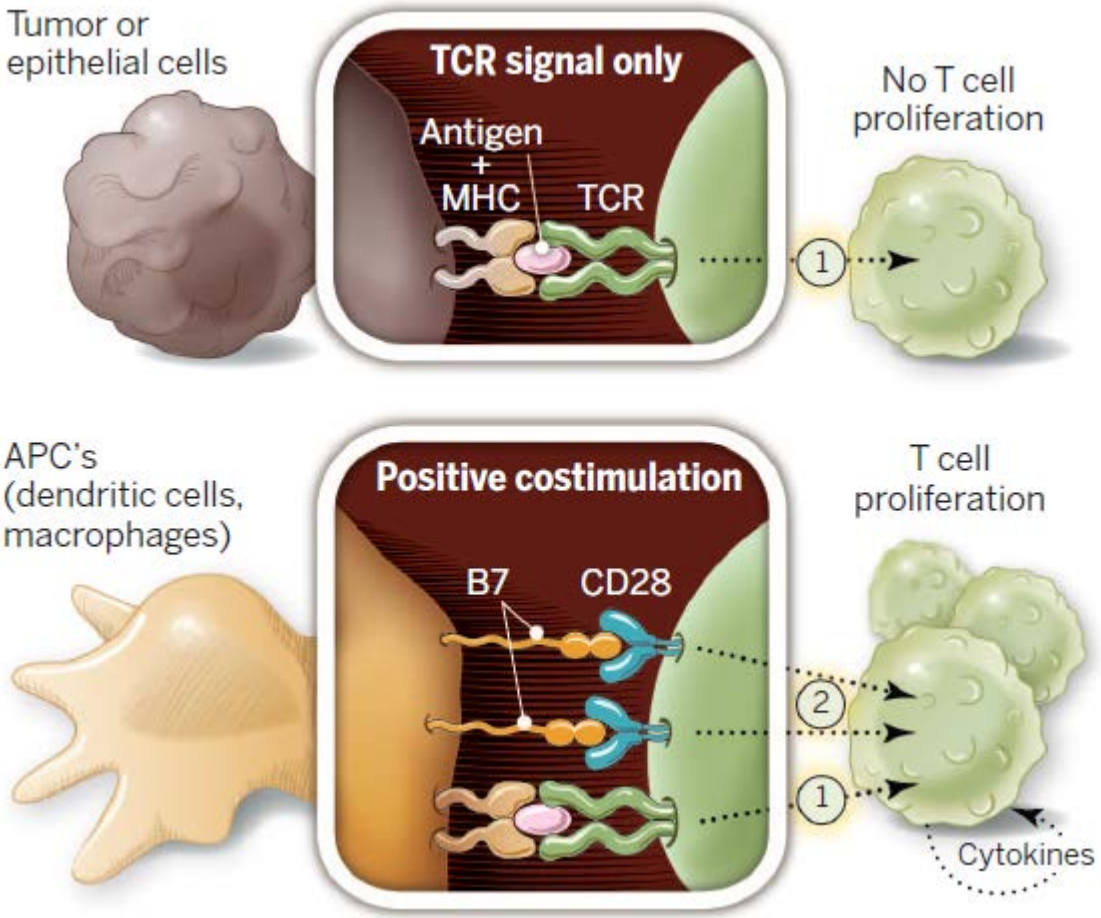
Иммунотерапия раковых опухолей с точки зрения системной биологии

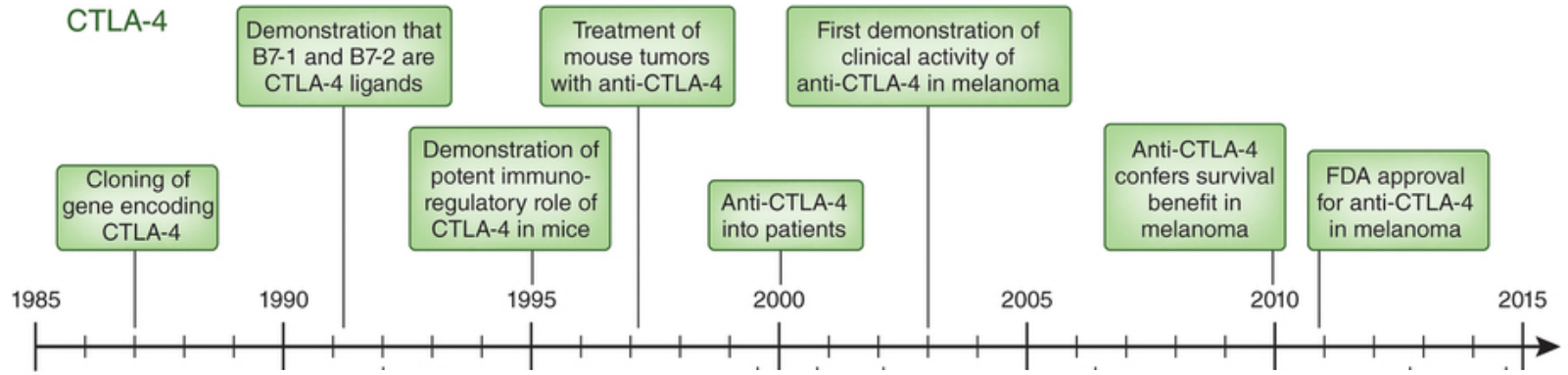
Максим Артёмов

25 мая 2015

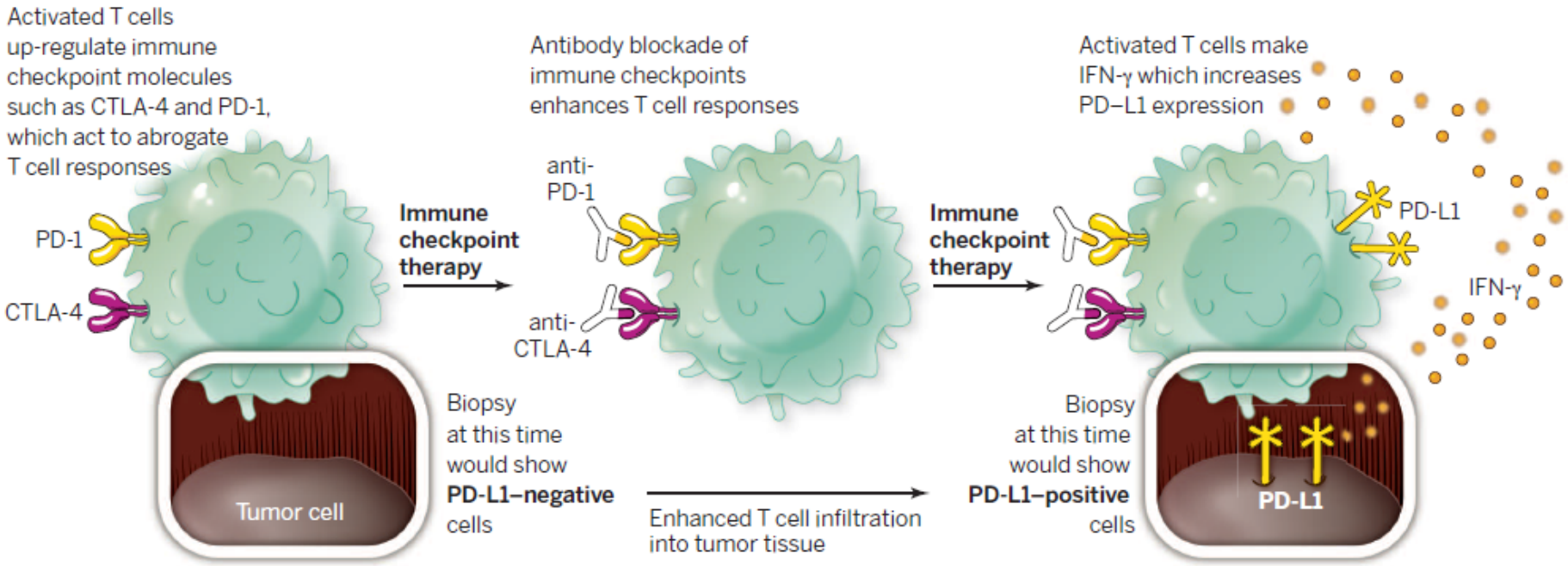
T cell activation requires 2 signals



CTLA4 and PD-1 are checkpoint regulatory molecules



Immunotherapy addresses second checkpoint



Huge success both in clinics and in academia

2011 -
ipilimumab approved by FDA
for melanoma

2013 -
breakthrough of the year

Paradigm shift

CONVENTIONAL THERAPIES	INHIBITORY IMMUNE SIGNALS
Chemotherapy	CTLA-4
Radiation	PD-1/PD-L1
Surgery	LAG-3
Genomically targeted	TIM-3
Anti-angiogenic	VISTA
Hormonal	BTLA

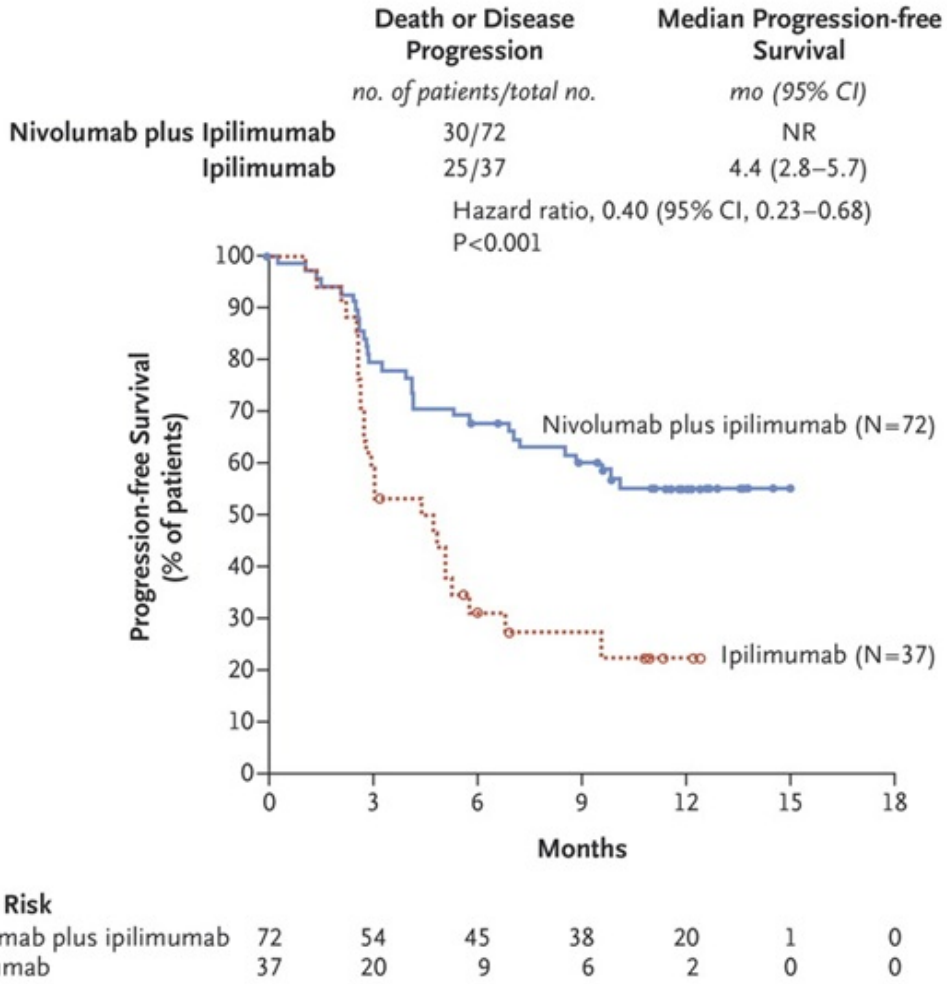
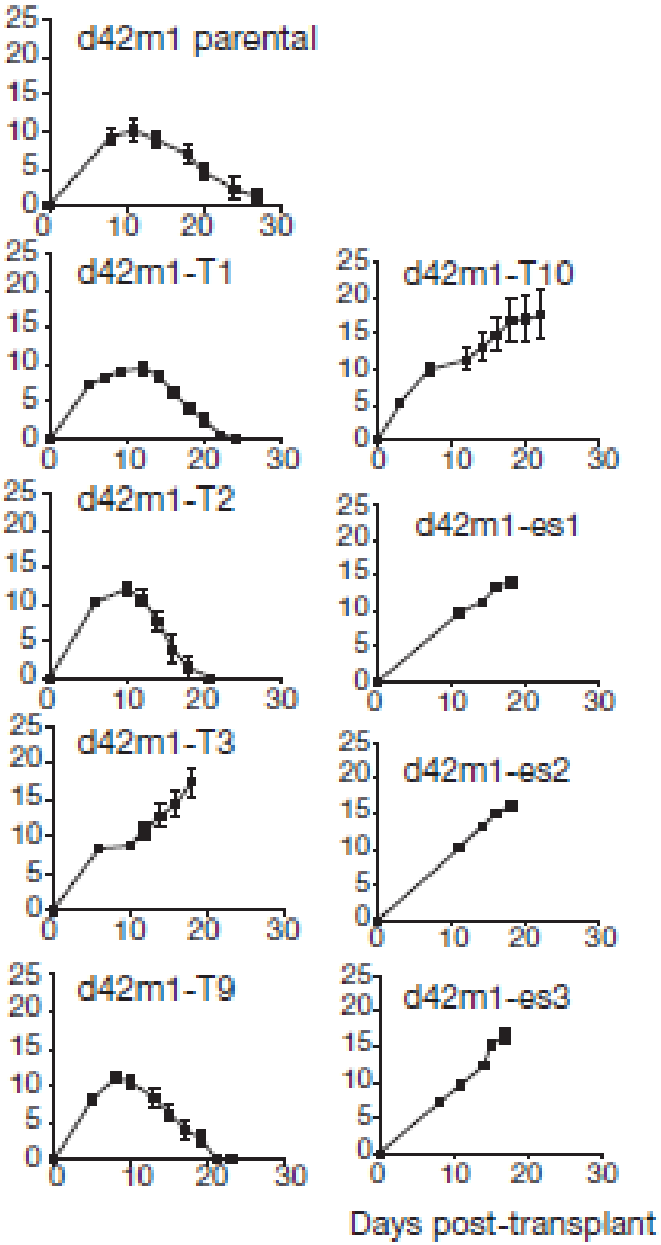


Image taken from: Postow MA et al. N Engl J Med 2015.
DOI: 10.1056/NEJMoa1414428

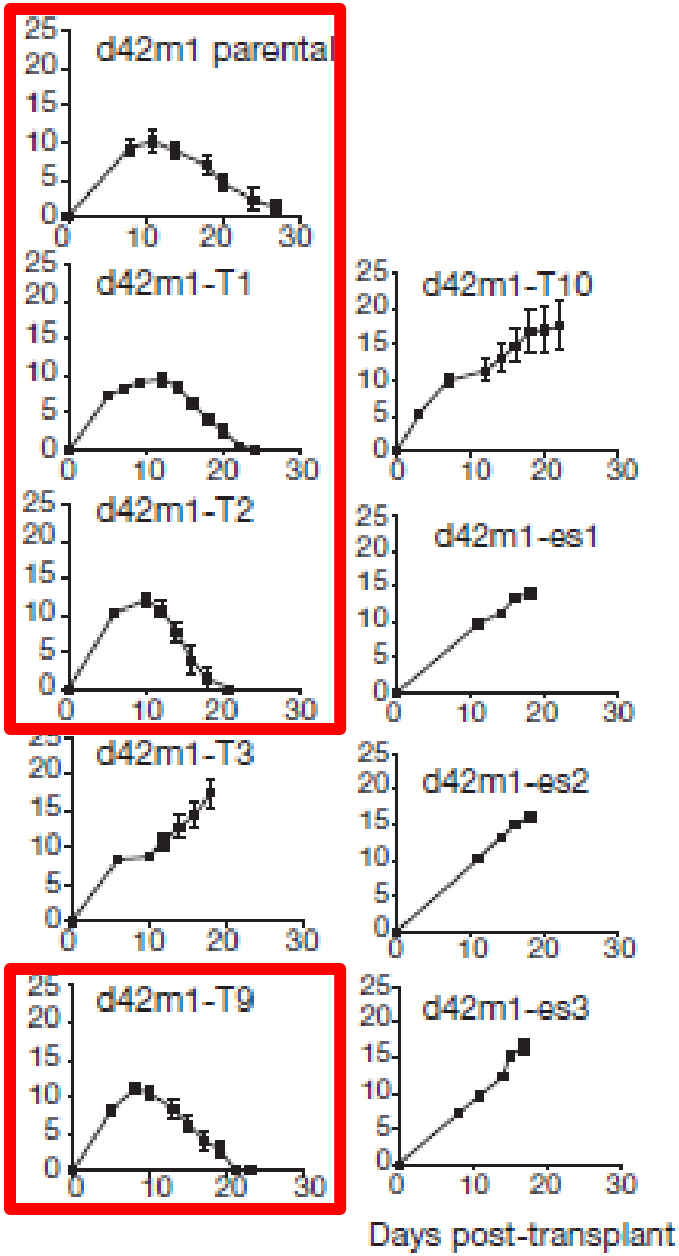
Mouse model of tumor rejection – panel of sarcomas



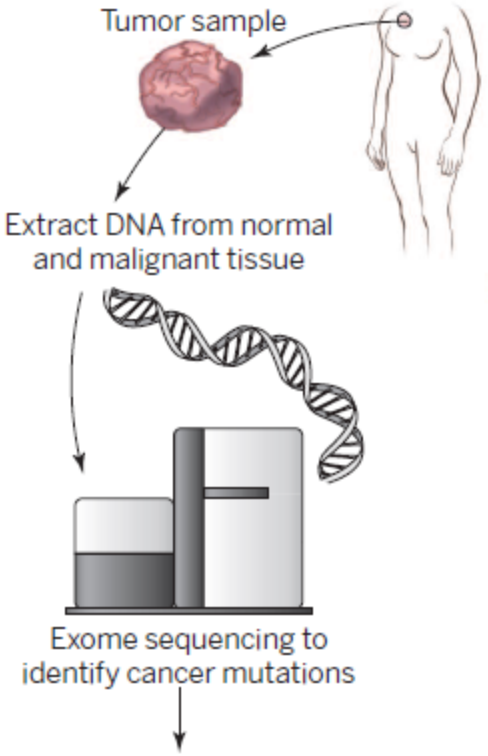
Some mouse sarcomas are naturally rejected while others grow out

Matsushita et al, Nature 2012

Mouse model of tumor rejection – panel of sarcomas



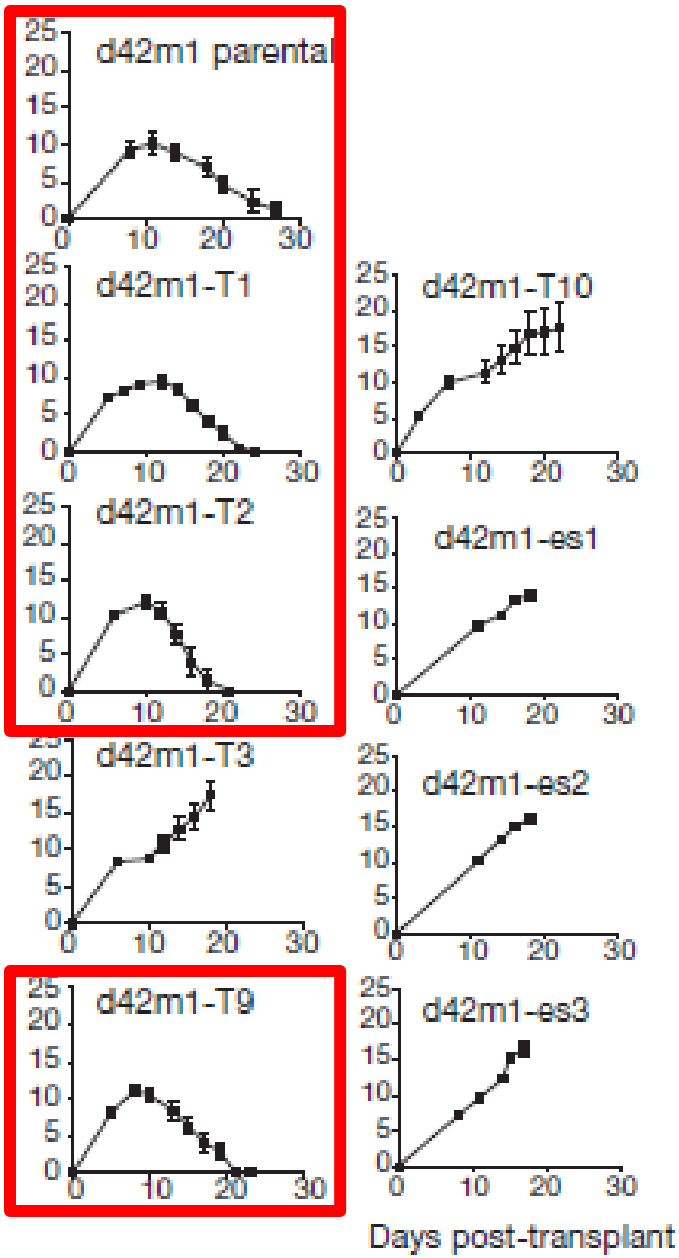
Hypothesis:
Tumor neoantigen is responsible for rejecion

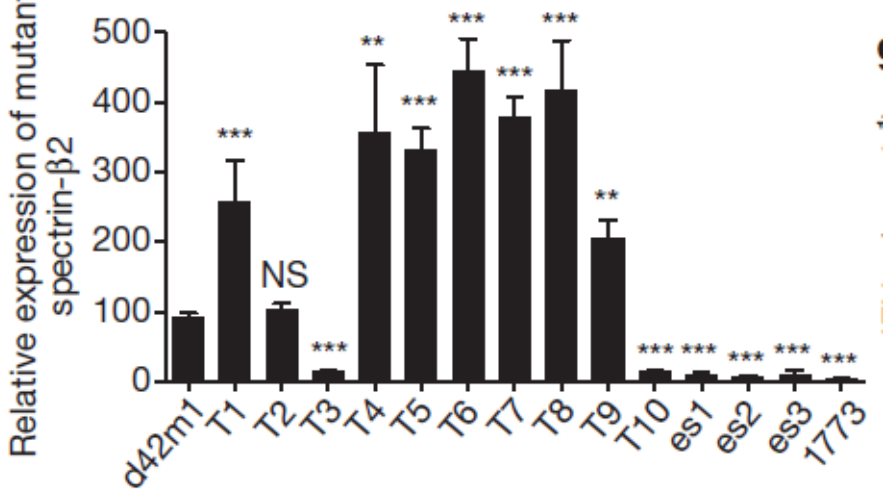


compare mutational landscape of regressor vs progressor tumors

Matsushita et al, Nature 2012

Only regressor tumors express spnb2 mutant!





Matsushita et al, Nature 2012

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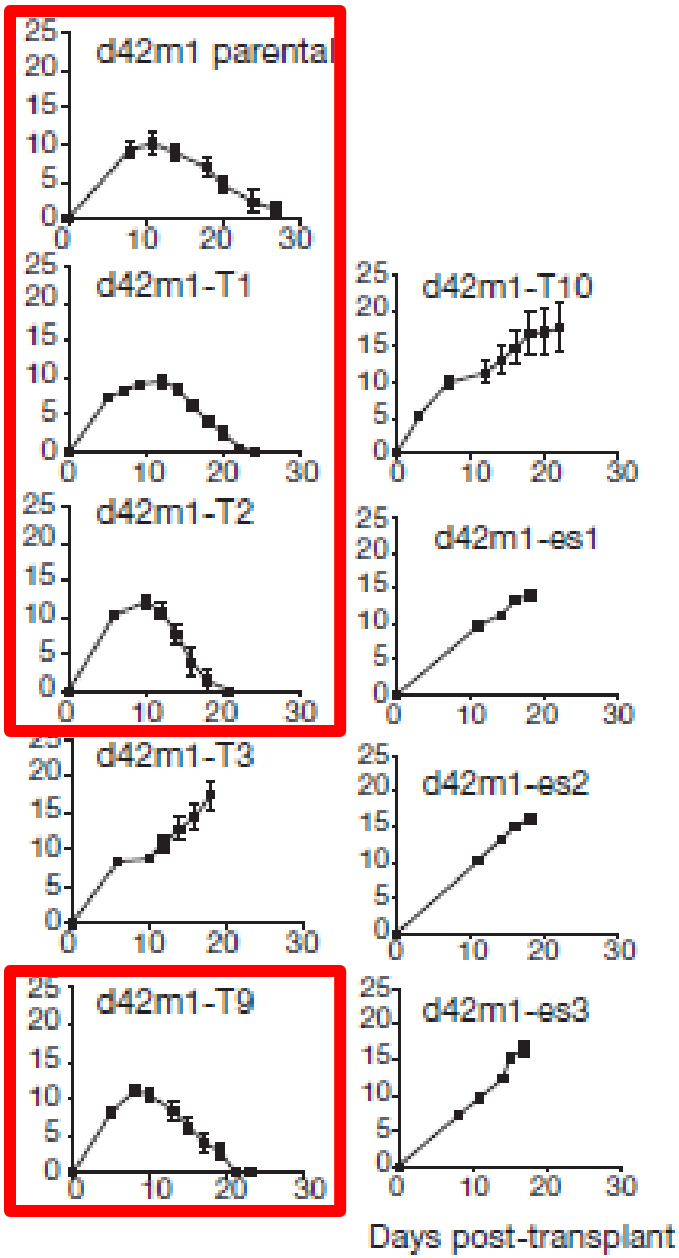
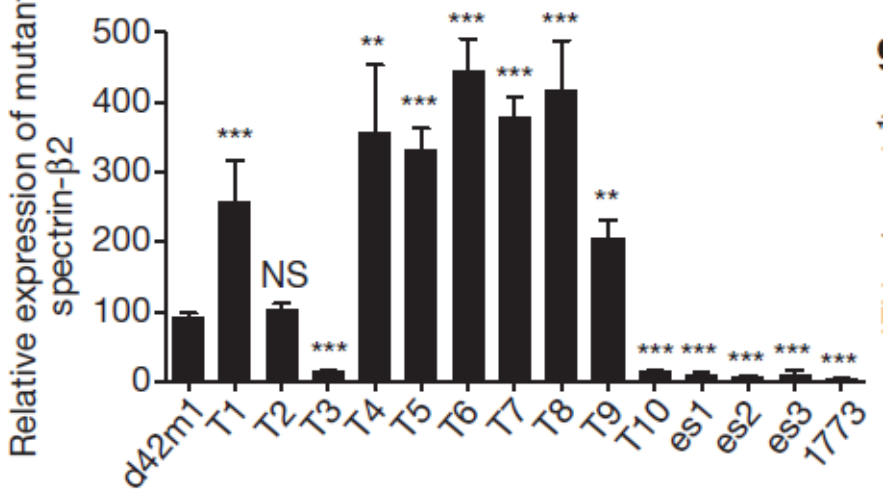




Spnb2 mutant is also computationally strong binder to MHC!

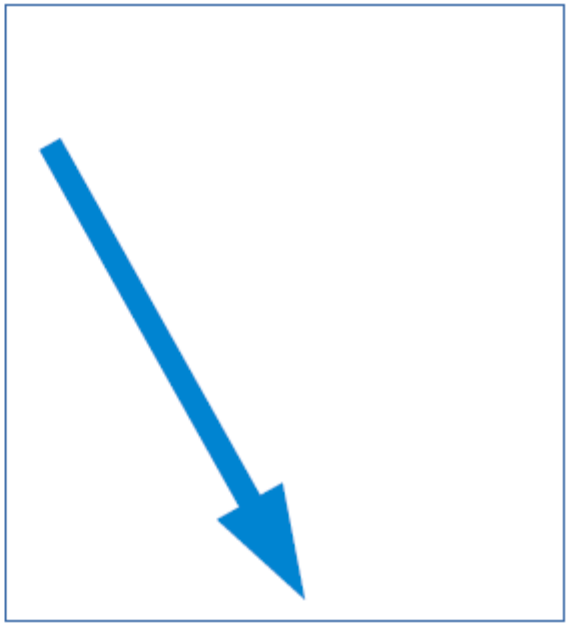
Matsushita et al, Nature 2012

Computational filtering allows to go identify neoantigens

cDNA Cap-Seq
Tumor vs Normal

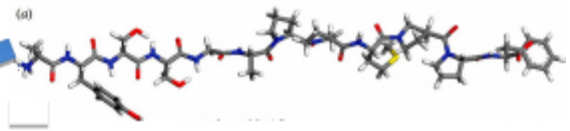
ID Expressed mutations

~40,000 peptides



~40,000 peptides

Synthesize Peptides



In Vitro/Ex Vivo Analyses

- *Mass Spec of eluted peptides*
- *Peptide binding to RMA-S*
- *Tetramer staining of TILs*

In Vivo Analyses

- *Vaccine induced T cell response*
- *Prevention of tumor outgrowth*

By Jeffrey Ward

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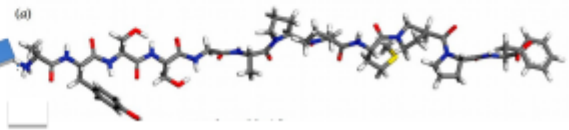

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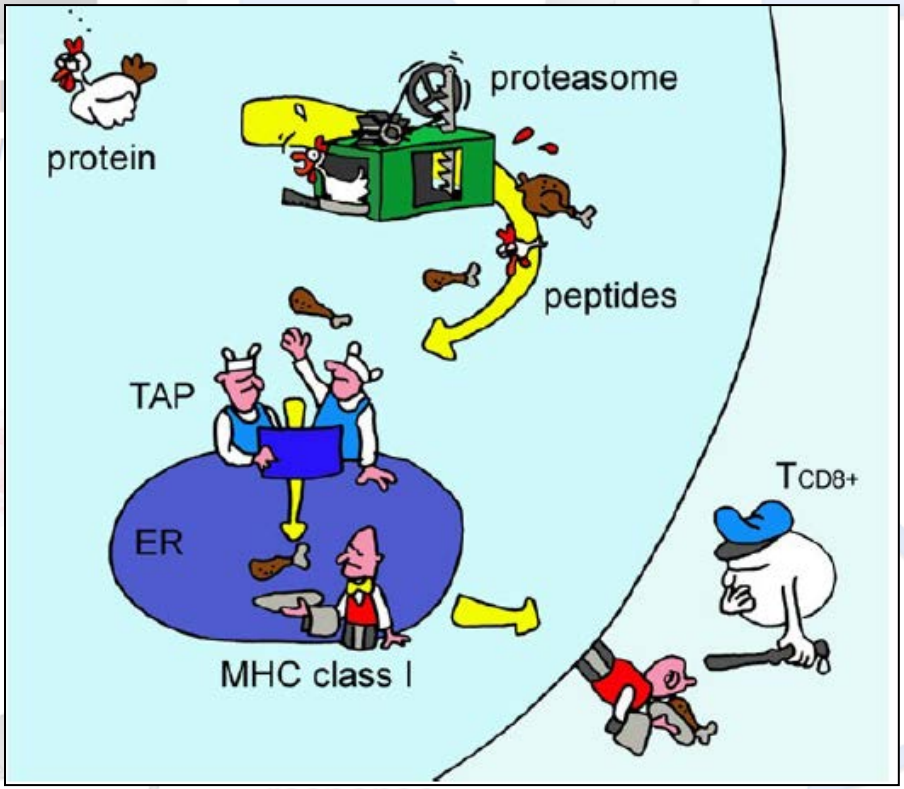


~40,000 peptides

~40 peptides

Synthesize Peptides

response

- Prevention of tumor outgrowth

By Jeffrey Ward

Computational filtering allows to go identify neoantigens

YTRGFQHTGIY**S**VMIQKVILRDL
~~YTRGFQHTGIY~~
TRGFQHTGIY**S**
RGFQHTGIY**S**V
GFQHTGIY**S**VM
FQHTGIY**S**VMI
QHTGIY**S**VMIQ
HTGIY**S**VMIQK
TGIY**S**VMIQKV
GIY**S**VMIQKVI
IY**S**VMIQKVIL
Y**S**VMIQKVILR
SVMIQKVILRD
~~VMIQKVILRDL~~

~40,000 peptides

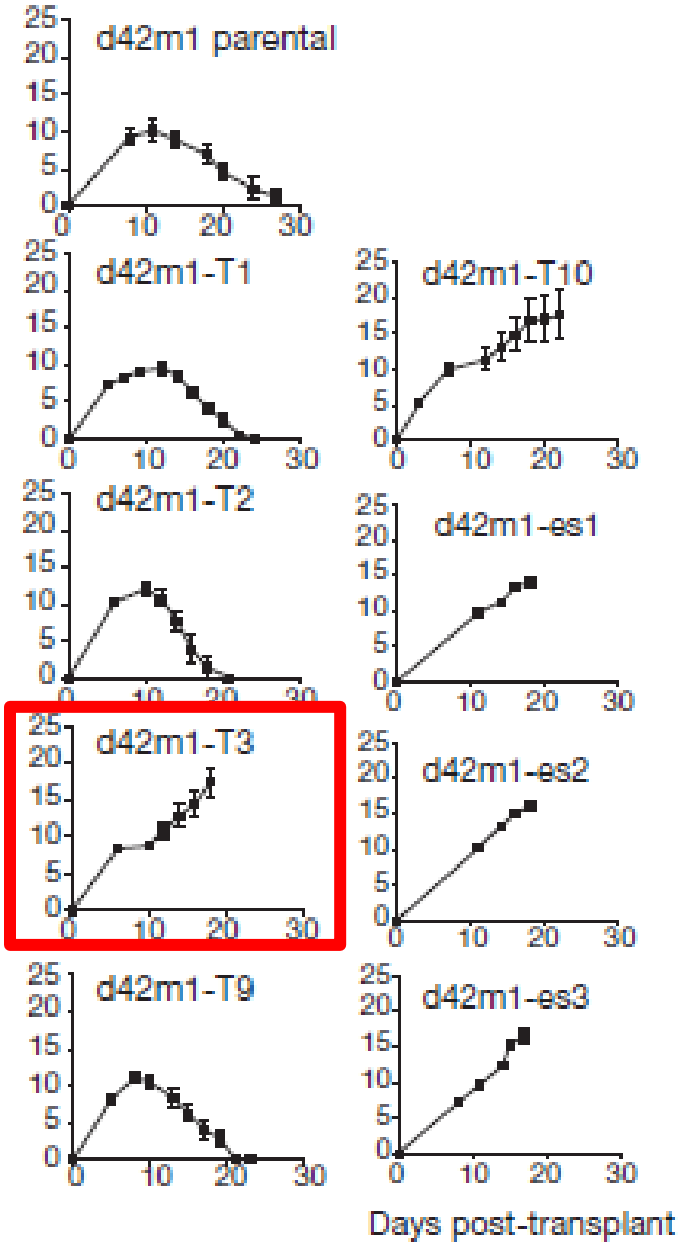
~40 peptides

Synthesize Peptides

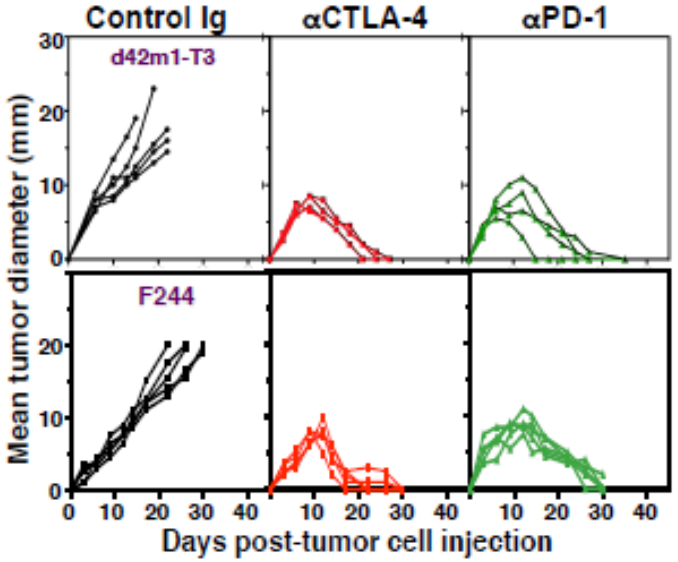
response
- Prevention of tumor outgrowth

By Jeffrey Ward

Checkpoint blockade works in progressor tumors



aCTLA4/aPD1 treatments “cure” the mice

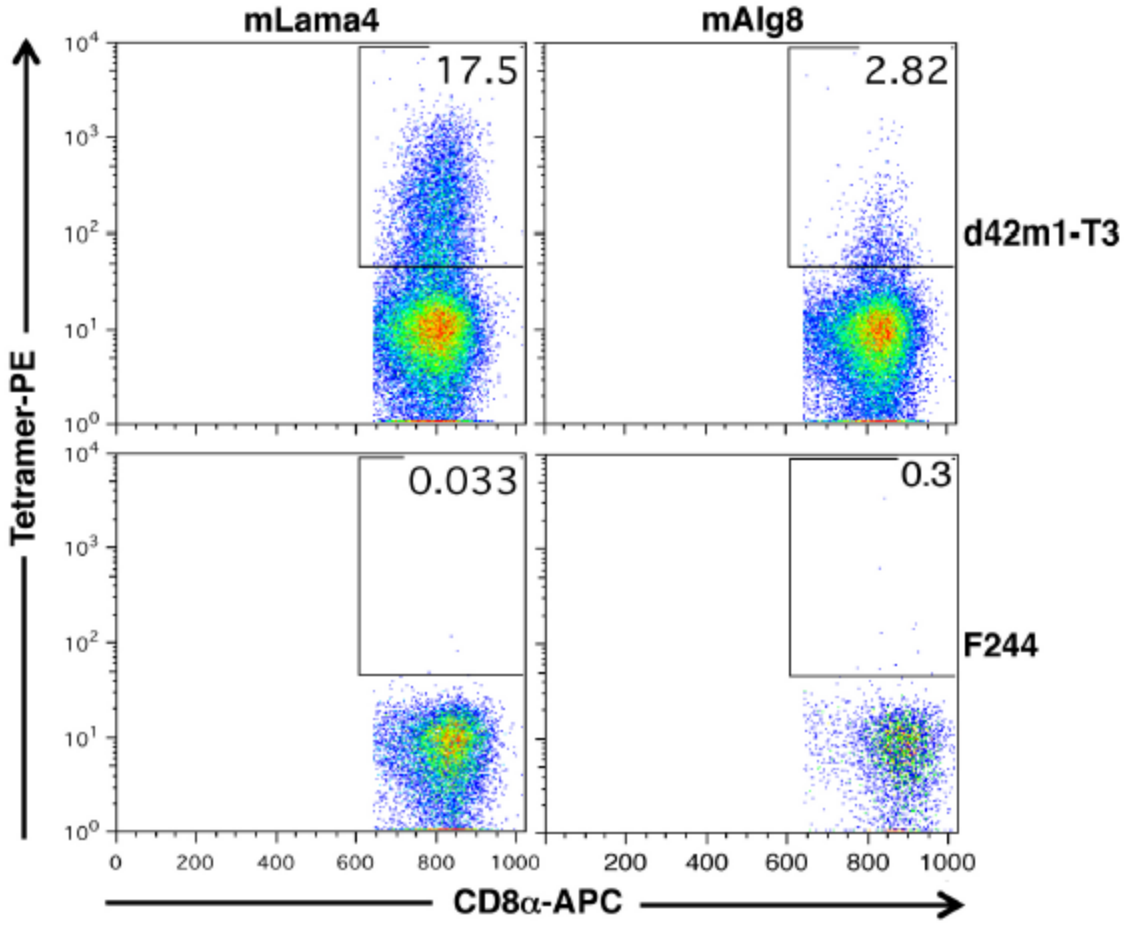


Gubin et al, Nature 2014

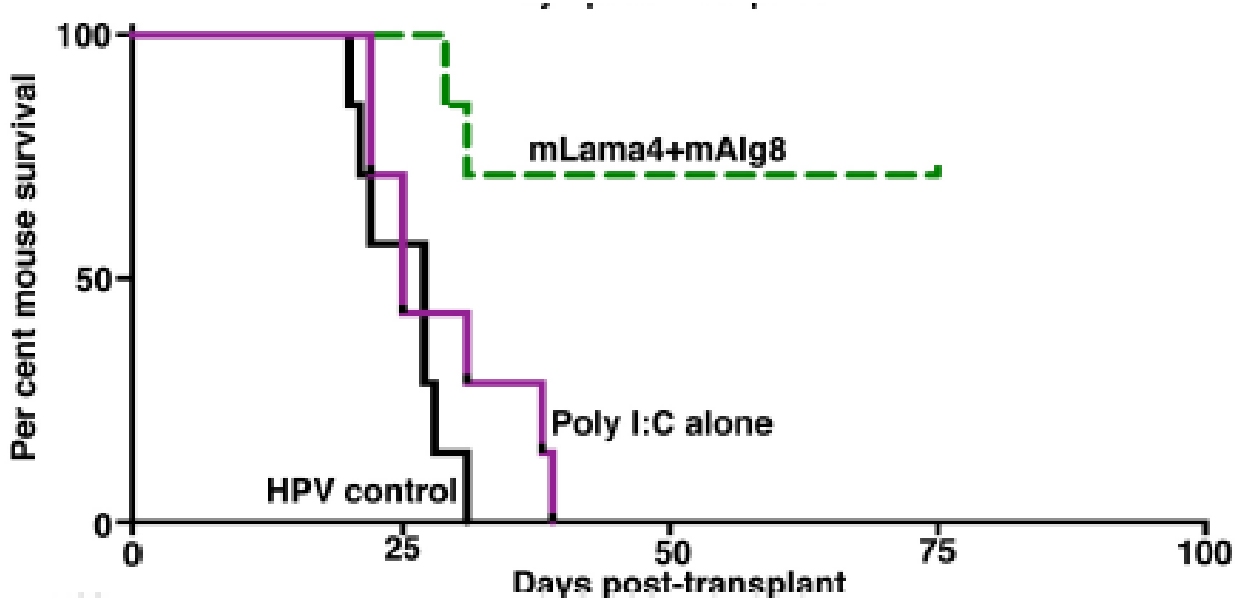
Potential Antigens identified for T3 tumor

Rank	Id	WT peptide	Mut peptide	Median mutant affnity (nm^{-1})	Cutting score	Neoepitope ratio
1	Sbf2_V511L	FNYLYSPV	FNYLYSPL	0.3998714058	0.542809	3.5952400634
2	Alg8_A506T	ITYAWTRL	ITYTWTRL	0.2223404132	0.954498	1.0616164751
3	Lama4_G1254V	GGFNFRTL	VGFNFRTL	0.2188577796	0.967372	12.8123805304
4	6430548M08Rik_H290R	KVYLYTHL	KVYLYTRL	0.1841609862	0.847512	1.2786360207
5	Apob_T1328S	STNVYSNL	SSNVYSNL	0.1027451056	0.870279	1.6649288887
6	Olfr168_P253H	VTFYYAPF	VTFYYAHF	0.0916300653	0.823354	0.667371074
7	Olfr1121_D127Y	MSYDRYVAI	MSYYRYVAI	0.0883059591	0.478287	1.4165113789
8	Olfr12_I133M	MAYDRFMAI	MAYDRFMAM	0.0818398718	0.975672	1.7597270663
9	Tpm2_I266T	ITLLFSFL	TTLLFSFL	0.0712708996	0.923923	0.3596749785
10	Olfr849_G208R	VSVLFFGV	VSVLFFRV	0.0698917259	0.368878	1.7065708369

Antigen-specific T-cells are present in tumor even before treatment!



Theraputic vaccination saves the mouse!



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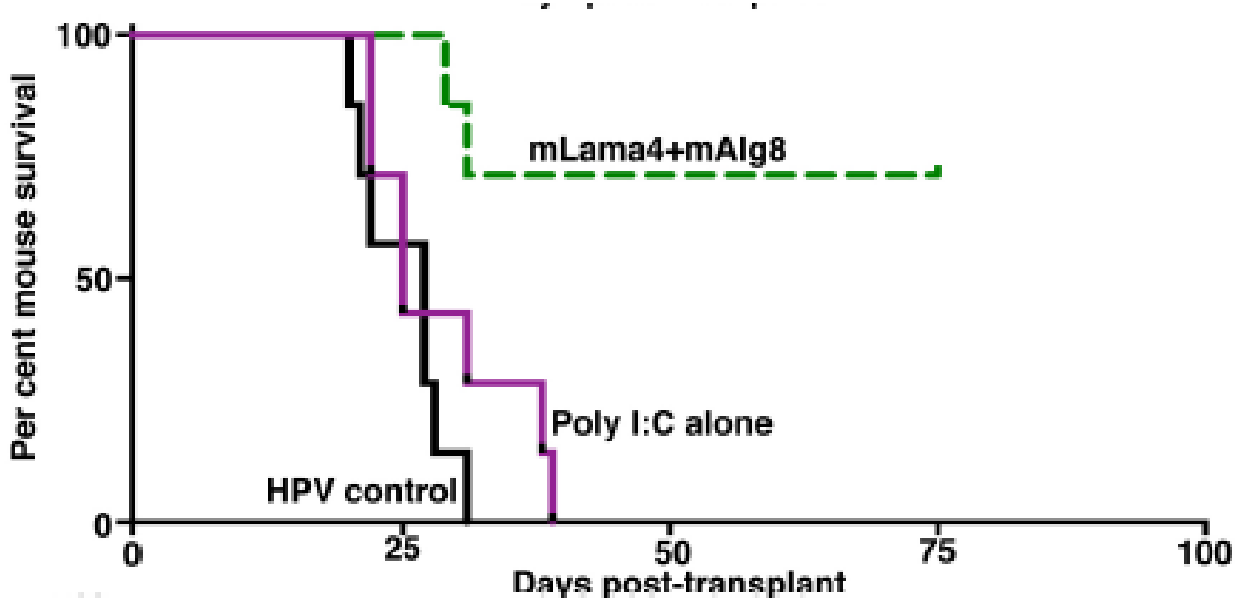
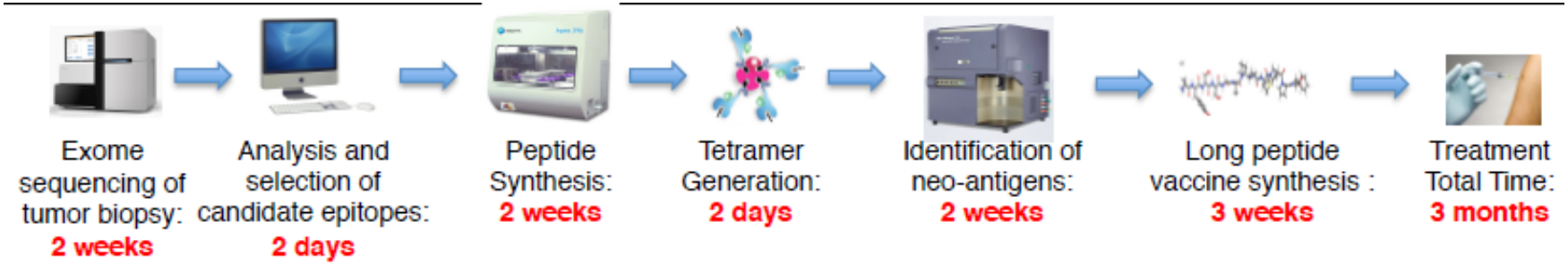




Independent validation in two additional tumors

Tumor	Epitope	Sequence	Allele	Anchor?	Rank
d42m1 (r)	Spectrin-b2	VAVVNQIAL	H-2D^b	Y	1
1969 (r)	Rabac1	VSFPFFCL	H-2K^b	N	1
	Gpd2	YSPENMELL	H-2D^b	N	1
T3 (p)	Alg8	ITYTWTRL	H-2K^b	N	1
	Lama4	VGFNFRTL	H-2K^b	N	2
F244 (p)	Pex14	IAFAFHQL	H-2K^b	N	1

Exome sequencing of tumor biopsy: **2 weeks**

Analysis and selection of candidate epitopes: **2 days**

Peptide Synthesis: **2 weeks**

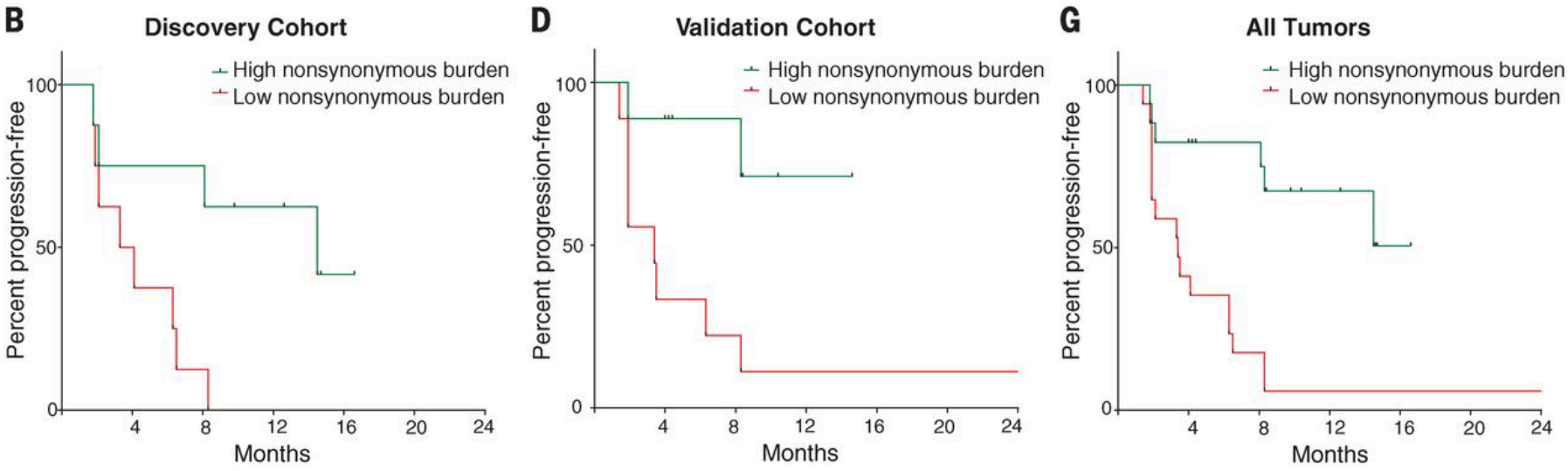
Tetramer Generation: **2 days**

Identification of neo-antigens: **2 weeks**

Long peptide vaccine synthesis : **3 weeks**

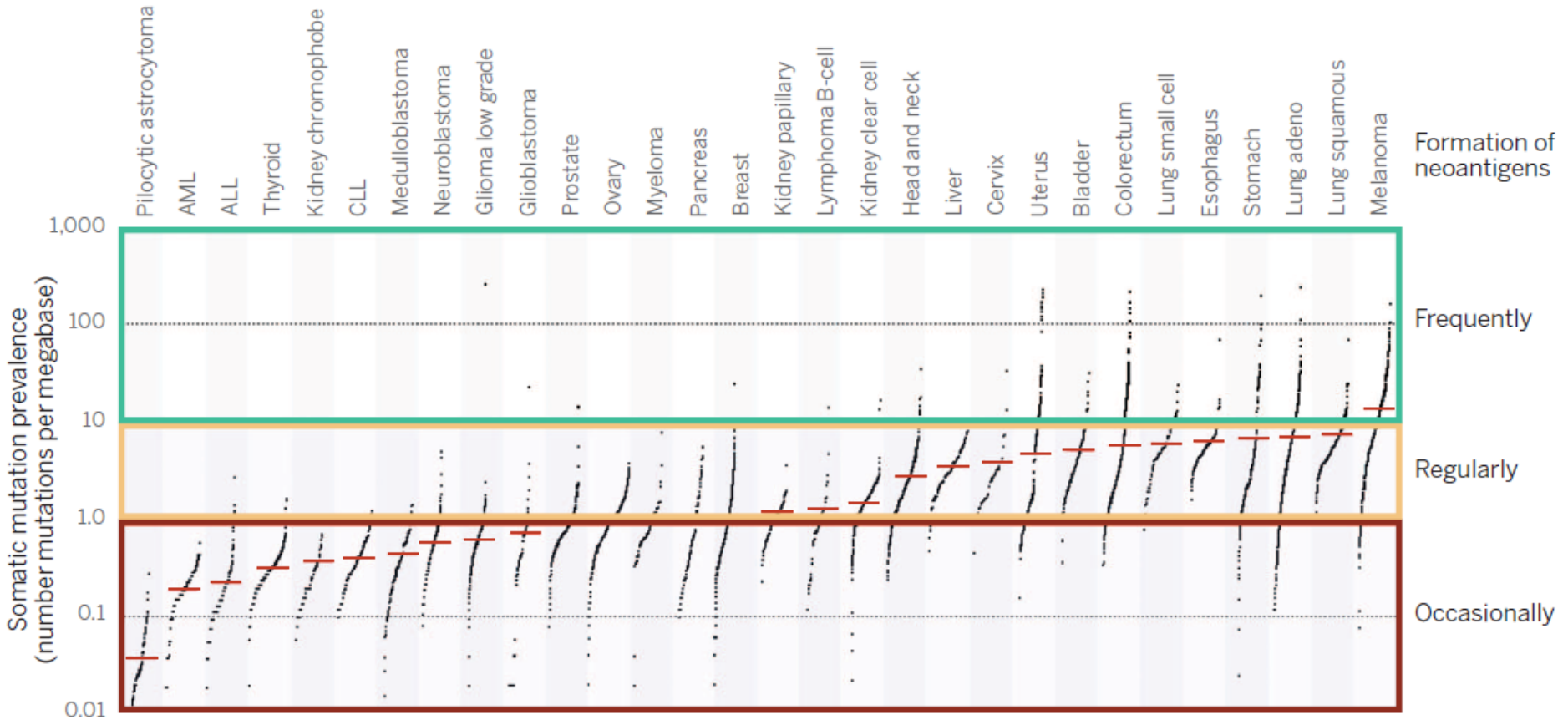
Treatment Total Time: **3 months**

Mutational load is predictive of immunotherapy response



Rizvi et al, Science 2015

This is why first successes of checkpoint blockade are in melanoma!!



It all started in 19th century

Fig. 1. Dr William B. Coley (active career 1891–1936).

Worked in New York Cancer Hospital
(later became a part of Memorial Sloan Kettering Cancer Center)

- Noticed that infection with erysipelas often leads to spontaneous regression of sarcomas

- Started therapeutically infection patients with inoperable sarcomas

"There can be no doubt that the influence of erysipelas upon malignant tumors is much more powerful than any other febrile disease." (Coley, 1931.)

It all started in 19th century

Fig. 1. Dr William B. Coley (active career 1891–1936).

Fig. 3. First patient Coley treated by deliberate induction of erysipelas (Coley, 1896a). Large lesion on neck broke down and disappeared under treatment; see text for description. Patient remained well for 8 years, then died of recurrence (Coley, 1909).

"There can be no doubt that the influence of erysipelas upon malignant tumors is much more powerful than any other febrile disease." (Coley, 1931.)

It turns out that *Streptococci* alone is not enough!

What we refer to as **Coley's toxins** is combination of two components:

- Streptococci – gram-positive bacterial infection (no endotoxins)
- Serratia – gram-negative bacteria (endotoxins)

"I wish at the outset to state what is known to every one who has read my previous papers, that the mixed toxins, prepared in the way described in these papers, have been shown to have a curative effect sufficient for practical purposes only in cases of sarcoma and not in cases of carcinoma." (Coley, 1908.)

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Note resemblance to classical vaccine formulation:

Adjuvant + Adaptive Immunity Target

Streptococci action is sarcoma specific!

"The curative action of erysipelas upon malignant tumors . . . is much more powerful in sarcoma than carcinoma." (Coley, 1894a.)

Survival

Table 2. *Summary of Patients Treated with Coley's Toxins before 1940*

Type of cancer	Total	A	B	C	D	E
Soft tissue sarcomas[1]	84	32	12	11	12	17
Lymphosarcomas (lymphomas)[2]	33	10	4	4	7	8
Osteosarcoma[3]	3	2	1	0	0	0
Ewing's tumor/reticulum cell sarcoma[4]	1	0	0	0	0	1
Ovarian carcinoma[5]	4	1	2	0	0	1
Cervical carcinoma[5]	2	0	1	0	0	1
Testicular[6]	14	5	3	3	2	1
Renal[7]	8	4	1	1	1	1
Multiple myeloma[8]	1	0	0	1	0	0
Colorectal carcinoma[9]	1	1	0	0	0	0
Breast carcinoma[10]	13	5	6	2	0	0
Melanoma[11]	6	2	3	0	1	0

Evaluation was restricted to those patients who were considered to be inoperable at the time of treatment, and who received no therapy other than the vaccine. Individual patient records are tabulated as follows: A, those making no beneficial response to the treatment; B, those making an initial response, but either known to relapse at any time or lost to follow-up in less than 5 years; C, those rendered free of disease, but lost to follow-up after at least 5, but less than 10, years; D, those rendered free of disease, but lost to follow-up after at least 10, but less than 20, years; E, those rendered free of any clinical evidence of disease for a period of time not less than 20 years. [1]Nauts, 1975c. [2]Nauts and Fowler, 1969. [3]Nauts, 1975b. [4]Nauts *et al.*, 1953. [5]Nauts, 1977. [6]Fowler, 1968. [7]Nauts, 1973. [8]Nauts, 1975a. [9]Fowler, 1969b. [10]Nauts, 1984. [11]Fowler, 1969a.

Sarcomas have characteristic genomic landscape

COSMIC
Catalogue of somatic mutations in cancer

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Cosmic » Fusion » Overview » EWSR1:FLI1

View in GRCh37 Archive

Fusions | Tissues | References

Genes EWSR1 ->FLI1

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF166	EWSR1	7	1112	-	FLI1	6	920	-	736	54.04%
COSF168	EWSR1	7	1112	-	FLI1	5	854	-	345	25.33%
COSF170	EWSR1	10	1364	-	FLI1	6	920	-	41	3.01%
COSF172	EWSR1	10	1364	-	FLI1	5	854	-	33	2.42%
COSF177	EWSR1	7	1112	-	FLI1	8	1046	-	15	1.1%
COSF181	EWSR1	7	1112	-	FLI1	7	986	-	9	.66%
COSF185	EWSR1	10	1364	-	FLI1	8	1046	-	4	.29%
COSF178	EWSR1	9	1331	-	FLI1	4	650	-	4	.29%
COSF184	EWSR1						986	-	3	.22%
COSF228	EWSR1						1094	-	3	.22%
COSF1303	EWSR1						986	-	2	.15%
COSF183	EWSR1						986	-	1	.07%
COSF205	EWSR1						920	-	1	.07%
COSF179	EWSR1						?	-	165	12%
									1362	100%

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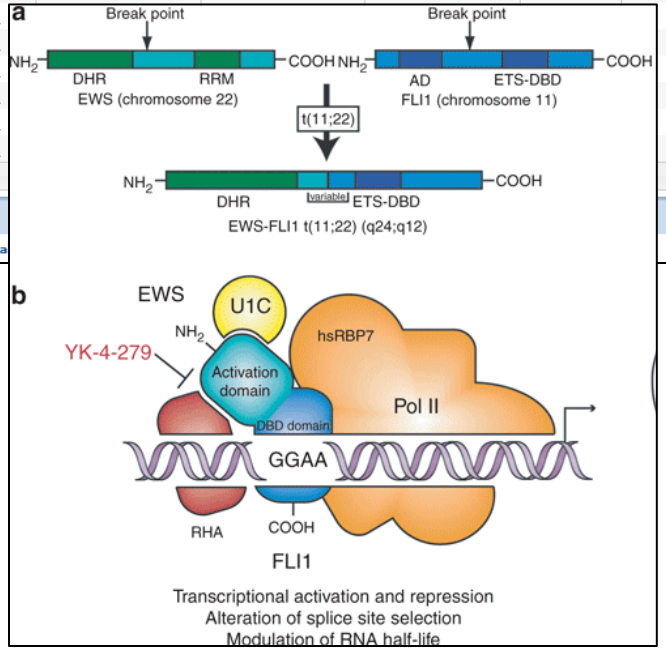
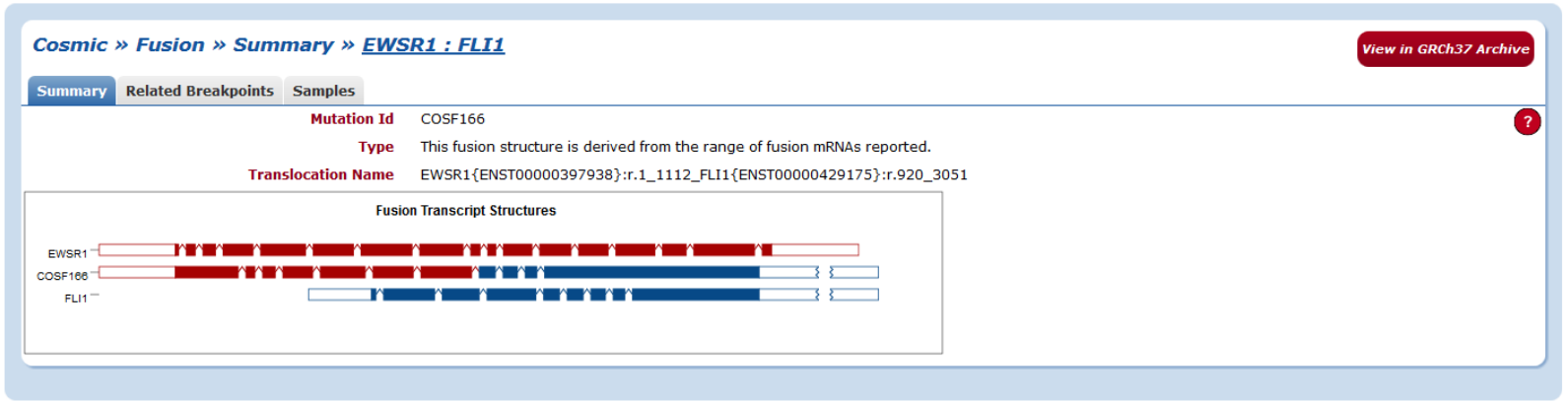
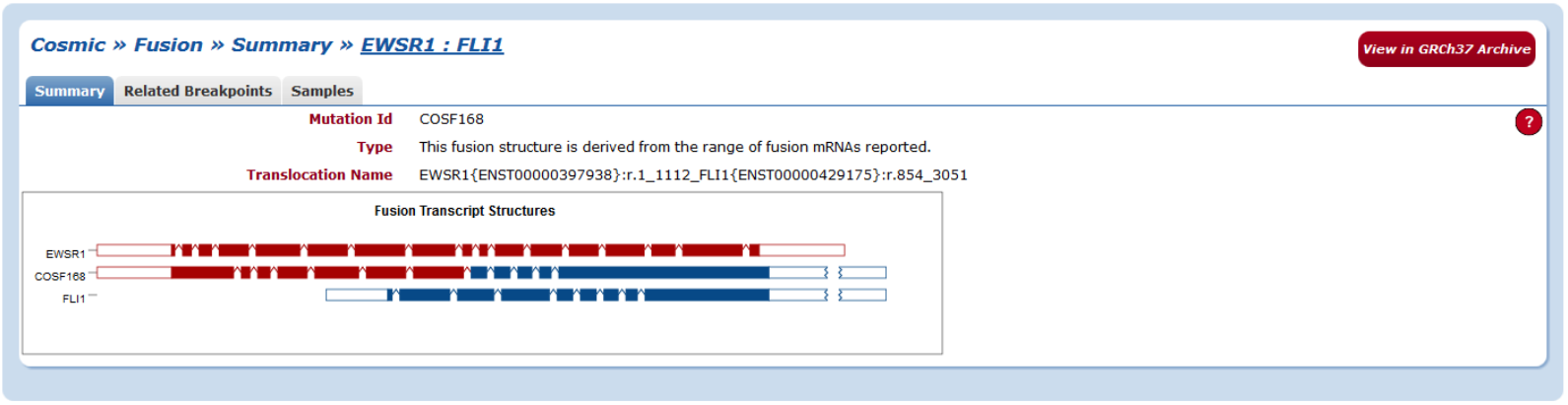


Fusion neoantigens are similar to *Streptococci* native protein epitopes!





Many thanks to lab members and collaborators

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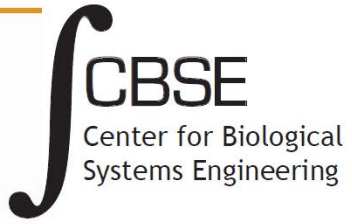
Fadi Towfic
(Immuneering)



Alex Predeus
Katya Loginicheva
Alexey Sergushichev
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Mike Orzel
Anton Alexandrov
Pavel Zakharov

Artyomov Lab members
(and associated members)