REOLYSIN® and Immune Therapy: Rationale for Combination Therapy

Matt Coffey, PhD
Royal Society of Medicine
15 April 2015
Forward Looking Statements

This presentation contains certain forward looking statements relating to the company’s financial results, business prospects and the development and commercialization of REOLYSIN®, a therapeutic reovirus. These statements are based on management’s current expectations and beliefs and are subject to a number of factors which involve known and unknown risks, delays, uncertainties and other factors not under the company’s control which may cause actual results, performance or achievements of the company to be materially different from the results, performance or other expectations implied by these forward looking statements.

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REOLYSIN®: Background
Oncolytics Biotech Inc.

- The Company is focused on the development of an oncolytic virus for use as a cancer therapeutic

- The Company’s product, REOLYSIN®, is a proprietary isolate of reovirus type 3 Dearing, currently being investigated in many randomised clinical studies across a variety of cancer indications

- USAN: pelareorep
Reovirus Replication

- Fully replication-competent
- Mammalian permissive which allows effective modeling in murine, canine, and non-human primate models
- Viral replication is exclusively cytoplastic

*Kras mutated colorectal cancer cell line infected with reovirus. Picture courtesy of Dr. Scott Wadler.*
Reovirus-Mediated Chemoimmunotherapy
A Historical Perspective

www.oncolyticsbiotech.com  TSX ONC  NASDAQ ONCY

Technology Changing Life®
Immunotherapy with Reovirus

- Kollmorgen et al (1976) demonstrates that mice implanted with EL4 lymphoma could be cured when treated with BCNU and reovirus.
- These animals developed tumour-specific immunity and were resistant to lethal tumour re-challenge with EL4 cells.
- Immunosuppressed mice derived no benefit from the combination therapy.
Further Refinements

- Steele et al (1995) demonstrate that BCNU/reovirus treated mice that reject their EL-4 tumours reject re-challenge with EL-4 but not another tumour (L1210).
- Cyclosporine abrogated BCNU/reovirus therapy (role for T-cells) but;
- Cyclosporine had no effect on re-challenge of cured mice.
REOLYSIN® Mechanism of Action

REOLYSIN® infects both tumour cells and normal, healthy cells

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

Healthy cell remains undamaged

REOLYSIN® replicates in Ras-activated tumour cells

Tumour cells rupture to release progeny virus

Replicated viruses repeat cell lysis cycle in nearby tumour cells

REOLYSIN® administered to patients via IV
REOLYSIN®: Clinical Development

- Phase I studies were conducted in various tumour types without reaching a maximum tolerated dose (MTD); highest total weekly dose reached was $1.5 \times 10^{11}$ TCID$_{50}$, administered intravenously.

- Synergistic with cytotoxic compounds or radiotherapy (RT), both of which promote delivery to the interior of a tumour, viral protein translation, and apoptosis of tumour cells.

- Objective responses are seen in tumours with known Ras pathway activation.

- Safety profile:
  - Minimal hematological or liver toxicity
  - Characteristic flu-like symptoms
  - Administered on an outpatient basis
  - BSL2 with no major precautions

*Picture courtesy of Drs. Mahalingham and Nuovo (REO 017 study of Gemcitabine and REOLYSIN® in pancreatic cancer with known KRAS mutation)*
<table>
<thead>
<tr>
<th>Trial</th>
<th>Phase</th>
<th>Sponsor</th>
<th>n</th>
<th>Enrollment Status</th>
</tr>
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<tbody>
<tr>
<td>IND 213: Intravenous REOLYSIN® in Combination with Paclitaxel in Patients with Advanced or Metastatic Breast Cancer</td>
<td>II</td>
<td>NCIC CTG</td>
<td>100</td>
<td>&gt;60% complete</td>
</tr>
<tr>
<td>IND 211: Intravenous REOLYSIN® in Combination with Docetaxel or Pemetrexed in Patients with Previously-Treated Advanced or Metastatic Non-Small Cell Lung Cancer (NSCLC)</td>
<td>II</td>
<td>NCIC CTG</td>
<td>150</td>
<td>&gt;90% complete</td>
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<tr>
<td>IND 210: Intravenous REOLYSIN® in Combination with FOLFOX-6 Plus Bevacizumab (Avastin®) in Patients with Advanced or Metastatic Colorectal Cancer</td>
<td>II</td>
<td>NCIC CTG</td>
<td>100</td>
<td>complete</td>
</tr>
<tr>
<td>IND 209: Intravenous REOLYSIN® in Combination with Docetaxel in Patients with Recurrent or Metastatic Castration-Resistant Prostate Cancer</td>
<td>II</td>
<td>NCIC CTG</td>
<td>80</td>
<td>&gt;90% complete</td>
</tr>
<tr>
<td>GOG-0186H: Intravenous REOLYSIN® in Combination with Paclitaxel for Patients with Persistent or Recurrent Ovarian, Fallopian Tube or Primary Peritoneal Cancer</td>
<td>II</td>
<td>NCI/GOG</td>
<td>110</td>
<td>complete</td>
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<tr>
<td>NCI-8601: Intravenous REOLYSIN® in Combination with Carboplatin and Paclitaxel for Patients with Metastatic Pancreatic Cancer</td>
<td>II</td>
<td>NCI</td>
<td>70</td>
<td>complete</td>
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REOLYSIN®, PD-1 and PD-L1 Initial Observation
Kelly et al (2011) notice that genes other than those related to endoplasmic reticular stress are induced in response to reovirus infection.
Reovirus Induces Expression of PD-L1 and PD-L2

<table>
<thead>
<tr>
<th></th>
<th>PD-L1 (CD274)</th>
<th>PD-L2 (PDCD1LG2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-Value</td>
<td>Fold Change</td>
</tr>
<tr>
<td>Panc + Reo 24h</td>
<td>0.0006619</td>
<td><strong>10.710</strong></td>
</tr>
<tr>
<td>Panc + Reo 48h</td>
<td>0.0011764</td>
<td>5.270</td>
</tr>
<tr>
<td>RPMI + Reo 24h</td>
<td>0.0217536</td>
<td>1.982</td>
</tr>
<tr>
<td>RPMI + Reo 48h</td>
<td>0.0167276</td>
<td>4.356</td>
</tr>
<tr>
<td>U266 + Reo 48h</td>
<td>0.0010705</td>
<td><strong>26.880</strong></td>
</tr>
<tr>
<td>HT1080 + Reo</td>
<td>0.0050015</td>
<td>2.090</td>
</tr>
<tr>
<td>SKLMS1 + Reo</td>
<td>0.0011008</td>
<td>3.489</td>
</tr>
</tbody>
</table>
Reovirus and PD-L1 *In Vitro*

- Reovirus upregulates PD-L1 on the surface of breast, kidney and lung cell lines.
- The combination of reovirus and sunitinib further enhances the expression of PD-L1 in breast and lung cancer cell lines.
- This combination increases cell killing in breast and lung cancer cell lines.

*Mostafa et al. 2014: Don Morris group, University of Calgary (AACR Tumour Immunology Conference poster)*
REOLYSIN®, PD-1 and PD-L1 *In Vivo*

- REOLYSIN® induces the up-regulation of PD-1 and PD-L1 in target tissues
- PD-L1 and PD-1 overexpression is strongly associated with productive reoviral infection
- PD-L1 expression appears to be enhanced when the virus is used in combination with cytotoxics
Mouse Melanoma Tumours Treated with Reovirus Showed Increased PD-L1 Expression

Reovirus + Paclitaxel

No Reovirus
Mouse Melanoma Tumours Treated with Reovirus Showed Increased PD-L1 Expression
PD-L1/PD-1 Expression in REOLYSIN®-Treated Patients
Evidence in Brain Lesions and Metastatic Pancreatic Cancer
REO 013b is an open-label, single arm, translational clinical study of REOLYSIN® as single intravenous infusion to nine patients with high grade glioma or metastatic brain tumours prior to planned surgical resection of the target tumours.
# Presence of Reoviral Protein, PD-1L, and PD-1 (REO 013b Study)

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Reoviral Protein</th>
<th>PD-L1</th>
<th>PD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glioblastoma</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>2</td>
<td>adenocarcinoma (colon metastasis)</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>3</td>
<td>glioma, grade 3</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>4</td>
<td>glioma, grade 3</td>
<td>negative</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>5</td>
<td>melanoma metastasis</td>
<td>negative</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>6</td>
<td>glioblastoma</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>7</td>
<td>glioblastoma</td>
<td>negative</td>
<td>weak</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>glioblastoma</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>9</td>
<td>melanoma metastasis</td>
<td>2+</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>10 (control)</td>
<td>adenocarcinoma (breast metastasis)</td>
<td>negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11 (control)</td>
<td>glioblastoma</td>
<td>negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 (control)</td>
<td>glioblastoma</td>
<td>negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13 (control)</td>
<td>glioblastoma</td>
<td>negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14 (control)</td>
<td>glioblastoma</td>
<td>negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15 (control)</td>
<td>adenocarcinoma (ovarian metastasis)</td>
<td>negative</td>
<td>0</td>
<td>weak</td>
</tr>
</tbody>
</table>
REOLYSIN® Increases PD-L1 Expression

- Glioblastomas treated with REOLYSIN®: productive reoviral infection showed increases in PD-L1 expression (brown is +)

GBM Treated with REOLYSIN®  Control GBM (Untreated)

Courtesy of Dr. Gerard Nuovo of the OSU Comprehensive Cancer Center and Phylogeny, Inc.
**REOLYSIN® Increases PD-1 Expression**

- Glioblastomas treated with REOLYSIN®: productive reoviral infection showed increases in PD-1 expression (brown is +)

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**GBM Treated with REOLYSIN®**

**GBM Treated with REOLYSIN®, But No Productive Infection**

*Courtesy of Dr. Gerard Nuovo of the OSU Comprehensive Cancer Center and Phylogeny, Inc.*
Adenocarcinoma: REOLYSIN® Treated Caspase-3 (red is +)

Case 2: Adenocarcinoma (CRC)
Metastatic to Brain
Reoviral +
Activation Kinetics of Human Natural Killer Cells *In Vivo*

- Window of opportunity studies allow for the investigation of a viral infection in a controlled setting
- Upon exposure to the virus, NK cell surface expression of IFN-inducible molecules CD69 and tetherin peaked 24 to 48 hours post infection
- Reovirus modulates NK cell activity *in vivo* and suggests that this may contribute to the therapeutic effect of the virus
Activation Kinetics of Human Natural Killer Cells In Vivo

(a) Patient Viruses Day 1 Day 2 Day 3 Day 4 Day 5 Surgery
P2, 3, 4, 5, 6, 9, 10 Blood 0 h 1 h 48 h 96 h Sgy 1 Mo 3 Mo
P7
P8
P1

(b) HC P3 P8 P1

<table>
<thead>
<tr>
<th>Time</th>
<th>CD69 (%) (total NK cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>5</td>
</tr>
<tr>
<td>1 h</td>
<td>n.s.</td>
</tr>
<tr>
<td>48 h</td>
<td>6</td>
</tr>
<tr>
<td>96 h</td>
<td>6</td>
</tr>
<tr>
<td>Sgy</td>
<td>8</td>
</tr>
<tr>
<td>1 Mo</td>
<td>10</td>
</tr>
<tr>
<td>3 Mo</td>
<td>11</td>
</tr>
</tbody>
</table>

(c) CD69 (% total NK cells) vs. Time post-infection

Clinical & Experimental Immunology
Volume 180, Issue 1, pages 98–107, 10 MAR 2015 DOI: 10.1111/cei.12562
Controlled Infection with a Therapeutic Virus Defines the Activation Kinetics of Human Natural Killer Cells *In Vivo*

![Graphs showing relative mRNA expression and fold change in expression for IFIT1 and IFI44L genes over time for different treatments.](http://onlinelibrary.wiley.com/doi/10.1111/cei.12562/full#cei12562-fig-0001)
But Are These Activated NK Cells Finding the Tumour?
REO 013b: Co-Expression of NK Cells and Productive Reoviral Infection

- Reoviral protein expression is seen in same areas as NK cells in brain metastases from colorectal cancer.

red = reovirus protein; brown = cKIT
red = reovirus protein; green = cKIT; blue = hematoxylin

Courtesy of Dr. Gerard Nuovo of the OSU Comprehensive Cancer Center and Phylogeny, Inc.
Co-Expression of NK Cells and Productive Reoviral Infection

- The lack of reoviral protein expression coincides with an absence of NK cells in colon cancer

red = reovirus protein; brown = cKIT; blue = hematoxylin
Gemcitabine and Reovirus – Mode of Action

- Gemcitabine potentiates oncolytic virotherapy-induced anti-tumour immune responses.
- Reovirus-based virotherapy and gemcitabine primarily target cancer cells by mediating direct oncolytic and pro-apoptotic effects, respectively.
- Additionally, both these interventions promote immunological events that support the development of beneficial anti-tumour immunity. The effects of the combination treatment include:
  - Inhibition of myeloid-derived suppressor cell (MDSC) recruitment to the tumour environment
  - Down-regulation of pro-MDSC factors
  - Accelerated development of anti-tumour T-cell responses

REO 017 Updated Survival Curves (PFS and OS)

- As of 20 February 2015, 34 patients recruited (29 evaluable patients for response)
- 1 PR, 23 patients SD and 5 patients PD
- 18 patients with clinical benefit ≥ 12 weeks (PR/SD)
- Of 33 patients (one patient censored for OS), median PFS of 4 months, OS of 10.2 months
- 1-year and 2-year survival of 45% and 24%, respectively
- Increased survival may be genetically linked and gemcitabine linked, and indicates OS as an endpoint for future studies
## Comparative Overview of REO 017, MPACT and ACCORD 11 Studies – Demographics and Extent of Disease

<table>
<thead>
<tr>
<th>Parameter</th>
<th>REO 017 (n=34)</th>
<th>MPACT (n=430)</th>
<th>ACCORD 11 (n=171)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>66</td>
<td>63</td>
<td>61</td>
</tr>
<tr>
<td>≥65 years</td>
<td>52%</td>
<td>44%</td>
<td>0%</td>
</tr>
<tr>
<td>Sex (M/F)%</td>
<td>53/47</td>
<td>60/40</td>
<td>61/39</td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 0-1</td>
<td>94%</td>
<td>92%</td>
<td>100%</td>
</tr>
<tr>
<td>• 2</td>
<td>6%</td>
<td>8%</td>
<td>0%</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Caucasian</td>
<td>71%</td>
<td>87%</td>
<td>N/A</td>
</tr>
<tr>
<td>• Asian</td>
<td>3%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Metastatic disease at baseline (%)</td>
<td>91</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Median no. of cycles (schedule)</td>
<td>4 (Q3wk)</td>
<td>3 (Q4wk)</td>
<td>6 (Q4wk)</td>
</tr>
<tr>
<td>Previous chemo/radiotherapy</td>
<td>5%</td>
<td>3%</td>
<td>N/A</td>
</tr>
<tr>
<td>Post-PD therapy</td>
<td>53%</td>
<td>42%</td>
<td>50%</td>
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## Comparative Overview of REO 017, MPACT and ACCORD 11 Studies – Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>CA-19.9 &gt;20% decrease from baseline</th>
<th>Median PFS (months)</th>
<th>Median OS (months)</th>
<th>1-year survival (%)</th>
<th>2-year survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemcitabine (ACCORD 11)</td>
<td>171</td>
<td>NA</td>
<td>3.3</td>
<td>6.8</td>
<td>20</td>
<td>2</td>
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<tr>
<td>(Conroy et al., 2011)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gemcitabine (MPACT)</td>
<td>430</td>
<td>44</td>
<td>3.7</td>
<td>6.6</td>
<td>22</td>
<td>5</td>
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<td>(Von Hoff et al., 2013; Goldstein et al., 2015)</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Gemcitabine/REOLYSIN® (REO 017)</td>
<td>33</td>
<td>70</td>
<td>4.0</td>
<td>10.2</td>
<td>45</td>
<td>24</td>
</tr>
</tbody>
</table>
REO 017: Partial Response in Pancreatic Mass and Liver Lesion

- 73-year old patient diagnosed with pancreatic adenocarcinoma in Dec 2011
- PD after cycle 24, patient currently surviving 38 months
Case report of a 54-year old patient diagnosed with pancreatic adenocarcinoma in Feb 2012
- Presence of KRAS (G12D) mutation
- Stable disease was achieved
- Following 25 cycles, patient biopsy samples revealed reovirus replication and co-localisation of active caspase-3
REO 017: Reovirus Replication in Patient Sample

- Single agent REOLYSIN® was associated with strong reoviral RNA proliferation
- Metastatic cancers treated with both reovirus and chemotherapy showed marked increase in reoviral capsid protein expression
REO 017: Gemcitabine and REOLYSIN® Induced PD-L1 Expression (25 cycles)

Baseline

Reolysin +Gem

PD-L1

Pancreatic Cancer Patient

Courtesy of Dr. Steffan T. Nawrocki, Department of Medicine, CRTC at University of Texas Health Science Center at San Antonio
In Vivo Use of the Virus with Checkpoint Inhibitors
REOLYSIN® and Anti-PD-1 Combination Therapy

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

Rajani et al. 2014: Richard Vile group (Conference poster)
REOLYSIN® and Anti-PD-1 Combination Therapy

- The combination of REOLYSIN® with an anti-PD-1 Ab augments tumour-specific NK responses and attenuates tumour-specific immunosuppression, resulting in significant survival benefits in C57BL/6 mice with established SC B16 tumours (melanoma).

Rajani et al. 2014: Richard Vile group (Conference poster)

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REOLYSIN® Mechanism of Action (Modified)

**Normal Cells**
- REOLYSIN® infects both tumour cells and normal healthy cells.
- REOLYSIN® does not replicate in cells that are not Ras activated.
- Healthy cells remain undamaged.

**Ras–Activated Cells**
- REOLYSIN® infects both tumour cells and normal healthy cells.
- REOLYSIN® replicates in Ras-activated tumour cells.
- Tumour cells rupture to release progeny virus.

Progeny viruses repeat cell infection cycle in nearby tumour cells.

Productively infected cells upregulate interferon, PD-1, PD-L1 and others, and induce an anti-tumour specific immune response mediated by NK and T cells.
Clinical Hypothesis

1- Oncolytic Virus

Infect and replicate in tumour cells

Cell Lysis and Virus replication

Lysis, TAA release, pro inflammatory and cytokines release

PDL1

+ 2- PDL1 Inhibitor

+ 3-Sunitinib or Gemcitabine

Enhanced Immune cell infiltration

Suppresser cells

Mostafa et al. 2014: Don Morris group, University of Calgary
(AACR Tumour Immunology Conference poster)
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REOLYSIN® and Immune Therapy: Rationale for Combination Therapy

Matt Coffey, PhD

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