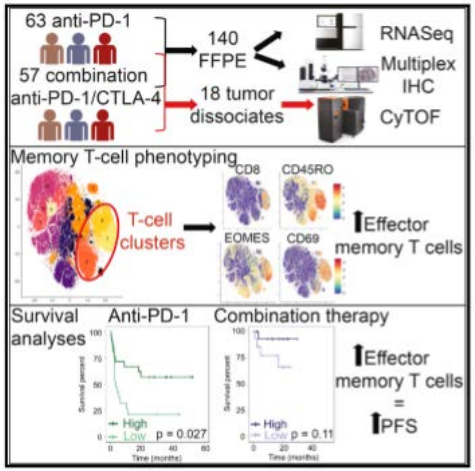
World
Hellenic
Biomedical
Association
WHBA, Inc.

8th International Summer School in
Medical & Biosciences Research & Management
May 17-24, 2019
Athens & Neo Itilo, Laconia – Greece
www.whba1990.org

Cancer Cell

Distinct Immune Cell Populations Define Response to Anti-PD-1 Monotherapy and Anti-PD-1/Anti-CTLA-4 Combined Therapy



Authors

Tuba N. Gide, Camelia Quek, Alexander M. Menzies, ..., Richard A. Scolyer, Georgina V. Long, James S. Wilmott

Dr. Karagiannis Sophia

Efi Chatziioannou

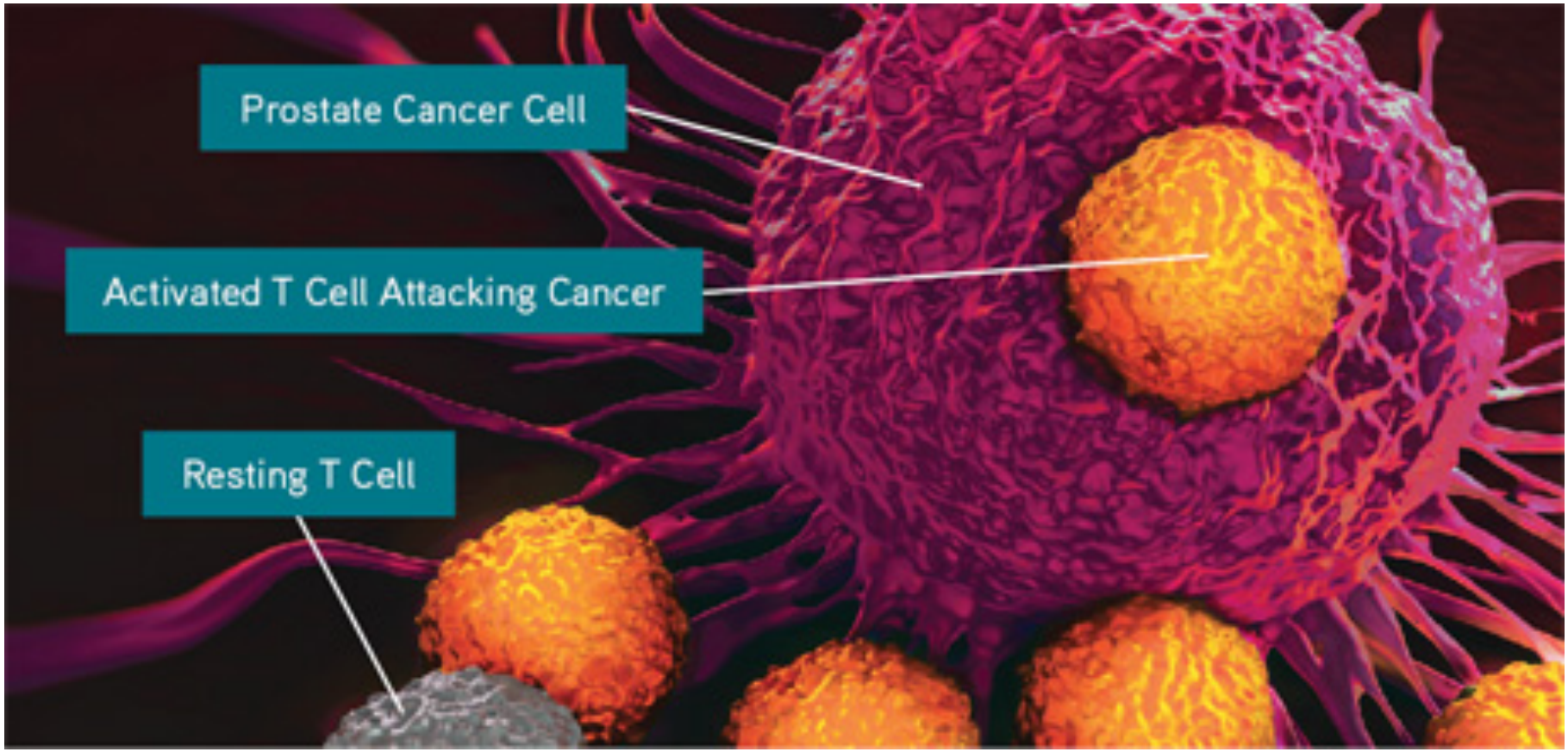
Athanasia Eleftheria Liapodimitri

Christina Niavi

Dionysis Nikolopoulos

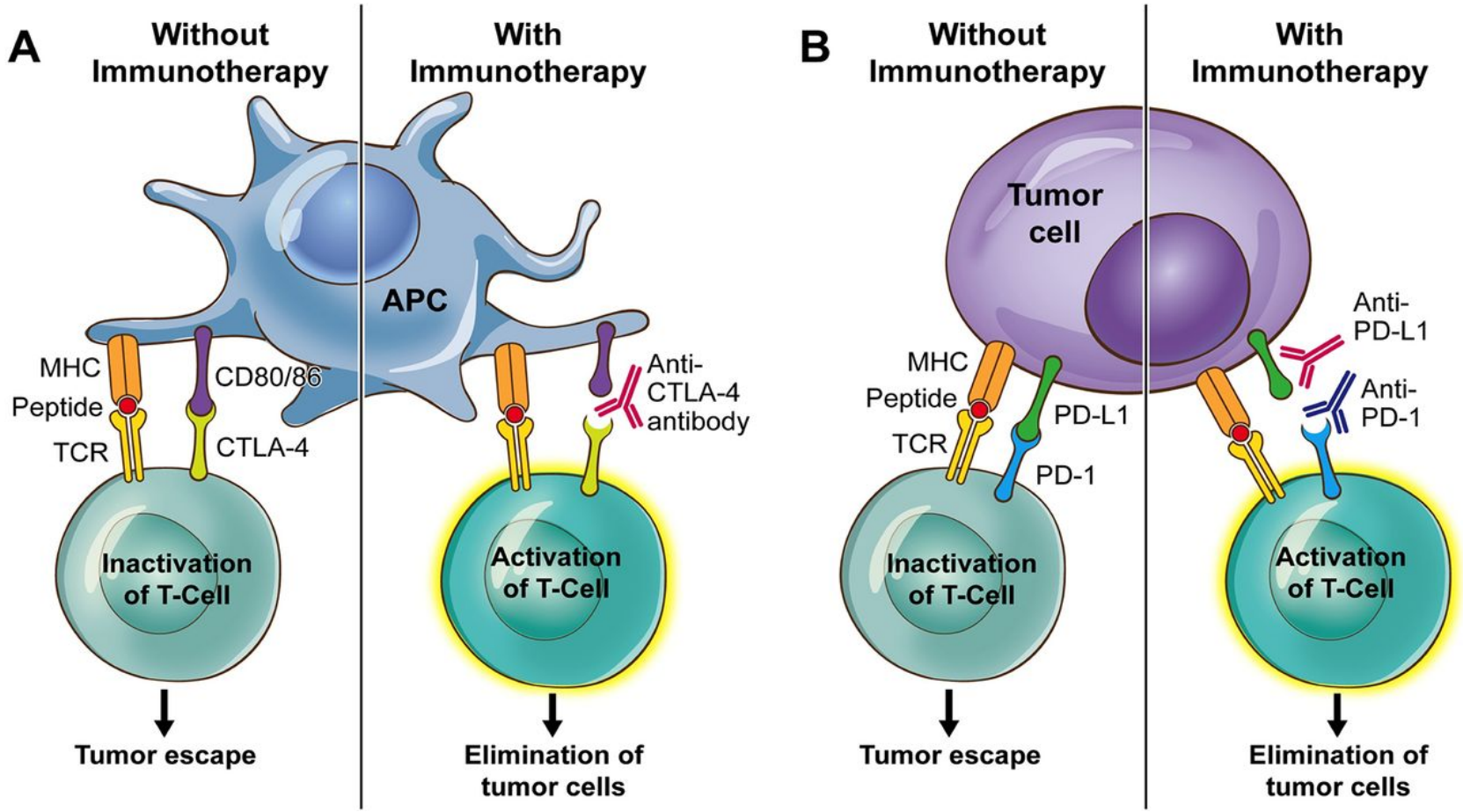
Cancer immunotherapy recognized with the 2018 Nobel Prize in Physiology or Medicine

HOW ?



http://www.mygenesishealth.com/treatment-options/genesis-prostate-cancer-center/castrate-resistant-prostate-cancer.html

HOW ?



https://gut.bmj.com/content/67/11/2056

Cancer Immunotherapy

- In metastatic melanoma, anti-PD-1 antibodies have become **standard care** providing high efficacy and minimal toxicity

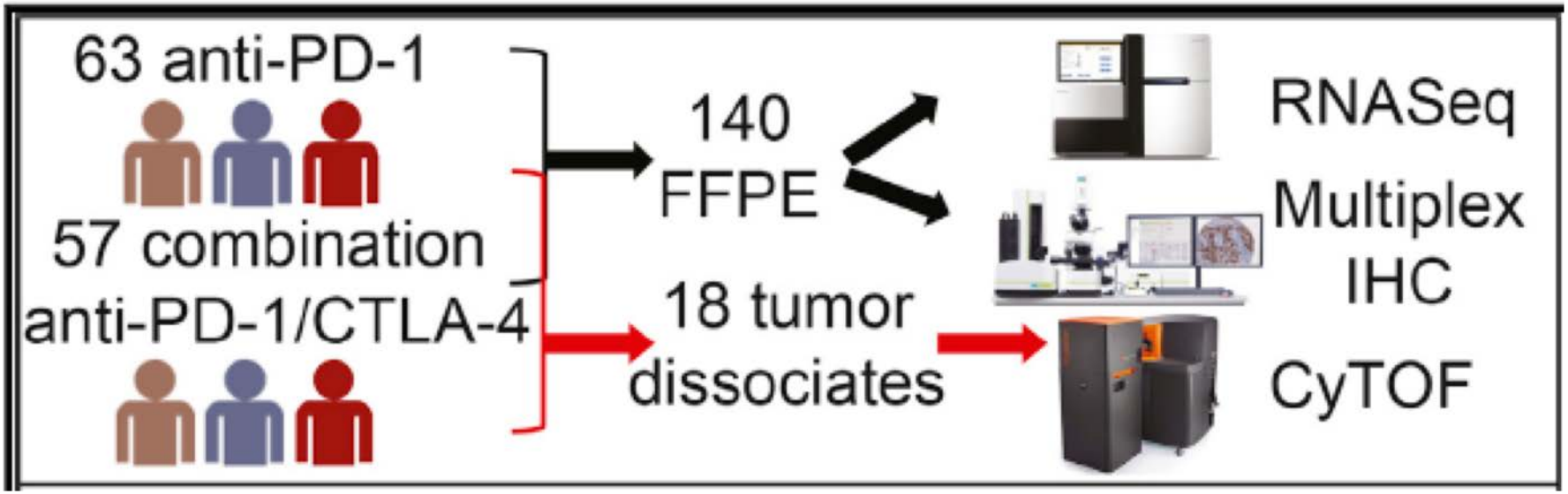
- the combination of the anti-CTLA-4 antibody with anti-PD-1 has been shown to have higher response rates than anti-PD-1 monotherapy, but at the cost of **significant toxicity**

- Several unmeet needs in the field of immunotherapy

- Why some patients respond to Tx, while some others not ? (different signaling pathways ? what cells are enabling the response ? What mechanisms prevent an effective immune response in non-responders ?)

- Need for baseline biomarkers in order to identify responders and non-responders…Is this important ?

Clinical characteristics



- PD1 inhibitors: nivolumab, pembrolizumab
- CTLA4 inhibitor: ipilimumab

➔ Response evaluated by RECIST classification

Key immune targets

- T cell exhaustion = dysregulation due to persistent stimulation

Progenitor Tex — TBET

Terminal Tex — EOMES

- TIGIT, LAG-3: immunosuppression, future checkpoints

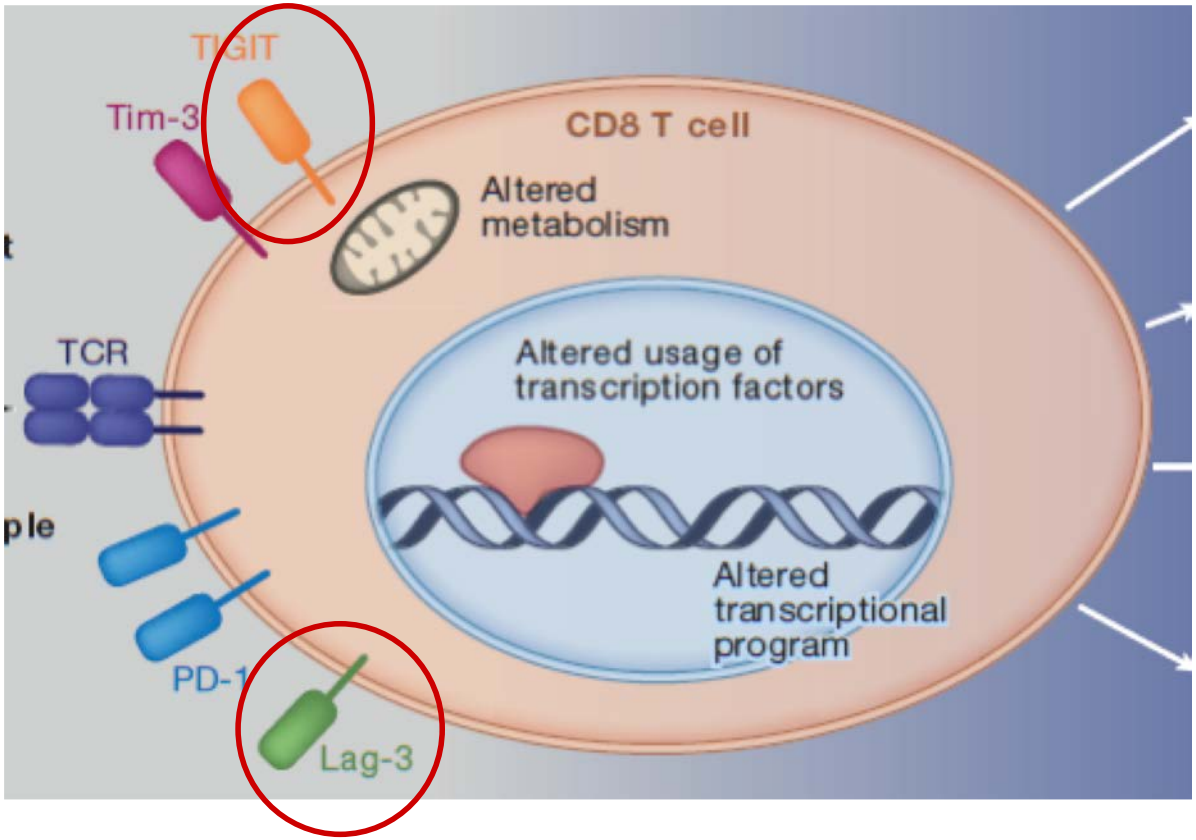
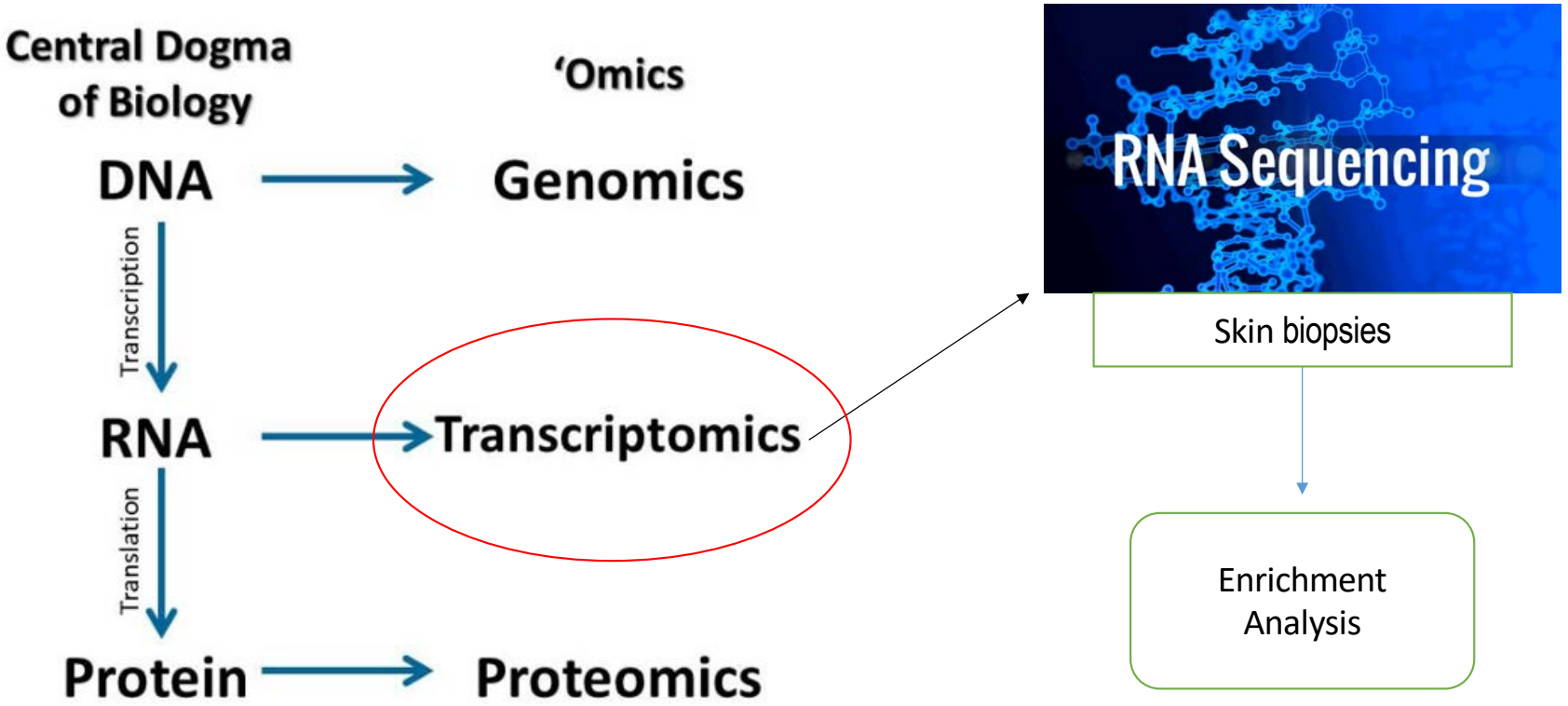


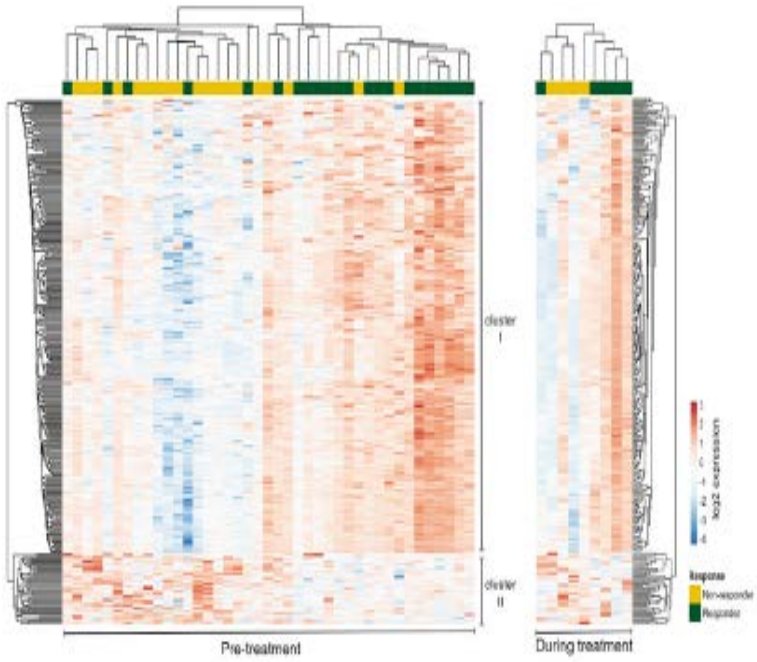
Figure . Pauken K., Wherry J. Snapshot: T cell exhaustion. Cell. November 2015

How can we detect non-responders ?



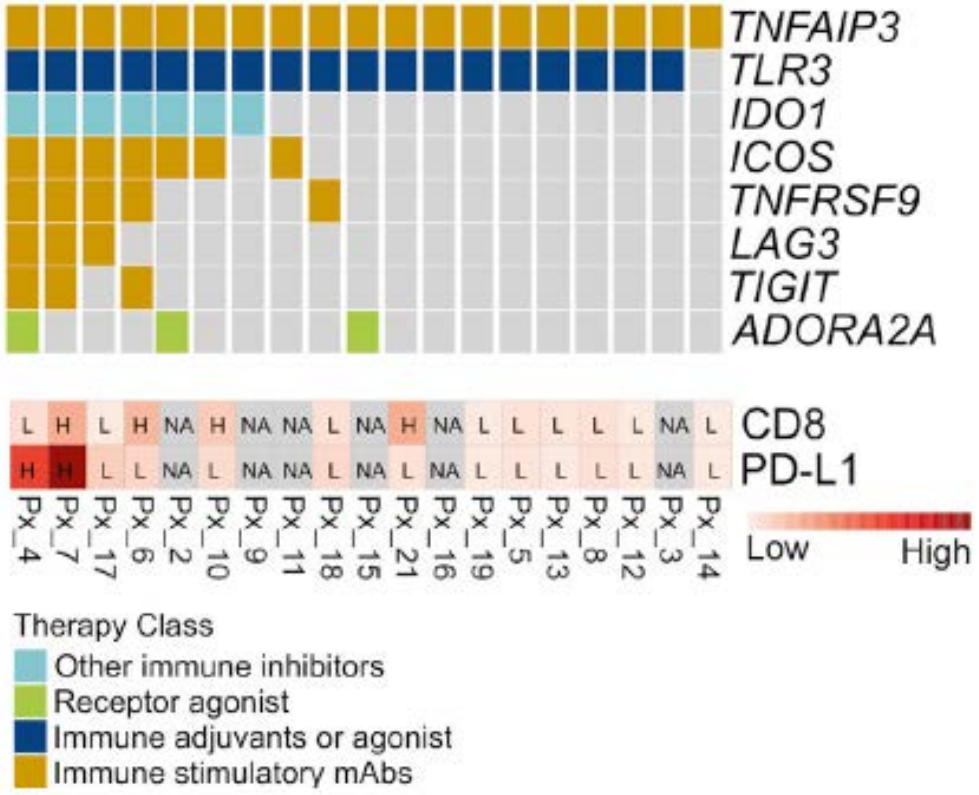
Differential Gene Signatures of patients to Anti-PD-1 demonstrate two distinct gene clusters

- Responders vs. non-responders at Tx (Monotherapy)
- This analysis identified 310 DEGs ($p < 0.05$)
- Two distinct gene clusters associated with **immune signaling** and **cellular signal transduction**
- IFN-related and tumor-infiltrating T cell genes were associated with better outcomes
- Responding patients also expressed higher levels of other immunosuppressive checkpoints and proteins in their tumors



The transcriptomic profiles of non-responders to monotherapy reveal decreased expression of immune checkpoint receptors

- ~ 50% of patients had low CD8+ and PDL1+ counts
- Fewer immune checkpoint receptors
- Decreased immune response
- The group of high CD8+ and PDL1+ displayed increased expression of immune targets

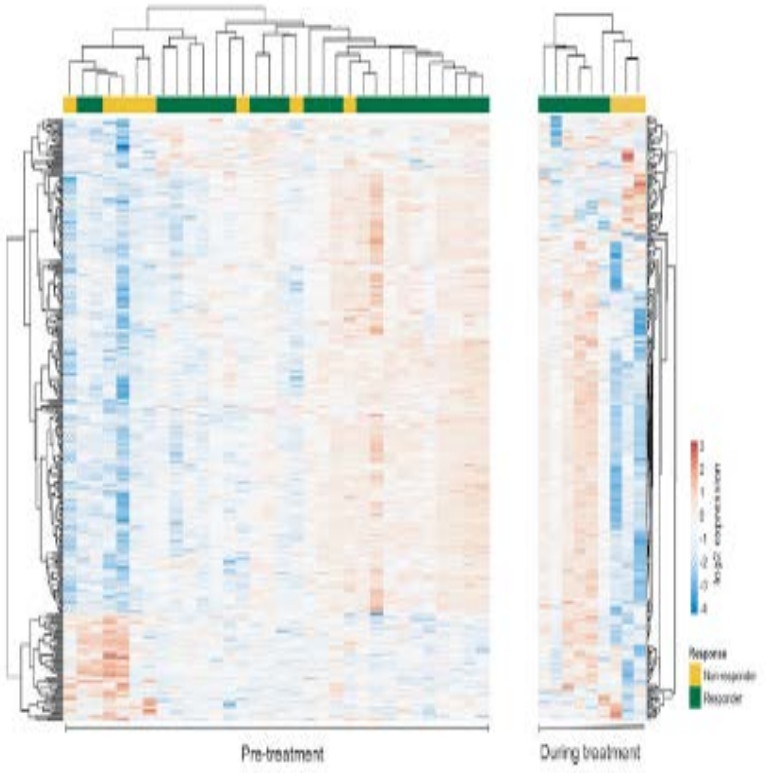


Differential Gene Signatures of non-responders to Combined Immunotherapy show T-cell & NK-cell mediated cytotoxicity

- A similar analysis on the combined anti-CTLA-4 and anti-PD-1
- This analysis identified 328 DEGs ($p < 0.05$)

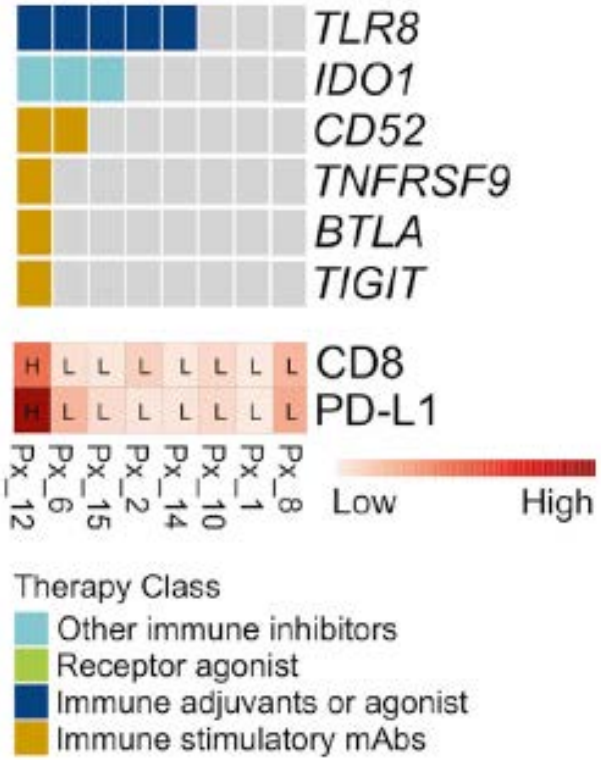
<u>Associations</u>

- T cell-related genes
- NK cell-mediated cytotoxicity
- T cell cytotoxicity
- Cytokine signaling



The transcriptomic profiles of non-responders to combined Therapy show lack of T-cell and PD-1L expression

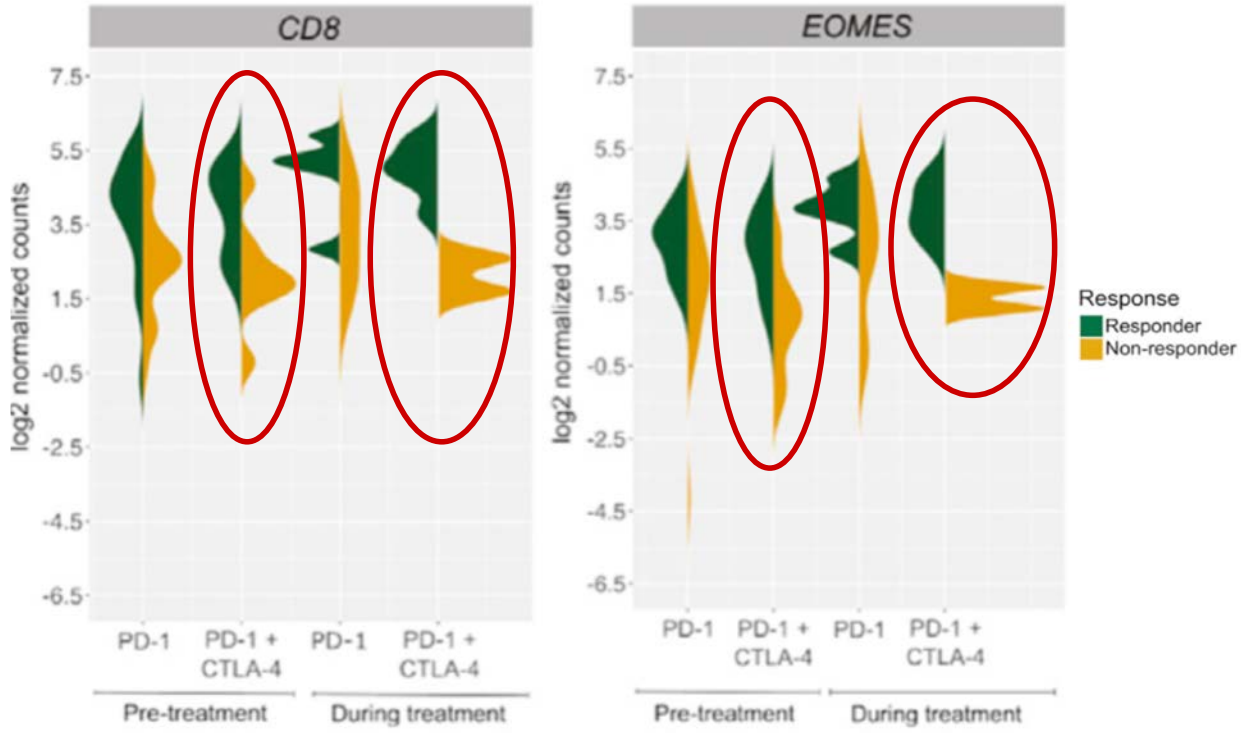
- One non-responding patient had high tumor-infiltrating lymphocytes (TILs) and PD-L1 expression
- Expression of all of the immune checkpoint markers
- The remaining non-responding patients expressed low levels of checkpoint markers and TILs
- Suggesting hypoxic and metabolic tumor microenvironment may play a role in underlying pathogenesis



Distinct CD8 and EOMES expression profiles

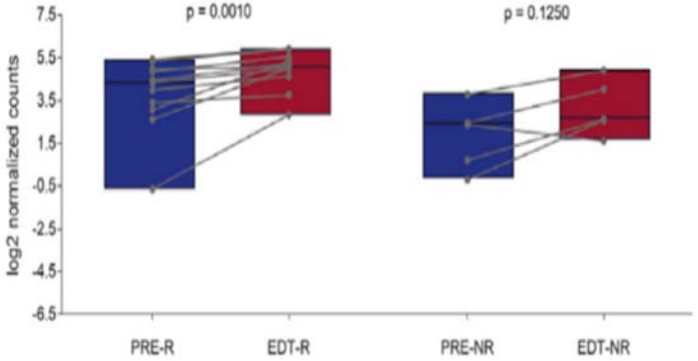
➔ ***CD8 and EOMES***

Distinct separation in expression profiles of both between responders and non-responders early during treatment in combined immunotherapy

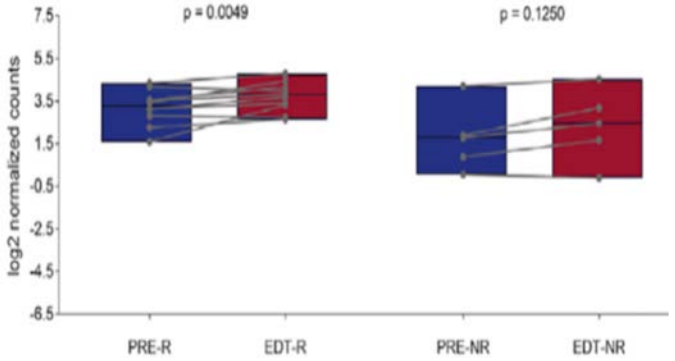


Increase in expression of immune markers from PRE to EDT

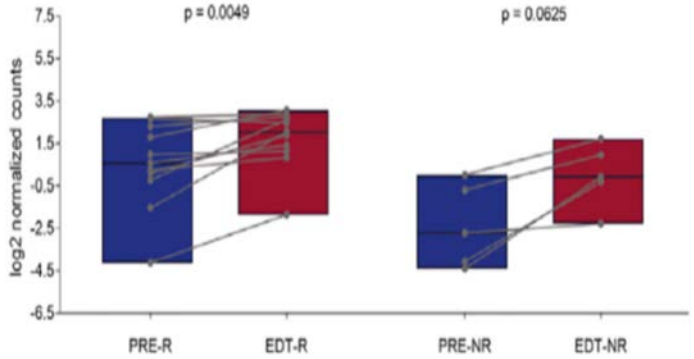
CD8



EOMES



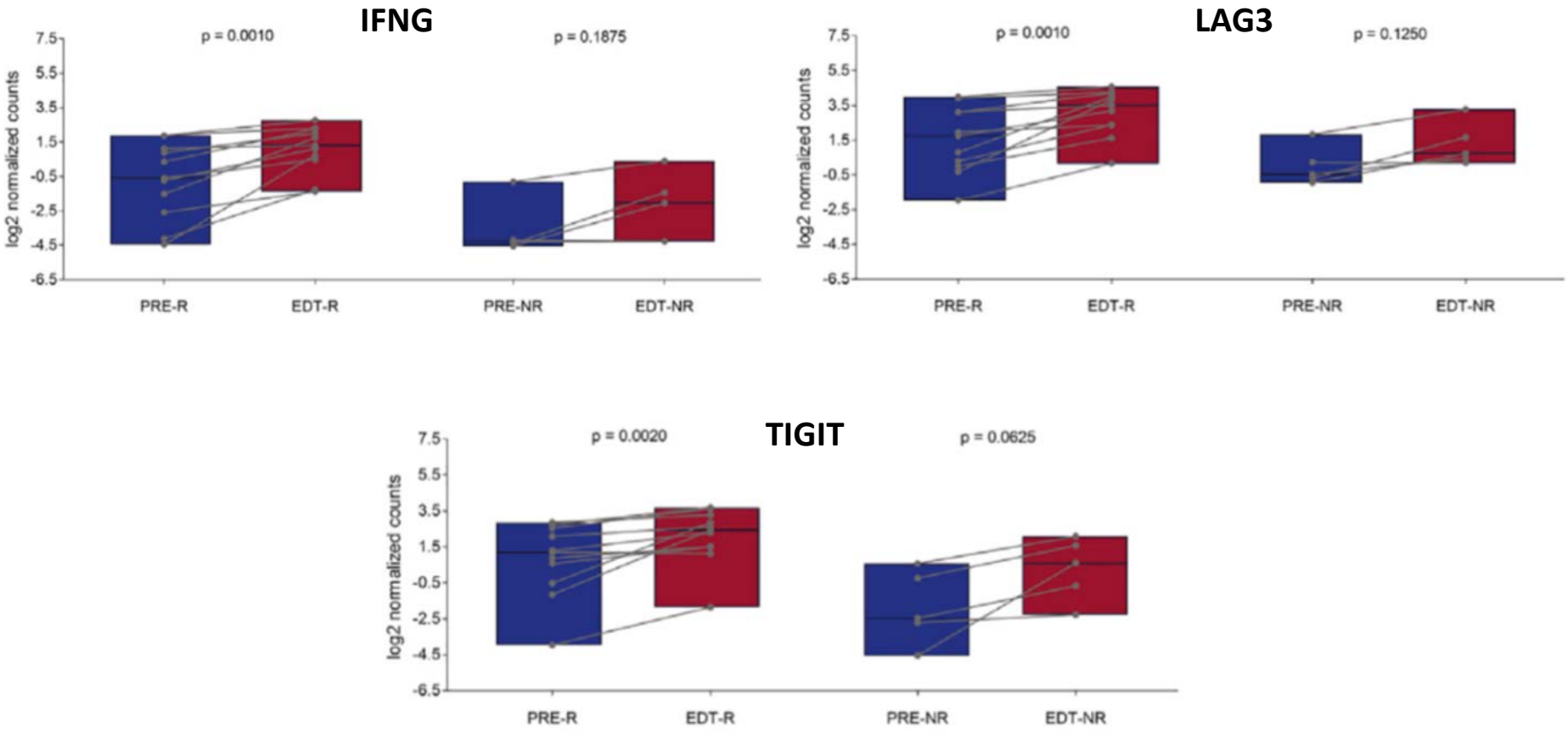
TBX21 (TBET)



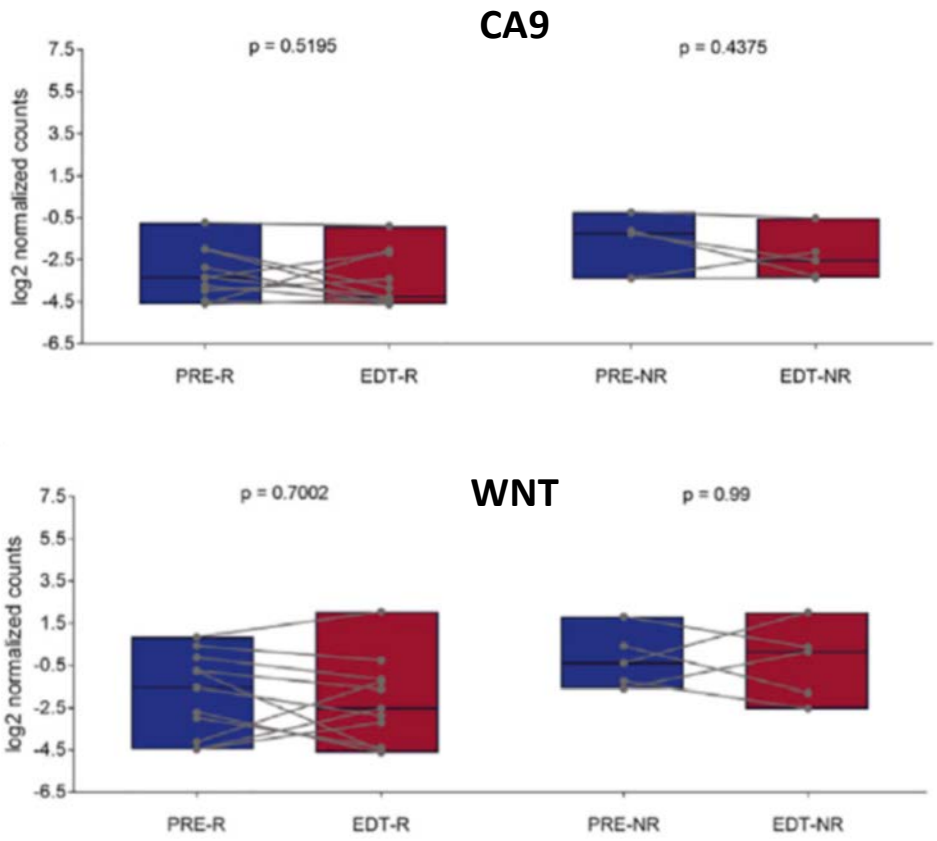
- Paired PRE and EDT biopsies
- Single- and combination- treated patient data were combined

➔ *<u>Responders</u>: significant **increase** in expression from PRE to EDT for immune genes*

Increase in expression of immune markers from PRE to EDT



No change in CA9 and WNT expression profile

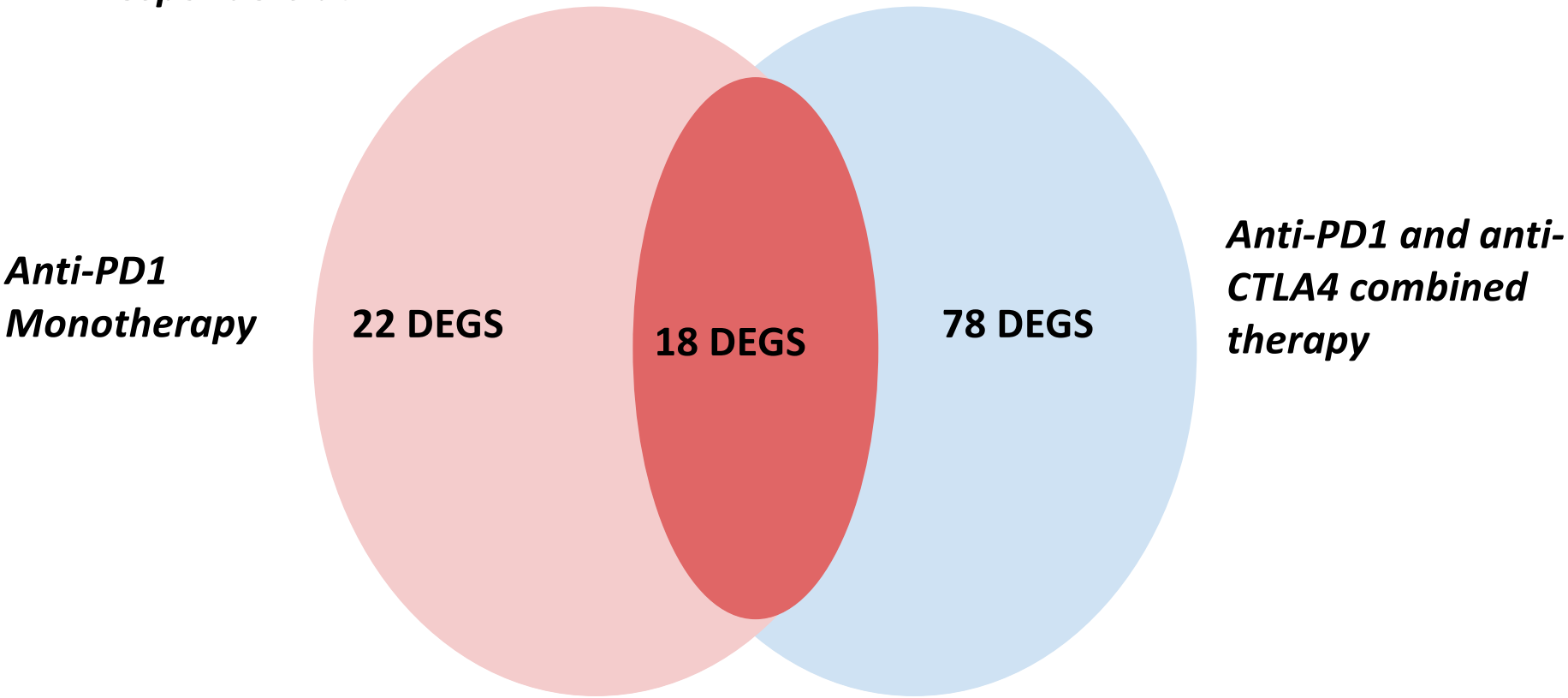


CA9 and WNT

- ➔ Both downregulated in responders to immunotherapy at baseline
- ➔ ***No significant difference*** in the change of expression level from PRE to EDT of either CA9 or WNT both for responders and non-responders

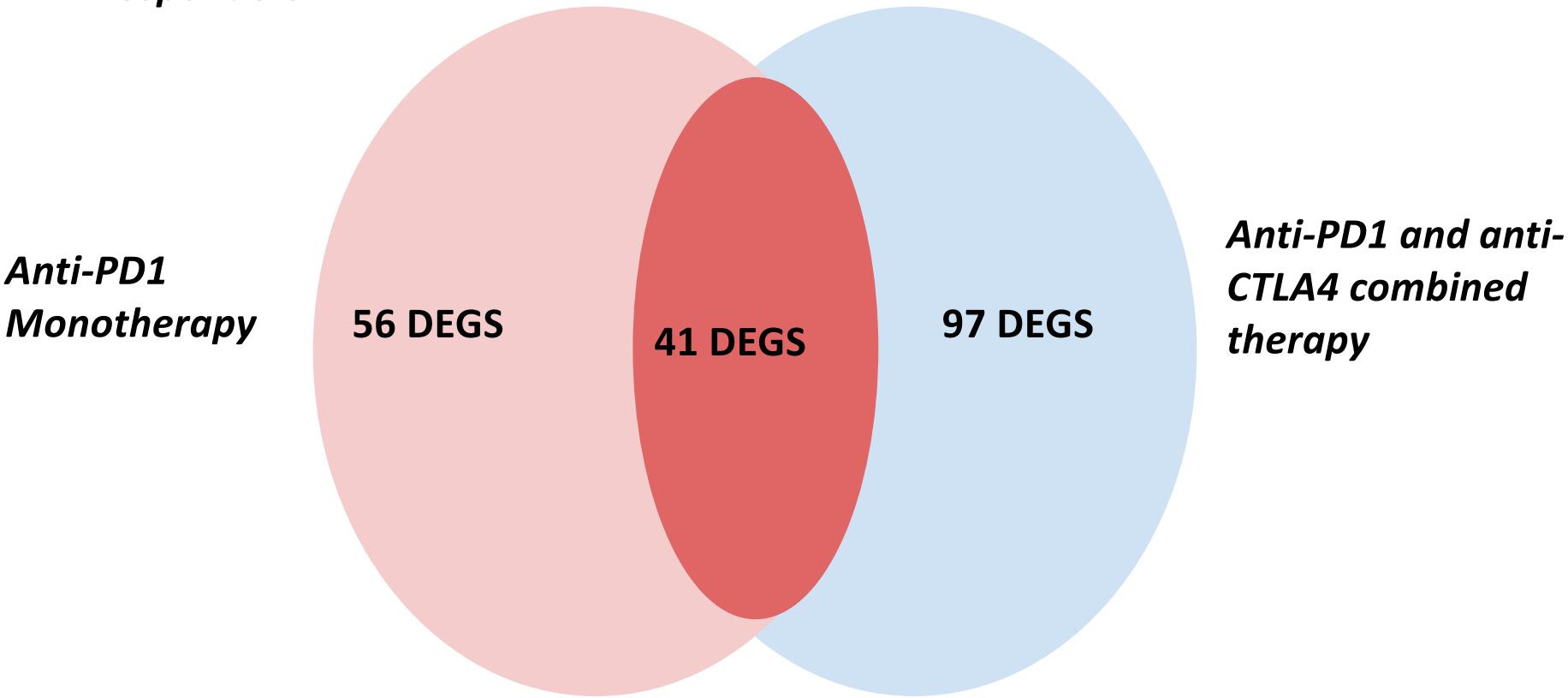
Transcriptomic profiles at PRE: similarities and differences

→ ***Responders at PRE***

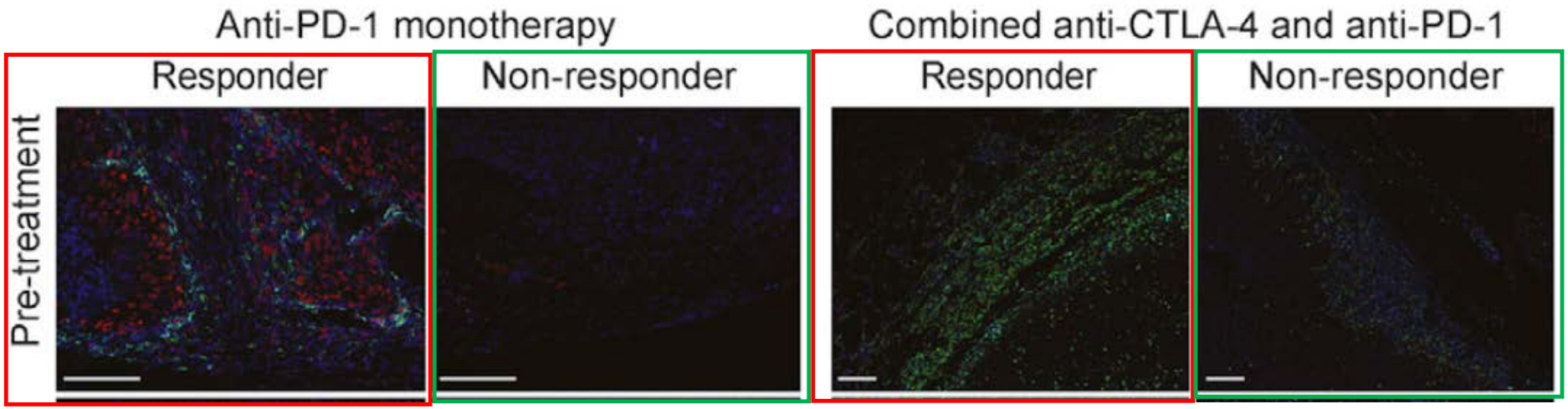


Transcriptomic profiles EDT: similarities and differences

→ ***Responders EDT***



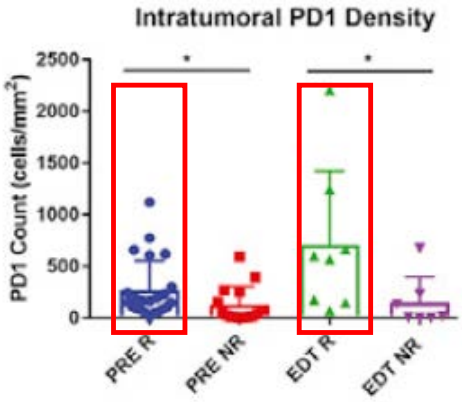
ALL Pre- treated responders showed increased CD8, PDL1.

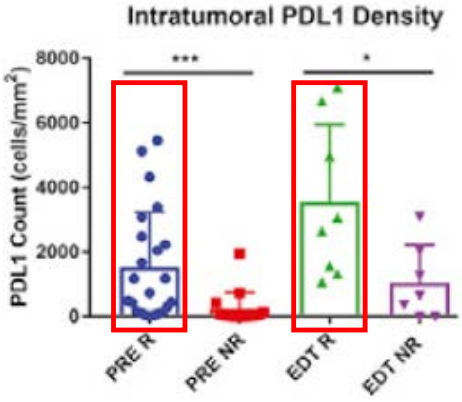


CD8
PDL1

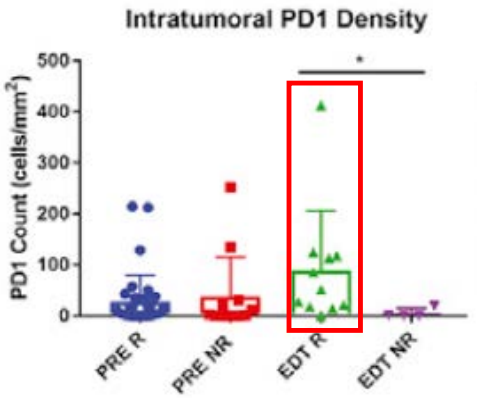
PRE, EDT responders showed increased CD8, PD1, PDL1.

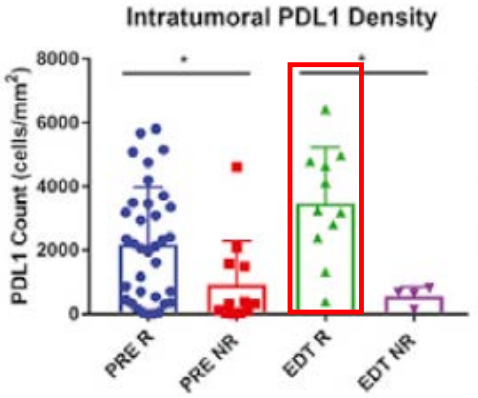
mono

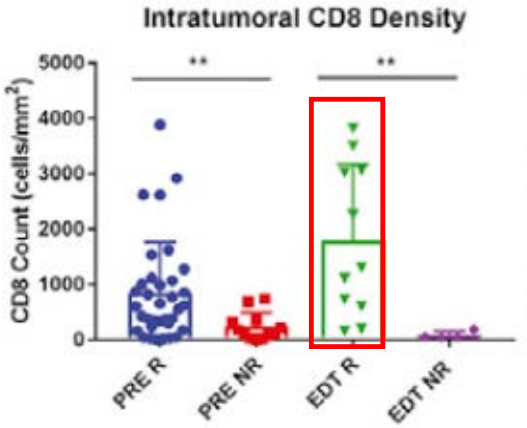




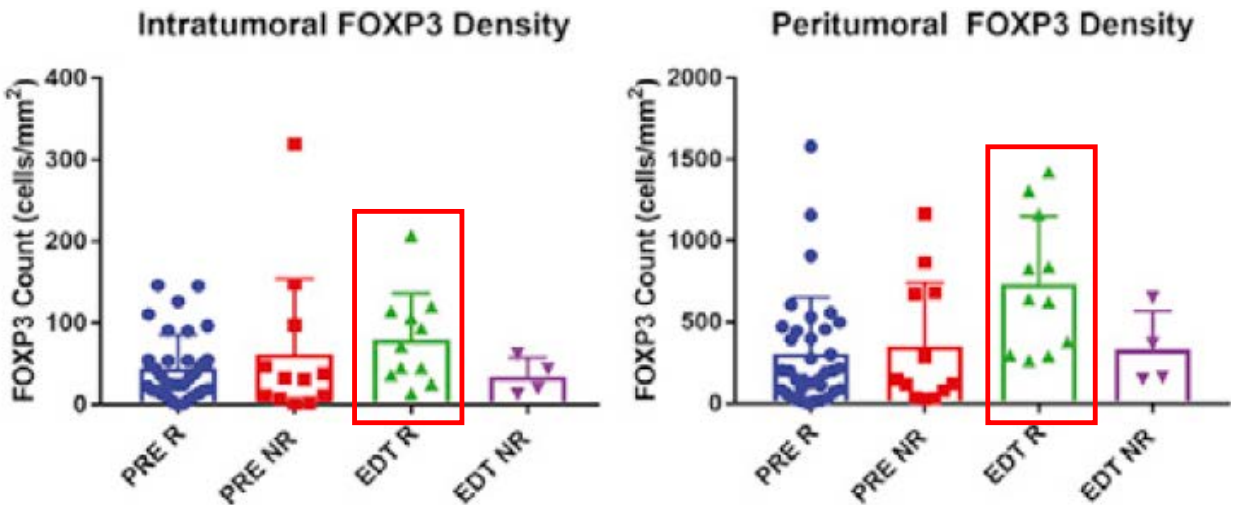
combo



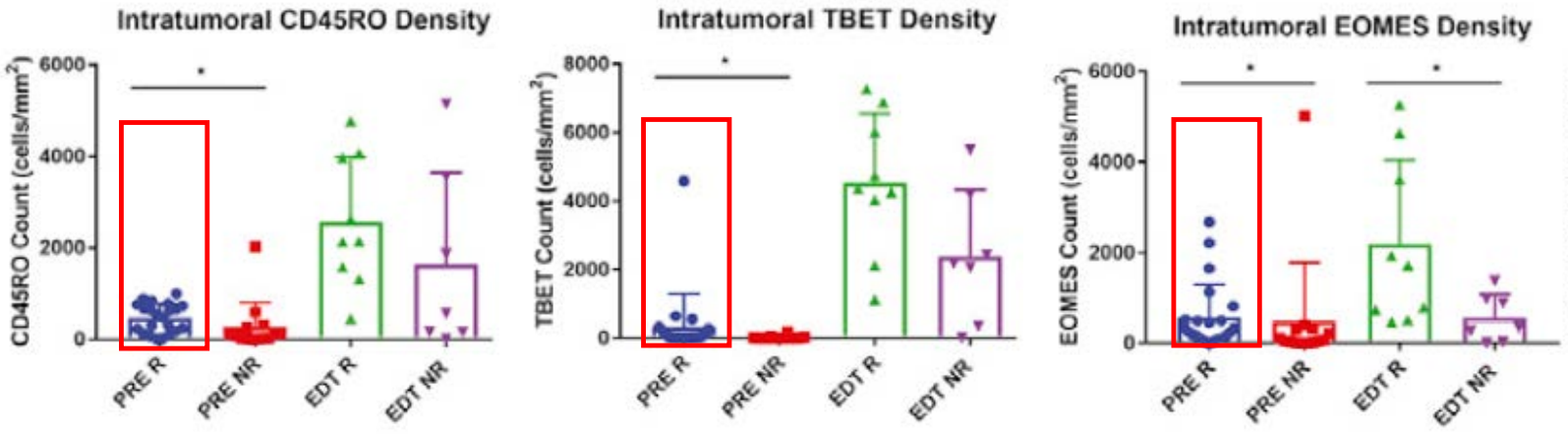




EDT combo responders showed increased FOXP3 suggesting overall increase in TILs.



PRE: Responders showed increased CD45RO, EOMES, TBET (anti-PD-1 monotherapy)



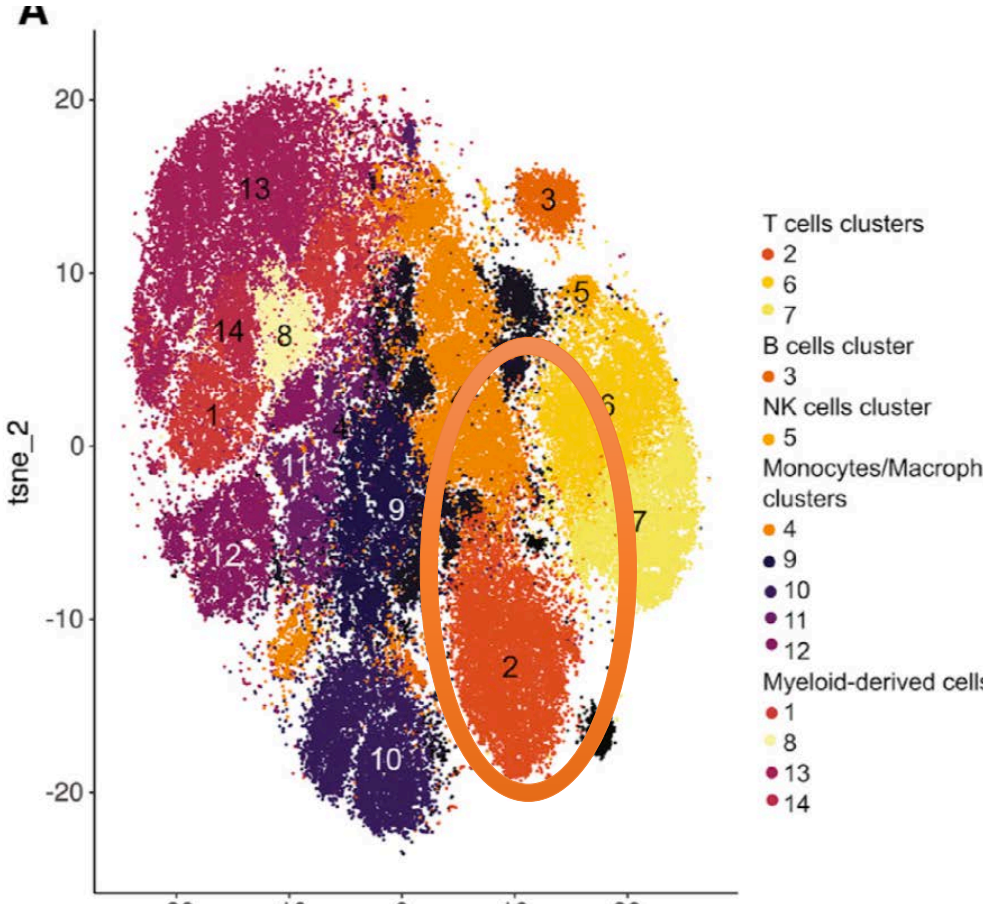
All in all

✓ agreement with transcriptome: increased T cell markers → responders (PRE+ EDT)

Responders have distinct subsets of T cells

- Tissue: all pre-treatment of mono- and combo-
- Method: mass cytometry
- Results: t-SNE of leukocytes

→ 3 T cell clusters (2,6,7)



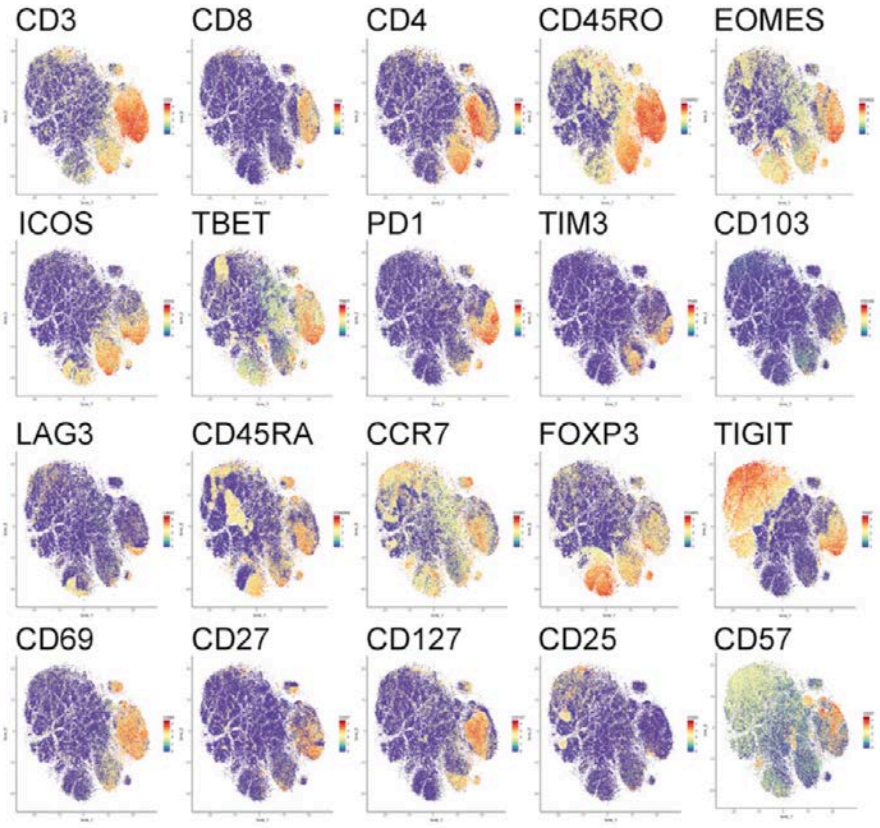
Responders have distinct biomarkers of T cells

t-SNE plots of markers of T cells

- $CD4^+$or $CD8^+$ of responders to both treatment:

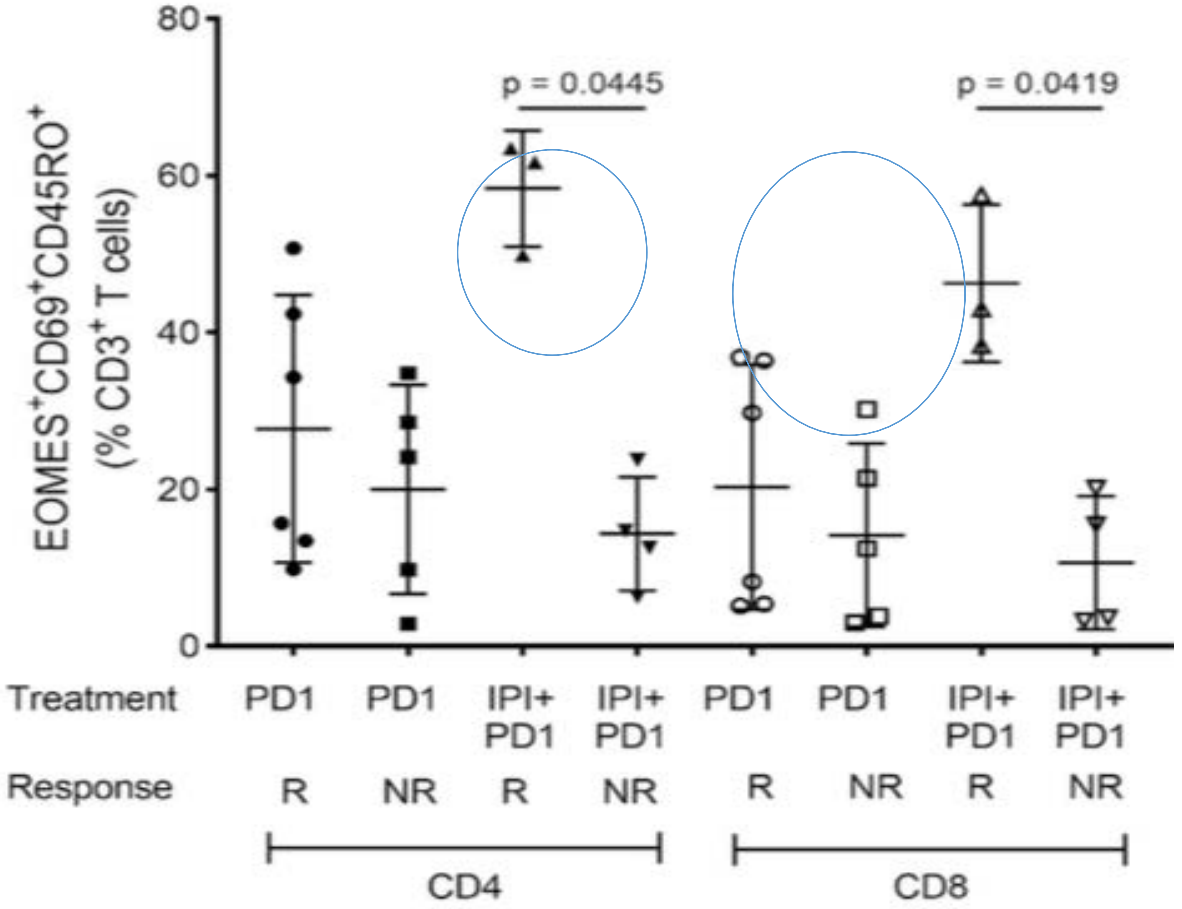
↑ CD69, EOMES, CD45R0, CD103, TBET, HLA-DR, PD-1, TIGIT

↓ CCR7, CD57



Expression of $CD8^+$/ $CD4^+$ $EOMES^+$ $CD69^+$ $CD45RO^+$ T cells

- High expression to responders vs. non- responders to **combo**
- High expression but NOT statistically significant difference responders vs. non-responders to **mono-**
- ! not large sample



Q: what is the **association** of high expression of $CD8^+$/ $CD4^+$ $EOMES^+$ $CD69^+$ $CD45RO^+$ T cells with PFS and tumor shrinkage?

$CD8^+$/ $CD4^+$ $EOMES^+$ $CD69^+$ $CD45RO^+$ T cells

PFS of responders and non-responders:

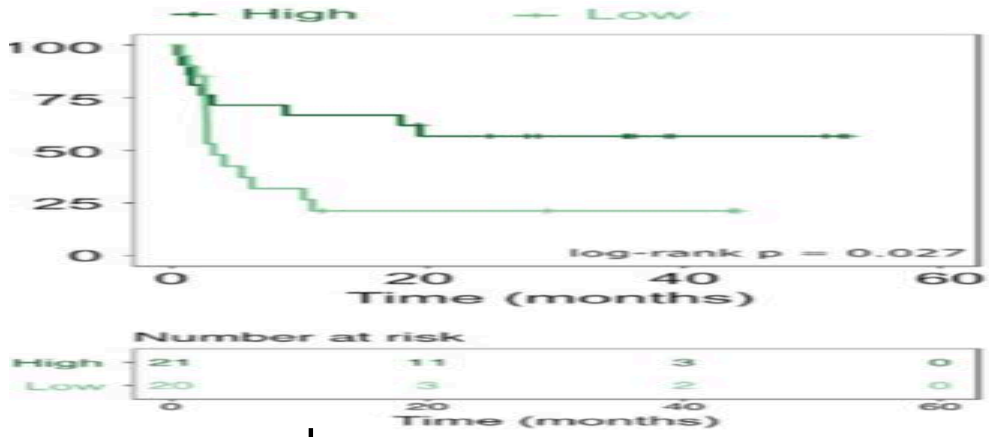
- ↑ to monotherapy

high vs. low expression: 24 vs. 3 months

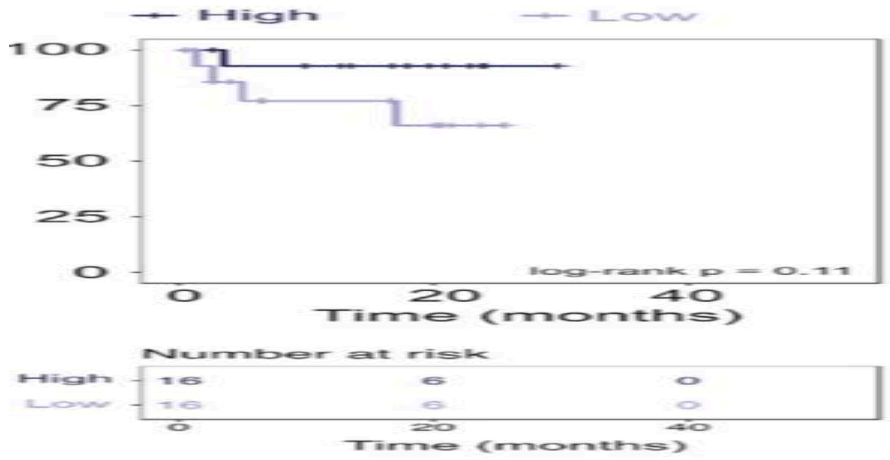
- Increase but not significant to combo (not enough sample)

high vs. low expression: 19 vs. 6 months

monotherapy:



combo:



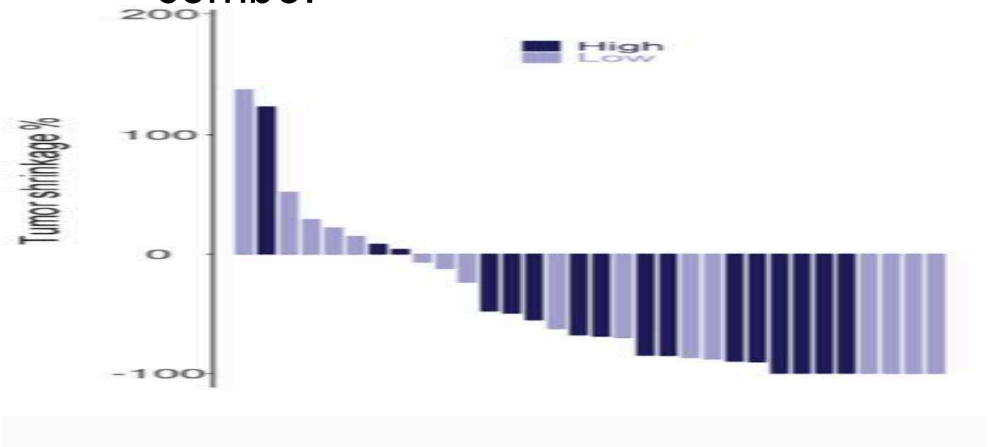
High expression of $CD8^+/CD4^+$ $EOMES^+$ $CD69^+$ $CD45RO^+$ T Cells is associated with tumor shrinkage

Tumor shrinkage of patients:

- 71% of monotherapy treated with high expression
- 81% of combo- treated with high expression

monotherapy:

combo:



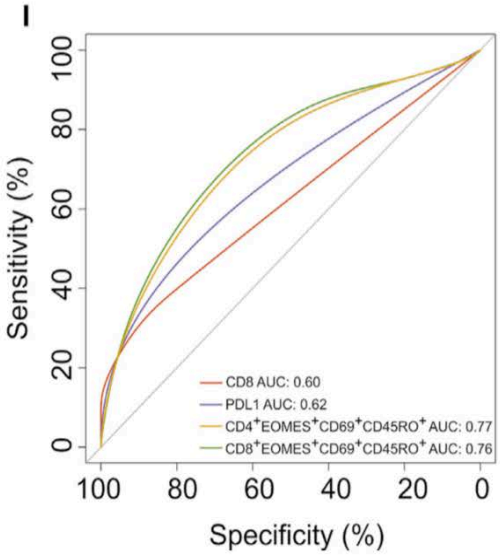
ROC curve for biomarkers: how much they predict response!!!

- $CD8^+$/ $CD4^+$ $EOMES^+$ $CD69^+$ $CD45RO^+$ **>** $CD8^+$ or PDL-1

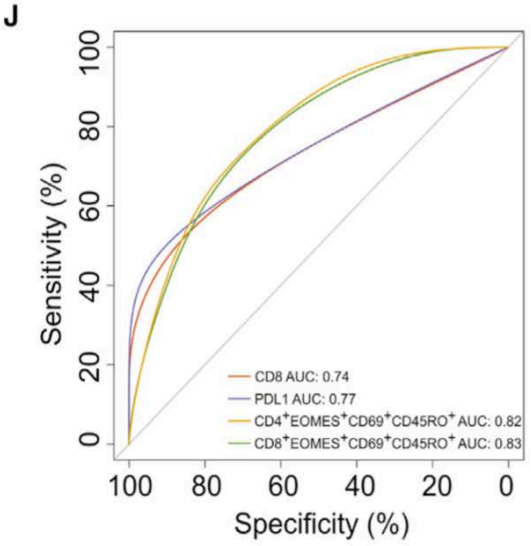
  - for $CD8^+$/ $CD4^+$ $EOMES^+$ $CD69^+$ $CD45RO^+$:

AUC = 0.7 for mono

AUC = 0.8 for combo



monotherapy

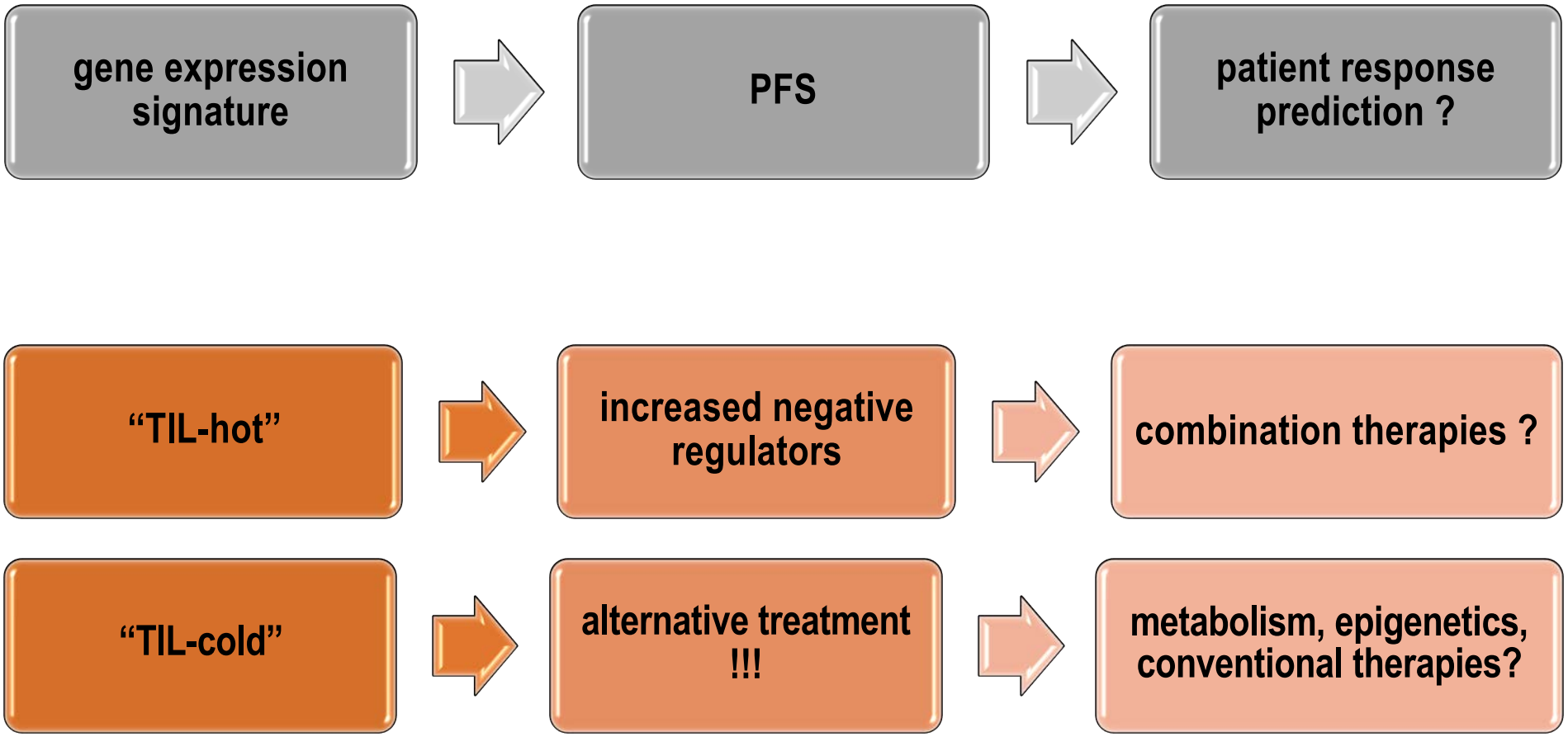


combo

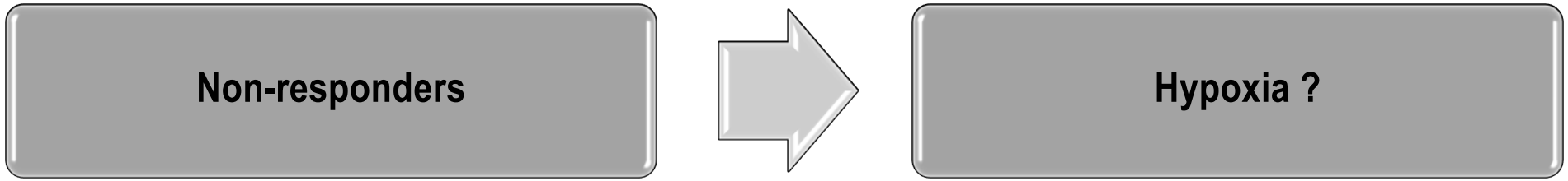
Summarizing and philosophizing

Phenotypes	Response to therapy	Additional comments
EOMES+CD69+CD45RO+ (effector memory)	both therapies	tumor shrinkage
TBET Hi EOMES Lo CD8+	a-PD1	reinvigoration
TBET Hi EOMES Hi CD8+	anti-CTLA-4	
EOMES+CD69+CD45RO+ CD57 Lo (responding tumors)	combo	not terminal + TBET Hi

We keep on summarizing and philosophizing …..



And we keep on summarizing and philosophizing …..



Q remains !! → Which non-responders to anti-PD-1 monotherapy would respond to combined therapy and vice versa ?

Acknowledgements

World
Hellenic
Biomedical
Association
WHBA, Inc.

Dr. Sophia Karagiannis

Thank you!!!