Management of Advanced Hepatocellular Carcinoma:
At the Dawn of a New Era?

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Challenges in Management of HCC

One patient with two diseases

- A highly malignant tumor
  - High propensity for venous invasion
  - Rapid growth (tumor volume doubling time 3 months)

- Associated cirrhosis (80%)
  - Impaired liver function

Multicentric hepatocarcinogenesis
Doxorubicin

TREATMENT OF HEPATOCELLULAR CARCINOMA WITH ADRIAMYCIN
Preliminary Communication

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EDWARD KATONGOLE-MBIDDE, MB, CHB,‡ JOSUA MUGERWA, MD,†
SEBASTIAN K. KYALWAZI, FRCS(Ed),¶ AND HERMAN COHEN, PhD**

In a Phase II clinical trial, 14 patients with histologically proven primary hepatocellular carcinoma were treated with Adriamycin administered intravenously at a dose of 75 mg/m² every 3 weeks. All 11 evaluable patients responded with 3 exhibiting complete tumor regression after two, three, and five courses of Adriamycin respectively. The remission durations for these 3 were 3, 6, and 7 months, and their survivals were 8, 9, and 13 months, respectively. The median survival of the evaluable patients is 8 months (range 1–13 months). The side effects encountered included myelosuppression, anorexia, nausea, vomiting, and alopecia. Adriamycin seems to be an effective agent in hepatocellular carcinoma. Further trials are underway to test its true efficacy both singly and in combination with other drugs in the management of this tumor.

Systemic Chemotherapy

- Response rate of monotherapy (epirubicin, doxorubicin, cisplatin, 5-FU) < 20%
- Significant toxicity
- No confirmed survival benefit in randomized controlled trials
Multiple Cellular Signaling Pathways Are Implicated in the Pathogenesis of HCC

Phase III SHARP and AP trials
Sorafenib vs placebo in advanced HCC

**SHARP**
- **Sorafenib (n=299)**: Median OS: 10.7 months
- **Placebo (n=303)**: Median OS: 7.9 months
- Survival probability: HR=0.69

**Asia-Pacific**
- **Sorafenib (n=150)**: Median OS: 6.5 months
- **Placebo (n=76)**: Median OS: 4.2 months
- Survival probability: HR=0.68

HR, hazard ratio; OS, overall survival; SHARP, Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol.
**Sorafenib: Indications**

- Sorafenib is the first-line treatment of advanced stage patients (portal vein invasion or extrahepatic spread) who are not suitable for locoregional therapy and with Child-Pugh A cirrhosis.

**Caveats**

- Response rate of only 3% and disease control rate of 28%
- Median survival of 5 months in Asian population
Approval scenario of key marketed drugs in CRC market

- **1996**: CAMPTOSAR
- **1998**: CAMPTOSAR + 5-FU/L, 1st line
- **1999**: AVASTIN + 5-FU/L, 1st line
- **2000**: ELOXATIN, 1st line
- **2001**: ELOXATIN, 1st line & Adjuvant, Stage III
- **2002**: ERBITUX + irinotecan & single agent, 2nd line
- **2003**: VECTIBIX
- **2004**: XELODA, Adjuvant in colon cancer
- **2005**: AVASTIN + 5-FU, 2nd line
- **2006**: Sorafenib
- **2007**: Regorafenib

Approval scenario of key marketed drugs in HCC market

- **1975**: Adriamycin
- **1996**: CAMPTOSAR 2nd line
- **2000**: ELOXATIN, 2nd line
- **2013**: Regorafenib
## Summary of Failed Phase III Trials for HCC

<table>
<thead>
<tr>
<th>Agent</th>
<th>MOA</th>
<th>Patient Population</th>
<th>Trial Design</th>
<th>Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brivanib[^1^-3]</td>
<td>VEGF and FGF inhibitor</td>
<td>• BRISK-FL: 1L unresectable; CP A</td>
<td>• 1L: briv vs sorafenib (N=1150)</td>
<td>• 1L: mOS=9.5 vs 9.9 mo (HR 1.06 [95.8% CI: 0.93–1.22])</td>
<td>• Did not improve survival over sorafenib in 1L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• BRISK-PS: 2L after sorafenib; CP A/B</td>
<td>• 2L: briv vs BSC(N=395)</td>
<td>• 2L: mOS=9.4 vs 8.2 mo (HR 0.89, P=0.3307)</td>
<td>• Did not meet primary endpoint (OS) in 2L or as adjuvant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• BRISK-TA: adjuvant after 1st TACE; CP A/B</td>
<td>• Adjuvant: briv vs placebo (N= 870)</td>
<td>• Adjuvant: mOS=26.4 vs 26.1 mo (HR 0.9, P=0.528)</td>
<td></td>
</tr>
<tr>
<td>Linifanib[^4]</td>
<td>VEGFR and PDGFR inhibitor</td>
<td>• 1L unresectable/metastatic HCC</td>
<td>Linifanib vs sorafenib (N=1035)</td>
<td>mOS=9.1 vs 9.8 mo (HR 1.046 [95% CI: 0.896–1.221])</td>
<td>• OS inferior to sorafenib</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• CP A</td>
<td></td>
<td></td>
<td>• Safety results favored sorafenib</td>
</tr>
<tr>
<td>Sunitinib[^5,6]</td>
<td>VEGFR, PDGFR, FLT3R, KIT, and RET inhibitor</td>
<td>• 1L advanced liver cancer CP A</td>
<td>Sunitinib vs sorafenib (N=1074)</td>
<td>mOS=7.9 vs 10.2 mo (HR 1.3, one-sided P=0.9990)</td>
<td>• OS inferior to sorafenib</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• CP A</td>
<td></td>
<td></td>
<td>• Associated with more frequent and severe toxicities</td>
</tr>
<tr>
<td>Orantinib[^7]</td>
<td>VEGFR2, FGFR2, and PDGFR inhibitor</td>
<td>Unresectable HCC</td>
<td>TACE + orantinib vs placebo (N=889)</td>
<td>mOS=NA</td>
<td>• Did not meet primary endpoint (OS)</td>
</tr>
</tbody>
</table>

Multiple multikinase inhibitors have failed to show survival benefit akin to sorafenib in HCC pts.
### Failed Phase III Trials for HCC (cont’d)

<table>
<thead>
<tr>
<th>Agent</th>
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Everolimus[1] (EVOLVE-1)</td>
<td>mTOR inhibitor</td>
<td>• Advanced HCC</td>
<td>Everolimus + BSC vs placebo + BSC (N=546)</td>
<td>mOS: 7.6 vs 7.3 mo (HR 1.05 [95% CI 0.86–1.27])</td>
<td>Did not meet primary endpoint (OS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sorafenib refractory/intolerant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• CP A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1 (TS-1 in Japan)[2] (S-CUBE)</td>
<td>Fluoropyrimidine trio (5-FU prodrug + modulators)</td>
<td>• Advanced HCC</td>
<td>TS-1 vs placebo (N=334)</td>
<td>mOS: 337.5 days vs 340 days</td>
<td>Did not meet primary endpoint (OS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sorafenib refractory/intolerant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Peretinoin[3]          | Synthetic retinoid; suppresses growth | • HCV-HCC with CR after curative tx                | Peretinoin lower dose and higher dose vs placebo (N=401) | 1-yr RFS: 63.6 vs 71.9 vs 66.0 3-yr RFS: 24.9 vs 43.7 vs 29.3 | • Did not meet primary endpoint (RFS)  
• Significant dose-response relationship shown in subgroup analysis |
|                        |                            | • CP class A/B                                    |                               |                                |                                                   |

No targeted agents other than sorafenib have demonstrated survival benefit in HCC patients[1-4]
RESORCE – 2L regorafenib after PD on sorafenib

Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial

Jordi Bruix, Shukui Qin, Philippe Merle, Alessandro Granito, Yi-Hsiang Huang, Gyorgy Bodoky, Marc Pracht, Osamu Yokosuka, Olivier Rosmorduc, Valeriy Breder, René Gérolami, Gianluca Masi, Paul J Ross, Tianqiang Song, Jean-Pierre Bronowicki, Isabelle Ollivier-Hourmand, Masatoshi Kudo, Ann-Li Cheng, Josep M Llovet, Richard SFinn, Marie-Aude LeBerre, Annette Baumhauer, Gerold Meinhardt, Guohong Han, on behalf of the RESORCE Investigators

mPFS: 3.1m vs 1.5m
HR 0.46 (95% CI: 0.37, 0.56)

mOS: 10.6m vs 7.8m
HR 0.63 (95% CI: 0.50, 0.79)

Bruix J et al., Lancet 2017
Lenvatinib non-inferior to sorafenib in OS… improvement in PFS, TTP, ORR

Results will be presented in ASCO 2017
The Future of Cancer Therapy: Targeting Multiple Pathways
Cancer is a genomic disease

Cancers with a driving genetic mutation can be effectively targeted with molecular inhibitors

Identification of such molecular aberrations can enable better matching of drug to patient
Somatic mutations found in cancers are either “drivers” or “passengers”

**Driver Mutations**
- Causally involved in the neoplastic process and are positively selected for during tumorigenesis
  - (cKIT in GIST)

**Passenger Mutations**
- Provide no positive or negative selective advantage to the tumor but are retained by chance during repeated rounds of cell division and clonal expansion
  - (KRAS mutation in mCRC)

# Overview of New Phase III Investigational Therapies for HCC

<table>
<thead>
<tr>
<th>Class</th>
<th>Agent</th>
<th>Target/Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted Therapies Therapies</td>
<td>Cabozantinib</td>
<td>VEGFR2 and MET TKI[^1,2^]</td>
</tr>
<tr>
<td></td>
<td>Lenvatinib</td>
<td>Multi TKI targeting angiogenesis and oncogenesis (VEGFR1–3, 3, FGFR1-4, RET, KIT, and PDGFRβ[^1,3^])</td>
</tr>
<tr>
<td></td>
<td>Ramucirumab</td>
<td>Anti-VEGFR2 mAb[^1,4^]</td>
</tr>
<tr>
<td></td>
<td>Regorafenib</td>
<td>Multi TKI targeting angiogenesis and oncogenesis (VEGFR1–3, 3, PDGFRβ, FGFR1, KIT, RET, and BRAF[^1,5^])</td>
</tr>
<tr>
<td></td>
<td>Tivantinib</td>
<td>MET TKI[^1,6^]</td>
</tr>
<tr>
<td></td>
<td>ADI-PEG20</td>
<td>Targets tumor cell growth by degrading arginine[^1,7^]</td>
</tr>
<tr>
<td></td>
<td>Muparfostat</td>
<td>Heparan sulfate mimic targeting angiogenesis (VEGF, FGF1 – 2) and spread[^1,8^]</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Doxorubicin</td>
<td>Nanoparticle doxorubicin delivered via hepatic artery[^1,9^]</td>
</tr>
<tr>
<td></td>
<td>TransDrug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ThermoDox</td>
<td>Liposomal-encapsulated doxorubicin delivered intravenously[^1,10^] intravenously[^1,10^]</td>
</tr>
</tbody>
</table>

FGFR, fibroblast growth factor receptor; mAb, monoclonal antibody; PDGFR, platelet-derived growth factor receptor; TKI, tyrosine-kinase inhibitor; VEGF, vascular endothelial growth factor.

[^1]: Clinicaltrials.gov.
[^3]: Mitsunaga S et al. ASCO GI Symposium 2013. 231.
Therapeutic Armamentarium

- EGFR inhibitors
- Cyclooxygenase-2 inhibitors
- Immune activating anti-CTLA4 mAb
- Aerobic glycolysis inhibitors
- Deregulating cellular energetics
- Sustaining proliferative signaling
- Evading growth suppressors
- Avoiding immune destruction
- Resisting cell death
- Proapoptotic BH3 mimetics
- Enabling replicative immortality
- Telomerase Inhibitors
- Genome instability & mutation
- Inducing angiogenesis
- Activating invasion & metastasis
- Selective anti-inflammatory drugs
- Inhibitors of VEGF signaling
- Inhibitors of HGF/c-Met
IMMUNOTHERAPY: Using the Body To Fight Cancer
Normal immune response

- Normally, the immune system can recognise many types of cancers and mount an active antitumour response
  - Through immunosurveillance, activated T cells remove tumours from the body

1. Tumour releases antigen through multiple mechanisms
2. Antigen-presenting cells (APCs) present these tumour antigens that activate T cells
3. These T cells proliferate, migrate to, and attack the tumour, recognising the tumour by the surface antigens presented
T-cell responses require 2 signals\cite{1,2}:
- TCR recognition of MHC-presented antigen
- Co-signaling interaction, which can be either co-stimulatory or co-inhibitory

T-cell function is thus regulated by a balance between co-stimulatory and co-inhibitory signals, which are also referred to as “checkpoint” pathways\cite{1,2}:

APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor.

Various tumors have been found to exploit immune checkpoint pathways to evade immune detection. APC, antigen-presenting cell; CTLA-4, cytotoxic T-lymphocyte antigen-4; LAG-3, lymphocyte activation gene-3; MHC, major histocompatibility complex; PD-1, programmed death-1; PD-L1, PD ligand-1; PD-L2, PD ligand-2; TCR, T-cell receptor.

Adapted from Pardoll 2012.1

Immunogenicity of the Liver

- The liver is the frontline immunological organ against gastrointestinal tract antigens as well as systemic antigens\[^1\]
  - Parenchymal cells: hepatocytes (approximately 80% liver cells)\[^1\]
    - Functional cells of the liver
    - Also involved in immune responses
  - Nonparenchymal cells: resident immune cells/constituents (approximately 20% cells)\[^1\]

HSC, hepatic stellate cells; LSEC, liver sinusoidal endothelial cells; NKT, natural killer T cell.

* Additional potential roles in antigen presentation.\[^1\]

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**Immune constituents of the liver**

- Hepatocyte
- LSEC: pathogen detection and capture*
- HSC: act as immune sentinels*
- Kupffer cells (Liver macrophages)*
- Intravascular lymphocyte (NKT cell)
Evidence of HCC as an Immunogenic Tumor

The rate of spontaneous regression is among the highest for solid tumors, and some of them are likely immunologic in nature\(^1,2\)

Spontaneous tumor-specific CD8 and CD4 cell responses have been reported\(^3,4\)

Several immunological features of HCC correlate with outcome\(^5\)

Presence of immune cells in tumor (eg, NK cells, T cells, DCs, macrophages)\(^3\)

HCC expression of TAAs (eg, AFP, GPC3, NY-ESO-1, MAGE-A)\(^4\)

AFP, alpha fetoprotein; CD, cluster of differentiation; DCs, dendritic cells; GPC3, glypican 3; HCC, hepatocellular carcinoma; MAGE-A, melanoma antigen gene-A; NK, natural killer; TAAs, tumor-associated antigens.

Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial


* Joint First Authors
CheckMate 040: Phase 1/2 Study of Nivolumab in Patients With Advanced HCC

Dose Escalation (N = 48)
3 + 3 design

- Uninfected
  - 0.1 mg/kg (n = 1)
  - 0.3 mg/kg (n = 3)
  - 1.0 mg/kg (n = 3)
  - 3.0 mg/kg (n = 3)
  - 10 mg/kg (n = 13)

- HCV infected
  - 0.3 mg/kg (n = 3)
  - 1.0 mg/kg (n = 4)
  - 3.0 mg/kg (n = 3)

- HBV infected
  - 0.1 mg/kg (n = 5)
  - 0.3 mg/kg (n = 3)
  - 1.0 mg/kg (n = 3)
  - 3.0 mg/kg (n = 4)

Dose Expansion (N = 214)
3 mg/kg

- Sorafenib naive/intolerant (n = 54)
- Sorafenib progressors (n = 58)
- HCV Infected (n = 51)
- HBV Infected (n = 51)

- Disease assessment imaging (CT or MRI) every 6 weeks
- Interim analysis data cutoff date: March 15, 2016

CT, computed tomography; MRI, magnetic resonance imaging; Q6W, every 6 weeks.
Key Eligibility Criteria and Study Endpoints

*CheckMate 040 Dose Escalation & Expansion*

**Eligibility criteria**

**Inclusion**
- Histologically confirmed advanced HCC not amenable to curative resection
- Child-Pugh scores ≤ 7 (escalation) or ≤ 6 (expansion)
- Progression on 1 prior line of systemic therapy, or intolerant of or refused sorafenib
- AST and ALT ≤ 5 × upper limit of normal; bilirubin ≤ 3 mg/dL
- For HBV-infected patients, viral load < 100 IU/mL and concomitant effective antiviral therapy

**Exclusion**
- Any history of hepatic encephalopathy
- Prior or current clinically significant ascites
- Active HBV and HCV co-infection

**Study endpoints**

**Primary**
- Safety and tolerability (escalation)
- Objective response rate\(^a\) (expansion)

**Secondary**
- Objective response rate (escalation)
- Disease control rate
- Time to response
- Duration of response
- Overall survival

**Exploratory**
- Biomarker assessments

\(^a\) RECIST v1.1 by BICR (blinded independent central review); BICR data are not yet available, and all efficacy assessments are per the local investigator analysis.
## Safety

### CheckMate 040 Dose Escalation & Expansion

<table>
<thead>
<tr>
<th></th>
<th>Uninfected (n = 135)</th>
<th>HCV Infected (n = 61)</th>
<th>HBV Infected (n = 66)</th>
<th>All Patients (n = 262)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Grade</td>
<td>Grade 3/4</td>
<td>Any Grade</td>
<td>Grade 3/4</td>
</tr>
<tr>
<td>Patients with any treatment-related AE, n (%)</td>
<td>91 (67)</td>
<td>24 (18)</td>
<td>45 (74)</td>
<td>21 (34)</td>
</tr>
<tr>
<td>Treatment-related AEs reported in ≥ 5% of all patients, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>32 (24)</td>
<td>2 (1)</td>
<td>7 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>14 (10)</td>
<td>0</td>
<td>12 (20)</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>19 (14)</td>
<td>1 (1)</td>
<td>9 (15)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>18 (13)</td>
<td>2 (1)</td>
<td>4 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>9 (7)</td>
<td>0</td>
<td>7 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>7 (5)</td>
<td>0</td>
<td>2 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Laboratory treatment-related AEs reported in ≥ 5% of all patients, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST increase</td>
<td>13 (10)</td>
<td>4 (3)</td>
<td>10 (16)</td>
<td>10 (16)</td>
</tr>
<tr>
<td>ALT increase</td>
<td>11 (8)</td>
<td>3 (2)</td>
<td>9 (15)</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Amylase increase</td>
<td>10 (7)</td>
<td>4 (3)</td>
<td>3 (5)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Lipase increase</td>
<td>10 (7)</td>
<td>7 (5)</td>
<td>5 (8)</td>
<td>4 (7)</td>
</tr>
</tbody>
</table>

- No treatment-related deaths occurred in either the escalation or expansion cohorts.
Objective responses were observed at all dose levels and in all etiologic subtypes
Overall Survival

*CheckMate 040 Dose Escalation & Expansion*

<table>
<thead>
<tr>
<th>Overall Survival Rate, % (95% CI)</th>
<th>Dose-Escalation Cohort (n = 48)</th>
<th>Dose-Expansion Cohort (n = 214)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>66 (51–78)</td>
<td>83 (76–88)</td>
</tr>
<tr>
<td>9 months</td>
<td>66 (51–78)</td>
<td>71 (57–81)^a</td>
</tr>
<tr>
<td>12 months</td>
<td>59 (44–72)</td>
<td>NC</td>
</tr>
<tr>
<td>18 months</td>
<td>44 (29–58)</td>
<td>NC</td>
</tr>
<tr>
<td><strong>Median OS, mo (95% CI)</strong></td>
<td><strong>14.3 (9.6–18.9)</strong></td>
<td>NC</td>
</tr>
</tbody>
</table>

^a Data cut-off March 15, 2016.

NC, not available/not calculated.
CHECKMATE-459: Phase III trial of Nivolumab vs Sorafenib in 1L Advanced HCC patients

Key Eligibility Criteria

N=726
- Advanced HCC not eligible for or progressive after surgical and/or locoregional therapies
- Child-Pugh A

Primary Endpoints:
- TTP, OS

Other Endpoints:
- ORR, PFS, biomarkers

Nivolumab

Sorafenib

HCC, hepatocellular carcinoma; ORR, objective response rate; OS, overall survival; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PK, pharmacokinetics; TTP, time to progression.

Partial Response to Nivolumab

- 63 year-old male, uninfected HCC, Child-Pugh score A5
- No prior sorafenib or other treatment for HCC

**Baseline**
AFP: 21,000 IU/mL

**Week 6**
AFP: 283 IU/mL
Durable Partial Response to Nivolumab

- 58-year-old white male with HCV-infected HCC, ECOG 0, Child-Pugh A5
- Progressed on sorafenib
Select Investigational Immunotherapies for HCC

<table>
<thead>
<tr>
<th>Class</th>
<th>Agent</th>
<th>Target/Mechanism of Action</th>
<th>Ph[^1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immuno-therapy*</td>
<td>Pexa-Vec (<em>JX-594</em>)</td>
<td>Oncolytic vaccinia virus</td>
<td>II⁺</td>
</tr>
<tr>
<td></td>
<td>Tremelimumab</td>
<td>Checkpoint inhibitor (anti-CTLA4 mAb)</td>
<td>I, II⁺</td>
</tr>
<tr>
<td></td>
<td>Durvalumab (<em>MEDI4736</em>)</td>
<td>Checkpoint inhibitor (anti-PD-L1 mAb)</td>
<td>I, I/II§</td>
</tr>
<tr>
<td></td>
<td>MEDI0680</td>
<td>Checkpoint inhibitor (anti-PD-1 mAb)</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Pembrolizumab</td>
<td>Checkpoint inhibitor (anti-PD-1 mAb)</td>
<td>I, I/II∥</td>
</tr>
<tr>
<td></td>
<td>MPDL3280A</td>
<td>Checkpoint inhibitor (anti-PD-L1 mAb)</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Nivolumab</td>
<td>Checkpoint inhibitor (anti-PD-1 mAb)</td>
<td>I</td>
</tr>
</tbody>
</table>

[^1]: 2 ongoing trials.  
[^2]: 1 ongoing trials in HCC, and 2 in advanced malignancies.  
[^3]: 6 ongoing trials in advanced malignancies.  
[^4]: 4 ongoing trials in advanced malignancies.  

[1]: clinicaltrials.gov.
Investigating Response to Immunotherapy

- PD-1 and CTLA-4 are distinct immune checkpoint proteins with complementary roles in regulating immune responses

- Anti-CTLA-4 agent, ipilimumab (IPI), and anti-PD-1 agent, nivolumab (NIVO) are approved for advanced melanoma alone or in combination (NIVO + IPI)\(^1,2\)

- In CheckMate 067, which compared NIVO and NIVO + IPI vs IPI, numerically higher

CTLA-4 pathway

CTLA-4, cytotoxic T-lymphocyte antigen-4; MHC, major histocompatibility complex; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; TCR, T-cell receptor.

CHECKMATE-040: Phase I/II trial of Nivolumab ± Ipilimumab in Advanced HCC patients

**Key Eligibility Criteria**

N=620
- HCC not amenable to curative resection
- CP ≤6; CP ≤7 for dose escalation; CP B cohort
- Progressed on at least 1 prior line of systemic therapy, intolerant of sorafenib, or refused sorafenib

**Noninfected/HBV/HCV Dose Escalation (n=48) Dose Expansion (n=214)**

CR, complete response; CP, Child-Pugh; DCR, disease control rate; DOR, duration of response; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ORR, objective response rate; OS, overall survival; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PK, pharmacokinetics; R, randomize; TTP, time to progression; TTR, time to response.

Rationale Behind I-O + Non–I-O Treatment Modalities\textsuperscript{1,2}

**Targeted Therapy**
(anti-angiogenic)

- Hypoxia
- Treg population
- ↑PD-L1 expression

**Localized Therapy**
(TACE/RFA/PEI)

- High antigen load
- Damage to liver cells
- Tumor-specific T-cell response

---

I-O, immuno-oncology; PD-L1, programmed death-ligand 1; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; TACE, transcatheter arterial embolization; Treg, regulatory T cell.

### On-going I-O Combo Treatment Modalities

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Phase</th>
<th>Primary Endpoint</th>
<th>Enrollmen t</th>
<th>Start Date</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab+ Young TIL+ Aldesleukin</td>
<td>II</td>
<td>Tumor regression rate</td>
<td>290</td>
<td>Jul 2010</td>
<td>Dec 2018</td>
</tr>
<tr>
<td>Galunisertib + Nivolumab</td>
<td>Ib/II</td>
<td>Maximum tolerated dose</td>
<td>100</td>
<td>Oct 2015</td>
<td>Apr 2018</td>
</tr>
<tr>
<td>Durvalumab+ Tremelimumab</td>
<td>II</td>
<td>SAE, toxicity</td>
<td>144</td>
<td>Oct 2015</td>
<td>Apr 2018</td>
</tr>
<tr>
<td>Nivolumab + Ipilimumab</td>
<td>I/II</td>
<td>Safety and tolerability, ORR</td>
<td>620</td>
<td>Sep 2012</td>
<td>Aug 2017</td>
</tr>
<tr>
<td>Durvalumab+ Tremelimumab+ TACE/RFA/Cryoablation</td>
<td>I/II</td>
<td>Efficacy</td>
<td>90</td>
<td>Jun 2016</td>
<td>April 2020</td>
</tr>
</tbody>
</table>

TIL, tumor-infiltrating lymphocytes; ORR, objective response rate; SAE, serious adverse events; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization

Nivo vs Nivo+Ipi
NIVOLUMAB—METASTATIC MELANOMA
1st LINE COMBINATION WITH IPILIMUMAB

- In combination with ipilimumab, is indicated for the treatment of patients with BRAF V600 wild-type, unresectable or metastatic melanoma.
CA209-069: Study Design

Eligible patients with unresectable stage III or IV melanoma
- Treatment-naïve
- BRAF WT (N = 100) or MT (N = 50)
- Stratified by BRAF status

R 2:1

NIVO 1 mg/kg + IPI 3 mg/kg

NIVO 3 mg/kg

Placebo + IPI 3 mg/kg

Placebo

Treat until: disease progression or unacceptable toxicity

Adapted from Hodi, FS et al. Presented at ASCO 2015; oral 9004.
Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>All randomized patients (N = 142)</th>
<th>NIVO + IPI (N = 95)</th>
<th>IPI (N = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (years)</td>
<td>64</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Age ≥65 years, %</td>
<td>50</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Male/female, %</td>
<td>66/34</td>
<td>68/32</td>
<td></td>
</tr>
<tr>
<td>AJCC stage IV, %</td>
<td>89</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>M1c stage, %</td>
<td>46</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>ECOG PS of 0, %(^a)</td>
<td>83</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Baseline LDH levels, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ULN</td>
<td>74</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>&gt;ULN</td>
<td>25</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>PD-L1 expression ≥5%(^b)</td>
<td>25</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>BRAF V600 MT, %</td>
<td>24</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Remaining patients had an ECOG PS of 1, except for 2 patients with a PS of 2 (NIVO + IPI).

\(^b\)Pretreatment tumor specimens were centrally assessed by PD-L1 immunohistochemistry (using a validated BMS/Dako assay).

AJCC = American Joint Committee on Cancer; ECOG PS = Eastern Cooperative Oncology Group performance status; IPI = ipilimumab; LDH = lactate dehydrogenase; MT = mutation; NIVO = nivolumab; PD-L1 = programmed death-ligand 1; ULN = upper limit of normal.
Tumor Burden Change From Baseline by RECIST v1.1 (BRAF WT Patients)

Median change: 68.1%

Patients
Confirmed responder

Database lock: January 30, 2015

Median change: 5.5% 30% reduction in tumor burden by RECIST v1.1


IPI = ipilimumab; MT = mutation; NIVO = nivolumab; RECIST = Response Evaluation Criteria In Solid Tumors.
**PFS Among BRAF WT Patients**

<table>
<thead>
<tr>
<th></th>
<th>Death or disease progression, n/N</th>
<th>Median PFS, mo (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIVO + IPI</td>
<td>30/72</td>
<td>NR</td>
</tr>
<tr>
<td>IPI monotherapy</td>
<td>25/37</td>
<td>4.4 (2.8-5.7)</td>
</tr>
</tbody>
</table>

CI = confidence interval; IPI = ipilimumab; MT = mutation; NIVO = nivolumab; NR = not reached; PFS = progression-free survival; WT = wild-type. Database lock: January 30, 2015

Similar PFS among BRAF MT patients (8.5 mo for NIVO + IPI, 2.7 mo for IPI alone)

Most Common Treatment-Related Select AEs

<table>
<thead>
<tr>
<th>Patients reporting event, %</th>
<th>NIVO + IPI (n = 94)</th>
<th>IPI (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any grade</td>
<td>Grade 3–4</td>
</tr>
<tr>
<td>Skin AEs</td>
<td>71</td>
<td>10</td>
</tr>
<tr>
<td>Rash</td>
<td>42</td>
<td>5</td>
</tr>
<tr>
<td>Pruritus</td>
<td>35</td>
<td>1</td>
</tr>
<tr>
<td>Gastrointestinal AEs</td>
<td>51</td>
<td>21</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>45</td>
<td>11</td>
</tr>
<tr>
<td>Colitis</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>Endocrine AEs</td>
<td>34</td>
<td>5</td>
</tr>
<tr>
<td>Thyroid disorder</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Hypophysitis</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Hepatic AEs</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>ALT increased</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>AST increased</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>Pulmonary AEs</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Renal AEs</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Creatinine increased</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

AEs = adverse events; ALT = alanine aminotransferase; AST = aspartate aminotransferase; IPI = ipilimumab; NIVO = nivolumab.

Conclusions

• Compared with IPI alone, the NIVO + IPI combination significantly improved ORR and PFS in all randomized patients
  – NIVO + IPI ORR (59%; CR: 22%) versus IPI ORR (11%; CR: 0%)
  – ORR and PFS benefit was observed irrespective of BRAF status, tumor PD-L1 status, and presence of poor prognostic factors

• Treatment-related AEs were reported more frequently with NIVO + IPI than with IPI alone

• Patients with poor prognostic factors had a similar safety profile to the entire population

• AEs were generally managed using established guidelines

• The NIVO + IPI regimen provided a favorable benefit-risk profile in treatment-naïve advanced melanoma patients, including those with poor prognostic factors

AEs = adverse events; IPI = ipilimumab; NIVO = nivolumab; ORR = objective response rate; PD-L1 = programmed death ligand 1; PFS = progression-free survival.
Immuno-Oncology Safety and Adverse Event Management
Organ Types Affected by AEs Are Similar With Anti–PD-1 and Anti–CTLA-4 Alone and in Combination$^{1-3}$

Safety Monitoring Overview

• Screen patients for AEs
  – Patient education: Reinforce to patients the importance of reporting any new or worsening symptom

• Early recognition and early intervention
  – Dose delay/discontinuation
  – Corticosteroids
  – Other immunosuppresants

• Monitor outpatients with ongoing AEs

• For patients admitted to an outside hospital for AEs
  – Frequent contact with admitting physician and subspecialist as appropriate
  – Provide guidance on detection and management of drug-related adverse events

• Select AE’s => refer to specific algorithms
Some Differences Observed....

- Single agent anti–PD-1 therapies are better tolerated than single agent IPI

- High-dose IPI 10 mg/kg in the adjuvant melanoma setting has more side effects than IPI 3 mg/kg in the metastatic setting

- Pulmonary AEs seem rare with IPI

- Hepatic AEs appear more frequently with combination than with monotherapy

- Colitis is rare with anti–PD-1 monotherapy, yet occurs more commonly with a regimen containing IPI

- Thyroiditis is more frequent with anti–PD-1-containing regimens than with IPI monotherapy

- Elevations of amylase and/or lipase may occur with the NIVO + IPI regimen, with some patients having symptoms of pancreatitis

*IPI = ipilimumab; NIVO = nivolumab.*
Endocrinopathy Take-Home Points

• Severe endocrine-related AEs are infrequent
  – Adrenal insufficiency and hypothyroidism < 1%
  – Hyperthyroidism and hypophysitis are rare (<0.1%)

• When encountering non-specific symptoms (fatigue, weakness), think of endocrinopathies

• Consider endocrine consult to interpret lab results and guide management
  – Treatment may be continued once appropriate hormone replacement initiated

• Subjects with endocrinopathy may require replacement dose steroids rather than high-dose steroids
Radiographic Findings for IPI-Associated Hypophysitis

6/30/04 Baseline (4.5 mm)  
12/3/04 After 5 doses (10.8 mm) headache/fatigue

Algorithm for Suspected Pulmonary Toxicity

Grade of Pneumonitis (NCI CTCAE v4)

Grade 1
Radiographic changes only

Management

- Consider delay of I-O therapy
- Monitor for symptoms every 2-3 days
- Consider Pulmonary and ID consults

Follow-up

- Re-image at least every 3 weeks
  If worsens:
  - Treat as Grade 2 or 3-4

Grade 2
Mild to moderate new symptoms

- Delay I-O therapy per protocol
- Pulmonary and ID consults
- Monitor symptoms daily, consider hospitalization
- 1.0 mg/kg/day methylprednisolone IV or oral equivalent
- Consider bronchoscopy, lung biopsy

Follow-up

- Re-image every 1-3 days
  If improves:
  - When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics
  If not improving after 2 weeks or worsening:
  - Treat as Grade 3-4

Grade 3-4
Severe new symptoms; New/worsening hypoxia; Life-threatening

- Discontinue I-O therapy per protocol
- Hospitalize
- Pulmonary and ID consults
- 2-4 mg/kg/day methylprednisolone IV or IV equivalent
- Add prophylactic antibiotics for opportunistic infections
- Consider bronchoscopy, lung biopsy

Follow-up

If improves to baseline:
- Taper steroids over at least 6 weeks
If not improving after 48 hours or worsening:
- Add additional immunosuppression (e.g. infliximab, cyclophosphamide, IVIG, or mycophenolate mofetil)

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Pulmonary Toxicity Take-Home Points

- Pulmonary toxicity has been infrequent across the nivolumab program
  - Nivo Monotherapy – Pneumonitis - 3% (all grades), 1% (Grades 3-4)

- At presentation: Grades 1-3, the majority are Grades 1-2

- Pulmonary toxicity may present with clinical symptoms or may be an incidental finding on scans

- Subjects have been successfully treated with prompt initiation of appropriate doses of corticosteroids

- Subjects with low-grade pulmonary toxicity may be re-challenged with study drug once off steroids

- Consider prophylactic antibiotics for opportunistic infections for those individuals receiving high dose steroids for greater than 4 weeks
Algorithm for Suspected GI Toxicity

Grade of Diarrhea/Colitis
(NCI CTC AE v4)

Grade 1
Diarrhea: < 4 stools/day over baseline; Colitis: asymptomatic

Management
• Continue I-O therapy per protocol
• Symptomatic treatment

Follow-up
• Close monitoring for worsening symptoms.
• Educate patient to report worsening immediately
  If worsens:
  • Treat as Grade 2 or 3/4

Grade 2
Diarrhea: 4-6 stools per day over baseline; IV fluids indicated < 24 hrs; not interfering with ADL; Colitis: abdominal pain; blood in stool

Management
• Delay I-O therapy per protocol
• Symptomatic treatment

Follow-up
If improves to grade 1:
• Resume I-O therapy per protocol
If persists > 3-5 days or recur:
• 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent
  • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol.
  • If worsens or persists > 3-5 days with oral steroids:
    • Treat as grade 3/4

Grade 3-4
Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥ 24 hrs; interfering with ADL
Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs
G4: life-threatening, perforation

Management
• Discontinue I-O therapy per protocol
• 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent
• Add prophylactic antibiotics for opportunistic infections
• Consider lower endoscopy

Follow-up
If improves:
• Continue steroids until grade 1, then taper over at least 1 month
If persists > 3-5 days, or recurs after improvement:
• Add infliximab 5 mg/kg (if no contraindication).
  Note: Infliximab should not be used in cases of perforation or sepsis

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g., prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
GI Toxicity Take-Home Points

• Most cases of diarrhea have been mild (Grade 1)
• Low grade diarrhea may be managed symptomatically + dose delay
• SAEs of colitis have been uncommon (<1.0%) in the nivolumab program overall
• Use results of diagnostic evaluation to guide management
  – A negative diagnostic evaluation may need to be repeated
• Initiate treatment early
• If steroids are begun, taper slowly
• Consider prophylactic antibiotics for opportunistic infections for those individuals receiving high dose steroids for greater than 4 weeks
PD-L1 as a Biomarker: Biological, Technical, and Logistical Complexity

**Biology: PD-L1**
- Inter and intratumor heterogeneity
- Inducible and dynamic (IFN, post-treatment)
- Cell type (immune cell versus tumor versus both)
- Location (membrane versus cytoplasm)

Expression of PD-L1 is heterogeneous\(^1\)

**Technical: Assay**\(^1,4,5\)
- Epitope stability
- Distribution (patchy versus diffuse)
- Different antibodies and platforms
- Different thresholds for expression
- Interobserver readability

Abs are not identical: >25% discordant\(^1,6,7\)

**Logistics: Tissue**\(^1,8,9\)
- Interval between tissue and treatment (archived versus fresh)
- Primary versus metastatic disease
- Some circumstances not amenable to obtaining any tissue
- Certain biopsy methods result in poor tissue quality/quantity

IFN = interferon; PD-L1 = programmed death ligand 1.

Biological Complexity of PD-L1: Dynamic PD-L1 Expression (1 of 1)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cell Type</th>
<th>Effect on PD-L1 Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation therapy</td>
<td>Colorectal, breast, melanoma</td>
<td>Up-regulated*</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Hepatoma4</td>
<td>Up-regulated*</td>
</tr>
<tr>
<td></td>
<td>HNSCC5</td>
<td></td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Breast6</td>
<td>Up-regulated*</td>
</tr>
<tr>
<td></td>
<td>Colorectal, hepatocellular carcinoma7</td>
<td>Up-regulated*</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Breast</td>
<td>Up-regulated*</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>Plasmacytoid dendritic cells</td>
<td>Up-regulated*</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Breast</td>
<td>Down-regulated*†§</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>NSCLC</td>
<td>Down-regulated* §</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Up-regulated*†‡</td>
</tr>
<tr>
<td>Sunitinib / pazopanib</td>
<td>Metastatic RCC</td>
<td>Down-regulated*</td>
</tr>
</tbody>
</table>

*PD-L1 expression determined by flow cytometry. †PD-L1 expression determined by qRT-PCR or transcriptomeic profiling. ‡PD-L1 expression determined by western blots. §PD-L1 expression determined by IHC. ¶ In tumors resistant to radiation + anti-CTLA-4.

HNSCC = head and neck squamous cell carcinoma; IHC = immunohistochemistry; NSCLC = non-small cell lung cancer; PD-L1 = programmed death ligand 1; RCC = renal cell carcinoma.

# Overview of PD-L1 Assays

<table>
<thead>
<tr>
<th>Drug</th>
<th>BMS*</th>
<th>Merck*1-3</th>
<th>Roche*4-8</th>
<th>AstraZeneca*9-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab clone/epitope</td>
<td>28-8 Abcam/Extracellular domain</td>
<td>22C3 Dako/Extracellular domain</td>
<td>SP142 Spring Bioscience/Intracellular domain</td>
<td>SP263 Spring Bioscience/Extracellular domain</td>
</tr>
<tr>
<td>IVD Class III diagnostic partner</td>
<td>Dako</td>
<td>Dako</td>
<td>Ventana</td>
<td>Ventana</td>
</tr>
<tr>
<td>Sample source</td>
<td>Archival or fresh tissue</td>
<td>Archival or fresh tissue</td>
<td>Archival or fresh tissue</td>
<td>Archival or fresh tissue</td>
</tr>
<tr>
<td>Staining location</td>
<td>Membrane</td>
<td>Membrane</td>
<td>Membrane</td>
<td>Membrane</td>
</tr>
<tr>
<td>Cell type scored</td>
<td>Tumor cells</td>
<td>Tumor cells</td>
<td>Tumor cells and immune cells</td>
<td>Tumor cells</td>
</tr>
<tr>
<td>Scoring method</td>
<td>% of cells with membrane staining at any intensity</td>
<td>% of cells with membrane staining at any intensity</td>
<td>Tumor cell (TC) score: staining % of tumor cells</td>
<td>% of cells with membrane staining</td>
</tr>
<tr>
<td></td>
<td>Tumor Proportion score (TPS): % of cells with membrane staining at any intensity</td>
<td>Immune cell (IC) score: staining % of tumor area</td>
<td>% of cells with membrane staining</td>
<td></td>
</tr>
<tr>
<td>Current IVD PD-L1 Threshold</td>
<td>&lt;1% or ≥1%</td>
<td>&lt;50% or ≥50%</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>PD-L1 Threshold Under Evaluation</td>
<td>≥1%, ≥5%, or ≥10%</td>
<td>≥1%, ≥50%</td>
<td>TC1/2/3 or IC1/2/3 ≥1%</td>
<td>≥25%</td>
</tr>
<tr>
<td>Trial Design</td>
<td>057: All comers 067: All comers</td>
<td>KN-001: PD-L1 ≥1% KN-010: PD-L1 ≥1%</td>
<td>POPLAR: all comers FIR: TC2/3 or IC2/3</td>
<td>NCT01693562: all comers</td>
</tr>
<tr>
<td>Testing Requirement</td>
<td>Complementary</td>
<td>Companion</td>
<td>Companion</td>
<td>Companion</td>
</tr>
</tbody>
</table>

*No head-to-head studies have been conducted and comparisons cannot be made between these assays or antibodies used therein. IC = immune cell; IVD = in vitro diagnostic; NSCLC = non-small cell lung cancer; PD-L1 = programmed death ligand 1; TC = tumor cell; TIIC = tumor-infiltrating IC.

**Gene Expression and Rearrangements**

- **RNA:** *CD274* gene expression can be assessed with mRNA levels by microarray. Association with PD-L1 protein levels (detected by IHC) was observed only for samples with greater than 80% staining\(^1\)

- **DNA amplification/translocation:** *CD274* overexpression can occur through gene amplification, or through translocation and fusion of *CD274* with a highly expressed promoter\(^2,3\)

---

**Protein Expression Levels**

- **Soluble PD-L1:** Serum levels of soluble PD-L1 can be assessed by ELISA. Soluble PD-L1 has been detected in patients with autoimmune disease\(^4\)

- **Circulating tumor cells:** Tumor cells extracted from peripheral blood can be assessed for PD-L1 expression\(^5\)

- **Peripheral blood mononuclear cells** PD-L1 expression can also be assessed in CD4 and CD8 T cells, B cells, plasmacytoid DC, natural killer cells, natural killer T cells, MDSC, monocytic MDSC, granulocytic MDSC, and lineage-negative MDSC\(^6\)

---

*The CD274 gene encodes for the PD-L1 protein.

DC = dendritic cell; ELISA = enzyme-linked immunosorbent assay; MDSC = myeloid-derived suppressor cell; PD-L1 = programmed death ligand 1.


Other Exploratory Biomarkers

<table>
<thead>
<tr>
<th>Tumor biomarkers&lt;sup&gt;1-7&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TILs/immunoscore</td>
</tr>
<tr>
<td>(eg, CD4 and CD8 T cells)</td>
</tr>
<tr>
<td>Gene expression signatures</td>
</tr>
<tr>
<td>(immune signatures/ inflamed phenotype)</td>
</tr>
<tr>
<td>Tumor immune marker expression</td>
</tr>
<tr>
<td>(eg, CTLA-4, PD-L1, PD-L2)</td>
</tr>
<tr>
<td>Mutational load/burden, neo-antigens</td>
</tr>
<tr>
<td>TCR sequencing</td>
</tr>
<tr>
<td>Other known oncogenic driver mutations</td>
</tr>
<tr>
<td>(eg, EGFR, KRAS, BRAF)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peripheral biomarkers&lt;sup&gt;2,3,8-12&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune monitoring: absolute lymphocyte count, circulating T-cell subsets/MDSCs</td>
</tr>
<tr>
<td>TCR sequencing</td>
</tr>
<tr>
<td>Gene expression profiling: peripheral blood</td>
</tr>
<tr>
<td>Serum chemokines/cytokines: interferons, interferon inducible factors, serum soluble factors</td>
</tr>
<tr>
<td>sPD-L1</td>
</tr>
<tr>
<td>Circulating miRNAs</td>
</tr>
<tr>
<td>Antitumor antibodies</td>
</tr>
<tr>
<td>SNPs (germline)</td>
</tr>
</tbody>
</table>

BRAF = serine/threonine-protein kinase B-Raf; CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; EGFR = epidermal growth factor receptor; KRAS = Kirsten rat sarcoma viral oncogene homolog; MDSC = myeloid-derived suppressor cells; PD-L1 = programmed death ligand 1; PD-L2 = programmed death ligand 2; SNP = single nucleotide polymorphism; sPD-L1 = soluble PD-L1; TCR = T-cell receptor; TILs = tumor-infiltrating lymphocytes.

Challenges for PD-L1 as a Biomarker¹-⁴

PD-L1, programmed death-ligand 1.

Conclusions

• Immunotherapy is a promising modality in the management of advanced HCC

• Nivolumab monotherapy has a manageable safety profile in patients with HCC, including those with HBV or HCV infection
  – The safety profile was similar to that observed in other tumor types

• Durable responses were observed across all dose levels and etiologic cohorts

• These preliminary data support the ongoing clinical development of nivolumab in HCC
Thank You