Development of an ADC Targeting TIM-1 for the Treatment of Ovarian and Renal Cell Carcinoma

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TIM-1 is Known by Several Names

TIM-1 = KIM-1 = HAVCR1

- TIM-1 (T-cell Immunoglobulin Domain and Mucin Domain Protein-1)
- KIM-1 (Kidney Injury Molecule-1)
- HAVCR1 (Hepatitis A Virus Cellular Receptor-1)
TIM-1 (T-cell Immunoglobulin Domain and Mucin Domain Protein-1)

- Also known as Transmembrane Immunoglobulin and Mucin Domain-1
- Co-stimulatory molecule
- TIM-1 expressed on activated T cells (along with other immune cells), and preferentially on CD4\(^+\) Th2 cells co-stimulating proliferation and IL-4 release\(^1\)
- Ligand is TIM-4 on APC including DC
- Agonist anti-TIM-1 mAb or TIM-4 co-stimulate proliferation and cytokine release
- Antagonist anti-TIM-1 mAb down-regulates immune responses
- Implicated in autoimmune diseases.

\(^1\) Nat Immunol 6: 447, 2005
KIM-1 (Kidney Injury Molecule-1)

- Biomarker of kidney injury
- KIM-1 absent in normal kidney and urine
- KIM-1 up-regulated and shed into urine in various renal injuries\(^1\)
  - Post-ischemic injury, nephrotoxicant renal injury (including drug-related toxicity), acute tubular necrosis, diabetic nephropathy, IgA nephropathy, SLE, polycystic kidney disease, acute and chronic allograft rejection, and others
- Appears to precede functional renal injury markers (creatinine, BUN)
- KIM-1 binds phosphatidylserine (PS)
  - Scavenger receptor
  - Transforms epithelial cells into phagocytes
- Associated with Clear Cell Carcinomas (renal, ovarian, uterine)\(^2,3\)

1) J Cell Physiol 228: 917, 1012
2) J Am Soc Nephrol 16: 1126, 2005
HAVCR1 (Hepatitis A Virus Cellular Receptor 1)

- HAVCR1 (TIM-1) was identified as a receptor for Hepatitis A virus, a non-enveloped *picornavirus*¹
- TIM-1 is a receptor for *Ebolavirus* (EBOV) and *Marburgvirus*²
  - Cell surface TIM-1 associated with EBOV infection of Vero cells
  - Anti-TIM-1 mAb block EBOV infection *in vitro*
- TIM-1 mediates entry of various enveloped viruses via binding phosphatidylserine on virus envelope³

2) *PNAS* 108: 8426, 2011
### Expression of Tim-1 in renal tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Positive Cases/Total Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional RCC</td>
<td>54/73 (74%)</td>
</tr>
<tr>
<td>Fuhrman nuclear grade I/II</td>
<td>36/49 (73%)</td>
</tr>
<tr>
<td>Fuhrman nuclear grade III/IV</td>
<td>18/24 (75%)</td>
</tr>
<tr>
<td>Papillary RCC</td>
<td>28/30 (93%)</td>
</tr>
<tr>
<td>Chromophobe RCC</td>
<td>0/54 (0)*</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>4/41 (9.75%)†</td>
</tr>
<tr>
<td>Metastatic RCC</td>
<td>35/45 (78%)</td>
</tr>
</tbody>
</table>


### Expression of Tim-1 in non-renal tumors

<table>
<thead>
<tr>
<th>Tumor Site</th>
<th>Tumor Type</th>
<th>Positive Cases/Total Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Adenocarcinoma</td>
<td>5/40 (12.5%)*</td>
</tr>
<tr>
<td></td>
<td>Well-moderately differentiated</td>
<td>5/28 (18%)</td>
</tr>
<tr>
<td></td>
<td>Poorly-undifferentiated</td>
<td>0/12 (0)</td>
</tr>
<tr>
<td>Breast</td>
<td>Invasive ductal carcinoma</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td></td>
<td>Invasive lobular carcinoma</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Ductal adenocarcinoma</td>
<td>0/13 (0)</td>
</tr>
<tr>
<td>Lung</td>
<td>Adenocarcinoma</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>Uterus</td>
<td>Endometrioid carcinoma</td>
<td>0/13 (0)</td>
</tr>
<tr>
<td></td>
<td>Clear cell carcinoma</td>
<td>6/18 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>Serous carcinoma</td>
<td>2/4 (0)</td>
</tr>
<tr>
<td>Ovary</td>
<td>Serous carcinoma</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td></td>
<td>Clear cell carcinoma</td>
<td>15/16 (93.8%)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Papillary carcinoma</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocellular carcinoma</td>
<td>0/13 (0)</td>
</tr>
<tr>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>Renal pelvis</td>
<td>Urothelial carcinoma</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>Mediastinum</td>
<td>Mesothelioma</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Testis</td>
<td>Seminoma</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>Site</td>
<td>Neuroendocrine carcinoma</td>
<td>0/10 (0)</td>
</tr>
</tbody>
</table>

Strong membranous/cytoplasmic staining of TIM-1+ renal clear cell carcinoma
Renal Cell Carcinoma (RCC)

- Kidney Cancer: 3.8% of all new cancers
- 3rd most common GU cancer after prostate and bladder
- 63,920 diagnosed with kidney cancer and 13,860 deaths in US (2014)
- The rate increased 1.6% per year (2002-2011)
- Male:Female = 3:2
- Peak incidence between 60-70 years of age
- 5-yr survival: 91.8% for early kidney cancer
- 5-yr survival: 12.3% for advanced disease (improved from 7.3%)
Renal Cell Carcinoma Histology

- Several histological subtypes of renal cell carcinoma
- Clear cell carcinoma is the most common subtype

<table>
<thead>
<tr>
<th>Type</th>
<th>Clear cell</th>
<th>Papillary type 1</th>
<th>Papillary type 2</th>
<th>Chromophobe</th>
<th>Oncocytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence (%)</td>
<td>75%</td>
<td>5%</td>
<td>10%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Associated mutations</td>
<td>VHL</td>
<td>c-Met</td>
<td>FH</td>
<td>BHD</td>
<td>BHD</td>
</tr>
</tbody>
</table>

- 54/73 (74%) of renal clear cell carcinoma tumors tested were TIM-1⁺

BHD=Birt-Hogg-Dubé; FH=fumarate hydratase; VHL=von Hippel-Lindau.

Ovarian Cancer

- 22,290 new cases; 14,180 deaths
- 5-yr survival: 45.6%
- 15/16 (93.8%) of ovarian clear cell carcinoma tumors tested were TIM-1+

Endometrial Cancer

- 54,870 new cases; 10,170 deaths
- 5-yr survival: 81.7%
- 33% of endometrial clear cell carcinoma tumors tested were TIM-1+

TIM-1 Immunohistochemistry of Normal Tissues

• Some normal tissues show cytoplasmic expression of TIM-1

• Plasma membrane expression of TIM-1 is limited
  • (e.g. bronchiolar epithelium in the lung)

• Supports the development of an anti-TIM-1 ADC
Anti-TIM-1 Human mAb, CR014

- Fully human mAb CR014 was produced by immunization of human Ig expressing mice (XenoMouse®) with the extracellular domain of human TIM-1
- Kd for human Tim-1 is $2.71 \times 10^{-9}$ M
- IgG1 and IgG4 versions

Modified from *J Immunol* 184: 2743, 2010
Immunohistochemistry Staining of Kidney Cancer Tissue Array

- 94 cases of malignant renal tumors / 10 normal tissues
- 77/94 (82%) of tumors stained pos. (>50% rare cells)

(Top Panel) Positive staining (plasma membrane) of clear cell carcinoma tissue with FITC-CR014-IgG1.
(Bottom Panel) No staining of same tumor with FITC-huIgG1

- One normal tissue had minor cytoplasmic staining
CR014-IgG1 mAb Binding

The indicated tumor cells were incubated with 5mg/mL antibody for 20 minutes at room temperature. Binding was detected with a PE-labeled goat anti-human IgG (Fc specific) antibody and analyzed on a BD FACSCanto II™ flow cytometer. CR011 is an IgG2 anti-GPNMB antibody acting as a negative control.

- Good expression levels of TIM-1 on all 3 tumor cell lines
CR014-IgG1 mAb Internalization

Internalization of isotype control (left panel) or CR014-IgG1 (right panel) was measured after incubation (40 min, 37°C) of IGROV-1 cells with FITC-labeled antibodies. The cells were counterstained with anti-Class I (red) and DAPI (blue) before analysis by confocal microscopy.

- Significant internalization of the anti-TIM-1 antibody observed
CR014 Antibody Drug Conjugate

- **Antibody:**
  - Assessed both IgG4 and IgG1 versions of the mAb
  - Full human antibody CR014-IgG4, CR014-IgG1

- **Cytotoxic drug:**
  - Monomethylauristatin (MMAE), a tubulin inhibitor

- **Linker:**
  - vc-linker (valine-citrulline)

- **CDX-014**
  - CR014-IgG1 selected for development
  - CR014-IgG1κ-vcMMAE=CDX-014

Same linker-toxin technology used in Adcetris™, brentuximab vedotin.
(Seattle Genetics)

CR014: fully-human IgG, targeting TIM-1

MMAE: dolastatin-like tubulin inhibitor
Characterization of IgG1 and IgG4 Versions of Anti-TIM-1 mAb CR014

Plates were coated with TIM-1-Fc and blocked with BSA/PBS, followed by an incubation with the mAb or ADC’s. The naked mAb’s were detected with an HRP labeled goat anti-human IgG F(ab’)₂ specific reagent. Bifunctional binding of the ADC’s was detected with a mouse anti-MMAE antibody, followed by an HRP labeled goat anti-mouse IgG antibody.

- No difference in binding patterns with IgG1 vs. IgG4
**In Vitro Cytotoxicity of CR014-vcMMAE (IgG1 and IgG4)**

- Good specific cytotoxicity observed in both cell lines
- Comparable activity between IgG1 and IgG4 versions

Frozen stocks of cells were thawed and allowed to adhere overnight at 37°C in 5% CO₂. ADC's were added and the plates were incubated for an additional 72 hours. Viability was measured with Alamar Blue reagent.
**In Vivo Efficacy of CDX-014: IGROV-1**

- Human Ovarian Carcinoma
- Animal Model: Nude (NCr) – Female
- Cell injection: $2 \times 10^6$ IGROV-1 cells s.c.
- Mice divided into two groups with equivalently sized tumors (~0.1-0.3cm$^3$)
- Dosed days 0, 4, 8, 12
- Treatment: CR014-IgG1-vcMMAE (CDX-014) or saline
In Vivo Efficacy of CR014 ADC: Caki-1

- Human Renal Clear Cell Carcinoma
- Animal Model: Nude (NCr) – Female
- Cell injection: $5.6 \times 10^5$ Caki-1 cells s.c.
- Mice divided into groups with equivalently sized tumors
- Dosed days 0, 4, 8, 12 with treatment.
**In Vivo Efficacy of CDX-014: A549**

- Human Lung Carcinoma
- Animal Model: SCID– Female
- Cell injection: $1 \times 10^7$ A549 cells s.c.
- Mice divided into two groups with equivalently sized tumors
- Dosed days 0, 4, 8, 12
- Treatment: CR014-IgG1-vcMMAE (CDX-014) or saline
**In Vivo Efficacy of CDX-014: A549**

- Multiple Cycles of Dosing of CR014-IgG1-vcMMAE (CDX-014)
  - Cycle 1: days 0, 4, 8, 12
  - Cycle 2: days 24, 28, 32, 36
  - Cycle 3: days 48, 52, 56, 60

[Graphs showing percent survival and mean tumor volume over study days with dosing cycle indicated.]
Moving Forward into Human Testing

Phase 1
- Initial ORR
- TIM-1 correlation

Phase 2
- Phase 1 first in human dose escalation study in Renal Cell Carcinoma (RCC) to determine MTD with expansion cohorts for initial efficacy (Overall Response Rate, ORR)

Phase 3
- Additional indications
- Clinical Development in Renal Cell Carcinoma (RCC) or other TIM-1 expressing tumors depending on results from Phase 1 study
CDX-014: Summary

- Anti-TIM-1 mAb IgG1κ-vcMMAE (CDX-014)
  - IgG1 and IgG4 versions comparable (target affinity, *in vitro* cytotoxicity, *in vivo* mouse tumor model)
  - Good specific *in vitro* cytotoxicity with IGROV-1 and Caki-1
  - Preclinical efficacy *in vivo* with IGROV-1, Caki-1, and A549
  - CR014-IgG1 selected for development: CDX-014
  - GMP production of CDX-014
  - GLP-compliant toxicology, TCR study nearing completion

- Phase 1 Clinical Trial for CDX-014 Planned (H12016)
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