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

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Abstract #B204

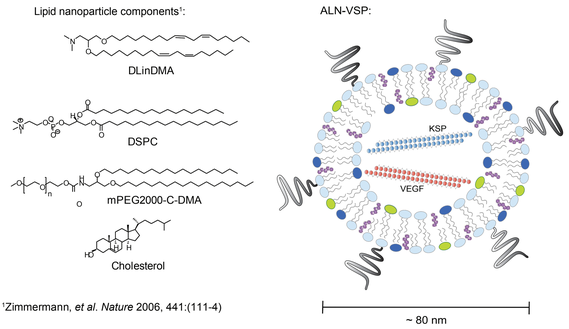
Malignancies of the liver, including primary (hepatocellular carcinoma) and secondary (metastatic) tumors, represent a significant unmet medical need. We are developing a therapeutic for solid tumors involving the liver that is comprised of lipid particle (SNALP)-formulated small interfering RNAs (siRNAs) targeting VEGF and the mitotic kinesin, KSP (Eg5). For each target, potent 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 demonstrate that ALN-VSP treatment leads to the formation of aberrant mitotic figures (monoasters) as a result of KSP silencing in both tumor types. Evidence of ALN-VSP efficacy in extra-hepatic tumors is shown by the formation of monoasters in intraperitoneal tumors (Hep3B) and lymph node metastases (HCT116). Further, we show that multi-dose administration of ALN-VSP leads to marked reductions in tumor microvessel density and intratumoral 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. Finally, we show that multi-dose administration of ALN-VSP significantly prolongs survival in the orthotopic Hep3B tumor model. A Phase 1 clinical trial of ALN-VSP has recently been initiated.

Tumor Models

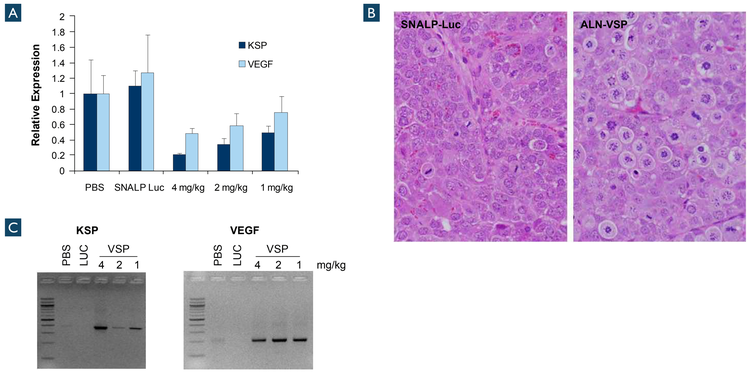
Orthotopic mouse liver tumor models have been developed comprising intrahepatic injection of human hepatoma cells (Hep3B) or human colorectal carcinoma cells (HCT116) into the left lateral lobe of scid/beige mice. Cells used for generating tumors express Luciferase which was monitored by *in vivo* imaging to assess tumor burden. Intraperitoneal HEP3B tumors were generated by IP injection of HEP3B cells.

Figure 1. ALN-VSP



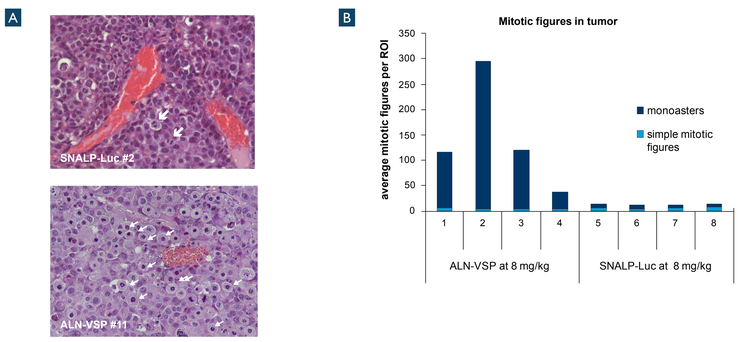
[1]Zimmermann, *et al. Nature* 2006, 441:(111-4)

Figure 2. Efficacy in Orthotopic HCC model



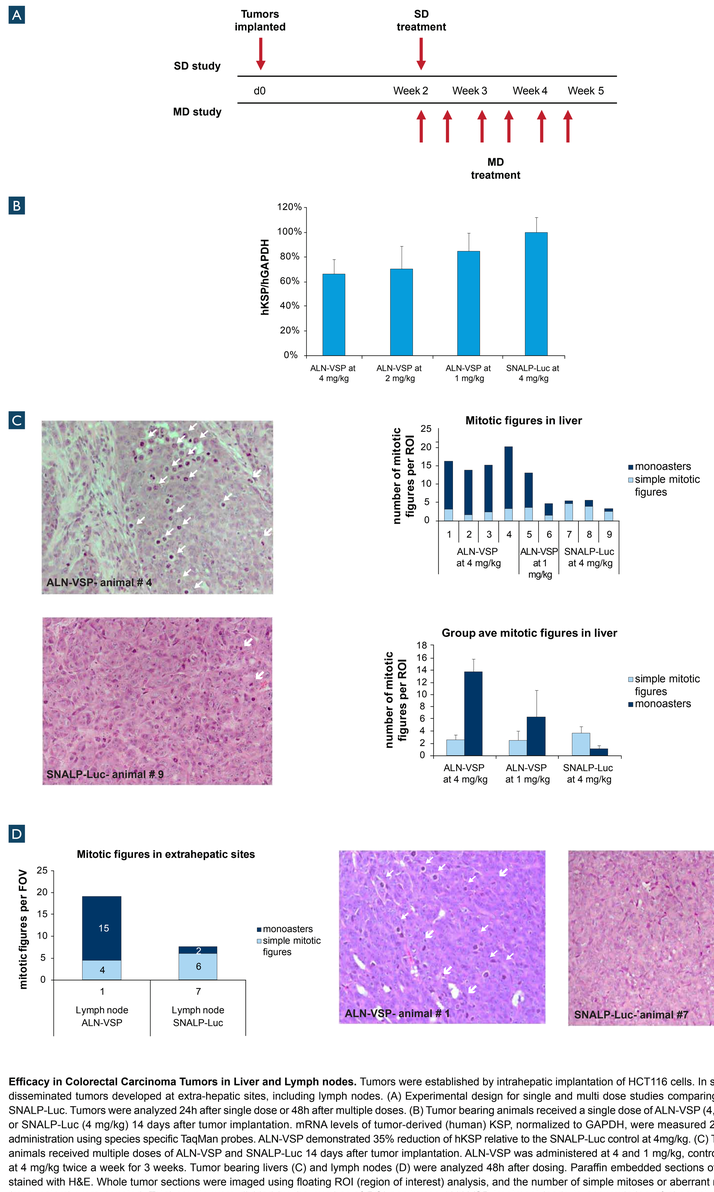
Efficacy in Orthotopic HCC model. (A) Tumor bearing animals received a single dose of ALN-VSP (4,2 or 1 mg/kg), SNALP-Luc (formulated siRNA targeting Luciferase; 4 mg/kg) or PBS 20 days after tumor implantation. mRNA levels of tumor-derived (human) VEGF and KSP, normalized to GAPDH, were measured 24h after drug administration using species specific TaqMan probes. ALN-VSP demonstrated 80% reduction of hKSP and 50% reduction of hVEGF relative to the PBS control at 4mg/kg. (B) Treatment with a single dose of ALN-VSP at 2 mg/kg resulted in accumulation of aberrant mitotic figures ("monoasters"), a hallmark of KSP inhibition. No aberrant mitoses were evident in control (SNALP-Luc) treated tumors. (C) A modified 5'-RACE assay was used to confirm the RNAi mechanism of action. Amplicons sizes consistent with site specific KSP and VEGF mRNA cleavage were detected in ALN-VSP treated tumors, but not in SNALP-Luc or PBS treated tumors.

Figure 3. Efficacy in Intraperitoneal HCC Model



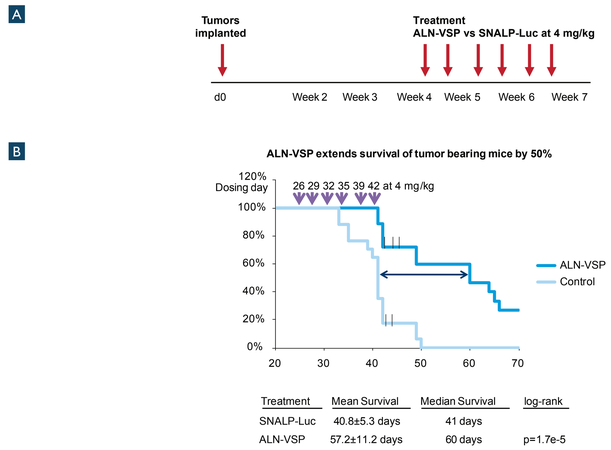
Efficacy in Intraperitoneal HCC Model. Animals bearing intraperitoneal HEP3B tumors received a single dose of ALN-VSP or SNALP-Luc at 8 mg/kg. Tumors were analyzed 48h after dosing. (A) Paraffin embedded sections of tumors were stained with H&E. Whole tumor sections were imaged using floating ROI (region of interest) analysis, and the number of simple mitoses or aberrant mitotic figures (monoasters) were counted. Total counts were divided by the number of ROI per tumor. Closed arrows highlight monasters; open arrows - simple mitotic figures. (B) ALN-VSP treatment leads to the accumulation of aberrant mitotic figures (monoasters).

Figure 4. Efficacy in Colorectal Carcinoma Tumors



Efficacy in Colorectal Carcinoma Tumors in Liver and Lymph nodes. Tumors were established by intrahepatic implantation of HCT116 cells. In some animals, disseminated tumors developed at extra-hepatic sites, including lymph nodes. (A) Experimental design for single and multi dose studies comparing ALN-VSP to SNALP-Luc. Tumors were analyzed 24h after single dose or 48h after multiple doses. (B) Tumor bearing animals received a single dose of ALN-VSP (4, 2 or 1 mg/kg) or SNALP-Luc (4 mg/kg) 14 days after tumor implantation. mRNA levels of tumor-derived (human) KSP, normalized to GAPDH, were measured 24h after drug administration using species specific TaqMan probes. ALN-VSP demonstrated 35% reduction of hKSP relative to the SNALP-Luc control at 4mg/kg. (C) Tumor bearing animals received multiple doses of ALN-VSP and SNALP-Luc 14 days after tumor implantation. ALN-VSP was administered at 4 and 1 mg/kg, control SNALP-Luc at 4 mg/kg twice a week for 3 weeks. Tumor bearing livers (C) and lymph nodes (D) were analyzed 48h after dosing. Paraffin embedded sections of tumors were stained with H&E. Whole tumor sections were imaged using floating ROI (region of interest) analysis, and the number of simple mitoses or aberrant mitotic figures (monoasters) were counted. Total counts were divided by the number of ROI per tumor. ALN-VSP treatment leads to accumulation of aberrant mitotic figures (monoasters) in liver tumors and lymph node metastases. Closed arrows highlight monasters; open arrows - simple mitotic figures.

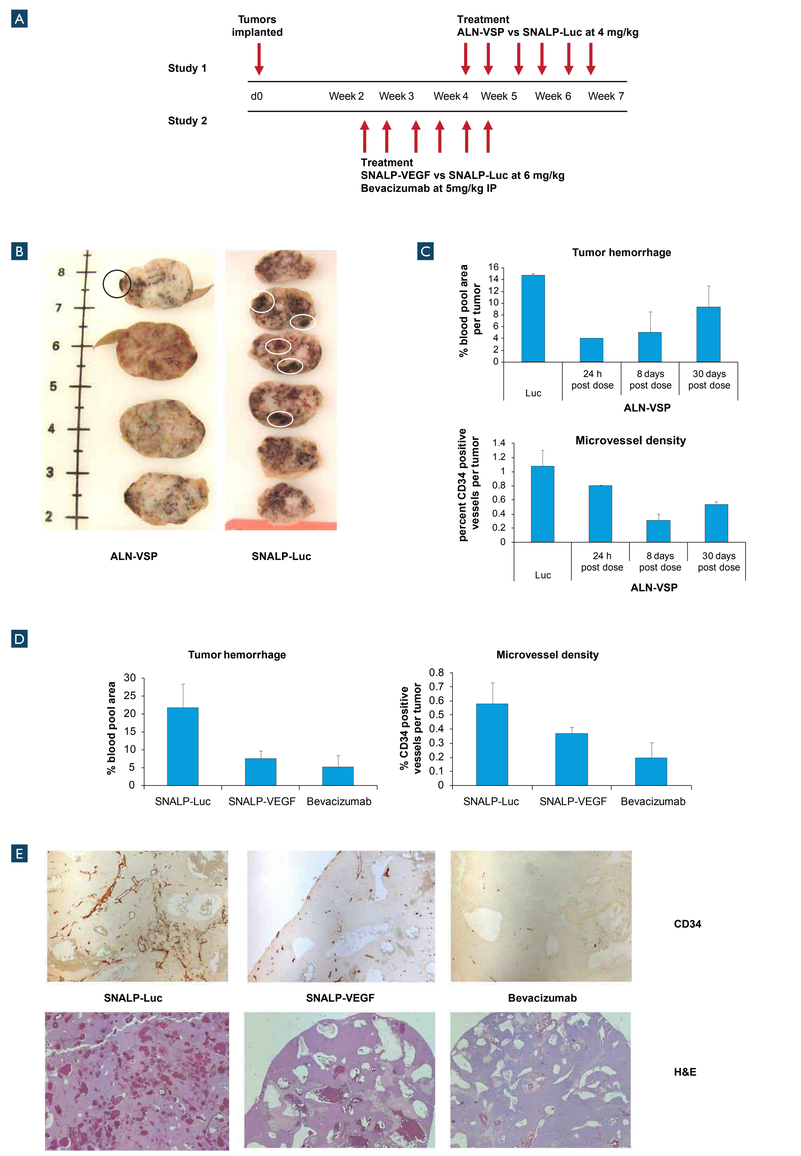
Figure 5. ALN-VSP Extends Survival



Treatment	Mean Survival	Median Survival	log-rank
SNALP-Luc	40.8±5.3 days	41 days	
ALN-VSP	57.2±11.2 days	60 days	p=1.7e-5

ALN-VSP extends survival. Tumors were established by intrahepatic implantation of Hep3B cells. (A) Experimental design for study comparing 4 mg/kg ALN-VSP vs SNALP-Luc administered twice per week for three weeks beginning 26 days after tumor implantation. Animals were euthanized based on humane surrogate endpoints. (B) Mean survival of ALN-VSP animals was extended by approximately 50% versus SNALP-Luc treated animals.

Figure 6. Effect on Tumor Vasculature



Effect on tumor vasculature. (A) Experimental designs. Study 1- comparison of 4 mg/kg ALN-VSP vs SNALP-Luc administered twice per week for three weeks beginning 26 days after Hep3B orthotopic tumor implantation. Animals were euthanized based on humane surrogate end points. Study 2- comparison of 6 mg/kg SNALP-VEGF vs SNALP-Luc administered twice per week for three weeks beginning 14 days after tumor implantation. Bevacizumab at 5 mg/kg administered IP was used as a positive control. Animals were euthanized 72h after the last dose. (B) ALN-VSP treated tumors exhibit far less intratumoral hemorrhage compared to SNALP-Luc treated animals. Areas of tumor hemorrhage in 2-4 mm tumor sections (slabs) are outlined. (C) ALN-VSP treatment reduces tumor hemorrhage and microvessel density. Paraffin embedded sections of tumors were stained with H&E to reveal regions of tumor hemorrhage, or with a CD34 antibody to detect tumor vasculature. Two whole tumor sections from distant tumor slabs were imaged using floating ROI (region of interest) analysis. Regions of intratumoral hemorrhage were outlined in H&E stained sections and total areas of hemorrhage were quantified in each tumor. To quantify microvessel density, CD34 stained areas were quantified as a percentage of total tumor area. (D) Vascular effects of ALN-VSP are attributable to the VEGF siRNA. SNALP-VEGF reduces tumor hemorrhage and microvessel density to the same extent as ALN-VSP. (E) Representative images of H&E and CD34 staining of tumors from study 2.

ALN-VSP Phase I Trial Design

- Multi-center, open label, dose escalation study in U.S.
- Expected enrollment of approximately 55 patients with advanced tumors with liver involvement
- Route of administration: intravenous infusion
- Objectives
  - Primary: Safety, tolerability and PK
  - Secondary: Efficacy
    - RECIST
    - DCE-MRI
    - Plasma biomarkers
    - Pharmacodynamic analyses of tumor biopsies

Conclusion

- ALN-VSP, a novel lipid particle formulation comprising KSP and VEGF siRNAs has been developed to treat solid tumors with liver involvement
- Efficacy has been demonstrated in mouse tumor models
  - Dose dependent target silencing in established HCC (Hep3B) and CRC (HCT116) tumors in liver
  - Efficacy (monoaster formation) in extra-hepatic tumors
  - Significant survival benefit
- ALN-VSP treatment significantly affects tumor vasculature
  - Reduced intratumoral hemorrhage
  - Reduced vessel density in tumor
- A Phase 1 Clinical study of ALN-VSP was initiated in March, 2009