IV Simposio

GETHI

Secuenciación masiva en la predicción de respuesta a inmunoterapia: del mutation burden a los neoantígenos y más allá

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CENTRO INTEGRAL ONCOLÓGICO hm CIOCC

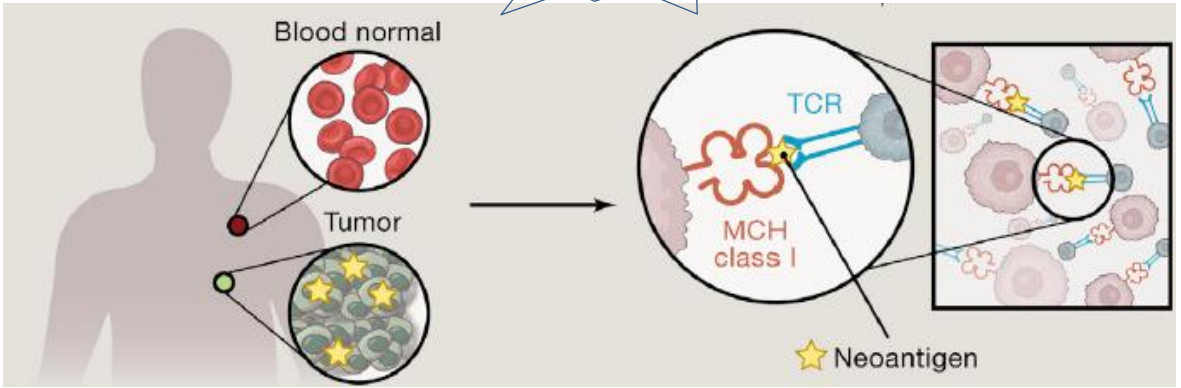
Cancer immunotherapy

Only a fraction of patients respond

Tumor
(neoantigens)

immune environment

Host
(immune response)



Biomarkers?

High mutation load (neoantigens)

Gut microbiota

Mutations in specific genes (MMR-MSI)

Clonality, intratumor heterogeneity

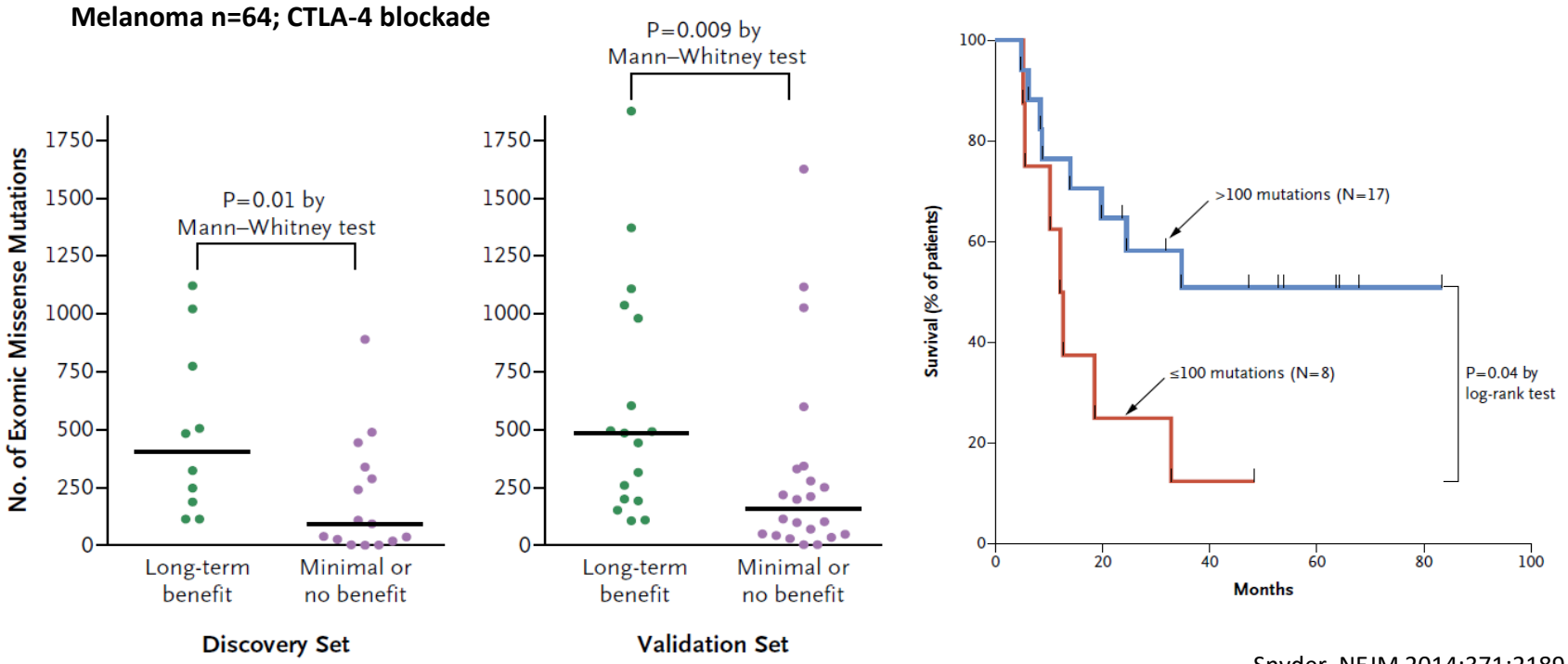
Genetic variation immune response genes

PD-L1 expression (tumor & normal tissues)

Tumor infiltrating lymphocytes

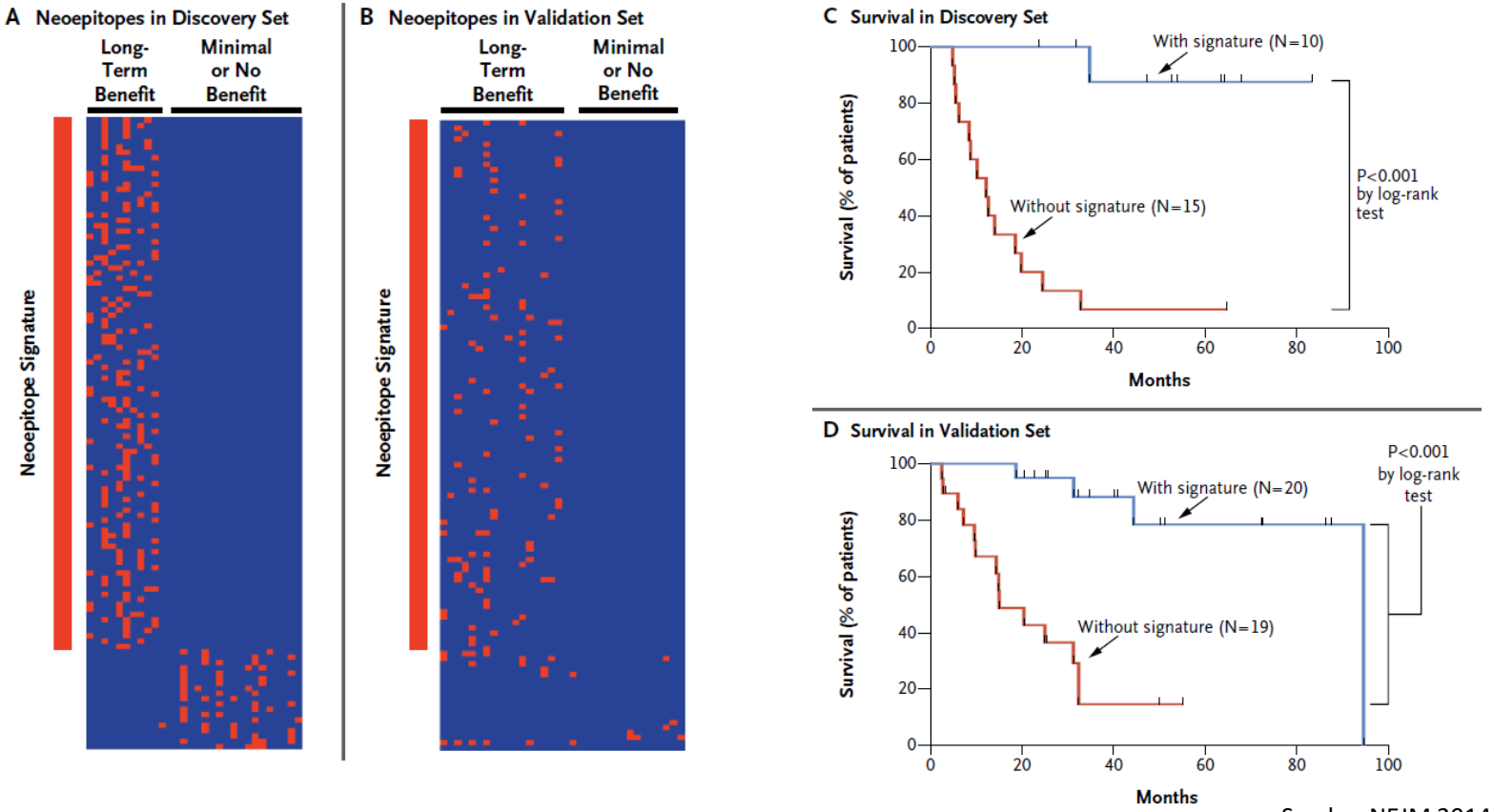
Tumor mutation burden (TMB)

Immune checkpoint inhibitors are active in tumors with high somatic mutation rates (melanoma, NSCLC, bladder cancer)



Snyder, NEJM 2014;371:2189

Tumor neoantigens

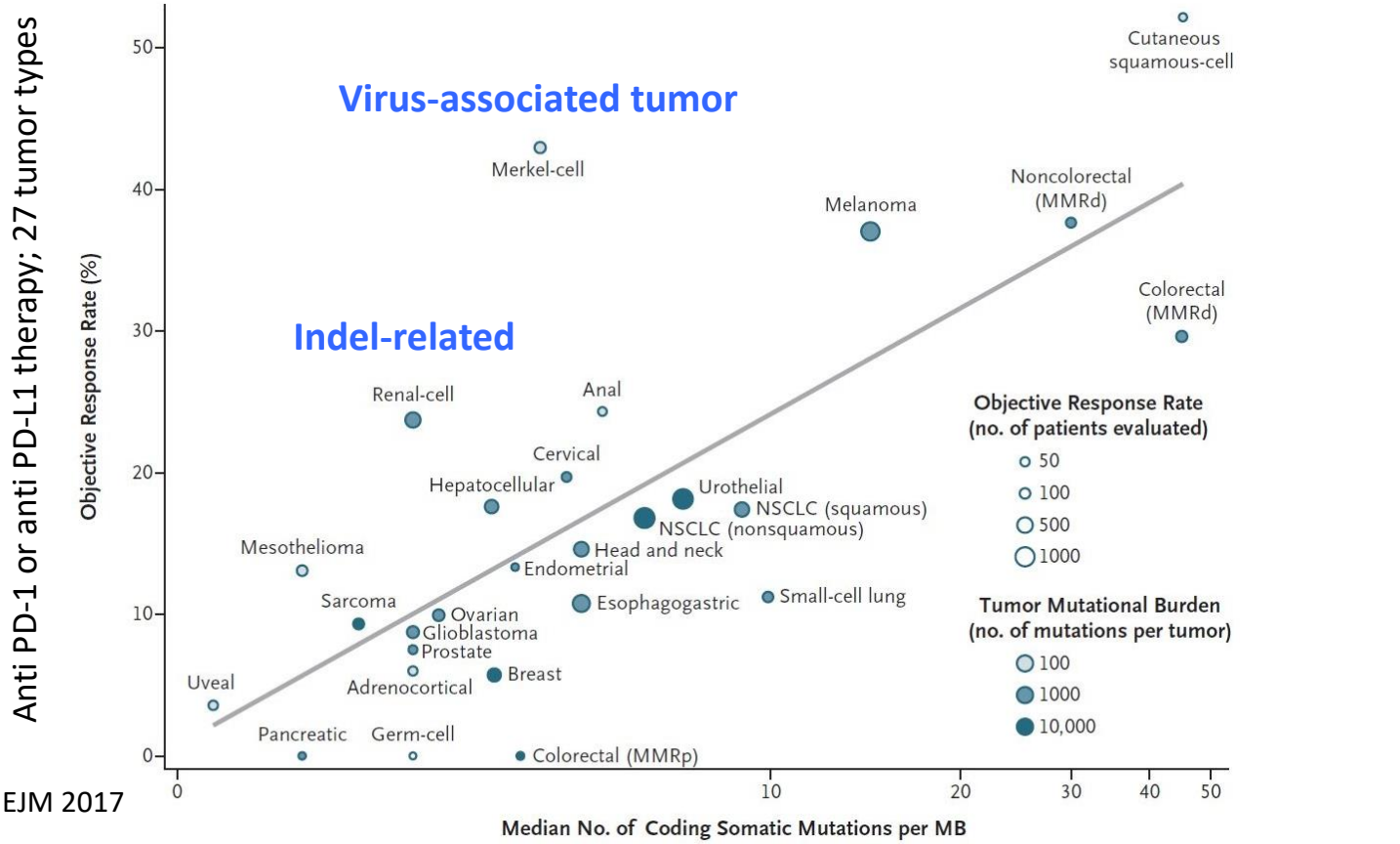


Snyder, NEJM 2014;371:2189

Replication: Van Allen, Science. 2015;350:207

TMB and checkpoint immunotherapy response

(TMB = nr of somatic mutations within the coding region of a tumor)



Yarchoan et al, NEJM 2017

TMB and checkpoint immunotherapy response

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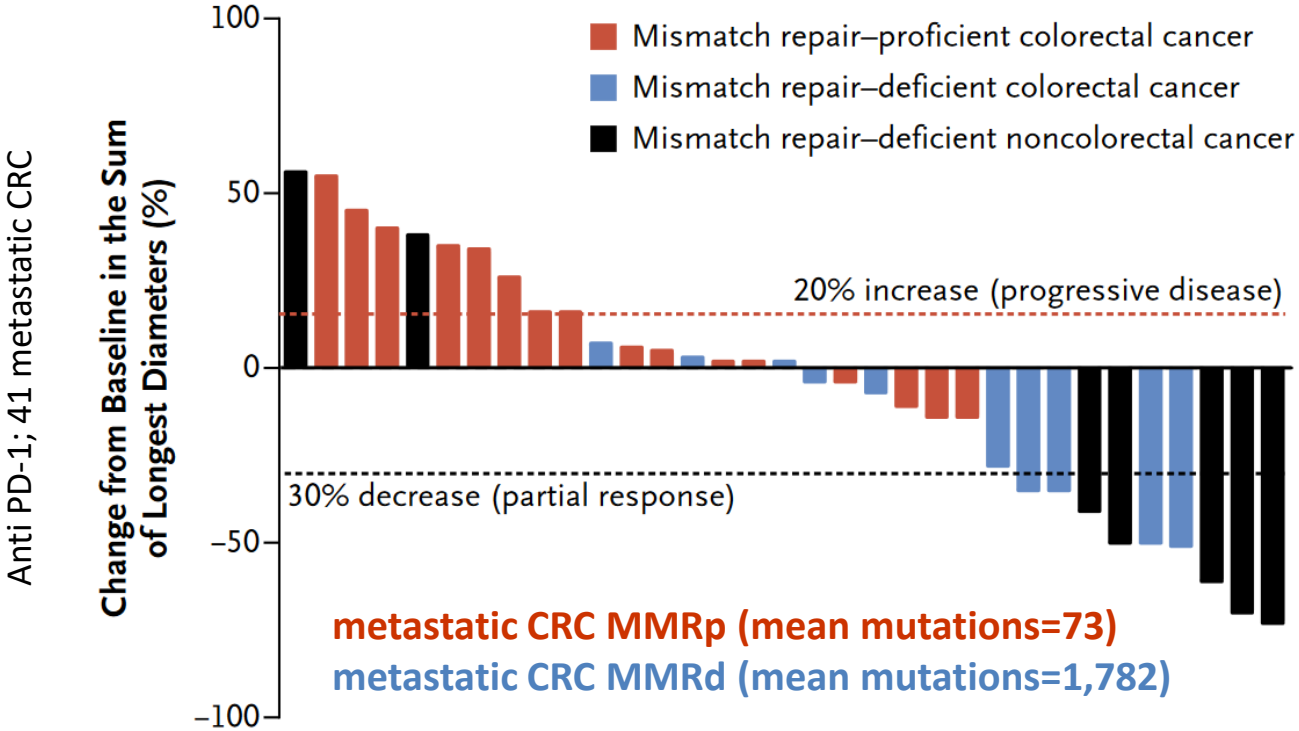
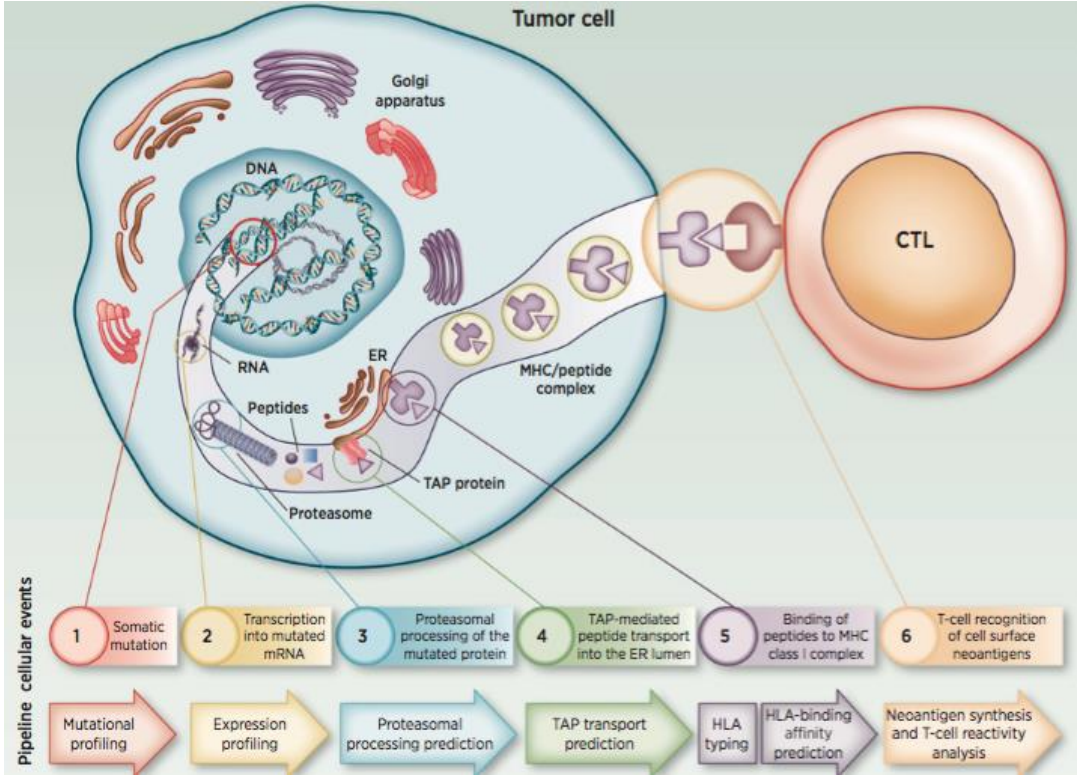


Le et al, NEJM 2015; 372(26): 2509

Rationale

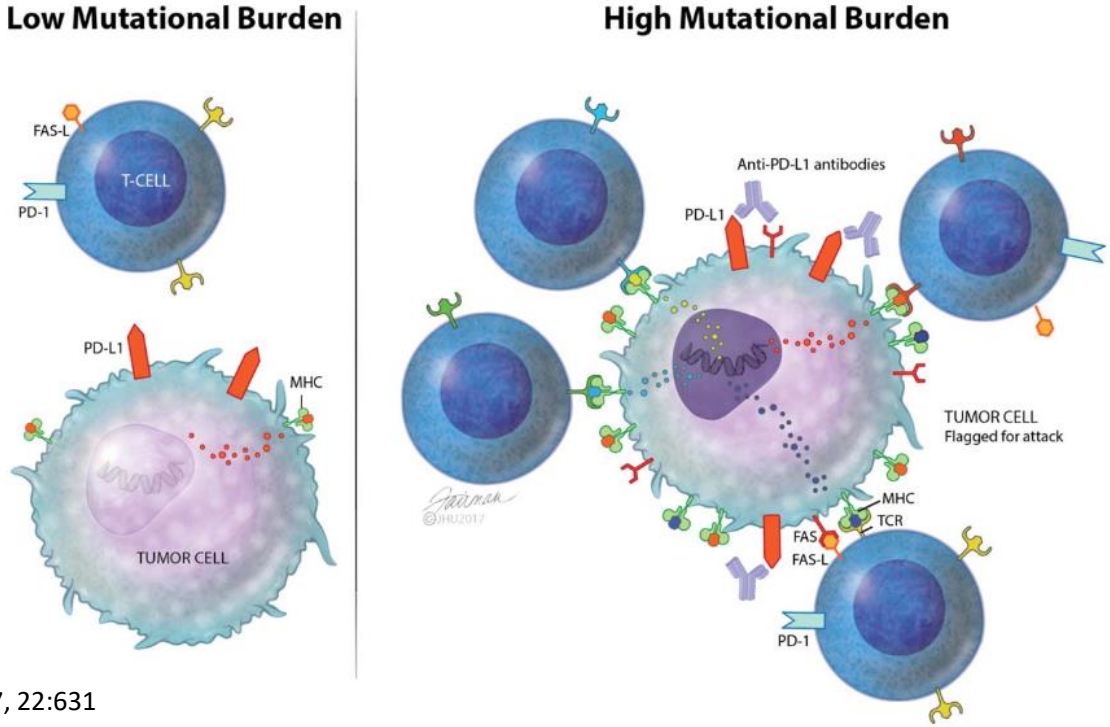
Nonsynonymous mutations can generate neoantigens recognized by the immune system, leading to antitumor immune response



CCR; 22(17), 2016

Rationale

High TMB tumors have the potential to generate larger nr of neoantigens, making them more immunogenic → **TMB is a surrogate for nr of neoantigens**



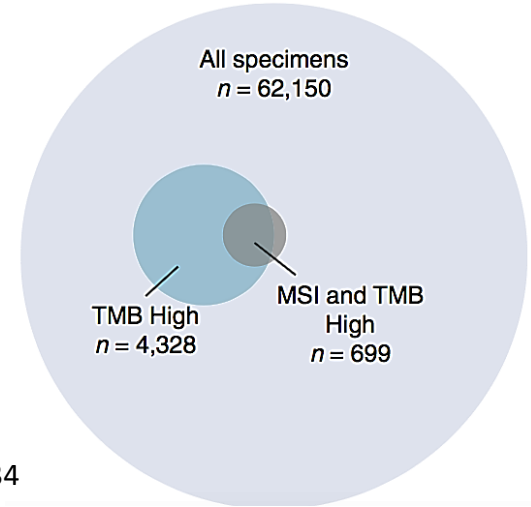
Sharabi, The Oncologist 2017, 22:631

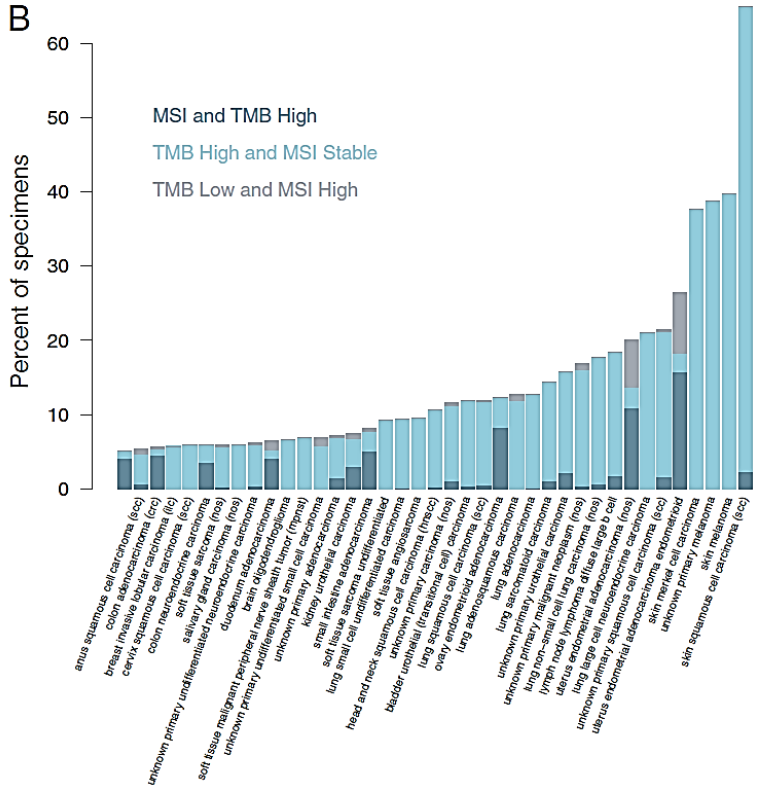
Causes for high TMB

- **Age** (2.4-fold increase 10 to 90 y)
- **Mutagens** (e.g. UV light, tobacco smoke)
- **Genetic defects: germline/ somatic (therapy)**

  DNA damage repair *PMS2, MLH1, MSH2, MSH6*

  DNA replication *POLE, POLD1*

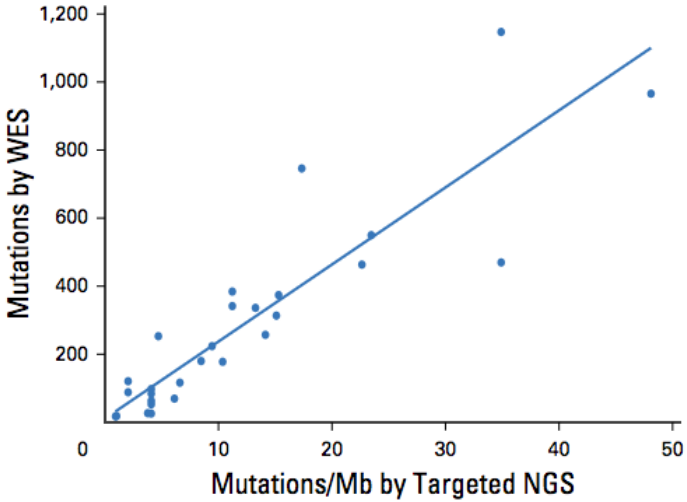




Chalmers, Genome Medicine 2017, 9:34

TMB as biomarker for immunotherapy response

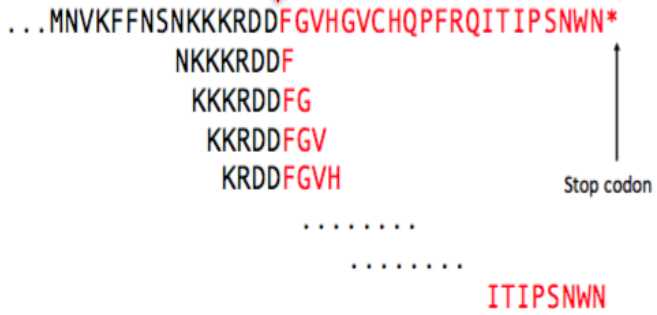
Comparable among labs? → **Standardization**



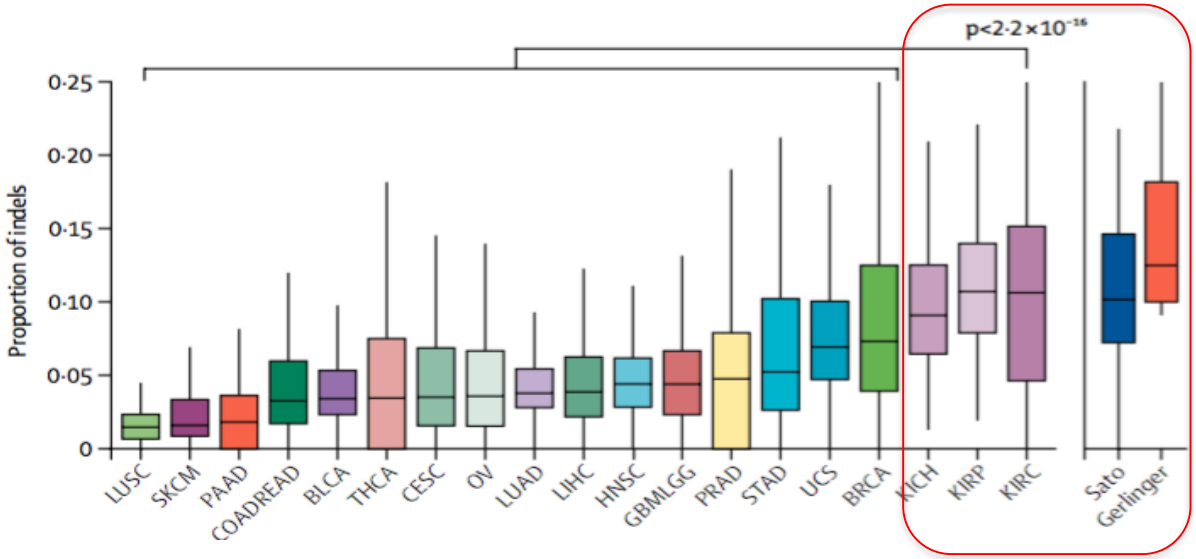
- WES (gold standard) vs NGS panel
- NGS panel: size (>1Mb), genes included
- Tumor content, sequencing depth
- Frozen vs FFPE (artifacts)
- Bioinformatic pipeline (germline?, non-synonymous only?, indels?, AF/clonality?…)
- Clinically relevant threshold (quantitative?, tumor type dependent?): >400 mut/20 per Mb?

Frameshift indels generate highly immunogenic neoantigens

Frameshift INDEL



Are these expressed?



pan-cancer TCGA cohort

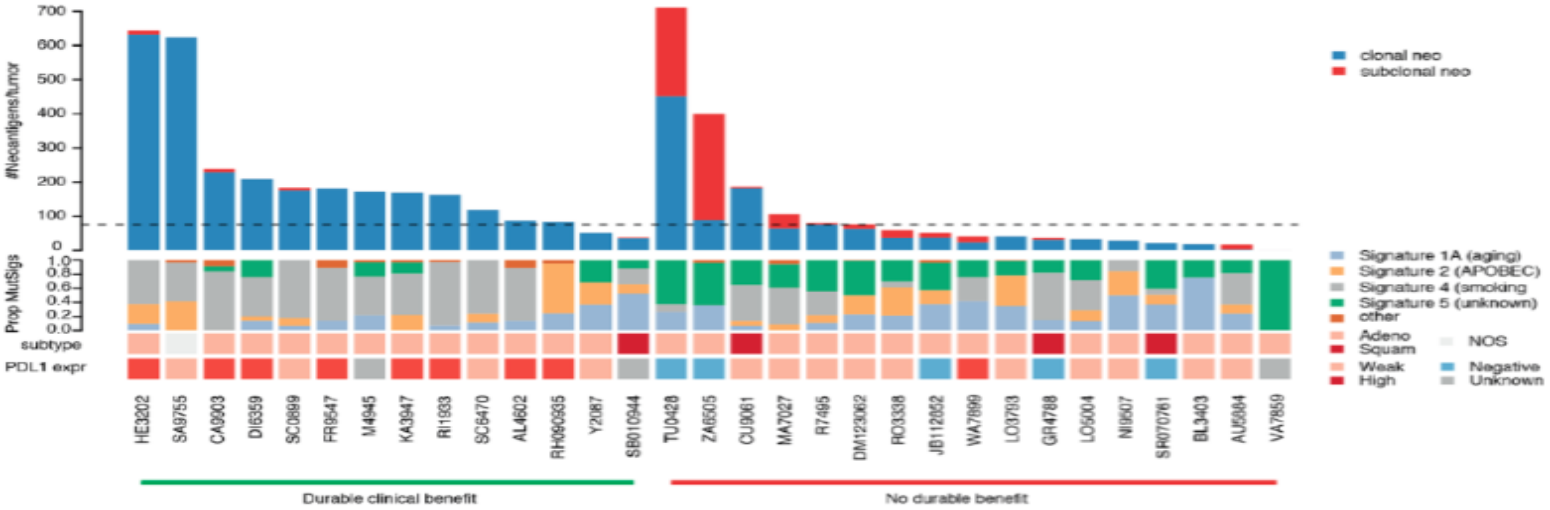
5777 solid tumors, 19 cancers

	Mutations (n)	Neoantigens (n)*	Mutant-specific neoantigens (n)†	Neoantigens per mutation	Mutant-specific neoantigens per mutation
nsSNVs	335 594	214 882	75 224	0·64	0·22
fs-indels	19 849	39 768	39 608	2·00	2·00
Enrichment	..	..	..	3·13	8·94

Turajlic et al, Lancet Oncology 2017

Intratumor heterogeneity

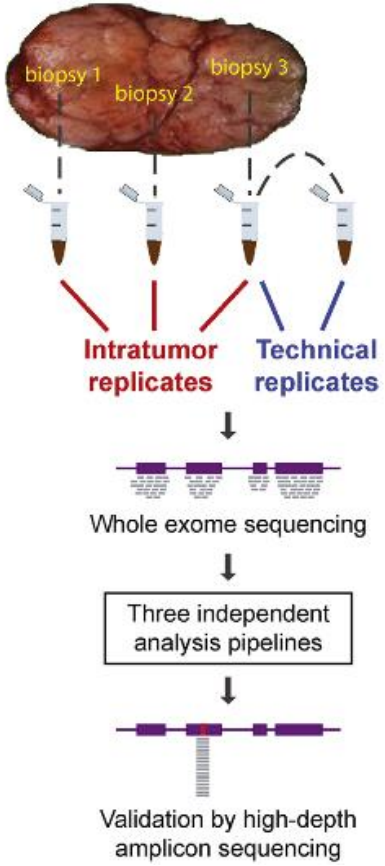
Checkpoint inhibitor response enhanced in tumors enriched for clonal neoantigens



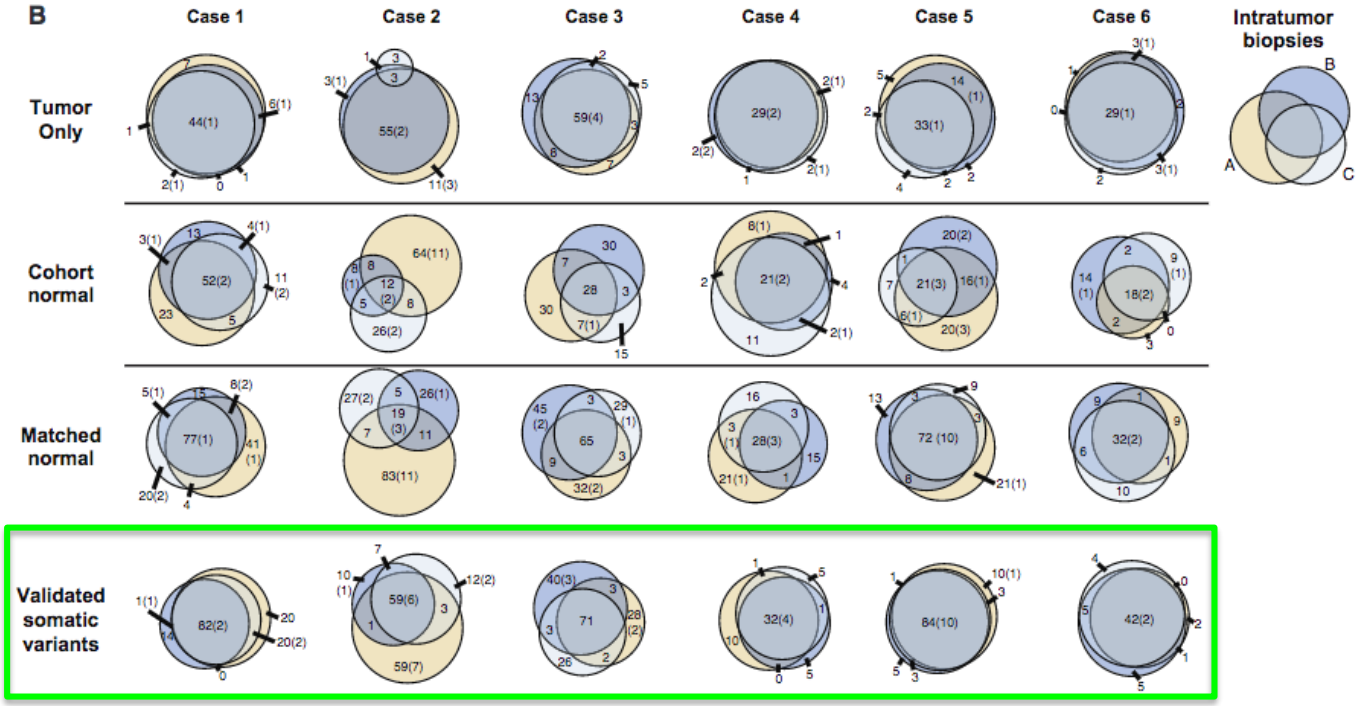
Neoantigen heterogeneity may influence immune surveillance and support therapeutic developments targeting clonal neoantigens

McGranahan, Science. 2016;351:1463

Intratumor heterogeneity hard to distinguish from sequencing artifacts

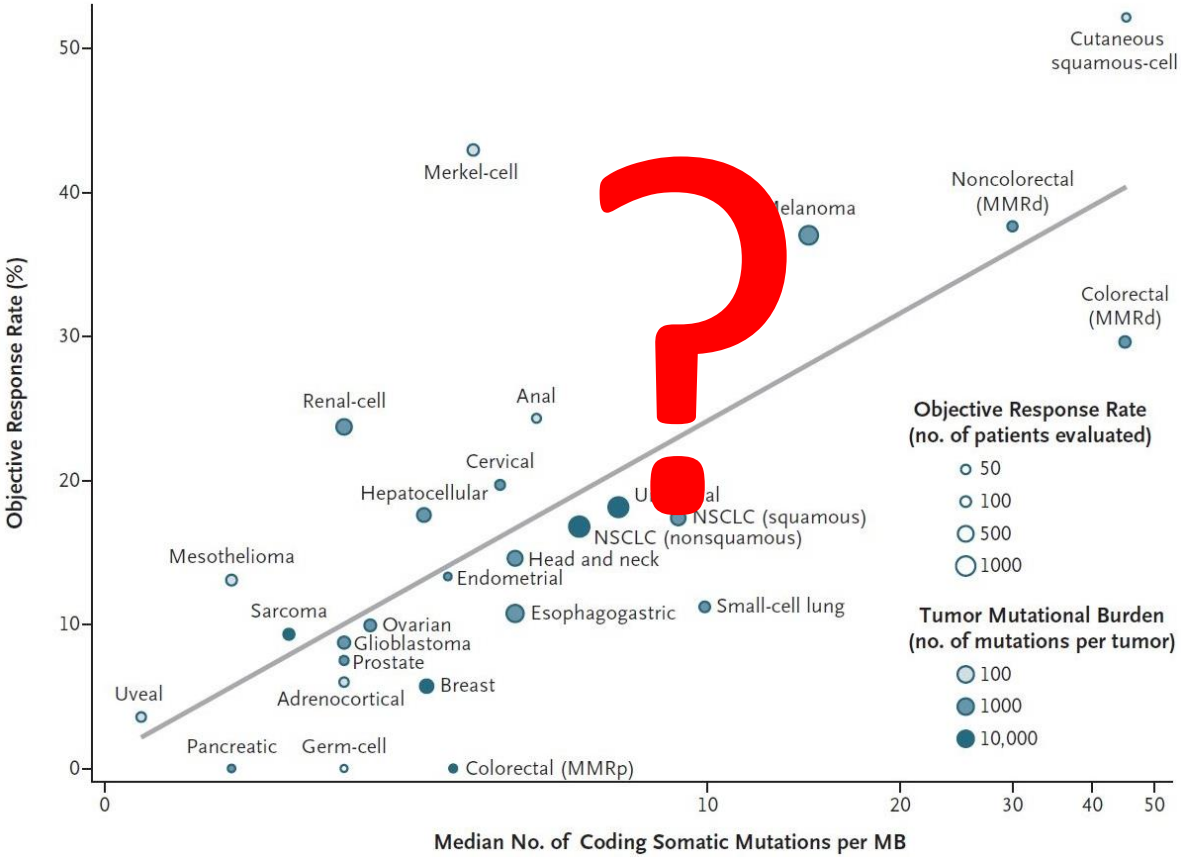


<u>34%</u> - 80% of somatic variants contributing to genetic heterogeneity are technical noise



Shi et al. Cell Rep. 2018 Nov 6;25(6):1446-1457

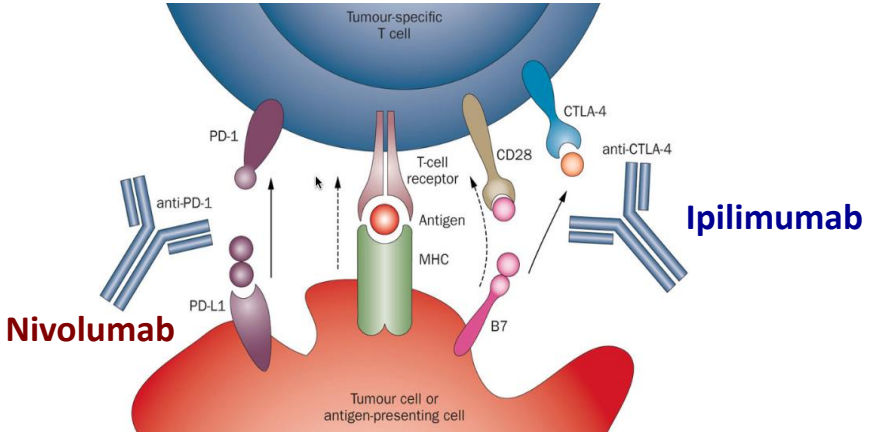
Where do rare cancers lie?. Will they respond to immunotherapy?



Nivorare study

Multicenter phase 2 study of nivolumab combined with ipilimumab in patients with pediatric solid tumors presenting in adulthood

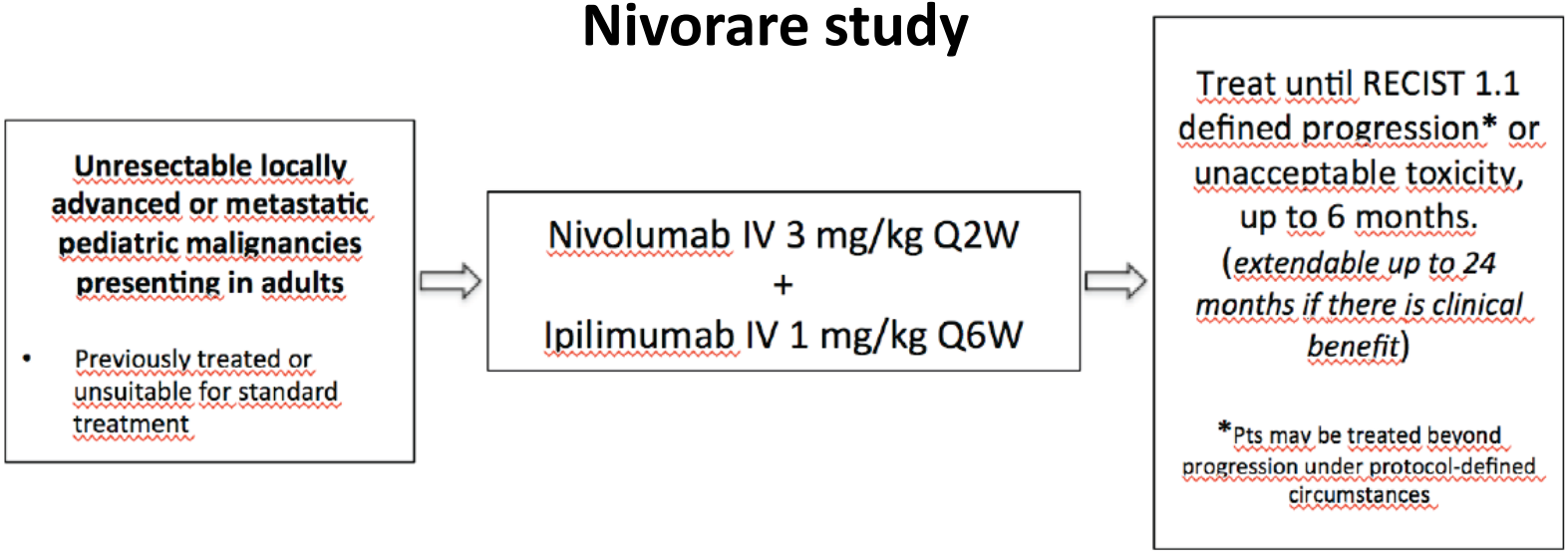
- o Medulloblastoma
- o Hepatoblastoma
- o Neuroblastoma
- o Wilms' tumor
- o Retinoblastoma
- o Pinealoblastoma
- o Pancreatoblastoma
- o Askin's tumor
- o Langerhans cell histiocytosis
- o Other pediatric malignancies



Trial conducted with support of the Spanish Group for Infrequent and Orphan Tumors

grupo Gethi
Grupo Español de Tumores Huérfanos e Infrecuentes

Nivorare study



Objectives

- Primary: activity in terms of objective response rate
- Secondary: PFS, OS, Disease control rate, Time to response, Duration of Overall Response, Time to progression, Time to Treatment Failure, Toxicity profile, QoL
- **Exploratory: identify molecular profiles able to predict patients response to treatment**

Number of patients: 30 and up to 89, depending on response rate

Nivorare exploratory objective: organization

Xabier Mielgo Rubio
Jesus Garcia-Donas

Clinical Database

- Tumor samples
- Lymphocytes
- Plasma

- Lymphocytes at:
  - Baseline
  - Week 9
  - Week 13
  - Disease Progr.

CNIO

Whole Exome Sequencing
Neoantigen prediction

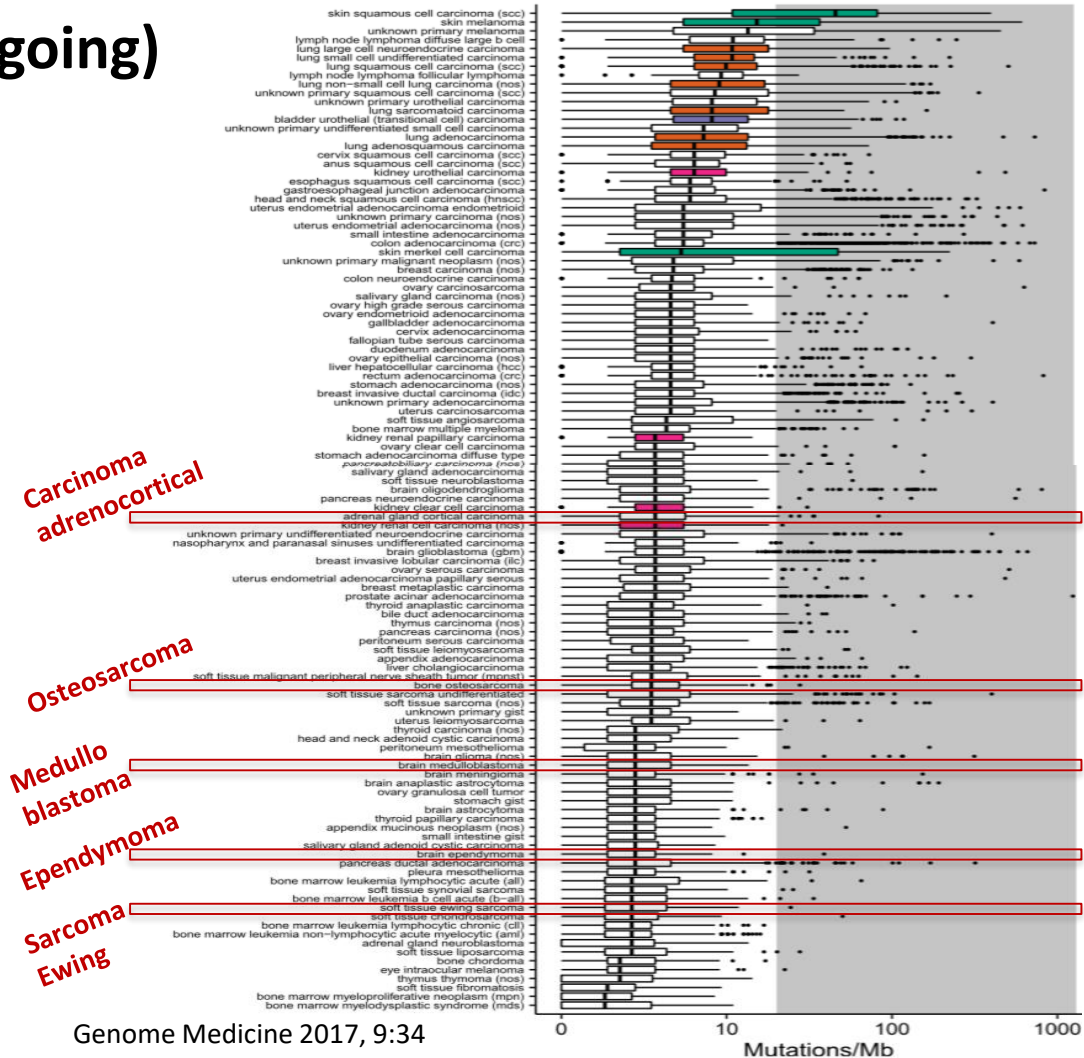
Central IHC (PD-1 and PD-L1)

CIMA

Functional assays
patients' lymphocytes

Whole exome sequncing (ongoing)

Patient	WES	Tumor type
06-001	Yes	Carcinoma adrenocortical
03-005	Yes	Carcinoma renal tubos colectores
03-002	Yes	Carcinoma suprarrenal
04-002	Yes	Cordoma
09-001	Yes	Ependimoma
12-003	Yes	Ependimoma
09-002	Yes	Estesionneuroblastoma
05-002	Yes	Meduloblastoma
12-004	Yes	Meduloblastoma
13-004	No	Meduloblastoma
03-004	Yes	Osteosarcoma (Li-Fraumeni)
12-001	Yes	Papiloma plexo coroideo
03-001	Yes	Paraganglioma
13-003	No	Rabdiomiosarcoma
05-001	Yes	Sarcoma Ewing
12-002	Yes	Teratoma quístico testículo
04-001	Yes	Tumor de células de Leydig



Genome Medicine 2017, 9:34

Preliminary data: WES patients' responses

Patient code	WES	Tumor type	Resp 1st control	Resp 2nd control	Resp 3rd control	Resp 4th control	Resp 5th control
06-001	Yes	Carcinoma adrenocortical	SD				
03-005	Yes	Carcinoma renal tubos colectores	NA paciente fallece antes de evaluacion tumoral				
03-002	Yes	Carcinoma suprarrenal	PD				
04-002	Yes	Cordoma	SD	SD			
09-001	Yes	Ependimoma	PD				
12-003	Yes	Ependimoma	SD	SD	SD	SD	
09-002	Yes	Estesionneuroblastoma	PR	PR	PR	PR	
05-002	Yes	Meduloblastoma	PD				
12-004	Yes	Meduloblastoma	PD				
13-004	No	Meduloblastoma					
03-004	Yes	Osteosarcoma (Li-Fraumeni)	SD				
12-001	Yes	Papiloma plexo coroideo	SD				
03-001	Yes	Paraganglioma	SD	PD			
13-003	No	Rabdiomiosarcoma					
05-001	Yes	Sarcoma de Ewing	NA paciente fallece antes de evaluacion tumoral				
12-002	Yes	Teratoma quistico testiculo	SD	PR	PR	SD	SD
04-001	Yes	Tumor de células de Leydig	NA paciente fallece antes de evaluacion tumoral				

WES: preliminary results

Blood + tumor; 100x

+

Somatic Mutations

No microsatellite instability detected

-

Somatic variant	Mutation type	Cordoma de sacro	Teratoma quistico de testiculo	Paraganglioma	Tumor de Células de Leydig	Papiloma atípico plexo coroideo	Meduloblastoma	Ependimoma	Meduloblastoma	Estesion-neuroblastoma		Carcinoma suprarrenal	Osteosarcoma (Li-Fraumeni)	Ependimoma	Sarcoma de Ewing	Renal cell carcinoma
SNV	missense	4026	2525	2329	522	265	225	183	161	108	93	100	88	62	46	25
	stopgain	85	174	101	35	16	12	21	14	6	7	10	6	6	0	2
	stoploss	4	0	1	0	3	0	0	0	0	0	0	0	0	0	0
	splicing	56	109	108	21	7	7	8	2	3	2	5	0	1	1	0
INDEL	frameshift_del	9	0	4	6	14	1	1	1	1	2	1	2	2	0	0
	frameshift_ins	8	0	2	0	1	1	0	3	2	2	1	1	4	0	0
	nonframeshift_de	28	7	2	12	2	3	1	3	0	1	4	1	1	3	1
	nonframeshift_ins	12	0	0	0	0	0	0	1	0	0	0	0	1	1	0
Total nr mut (ns+ indel)		**4083**	**2532**	**2337**	**540**	**282**	**230**	**185**	**169**	**111**	**98**	**106**	**92**	**70**	**50**	**26**
TMB (mut/Mb)		**215**	**240**	**107**	**54**	**12**	**9**	**8**	**6**	**4**	**3**	**6**	**4**	**3**	**2**	**1**

Paraganglioma metastatic patient

- PCPG are amongst the tumors with lowest mutation burden
- Non-synonymous mutations: **median=14 (min=4 max=44);** TCGA (n=179)

Outlier patient?

Does TCGA correctly reflects the disease?

→ TMB/MSI routinely determined

- Nivorare mtx PGL has **2439 mutations**
- WES analysis confirms ***SDHB* germline** mutation (splicing defect)
- Somatic mutation in ***MLH1* p.G147E** (VUS)

Take home messages

• TMB is a promising biomarker, however, standardization is needed. Targeted NGS panels can reproduce WES data. Clinically relevant thresholds (“TMB-high”) need to be established, and may vary across tumor types

• Uncertainties include: how indels should be taken into account?, intratumor heterogeneity?, RNAseq and neoantigen predictions?

• Nivorare data suggest higher TMB than initially expected. It may indicate differences with TCGA cases or reflect outliers. TMB/MSI routinely determined?

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