Development of Talimogene Laherparepvec (T-VEC, IMLYGIC®), First FDA Approved Genetically Modified Oncolytic Virus

- Perspectives of Clinical Pharmacology

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Sumita Bhatta, Executive Medical Director, Global Development, Amgen
Outline

• Introduction of genetically modified virus-based products
• Overview of talimogene laherparepvec (T-VEC) development pathway
  • Unique construct of T-VEC design
  • Mechanism of action and indications
  • Dose and administration
  • Filing history and current status
• Clinical pharmacology support of T-VEC development
  • Preclinical/clinical study design
  • Biodistribution/shedding study results
  • Bioanalytical support
  • Prescribing information and medication guide
• Regulatory advances and perspectives
Human Gene Therapy

Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use.

Gene therapies can work by several mechanisms:
- Replacing a disease-causing gene with a healthy copy of the gene
- Inactivating a disease-causing gene that is not functioning properly
- Introducing a new or modified gene into the body to help treat a disease

Gene therapy products are being studied to treat diseases including cancer, genetic diseases, and infectious diseases.

Types of Human Gene Therapy Products

<table>
<thead>
<tr>
<th><strong>IMLYGIC</strong>®</th>
<th><strong>Indication</strong></th>
<th><strong>Vector</strong></th>
<th><strong>Route</strong></th>
<th><strong>Dose</strong></th>
<th><strong>Treatment duration</strong></th>
<th><strong>Nonclinical Biodistribution</strong></th>
<th><strong>Clinical biodistribution</strong></th>
<th><strong>Clinical shedding</strong></th>
<th><strong>Key safety warning</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>HSV-1</td>
<td>Intra-lesional injection</td>
<td>$10^6$, $10^8$ plaque-forming units (PFU) per mL</td>
<td>Q3W (first dose) then Q2W</td>
<td>Tumor, blood, spleen, liver, lymph node, kidney, heart, lung, gonads, salivary gland</td>
<td>Blood</td>
<td></td>
<td>Urine, oral mucosa, anogenital, inj. lesion, ext. dressing</td>
<td>Herpetic infection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>LUXTURNA</strong>®</th>
<th><strong>Indication</strong></th>
<th><strong>Vector</strong></th>
<th><strong>Route</strong></th>
<th><strong>Dose</strong></th>
<th><strong>Treatment duration</strong></th>
<th><strong>Nonclinical Biodistribution</strong></th>
<th><strong>Clinical biodistribution</strong></th>
<th><strong>Clinical shedding</strong></th>
<th><strong>Key safety warning</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE65 mutation-associated retinal dystrophy</td>
<td>AAV2</td>
<td>Subretinal injection</td>
<td>$1.5 \times 10^{11}$ vector genomes (vg) per eye</td>
<td>Single dose</td>
<td>Eyes, spleen, liver, lymph node Not in gonads</td>
<td>Blood, tears</td>
<td>Blood, tears</td>
<td>Tears</td>
<td>Retinal infection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>ZOLGENSMA</strong>®</th>
<th><strong>Indication</strong></th>
<th><strong>Vector</strong></th>
<th><strong>Route</strong></th>
<th><strong>Dose</strong></th>
<th><strong>Treatment duration</strong></th>
<th><strong>Nonclinical Biodistribution</strong></th>
<th><strong>Clinical biodistribution</strong></th>
<th><strong>Clinical shedding</strong></th>
<th><strong>Key safety warning</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric spinal muscular atrophy</td>
<td>AAV9</td>
<td>IV infusion</td>
<td>$1.1 \times 10^{14}$ vg per kg</td>
<td>Single dose</td>
<td>CNS (brain, spinal cord), muscle</td>
<td>Liver, spleen, heart, lung, pancreas, lymph node, muscles, nerves, kidney, intestines, spinal cord, brain, thymus</td>
<td>Saliva, urine, stool</td>
<td>Acute serious liver injury</td>
<td></td>
</tr>
</tbody>
</table>
Overview of T-VEC Development Pathway
Talimogene Laherparepvec

• Innovative oncolytic immunotherapy based on herpes simplex virus type 1 (HSV-1)
• Deletion of both copies of ICP34.5 attenuates neurovirulence
• Efficiently replicates in tumors but not normal tissues
• Retains sensitivity to anti-viral agents
• Results in tumor cell lysis for local control
• Results in release of tumor-derived antigens and GM-CSF to initiate a systemic anti-tumor immune response

GM-CSF: granulocyte macrophage colony stimulating factor
Talimogene Laherparepvec
An HSV-1 Derived Oncolytic Immunotherapy

ICP34.5-/ICP47-/hGM-CSF

Envelope
dsDNA
Capsid
Tegument

GM-CSF
Deletion of ICP34.5: Tumor-Selective Replication

Viral Safety Considerations

• Deletion of ICP34.5 markedly reduces neurovirulence compared to wild-type HSV-1 in mouse models\textsuperscript{1,2}

• Administered intracerebrally, the LD$_{50}$ was $10^5$, ~10,000-fold reduction in virulence\textsuperscript{1,2}

• Functional viral thymidine kinase maintains susceptibility to acyclovir\textsuperscript{3}

Systemic Effect of Murine Talimogene Laherparepvec (mT-VEC) with GM-CSF

Implant A20 tumor cells, grow to ~0.5 cm

Day 0

Days 7, 9, and 11

Tumor Measurements

Injected Tumor

Contralateral Uninjected Tumor

IMLYGIC® is injected directly into tumor

**Local Oncolysis**
- IMLYGIC® selectively replicates in tumor cells resulting in lysis and the release of tumor-derived antigens (TDAs)

**Local Immune Response**
- GM-CSF from IMLYGIC® promotes the maturation of dendritic cells which in turn, in conjunction with TDAs, activate T cells

**Distant Immune Response**
- Activated T cells proliferate and migrate to distant tumor sites for T cell-mediated tumor cell death, causing a systemic antitumor response*

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*This figure depicts the proposed mechanism of action and is not meant to imply clinical efficacy.
### Clinical Studies with T-VEC

#### Monotherapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>Success</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Melanoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 001/01</td>
<td>Phase 1 (N=30)</td>
<td>Safety/BD</td>
<td>Completed*</td>
</tr>
<tr>
<td>Study 20120324</td>
<td>Phase 2 (N=61)</td>
<td>Biodistribution+ Shedding</td>
<td>Completed</td>
</tr>
<tr>
<td>Study 002/03</td>
<td>Phase 2 (N=50)</td>
<td>Unresectable</td>
<td>Completed*</td>
</tr>
<tr>
<td>Study 005/04</td>
<td>Phase 1 (N=17)</td>
<td>Pancreatic</td>
<td>Completed</td>
</tr>
<tr>
<td>Study 005/05</td>
<td>Phase 3 (N=436)</td>
<td>Unresectable</td>
<td>Completed*</td>
</tr>
<tr>
<td>Study 20110266</td>
<td>Phase 2 (N=150)</td>
<td>Neoadjuvant</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Study 20120325</td>
<td>Phase 2 (N=112)</td>
<td>Biomarker</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Study 20140270</td>
<td>Phase 1 (N=18)</td>
<td>Japan</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

| **Non-Melanoma** | | | |
| Study 005/04 | Phase 1 (N=17) | | Completed |
| Study 20110261 | Phase 1b (N=18) | Pediatric Solid Tumors | Ongoing |

#### Combination

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>Success</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Melanoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 20110264</td>
<td>Phase 1/2 (N=217)</td>
<td></td>
<td>Ongoing</td>
</tr>
<tr>
<td>Study 20110265</td>
<td>Phase 1b/3 (N=21/692)</td>
<td></td>
<td>Ongoing</td>
</tr>
<tr>
<td>Study 004/04</td>
<td>Phase 1/2 (N=17)</td>
<td>SCCHN +CT &amp; RT</td>
<td>Completed</td>
</tr>
<tr>
<td>Study 002/03</td>
<td>Phase 2 (N=50)</td>
<td></td>
<td>Completed</td>
</tr>
<tr>
<td>Study 20110261</td>
<td>Phase 1b (N=18)</td>
<td></td>
<td>Ongoing</td>
</tr>
<tr>
<td>Study 20140299</td>
<td>Phase 1b</td>
<td>TNBC/mCRC (N=36)</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Study 20140318</td>
<td>Phase 1b/2 (N=244)</td>
<td>Alternative Injection Multi-Tumor Basket Study</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

| **Non-Melanoma** | | | |
| Study 20130232 | Phase 1b | SCCHN (N=36) + pembrolizumab | Ongoing |
| Study 20180115 | Phase 2 (N=100) | | Pending |
| Study 20140299 | Phase 1b | TNBC/mCRC (N=36) | Ongoing |
| Study 20140318 | Phase 1b/2 (N=244) | Alternative Injection Multi-Tumor Basket Study | Ongoing |

Registry and extension studies not included in this figure. Clinical trials information available at: www.clinicaltrials.gov.
Key Studies Contributing to the BLA

<table>
<thead>
<tr>
<th>Monotherapy</th>
<th>Melanoma</th>
</tr>
</thead>
</table>
| **Clinical Pharmacology** | **Study 001/01**<sup>1</sup>  
   Phase 1 (N=30)  
   Safety/BD |  
   Completed* |
| **Study 002/03**<sup>3</sup>  
   Phase 2 (N=50)  
   Unresectable |  
   Completed* |
| **Study 005/05**<sup>4</sup>  
   Phase 3 (N=437)  
   Unresectable |  
   Completed* |

Registry and extension studies not included in this figure. Clinical trials information available at: www.clinicaltrials.gov.
Key Regulatory Interactions for BLA

- **Type B End of P2**
  - Discuss Phase 3 Protocol
  - Jan 2008

- **Type B End of P2**
  - CMC
  - Apr 2008

- **Type B**
  - Pharmacovigilance
  - Aug 2008

- **Type B**
  - Pre-BLA
  - May 2013

- **Type B**
  - BLA Submission
  - Oct 2013

- **Type B**
  - SPA
  - Study design, DRR endpoint, comparator
  - Jul 2014

CMC: chemistry, manufacturing, and control; SPA: special protocol assessment; BLA: Biologics License Application
Method of Administration

- Talimogene laherparepvec administered into cutaneous, SC, or nodal lesions (+/- ultrasound guidance)\(^1,2\)
- No injections of visceral lesions permitted\(^1\)
- Limits on amount to be injected per lesion by size (see table)\(^1,2\)
- No specific limits on number of lesions injected per visit\(^1\)
- Precedence to be given to new lesions, then larger lesions\(^1\)

<table>
<thead>
<tr>
<th>Lesion Size (Diameter)(^1,2)</th>
<th>Talimogene Laherparepvec Injection Volume(^1,2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 5.0 cm</td>
<td>≤ 4.0 mL</td>
</tr>
<tr>
<td>&gt; 2.5 cm to 5.0 cm</td>
<td>≤ 2.0 mL</td>
</tr>
<tr>
<td>&gt; 1.5 cm to 2.5 cm</td>
<td>≤ 1.0 mL</td>
</tr>
<tr>
<td>&gt; 0.5 cm to 1.5 cm</td>
<td>≤ 0.5 mL</td>
</tr>
<tr>
<td>≤ 0.5 cm</td>
<td>≤ 0.1 mL</td>
</tr>
</tbody>
</table>

This total dose administered in any one treatment session should not exceed 4.0 mL\(^1\)

IMLYGIC® (Talimogene Laherparepvec) is the first and only oncolytic virus therapy approved by FDA

- IMLYGIC® is the first and only modified oncolytic virus (HSV-1 based) immunotherapy approved in melanoma in the US and approved in EU
- Approval based on the Phase 3 OPTiM trial in unresectable metastatic melanoma
  - Eightfold improvement in durable response (CR/PR maintained ≥6 months) with IMLYGIC® vs GM-CSF (16.3% vs 2.1%, p<0.0001)
- Clinical proof of concept has been demonstrated with IMLYGIC® in combination with Checkpoint inhibitors
  - A large randomized phase 2 study demonstrated a doubling of overall response (38% vs.18%) and complete response (13% vs 7%) with the combination of IMLYGIC® plus Yervoy vs. Yervoy alone in patients with stage IIIB-IVM1c unresectable melanoma
  - A single arm phase 1b study of IMLYGIC® plus Keytruda demonstrated a overall response rate of 67% with a complete response rate of 43%
  - In studies combining with checkpoint inhibitors, IMLYGIC® was well tolerated and had no overlapping toxicities
- A phase 3 study (n=696) of IMLYGIC® plus Keytruda® vs. Keytruda® alone for first line treatment of advanced melanoma patients is fully enrolled and results will be available based on event driven analyses
- Neoadjuvant clinical data is available for IMLYGIC® in patients with advanced resectable melanoma
  - In an interim one year landmark analysis, IMLYGIC® demonstrated a recurrence free survival (RFS) of 55.8% vs. 39.3% for the upfront surgery arm

2. Chesney J et al, Clin Oncol 2017
3. Ribas A, Cell 2017
T-VEC Specific Development Consideration

MOA/Efficacy
- Viral replication and oncolytic effect in tumor cells
- Systemic anti-tumor immune response due to GM-CSF expression
- Effectiveness of the virus due to previous HSV-1 exposure
- Efficacy upon re-treatment

Safety
- Viral shedding and accidental exposure to healthcare providers and close patient contacts
- Risk of herpetic infection in immunocompromised individuals
- Risk of viral latency and reactivation
- Viral sensitivity toward acyclovir upon infection

PK
- Kinetics of viral clearance in the blood and urine
- Biodistribution in different tissues
- Development of a specific assay for T-VEC DNA
- Development of an assay for infectivity due to live virus
Key Nonclinical Pharmacology Studies Results

• Tumor killing effect observed in multiple cancer cell lines
• Anti-tumor effect observed on un-injected tumors
• Systemic activation of tumor specific T cells
• Anti-tumor effect in mice previously exposed to HSV
• Protection against tumor cell re-challenge following clearance of established tumors with T-VEC
• Demonstrated susceptibility to acyclovir
Key Nonclinical Toxicology Studies Results

- High and multiple doses of T-VEC (>60-fold of clinical dose) are well tolerated following SC, IV, or IT administration.
- Toxicology findings consistent with normal anti-viral immunity following HSV infection.
- Systemic viremia was observed in immunodeficient mice.
- Does not accumulate or persist in reproductive tissues.
- No effect on embryo-fetal viability and development.
- Negligible transfer of viral DNA from mother to fetus (<0.001%).
## Nonclinical Biodistribution Studies in Mice

<table>
<thead>
<tr>
<th>Study</th>
<th>Route</th>
<th>Dose Frequency</th>
<th>Dose (PFU)</th>
<th>Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>4648-00030</td>
<td>SC, IV</td>
<td>Single</td>
<td>0.6x10⁷ (n=15/sex/route)</td>
<td>blood, urine, injection site, spleen, lung, liver, heart, kidney, gonads, eyes, brain, trigeminal ganglion, nerve</td>
</tr>
<tr>
<td>4648-00027</td>
<td>SC</td>
<td>Multiple (Q3Dx5)</td>
<td>1x10⁷ (n = 9/sex)</td>
<td>blood, urine, injection site, spleen, lung, liver, heart, kidney, gonads, eyes, brain, trigeminal ganglia, duodenum</td>
</tr>
<tr>
<td>4648-00028</td>
<td>SC</td>
<td>multiple (QWx5)</td>
<td>1x10⁷ (n = 15/sex)</td>
<td>blood, urine, tissues (same as for 4648-00027)</td>
</tr>
<tr>
<td>115857</td>
<td>Intra-tumoral</td>
<td>Multiple (Q3Dx3)</td>
<td>1x10⁵ or 5x10⁵ (n=24/sex/dose)</td>
<td>blood, injection site, spleen, lung, liver, heart, kidney, gonads, eyes, brain, trigeminal ganglion, bone marrow, lymph node, additional specimens from possible sources for viral shedding (feces, lachrymal glands, nasal mucosa, and salivary glands)</td>
</tr>
</tbody>
</table>
# Intratumoral Biodistribution/Shedding Study in Mice

**Treatment Frequency**: Once every 3 days (Days 1, 4 and 7) for a total of 3 doses

**Treatment Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose Volume (mL/mouse)</th>
<th>Concentration (PFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>0.05</td>
<td>0 (vehicle control article)</td>
</tr>
<tr>
<td>3-4</td>
<td>0.05</td>
<td>$2 \times 10^6$</td>
</tr>
<tr>
<td>5-6</td>
<td>0.05</td>
<td>$1 \times 10^7$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Animals</th>
<th>Dose (PFU/mouse)</th>
<th>Scheduled Euthanasia (Study Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14 (Female)</td>
<td>Vehicle(^a)</td>
<td>8, 14</td>
</tr>
<tr>
<td>2</td>
<td>14 (Male)</td>
<td>Vehicle(^a)</td>
<td>8, 14</td>
</tr>
<tr>
<td>3</td>
<td>24 (Female)</td>
<td>$1 \times 10^5$</td>
<td>8, 14, 91</td>
</tr>
<tr>
<td>4</td>
<td>24 (Male)</td>
<td>$1 \times 10^5$</td>
<td>8, 14, 91</td>
</tr>
<tr>
<td>5</td>
<td>24 (Female)</td>
<td>$5 \times 10^5$</td>
<td>8, 14, 91</td>
</tr>
<tr>
<td>6</td>
<td>24 (Male)</td>
<td>$5 \times 10^5$</td>
<td>8, 14, 91</td>
</tr>
</tbody>
</table>
Incidence Rate of T-VEC DNA Detection

- Rate of detection highest in tumor tissue
- Levels of viral DNA in tumor tissue were over 50-fold or higher compared to other tissues
- Tissues associated with viral clearance: spleen (16%), liver (8%) and lymph node (8%)
- Highly perfused tissues: heart (5%), kidney (3%) and lung (3%)
- Brain (2%, 2/91 samples)
Nonclinical Results Guided the Design of Clinical Biodistribution/Shedding Study

• Blood
  • 13% of samples are positive
  • last positive sample occurred 43 days after the last dose
  • viral levels in blood are a small fraction of those found in tumor (~0.006%)
• Urine
  • detected in 22% of animals within 24 hours post-dose
• Salivary gland
  • one sample had detectable viral DNA on Day 42
• T-VEC DNA not detected in lachrymal glands, nasal mucosa or feces
Objectives of Clinical Pharmacology Program

• Evaluate the dosing regimen
• Evaluate anti-HSV-1 seroconversion
• Measure GM-CSF expression in tumor tissue and blood
• Understand the kinetics of viral clearance through biodistribution in the blood and urine
• Assess shedding from injected tumors, exterior of the dressing, oral mucosa, anogenital area and suspicious herpetic lesions
## Clinical Biodistribution/Shedding Assessment

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>N</th>
<th>Dose</th>
<th>T-VEC DNA in blood and urine</th>
<th>Live Viral Shedding From Tumor and Exterior of Dressing Swabs</th>
<th>Suspicious herpetic lesion swabs</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>001/01</td>
<td>1</td>
<td>30</td>
<td>Part 1: $10^6$, $10^7$, or $10^8$ PFU/mL single dose Part 2: $10^6$, $10^7$, or $10^8$ PFU/mL multiple doses</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>002/03</td>
<td>2</td>
<td>50</td>
<td>$10^6$ and $10^8$ PFU/mL, multiple doses</td>
<td>√</td>
<td>√</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>005/05</td>
<td>3</td>
<td>436</td>
<td>$10^6$ and $10^8$ PFU/mL, multiple doses</td>
<td></td>
<td></td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>324</td>
<td>2</td>
<td>60</td>
<td>$10^6$ and $10^8$ PFU/mL, multiple doses</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
</tbody>
</table>
Phase 1 First-In-Human Study

Part 1: Single Dose

N = 13

Patients with refractory cutaneous or subcutaneous metastases from solid tumors, independent of HSV status

T-VEC 10^6 PFU/mL

T-VEC 10^7 PFU/mL

T-VEC 10^8 PFU/mL

Part 2: Multidose (Three Injections)

N = 17

HSV-seronegative patients

T-VEC 10^6, 10^7, 10^7 PFU/mL

T-VEC 10^6, 10^8, 10^8 PFU/mL

T-VEC 10^8, 10^8, 10^8 PFU/mL

HSV-seropositive patients
GM-CSF mRNA in Tumor Biopsies After Injection

- mRNA detected 48 hours after administration
- Detected in both seronegative and seropositive subjects
- Did not seem dose dependent

Dosing Regimen and Seroconversion

• In HSV seronegative patients, more pronounced local reactions and febrile influenza-like syndromes were observed with doses > $10^6$ pfu/mL

• Majority seronegative patients seroconverted in 3 weeks

• The initial dose of $10^6$ pfu/mL was sufficient for seroconversion

• The $10^8$ pfu/mL was well tolerated and was the highest dose studied in clinical studies

• $10^6$ pfu/mL followed by $10^8$ pfu/mL was found to be tolerable in both seronegative and seropositive patients
A Phase 2, Multicenter, Single-arm Biodistribution and Shedding Study in Subjects with Melanoma

Primary Objective: To estimate the proportion of subjects with detectable T-VEC DNA in the blood and urine any time after administration of T-VEC within the first 3 cycles

<table>
<thead>
<tr>
<th>Day</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4a</th>
<th>Safety Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour</td>
<td>1</td>
<td>2 3 8 15</td>
<td>1 2 3 8</td>
<td>1 8</td>
<td>30 60</td>
</tr>
<tr>
<td>Predose</td>
<td>1 4 8</td>
<td>Predose</td>
<td>1 4 8</td>
<td>Predose</td>
<td></td>
</tr>
</tbody>
</table>

Sample Collection

- **Blood/Urine**: ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ●.
Assay Summary for T-VEC DNA and Infectivity

Quantitative polymerase chain reaction (qPCR) assay
  • Validated and specific to T-VEC DNA
  • Sensitive with LLOQ at 24, 18, and 1.76 copies/µg DNA for urine, swab, and blood samples, respectively

Plaque assay
  • Considered gold standard as viral infectivity assay
  • Detect live virus
  • Formation of plaques in cells are quantified and expressed as plaque forming unit (PFU) per mL

TCID\textsubscript{50} assay
  • Detect live virus
  • Quantify the amount of virus required to create a cytopathic effect in 50% of inoculated Vero cells; virus titer is calculated as TCID\textsubscript{50}/mL
  • Sensitive and more robust
## T-VEC DNA Detection and Infectivity Results

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Subjects, n/N (%)</th>
<th>Samples, n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>59/60 (98%)</td>
<td>383/1094 (35%)</td>
</tr>
<tr>
<td>Urine</td>
<td>19/60 (32%)</td>
<td>31/1088 (3%)</td>
</tr>
<tr>
<td>Swabs of Injected Lesions</td>
<td>60/60 (100%)</td>
<td>741/1520 (49%)</td>
</tr>
<tr>
<td>Viral Infectivity</td>
<td>7/60 (12%)</td>
<td>8/740 (1.1%)</td>
</tr>
<tr>
<td>Exterior of Occlusive Dressing</td>
<td>48/60 (80%)</td>
<td>212/1085 (20%)</td>
</tr>
<tr>
<td>Viral Infectivity</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>8/60 (13%)</td>
<td>12/964 (1.2%)</td>
</tr>
<tr>
<td>Viral Infectivity</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anogenital system</td>
<td>5/26 (19%)</td>
<td>7/448 (1.6%)</td>
</tr>
<tr>
<td>Viral Infectivity</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Suspicious Herpetic Lesions</td>
<td>3/19 (16%)</td>
<td>4/37 (11%)</td>
</tr>
<tr>
<td>Viral Infectivity</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
T-VEC Biodistribution in Blood and Urine by Cycles

Blood

Patient Incidence of Detectable T-VEC DNA

- HSV-1 seronegative (N = 17)
- HSV-1 seropositive (N = 40)
- All (N = 60)

<table>
<thead>
<tr>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Safety Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>82%</td>
<td>68%</td>
<td>100%</td>
<td>38%</td>
<td>0%</td>
</tr>
<tr>
<td>72%</td>
<td>95%</td>
<td>95%</td>
<td>5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Urine

Patient Incidence of Detectable T-VEC DNA

- HSV-1 seronegative (N = 17)
- HSV-1 seropositive (N = 40)
- All (N = 60)

<table>
<thead>
<tr>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Safety Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>6%</td>
<td>5%</td>
<td>24%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>5%</td>
<td>30%</td>
<td>27%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
T-VEC DNA Levels in Blood and Urine by Cycles

Blood

Urine

Summary of Biodistribution and Shedding Results

**Biodistribution (within the body)**

- Higher incidence of positive T-VEC DNA in blood in HSV-1 seronegative patients
- The amount of DNA in blood was highest during cycle 2
- Rapid decline potentially due to immune responses and cleared from blood by the end of treatment

**Viral shedding (excretion/secretion)**

- The incidence of positive urine samples was low and DNA was detected highest during cycle 2
- No DNA detection in urine at Cycle 4 and at the follow-up visit
- Overall incidence in swabs of the oral mucosa and anogenital area was low (1-2% of samples)

**Viral detection on the surface of injected lesions and exterior dressing**

- DNA detection was high on the surface of injected lesions and 14% subjects tested positive during the safety follow-up
- 1.1% samples from the surface of injected lesions tested positive for live virus. No samples were positive for viral infectivity after cycle 2 or during safety follow-up (up to day 60)
- Live virus was NOT detected on the exterior surface of occlusive dressing
Detection methods

- IMLYGIC® viral DNA levels in various tissues and secretions were determined using a quantitative polymerase chain reaction (qPCR) assay.
- Infectious IMLYGIC® at the injection sites and at some potential herpetic lesions was also quantified using viral infectivity assays.

Biodistribution/shedding in blood and urine

- IMLYGIC® DNA was present in the blood of 17 (85%) patients and in urine of 4 (20%) patients.
- The peak levels of IMLYGIC® DNA in the urine were detected on the day of treatment.

Viral detection on the surface of injected lesions and exterior dressing

- Infectious IMLYGIC® virus was detected at the site of injection in 3 (15%) patients at a single time point each and all within the first week after the initial injection.
- The exterior of the occlusive dressings was positive in 14 (70%) patients. However, no infectious virus was detected on the exterior of the occlusive dressing.
- The number of patients with measurable levels of DNA on the exterior of occlusive dressings declined over time with no measurable DNA by the third treatment in 13 patients tested.
Herpetic Lesions and Viral Transmission

Suspicous herpetic lesions

- Of 19 patients with lesions of suspected herpetic origin, four of 37 swabs taken from three patients had detectable T-VEC DNA
- None were positive for infectivity based on TCID$_{50}$ assay

Viral transmissibility

- 3 close contacts and 2 investigators reported exposure or signs and symptoms of suspected herpetic origin
- None had detective T-VEC DNA
Medication Guide on IMLYGIC® Handling

What should I avoid while getting IMLYGIC®?
IMLYGIC® virus can spread to other areas of your body or to your close contacts (household members, caregivers, sex partners, or persons sharing the same bed).

Do the following to avoid spreading IMLYGIC® to other areas of your body or to your close contacts:

- Avoid direct contact between your treatment sites, dressings, or body fluids and close contacts (for example, use condoms when engaging in sexual activity, avoid kissing close contacts if either has an open mouth sore).
- Wear gloves while putting on or changing your dressings.
- Keep treatment sites covered with airtight and watertight dressings for at least 1 week after each treatment (or longer if the treatment site is weeping or oozing).
- If the dressing comes loose or falls off, replace it right away with a clean dressing.
- Place all used dressings and cleaning materials in a sealed plastic bag and throw them away in the garbage.
- Do not touch or scratch the treatment sites.

With proper handling, administration, and post-injection care, T-VEC can be administered safely in patients with minimal risk of transmission to close contacts.
Outline

- Introduction to Oncolytic Virus (OV)
- Clinical Pharmacology Considerations of OV Therapy
- Future Perspectives of OV Therapy
- Regulatory Resources for OV Product Development
Oncolytic Virus

- Virus that infects and lyses cancer cells but not normal cells*

- Types
  - Naturally occurring (unmodified)
  - Genetically engineered
    - Conditional viral replication (selectively infect and replicate in tumor cells)
    - Expression of transgene with therapeutic or immune modulating effects

*NCI Dictionary of Cancer Terms
Clinical Pharmacology Considerations

- Study Design Considerations

- Pharmacokinetic, Pharmacodynamic and Immunogenicity Assessments

- Biodistribution

- Viral Shedding
Study Design Considerations

- Route of Administration
  - Intratumoral, intravenous, intraperitoneal, etc.

- Dose and Dosing Schedule
  - Dose: preclinical data, available clinical data from similar OVs, available immunity information, dose ranging study data
  - Dosing schedule: preclinical data (anti-tumor effect) and available OV pharmacokinetic information
Pharmacokinetic, Pharmacodynamic & Immunogenicity Assessments

- Pharmacokinetic (PK) Assessment
  - Systemic (blood) viral concentration-time profile
  - Viral presence within tumor sites (if possible)
  - Measure both viral transgene and infectivity using qPCR and infectivity assays
  - Evaluate PK profiles for co-transgene expression (if applicable)

- Pharmacodynamic Assessment: Biomarkers
  - Tumor lysis/Cytotoxic biomarkers
  - Tumor microenvironment modulation biomarkers

- Immunogenicity Assessment
Biodistribution Assessment
– Use of Animal Data

- Determines distribution and persistence of the product in both target and non-target tissues

- Study Design
  - Product formulation
  - Animal sex
  - Sample size
  - Appropriate safety endpoints
  - Sampling schedule
    - Expected time of peak levels
    - Several later time points to evaluate the persistence and clearance of product sequences from tissues
Biodistribution Assessment
- Use of Animal Data (cont’d)

- Tissue Collection
  - Recommended sample tissues, at a minimum: blood, injection site(s), gonads, brain, liver, kidneys, lung, heart, and spleen
  - Other sampling tissues considerations

- Tissue Analysis
  - A quantitative, sensitive assay such as qPCR
Viral Shedding Assessment

- **Viral Shedding** – virus excretes outside of body (urine, fecal, dressing, swabs, saliva, etc.) - Elimination

- **Viral Shedding Evaluation in Clinical Studies**
  - Collect shedding data in Phase 1 trials: OV products are classified as replication competent. The data collection may continue in Phase 2 and Phase 3 studies.
  - Shedding study design
    - Biological characteristics
    - Route of administration
  - Sample collection
    - Frequency, duration, types of samples collected, storage conditions
  - Analytical assays
    - a quantitative, sensitive assay like qPCR, and
    - an infectivity or growth-based assay
Future Considerations

- Combination with check-point inhibitors, chemotherapy agents, and CAR T-cell therapy.
- Bioanalytical assays method development and validation
- Biomarkers qualification
- PK/PD modeling for OV therapy
Opportunities for Interaction During Product Development

- **Pre-IND Meeting** (INTERACT *)
  - (Informal)
  - Pre-Pre IND
  - (Not PDUFA VI)

- **End of Ph 1 Meeting**
- **End of Ph 2 Meeting**
- **Pre-BLA Meeting**
- **PDUFA VI “The Program” Meetings**

- **IND submission**

- **Development**
- **Preclinical**
- **Clinical Trials**
  - Phase 1
  - Phase 2
  - Phase 3
- **BLA**
- **Marketing Application**
- **Post-marketing**
FDA Guidances

- Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products (June 2015)
  https://www.fda.gov/media/106369/download

- Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products (August 2015)
  https://www.fda.gov/media/89036/download

- Long Term Follow-Up After Administration of Human Gene Therapy Products (Draft, July 2018)
  https://www.fda.gov/media/113768/download

- Biomarker Qualification: Evidentiary Framework (Draft, December 2018)
  https://www.fda.gov/media/122319/download
References

- ICH Considerations – Oncolytic Viruses (October 2009)

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- OTAT Learn Webinar Series:
  http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm

- CBER website: www.fda.gov/BiologicsBloodVaccines/default.htm

- Phone: 1-800-835-4709 or 240-402-8010

- Consumer Affairs Branch: ocod@fda.hhs.gov

- Manufacturers Assistance and Technical Training Branch: industry.biologics@fda.hhs.gov

- Follow us on Twitter: https://www.twitter.com/fdacber
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