Locoregional immunomodulation with oncolytic viruses for systemic cancer immunotherapy

Igor Puzanov, M.D., M.S.C.I., F.A.C.P.
Director, Melanoma Clinical Research
Vanderbilt University, Nashville, Tennesee
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Cancer therapy in the 21st century

- Many new ‘molecularly-targeted’ therapies have been approved
- These drugs work in patients with targeted mutation, patients ultimately develop resistance
- A new classes of therapies (checkpoint inhibitors, oncolytic virus) were recently been approved by the FDA.
  - These drugs produce longer durable remissions in some patients
  - They engage the immune system against the tumor – frequently employing T cells
- Will there be a synergy?
- Agent specific vs. class synergy?
Locoregional therapies in development

- Oncolytic viruses (Talimogene laherparepvec, Newcastle disease virus, Coxsackie virus A21, HF10-oncolytic HSV1, Reovirus)
- Non-viral based therapies (PV-10/Rose Bengal disodium)
- These drugs work in patients with or without specific targeted mutation
- Will there be a synergy?
- Agent specific vs. class synergy?
Pitfalls of injectable therapies

• Need for direct injection-limits the type of patients able to receive the therapy and thus benefit

• Patient selection-lack of easily obtainable biomarkers
Immune-active microenvironment and type I IFN transcriptional signature are associated with clinical benefit from immunotherapies

Positive correlation between increased T cell infiltration and type I IFN-induced transcripts

Fuertes M.B. et al., JEM. 208:2005-16 (2011)

Could strategies targeting type I IFN pathway sensitize tumors to immune checkpoint blockade?


Positive correlation between increased T cell infiltration and type I IFN-induced transcripts

Fuertes M.B. et al., JEM. 208:2005-16 (2011)
Tumor infection with oncolytic viruses results in type I IFN production and immunogenic cell death

- DC maturation
- ↑MHC
- ↑Costimulatory ligands
- ↑Cytokines, chemokines

- ↑MHC,
- ↑Costimulatory ligands
- ↑Surface HSPs and ecto-CRT
- ↑Type I IFN

- 1. TAA’s, 2. DAMPS
- 1. DC maturation
- 2. ↑MHC
- 3. ↑Costimulatory ligands
- 4. ↑Cytokines, chemokines

D Zamarin, JD Wolchok - Molecular Therapy—Oncolytics, 2014
Newcastle disease virus (NDV) as an anti-cancer agent

- Birds are a natural host, thus humans do not have pre-existing immunity to the virus.
- Readily infects the majority of cancer cells due to ubiquity of the receptor (sialic acid).
- Strong inducer of type I interferon and dendritic cell maturation.
- Specificity for cancer cells is mediated by selective viral replication in cells with deficient innate immune responses and cells resistant to apoptosis.
- Clinical trials with systemically-administered NDV in humans demonstrated safety and durable clinical benefit in different cancer types.
NDV infection upregulates MHC and co-stimulatory molecules on the surface of tumor cells
Intratumoral NDV treatment results in immune infiltration in both virus-injected and distant tumors

B16-F10 melanoma model

NDV

Virus replicates only in treated tumors

NDV induces distant B16 tumor growth delay, but few complete regressions

![Graphs showing tumor size over days for treated and distant tumors treated with PBS and NDV. The p-values are p<0.008 for treated tumors and p<0.0007 for distant tumors.](image-url)
TILs from distant tumors upregulate CTLA-4
Combination therapy with NDV and CTLA-4 blockade leads to rejection of the treated and distant B16-F10 tumors and long-term survival

Talimogene laherparepvec

- Talimogene laherparepvec
  - Genetically modified HSV-1-based oncolytic immunotherapy
  - Approved in the US for local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery*

- Pembrolizumab
  - Human anti-PD-1 Ab that restores the function of tumor antigen-specific T cells and improves tumor control
  - Approved for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor

*Talimogene laherparepvec has not been shown to improve overall survival or have an effect on visceral metastases (Imlygic™ [talimogene laherparepvec]; USPI, Amgen 2015)
Key Melanoma Studies

• OPTiM: Ph 3 T-VEC vs GM-CSF
  — Improved Durable Response Rate (response for ≥6 months)
  — 16% vs 2%, p<0.0001

• KEYNOTE 006: Ph 3 pembrolizumab (Q2W/Q3W) vs ipilumumab
  — Improved PFS (HR: 0.58, p<0.001)
  — Improved OS (Q2W HR: 0.63, p=0.0005; Q3W HR: 0.69, p=0.0036)
  — Improved ORRRECIST (Q2W 33.7%, Q3W 32.9% vs 11.9% ipi, p<0.001)

• 20110264: Phase 1b T-VEC + ipilimumab
  — ORRIRRC 50%
  — Durable Response Rate of 44%
  — Tolerable safety profile

irRC = immune-relate response criteria; T-VEC = talimogene laherparepvec
Phase 1b results from a multicenter trial of talimogene laherparepvec + ipilimumab (T-VEC+ipi) in previously untreated, unresected stage IIIB-IV melanoma

Igor Puzanov,¹ Mohammed Milhem,² Robert H. I. Andtbacka,³ David Minor,⁴ Omid Hamid,⁵ Ai Li,⁶ Jeffrey Chou,⁶ and Howard Kaufman⁷

¹Vanderbilt University, Nashville, TN; ²University of Iowa, Iowa City, IA; ³Huntsman Cancer Institute, Salt Lake City, UT; ⁴California Pacific Melanoma Center, San Francisco, CA; ⁵The Angeles Clinic and Research Institute, Los Angeles, CA; ⁶Amgen Inc., Thousand Oaks
Background: T-VEC Mechanism of Action
20110264: Study Schema – Phase 1b Portion

Unresectable Stage IIIB-IV Melanoma
- Injectable
- Treatment naïve
- ECOG PS 0 or 1
- No evidence of CNS mets

T-VEC Intralesional
$10^6$ PFU/mL, after 3 weeks $10^8$ PFU/mL Q2W

Ipilimumab 3mg/kg IV Q3W x 4

Week 1

Week 6

N = 19

- T-VEC dosing until CR, all injectable tumors disappear, PD per irRC, or intolerance, whichever is first
- Safety follow-up occurs 30 (+7) days after last dose of T-VEC or 60 (+7) days after last dose of ipilimumab, whichever is later

Primary Endpoint: Incidence of dose-limiting toxicities (DLTs)
Key Secondary Endpoints: ORR$^{irRC}$, Safety
### Results: Patient Demographics

<table>
<thead>
<tr>
<th></th>
<th>Total (N = 19*)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex – n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 (42)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (58)</td>
</tr>
<tr>
<td><strong>Age, median (min, max) – years</strong></td>
<td>61 (29, 84)</td>
</tr>
<tr>
<td><strong>ECOG PS – n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14 (74)</td>
</tr>
<tr>
<td>1</td>
<td>5 (26)</td>
</tr>
<tr>
<td><strong>Disease stage – n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>1 (5)</td>
</tr>
<tr>
<td>IIIIC</td>
<td>3 (16)</td>
</tr>
<tr>
<td>IV M1a</td>
<td>4 (21)</td>
</tr>
<tr>
<td>IV M1b</td>
<td>5 (26)</td>
</tr>
<tr>
<td>IV M1c</td>
<td>6 (32)</td>
</tr>
<tr>
<td><strong>BRAF mutation status – n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>11 (58)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>8 (42)</td>
</tr>
<tr>
<td><strong>HSV-1 status – n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5 (26)</td>
</tr>
<tr>
<td>Positive</td>
<td>13 (68)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

*One patient received one dose of T-VEC only before withdrawing consent and enrolling in hospice care*
## Treatment-Emergent Adverse Events*

<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>Total N (%)</th>
<th>Grade 3 N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any event</td>
<td>19 (100)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>Any attributed to T-VEC</td>
<td>17 (90)</td>
<td>3 (16)†</td>
</tr>
<tr>
<td>Any attributed to ipilimumab</td>
<td>15 (80)</td>
<td>3 (16)†</td>
</tr>
<tr>
<td>Chills</td>
<td>11 (58)</td>
<td>-</td>
</tr>
<tr>
<td>Fatigue</td>
<td>11 (58)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>11 (58)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Nausea</td>
<td>9 (47)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Rash</td>
<td>9 (47)</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>8 (42)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Headache</td>
<td>8 (42)</td>
<td>-</td>
</tr>
<tr>
<td>Pruritis</td>
<td>8 (42)</td>
<td>-</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>4 (21)</td>
<td>-</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>4 (21)</td>
<td>-</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (21)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>ALT increased</td>
<td>3 (16)</td>
<td>-</td>
</tr>
<tr>
<td>Back pain</td>
<td>3 (16)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Influenza-like illness</td>
<td>3 (16)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Pain</td>
<td>3 (16)</td>
<td>-</td>
</tr>
<tr>
<td>Vision blurred</td>
<td>3 (16)</td>
<td>-</td>
</tr>
</tbody>
</table>

- Nausea was the only grade 3 event occurring in > 1 patient
- Two grade 4 events occurred in one patient with elevated amylase and lipase (attributed to ipilimumab)
- There was one grade 5 event of metastases to central nervous system (preferred term)
- The overall rate of grade 3 or higher treatment-emergent events was 32%

*All events of any grade occurring in > 15% of patients during treatment or up to 30 days after last T-VEC or 60 days after last ipilimumab, whichever was later; †Grade 3 events in these patients: pyrexia attributed to T-VEC; hypophysitis and abdominal distention attributed to ipilimumab; and nausea, diarrhea, fatigue, influenza-like illness, vomiting, adrenal insufficiency, and dehydration attributed to both; ALT: alanine aminotransferase.
Changes in Tumor Burden by Disease Stage

Tumor follow-up time is the duration from the first dose of T-VEC to the last tumor assessment.
Best Overall Response

The waterfall plot shows best reductions in tumor burden at a single time point. For the irRC response table, CR, PR, and PD needed to be confirmed by consecutive assessments no less than 4 weeks apart to be considered confirmed with the following exception: if PD was the last tumor assessment, it was considered as confirmed.

* ≤ -98%, but > -100%
** unconfirmed CR

<table>
<thead>
<tr>
<th>Best irRC Confirmed Response</th>
<th>Per irRC n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall response rate</td>
<td>9 (50)</td>
</tr>
<tr>
<td>Complete response (CR)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Stable disease (SD)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Progressive disease (PD)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Disease control rate (CR+PR+SD)</td>
<td>13 (72)</td>
</tr>
</tbody>
</table>
Responses in Injected Lesions

- 74% of injected index lesions regressed ≥ 50%
- 31% completely regressed

Tumor area change:
- ≥ 25%
- > -50% to <25%
- > -100% to ≤ -50%
- -100%

N = 35
Both uninjected non-visceral and visceral lesions regressed
52% of uninjected index lesions regressed ≥ 50%
39% completely regressed
Progression-Free Survival

- Kaplan-Meier estimate of median PFS was not yet reached (range: 2.6 to 25.4+ months)
- 12-month and 18-month PFS was 50%
Overall Survival

- Kaplan-Meier estimate of median OS was not yet reached
  - 12-month survival was 72%
  - 18-month survival was 67%
Total and activated CD8 T-cells* increase after T-VEC and combination treatment

- Total and activated CD8 T cells in the peripheral blood increased from baseline after T-VEC administration at weeks 4 and 6 and further increased at weeks 9 and 15 after combination T-VEC and ipilimumab.

Data points are overlaid on the box plots. Each box plot shows the range between 25th percentile (q1) and 75th percentile (q3) as a yellow box, with a pink line showing the 50th percentile. The whiskers on each box are q3 ± 1.5* (q3 – q1). A red plus sign indicates outlier data within a subset. P-values below each post week 1 subset indicate significant changes from baseline level on week 1, and those above week 9 subset indicate significant changes from week 6 to week 9.

*Activated CD8 T cells are defined as HLA-DR+CD3+CD4- cells. Puzanov I et al. J Clin Oncol. 2014;suppl: abstr 9029^
Changes in Activated CD8* T Cell Count After Administration of T-VEC and Ipilimumab According to Best Response

- At week 6 after receiving 2 doses of T-VEC, 10 of 12 patients with disease control (SD+PR +CR) had > 1.4x increase in activated CD8 T cell count while 4 of 5 patients with tumor growth (PD) did not
- This pattern was no longer evident after ipilimumab was given

*Activated CD8 T cells are defined as HLA-DR+CD3+CD4- cells.  

20110264: Study Schema - Phase 2 Portion

- **Primary Endpoint**: ORR
  - irRC
- **Secondary Endpoints**: PFS, OS, DRR, BOR, DCR, DoR, TTR, resection rate

**Unresectable Stage IIIB-IV Melanoma**
- Injectable
- ≤ 1 line of systemic therapy for BRAF wt, or ≤ 2 lines of systemic therapy including BRAFi regimen for BRAF mutated
- ECOG PS 0 or 1
- No evidence of active CNS mets

**Ipilimumab 3mg/kg IV Q3W x 4**
- Week 1
- Week 6

**T-VEC Intralesional**
- 10^6 PFU/mL, after 3 weeks 10^8 PFU/mL Q2W
- N = 100

- T-VEC dosing until CR, all injectable tumors disappear, PD per irRC, or intolerance, whichever is first
- Safety follow-up occurs 30 (+7) days after last dose of T-VEC or 60 (+7) days after last dose of ipilimumab, whichever is later
Primary analysis of MASTERKEY-265 phase 1b study of talimogene laherparepvec and pembrolizumab for unresectable stage IIIB-IV melanoma


1Melanoma Institute Australia and The University of Sydney, Sydney, Australia; 2University Hospital of Zurich, Zurich, Switzerland; 3University of California at Los Angeles Medical Center, Los Angeles, CA; 4Vanderbilt University Medical Center, Nashville, TN; 5Centre Hospitalier Universitaire Vaudois; Lausanne, Switzerland; 6The West Clinic, Memphis, TN; 7University of Utah Huntsman Cancer Institute, Salt Lake City, UT; 8Austin Health, Austin Hospital, Heidelberg, Australia; 9Hopitaux Universitaires de Geneve; Geneva, Switzerland; 10Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; 11Fox Chase Cancer Center, Philadelphia, PA; 12The University of Chicago Medicine, Chicago, IL; 13University of Pittsburgh Cancer Institute, Pittsburgh, PA; 14Merck & Co., Inc., Kenilworth, NJ; 15Amgen Inc., Thousand Oaks, CA; 16Dana-Farber Cancer Institute, Boston, MA
Pitfalls of injectable therapies

- Need for direct injection-limits the type of patients able to receive the therapy and thus benefit

- Patient selection-lack of easily obtainable biomarkers
Respiratory Enteric Orphan Virus (pelareorep)

Pelareorep infects both tumor cells and normal, healthy cells

Pelareorep is a virus whose replication is stopped in a non-Ras-activated cell

Healthy cell remains undamaged

Pelareorep replicates in Ras-activated tumor cells

Tumor cells rupture to release progeny virus

Replicated viruses repeat cell lysis cycle in nearby tumor cells

Pelareorep administered to patients via IV
Mouse Melanoma Treated with Reovirus Showed Increased PD L1 Expression

Reovirus + paclitaxel  No reovirus
Pelareorep and Anti-PD-1 Combination Therapy

- The combination of Pelareorep with an anti-PD-1 Ab results in prolonged survival of mice with melanoma.

Rajani et al. 2014
PV-10

- PV-10 is a sterile, non-pyrogenic solution of Rose Bengal disodium (10% RB) for intralesional injection
  - 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®
    - Topical ophthalmic diagnostic: Rosettes® and Minims®
  - Established safety history
    - Not metabolized
    - Short circulatory half-life (ca 30 min)
    - Excretion via bile
  - Radiopaque with prolonged retention in tumors
  - Intralesional injection results in ablative immunotherapy
Direct liver injections:
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
Pitfalls of injectable therapies

• Need for direct injection-limits the type of patients able to receive the therapy and thus benefit

• Patient selection-lack of easily obtainable biomarkers
Tumor infiltrating T-cells are promising biomarkers of immunotherapy

- T-cells (CD8+) can attack and kill tumor cells
- Influx of T cells into the tumor prior to response
- Repeat biopsies burdensome
Radiographic imaging

- Anatomical
  - MRI
  - XRAY
  - CT
- Show anatomy (density, structure)

- Functional
  - SPECT
  - PET
- Show function or other features

Xray

FDG-PET
Development of $^{124}$I–labelled anti-CD8 antibody

- Radioactive Iodine-124 is attached chemically to an antibody that binds to CD8 on T-cells.
- This anti-CD8-$^{124}$I conjugate is then delivered to the patient or subject by IV injection (microdose).
- The anti-CD8-$^{124}$I binds to T-cells and ‘labels’ them in the body.
- PET scanning is then performed, focusing on the tumor site.
- Measuring the signal in the tumor region gives an estimation on the amount of T-cells present in the tumor.
- This could be at baseline and after 1-2 doses of immunotherapy.
- Data analysis defining “Immuno-PET signature or response/non-response”
Imaging T cells – initial experience
Summary

• Combination of locoregional therapy with systemic immunotherapy seems to be safe with several multiple agents and combinations in development

• Expanding the definition of “injectable lesion” to liver, deep lymph nodes and other locations would increase the potential of such therapies

• Preliminary efficacy from Phase Ib talimogene laherparepvec in combinations with either ipilimumab or pembrolizumab is promising with minimal added toxicity

• Other agents are planning or undergoing similar development

• The optimal selection of both patients and agents is unclear, unlikely to be answered in randomized trials only across tumor types

• Development of biomarkers for selection is of extreme importance
Acknowledgements

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