

# Patolog bakış açısı ile immunoterapi

Dr Büge Öz

İÜ Cerrahpaşa Tıp Fakültesi  
Patoloji AD



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# *Konuřmanın akıřı;*

- Tmr- immunit fizyopatolojisi
- Kuř bakıřı İmmunterapi
- İmmunoterapide patolojinin yeri



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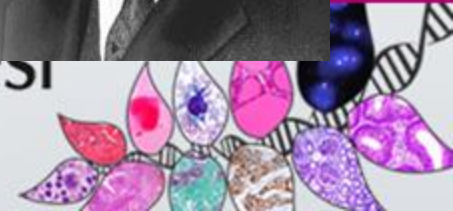


- 1890’li yıllarda bakteri toksini kullanılarak immunoterapi (Coley toksinleri);
- Sir Macfarlane Burnet «1960 Nobel ödülü» “Az sayıda oluşan *tümör* hücreleri yüzeylerinde *antijenik* yapılar buldukları için etkili bir immün cevaba ve tümör gerilemesine neden olurlar”
- Tümör karşı immünite : Naif ve **adaptif immünite**

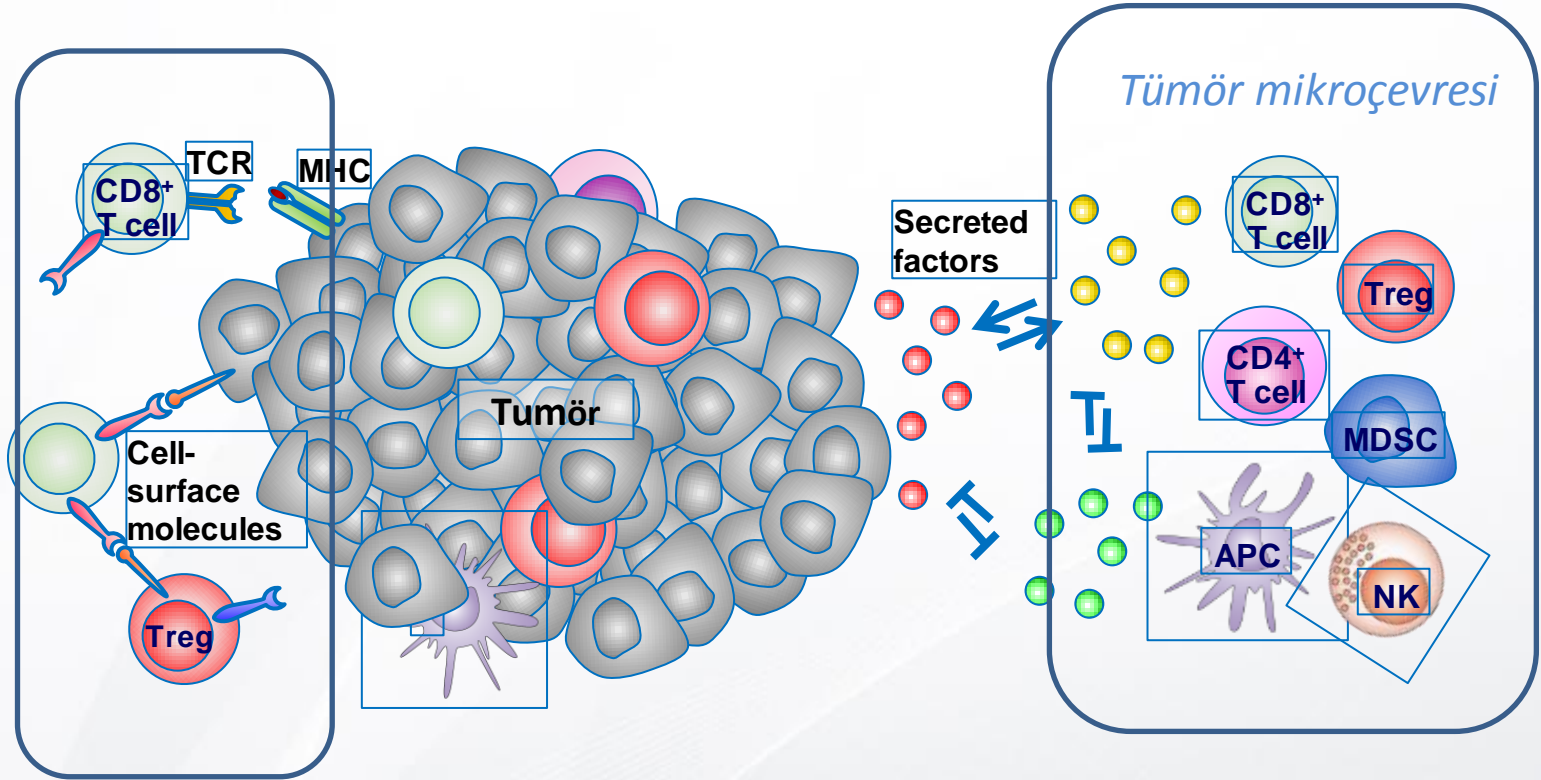


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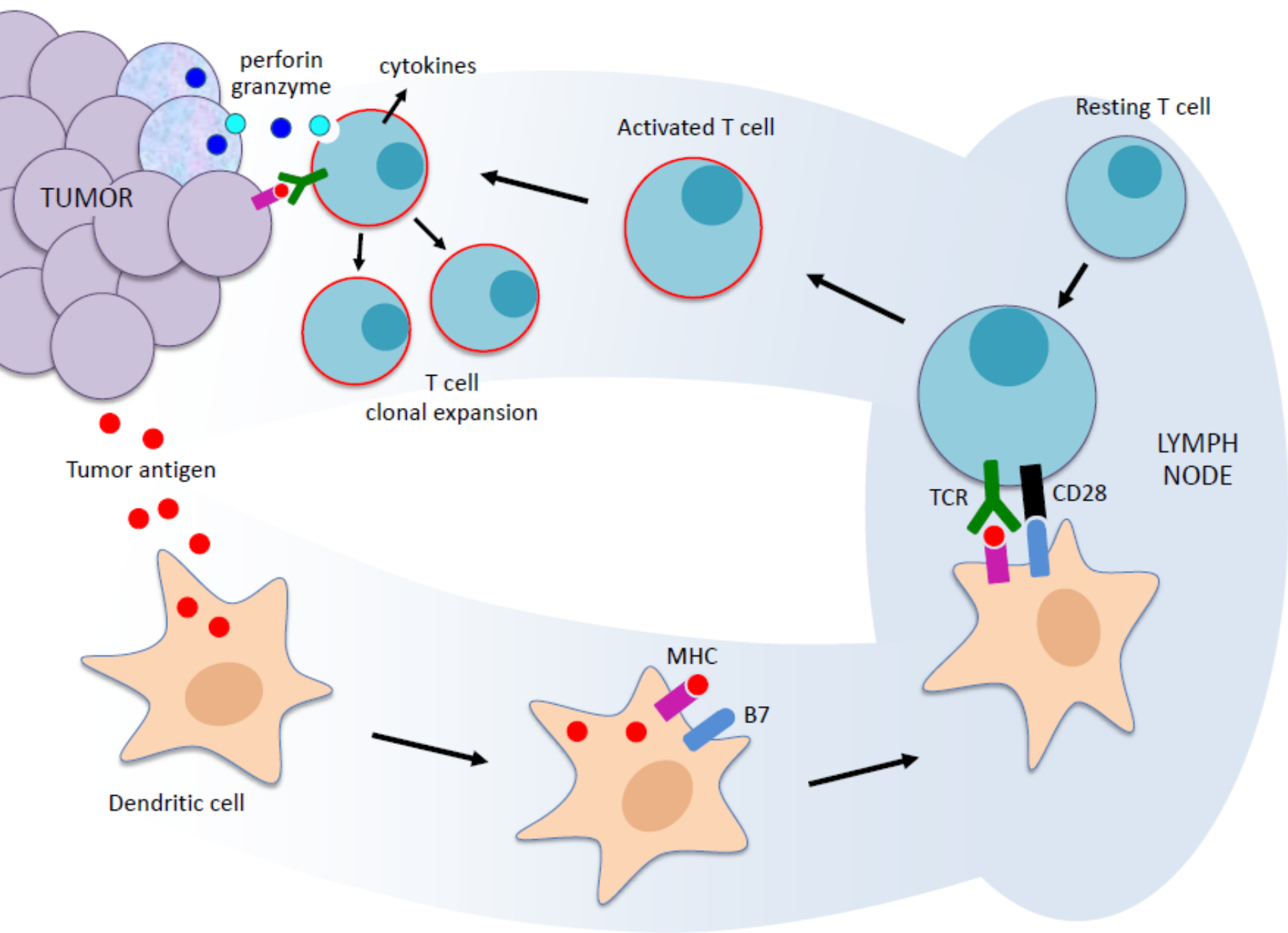
# Tümör –/Adaptif immun yanıt

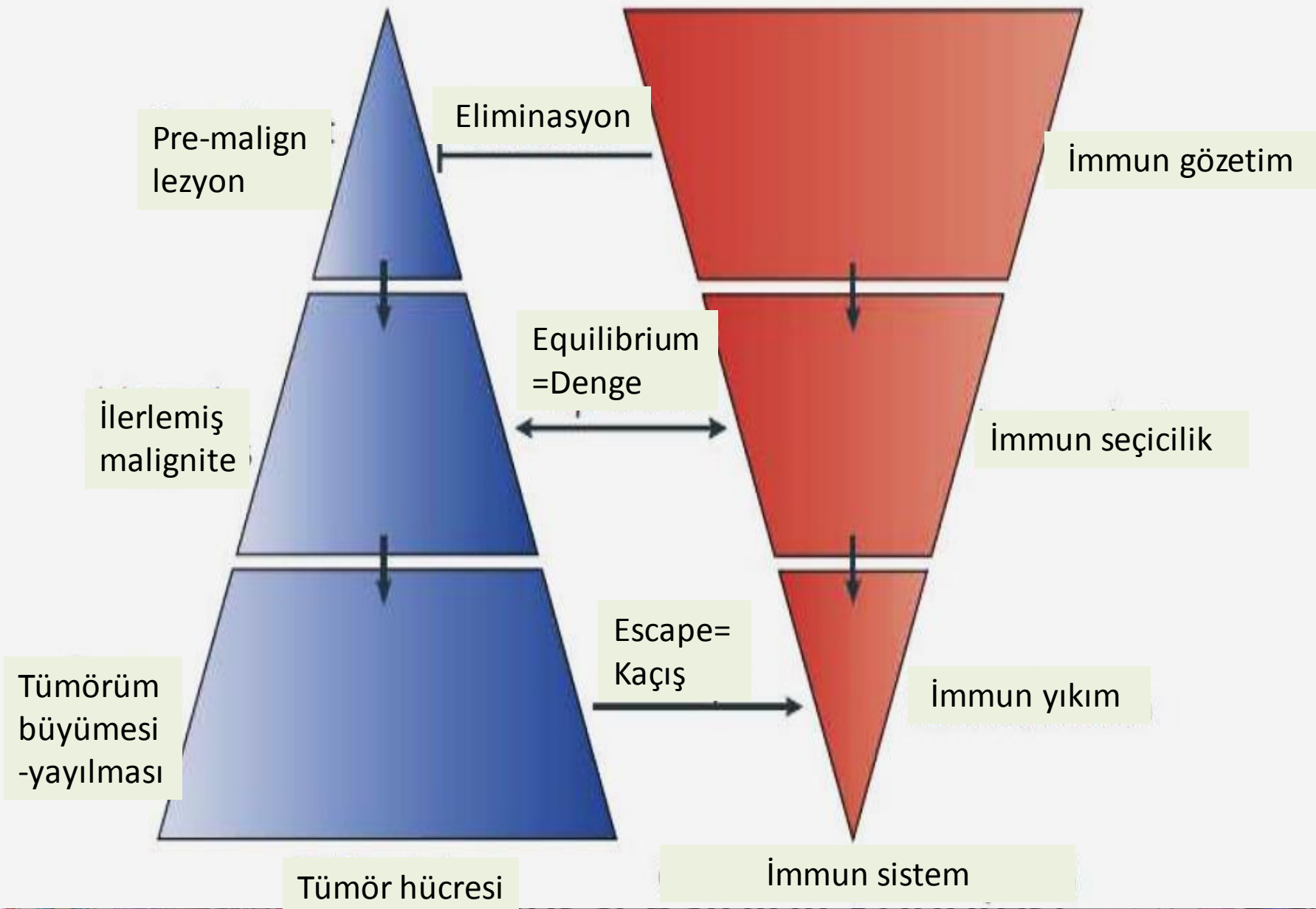


1. Whiteside TL. *Front Oncol.* 2013;3:1–8.

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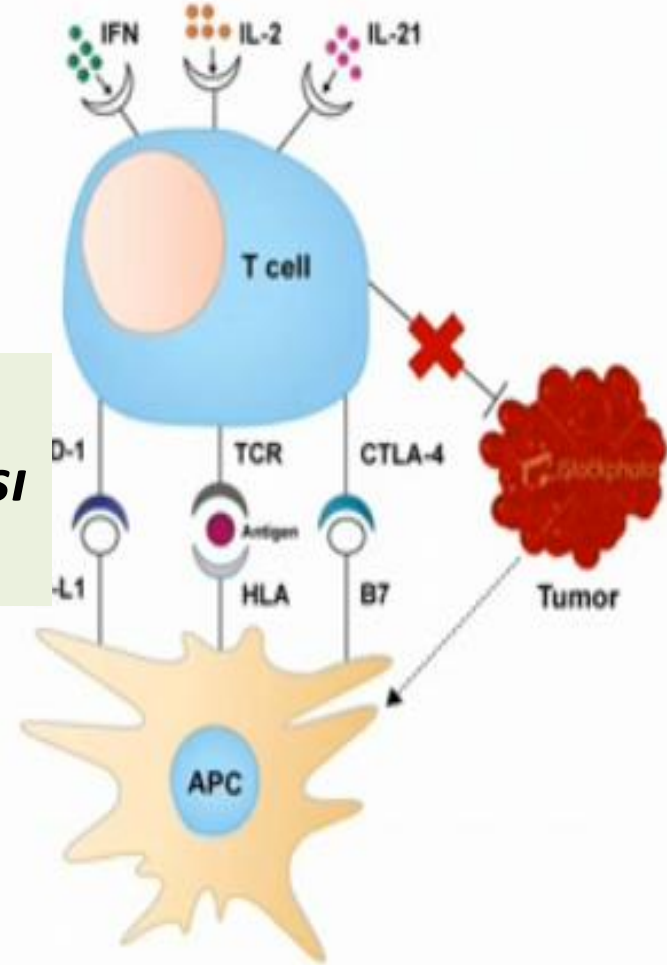




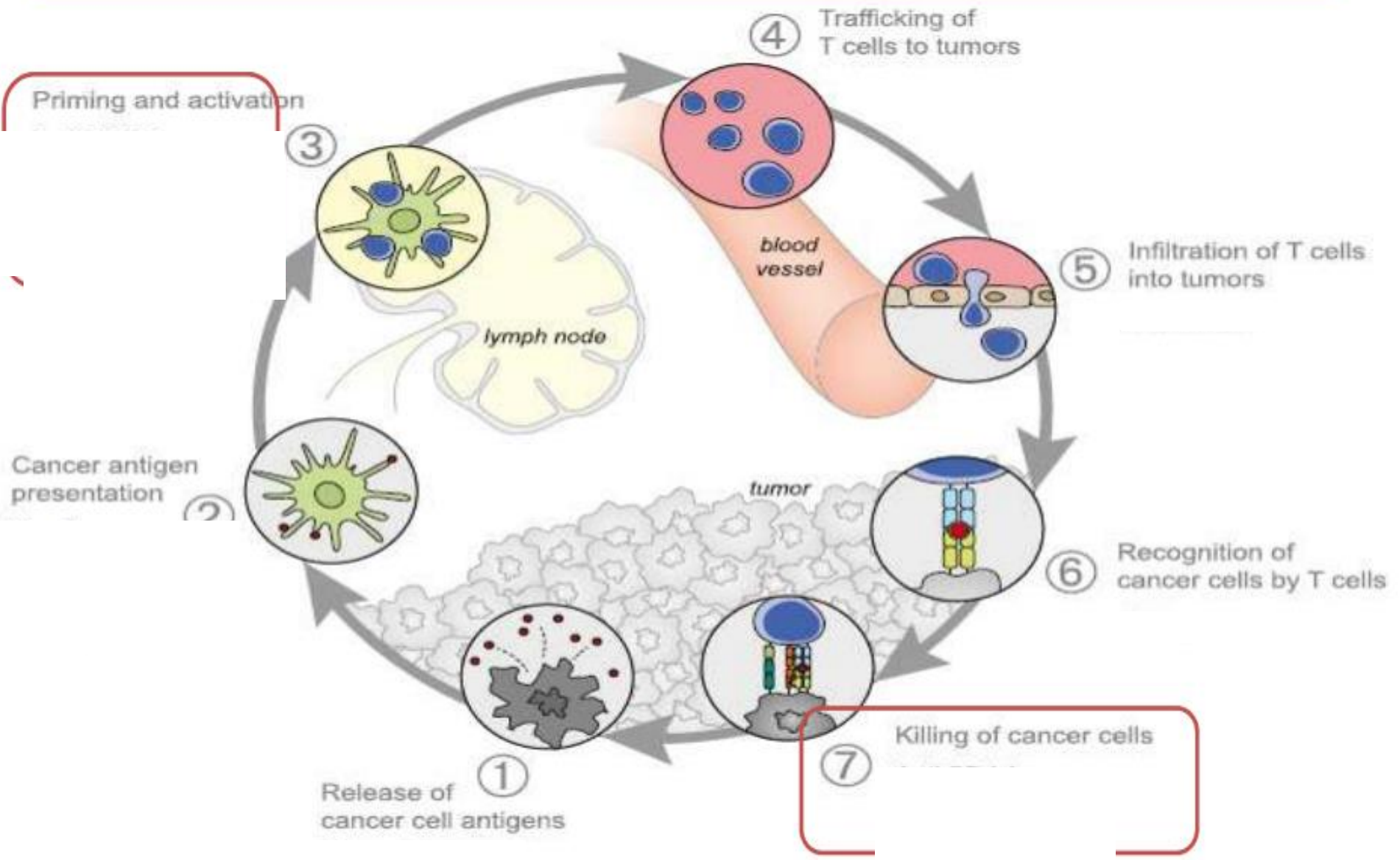
**İmmun atağa  
karşı direnç kazanma**

**PD-L 1 ligandının  
PD-1 inhibitör reseptörüne bağlanması  
T hücre aktivasyonunu bloke eder**

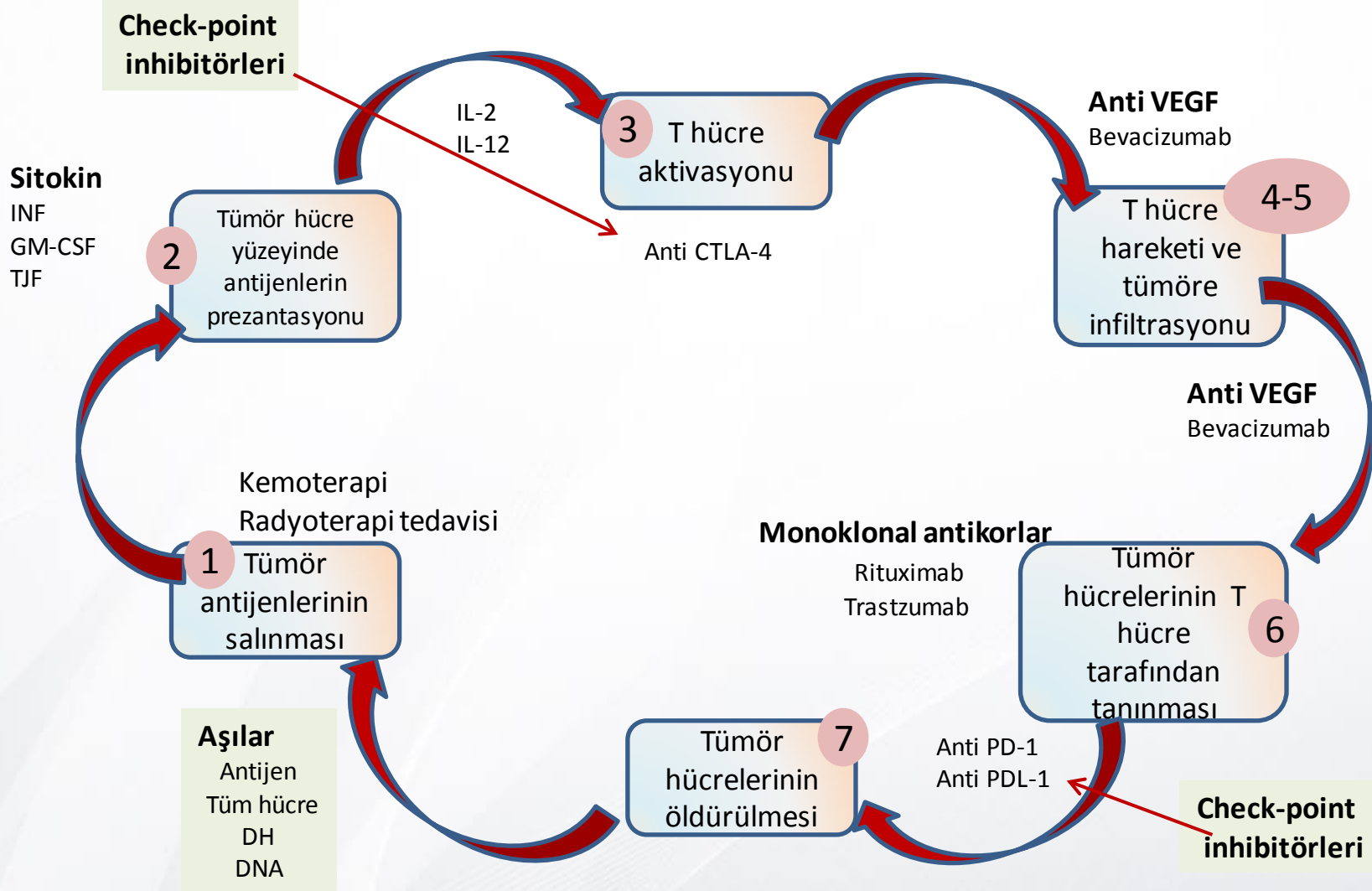
**TH1 hücrelerinde anerji  
ve tükenmişlik gelişir**

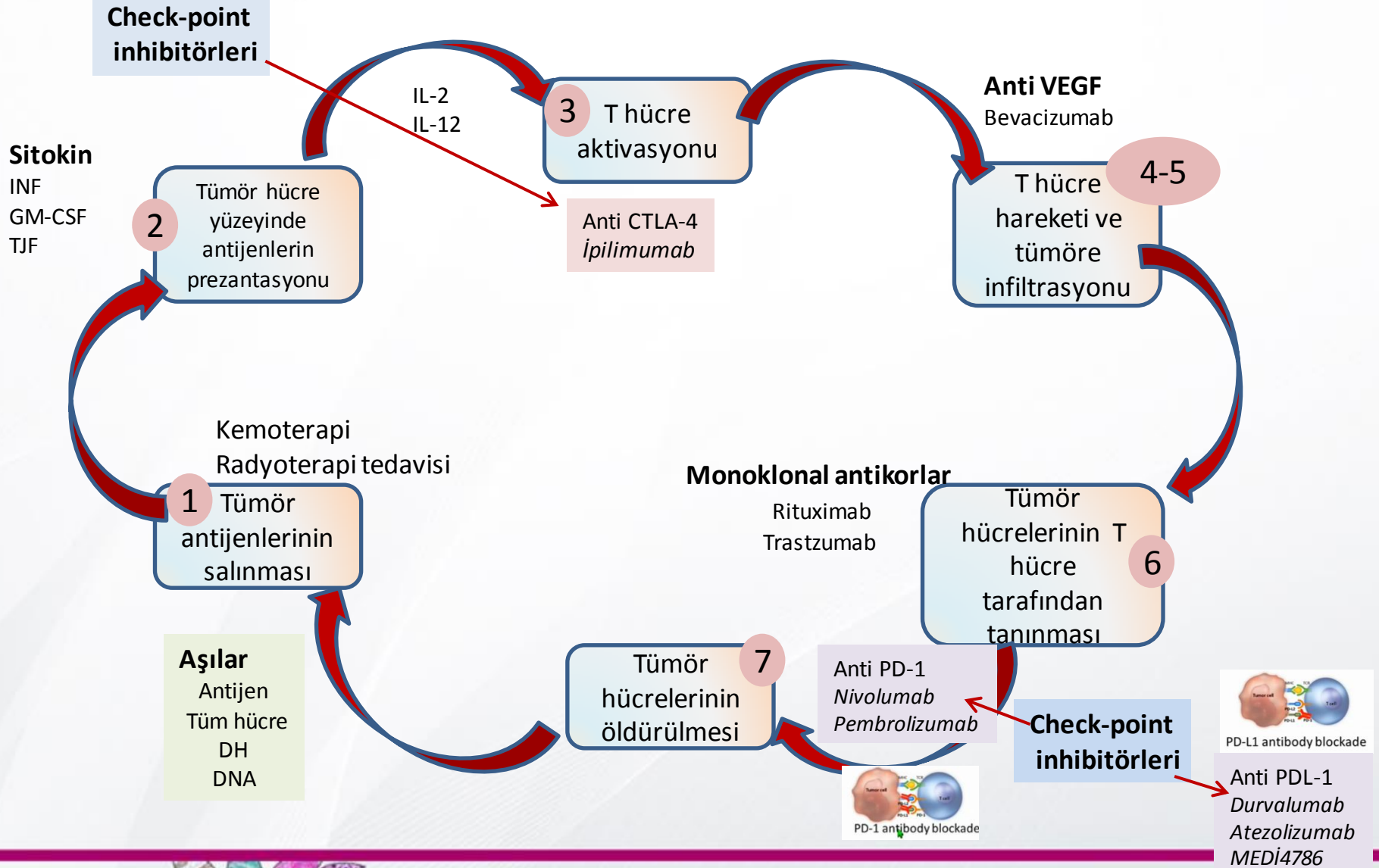


# Oluşması istenen Kanser-immunite Döngüsü









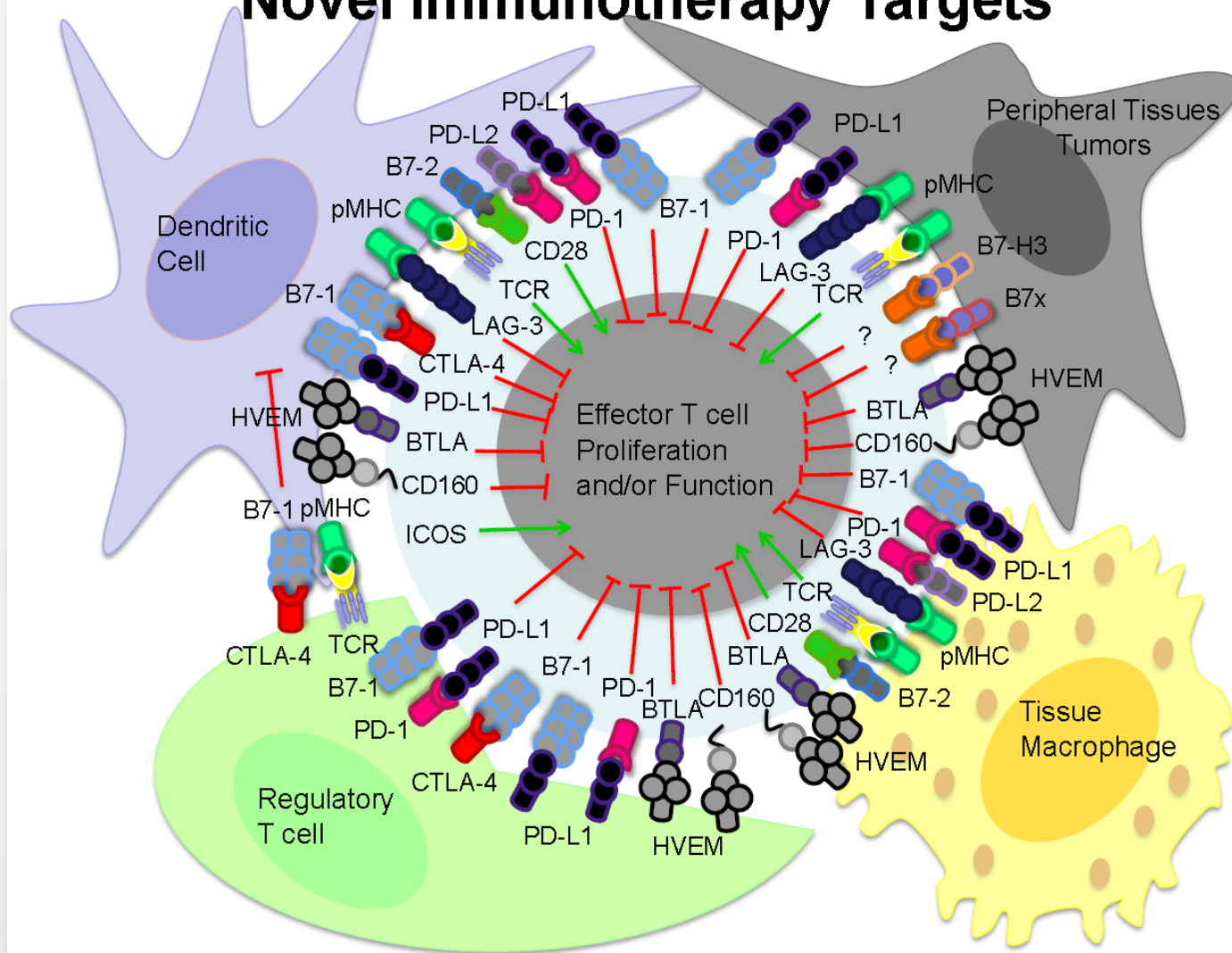
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# Novel Immunotherapy Targets



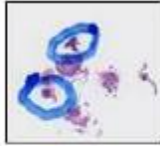
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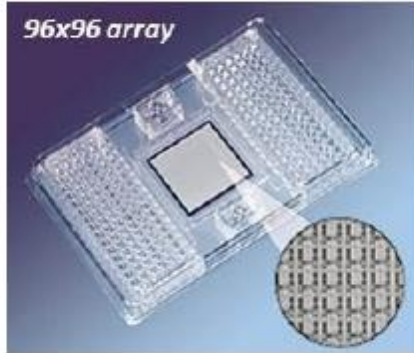


# Genentech Gene Expression Assay (ImmunoChip): Exploratory assay optimized to evaluate tumor immunobiology in FFPE samples

Macrodissected FFPE tumor



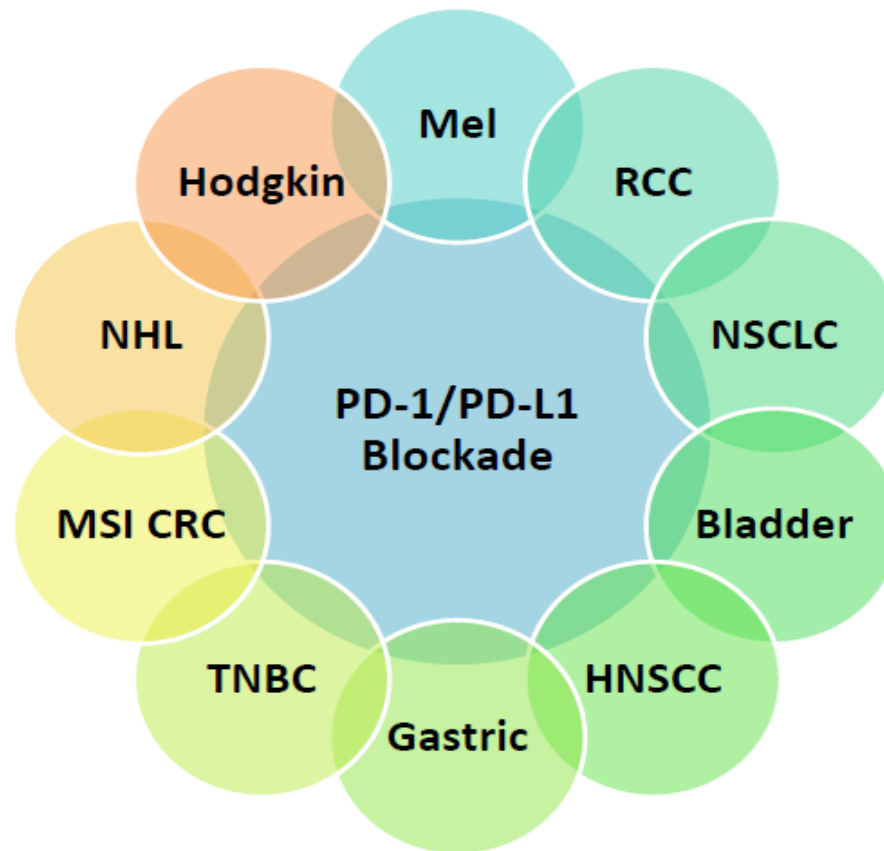
Immune Biomark Fluidigm



- Immune Biomark Fluidigm platform
- RNA extracted from FFPE
- ≈ 90 unique transcripts (+ controls)

## Tümör immün profili ile ilişkili gen-belirteçler

- T hücre alt tip belirleyiciler  
(CD4, CD8, FoxP3, GzA, CD69 vb.)
- Sitokin ve kemokinler  
(INFg, TNFa, CXCL9 vb)
- **Immün düzenleyiciler**  
(PDL-1, PDL-2, CTLA4, vb)
- Immün hücre populasyon belirleyiciler  
(NK, DH, B hüç, vb)



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RESEARCH ARTICLE

## PD-L1 and Survival in Solid Tumors: A Meta-Analysis

Pin Wu<sup>1,3\*</sup>, Dang Wu<sup>3\*</sup>, Lijun Li<sup>4</sup>, Ying Chai<sup>1\*</sup>, Jian Huang<sup>2,3\*</sup>

- **Meta analiz :**
- 28 çalışmada 3107 hastada PD-L1 ekspresyonu
- PD-L1 «+» olumsuz prognostik faktör
- özellikle özefagus, hepatosellüler, gastirik, üroteliyal Ka



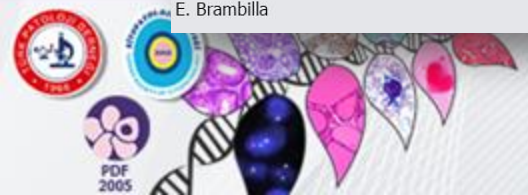
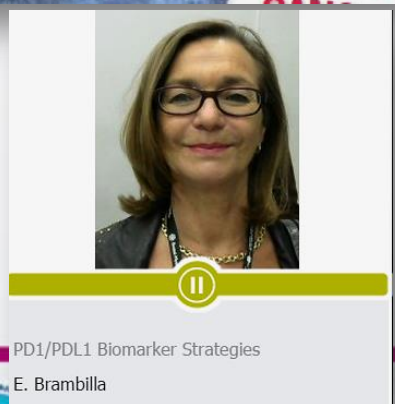
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# IASLC 2015- Denver PATOLOJİ



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STATE OF THE ART: CONCISE REVIEW

*J Thorac Oncol.* 2015;10:985–989)

## Programmed Death-Ligand 1 Immunohistochemistry in Lung Cancer

*In what state is this art?*

*Keith M. Kerr, MBChB, FRCPath,\* Ming-Sound Tsao, MD, PhD,† Andrew G. Nicholson, DM, FRCPath,‡  
Yasushi Yatabe, MD, PhD,§ Ignacio I. Wistuba, MD, PhD,|| and Fred R. Hirsch, MD, PhD,¶  
On behalf of the IASLC Pathology Committee*

STATE OF THE ART: CONCISE REVIEW

*(J Thorac Oncol.* 2015;10: 974–984)

## Non–Small-Cell Lung Cancer *Role of the Immune System and Potential for Immunotherapy*

*David P. Carbone, MD, PhD,\* David R. Gandara, MD,† Scott J. Antonia, MD, PhD,‡  
Christoph Zielinski, MD,§ and Luis Paz-Ares, MD, PhD||*



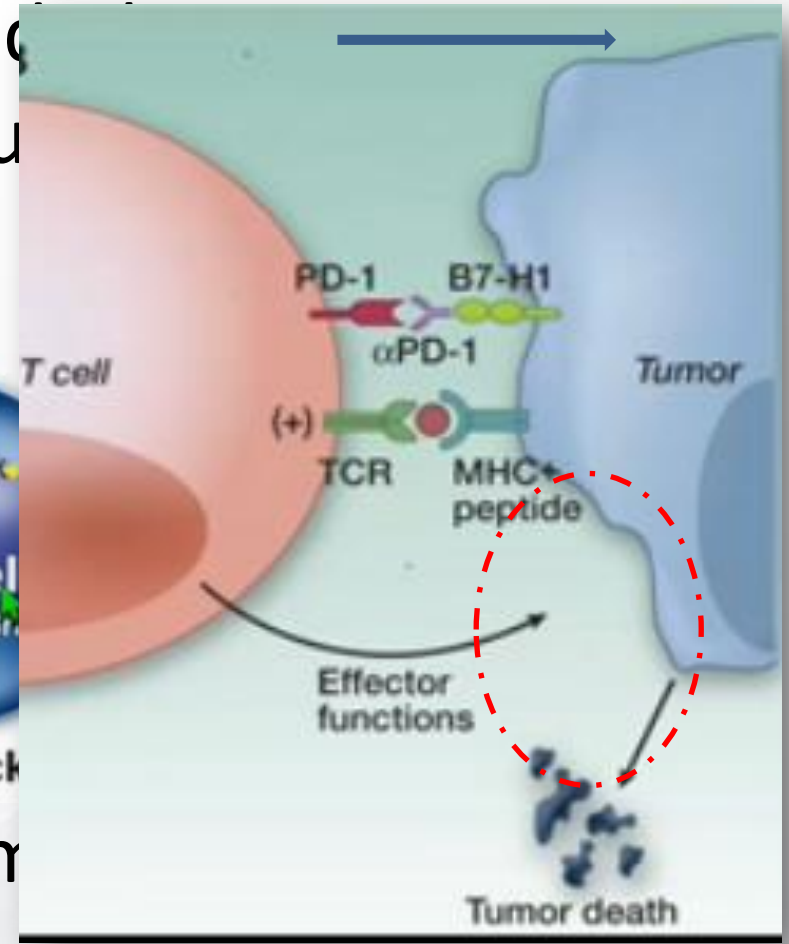
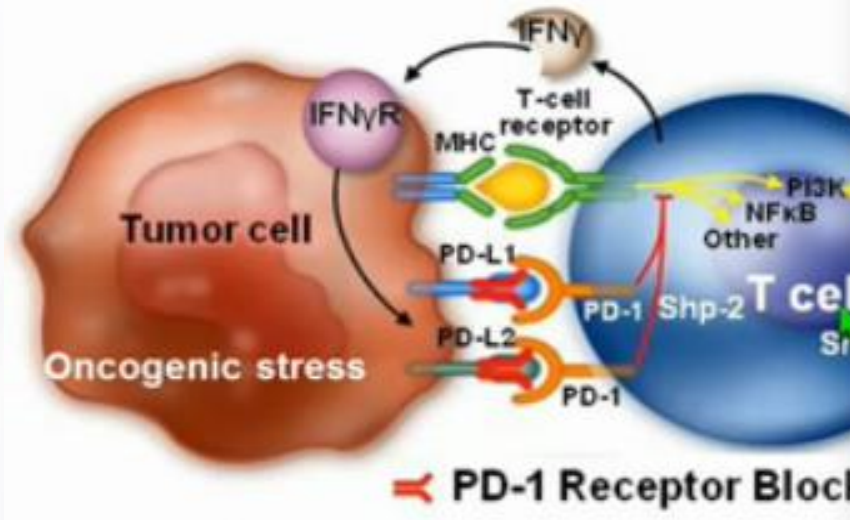
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- İki sinaps bölgesinde (PD-1/PDL-1) Anti-tümör T hücre inhibisyonunun kaldırılması, tümör –immün yanıt oluşturmaya ve tümör regresyonu



- PDL-1 eksprese eden tümörlerin immün cevabı daha iyi



**İmmunhistokimya ile PDL-1 araştırması prediktif biyo-belirleyici olarak kullanılabilir mi?**

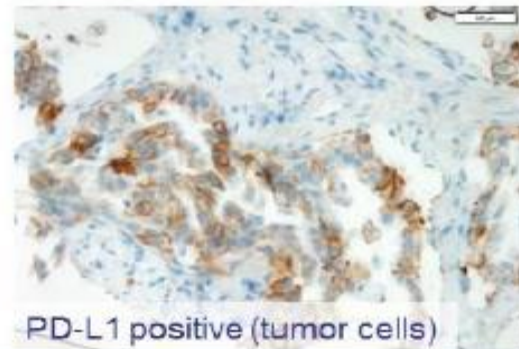
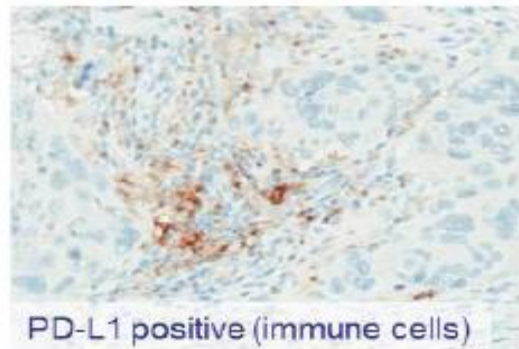
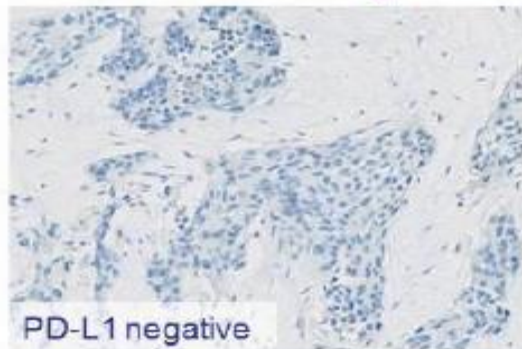


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# Proprietary Genentech/Roche Dx PD-L1 IHC Reagent – a Robust Assay to Measure PD-L1 in Human Tissues



## PD-L1 IHC (Genentech/Roche Dx):

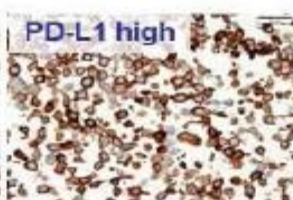
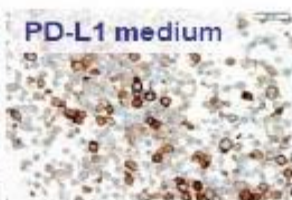
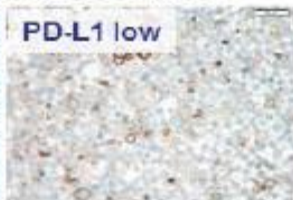
- Monoclonal Ab against human PD-L1
- High sensitivity and specificity
- No background
- Recognizes PD-L1 in tumor cells and tumor infiltrating immune cells

Available PD-L1 IHC reagents are not reliable Dx reagents



Intensive effort by Genentech and Roche Dx to generate a clinical grade IHC reagent

## PD-L1 expression in control cell lines



## Positive tissue control



PRESENTED AT: ASCO® Annual '13 Meeting

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## PD-L1 Assays Are Being Developed for Selection of Patients With Melanoma or NSCLC

PD-L1 Immune Marker Competition				
Drug (company)	Nivolumab (BMS)	Pembrolizumab (Merck)	Atezolizumab (Roche)	MEDI4736 (AstraZeneca)
IVD partner (advanced staining market share)	Dako (20%)	Dako (20%)	Ventana (45%)	Ventana (45%)
Antibody	28-8	22C3	SP142	SP263
Cells scored	Tumor cells	Tumor cells	Tumor cells and TILs	Tumor cells
Cut-off	1%, 5%, 10%	50%	1+, 2+, 3+	25%
Development position relative to BMS		Ahead	Likely ahead	Class I IVD Launched Jun '15

### Implications

- Merck (and possibly others) is ahead of BMS in PD-L1 immune marker development and regulatory filings
- Ventana's dominant market size may create a barrier to market entry for Dako/BMS
- AbCam 28-8 release will be discussed on a reactive basis by the medical team only

IVD=in-vitro diagnostic; TIL=tumor-infiltrating lymphocyte

Source: ASCO 2015, BMS Internal Document, "ASCO Biomarker Summary v Shared."



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# PDL-1 pozitifliği deęerlendirmesi

- PDL-1 pozitifliğinin devamlı ve yaygın olması (intensite yorum -)

Goron et al. NEJM 2015

- Ekspresyon % ile (intensite yorum -)

≥%1+ Skor 1

≥%5 + Skor 2

≥%10+ Skor 3

*Spira et al ;J.C.O. 2015*

*Brahmer J. et al., N.E.J.M. 2015*

- Kombine skorlama (yaygınlık +intensite) (linear H skor)

Kim M et al. Lung Cancer 2014

- Membranöz/ ve veya sitoplazmik boyanma (AQUA floresan teknik)

*Velcheti V. et al.,Lab. Invest, 2014*

*Schalper K. et al. JNCI, 2015*



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# İmmunhistokimya ile PDL-1 araştırması prediktif biyo-belirleyici olarak kullanılabilir mi?

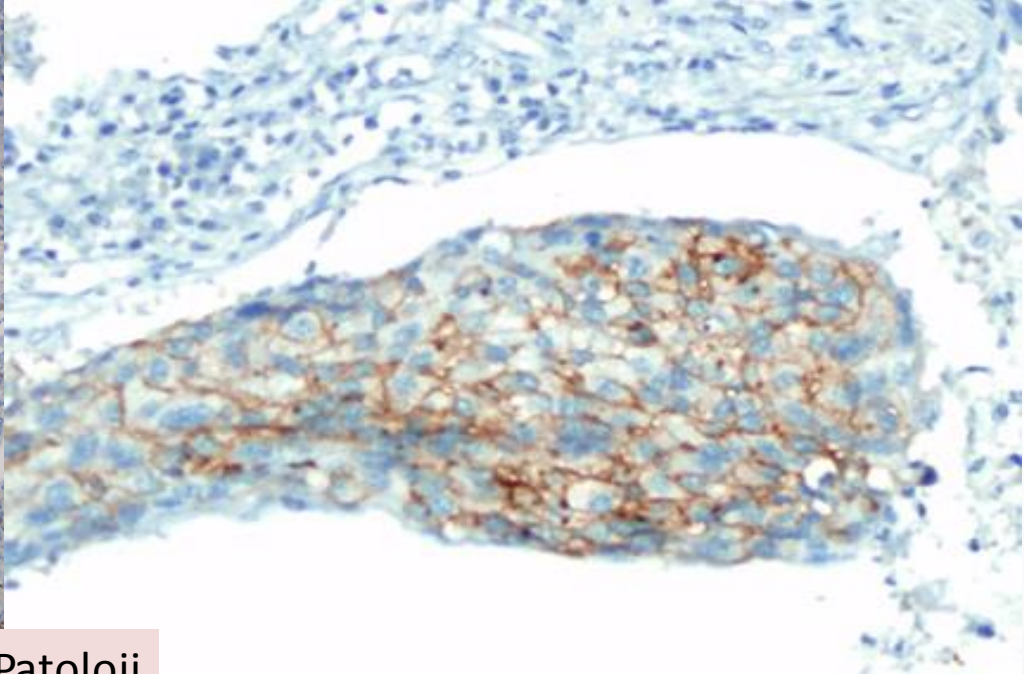
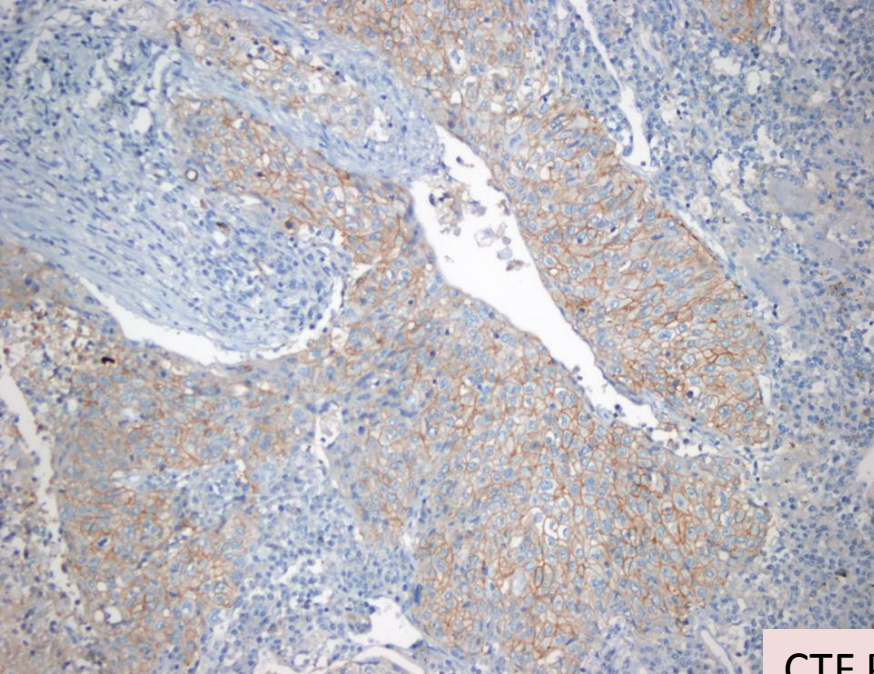
- Farklı antijen klonu ve farklı uygulama protokolleri
- Birbirinden farklı PDL-1 pozitiflik eşik değerleri
- Materyal farklılıkları (primer tm/ met LN vb)
- Farklı örnekleme zamanları ( PDL-1 exp. Dinamik bir olay)
- PDL-1/PD-1 iletişimi kompleks ; farklı alternatif biyo-belirleyiciler olabilir mi?



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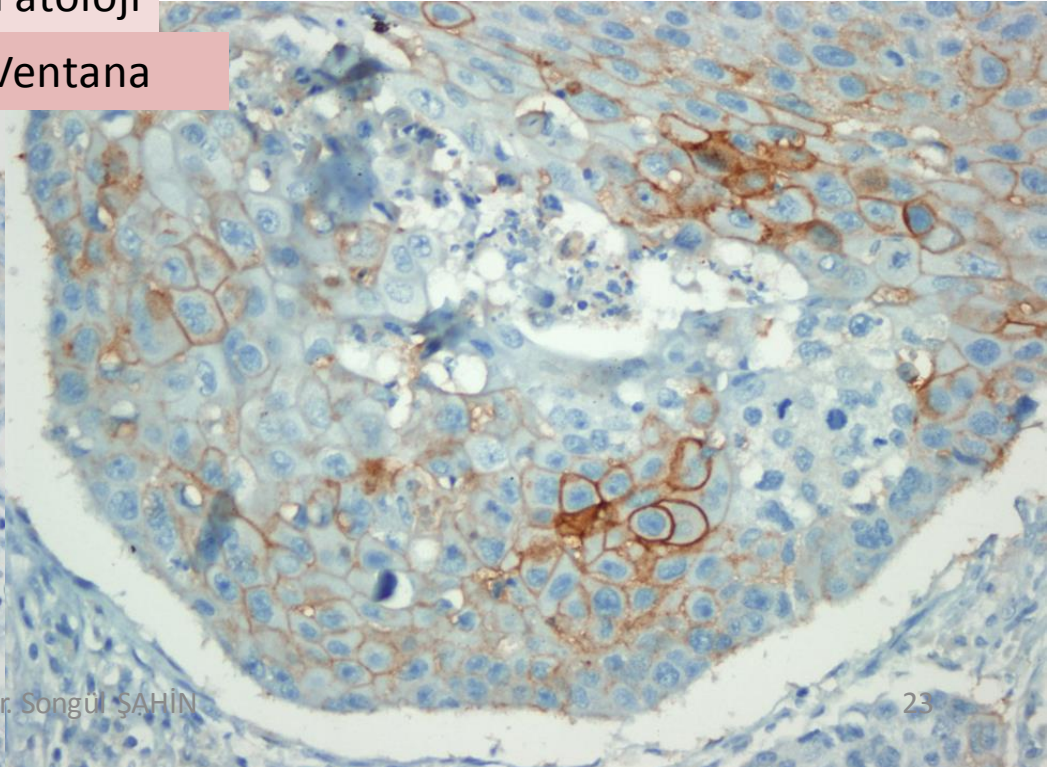
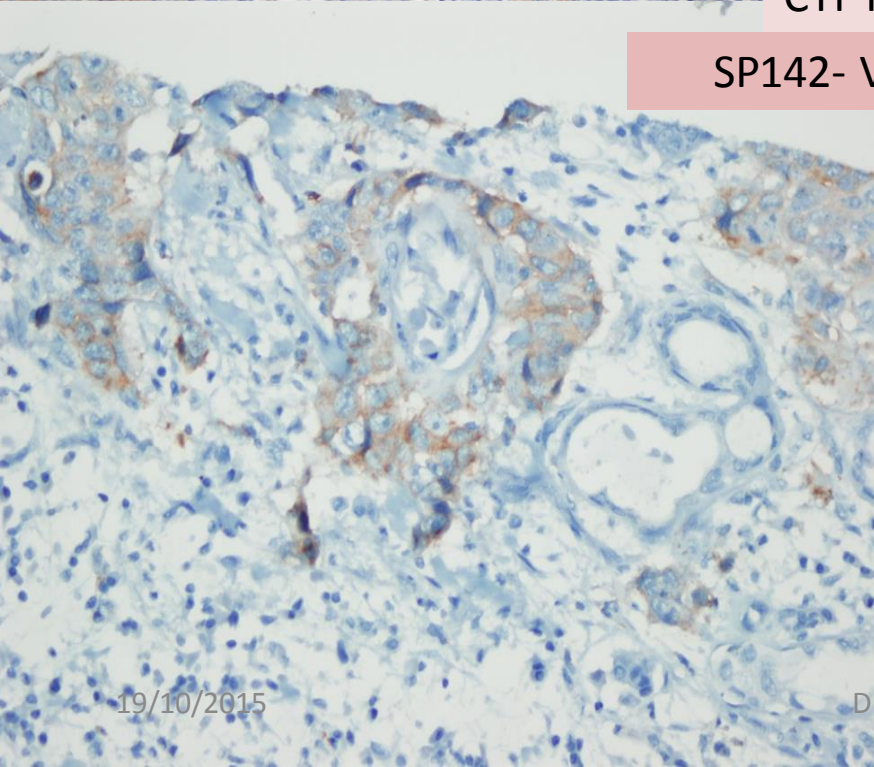
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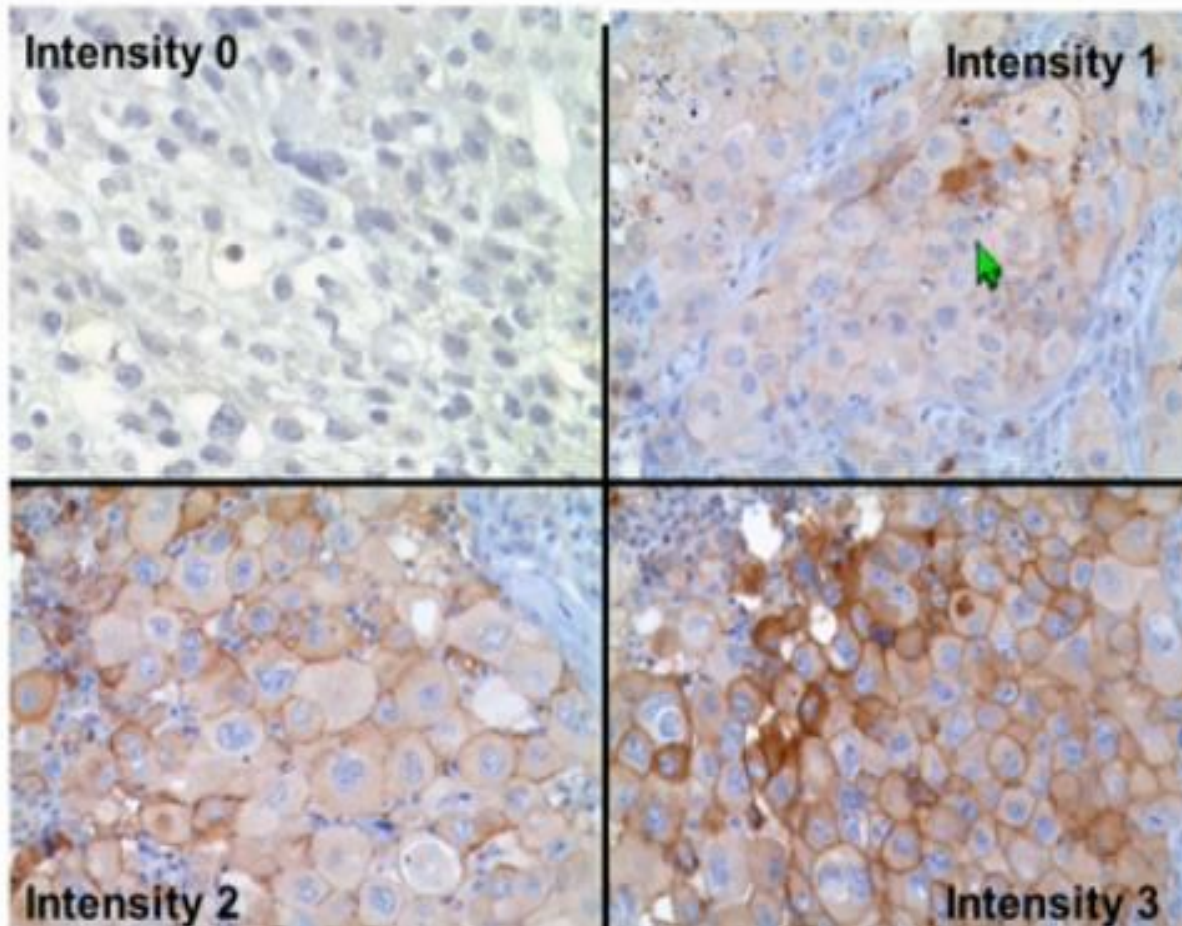


CTF Patoloji

SP142- Ventana



# PD-L1 positivity assessment :intensity scale 0-3



Best seen at  
magnification  
x100

Best seen at  
magnification  
X50



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**TABLE 1.** Summary of Published Findings for PD-L1 Immunohistochemistry in Therapeutic Trials

Drug	Blomarker Antibody	Rx Line	Definition of "Positive" <sup>a</sup> (%)	N Positive (%)	Positive Predictive Outcome	ORR % IHC pos. Cases	ORR % IHC neg. Cases	Ref.
Nivolumab	Dako 28-8	1st	≥5 in >100 cells	59	Yes	31 <sup>b</sup>	10	7,8 <sup>f</sup>
Nivolumab	Dako 28-8	≥2nd	≥5, ≥1	49, 56	No	15, 13	14, 17	9,10
Nivolumab + Ipilimumab	Dako 28-8	1st	≥5 in >100 cells	42	No	19	14	11
Nivolumab	Dako 28-8	≥2nd	≥5	33 <sup>c</sup>	Yes	24	14	12 <sup>f</sup>
Nivolumab	5H1 <sup>d</sup>	≥2nd	≥5, also studied TIIcs	67	Yes	No data for lung	No data for lung	13
Pembrolizumab	Dako 22C3	Any	"Strong" ≥50, "Weak" 1-49	25, 70	Yes, Yes	37, 17	9	14
Pembrolizumab	Dako 22C3	1st	≥50, ≥1	?	Yes	47, 26	?	15
MPDL3280A	Roche Ventana, SP142	≥2nd	≥10, <sup>e</sup> ≥5, ≥1 TIIcs	13, 28, 56	Yes	83, 46, 31	18, 18, 20	16-18
MEDI-4736	Roche Ventana, SP263	≥2nd	Data not available	41	Yes	25	3	19,20

*K Kerr .J Thorac Oncol. 2015;10: 985-989*



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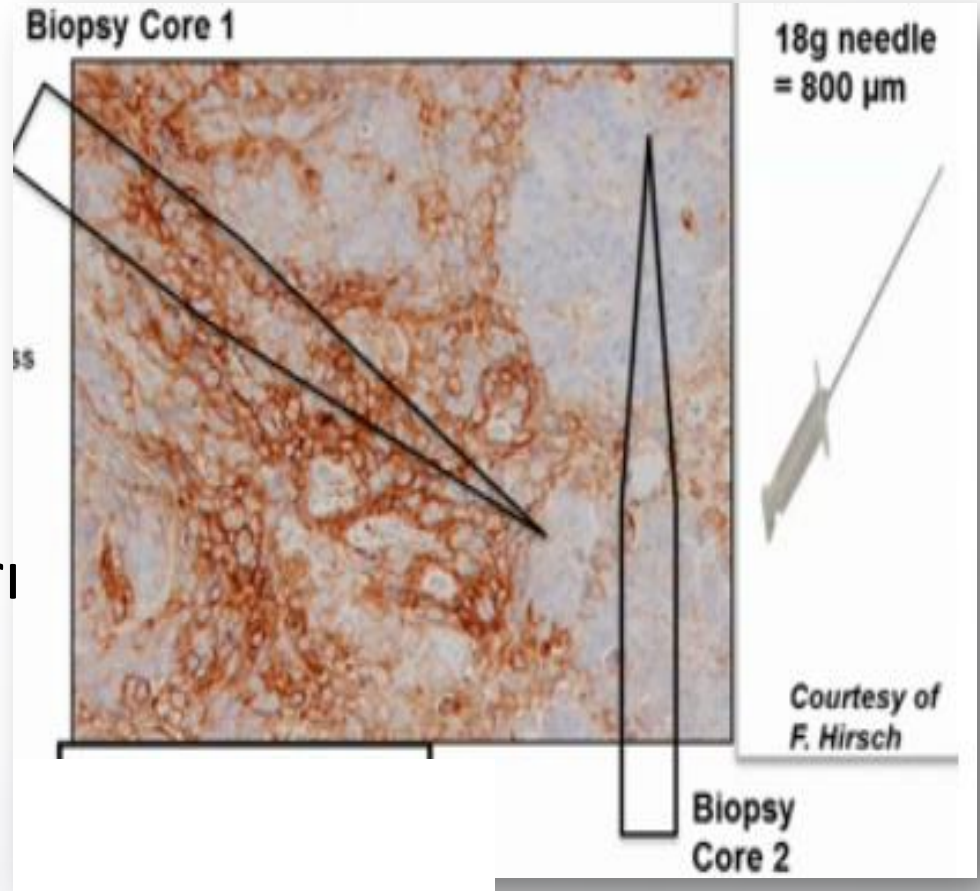
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# Tümör heterojenitesi

- Rezeksiyon mat. ile yapılan çalışmalarda
- (★CTF patoloji)
- 6 aydan daha eski kesitlerde IHK sonuçları problemlidir

Garon E. et al. N.E.J.M. 2015



PDL-1 «-» olguların %5-20'si tedaviye iyi yanıt veriyor

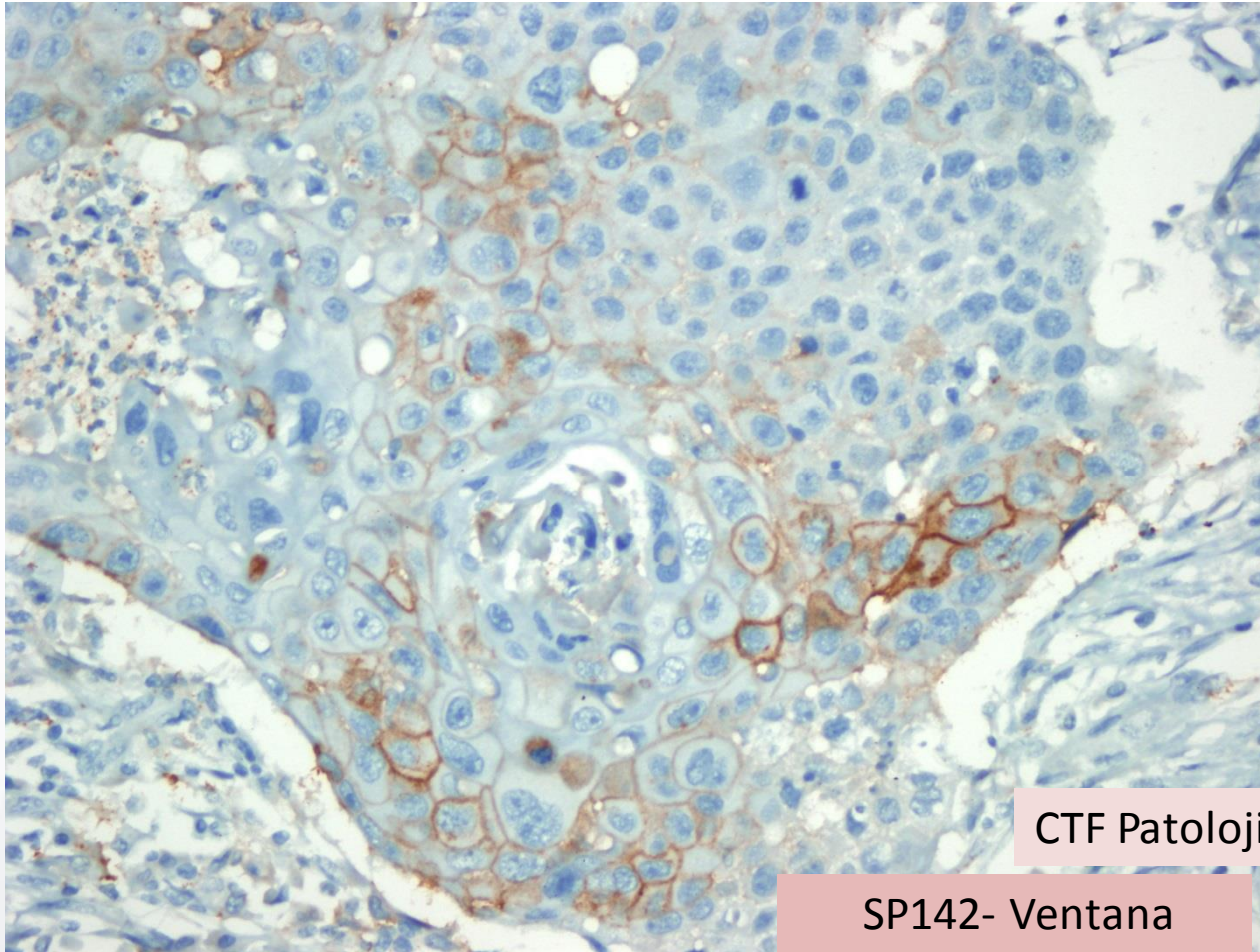
Tümör heterojenitesi nedeniyle küçük biyopsilerde yalancı negatiflik



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CTF Patoloji

SP142- Ventana



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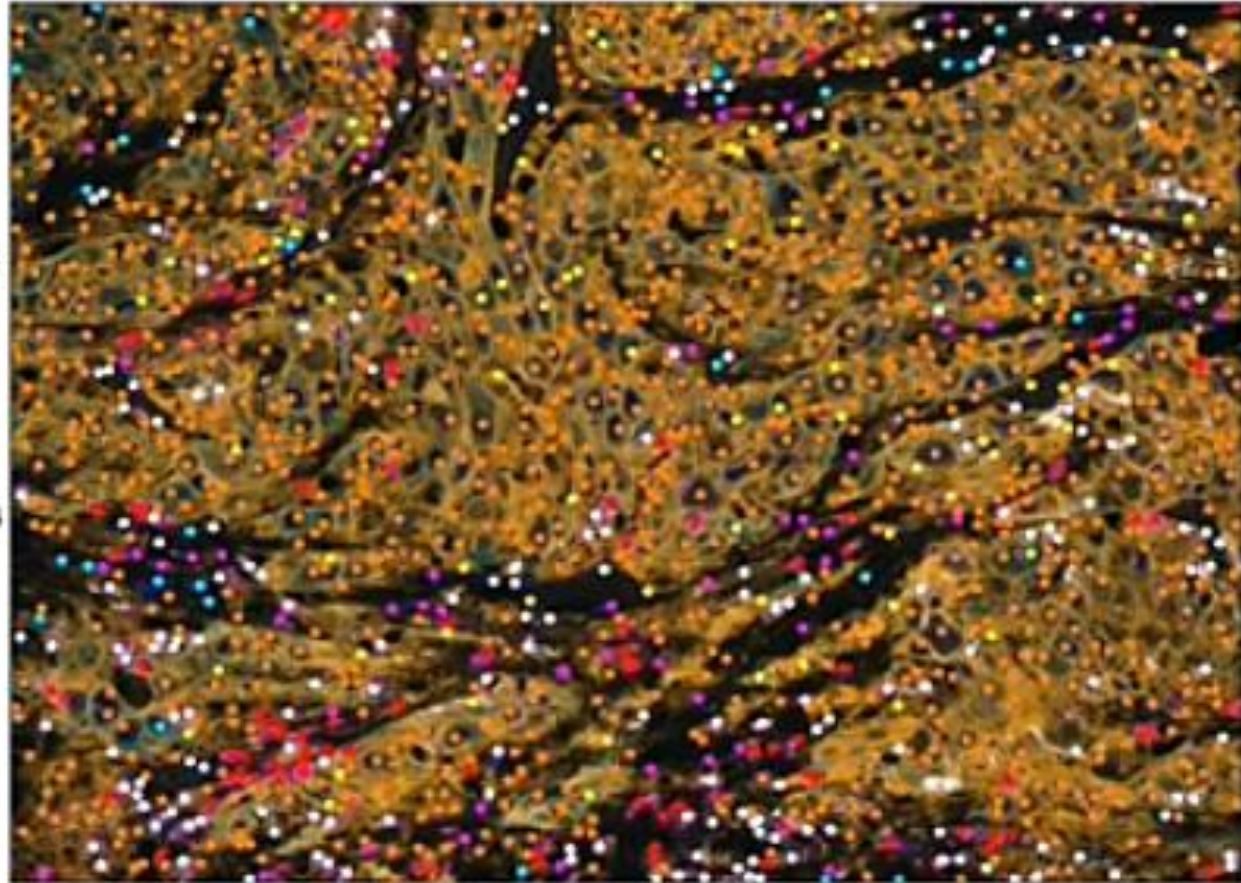
- KHDAK immunolojik mikro çevreye sahiptir.
- Özellikle rezeksiyon materyallerinde yapılan arařtırmalar da Tumor ilişkili iltihabi hücre (=TAIC) oranının önemi ???
- Multipl IF gibi yeni yöntemlerle tümör dokusundaki TAIC kantitatif olarak değerlendirilebilir.
- TAIC (TIL / TAM) de biyo-belirteç mi ??
- TAIC sayılması ve değerlendirmesi için hangi yöntem uygun ?????



# Immuno-profiling Multiplex IF Vectra™ Panels

## Phenotyping Training - Inform Software

- Malignant Cells
- PD-L1 Malignant Cells
- PD-L1 Macrophages
- CD3+/CD8+ Cytotoxic T Cells
- CD3+/CD4+ Helper T Cells
- Other T cells



*E. Parra and J. Rodriguez, MD Anderson Cancer Center, 2015*

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16<sup>TH</sup> WORLD CONFERENCE ON LUNG CANCER

SEPTEMBER 6-9, 2015 • DENVER, COLORADO, USA

INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

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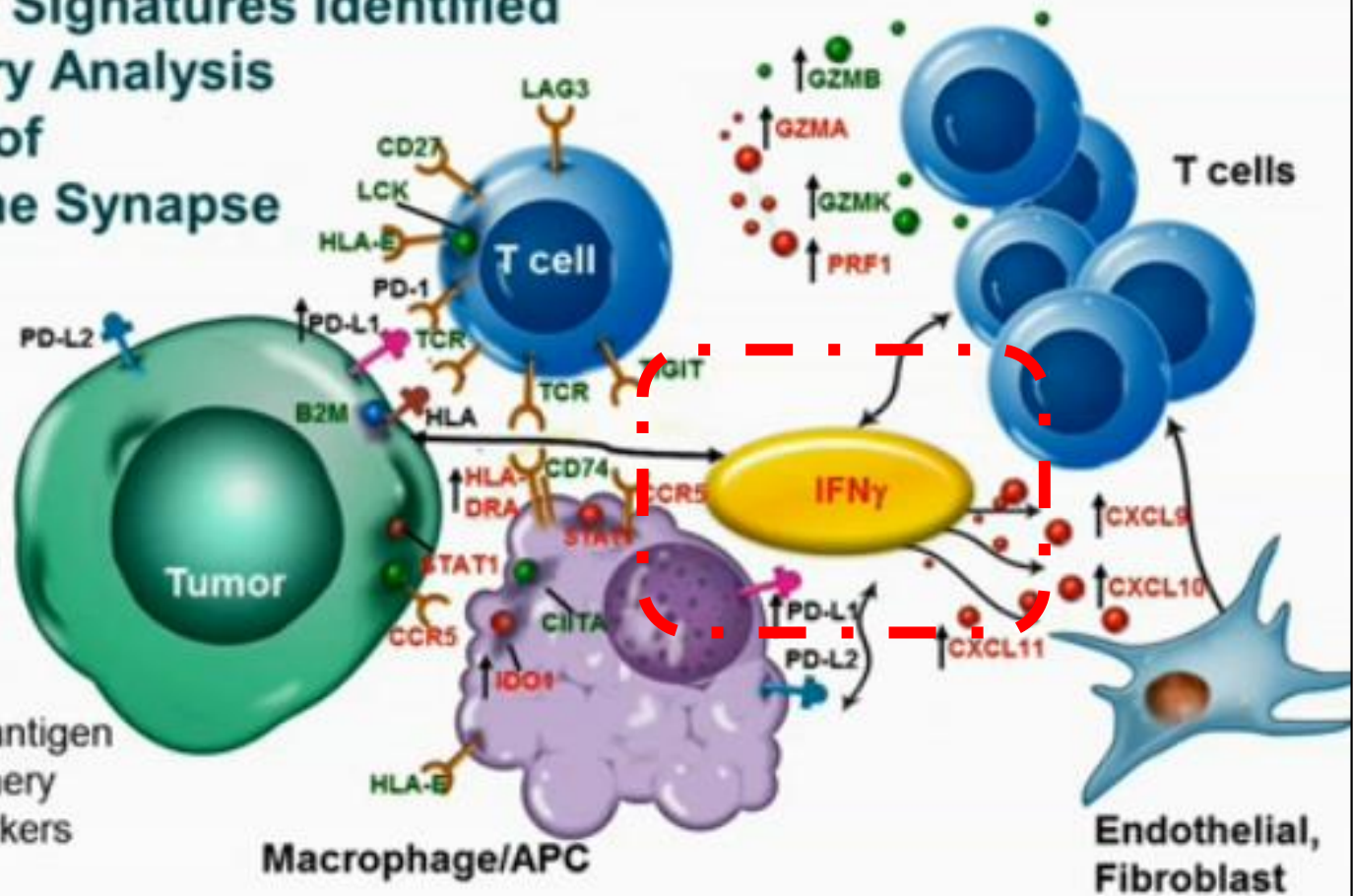
PDF  
2005



# Expanded Gene Signatures Identified During Discovery Analysis Reveal Biology of Complex Immune Synapse

Discovery analysis of entire NanoString melanoma data set led to identification of new genes:

- IFN $\gamma$  signaling
- MHC class I and II antigen presentation machinery
- T-cell activation markers



Ribas, ASCO 2015

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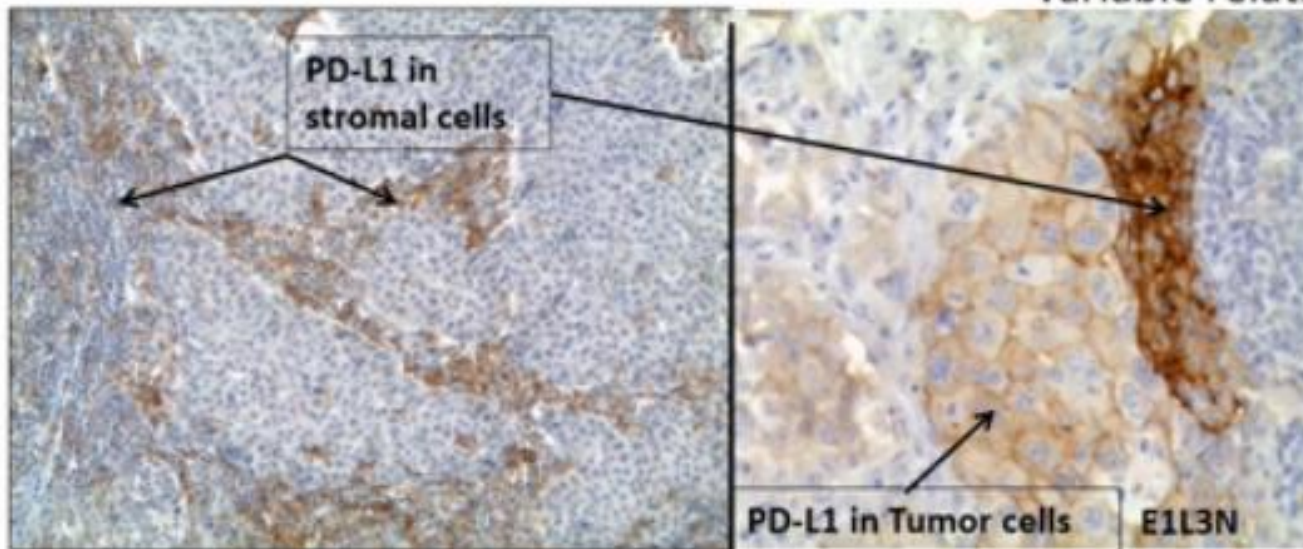


# Biomarkers assays in development

- Each test requires proprietary platforms
- 5 Different antibodies
- Different definitions of a positive test for PD-L1

- **Expression on tumor cells and/or stromal immune cells** : variably appreciated

Variable relation: associated or no



Not in our study

9% both positive  
(TC + IC)

20% TC PDL1+  
29% IC PDL1+



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# *Tümördeki mutasyon sıklığı ile immunoterapi arasında ilişki var mı?*



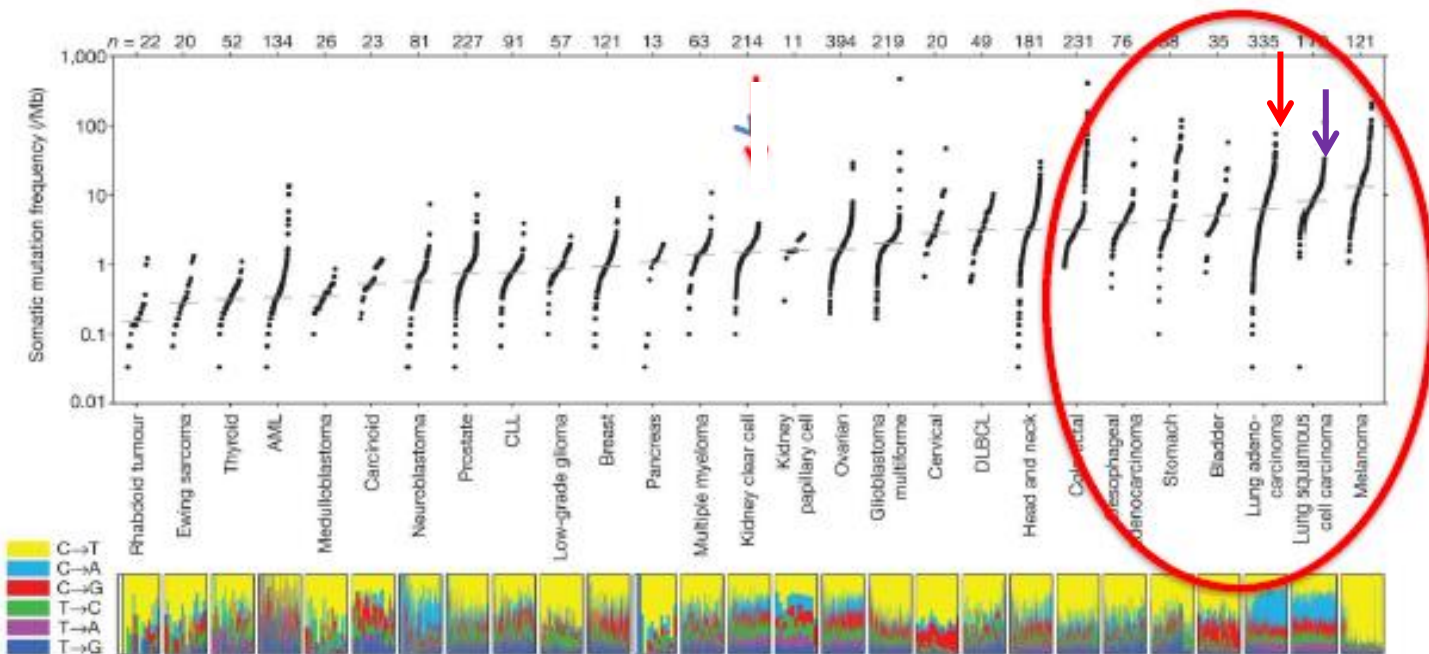
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# Somatic mutation frequencies observed in exomes from 3,083 tumour–normal pairs.

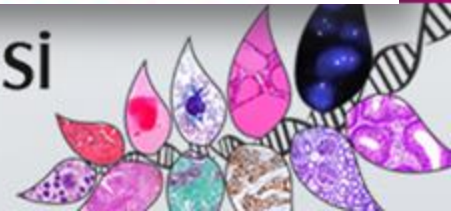


MS Lawrence *et al.* *Nature* 000, 1-5 (2013) doi:10.1038/nature12213

nature

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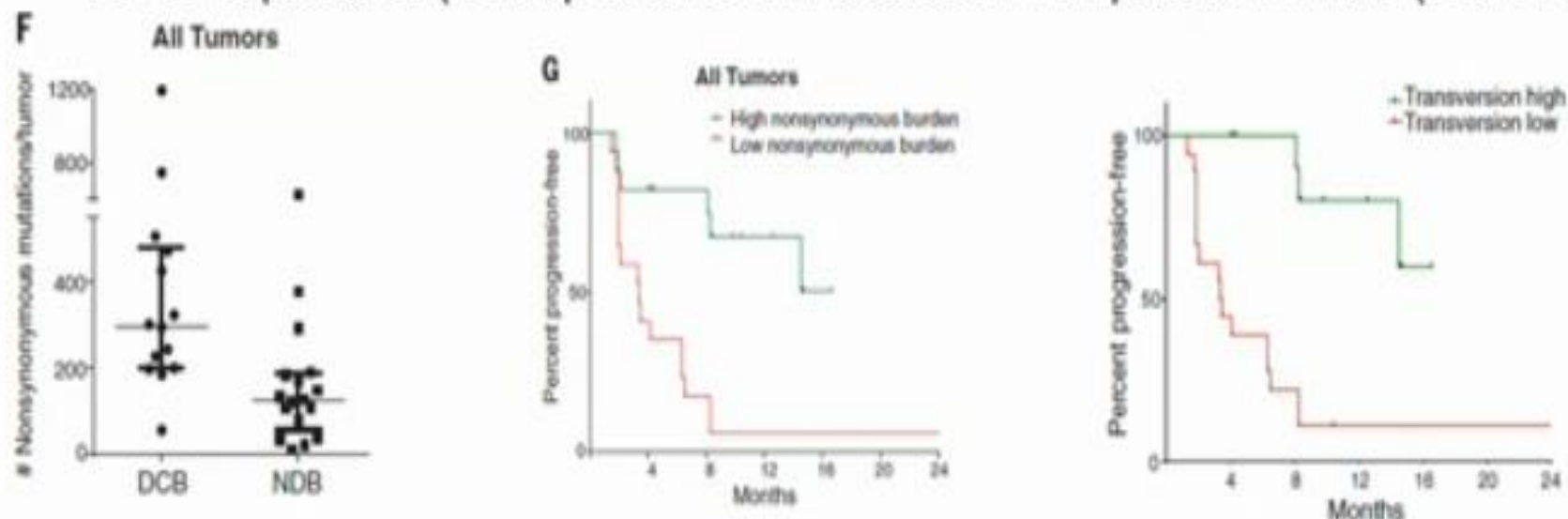
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# Mutational landscape and PD-1 blockade in NSCLC

Rizvi N.A. et al.; Science 2015

Mutational landscape determines sensitivity to PD-1 blockade in NSCLC independent of PD-L1 expression (2 independent cohorts treated with pembrolizumab (Anti PD-1))

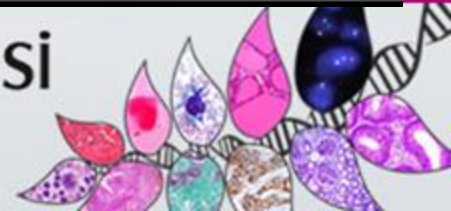


- Efficacy correlated to:
  - the molecular smoking signature,
  - higher neoantigen burden with expansion of specific CD8+T cells
  - DNA repair pathway mutation



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# PD-1/PDL-1 exp. KHDAK'da moleküler-genetik verilerle tedavi cevabında prediktif değeri

- Onkogen ilişkili KHDAK'da PD-1 ve PDL-1 farklı oranlarda pozitif ; Check- point inhibisyonu daha etkili

– EGFR pozitifliği

*Abkay E.A. et al.; Can. Disc. 2013*

*Azuma K. et al.; Ann. Oncol. 2014*

*D'Incecco A. et al. B.J.C. 2015*

– EML4-ALK ,MEK-ERK

*Ota K. et al., C.C.R. 2015*

– KRAS PDL-1 eks .ile korele ancak check point inhibisyonu tedavi sonuçları değişken

*M.MinoKenudson IASLC oral 13.0*



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INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

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SEPTEMBER 6-9, 2015 • DENVER, COLORADO, USA

• Sitopatoloji Kongresi

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PDF  
2005

# Aklımızda kalanlar;

- Klinik arařtırmaların sonuçları , PDL-1'in IHK ile deęerlendirilmesinin «check point inhibitörleri» tedavisinde önemli bir prediktif biyo-belirteç olduęu yönündedir.
- Ancak KHDAK'larında çok kompleks immunité vardır; patolojik çalıřmalar sadece biyo-belirteç olarak PDL-1 +/- yeterli olmayabileceęini göstermektedir.
- CD8, PD-1, CD4/T hüç oranı, TIL, INF- $\gamma$  vb. gibi dięer fonksiyonel belirteçlerin birlikte deęerlendirmesi tedavi seęiminde daha yararlı olabilir.
- PDL-1 genetik upregölasyonunun arařtırılması???
- **Farklı PDL-1 klonları, farklı boyama cihazlarının řart kořulması pratik uygulama sorunu oluřturmakta; Çok merkezli karřılařtırma çalıřmalarına ihtiyaç var .**





## IASLC: PD-L1 CHARACTERIZATION PROJECT ("Blueprint Project")

- To achieve a better understanding of the existing PD-L1 assays and their characterization/comparison (Phase 1)
- To produce a **PD-L1 ATLAS** describing the background, feasibility, and comparability of the assays with corresponding illustrations (Phase 2)
- Eventually: to prospectively apply one or more assays to a clinical cohort (Phase 2)

Courtesy of F.Hirsch



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