

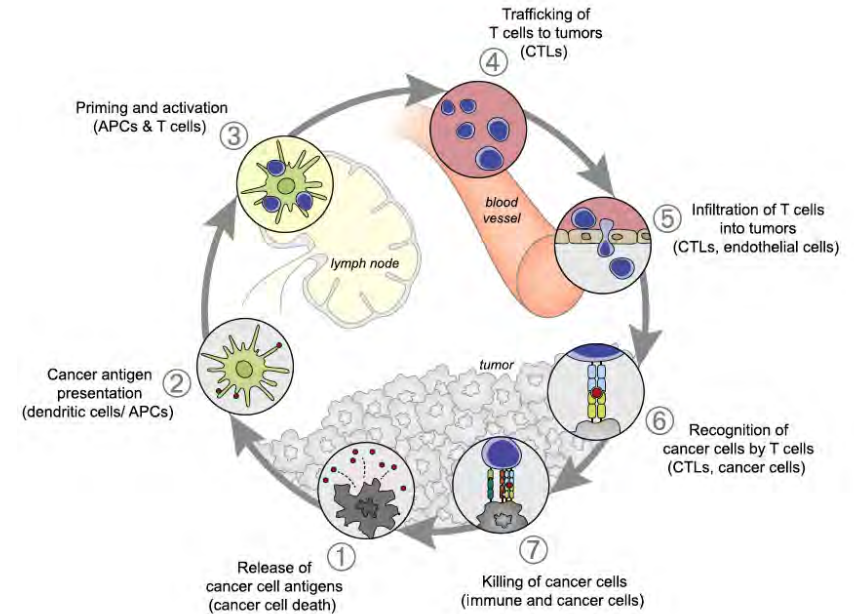
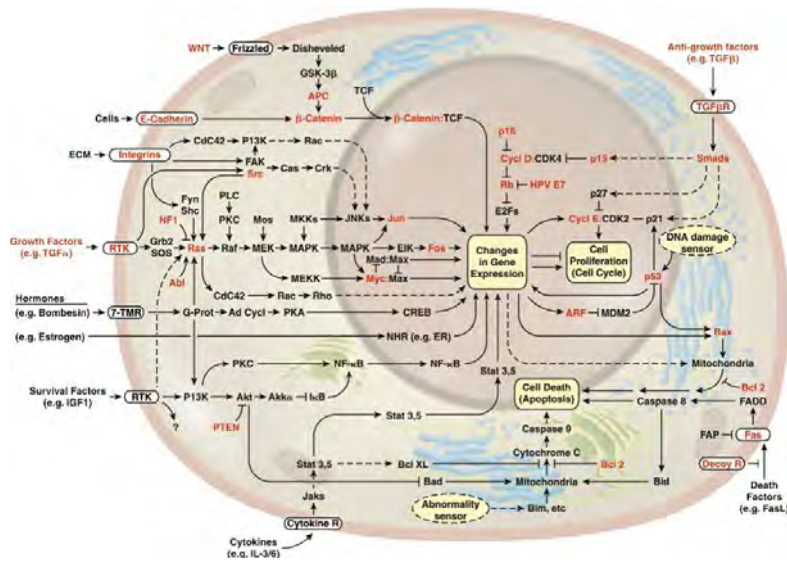
Les marqueurs prédictifs en immunothérapie : des AMM conditionnées aux marqueurs en développement



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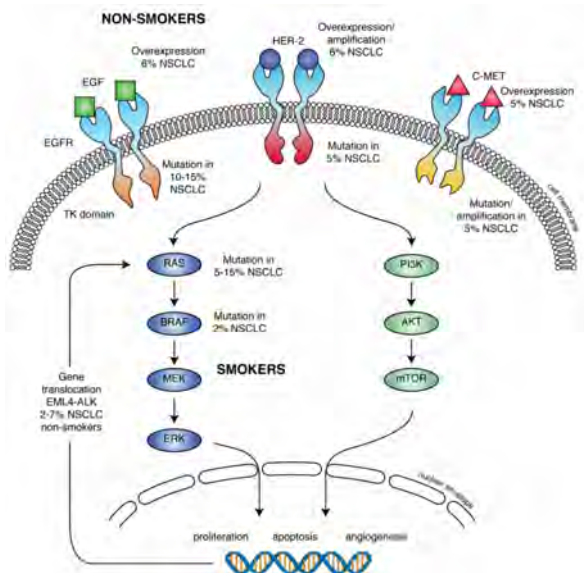
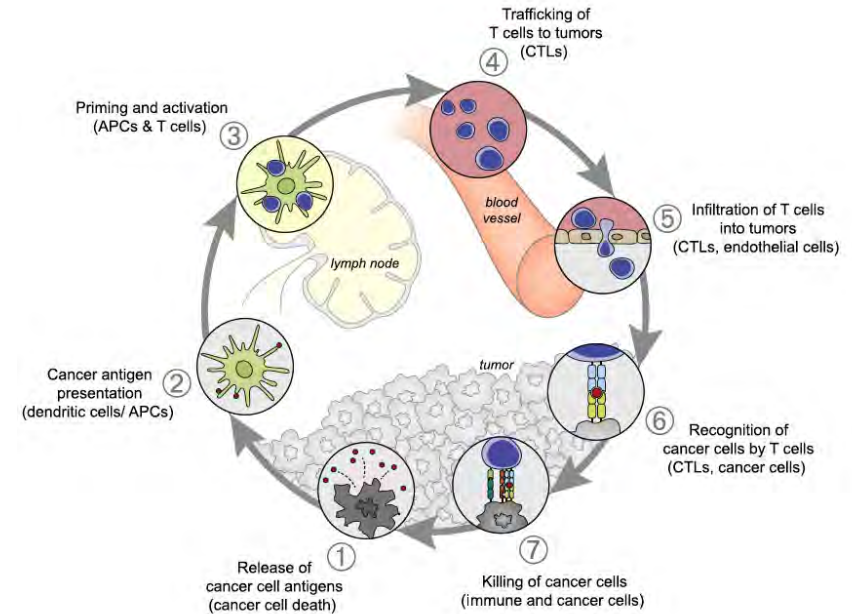
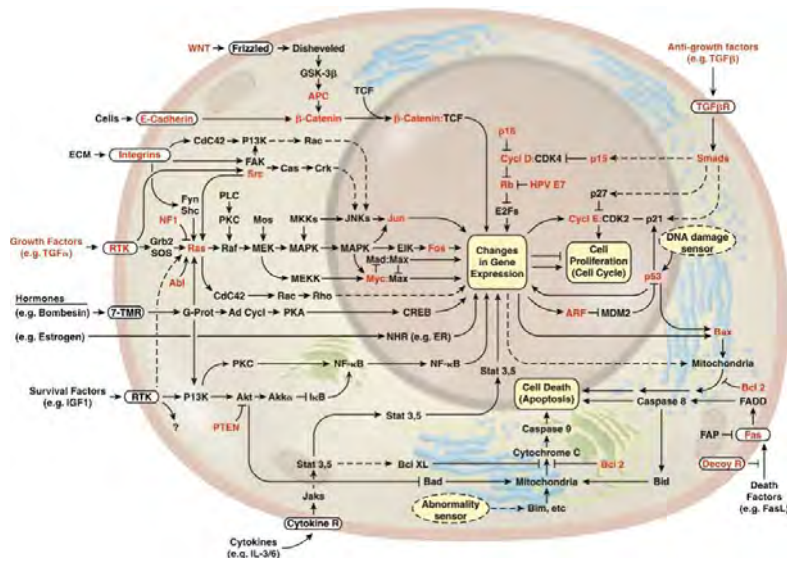
Quelques idées reçues

L'immunologie est trop compliquée pour trouver un biomarqueur



Quelques idées reçues

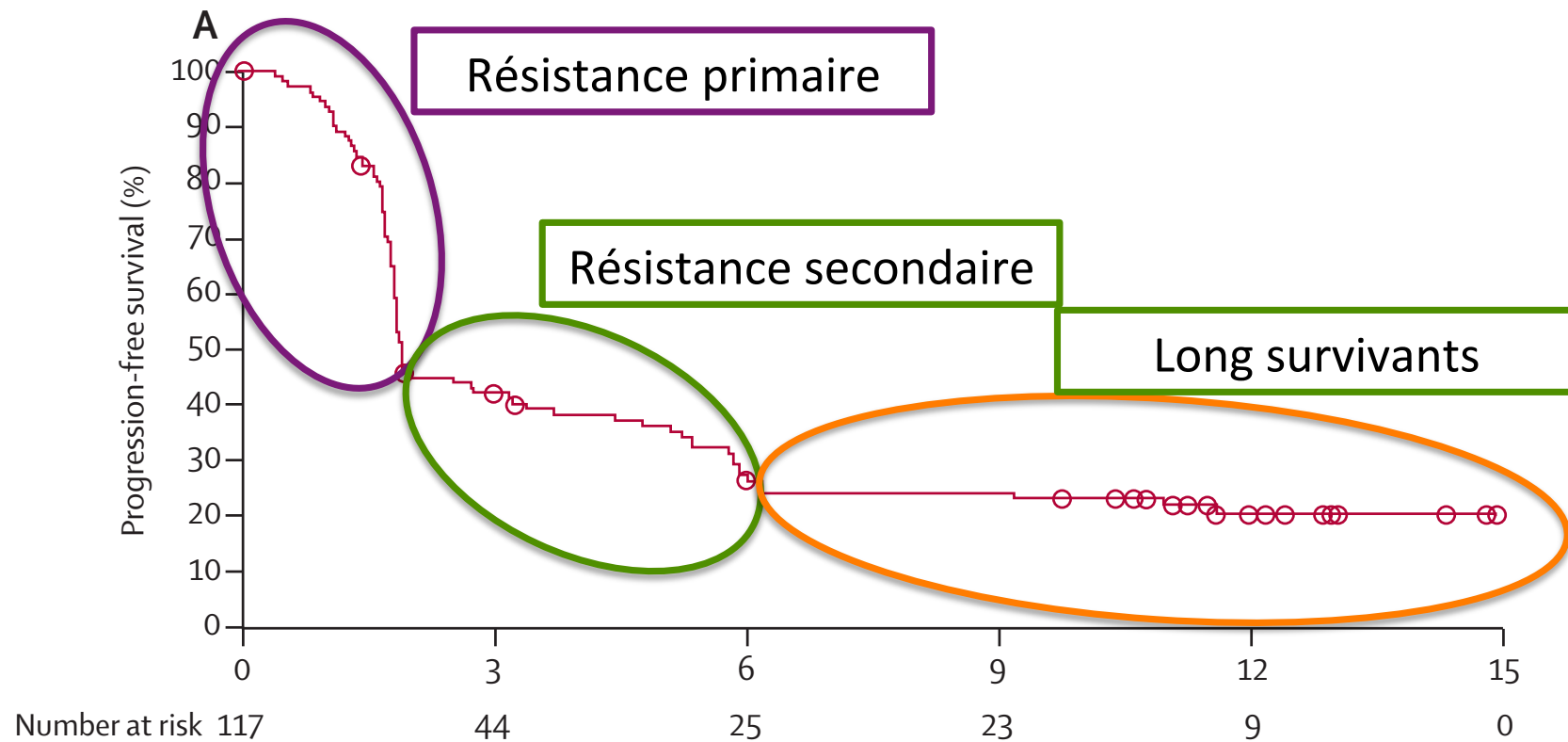
L'immunologie est trop compliquée pour trouver un biomarqueur



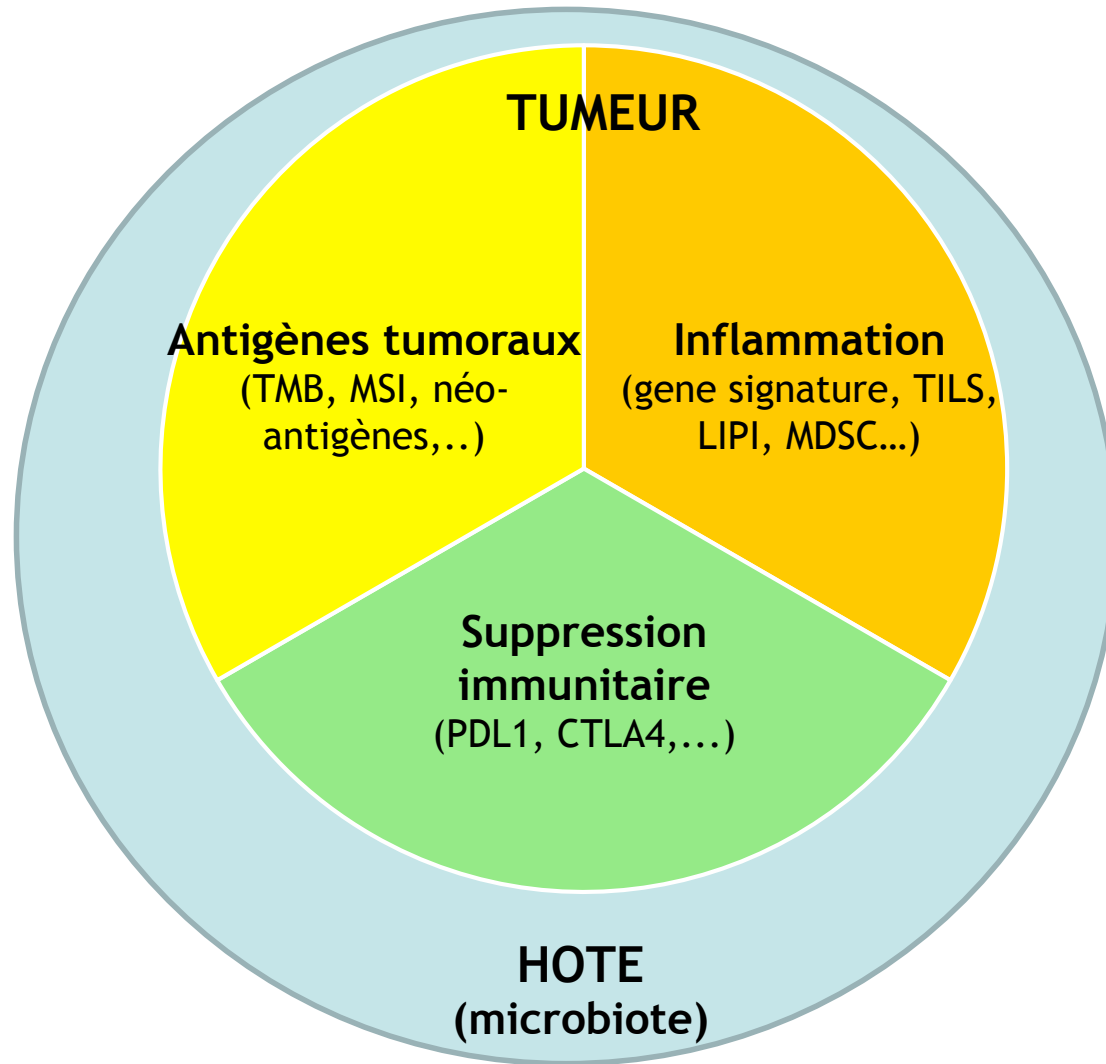
Immunothérapie en oncologie thoracique

Comment mieux faire ?

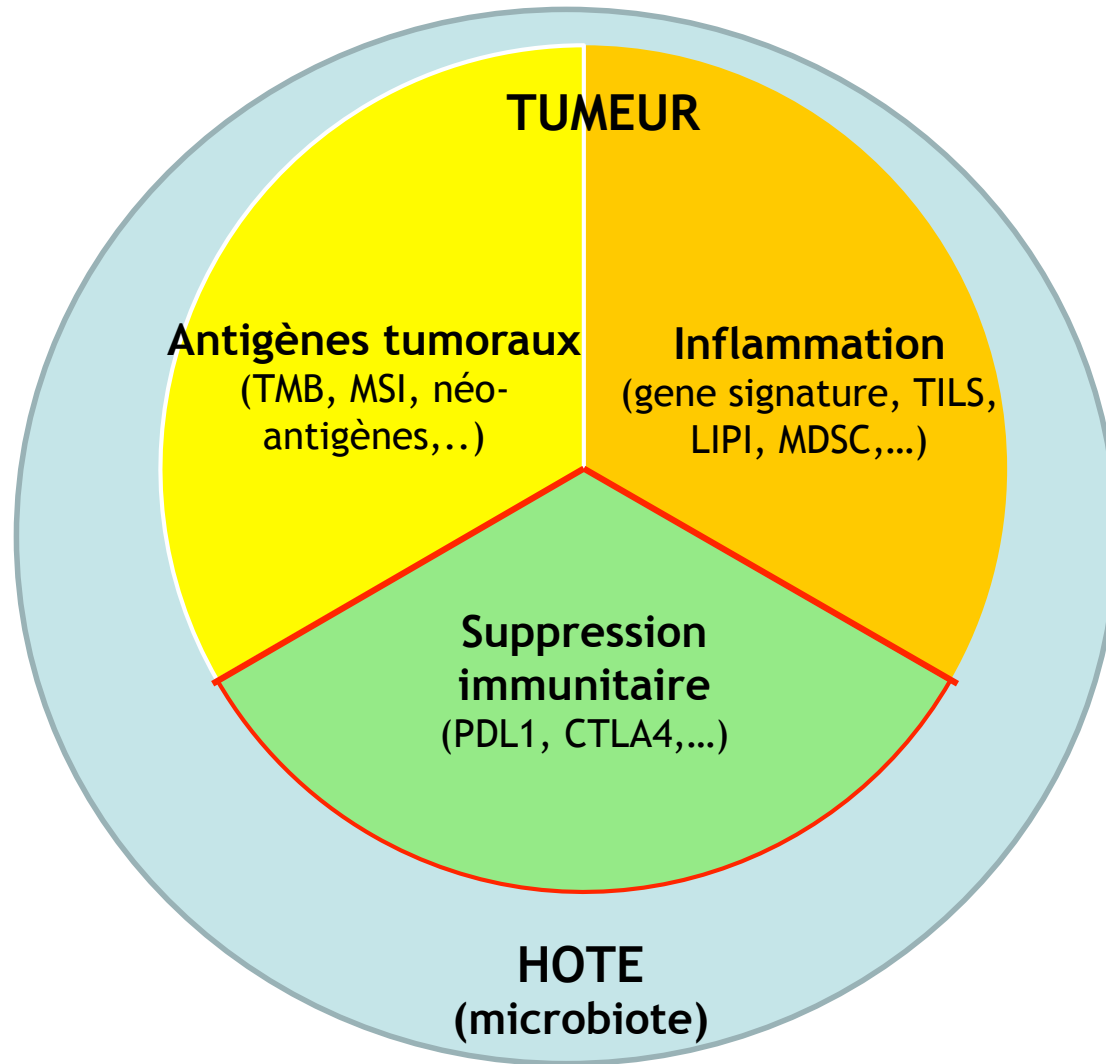
Etude CHECKMATE 063 (nivolumab epidermoïdes prétraités)



Les biomarqueurs en immunothérapie

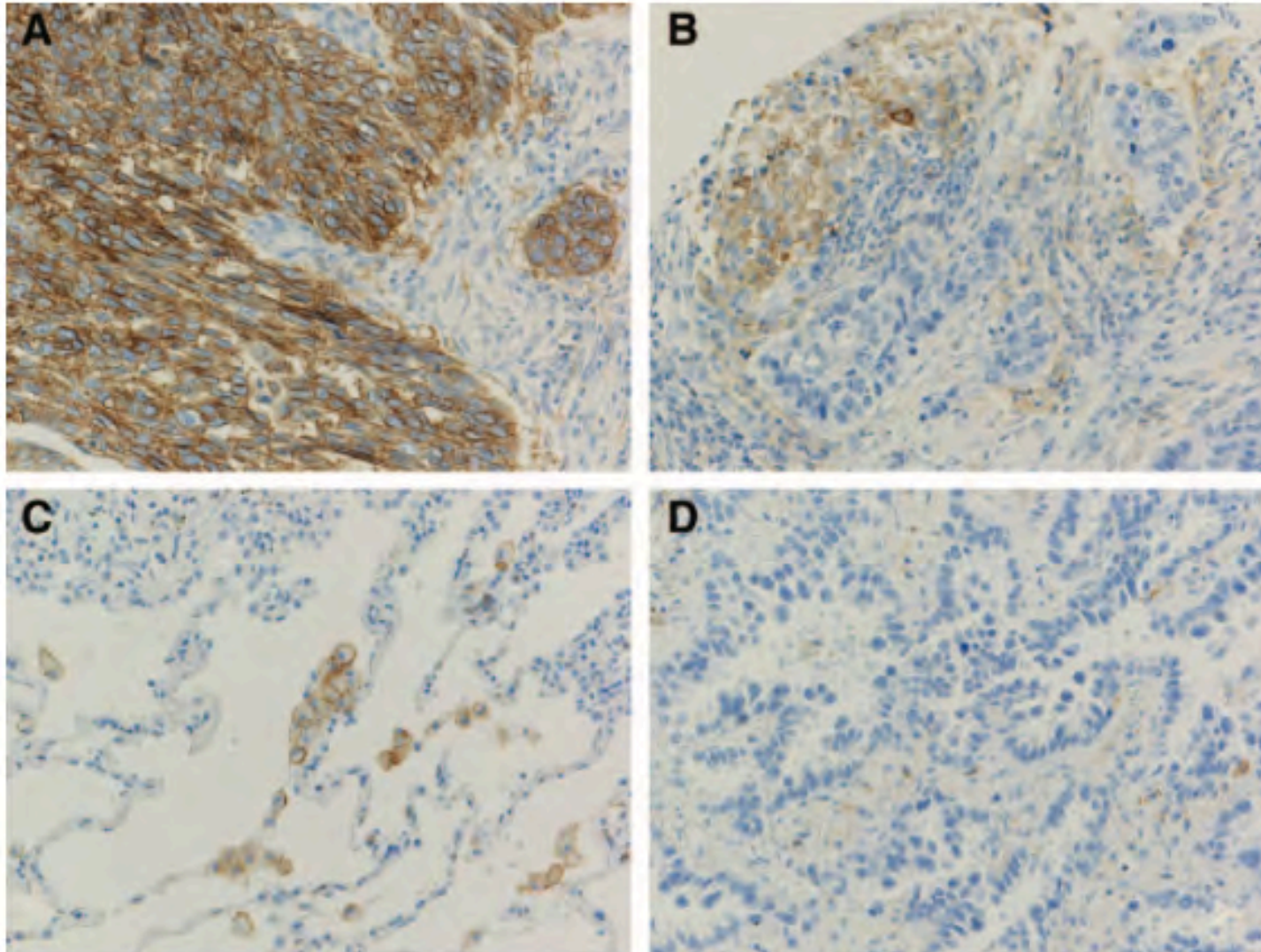


Les biomarqueurs en immunothérapie

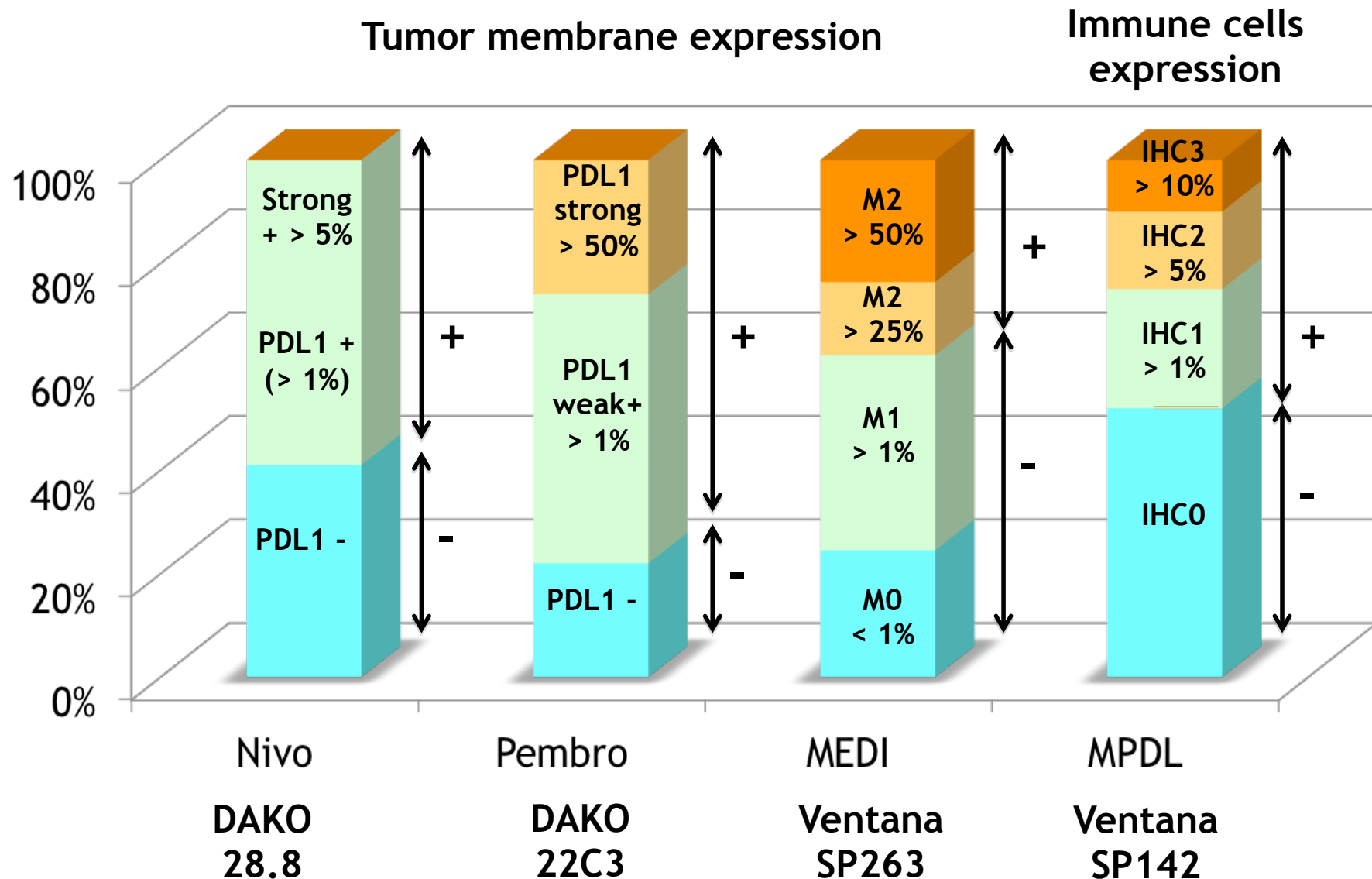


Biomarqueurs : expression de PD-L1

- Expression de PDL1 dans le CBNPC

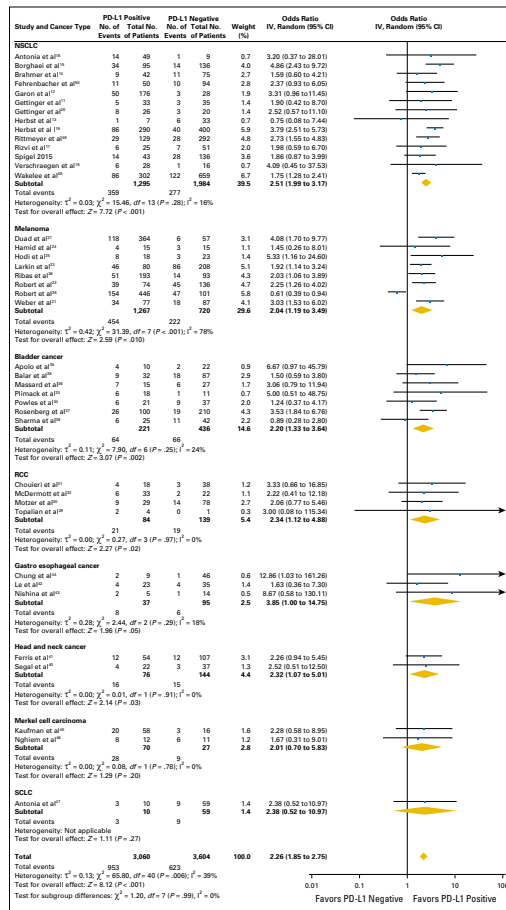


Biomarqueurs : expression de PD-L1



Biomarqueurs : expression de PD-L1







- Expression de PDL1 dans les tumeurs solides et réponses aux ICI : méta-analyse



| Study and Cancer Type | PD-L1 Positive No. of Events of Patients | Total No. | PD-L1 Negative No. of Events of Patients | Total No. | Weight (%) | Odds Ratio IV, Random (95% CI) | Odds Ratio IV, Random (95% CI) |
|---|--|-----------|--|-----------|---------------|-----------------------------------|-----------------------------------|
| NSCLC | | | | | | | |
| Antonia et al ¹⁶ | 14 | 49 | 1 | 9 | 0.7 | 3.20 (0.37 to 28.01) | |
| Borghaei et al ¹⁵ | 34 | 95 | 14 | 136 | 4.0 | 4.86 (2.43 to 9.72) | |
| Fehrenbacher et al ¹⁰ | 9 | 42 | 11 | 75 | 2.7 | 1.59 (0.60 to 4.21) | |
| Garon et al ¹² | 50 | 176 | 3 | 28 | 1.9 | 3.31 (0.96 to 11.45) | |
| Gettinger et al ¹¹ | 5 | 33 | 3 | 35 | 1.4 | 1.90 (0.42 to 8.70) | |
| Herbst et al ¹⁹ | 86 | 290 | 40 | 400 | 5.9 | 3.79 (2.51 to 5.73) | |
| Rittmeyer et al ⁴⁹ | 29 | 129 | 28 | 292 | 4.8 | 2.73 (1.55 to 4.83) | |
| Rizvi et al ¹⁷ | 6 | 25 | 7 | 51 | 2.0 | 1.98 (0.59 to 6.70) | |
| Spigel 2015 | 14 | 43 | 28 | 136 | 3.6 | 1.86 (0.87 to 3.99) | |
| Verschraegen et al ¹⁸ | 6 | 28 | 1 | 16 | 0.7 | 4.09 (0.45 to 37.53) | |
| Wakelee et al ⁴⁸ | 86 | 302 | 122 | 659 | 6.7 | 1.75 (1.28 to 2.41) | |
| Subtotal | 1,295 | | 1,984 | | 39.5 | 2.51 (1.99 to 3.17) | |
| Total events | 359 | | 277 | | | | |
| Heterogeneity: $\tau^2 = 0.03$; $\chi^2 = 15.46$, $df = 13$ ($P = .28$); $I^2 = 16\%$ | | | | | | | |
| Test for overall effect: $Z = 7.72$ ($P < .001$) | | | | | | | |

Biomarqueurs : expression de PD-L1

PD-L1 and evidence-based thresholds to use

| Immunotherapy (IO) | Nivolumab | Pembrolizumab | Durvalumab | Avelumab | Atezolizumab |
|--|---|--|---|----------|--|
| Detection antibody | 28-8 | 22C3 | SP263 | 73-10 | SP142 |
| IHC platform | Dako | Dako | Ventana | Dako | Ventana |
| Cell types scored for NSCLC | TC | TC | TC | TC | TC & IC |
| Cut-off definitions for positivity (complementary vs companion) | >5% | First line: PD-L1+ ≥50% Late lines: PD-L1+ ≥1% | >25% | None | None |
| Estimated PD-L1 biomarker positivity in 2 nd line at registration level |  |  | | |  |
| Estimated PD-L1 biomarker positivity in 1 st line at registration level |  |  |  | | |

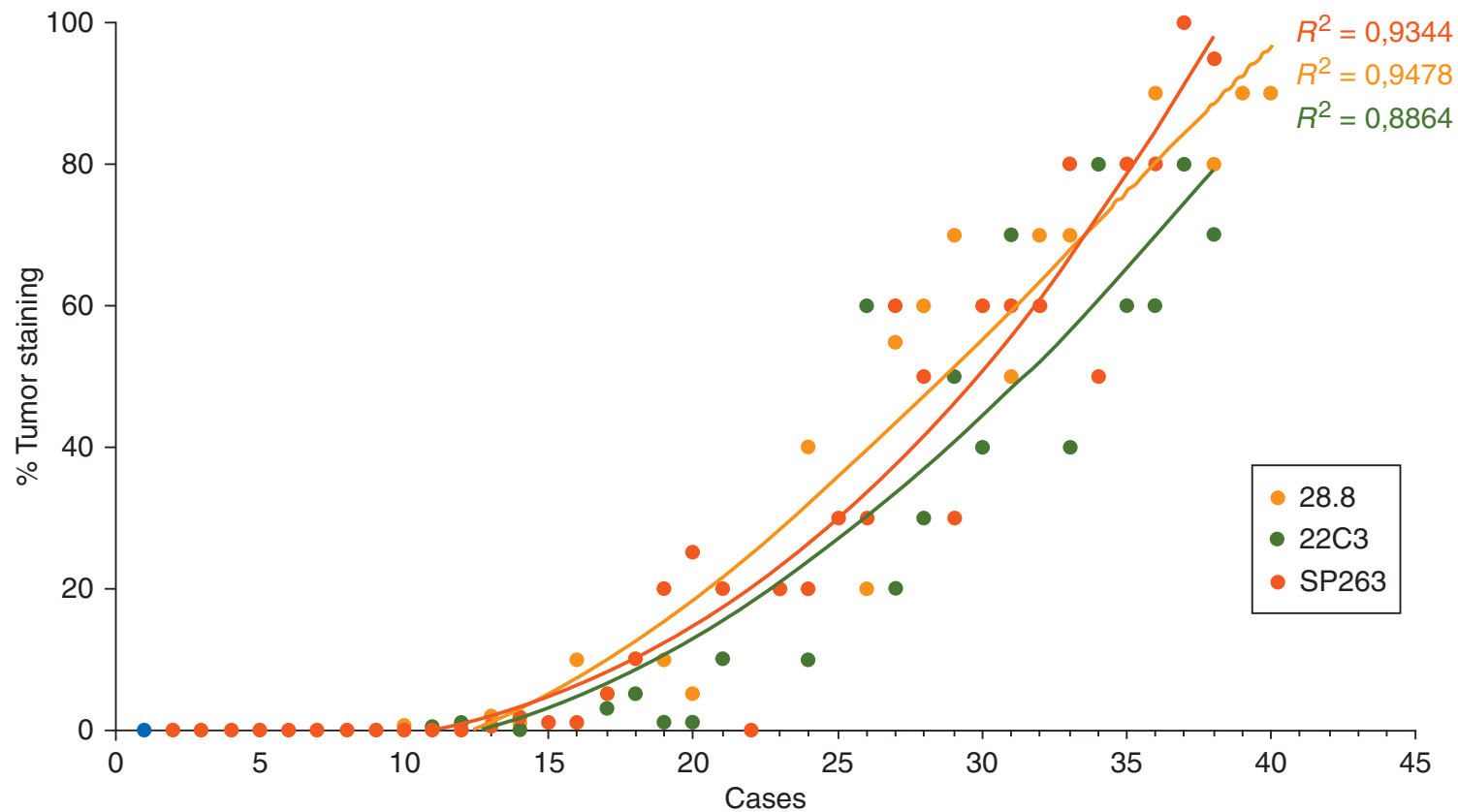
Biomarqueurs : expression de PD-L1

- Harmonisation du test PDL1 dans différents centres avec différents tests.
- Les tests 28-8, 22C3 et SP263 ont des performances proches.
- Possibilité de développer des tests locaux.

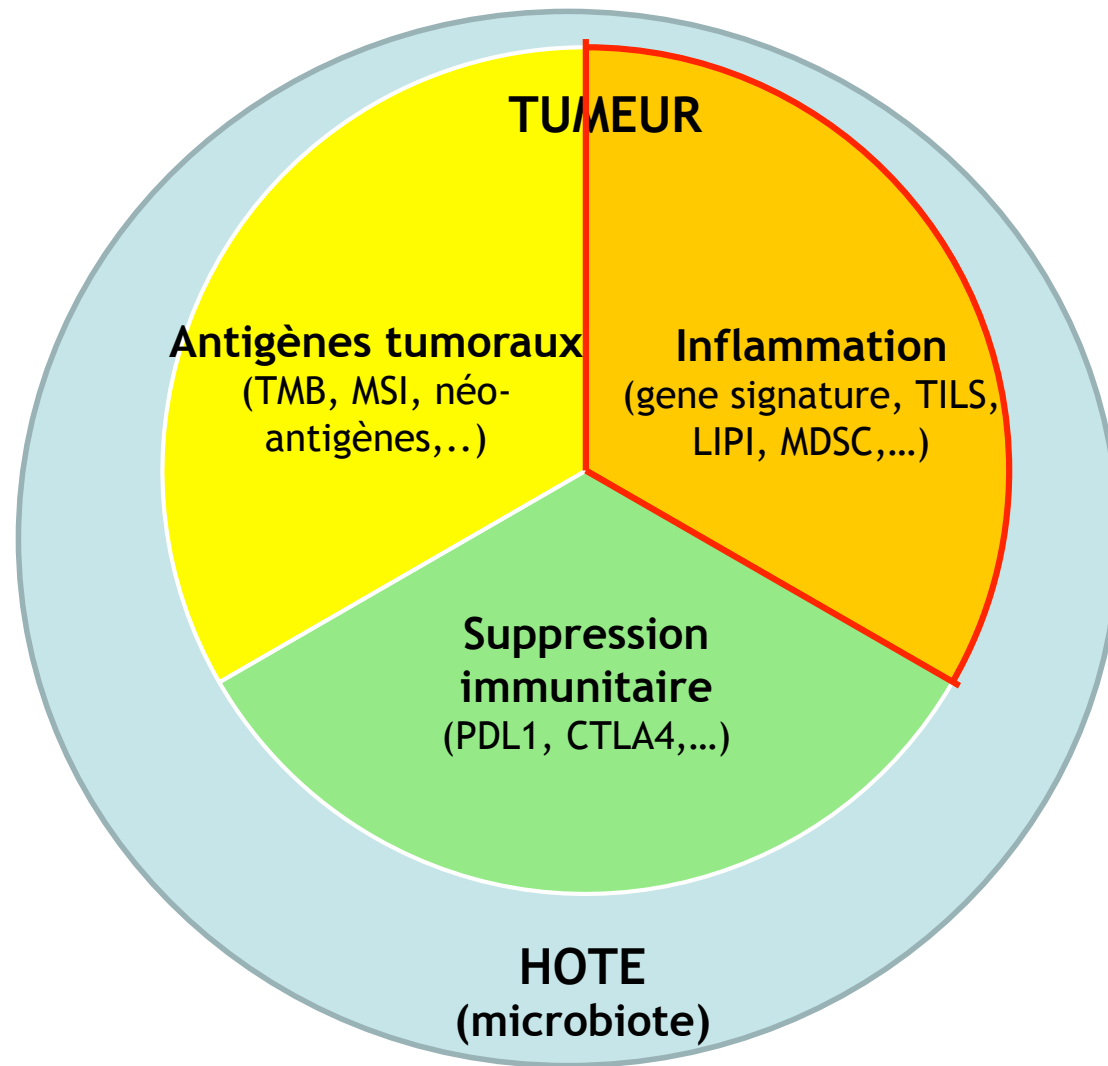
| | Dako | | | Ventana | | Leica | | |
|-------|-------------|----------|----------|----------|-------------|----------|----------|------------------|
| | Center 1 | Center 2 | Center 3 | Center 4 | Center 5 | Center 6 | Center 7 | Reference |
| 28-8 | <i>Ref.</i> | 0.94 | 0.79 | 0.8 | 0.73 | 0.6 | 0.58 | 28-8 (center 1) |
| 22C3 | <i>Ref.</i> | 0.91 | 0.82 | 0.81 | 0.77 | 0.5 | 0.62 | 22C3 (center 1) |
| SP263 | 0.83 | 0.83 | 0.86 | 0.81 | <i>Ref.</i> | 0.83 | 0.86 | SP263 (center 5) |
| SP142 | 0.68 | 0.38 | 0.61 | 0.43 | 0.45 | 0.78 | 0.81 | SP263 (center 5) |
| E1L3N | 0.63 | 0.65 | 0.77 | 0.6 | 0.81 | 0.75 | 0.78 | SP263 (center 5) |

Biomarqueurs : expression de PD-L1

- Harmonisation du test PDL1 dans différents centres avec différents tests.

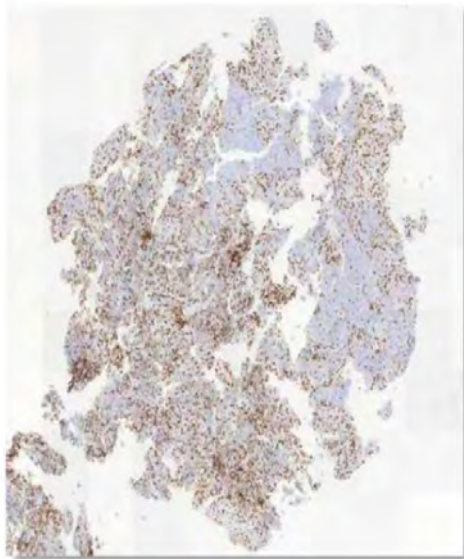


Les biomarqueurs en immunothérapie



Microenvironnement

Les trois phénotypes histologiques observés dans les cancers



INFLAMMATOIRE
Cellules-T CD8+
infiltrées mais non-
fonctionnelles



EXCLUSION IMMUNE
Cellules-T CD8+
accumulées mais n'ont
pas infiltré la tumeur

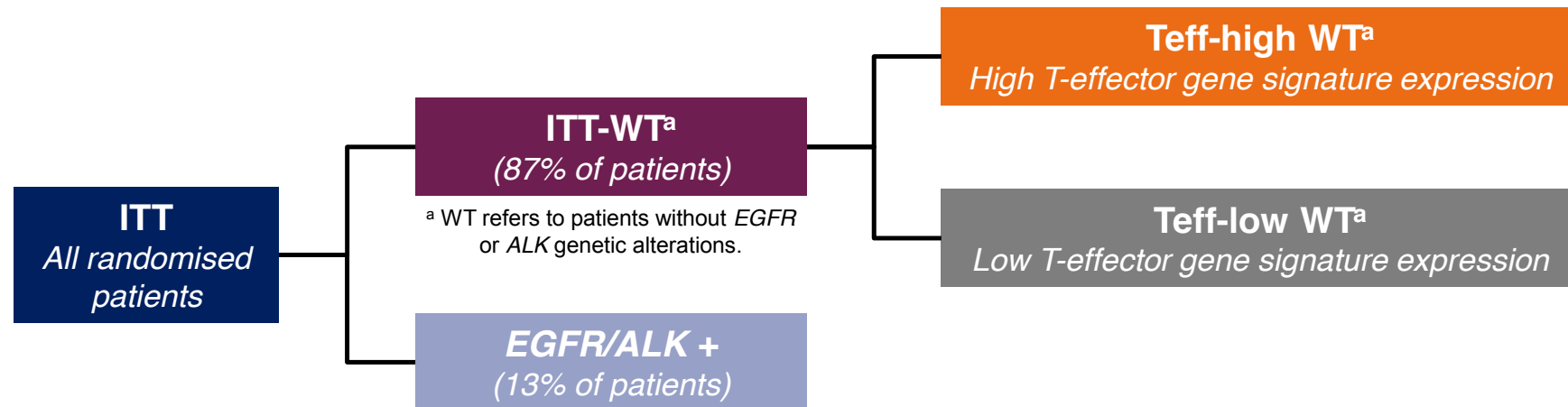


DESERT IMMUN
Cellules-T CD8+
absentes de la tumeur
et de sa périphérie

Statut immunologique

- Etude IMPower Chimio + Beva +/- atezo
- Intérêt du Teff: The T-effector (Teff) gene signature is defined by expression of PD-L1, CXCL9 and IFN γ .

IMpower150 study populations and objectives

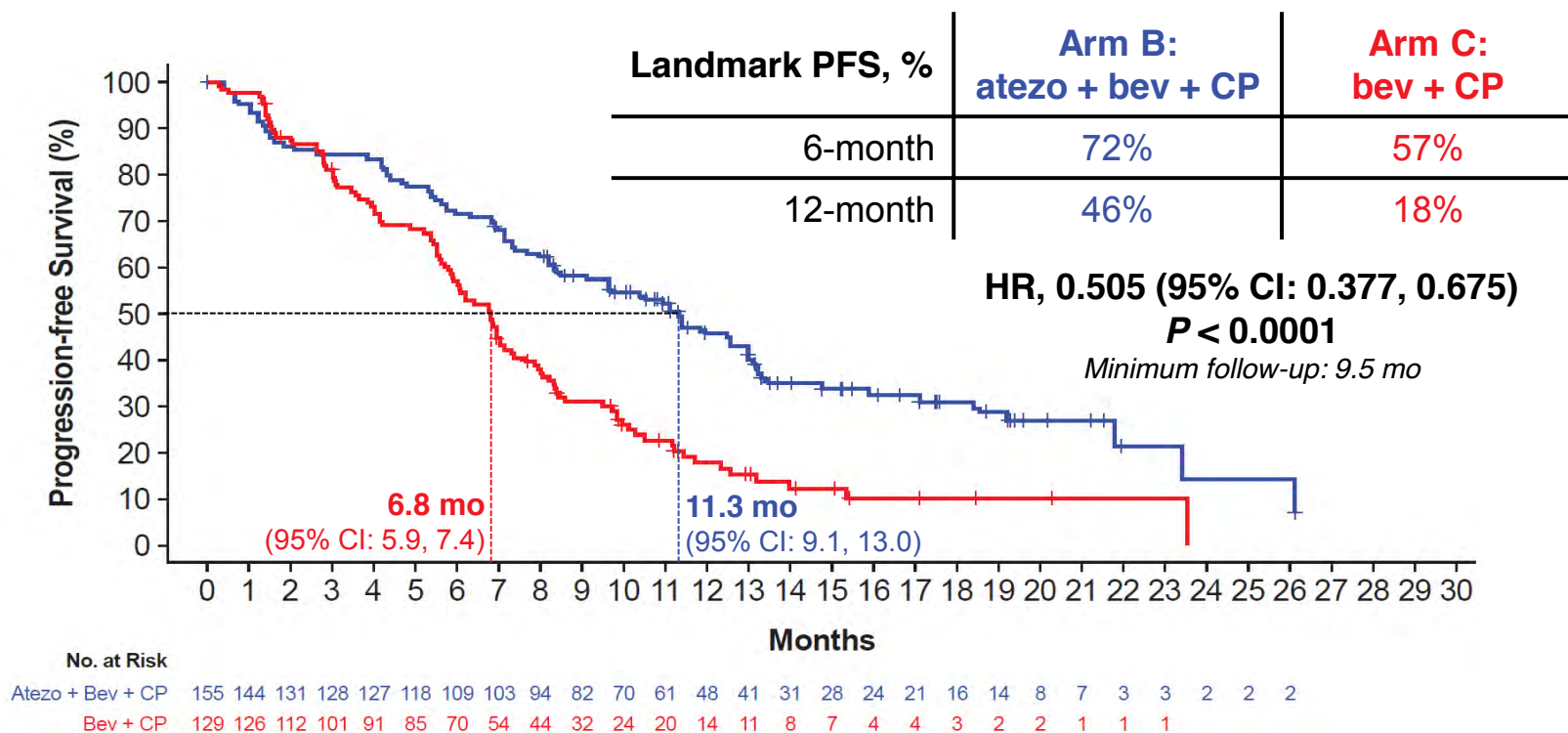


(Fehrenbacher L, Lancet 2016)

Statut immunologique

- Intérêt du Teff: The T-effector (Teff) gene signature is defined by expression of PD-L1, CXCL9 and IFN γ .

INV-assessed PFS in Teff-high WT (Arm B vs Arm C)



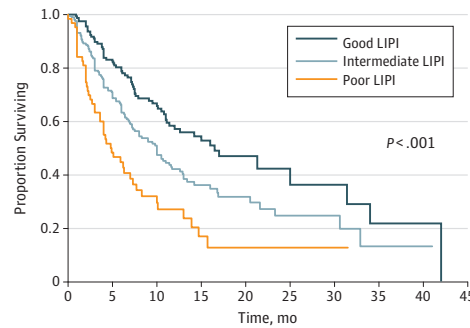
(Fehrenbacher L, Lancet 2016)

Statut immunologique

- LIPI score (Lung Immune Prognostic Index) : dLNR (Leucocytes/Leuco-Neutro et LDH)

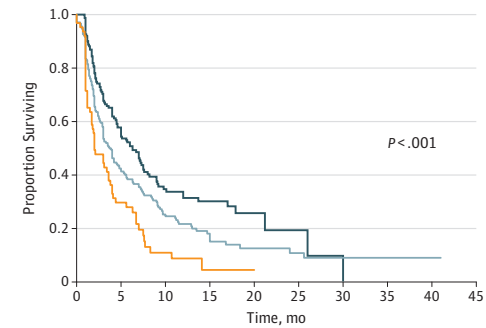
Immunothérapie

A OS in the immunotherapy pooled cohort



| No. at risk | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 |
|-------------------|-----|-----|----|----|----|----|----|----|----|----|
| Good LIPI | 162 | 118 | 69 | 34 | 12 | 7 | 5 | 3 | 3 | 0 |
| Intermediate LIPI | 206 | 125 | 72 | 28 | 15 | 9 | 5 | 2 | 1 | 0 |
| Poor LIPI | 63 | 29 | 13 | 5 | 2 | 1 | 1 | 0 | 0 | 0 |

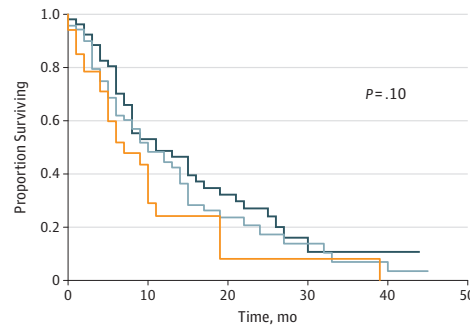
B PFS in the immunotherapy pooled cohort



| No. at risk | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 |
|-------------------|-----|----|----|----|----|----|----|----|----|----|
| Good LIPI | 162 | 84 | 36 | 20 | 6 | 2 | 1 | 0 | 0 | 0 |
| Intermediate LIPI | 206 | 75 | 38 | 18 | 8 | 6 | 2 | 2 | 1 | 0 |
| Poor LIPI | 63 | 18 | 5 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |

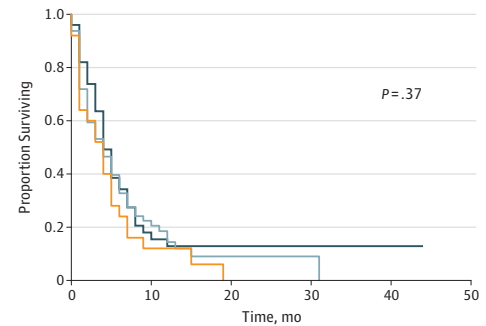
Chimiothérapie

C OS in the chemotherapy cohort



| No. at risk | 0 | 10 | 20 | 30 | 40 | 50 |
|-------------------|----|----|----|----|----|----|
| Good LIPI | 53 | 24 | 13 | 3 | 2 | 0 |
| Intermediate LIPI | 70 | 30 | 9 | 4 | 2 | 0 |
| Poor LIPI | 34 | 9 | 1 | 1 | 0 | 0 |

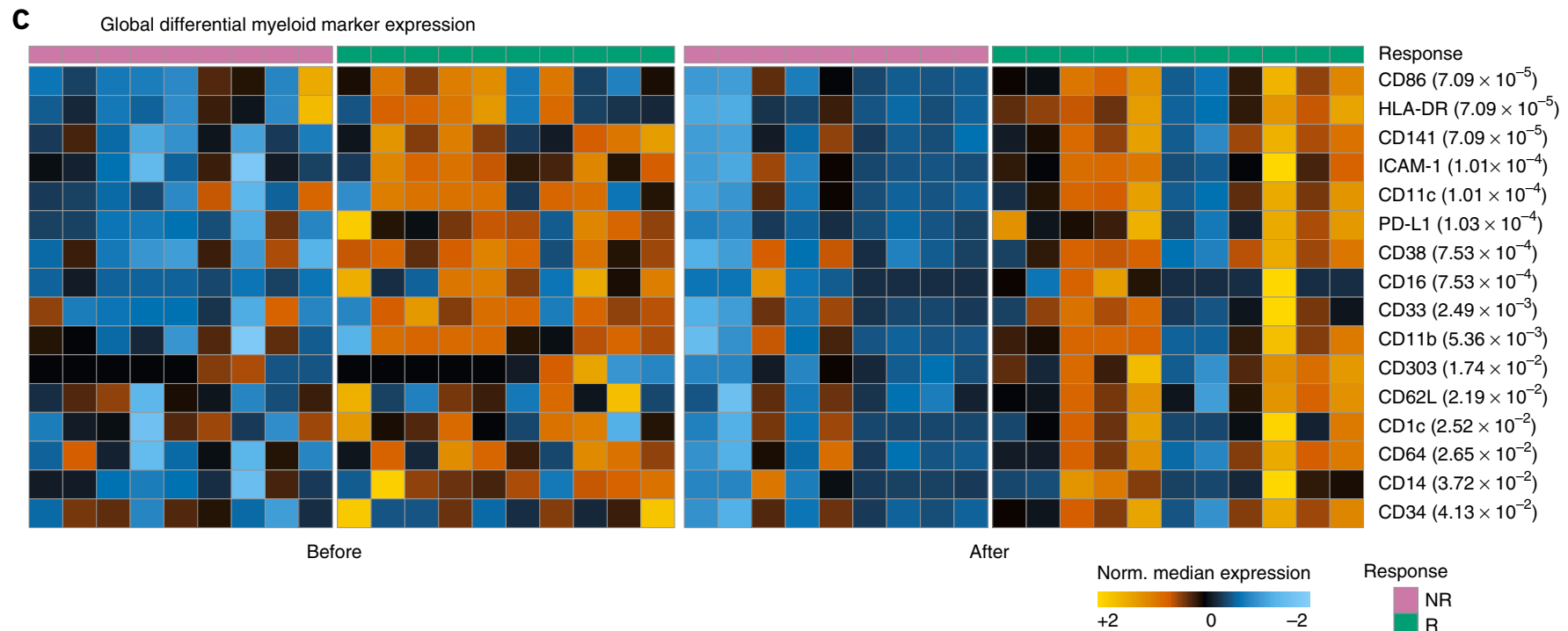
D PFS in the chemotherapy cohort



| No. at risk | 0 | 10 | 20 | 30 | 40 | 50 |
|-------------------|----|----|----|----|----|----|
| Good LIPI | 50 | 7 | 4 | 1 | 1 | 0 |
| Intermediate LIPI | 64 | 12 | 2 | 1 | 0 | 0 |
| Poor LIPI | 25 | 2 | 0 | 0 | 0 | 0 |

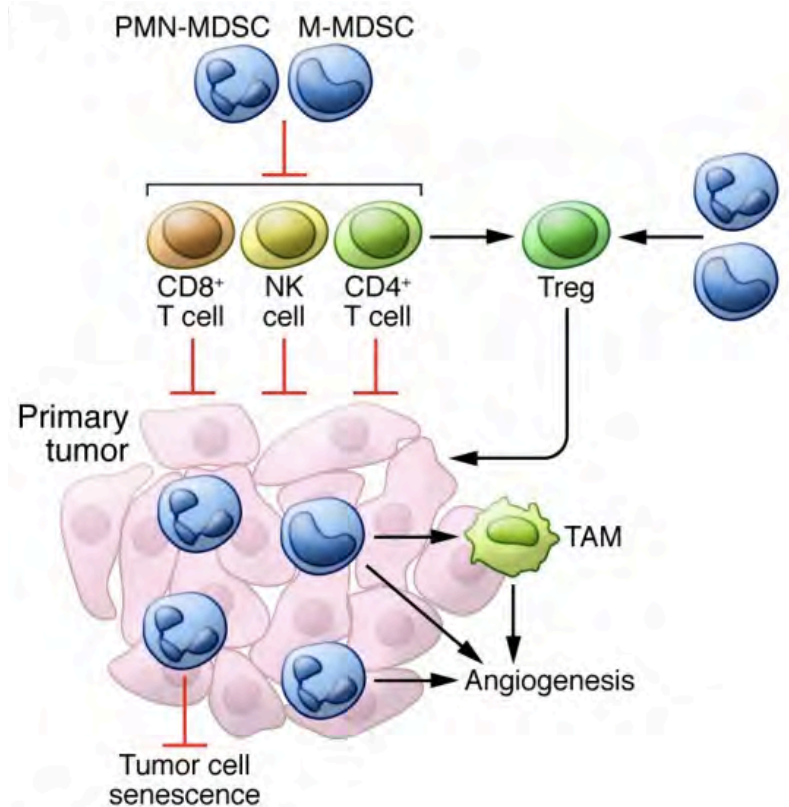
Statut immunologique

- Analyse single-cell et prédiction de la réponse aux anti PD1
- Caractérisation de l'infiltrat immunitaire dans le sang périphérique
- Réponse au niveau du compartiment lymphocytaire

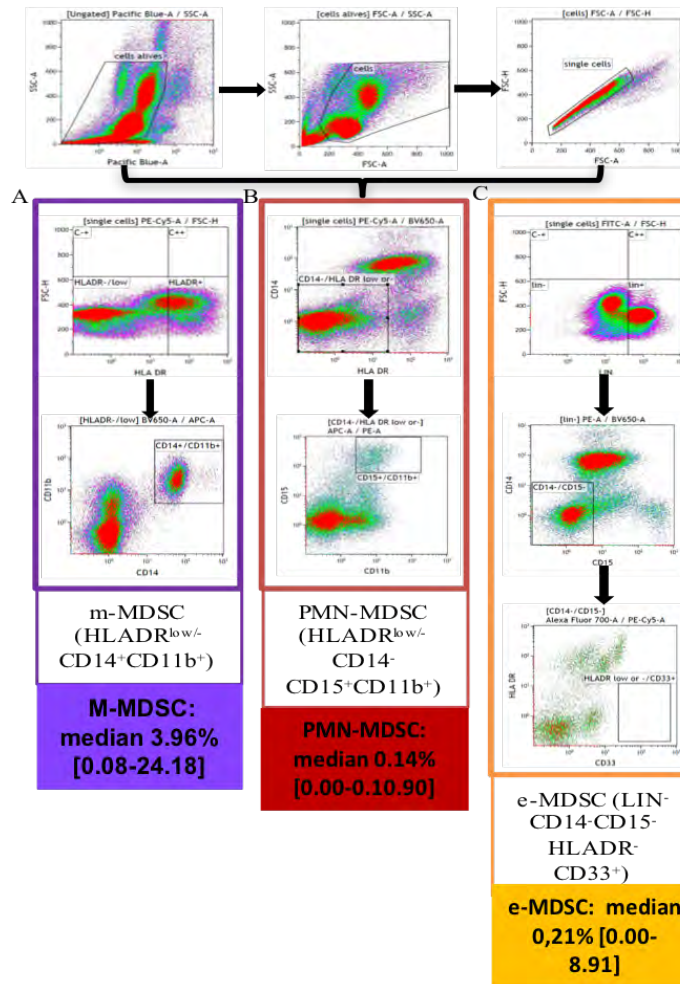


Statut immunologique

- Cellules myéloïdes circulantes: m-MDSC

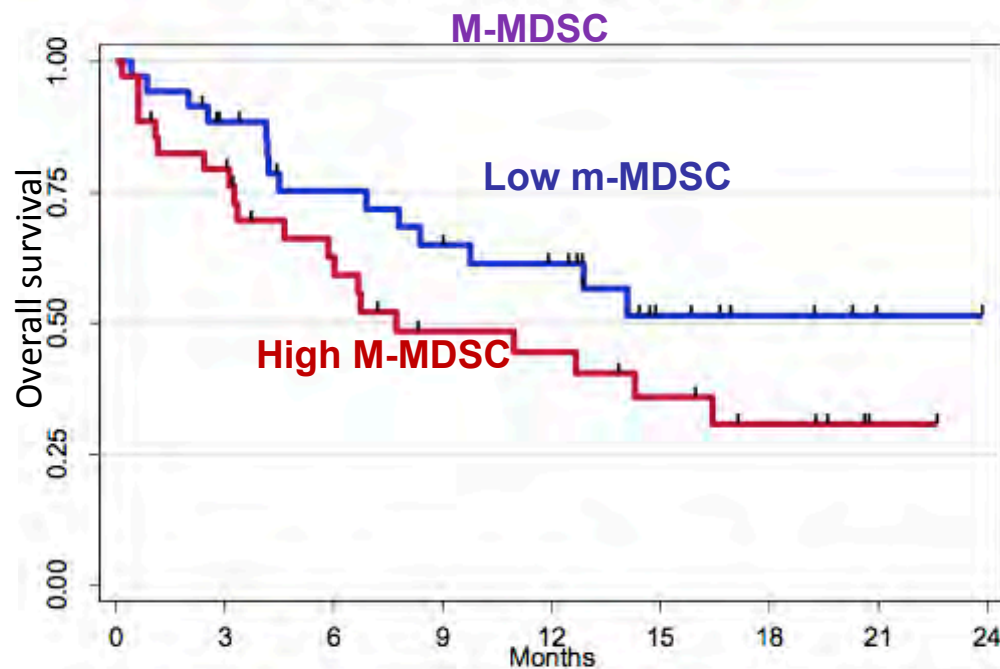


Marvel and Gabilovich, *J clin invest* 2015



Statut immunologique

- Cellules myéloïdes circulantes: m-MDSC
- Faible taux de m-MDSC associé à une meilleure survie des patients traités par immunothérapie



% M-MDSC at baseline (Cox proportional hazards model for continuous variables)

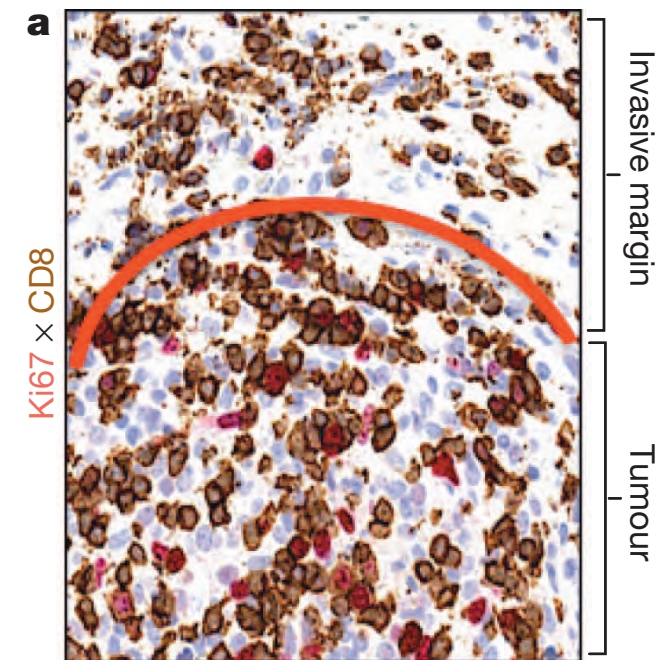
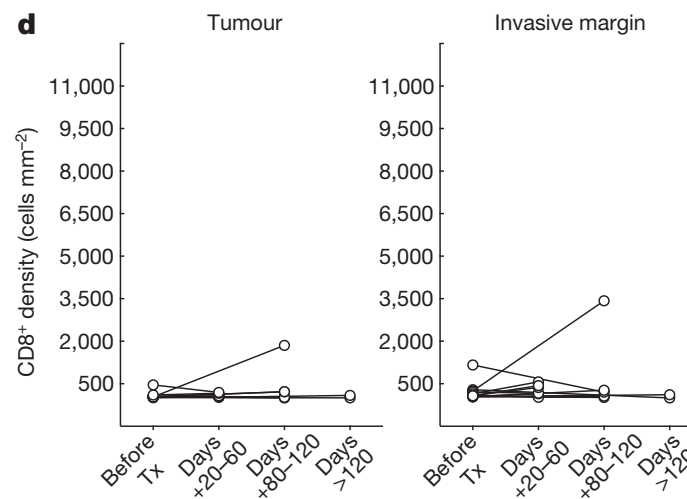
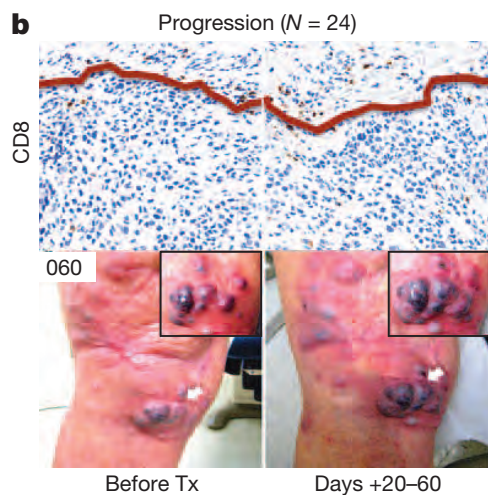
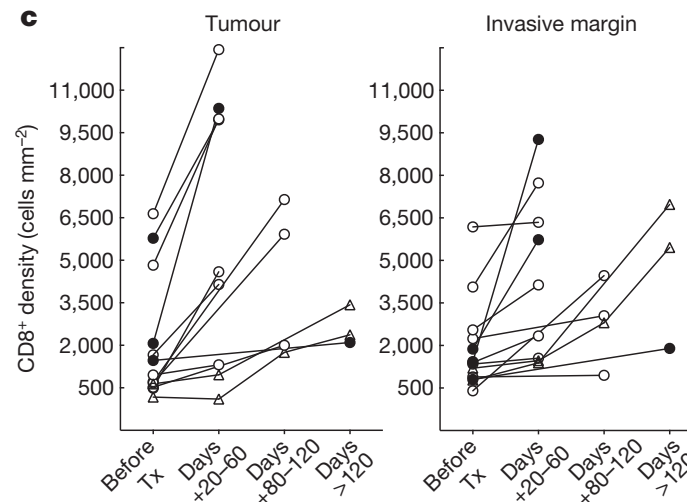
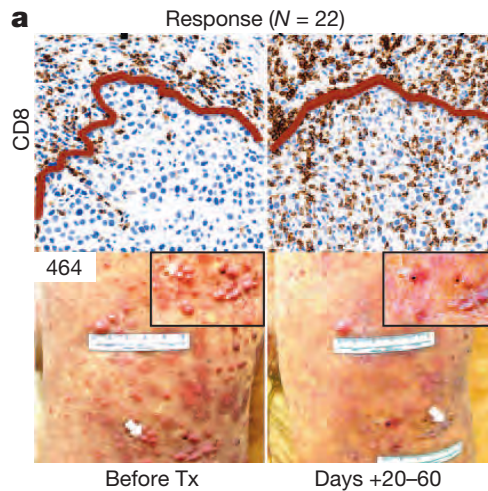
Overall survival HR [95% CI] 1.08 [1.01; 1.14] P=0.02

Progression free-survival HR [95% CI] 0.8 [1.03; 1.15] P= 0.004

Delaunay M, ASCO-SITC 2018

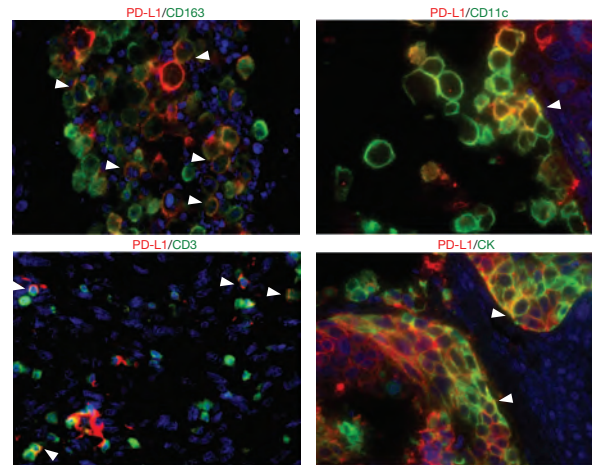
Microenvironnement

- Importance de la présence de lymphocytes CD8 à proximité de la tumeur (Exemple dans le mélanome traité par pembrolizumab)



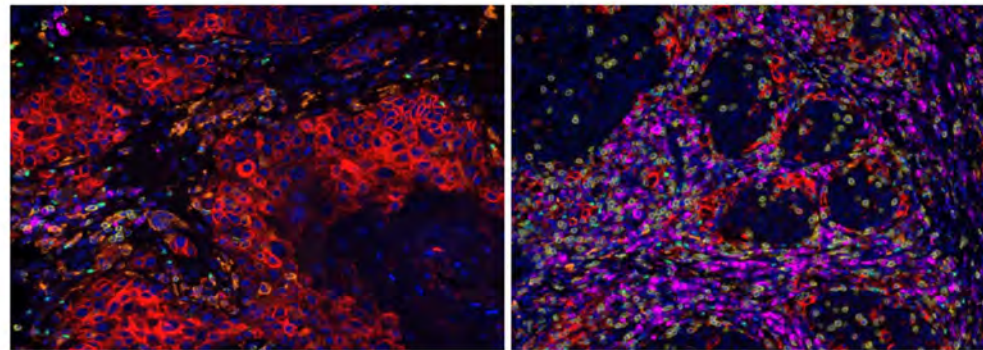
Microenvironnement

- Co-infiltration de cellules immunitaires dans les tumeurs PDL1 +.
(Exemple dans le CBNPC traité par atezolizumab)



- Exemple K ORL

Carcinome malpighien ORL



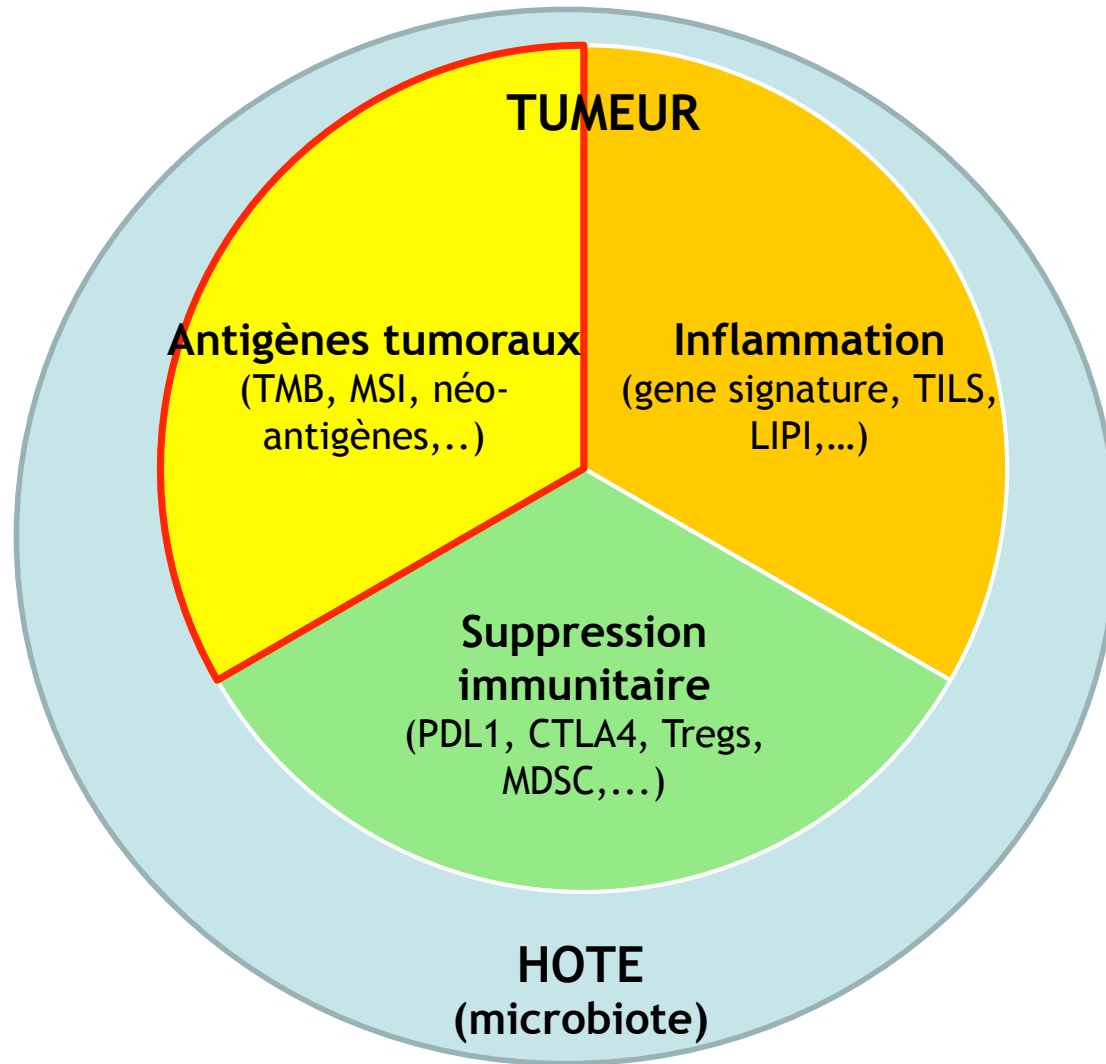
Faible infiltrat lymphocytaire
PD-L1 élevé (rouge)

Fort infiltrat lymphocytaire
PD-L1 bas (rouge), violet

CD8 jaune, FOXP3 vert, CD79a (B) violet, Macrophages CD163 orange

Herbst R, Nature 2014

Les biomarqueurs en immunothérapie



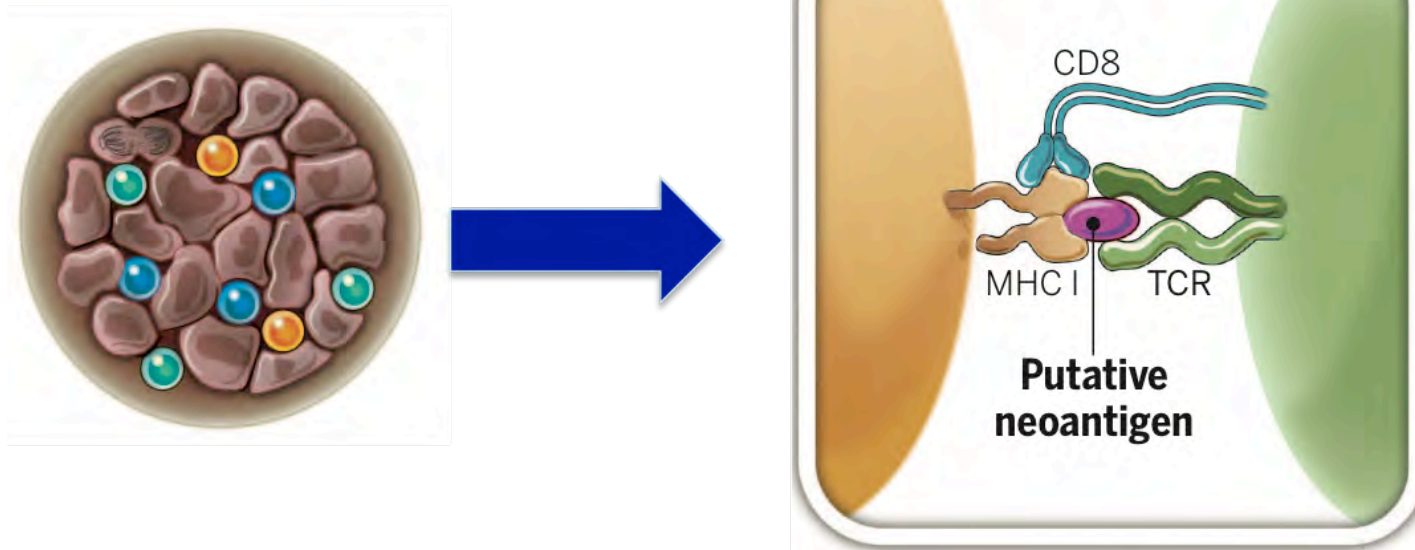
Charge mutationnelle et néo-antigènes

- Les peptides mutés issus de mutations de l'ADN sont reconnus par les lymphocytes T CD8+ et CD4+

*Robbins PF, Nat Med 2013
Linneman C, Nat Med 2014*

- La réponse des lymphocytes T à ces néo-antigènes contribue à la réponse clinique à l'immunothérapie

Schumacher TN, Cancer Cell 2015



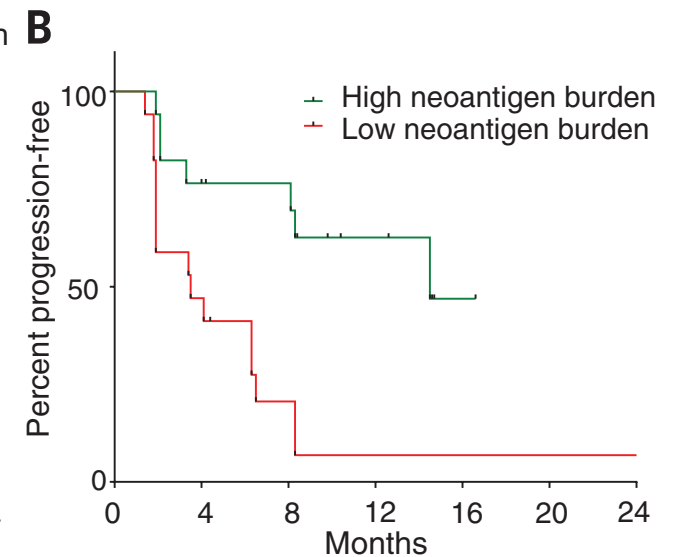
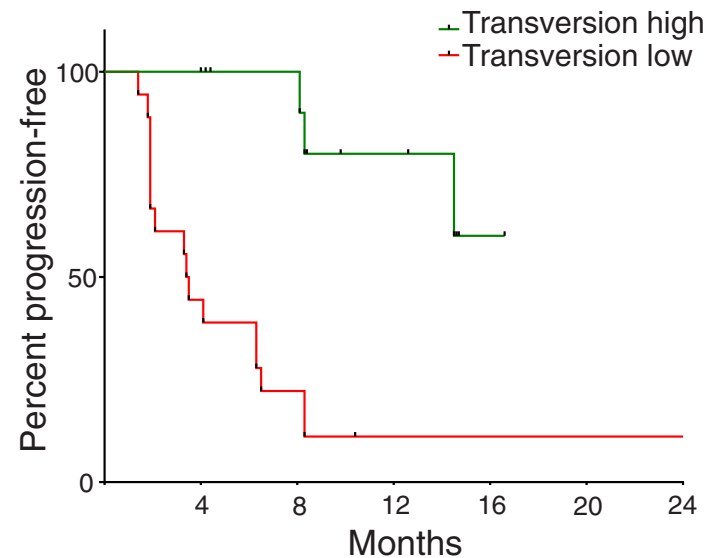
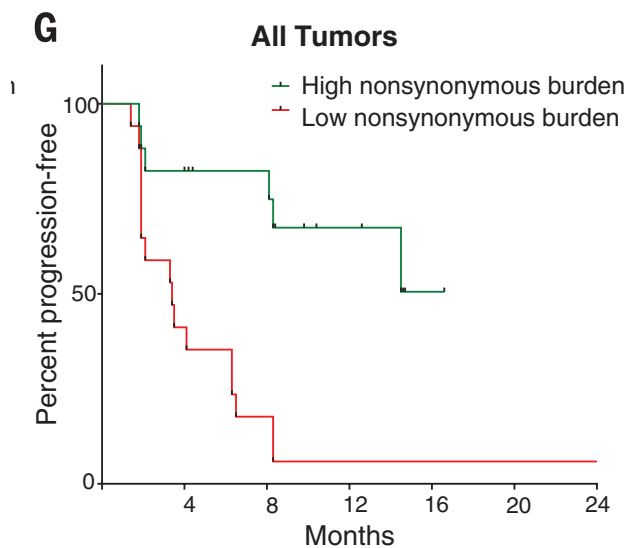
Charge mutationnelle et néo-antigènes

- Analyse de la réponse au pembrolizumab dans le CBNPC selon la charge mutationnelle.

PFS selon la charge mutationnelle
« non synonyme »

PFS selon le taux de transversion
(lié au tabagisme)

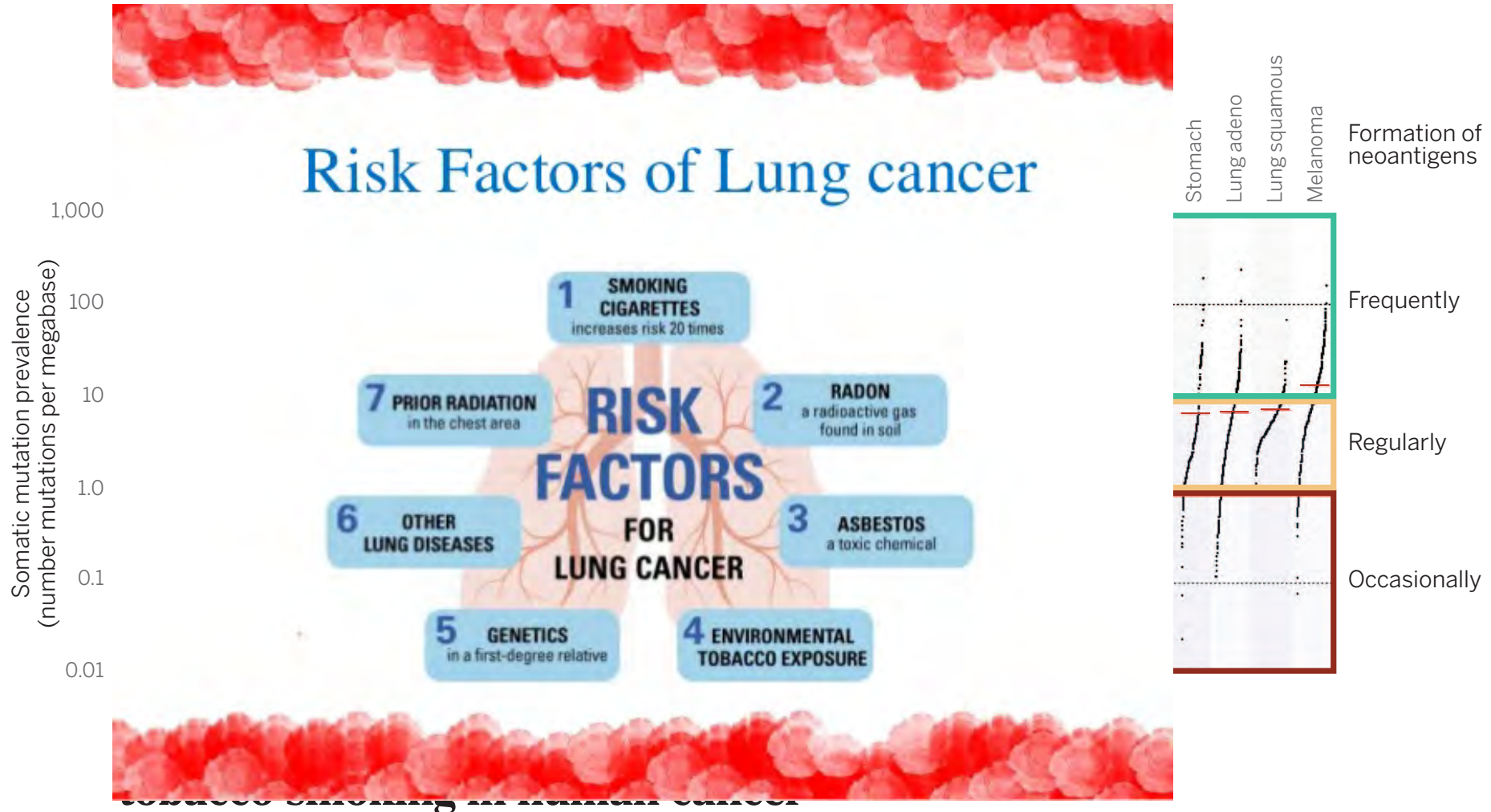
PFS selon les néo-antigènes



- Mise en évidence d'une signature de néo-épitopes prédictive de la survie

Charge mutationnelle et néo-antigènes

- Mutations et néo-antigènes selon les cancers.

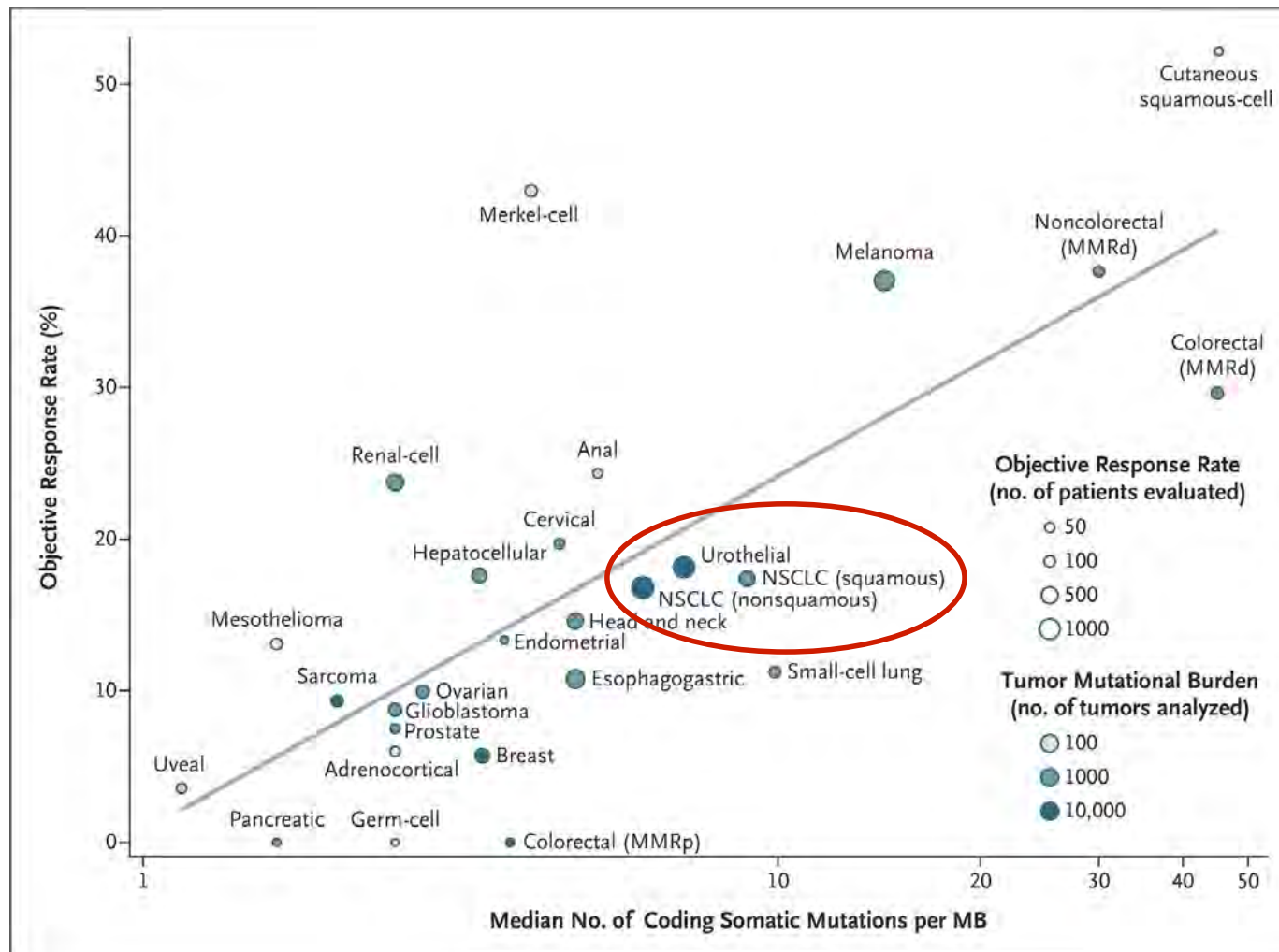


Ludmil B. Alexandrov,^{1,2,3*} Young Seok Ju,⁴ Kerstin Haase,⁵ Peter Van Loo,^{5,6}
 Iñigo Martincorena,⁷ Serena Nik-Zainal,^{7,8} Yasushi Totoki,⁹ Akihiro Fujimoto,^{10,11}
 Hidewaki Nakagawa,¹⁰ Tatsuhiro Shibata,^{9,12} Peter J. Campbell,^{7,13} Paolo Vineis,^{14,15}
 David H. Phillips,¹⁶ Michael R. Stratton^{7*}

Alexandrov L, Nature 2011
 Alexandrov L, Science 2016

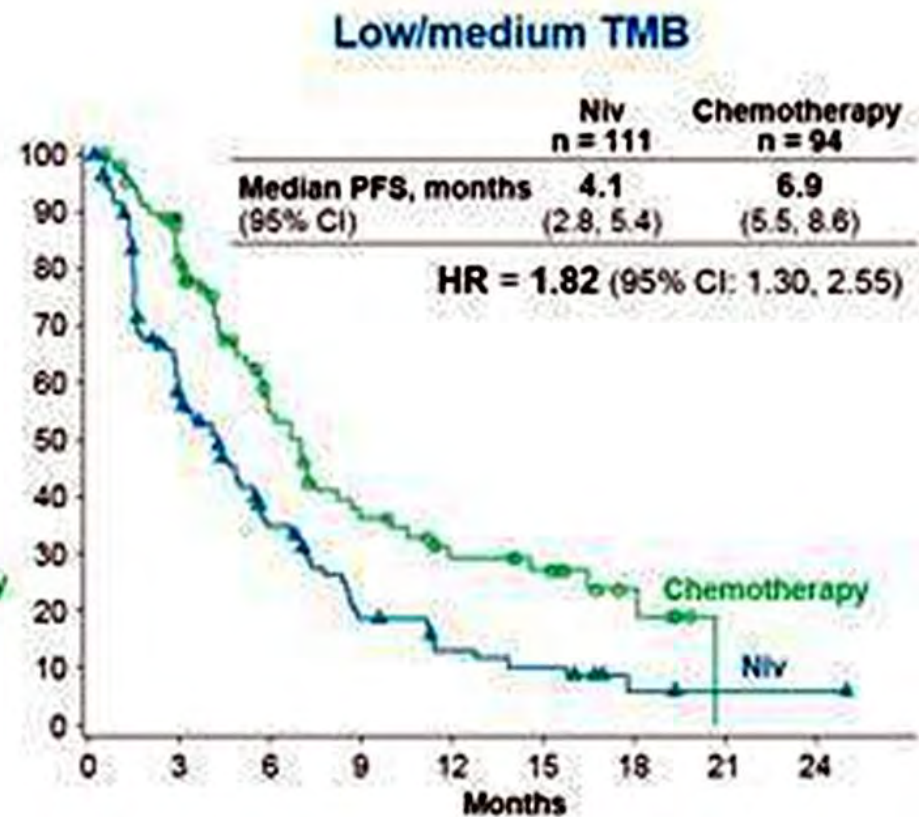
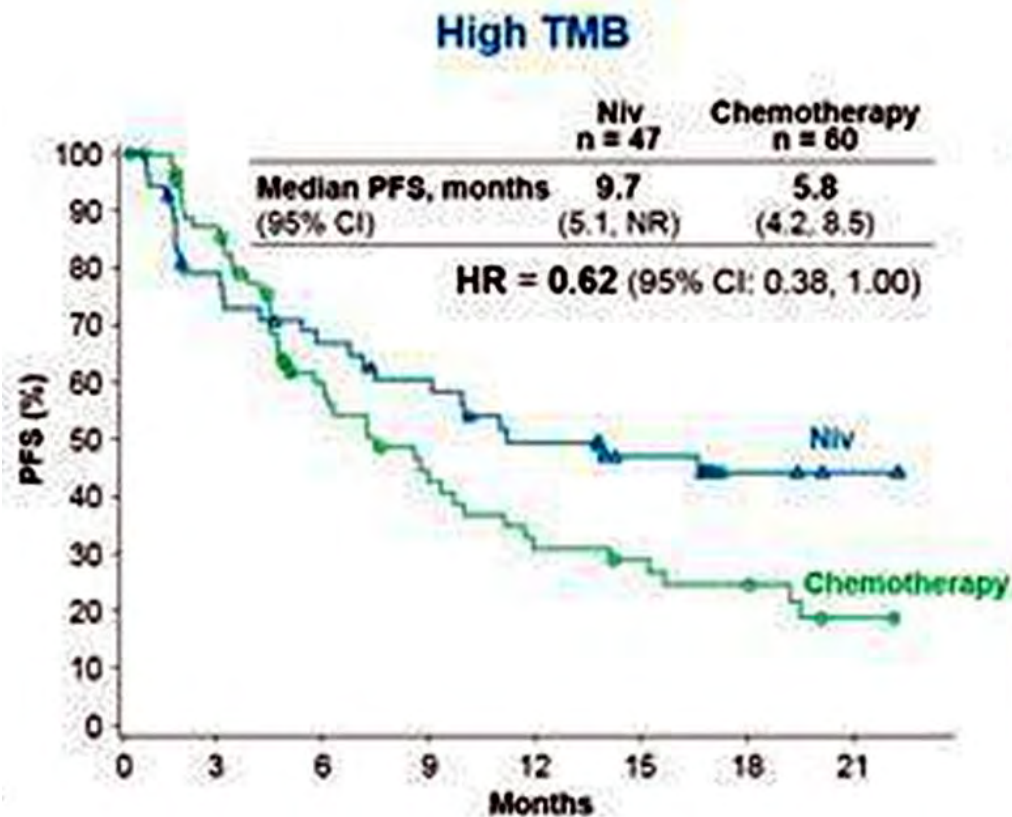
Charge mutationnelle et néo-antigènes

- Charge mutationnelle et réponse à l'immunothérapie



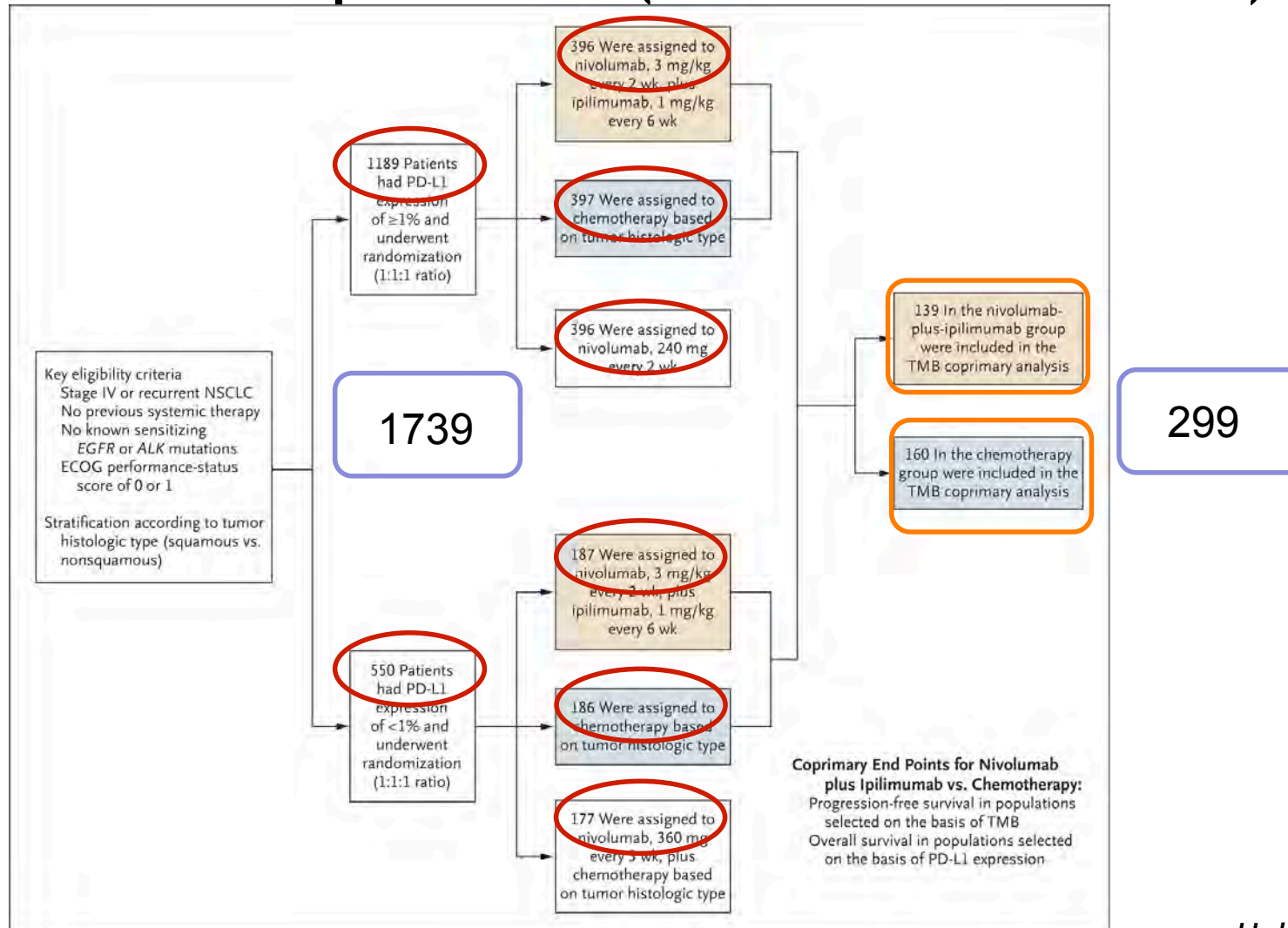
Charge mutationnelle et néo-antigènes

- Impact de la charge mutationnelle sur la réponse au nivolumab (essai CHECKMATE 026)



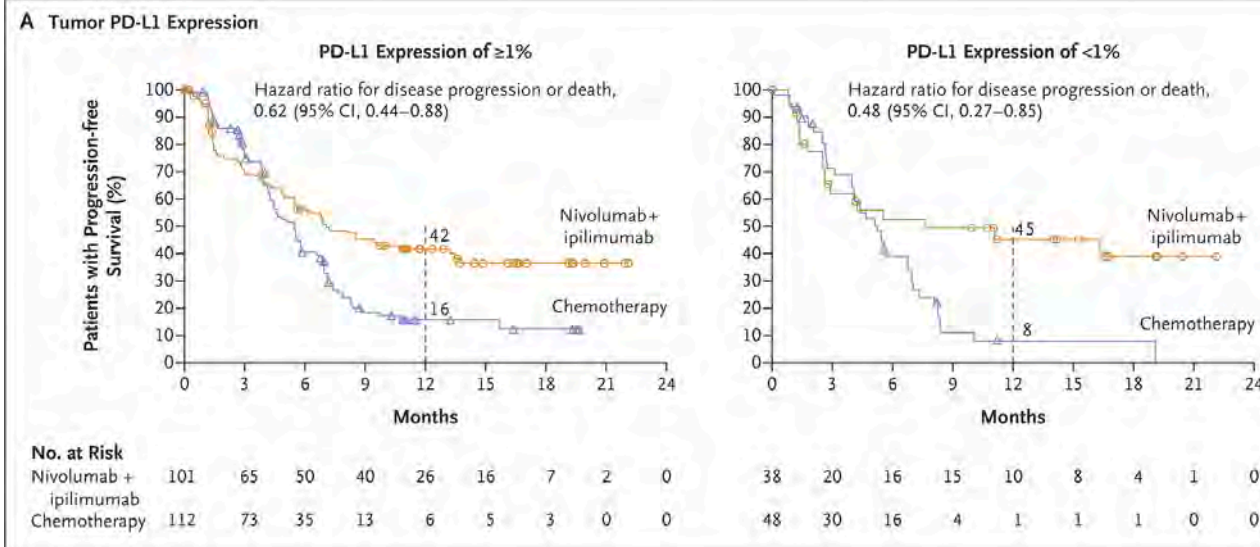
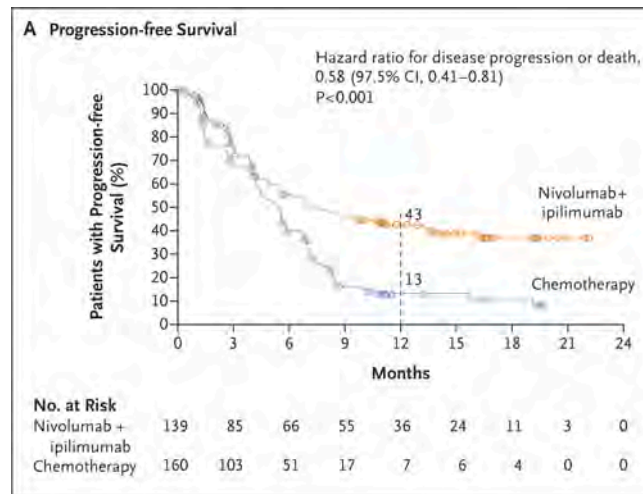
Charge mutationnelle et néo-antigènes

- Impact de la charge mutationnelle sur la réponse à nivolumab + ipilimumab (essai CHECKMATE 227)



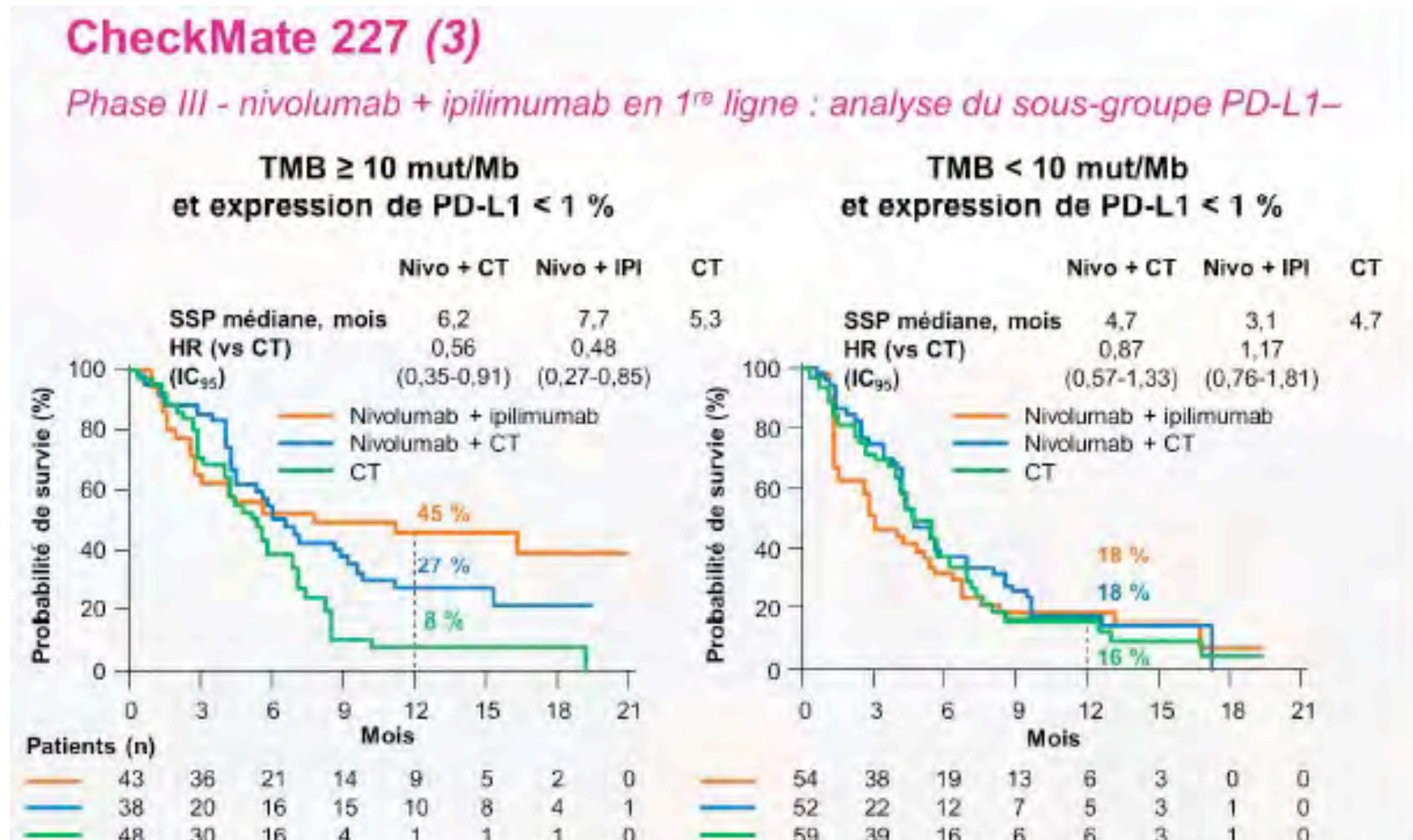
Charge mutationnelle et néo-antigènes

- Impact de la charge mutationnelle sur la réponse à nivolumab + ipilimumab (essai CHECKMATE 227)



Charge mutationnelle et néo-antigènes

- Impact de la charge mutationnelle sur la réponse à nivolumab + ipilimumab (essai CHECKMATE 227)

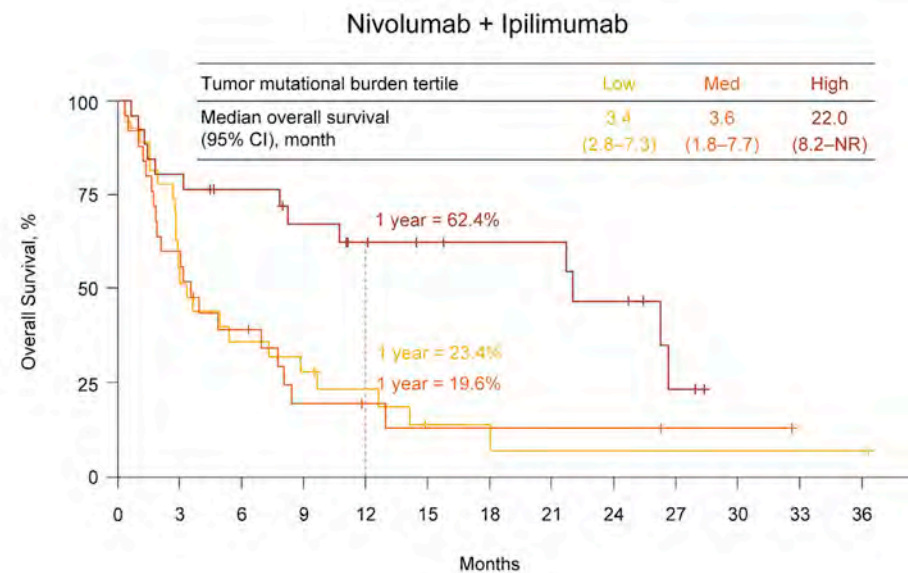
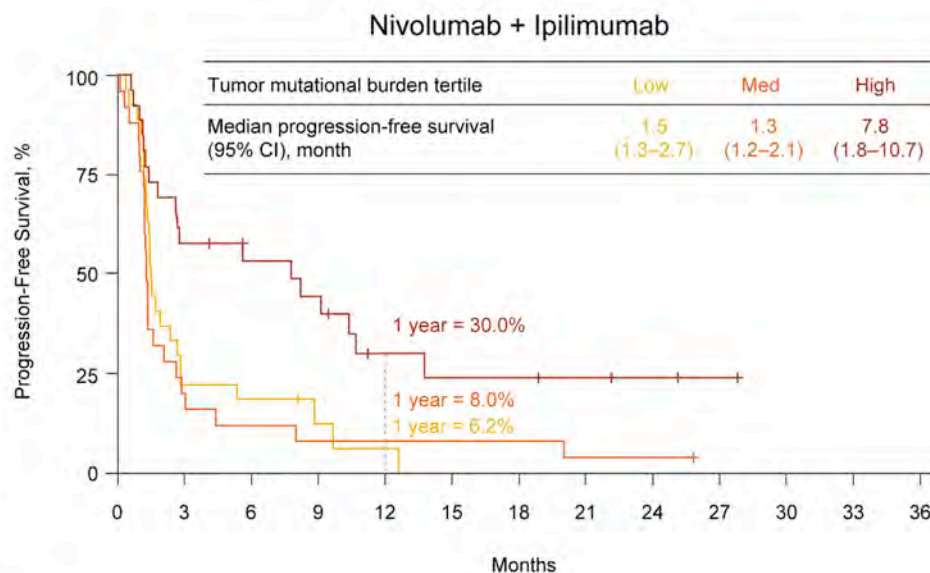


Charge mutationnelle et néo-antigènes

- Impact de la charge mutationnelle sur la réponse à nivolumab + ipilimumab dans le CPC

Tumor Mutational Burden and Efficacy of Nivolumab Monotherapy and in Combination with Ipilimumab in Small-Cell Lung Cancer

Matthew D. Hellmann,^{1,11,12,*} Margaret K. Callahan,^{1,11} Mark M. Awad,² Emiliano Calvo,³ Paolo A. Ascierto,⁴ Akin Atmaca,⁵ Naiyer A. Rizvi,⁶ Fred R. Hirsch,⁷ Giovanni Selvaggi,⁸ Joseph D. Szustakowski,⁹ Ariella Sasson,⁹ Ryan Golhar,⁹ Patrik Vitazka,⁹ Han Chang,⁹ William J. Geese,⁹ and Scott J. Antonia¹⁰



Charge mutationnelle et néo-antigènes

- Comment analyser la charge mutationnelle ?

- NGS

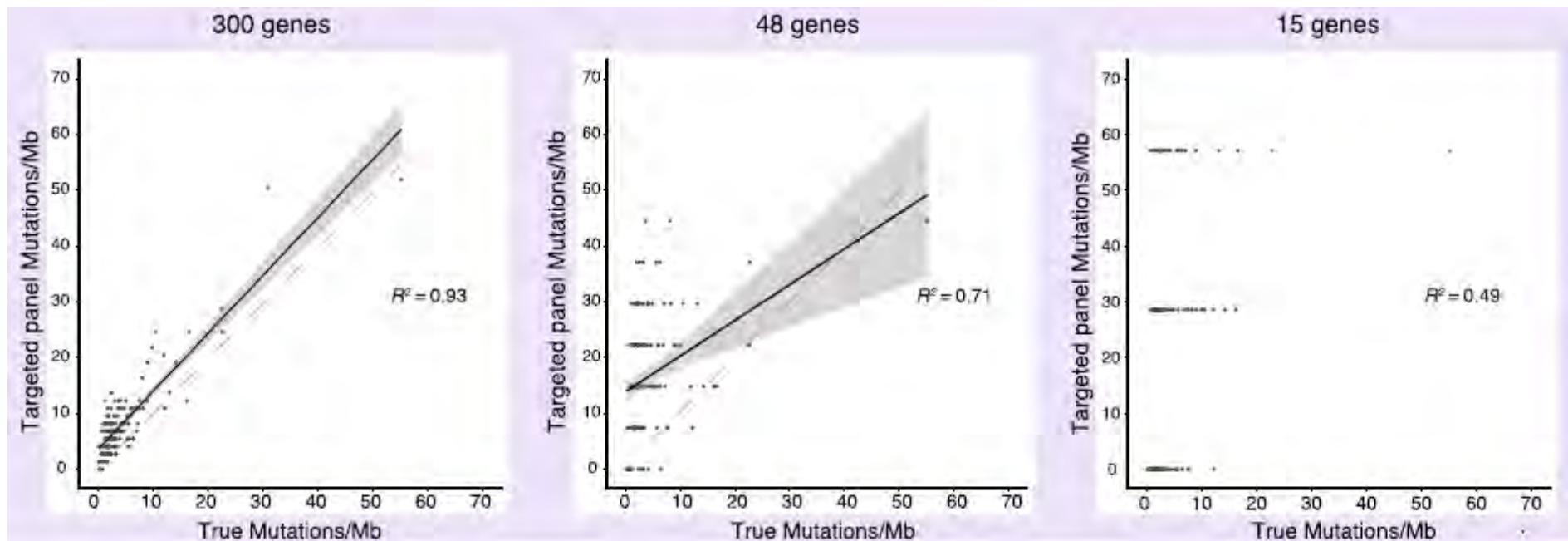
- Whole exome
- RNAseq
- Panel ciblé de gènes: quantification des mutations somatiques après exclusion des drivers oncogéniques et des polymorphismes de la lignée germinale. Ex. FoundationOne

- Etude plus qualitative par analyse des épitopes néo-antigéniques



Charge mutationnelle et néo-antigènes

- Combien de gènes doit comporter le test TMB ?
- Analyse modélisée en faveur de panel 300+ genes pour la détermination de la TMB



Charge mutationnelle et néo-antigènes

- Combien de gènes doit comporter le test TMB ?



MSK-Impact



Memorial Sloan Kettering
Cancer Center

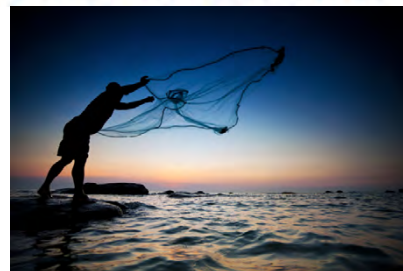
Oncomine TML

ThermoFisher
S C I E N T I F I C

- | | |
|------------------------|---|
| • FDA cleared | + |
| • Actionable mutations | + |
| • Outsource service | - |
| • High DNA input | - |

- | | |
|------------------|---|
| • RUO/LDT | - |
| • Separate test | - |
| • In house | + |
| • DNA input 20ng | + |

Good correlation with WES



Charge mutationnelle et néo-antigènes

- Exigence de l'analyse de la charge mutationnelle



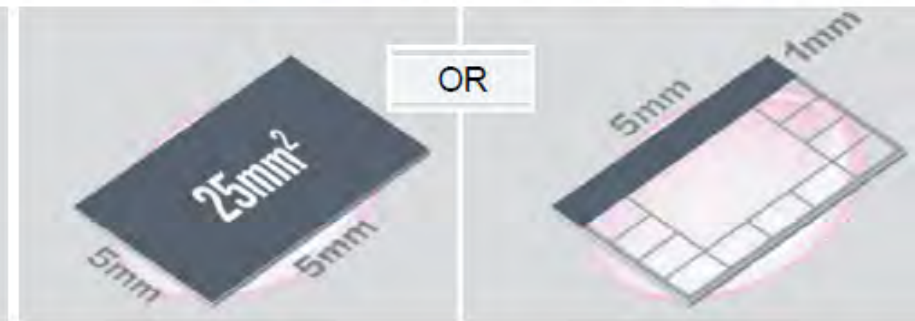
Type of probes

Size of probe surface



FFPE block & HE

10 unstained sections



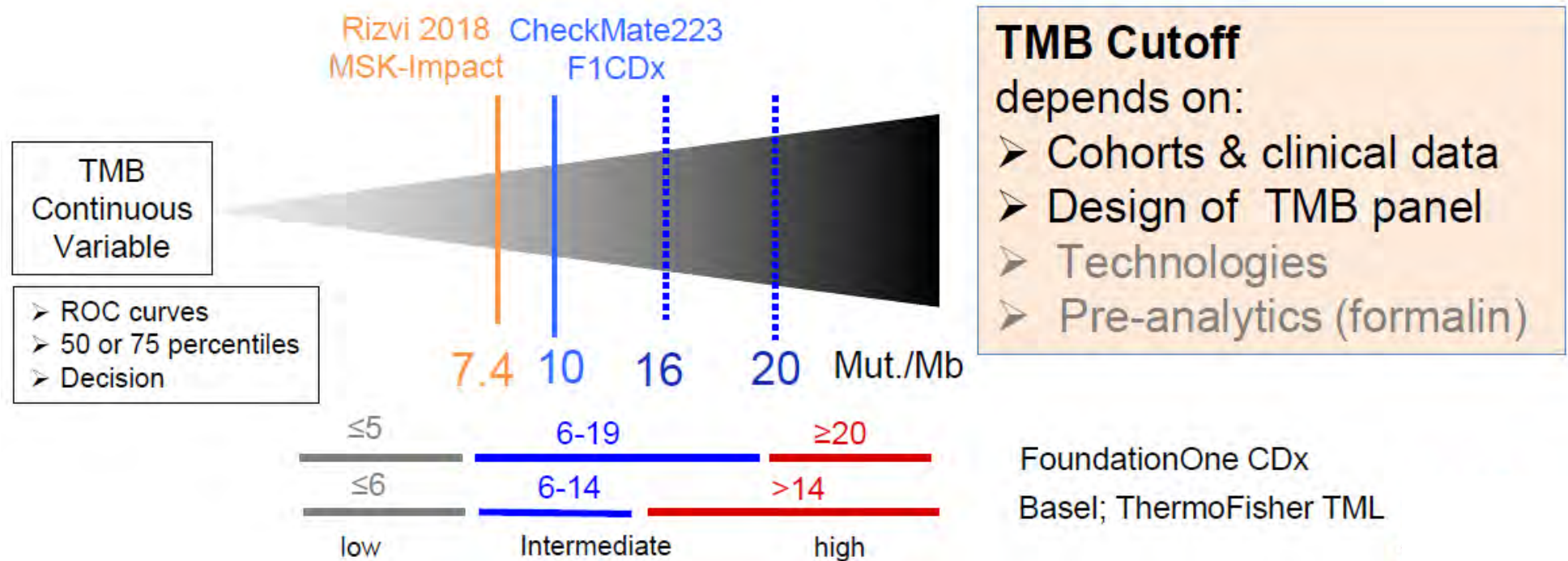
If 5-25mm² → more sections

CheckMate 227: Rejection rate 42%

Charge mutationnelle et néo-antigènes

- Le problème du seuil dans le TMB

The threshold issue



Charge mutationnelle et néo-antigènes

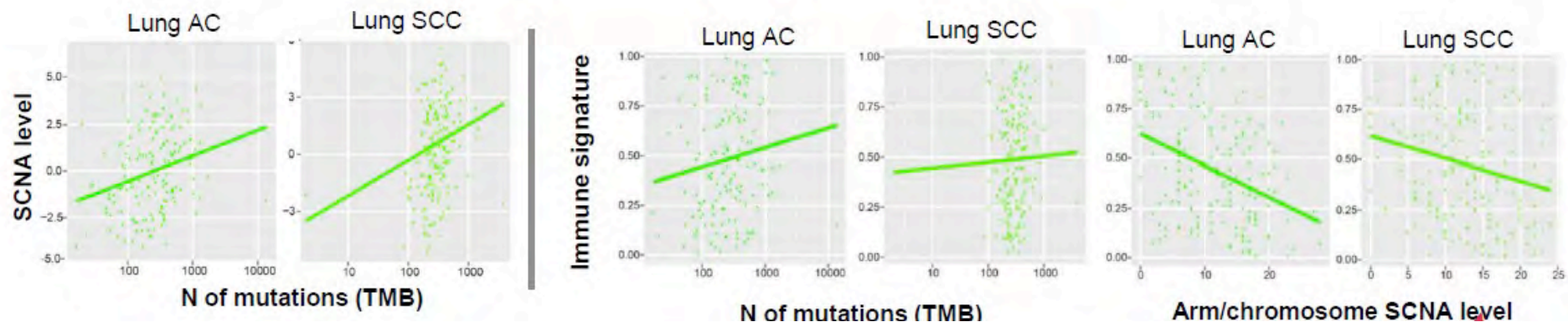
- Attention au faux positif de l'aneuploidie

Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy

Teresa Davoli, Hajime Uno, Eric C. Wooten, Stephen J. Elledge*

Aneuploidy in NSCLC correlates with

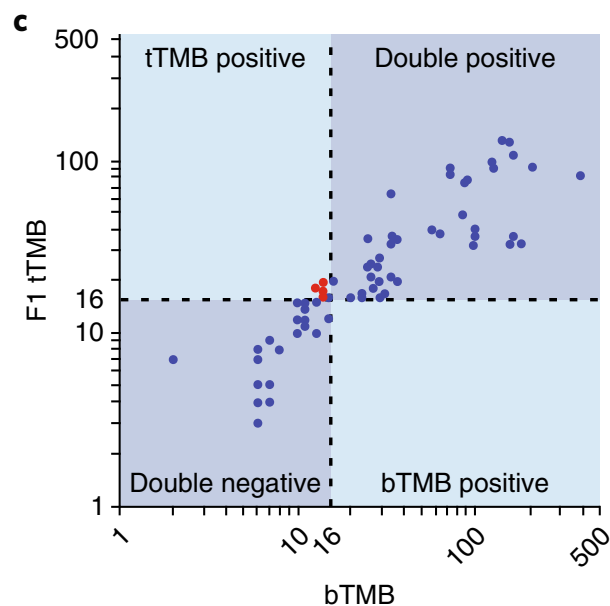
- High TMB
- Decreased immune signature



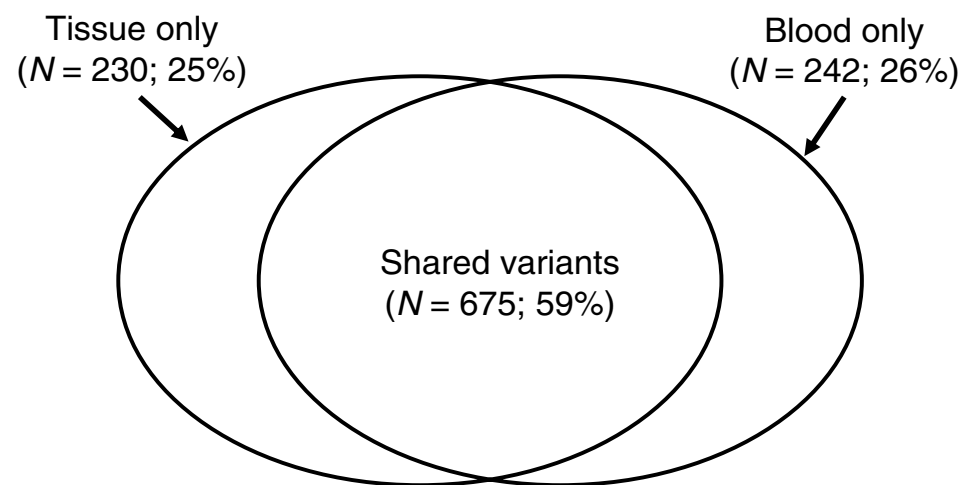
Davoli, Science 2017

Charge mutationnelle et néo-antigènes

- TMB dans le sang circulant ?
- TMB sérique et bénéfice clinique dans le CBNPC traité par atezolizumab
- Lien tissu vs sang

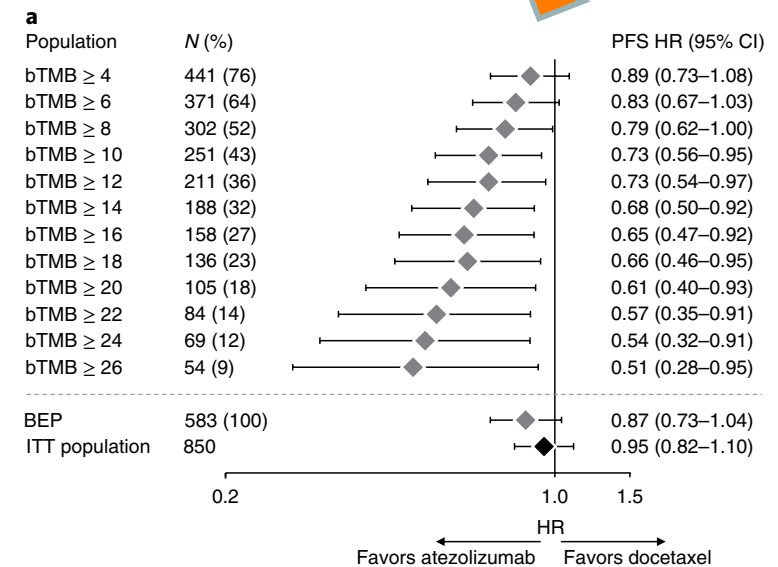
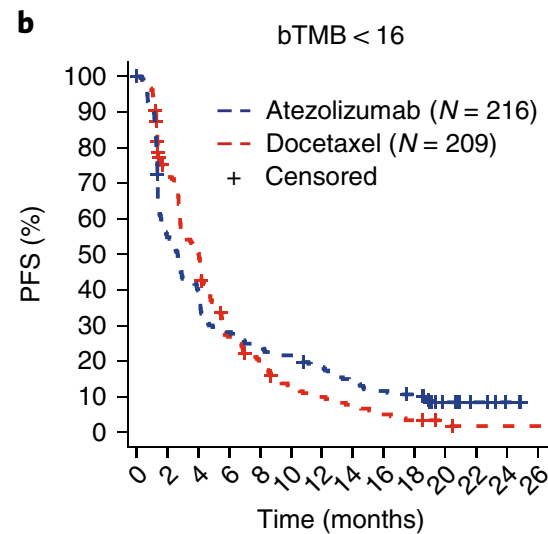
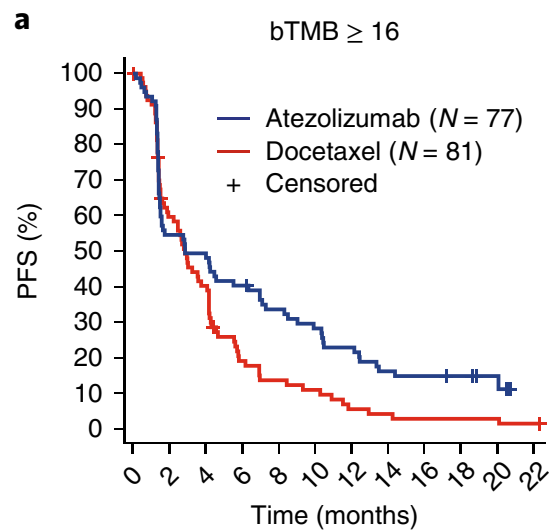


Spearman correlation = 0.93



Charge mutationnelle et néo-antigènes

- TMB dans le sang circulant ?
- TMB sérique et bénéfice clinique dans le CBNPC traité par atezolizumab



Charge mutationnelle et néo-antigènes

- TMB sérique et bénéfice clinique dans le CBNPC traité par atezolizumab

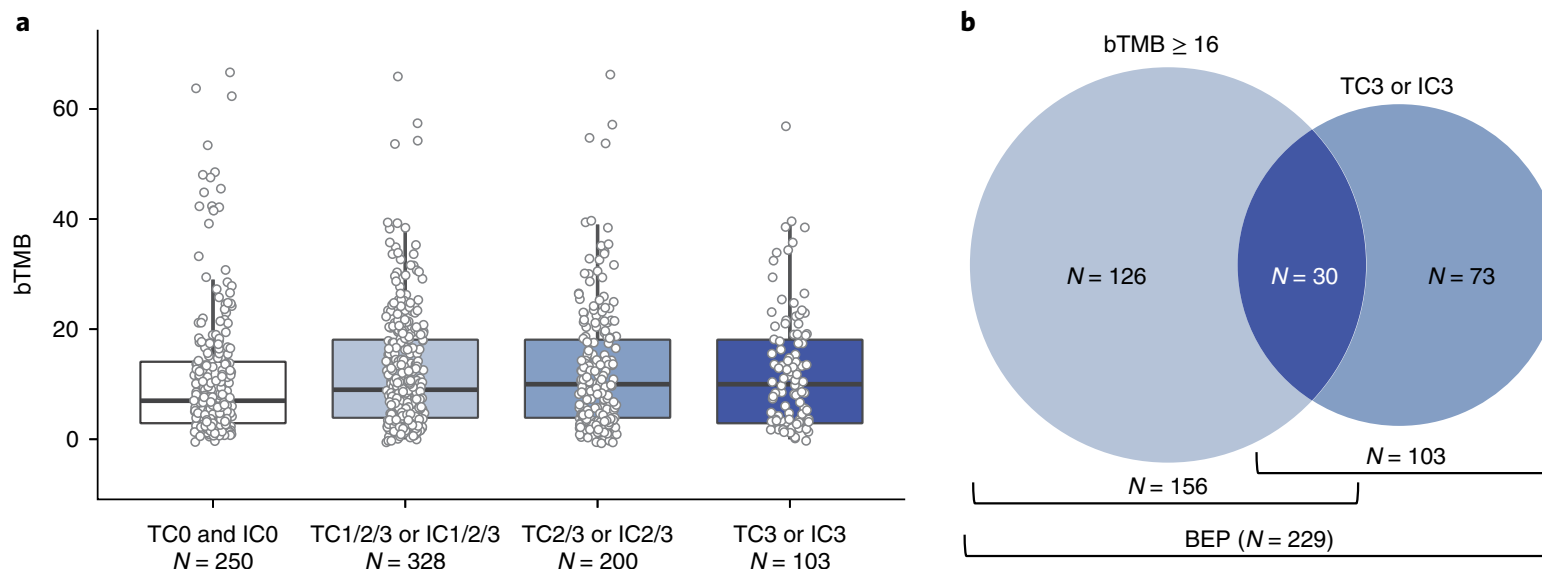


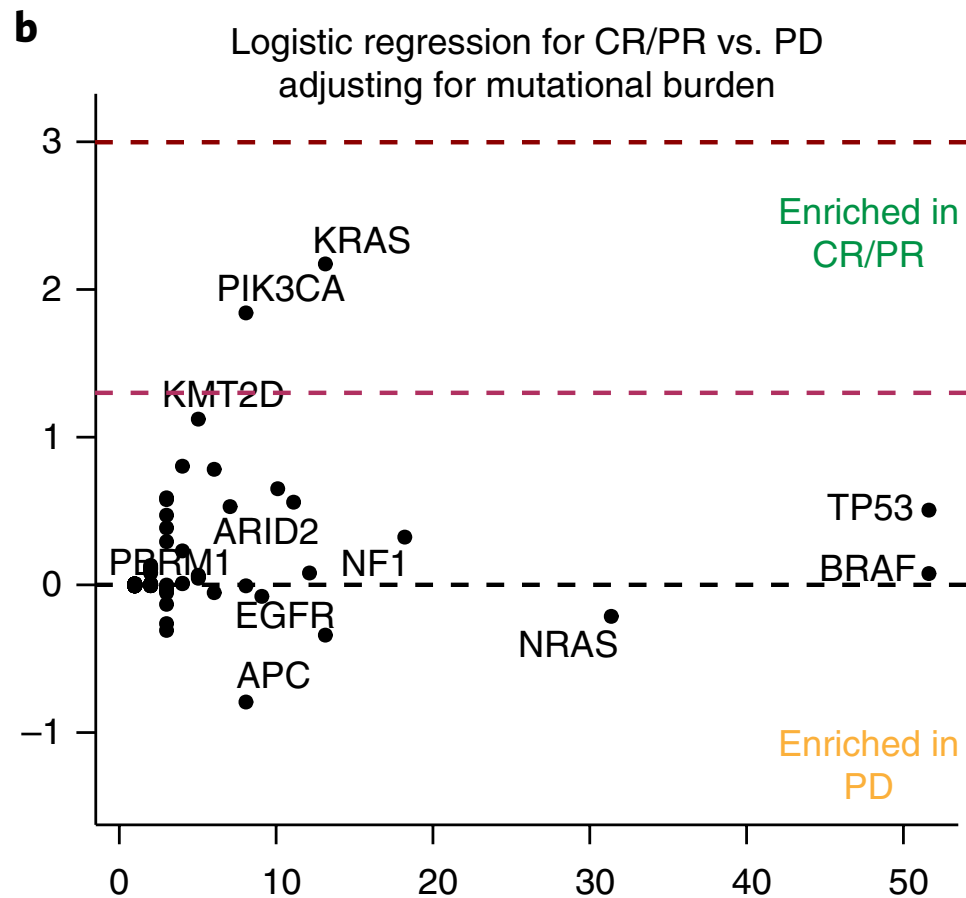
Table 1 | OS and PFS HRs in the OAK BEP with valid bTMB and PD-L1 IHC results

| | N | PFS HR (95% CI) | OS HR (95% CI) |
|-------------------------------|-----|------------------|------------------|
| bTMB ≥ 16 | 156 | 0.64 (0.46-0.91) | 0.64 (0.44-0.93) |
| TC3 or IC3 | 103 | 0.62 (0.41-0.93) | 0.44 (0.27-0.71) |
| bTMB ≥ 16 and TC3 or IC3 | 30 | 0.38 (0.17-0.85) | 0.23 (0.09-0.58) |

N represents the number of patients in each subgroup. IC3 or IC3, $\geq 50\%$ of tumor cells or $\geq 10\%$ of tumor-infiltrating immune cells expressing PD-L1.

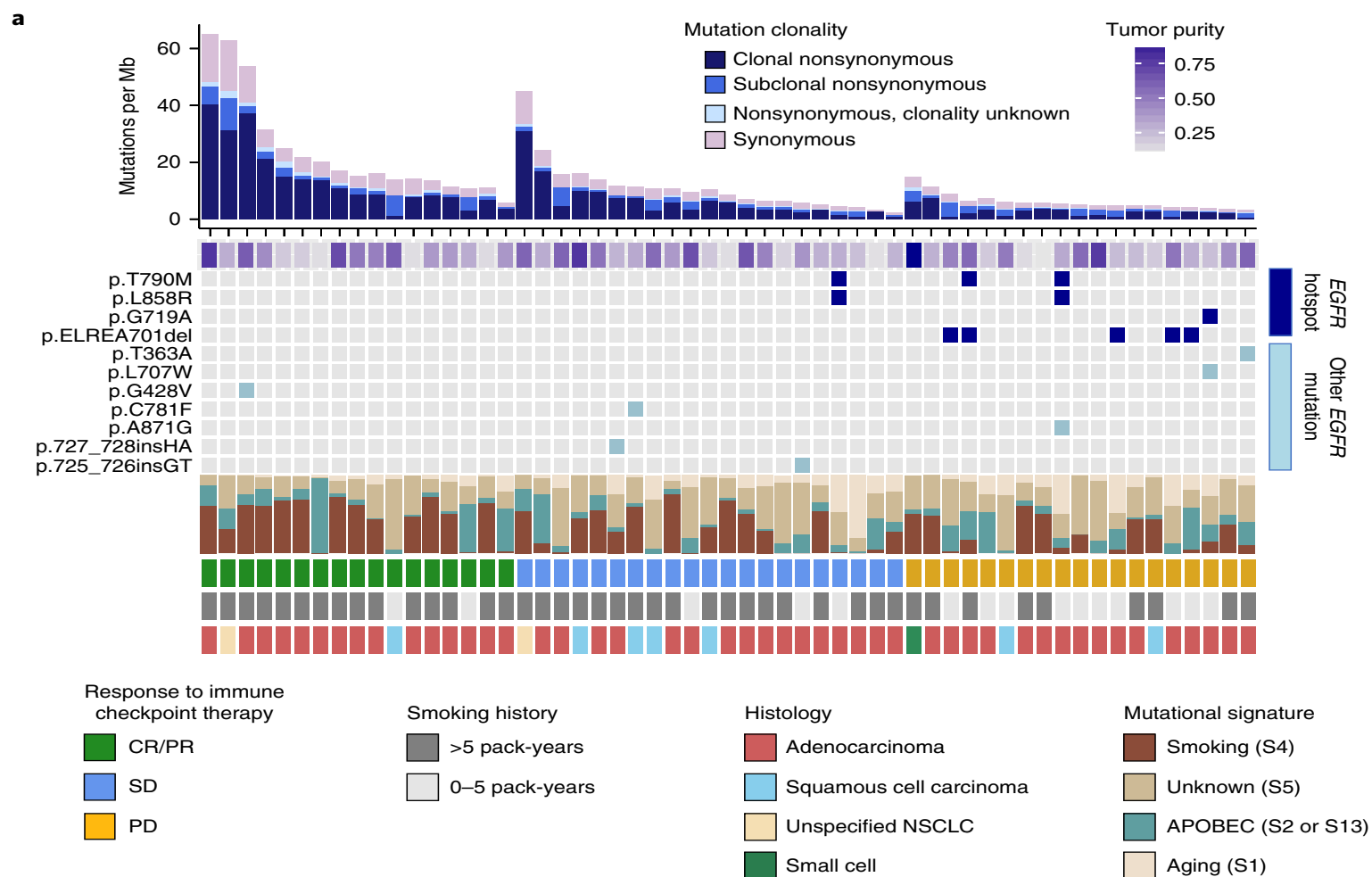
Génétique et immunothérapie

- Corrélation altérations génomiques et réponse aux anti PD1 / PDL1 dans les tumeurs solides



Génétique et immunothérapie

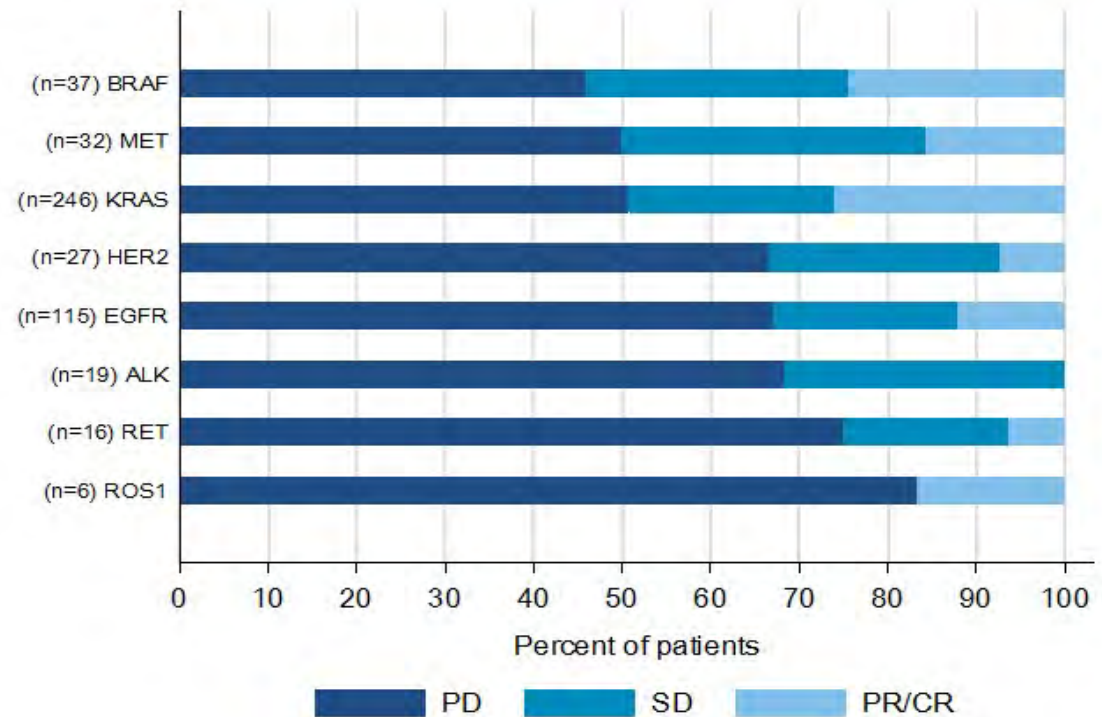
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Génétique et immunothérapie

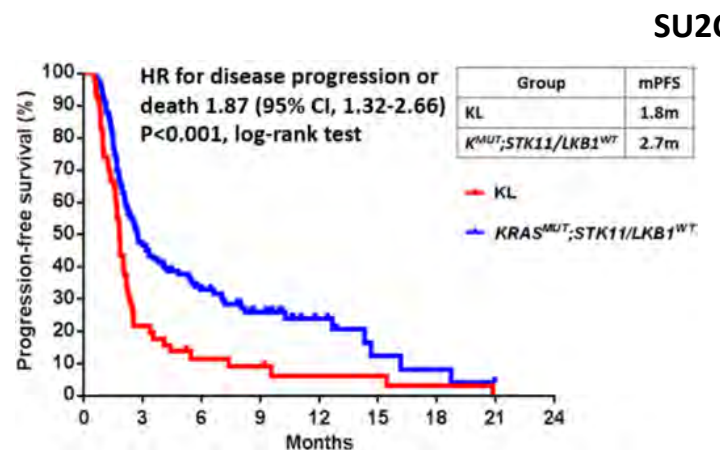
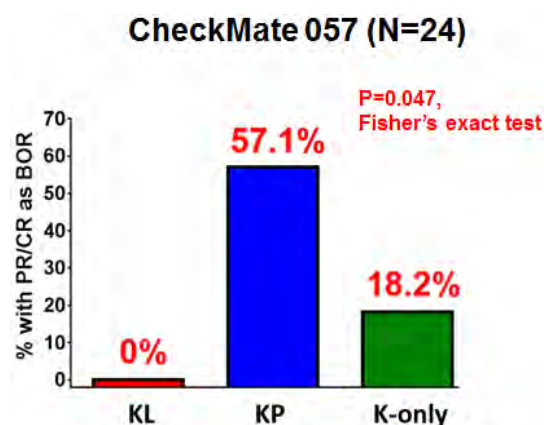
- Et les addictions oncogéniques ?

| Driver | PD | SD | CR/PR |
|--------|-----|-----|-------|
| BRAF | 46% | 30% | 24% |
| MET | 50% | 34% | 16% |
| KRAS | 51% | 23% | 26% |
| HER2 | 67% | 26% | 7% |
| EGFR | 67% | 21% | 12% |
| ALK | 68% | 32% | 0 |
| RET | 75% | 19% | 6% |
| ROS1 | 83% | 0 | 17% |
| TOTAL | 57% | 24% | 19% |



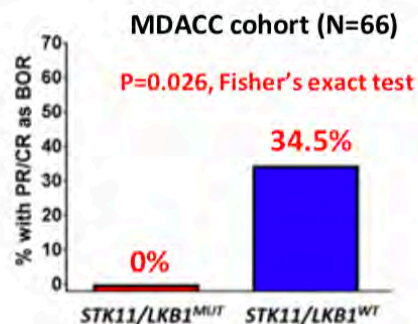
Génétique et immunothérapie

- Impact de STK11-LKB1
- Démonstré en co-mutation de KRas

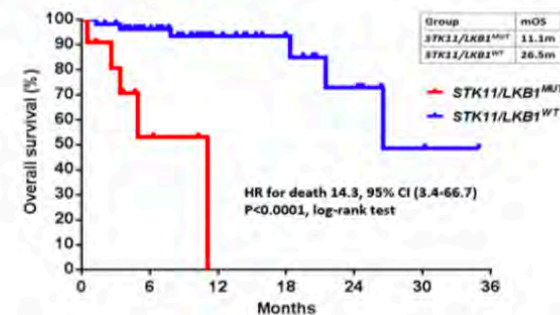
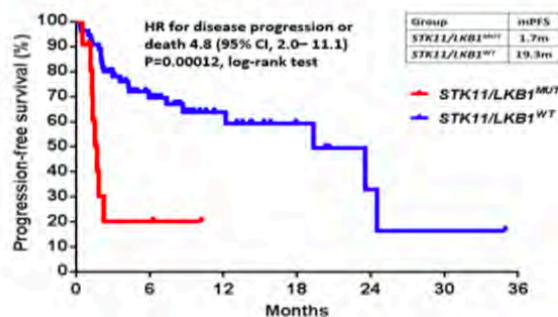


- Dans les CBNPC PDL1 +

Skoulidis F et al., *Cancer Discovery*, 2018



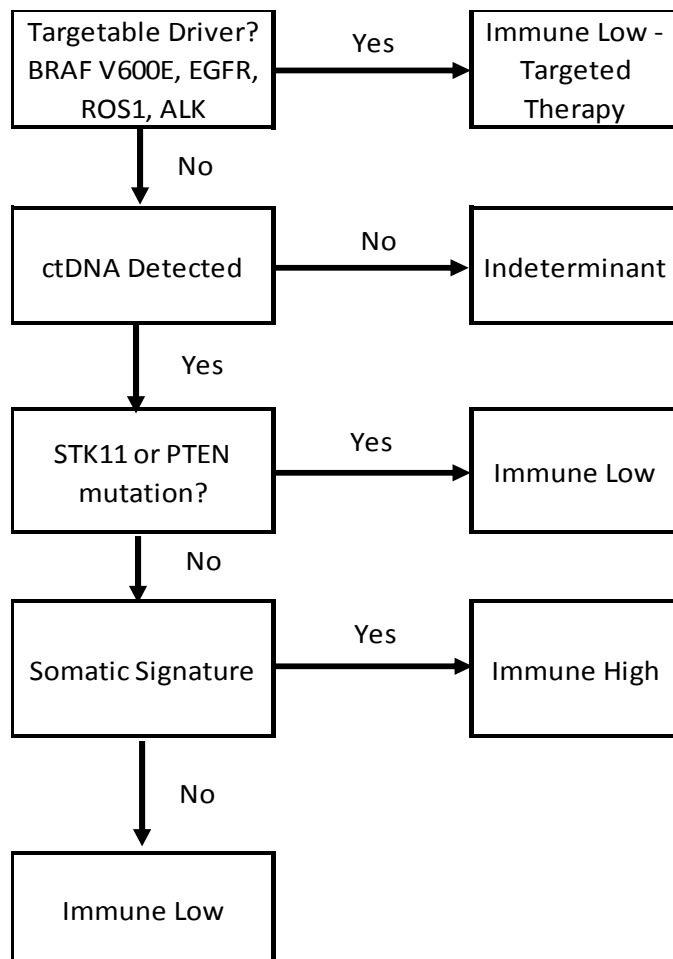
PD-L1 expressing non-squamous NSCLC



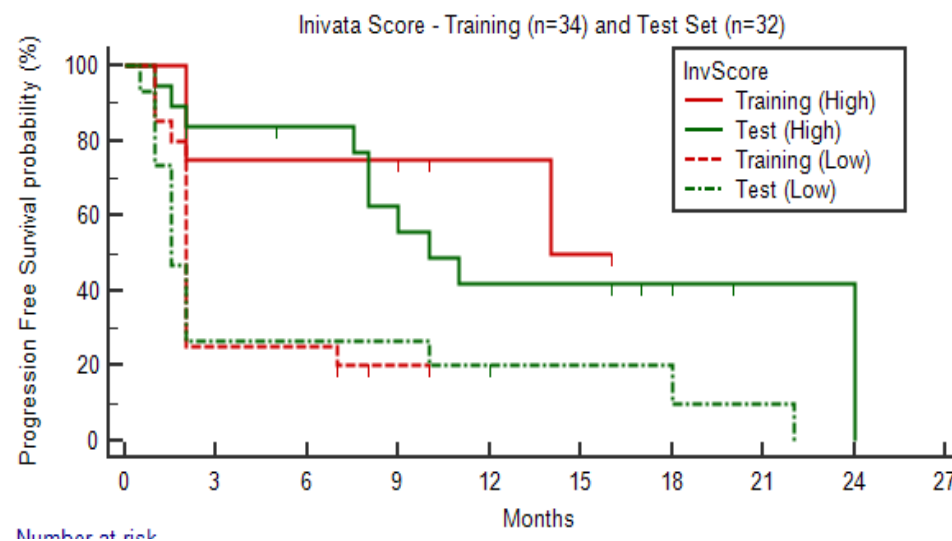
Skoulidis, *Cancer Disc* 2018

Génétique et immunothérapie

- Score composite avec 5 éléments sur biopsie liquide



Hazard Ratio - **Training** 3.582 (1.50-8.57)
 Hazard Ratio - **Test** 2.866 (1.22-6.73)

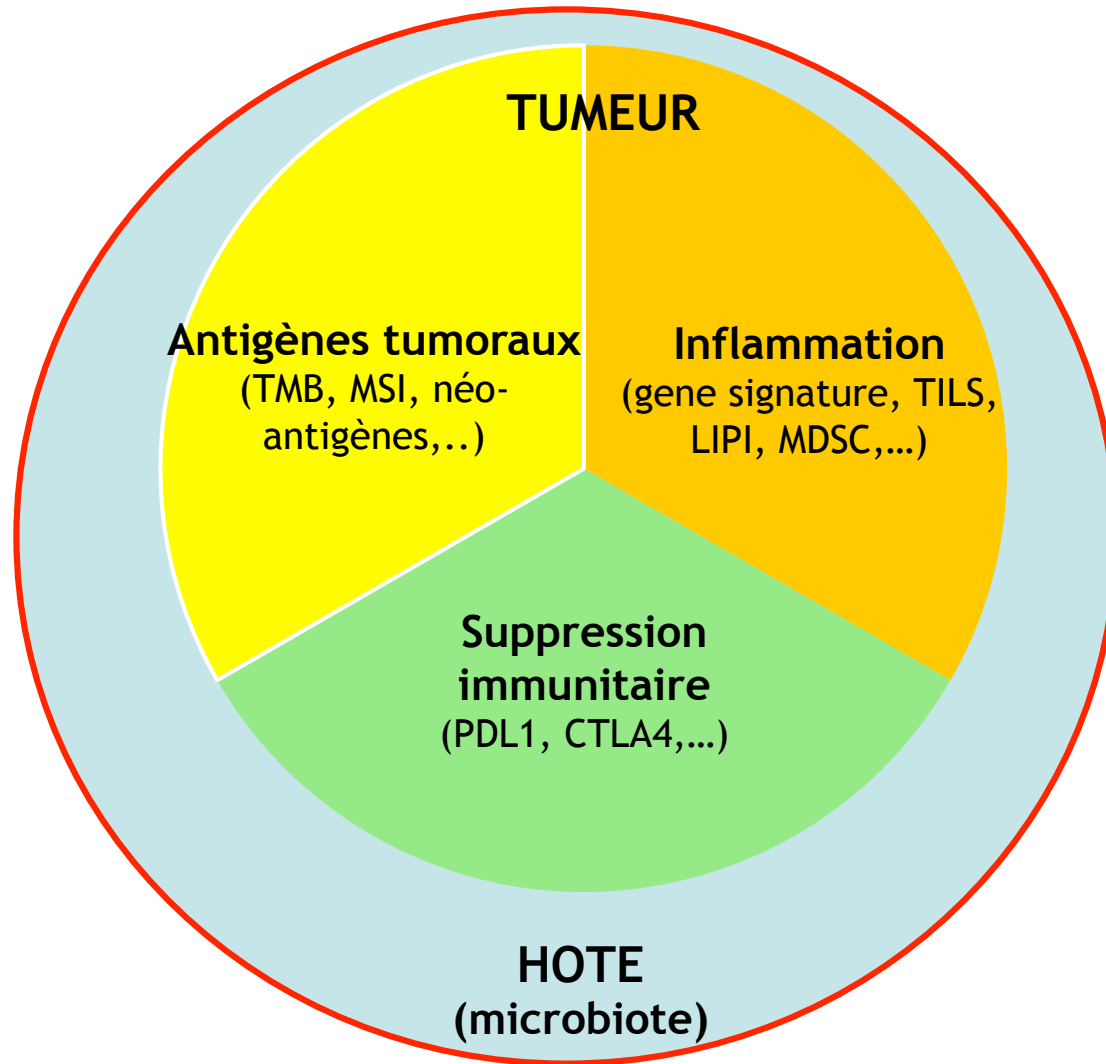


Comparison of survival curves
 (Logrank test) P = 0.0009

PFS

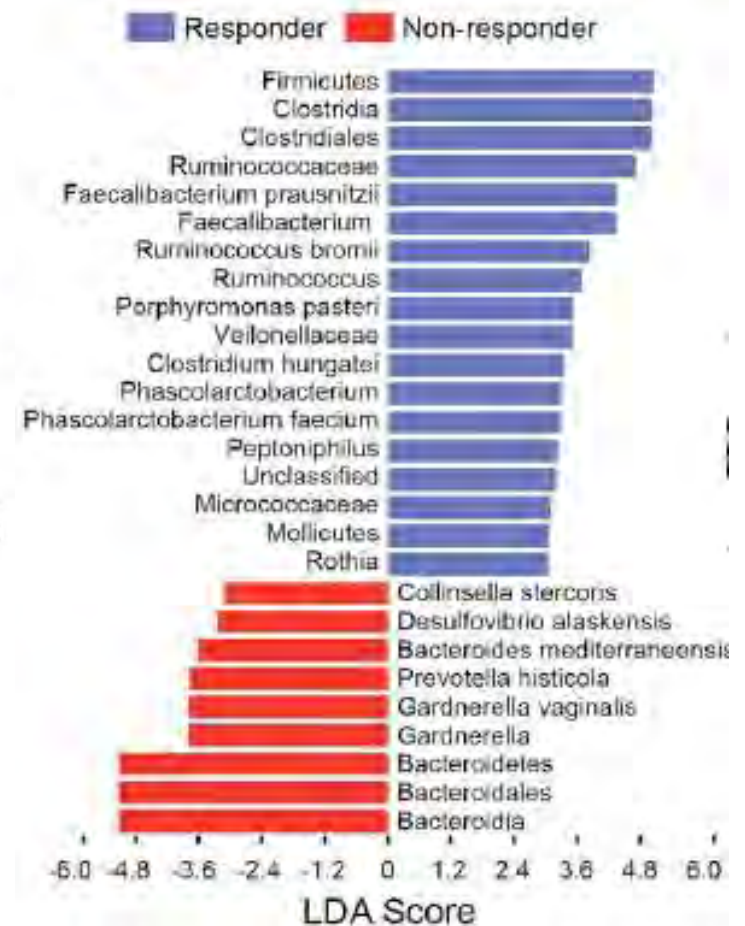
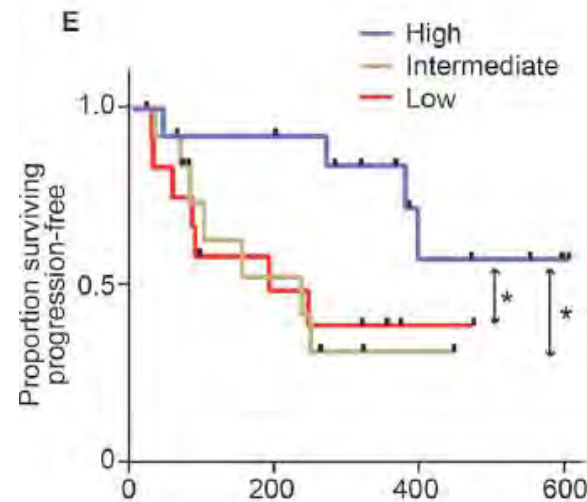
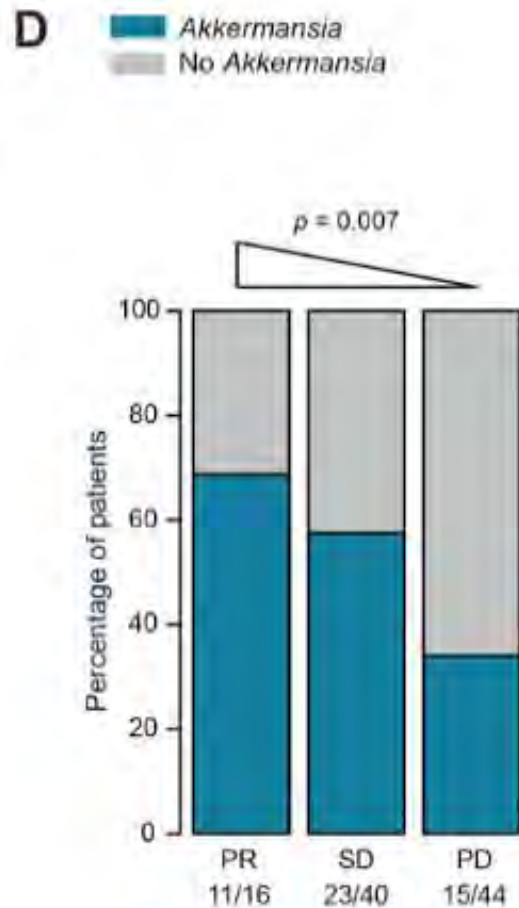
| | |
|-----------------|---|
| Training | Inivata Immune High = 14 mos (14 - 14 mos) |
| | Inivata Immune Low = 2 mos (2 - 2 mos) |
| Test Set | Inivata Immune High = 10 mos (8 - 24 mos) |
| | Inivata Immune Low = 1.5 mos (1.5 - 2 mos) |

Les biomarqueurs en immunothérapie



Le microbiote

- Intérêt du microbiome en oncologie: marqueur et ciblé thérapeutique

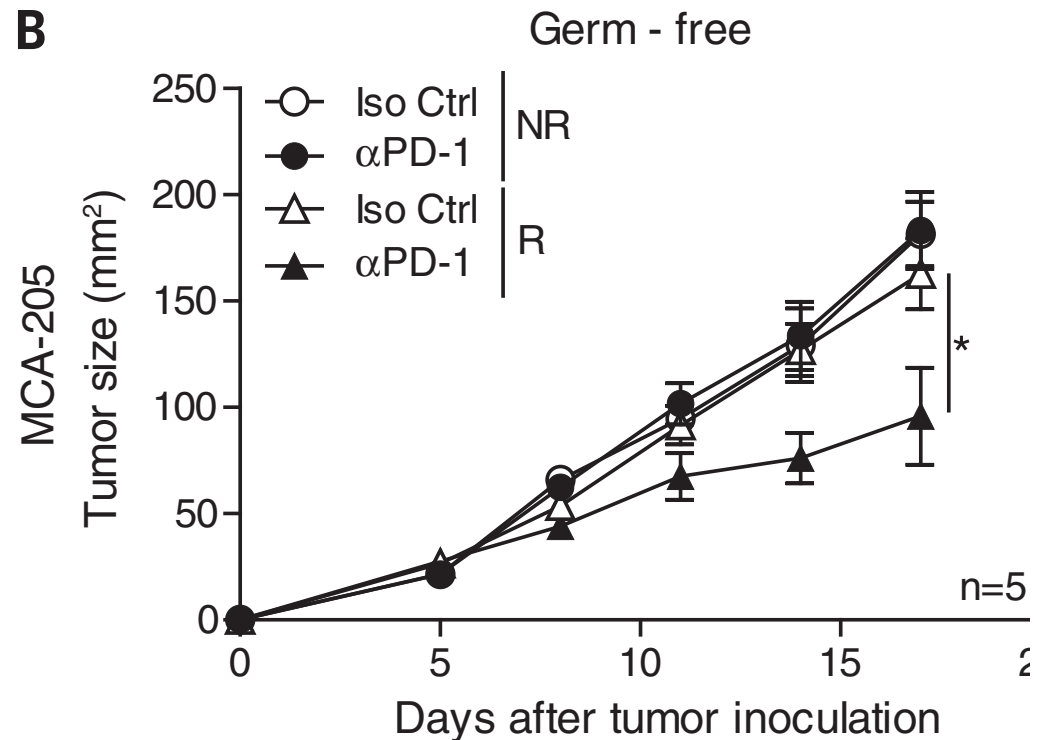
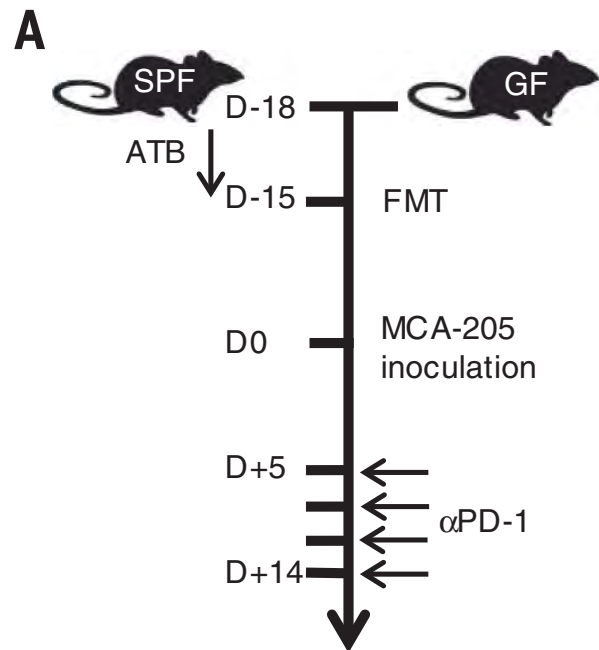


Routy et al, Science 2017

Routy B, Science 2018

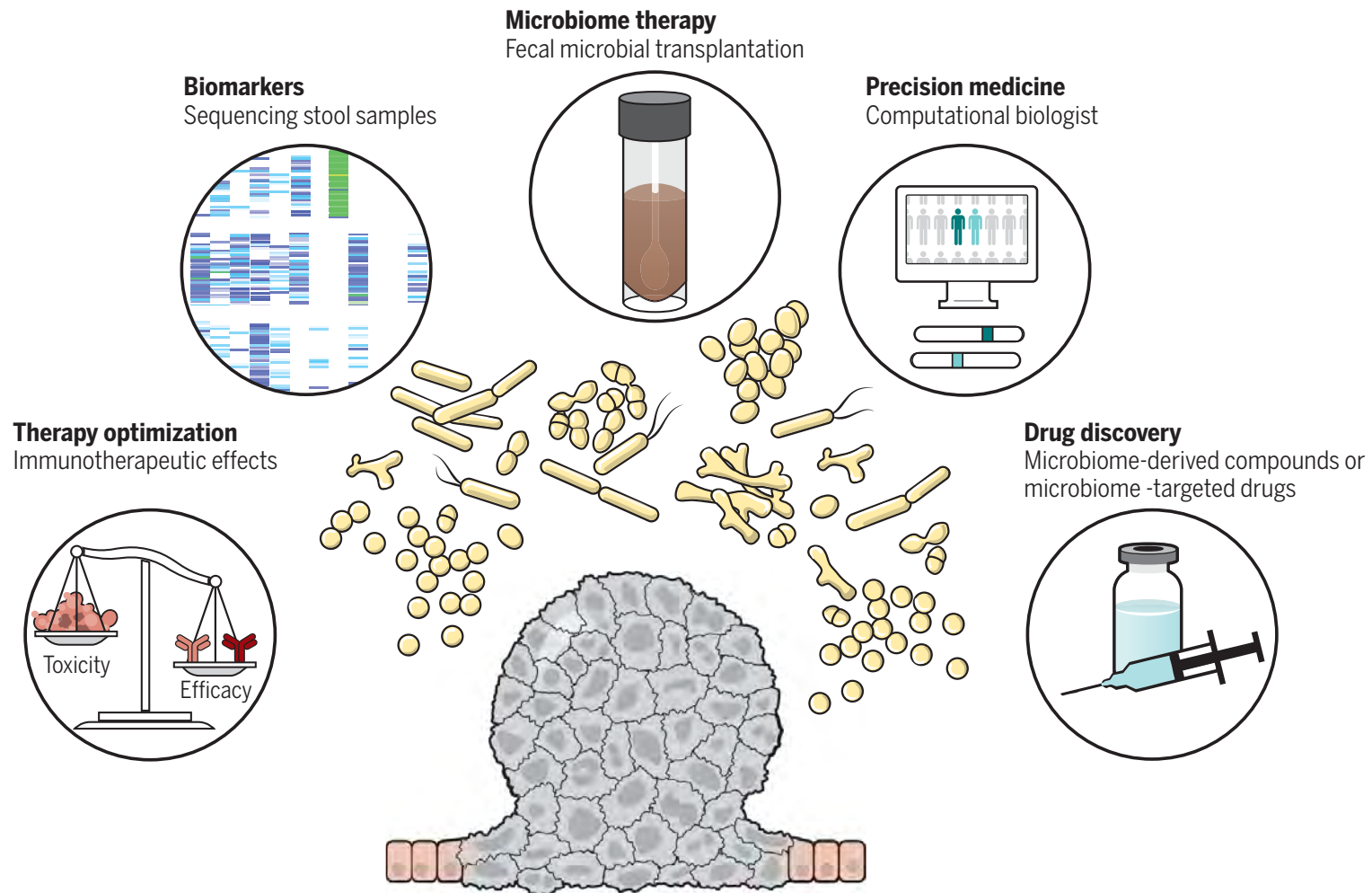
Le microbiote

- Intérêt du microbiome en oncologie: marqueur et ciblé thérapeutique



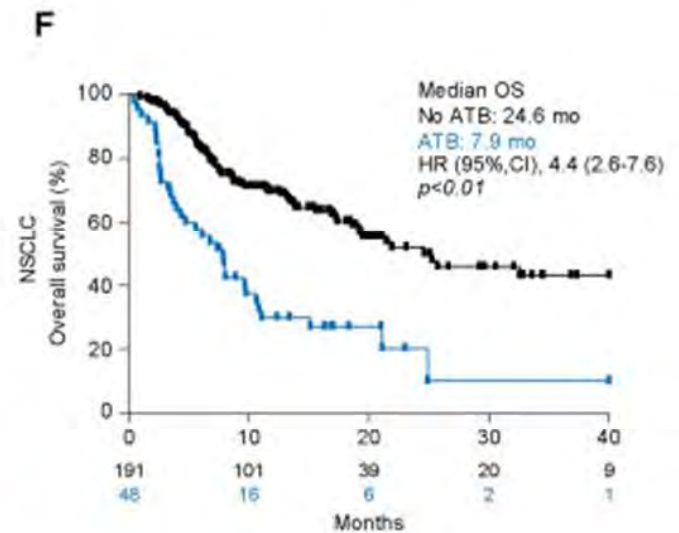
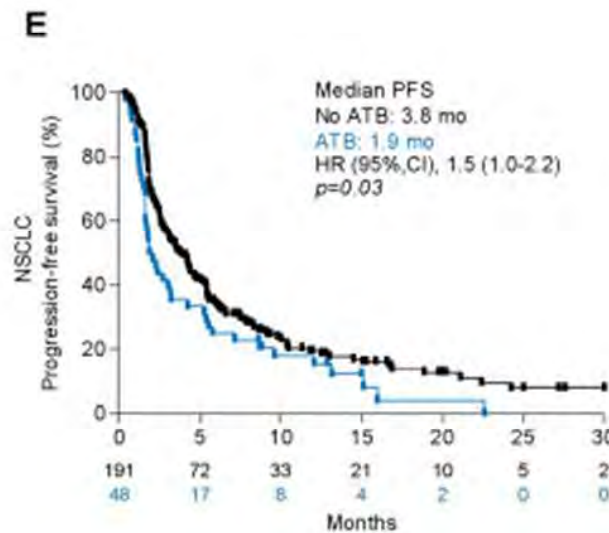
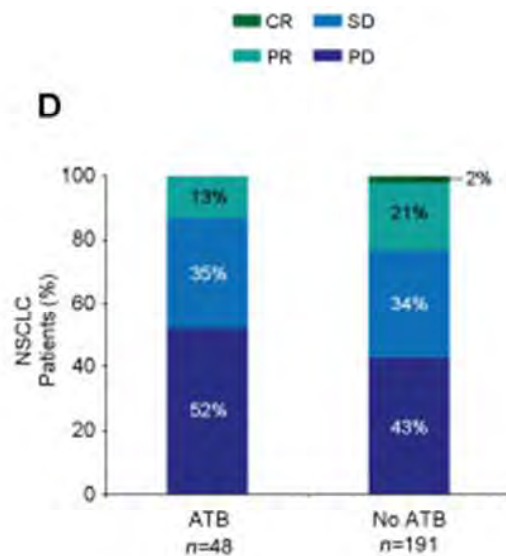
Le microbiote

- Intérêt du microbiome en oncologie: marqueur et ciblé thérapeutique

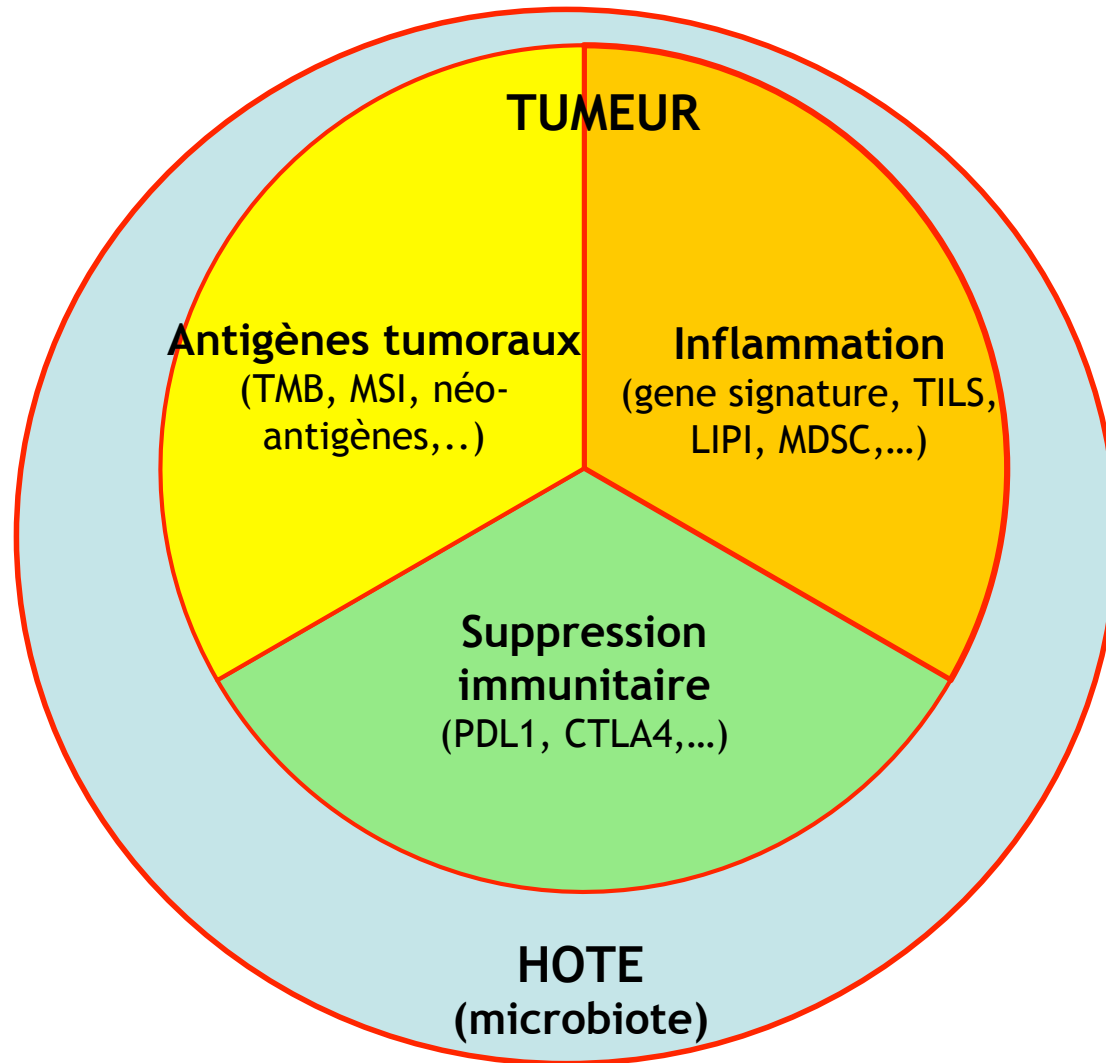


Le microbiote

- Impact de la prescription d'antibiotiques sur la réponse aux ICI
- Effet négatif sur la PFS et la survie globale de patients traités par immunothérapie



Les biomarqueurs en immunothérapie

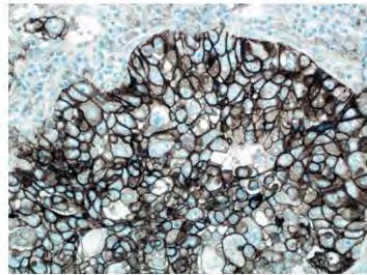


Synthèse des tests prédictifs

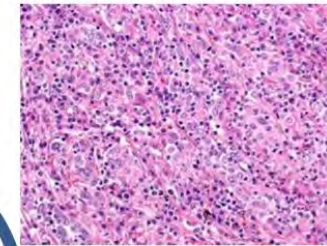
| | Expression de PD-L1 | Charge mutationnelle | Signature inflammatoire |
|---------------------|---------------------|------------------------------------|----------------------------|
| Technique | IHC | Séquençage | Signature d'expression |
| Matériel | 1-2 lames FFPE | Tissu congelé. FFPE (6-10 lames ?) | 2-3 lames FFPE |
| Technique | IHC sur lames | NGS, RNAseq, bioinformatique | Extraction ARN. Nanostring |
| Complexité | Faible | Elevée | Modérée |
| Délai | 3-5 jours | 2 semaines | 10 jours |
| Prédiction | modérée | Modérée-forte | Modérée-forte |
| Commentaires | Méthodes variables | Complexe (difficile en routine) | Quantitatif. Automatisé |

Synthèse des tests prédictifs

Biomarkers currently applied for NSCLC immunotherapy



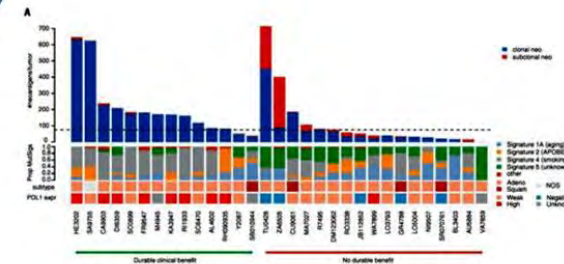
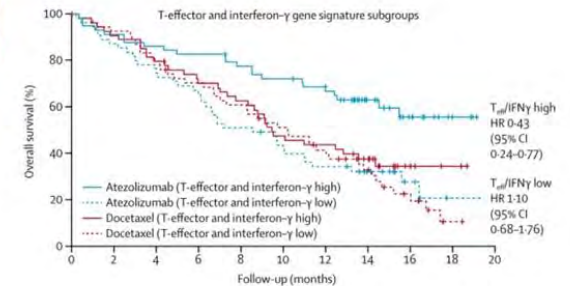
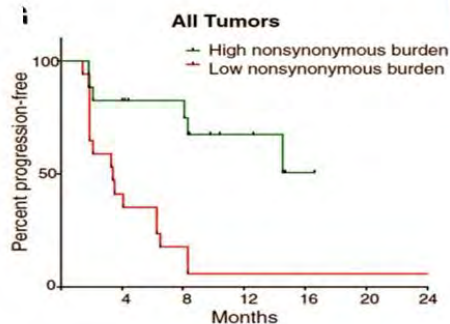
PD-L1 IHC



Surrogates of immune response
Immune cells
Gene signature

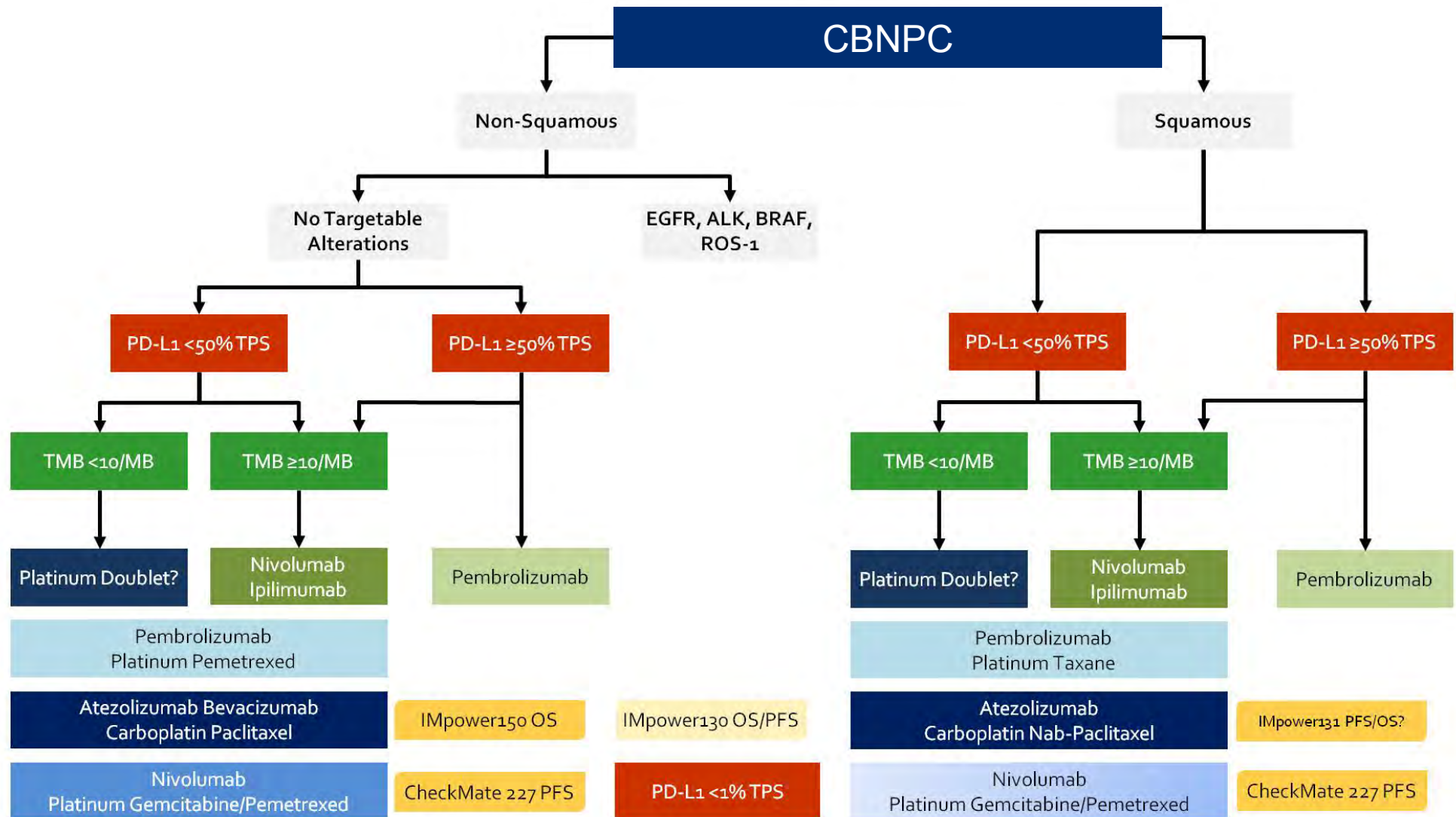
Mutational burden
or surrogate

Microsatellite instability



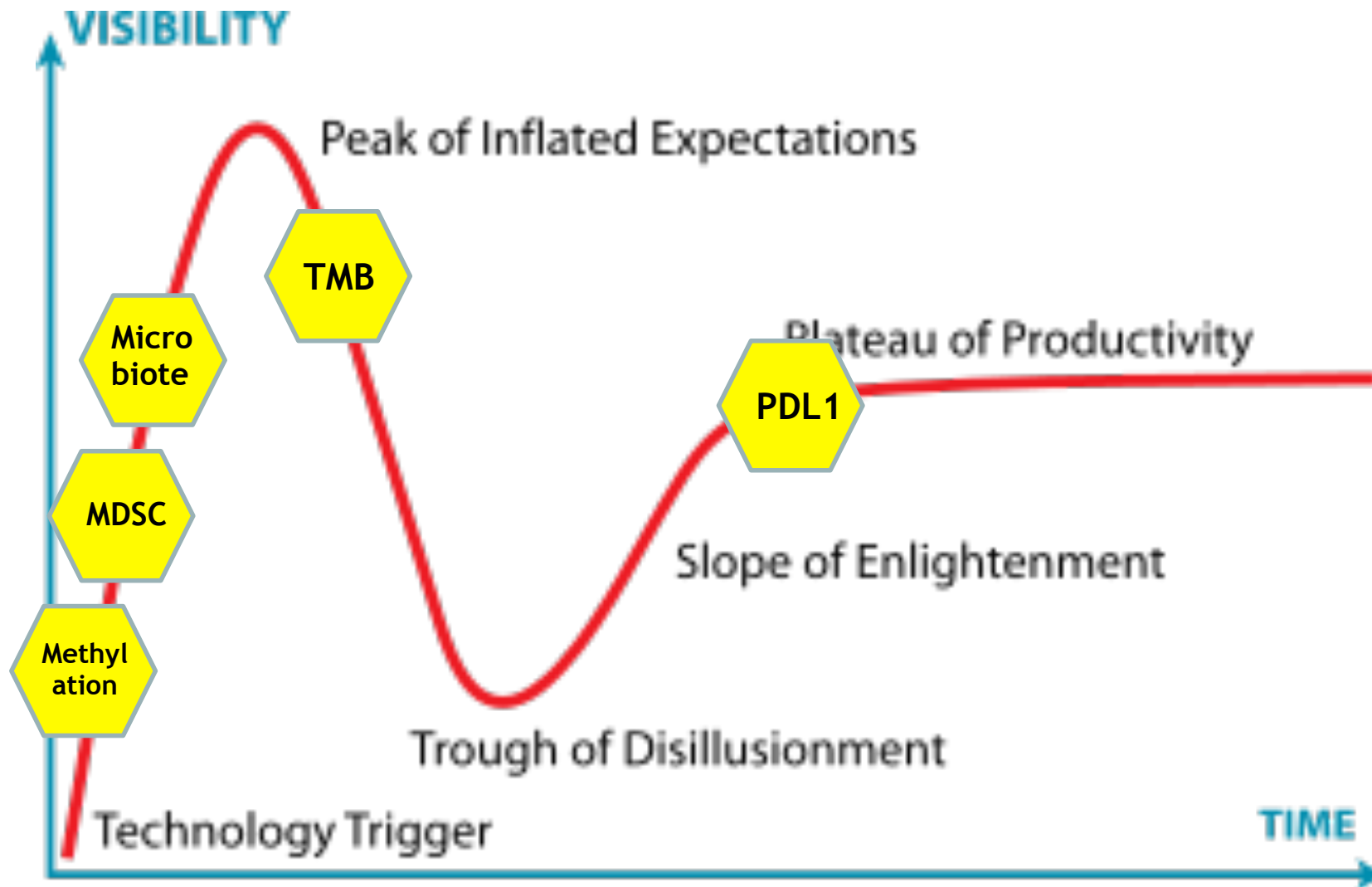
Rizvi, Science 2015; Fehrenbacher, Lancet 2016; McGranahan, Science 2016; Kerr, ASCO 2016

Conclusion



D'après Peters S, ASCO 2018

Conclusion



COURS DU GOLF

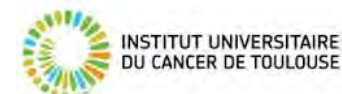
7 AU 10 OCTOBRE 2019

Groupe d'Oncologie de la Société de
Pneumologie de Langue Française



Comité d'organisation

J. Mazieres
L. Bigay-Game
L. Brouchet
A. Rabeau
I. Rouquette
J. Khalifa
C. Massabeau



TOULOUSE

Amphithéâtre Pierre Paul Riquet,
Hôpital Purpan.

Inscription
brunon.i@chu-toulouse.fr

Back-up

QUIZZ 1

- **Question 1: Quels tests ont montré leur intérêt dans un essai clinique d'immunothérapie en oncologie thoracique.**
- 1. Charge mutationnelle**
 - 2. LIPI score**
 - 3. Expression de PDL1**
 - 4. Lymphocyte T gene signature (Teff)**
 - 5. Microbiote digestif**

QUIZZ 1

- **Question 1: Quels tests ont montré leur intérêt dans un essai clinique d'immunothérapie en oncologie thoracique.**

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QUIZZ 2

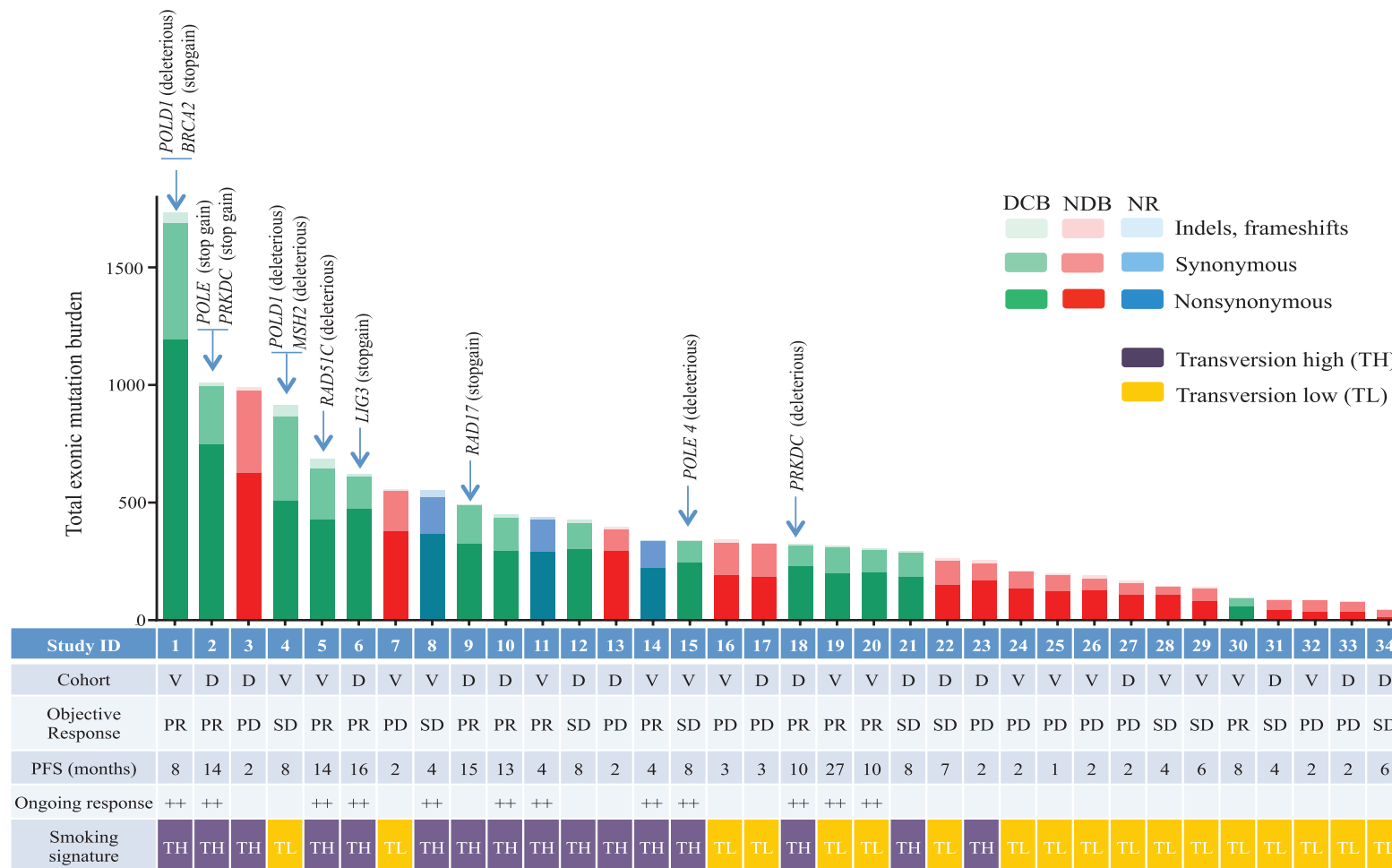
- **Question 2: Quel(s) test(s) est/sont validés en routine en oncologie thoracique.**
- 1. Charge mutationnelle**
 - 2. LIPI score**
 - 3. Expression de PDL1**
 - 4. Test Teff**
 - 5. Microbiote digestif**

QUIZZ 2

- **Question 2: Quel(s) test(s) est/sont validés en routine en oncologie thoracique.**
 1. Charge mutationnelle
 2. LIPI score
 - 3. Expression de PDL1**
 4. Test Teff
 5. Microbiote digestif

Charge mutationnelle et néo-antigènes

- Analyse de la réponse au pembrolizumab dans le CBNPC selon la « signature » mutationnelle.



Phase 2 data defining TMB threshold

| Cancer | Trial and treatment | Method | Threshold Defined | RR | PFS | OS | Ref. |
|------------|--------------------------------------|-----------|---|------------------------|---------------------------------|--------------------|---------------------------|
| NSCLC | KN 001 Phase 1/2 Pembrolizumab | WES | 200 mutations (median) | 63% vs. 0% 73% vs. 13% | 14.5 vs. 3.7 mo. NR vs. 3.4 mo. | | (Rizvi et al., 2015) |
| NSCLC | POPLAR Randomized Ph. 2 Atezolizumab | FM NGS | Atezolizumab vs. docetaxel in ≥ 9.9 Mut/Mb | 20% vs. 4% | 7.3 vs. 2.8 mo. | 16.2 vs. 8.3 mo. | (Kowanetz et al., 2016) |
| NSCLC | MSKCC: various immunotherapies | MSKCC NGS | 7.4 mut/MB (Median) | | 38.6 vs. 25% | | (H. Rizvi et al., 2018) |
| NSCLC | CM 588 Nivolumab/ipilimumab | FM NGS | 10 mut/Mb | 44% vs. 12% | 7.1 vs. 2.6 mo. | | (Ramalingam et al., 2018) |
| Urothelial | CM 275 Phase 2 Nivolumab | WES | ≥ 170 vs. < 85 mutations | 31.9% vs. 10.9% | 3 vs. 2 mo. | 11.63 vs. 5.72 mo. | (Galsky et al., 2017) |
| Urothelial | IMvigor210 Phase 2 Atezolizumab | FM NGS | 16 mut/Mb (Upper quartile) | | | OS advantage | (Balar et al., 2017) |
| HNSCC | KN 012 and KN 055 Pembrolizumab | WES | 175 mutations | | 0.64 | 0.98 | Seiwert et al, 2018 |

| Parameter | WES | FM NGS (F1CDx) | MSKCC NGS (MSK-IMPACT) |
|-----------------------|------------------------|----------------|------------------------|
| Validation in phase 2 | 200 missense mutations | 10 mut/MB | 7.4 mut/MB |

Issues with TMB in Routine Clinical Practice

- **Tissue requirements**
- **Turnaround time**
- **Lack of gold standard/validation**
- **Lack of a significant improvement in OS**
- **Cost (included on FM, some other panels)**
- **Is it specific or just predictive of utility with any immunotherapy?**

Not a standard of care...yet

@JackWestMD



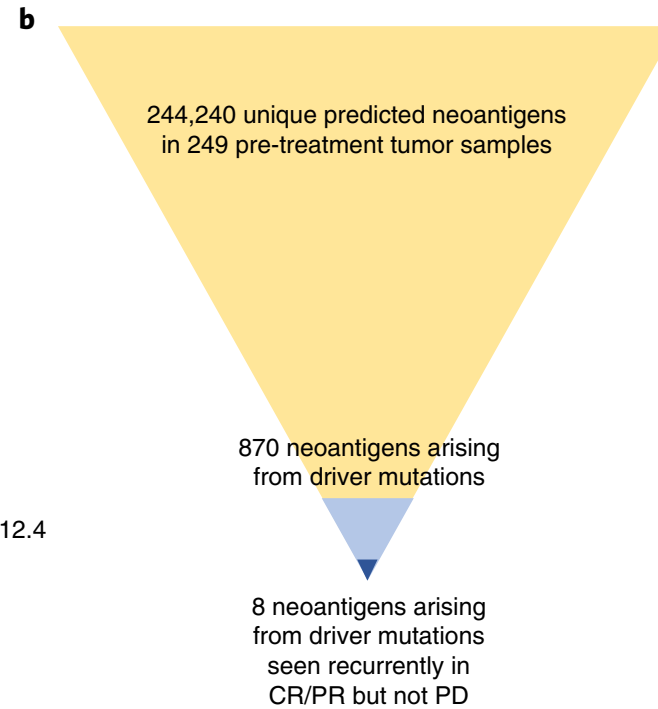
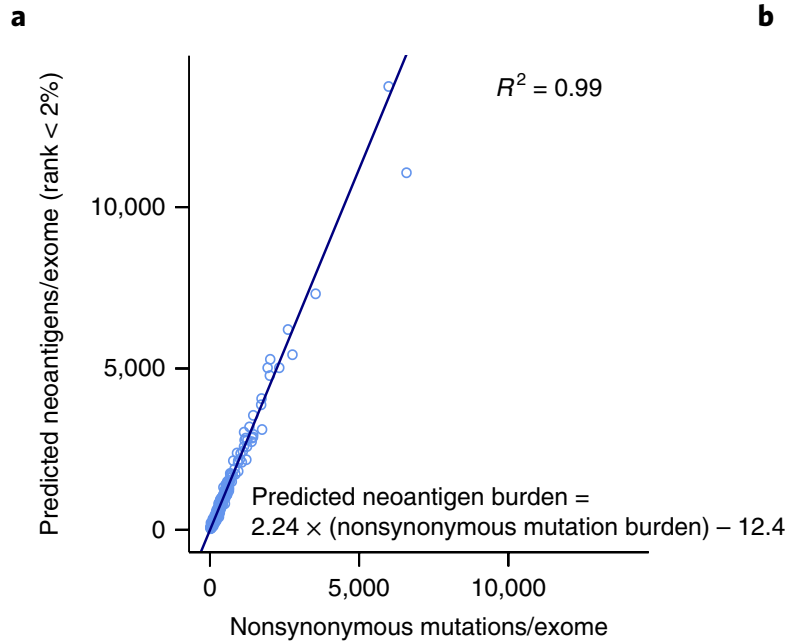
Charge mutationnelle et néo-antigènes

- Combien de gènes doit comporter le test TMB ?

| Company | Test name | Test description |
|--|---|---|
| Caris Life Sciences Inc. | Caris Molecular Intelligence CGP+ | Assay profiling mutations in tumor tissue using a 592-gene panel |
| Foundation Medicine Inc. (NASDAQ:FMI) (A) | FoundationOne CDx | Assay profiling mutations in tumor tissue using a 324-gene panel |
| Foundation Medicine Inc.; Roche (SIX:ROG; OTCQX:RHHBY) | bTMB assay | Assay profiling mutations in cell-free DNA in plasma using a 394-gene panel |
| Illumina Inc. (NASDAQ:ILMN) (A) | TruSight Tumor 170 | Assay profiling mutations in tumor tissue using a 170-gene panel |
| KEW Group Inc. | Cancerplex | Assay profiling mutations in tumor tissue using a >400-gene panel |
| Memorial Sloan Kettering Cancer Center (A) | MSK-IMPACT | Assay profiling mutations in tumor tissue using a 468-gene panel |
| NeoGenomics Inc. (NASDAQ:NEO) | NeoTYPE Discovery Profile | Assay combining NGS testing of 315 molecular markers and Tumor Mutation Burden (TMB) analysis |
| Personal Genome Diagnostics Inc. (A) | Unnamed panel | Assay profiling mutations in tumor tissue using a >500 gene panel |
| Qiagen N.V. (Xetra:QIA; NYSE:QGEN) (A) | GeneRead DNAseq Mix-n-Match Panels | Customizable assay profiling mutations using 570 primer sets |
| Thermo Fisher Scientific Inc. (NYSE:TMO) (A) | Ion Torrent OncoPrint Tumor Mutational Load Assay | Assay profiling mutations in tumor tissue using a 409-gene panel |

Biocentury, Mar 15, 2018

Garofalo et al. Genome Medicine. 2016



c

| Peptide | Mutation | Cancer type | HLA | Response |
|-------------|------------------------|---------------------|----------------|------------------|
| VVVGADGVGK | <i>KRAS</i> p.G12D | Bladder, lung | A03:01, A11:01 | 3 PR |
| GSFATVYK GK | <i>BRAF</i> p.G469A | Bladder, lung | A11:01, A03:01 | 2 PR |
| GSGSFATVYK | <i>BRAF</i> p.G469A | Bladder, lung | A11:01, A03:01 | 2 PR |
| SGSFATVYK | <i>BRAF</i> p.G469A | Bladder, lung | A11:01, A03:01 | 2 PR |
| SEITKQEKDF | <i>PIK3CA</i> p.E545K | Bladder, lung, anal | B44:02, B44:03 | 1 CR, 1 PR, 1 SD |
| RHGGWTTKM | <i>PIK3CA</i> p.H1047R | Melanoma, HNSCC | C07:01, C07:02 | 2 PR, 1 SD |
| ARHGGWTTKM | <i>PIK3CA</i> p.H1047R | Melanoma, HNSCC | C07:01, C07:02 | 2 PR, 1 SD |
| KLVVVGACGV | <i>KRAS</i> p.G12C | Lung | A02:01 | 2 PR, 2 SD |

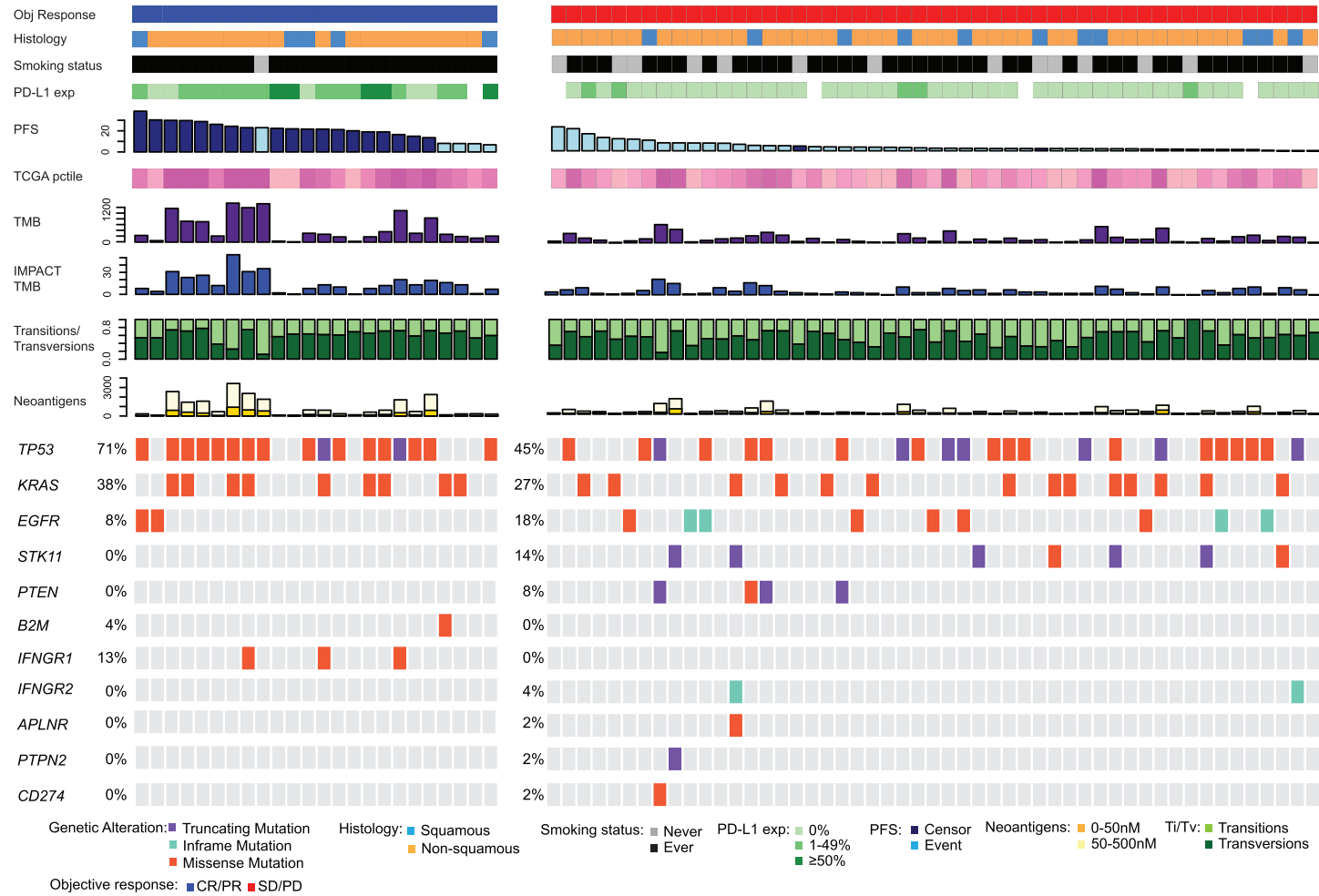


Figure 2. Summary of Clinical and Molecular Features Associated with Response or Non-response in Patients with NSCLC Treated with Nivolumab Plus Ipilimumab

Génétique et immunothérapie

Cancer Therapy: Clinical

Clinical
Cancer
Research

EGFR Mutations and ALK Rearrangements Are Associated with Low Response Rates to PD-1 Pathway Blockade in Non-Small Cell Lung Cancer: A Retrospective Analysis

Justin F. Gainor¹, Alice T. Shaw¹, Lecia V. Sequist¹, Xiujun Fu², Christopher G. Azzoli¹, Zofia Piotrowska¹, Tiffany G. Huynh², Ling Zhao², Linnea Fulton¹, Katherine R. Schultz¹, Emily Howe¹, Anna F. Farago¹, Ryan J. Sullivan¹, James R. Stone², Subba Digumarthy³, Teresa Moran⁴, Aaron N. Hata¹, Yukako Yagi², Beow Y. Yeap¹, Jeffrey A. Engelman¹, and Mari Mino-Kenudson²

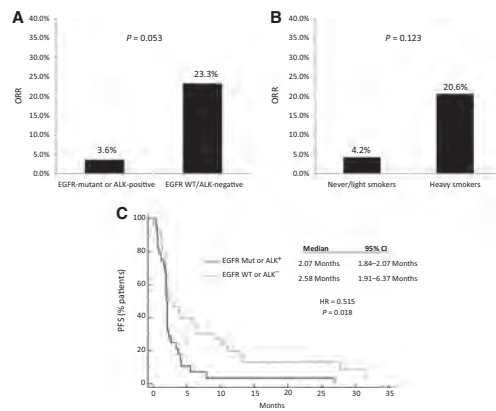


Figure 1. **A**, unconfirmed/confirmed ORRs to PD-1/PD-L1 inhibitors comparing EGFR-mutant (Mut) or ALK-positive NSCLC patients with EGFR wild-type and ALK-negative/unknown patients. **B**, ORRs to PD-1/PD-L1 inhibitors of never- or light-smokers versus heavy smokers (>10 pack years). **C**, PFS on PD-1/PD-L1 inhibitors based upon EGFR mutation or ALK rearrangement status.

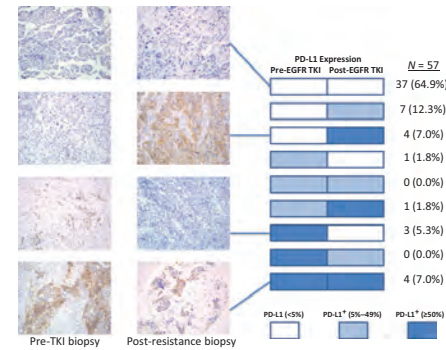
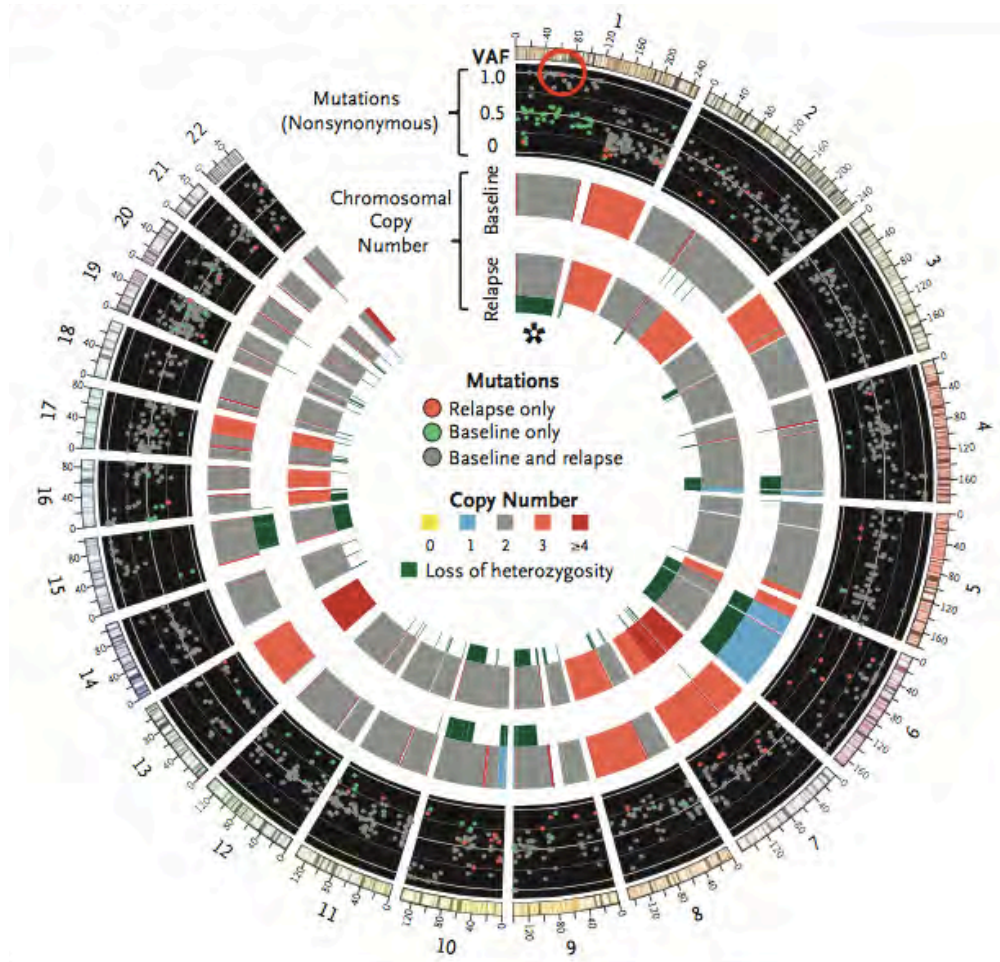


Figure 2. PD-L1 expression levels in paired, pre- and post-TKI biopsies among EGFR-mutant patients along with representative PD-L1 immunohistochemical images. A majority of EGFR-mutant patients (72%) exhibited consistent PD-L1 staining across both specimens, but 16 (28%) patients demonstrated variable staining across biopsies.

Mutations de résistance aux IO

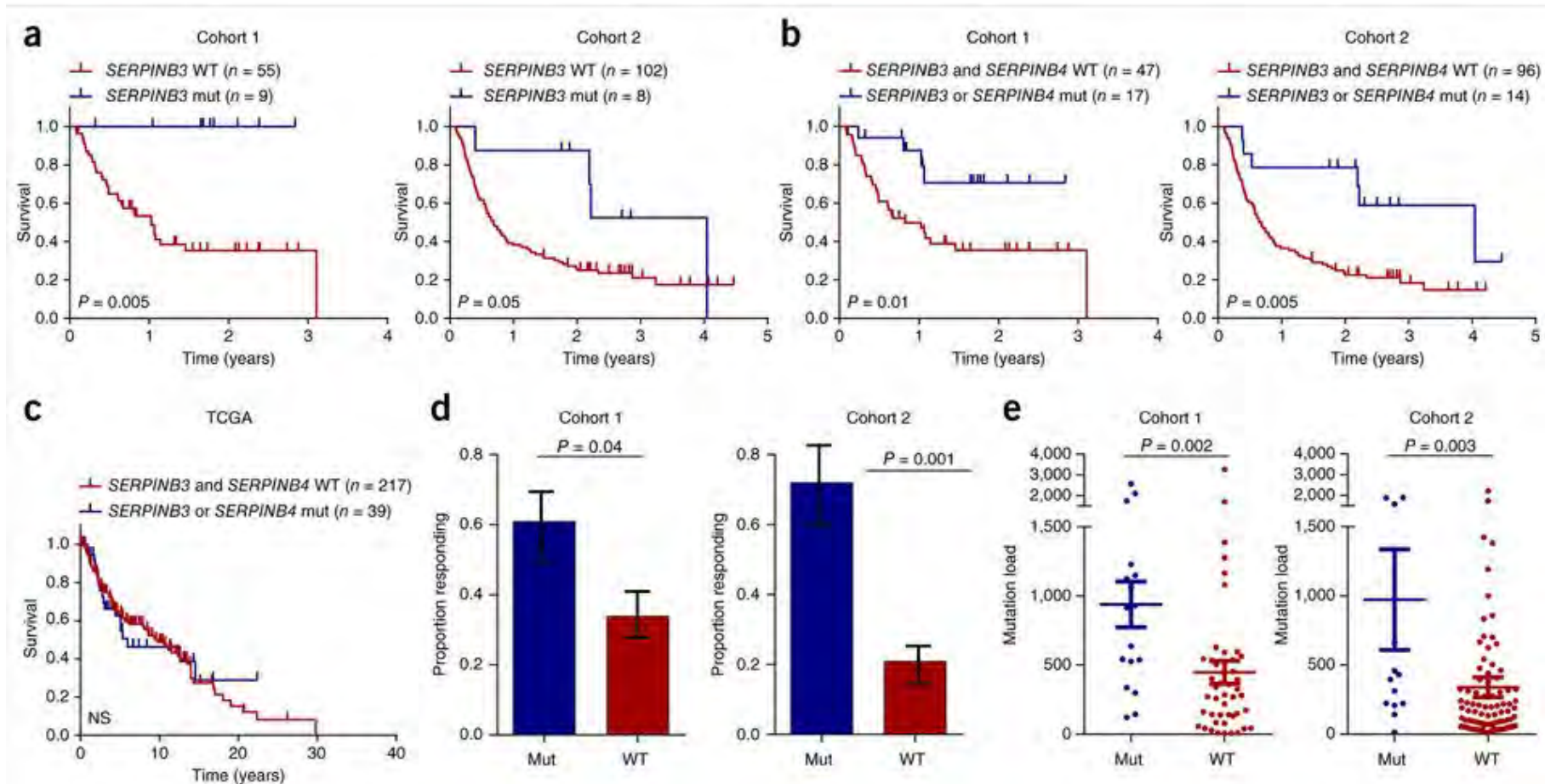
- Analyse de la résistance au pembrolizumab dans le CBNPC selon la « signature » mutationnelle (mélanome).



- Apparition de néo-mutations
- Acquisition de la mutation JAK1/JAK2
- Induction de la résistance à l'interféron gamma et la présentation antigénique

Mutations de résistance aux IO

- Rôle prédictif de la SERPIn B3 et B4 dans la réponse aux anti-CTLA4 (mélanome).
- Serpin: homologues de l'antigène ovalbumine (auto-immunité)



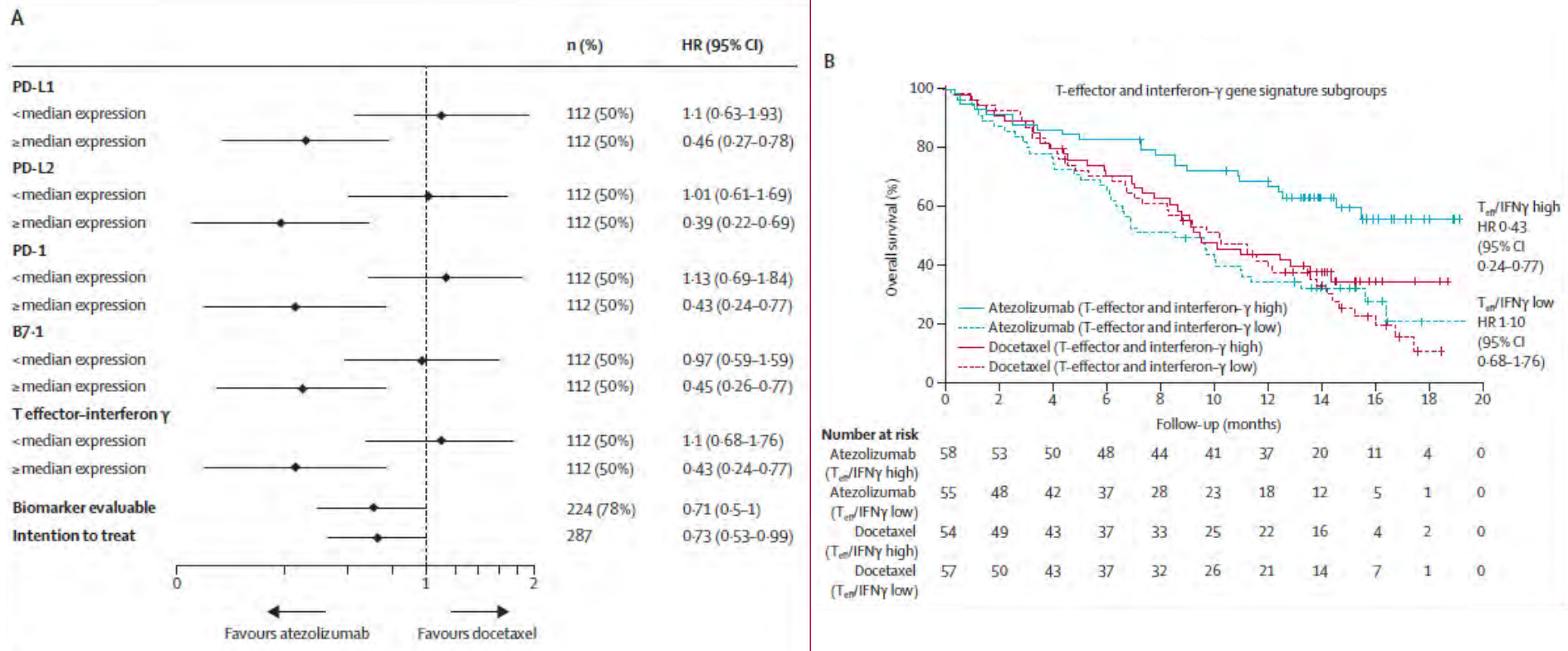
(a) Overall survival of patients with *SERPINB3* mutations in cohort 1 ($n = 64$) and cohort 2 ($n = 110$). WT, wild type; mut, mutant. (b) Overall survival of patients

Statut immunologique

- Analyse de différents marqueurs de la réponse immunologique (présentation des Ag, priming et activation)
 - Cytokines proinflammatoires (e.g., TNF- α , IL1, IFN- α)
 - Cofacteurs cellulaires: CD40L/CD40
 - Adjuvants endogènes libérés par les tumeurs: CDN (STING ligand), ATP, HMGB1
 - Produits du microbiomes digestif: TLR ligands
 - Interleukines : IL-10, IL-4, IL-13
 - Prostaglandines
 - Maturité des cellules dendritiques

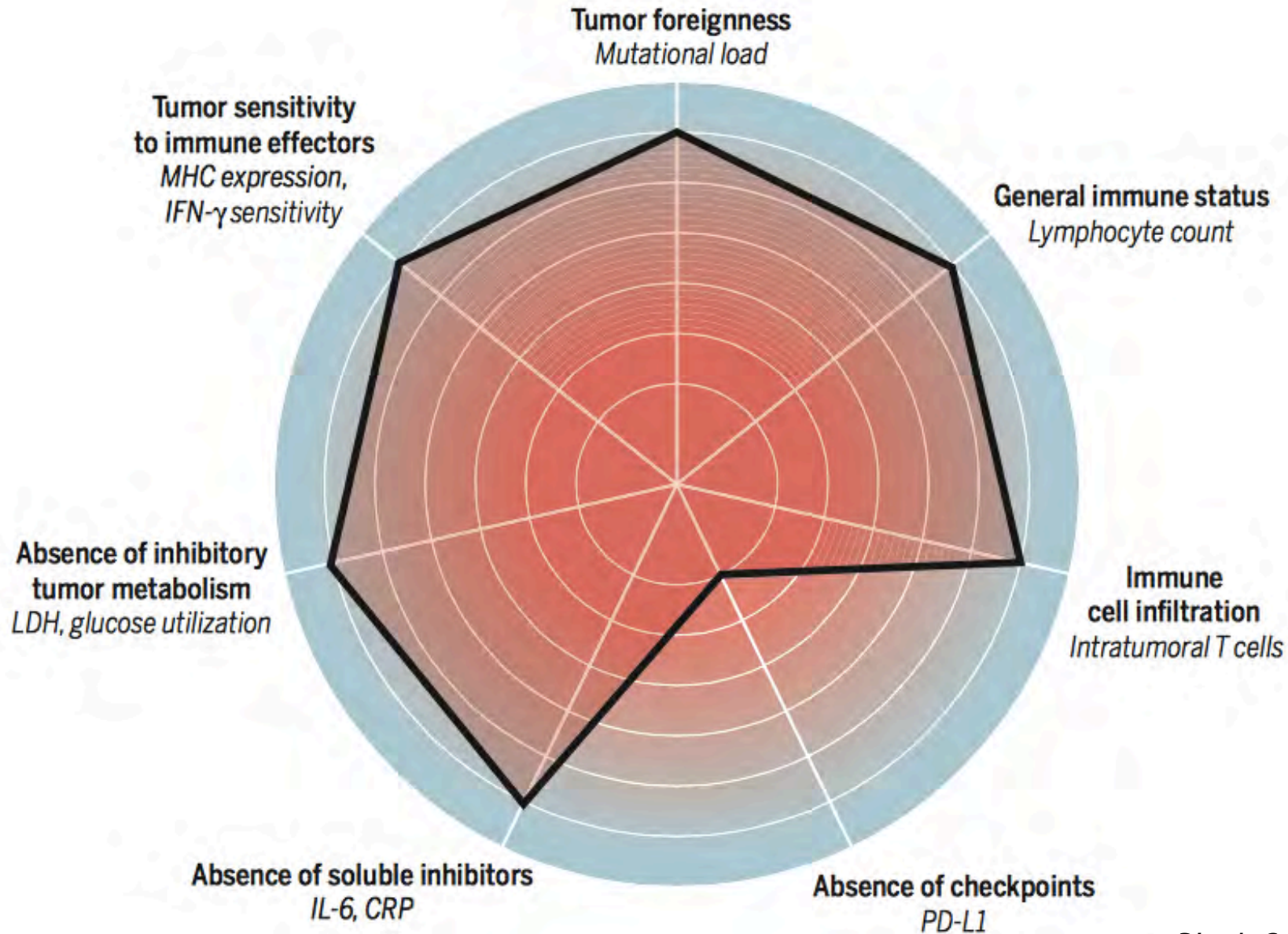
Statut immunologique

- Analyse de biomarqueurs sériques dans le sang
- Etude de phase 2 atezolizumab vs docetaxel
- Intérêt de l'interféron gamma

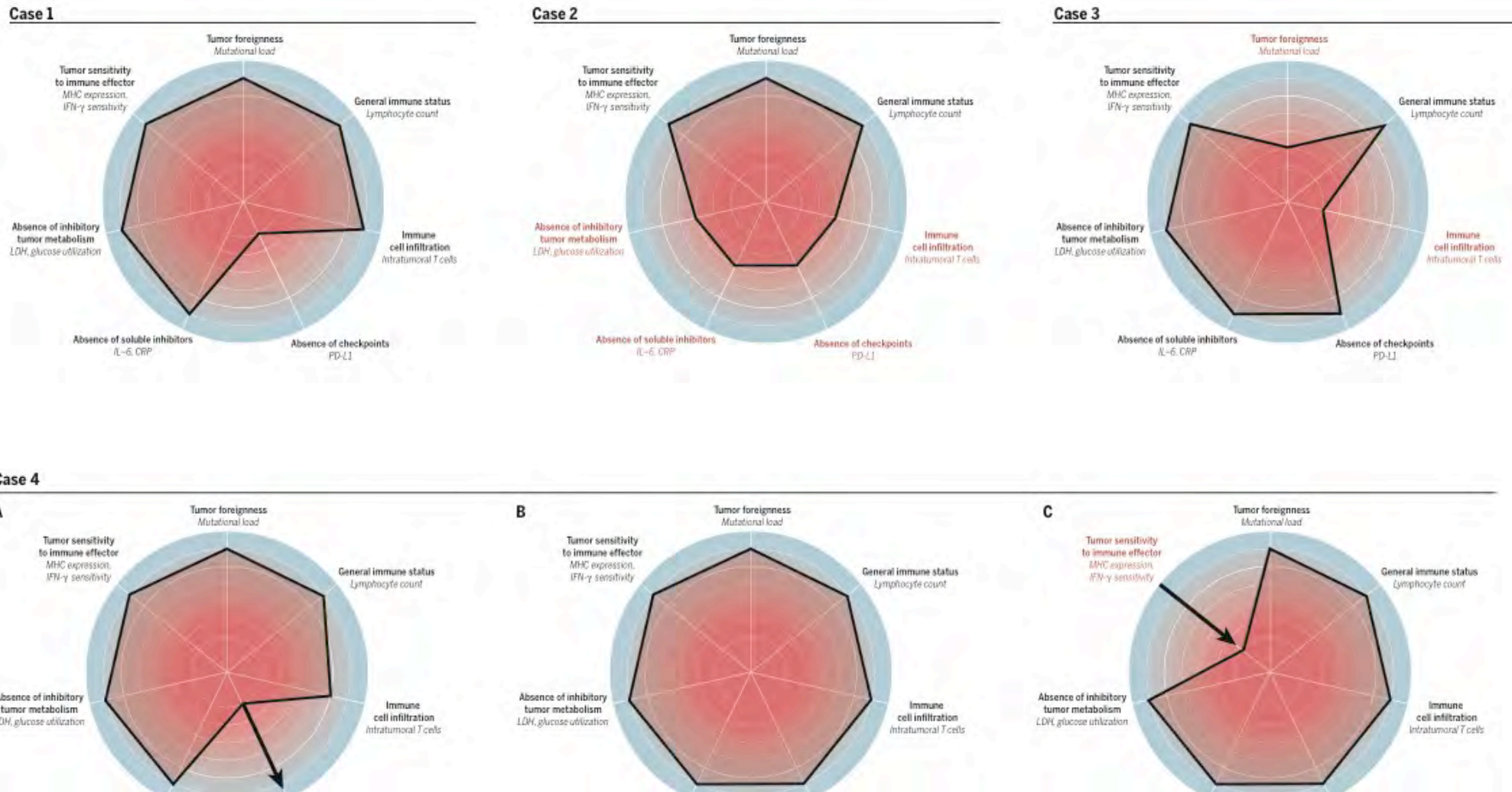


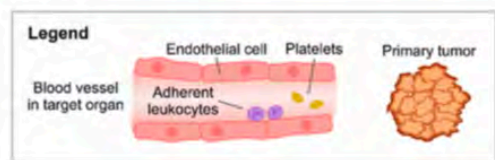
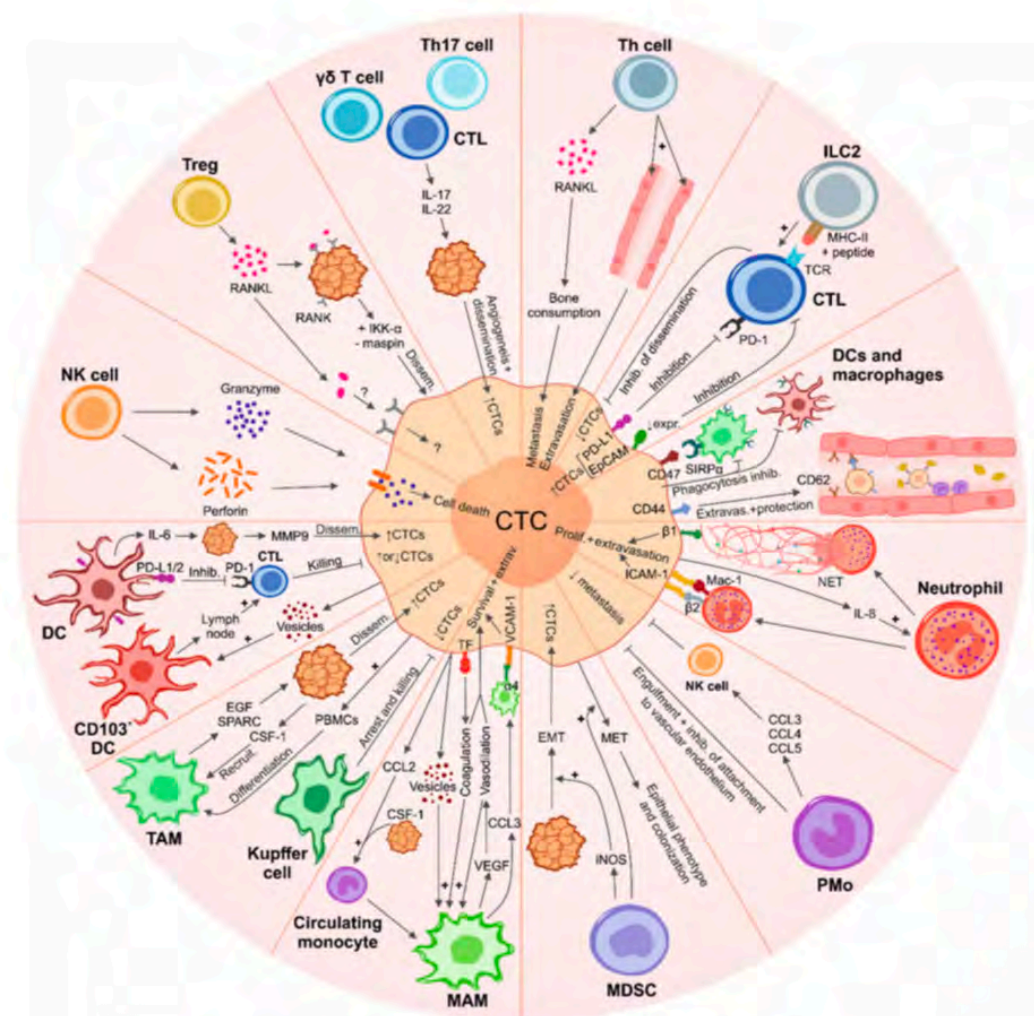
(Fehrenbacher L, Lancet 2016)

« The cancer immunogram »



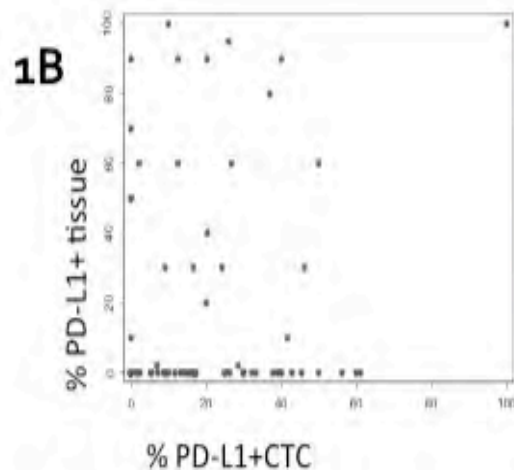
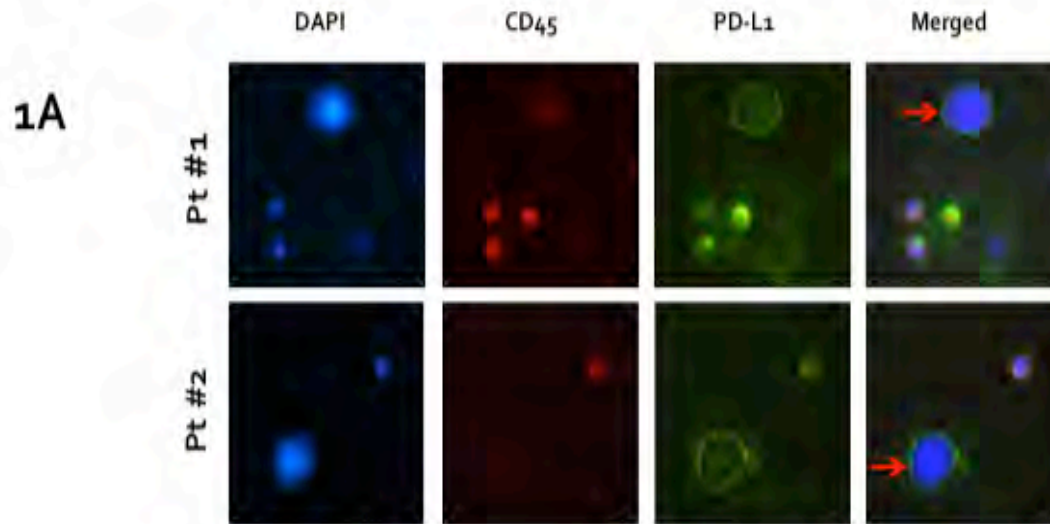
« The cancer immunogram »





Immunothérapie en oncologie thoracique

PDL1 dans le sang: CTC



1C

| Baseline PD-L1 expression on Tissue (IHC) (n=69) | | | Baseline PD-L1 expression on CTCs (IF) (n=89) | | |
|---|----|-----|--|-------|-----|
| % PD-L1+ tissue | n | % | % PD-L1+ CTC | n | % |
| 0% | 41 | 59% | 0% | 15 | 17% |
| ≥1% | 28 | 41% | ≥1% | 74 | 83% |
| ≥5% | 25 | 36% | ≥5% | 71 | 80% |
| ≥50% | 16 | 23% | ≥10% | 61 | 68% |
| Correlation Tissues/CTCs | | | | | |
| PD-L1 tissue (%) | | | | | |
| PD-L1 CTC (%) | 0% | ≥1% | ND | Total | |
| 0% | 8 | 5 | 2 | 15 | |
| ≥1% | 31 | 22 | 21 | 74 | |
| ND | 2 | 1 | 4 | 7 | |
| Total | 41 | 28 | 27 | 96 | |

Immunothérapie en oncologie thoracique

PDL1 dans le sang: CTC

