Phase I Dose Escalation Study of ALN-VSP02
A Novel RNAi Therapeutic for Solid Tumors with Liver Involvement

June 4, 2011
Mechanism for RNA Interference (RNAi)

- Synthetic siRNA
- dsRNA
- dicer
- Cleavage
- Strand separation
- RISC
- Complementary pairing
- mRNA
- mRNA degradation

Targeted Gene Silencing

ASCO, June 2011
ALN-VSP02 Scheme

Lipid nanoparticle* (LNP) components:

- **DLinDMA**
- **DPPC**
- **mPEG2000-C-DMA**
- **Cholesterol**

*Lipid nanoparticle formulation from Tekmira Pharmaceuticals*
ALN-VSP02 Phase I Study

Objectives

Primary
- Evaluate the safety and tolerability of ALN-VSP02

Secondary
- Characterize PK
- Assess for evidence of antitumor/antiangiogenic activity
  » Tumor response rate, change in tumor blood flow on DCE-MRI

Exploratory
- Analyze voluntary tumor biopsies for drug levels and for evidence of RNA interference using 5’ RACE assay
### Key Inclusion Criteria

<table>
<thead>
<tr>
<th>Each patient must meet the following criteria within 14 days of C1W1D1 to be enrolled in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Patients must have histologically or cytologically confirmed advanced solid tumors that have recurred or progressed following standard therapy, or that have not responded to standard therapy, or for which there is no standard therapy, or who are not candidates for standard therapy.</td>
</tr>
<tr>
<td>• Patient has measurable tumor in the liver (≥ 1 cm by spiral CT or ≥ 2 cm by standard CT).</td>
</tr>
<tr>
<td>• Patient has an Eastern Cooperative Oncology Group (ECOG) performance status score of 0-1.</td>
</tr>
<tr>
<td>• Patient has adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 2.5 × upper limit of normal (ULN), total bilirubin within normal limits, albumin &gt; 3.0 g/dL, international normalized ratio (INR) ≤ 1.2, and Child-Pugh Class A (for hepatocellular carcinoma [HCC] patients).</td>
</tr>
<tr>
<td>• Patient has adequate renal function: serum creatinine ≤ 1.5 × ULN.</td>
</tr>
</tbody>
</table>

### Key Exclusion Criteria

<table>
<thead>
<tr>
<th>Patients meeting any of the following criteria within 14 days of C1W1D1 will be excluded from the study:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Patient has brain or leptomeningeal metastases. (Note: Patients with completely resected brain metastases and no evidence of residual disease on CT or MRI scan of the brain will be considered eligible).</td>
</tr>
<tr>
<td>• Patient has known HBV, HCV, or human immunodeficiency virus (HIV) infection.</td>
</tr>
<tr>
<td>• Patient has &gt;50% involvement of the liver by tumor.</td>
</tr>
<tr>
<td>• Patient has undergone splenectomy.</td>
</tr>
</tbody>
</table>
### ALN-VSP02 Phase I Study Design

**Dose levels and dosing schedule**

- 0.1, 0.2, 0.4, 0.7, 1.0, 1.25, 1.5, 1.7 mg/kg
- 3 + 3 cohort design, expansion phase of 10 pts at MTD
- 15-min IV infusion q2 wks; premed with steroids, H1 and H2 blockers, acetaminophen
- Cycle = 2 doses (1 month), tumor measurements after every 2 cycles, treat until disease progression
  - ALN-VSP02-002 extension study for pts remaining on study beyond 4 cycles (8 doses)
Demographics and Dosing

- N=41
  - Dose escalation (0.1-1.5 mg/kg): N=31
  - Expansion phase at 1.0 and 1.25 mg/kg: N=10 (5 per dose level)
- Median age 57 (range 34-78)
- Male: Female = 17:24
- ECOG performance status 0/ 1 (%): 44 / 56
- Average # of prior regimens for metastatic disease: 4.3 (range 1-13)
- Prior chemotherapy/anti-VEGF therapy (%): 88 / 61
- Liver/Extrahepatic metastases (%): 98 / 88
- Tumor types
  - GI (N=24)
  - GYN (N=9)
  - GU (N=3)
  - Sarcoma (N=2)
  - Other (N=3)
- Total of 182 doses administered to date
- Average # of doses/patient: 4.4 (range 1-24)
  - One patient continues on study after full year of dosing
### TreatmentEmergent Adverse Events

**Number of Patients with Grades 1, 2, 3, 4**

<table>
<thead>
<tr>
<th>Toxicity**</th>
<th>% of Patients (n=41)</th>
<th>ALN-VSP02 Dose Level (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.10 (n=3)</td>
<td>0.20 (n=3)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>24%</td>
<td>1, 0, 0, 0</td>
</tr>
<tr>
<td>Nausea</td>
<td>17%</td>
<td>1, 0, 0, 0</td>
</tr>
<tr>
<td>Fever</td>
<td>15%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Infusion-Related Reaction</td>
<td>15%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>12%</td>
<td>2, 0, 0, 0</td>
</tr>
<tr>
<td>AST Elevation</td>
<td>12%</td>
<td>0, 1, 0, 0</td>
</tr>
<tr>
<td>Rash/Flushing</td>
<td>12%</td>
<td>1, 0, 0, 0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>12%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Chills/Rigors</td>
<td>10%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>7%</td>
<td>1, 0, 0, 0</td>
</tr>
<tr>
<td>Headache</td>
<td>7%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>ALT Elevation</td>
<td>5%</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>5%</td>
<td>1, 0, 0, 0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>5%</td>
<td>1, 0, 0, 0</td>
</tr>
</tbody>
</table>

**Includes only those toxicities deemed related or possibly related to study drug in 5% or more pts

*Patient with extensive liver metastases died from liver failure, possibly related to study drug

†1 patient reduced to 0.40 mg/kg after dose 1
‡2 patients reduced to 1.00 mg/kg after dose 1
## Serious Adverse Events

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Tumor Type</th>
<th>Dose Level (mg/kg)</th>
<th>Event(s)</th>
<th>Relatedness</th>
</tr>
</thead>
</table>
| 003-012   | Pancreatic neuroendocrine | 0.70              | 1. Liver failure  
2. Hepatic encephalopathy  
Both arising 5 days post-dose 2, resulting in death | Possibly related  |
| 001-021   | Endometrial           | 0.70              | 1. Elevated WBC  
2. Fever  
Both occurred post-dose 23. Hospitalized to rule out infection and then resumed dosing on study. | Possibly related  |
| 001-030   | Endometrial           | 1.25              | Elevated WBC post-dose 3. Hospitalized to rule out infection and then resumed dosing on study. | Possibly related  |

- 5 possibly related SAEs in 3 patients
- 23 unrelated SAEs in 10 patients
**Plasma PK Data Summary**

**VEGF and KSP siRNAs**

- **AUC and $C_{\text{max}}$ dose proportional**
- **Same PK profile for Cycles 1 and 2 with no evidence of drug accumulation**

### KSP siRNA

<table>
<thead>
<tr>
<th>ALN-VSP</th>
<th>Cycle 1 (mg/kg)</th>
<th>0.10 (n=3)</th>
<th>0.20 (n=3)</th>
<th>0.40 (n=5)</th>
<th>0.70 (n=7)</th>
<th>1.00 (n=4)</th>
<th>1.25 (n=5)</th>
<th>1.50 (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>0.76 ± 0.36</td>
<td>2.26 ± 0.54</td>
<td>3.92 ± 1.12</td>
<td>7.99 ± 3.39</td>
<td>15.31 ± 5.35</td>
<td>21.94 ± 7.64</td>
<td>18.04</td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{0-\text{last}}$ (min*µg/mL)</td>
<td>30.83 ± 20.86</td>
<td>133.02 ± 46.66</td>
<td>252.38 ± 97.9</td>
<td>523.18 ± 206.18</td>
<td>923.59 ± 360.13</td>
<td>2035.74 ± 1100.08</td>
<td>1491.03</td>
<td></td>
</tr>
</tbody>
</table>

### VEGF siRNA

<table>
<thead>
<tr>
<th>ALN-VSP</th>
<th>Cycle 1 (mg/kg)</th>
<th>0.10 (n=3)</th>
<th>0.20 (n=3)</th>
<th>0.40 (n=5)</th>
<th>0.70 (n=7)</th>
<th>1.00 (n=4)</th>
<th>1.25 (n=5)</th>
<th>1.50 (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>0.86 ± 0.42</td>
<td>2.51 ± 0.56</td>
<td>4.31 ± 1.10</td>
<td>8.81 ± 2.75</td>
<td>15.84 ± 7.01</td>
<td>21.58 ± 6.80</td>
<td>18.19 ± 1.09</td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{0-\text{last}}$ (min*µg/mL)</td>
<td>68.95 ± 66.12</td>
<td>143.11 ± 58.58</td>
<td>272.21 ± 98.16</td>
<td>626.37 ± 272.66</td>
<td>984.26 ± 436.81</td>
<td>2006.58 ± 1113.54</td>
<td>1514.63 ± 150.24</td>
<td></td>
</tr>
</tbody>
</table>
Safety Summary

- ALN-VSP02 was generally well-tolerated
  - 1 patient has so far received 24 doses over 1 year
- No dose-dependent changes in liver function tests
- Grade 1-2 fatigue (24%), nausea (17%) and fever (15%) most common AEs with no clear dose dependence
- Grade 2 infusion-related reactions seen in 15% of patients or 3% of doses administered
  - Responded to slowing of infusion
  - No patients discontinued from study because of infusion reaction
- Grade 1-2 chills/rigors seen at 1.25 mg/kg dose level
  - Occurred after completion of dosing in 3 of 11 patients and associated with higher levels of transient IL-6 induction (peak at 6 hrs post-dose, resolution by 24 hrs)
    - In 2 patients, symptoms re-occurred after dose reduction to 1.0 mg/kg
- Dose-limiting toxicities included:
  - Liver failure and death in patient with extensive hepatic metastases and prior splenectomy/partial hepatectomy at 0.7 mg/kg (possibly related),
  - Transient grade 3 thrombocytopenia in 2 patients at 1.25 mg/kg,
  - Grade 3 hypokalemia in 1 patients at 1.5 mg/kg
- Recommended Phase II dose is 1.0 mg/kg IV q2 weeks
# Tumor Response Summary

**Dose Level (mg/kg)** | **N (evaluable for response)** | **Avg # of Doses Received (range)** | **# Pts with Stable Disease or Better for ≥ 2 mos** | **# Pts Who Went on to Extension Study (>8 doses) to Date**
--- | --- | --- | --- | ---
0.10 | 3 | 3 (2-4) | 0 | 0
0.20 | 3 | 4 (4-4) | 0 | 0
0.40 | 7* | 4.6 (2-11) | 1 | 1
0.70 | 5 | 9.6 (3-23) | 3 (includes 1 PR with ~70% tumor reduction) | 2
1.00 | 11† | 4.8 (2-8) | 7 | 1
1.25 | 7 | 2.4 (1-6) | 2 | 0
1.50 | 1 | 4 | 0 | 0

PR: partial response
*Includes 1 patient whose first dose was given at 0.7 mg/kg
†Includes 2 patients whose first dose was given at 1.25 mg/kg

**Stable disease or better in:**
- 1/13 pts (8%) at ≤ 0.4 mg/kg
- 12/24 pts (50%) at ≥ 0.7 mg/kg
## Characteristics of Patients with SD or PR

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Dose Level (mg/kg)</th>
<th>Tumor Type</th>
<th>Best Response</th>
<th># of Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>016</td>
<td>0.40</td>
<td>Squamous Cell Head and Neck</td>
<td>SD</td>
<td>11</td>
</tr>
<tr>
<td>019</td>
<td>0.70</td>
<td>Colorectal</td>
<td>SD</td>
<td>8</td>
</tr>
<tr>
<td>020</td>
<td>0.70</td>
<td>Angiosarcoma</td>
<td>SD</td>
<td>10</td>
</tr>
<tr>
<td>021</td>
<td>0.70</td>
<td>Endometrial</td>
<td>PR</td>
<td>24 (ongoing)</td>
</tr>
<tr>
<td>022</td>
<td>1.00</td>
<td>Colorectal</td>
<td>SD</td>
<td>8</td>
</tr>
<tr>
<td>025</td>
<td>1.00</td>
<td>Synovial Sarcoma</td>
<td>SD</td>
<td>7</td>
</tr>
<tr>
<td>037</td>
<td>1.00</td>
<td>Renal Cell</td>
<td>SD</td>
<td>8 (ongoing)</td>
</tr>
<tr>
<td>040</td>
<td>1.00</td>
<td>Pancreatic Neuroendocrine</td>
<td>SD</td>
<td>5 (ongoing)</td>
</tr>
<tr>
<td>041</td>
<td>1.00</td>
<td>Renal Cell</td>
<td>SD</td>
<td>4 (ongoing)</td>
</tr>
<tr>
<td>042</td>
<td>1.00</td>
<td>Uveal Melanoma</td>
<td>SD</td>
<td>4 (ongoing)</td>
</tr>
<tr>
<td>044</td>
<td>1.00</td>
<td>Pancreatic Neuroendocrine</td>
<td>SD</td>
<td>4 (ongoing)</td>
</tr>
<tr>
<td>028</td>
<td>1.25</td>
<td>Esophageal Adenocarcinoma</td>
<td>SD</td>
<td>6</td>
</tr>
<tr>
<td>030</td>
<td>1.25</td>
<td>Endometrial</td>
<td>SD</td>
<td>4</td>
</tr>
</tbody>
</table>

SD: stable disease  
PR: partial response
Major Response in Endometrial Cancer
Patient 021, 70% Regression of Liver Metastases

Pre-Treatment

After 12 Doses ALN-VSP02*

*Response ongoing after 24 doses

ASCO, June 2011
• 46% (13/28) of patients with average Ktrans ↓ of ≥40%
DCE-MRI in PNET Patients with Liver Metastases

**Pre-Treatment**

- **Pt 012** (0.7 mg/kg)
- **Pt 044** (1.0 mg/kg)

**Day 7 Post-Dose 1**

- Avg $K_{trans}$↓=49%
  (n=3 tumors, T1-T3)
- Avg $K_{trans}$↓=86%
  (n=3 tumors, only T1 shown)

T: liver tumor
PNET: pancreatic neuroendocrine tumor

ASCO, June 2011
Voluntary Tumor Biopsies

- CT-guided core needle biopsies obtained pre- and post-dose 1 in patients on voluntary basis
  - Analyses:
    - Drug levels
    - 5' RACE
    - qPCR
- 29 Tumor biopsies obtained from 15 patients across multiple dose levels
  - 0.4 mg/kg (n=3), 0.7 mg/kg (n=2), 1.0 mg/kg (n=6), 1.25 mg/kg (n=3), 1.5 mg/kg (n=1)
  - Liver tumor biopsies in 11 patients
  - Extrahepatic tumor biopsies in 4 patients
- Histological exam reveals high degree of variability in proportion of tumor, fibrotic/necrotic tissue, and normal tissue in biopsy samples
  - Impacts certain quantitative interpretations of molecular results
Drug Levels in Tumor Biopsies Post-Dose 1

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Dose (mg/kg)</th>
<th>Tumor Type (Biopsy Site, Day of Post-Dose Biopsy)</th>
<th>Post-Dose Biopsy (%)</th>
<th>Drug Levels** (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Viable Tumor</td>
<td>Liver</td>
<td>Fibrosis/Necrosis</td>
</tr>
<tr>
<td>007</td>
<td>0.40</td>
<td>Colorectal (liver, d7)</td>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td>017</td>
<td>0.40</td>
<td>Ovarian (liver, d2)</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>019</td>
<td>0.70</td>
<td>Colorectal (liver, d2)</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>022</td>
<td>1.00</td>
<td>Colorectal (adrenal, d2)</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>025</td>
<td>1.00</td>
<td>Sarcoma (muscle, d2)</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>033</td>
<td>1.00</td>
<td>Colorectal (liver, d3)</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>041</td>
<td>1.00</td>
<td>RCC (liver, d2)</td>
<td>70</td>
<td>15</td>
</tr>
<tr>
<td>042</td>
<td>1.00</td>
<td>Uveal mel (liver, d2)</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>026</td>
<td>1.25</td>
<td>Colorectal (liver, d6)</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>031</td>
<td>1.25</td>
<td>Ovarian (abdomen, d4)</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>035</td>
<td>1.25</td>
<td>Ovarian (L. node, d5)</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>032</td>
<td>1.50</td>
<td>Small bowel (liver, d6)</td>
<td>42</td>
<td>0</td>
</tr>
</tbody>
</table>

**Measured by qPCR, LLOQ (lower limit of quantitation)=0.14 ng/g tissue. All pre-treatment biopsy samples were <LLOQ.

*Remaining 12% was normal adrenal (6%) and fat (6%)
†Remaining 9% was skeletal muscle
‡Remaining 15% was skeletal muscle
VEGF 5' RACE Assay
Method for Demonstrating RNAi

**Pre-treatment**
1.4%

**Post-treatment**
29.2%

p<0.001

Illustrative Example: Patient 016

**Step 1**
*In vivo RNAi*

**Step 2**
Ligation of adaptor

**Step 3**
Amplification of RNAi-specific product

Sequence Analysis

VEGF mRNA

RNA cleavage site

RNA adaptor

GR5N VEGF Rev2

% Specific Cleavage

Illustrative Example: Patient 016

VEGF Rev2

p<0.001
Tumor Biopsies Positive By VEGF 5’ RACE

- 15 patients with post-treatment biopsy evaluable by 5’ RACE:
  - 0.4 mg/kg (n=3), 0.7 (n=2), 1.0 (n=6), 1.25 (n=3), 1.5 (n=1)
- 3 of 15 positive for VEGF 5’ RACE:
  - 2 liver tumor biopsies at 0.4 mg/kg
  - 1 extrahepatic tumor biopsy at 1.25 mg/kg
- Assay development in progress for KSP 5’ RACE:
  - Required due to low KSP mRNA levels

**Table:**

<table>
<thead>
<tr>
<th>Pt #</th>
<th>Dose (mg/kg)</th>
<th>Tumor Type (Biopsy Site, Day of Post-Dose Biopsy)</th>
<th>Pre-Dose Biopsy (%)</th>
<th>Post-Dose Biopsy (%)</th>
<th>VEGF 5’ RACE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Viable Tumor</td>
<td>Liver</td>
<td>Fibrosis/Necrosis</td>
</tr>
<tr>
<td>016</td>
<td>0.40</td>
<td>SCC H&amp;N (liver, d2)</td>
<td>10</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>017</td>
<td>0.40</td>
<td>Ovarian (liver, d2)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>031</td>
<td>1.25</td>
<td>Ovarian (abdomen, d4)</td>
<td>25</td>
<td>0</td>
<td>75</td>
</tr>
</tbody>
</table>

SCC H&N: Squamous cell cancer of head and neck
N/A: No sample for analysis

*p<0.001
Clinical Activity/PD Summary

Evidence of antitumor activity in heavily pre-treated patients at doses ≥0.7 mg/kg

- Major ongoing response (PR with ~70% tumor reduction) in patient with endometrial cancer and multiple liver metastases treated at 0.7 mg/kg
  » Patient treatment continuing after one full year
- 64% (7 of 11) of patients with stable disease ≥2 months at recommended Phase II dose of 1.0 mg/kg
  » 45% (5 of 11) of patients continue receiving drug on study

DCE-MRI results suggestive of anti-VEGF effect

- ~50% of pts showing ≥40% drop in Ktrans in liver tumors, including endometrial cancer patient with major response (PR) and two PNET pts

siRNA delivery and RNAi proof of mechanism shown in tumor biopsies

- Pharmacologically relevant concentrations of VEGF and KSP siRNAs in hepatic and extrahepatic tumors
- Molecular evidence of RNAi-mediated VEGF mRNA cleavage in biopsies of hepatic and extrahepatic tumors by 5’ RACE