L-DOS47
Single Domain Antibody Conjugated Anticancer Therapeutic
Forward Looking Statements

This presentation contains certain forward-looking statements and information (collectively, “forward-looking statements”) within the meaning of applicable Canadian securities laws, including, without limitation, those relating to the potential benefits of Helix’s DOS47 platform in the treatment of cancer. Forward-looking statements, which may be identified by words including, without limitation, “will” and other similar expressions, are intended to provide information about management’s current plans and expectations regarding future operations.

Although Helix believes that the expectations reflected in such forward-looking statements are reasonable, such statements involve risks and uncertainties that may cause actual results or events to differ materially from those anticipated and no assurance can be given that these expectations will be realized, and undue reliance should not be placed on such statements. Risk factors that could cause actual results or events to differ materially from the forward-looking statements include, without limitation, (i) the inherent uncertainty involved in scientific research and drug development; (ii) the risks associated with delay or inability to complete clinical trials successfully; (iii) need to secure additional financing on terms satisfactory to Helix or at all; (iv) clinical trials that yield negative results, or results that do not justify future clinical development, including that the Polish Phase I/II clinical trial for L-DOS47 will yield negative results; and (v) those risks and uncertainties affecting the company as more fully described in Helix’s most recent Annual Report, including under the headings “Forward-Looking Statements” and “Risk Factors”, filed with the Canadian Securities Administrators at www.sedar.com (together, the “Helix Risk Factors”). Certain material factors or assumptions are applied in making the forward-looking statements, including, without limitation, that the Helix Risk Factors will not cause Helix’s actual results or events to differ materially from the forward-looking statements.

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Single Domain Antibody (sdAb)

• sdAb is a term often used to describe antibody fragments derived from IgGs, camelid heavy chain antibodies or the variable domains (VNARs) of a shark immunoglobulin

• Small with stable structure; high affinity and some with specificity to epitopes inaccessible by conventional antibodies make them interesting research tools and attractive therapeutic development candidates
Llama Antibody
Outline

• AFAFIKL2 (L) – a unique llama derived sAb
• DOS47 (urease) – microenvironement modifier and therapeutic agent
• L-DOS47 – Phase I clinical testing Candidate for non small cell lung cancer
Llama Antibody
Cell Surface Specific

A549 cell

A549 tumour slide
# Antibody Specificity

<table>
<thead>
<tr>
<th>categories</th>
<th>#</th>
<th>Results of immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><strong>A) THORACIC LESIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, nos</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Bronchiolo-alveolar carcinoma</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Large cell Undifferentiated carcinoma</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Carcinoid tumor</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Atypical adenomatus hyperplasia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>B) NON-LUNG TUMORS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colonic adenocarcinoma</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Colonic adenoma</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Gall bladder adenocarcinoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder adenocarcinoma</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Breast ductal carcinoma</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Tracheal adenoid cystic carcinoma</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

CEACAM6 (CD66c or NCA)

- Glycosylated 90 kDa (286aa) GPI-linked membrane protein
- Intercellular adhesion molecule forming homotypic and heterotypic bonds with CEACAM-1, 5 and -8
- Azurophilic granules and epithelia
Figure 1
CEACAM5 and CEACAM6 staining of colon, ovarian, breast, lung, prostate and pancreatic tissue core specimens as a function of tumor histotype is summarized. The results graphed represent the mean ± standard deviation for each histotype and antigen.
Tumour Microenvironment

Acidosis – Warburg effect

Differentiated tissue

\[ \text{Glucose} \xrightarrow{+O_2} \text{Pyruvate} \xrightarrow{O_2} \text{CO}_2 \]

\[ \text{Glucose} \xrightarrow{-O_2} \text{Pyruvate} \xrightarrow{\text{Lactate}} \]

Oxidative phosphorylation
-36 mol ATP/mol glucose

Anaerobic glycolysis
2 mol ATP/mol glucose

Proliferative tissue

\[ \text{Glucose} \xrightarrow{O_2} \text{Pyruvate} \xrightarrow{\text{Lactate}} \]

Aerobic glycolysis (Warburg effect)
-4 mol ATP/mol glucose

Tumor

\[ \text{Glucose} \xrightarrow{O_2} \text{Pyruvate} \xrightarrow{5\%} \text{CO}_2 \]

\[ \text{Glucose} \xrightarrow{\text{Lactate}} \]

\[ \text{or} \]

\[ \text{Glucose} \xrightarrow{+/-O_2} \text{Pyruvate} \xrightarrow{85\%} \text{Lactate} \]

Acidic and Hypoxic Environment

MCF-7 fluorescent ratio imaging

$^1$H MRS

Robert A. Gatenby and Robert J. Gillies Nature Reviews Cancer 4:891 2004
## Acidosis and Hypoxia

<table>
<thead>
<tr>
<th>Hypoxia</th>
<th>Acidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioresistance</td>
<td>Increased radioresistance</td>
</tr>
<tr>
<td>Drug resistance</td>
<td>Resistance to anthracyclines</td>
</tr>
<tr>
<td>Metastasis and Invasion</td>
<td>Increased metastases</td>
</tr>
<tr>
<td>Increased mutation rate</td>
<td>Increased migration and invasion</td>
</tr>
<tr>
<td>Gene expression induced hypoxia-inducible factor</td>
<td>Mutagenesis / clastogenesis</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Apoptosis</td>
</tr>
</tbody>
</table>

Robert A. Gatenby and Robert J. Gillies Nature Reviews Cancer 4:891 2004
DOS47 – Proposed MOA

- Reverse Tumour Acidity
- Apply Natural Metabolic Toxin
- Induce Chemo-optimized Environment

Urea $\xrightarrow{\text{Urease}}$ \(2\text{NH}_4\text{OH} + \text{CO}_2\)
pH and Ammonium

$$\text{H}_2\text{O} + \text{NH}_3 \leftrightarrow \text{NH}_4^+ + \text{OH}^-$$

Ammonia \quad \text{Ammonium}

% Survival vs. [NH$_4$Cl] mM for different pH conditions:

- pH 7.4
- pH 8.4
- pH 9.0
- pH 9.6
- pH 10.0

% Survival decreases with increasing [NH$_4$Cl] mM for all pH conditions.
DOS47 Activity Inhibition

% Survival vs [AHA] mM

- No Urea
- 5 mM Urea
- 25 mM Urea (no DOS47)

Acetohydroxamic acid (AHA)
DOS47 MCF7 and A549 Xenograft

MCF-7

A

Control
Urease (10 U)

Days

Tumor Size (mg)

0
10
20
30
40
50
60
70
80

5 7 9 11 13 16

A549

B

Control
Urease (1 U)
Urease (4 U)

Days

Tumor Size (mg)

0
100
200
300
400
500
600
700
800

5 6 10 13 17 20 24 27

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L-DOS47

Antibody “L”

urease
Conjugation

SIAB (N-succinimidyl(4-iodoacetyl]aminobenzoate)

antibody <-> urease
amine <-> Sulfhydryl
Full Body Scan
A549 tumour (8 x 7 mm)
L-DOS47-Cy5.5

Filtered Scan
L-DOS47-Cy5.5
Cy5.5 emission max @710nm

Tumour specific localization
L-DOS47 Imaging

![Graph showing normalized fluorescence intensity over time in hours. The graph indicates the following time points: 12 hours, 24 hours, 48 hours, and 72 hours. The y-axis represents normalized fluorescence intensity, and the x-axis represents time post injection (hours). Each time point is represented by a different color: 12 hours in orange, 24 hours in dark blue, 48 hours in green, and 72 hours in purple.]

Normalized Fluorescence Intensity

Time post injection (hours)
L-DOS47 A549 Xenograft

%Change in Tumour Volume vs Days

- Vehicle
- Cisplatin
- L-DOS47 (35U/kg)
- L-DOS47 (20U/kg)
- L-DOS47 (10U/kg)
## Human Cancer Tissue Screening

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tumour Tissue</th>
<th>Age-matched Normal Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Kidney carcinoma</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Parathyroid adenoma</td>
<td>1/1</td>
<td>n/a</td>
</tr>
<tr>
<td>Plaenta, umbilical cord, allantois</td>
<td>n/a</td>
<td>1/1</td>
</tr>
<tr>
<td>Myofibroblastic tumor</td>
<td>1/1</td>
<td>n/a</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Pancreas adenocarcinoma</td>
<td>7/57 weak</td>
<td>42/57</td>
</tr>
<tr>
<td></td>
<td>8/57 v. weak</td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine tumors</td>
<td>9/9</td>
<td>n/a</td>
</tr>
<tr>
<td>Brain, heart muscle, testis, spleen</td>
<td>n/a</td>
<td>30/30</td>
</tr>
<tr>
<td>Testis - teratoma and seminoma</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Parotis tumor</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Cervix squamous carcinoma</td>
<td>2/2</td>
<td>n/a</td>
</tr>
<tr>
<td>Thymoma</td>
<td>2/2</td>
<td>n/a</td>
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<tr>
<td>Colon adenocarcinoma</td>
<td>14/24 weak</td>
<td>10/24</td>
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<tr>
<td>- lymph node metastasis</td>
<td>3/3</td>
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<tr>
<td>Breast adenocarcinoma</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>- lymph node metastasis</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>Leiomoma - lung metastasis</td>
<td>1/1</td>
<td>n/a</td>
</tr>
<tr>
<td>Ovary carcinoma</td>
<td>4/4</td>
<td>n/a</td>
</tr>
<tr>
<td>Bladder carcinoma</td>
<td>42/42</td>
<td></td>
</tr>
<tr>
<td>- lymph node metastasis</td>
<td>1/1 strong</td>
<td></td>
</tr>
<tr>
<td>- squamous carcinoma metastasis</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>Lung - small cell carcinoma</td>
<td>1/1</td>
<td>5/5</td>
</tr>
<tr>
<td>- adenocarcinoma</td>
<td>5/5 strong</td>
<td></td>
</tr>
<tr>
<td>Stomach adenocarcinoma</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Liver carcinoma</td>
<td>4/4</td>
<td>4/4</td>
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<tr>
<td>Soft tissue tumors</td>
<td>3/3</td>
<td>n/a</td>
</tr>
<tr>
<td>Melanoma</td>
<td>48/48</td>
<td>18/18</td>
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<tr>
<td>- metastasis</td>
<td>18/18</td>
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# Tumour Formation Inhibition

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell Treatment</th>
<th>Dose (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Isoptype</td>
<td>17.5</td>
</tr>
<tr>
<td>3</td>
<td>L-DOS47</td>
<td>12.5</td>
</tr>
<tr>
<td>4</td>
<td>L-DOS47</td>
<td>17.5</td>
</tr>
<tr>
<td>5</td>
<td>L-DOS47</td>
<td>25.0</td>
</tr>
</tbody>
</table>
# Tumour Formation Inhibition

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell Treatment</th>
<th>Dose U/mL</th>
<th>Mean number 3 weeks</th>
<th>Mean number 10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated</td>
<td>-</td>
<td>103.8 ± 30.0</td>
<td>110.6 ± 50.0</td>
</tr>
<tr>
<td>2</td>
<td>Isoptype</td>
<td>17.5</td>
<td>44.6 ± 5.1</td>
<td>60.4 ± 14.3</td>
</tr>
<tr>
<td>3</td>
<td>L-DOS47</td>
<td>12.5</td>
<td>104.6 ± 35.5</td>
<td>140 ± 52.5</td>
</tr>
<tr>
<td>4</td>
<td>L-DOS47</td>
<td>17.5</td>
<td>28.0 ± 7.2</td>
<td>50.0 ± 17.7</td>
</tr>
<tr>
<td>5</td>
<td>L-DOS47</td>
<td>25.0</td>
<td><strong>18.2 ± 7.8</strong> *</td>
<td>112.2 ± 52.5</td>
</tr>
</tbody>
</table>

* p < 0.05
Tumour Formation Inhibition

Control

Treated
Weakly Basic Drug

Mitoxantrone pKa 8.13

Doxorubcin pKa 8.34

Vinblastine pKa 7.4
Vincristine
Vinorelbine
Effect of pH on Drug’s Efficacy

A549 cells
doxorubicin (100 µM), mitoxantrone (75 µM), and vinblastine (150 µM)
Effect of L-DOS47 and Vinorelbine on A549

% Viable Cancer Cell

Vinorelbine only

Vinorelbine + L-DOS47

Vinorelbine (µM)

0 20 40 60 80 100 120 140 160
Toxicology Program

• Tissue Cross Reactivity Studies
• GLP animal toxicology studies
• Cytokine release studies
• Immunogenicity studies
Planned Phase I/II Study (EU - Poland)

- Open-label safety, tolerability study and preliminary efficacy study
- Standalone, with chemo or with radiation
  - L-DOS47 monotherapy (active)
- Inoperable, locally advanced, recurrent or non-squamous NSCLC
- Start dose: 0.12µg/kg, ascending
- 3-6 patient cohorts
- Treatment cycle: once weekly for 3 wks, rest 1 wk; 4 treatment cycles
Phase I Study (US)

- Open-label first-in-human safety and tolerability study
- Stage IV, solid tumour, unresponsive to treatment
- 3-6 patient cohorts
- Treatment cycle: once weekly for 3 wks, rest 1 wk; 4 treatment cycles
Acknowledgement

• Helix BioPharma Research Team
  – Wah Wong, Baomin Tian, Iain Wilson, Sharon Molund, Kim Gaspar, Frederic Morneau, Terry Cochrane

• National Research Council of Canada
  – Roger Mackenzie, Jianbing Zhang and research team (antibody discovery)
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