Development of ALN-VSP: an RNAi Therapeutic for Liver Malignancies

Abstract #B204

Mean survival of the mice bearing primary hepatocellular carcinoma and secondary (stereotactic) tumors, represent a significant unmet medical need. We developed a therapeutic for solid tumors involving the liver that is composed of lipid particle (SNALP)-formulated small interfering RNA (siRNA) targeting VEGF and the mitosis kinase, KSP. For each target, several siRNA duplexes were selected following extensive screening in tissue culture cells. A SNALP-formulated combination of the KSP and VEGF siRNAs (referred to as ALN-VSP) was tested in orthotopic liver tumor models in which human hepatoma cells (Hep3B) or human colorectal carcinoma cells (HCT116) are implanted directly into the livers of immunocompromised mice. We have demonstrated that ALN-VSP treatment leads to a reduction in tumor volume of 60% to 80% in intrasplenic HCC tumors (Hep3B) and human liver metastases (HCT116). Further, we show that multi-dose administration of ALN-VSP leads to sustained reductions in tumor microvessel density and enhanced hemorrhage in orthotopic Hep3B tumors. Similar results were obtained with a SNALP formulation of the VEGF siRNA alone. Thus, each siRNA in ALN-VSP makes a distinct contribution to efficacy.

Figure 1. ALN-VSP

**Efficacy in Orthotopic HCC Model**

- **A** Tumor bearing animals received a single dose of ALN-VSP (4 mg/kg) or SNALP-Luc (vehicle control). Tumor bearing liver and lymph nodes were analyzed 24 h after dosing. ALN-VSP significantly affects tumor vasculature and target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.
- **B** Dose dependent target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.

Figure 2. Efficacy in Intraperitoneal HCC Model

- In some animals, intraperitoneal tumors were treated with ALN-VSP (4 mg/kg, twice per week) beginning 26 days after tumor implantation. In some animals, tumors were treated with ALN-VSP at 4 mg/kg twice a week for 3 weeks. Tumor bearing livers and lymph nodes were analyzed 48 h after dosing. Paraffin embedded tissues of tumors were stained with H&E to reveal regions of tumor hemorrhage, or with a CD34 antibody to detect tumor microvessels.

Figure 3. Efficacy in Intraportal Cinical Model

- A Phase 1 Clinical study of ALN-VSP was initiated in March, 2009.

Figure 4. Efficacy in Colorectal Carcinoma Tumors

- **A** Tumor bearing animals received two doses of ALN-VSP (4 mg/kg) or SNALP-Luc (vehicle control). Tumor bearing liver and lymph nodes were analyzed 24 h after dosing. ALN-VSP significantly affects tumor vasculature and target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.
- **B** Dose dependent target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.

Figure 5. ALN-VSP Extends Survival

- **A** Tumor bearing animals received two doses of ALN-VSP (4 mg/kg) or SNALP-Luc (vehicle control). Tumor bearing liver and lymph nodes were analyzed 24 h after dosing. ALN-VSP significantly affects tumor vasculature and target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.
- **B** Dose dependent target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.

Figure 6. Effect on Tumor Vascularity

- **A** Tumor bearing animals received one dose of ALN-VSP (4 mg/kg) or SNALP-Luc (vehicle control). Tumor bearing liver and lymph nodes were analyzed 24 h after dosing. ALN-VSP significantly affects tumor vasculature and target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.
- **B** Dose dependent target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver.

Conclusion

- ALN-VSP is a novel lipoid particle formulation comprising KSP and VEGF siRNAs that has been developed to treat solid tumors with liver involvement.
- Efficacy has been demonstrated in mouse tumor models.
- Phase 1 clinical study of ALN-VSP was initiated in March, 2009.