Cancer immunotherapy: from translational research to clinical application of checkpoint inhibitors

John Haanen MD PhD

ESMO IO Preceptorship Zürich, May 2019
MY DISCLOSURES

• I have provided consultation, attended advisory boards, and/or provided lectures for: Amgen, AZ/Medimmune, Bayer, BMS, GSK, Ipsen, Merck Serono, MSD, Novartis, Pfizer, Roche/Genentech, Sanofi, Seattle Genetics for which NKI received honoraria.

• I am on the SAB of AIMM, Celsius Therapeutics, Immunocore and Neon Therapeutics.

• Through my work NKI received grant support from Bayer, BMS, MSD, Novartis, Neon Therapeutics, Pfizer.

• I am member of ESMO, ASCO, AACR.

• I am member of ESMO W4O committee, ESMO Press committee, ESMO Educational Committee.

• I am scientific (co-)chair of ESMO IO congress 2019, MAP 2019, and ESMO congress 2020.

• I am member of the scientific committee of ECIC 2019 and ITOC6 2019.

• I am faculty chair of ESMO Immunotherapy of Cancer.

• I am Editor-in-Chief of ESMO IOTECH.

• I am member of the advisory board of “Stichting Melanoom”.

• I am on the board of the Dutch Melanoma Treatment Registry (DMTR).
Cancer Immunotherapy

...fighting cancer but ignoring the tumor...

...unleashing or harnessing the immune system to combat cancer!
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency


Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma


Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC

IMMUNOTHERAPY
THE BIG HOPE FOR CANCER TREATMENT?
The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation."
A Believer’s Overview of Cancer Immunosurveillance
A Believer’s Overview of Cancer Immunotherapy
Cancer immunity cycle

- No immunogenic cell death
- No STING or cGAS activation
- No strong antigens
- No DCs
- Immature DCs
- No LN trafficking
- No T cell repertoire
- T regs
- No activation of tumor-reactive T cells (CTLA-4)
- Tumor T cells trafficking
- Blockade to infiltrate into tumor (vasculature, stroma)
- T cells undergo apoptosis at arrival (Fas-induced)
- No killing because PD-L1 or PD-L2 expression
- Tumor cells lost MHC or are insensitive to IFN-γ or TNF-α
- Other immunosuppressive factors: TGF-b, IL-10, Tregs, Arg-1, TAM-2, IDO, arginase, low pH, adenosine, etc
Mechanism of action of immune checkpoint blockade

- CTLA-4 suppresses T cell activation and inhibits T cell function
- CTLA-4 regulates T cell tolerance
  -- CTLA-4 KO mice develop lethal lympho-proliferative syndrome
Negative co-stimulation not only attenuates T cell activation, but also constraints T cell differentiation

CTLA-4 constraints the formation of phenotypic and functional T cell states

CTLA-4 does this primarily for CD4 T cells

CTLA-4 limits amount expression of produced cytokines

CTLA-4 blockade allows peripheral oligoclonal T cells expansion

Spencer et al., Immunity 2019
T cell activating and inhibiting molecules and signals

Combination Immunotherapy of B16 Melanoma Using Anti-Cytotoxic T Lymphocyte-associated Antigen 4 (CTLA-4) and Granulocyte/Macrophage Colony-Stimulating Factor (GM-CSF)-producing Vaccines Induces Rejection of Subcutaneous and Metastatic Tumors Accompanied by Autoimmune Depigmentation

By Andrea van Elsas, Arthur A. Hurwitz, and James P. Allison
Prevention of outgrowth and rejection of aggressive B16 melanoma using CLTA-4 blockade and vaccine
Combination of anti-CTLA-4 + vaccine leads to rejection of pulmonary metastases and to immune infiltrates

van Elsas et al., J Exp Med 1999
Melanoma bearing mice successfully treated with anti-CTLA4 and vaccine develop vitiligo
Human CTLA4 blockade

- **Ipilimumab, IgG1 human mAb, selected for its lack of ADCC/CDC activity**
  - Administered every 3 weeks x 4, 3 mg/kg
  - Showed improvement in mOS of 4 months in metastatic melanoma
  - Approved in 2011

- **Tremelimumab, IgG2 human mAb**
  - Administered every 3 months, 15 mg/kg
  - Failed to show improvement in mOS
Efficacy of ipilimumab as first line treatment

ORR: 11.9%
CTLA-4 blockade (ipilimumab) can induce long-term survival
(pooled overall survival analysis including Expanded Access Program data from 4846 patients)

Median OS (95% CI): 9.5 months (9.0–10.0)

3-year OS rate (95% CI): 21% (20%–22%)
Treatment with anti-CTLA-4 mAb
Immune related adverse events upon anti-CTLA-4 mAb treatment

Haanen, unpublished
How does CTLA4 blockade lead to anti-tumor immune responses?

Two not mutually exclusive mechanisms have been proposed:

1. Priming of tumor-reactive T cells
   - Against shared tumor associated antigens
   - Against mutated (neo) antigens

2. Depletion of regulatory FoxP3+ T cells from the tumor microenvironment
What could tumor-specific cytotoxic T cells detect on human cancer?

1. Self antigens (to which tolerance is incomplete)
   *Shared between patients*

2. ‘Neo-antigens’, epitopes that arise as a consequence of tumor-specific mutations
   *In large part patient-specific, hence generally ignored*
TWO MODELS PROPOSED

Kvistborg et al., Science Transl Med 2014
Analysis of PBMC from 40 ipilimumab treated melanoma patients for 75 tumor associated antigens

75 HLA-A*0201 binding epitopes coming from:

- Lineage antigens
- Cancer/Germlines antigens
- Overexpressed antigens
- Few shared mutated antigens
- Few others

Kvistborg et al., Science Transl Med 2014
Flow results…

Pretherapy

MART-1$_{ELA}$

Posttherapy

MAGE-C2$_{ALK}$

GNT-V$_{YLP}$

Kvistborg et al., Science Transl Med 2014
Ipilimumab treatment leads to broadening of the anti-cancer IR

| Patient | Pt21 | Pt22 | Pt23 | Pt24 | Pt25 | Pt26 | Pt27 | Pt28 | Pt29 | Pt30 | Pt31 | Pt32 | Pt33 | Pt34 | Pt35 | Pt36 | Pt37 | Pt38 | Pt39 | Pt40 |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Time point | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| MART-1 ELA | | | | | | | | | | | | | | | | | | | | |
| Tnpz 100 | | | | | | | | | | | | | | | | | | | | |
| gp100 M20 | | | | | | | | | | | | | | | | | | | | |
| gp100 YF | | | | | | | | | | | | | | | | | | | | |
| gp100 F2W | | | | | | | | | | | | | | | | | | | | |
| MAGE C2 | | | | | | | | | | | | | | | | | | | | |
| MAGE C2 | | | | | | | | | | | | | | | | | | | | |
| MAGE C2 | | | | | | | | | | | | | | | | | | | | |
| MAGE A2 | | | | | | | | | | | | | | | | | | | | |
| LAGE-1 NM | | | | | | | | | | | | | | | | | | | | |
| LAGE-1 SL | | | | | | | | | | | | | | | | | | | | |
| SSX-2 K1S | | | | | | | | | | | | | | | | | | | | |
| RAGE-1 NL | | | | | | | | | | | | | | | | | | | | |
| NY-ESO-1 | | | | | | | | | | | | | | | | | | | | |
| Gntv VLP | | | | | | | | | | | | | | | | | | | | |
| PRDXS3 MA | | | | | | | | | | | | | | | | | | | | |
| Meloe TelN | | | | | | | | | | | | | | | | | | | | |
| LGAL3 RD | | | | | | | | | | | | | | | | | | | | |
| Bing-4 CW | | | | | | | | | | | | | | | | | | | | |
| ATIC RL | | | | | | | | | | | | | | | | | | | | |
| tsstb | | | | | | | | | | | | | | | | | | | | |

Kvistborg et al., Science Transl Med 2014
T cell responses against shared tumor antigens
What could tumor-specific cytotoxic T cells detect on human cancer?

1. Self antigens (to which tolerance is incomplete)
   *Shared between patients*

2. ‘Neo-antigens’, epitopes that arise as a consequence of tumor-specific mutations
   *In large part patient-specific, hence generally ignored*
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

1. Generate map of tumor-specific mutations (ExomeSeq)
2. Determine which mutated genes are expressed (RNASeq)
3. Predict epitopes for each mutation/each HLA-allele in silico
4. Screen for T cell recognition of mutated epitopes
Pt NKI-002: Partial response upon anti-CTLA4 treatment

Van Rooij et al., J Clin Oncol 2013
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

Resected tumor material → Isolate tumor cells → Identify tumor-specific mutations

Graph showing the percentage of non-synonymous mutations with different mutation types (n=1036)
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

- Resected tumor material
- Isolate tumor cells
- Isolate tumor-infiltrating T cells
- Identify tumor-specific mutations
- Predict potential epitopes
- Screen with MHC multimer technology
Strong T cell response against an ATR\textsubscript{S>L} neo-epitope within the tumor

Resected tumor material

Isolate tumor cells

Isolate tumor-infiltrating T cells

Identify tumor-specific mutations

Predict potential epitopes

Screen with MHC multimer technology
Increased magnitude of neo-antigen-specific T cell response under anti-CTLA4

van Rooij et al., J Clin Oncol 2013
How does CTLA4 blockade lead to anti-tumor immune responses?

Two not mutually exclusive mechanisms have been proposed:

1. Priming of tumor-reactive T cells
   • Against shared tumor associated antigens
   • Against mutated (neo) antigens
2. Depletion of regulatory FoxP3+ T cells from the tumor microenvironment
Anti–CTLA-4 therapy requires an Fc domain for efficacy

Jessica R. Ingram, Olga S. Blomberg, Mohammad Rashidian, Lestat Ali, Scott Garforth, Elena Fedorov, Alexander A. Fedorov, Jeffrey B. Bonanno, Camille Le Gall, Stephanie Crowley, Camilo Espinosa, Tamara Biary, Edmund J. Keliher, Ralph Weissleder, Steven C. Almo, Stephanie K. Dougan, Hidde L. Ploegh, and Michael Dougan

Department of Cancer Immunology and Virology, Dana–Farber Cancer Institute, Boston, MA 02215; Program in Cellular and Molecular Medicine, Children’s Hospital Boston, Boston, MA 02115; Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461; Division of Gastroenterology, Massachusetts General Hospital, Boston, MA 02114; and Department of Radiology, Massachusetts General Hospital, Boston, MA 02114

Contributed by Hidde L. Ploegh, March 5, 2018 (sent for review January 29, 2018; reviewed by John B. A. G. Haanen and Karl Dane Wittrup)
VHH H11 binds to a conformational epitope on the mCTLA-4 surface and interferes with B7 binding

Ingram et al., PNAS 2018
Anti-CTLA-4 VHH or VHH-dimer as PET tracer shows uptake in tumor but not in lymphoid tissue, but does not exert anti-tumor effects.
PD1 and PD-L1 checkpoint

Freeman & Sharpe. Nat Immunol 2013
Programmed Death-1 receptor (PD1)

- Discovered in 1992 by Honjo and coworkers
  - Upregulated gene in relation to apoptosis
- Member of the Ig superfamily
- Cytoplasmic domains with ITIM and ITSM
  - Recruits phosphatases
  - Inhibits PI3K and AKT activity
- Inducibly expressed by CD4 and CD8 T cells, NKT cells, B cells, monocytes and subtypes of DC
- Expressed by both effector and regulatory T cells
- PD1/PD-L1 interaction involved in tolerance and chronic inflammation
- PD1/PD-L1 contributes to functional T cell exhaustion during chronic infection and cancer
Development of Lupus-like Autoimmune Diseases by Disruption of the PD-1 Gene Encoding an ITIM Motif-Carrying Immunoreceptor

Hiroyuki Nishimura,1 Masato Nose,9 Hiroshi Hiai,7 Nagahiro Minato,5 and Tsukui Honjo4

Autoimmune Dilated Cardiomyopathy in PD-1 Receptor–Deficient Mice

Hiroyuki Nishimura,1 Taku Okazaki,1 Yoshimasa Tanaka,2 Kazuki Nakatani,6 Masatake Hara,3 Akira Matsumori,3 Shigetake Sasayama,3 Akira Mizoguchi,4 Hiroshi Hiai,5 Nagahiro Minato,2 Tsukui Honjo1

Engagement of the PD-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation

By Gordon J. Freeman,* Andrew J. Long,† Yoshiko Iwai,§ Karen Bourque,¶ Tatyana Chernova,* Hiroyuki Nishimura,§ Lori J. Fitz,† Nelly Malenkovich,* Taku Okazaki,§ Michael C. Byrne,§ Heidi E. Horton,† Lynette Fouser,‡ Laura Carter,‡ Vincent Ling,‡ Michael R. Bowman,‡ Beatriz M. Carreno,‡ Mary Collins,‡ Clive R. Wood,‡ and Tsukku Honjo§

[Graphs showing data on lymphocyte activation and regulation with anti-CD3 and PD-L1 antibodies.]

Freeman et al., J Exp Med 2000
Adoptive cell transfer of tumor-specific TCR transgenic 2C PD-1/- T cells rejected tumor cells

Blank et al., Cancer Res 2004
PD-1 pathway inhibits T cell response directly downstream of the TCR

Freeman PNAS 2008
PD1/PD-L1 play a role at the tumor/effector phase

Checkpoint molecules PD-1/PD-L1

- Nivolumab: anti-PD-1
- Pembrolizumab: anti-PD-1
- Cemiplimab: anti-PD-1
- Spartalizumab: anti-PD-1
- Atezolizumab: anti-PD-L1
- Durvalumab: anti-PD-L1
- Avelumab: anti-PD-L1
## Expression of PD1 ligands

<table>
<thead>
<tr>
<th></th>
<th>PD-L1 (B7-H1)</th>
<th>PD-L2 (B7-DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematopoietic cells</strong></td>
<td>DC, macrophages, B cells, T cells, BM-derived mast cells</td>
<td>DC, macrophages, B cells, Th2 cells, BM-derived mast cells</td>
</tr>
<tr>
<td><strong>Non-hematopoietic cells</strong></td>
<td>Vascular endothelium, epithelia, muscle, liver, pancreatic islets, placenta, eye</td>
<td>Few, airway epithelia</td>
</tr>
<tr>
<td><strong>Stimuli</strong></td>
<td>Interferons (a, b, g)</td>
<td>IL-4 + GM-CSG</td>
</tr>
<tr>
<td><strong>Binding partners</strong></td>
<td>PD1, B7.1</td>
<td>PD1</td>
</tr>
<tr>
<td><strong>Expression by tumors</strong></td>
<td>Melanoma, RCC, HNSCC, ovary, NSCLC</td>
<td>Expression also found on tumors</td>
</tr>
</tbody>
</table>
PD-L1 on human tumor cells mediates T cell inhibition

Pardoll DM, Nat Rev Cancer 2012
Expression of PD-L1 co-localizes with TILs

Taube et al. Science Transl Med 2012
1 PEMBROLIZUMAB

Baseline: April 13, 2012

April 9, 2013

72-year-old male with symptomatic progression after bio-chemotherapy, HD IL-2, and ipilimumab
Anti-PD1 Demonstrates Broad Antitumor Activity

### Major indications of anti-PD1/PDL1 therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Indication</th>
<th>Objective response rate (%)</th>
<th>Agents approved*</th>
<th>Main driver of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>High response rate</td>
<td>Hodgkin’s disease</td>
<td>87</td>
<td>nivolumab, pembrolizumab</td>
<td>PDJ amplicon</td>
</tr>
<tr>
<td>Desmoplastic melanoma</td>
<td>70</td>
<td>nivolumab, pembrolizumab</td>
<td></td>
<td>Mutations from chronic sun exposure</td>
</tr>
<tr>
<td>Merkel cell</td>
<td>56</td>
<td>pembrolizumab</td>
<td></td>
<td>Merkel cell virus</td>
</tr>
<tr>
<td>MSI-h cancers</td>
<td>53</td>
<td>nivolumab, pembrolizumab</td>
<td></td>
<td>Mutations from mismatch-repair deficiency</td>
</tr>
<tr>
<td>Intermediate response rate</td>
<td>Skin melanoma</td>
<td>35 to 40</td>
<td>nivolumab, pembrolizumab</td>
<td>Mutations from intermittent sun exposure</td>
</tr>
<tr>
<td>NSCLC</td>
<td>20</td>
<td>atezolizumab, nivolumab, pembrolizumab</td>
<td></td>
<td>Mutations from cigarette smoking</td>
</tr>
<tr>
<td>Head and neck</td>
<td>15</td>
<td>nivolumab, pembrolizumab</td>
<td></td>
<td>Mutations from cigarette smoking</td>
</tr>
<tr>
<td>Gastroesophageal</td>
<td>15</td>
<td>pembrolizumab</td>
<td></td>
<td>Mutations from cigarette smoking</td>
</tr>
<tr>
<td>Bladder and urinary tract</td>
<td>15</td>
<td>atezolizumab, avelumab, durvalumab, nivolumab, pembrolizumab</td>
<td></td>
<td>Mutations from cigarette smoking</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>25</td>
<td>nivolumab, pembrolizumab</td>
<td></td>
<td>Insertions and deletions (indels)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>20</td>
<td>nivolumab, pembrolizumab</td>
<td></td>
<td>Hepatitis virus</td>
</tr>
</tbody>
</table>

*In alphabetical order

Ribas & Wolchok Science 2018
Nivolumab in Previously Untreated Melanoma without BRAF Mutation

Caroline Robert, M.D., Ph.D., Georgina V. Long, M.D., Ph.D., Benjamin Brady, M.D., Caroline Dutriaux, M.D., Michele Maio, M.D., Laurent Mortier, M.D., Jessica C. Hassel, M.D., Piotr Rutkowski, M.D., Ph.D., Catriona McNeil, M.D., Ph.D., Ewa Kalinka-Warzocha, M.D., Ph.D., Kerry J. Savage, M.D., Micaela M. Hernberg, M.D., Ph.D., Celeste Lebbé, M.D., Ph.D., Julie Charles, M.D., Ph.D., Catalin Mihalciou, M.D., Vanna Chiarion-Sileni, M.D., Cornelia Mauch, M.D., Ph.D., Francesco Cognetti, M.D., Ana Arance, M.D., Ph.D., Henrik Schmidt, M.D., D.M.Sc., Dirk Schadendorf, M.D., Helen Gogas, M.D., Lotta Lundgren-Eriksson, M.D., Christine Horak, Ph.D., Brian Sharkey, Ph.D., Ian M. Waxman, M.D., Victoria Atkinson, M.D., and Paolo A. Ascierto, M.D.
Nivolumab improves PFS and OS compared to dacarbazine
Are there any predictive markers for PD1/PDL1 blockade?

- PD-L1 expression
- Tumor mutational burden
- T cell gene expression profile
Requirements for a response to anti-PD1: CD8+ TILs

IHC Analysis of CD8+ T-cells in samples obtained before and during anti-PD1 treatment

Tumeh et al. Nature 2014
Predictive biomarkers of response to IO

Hodgkin lymphoma

9p24 amplification

↑ PD-L1

↑ JAK2

↑ Immune evasion

↑ Proliferation

PD-L1 expression RS cells

Antibody to PD1

Nivolumab in Hodgkin’s disease

Ohad Benjamini et al. Blood 2016; Kasamon et al. The Oncologist 2017
Predictive biomarkers of response to IO

>1,000 DNA mutations (>26 mut/MB)

↑ Neoantigens

↑ T cell response

dMMR cancers

Ipilimumab + nivolumab in MSI-H CRC

Overman et al. J Clin Oncol 2018
PD-L1 and blood TMB are Independent Biomarkers

Results from the MYSTIC trial in NSCLC

bTMB values did not correlate with tumor PD-L1 expression levels

*Spearman’s rho = 0.05, Pearson’s r = 0.01, N=809

*Percentages are calculated from the ITT (n=1118). Reference line in correlation plot is from linear regression.
Predictive biomarkers of response to IO: A complex continuum

Garon, AACR 2015; Lopes, ASCO 2018; Peters, AACR 2019
Predictive biomarkers of response to IO: A complex continuum

PD-L1

CheckMate-066  
CheckMate-067

NIVO+IPI  
NIVO  
IPI

Melanoma

HR for OS by TMB status

HR (95% CI)
NIVO+IPI  
High vs low
NIVO  
High vs low
IPI  
High vs low

-0.5 0.5 1.5
HR

TMB

NIVO PD-L1  
NIVO+IPI PD-L1  
DTIC PD-L1

NIVO PD-L1  
NIVO+IPI PD-L1  
DTIC PD-L1

TMB high  
TMB low

NIVO  
IPI

Hazard ratios expressed as PD-L1 ≥5% over PD-L1 <5%
CI = confidence interval; HR = hazard ratio; mo = month; NR = not reached
IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade

CheckMate 067: Relationship Between TMB and BMS 4-Gene Inflammatory Signature Score

The BMS 4-gene inflammatory signature comprises CD274 (PD-L1), CD8A, LAG3, and STAT1; includes patients not evaluable for best overall response. 

- TMB and inflammatory signature score were not correlated (r = 0.27 [95% CI, 0.15–0.38], all arms combined) and appear to be independent markers of response to I-O therapy.

- High TMB and high inflammatory signature score were each associated with increased CR/PR for NIVO+IPI and NIVO, relative to IPI alone.

Presented by Hodi at AACR 2019

\*The BMS 4-gene inflammatory signature comprises CD274 (PD-L1), CD8A, LAG3, and STAT1; includes patients not evaluable for best overall response. 

r, correlation coefficient.
The Cancer Immunogram

Describing the state of Cancer - Immune interaction

- Tumor foreignness
  - Mutational load
  - Tumor sensitivity to immune effectors
    - MHC expression
    - IFN-γ sensitivity
- General immune status
  - Lymphocyte count
- Absence of inhibitory tumor metabolism
  - LDH, glucose utilization
- Absence of soluble inhibitors
  - IL6->CRP/ESR
- Immune cell infiltration
  - Intratumoral T cells
- Absence of Checkpoints
  - PD-L1

Blank et al. Science 2016
THANK YOU FOR YOUR ATTENTION!

QUESTIONS?