Combination strategies with checkpoint-inhibitors

Combination with chemotherapy and targeted agents

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Outline

- Rational for combination of immunotherapy and chemotherapy
- Molecular characterization of BC immune-phenotypes
- Evidences from clinical trials
- Future perspectives
## Immune-Signatures

<table>
<thead>
<tr>
<th>Reference</th>
<th># of Patients</th>
<th>Signatures</th>
<th>ER-</th>
<th>HER2+</th>
<th>ER+ Lum B</th>
<th>ER+ Lum A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teschendorff et al. 2007</td>
<td>1056</td>
<td>7-gene immune module</td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>Alexe et al. 2007</td>
<td>286</td>
<td>651 lymphocyte-associated genes</td>
<td></td>
<td></td>
<td>+</td>
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</tr>
<tr>
<td>Schmidt et al. 2008</td>
<td>788</td>
<td>B-cell metagene</td>
<td>+</td>
<td></td>
<td>+</td>
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<tr>
<td>Desmedt et al. 2008</td>
<td>1605</td>
<td>Stat1 metagene</td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>Rody et al. 2009</td>
<td>1781</td>
<td>Lymphocyte-specific kinase (LCK)</td>
<td>+</td>
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<tr>
<td>Bianchini et al. 2010</td>
<td>684</td>
<td>B-cell/plasma cell metagene</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Criscitiello et al 2018</td>
<td>99</td>
<td>4-gene signature</td>
<td>+</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

# TILs in TNBC

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Trial</th>
<th>Endpoint</th>
<th>Subtype Analyzed</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denkert et al. 2010</td>
<td>840</td>
<td>GBG G-3</td>
<td>pCR</td>
<td>All</td>
<td>pCR: 41% in TIL+ BC Validated in G-5</td>
</tr>
<tr>
<td>Loi et al. 2013</td>
<td>2009</td>
<td>BIG 2-98</td>
<td>DFS</td>
<td>Preplanned analysis of molecular subtypes</td>
<td>Prognostic impact in TNBC (n = 256): HR: 0.31 (0.11-0.84)</td>
</tr>
<tr>
<td>Loi et al. 2014</td>
<td>935</td>
<td>FinHer</td>
<td>DFS</td>
<td>Preplanned analysis of molecular subtypes</td>
<td>Prognostic impact in TNBC (n = 134): HR: 0.31 (0.12-0.8)</td>
</tr>
<tr>
<td>Adams et al. 2014</td>
<td>506</td>
<td>ECOG 2197</td>
<td>DFS</td>
<td>TNBC</td>
<td>HR: 0.84 (0.74-0.95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ECOG 1199</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dieci et al. 2014</td>
<td>278</td>
<td>MFS</td>
<td>TNBC</td>
<td></td>
<td>HR: 0.86 (0.77 -0.96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS</td>
<td></td>
<td></td>
<td>HR: 0.86 (0.77 -0.97)</td>
</tr>
<tr>
<td>Denkert et al. 2015</td>
<td>580</td>
<td>Gepar-Sixto</td>
<td>pCR</td>
<td>TNBC and HER2</td>
<td>pCR rate was 59.9% in LPBC and 33.8% for non-LPBC (P&lt;.001)</td>
</tr>
</tbody>
</table>

Immunologic Constant of Rejection

Immunologic Constant of Rejection

TCGA

Overall survival

Validation cohort

Distant metastasis free survival

Combination of CT and I-O

Immunotherapy & radiotherapy

Immunotherapy alone

Immunotherapy & Targeted therapy

Immunotherapy & chemotherapy

Ongoing combinations
Combination of CT and I-O

Galluzzi L, Cell, 2015
Tumour-associated infiltrate

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Sub-Type</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th2-CD4 T cells, Treg, B Cells</td>
<td>Mitogenic, recruit/activate myeloid cells, suppress CTLs</td>
<td></td>
</tr>
<tr>
<td>CD8 T Cells (CTL), NK T cells</td>
<td>Cancer cell killing: direct and via helping CTLs</td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>Cancer cell killing</td>
<td></td>
</tr>
<tr>
<td>Inflammatory Monocytes, MDSCs</td>
<td>Pro-angiogenic, pro-invasive, pro-metastatic, pro-inflammatory, inhibit apoptosis, suppress killing by CTL</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Cancer cell killing; antigen-presenting</td>
<td></td>
</tr>
<tr>
<td>Mast Cells</td>
<td>Pro-angiogenic, pro-invasive, pro-metastatic, suppress CTL and NK T cell cytotoxic activity</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>Metastatic, anti-metastatic, cancer cell killing</td>
<td></td>
</tr>
<tr>
<td>αSMA* Myofibroblasts &amp; MSCs</td>
<td>Mitogenic, pro-angiogenic, pro-invasive, recruit other ILCs, suppress CTL &amp; NK T cell cytotoxic activity</td>
<td></td>
</tr>
<tr>
<td>Activated Tissue Fibroblasts</td>
<td>Pro-angiogenic, pro-invasive, support cancer stem cells</td>
<td></td>
</tr>
<tr>
<td>Activated Adipocytes</td>
<td>Mitogenic, pro-survival, pro-angiogenic, recruit/activate ILCs, pro-invasive, pro-metastatic, metabolic support</td>
<td></td>
</tr>
<tr>
<td>Endothelial Tip, Stalk, Tube Cells</td>
<td>Angiogenesis for blood supply of oxygen &amp; nutrients, Produce paracrine trophic factors, recruit ILCs, Modulate cancer cell dissemination &amp; seeding, Limit CTL and NK T cell infiltration</td>
<td></td>
</tr>
<tr>
<td>Pericytes (immature &amp; mature)</td>
<td>Modulate angiogenesis &amp; cell transit, support vascular functionality</td>
<td></td>
</tr>
</tbody>
</table>

Pro-tumour

Anti-tumour

Hanahan & Coussens, 2012
Chemotherapy and tumour-associated infiltrate

Chemotherapy is a main treatment for breast cancer (consider triple negative and HER2 positive BC)

Chemotherapy *induces DNA damage* to which cancer cells are particularly sensitive because of mutations in DNA-repair pathways

Chemotherapy *induces an inflammatory response in which immune cells regulate tissue repair*
Chemotherapy and tumour-associated infiltrate

Chemotherapy and tumour-associated infiltrate

Mouse MC38 tumours: platinum salts

before CT  7 d after CT

Sarcoma patient
Paired paraffin sections before and after anthracyclines
Chemotherapy and tumour-associated infiltrate

- IgM
- Factor B
- C1q
- C3
- C3a, C3aR1
- C5a, C5aR1
- IFN-γ

CT

Necrosis  C3a, C5a production  DC activation  CD8+ eff Tumor control

- Tumor cell
- iDC
- mDC
- CD8+
- CD8+ eff
- CD4+
- Treg

- Necrosis - 4–18 h
- 24 h
- 48 h
- 196 h

Tumor progression
How to Enhance Immunogenicity?

- Translocation of Calreticulin to the cell surface
- Activation of HSP90
- Release of High Mobility Group Box 1 protein

- Cytotoxic agent or radiotherapy
- Immature dendritic cells
- Live tumor cell
- Stressed tumor cell
- Dying tumor cell

- «Eat-me» signal
- DC maturation
- IFN-I, IL-1, TLR-4

- HSP90
- HMGB1
- Calreticulin (Calr)
- HMGB1
How to Enhance Immunogenicity?
How to Enhance Immunogenicity?

How to Enhance Immunogenicity?

A

Lethal trigger

Lethal trigger

Lethal trigger

RCD

RCD

RCD

B

Lethal trigger

Lethal trigger

Lethal trigger

RCD

RCD

RCD

Targeted inhibition

C

Lethal trigger

RCD

RCD

Targeted inhibition

D

Lethal trigger

RCD

RCD

Galluzzi L. et al Cell Death & Differentiation, 2018, 25, 486–541
<table>
<thead>
<tr>
<th>DRUG</th>
<th>EFFECT ON IMMUNE SYSTEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>Induces immunogenic cell death</td>
</tr>
<tr>
<td></td>
<td>Increases proliferation of CD8 T cells</td>
</tr>
<tr>
<td></td>
<td>Stimulates antigen presentation by DCs</td>
</tr>
<tr>
<td></td>
<td>Stimulates MCP1 and M6PR</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Induces immunogenic cell death</td>
</tr>
<tr>
<td></td>
<td>Suppressed Treg inhibitory functions and restoration of the proliferative capacity of effector T cells and NK cell cytotoxicity</td>
</tr>
<tr>
<td>Taxanes</td>
<td>Enhance T-cell and NK-cell function</td>
</tr>
<tr>
<td></td>
<td>Increase recruitment of TIL</td>
</tr>
<tr>
<td></td>
<td>Increase efficacy of immunostimulatory agents</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>Reduce the number of myeloid suppressor cells</td>
</tr>
<tr>
<td></td>
<td>Increase the antitumor activity of CD8(+) T cells and activated NK cells</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>Induces immunogenic cell death</td>
</tr>
<tr>
<td></td>
<td>Increases MHC I complex</td>
</tr>
<tr>
<td></td>
<td>Inhibits PD-L2</td>
</tr>
</tbody>
</table>

DC, dendritic cells; MHC, major histocompatibility complex; NK, natural killer
How to Enhance Immunogenicity?

**Immunogenic cell death**

1. Tumour cell death
2. Antigen-release
3. Type I interferon
4. Upregulation of MHC-I and tumour-associated antigens

**Improved antigen presentation**

1. DC maturation
2. Improved cross-presentation (type I IFN)
3. Acute inflammation

**Checkpoint blockade**

1. Infiltration
2. Intratumoural survival
3. Maintenance of effector function
Evidence from clinical trials

**Nivolumab** HUMAN IgG4 ANTI–PD-1 antibody

**Pembrolizumab** Humanized IgG4 anti–PD-1 antibody

**Atezolizumab** Engineered human IgG1 anti–PD-L1 antibody

**Durvalumab** Human IgG1 anti–PD-L1 antibody

**Tremelimumab** Human IgG2 anti–CTLA-4 antibody
Pembrolizumab in TNBC

- Recurrent or metastatic ER-/PgR-/HER2- breast cancer
- ECOG PS 0-1
- PD-L1+ tumor
- No systemic steroid therapy
- No autoimmune disease (active or history of)
- No active brain metastases

Pembro 10 mg/kg Q2W

CR
Discontinuation permitted

PR/SD
Treat for 24 months or until PD or toxicity

Confirmed PD

Discontinue

- PD-L1 positivity: 58% of all patients screened had PD-L1–positive tumors
- Treatment: 10 mg/kg IV Q2W
- Response assessment: Performed every 8 weeks per RECIST v1.1

*PD-L1 expression was assessed in archival tumor samples using a prototype IHC assay and the 22C3 antibody. Only patients with PD-L1 staining in the stroma or in ≥1% of tumor cells were eligible for enrollment.

**If clinically stable, patients are permitted to remain on pembrolizumab until progressive disease is confirmed on a second scan performed ≥4 weeks later. If progressive disease is confirmed, pembrolizumab is discontinued. An exception may be granted for patients with clinical stability or improvement after consultation with the sponsor.

CR, complete response; ECOG PS, Eastern Cooperative Oncology Group performance status; PD, progressive disease; PgR, progesterone receptor; PR, partial response; SD, stable disease
Pembrolizumab in TNBC

Objective response rate: 18.5%
Stable disease: 25.9%

n = 32

Confirmed complete response (nodal disease)
Confirmed partial response
Stable disease
Progressive disease
Pembrolizumab in TNBC

Efficacy (N = 27)

- **ORR**: 18.5%
- **Stable disease**: 25.9%
- **Median duration of response**: NR (15.0 to ≥47.3 weeks)
- **Median PFS**: 1.9 months
- **6-month PFS**: 24.4%
- **Median OS**: 11.2 months
- **12-month OS**: 43.1%

ORR, overall response rate; OS, overall survival; PFS, progressive-free survival

# Pembrolizumab in TNBC

<table>
<thead>
<tr>
<th></th>
<th>Total Population N = 170</th>
<th>PD-L1 Positive n = 105</th>
<th>PD-L1 Negative n = 64</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, n (%) [95% CI]</td>
<td>8 (4.7) [2.3-9.2]</td>
<td>5 (4.8) [1.8-10.9]</td>
<td>3 (4.7) [1.1-13.4]</td>
</tr>
<tr>
<td>DCR, n (%) [95% CI]</td>
<td>13 (7.6) [4.4-12.7]</td>
<td>10 (9.5) [5.1-16.8]</td>
<td>3 (4.7) [1.1-13.4]</td>
</tr>
<tr>
<td>Best overall response, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>1 (0.6)</td>
<td>1 (1.0)</td>
<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>7 (4.1)</td>
<td>4 (3.8)</td>
<td>3 (4.7)</td>
</tr>
<tr>
<td>SD</td>
<td>35 (20.6)</td>
<td>22 (21.0)</td>
<td>12 (18.8)</td>
</tr>
<tr>
<td>PD</td>
<td>103 (60.6)</td>
<td>66 (62.9)</td>
<td>37 (57.8)</td>
</tr>
<tr>
<td>Not evaluable, n (%)</td>
<td>5 (2.9)</td>
<td>2 (1.9)</td>
<td>3 (4.7)</td>
</tr>
<tr>
<td>Not able to be assessed, n (%)</td>
<td>19 (11.2)</td>
<td>10 (9.5)</td>
<td>9 (14.1)</td>
</tr>
</tbody>
</table>

DCR, disease control rate
Pembrolizumab in TNBC

<table>
<thead>
<tr>
<th>Events/Patients, n</th>
<th>Median (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Totala</td>
<td>148/170</td>
</tr>
<tr>
<td>PD-L1 positive</td>
<td>90/105</td>
</tr>
<tr>
<td>PD-L1 negative</td>
<td>57/64</td>
</tr>
</tbody>
</table>

Cohort A (N = 170): 
**Previously Treated, Regardless of PD-L1 Expression**

- Complete response: 7.6%
- Partial response: 9.5%
- Stable disease ≥24 wk: 4.7%

Cohort B (N = 52):
**Previously Untreated, PD-L1 Positive**

- Complete response: 23.1%

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Monotherapy in TNBC

Anti–PD-L1/PD-1 Single Agent in mTNBC

ORR by sTIL Level ≥Median vs <Median

Data cutoff date: Nov 10, 2016.
Eribulin and Pembrolizumab

Percentage change in total sum of target lesion diameters from baseline

<table>
<thead>
<tr>
<th></th>
<th>ALL (n = 17)</th>
<th>1L (n = 17)</th>
<th>2L/3L (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>34.4%</td>
<td>41.2%</td>
<td>27.3%</td>
</tr>
<tr>
<td>CBR</td>
<td>40.6%</td>
<td>47.1%</td>
<td>36.4%</td>
</tr>
</tbody>
</table>

Duration of treatment

<table>
<thead>
<tr>
<th></th>
<th>PD-L1+ (n = 17)</th>
<th>PD-L1– (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>29.4%</td>
<td>33.3%</td>
</tr>
<tr>
<td>CBR</td>
<td>35.8%</td>
<td>44.4%</td>
</tr>
</tbody>
</table>

1L, first line; 2L/3L, second/third line; BOR, best overall response; CBR, clinical benefit rate; IC, tumor-infiltrating immune cell
Targeting stroma and inflammation

- **PD-L1 positivity**: Stratification factor
- **Treatment**: metronomic CT plus pembrolizumab
- **Response assessment**: Performed every 8 weeks per RECIST v1.1

PI G. Curigliano et al.
TONIC Trial: Study Design

- Radiation: 3 x 8 Gy
- Doxorubicin: 15 mg x2
- Cyclophosphamide: 50 mg daily
- Cisplatin: 40 mg/m² x2
- No treatment
- Nivolumab

# Efficacy of Nivolumab After Induction

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 50)</th>
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<tbody>
<tr>
<td><strong>Best ORR (CR + PR) iRECIST</strong></td>
<td>24%</td>
</tr>
<tr>
<td><strong>CBR (CR + PR + SD)</strong></td>
<td>26%</td>
</tr>
<tr>
<td><strong>CR</strong></td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>11 (22%)</td>
</tr>
<tr>
<td><strong>SD ≥24 weeks</strong></td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>ORR RECIST1.1</strong></td>
<td>22%</td>
</tr>
<tr>
<td><strong>Median PFS [95% CI]</strong></td>
<td>3.4 months [2.5-3.7]</td>
</tr>
<tr>
<td><strong>Median time to response [range]</strong></td>
<td>2.1 months [0.5-3.5]</td>
</tr>
<tr>
<td><strong>Median duration of response [95% CI]</strong></td>
<td>9.0 months [5.5-NA]</td>
</tr>
</tbody>
</table>

NA, not available  
### Atezolizumab and nab-Paclitaxel in mTNBC

<table>
<thead>
<tr>
<th>Best Overall Response</th>
<th>1L (n = 9)</th>
<th>2L (n = 8)</th>
<th>3L+ (n = 7)</th>
<th>All Patients N = 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed ORR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.7% (29.9, 92.5)</td>
<td>25% (3.2, 65.1)</td>
<td>28.6% (3.7, 71.0)</td>
<td>41.7% (22.1, 63.4)</td>
</tr>
<tr>
<td>ORR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.9% (51.7, 99.7)</td>
<td>75.0% (34.9, 96.8)</td>
<td>42.9% (9.9, 81.6)</td>
<td>70.8% (48.9, 87.4)</td>
</tr>
<tr>
<td>CR</td>
<td>11.1%</td>
<td>0</td>
<td>0</td>
<td>4.2%</td>
</tr>
<tr>
<td>PR</td>
<td>77.8%</td>
<td>75.0%</td>
<td>42.9%</td>
<td>66.7%</td>
</tr>
<tr>
<td>SD</td>
<td>11.1%</td>
<td>25.0%</td>
<td>28.6%</td>
<td>20.8%</td>
</tr>
<tr>
<td>PD</td>
<td>0</td>
<td>0</td>
<td>28.6%</td>
<td>8.3%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Confirmed ORR defined as at least 2 consecutive assessments of complete or partial response.

<sup>b</sup> Including investigator-assessed unconfirmed responses.

Efficacy-evaluable patients were dosed by June 1, 2015, and were evaluable for response by RECIST v1.1. Minimum efficacy follow up was ≥ 3 months.

Response rates were higher for patients who received atezolizumab/nab-paclitaxel treatment as 1L therapy compared to 2L+.
Atezolizumab and \textit{nab}-Paclitaxel in mTNBC

- 11 of 17 responses (65\%) continued on treatment at time of data cut off

Phase Ib Study of Atezolizumab and Nab-Paclitaxel in mTNBC

<table>
<thead>
<tr>
<th></th>
<th>IC0 (n = 7)</th>
<th>IC1/2/3 (n = 9)</th>
<th>Unknown (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR (95% CI)</td>
<td>57.1% (18.4, 90.1)</td>
<td>77.8% (40.0, 97.2)</td>
<td>75% (34.9, 96.8)</td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
<td>0</td>
<td>12.5%</td>
</tr>
<tr>
<td>PR</td>
<td>57.1%</td>
<td>77.8%</td>
<td>62.5%</td>
</tr>
<tr>
<td>SD</td>
<td>42.9%</td>
<td>22.2%</td>
<td>0</td>
</tr>
<tr>
<td>PD</td>
<td>0</td>
<td>0</td>
<td>25%</td>
</tr>
</tbody>
</table>

Including investigator-assessed unconfirmed responses.

- Responses were observed in both IC0 and IC1/2/3 patients

Phase Ib Study of Atezolizumab and Nab-Paclitaxel in mTNBC

- Proliferating activated CD8+ T cells transiently peaked at the end of the first cycle of atezolizumab treatment

**Atezolizumab and nab-Paclitaxel in mTNBC**

Randomized, double-blind, placebo-controlled Phase 3 trial of nab-paclitaxel ± atezolizumab as 1st line therapy in mTNBC (NCT02425891)

**Study design**

- Histologically documented locally advanced or metastatic TNBC
- No prior therapy for advanced disease
- ECOG PS 0-1
- Measurable disease per RECIST v1.1
- Patients with significant CV or CNS disease (except asymptomatic brain metastases), autoimmune disease or prior checkpoint inhibitor therapy are excluded
- Target accrual: ~350 patients

**Co-primary endpoints:**
- PFS in all patients
- PFS according to PD-L1 expression

**Secondary endpoints:**
- OS
- ORR
- Response duration
- Safety/tolerability
- Pharmacokinetics (PK)
- Health-related quality of life (HR QoL)

**Stratification factors:**
- Presence of liver metastases
- Prior taxane therapy
- PD-L1 expression status (centrally evaluated by IHC using the SP142 assay)

PARP Inhibitors and Immuno-Oncology
TOPACIO Trial of Niraparib and Pembrolizumab

**Phase I**

Patients with OC or TNBC

- **Dose level 1**
  - Niraparib 200 mg + pembrolizumab 200 mg

- **Dose level 2**
  - Niraparib 300 mg + pembrolizumab 200 mg

**Endpoint assessment**

**Phase II**

Patients with OC (target n = 48) or TNBC (target n = 48) RP2D

**Endpoint assessment**

Preliminary Best Percentage Change in Lesion Size in Patients Enrolled in Phase 2 with TNBC

Immunotherapy in HER2 positive

- Centrally confirmed HER2+
- ECOG 0-1
- Tumor biopsy sample <1yr
- Measurable disease RECIST 1.1
- No limit of prior systemic treatment
- Documented PD on trastuzumab or TDM-1

### Phase Ib Pembrolizumab

- Pembrolizumab 2mg/kg and 10mg/kg IV + trastuzumab Q3W

### Phase II

- Pembrolizumab 200mg IV + trastuzumab Q3W

Protocol specified follow-up. Treatment until progression, toxicity, patient withdrawal or investigator decision of maximum 2 years

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## Immunotherapy in HER2 positive

### Characteristic

<table>
<thead>
<tr>
<th></th>
<th>Phase Ib PD-L1 positive</th>
<th>Phase II PD-L1 positive;</th>
<th>Phase II PD-L1 negative;</th>
<th>Overall n=58</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=6</td>
<td>N=40</td>
<td>N=12</td>
<td></td>
</tr>
<tr>
<td>Age yrs. median (range)</td>
<td>49 (38-57)</td>
<td>49 (28-72)</td>
<td>56.5 (43-61)</td>
<td>50.5 (28-72)</td>
</tr>
<tr>
<td>ER negative</td>
<td>4 (66%)</td>
<td>23 (57.5%)</td>
<td>6 (50%)</td>
<td>33 (56.9%)</td>
</tr>
<tr>
<td>positive (≥1%)</td>
<td>2 (33%)</td>
<td>17 (42.5%)</td>
<td>6 (50%)</td>
<td>25 (43.1%)</td>
</tr>
<tr>
<td>Prior trastuzumab-containing therapy</td>
<td>6 (100%)</td>
<td>40 (100%)</td>
<td>12 (100%)</td>
<td>58 (100%)</td>
</tr>
<tr>
<td>Additional anti-HER2 therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1 (16.7%)</td>
<td>6 (15%)</td>
<td>0 ( 0%)</td>
<td>7 (12.1%)</td>
</tr>
<tr>
<td>Yes</td>
<td>5 (83.3%)</td>
<td>34 (85%)</td>
<td>12 (100%)</td>
<td>51 (87.9%)</td>
</tr>
<tr>
<td>T-DM1</td>
<td>4</td>
<td>29</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>17</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td>Prior endocrine therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (33%)</td>
<td>13 (32.5%)</td>
<td>7 (58%)</td>
<td>22 (38%)</td>
</tr>
<tr>
<td>Prior chemotherapy (Anth/Taxane)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 (100%)</td>
<td>40 (100%)</td>
<td>12 (100%)</td>
<td>58 (100%)</td>
</tr>
</tbody>
</table>

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# Best Overall Response (RECIST 1.1)

<table>
<thead>
<tr>
<th></th>
<th>PD-L1 Positive Phase Ib, n=6</th>
<th>PD-L1 Positive Phase II, n=40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORR n (%) [90%CI]</strong></td>
<td>1 (17%) [1-58]</td>
<td>6 (15%) [7-29]</td>
</tr>
<tr>
<td><strong>DCR(^1) n (%) [90%CI]</strong></td>
<td>1 (17%) [1-58]</td>
<td>10 (25%) [14-49]</td>
</tr>
</tbody>
</table>

**Best overall response, n (%)**

<table>
<thead>
<tr>
<th>Response Type</th>
<th>PD-L1 Positive Phase Ib, n=6</th>
<th>PD-L1 Positive Phase II, n=40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response</td>
<td>1 (17%)</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>Partial Response</td>
<td>-</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>-</td>
<td>7 (17.5%)</td>
</tr>
<tr>
<td>Progressive Disease</td>
<td>5 (83%)</td>
<td>25 (62.5%)</td>
</tr>
<tr>
<td>Not Evaluable</td>
<td>-</td>
<td>2 (5.0%)</td>
</tr>
</tbody>
</table>

**Overall PD-L1 +**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORR</strong></td>
<td>15.2% [7-27]</td>
</tr>
<tr>
<td><strong>DCR</strong></td>
<td>24% [14-36]</td>
</tr>
</tbody>
</table>

\(^1\)DCR: CR, PR, or SD ≥ 6 months

S. Loi et al San Antonio 2017
### Best Overall Response (RECIST 1.1)

<table>
<thead>
<tr>
<th></th>
<th>PD-L1 Positive Phase Ib, n=6</th>
<th>PD-L1 Positive Phase II, n=40</th>
<th>PD-L1 Negative Phase II, n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORR n (%) [90%CI]</strong></td>
<td>1 (17%) [1-58]</td>
<td>6 (15%) [7-29]</td>
<td>0 (0%) [0-18]</td>
</tr>
<tr>
<td><strong>DCR¹ n (%) [90%CI]</strong></td>
<td>1 (17%) [1-58]</td>
<td>10 (25%) [14-49]</td>
<td>0 (0%) [0-18]</td>
</tr>
<tr>
<td><strong>Best overall response, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete Response</td>
<td>1 (17%)</td>
<td>1 ( 2.5%)</td>
<td>-</td>
</tr>
<tr>
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<tr>
<td>Stable Disease</td>
<td>-</td>
<td>7 (17.5%)</td>
<td>2 (16.7%)</td>
</tr>
<tr>
<td>Progressive Disease</td>
<td>5 (83%)</td>
<td>25 (62.5%)</td>
<td>9 (75.0%)</td>
</tr>
<tr>
<td>Not Evaluable</td>
<td>-</td>
<td>2 ( 5.0%)</td>
<td>1 ( 8.3%)</td>
</tr>
</tbody>
</table>

Overall PD-L1 +

**ORR 15.2% [7-27]**

**DCR 24% [14-36]**

Disease Control Rate¹DCR: CR, PR, or SD ≥ 6 months

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Maximum Change from Baseline in Target Lesions PD-L1 Positive Cohorts

Maximum Change from Baseline in Sum of Target Lesions
PD-L1 Positive (N=44)

Excludes 2 patients not evaluable for response

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PFS and OS

Progression-free Survival By PD-L1 Status

Median, months (90% CI):
PD-L1 Pos: 2.7 (2.6 to 4.0)
PD-L1 Neg: 2.5 (1.4 to 2.7)

P = 0.07

Overall Survival By PD-L1 Status

Median, months (90% CI):
PD-L1 Pos: 16.1 (13.1 to ∞)
PD-L1 Neg: 7.0 (4.9 to 9.8)

P = 0.0006

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Stromal TILs Median 1%, Mean 4.8%, IQR 0-5%, all fresh biopsies of heavily pretreated pts

**Baseline TILs by PD-L1 status**

**Baseline TIL by site of biopsy**

`P=0.0004`

`P=0.0003`
Higher sTILs Associated with Higher ORR and DCR PD-L1 Positive Cohorts

Baseline TILs and ORR

Baseline Stromal TILs by Response

P=0.006

Baseline Stromal TILs by Disease Control

P=0.0006

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sTIL ≥5% as Potential Predictive Marker, PD-L1 Positive

- 44% of PD-L1 positive had sTIL ≥5%
- For sTIL≥5% v. sTIL<5%

**ORR**
- 39% vs. 5%
  - Sensitivity: 85.7%
  - Specificity: 61.8%
  - NPV: 95.5%
  - PPV: 31.6%

**DCR**
- 47% vs. 5%
  - Sensitivity: 90.0%
  - Specificity: 67.7%
  - NPV: 95.5%
  - PPV: 47.4%
Summary and Conclusions

• PANACEA study of pembrolizumab added to trastuzumab in trastuzumab-resistant mHER2+ patients met its primary objective in the PD-L1 positive cohort (n=46: ORR 15%, DCR 25%)
  – No responses observed in PD-L1 negative patients
  – Stromal TIL levels associated with response: high TIL≥5% patients (ORR 39%, DCR 47%)
  – For responders: pembrolizumab monotherapy with trastuzumab offers excellent QoL and durable control without chemotherapy

• Metastatic HER2+ disease in the heavily pretreated setting is a poorly immunogenic (majority of patients had low TILs in fresh biopsies from metastatic lesions)
  – Higher ORR observed than in metastatic TNBC (KN-086 CohortA)

• Future directions in IO in mHER2+ low TIL patients should focus on combinations with effective anti-HER2 therapy
Immunotherapy in TNBC: Neoadjuvant Setting

N = 272

Primary endpoint: Event-free survival (EFS)
Secondary endpoint: pCR (ypT0-ypTis ypN0)

nab-Paclitaxel 125 mg/m²
+ CBDCA AUC2

Surgery

+/- ATEZOLIZUMAB 1200 mg
Immunotherapy in TNBC: Neoadjuvant Setting

N = 174  
Primary endpoint: pCR (ypT0 ypN0)

- nab-Paclitaxel
- nab-P = 125 mg/m²
- Epirubicin 90 mg/m² + cyclophosphamide 600 mg/m²
- MEDI 4736/durvalumab 2g total q4w

Window of opportunity 2 weeks

EC

Surgery
Immunotherapy in TNBC: Neoadjuvant Setting

KEYNOTE-173 Phase I/II Trial

- **Cohort A (no platinum)**
  - Chemotherapy + anti-PD-1
  - Paclitaxel Q1W x 12 ± carboplatin Q1W x 12 + pembrolizumab Q3W x 4 → AC Q3W x 4 + pembrolizumab Q3W x 4
  - pCR = ypT0 ypN0
  - 60%

- **Cohort B (platinum)**
  - Chemotherapy + anti-PD-1
  - Paclitaxel Q1W x 12 ± carboplatin Q1W x 12 + pembrolizumab Q3W x 4
  - pCR = ypT0 ypN0
  - 80%

I-SPY-2 Trial

- **Control (no immunotherapy)**
  - Chemotherapy ± anti-PD-1
  - Paclitaxel Q1W x 12 + pembrolizumab Q3W x 4 → AC Q3W x 4
  - pCR = ypT0/is ypN0
  - 20%

- **Immunotherapy (no platinum)**
  - Chemotherapy ± anti-PD-1
  - Paclitaxel Q1W x 12 + pembrolizumab Q3W x 4
  - pCR = ypT0/is ypN0
  - 60%

Immunotherapy in TNBC: Adjuvant Setting

Immunotherapy in TNBC: Adjuvant Setting

**BRAVE Protocol**

- **TNBC** → **Neoadj Chemo** → **Surgery**
  - pCR: 40%
  - No pCR: 60%

- Options after Surgery:
  - 1. Placebo
  - 2. Radiotherapy
    - Option 2 can be followed by Avelumab.
Immunotherapy in BC

High risk breast cancer

High TILs/immune activation signature / PL1+/ High TMB

I-O as monotherapy or combination of I-O

High TMB

Add CT to enhance immunogenicity or STING, TIGIT, RT

Low TILs/immune activation signature / PDL1-

Low TMB

No I-O
Immunotherapy in BC

**Stage I-III**

**Neoadj**
- Chemo (ACT, AC)
- Aromatase Inhibitor (AI)

**Adjuvant**
- PARP (BRCA+)
- Next-gen SERM / SERDs (ESR1+)
- Next-gen SERM / SERDs (ESR1+)
- Herceptin + Chemo
- Perjeta + Herceptin + Chemo
- Herceptin + Chemo
- Perjeta + Herceptin + Chemo

**Stage IV**

**Met 1L**
- Next-gen SERM / SERDs (ESR1+)
- PD-(L)1 + HER2 + chemo
- CDK4/6 + AI or SERD
- Hormone therapy
- mTOR + AI

**Met 2L**
- PD-(L)1 + chemo?

**Met 3L+**
- PD-(L)1 + chemo?

**HR+ / HER2-**

**HR+ / HER2+**

**HR- / HER2+**

**HR- / HER2-**

- PARP (BRCA+)
- AKT?
- PD-(L)1 mono
**Triple-negative BC**

**Mutational load**

- Tumor sensitivity to immune effector
  - MHC expression, IFN-γ sensitivity

- Immune cell infiltration
  - Intratumoral T cells

- Immune competent patient
  - Lymphocyte count

- Absence of T cell checkpoints
  - PD-L1

- Absence of soluble inhibitors
  - Less inflammation
  - IL-6, CRP

- Limited tumor burden
  - LDH, glucose utilization

**Early Breast Cancer?**

Conclusions

- Is there a rationale for immune-based therapy in BC? **YES**
- Evidence from clinical data? **LIMITED**
- Can you enhance immunogenicity? **YES**
- Which the most promising setting? **EBC or 1st-Line MBC**
Thank You

Giuseppe Curigliano MD, PhD

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