Overview of Immune Checkpoint Inhibitors for Breast Cancer Treatment: Beyond PD-1 Blockade

Sang-Jun Ha

Department of Biochemistry
College of Life Science and Biotechnology
Yonsei University
Journey from targeted therapy to immunotherapy for cancers

Only tumor cells  
Tumor microenvironment
Question

Why cannot our immune system treat efficiently chronic infection and cancer?
Repeated TCR stimulation by antigen persistence → T cell exhaustion
“Driving” an Immune Response

**Starter**

TCR: antigen-MHC

Signal 1

**Accelerator**

CD28: B7

Signal 2

**Brake**

CTLA4: B7
PD-1: PD-L1

Blockade of Signal 2
Taking off the brakes!!!
Parallel timelines and milestones of αCTLA4 & αPD-1 development

CTLA-4
- Cloning of gene encoding CTLA-4
- Demonstration that B7-1 and B7-2 are CTLA-4 ligands
- Demonstration of potent immunoregulatory role of CTLA-4 in mice
- Treatment of mouse tumors with anti-CTLA-4
- First demonstration of clinical activity of anti-CTLA-4 in melanoma
- Anti-CTLA-4 confers survival benefit in melanoma
- FDA approval for anti-CTLA-4 in melanoma

PD-1
- Cloning of gene encoding PD-1
- Demonstration of organ-specific autointolerance in PD-1-deficient mice
- PD-L1 expression in mouse tumors confers immune resistance and high PD-L1 expression in human tumors
- PD-L1 and PD-L2 identified as PD-1 ligands
- Anti-PD-1 into patients
- First demonstration of clinical activity of anti-PD-1
- Anti-PD-1 and anti-PD-L1 induce durable tumor responses in melanoma, kidney and lung cancer

Pardoll DM. *Nat Immunol* 2012 13:1129
Expression of immune checkpoint ligands on tumor cells and immune cells is required to trigger immune checkpoint receptor signaling
Checkpoints in the cancer-immunity cycle

1. Release of cancer cell antigens
   - Stimulatory factors
   - Inhibitors
   - Immunogenic cell death
   - Tolerogenic cell death

2. Cancer antigen presentation
   - TNF-α
   - IL-1
   - IFN-α
   - CD40L/CD40
   - CDN
   - ATP
   - HMGB1
   - TLR
   - IL-10
   - IL-4
   - IL-13

3. Priming and activation
   - CD28/B7.1
   - CD137/CD137L
   - OX40/OX40L
   - CD27/CD70
   - HVEM
   - GITR
   - IL-2
   - IL-12
   - CTLA4/B7.1
   - PD-L1/PD-1
   - PD-L1/B7.1
   - Prostaglandins

4. Trafficking of T cells to tumors
   - CX3CL1
   - CXCL9
   - CXCL10
   - CCL5

5. Infiltration of T cells into tumors
   - LFA1/ICAM1
   - Selectins
   - VEGF
   - Endothelin B receptor

6. Recognition of cancer cells by T cells
   - T cell receptor
   - Reduced pMHC on cancer cells

7. Killing of cancer cells
   - IFN-γ
   - T cell granule content
   - PD-L1/PD-1
   - LAG-3
   - PD-L1/B7.1
   - Arginase
   - IDO
   - MICA/MICB
   - TGF-β
   - B7-H4
   - BTLA
   - TIM-3/phospholipids

Chen DS & Mellman I. *Immunity* 2013 39:1
CTLA-4/B7-1 and PD-1/PD-L1 checkpoint blockade for cancer treatment

Priming phase (lymph node)

Effector phase (peripheral tissue)

T-cell migration

Adapted from Ribas A. *N Engl J Med* 2012 366:2517
PD-1 blockade, when and where?

Window when PD-1/L1 blockade is effective for cancer

T cell activation stage
- Mature APC
- Naïve CD8+ T cell

T cell effector stage
- Mature APC
- Activated CD8+ T cell

T cell “exhaustion” stage
- Activated CD8+ T cell
- Cell division (multiple rounds)
- Exhausted CD8+ T cell

T cell migration

Clouthier DL & Ohashi PS. Science 2017 355:1373
Why do we need more checkpoint blockers?

Pembrolizumab monotherapy in 20 tumor types (Merck)

Expanding scope of checkpoint inhibitors

**FDA-APPROVED AS OF AUGUST 1, 2016:**

- Hodgkin lymphoma: nivolumab (Opdivo)
- Lung cancer: nivolumab (Opdivo) and pembrolizumab (Keytruda)
- Kidney cancer: nivolumab (Opdivo)
- Bladder cancer: atezolizumab (Tecentriq)
- Melanoma: ipilimumab (Yervoy), nivolumab (Opdivo), pembrolizumab (Keytruda), and combination of ipilimumab and nivolumab

**FDA-APPROVED AS OF JULY 31, 2017:**

- Head and neck cancer: nivolumab (Opdivo) and pembrolizumab (Keytruda)
- Solid tumors that are microsatellite instability-high or mismatch repair-deficient: pembrolizumab (Keytruda)
- Hodgkin lymphoma: nivolumab (Opdivo) and pembrolizumab (Keytruda)
- Lung cancer: nivolumab (Opdivo), pembrolizumab (Keytruda), and atezolizumab (Tecentriq)
- Kidney cancer: nivolumab (Opdivo)
- Bladder cancer: atezolizumab (Tecentriq), avelumab (Bavencio), durvalumab (Imfinzi), nivolumab (Opdivo), and pembrolizumab (Keytruda)
- Melanoma: ipilimumab (Yervoy), nivolumab (Opdivo), pembrolizumab (Keytruda), and combination of ipilimumab and nivolumab
- Merkel cell carcinoma: avelumab (Bavencio)
### List of PD-1/PD-L1 blockers and most advanced trial phase

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>anti-PD-L1</th>
<th>anti-PD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Atezolizumab (Tecentriq®)</td>
<td>Avelumab (Bavencio®)</td>
</tr>
<tr>
<td><strong>Roche</strong></td>
<td>Engineered human IgG1</td>
<td>Fully human IgG1</td>
</tr>
<tr>
<td><strong>Merck-Pfizer</strong></td>
<td>Approved</td>
<td>Approved</td>
</tr>
<tr>
<td><strong>AstraZeneca</strong></td>
<td>Approved</td>
<td>Approved</td>
</tr>
<tr>
<td><strong>Merck</strong></td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td><strong>BMS</strong></td>
<td>II</td>
<td>II</td>
</tr>
</tbody>
</table>

- **AML**
- **B cell HL**
- **B cell NHL**
- **Bladder**
- **Breast**
- **CLL**
- **CML**
- **Colorectal**
- **Gastric**
- **GBM**
- **HCC**
- **Head&Neck**
- **Merkel cell carcinoma**
- **Melanoma**
- **Multiple Myeloma**
- **NSCLC**
- **Ovarian**
- **Pancreatic**
- **Prostate**
- **RCC**
Clinical data on the use of immune checkpoint inhibitors for patients with breast cancer
Breast cancer subtype

**All Cases**

**Non-luminal**
- **HR- (ER- PR-)**
  - **HR-/HER2-**
    - "Triple Negative"
    - 13%
  - **HR-/HER2+**
    - (Least Common)
    - 5%

**Luminal**
- **HR+ (ER+ or PR+)**
  - **HR+/HER2+**
    - "Triple Positive"
    - 10%
  - **HR+/HER2-**
    - (Most Common)
    - 73%
One of limitation of cancer immunotherapy: Cold tumor

**IMMUNE INFLAMED**
CD8+ T cells infiltrated, but non-functional
Accelerate or remove brakes on T-cell response
- e.g. aPD-1, aCTLA-4, aTIM-3, aLAG-3, aTIGIT, aCSF-1R, IDOi, aOX40, TCBs

**IMMUNE EXCLUDED**
CD8+ T cells accumulated, but have not efficiently infiltrated
Bring T cells in contact with cancer cells
- e.g. aVEGF, TCBs

**IMMUNE DESERT**
CD8+ T cells absent from tumor and periphery
Increase number of Ag-specific T cells or antigen presentation
Chemotherapy, radiotherapy, targeted therapy, aCD40, TCBs
Characteristics of breast cancer for immunotherapy

- T cell infiltration: relatively low (but relatively high in TNBC & HER2+)
- Nonsynonymous mutation: low (but TNBC > non-TNBC)
- PD-L1 expression: modest & variable (modest & variable) on tumor cells (but high on myeloid cells in the stroma of TNBC)
- Clinical trials using immunotherapies: ~ 70 ongoing

Updated from Pusztai L et al. *Clin Cancer Res* 2015 22:2105
### Summary of status of breast cancer immunotherapy

<table>
<thead>
<tr>
<th>Modality</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Approved?</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTLA-4</td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Minimal activity</td>
</tr>
<tr>
<td>PD-1/PD-L1</td>
<td>![arrow]</td>
<td>![arrow]</td>
<td></td>
<td>No</td>
<td>ORR &lt;20% in TNBC; combinations being tested</td>
</tr>
<tr>
<td>Therapeutic vaccines</td>
<td>![arrow]</td>
<td>![X]</td>
<td></td>
<td>No</td>
<td>Negative randomized studies; combinations critical</td>
</tr>
<tr>
<td>Prevention vaccines</td>
<td>![arrow]</td>
<td></td>
<td></td>
<td>No</td>
<td>First-in-human studies underway</td>
</tr>
<tr>
<td>T-cell agonists</td>
<td>![arrow]</td>
<td></td>
<td></td>
<td>No</td>
<td>Unlikely to offer single-agent activity in breast cancer</td>
</tr>
<tr>
<td>Adoptive T cells</td>
<td>![arrow]</td>
<td></td>
<td></td>
<td>No</td>
<td>Initial CAR T-cell studies underway</td>
</tr>
</tbody>
</table>

### Ongoing clinical trials with immunotherapies in breast cancer

<table>
<thead>
<tr>
<th>Phase</th>
<th>ClinicalTrials.gov ID</th>
<th>Disease setting</th>
<th>Type of disease</th>
<th>Breast cancer subtype</th>
<th>Immunotherapies</th>
<th>Combined treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NCT02303366</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>All</td>
<td>Pembrolizumab</td>
<td>Stereotactic ablation radiosurgery</td>
</tr>
<tr>
<td>I</td>
<td>NCT02605915</td>
<td>Metastatic and neoadjuvant</td>
<td>Only BC</td>
<td>HER2+</td>
<td>Atezolizumab</td>
<td>Trastuzumab/pertuzumab or T-DM1 or trastuzumab/pertuzumab/carboplatin/docetaxel</td>
</tr>
<tr>
<td>I</td>
<td>NCT02649686</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>HER2+</td>
<td>Durvalumab</td>
<td>Trastuzumab</td>
</tr>
<tr>
<td>I/I</td>
<td>NCT02129556</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>HER2+</td>
<td>Pembrolizumab</td>
<td>Trastuzumab</td>
</tr>
<tr>
<td>I/I</td>
<td>NCT02513472</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Pembrolizumab</td>
<td>Erbulin mesylate</td>
</tr>
<tr>
<td>I/I</td>
<td>NCT02628132</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Durvalumab</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td>II</td>
<td>NCT02411656</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC or ER+/HER2−</td>
<td>Pembrolizumab</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td>II</td>
<td>NCT02447003</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Pembrolizumab</td>
<td>Doxorubicin (low dose), cyclophosphamide, metronomic, radiotherapy, or cisplatin</td>
</tr>
<tr>
<td>II</td>
<td>NCT02499367</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Pembrolizumab</td>
<td>Metronomic, Erbitux</td>
</tr>
<tr>
<td>II</td>
<td>NCT02411656</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>HER2−</td>
<td>Pembrolizumab</td>
<td>Vorinostat and tamoxifen</td>
</tr>
<tr>
<td>II</td>
<td>NCT02447003</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Pembrolizumab</td>
<td>Vorinostat and tamoxifen</td>
</tr>
<tr>
<td>II</td>
<td>NCT02395627</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>HR+ (endocrine-resistant BC)</td>
<td>Pembrolizumab</td>
<td>Vorinostat and tamoxifen</td>
</tr>
<tr>
<td>II</td>
<td>NCT02536794</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC ER+/HER2−</td>
<td>Durvalumab and tremelimumab</td>
<td>Brain radiotherapy or stereotactic</td>
</tr>
<tr>
<td>II</td>
<td>NCT02563925</td>
<td>Metastatic (brain)</td>
<td>Only BC</td>
<td>All</td>
<td>Tremelimumab</td>
<td>Brain radiotherapy or stereotactic</td>
</tr>
<tr>
<td>II</td>
<td>NCT00083278</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>All</td>
<td>Ipilimumab</td>
<td>Brain radiotherapy or stereotactic</td>
</tr>
<tr>
<td>II</td>
<td>NCT02648477</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC and ER+/HER2−</td>
<td>Pembrolizumab</td>
<td>Brain radiotherapy or stereotactic</td>
</tr>
<tr>
<td>III</td>
<td>NCT02555567</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Pembrolizumab</td>
<td>Nab-paclitaxel</td>
</tr>
<tr>
<td>III</td>
<td>NCT02425891</td>
<td>Metastatic</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Atezolizumab</td>
<td>Nab-paclitaxel → AC or nab-paclitaxel/carboplatin → AC</td>
</tr>
<tr>
<td>I</td>
<td>NCT02622074</td>
<td>Neoadjuvant</td>
<td>Only BC</td>
<td>TNBC (LABC)</td>
<td>Pembrolizumab</td>
<td>Nab-paclitaxel → ddAC</td>
</tr>
<tr>
<td>I/I</td>
<td>NCT02489448</td>
<td>Neoadjuvant</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Durvalumab</td>
<td>Nab-paclitaxel → ddAC</td>
</tr>
<tr>
<td>II</td>
<td>NCT01042379</td>
<td>Neoadjuvant</td>
<td>Only BC</td>
<td>All</td>
<td>Pembrolizumab</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td>II</td>
<td>NCT02530489</td>
<td>Neoadjuvant</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Atezolizumab</td>
<td>Nab-paclitaxel</td>
</tr>
<tr>
<td>II</td>
<td>NCT02620280</td>
<td>Neoadjuvant</td>
<td>Only BC</td>
<td>TNBC</td>
<td>Atezolizumab</td>
<td>Nab-paclitaxel/carboplatin</td>
</tr>
<tr>
<td>II</td>
<td>NCT01502592</td>
<td>Presurgical</td>
<td>Only BC</td>
<td>All</td>
<td>Ipilimumab</td>
<td>Cryoablation</td>
</tr>
</tbody>
</table>

Polk A et al. *Cancer Treat Rev* 2018 63:122
- Disease setting: Metastatic and/or neoadjuvant
- Breast cancer subtype: TNBC or HER2+
- Immunotherapies: aPD-1, aPD-L1, aCTLA4, IDO inhibitor, aCSF1R, CD27 agonist, or Poly-ICLC
- Combined treatments: targeted agent, chemotherapy, or radiotherapy
Synergistic stimulation by cytotoxic agent & IC inhibitor

Belvin MB & Mellman I. *Sci Transl Med* 2015 7:315fs48

Cold to hot immunologic conversion

Cold tumor → Vaccine → New T cells → Hot tumor

Cold tumor

Checkpoint blockade

PD-1
PD-L1
CTLA-4

No response

Checkpoint blockade

Hot tumor

Adjuvant

Cytokines
CD40
STING
TLR ligands
Other

Classic vaccine
Radiation
Chemotherapy
Antitumor Ab
Targeted therapy
Hormone therapy
Gene therapy

Response

KEYNOTE-086 (Cohort A): Pembrolizumab in pts with mTNBC and ≥ 1 systemic therapy

- International, multicohort phase II study

**Cohort A:**
Pts with PD after ≥ 1 prior systemic tx for mTNBC; tumor biopsy available for PD-L1 evaluation

**Cohort B:**
Pts with previously untreated, PD-L1-positive mTNBC

Pembrolizumab 200 mg IV Q3W
(N_{cohort 1} = 170; N_{cohort 2} = 52)

Tx 2 yrs or until PD, unacceptable toxicity, consent withdrawal, or investigator decision

**Endpoints**
- Primary: ORR in overall and PD-L1+ pts; safety
- Secondary: DoR, DCR, PFS, OS in overall and PD-L1+ pts

**Assessments**
- Imaging: every 9 wks for 1 yr, then every 12
- Response: RECIST v1.1 by ICR
- PD-L1 positive: CPS ≥ 1% by IHC at central lab

KEYNOTE-086: Best overall response

<table>
<thead>
<tr>
<th>Response</th>
<th>Cohort A: ≥ 1 Previous Tx*[1]</th>
<th>Cohort B: Untreated (N = 52)*[2]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Pts (N = 170†)</td>
<td></td>
</tr>
<tr>
<td><strong>ORR, % (95% CI)</strong></td>
<td>4.7 (2.3-9.2)</td>
<td>23 (14-36)</td>
</tr>
<tr>
<td><strong>DCR, ‡ % (95% CI)</strong></td>
<td>7.6 (4.4-12.7)</td>
<td>23 (14-36)</td>
</tr>
<tr>
<td>Best overall response, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ CR</td>
<td>0.6</td>
<td>4</td>
</tr>
<tr>
<td>▪ PR</td>
<td>4.1</td>
<td>19</td>
</tr>
<tr>
<td>▪ SD</td>
<td>20.6</td>
<td>17</td>
</tr>
<tr>
<td>▪ PD</td>
<td>60.6</td>
<td>58</td>
</tr>
<tr>
<td>Median TTR, mos (range)</td>
<td>3.0 (1.9-8.1)</td>
<td>2.0 (1.9-4.1)</td>
</tr>
<tr>
<td>Median DoR, mos (range)</td>
<td>6.3 (1.2+ to 10.3+)</td>
<td>8.4 (2.1+ to 8.4)</td>
</tr>
</tbody>
</table>

*Median follow-up: 10.9 mos for cohort A; 7.0 mos for cohort B.
†n = 1 pt with unknown PD-L1 status. ‡DCR = SD ≥ 24 wks + CR + PR.

Numerically lower ORR in poor prognosis pt subgroups in cohort A (not assessed in Cohort B): LDH > ULN, ≥ 3 metastatic organ sites, liver metastases, visceral disease
Atezolizumab + nab-paclitaxel in mTNBC: phase Ib study design

- GP28328: a multicenter, multicohort phase Ib study; arm F includes pts with TNBC (metastatic or unresectable, locally advanced)\(^1,2\)

\textbf{Atezolizumab} (800 mg) + \textbf{nab-paclitaxel} (125 mg/m\(^2\)), as long as clinical benefit received; 
\textit{Nab-paclitaxel for at least 4 cycles, unless PD or unacceptable AE; if discontinued, atezolizumab as monotherapy}

- Pts with TNBC ≤ 2 previous lines of systemic chemo; ECOG PS 0/1; no CNS cancer or untreated/active CNS mets; available tumor sample

- Primary endpoint: safety and tolerability
- Secondary endpoints: response per RECIST v1.1 (ORR, DoR, PFS) and immune-modified response criteria; pharmacokinetics, biomarker analyses

**Atezolizumab + nab-paclitaxel in mTNBC: Efficacy (secondary endpoints)**

<table>
<thead>
<tr>
<th>Best Overall Response (RECIST v1.1)</th>
<th>First Line (n = 13)</th>
<th>Second Line (n = 9)</th>
<th>Third Line+ (n = 10)</th>
<th>All (N = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed ORR, % (95% CI)</td>
<td>46 (19-75)</td>
<td>22 (3-60)</td>
<td>40 (12-74)</td>
<td>38 (21-56)</td>
</tr>
<tr>
<td>CR, %</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>PR, %</td>
<td>38</td>
<td>22</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>SD, %</td>
<td>38</td>
<td>67</td>
<td>30</td>
<td>44</td>
</tr>
<tr>
<td>PD, %</td>
<td>15</td>
<td>0</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Missing or not estimable, %</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Median DoR, mos (range)</td>
<td>NE (2.9 to 11.5+)</td>
<td>NE (9.1 to 13.1+)</td>
<td>NE (1.9+ to 5.6+)</td>
<td></td>
</tr>
</tbody>
</table>

- Among 12 responders, 6 (50%) remain on atezolizumab; 1 for > 17 mos
- Median DoR not reached; PFS and OS data not yet mature
- Responses observed in pts regardless of PD-L1 expression level
- Trend toward increase in baseline TILs for responding pts

Phase II TONIC: nivolumab after induction radiotherapy or low-dose chemo in mTNBC

• Adaptive, nonrandomized, noncomparative trial

Primary endpoint: PFS

Secondary endpoints: ORR, CBR, safety

Pts with mTNBC, ≤ 3 lines of palliative chemotherapy (Planned N = 84)

Induction Treatment

8 Gy irradiation of 1 metastatic lesion
3 cycles over 2 wks

Doxorubicin 15 mg/wk
one 2-wk cycle

Cyclophosphamide PO 50 mg/day
2 wks

Cisplatin 40 mg/m²
two 2-wk cycles

No Induction

Nivolumab 3 mg/kg
Q2W until PD

Nivolumab after induction radiotherapy or low-dose chemo in mTNBC (TONIC): Results

- Interim results at median follow-up of 10.8 mos (N = 50 evaluable)
- **ORR for entire cohort: 22%** (24% per iRECIST)
  - CR: 1 (2%), PR: 11 (22%), SD > 24 wks: 1 (2%)
  - Median DoR: 9 mos (95% CI: 5.5-NR)
- Preliminary analyses suggest higher responses in subgroups
  - Doxorubicin or cisplatin induction
  - Higher leukocyte infiltration and CD8+ T-cell counts in tumor biopsies
- **Median PFS: 3.4 months**

# Ongoing trials of PD-1/PD-L1 inhibitors in mTNBC

<table>
<thead>
<tr>
<th>Phase III Trial</th>
<th>Population</th>
<th>Investigational</th>
<th>Comparator</th>
<th>Primary Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>KEYNOTE-119</td>
<td>TNBC after 1-2 prior systemic tx for MBC</td>
<td>Pembrolizumab</td>
<td>Physician’s choice Single-agent chemo</td>
<td>OS</td>
</tr>
<tr>
<td>KEYNOTE-355</td>
<td>TNBC with no previous chemo for MBC</td>
<td>Pembrolizumab + chemo</td>
<td>Placebo + chemo</td>
<td>Part 1: safety Part 2: PFS, OS</td>
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<tr>
<td>IMpassion130</td>
<td>TNBC not previously treated for MBC</td>
<td>Atezolizumab + nab-paclitaxel</td>
<td>Placebo + nab-paclitaxel</td>
<td>PFS and OS</td>
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<tr>
<td>IMpassion131</td>
<td>TNBC not previously treated for MBC</td>
<td>Atezolizumab + paclitaxel</td>
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</table>

## Select Phase II Studies

<table>
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<tr>
<th>Study</th>
<th>Population</th>
<th>Investigational</th>
<th>Primary Endpoint</th>
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<tbody>
<tr>
<td>DORA</td>
<td>mTNBC following clinical benefit with platinum-based tx</td>
<td>Durvalumab + olaparib and durvalumab</td>
<td>PFS</td>
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<tr>
<td>Study 2151-169</td>
<td>PD-L1+ mTNBC</td>
<td>Durvalumab + paclitaxel</td>
<td>AEs</td>
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<tr>
<td>NCI Trial</td>
<td>HDR-deficient mTNBC (with known BRCA status)</td>
<td>Veliparib, atezolizumab, or veliparib + atezolizumab</td>
<td>PFS</td>
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<tr>
<td>SNDX-275-0602</td>
<td>mTNBC with 1-2 previous lines of Tx</td>
<td>Entinostat + atezolizumab, or placebo + atezolizumab</td>
<td>MTD, PFS</td>
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<tr>
<td>MORPHEUS</td>
<td>An open-label, multicenter, randomized umbrella study evaluating multiple immunotherapy-based combinations</td>
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</table>
Conclusions

• Immune checkpoint inhibitors have shown promising efficacy in patients with TNBC, particularly in previous untreated patients and in combination with chemotherapy.

• Responsiveness seems to be correlated with TIL frequency rather than PD-L1 expression.
How can we predict response to certain immune checkpoint inhibitors in cancer patients?

Can we know which combination of immune checkpoint inhibitors increases the responsiveness?
PD-L1 expression enriches for responses to αPD-1 therapy

PD-L1 is not be the only factor to control PD-1 pathway

Need another biomarker to further categorize the population

Sunshine J & Taube JM. Curr Opin Pharmacol 2015 23:32
How can we distinguish the responders and non-responders to α-PD-1/PD-L1 therapy?
Predictive biomarkers for anti-PD-1/PD-L1 blockade

1) TIL frequency
2) PD-L1 expression level on tumors
3) Nonsynonymous mutation rate
4) Gut microbiota
5) Other immune checkpoint signaling
**In silico** analysis & clinical validation

**Cancer cells**
- Cancer
- Cytokine receptors
- **immune checkpoint ligands**
  - Preferential expression of specific set of immune checkpoint ligands

**Cancer-infiltrating T cells**
- **immune checkpoint receptors**
  - Co-expressions of various immune checkpoint receptors
- TCR

**Suppression of T cell function & Cancer progression**
Hypothesis for IC-related immune regulation in TME

**Individual difference**
1. Tumor microenvironment
2. TME-derived factors
3. Signaling network
4. Immune checkpoint ligand expression

**Signaling network (TF.. etc)**

**Cancer/APC**
- B7-1, B7-2
- PD-L1, PD-L2
- PVR
- Galectin 9, Ceacam1
- MHC
- B7-H3
- B7-H4

**Differential expression of Immune checkpoint ligand**

**T cell**
- CTLA-4
- PD-1
- TIGIT
- TIM-3
- LAG-3
- **Individually different levels of T cell exhaustion**

**APC-T interaction + TME-derived factors**

**Tumor microenvironment**
Other immune checkpoints than PD-1

Anderson AC et al. *Immunity* 2016 44:989
Simultaneous expression of immune checkpoints in tumor lymphocytes of individual patients


LUAD correlation between receptors

<table>
<thead>
<tr>
<th></th>
<th>PDCD1</th>
<th>CTLA4</th>
<th>TIGIT</th>
<th>HAVCR2</th>
<th>CD244</th>
<th>CD160</th>
<th>BTLA</th>
<th>CD200R1</th>
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<tr>
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<td>TIGIT</td>
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<td>1.0</td>
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<td>HAVCR2</td>
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<td>BTLA</td>
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</table>

Modified from Anderson AC et al. *Immunity* 2016 44:989
Tumor expression for the ligands

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Tumor Expression</th>
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<tbody>
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<td>PDL1</td>
<td>Melanoma, renal cell, head and neck, cervical, glioblastoma, bladder, oesophageal, breast, hepatocellular, Hodgkin lymphoma, mediastinal large B-cell lymphoma, among others</td>
</tr>
<tr>
<td>PDL2</td>
<td>Oesophageal, ovarian, pancreatic, hepatocellular, breast, Hodgkin, mediastinal large B-cell lymphoma, among others</td>
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<tr>
<td>B7-H3</td>
<td>Prostate, renal cell, non-small cell lung, pancreatic, gastric, ovarian, colorectal, urothelial cell, among others</td>
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<tr>
<td>B7-H4</td>
<td>Breast, renal cell, ovarian, oesophageal, gastric, pancreatic, melanoma, among others</td>
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<tr>
<td>HHLA2</td>
<td>Breast, lung, thyroid, melanoma, pancreas, ovary, liver, bladder, colon, prostate, kidney, oesophagus</td>
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<td>Galectins</td>
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<td>CD70</td>
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<tr>
<td>ICOSL</td>
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</tr>
<tr>
<td>CD155</td>
<td>Kidney, prostate, pancreatic, glioblastoma</td>
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Mahoney KM et al. Nat Rev Drug Discov 2015 14:561
Clinical significance of differential response of populations stratified by PD-L1 and PVR expression to anti-PD-1 therapy
**In silico** analysis for ICs & their ligands

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Gene ID</th>
<th>Aliases</th>
<th>Common Name</th>
<th>Gene ID</th>
<th>Aliases</th>
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<tr>
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<td>PDCD1LG2</td>
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<td>CD96</td>
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<td>B7-2</td>
<td>CD86</td>
<td>CD28LG2, CLS1</td>
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<td>TIM-3</td>
<td>HAVCR2</td>
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<td>HVED, NECL5</td>
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<td>CD160</td>
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**Immune Checkpoint (Receptor)**

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<th>Common Name</th>
<th>Gene ID</th>
<th>Aliases</th>
</tr>
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<tbody>
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<tr>
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<td>VSIG9</td>
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<td>TIM-3</td>
<td>HAVCR2</td>
<td>CD366, HAVcr-2</td>
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<tr>
<td>CD160</td>
<td>CD160</td>
<td>NK1, NK28</td>
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<tr>
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<td>BTLA</td>
<td>CD272, BTLA1</td>
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<td>CD200R</td>
<td>CD200R1</td>
<td>HCRTR2, MOX2R, OX2R</td>
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**Immune Checkpoint Ligand**

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<td>PDCD1LG2</td>
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<td>B7-1</td>
<td>CD80</td>
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<td>B7-2</td>
<td>CD86</td>
<td>CD28LG2, CLS1</td>
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<tr>
<td>CD155</td>
<td>PVR</td>
<td>HVED, NECL5</td>
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<tr>
<td>CD112</td>
<td>PVRL2</td>
<td>PRR2, PVRR2, NECTIN-2, HVEB</td>
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<tr>
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<td>HAVCR1</td>
<td>CD365</td>
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<tr>
<td>TIM-4</td>
<td>TIMD4</td>
<td>SMUCKLER</td>
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# Collected datasets for lung cancer

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<tr>
<th>Sample Type</th>
<th>Sample Numbers</th>
<th>Source</th>
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<tbody>
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<td>Lung Adenocarcinoma</td>
<td>515</td>
<td>TCGA</td>
</tr>
<tr>
<td>Lung Adenocarcinoma</td>
<td>85</td>
<td>GSE30219</td>
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<tr>
<td>Lung Adenocarcinoma</td>
<td>106</td>
<td>GSE37745</td>
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<tr>
<td>Lung Adenocarcinoma</td>
<td>226 (127 with EGFR mutation, 20 with KRAS mutation, 11 with EML4-ALK fusion and 68 triple negative cases)</td>
<td>GSE31210</td>
</tr>
<tr>
<td>Lung Adenocarcinoma</td>
<td>40</td>
<td>GSE10245</td>
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</table>
Correlation between Ligands
Lung Adenocarcinoma (LUAD)
in TCGA

Differential expression of immune checkpoint ligands in tumors of individual patients

Ligand for Immune Checkpoint TIGIT
Uncorrelated expression

Ligands for immune Checkpoints PD-1
Differential expression of immune checkpoint ligands in 75 NSCLC patients who received aPD-1 therapy

<table>
<thead>
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<th>H&amp;E</th>
<th>CD8</th>
<th>PD-L1</th>
<th>PVR</th>
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<td>PD-L1+/PVR-</td>
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<tr>
<td>PD-L1+/PVR+</td>
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<tr>
<td>PD-L1-/PVR-</td>
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<td>PD-L1+/PVR+</td>
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<td></td>
</tr>
</tbody>
</table>

- PD-L1-/PVR+
- PD-L1+/PVR-
Different efficacy for aPD-1 therapy in 75 NSCLC patients with differential expression of IC ligands

Definition of Responder: PR or SD>6 mon
Different efficacy for aPD-1 therapy in 75 NSCLC patients with differential expression of IC ligands

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>PD-L1-/PVR+</th>
<th>PD-L1-/PVR-</th>
<th>PD-L1+/PVR+</th>
<th>PD-L1+/PVR-</th>
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<td>SD</td>
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<td>30.0</td>
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</tr>
<tr>
<td>PD</td>
<td>100.0</td>
<td>55.2</td>
<td>20.0</td>
<td>8.3</td>
</tr>
</tbody>
</table>
Survival evaluation of PD-1 blockade in 75 NSCLC patients (Swimmer’s plot)
Responses to $\alpha$PD-1 therapy by the expressions of PD-L1 & PVR in 75 NSCLC patients

% Progression free survival

% Overall survival

Months
Modes of action of TIGIT

TIGIT signaling
- Tumor cell or DC
- PVR-TIGIT
- Treg
- Teff
- NK

APC modulation
- Tumor cell or DC
- PVR-TIGIT
- IL-10
- Teff
- Treg

CD226 interference
- Tumor cell or DC
- PVR-TIGIT
- CD226
- Teff

Multiple immune checkpoint pathways

Sakuishi K et al. *J Exp Med* 2010 207:2187


### TIGIT targeting antibodies for clinical trials

<table>
<thead>
<tr>
<th>Agents targeting human TIGIT</th>
<th>Antibody</th>
<th>Mode of Action</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC, tumor cell, infected cell</td>
<td>MTIG7192A (PI)</td>
<td>Blocking ligand binding X-reactive to human/mouse</td>
<td>Genentech</td>
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<tr>
<td>CD112, CD155, CD226, TIGIT, CD96</td>
<td>BMS-986207 (PI)</td>
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<td>BMS</td>
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<tr>
<td>LFA-1</td>
<td>OMP-313M32 (PI)</td>
<td>Blocking ligand binding + Fc effector function No X-reactive</td>
<td>OncoMed</td>
</tr>
<tr>
<td>?</td>
<td>Blocking ligand binding</td>
<td>Merck</td>
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</tr>
</tbody>
</table>
Summary

1. Unlike expressions of immune checkpoints, the ligands for PD-1 and TIGIT were expressed in uncorrelated manner from tumor tissues of NSCLC patients.

2. Prediction power for the efficacy of PD-1 blocker in NSCLC patient could be dramatically improved by using PD-L1 and PVR dual expression pattern analysis.

3. Use of TIGIT blocker combined with PD-1 blocker might extend therapeutic benefit for the NSCLC patients with co-expressions of PD-L1 and PVR.

4. Comprehensive analysis for the expressions of immune checkpoint ligands will open the field of personalized immunotherapy.
Future direction of cancer immunotherapy
Personalized cancer medicine

Stratified targeted medicine

In vitro diagnostic

Neg  Pos  Neg

Invariant drug off the shelf

Personalized mutanome vaccine

Mutanome vaccine design

Just-in-time production

Individually tailored drug on demand
Acknowledgement

Boryeong Lee  Prof. Hye Ryun Kim
Hyo-Jin Park  Prof. Hyo Sup Shim
Jimin Son  Prof. In Suk Lee
Jihyun Moon

DGIST
Prof. Dahee Hwang
Dr. Sehyun Chae