Current Clinical Trials with PV-10 (Rose Bengal)

The First Small Molecule Oncolytic Immunotherapy

Sanjiv S. Agarwala, MD

Professor of Medicine
St. Luke’s Cancer Center and Temple University
Bethlehem, Pennsylvania  USA
Oncolytic Immunotherapy with PV-10

- **PV-10** is an **injectable formulation** of **rose bengal disodium** (10% RB)
  - RB is a **small molecule** fluorescein derivative attributed to Gnehm in 1882
  - **Prior human use** of RB
    - IV hepatic diagnostic, $^{131}$I radiolabeled RB: Robengatope®
    - Neonatal use for hepatobiliary diagnosis
    - Topical ophthalmic diagnostic: Rosettes® and Minims®
    - Currently used as a food dye: FR-105 (Japan)
  - **Established safety history**
    - Not metabolized
    - Short circulatory half-life (ca 30 min)
    - Excreted via bile
  - **Stable at room temperature**
  - **Intrallesional injection** can yield **immunogenic cell death (ICD)** resulting in tumor-specific reactivity in circulating CD8+ T cells
PV-10 accumulation in lysosomes of cancer cells

- Lysosomal disruption yields Immunogenic Cell Death (ICD)
Oncolytic Immunotherapy with PV-10

Primary Oncolysis

- Intrallesional Injection
- Lysosomal Accumulation
- Lysosomal Disruption
- Immunogenic Cell Death (ICD)

Secondary Adaptive Immunity

- DAMP and Antigen Release
- APC Recruitment and Antigen Uptake
- T-cell Activation
- Functional T-cell Activity

References:
- Wachter et al., SPIE 4620, 143, 2002 (lysosomal accumulation and rupture in tissue culture)
- Thompson et al., Mel Res 18, 405, 2008 (ablation of injected tumors and bystander regression in recurrent patients)
- Toomey et al., PLoS One 8, e68561, 2013 (tumor-specific immune response in mice)
- Liu et al., Oncotarget 7, 37893, 2016 (DAMPs, DC recruitment/activation, T-cell activation in mouse and man)
- Qin et al., Cell Death and Disease 8, e2584, 2017 (immunogenic cell death in colon cancer)
Phase 2: Subgroups by Baseline Disease Burden

ITT Population
N = 80
26% CR (CI 17-37%)
51% ORR (CI 40-63%)
TTR = 1.9 months

Stable Visceral Disease with Cutaneous Lesions
N = 18

Cutaneous Disease Only
N = 62

Some Disease Not Followed (TNTC or > 1 Lesion Not Measured)
N = 8

All Melanoma Followed
N = 54
37% CR (CI 24-51%)
63% ORR (CI 49-76%)
TTR = 1.8 months

Bystanders Untreated
N = 26
23% CR (CI 9-44%)
54% ORR (CI 33-73%)
TTR = 2.5 months

All Lesions Treated
N = 28
50% CR (CI 31-70%)
71% ORR (CI 51-87%)
TTR = 1.8 months

Per-patient response maximized when all baseline disease treated

Agarwala et al., ASCO 2014
Response of Injected Lesions

56% of lesions achieved CR after 1-2 injections
All Melanoma Followed Sub-Group (N = 54 Patients)

74% CR when all lesions injected
All Melanoma Treated Subgroup (N = 28 patients / 181 lesions)

Agarwala et al., ASCO 2014
Male age 73, Stage IIIB in-transit melanoma of the left lower extremity recurrent after surgical intervention (PV-10 started 1.6 months after resection). Eleven lesions (6.0 cm sum diameter) injected with 1.1 mL PV-10 at Day 0 (single bystander lesion not injected); 7 lesions injected with 1.0 mL PV-10 at Week 8 (5.1 cm sum diameter); and 3 lesions injected with 1.3 mL PV-10 at Week 16 (3.4 cm sum diameter). Subject achieved CR in all injected lesions at Week 36 and confirmed CR in all lesions (including uninjected bystander) at Week 52. Reproduced with permission of Pro vectus Biopharmaceuticals, Inc.

Agarwala et al., ASCO 2010
Clinical Examples

Day 0 – Pre-Rx

Day 1

Week 36

Male age 57, Stage IIIIB melanoma recurrent after 3 interventions. Six lesions injected with PV-10 on Day 0, 3 lesions injected at Week 8 and 3 injected at Week 16. CR at Week 24 with NED at Week 52.

Agarwala et al., ASCO 2014
Distant Tumor Response

Subject 0907: Male, age 40, Stage IV (M1c) since 2006
Multiple Sx, CLND, whole brain XRT, stereotactic radiosurgery, DTIC, IV- and SQ-IFN
Four treatments (Day 0, Week 8, Week 12 and Week 16) with PV-10 to cutaneous lesions

PR of injected cutaneous lesions; 9 of 10 pulmonary lesions resolved at Week 12 (PR of 10\textsuperscript{th} nodule)

Agarwala et al., ESMO 2012
# Locoregional Adverse Events

<table>
<thead>
<tr>
<th>System Organ Class&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adverse Events&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred Term&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(ITT Population, N = 80)</td>
</tr>
<tr>
<td><strong>General Disorders and Administration Site Conditions</strong></td>
<td><strong>CTCAE Grade</strong></td>
</tr>
<tr>
<td>Injection Site Pain</td>
<td></td>
</tr>
<tr>
<td>Injection Site Edema</td>
<td></td>
</tr>
<tr>
<td>Injection Site Vesicles</td>
<td></td>
</tr>
<tr>
<td>Injection Site Discoloration</td>
<td></td>
</tr>
<tr>
<td>Injection Site Swelling</td>
<td></td>
</tr>
<tr>
<td>Injection Site Pruritus</td>
<td></td>
</tr>
<tr>
<td>Injection Site Erythema</td>
<td></td>
</tr>
<tr>
<td>Injection Site Infection</td>
<td></td>
</tr>
<tr>
<td>Peripheral Edema</td>
<td></td>
</tr>
<tr>
<td>Injection Site Cellulitis</td>
<td></td>
</tr>
<tr>
<td>Injection Site Necrosis</td>
<td></td>
</tr>
<tr>
<td><strong>Gastrointestinal Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Dysphagia</td>
<td></td>
</tr>
<tr>
<td><strong>Nervous System Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
</tr>
<tr>
<td><strong>Skin and Subcutaneous Tissue Disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Photosensitivity Reaction</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>System Organ Class and Preferred Term are based on the MedDRA<sup>®</sup> version 13.0 terminology dictionary. Locoregional adverse events were coded to “injection site” Preferred Terms to differentiate these from systemic events.

<sup>b</sup>Includes all AEs with an incidence of 10% or higher and all CTCAE Grade 3 and higher AEs; there were no treatment-related Grade 4 or 5 AEs reported.

<sup>c</sup>If a patient experienced an AE more than once during the study the greatest severity is presented.

<sup>d</sup>Discoloration locoregional to injected lesions.

Thompson et al., *Ann Surg Oncol* 2015
Phase 3 Design

- **PV-10-MM-31: International Pivotal RCT**
  - 225 patients with locally advanced cutaneous melanoma
    - Stage III B - IVM1a
  - Randomized 2 : 1 to PV-10 or comparator
    - Investigator’s choice of Imlygic or DTIC / TMZ
  - RECIST 1.1 by Independent Review Committee (IRC)
    - Primary: PFS
    - Secondary: Complete Response Rate, Duration of Complete Response
    - Exploratory: QoL, Investigator-Assessed Lesion Symptoms
  - Competent Authority (CA) approval in USA, AUS, Germany, Italy, France, Mexico and Argentina
**Phase 3 Schematic**

- **Patients with Locally Advanced Cutaneous Melanoma**
  - Randomize (2 : 1)³
  - Investigational Arm
    - PV-10 q4w
  - RECIST q12w
    - PR/SD
    - CR/PDe

- **Comparator Arm**
  - DTIC or TMZ q4w
  - IMLYGIC q2w³

---

*Footnotes:*

a. 225 patients randomized 2:1 (stratified for prior immune checkpoint inhibition)
b. IMLYGIC repeated after 3 weeks then q2w
c. Cross-over allowed upon documented PD in comparator arm
Phase 1b / 2 Combination Study

- **Protocol PV-10-MM-1201**
- **Phase 1b**
  - Safety of PV-10 + pembrolizumab in up to 24 subjects with unresectable metastatic melanoma (i.e., Stage IV) having at least 1 injectable cutaneous melanoma lesion and who are candidates for pembrolizumab.
  - PV-10 administered by intralesional injection to all injectable cutaneous and subcutaneous lesions (up to 5 cycles); pembrolizumab administered by intravenous infusion (up to 24 mo).
  - Preliminary efficacy (best objective response rate and progression free survival at week 16 based on RECIST 1.1; and overall survival).
  - Phase 1 accrual completed May 2018 (24 subjects in ITT population).
- **Phase 2**
  - Expanded phase 2 RCT of PV-10 + pembrolizumab vs. pembrolizumab alone (i.e., PV-10 + Standard of Care vs. Standard of Care).
  - PFS, ORR and OS key endpoints.
# Phase 1b / 2 Combination Study

## Subject Characteristics

(Safety Population, N = 12)

<table>
<thead>
<tr>
<th>ID / Age / Gender</th>
<th>Stage</th>
<th>Number of CUT/SQ Lesions</th>
<th>Location(s) of Target Lesions</th>
<th>Site(s) of Non-Target Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0101 / 81 / M</td>
<td>M1c</td>
<td>3</td>
<td>SQ (x2), Axillary LN</td>
<td>Liver, Lung</td>
</tr>
<tr>
<td>0102 / 47 / M</td>
<td>M1b</td>
<td>4</td>
<td>Scalp</td>
<td>Lung</td>
</tr>
<tr>
<td>0104 / 79 / M</td>
<td>M1b</td>
<td>3</td>
<td>LLE</td>
<td>Bilateral Lung</td>
</tr>
<tr>
<td>0105 / 69 / M</td>
<td>M1c</td>
<td>4</td>
<td>RUE</td>
<td>Bone, Liver, Lung</td>
</tr>
<tr>
<td>0106 / 78 / M</td>
<td>M1a</td>
<td>1</td>
<td>RLE (x2)</td>
<td>In-transit or satellite with nodal mets (N3)</td>
</tr>
<tr>
<td>0202 / 52 / M</td>
<td>M1a</td>
<td>1</td>
<td>Chest Wall</td>
<td>Chest Wall, Axillary LN</td>
</tr>
<tr>
<td>0203 / 76 / M</td>
<td>M1b</td>
<td>2</td>
<td>RLE (x2)</td>
<td>Lower Extremity and Lung</td>
</tr>
<tr>
<td>0204 / 28 / M</td>
<td>M1a</td>
<td>40</td>
<td>Jaw, LUE</td>
<td>Scalp, Face, Neck, Torso, Upper Extremity, LN (x20)</td>
</tr>
<tr>
<td>0205 / 50 / F</td>
<td>M1a</td>
<td>3</td>
<td>Chest Wall</td>
<td>Back</td>
</tr>
<tr>
<td>0206 / 73 / M</td>
<td>M1b</td>
<td>1</td>
<td>RUE</td>
<td>Lung</td>
</tr>
<tr>
<td>0401 / 70 / M</td>
<td>M1c</td>
<td>1</td>
<td>Lung, Shoulder</td>
<td>Liver, Lung</td>
</tr>
<tr>
<td>0402 / 79 / M</td>
<td>M1c</td>
<td>2</td>
<td>Axilla, Flank</td>
<td>Liver</td>
</tr>
</tbody>
</table>

Patients had extensive uninjected tumor burden

Agarwala et al., SMR 2017
# Preliminary Safety Results

<table>
<thead>
<tr>
<th>Treatment-Emergent Adverse Events (TEAEs)</th>
<th>All TEAEs</th>
<th>TEAEs Related to PV-10</th>
<th>TEAEs Related to Pembrolizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site pain</td>
<td>7 0</td>
<td>7 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4 0</td>
<td>0 0</td>
<td>3 0</td>
</tr>
<tr>
<td>Injection site discolouration</td>
<td>3 0</td>
<td>3 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Chest pain</td>
<td>2 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Injection site discharge</td>
<td>2 0</td>
<td>2 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Injection site haemorrhage</td>
<td>2 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Injection site photosensitivity reaction</td>
<td>2 0</td>
<td>2 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Oedema peripheral</td>
<td>2 0</td>
<td>2 0</td>
<td>0 0</td>
</tr>
<tr>
<td><strong>SKIN AND SUBCUTANEOUS TISSUE DISORDERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>2 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td>Rash maculo-papular</td>
<td>2 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td><strong>ENDOCRINE DISORDERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>2 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td><strong>GASTROINTESTINAL DISORDERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td><strong>MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>3 0</td>
<td>0 0</td>
<td>2 0</td>
</tr>
<tr>
<td><strong>METABOLISM AND NUTRITION DISORDERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycaemia</td>
<td>1 1</td>
<td>0 0</td>
<td>1 1*</td>
</tr>
<tr>
<td><strong>NERVOUS SYSTEM DISORDERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>1 1</td>
<td>0 0</td>
<td>1 1*</td>
</tr>
</tbody>
</table>

AEs coded using MedDRA v19.0 for system organ class (SOC) and preferred term (PT). Subjects with more than one occurrence of the same AE are counted once based on maximum severity. Single occurrences of decreased appetite (Grade 1) and fatigue (Grade 1) were deemed possibly related to the combination. Single occurrences of hyperglycaemia (Grade 3) and exacerbation of myasthenia gravis (Grade 5) were deemed possibly related and certain related to pembrolizumab, respectively.

PV-10 AEs Equivalent to Single-Agent  
Pembro AEs Non-overlapping  

Agarwala et al., SMR 2017
Preliminary Efficacy Results

- Most subjects had robust response in their Target Lesions
- These included injected cutaneous/subcutaneous lesions and non-injected nodal/visceral lesions

- Response of Target Lesions coincided with combination regimen
- Subjects received a median of 5 cycles of PV-10 (mean 3.8, range 1 – 5)

Patients had rapid, deep response

Agarwala et al., SMR 2017
Beyond Melanoma: Hepatic Tumors

- Percutaneous PV-10 to Hepatic Tumors
  - Protocol PV-10-LC-01
    - “Basket study” design allows assessment in HCC and metastatic tumors
  - PV-10-NET-01
    - Symptomatic gastrointestinal neuroendocrine tumors (GI-NET) metastatic to liver

PV-10 is radiopaque, facilitating delivery and follow-up
Beyond Melanoma: Hepatic Tumors

Protocol PV-10-LC-01 (Phase 1): Initial 18 Subjects

- “Basket study” design allows assessment in HCC and metastatic tumors
  - Currently enrolling cutaneous and uveal melanoma patients

- Single intralesional injection into center of a single study lesion
  - Percutaneous delivery
  - CT or U/S guided

- Follow-up
  - 23 hour admission for initial safety observation
  - Primary follow-up at 28 days to 3 months
  - Extended follow-up every 3 months beginning at Month 6

- Observe treated lesion and any untreated lesions
  - Outcome scored using RECIST (amended to 2D EASL)
Beyond Melanoma: Hepatic Tumors

- Protocol PV-10-LC-01 (Phase 1): Initial 18 Lesions (16 Patients)
  - Median Age 68 years (range 51 – 89)
  - HCC – 7 lesions (6 patients) / Metastases – 11 lesions (10 patients)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Disease and History</th>
<th>Survival Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0005 M 68</td>
<td>HCC (3 tu + Chest Wall and Adrenal Mets, HBV and Cirrhosis)</td>
<td>Alive (NED, 75 mon) a</td>
</tr>
<tr>
<td>0001 F 71</td>
<td>HCC (3 tu, Lobectomy, RFA) b</td>
<td>Alive (with Disease, 58 mon, lost to follow-up)</td>
</tr>
<tr>
<td>0004 F 73</td>
<td>HCC (4 tu, HCV, Cirrhosis, Portal Hypertension, RFA, TACE)</td>
<td>Expired (DP, 48 mon)</td>
</tr>
<tr>
<td>0008 F 66</td>
<td>HCC (3 tu, HCV, Cirrhosis, Portal Hypertension, TACE)</td>
<td>Expired (DP, 12 mon)</td>
</tr>
<tr>
<td>0007 M 67</td>
<td>HCC (1 tu Penetrating Diaphragm)</td>
<td>Expired (Cardiac Comorbidity, 2 mon)</td>
</tr>
<tr>
<td>0101 F 89</td>
<td>HCC (1 tu 8.9 cm)</td>
<td>Expired (SAE, suspected thromboembolism)</td>
</tr>
<tr>
<td>0006 M 61</td>
<td>mCRC (3 tu + Extensive Abdominal Mets, FOLFOX, Avastin, Erbitux)</td>
<td>Alive (NED, 73 mon)</td>
</tr>
<tr>
<td>0204 F 67</td>
<td>mCRC (2 tu, RFA, FOLFOX, Liver Resections)</td>
<td>Alive (24 mon)</td>
</tr>
<tr>
<td>0009 M 85</td>
<td>mCRC (Numerous Metabolically Active Hepatic tu)</td>
<td>Alive (18 mon) c</td>
</tr>
<tr>
<td>0010 F 53</td>
<td>mCRC (3 tu, FOLFOX, Avastin, Irinotecan, Partial Hepatectomy)</td>
<td>Alive (9 mon)</td>
</tr>
<tr>
<td>0206 F 67</td>
<td>mCRC (≥ 6 tu, FOLFOX, FOLFIRI, ZALTRAP, Regorafinib)</td>
<td>Expired (DP, 3 mon)</td>
</tr>
<tr>
<td>0203 M 69</td>
<td>Lung (≥ 4 tu, Nivo, SNX-5422 and Carbo/Paclitaxel)</td>
<td>Expired (DP, 12 mon)</td>
</tr>
<tr>
<td>0202 M 83</td>
<td>Lung (≥ 6 tu, Carbo/Abraxane)</td>
<td>Expired (DP, 4 mon)</td>
</tr>
<tr>
<td>0205 M 83</td>
<td>Pancreatic (2 tu)</td>
<td>Alive (12 mon)</td>
</tr>
<tr>
<td>0102 F 53</td>
<td>Melanoma (≥ 4 tu + Lung Mets, Hepatitis, Biochemo, Nivo + Ipi) b</td>
<td>Expired (DP, 18 mon)</td>
</tr>
<tr>
<td>0201 F 51</td>
<td>Ovarian (≥ 35 tu, Carbo/Paclitaxel)</td>
<td>Expired (DP, 15 mon)</td>
</tr>
</tbody>
</table>

a commenced sorafenib 11 months after PV-10 for residual adrenal and chest wall nodules (continued for 28 months until NED)
b 2 lesions injected with PV-10 (requiring re-enrollment under separate subject number)
c commenced Avastin, 5-FU and Fusilev two months after PV-10

Goldfarb et al., APASL February 2017
PV-10-LC-01 – Subject 0001/0003 (HCC #1) – CT

- Female, age 71, 3.4 cm HCC lesion injected once with 5.1 mL PV-10

Goldfarb et al., ESMO-GI 2015
Beyond Melanoma: Hepatic mNETs

- **Protocol PV-10-NET-01 (Phase 1)**
  - Patients with Symptomatic Neuroendocrine Tumors (mNET) metastatic to liver
  - Study design similar to PV-10-LC-01 protocol
  - Assessment of multiple dimensions of response
    - Objective Response of Injected and Bystander Tumors
      - Ga-DOTATA PET/CT
    - Change in Tumor Biomarkers (CgA and LFT)
    - Change in Tumor Symptoms (QoL and Symptom Log)
  - Single intralesional injection into center of study lesion
    - Percutaneous delivery
    - CT or U/S guided
    - Repeat injection of additional tumors allowed after 6 weeks
    - Cohort 1: single lesion injected / treatment
    - Cohort 2: 1-3 lesions injected / treatment
Beyond Melanoma: Hepatic mNETs

SUV max 37.5 (Injected Tumor)  SUV max 16.7

Baseline  Month 3

SUV max 34.5 (Non-injected Tumor)  SUV max 31.2
Planned Studies for Metastatic Disease

- Continue Basket and NET Studies
  - Open Additional Centers
    - All-comers to expand numbers for important tumor types
    - Focused enrollment of high-priority tumor types (e.g., uveal melanoma, CRC)
    - Expand NET program within AUS and to USA and/or EU

- Leverage LC-01 and MM-1201 Protocols
  - Combination therapy for demonstrated combinations
    - PV-10 + Gemcitabine for pancreatic adenocarcinoma
    - PV-10 + PD-1
    - PV-10 + PD-L1
    - Path to relevance for lagging class of checkpoint inhibitor

Conclusions

• PV-10 is a non-viral oncolytic immunotherapy with activity in multiple tumor types
• PV-10 shows robust responses as a monotherapy in a large melanoma phase II trial
• Randomized Phase III international trial underway
• Combination data with PV-10 and anti-PD1 (pembrolizumab) appears promising
• Non-melanoma data is intriguing and warrants further investigation