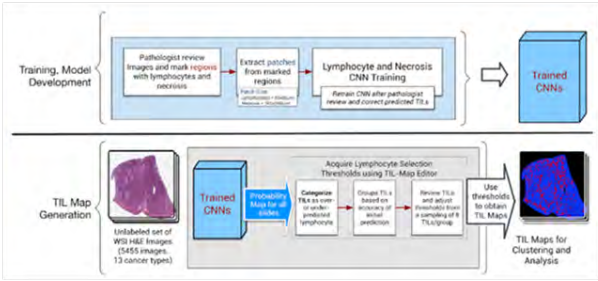



*Mezquita et al. WCLC 2019*

Cell Reports

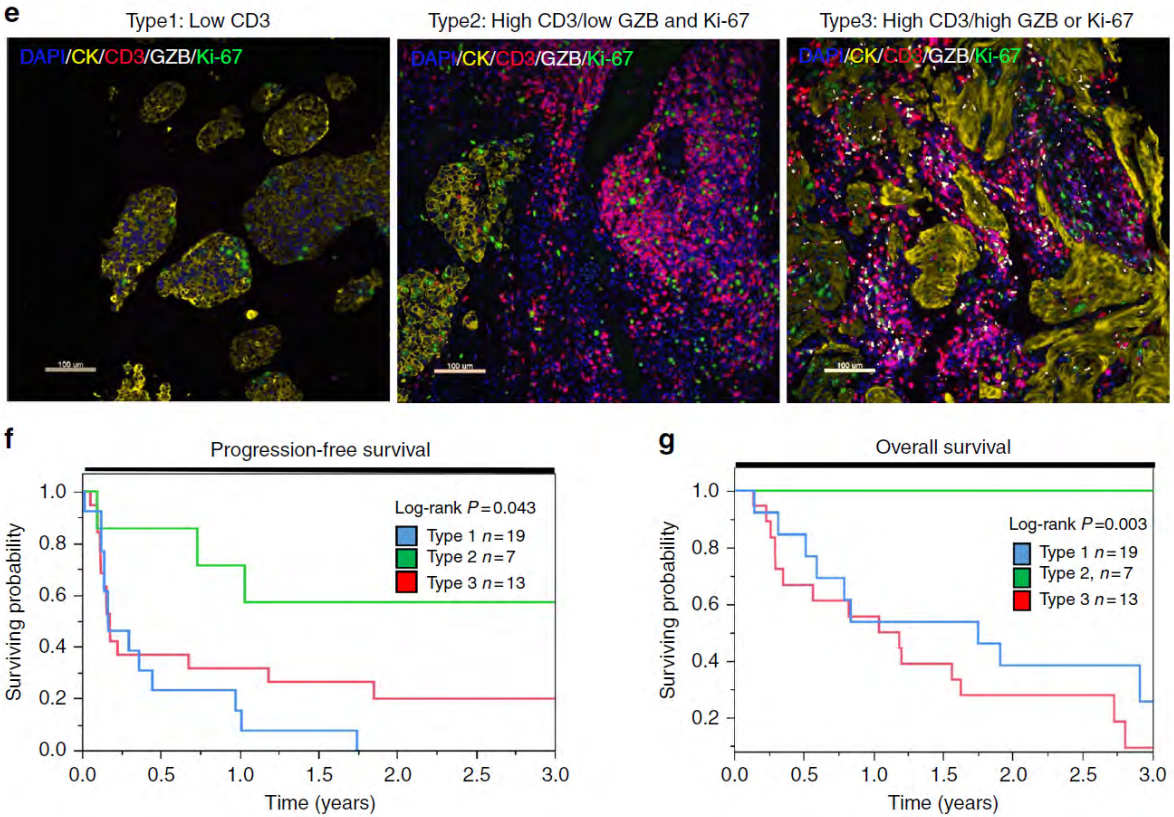
**Spatial Organization and Molecular Correlation of Tumor-Infiltrating Lymphocytes Using Deep Learning on Pathology Images**

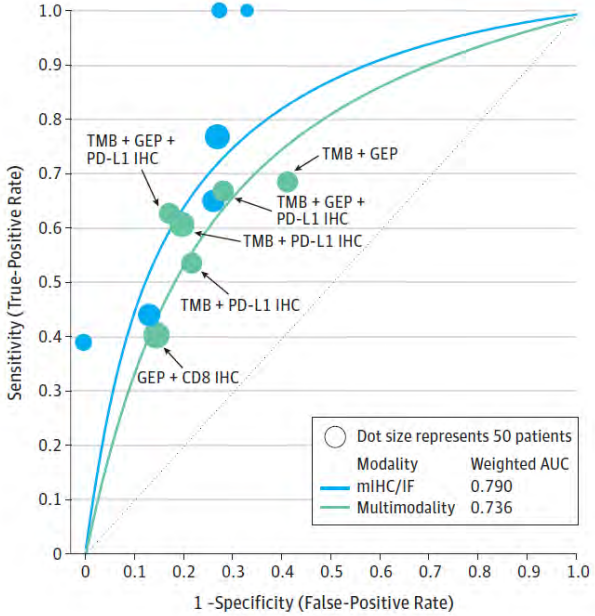
Joel Saltz,[1,*] Rajarsi Gupta,[1,4] Le Hou,[2] Tahsin Kurc,[1] Pankaj Singh,[3] Vu Nguyen,[2] Dimitris Samaras,[2] Kenneth R. Shroyer,[4] Tianhao Zhao,[4] Rebecca Batiste,[4] John Van Arnam,[5] The Cancer Genome Atlas Research Network, Ilya Shmulevich,[6] Arvind U.K. Rao,[3,7] Alexander J. Lazar,[8] Ashish Sharma,[9] and Vésteinn Thorsson[6,10,*]


Saltz et al., Cell Reports 2017

# Phénotypage in situ (F-IHC multiplex)




*Gettinger S et al. Nature Communications 2018*

*Lee et al. JAMA Oncol 2019*

# Signatures ARN : IFNgamma, Teff, Th1,…

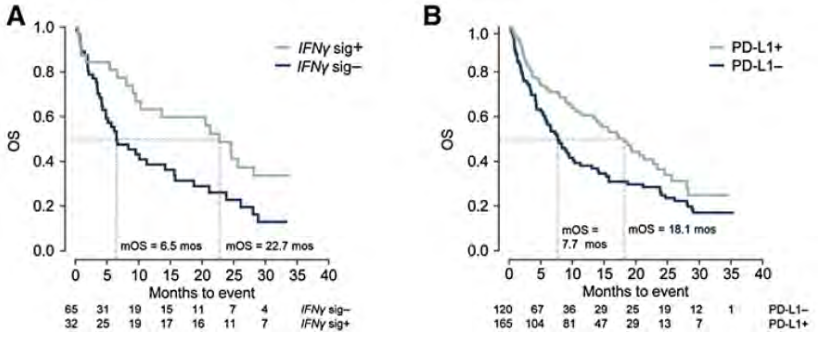



**Table 5.** Concordance between tumor IFNγ gene signature and PD-L1 status in NSCLC and urothelial cancer patients

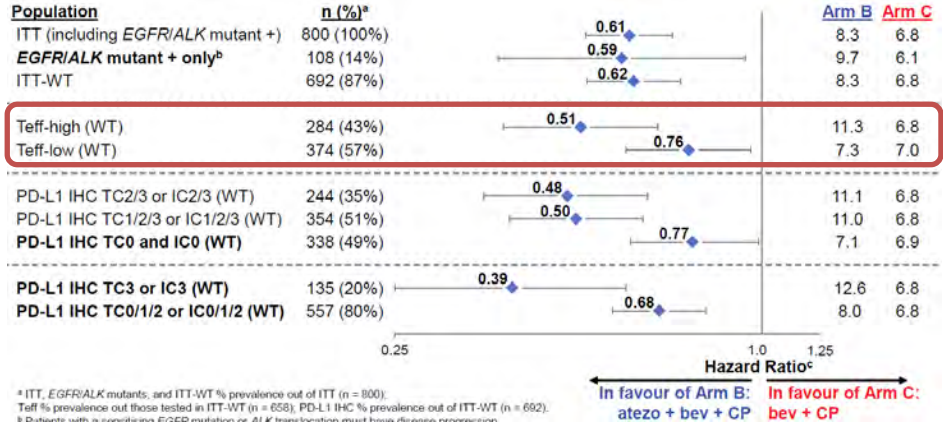
| NSCLC | IFNγ sig$^+$ mOS (*n*) | IFNγ sig$^-$ mOS (*n*) |
|---|---|---|
| PD-L1$^+$, mOS (*n*) | 23 mo (19) | 6 mo (18) |
| PD-L1$^-$, mOS (*n*) | 25 mo (11) | 7 mo (43) |

Fisher's exact test for count data; $P = 0.003$; OR = 4.1.

| UC[a] | IFNγ sig$^+$ mPFS (*n*) | IFNγ sig$^-$ mPFS (*n*) |
|---|---|---|
| PD-L1$^+$, mPFS (*n*) | NR (14) | NR (11) |
| PD-L1$^-$, mPFS (*n*) | NR (1) | NR (16) |

NOTE: Fisher's exact test for count data; $P = 0.001$; OR = 19.0.
Abbreviations: mPFS, median progression-free survival; NR, median not reached; UC, urothelial carcinoma.
[a]UC OS data are not fully mature for subgroup analysis.

| Teff Gene Signature | |
|---|---|
| *PDL1* | PD-L1 expression on TC and IC |
| *IFNG* | Pre-existing immunity |
| *CXCL9* | |




*Higgs et al. Clin Cancer Res 2018; Kowanetz et al. ESMO 2017*

# Infiltration immunitaire intratumorale

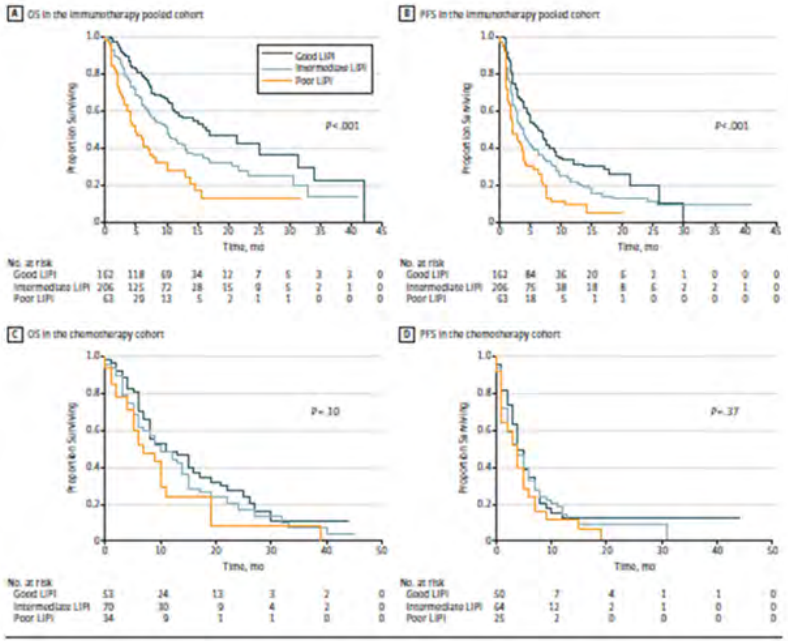
- Beaucoup de technique différentes :
  - Identifie-t-on les même tumeurs ?
- Validation clinique restreinte (analyses exploratoires)
- Limitations :
  - Hétérogénéité tumorale et représentativité des prélèvements
  - Valeur pronostique
  - Corrélation aux paramètres moléculaires
- Données complexes (nombre de paramètre, paramètres spatiaux) : champ d'application pour l'IA+++

# Marqueurs sanguins ? Paramètres biologiques

**LIPI score**: Derived neutrophils/(leukocytes minus neutrophils) ratio (dNLR) and lactate dehydrogenase (LDH) levels

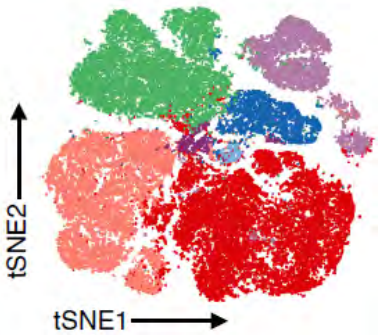
Immunothérapie

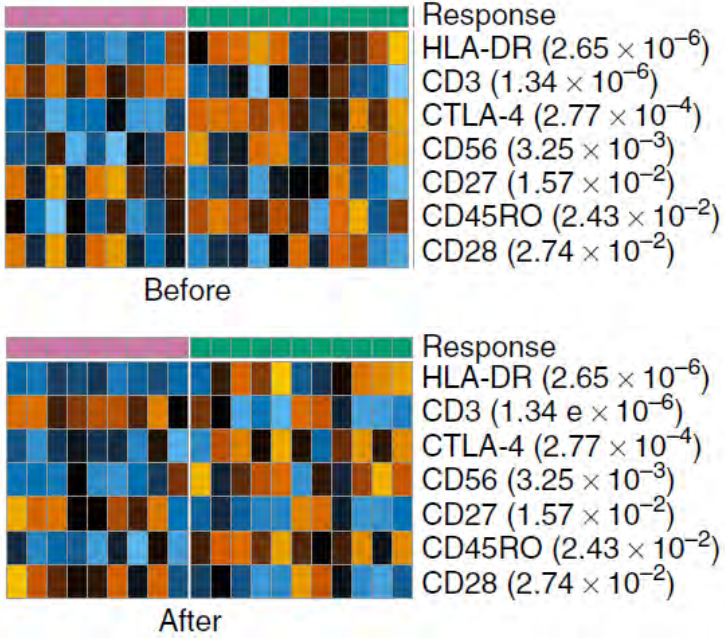
Chimiothérapie

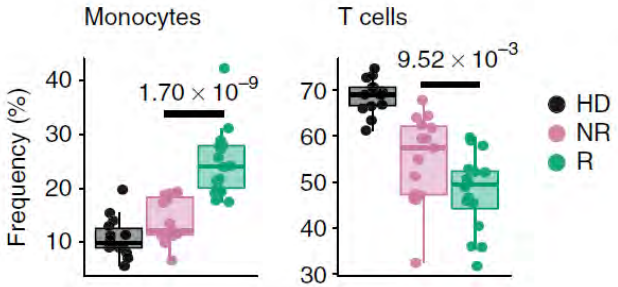



*Mezquita L, JAMA Oncol 2018*

# Marqueurs sanguins ? Cellules circulantes

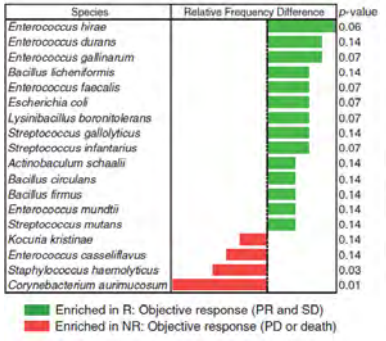


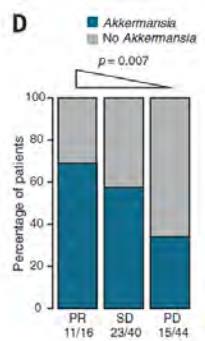



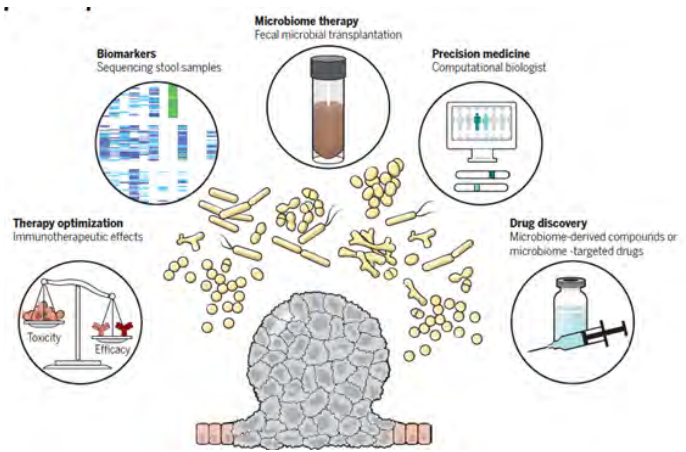



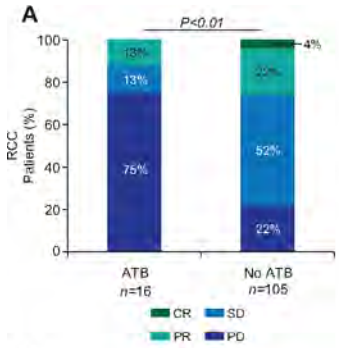

*Krieg C et al., Nat Med 2018*

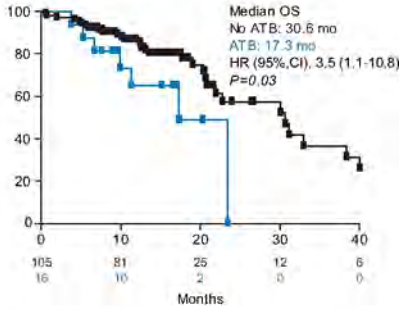
# Microbiote intestinal

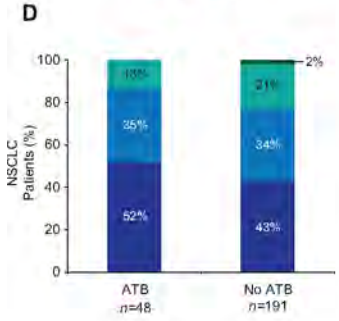


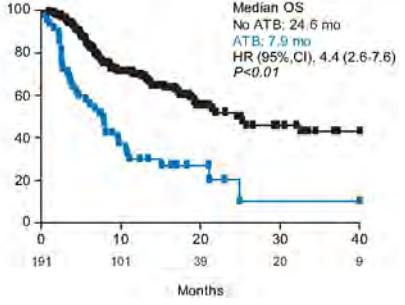



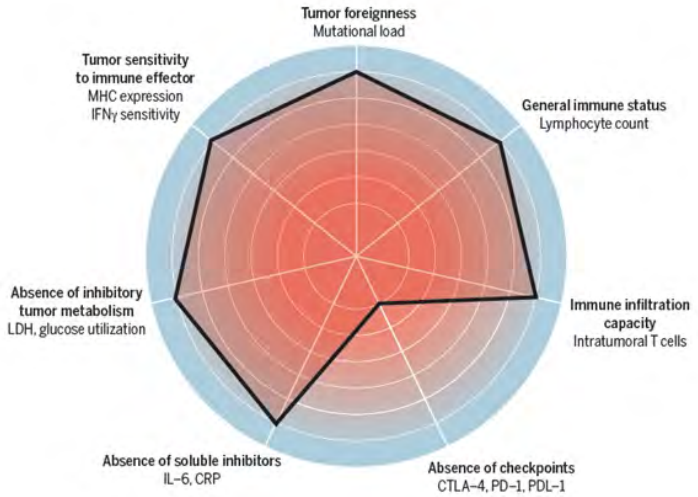



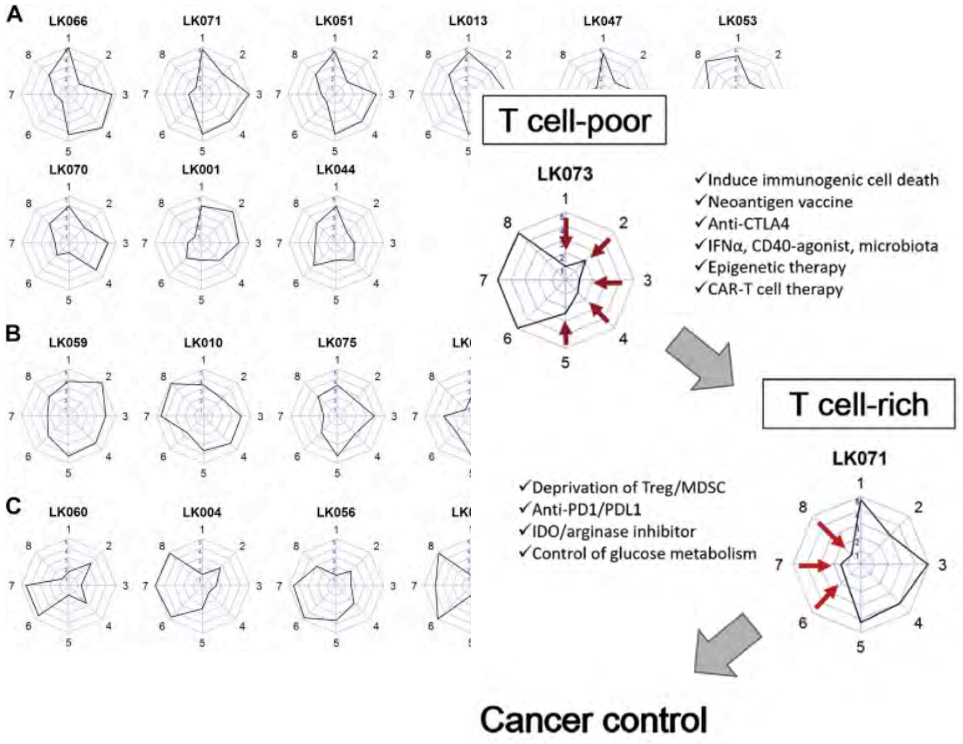

*Routy et al Science 2018; De Rosa et al Ann Oncol 2018; Zitvogel Nat Med 2018*

# Combiner les biomarqueurs ?

« Cancer immunogram »

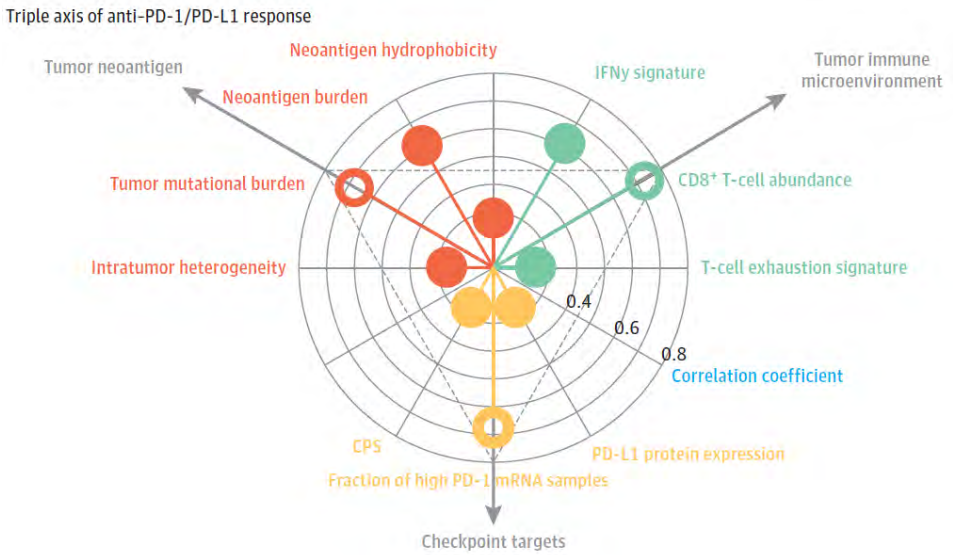


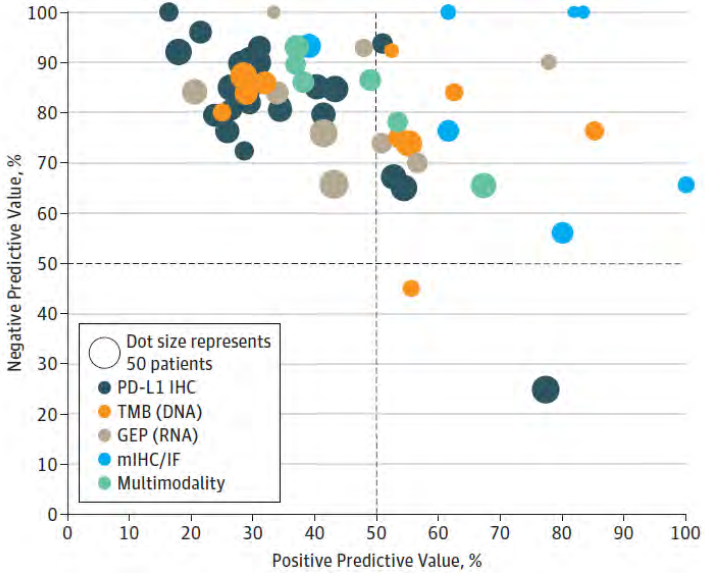

*Blank et al. Science 2016*




*Karasaki et al., J Thor Oncol2017*

# Combiner les biomarqueurs ?

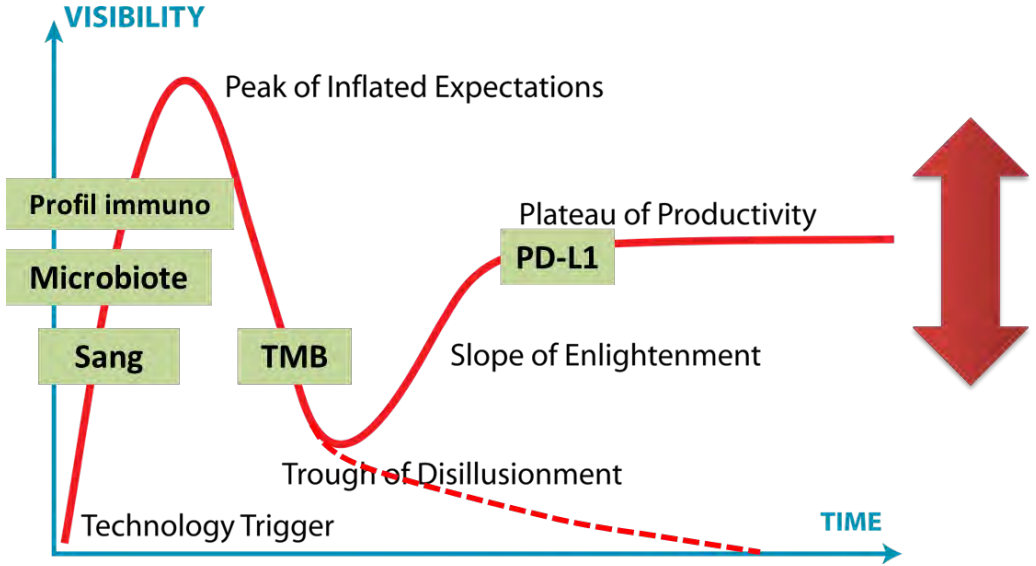


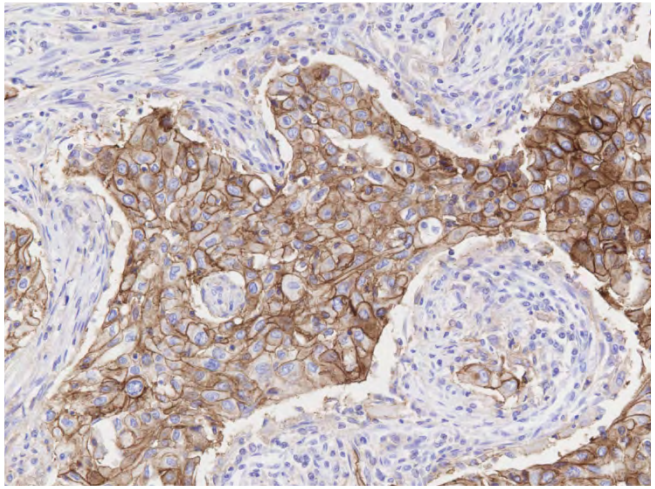

*Lee et al. JAMA Oncol 2019 (a,b)*

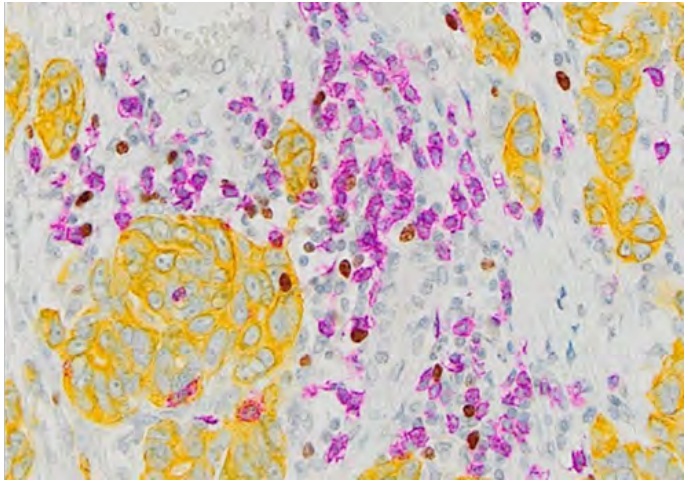
# Conclusions

- Biomarqueurs imparfaits
- Intégration des données
- Analyses exploratoires ⇨ validation prospective



GUSTAVE ROUSSY
CANCER CAMPUS
GRAND PARIS





# Merci pour votre attention

*julien.adam@gustaveroussy.fr*