Development of Adenovirus vectors – from preclinical to Phase III
iSBTc Oncology Biologics Development Primer
Sunil Chada, Ph.D.
s.chada@introgen.com
<table>
<thead>
<tr>
<th>Product (Target)</th>
<th>Pre-Clinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
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<tbody>
<tr>
<td>ADVEXIN (p53)</td>
<td></td>
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<tr>
<td>Head and Neck (monotherapy)</td>
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<tr>
<td>Head and Neck (combo/chemo)</td>
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<tr>
<td>Lung Cancer</td>
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<tr>
<td>Breast Cancer</td>
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<tr>
<td>Esophageal Cancer</td>
<td></td>
<td></td>
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<tr>
<td>+ 4 additional solid cancers</td>
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<td>INGN 241 (mda-7/IL-24)</td>
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<tr>
<td>Solid Tumors + XRT</td>
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<tr>
<td>Melanoma</td>
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<tr>
<td>INGN 225 (p53 Immunotherapy)</td>
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<tr>
<td>Small-cell Lung Cancer</td>
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<tr>
<td>Breast Cancer</td>
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<tr>
<td>INGN 401 (Nanoparticle-FUS-1)</td>
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<td>Lung Cancer</td>
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</tr>
<tr>
<td>INGN 234 (Mouthwash)</td>
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<td></td>
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<tr>
<td>Oral cancers</td>
<td></td>
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</tr>
</tbody>
</table>
ADVEXIN® Construct

35.4 kb Adenovirus genome

- p53
- Adenovirus Structural Proteins
  - E1A/E1B Deleted
  - (E1)
  - E2
  - E3
- E4

CMV Promoter

Human wt p53 cDNA

SV40 Poly A

2.3 kb Expression cassette insert

Hexon
Penton
Fiber
Core

Core
Hexon associated
ADVEXIN®
p53 Tumor Suppressor Therapy

- Selectively kills cancer cells, safe to normal cells
- Pharmacologic intervention with p53 protein - targets fundamental molecular defect in cancer
- Non-replicating adenovirus; well tolerated >600 patients; >30 trials
- Excellent safety profile
- Useful alone and in combination with local and systemic modalities — radiotherapy, surgery, chemotherapy, biotherapy
Mechanisms of ADVEXIN® Activity

1. Cell cycle arrest
   - CDK
   - BAX
   - BAK

2. Apoptosis
   - FasL
   - Fas
   - Akt
   - p53
   - MDM2
   - BAX
   - BAK
   - Caspase Recruitment and Activation

3. Anti-angiogenesis
   - VEGF
   - Angiogenic Factors
   - Anti-angiogenic Factors
   - GD-AIF
   - BAI-1
   - TSP

Regulatory Feedback Loop
- p21
- p53
- MDM2
Preclinical studies

SiHa tumors
1E11 vp IT; 6 injections
Hamada et al, Cancer Res 56: 3047, 1996

HeLa cells
Trypan blue assay

MOI (vp/cell)

Cell Viability

0 2000 4000 6000 8000 10000 12000

0 50 100 150 200 250

Ad- empty

Advexin

Tumor Volume (mm^3)

PBS
AdSCMV-poly A
AdSCMV-p53

Day

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

0 500 1000 1500 2000 2500
Advexin exhibits tumor-selectivity

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Killing in vitro</th>
<th>Killing in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCHN</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>NSCLC</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Breast</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Prostate</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Colorectal</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Ovarian</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>HCC</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Glioma</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Melanoma</td>
<td>√</td>
<td>n.d.</td>
</tr>
<tr>
<td>Cervical</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Bladder</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Myeloma</td>
<td>√</td>
<td>n.d.</td>
</tr>
<tr>
<td>Normal cells</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
ADVEXIN® Inhibits Tumor Growth in Combination with Other Cancer Therapies

Additive or synergistic effects

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCHN</td>
<td>XRT</td>
</tr>
<tr>
<td>NSCLC</td>
<td>CDDP</td>
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<tr>
<td>Breast</td>
<td>5FU</td>
</tr>
<tr>
<td>Prostate</td>
<td>Taxanes</td>
</tr>
<tr>
<td>Colorectal</td>
<td>CPT-11</td>
</tr>
<tr>
<td>HCC</td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>Glioma</td>
<td>etc</td>
</tr>
</tbody>
</table>
Additional preclinical studies

• 8 GLP toxicology studies: mice, rats, cotton rats
  – Advexin well tolerated: sq; oral; iv; ip; ia
  – The liver is affected at very high doses with iv route, but see no liver effects in the clinic

• Biodistribution (PK) studies
  – $t_{1/2} = 10$ minutes; no gonadal persistence

• Other safety studies
  – Little/ no effect on normal cells; lack of replication or integration
    
    Therapeutic index $> 3$ logs
Clinical Studies
Advexin® Clinical Program

• > 600 patients treated with > 3,000 doses
• First trial conducted in 1995; published results in 1996
• > 30 active or completed trials
• Most patients treated with intratumoral injection
• Four additional routes of administration: IV, IP, BAL, intravesicle
• Randomized controlled Phase III multinational studies ongoing
Advexin® Clinical Program

- **Phase I Trials** – **US**; EU; Japan
  - Head & Neck, Lung, Breast, Prostate, Colorectal, Bladder, Ovarian, Brain, Lung + Chemotherapy, Solid Tumors (IV), Oral Premalignancy

- **Phase II Trials**
  - Head & Neck, Lung + Radiation, Breast + Chemotherapy, Esophageal

- **Phase III Trials**
  - Head & Neck ± Chemotherapy
ADVEXIN® Well Tolerated Safety Data in >600 Treated Patients

<table>
<thead>
<tr>
<th>Body System</th>
<th>EVENT</th>
<th>All Serious Adverse Events Occurring in &gt; 1% of Patients</th>
<th>SAE - Investigator Related</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Body as a Whole</td>
<td>Fever</td>
<td>13</td>
<td>2.0</td>
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<tr>
<td></td>
<td>Pain</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Asthenia</td>
<td>7</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Infection local</td>
<td>16</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Tumor hemorrhage</td>
<td>28</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Procedure (Inpatient scheduled)</td>
<td>8</td>
<td>1.3</td>
</tr>
<tr>
<td>Digestive</td>
<td>Vomit</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Dysphagia</td>
<td>8</td>
<td>1.3</td>
</tr>
<tr>
<td>Respiratory System</td>
<td>Pneumonia</td>
<td>38</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Dyspnea</td>
<td>24</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Apnea</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td>Cardiovascular System</td>
<td>Hypotension</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Heart arrest</td>
<td>9</td>
<td>1.5</td>
</tr>
<tr>
<td>Metabolic and Nutritional Systems</td>
<td>Dehydration</td>
<td>26</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Kidney Failure</td>
<td>7</td>
<td>1.1</td>
</tr>
</tbody>
</table>
ADVEXIN® Monotherapy Results in Long Term Survival (> 8 years) in SCCHN

Age=48

Baseline: 27 May 1998

Cycle 13: 21 June 1999
39 injections
≈ $6 \times 10^{13}$ vp

8 June 2006
276 injections
≈ $4 \times 10^{14}$ vp
Objective response after Advexin injection in NSCLC

Molecular pharmacology and biomarker development
**Upstream Regulators**

- p14ARF
- HDM2

**Downstream Effectors**

**CELL CYCLE**
- p21
- Cdk
- G1
- PCNA
- G1/S

**ANGIOGENESIS**
- BAI-1
- TSP-1
- VEGF

**APOPTOSIS**
- Bax
- Bak
- Bcl2

**TUMOR IMMUNITY**
- CD95L/FasL

**Genomic integrity**

**Growth control**
Representative images from Advexin Phase II SCCHN patient tumors depicting examples of positive and negative immunostaining with each antibody.
**Interrogation of p53 pathway markers for tumor response and survival**

<table>
<thead>
<tr>
<th>Tumor Marker*</th>
<th>N</th>
<th>P-value</th>
<th>Overall Survival (Months)</th>
<th>Log-Rank Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor Marker*</td>
<td>N</td>
</tr>
<tr>
<td>p53</td>
<td>18</td>
<td>0.03</td>
<td>positive</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>7</td>
</tr>
<tr>
<td>p53-Ser15</td>
<td>18</td>
<td>0.07</td>
<td>positive</td>
<td>8</td>
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<td>p14^{ARF}</td>
<td>18</td>
<td>0.28</td>
<td>positive</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>14</td>
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<tr>
<td>HDM2</td>
<td>17</td>
<td>0.10</td>
<td>positive</td>
<td>8</td>
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<tr>
<td></td>
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<td>negative</td>
<td>9</td>
</tr>
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<td>Bcl-2</td>
<td>17</td>
<td>0.17</td>
<td>positive</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>12</td>
</tr>
<tr>
<td>survivin</td>
<td>13</td>
<td>0.62</td>
<td>positive</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>negative</td>
<td>3</td>
</tr>
</tbody>
</table>

* ≥20% is positive for p53, p14^{ARF}, HDM2, Bcl2, and survivin; ≥5% is positive for p53-Ser15
--- could not be calculated reliably variability in the data
Imaging technology: PET-CT response in LFS patient

Pre-Treatment

Post-Treatment

SUV: 80% decrease in injected lesion. Non-injected lesion; 130% increase
Case Studies: Lessons and Issues

• **Key Strategic Decisions**
  
  Early decision on r & D development company
  
  Rely upon academic collaborations for “r”, animal data, etc.
  
  Focus resources on generation of clinical data
  
  Important to control supply of clinical-grade materials
  
  Decision made early to create manufacturing infrastructure
  
  Develop parallel regulatory development paths with FDA and EMEA
  
  Take industrialized approach to clinical design and biostatistics – avoid repeating studies

• **Impact of Regulatory Interactions**
  
  Provided valuable guidance
  
  Early interactions important to avoid surprises
  
  Regulators don’t have all the answers
  
  Be collaborative, not combative
Case Studies: Lessons and Issues

• **Financial Considerations: Projected Costs vs. Reality**
  
  Everything is more expensive and takes longer…..
  
  Heavy price for first-in-class development
  
  - No regulatory precedents
  - Investor reluctance (no comparables)
  - Pharma partner caution
  
  Avoid temptation to cut corners on required GLP studies
  (more expensive to do it twice!!)

  Outsourcing/ consultants
  
  - need careful oversight
  - do not assume they are “experts” in your area
  - monitor timelines

• **People matter**
  
  - Flexible, non-silo people key in the early days
  - Research mindset needs to evolve to industrial/b business approach
Case Studies: Lessons and Issues

• **Impact of long development timelines**
  – Early studies may not meet current standards (e.g., PCR sensitivity; RCA levels)
  – Evolution of clinical standard of care
  – New drug approvals
Lessons learned

1. Early deployment of Clinical Development Plan
   - Synchronize research and clinical studies
   - Enhances iterative translation-based development program
   - Challenges of using CROs:
     • Databases
     • Monitoring
     • Need oversight
   - Tough to modify protocols/ CRFs during study
   - Stick to the plan!! Avoid tempting, incremental research
Lessons learned

2. Biomarker development
   – Goal is to identify responding and non-responding patient populations
   – Limited by patient #/ samples/ informed consent/ etc
   – Preparation is critical – work closely with PI’s on informed consent, CRF’s, sample logistics
Words to the Wise

– Have the courage to kill a project
– Impact of having pharma partner early
  • Differences in cultures and risk assessment
  • Keep your eye on the clinical development plan
  • Control your company’s/project’s destiny

– What is your backup plan ???
  • Financially
  • Balance need for product pipeline with “all eyes on the prize”
Questions?