The Immune System and Ovarian cancer : New Horizon for Treatment

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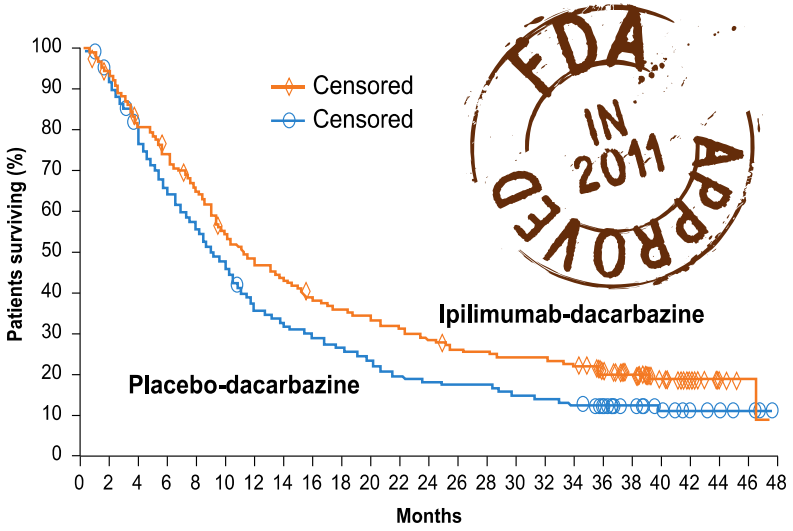
Potential of immunotherapy

- Mechanism of action different from chemotherapy: can circumvent primary or acquired **resistance to cytotoxic drugs**

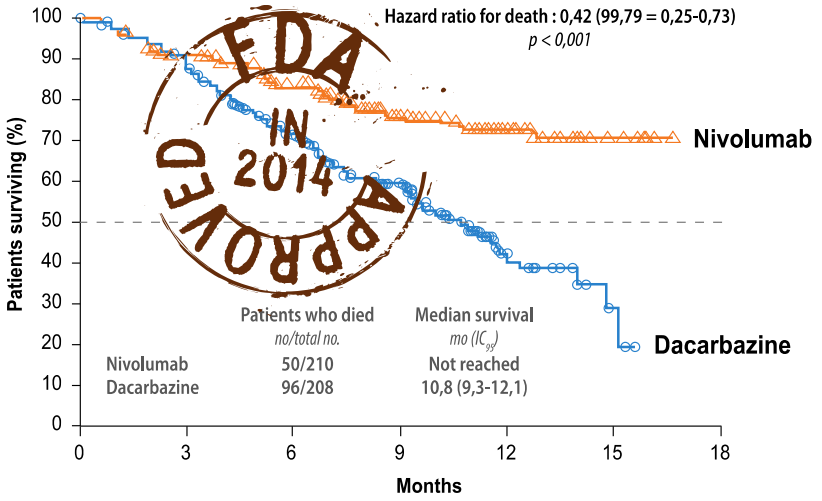
- Efficacy of immune-based therapy can be **durable** due to immunologic memory

Checkpoint inhibitors

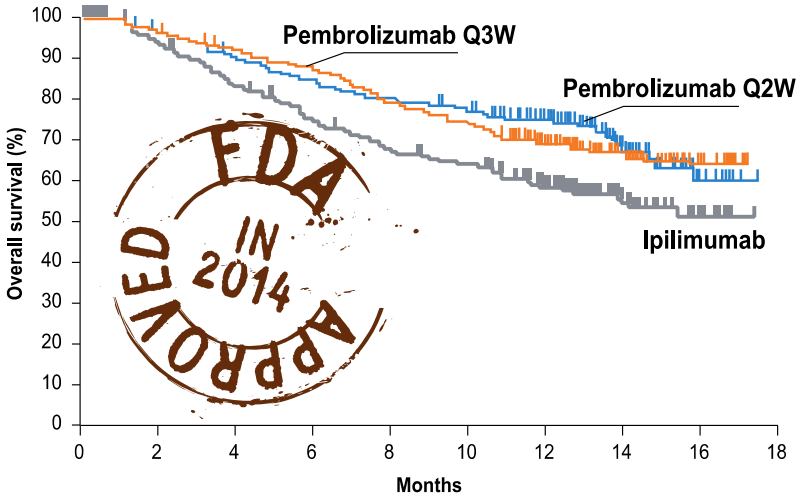
Anti-CTLA-4 (ipilimumab) in melanoma



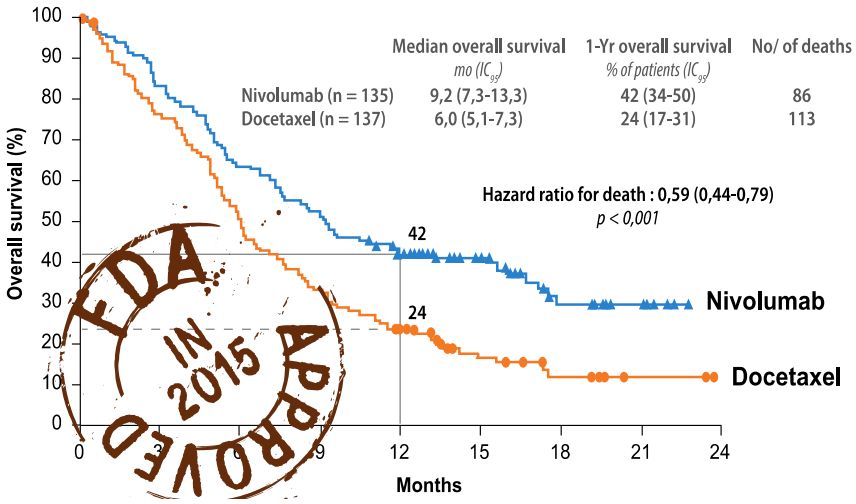
Anti-PD-1 (nivolumab) in melanoma



Anti-PD-1 (pembrolizumab) in melanoma



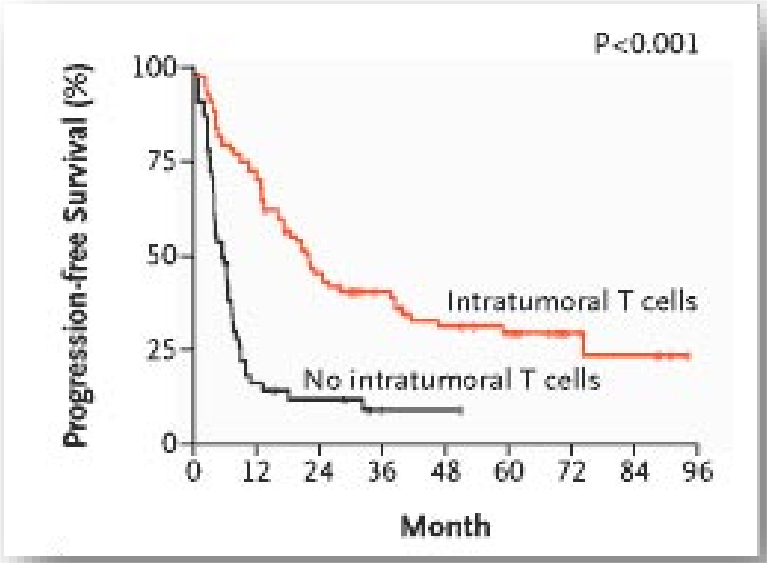
Anti-PD-1 (nivolumab) in NSCLC



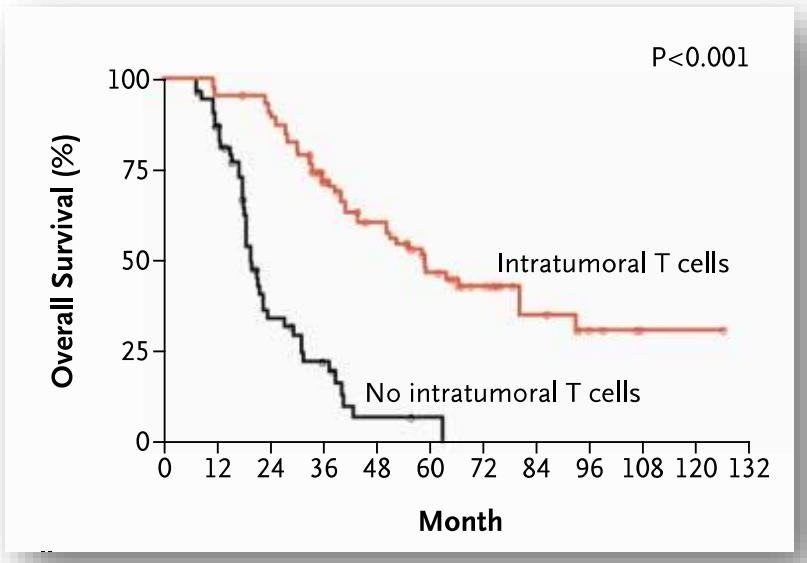
Ovarian Cancer is immunogenic

The NEW ENGLAND JOURNAL of MEDICINE

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer



PFS



OS

Zhang et al. NEJM 2003

Intraepithelial TILs are a robust predictor of outcome in ovarian cancer and define a specific class of patients

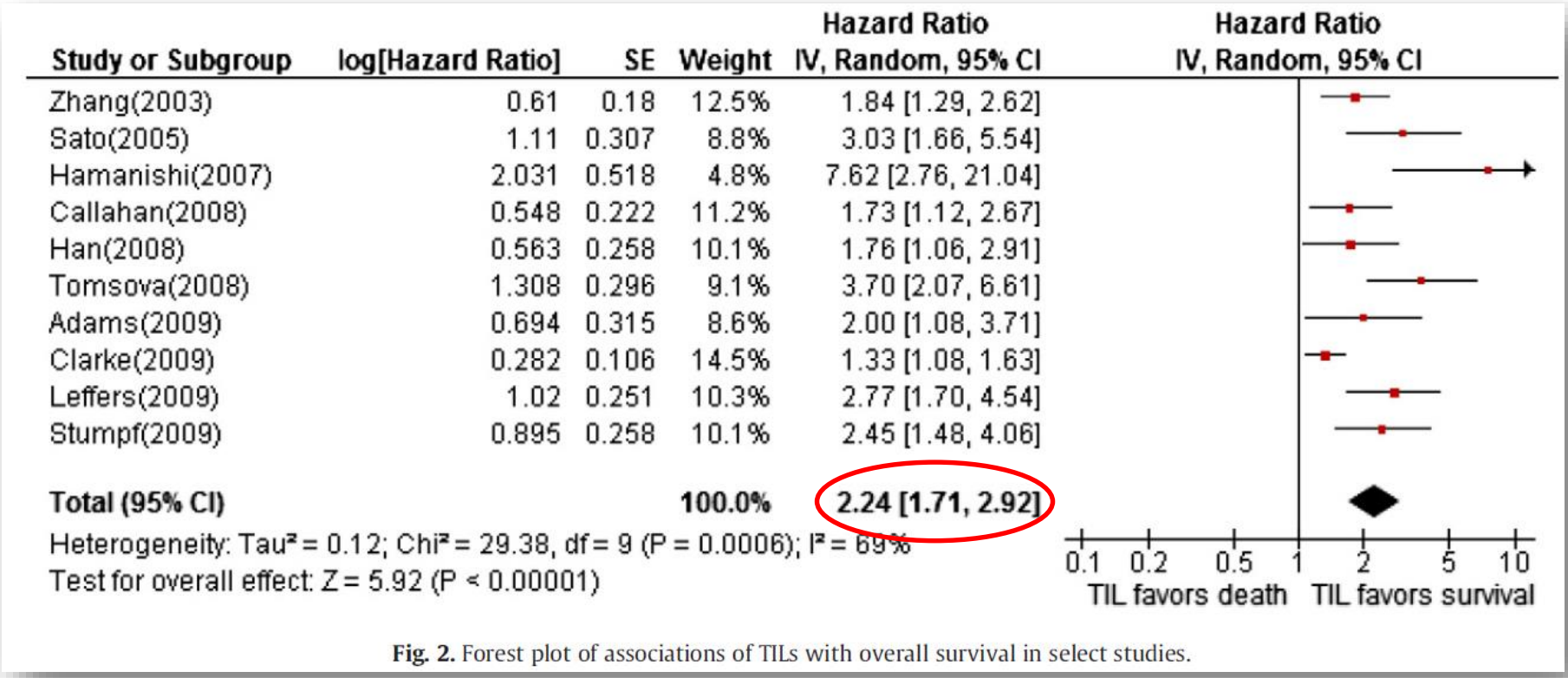
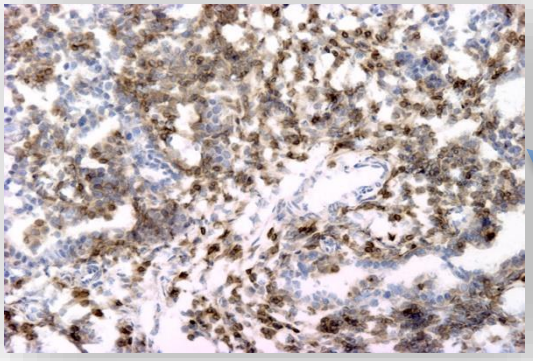


Fig. 2. Forest plot of associations of TILs with overall survival in select studies.

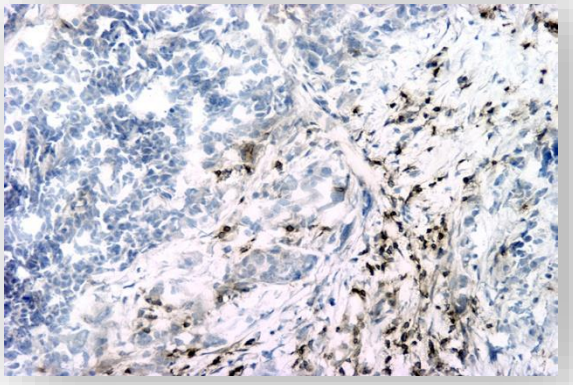
Hwang WT et al. Gynecol Oncol. 2012;124(2):192-8.

Intraepithelial TILs define two specific subset of ovarian cancer patients



TIL-rich
55%

TIL



TIL-poor
45%

T-lymphocyte are present , but not working

T-lymphocyte are just absent in the tumor!

Let's activate globally the immune system! The recombinant cytokines story

The Intra-Peritoneal Cytokines

Intraperitoneal recombinant alpha-interferon for "salvage" immunotherapy in stage III epithelial ovarian cancer: a GOG Study. Berek JS, et al. Cancer Res. 1985.

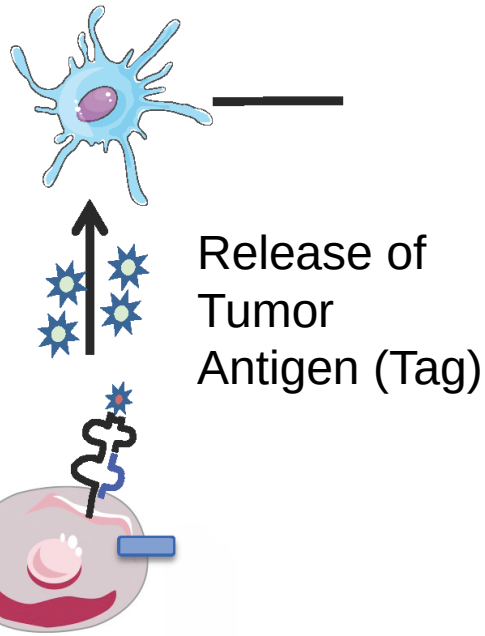
14 pts. 4CR, 1 PR

Intraperitoneal interferon-gamma in ovarian cancer patients with residual disease at second look laparotomy. Pujade-Lauraine E, et al. J Clin Oncol. 1996

108 pts. ORR: 32%. Median response duration of 20 months

The anti-tumor immune response

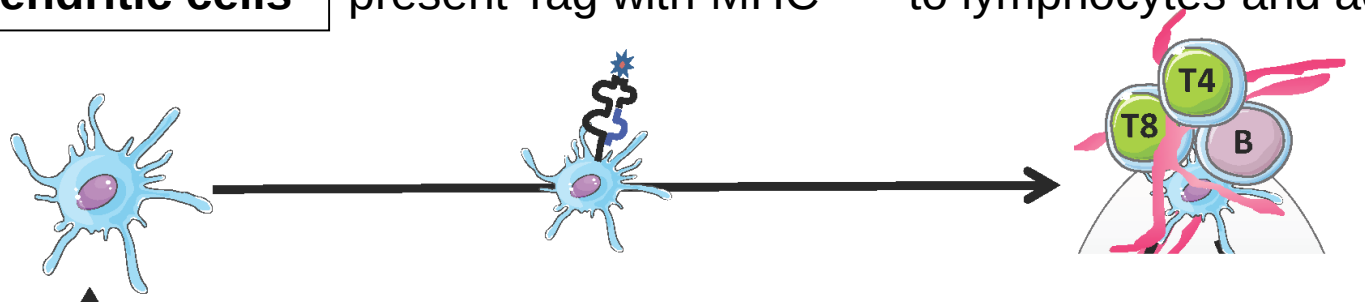
2-Dendritic cells



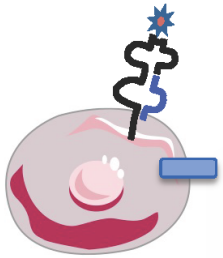
1-Tumor cell

The anti-tumor immune response

2-Dendritic cells present Tag with MHC to lymphocytes and activate

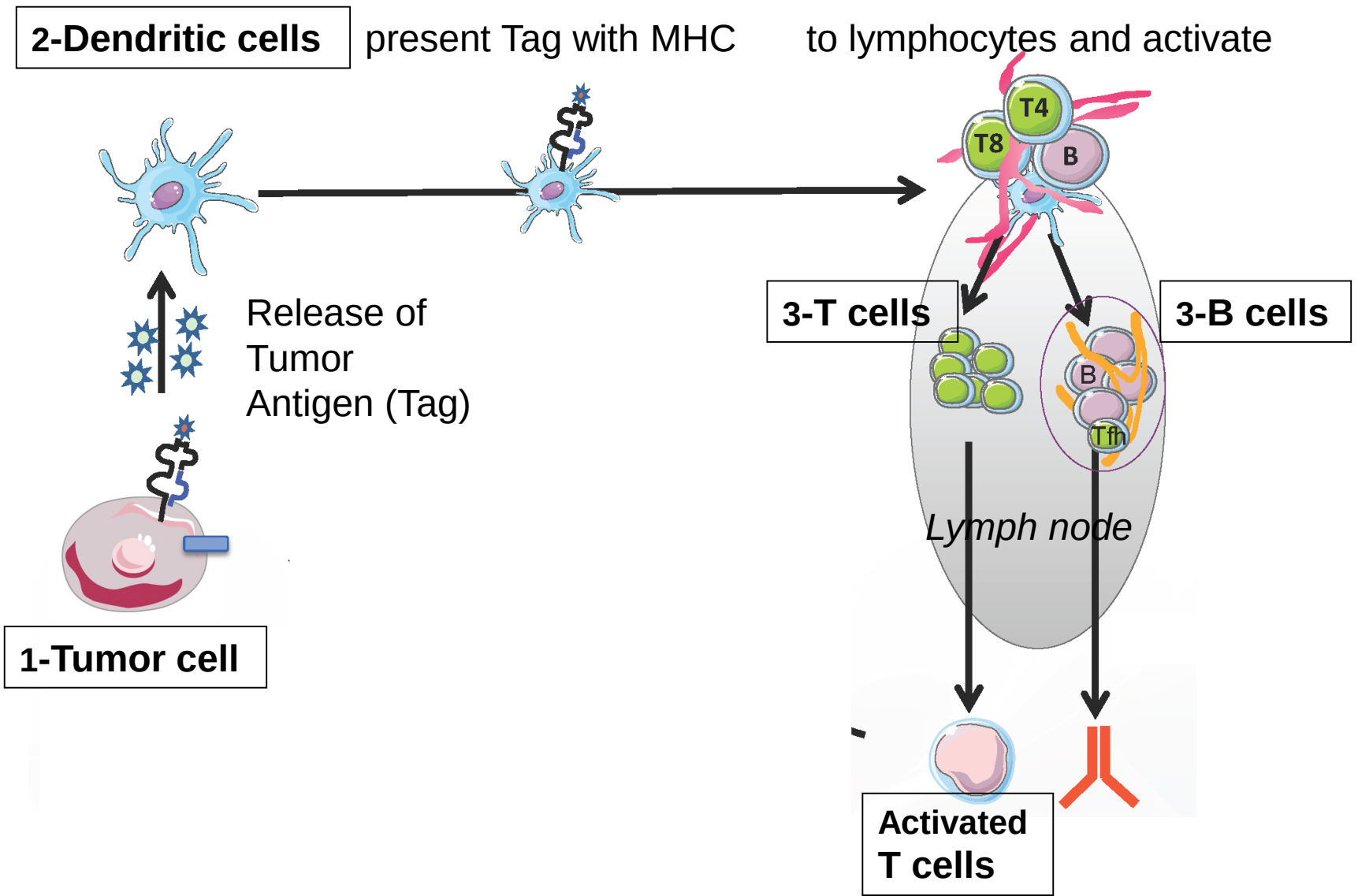


Release of Tumor Antigen (Tag)

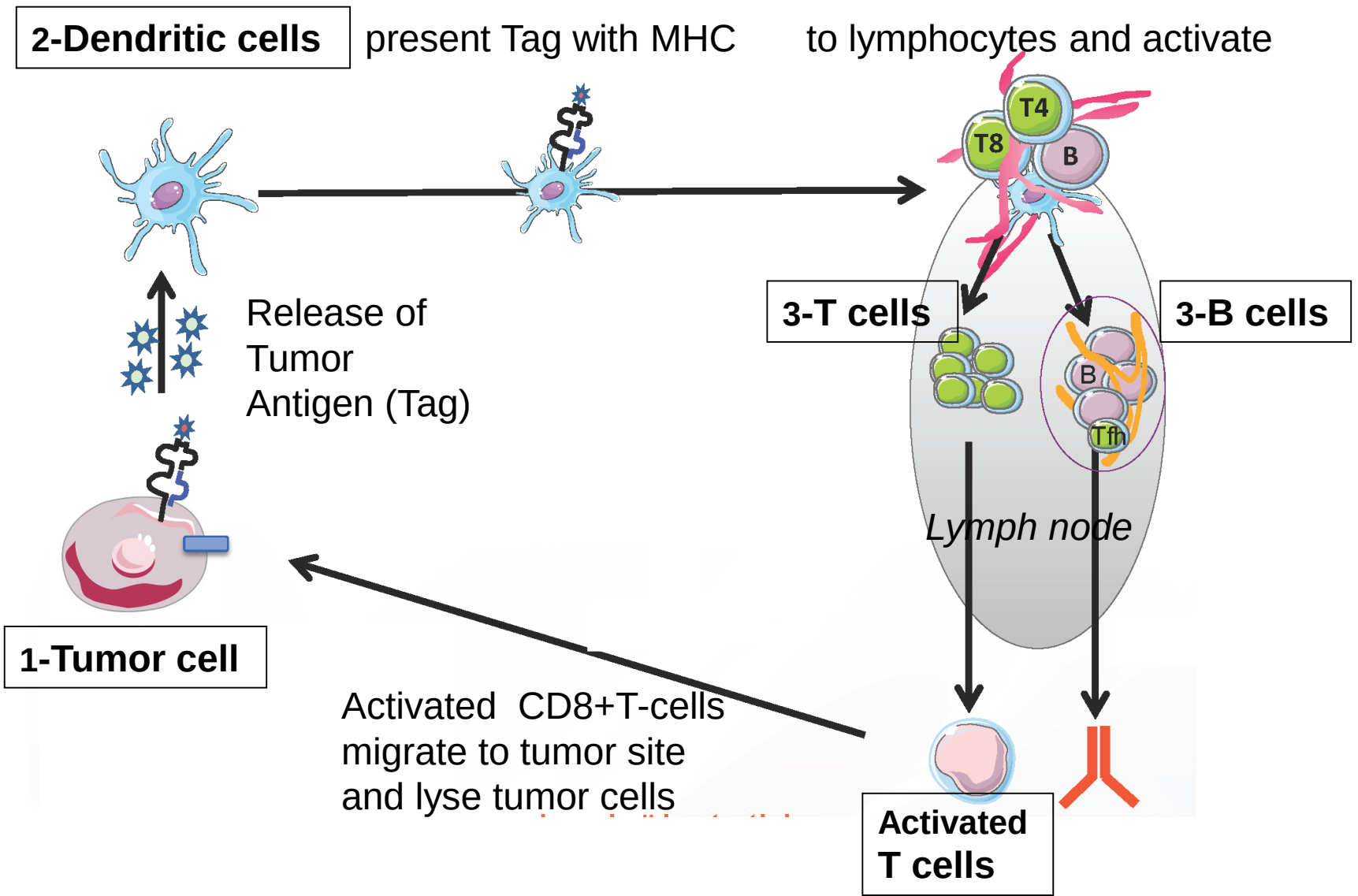


1-Tumor cell

The anti-tumor immune response

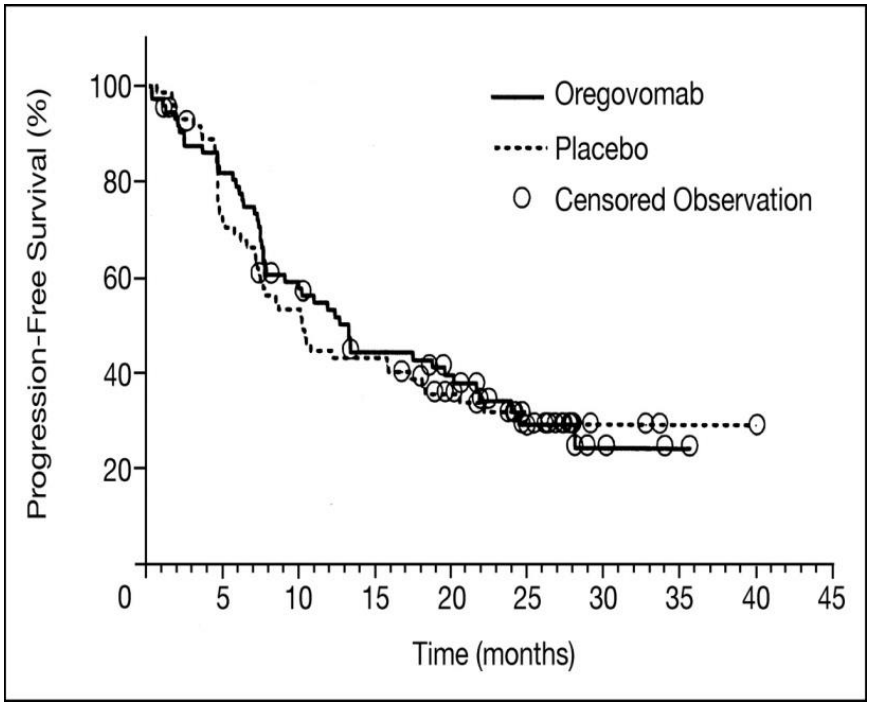


The anti-tumor immune response



Antigen-specific immunotherapy

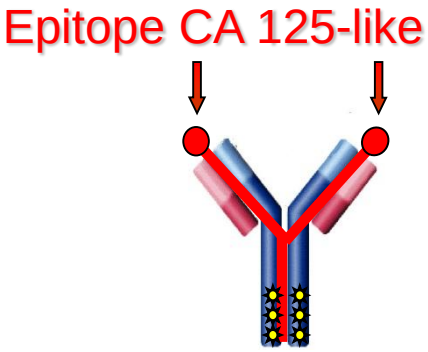
Oregovomab: murine antibody targeting CA 125

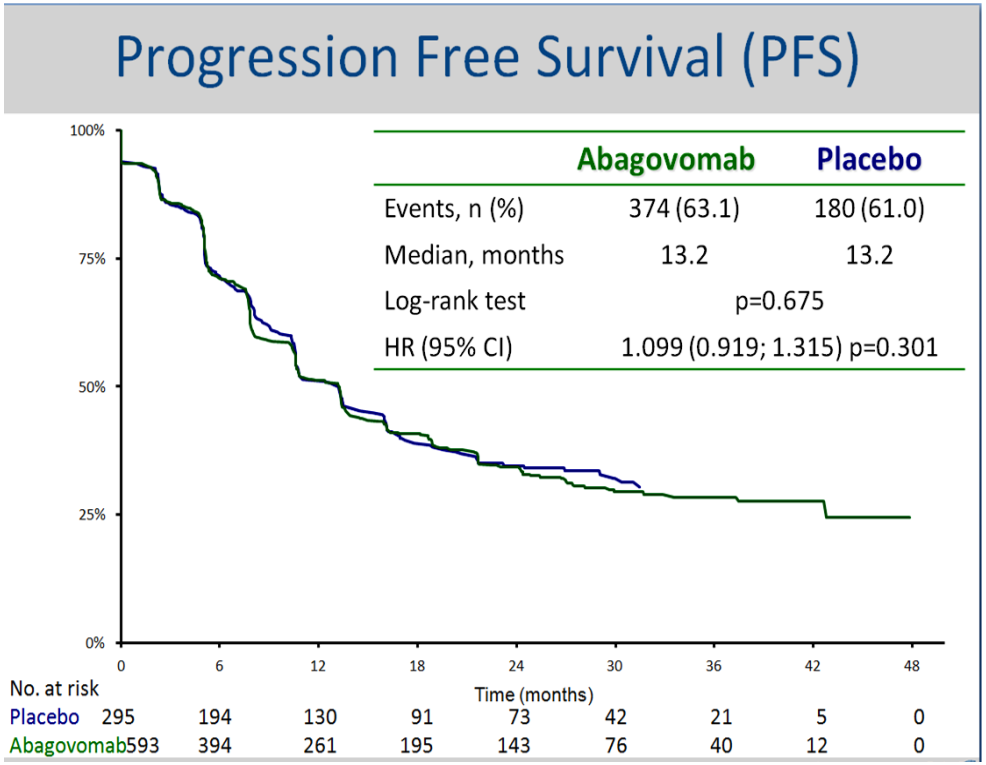


Berek JS et al.: J Clin Oncol 2004; 22:3507-16

Antigen-specific immunotherapy

Vaccination with abagovomab: anti-idiotypic antibody mimicking CA 125



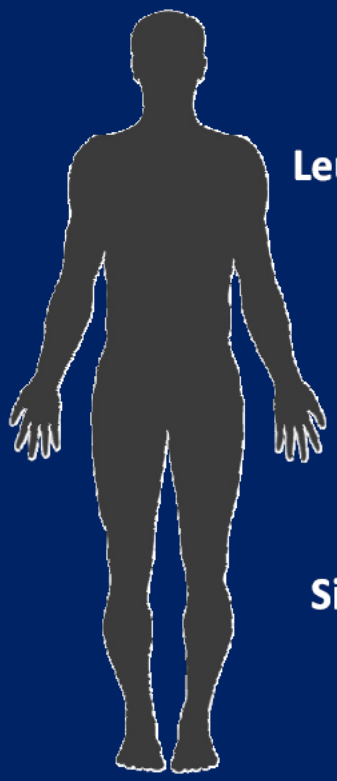


Sabbatini P et al.:J Clin Oncol 2013;31:1554-61

Mononuclear cells trained to detect tumor antigen and stimulated to kill tumor cells

Sipuleucel-T

- Sipuleucel-T: an autologous cellular immunotherapy targeting prostate cancer
- Patient-derived peripheral blood mononuclear cells are co-cultured with Prostate Acid Phosphatase (PAP)-GM-CSF fusion protein.
- Cultured cells are reinfused.



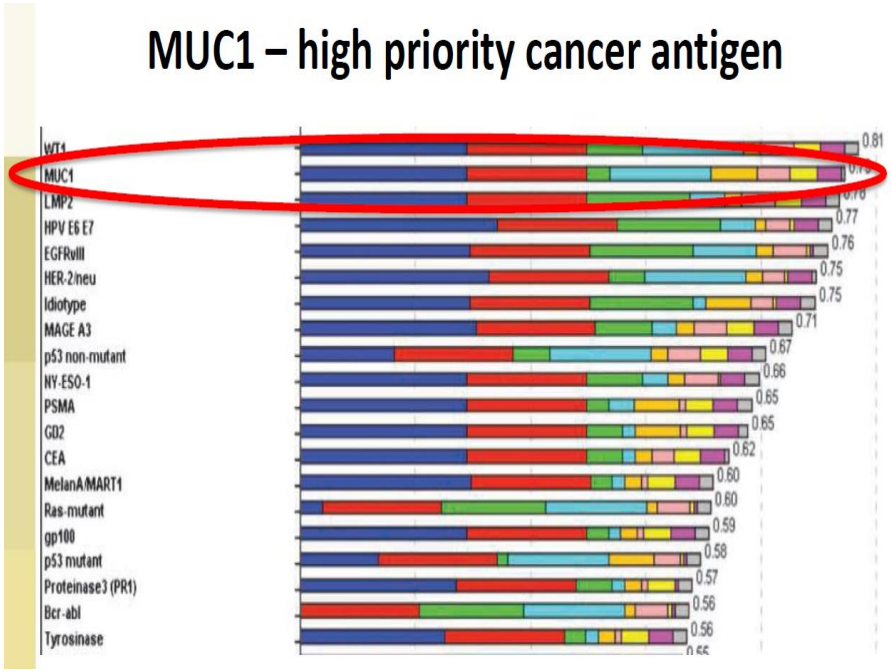
Leukapheresis

PBMC co-incubated with PAP/GM-CSF* fusion X 3

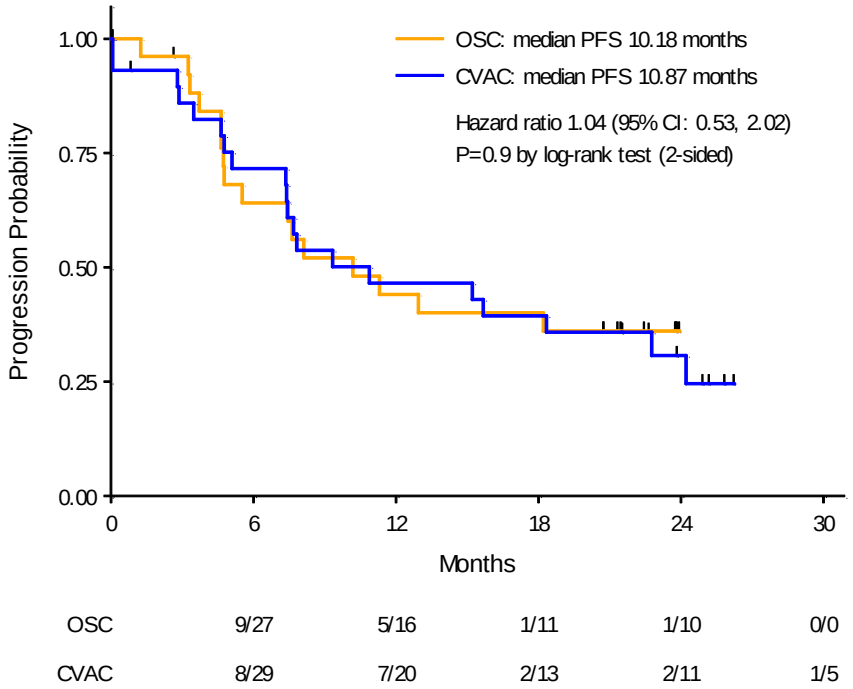
Sipuleucel-T

*PAP/GM-CSF = "PA2024"

A Randomized, Open-Label Phase IIb Trial of Maintenance Therapy with a MUC1 Dendritic Cell Vaccine (Cvac™) for Epithelial Ovarian Cancer Patients in 1st or 2nd Remission

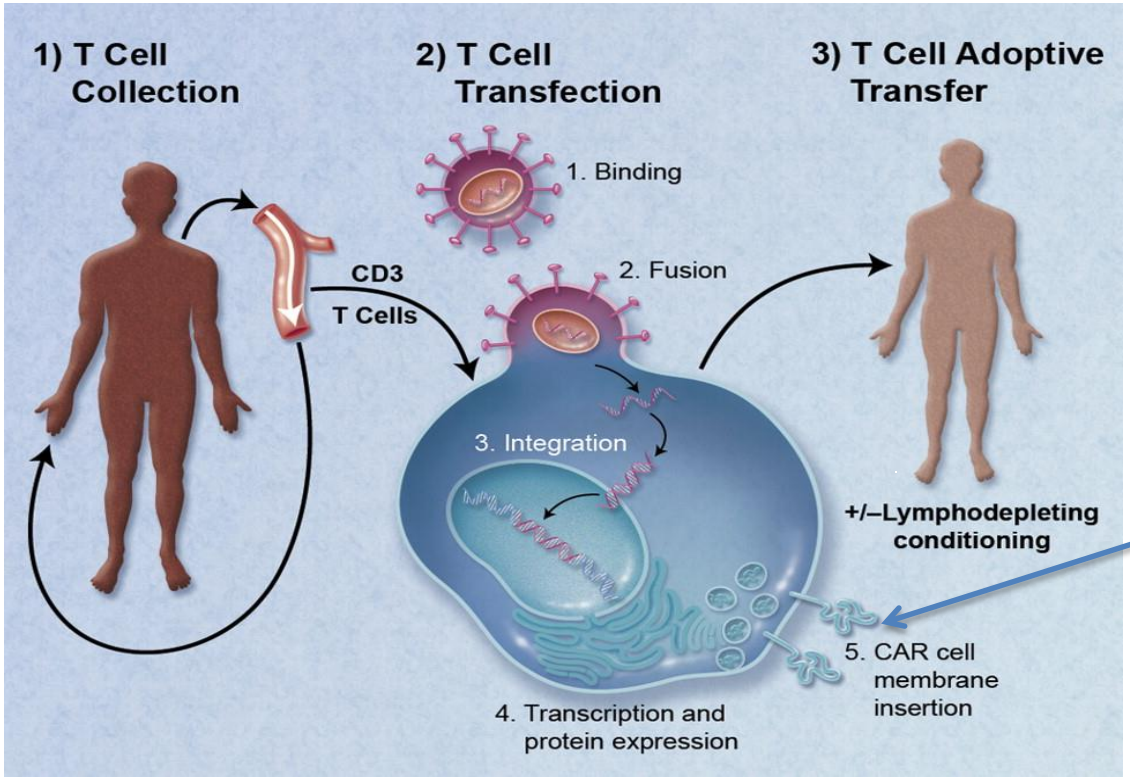


Cheever et al. Clin Cancer Res 2009

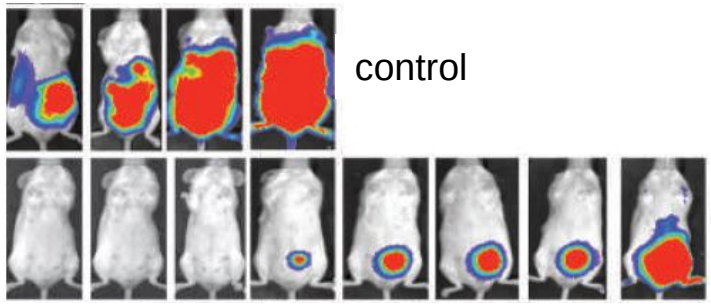


Gray H et al. ASCO 2014

Adoptive T-cells with modified T-cell receptor for MUC16



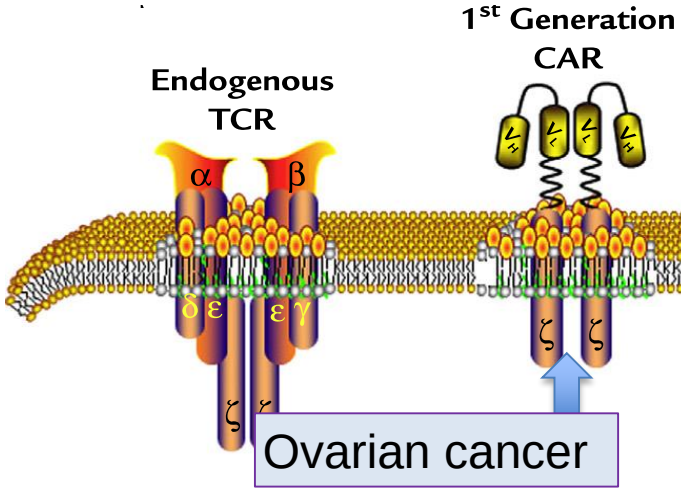
chimeric antigen receptors (CARs) specific for malignant cells



control

CAR MUC16

Chekmasova et al. Clin Cancer Res 2010



Kershaw MH et al. Clin Cancer Res 2010

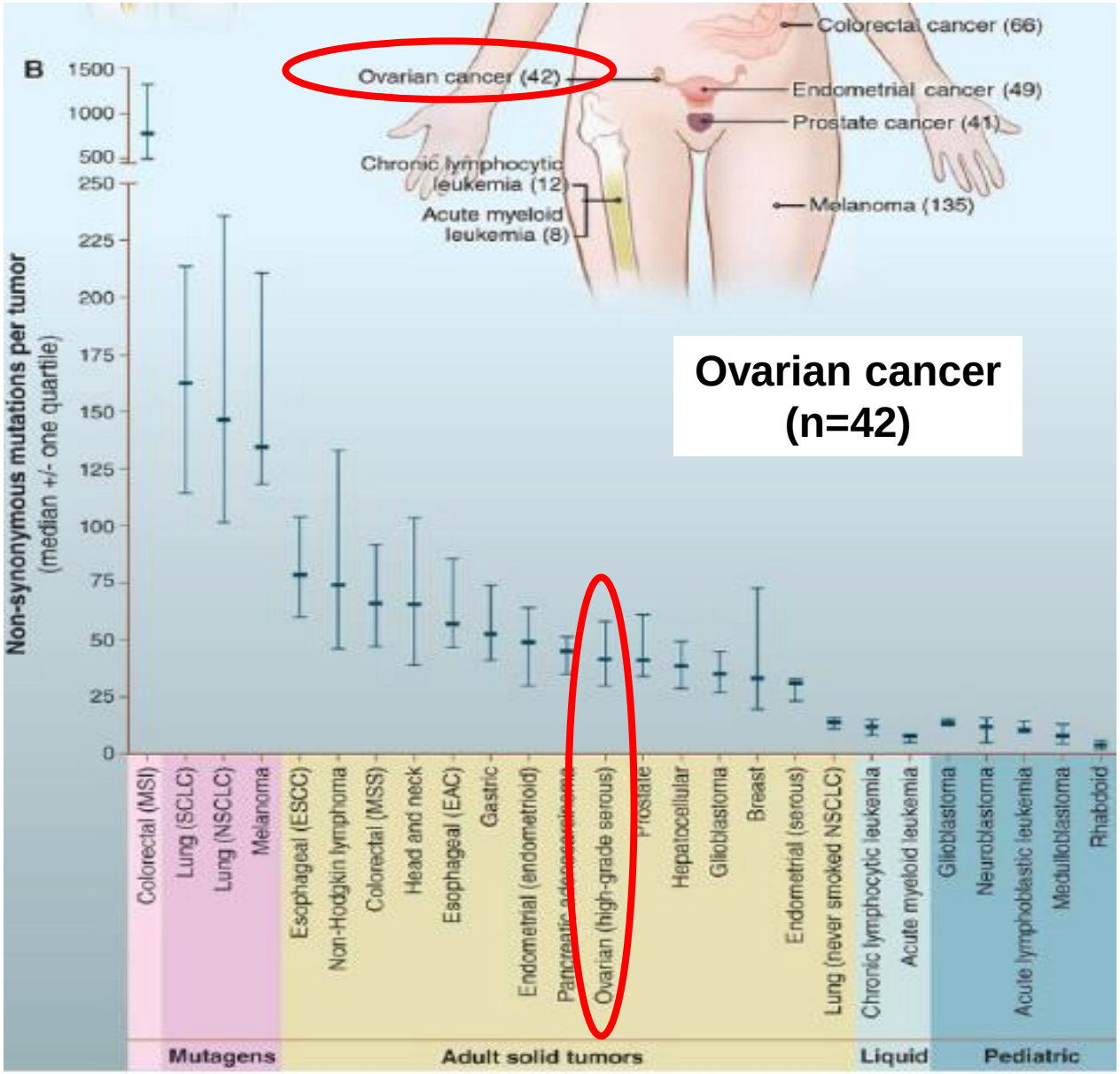
Antigen-specific active immunotherapy for ovarian cancer (Review)

Leffers N, Daemen T, Helfrich W, Boezen HM, Cohlen BJ, Melief CJM, Nijman HW

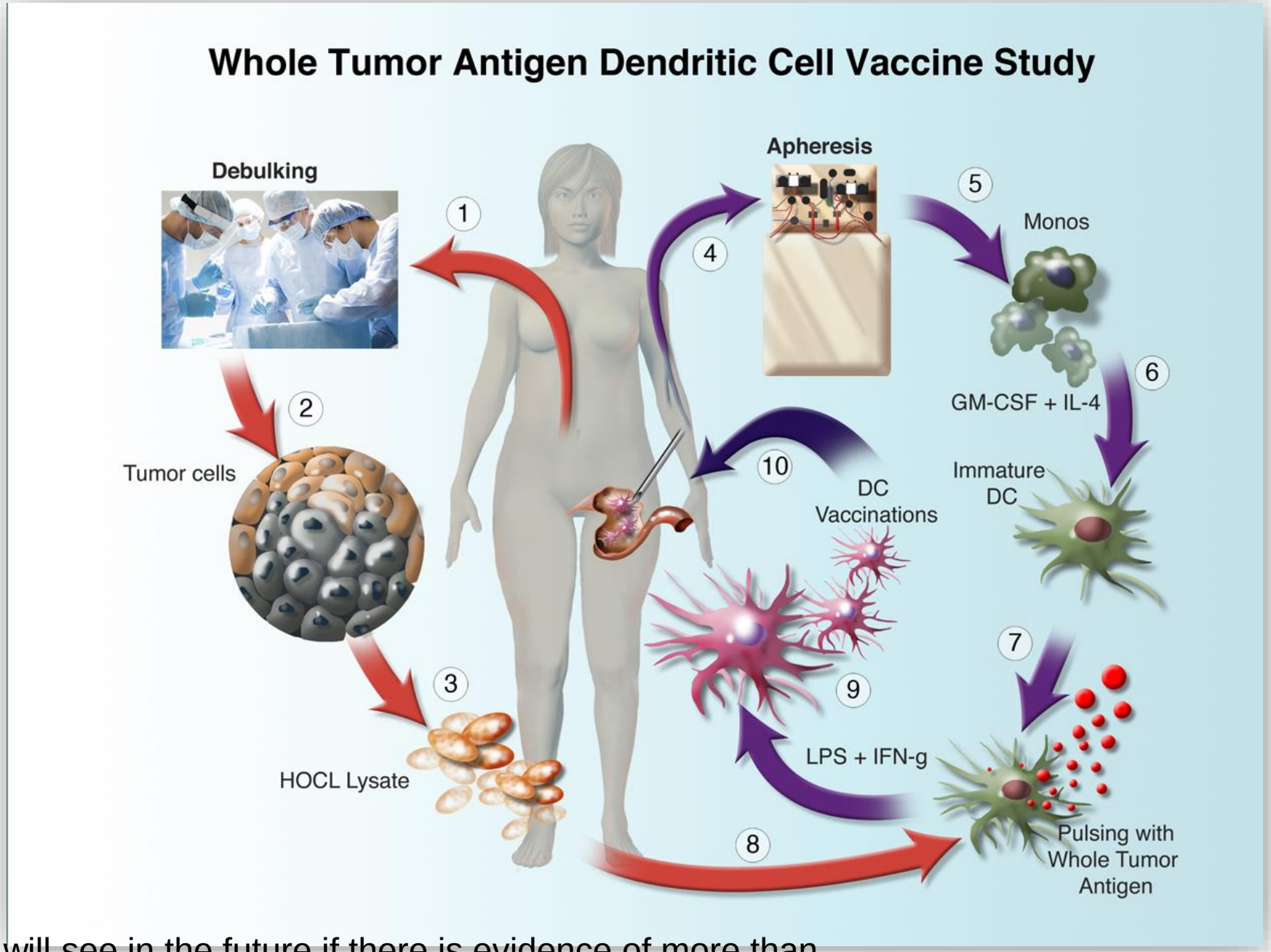
- **55 studies included = 3051 women with epithelial ovarian cancer**
- **all strategies are capable of inducing immunological response, be it humoral or cellular**
- **No clinically effectiveness demonstrated**

Leffers N, et al. Cochrane Database Syst Rev. 2014

Non-synonymous mutations (which change the resulting protein that is expressed) in OC



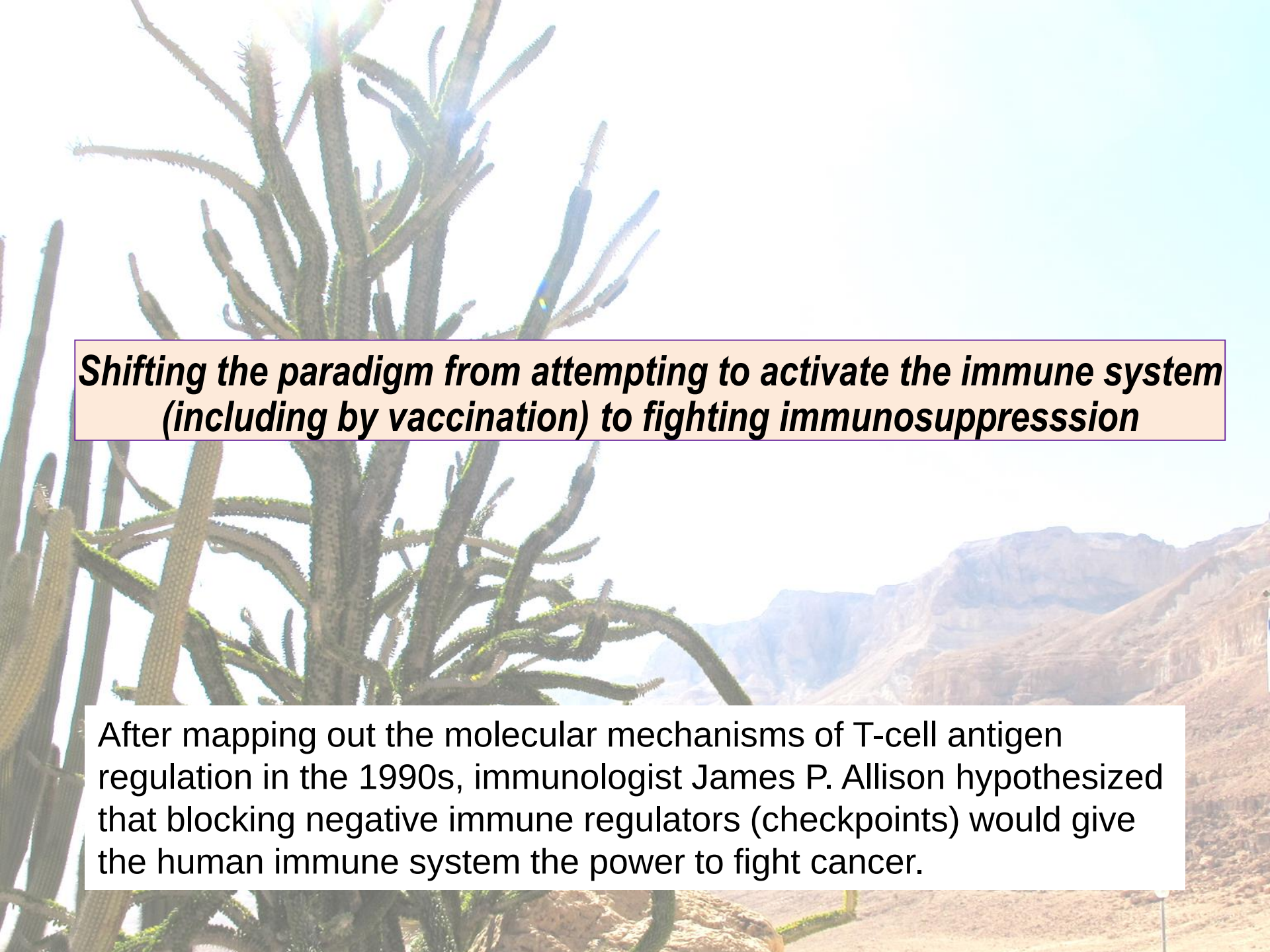
Vogelstein B, Science 2013



We will see in the future if there is evidence of more than anecdotal clinical responses to these approaches inOC

Courtesy George Coukos

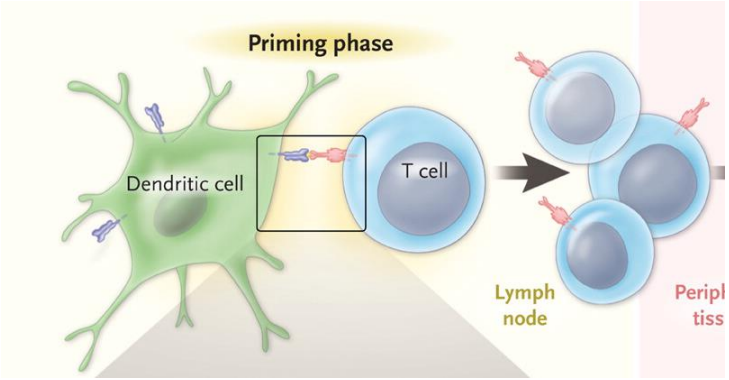
One drug for each patient: Logistic complexity and budget burden

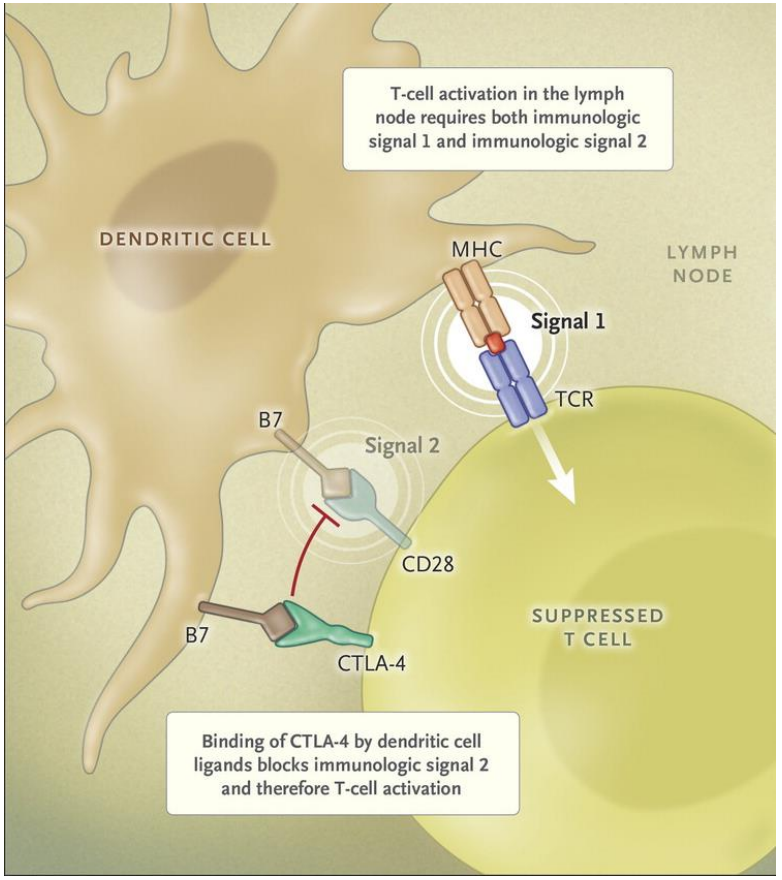


Shifting the paradigm from attempting to activate the immune system (including by vaccination) to fighting immunosuppresssion

After mapping out the molecular mechanisms of T-cell antigen regulation in the 1990s, immunologist James P. Allison hypothesized that blocking negative immune regulators (checkpoints) would give the human immune system the power to fight cancer.

CTLA-4 and PD-1/L1 Checkpoint Blockade

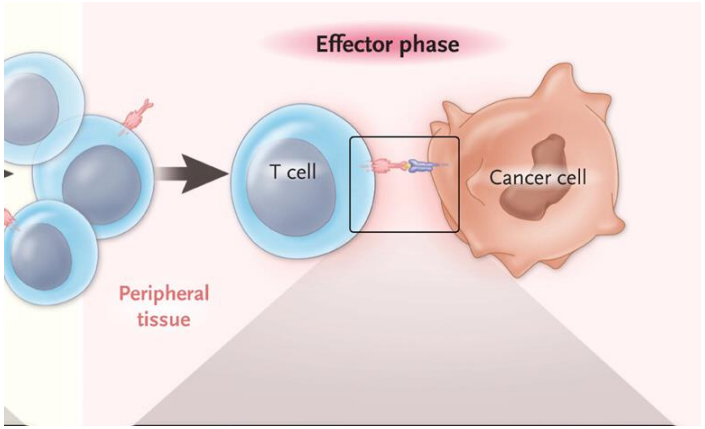


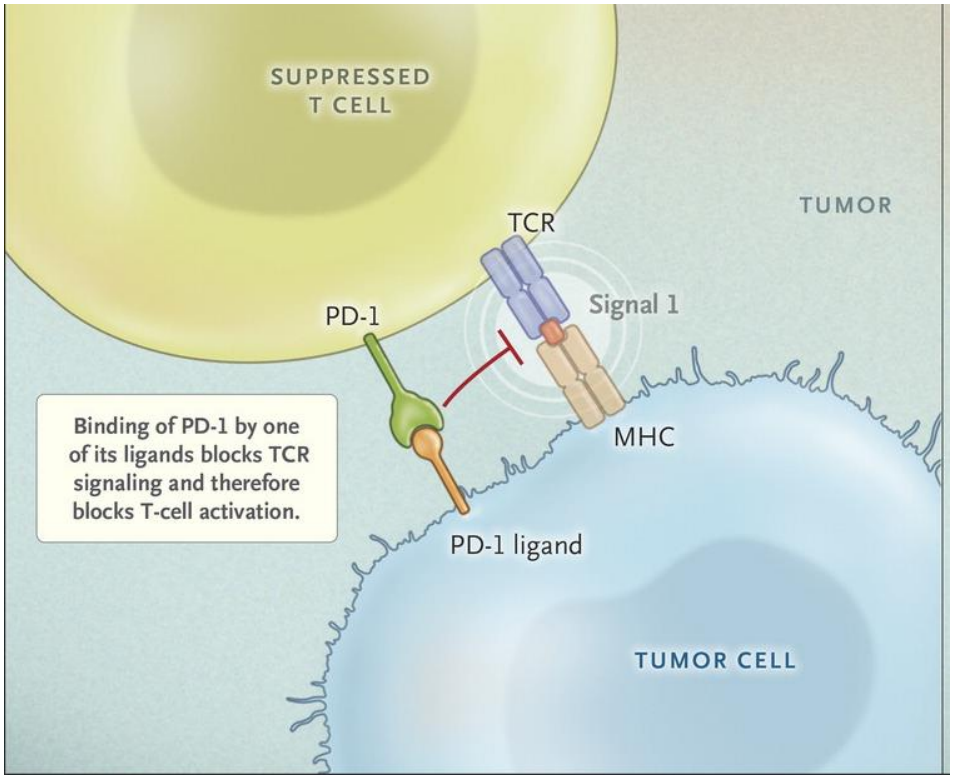


Place of anti-CTLA4

Binding of the B7 costimulatory molecules to CTLA-4 blocks immunologic signal n°2 (stimulation of CD28 by the B7 costimulatory molecule),

CTLA-4 and PD-1/L1 Checkpoint Blockade

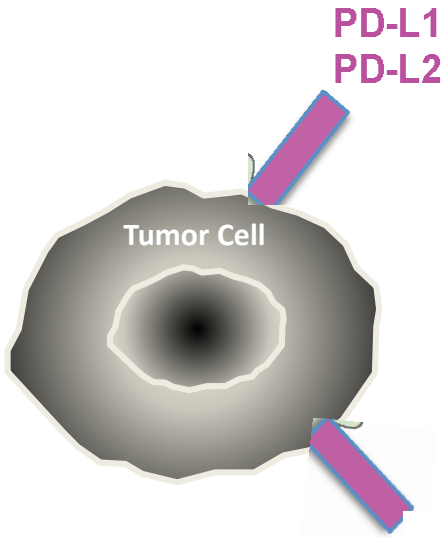




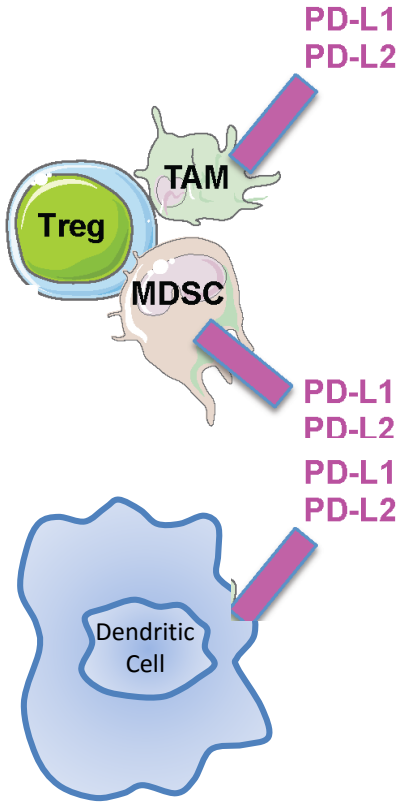
Place of anti-PD1/PDL-1

During long-term antigen exposure, such as occurs in the tumor milieu, the programmed death 1 (PD-1) inhibitor receptor is expressed by T cells

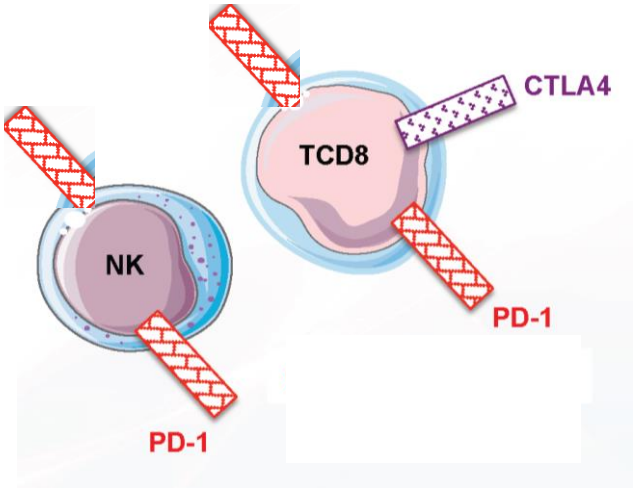
The PD1/PDL-1 pathway



PD-L1/PD-1–mediated inhibition of tumor cell killing

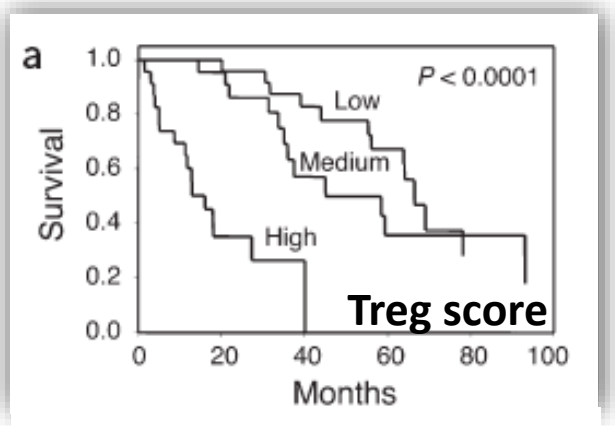


Stromal PD-L1 modulation of T cells



MDSCs: myeloid derived suppressor cells
TAM: M2 Tumor associated Macrophages
T reg: regulatory T cells
NK: Natural Killer cells

Others Immune factors correlated with bad prognosis



Curiel, Nat Med (2004) 10: 942

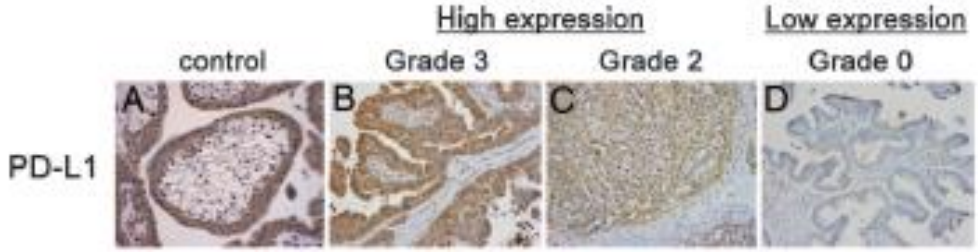
- ✓ **Presence of Treg in the tumor**
  - Curiel, Nat Med (2004) 10: 942; Wolf, Clin Cancer Res (2005) 11:8326; Redjimi, Cancer Res. (2012) 72:4351; Govindaraj, Clin Immunol. (2013) 149:97
- ✓ **Accumulation of plasmacytoid dendritic cells (pDC)**
  - Zou, Nat Med (2001) 7: 133; Wei Cancer Res. (2005) 65: 5020; Labidi-Galy Cancer Res. (2011)
- ✓ **Presence of immunosuppressive macrophages expressing B7-H4**
  - Kryczek, Cancer Res. (2007) 67: 8900, Zhang QW, PlosOne (2012)

PD-L1 expression in Ovarian Cancer

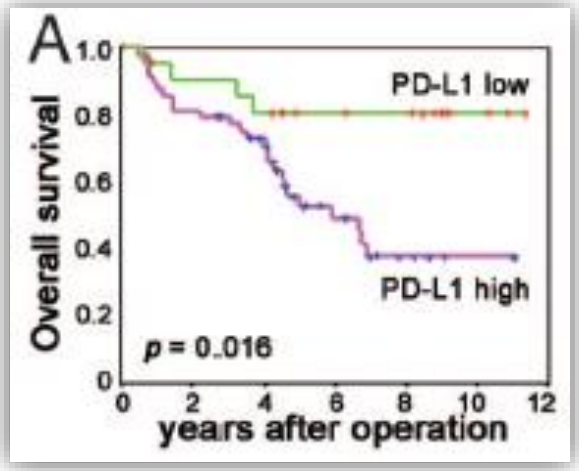
Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer

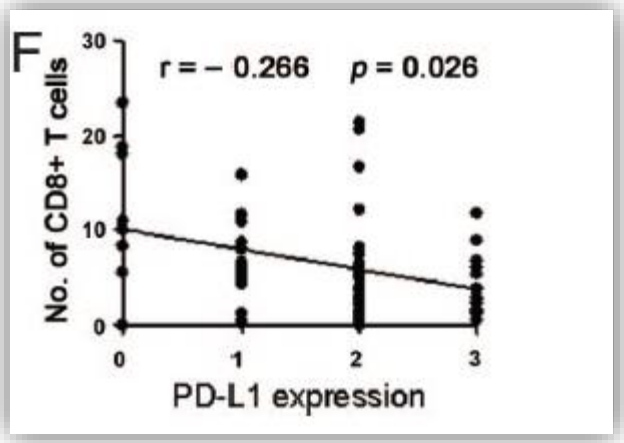
Junzo Hamanishi*, Masaki Mandai*†, Masashi Iwasaki‡, Taku Okazaki§, Yoshimasa Tanaka‡, Ken Yamaguchi*, Toshihiro Higuchi*, Haruhiko Yagi*, Kenji Takakura*, Nagahiro Minato‡, Tasuku Honjo†§, and Shingo Fujii*

PNAS USA, 2007



- PD-L1 evaluation by IHC with local non commercial antibody in 70 ovarian cancer patients





- Inverse correlation between PD-L1 and TIL expression

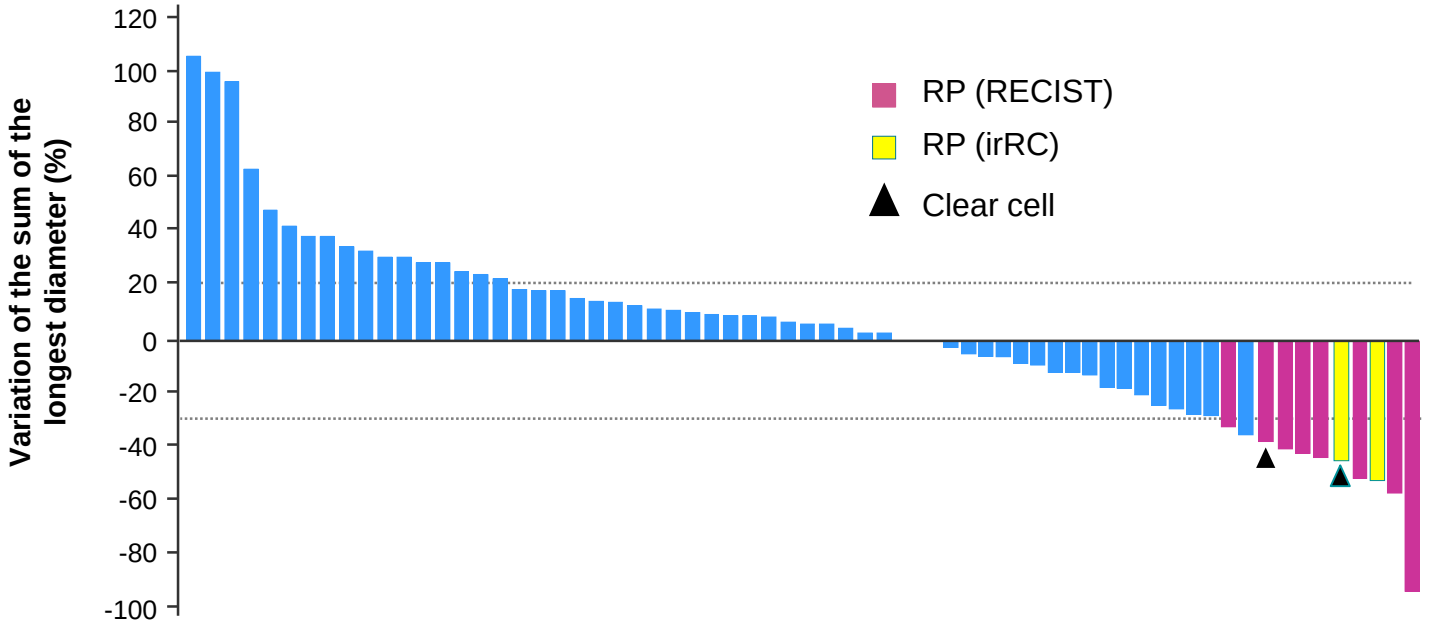
Overview of several anti-PDL1/PD1 therapies currently in development

Therapies are currently in development that target both PD-L1 and PD-1

Therapeutic	Lead company	Antibody type	Affinity/K_2*	Reference
Anti-PDL1				
Atezolizumab	Roche	Engineered IgG1 (no ADCC)	0.4nM	Herbst, et al. ASCO 2013
Durvalumab	AstraZeneca	Modified IgG1 (no ADCC)	Not available	Stewart, et al. Cancer Res 2011
Avelumab	Pfizer	IgG1	Not available	Lachman, et al. Nat Immuno 2001
Anti-PD1				
Nivolumab	Bristol-Myers Squibb	IgG4	2.6nM	Brahmer, et al. J Clin Oncol 2010
Pembrolizumab	Merck & Co	IgG4 (humanised)	29pM	Patnaik, et al. J Clin Oncol 2012
AMP-224	GlaxoSmithKline	PD-L2 IgG1 Fc fusion	Not available	Smothers, et al. Ann Oncol 2013

*Affinity/K_2 describes the strength of binding of an antibody to PD-L1 or PD-1; the lower the value, the higher the affinity

Phase Ib trial of avelumab (anti-PD-L1) in resistant or relapsing OC



- **A tumor size decrease ≥ 30 % has been observed in 11/75 patients (14,7 %)**
  - 8 patients with RP according to RECIST, 1 not confirmed
  - 2 additional RP according to irRC (*Immune-related Response Criteria*)

Disis ML. et al., ASCO 2015 abstr. 5509

KEYNOTE-028 : multicohort phase Ib trial of pembrolizumab (anti-PD-1)

Results in advanced ovarian cancer cohort

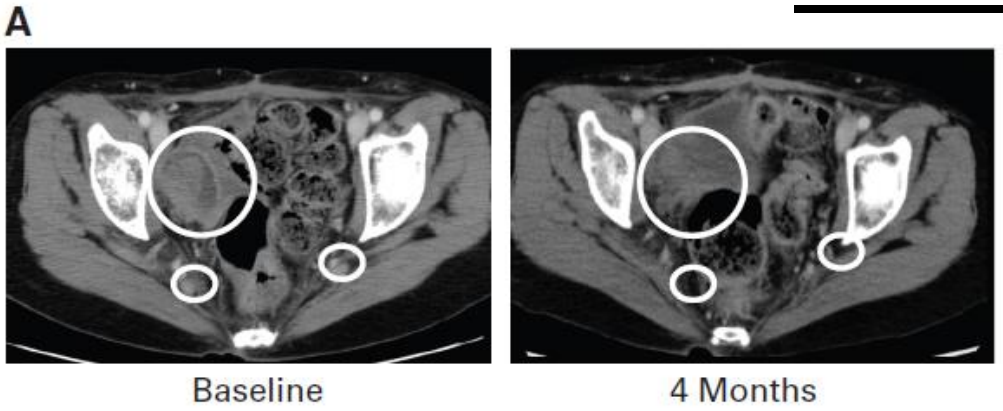
Antitumoral activity

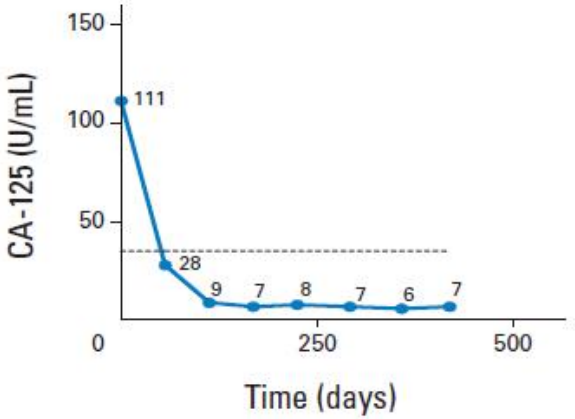
	Patients (n = 26)		
Best response	**n**	**%**	**IC_{95}**
Response rate	**3**	**11,5**	2,4-30,2
RC	1	3,8	0,1-19,6
RP	2	7,7	0,9-25,1
Stable disease	**6**	**23,1**	9,0-43,6
Disease Progression	17	65,4	44,3-82,8
Disease control rate	9	34,6	17,2-55,7

Varga A et al., ASCO 2015 abstr. 5510,

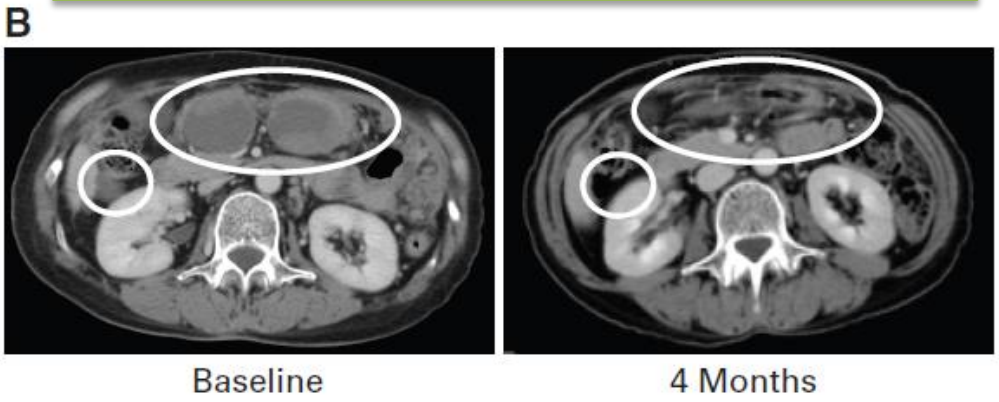
Nivolumab (anti-PD1) in OC

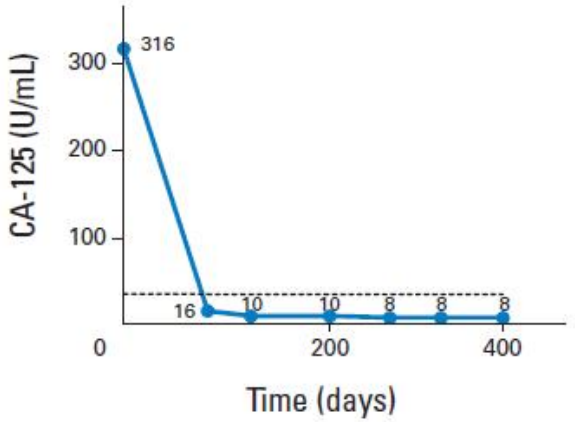
Nivolumab dose	Number of OC patients	Response
1mg/kg	10	1 PR (10%)
3mg/kg	10	2 CR (20%)





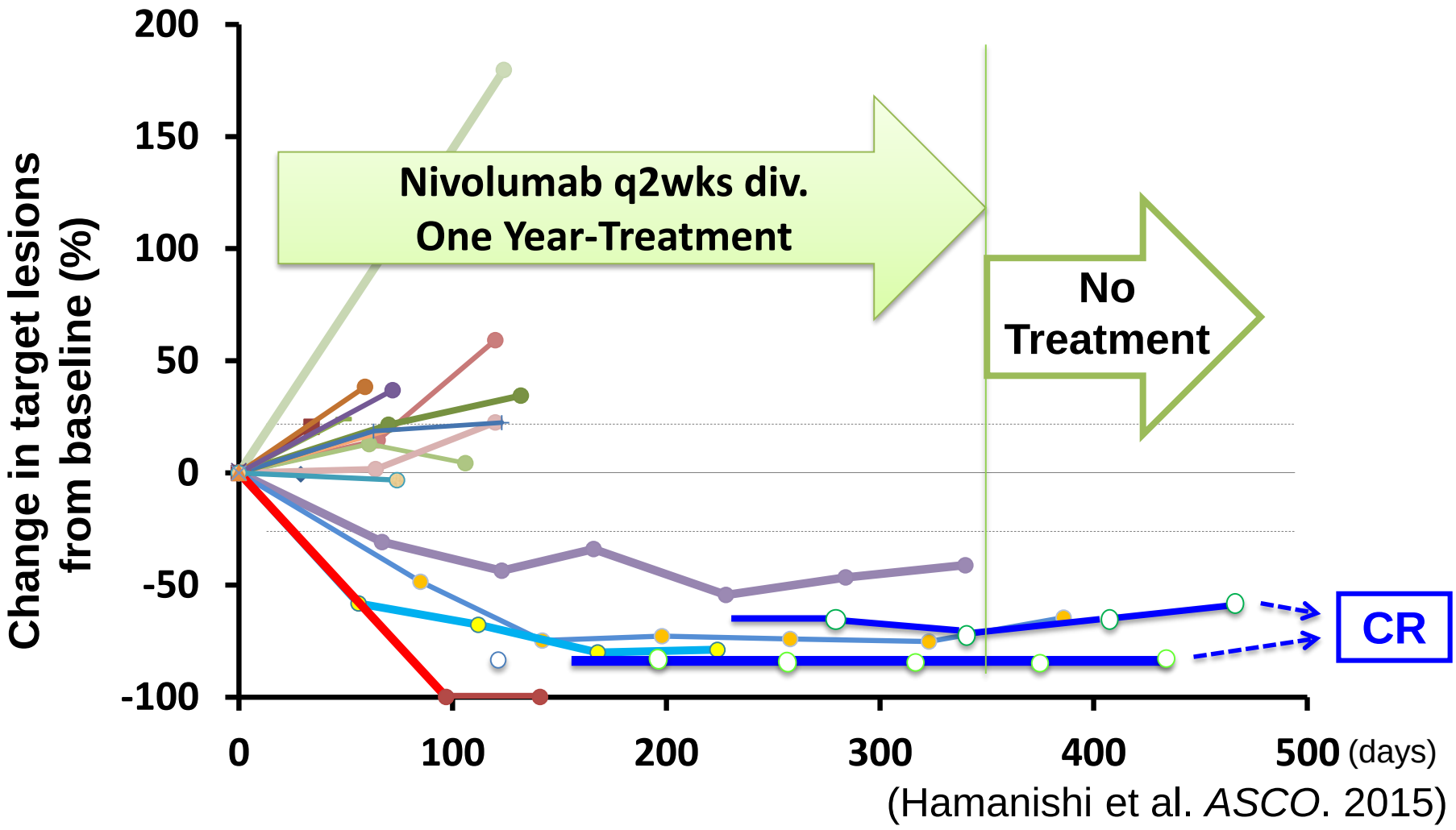
2 cases with a Complete response





Hamanashi J. J Clin Oncol 2015 Dec 1;33(34):4015-22

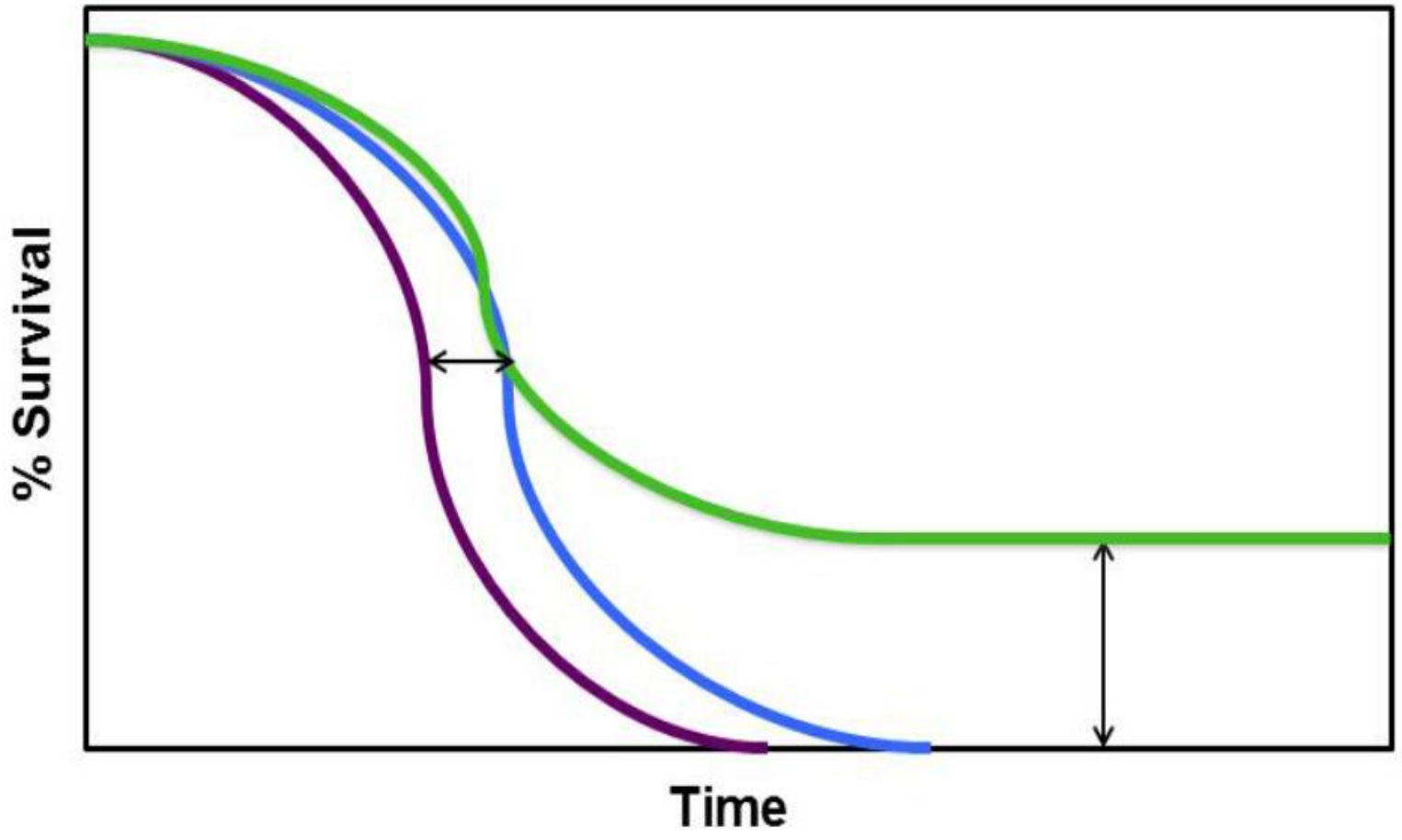
Follow-up Study (on going)



(Hamanishi et al. *ASCO*. 2015)

Durable response without treatment

Getting durable responses and improving survival with check-point inhibitors



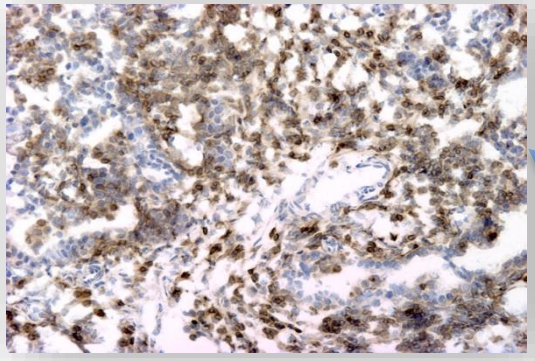
Anti-PD1 & anti-PDL-1: the 3 issues

1. **They have unique toxicities: we are dealing with « itises »: colitis, pneumonitis, hepatitis..**
2. **Selection of patients:Predictive biomarker is not yet ready: each company develop their own marker with their own cut-off, issues of tumor heterogeneity, timing of the biopsy, ….**

Correlation between PDL-1 expression and response to anti-PDL1/PD1 in Lung Cancer

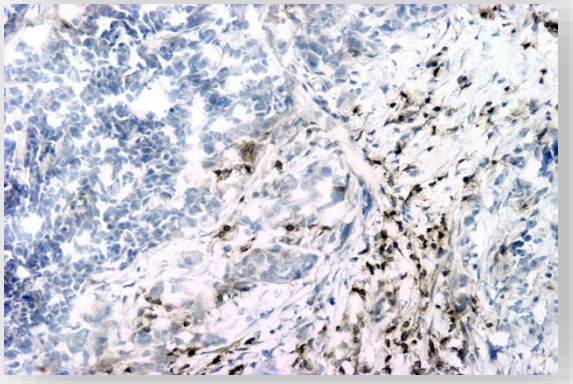
Agent	Phase	ORR	ORR (Tumor PD-L1 +)	ORR (Tumor PD-L1 –)	ORR (TIL PD-L1 +)
Nivolumab (BMS-936558)	I	18 %	15 %	0 %	–
Pembrolizumab (MK-3475)	I	21 %	37 %	11 %	–
MPDL3280A	I	23 %	38 %	24 %	83 %
MEDI4736	I	16 %	25 %	3 %	–

IHC = immunohistochemistry; ORR = overall response rate; PD-L1 = programmed death-ligand 1.



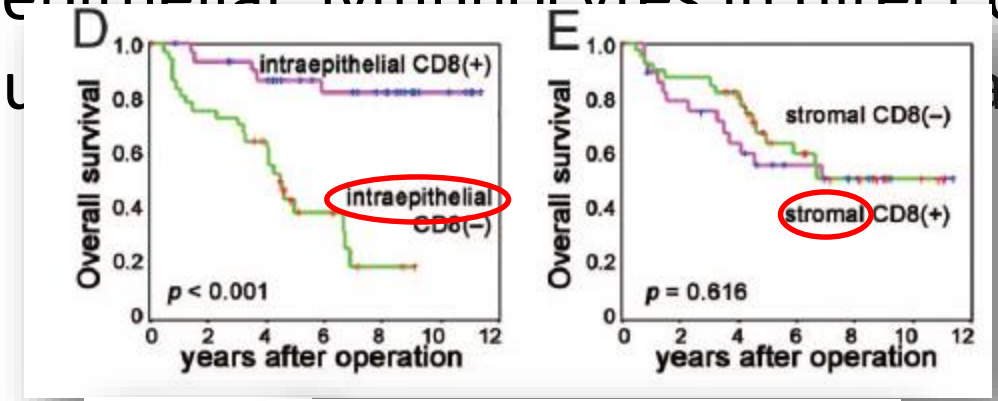
TIL-rich
55%

TIL



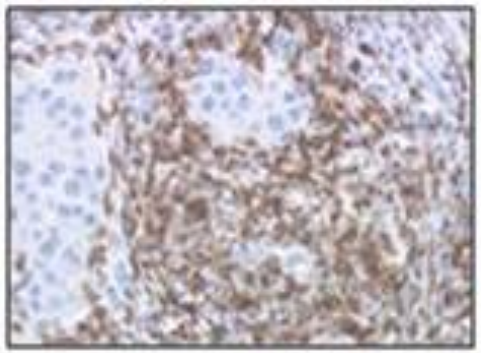
TIL-poor
45%

- IHC, IHC with score, QuanTILfy (PCR)
- CD3, CD4, CD 8, ratio CD8/Treg, ratio CD3/Treg
- Core biopsy vs tumor sample
- Intraepithelial: lymphocytes in direct contact with the tu ative BC)

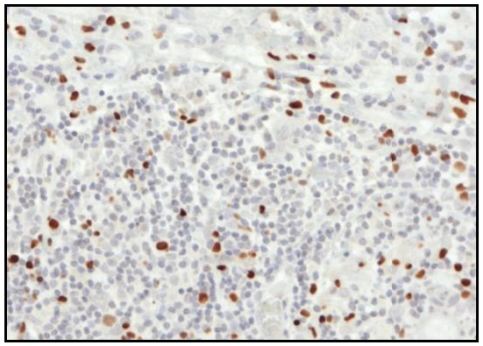


IHC staining to identify T cells, Treg, DC subsets, NK, and macrophages in OC

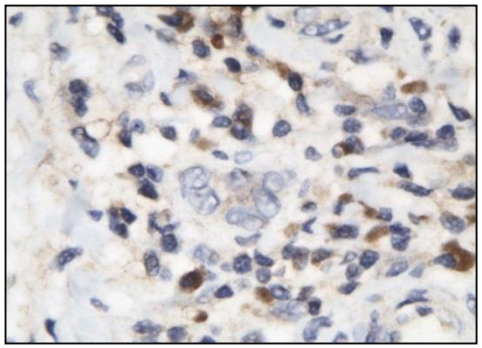
CD3



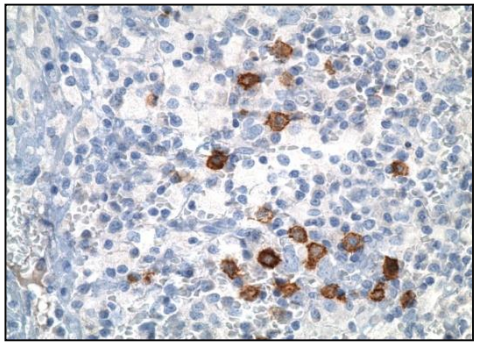
FoxP3



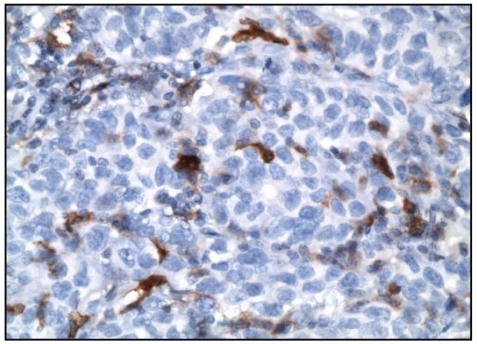
CD163



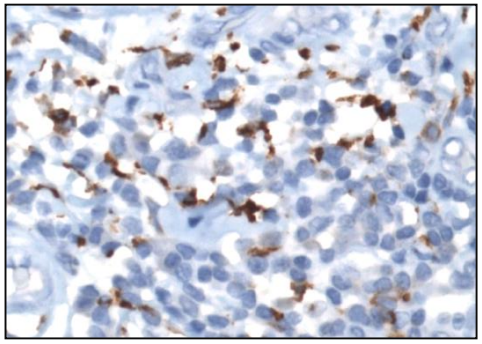
BDCA2



ICOS



NKp46

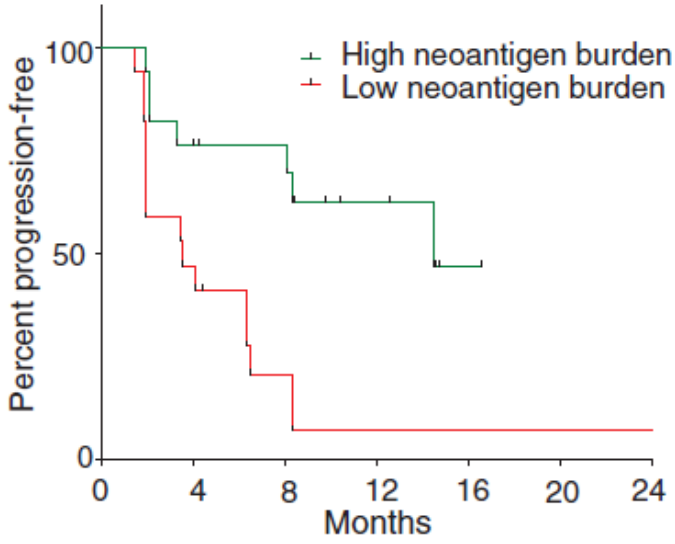


By courtesy of C. Caux

Mutational load predicts immune checkpoint inhibitor activity

Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer

Naiyer A. Rizvi,[1,2*†] Matthew D. Hellmann,[1,2*] Alexandra Snyder,[1,2,3*] Pia Kvistborg,[4] Vladimir Makarov,[3] Jonathan J. Havel,[3] William Lee,[5] Jianda Yuan,[6] Phillip Wong,[6] Teresa S. Ho,[6] Martin L. Miller,[7] Natasha Rekhtman,[8] Andre L. Moreira,[8] Fawzia Ibrahim,[1] Cameron Bruggeman,[9] Billel Gasmi,[10] Roberta Zappasodi,[10] Yuka Maeda,[10] Chris Sander,[7] Edward B. Garon,[11] Taha Merghoub,[1,10] Jedd D. Wolchok,[1,2,10] Ton N. Schumacher,[4] Timothy A. Chan[2,3,5‡]



DNA repair deficiency is correlated to mutational load

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

Dung T. Le, M.D., Jennifer N. Uram, Ph.D., Hao Wang, Ph.D., Bjarne R. Bartlett, B.S., Holly Kemberling, R.N., Aleksandra D. Eyring, M.Pharm., Andrew D. Skora, Ph.D., Brandon S. Luber, Sc.M., Nilofer S. Azad, M.D., Dan Laheru, M.D., Barbara Biedrzycki, Ph.D., C.N. Fisher, M.D., Ph.D., Todd S. Crocenzi, M.D., James erg, M.D., Albert de la Chapelle, M.D., Ph.D., Minori Kos , Ralph H. Hruban, M.D., Laura D. Wood, M.D., Ph.D., Nat dopoulos, Ph.D., Kenneth W. Kinzler, Ph.D., Shibin Zhou, M.D. , Robert A. Anders, M.D., Ph.D., James R. Eshleman, M.D., Ph.D., Bert Vogelstein, M.D., and Luis A. Diaz, Jr., M.D.

Objective response rates to anti-PD1
MSI positif = 60%
MSI negatif = 0%

N Engl J Med 2015; 372:2509-2520 | June 25, 2015 | DOI: 10.1056/NEJMoa1500596

JAMA Oncol. 2015 Jul 9. doi: 10.1001/jamaoncol.2015.2151. [Epub ahead of print]

Association of Polymerase e-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1.

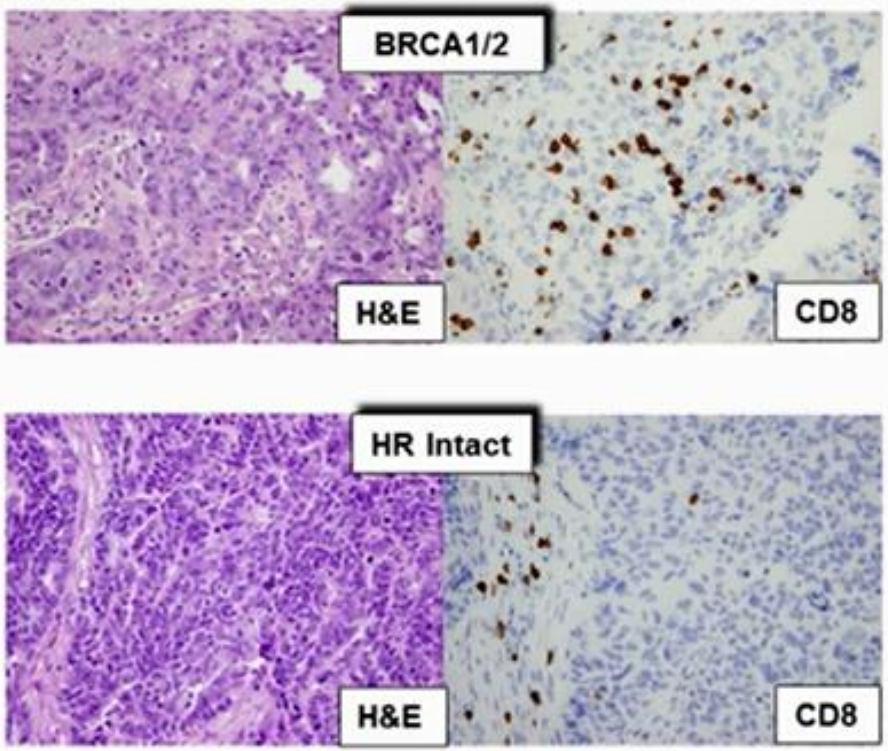
Howitt BE[1], Shukla SA[2], Sholl LM[1], Ritterhouse LL[1], Watkins JC[1], Rodig S[1], Stover E[3], Strickland KC[1], D'Andrea AD[4], Wu CJ[2], Matulonis UA[3], Konstantinopoulos

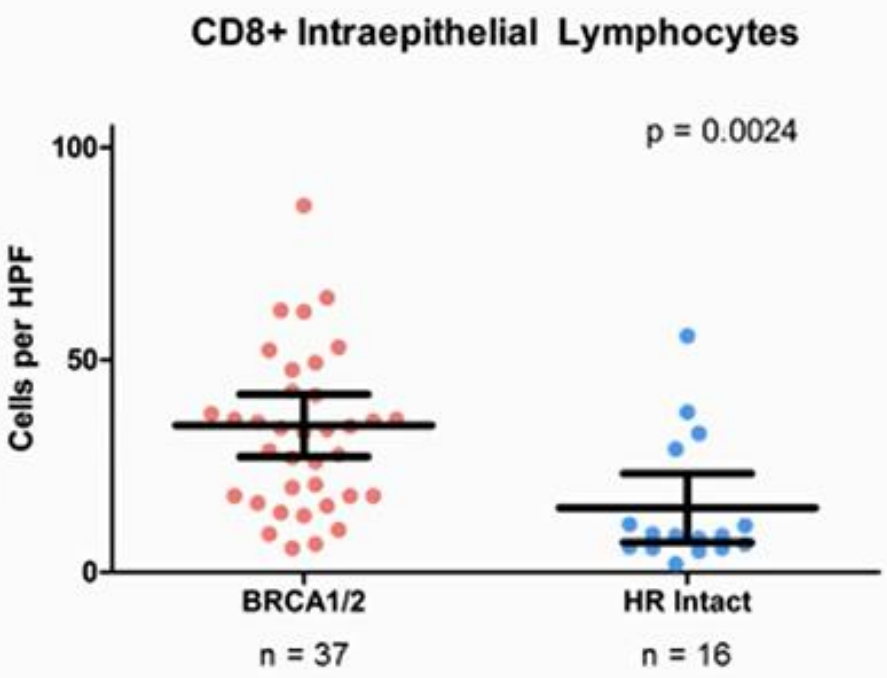
Kyle Strickland et al. ASCO 2015

DANA-FARBER CANCER INSTITUTE

HARVARD MEDICAL SCHOOL

CD8+ TILs were increased in the BRCA1/2 cohort





PRESENTED AT: ASCO Annual '15 Meeting

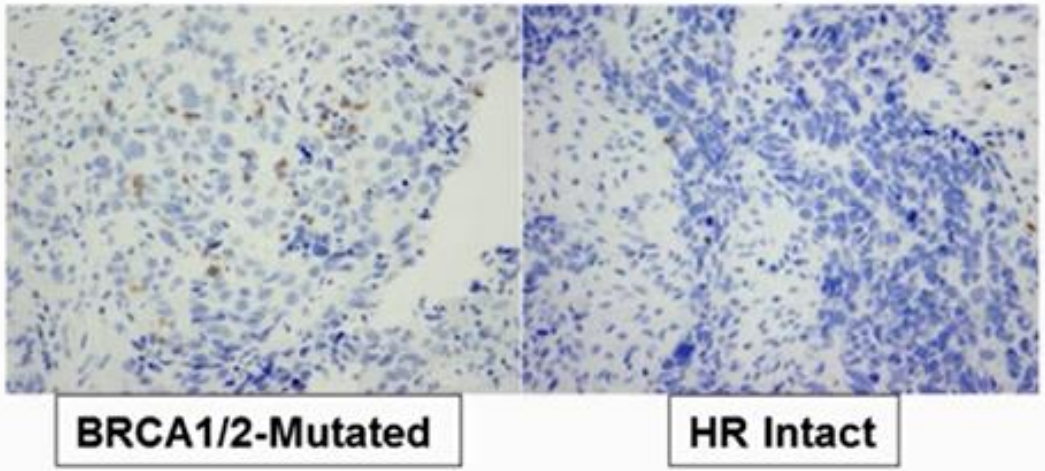
No correlation of CD4+ and CD20+ cells was shown to BRCA 1/2 status

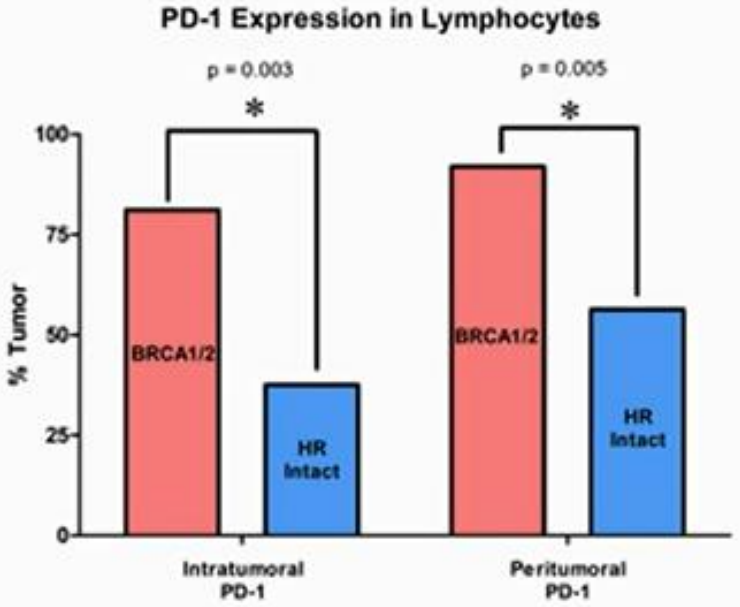
Kyle Strickland et al. ASCO 2015

DANA-FARBER CANCER INSTITUTE

HARVARD MEDICAL SCHOOL

PD-1 is expressed more frequently in TILs and PTLs of BRCA1/2-mutated tumors



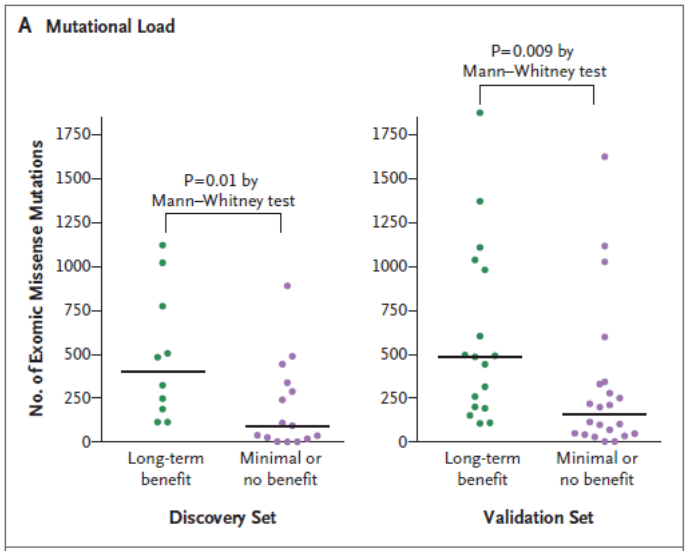


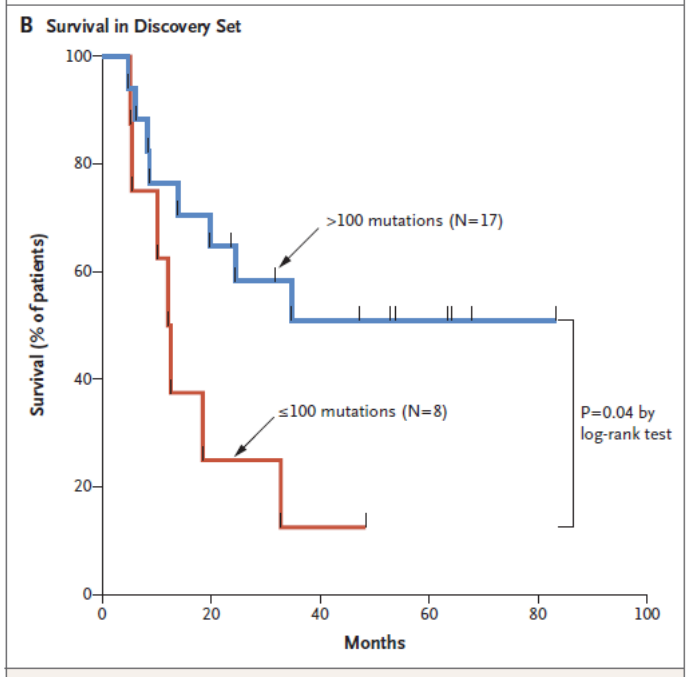
PRESENTED AT: ASCO Annual '15 Meeting

Increased PDL1 expression in IC but <u>not</u> in TC

Melanoma et anti-CTLA-4

- Mutational load is predictive , but only statistically

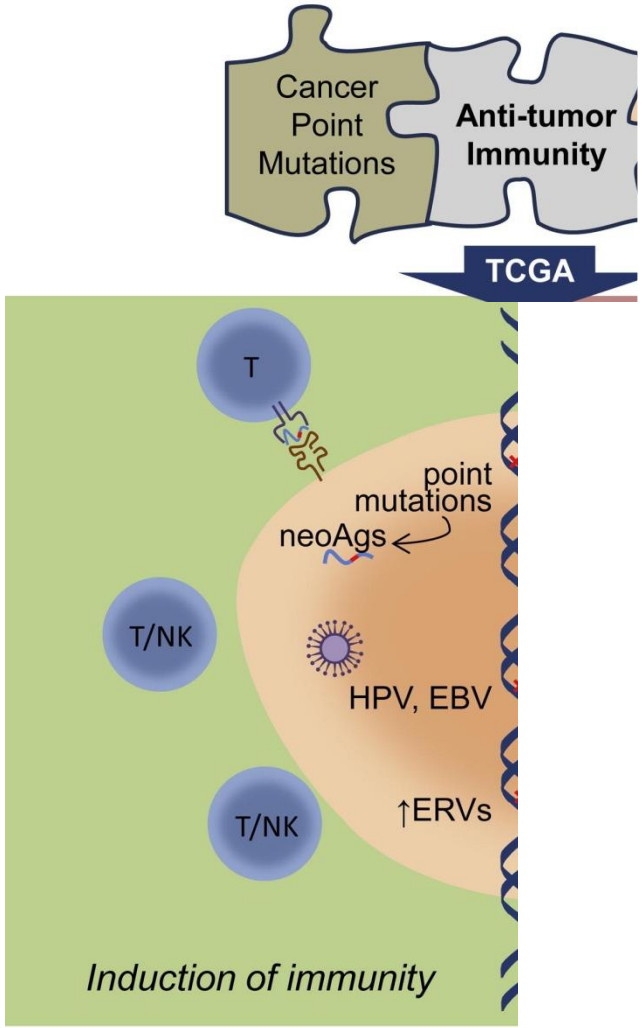




Snyder A , et al; N Engl J Med 2014;371:2189-99

Cytolytic T-cell is activity is the fruit of a balance

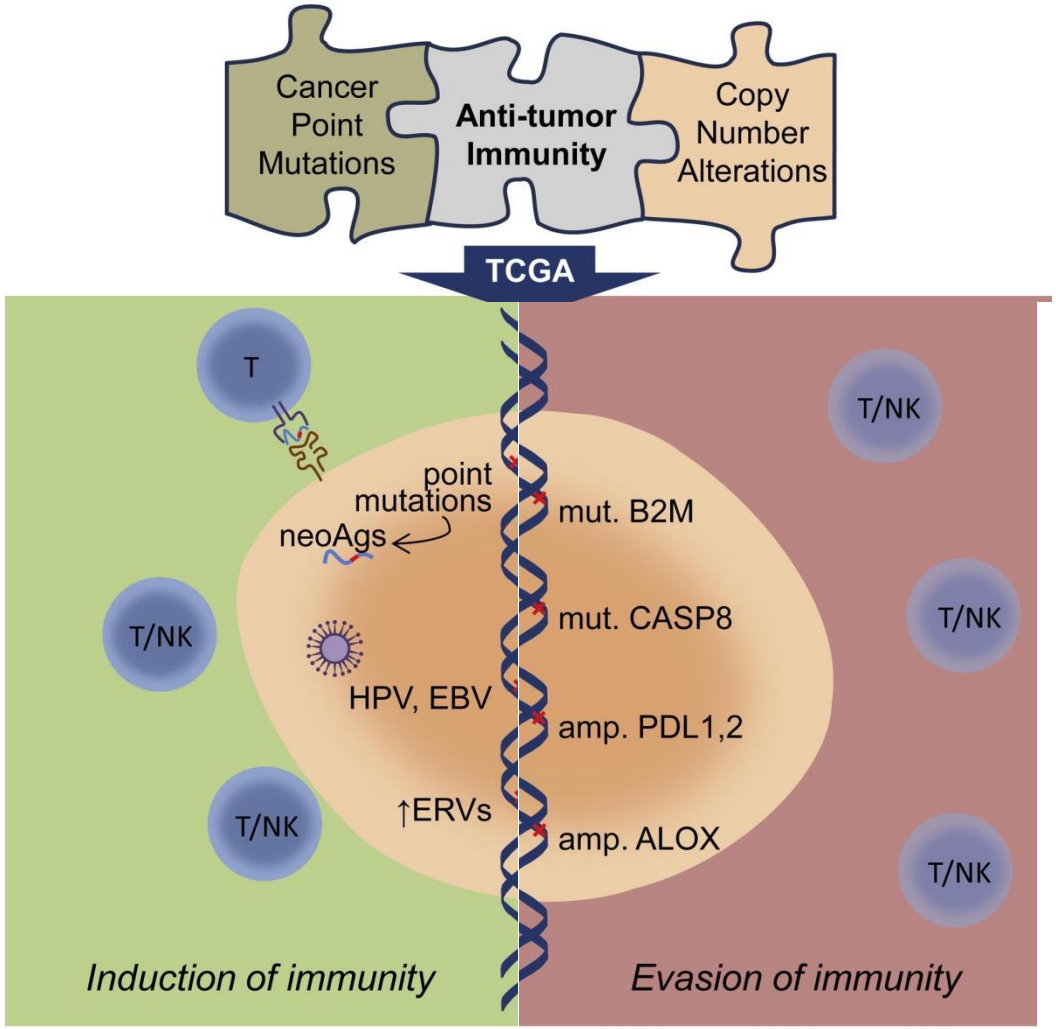
Induction of immunity



Rooney MS, et al. Cell 2015; 48-61

Cytolytic T-cell is activity is the fruit of a balance

Induction of immunity



Evasion of immunity

Rooney MS, et al. Cell 2015; 48-61

The 3 issues
on anti-PD1 & anti-PDL-1

3- Tumor immune landscape is changing with treatments and disease evolution

Profiling cancer gene mutations in longitudinal epithelial ovarian cancer biopsies by targeted next-generation sequencing: a retrospective study

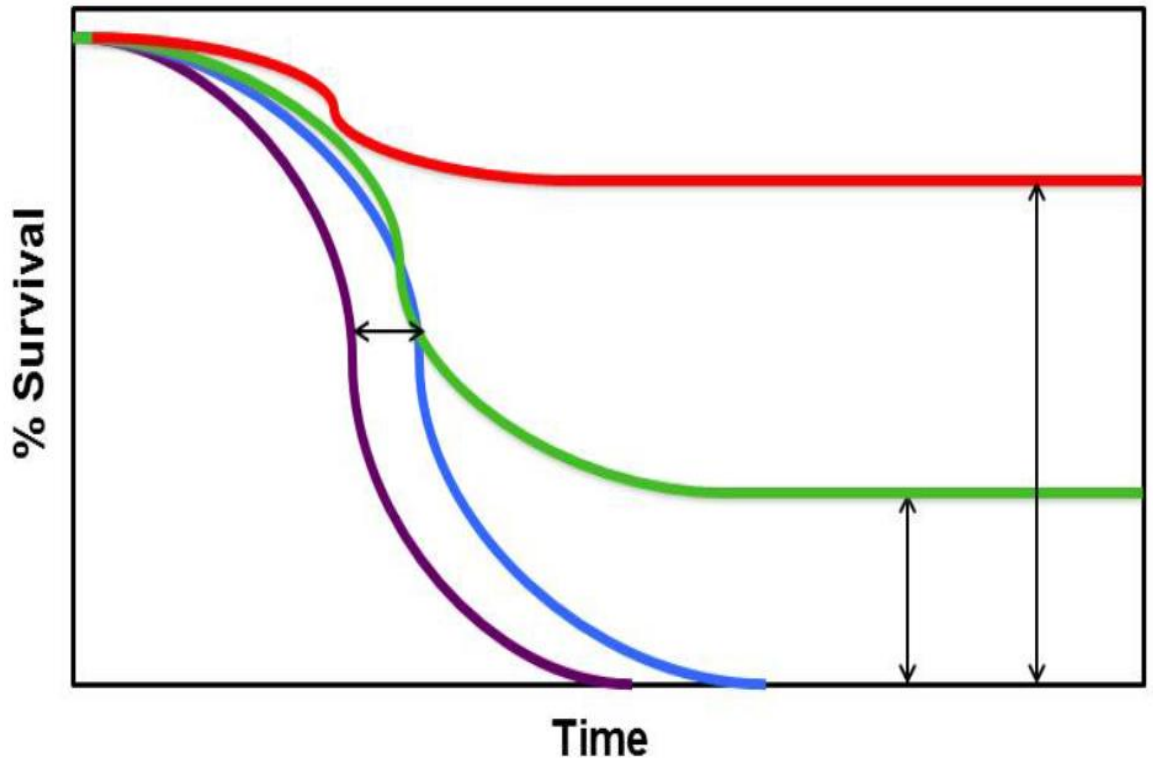
L. Beltrame[1,†], M. Di Marino[1,†], R. Fruscio[3,‡], E. Calura[5], B. Chapman[6], L. Clivio[1], F. Sina[3], C. Mele[2], P. Iatropoulos[2], T. Grassi[3], V. Fotia[7], C. Romualdi[5], P. Martini[5], M. Noris[2], L. Paracchini[1], I. Craparotta[1], M. Petrillo[8], R. Milani[3], P. Perego[4], A. Ravaggi[9], A. Zambelli[10], E. Ronchetti[11], M. D'Incalci[1,‡§*] & S. Marchini[1,§]

Annals of Oncology 26: 1363–1371, 2015

Somatic mutations	Per patient	Interindividual range	Nonsynonymous mutations
Front-line surgery	62 ± 40	25-178	350
Secondary surgery	38 ± 51	4-253	345
Shared (concordant)			**20 (11)**

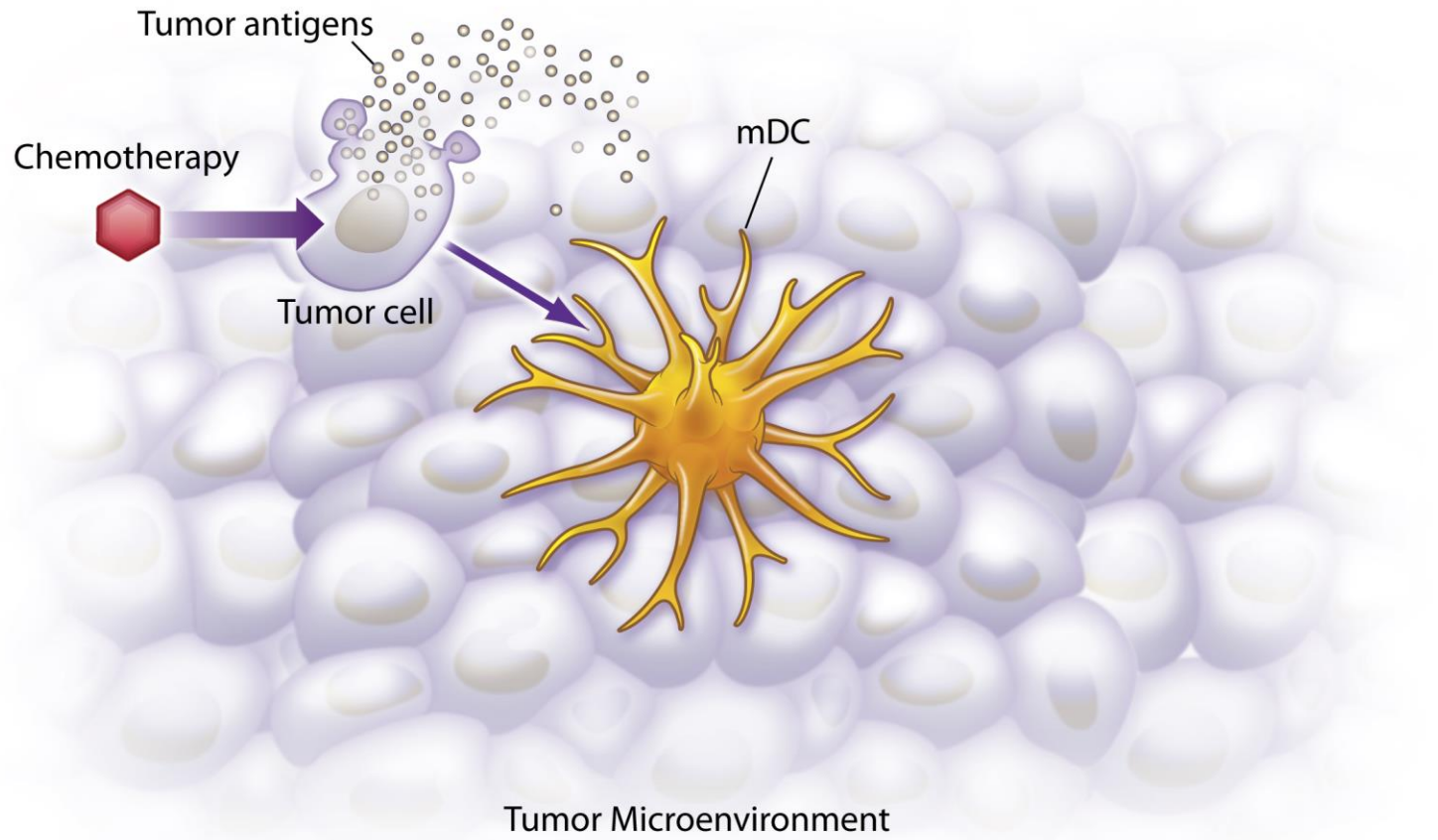
Biopsies should be done at relapse

Improving survival with combinations



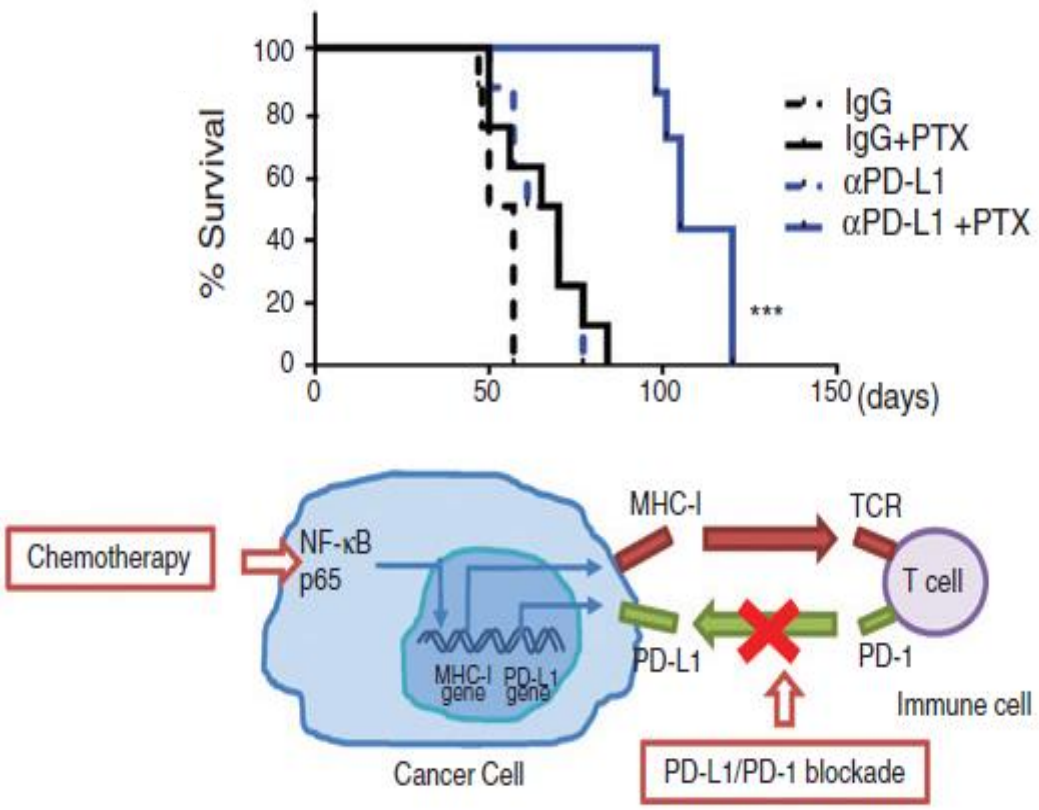
Combination with chemotherapy

- **Chemotherapy** (anthracyclin, oxaliplatin, cyclophosphamide, 5-Aza)



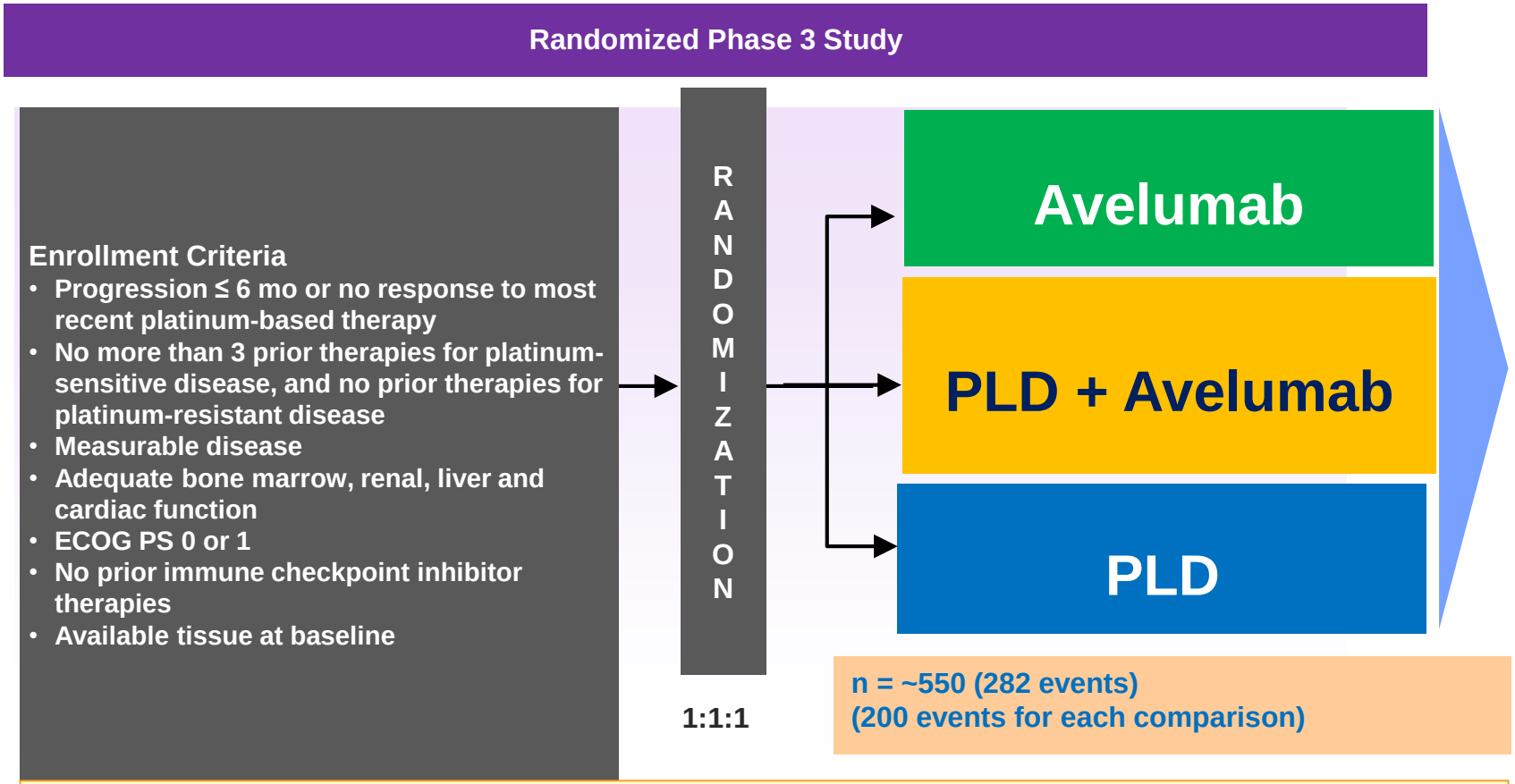
Combination with chemotherapy

- **Paclitaxel upregulates PDL-1 and MHC-1 on tumor cells (mouse model)**



Peng J et al. Cancer Res 2015

Phase III study: JAVELIN 200
Avelumab in Platinum Resistant/Refractory Ovarian Cancer
PI: E Pujade-Lauraine



PLD: pegylated liposomal doxorubicin

Combination with anti-VEGF therapy

VEGF exerts an immunosuppressive effect in cancer

- **Inverse correlation between VEGF levels and presence of TILs**

  Zhang L et al N Engl J Med 2003;348:203-13.

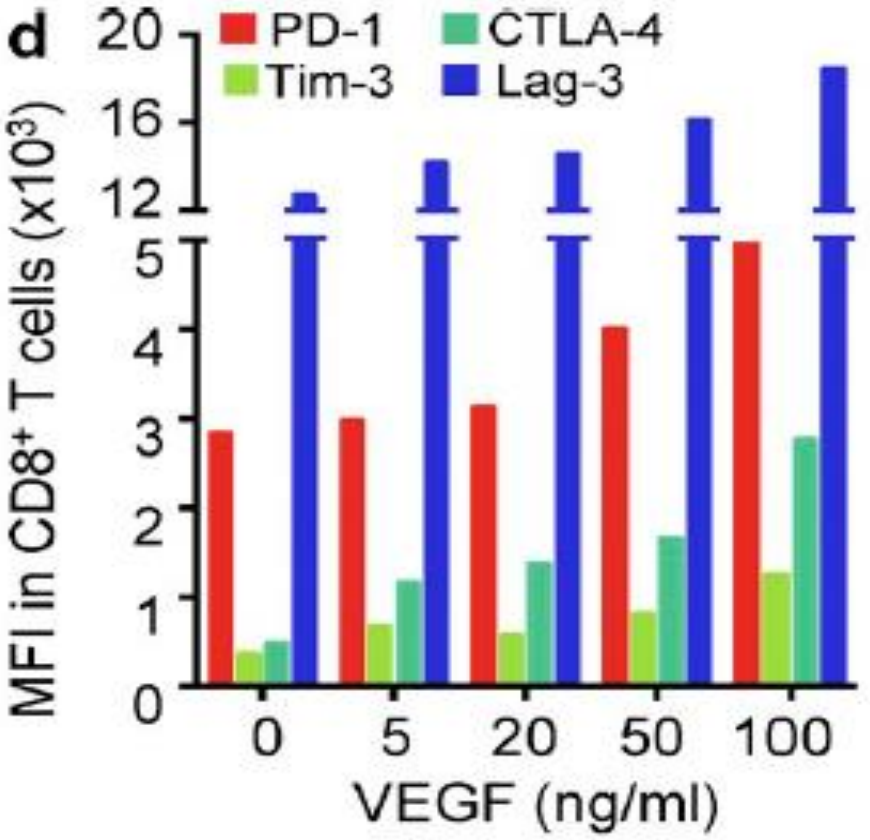
- VEGFR2 is selectively expressed in Treg CD4+FoxP3 + cells and VEGF directly **suppresses activation of T Cells**

  H. Suzuki Eur J of Immunology, vol. 40, no. 1,2010; Gavalas NG et al British Journal of Cancer (2012) 107, 1869

- **In response to VEGF**, **immature DCs** acquire a pro-angiogenic phenotype and contribute to ovarian cancer progression

  Coukos G Br J Cancer. 2005;92:1182–1187.

VEGF-A enhances co-expression of inhibitory receptors involved in CD8+ T cell exhaustion



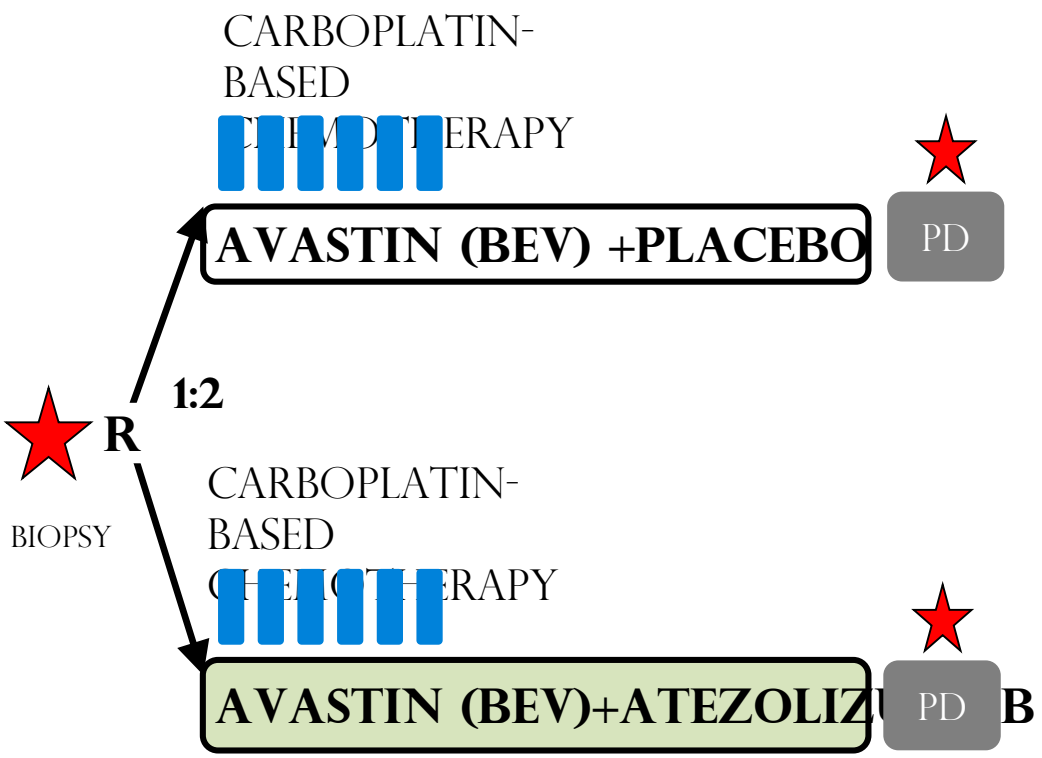
Voron et al; JEM 2015

ATALANTE phase III trial design in OC patients with late relapse (TFIp > 6 months)

PI: E Pujade-Lauraine

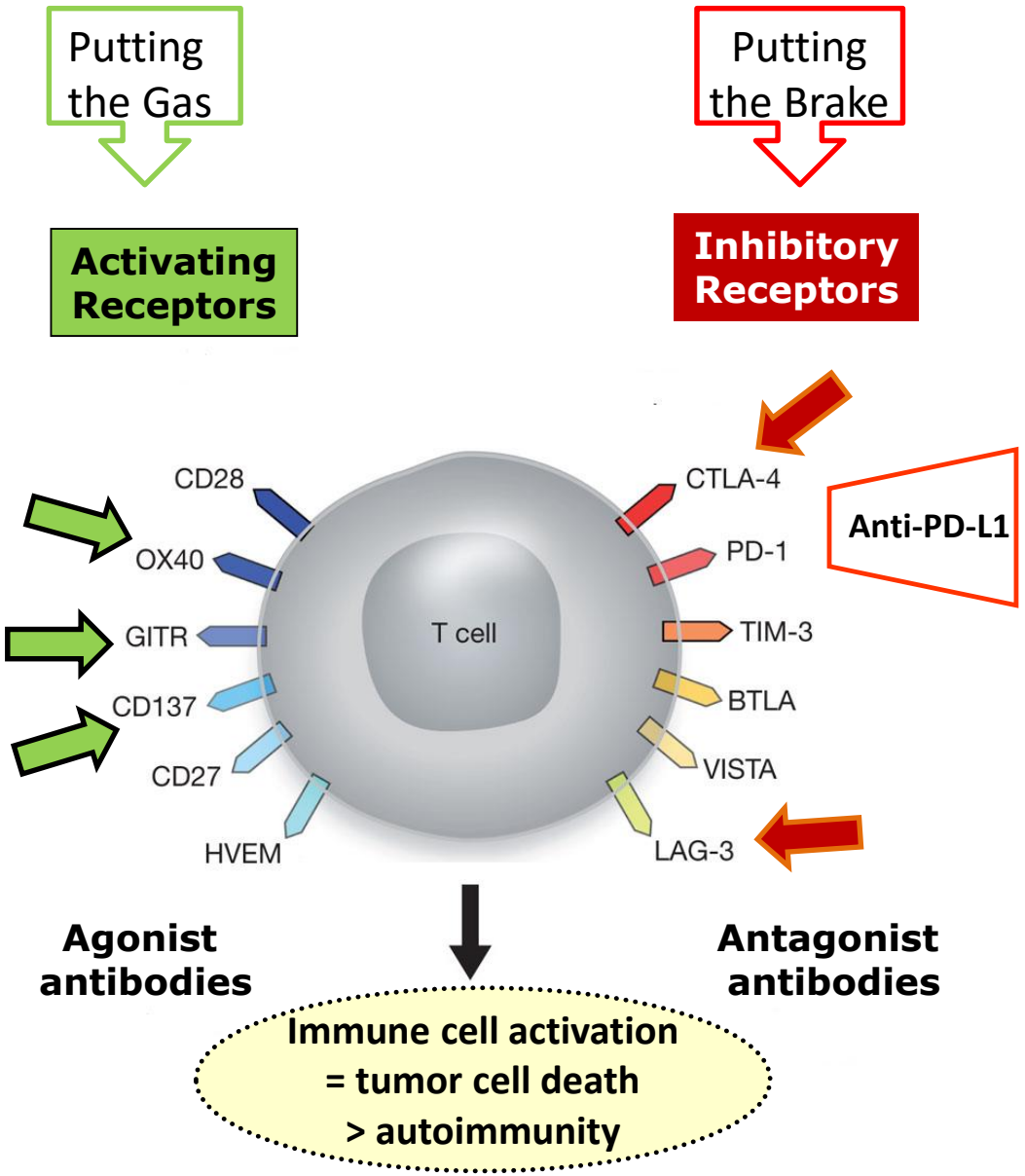
RECURRENT PLATINUM-SENSITIVE N=405

- NON-MUCINOUS HISTOLOGY
- PFI >6 MONTHS
- ONE OR 2 PRIOR LINES OF CX
- MEASURABLE DISEASE
- ECOG ≤1



PRIMARY OBJECTIVE:
PFS SUPPORTED BY PROS ET TIME TO SUBSEQUENT SECOND TREATMENT (PROXY OF PFS 2)

Combination with immunomodulators



Immune activators and checkpoint inhibitors

Issue of combo:
auto-immunity

Cible	Ac	Companie
CTLA-4	Ipilimumab	BMS
	Tremelimumab	Medimmune/AZ
PD-1	Nivolumab	BMS
	Pembrolizumab	Merck
	Pidilizumab	Medivation
	MEDI0680	Medimmune/AZ
PL-L1	BMS-936559	BMS
	Atezolizumab	Genentech
	Durvalumab	Medimmune/AZ
	Avelumab	Merck
LAG-3	BMS-986016	BMS
	IMPS321	Immutep
KIR	Lirilumab	BMS/Innate Pharma
CD40	CP-870,893	Pfizer
	Dacetuszumab	Seattle Genetics
	Lucatumumab	Novartis (antagonist)
CD137	Urelumab	BMS
	PF-05082566	Pfizer
OX40	ANTI-OX40	Providence H&S
	MEDI6383	Medimmune/AZ
	RG7888	Genentech
CD27	Varlilumab	Celldex
GITR	TRX518	GITR Inc.
SLAMF7	Elotuzumab	BMS

Mellman et al. Nature 2011;480:480-9.

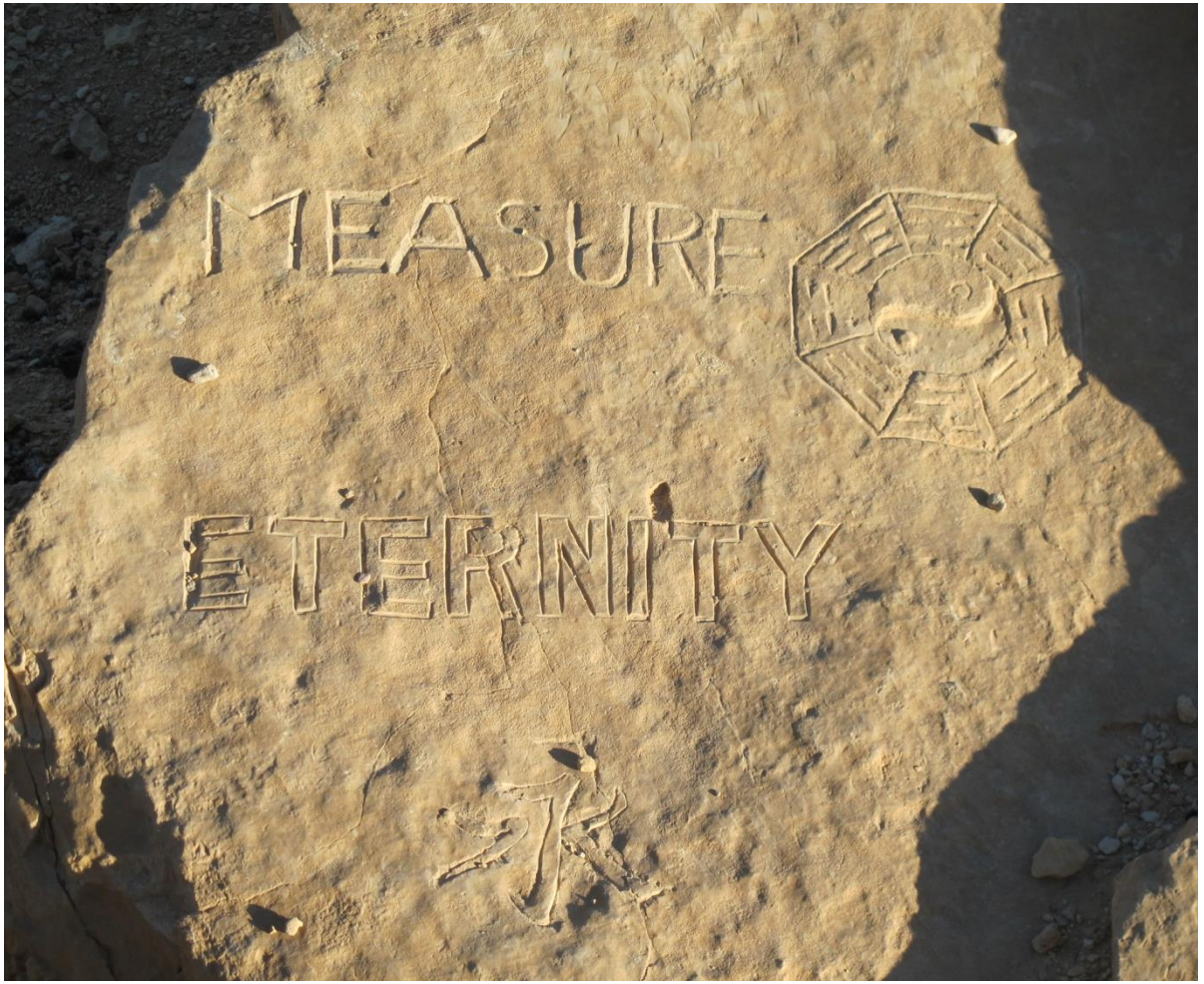
Conclusions

- Checkpoint-blockade immunotherapy has been the most exciting advance made in cancer treatment in recent years.

- It has joined the ranks of radical surgery, radiation therapy, chemotherapy, endocrine therapy, and targeted oncogene therapies.

- Immunotherapy has come to age in GYN oncology

Thank you!



Author's Conclusion

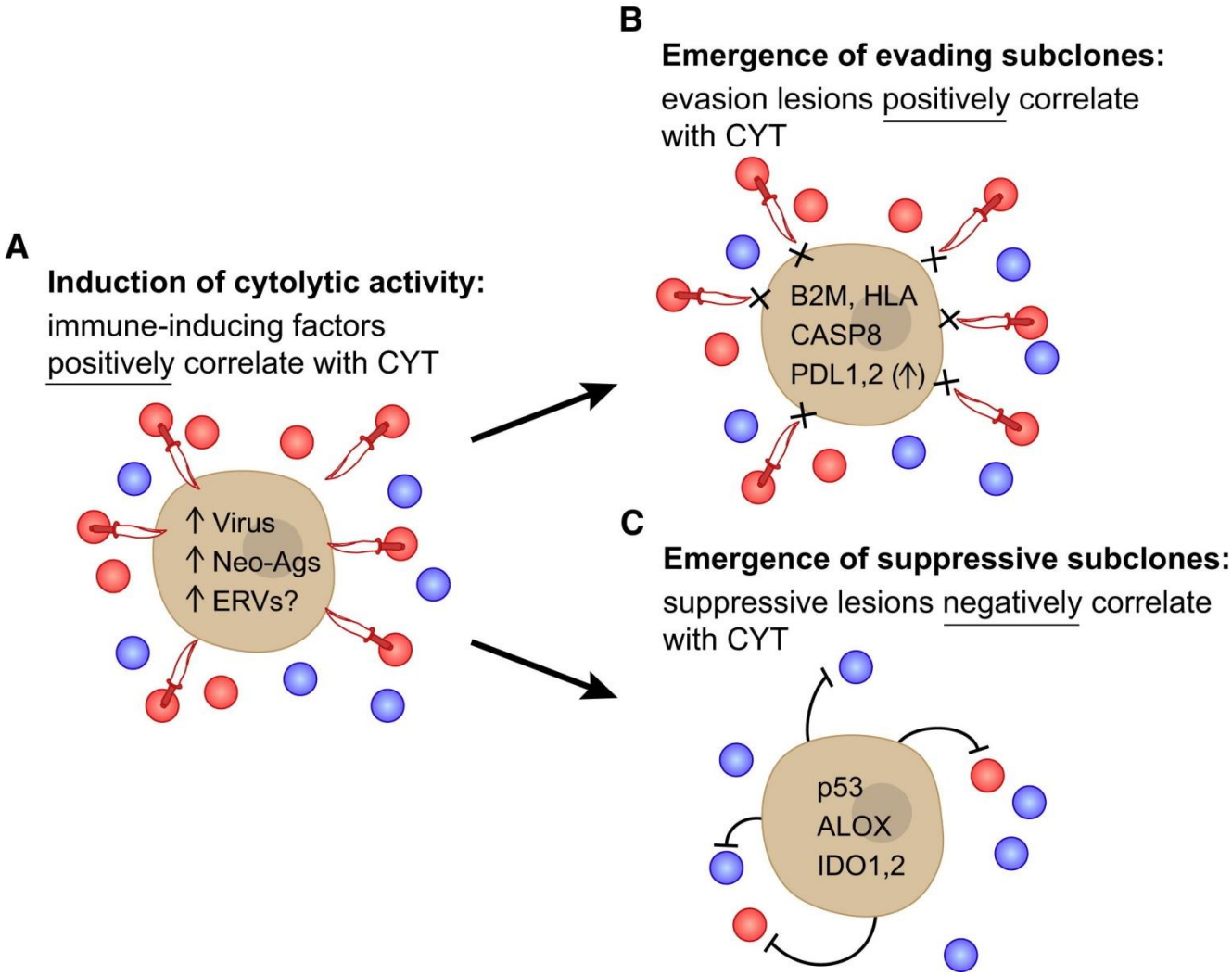
At this point in time, there is no evidence of effective immunotherapy for ovarian cancer. Although promising immunological responses have been observed for most strategies evaluated, these do not coincide with clinical benefits for women with ovarian cancer. Furthermore, there are currently no immunological surrogate markers that correlate with clinical outcomes. Until evidence of true clinical effectiveness is available, immunotherapy should therefore not be offered as an alternative to standard therapy for primary or recurrent ovarian cancer.

Intrinsic resistance often occurs in patients with global immunosuppression (for example, patients with HIV and some elderly patients), in tumours that express few molecular cues that can be recognized as foreign to the immune system (for example, non-viral tumours with a low mutational load) or in tumours that display intrinsic resistance to immune-mediated killing mechanisms.

Cancer immunoediting is the process by which the immune system controls tumour outgrowth and shapes tumour immunogenicity, and it comprises three phases: elimination, equilibrium and escape. there is substantial evidence for immunosurveillance in humans… The strongest arguments against immunoediting are the powerful, complete and durable responses we observe in the clinic. These responses seem to be the result of T cells recognizing mutated antigens…"

three problems. It is clear that most successful immunotherapies to date depend on T cells, but the characteristics of highly effective T cells remain largely unknown…The second problem concerns elucidation of the realm of structures that can serve as appropriate target antigens on tumour cells… third major problem :understanding the nature of the target structures recognized by naturally occurring T cells.

A large proportion of tumours with an 'immune-ignorant' phenotype (type II; PDL1 negative with no TILs)= prolemess



Rooney cell2015

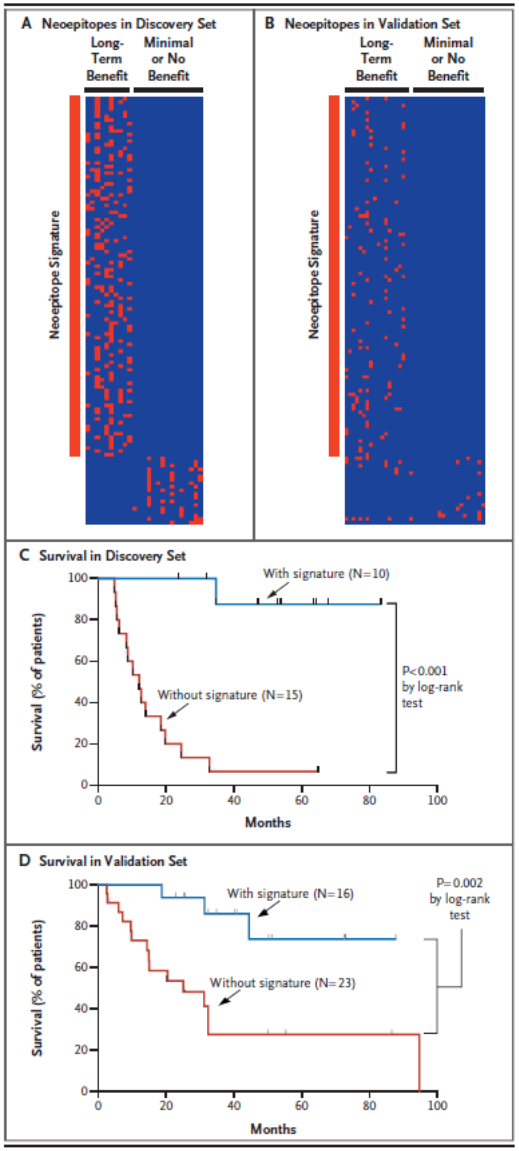


Figure 3. Association of a Neoepitope Signature with a Clinical Benefit from CTLA-4 Blockade.

Candidate neoepitopes were identified by means of mutational analysis, as described in the Methods section in the Supplementary Appendix. Panel A shows a heat map of candidate tetrapeptide neoantigens that were present in patients with a long-term clinical benefit but absent in patients with a minimal benefit or no benefit in the discovery set (comprising 25 patients). Each row represents a neoepitope; each column represents a patient. The vertical red line indicates the tetrapeptide signature associated with a response to blockade of cytotoxic T-lymphocyte antigen 4 (CTLA-4). The exact tetrapeptides, chromosomal loci, and nonmutant and mutant nonamers in which they occur are listed in Table S6 in the Supplementary Appendix. Panel B shows the same information for the validation set (comprising 39 patients). Panel C shows the Kaplan–Meier curves for overall survival in the discovery set for patients with the signature and those without the signature. Panel D shows the same data for the validation set.

Snyder A , et al; N Engl J Med 2014;371:2189-99