Development of Small Molecule Checkpoint Inhibitors

David Tuck, M.D.
CMO Curis Inc
why small molecule immune checkpoint blockade?

- small molecule PK profile allows optimization of dose and administration schedule
  - Usually less than 24 hour half-life ($T_{1/2}$) permits flexibility with dosing schedule – daily or intermittent dosing
  - Permits flexible adjustment of exposure as measured by $C_{\text{max}}$ and AUC – both parameters can be adjusted to match mechanism-of-action
  - Flexibility to adjust dose and schedule to address emergent adverse events
  - Flexibility to adjust for combinations
  - Untether patient from infusion chair – cost, convenience, access

Typical Small Molecule Drug PK Profile

Typical Antibody Drug PK Profile
Structural Features of PD-1 / PD-L1 Interaction

Lin et al., 2008; PNAS. 105: 3011

Zak et al., 2015; Structure. 23: 2341
### Small molecules, peptides, non-blocking antibodies

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mechanism</th>
<th>Reference</th>
<th>Source</th>
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<tbody>
<tr>
<td>ENUM-C8 PD1</td>
<td>Non-blocking antibody</td>
<td>AACR 2016</td>
<td>Enumeral</td>
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<tr>
<td>MQ-209,MQ-210</td>
<td>Non-blocking (Allosteric) antibody</td>
<td>ASCO 2016 Fenwick Abstract 3072</td>
<td>Mabquest</td>
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<td>NP12</td>
<td>Peptide targeting PDL1</td>
<td>AACR 2013</td>
<td>Aurigene</td>
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<td>(D) PPA-1</td>
<td>D peptides blocking PDL1-PD1 interaction</td>
<td>Chang 2015</td>
<td>Tsinghua Univ</td>
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<tr>
<td>high-affinity PD-1</td>
<td>Small non-antibody peptides</td>
<td>Maute PNAS 2015</td>
<td>Stanford</td>
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<td>Small molecule BMS-202, BMS-8</td>
<td>Small molecule targeting PDL1</td>
<td>Zak et al 2016</td>
<td>BMS</td>
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<tr>
<td>CA170,CA327</td>
<td>Small molecules targeting immune checkpoints</td>
<td>AACR 2016 SITC 2016</td>
<td>Curis/Aurigene</td>
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</tbody>
</table>
Peptides, small molecule and mAbs ICI may have different mechanisms

Discovery, Characterization and Development of a New Class of Therapeutic Anti-PD-1 Antibody - Amirinia-Nave et al AACR 2016
Discovery process for small molecule immune checkpoint blockade

Aurigene discovery platform
Small molecule design based on structure of interaction hotspots

Small molecule library
Pharmacophore derived small molecule mimics

Functional screening to identify compounds capable of selectively rescuing T cell proliferation and activation in the presence of inhibitory checkpoints

PD-1
PD-L1

VISTA TIM3
PD-L1

Graph showing Rescue (%) vs. (Log nM)
Antagonism of PD-L1 and VISTA by CA-170

Ex-vivo activation of human PBMC

- Potent and selective rescue of human T cell activation (proliferation or IFN-γ production)
  - Dose-dependent activation of T cells inhibited by exogenous PD ligands or by VISTA
    - Similar to that observed using anti-PD1 or anti-VISTA antibodies as control
  - No rescue of T cells inhibited by CTLA-4, TIM3, BTLA, LAG3

- Dose dependent activation of PD-L1 or VISTA-inhibited T cells ex-vivo
- No activation of CTLA4-inhibited T cells
- No activation of TIM3-inhibited T cells

IFN-γ production used as a marker for T cell activation
CA-170 is Orally Bioavailable
*Multiple species*

- Oral exposure in all non-clinical species examined
  - At 10mg/kg: $C_{\text{max}} \sim 500\text{ng/ml}$, $AUC \sim 3,500\text{ng*hr/ml}$ in mouse (efficacy animal model)

- Proportional increase in $C_{\text{max}}$ and $AUC$ with increased oral dosing of CA-170
  - From 10mg/kg to 1000mg/kg dose tested in mouse and monkey (safety animal model)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oral PK Profile</th>
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<tbody>
<tr>
<td>Species</td>
<td>Mice</td>
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<tr>
<td>$T_{1/2}$ (hr)</td>
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<tr>
<td>Tmax (hr)</td>
<td>1.0</td>
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<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
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<tr>
<td>$AUC_{(0-t)}$ (µg*hr/ml)</td>
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CA-170 Anti-Tumor Activity In Vivo
Syngeneic mouse tumor models sensitive to anti-PD1 antibodies

In vivo activity in tumor models grown in immuno-competent C57BL/6 mouse strain
- CT26 and MC-38 CRC tumors grown subcutaneously and change in tumor size measured
- B16/F10 melanoma tumor cells injected intravenously and number of lung metastatic nodules measured

CT26 Colon Carcinoma Model
- Vehicle
- anti-PD1 (J43) 100 μg
- CA-170 10 mpk

MC38 Colon Carcinoma Model
- Vehicle
- anti-PD1 (J43) 100 μg
- CA-170 10 mpk

B16/F10 Melanoma Model
- Vehicle
- anti-PD1 (J43)
- CA-170 10 mpk

Day 13 post tumor cell inoculation

*p<0.05, **p<0.01, ***p<0.001
J43 at 100ug/animal, ip, q7d
**In vivo Activity Requires Intact Immune System**

- No anti-tumor activity observed in immune-deficient SCID/beige mice
- Provides evidence for immune response-mediated anti-tumor mechanism of CA-170
CA-170 is Active In Vivo

Syngeneic mouse tumor models where anti-PD1 antibodies are inactive

- **In vivo** activity in 4T1 breast cancer tumor model
  - Dose dependent response to CA-170
  - Tumor model is non-responsive to anti-PD1 antibody treatment
CA-170 is Active *In Vivo*

*Syngeneic mouse tumor models insensitive to anti-PD1 antibodies*

- *In vivo* activity in B16/F1 melanoma tumor model
  - Dose dependent response to CA-170
  - Tumor model is non-responsive to anti-PD1 antibody treatment

**CA-170 Daily Oral Treatment**

**Anti-PD1 Twice-weekly Injection**
Predictable Dose Exposure, T cell Activation and Efficacy in anti-PD-1 Non-responsive Mouse Models with CA-170

Mouse single dose exposure

T Cell Activation – In Tumor

Efficacy in the B16/F1 Model

T Cell Activation – In Blood
CA-170 *In vivo* Effect on Immune Profile

- **Increased T cell activation in tumor-bearing animals following CA-170 administration**
  - Increase in CD8+ T cell activation markers (CD69) in tumor and in tumor draining lymph nodes
  - Increase in peripheral circulation of active CD4+ and CD8+ T cells

↑ Activation – Tumor Infiltrating CD8+ T cells

↑ Activation – Tumor-draining Lymph Node CD8+ T cells

↑ Number – Peripheral Circulating CD8+ & CD4+ T cells

↑ Activation – Peripheral Circulating CD8+ & CD4+ T cells
CA-170 has Clean Preclinical Safety Profile

GLP 28-day repeat oral dosing safety profile – mice and monkeys

- Mortality: No mortality
- Clinical Signs of Toxicity: No abnormalities detected
- Body weight: No test item related changes
- Food Consumption: No test item related changes
- Hematology: No test item related changes
- Clinical Chemistry: No test item related changes
- Gross Pathology: No treatment related changes
- Organ weights: No treatment related changes
- Histopathology: No treatment related changes
- NOAEL at over 1,000 mg/kg/day: \textit{In vivo} active dose = 10 mg/kg/day
CA-327 Selectively Inhibits PDL1 and TIM3

Activation of human T cells in culture

- Potent and selective rescue of human T cell activation ex-vivo (proliferation or IFN-γ production)
  - Dose-dependent activation of isolated human T cells inhibited by exogenous PD-ligands or by TIM3
  - Specificity: no rescue of T cells inhibited by CTLA-4, LAG3, VISTA

Activation of PD-L1 or VISTA-inhibited T cells ex-vivo

No activation of CTLA4-inhibited T cells

No activation of VISTA-inhibited T cells
CA-327 is Orally Bioavailable
Multiple species

PK parameters at 10 mpk PO dose using water as vehicle under fed condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>BALB/c Mouse</th>
<th>Wistar Rat</th>
<th>Beagle Dog</th>
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<tbody>
<tr>
<td>$AUC_{0-LAST}$</td>
<td>h*ng/ml</td>
<td>5069</td>
<td>4631</td>
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<td>$AUC_{0-inf}$</td>
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<td>Beta $t_{1/2}$</td>
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<td>$C_{max}$</td>
<td>ng/ml</td>
<td>755</td>
<td>481</td>
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<td>$T_{max}$</td>
<td>h</td>
<td>1.0</td>
<td>3.3</td>
<td>2.6</td>
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</table>
CA-327 can inhibit tumor growth of multiple syngeneic tumor models in an immune dependent manner.

**MC38 colon carcinoma**

**CT26 colon carcinoma**

**B16F10 melanoma**

*p < 0.05*
CA-327 Effect on Immune Profile In Vivo
CT-26 syngeneic colorectal cancer mouse model

- Increased T cell activation in tumor-bearing animals with oral CA-327 administration
  - Increased percentage of activated (CD69+ marker) CD8+ and CD4+ T cells in circulation and in tumors

↑ Percent Active – Circulating CD8+ & CD4+ T cells

Blood CD8+/CD69+ T cells  Blood CD4+/CD69+ T cells

↑ Percent Active – Tumor Infiltrating CD8+ & CD4+ T cells

Tumor CD8+/CD69+ T cells  Tumor CD4+/CD69+ T cells
Additional compounds with distinct targets in preparation for clinical study demonstrate the robustness of this discovery platform

**Potent and selective, oral small molecule immune checkpoint inhibitor ex-vivo**
- Targets PDL1 and TIM3 immune checkpoints
- Activates isolated T cells that are inhibited by PDL1 or TIM3 in culture

**Potent immune checkpoint inhibitor in vivo in mouse tumor models with oral dosing**
- Biologically active in mouse: Oral administration results in T cell activation in circulation and in tumor tissue
- Significant anti-tumor activity in multiple syngeneic tumor models

**IND-enabling studies ongoing**
CA-170 Phase 1 Trial (CA-170-101)

Dose escalation design

Open-label, dose escalation and dose expansion trial

- Up to 300 patients with advanced solid tumors or lymphoma
- 6 centers in US enrolling patients for dose escalation stage
- Currently in 3+3 stage of dose escalation

Objectives

- Safety, tolerability, PK, PD, clinical effects, and recommended Phase 2 dose (RP2D)

Treatment

- Oral, once daily administration in continuous 21-day cycles
Dose proportional increase in exposure with increasing doses in patients

- Near-linear doubling in Cmax and AUC with dose doubling: 50mg – 400mg daily dose
First dose levels in patients are consistent with Preclinical Predictable Dose Exposure and T Cell Activation (NCT02812875)

Human PK (200 mg/day)

![Plasma Concentration vs Time Graph]

CA-170 exposure in humans

![AUC_last vs CA-170 Dose Graph]

Change in the percentage of circulating CD8+ T cells expressing:

- **CD69+**
  - PT-3
  - PT-1
  - PT-2

- **CD134+**
  - PT-3
  - PT-1
  - PT-2

- **Granzyme B+**
  - PT-3
  - PT-1
  - PT-2
Summary

- CA170 is a potent and selective, oral small molecule checkpoint inhibitor, and the first to enter the clinic
- Preclinical data demonstrate dose-dependent oral exposure, immune modulation and anti-tumor activity
- Clinical PK profile is similar to non-clinical and human exposure appears predictable on oral dosing
- CA-170 appears to be biologically active in patients, supporting continued clinical development
First-in-Class orally available small molecule antagonist of PD-L1 and VISTA

Induces proliferation of T-cells and IFN-gamma production
- Selective rescue in response to PD-L1, PD-L2, and VISTA inhibition and not other immune checkpoint molecules

Clean off-target profile (enzyme, receptors, ion channels, kinases)

Orally bioavailable with good PK properties in mice & monkeys

Anti-tumor activity in multiple syngeneic tumor models

Clean in vivo safety profile – GLP toxicology in mice and monkeys

Phase 1 trial underway
Immune checkpoints of the immunoglobulin superfamily are amenable to small molecule targeting

- Transient interaction, not pre-formed complexes
- Structural information for receptor-ligand interaction is available
- Defined regions provide contact sites for small molecule interaction

This chemistry has identified key interaction regions for targeting

- Proposed interaction model is consistent with the structure-based design

This chemistry appears to extend to multiple checkpoint inhibitors

- PDL1, VISTA and TIM3 targeting compounds identified

Chemistry has translated to multiple drug candidates

- Successful conversion of design to an orally available, active and safe molecule