Expression of Novel Immunotherapeutic Targets in Triple Negative Breast Cancer

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ASCO 50th ANNUAL MEETING SCIENCE & SOCIETY

Diclosures

- Barbara A. Pockaj, MD
  - Nothing to disclose

- Collaborators are employees at Caris Life Sciences

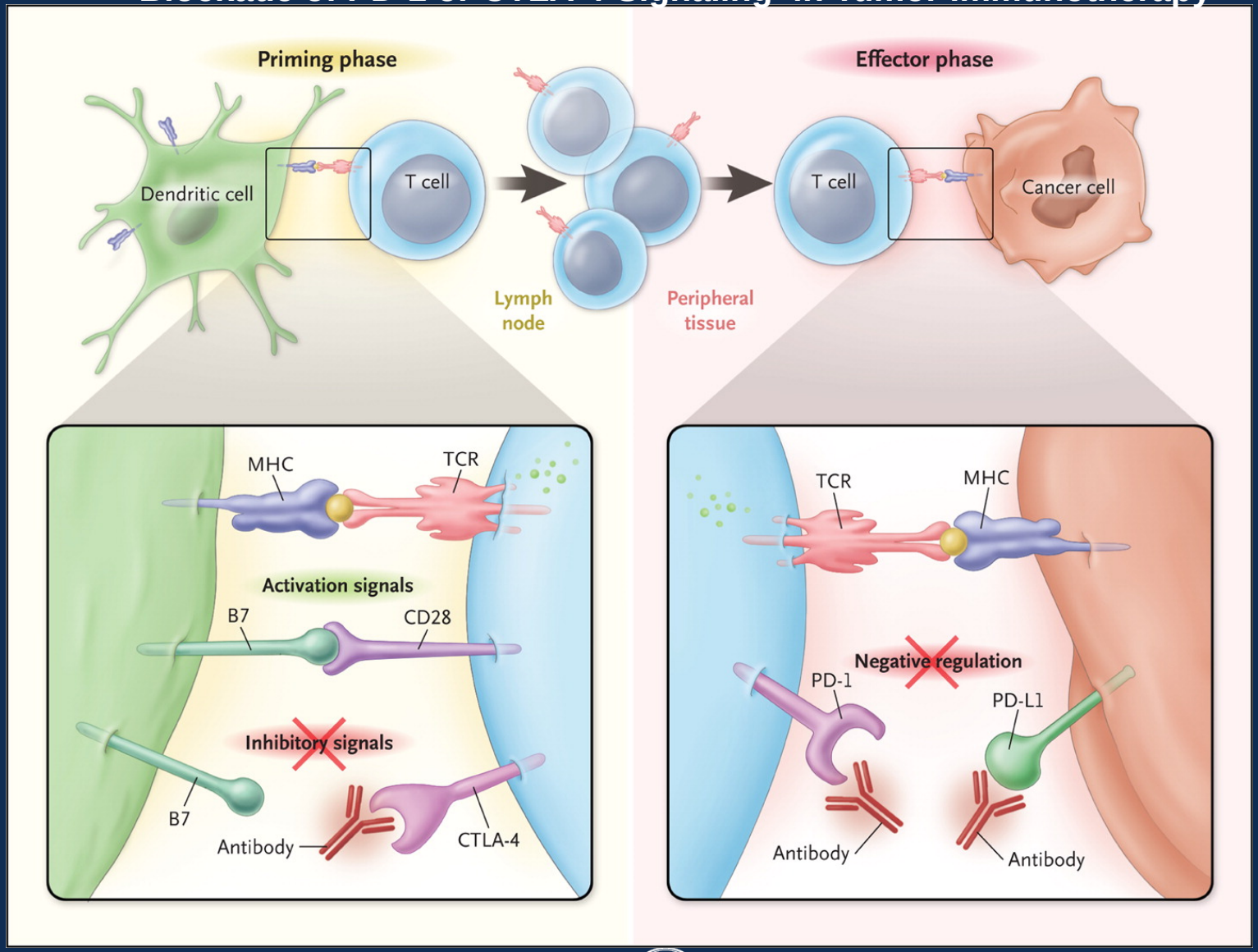
Learning Objectives

- Discuss immune checkpoints and their ramifications in human cancers
- Evaluate the presence of PD-L1 expression in a large breast cancer population
- Identify immune and molecular pathway associations with PD-L1
- Review preliminary validation data

Introduction – Immune Checkpoints

- Immune checkpoints regulate the duration and level the T-cell response
  - Cytotoxic T-lymphocyte Antigen-4 (CTLA-4) functions as an “off” switch to T-cell activity in the priming phase
  - Programmed Death (PD-1) regulates T-cell activity during the effector phase and can shut down antigen-specific T cells in the tumor micro-environment
    - Tumor cells can block the immune response via the PD-1 checkpoint by expressing programmed death ligands (PD-L1) and inactivating T-cells

Blockade of PD-1 or CTLA-4 Signaling in Tumor Immunotherapy



Ribas A. N Engl J Med 2012;366:2517-2519.

The NEW ENGLAND JOURNAL of MEDICINE

Presented by: Barbara A. Pockaj, MD

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Introduction – Immune Mechanisms

- IDO1 indoleamine 2,3-dioxygenase 1 (IDO-1) – catalyzes the first and rate-limiting step in tryptophan catabolism
  - Important to immune tolerance and immunosuppression
  - IDO-1 inhibitors are available
    - Current trials are now underway

Clinical Application of Immunotherapy

- Several drugs have been developed which block the CTLA-4 and PD1 Immune Checkpoints
  - Anti-CTLA-4
    - Ipilumunab
    - Tremelimumab
  - Anti-PD1
    - Nivolumab (BMS936558/MDX-1106)
    - Lambrolizumab (MK-3475)
  - Anti-PD-L1
    - BMS-936559, MDX-1105
    - MPDL3280A/RG7446
    - MEDI4736
    - AMP-224
    - Pidilizumab (CT-011)

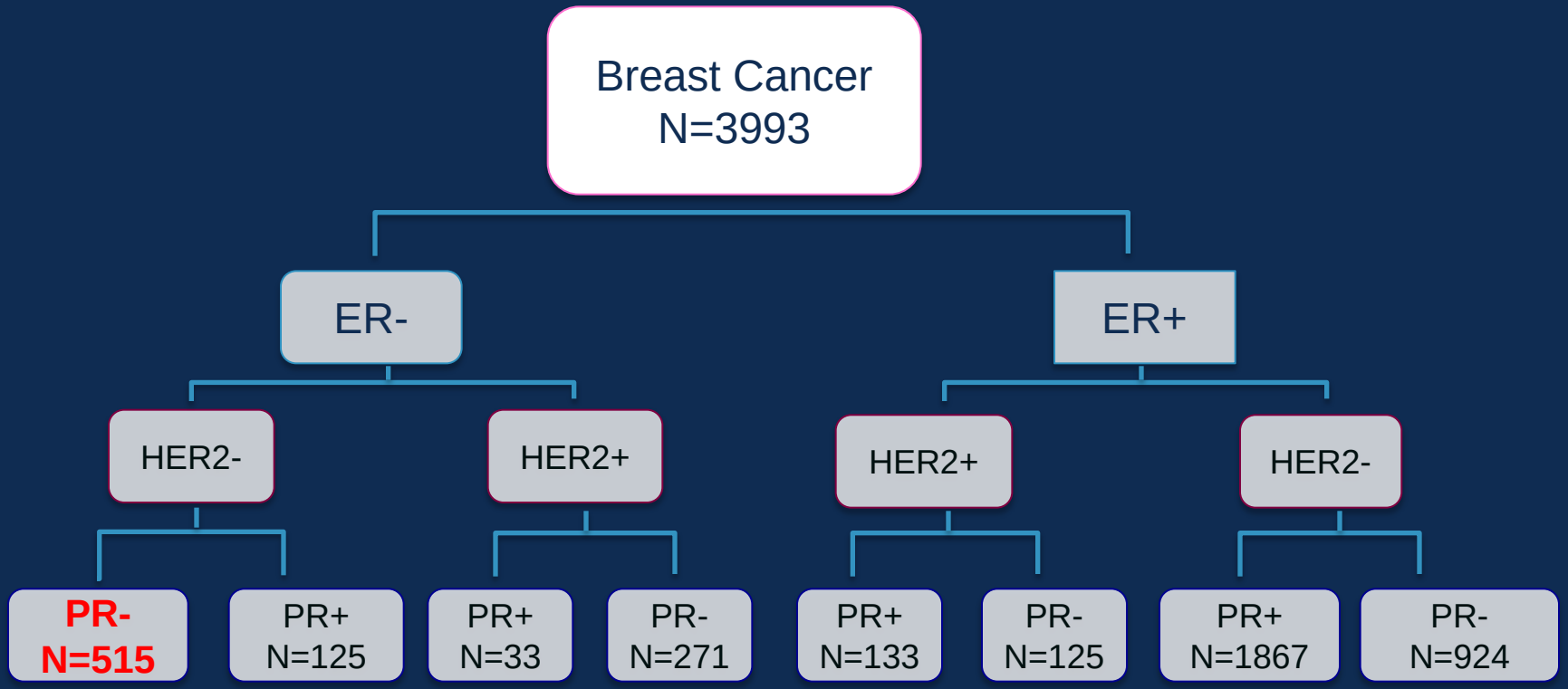
Clinical Trials

Trial	Drug	Tumor Type	Response Rate	Immune Correlates
Topalian, NEJM, 2012	Nivolumab (BMS-936558) Anti-PD-1	Melanoma, NSCLC, Renal Cell, Colorectal, Prostate	18% NSCLC 28% Melanoma 27% Renal	Response related to PD-L1 tumor expression 36% Response PD-L1+ 0% Response PD-L1-
Wolchok, NEJM, 2013	Nivolumab + Ipilimumab Anti-PD-1 and Anti-CTLA-4	Melanoma	40% Concurrent Therapy 20% Sequential Therapy	PD-L1 Expression did not correlate to response for concurrent therapy but did for sequential therapy
Daud, AACR, 2014	MK-3475 Anti-PD-L1	Melanoma	ORR 41%	ORR correlated with PD-L1 expression 52% ORR PD-L1+ 6% PD-L1-

Methods

- 3993 formalin fixed, paraffin embedded breast cancer samples (Caris Life Sciences)
- Gene expression was performed using Illumina HumanHT-12 v4 BeadChip
- The Comprehensive R Archive Network was used for statistical computing and graphics
- The study was IRB approved

Human Ref-12 Expression Array
Transcripts 29,526
Genes 18,401
Probe Beads ~1,000,000
Probe Beads/Transcript ~41
Control Probes~850


Breast Cancer
N=3993
ER-
ER+
HER2-
HER2+
HER2+
HER2-
PR-
N=515
PR+
N=125
PR+
N=33
PR-
N=271
PR+
N=133
PR-
N=125
PR+
N=1867
PR-
N=924

Methods

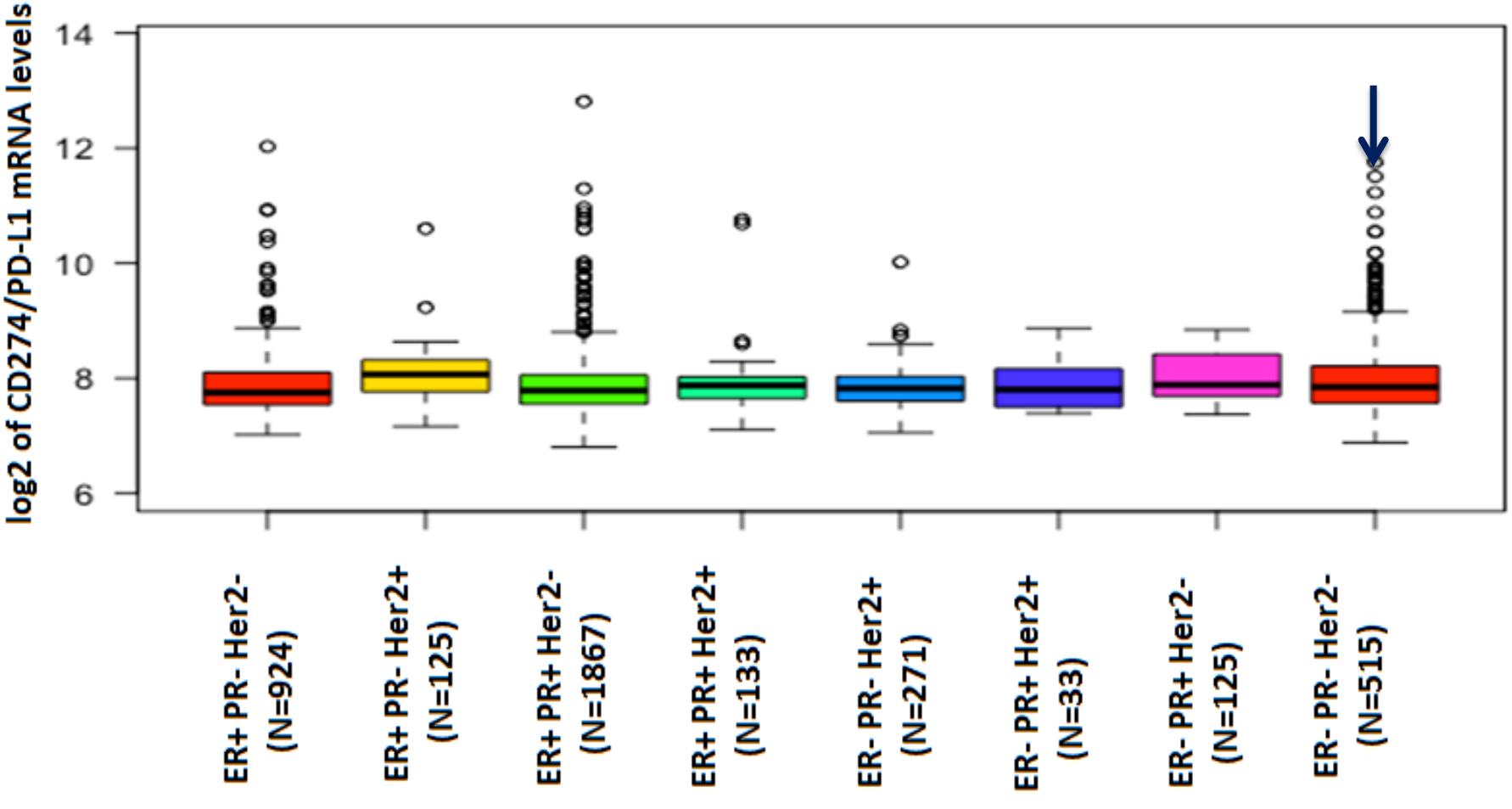
- Validation:
  - Immunohistochemistry (Caris Life Sciences)
    - Slides were stained using an automated system (Ventana Medical Systems, Tucson, AZ) as per manufacturer's protocol with proprietary reagents.
    - IHC stained slides were scored by pathologists.
      - Tumor staining was scored for all markers except for PD1 which was scored in the tumor infiltrating lymphocytes.
  - BRCA 1 somatic mutation testing was performed by Next Gen Sequencing (Illumina Miseq platform)
    - Sequencing plots were read by board certified geneticists.

Methods

- Validation
  - 18 TNBC cases analyzed at Mayo Clinic in Arizona using array-based comparative genomic hybridization (aCGH) to evaluate genomic amplifications and deletion

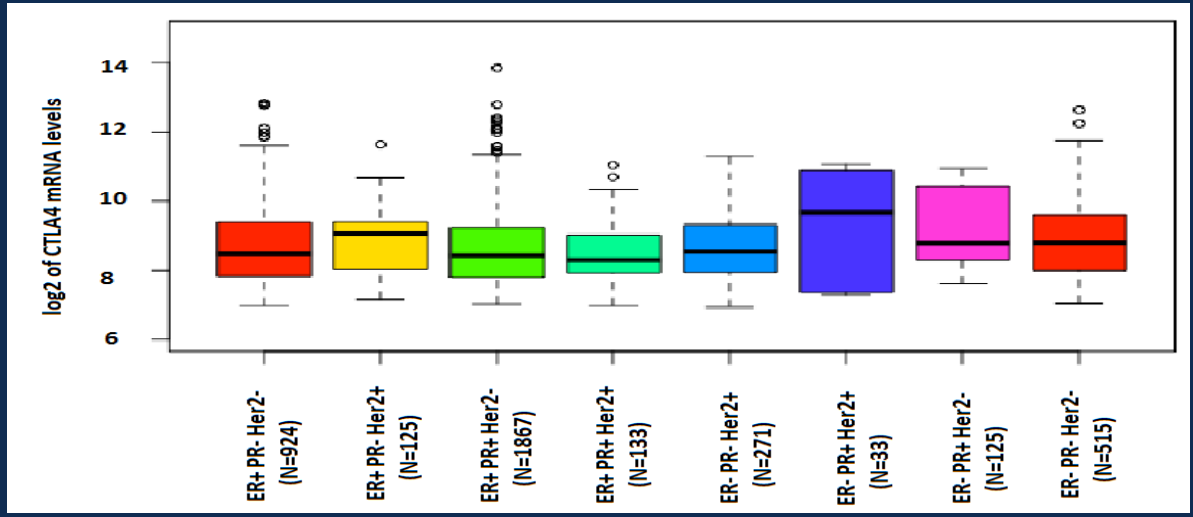
RESULTS

PD-L1 Levels

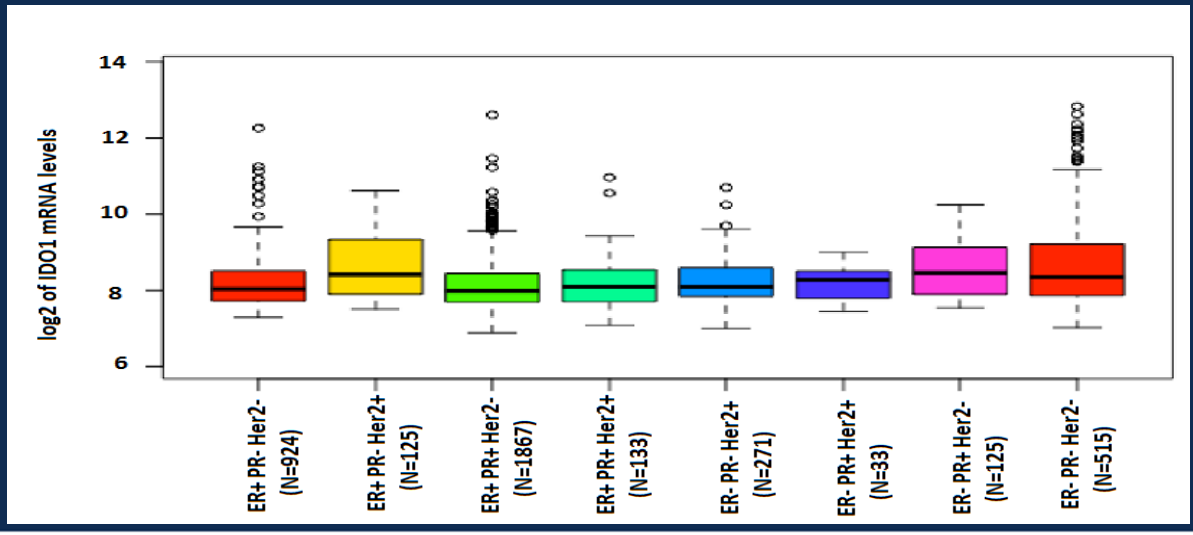


Data was normalized by doing mean normalization (using mean value from control normal breast tissue)

CTLA-4 and IDO1 Levels



CTLA-4



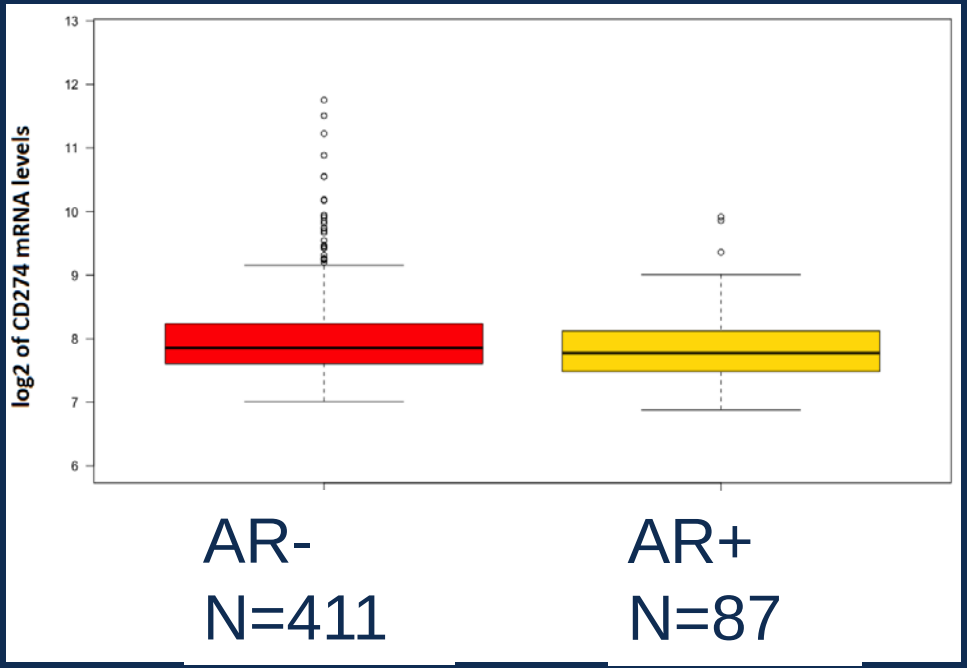
IDO1

PD-L1 Associations

- Spearman correlation test
  - Positive correlation with immune regulators:
    - CTLA-4 correlation coefficient 0.528
    - IDO1 correlation coefficient 0.481
  - Mixed results with the Phosphatidylinositol 3-kinase (PI3-kinase) Pathway
    - PIK3CA correlation coefficient 0.39
    - PTEN correlation coefficient 0.11

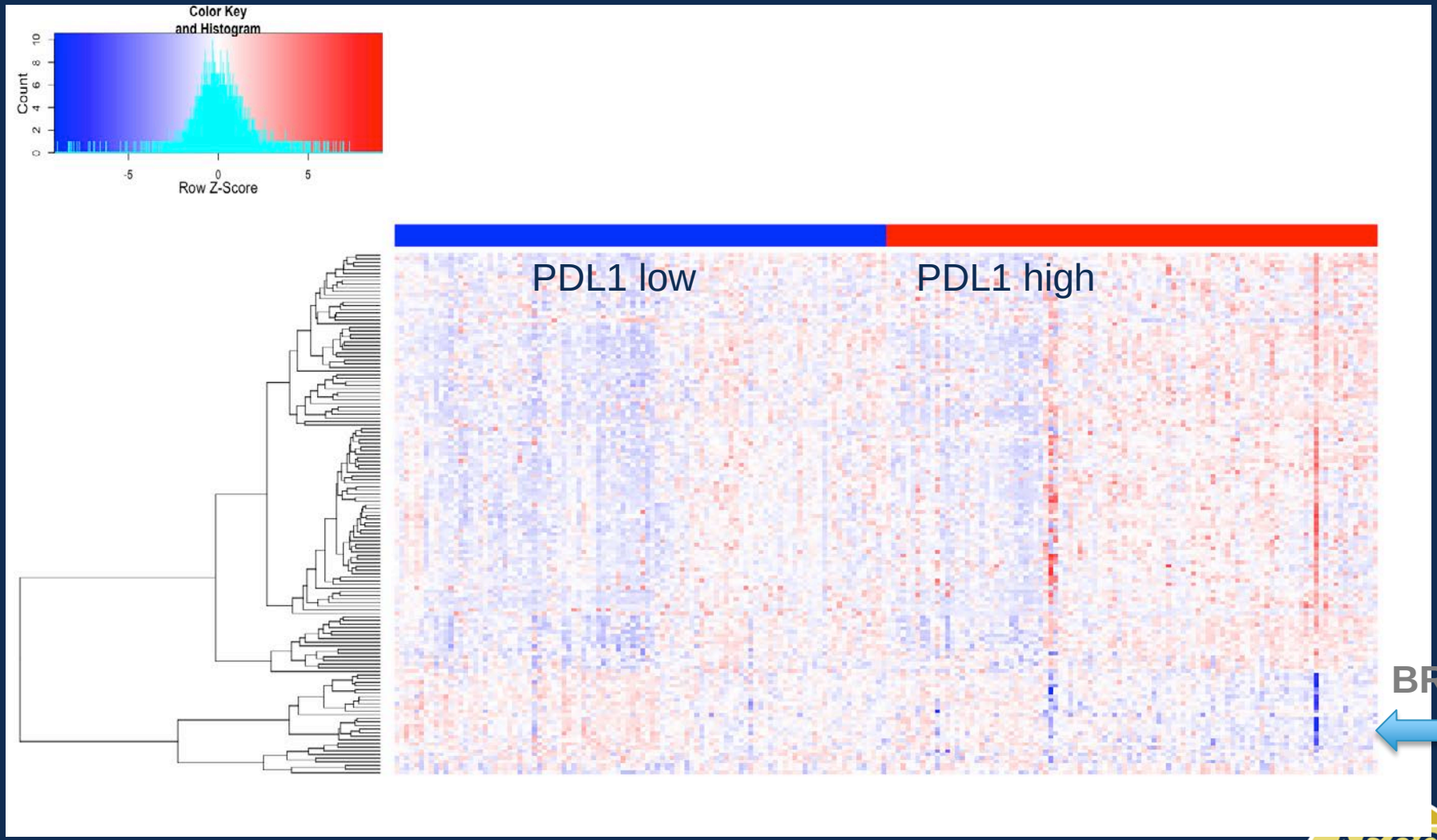
AR Expression and PD-L1

- Anova p value=.05
- Suggests that there is a relationship between AR expression and PDL1 expression
  - AR- higher likelihood of expressing
- Similar finding with IDO1 and CTLA-4



Differential expression of 144 genes based on T test between the high and low PD-L1 expressers:

- 4 distinct clusters noted in the PD-L1 low vs high population



Heatmap Analsis

- WebGestalt a "WEB-based GEne SeT AnaLysis Toolkit“ was used to do enrichment analysis of the heatmap data
  - DNA repair genes were significant (adjusted p value=0.02)
    - BRCA1
    - Fanconi anemia, complementation goup A
    - HUS1 checkpoint homolog (S. Pombe)

Validation

- 36 TNBC patients were profiled for PD1, PD-L1, AR, BRCA1 mutation.
  - PD-L1 expression 10 patients (28%)
  - PD-1 expression is present in 22 patients (61%)
  - Co-expression of PD-1 and PD-L1 was found in 7/10 pateints (70%)

Validation

- AR expression found in 9 (25%) patients
  - Only 1 patient (11%) to be PD-L1+
- 33% of AR- TNBC were PD-L1+
- 90% PD-L1+ were AR-

- 3/3 BRCA1 mutated patients were PD-L1+

- 4/4 BRCA1+ (Mayo Samples) were PD-L1+

PI-3 Kinase Pathway

- Loss of PTEN expression was present in 19 patients (54%)
  - Only 4 of these patients were PD-L1+ (21%)
- PI3K mutation was present in 5 patients (14%)
  - Only 1 patient (20%) was PD-L1+

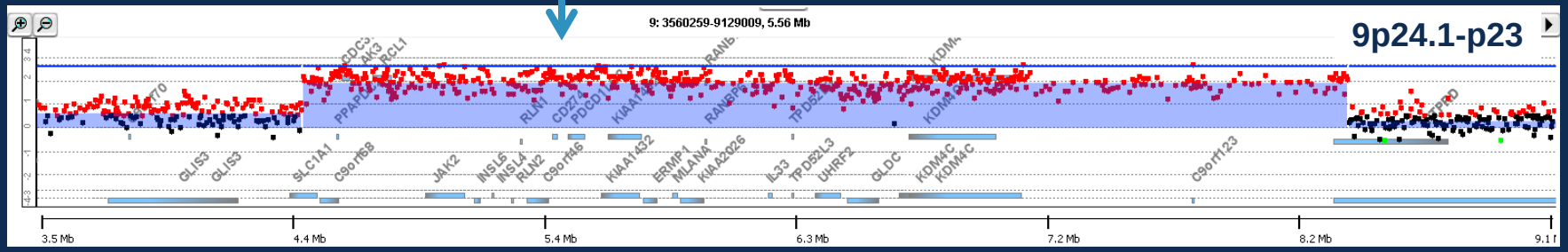
Validation

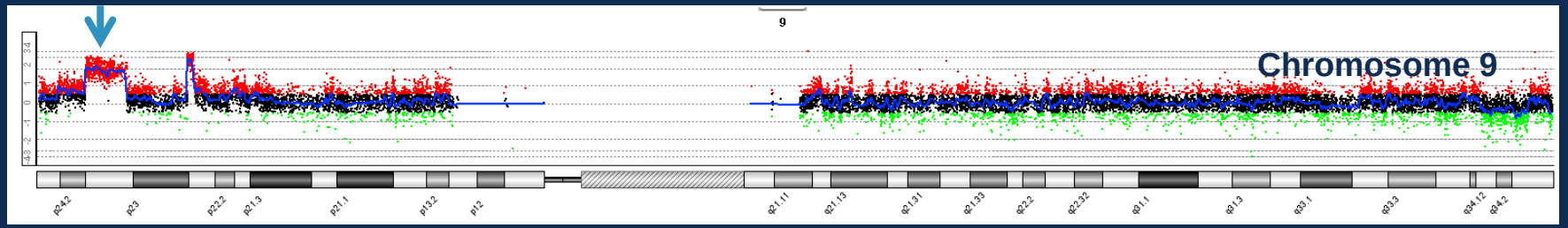
- cGH revealed over-expression of PD-L1 in 3/18 patients (17%)
  - All patients were AR- 3/11 (27%)
    - Mixed PI-3 Kinase pathway changes

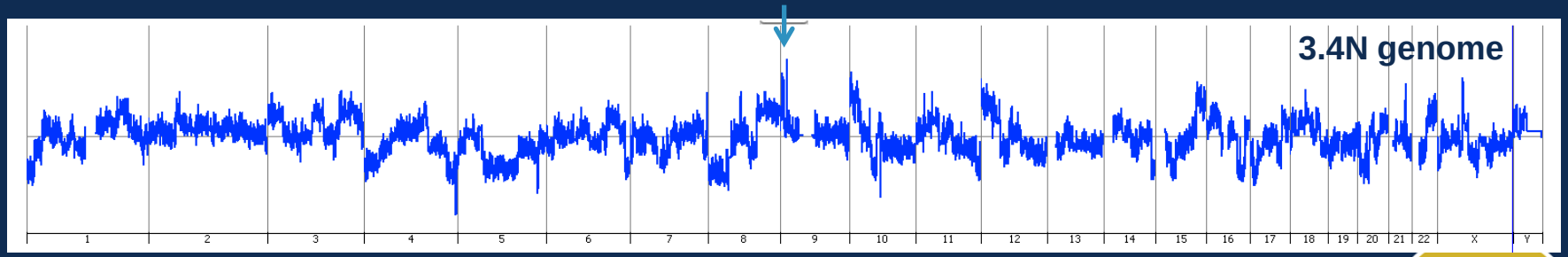
Validation

CD274 (PD-L1)

Data provided by Michael Barrett, PhD







Conclusions

- A subset of TNBC patients express immune regulatory targets suggesting immunotherapy may be an effective option
  - PD-L1+ appears to be associated with
    - AR- TNBC
    - BRCA1 mutated TNBC
- High expression of PD-L1 in BRCA1 deficient as well as BRCA1 mutated patients indicate that anti PD-1/PD-L1 therapy in combination with platinum salts and/or PARP inhibitors may be a synergistic treatment strategy that warrants further study.

Conclusions

- Consistent association with the PI-3 Kinase pathway are not found
- Further validation of findings are ongoing
  - Does PD-L1 overexpression by cGH lead to PD-L1 expression as seen by IHC?
  - Is PD-L1 expression correlated with BRCA1 mutation?
    - Would BRCA1 mutated patients benefit from immunotherapy?

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– 6,400 referring physicians
– All 50 states
– 30 countries

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– Pathologists and PAs
–Molecular Geneticists
– Consulting Medical Oncologists
– scientists
-- Entire lab staff

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