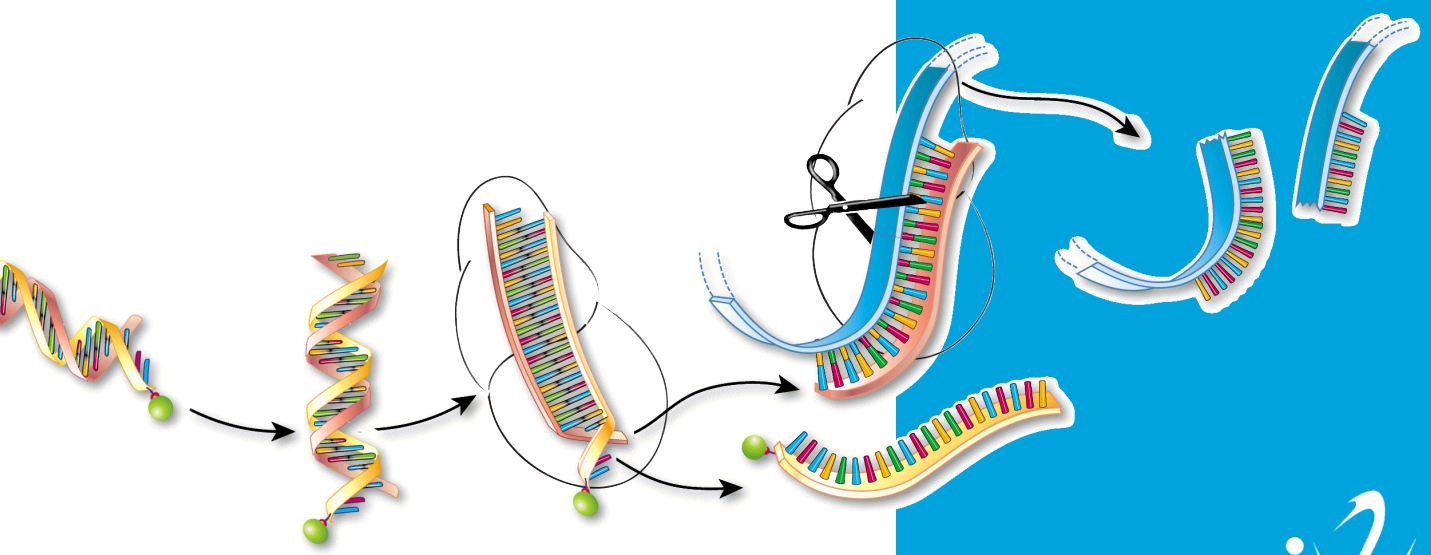


Alnylam® PHARMACEUTICALS

ALN-VSP02 Phase 1 Trial Update

Demonstrating RNAi in Man

Jared A. Gollob, M.D.

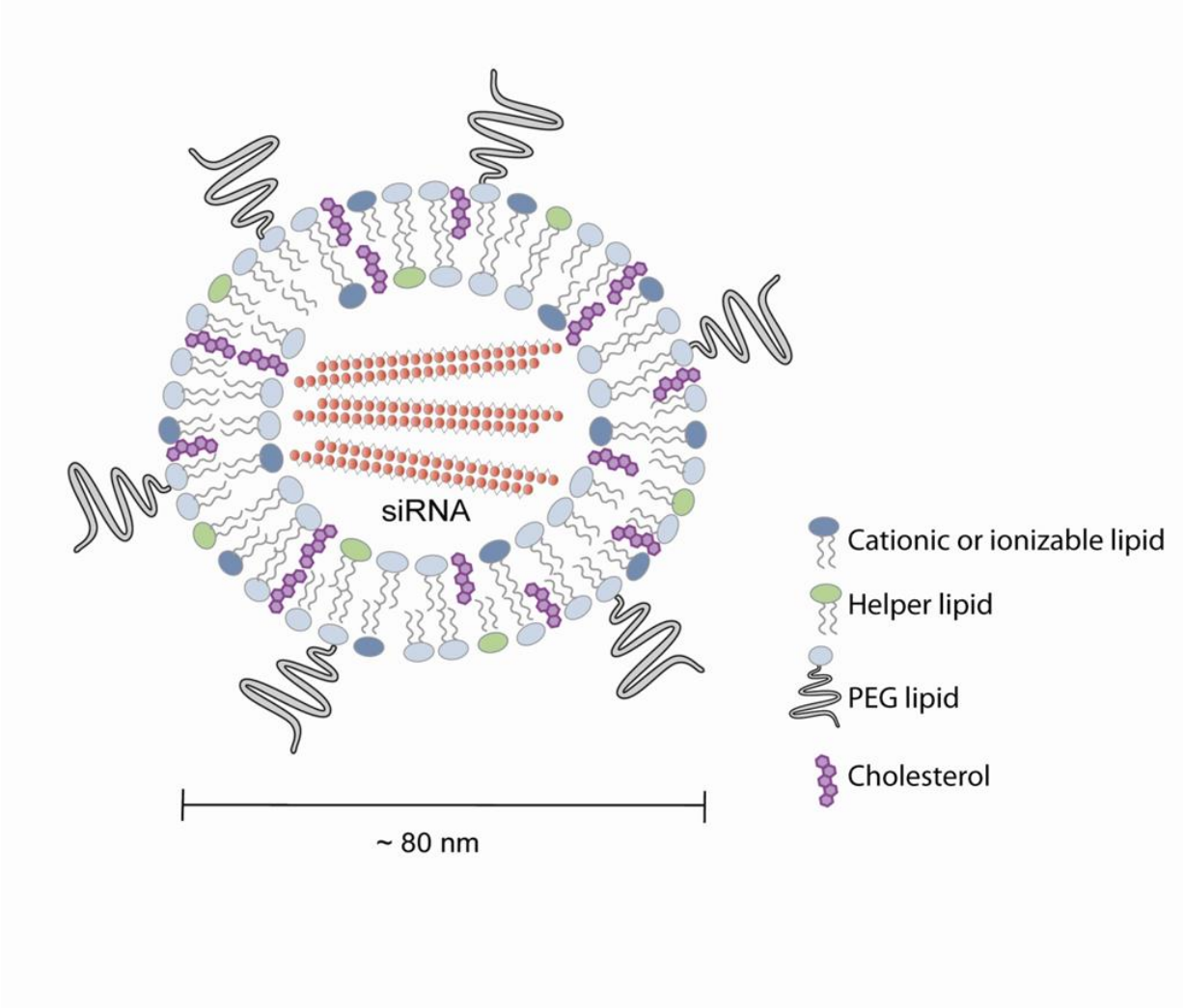
January 4, 2011

Dana-Farber Cancer Institute

Agenda

- LNPs and ALN-VSP02: Background
- Phase 1 Trial Safety Data
- Pharmacodynamic Data
  - 5’ RACE
  - DCE-MRI
- Summary

Lipid Nanoparticles for Systemic Delivery

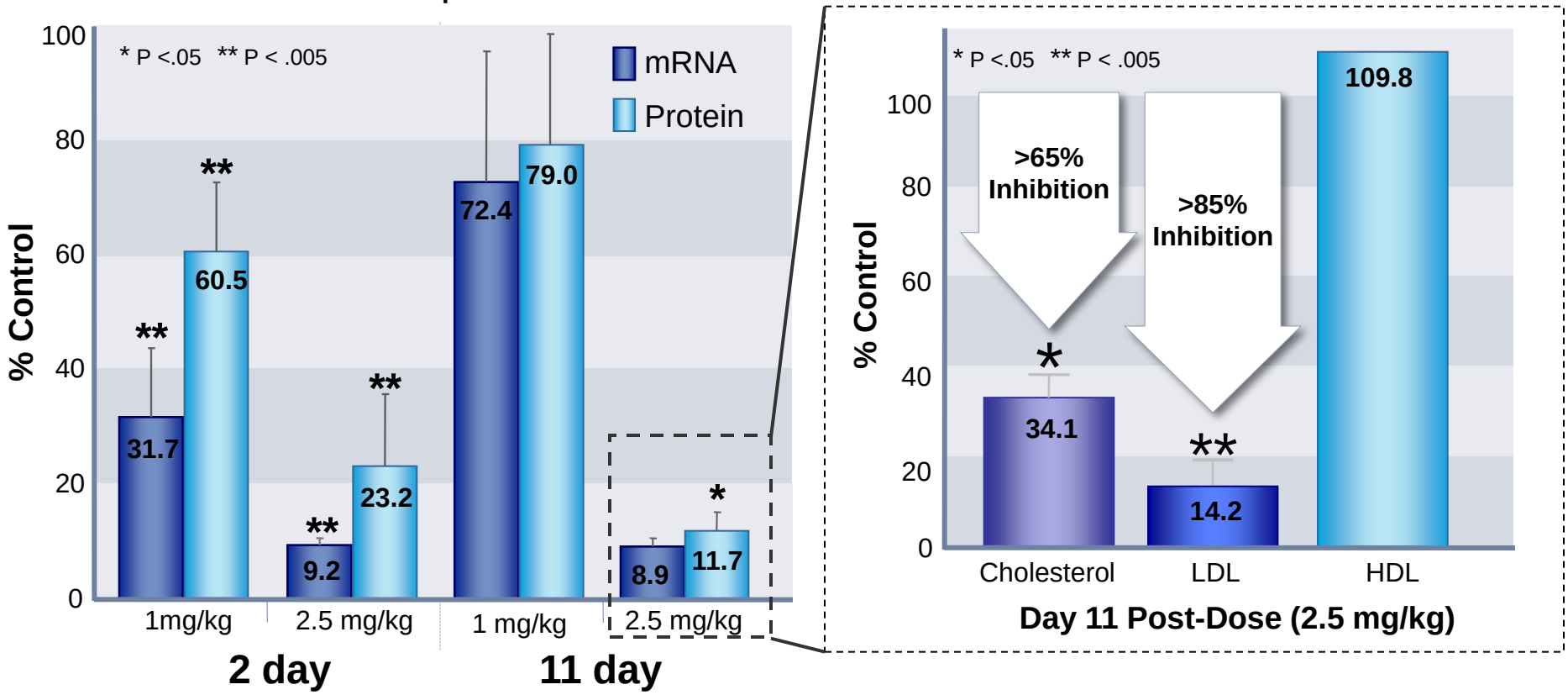


Liver Targeting

In Vivo Silencing of ApoB in Non-Human Primates

Efficacy in primates with Systemic RNAi after single IV injection

- Rapid, potent, dose-dependent and durable effects
- RNAi specific and leads to measurable therapeutic benefit
- RNAi mechanism proven *in vivo*



Nature, 441, 111-114, Mar 2006

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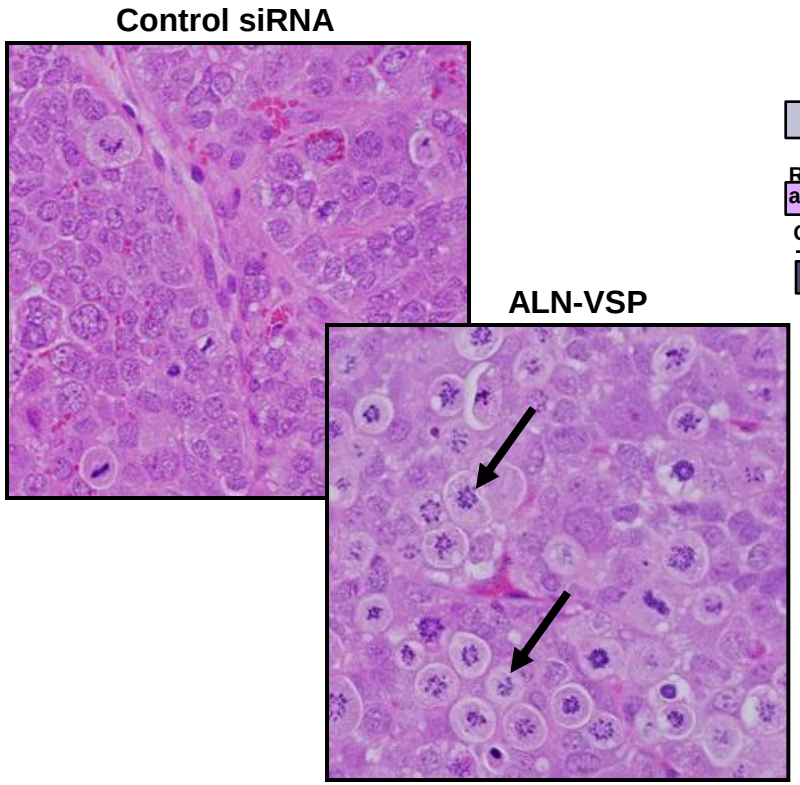
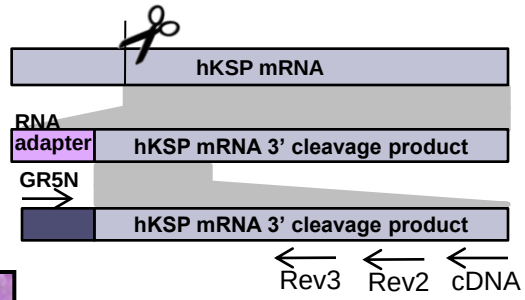
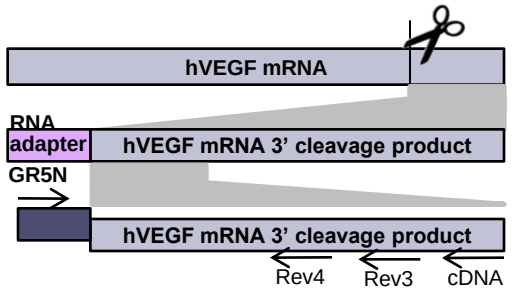
Tumor Targeting
Murine Liver Cancer Model

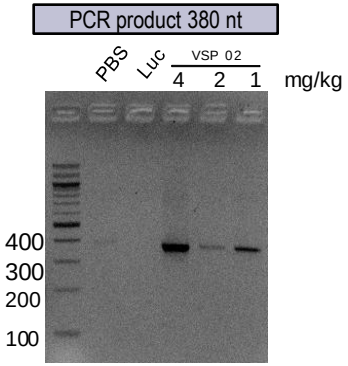
Orthotopic tumor model with intrahepatic Hep3B seeding in SCID mice

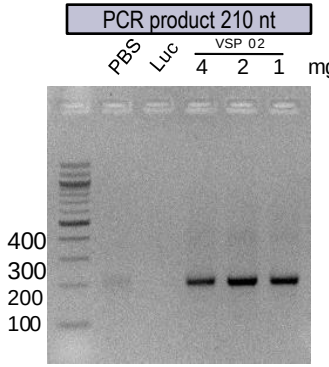
- Single IV bolus injection of ALN-VSP or control siRNA
- Mitotic arrest (monoasters) clearly detected in VSP-treated animals
- KSP and VEGF target mRNAs cleaved in tumors confirming RNAi mechanism











Keystone: RNAi, Feb 2009

Does Animal Pharmacology Translate to Man?

VSP02: First Opportunity to Show RNAi POC/POM in Man with LNP

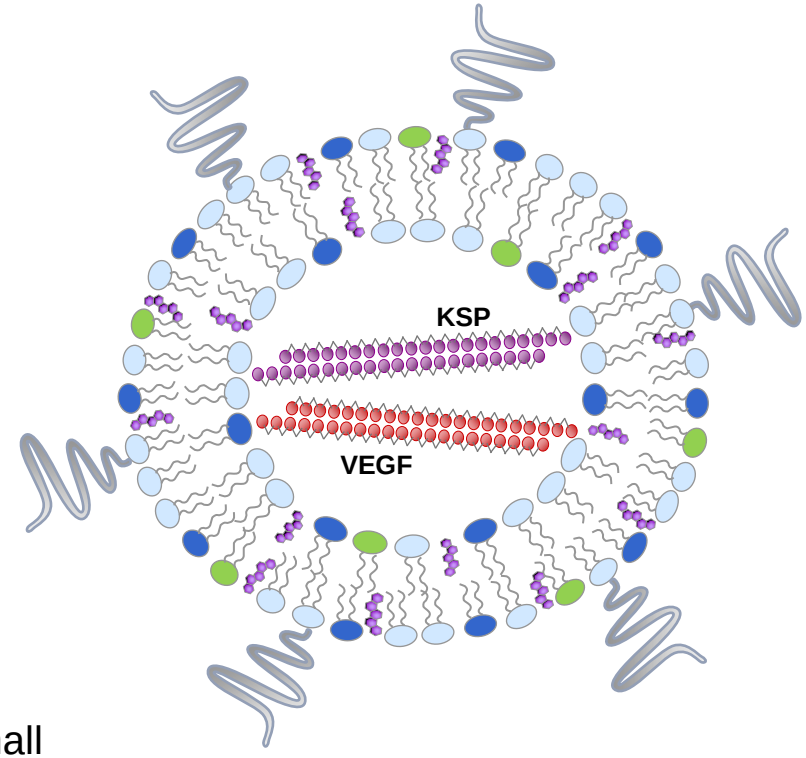
		VSP02	TTR01
POC	**Target kd in humans**	•VEGF: DCE-MRI and/or angiogenesis biomarker changes	•TTR lowering in blood
	Clinical effect of target kd	•Tumor response on CT scans	•Improvement in neuropathy score •Improvement in cardiac imaging
	Improved clinical outcomes with target kd	•Improved PFS	•Improved PFS
	NDA	•Improved PFS •Improved survival	•Improved PFS •Improved QOL
POM	**5'-RACE**	•Tumor biopsies	• N/A
	Target mRNA kd	•Tumor biopsies	• N/A
	Specificity of target kd	•Tumor biopsies	• N/A

Liver Cancer Program

ALN-VSP02

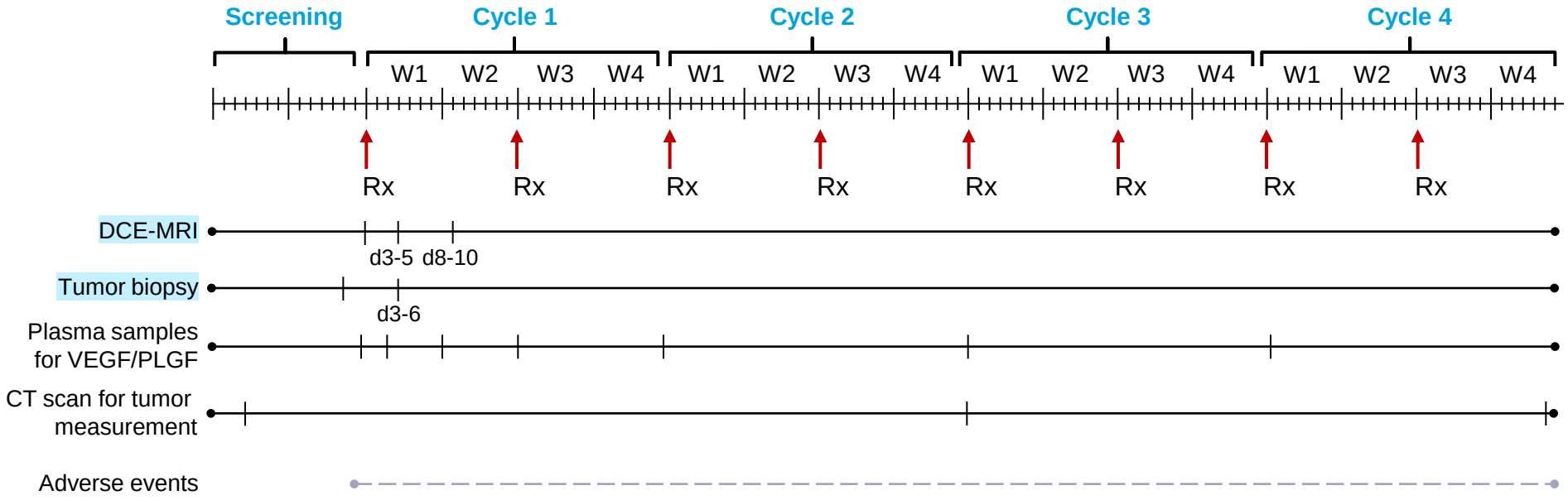
RNAi to treat primary and secondary liver cancers

- Prevalent solid tumor and common site of metastatic disease
  - » ~700,000/yr: Incidence of HCC worldwide
  - » ~500,000/yr: Patients with liver metastasis
- ALN-VSP is first dual-targeted RNAi drug
  - » Targeting 2 pathways with 2 different siRNAs increases potential therapeutic impact
    - – Proliferation: Kinesin Spindle Protein (KSP)
    - – Angiogenesis: VEGF
  - » Lipid nanoparticle (LNP) formulation
    - – From Tekmira Pharmaceuticals
- Preferential biodistribution of LNPs to liver, spleen, tumors
  - » May be able to avoid dose-limiting on-target toxicities associated with systemic delivery of small molecules and antibodies:
    - – KSP: myelosuppression, gastro-intestinal toxicity
    - – VEGF: Hypertension, bleeding, thrombosis, proteinuria, bowel perforation



ALN-VSP02

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, planned expansion phase of up to 20 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

Agenda

- LNPs and ALN-VSP02: Background
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ALN-VSP02 Phase I Study Status
As Reported at Chemotherapy Foundation Symposium

- N=28 Enrolled to date; Cohorts 1-6
- Total of 127 ALN-VSP02 doses administered
  - » Range of doses per patient: 2-13
- Dose escalation ongoing
  - » Current dose 1.25 mg/kg
  - » MTD not yet reached
- Key demographics
  - » Median age 56 yrs (range 34-78)
  - » 13 males, 15 females
  - » Tumor types
    - – Colorectal cancer (N=16)
    - – Pancreatic neuroendocrine tumor (N=1)
    - – Papillary renal cell cancer (N=1)
    - – Squamous cell cancer of head and neck (N=1)
    - – Pancreatic cancer (N=1)
    - – Esophageal cancer (N=1)
    - – Endometrial cancer (N=2)
    - – Angiosarcoma (N=1)
    - – Ovarian cancer (N=2)
    - – Synovial sarcoma (N=1)
    - – Mullerian stromal tumor (N=1)
- All patients treated with multiple prior anti-angiogenic and/or chemotherapy regimens

Chemotherapy Foundation Symposium, November 2010

ALN-VSP02 Phase I Safety Summary

- ALN-VSP02 generally well tolerated to date
  - 127 doses administered to 28 patients across 6 dose levels
  - Up to 13 doses given to single patient
- No dose-dependent trends in clinical or laboratory adverse events
- No dose-dependent changes in LFTs
- Human plasma PK showed dose-proportional Cmax and AUC with no evidence of drug accumulation
  - Animal PK studies accurately predicted for human
- Two dose-limiting toxicities
  - 0.7 mg/kg: Liver failure and death after 2 doses (possibly related to study drug) in patient with near complete replacement of liver by tumor and prior partial hepatectomy and splenectomy
  - 1.25 mg/kg: Grade 3 thrombocytopenia after dose 1 (related to study drug), resolved within 5 days
- Three grade 2 infusion reactions (one each at 0.4, 0.7 and 1.25 mg/kg), all 3 tolerated further treatment with prolongation of infusion duration
- MTD not yet reached, dose escalation continuing

Chemotherapy Foundation Symposium, November 2010

Agenda

- LNPs and ALN-VSP02: Background
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- Summary

ALN-VSP02 Pharmacology
Tumor Biopsies

- 17 Tumor biopsies obtained from 9 patients
  - » 3 at 0.4 mg/kg
  - » 2 each at 0.7, 1.0 and 1.25 mg/kg
  - » Liver tumor biopsies in 6 patients
  - » Extrahepatic tumor biopsies in 3 patients
- CT-guided core needle biopsies obtained pre- and post-dose 1
  - » Analyses ongoing:
    - – 5’ RACE
    - – qPCR
    - – Drug levels

Drug Levels for All Biopsies to Date

Pt #	Dose mg/kg	Tumor Type (site of metastasis)	siRNA Levels (ng/g) VEGF	KSP
007	0.4	Colorectal (liver)	25.7	12.5
016	0.4	SCC H&N (liver)	N/A	N/A
017	0.4	Ovarian (liver)	28.9	17.2
018	0.7	Pancreatic (liver)	N/A§	N/A§
019	0.7	Colorectal (liver)	142	73.3
022	1.0	Colorectal (adrenal)	9.8	3.8
025	1.0	Sarcoma (gluteal muscle)	0.45	0.4
026	1.25	Colorectal (liver)	0.32	<LLOQ
031	1.25	Ovarian (peri-umbilical)	4.9	3.6

N/A: Assay not performed
§ Very little tissue available for analysis
LLOQ: lower limit of quantitation

ALN-VSP02 Pharmacology
Tumor Biopsies

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  - » 3 at 0.4 mg/kg
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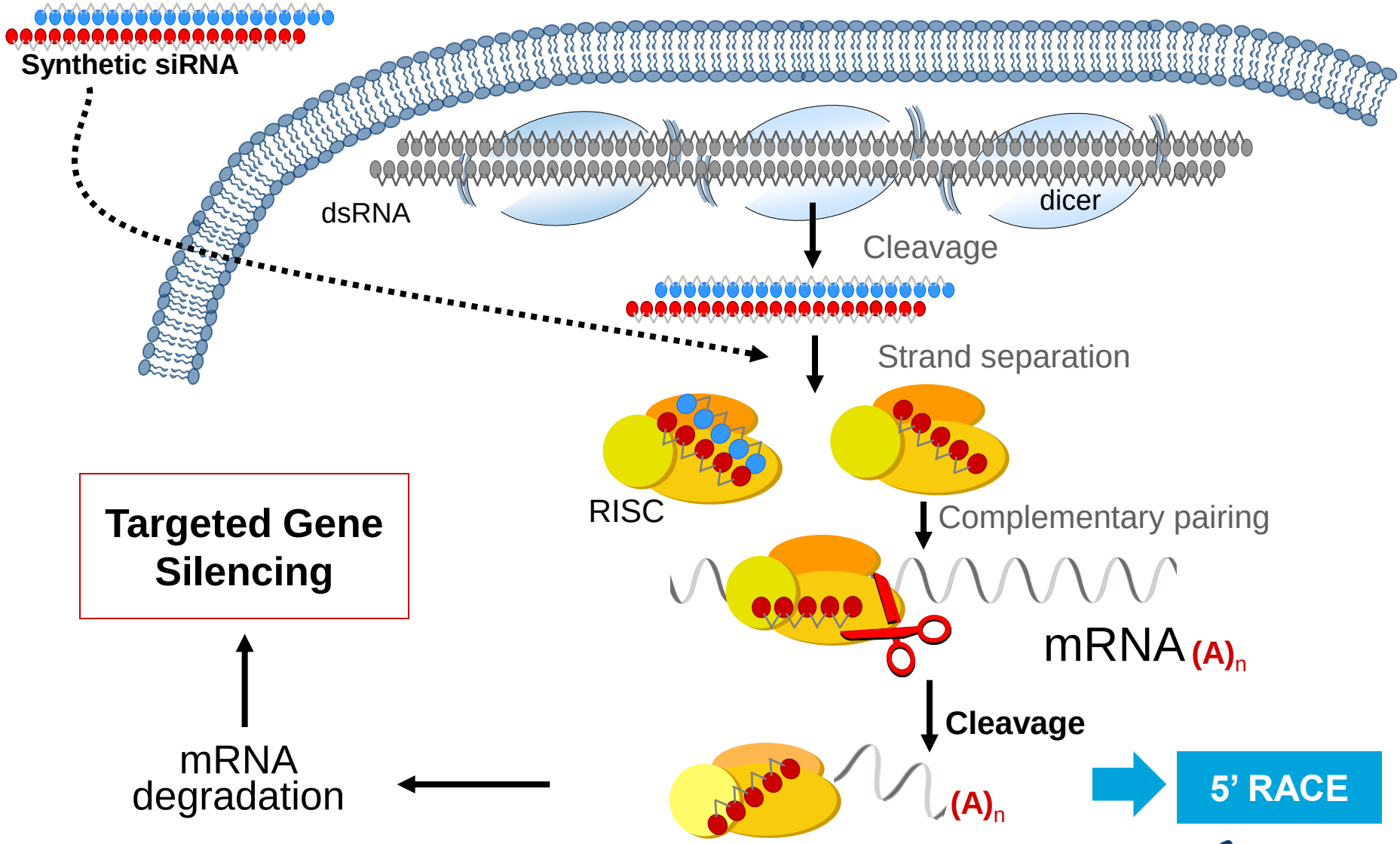
  - » Analyses ongoing:
    - – 5’ RACE
    - – qPCR
    - – Drug levels

RNAi Produces Precise Cleavage of Target mRNA



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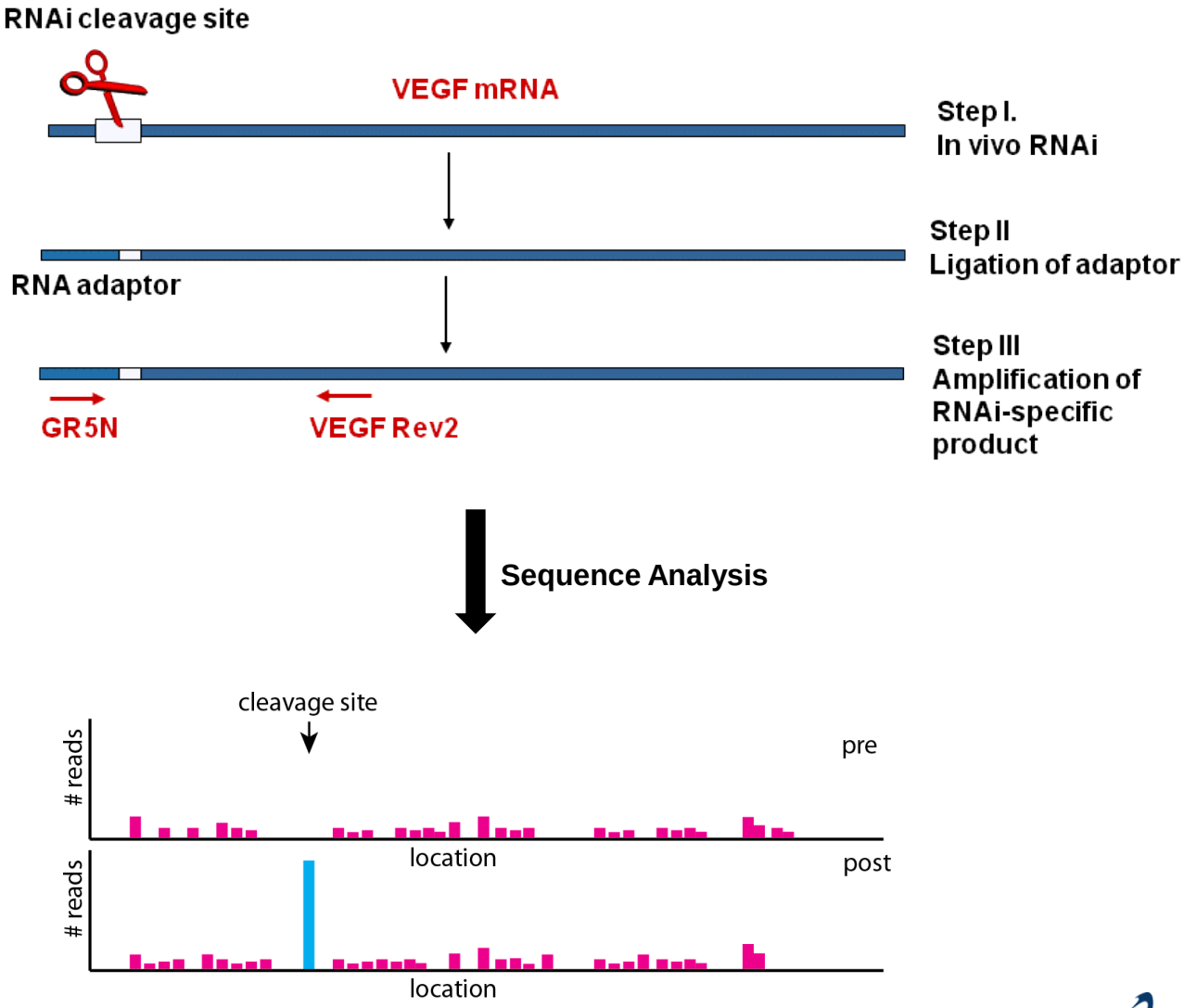
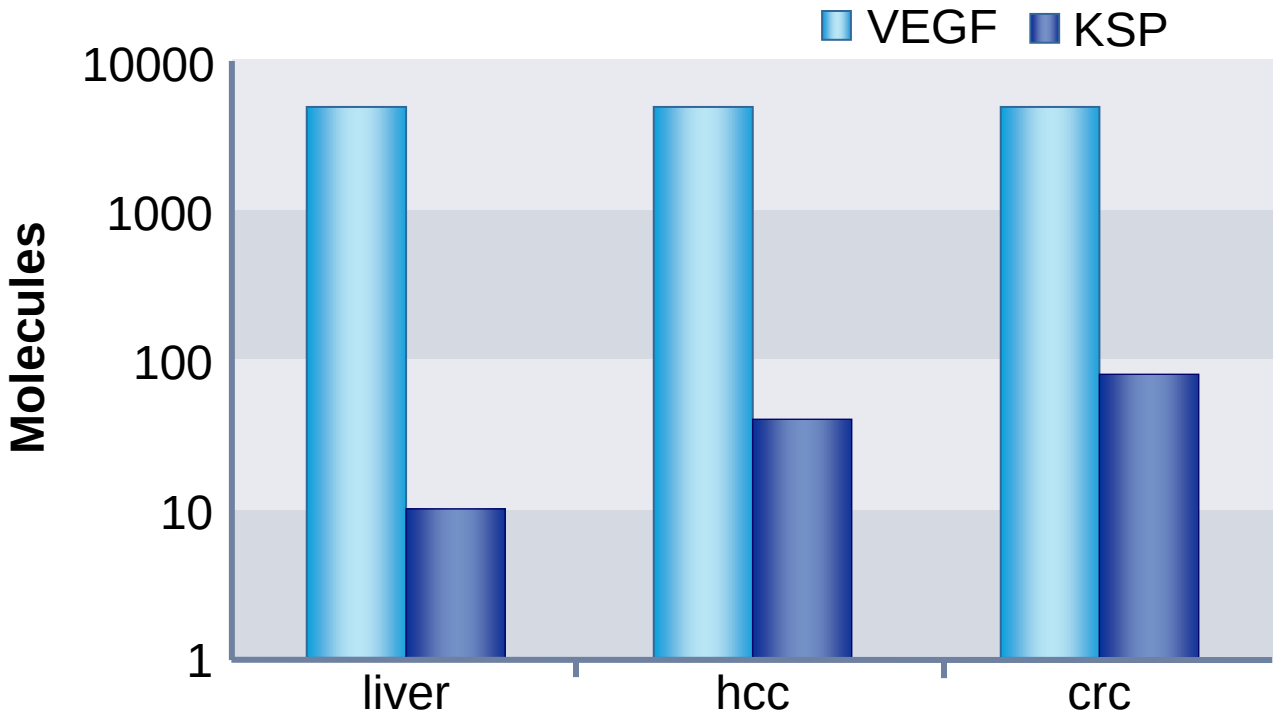
5’ RACE Assay
Method for Demonstrating RNAi



Factors Influencing Ability to Show RNAi By 5’ RACE in Tumor Biopsies

- Expression level of target mRNA
- Amount of tissue in biopsy that expresses target

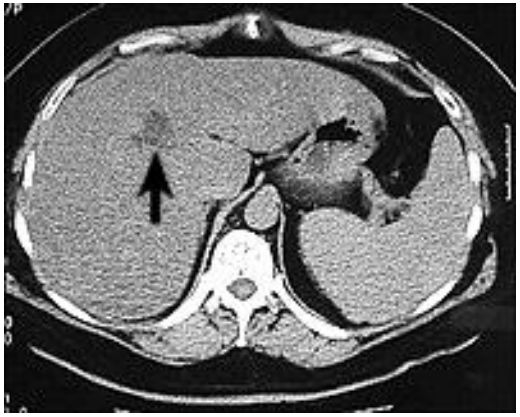
VEGF-A and KSP mRNA Levels
in Normal Liver and Tumors

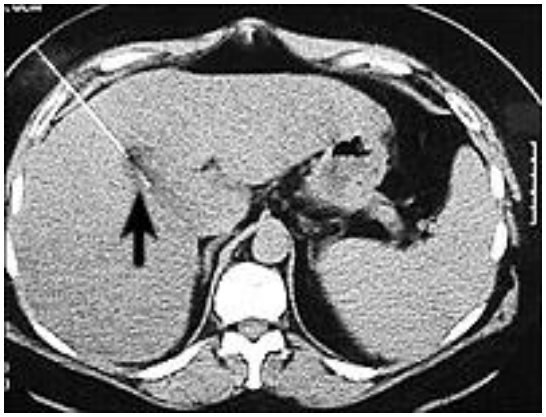


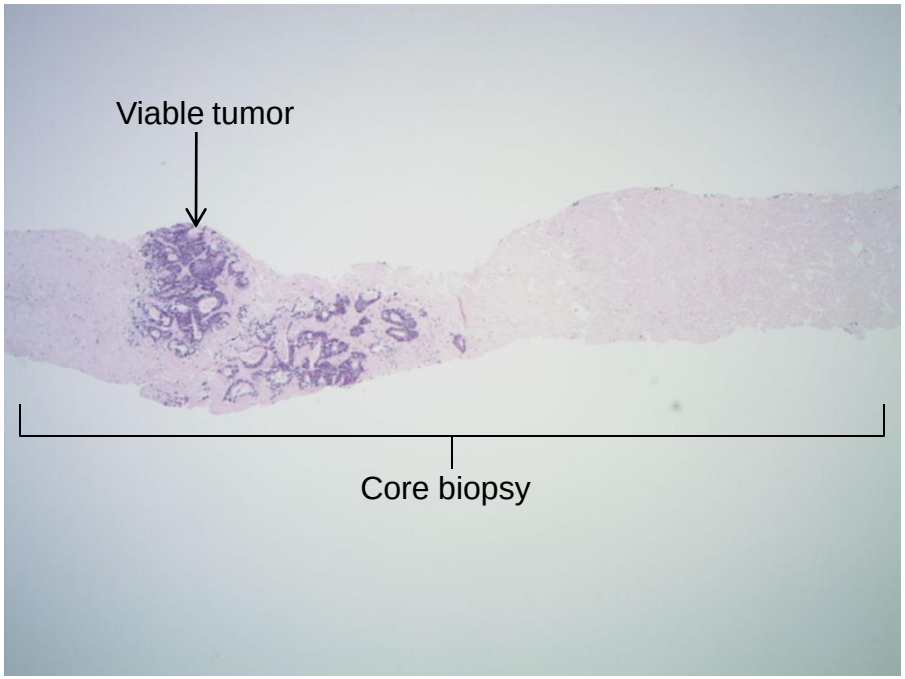
- Very low expression of KSP mRNA in normal liver and tumors
- Relatively abundant expression of VEGF-A mRNA in both normal liver and tumor

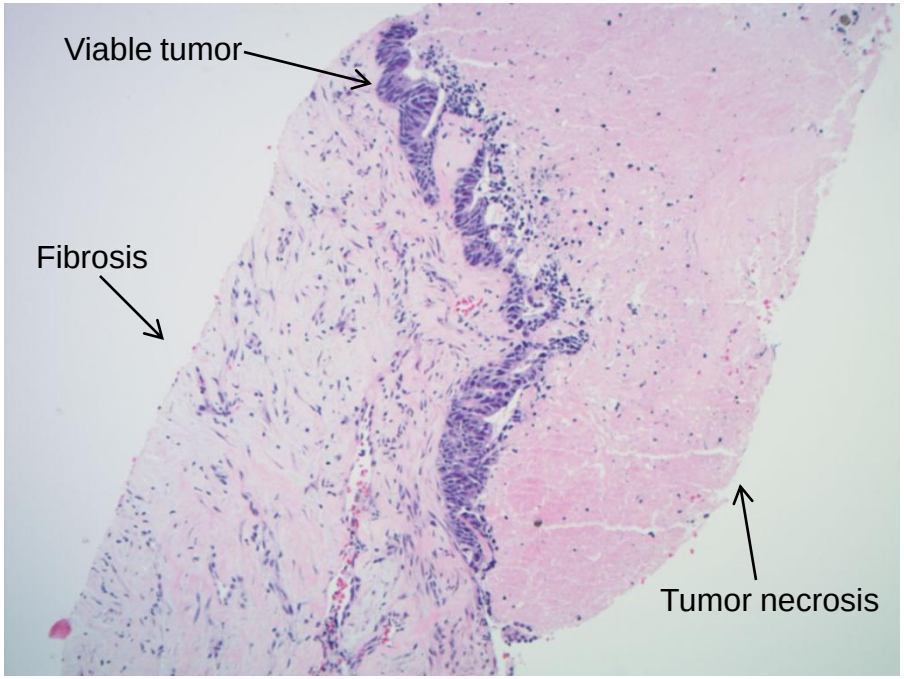
Tumor Core Biopsies

CT-guided Core Needle Biopsy

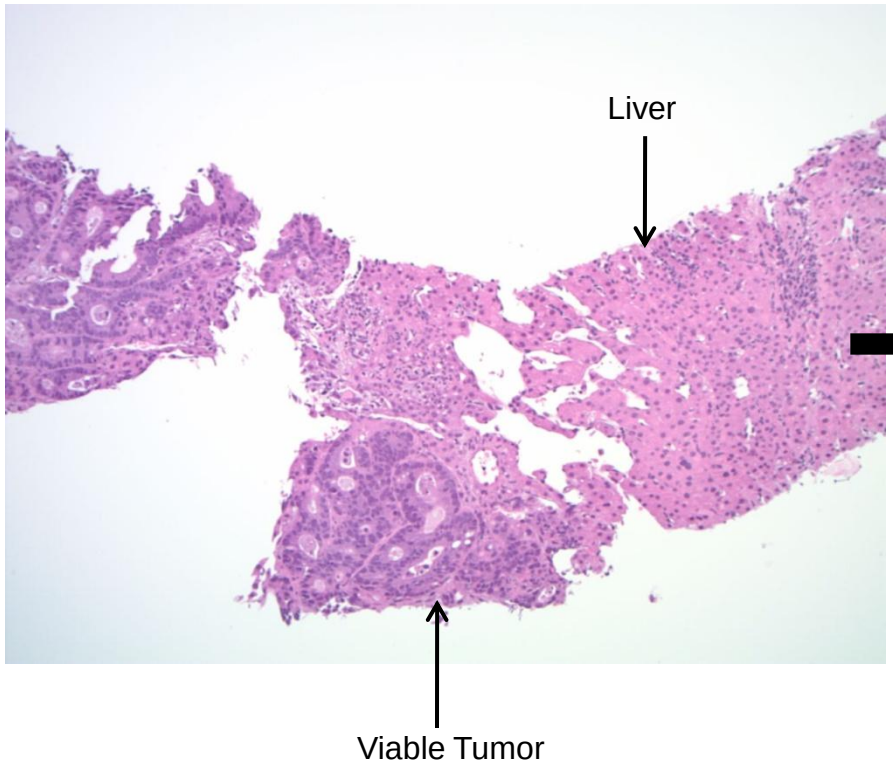




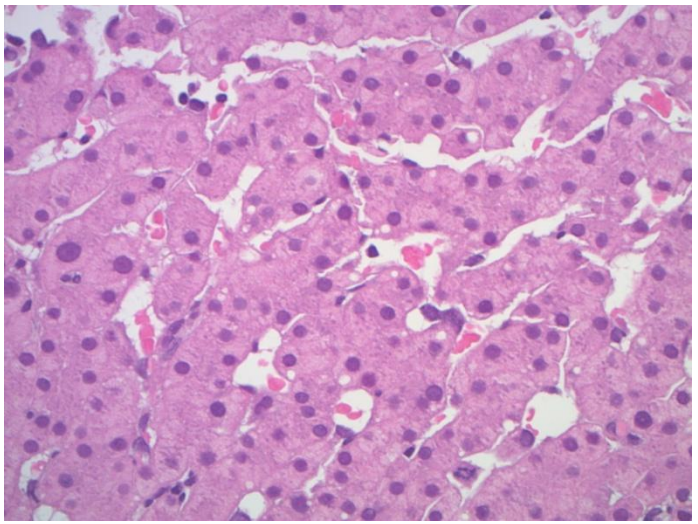




Tumor Core Biopsies



Liver



Tissue Components of Biopsies Analyzed to Date

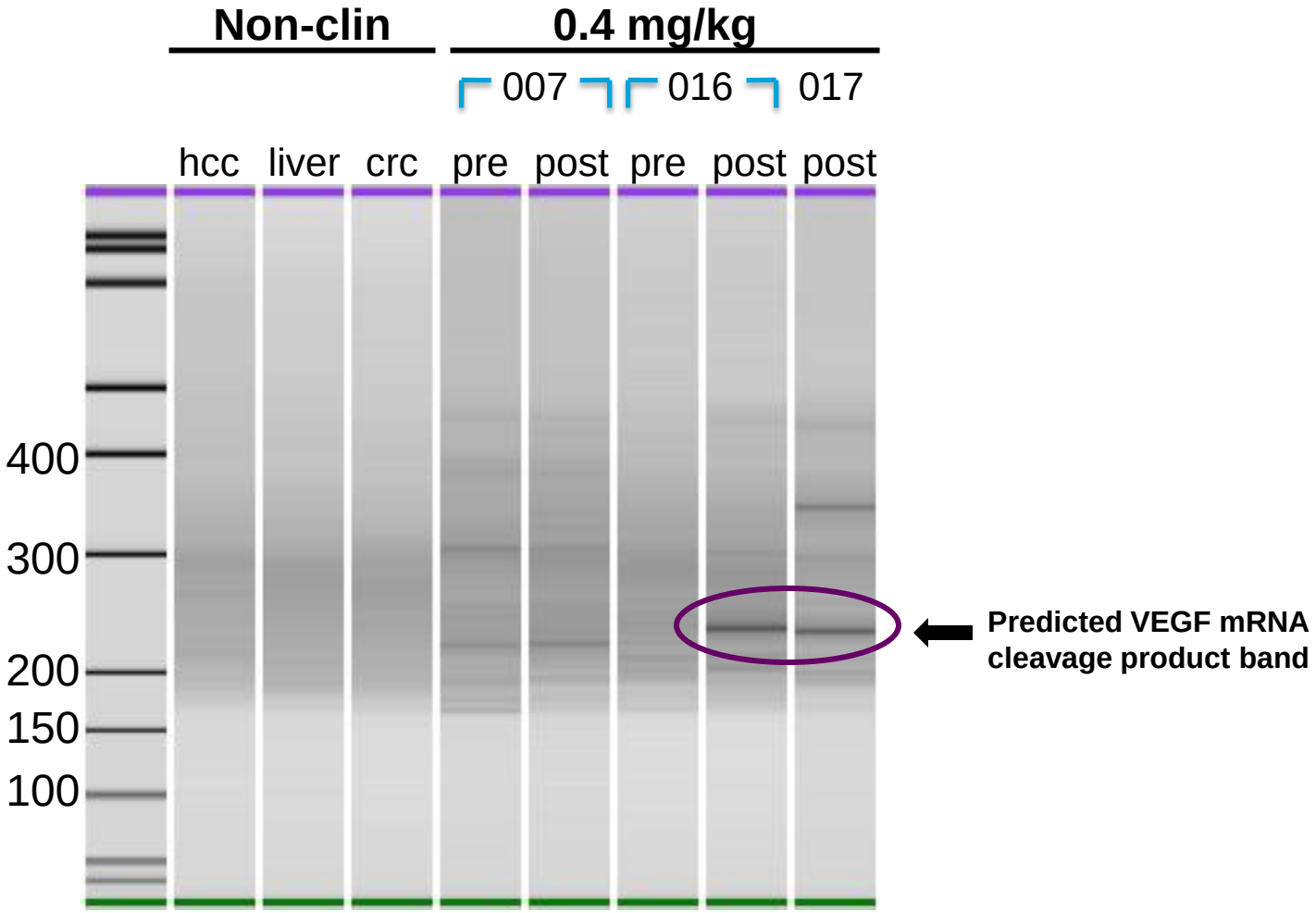
Pt#	Dose mg/kg	Tumor Type site of metastasis	Pre-Treatment Biopsy (%)			Post-Treatment Biopsy (%)		
			Tumor	Liver	Necrosis	Tumor	Liver	Necrosis
007	0.4	Colorectal (liver)	14	0	86	17	80	3
016	0.4	SCC H&N (liver)	10	0	90	0	100	0
017	0.4	Ovarian (liver)	N/A	N/A	N/A	0	95	0
018	0.7	Pancreatic (liver)	40	0	0	8	5	52
019	0.7	Colorectal (liver)	0	100	0	20	5	60

N/A: Biopsy not performed

- Tumor biopsies from liver very heterogeneous with regards to amounts of tumor, normal liver, and dead tissue
- Post-treatment biopsies from 3 patients at 0.4 mg/kg:
  - » Little to no tumor, abundant normal liver
  - » Can be used for VEGF 5' RACE, since robust VEGF expression in liver
  - » Not informative for KSP 5' RACE, as very little expression in normal liver
- Post-treatment biopsies from 2 patients at 0.7 mg/kg:
  - » Little tumor, very little normal liver
  - » Not informative for either VEGF or KSP 5' RACE

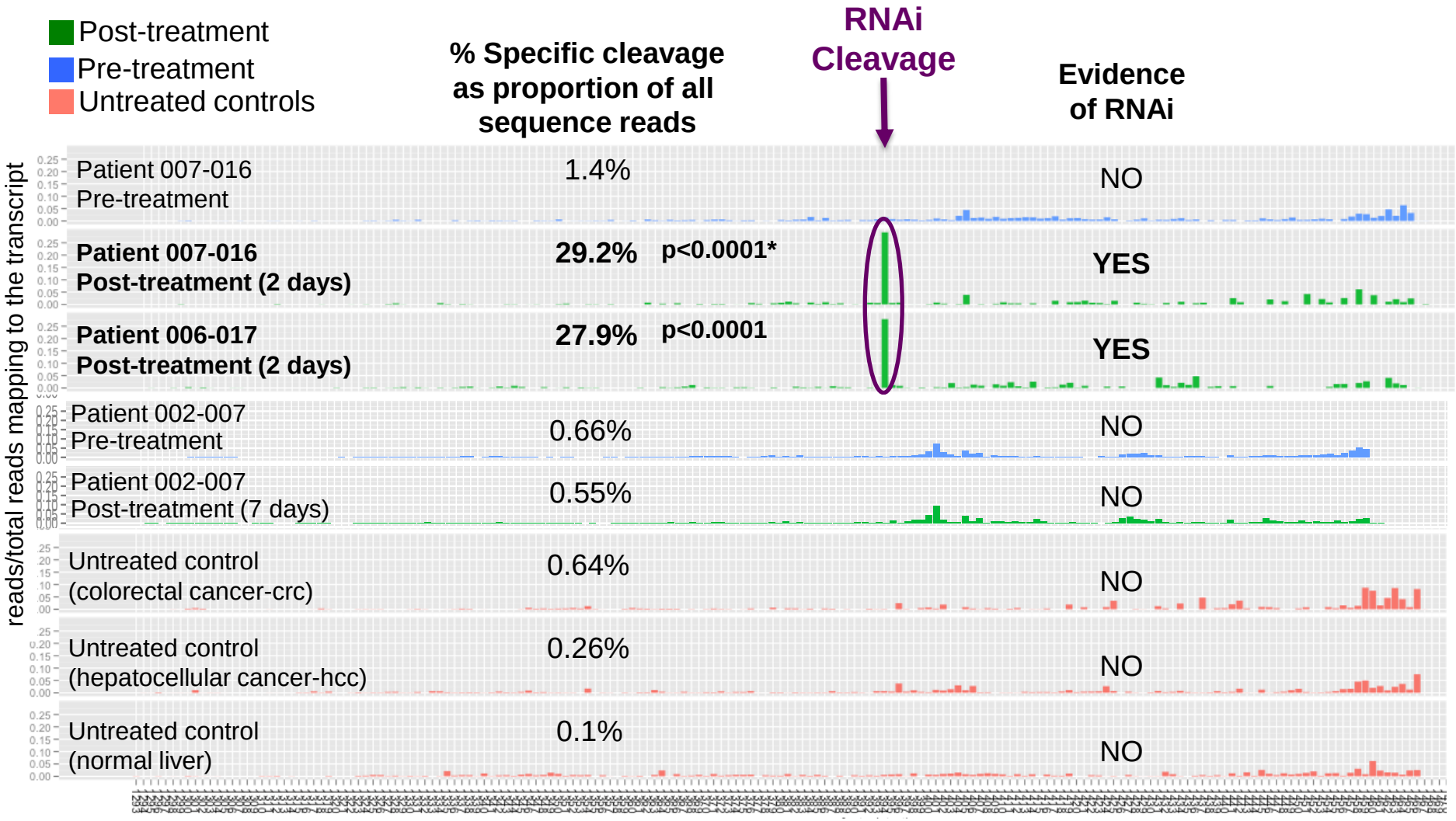
Dominant Band Seen in 5' RACE for VEGF

in Two Post-Dose Clinical Samples



Human RNAi Proof of Mechanism

Results from Blinded Molecular Analysis of Human Biopsy Samples



*T-test

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5’ RACE Tumor Biopsy Data
Conclusions

- In first 5 patients analyzed, 3 had abundant normal liver/total mRNA that permitted VEGF 5’ RACE analysis
- Predicted VEGF mRNA cleavage product seen post-treatment in livers of 2/3 patients
  - » $p<0.0001$
  - » Biopsy from negative patient was obtained 7 days post treatment
- First demonstration of RNAi in man with LNP-formulated siRNA

Agenda

- LNPs and ALN-VSP02: Background
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- Pharmacodynamic Data
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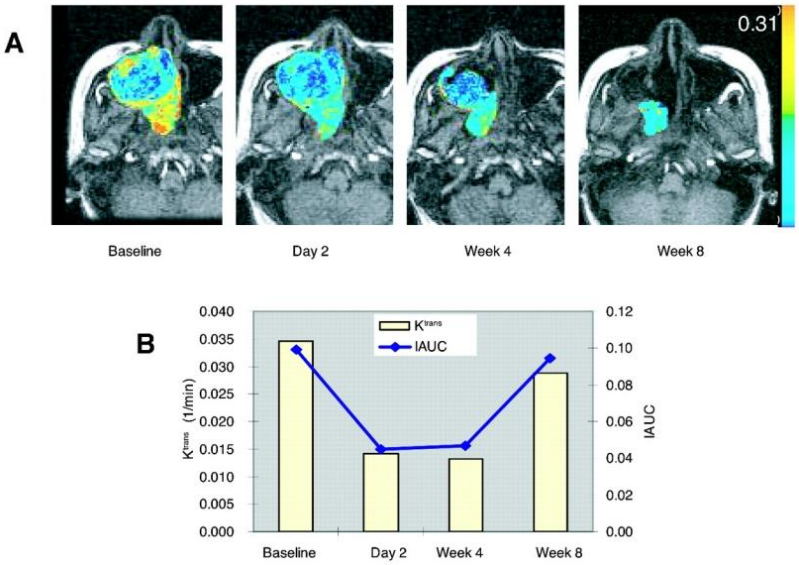
- Summary

ALN-VSP02 Pharmacology
DCE-MRI

- DCE-MRI (dynamic contrast-enhanced MRI) established as radiologic test for assessing anti-VEGF effect of novel drugs in clinical trials
- Ktrans is key parameter
  - » Measure of blood flow and blood vessel permeability in tumors
  - » 40% or greater drop in Ktrans post-treatment considered a significant drop in tumor blood flow



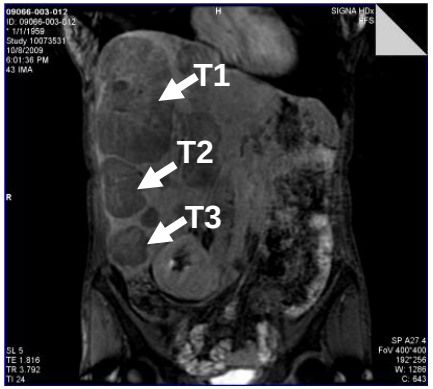
Example of Ktrans drop in patient treated with oral anti-VEGFR drug AG-013736

Liu *G et al. JCO* 2005;23:5464-5473

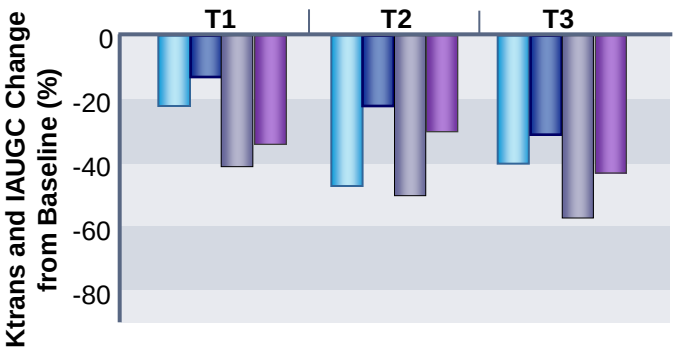
DCE-MRI Result at 0.7 mg/kg

Patient 012: Pancreatic Neuroendocrine Tumor

Baseline MRI, coronal view



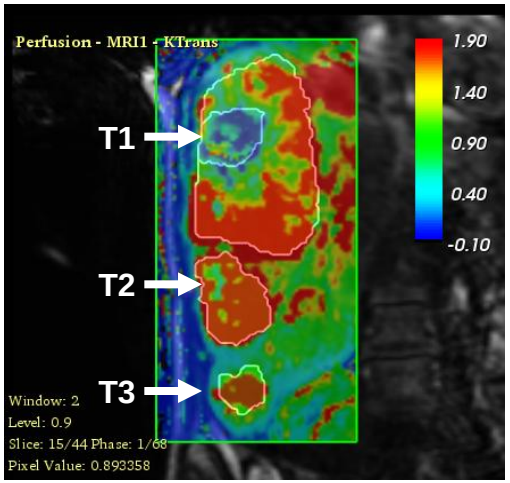
Patient 003-012



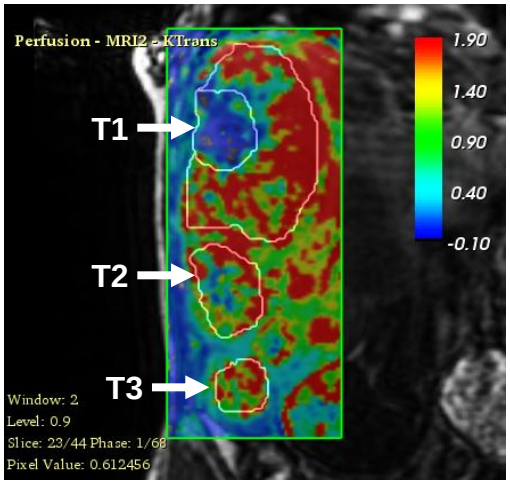
T: liver tumor number
Ktrans2/IAUGC2: Δ DCE-MRI #1 (BL) to DCE-MRI #2 (Day 4)
Ktrans3/IAUGC3: Δ DCE-MRI #1 (BL) to DCE-MRI #3 (Day 7)

Ktrans

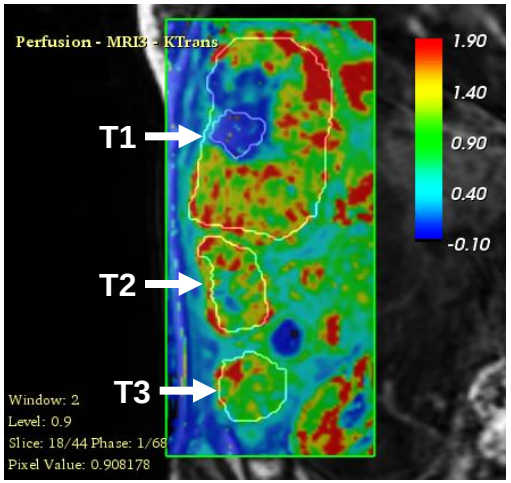
Baseline (BL) Pre-Dose



Day 4 Post-Dose 1



Day 7 Post-Dose 1



ASCO, June 2010

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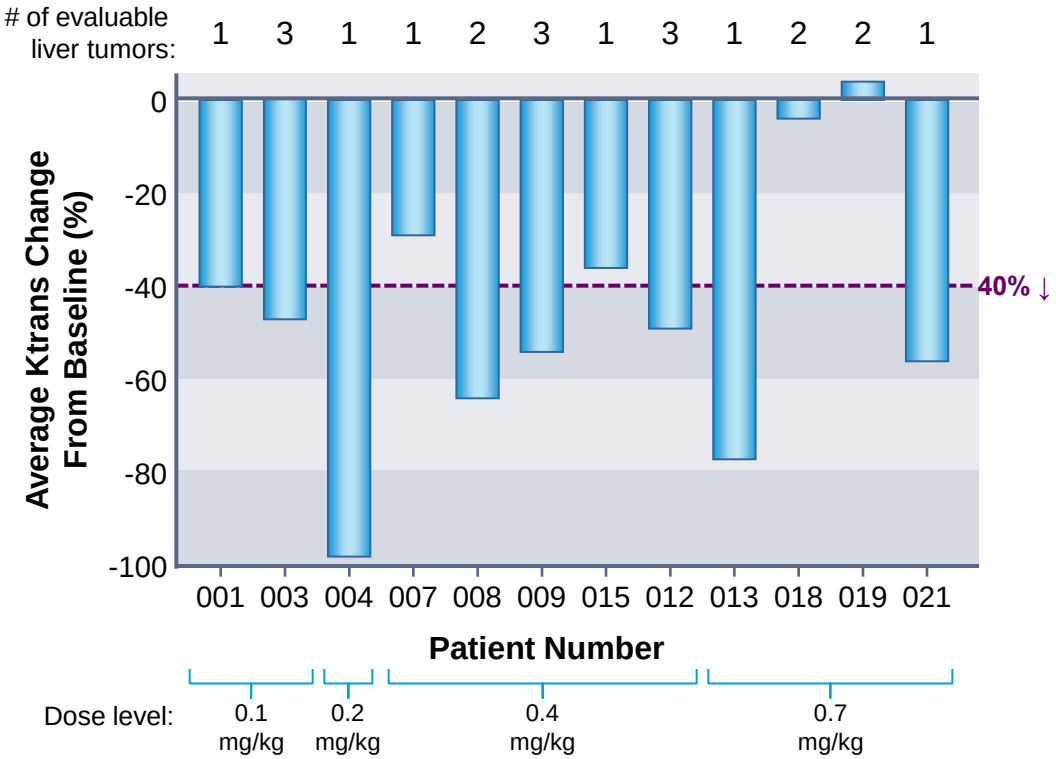
DCE-MRI Results
Summary of Cohorts 1-4



- 21 evaluable liver tumors in 12 patients
- 19/21 tumors (90%) showed decline in Ktrans
- 13 of 21 tumors (62%) had ↓Ktrans of ≥40%
- 8 of 12 patients (67%) had average ↓Ktrans of ≥40%

ASCO, June 2010

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Agenda

- LNPs and ALN-VSP02: Background
- Phase 1 Trial Safety Data
- Pharmacodynamic Data
  - » 5’ RACE
  - » DCE-MRI
- Summary

Summary

- Safety of LNP delivery to liver established in ALN-VSP02 Phase 1 liver cancer trial
- Liver delivery and VEGF mRNA target engagement with LNP-formulated siRNA demonstrated through 5' RACE assay on liver tumor biopsies
  - » Clear proof of RNAi activity in man
- Preliminary DCE-MRI data from imaged liver tumors further supportive of anti-VEGF pharmacology with ALN-VSP02
- Translatability of safety and pharmacology from NHP to man greatly de-risks the LNP delivery platform
  - » Also highlights potential of liver-directed programs currently in development including ALN-TTR and ALN-PCS

Acknowledgements

ALN-VSP02 Principal Investigators

- Sarah Cannon Cancer Center
  - Howard “Skip” Burris
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Tekmira