key objectives

• Provide an overview of the current pharmacological approaches for harnessing the immune system to attack cancer

• Describe mechanisms by which chemotherapy can increase tumor immunity

• Introduce the concept of priming to maximize efficacy of immune checkpoint blockade, particularly in tumors with low immunogenicity
... over the last years, there has been a wave of successes with immunotherapy ...
Immune evasion is an emerging hallmark of cancer.
The cancer - immunity cycle

A) Release of cancer cells antigens (tumor cell death)
B) Cancer antigens presentation (fundamental role of dendritic antigen-presenting cells and professionals – APC)
C) APC and T cells priming and activation
D) Trafficking of cytotoxic T-cells to tumor
E) T lymphocyte infiltration into the tumor (cytotoxic T lymphocytes, endothelial cells)
F) Recognition of tumor cells by T lymphocytes
G) Death of the tumor cells

Therapies that target the cancer-immunity cycle

1. Release of cancer cell antigens
   - Chemotherapy
   - Radiation therapy
   - Targeted therapy

2. Cancer antigen presentation
   - Vaccines
   - IFN-α
   - GM-CSF
   - Anti-CD40 (agonist)
   - TLR agonists

3. Priming and activation
   - Anti-CTLA4
   - Anti-CD137 (agonist)
   - Anti-OX40 (agonist)
   - Anti-CD27 (agonist)
   - IL-2
   - IL-12

4. Trafficking of T cells to tumors

5. Infiltration of T cells into tumors
   - Anti-VEGF

6. Recognition of cancer cells by T cells
   - CARs

7. Killing of cancer cells
   - Anti-PD-L1
   - Anti-PD-1
   - IDO inhibitors

Chen and Mellman, Cell 2013
## Characteristics of immuno-oncology modalities

<table>
<thead>
<tr>
<th>Modality</th>
<th>Status</th>
<th>Pre-clinical findings</th>
<th>Pharmacokinetics</th>
<th>Pharmacodynamics</th>
<th>Clinical Efficacy</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines</td>
<td>IL-2 and IFNα approved but uncommonly used owing to high toxicity and low efficacy</td>
<td>Moderate effects</td>
<td>Clear kinetics</td>
<td>Multiple effects, MoA is complex and hard to attribute to one mechanism</td>
<td>Low</td>
<td>High unspecific toxicity (for example, whole body oedema)</td>
</tr>
<tr>
<td>Cellular therapies (CAR-Ts and TCR-Ts)</td>
<td>Multiple CAR-Ts and TCR-Ts in clinical trials; high complexity of manufacture and supply chain; strong target dependency; and few clinically effective targets (for example, CD19 and NY-ESO-1)</td>
<td>Moderate-to-strong effects</td>
<td><em>In vivo</em> tracing and longevity of infused cells</td>
<td>Clear MoA; target-dependent effects</td>
<td>High response rates depending on the target (up to 90% for CD19, 50–60% for NY-ESO-1)</td>
<td>Cytokine release syndrome; target-dependent cross-reactivity with healthy tissue</td>
</tr>
<tr>
<td>Vaccines</td>
<td>Many types of cancer vaccines in clinical trials (including peptides, proteins, viruses and cells)</td>
<td>Clear effects in mice, but these do not directly translate to humans</td>
<td>No direct pharmacokinetics for peptide- or protein-based vaccines</td>
<td>Measurable immune responses</td>
<td>Minimal as monotherapy; combinations to be explored</td>
<td>Minimal toxicity</td>
</tr>
<tr>
<td>Checkpoint-modulatory antibodies</td>
<td>Ipilimumab (targeting CTLA4), pembrolizumab (targeting PD1) and nivolumab (targeting PD1) approved; many compounds (including PDL1 blockers) in clinical investigation</td>
<td>Moderate effects</td>
<td>Clear kinetics</td>
<td>Universal mechanism not bound to histology, specific mutations or cancer antigens; multiple downstream effects after target engagement</td>
<td>Strong effects on survival with long-term survival in a subset of patients</td>
<td>Distinct irAEs; manageable with treatment algorithms</td>
</tr>
</tbody>
</table>

*Hoos, Nat Rev Drug Discov 2016*
Immunotherapy drug-development milestones (...from 6 months ago, but already outdated)

- Ipilimumab (Bristol-Myers Squibb)
  - Sipuleucel-T (Dendreon, now Valeant Pharmaceuticals)
- Pembrolizumab (Merck)
- T-vec (Amgen)
- Atezolizumab (Genentech/Roche)
- Multiple therapies under development
- Nivolumab (Bristol-Myers Squibb)
- Blinatumomab (Amgen)
- CAR-Ts (Novartis)
- Durvalumab (AstraZeneca)

FDA-Approved, 30th August 2017
FDA-Approved, PACIFIC phase III trial NEJM 8th September 2017

Hoos, 2016

Nature Reviews | Drug Discovery
Check-point inhibition

CTLA-4 Pathway Inhibition

PD-1 Pathway Inhibition
Immuno-oncology has driven recent volume of clinical activity (focus on checkpoint inhibitors)

SOURCE: McKinsey MIOSS, clinicaltrials.gov as of 6/30/2017, FDA, ACSO
A “combinatorial explosion”

Ipilimumab, the first approved checkpoint inhibitor, has been tested in dozens of clinical trials since 2001. And like many other drugs in its class, it is increasingly being tested in combination with other therapies.

Studies show improved survival in people with advanced melanoma.

US regulators approve ipilimumab for treatment of advanced melanoma.

Ledford, Nature 2016
Increasing role of combination therapies

Number of experiments across MOAs being tested in combination with PD-(L)1 or CTLA-4 therapies in registration or proof-of-concept trials¹

- Release of cancer cell antigens (cell death)
- Priming and activation of APCs and T-cells
- Cancer antigen presentation by APCs
- Trafficking / Infiltration of T-cells to tumors
- Recognition and killing of cancer cells by T-cells

- 85 unique mechanisms explored in combination with PD-(L)1 or CTLA-4
- In 2016, 353 unique experiments tested across these 85 MOAs

SOURCE: McKinsey Center for Asset Optimization MIOSS; Data as of 1/31/2017
NEW DRUGS for a more complex picture:

Multiple co-stimulatory and inhibitory interactions regulate T cell responses

Multiple new immunotherapies are being developed

- Targeted to tumour-specific antigens
- Rapid activation of the immune response
- Adaptable as the tumour mutates and evolves
- Self-propagating with each revolution of the cancer immunity cycle

Pardoll, 2012
...but not all cancers are created equal
Resistance to Immune-Checkpoint Blockade

Mutational landscape and sensitivity to PD-1 blockade

Rizvi et al. Science 2015

Tumor microenvironment

Pitt et al. Cell 2016

Junmtilla & de Sauvage, Nature 2013
Tumor immunogenicity and mutation load

Tumor with best outcome to immunotherapeutic approaches

Alexandrov et al. Nature 2013
Tumor immunogenicity and mismatch repair

- Sequencing of 385 unselected sporadic pancreas cancers defined a mean mutation load of 1.1-1.8 mutation/Mb
- 5 extreme outliers were classified as hypermutated as they contained ≥12 mutations/Mb
- IHC for mismatch repair proteins (MSH2, MSH6, MLH1, and PMS2) identified 4 MMR-deficient tumors, all hypermutated
Multifactorial biomarkers of clinical response to PD1 pathway blockade

PDL-1

PDL-2

CD3, CD8 and FoxP3 IF

Jordanova, Giovannetti et al. Unpublished data

Spatial computation of intratumoral cytotoxic T cells correlates with survival of patients with pancreatic cancer

Carstens et al. Nat Commun 2017
The stromal component

1. Anti-tumor T cell infiltration

Dense stroma
Chemokines

2. Tumor antigen recognition

Antigen loss
Off-target side effects

3. T cell activation, effector functions and persistence

Immunostimulating cytokines
e.g. IL-2, IL-7, IL-12, IL-15, IL-21

Immunosuppressive cells
e.g. MDSCs, T_{reg}

Immune checkpoints
e.g. CTLA-4, PD-1

Wayteck et al. Cancer Letters 2013
Lack of efficacy of check-points inhibitors in pancreatic cancer

• In a Phase I trial, 207 patients with solid tumours, including 14 with pancreatic cancer, were treated with the anti-PD-L1 antibody, nivolumab¹
  – Objective responses seen in NSCLC, RCC, ovarian cancer and melanoma
  – No response seen in pancreatic cancer

• In a Phase 2 trial, 27 patients with advanced/metastatic pancreatic cancer were treated with the anti-CTLA4 antibody, ipilimumab (3 mg/kg)²
  – No objective responses reported
  – 1 delayed response after PD observed
  – Unclear if sub-optimal dosing contributed to the poor efficacy

Disappointing tumour response to ipilimumab in patients with advanced pancreatic cancer²

1. Brahmer et al. NEJM 2012
2. Royal et al. J Immunother 2010
Targeting pancreatic cancer associated fibroblasts: A viable target to reduce immunosuppression?

Mace et al. OncoImmunology 2013
Enhanced efficacy with JAK1/2 inhibition in ‘inflammatory’ pancreatic cancer

### Overall survival (ITT)

<table>
<thead>
<tr>
<th></th>
<th>Ruxolitinib + cape (n=54)</th>
<th>Placebo + cape (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS, days</td>
<td>137</td>
<td>130</td>
</tr>
<tr>
<td>Survival rate, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>64</td>
<td>58</td>
</tr>
<tr>
<td>6 months</td>
<td>42</td>
<td>35</td>
</tr>
<tr>
<td>12 months</td>
<td>22</td>
<td>11</td>
</tr>
</tbody>
</table>

**Graph:**

- **Overall survival probability**
  - Ruxolitinib: Blue
  - Placebo: Red
  - HR=0.79 (95% CI:0.53-1.18)
  - 2-sided p=0.25

### Overall survival in patients with CRP >13 mg/L

<table>
<thead>
<tr>
<th></th>
<th>Ruxolitinib + cape (n=31)</th>
<th>Placebo + cape (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS, days</td>
<td>83</td>
<td>55</td>
</tr>
<tr>
<td>Survival rate, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>48</td>
<td>29</td>
</tr>
<tr>
<td>6 months</td>
<td>42</td>
<td>11</td>
</tr>
<tr>
<td>12 months</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

**Graph:**

- **Overall survival probability**
  - Ruxolitinib: Blue
  - Placebo: Red
  - HR=0.47 (95% CI:0.26-0.85)
  - 2-sided p=0.01

---

**Hurwitz et al. JCO 2015**
Phase 3 trials of 2nd line ruxolitinib + capecitabine in MPC with evidence of a systemic inflammatory response (JANUS 1 & 2)

Eligible Patients
- JANUS 1 = 310
- JANUS 2 = 270

Stratification
- mGPS 1 vs 2
- ECOG 0/1 vs 2

Treatment phase
- Treatment A
  - capecitabine 2000 mg/m²/d (d1-14) + ruxolitinib 15 mg bid q21d
- Treatment B
  - capecitabine 2000 mg/m²/d (d1-14) + placebo bid q21d

Follow-up phase
- Follow-up for anticancer therapies and OS at least every 6 weeks

Modified Glasgow Prognostic Score (mGPS)

<table>
<thead>
<tr>
<th>CRP or Albumin Value</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP ≤ 10 mg/L</td>
<td>0</td>
</tr>
<tr>
<td>CRP &gt;10 mg/L and albumin ≥35 g/dL</td>
<td>1</td>
</tr>
<tr>
<td>CRP &gt;10 mg/L and albumin &lt;35 g/dL</td>
<td>2</td>
</tr>
</tbody>
</table>

Primary endpoint: OS
Secondary endpoints: PFS, RR, DoR

O’Reilly ASCO 2015 abstract TPS4146
Hurwitz ASCO 2015 abstract TPS4147
Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival

Gore and Korc, Cancer Cell 2014
Özdemir et al. Cancer Cell 2014
Targeting CXCL12 from FAP-expressing carcinoma associated fibroblasts synergizes with anti-PD-L1

- In murine models, depleting FAP+ CAFs restored (1) immune control of PDAC growth and (2) antitumor effects of α-CTLA-4 and α-PD-L1
- Chemokine ligand 12 (CXCL12) may be responsible for immunosuppression by FAP+ cells:
  - Cancer cells were coated with CXCL12
  - FAP+ CAF was the principal source of CXCL12 in the tumour
- **AMD3100**, a CXCL12 receptor chemokine (C-X-C motif) inhibitor, induced rapid T-cell accumulation among cancer cells and acted synergistically with α-PD-L1
Emerging evidence for combination strategies with immune checkpoint inhibitors

• Despite the recent “misstep” with the MYSTIC trial (...and waiting for the final data from the CHECK MATE trials)

• Various combinations with immune checkpoint inhibitors are being explored, including:
  – CXCL12 receptor chemokine inhibitors
  – Vaccines
  – CD40 agonists
  – Chemotherapy (already with a very successful story: KEYNOTE-021g trial)
Combination of immunotherapy with chemo- and targeted therapy
Chemotherapy and targeted therapy modulate the key players in the immune regulation of tumor growth.
Immunological effects of anticancer therapies

A

Immunosuppressive cells
- MDSCs
- T_{reg} Cells
- M2 TAMs

Indirect immunostimulation
- 5-Fluorouracil
- Cyclophosphamide
- Docetaxel
- Gemcitabine
- Oxaliplatin
- Paclitaxel
- Bevacizumab
- Dasatinib
- Decitabine
- Lapatinib
- Sorafenib
- Sunitinib

Direct immunostimulation
- Immune effector cells
- NK Cells
- CTLs

Increased immunogenicity
- Poorly immunogenic tumor
- 5-Fluorouracil
- Doxorubicin
- Gemcitabine
- Idarubicin
- Oxaliplatin
- Radiation
- Cetuximab
- Dabrafenib
- Decitabine
- Erlotinib
- Gefitinib
- Trametinib

B

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Galluzzi et al. 2017
Effects of **chemotherapy** on tumor-specific immune response

![Diagram showing the effects of chemotherapeutic agents on the immune system and cancer cells.](image)
Chemotherapy stimulates immune-based anti-cancer activity through multiple mechanisms

Chemotherapy mediated activation of anticancer immune responses

Chemotherapy may stimulate the immune system by:
- Lysing tumor cells to create an endogenous cancer vaccine\(^1,\)\(^2\)
- Activating dendritic cells\(^3\)
- Depleting immunosuppressive Tregs at low doses\(^4\)
- Increasing tumor-infiltrating lymphocytes (TILs)\(^5\)

Chemotherapy increased TIL number following neoadjuvant therapy in 278 patients with TNBC\(^5\)
- Higher numbers of TILs were significantly associated with longer 5-year survival rates\(^5\)

# Immune response and chemotherapy: an example of a potentially successful combination strategy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect on immune system</th>
</tr>
</thead>
</table>
| **Taxanes**    | • Enhances T cell and NK cell function  
                    • Increases recruitment of TIL  
                    • Increase efficacy of immuno-stimulatory agents                                                      |
| **Doxorubicin**| • Induces immunogenic cell death  
                    • Increases proliferation of CD8 T cells  
                    • Stimulates antigen presentation by DCs  
                    • Stimulates MCP1 and M6PR                                                                        |
| **Cyclophosphamide** | • Induces immunogenic cell death  
                                      • Suppresses Treg inhibitory functions and restores the proliferative capacity of effector T cells and NK cell cytotoxicity |
| **Gemcitabine** | • Reduces the number of myeloid suppressor cells  
                                   • Increases the antitumor activity of CD8(+) T cells and activated NK cells                     |
| **Oxaliplatin** | • Induces immunogenic cell death  
                                      • Increases MHC I complex  
                                      • Inhibits PD-L2                                                                                 |
**Checkpoint inhibition + CD40 agonist + nab-paclitaxel/gemcitabine: Preclinical data**

- T cell stimulation with CD40 agonist mAB + nab-paclitaxel/gemcitabine renders PDAC highly sensitive to immune checkpoint blockade
- CD40/nab-paclitaxel/gemcitabine/PD-1/CTLA-4 leads to complete tumour rejection and long-term tumour-free survival in a KPC mouse model
  - PD-1/CTLA-4: 5% long-term survivors
  - CD40/nab-paclitaxel/gemcitabine: 12% long-term survivors
  - CD40/nab-paclitaxel/gemcitabine/PD-1/CTLA-4: 39% long-term survivors

Combining DNA damaging therapeutics with immunotherapy: more haste, less speed

- **Choice of agent.** DNA damaging agents are not equally immunogenic

- **Dose.** Carefully designed trials need to consider testing whether maximal tumor cell death (at the MTD) should be compromised in an effort to spare immunoreactive T-cell populations

- **Scheduling and sequencing of combinations.** It might be advantageous to prime the immune system, administering DNA damaging agents up front, followed by a period of concurrent treatment

- **Toxicity.** Most toxicities do not overlap, but many DNA damaging chemotherapy regimens incorporate significant doses of corticosteroids, whose immuno-suppressive effects have the potential to attenuate the effects of the immune checkpoint inhibitors

- **Biomarkers.** Utilizing PD biomarkers should be a compulsory component of early-phase combination studies in order to determine optimal doses and scheduling

*Brown et al. Br J Cancer 2018*
Chemotherapy combination trials with current PD-1 and PD-L1 checkpoint inhibitors are actually testing every standard of care chemotherapy regimen in every tumour type, as registered with www.clinicaltrials.gov.

Inflamed tumors might demonstrate high levels of effector T cells (green), APCs (orange) and MDSCs (purple), with low PD-L1 expression and may respond to immune checkpoint inhibitor (ICI) monotherapy, requiring combination treatment with DNA damaging (DD) agents on progression only.

Conversely immune desert tumors that may require **priming with DD agents** followed by concurrent treatment with an ICI.
Biological rationale behind combining immune checkpoint blockade with targeted therapies in melanoma and NSCLC

Karachaliou et al. ATM 2017
A thorough preclinical assessment of the mechanism of action and risks associated with each potential combination of targeted therapy and immunotherapy may help limit the severity and incidence of toxicities in the clinic, as well as inform dose-sequencing and clinical-monitoring paradigms.
A survey of **13,349 genomic profiles** from public databases for cases with specific mutations targeted by current agents or a burden of exome-wide nonsynonymous mutations (NsM) that exceed a proposed threshold for response to checkpoint inhibitors.
Future prospects: rationally designed combinations and biomarkers

The approval of several immunotherapies has engendered a new-found awareness of the potential antitumour activity of a patient's endogenous immune system once the 'brakes' elicited by the immune system have been released (Pardoll, Nat Rev Cancer 2012)

Immune checkpoints are a tiny fraction of the receptors/ligands that inhibit immune responses at various levels. The opportunities to explore the plethora of potential immunotherapy targets brings forth 2 challenges:

1) the clinical development (based on strong preclinical studies) of optimal pharmacological targeting and combinatorial approaches
2) the definition of potential biomarkers that can guide the therapeutic choice

Zitvogel et al. Immunity 2013
New biomarkers for mono-/combination therapies:
1) the cancer immunogram
2) Swarm intelligence-enhanced algorithm of RNA-seq data using blood-based liquid biopsies

Blank et al. Science 2016
Best et al. Cancer Cell 2017