Presenter Disclosure:
John Nemunaitis, MD

The following relationships exist with this disclosure.

Gradalis, Inc . - shareholder

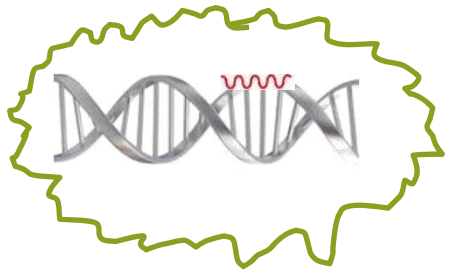
MARY CROWLEY
CANCER RESEARCH CENTERS
HOPE LIVES HERE.

Clinical Update of bifunctional (bi)-shRNAi Nanoplex Technology in Cancer

gradalis

Traditional non-targeted therapeutics (e.g. chemotherapy) shifts the signal fitness landscape of cancer enabling generation of a new resistance strategy; in essence, acting as a selection factor allowing tumor cell rebound.

Molecular Signal Strategy Mechanism



DNA

Cancer Abnormal/Over expressed Protein

RNA

Protein

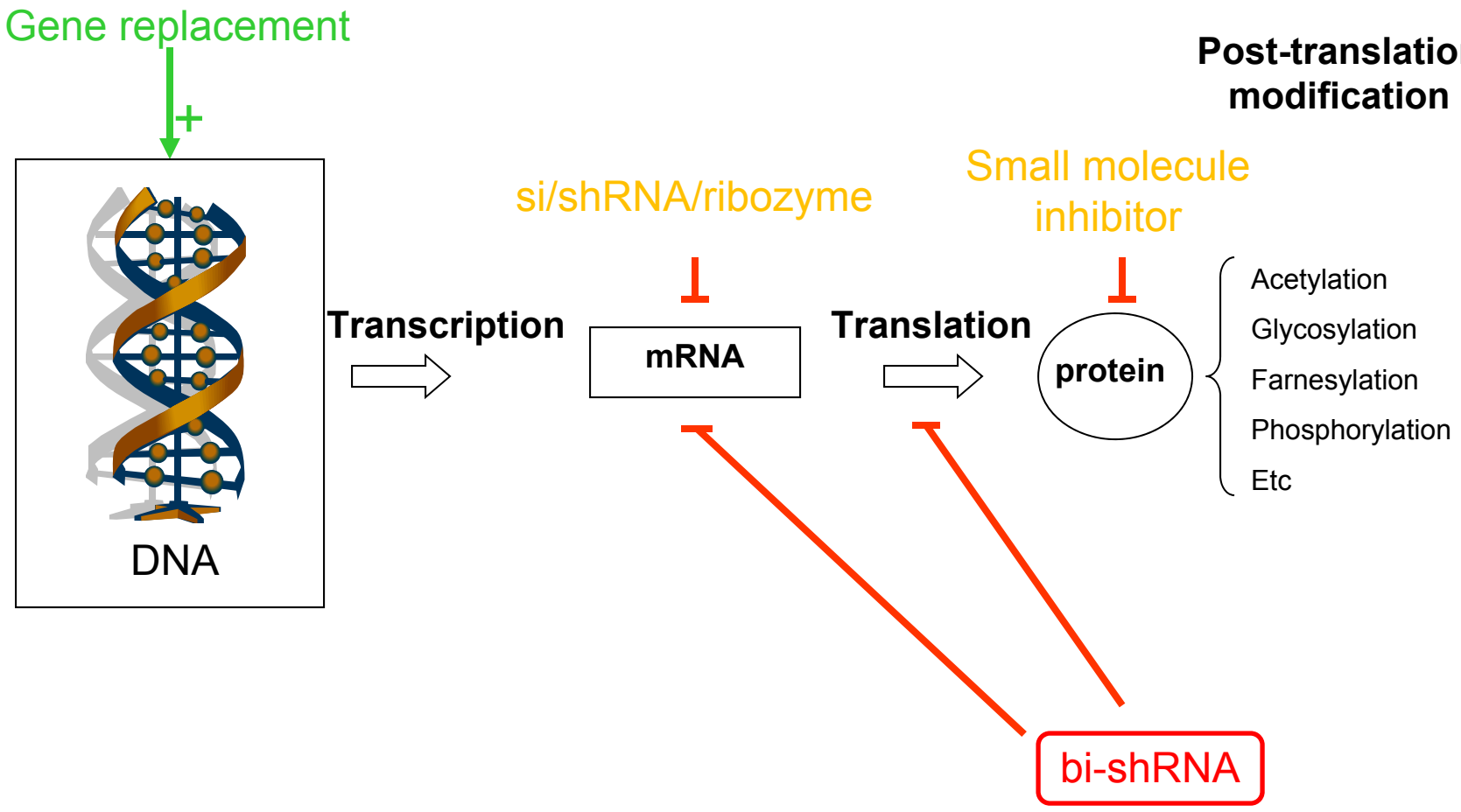
Cancer Abnormal Growth

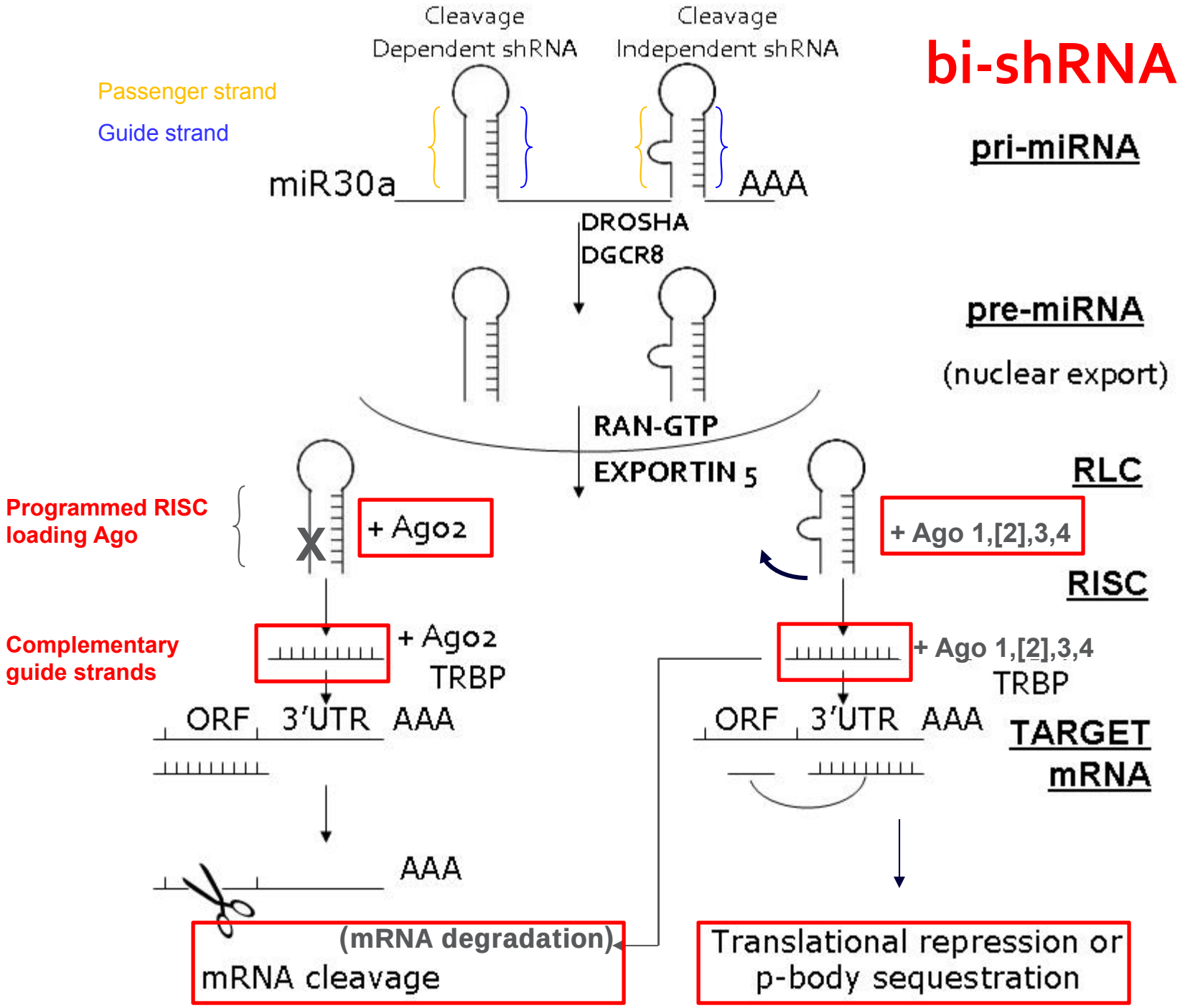
However Targeted therapeutics could have similar limitations

- Wrong primary target
- Not all pertinent targets are addressed
- Insufficient target control
- Inadequate PK
  - Inadequate delivery to tumor
  - Limited intra-tumoral penetration
  - Limited homogenous distribution
  - Limited tumor cellular uptake

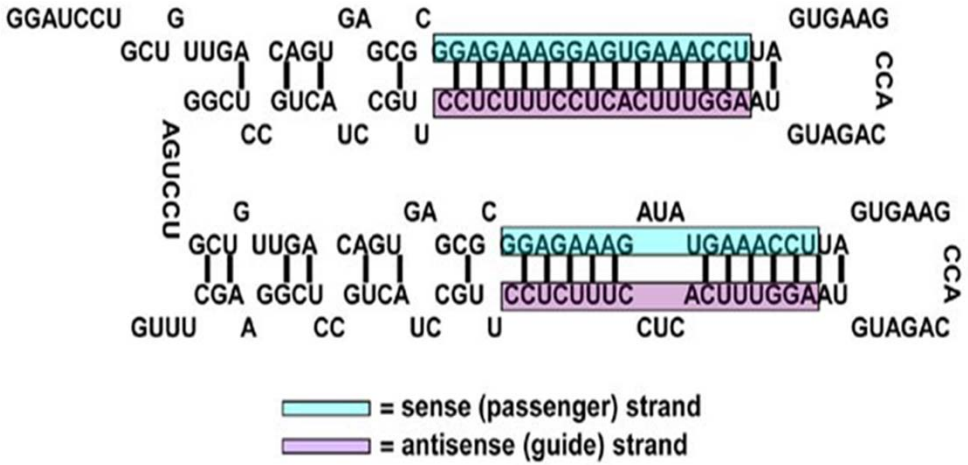
Clinical Trials of RNAi-Based Cancer Therapeutics							
Drug	**CALAA-01**	**ALN-VSP02**	**TKM-080301**	**siRNA-tenacinC**	**Atu027**	**FANG™ vaccine**	**bi-sh RNAi STMN1 Nanoplex**
Phase	I	I	I	1	I	I, II	I
Cleavage Products	Yes	Yes VEGF No KSP	Yes	Unk	Unk	N/A	Yes
Delivery	cyclodextrin polymer	lipid nanoparticle	lipid nanoparticle	naked	lipoplex	ex-vivo by electroporation	Bilamellar invaginated vesicle
Route	IV	IV	IV	IT	IV	ID	IT
Target	RRM2	KSP/VEGF	polo-like kinase 1 (PLK1)	tenascin-C	protein kinase N3	furin	STMN1
RNAi	unmodified siRNA	chemically modified siRNA	chemically modified siRNA	160bp double-stranded RNA	chemically modified siRNA	bifunctional-short hairpin	bifunctional-short hairpin
Current Enrollment	(36)	(41)	(24)	(53)	(34)	(121)	(8)
References	Davis et al *Nat 464: 1067-1070* (2010), a	Cervantes et al *J Clin Oncol* **29**: (2011), a	Ramanathan et al AACR 2013 Annual Meeting, a	Rolle et al *Cancer Biol Ther* **9**: 396-406 (2010)	Santel et al *Clin Cancer Res* **16**: 5469-80 (2010), a	Senzer NN, et al. *Mol Ther.* 2012:20(3):679-686.	N/A

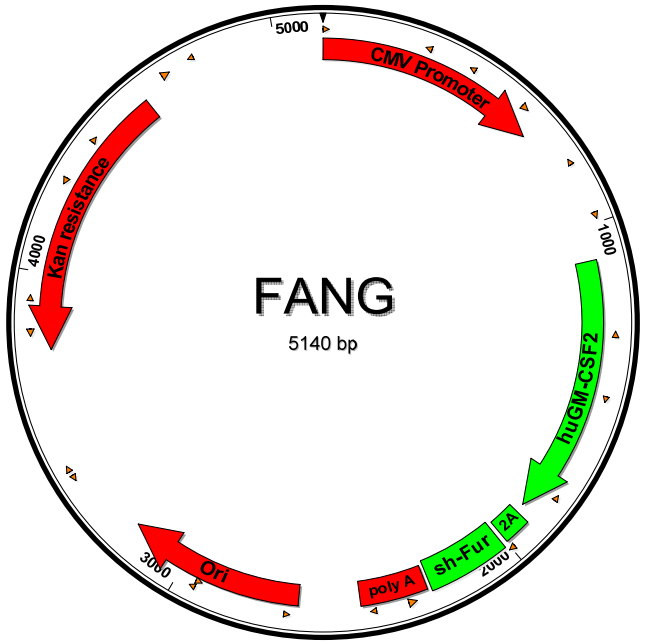
bi-shRNAi Platform
Targeting Attack Sites: 2-Front Attack




bi-shRNA
Cleavage Dependent shRNA
Cleavage Independent shRNA
Passenger strand
Guide strand
miR30a
AAA
pri-miRNA
DROSHA
DGCR8
pre-miRNA
(nuclear export)
RAN-GTP
EXPORTIN 5
RLC
Programmed RISC loading Ago
+ Ago2
+ Ago 1,[2],3,4
RISC
Complementary guide strands
+ Ago2
TRBP
+ Ago 1,[2],3,4
TRBP
ORF
3'UTR
AAA
TARGET mRNA
AAA
(mRNA degradation)
mRNA cleavage
Translational repression or p-body sequestration

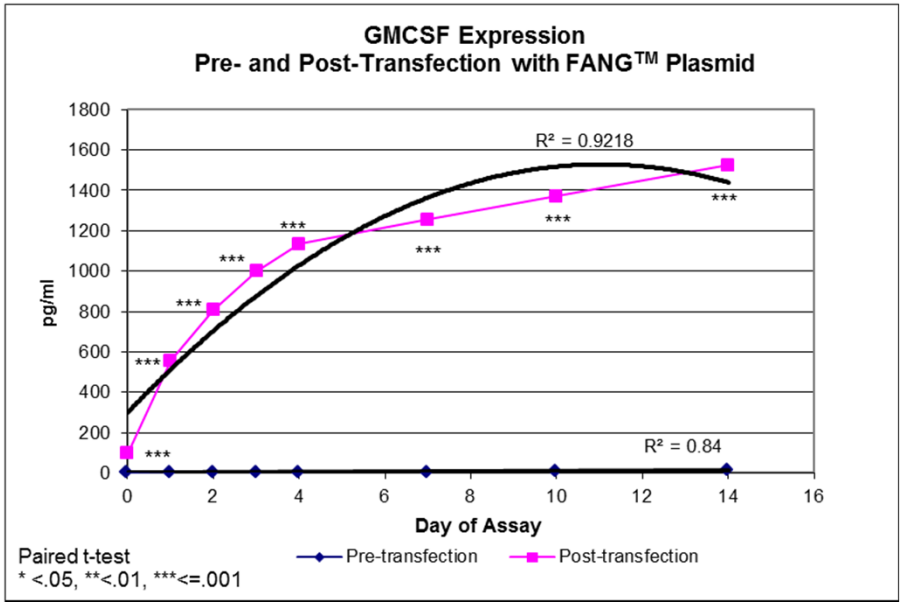
First Clinical Experience with bi-shRNAi Platform FANG™ Phase I Trial Design (BB-IND-14205)

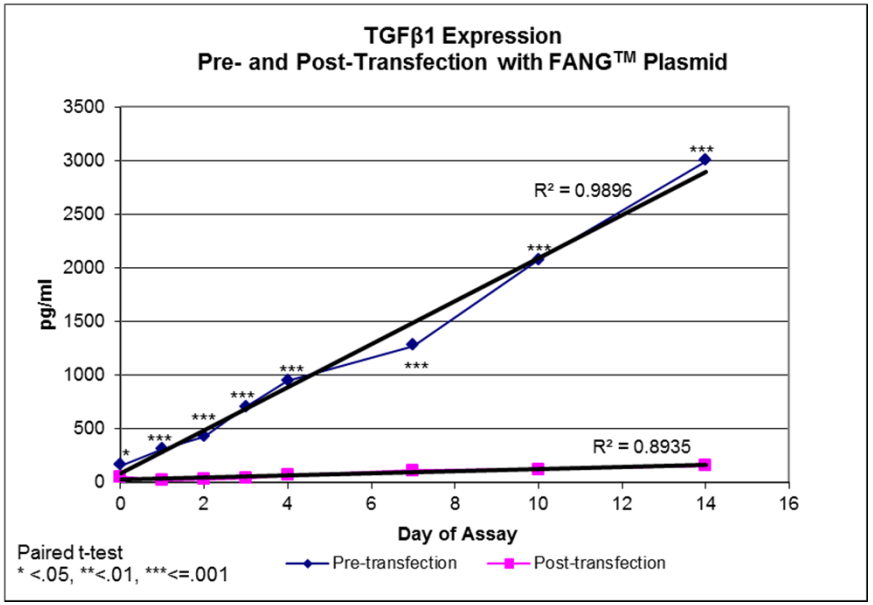


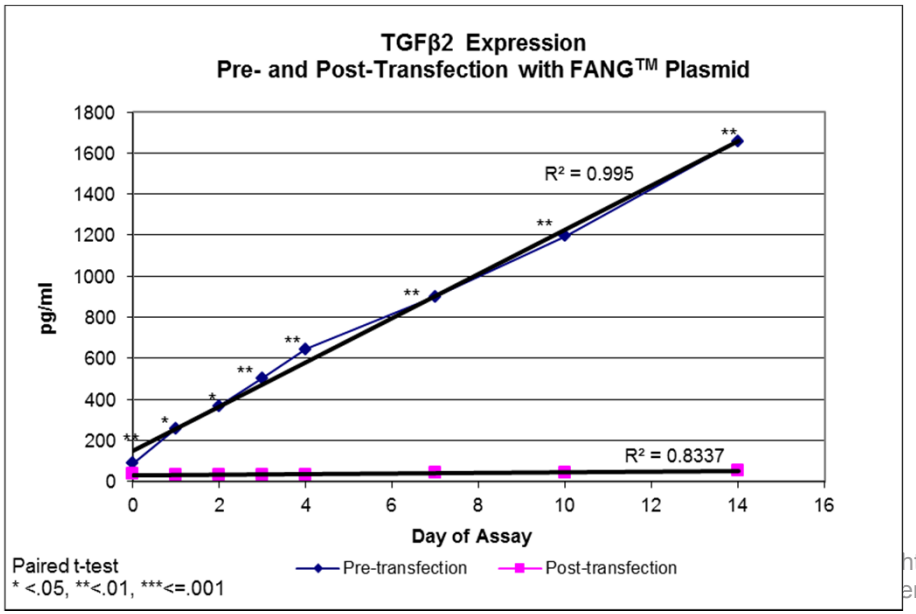


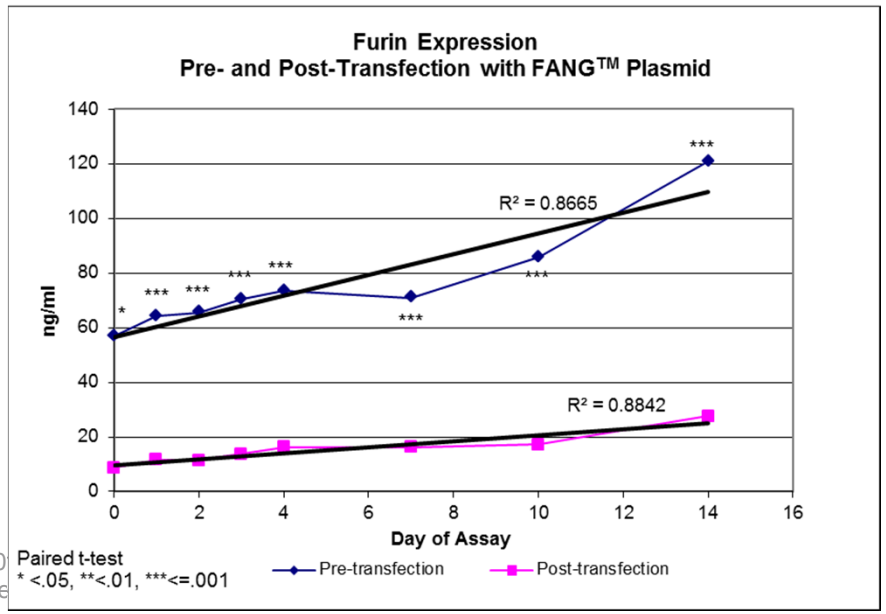
- 2 dose levels ($1x10^7$ / $2.5x10^7$ cells/inj)
- Monthly ID injection (maximum of 12 months)
- Two groups of patients: other options prior to FANG™ vs. no options → FANG™
- ELISPOT for T-cell activation at baseline and follow up time points

Target Activity of FANG Vector

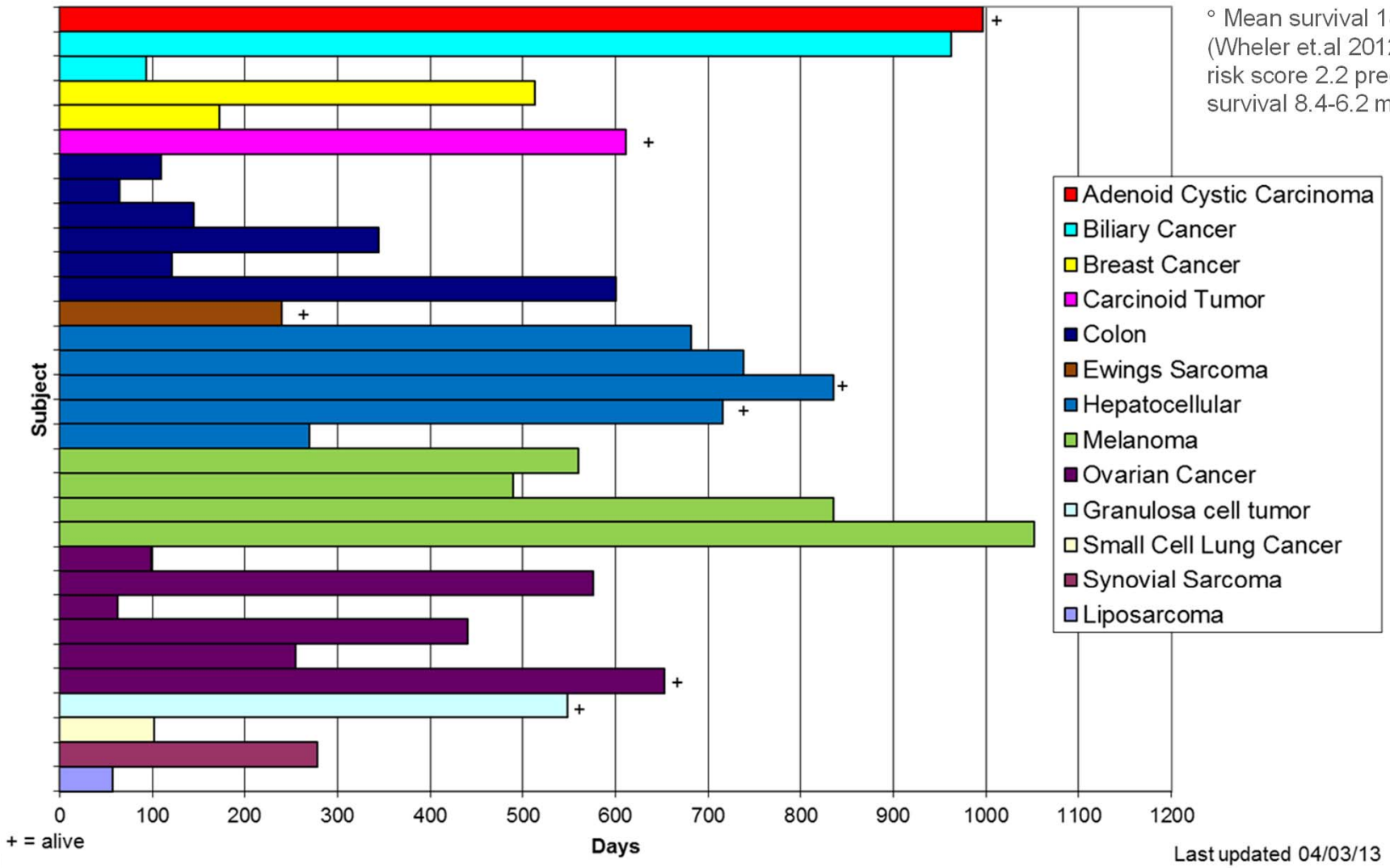






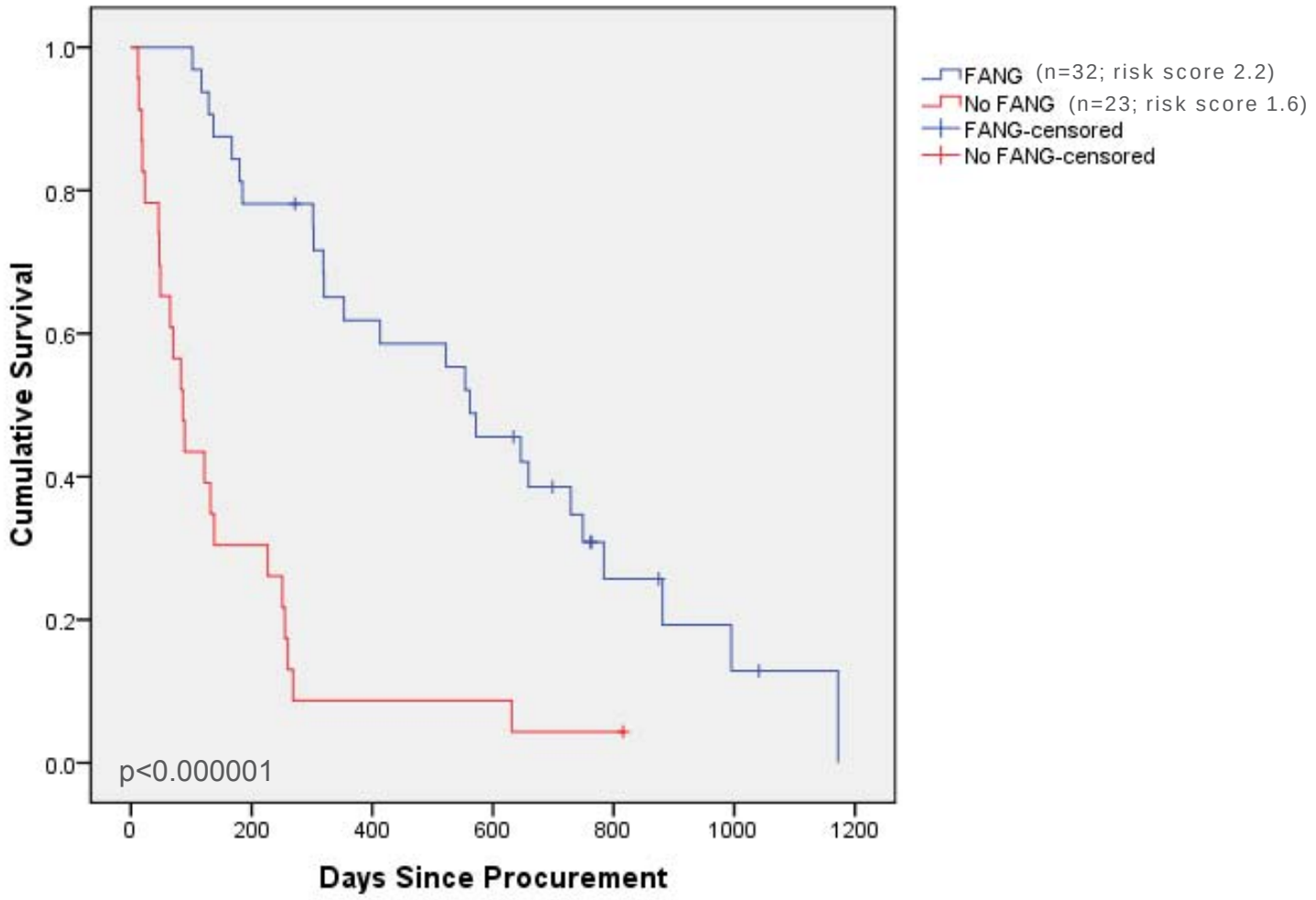


Survival of Treated Patients Since Treatment Start on FANG™ Phase I Protocol°



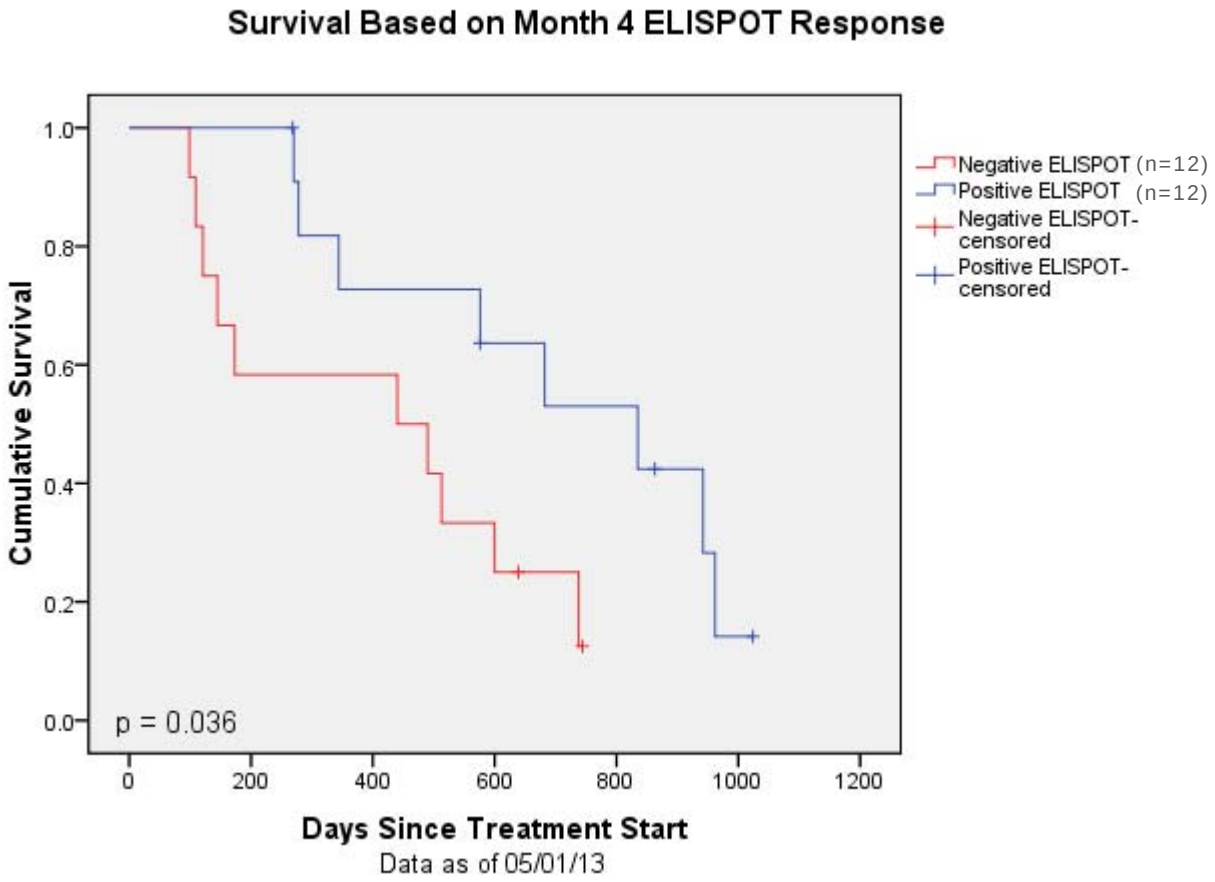
° Mean survival 18.7 months (Wheler et.al 2012) Phase I risk score 2.2 predicated survival 8.4-6.2 months.

Phase I FANG vs. No-FANG Survival

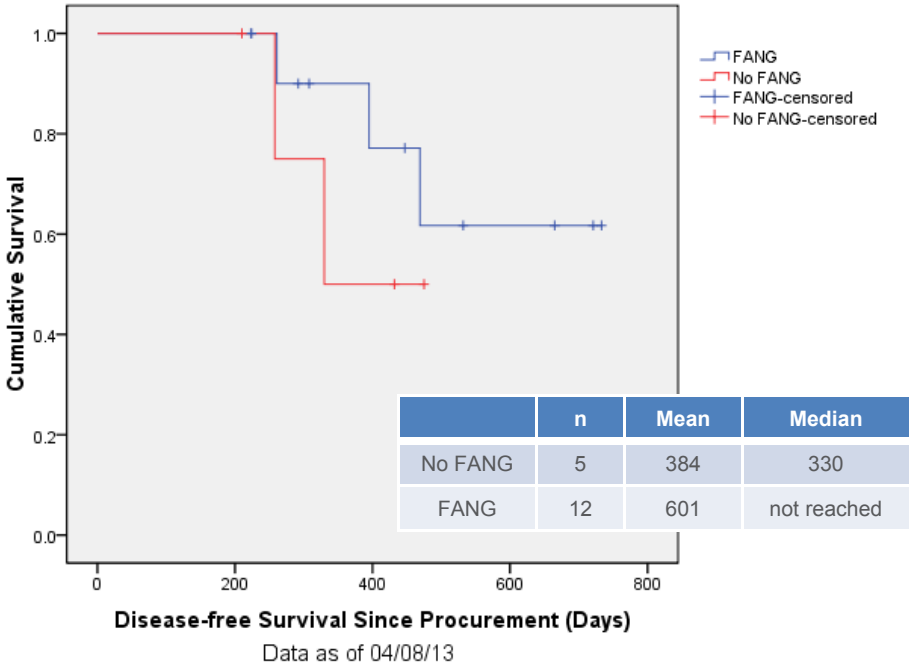


Data as of 04/26/13

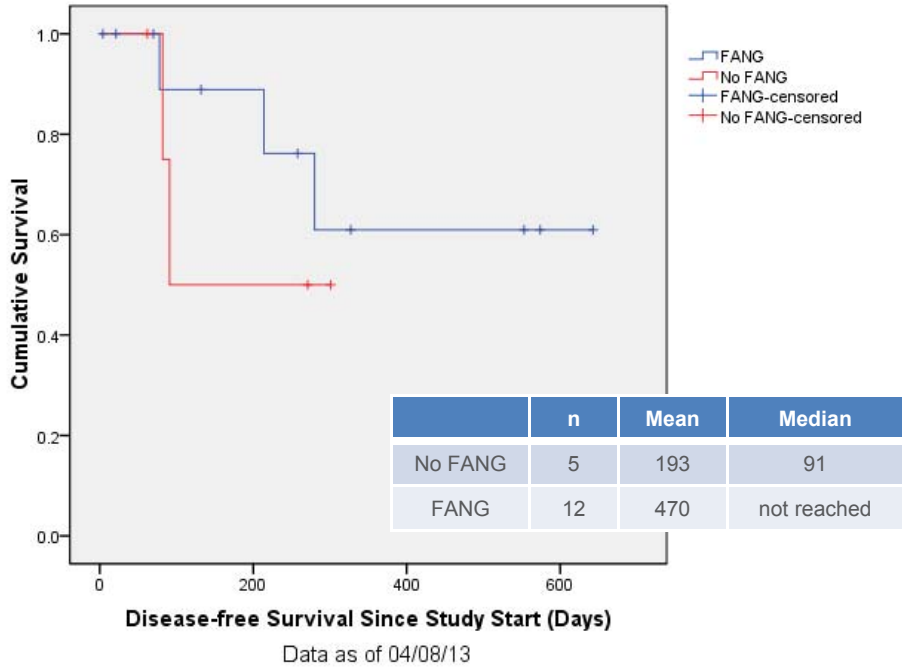
Survival Relationship to Immune Response



Preliminary Disease-Free Survival Interval Demonstrated in Phase II Trial of III/IV Ovarian Cancer

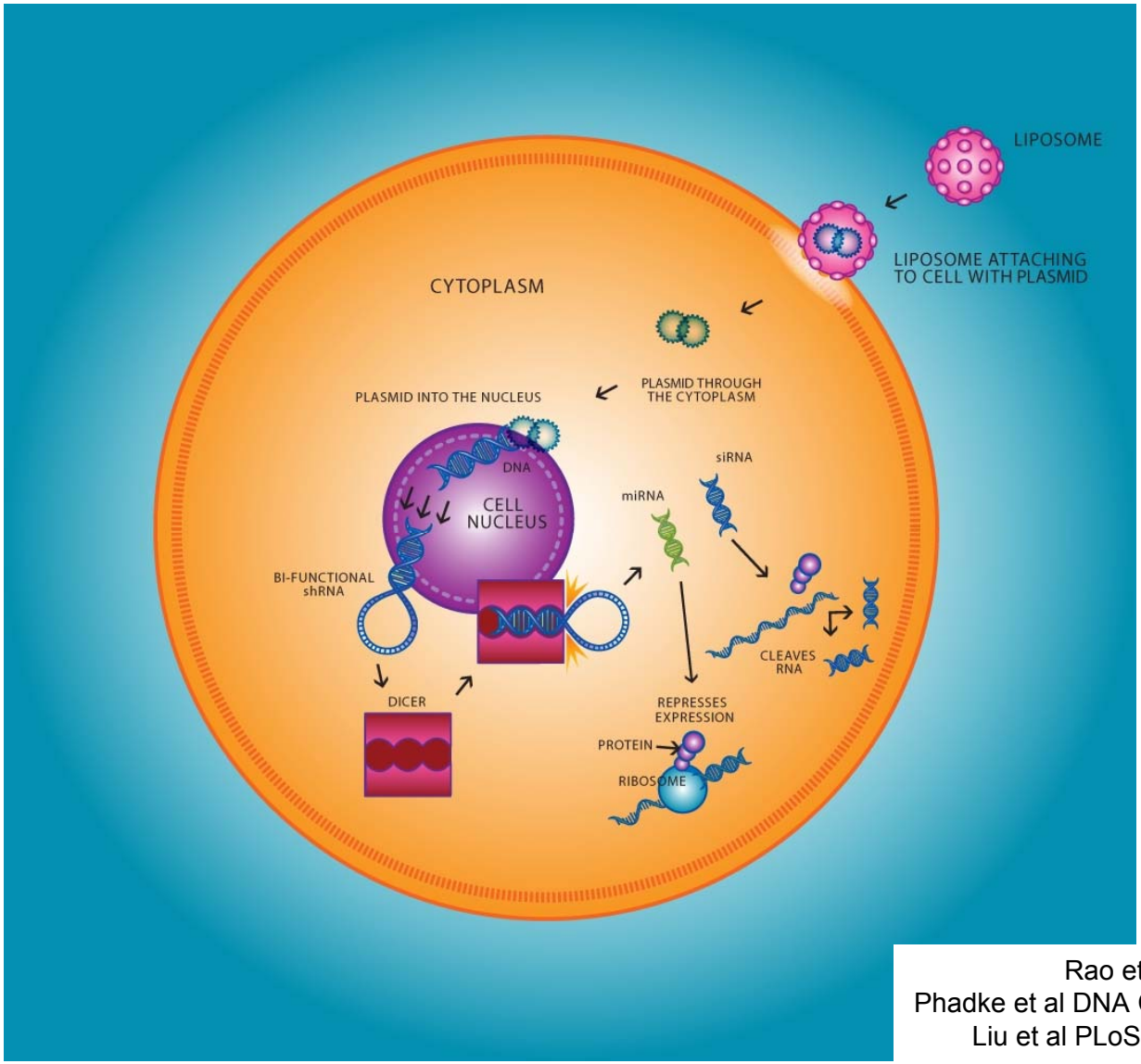


	n	Mean	Median
No FANG	5	384	330
FANG	12	601	not reached



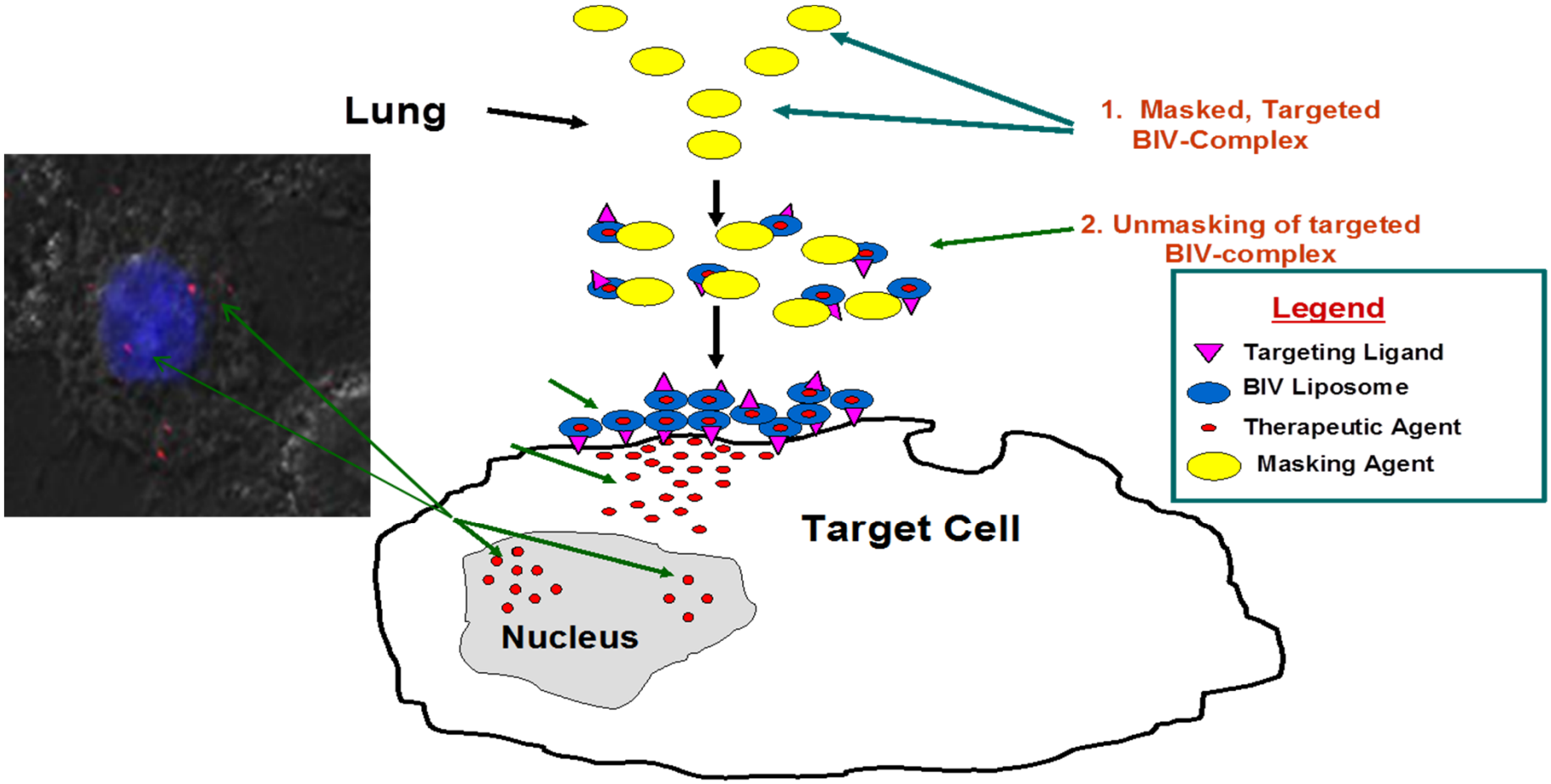
	n	Mean	Median
No FANG	5	193	91
FANG	12	470	not reached

DNA based “knockdown” attack



Rao et al CGT 17:780, 2010
Phadke et al DNA Cell Biol 30:715, 2011
Liu et al PLoS ONE 7:e40452, 2012

Bilamellar Invaginated Vesicle (BIV) Liposome with Reversible Masking and Peptidomimetic Decoration

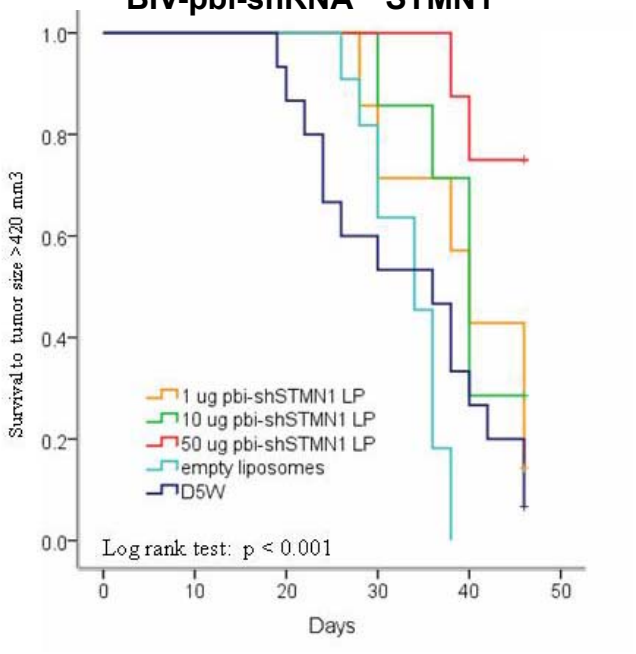


pbi-shRNA™ *in vivo* studies

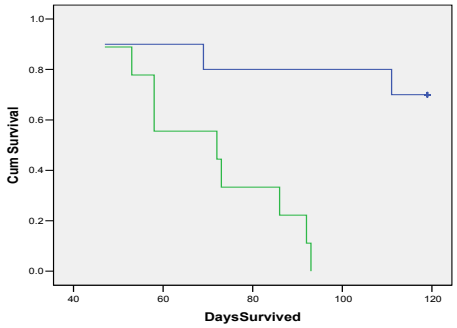
with IV delivery of BIV pbi-shRNA™ to STMN1 and PDX-1

Kaplan-Meier Estimator for Time to Progression

BIV-pbi-shRNA™STMN1

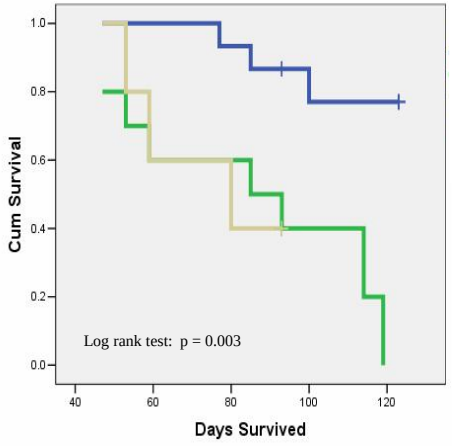


pbi-shRNA™PDX-1
TC6 cells



BIV pbi-shRNA™ mPDX-1
Empty Vector

pbi-shRNA™PDX-1
PANC1



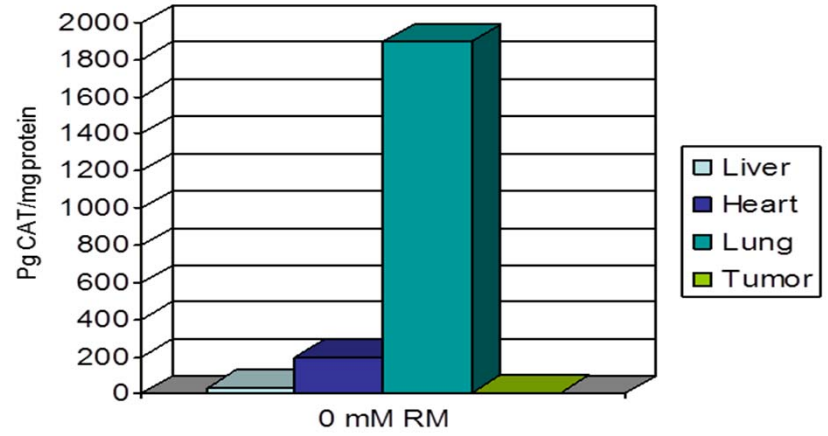
BIV-pbi-shRNA™ hPDX-1

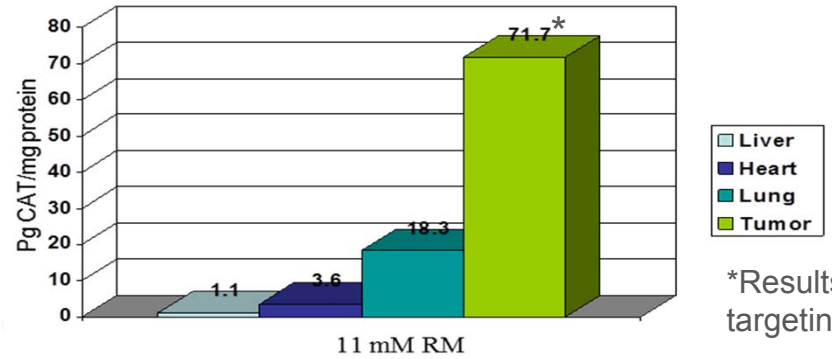
pUMVC3

Not injected

Phadke et al., DNA & Cell Biol 2011 May 25
Liu et al., Cancer 117(4):723-3, 2011.

Focused biodistribution and 200-fold increased targeted expression with in vivo targeting and reversible masking 14 h after IV injection



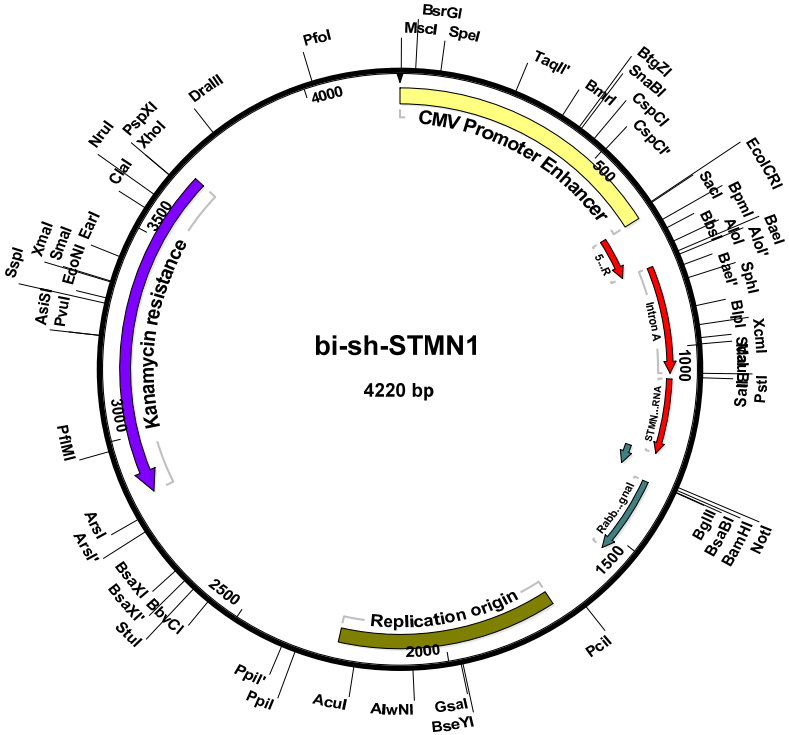


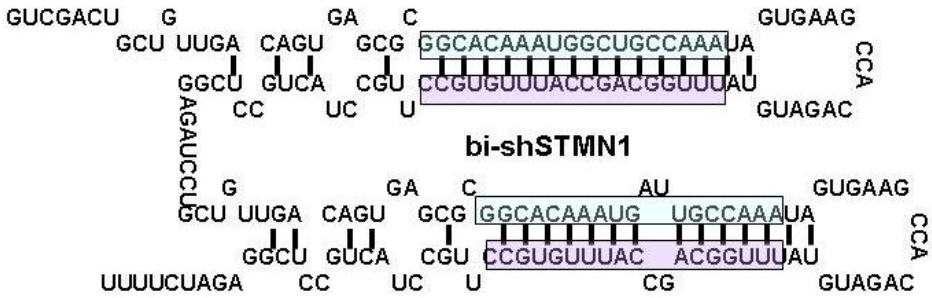
*Results represent tumor vasculature targeting that comprises ~5% tumor

Shi Q, Templeton N, et al: Gene Therapy 2010. 17(9): 1085-97 (modified)

Pre-Clinical Development

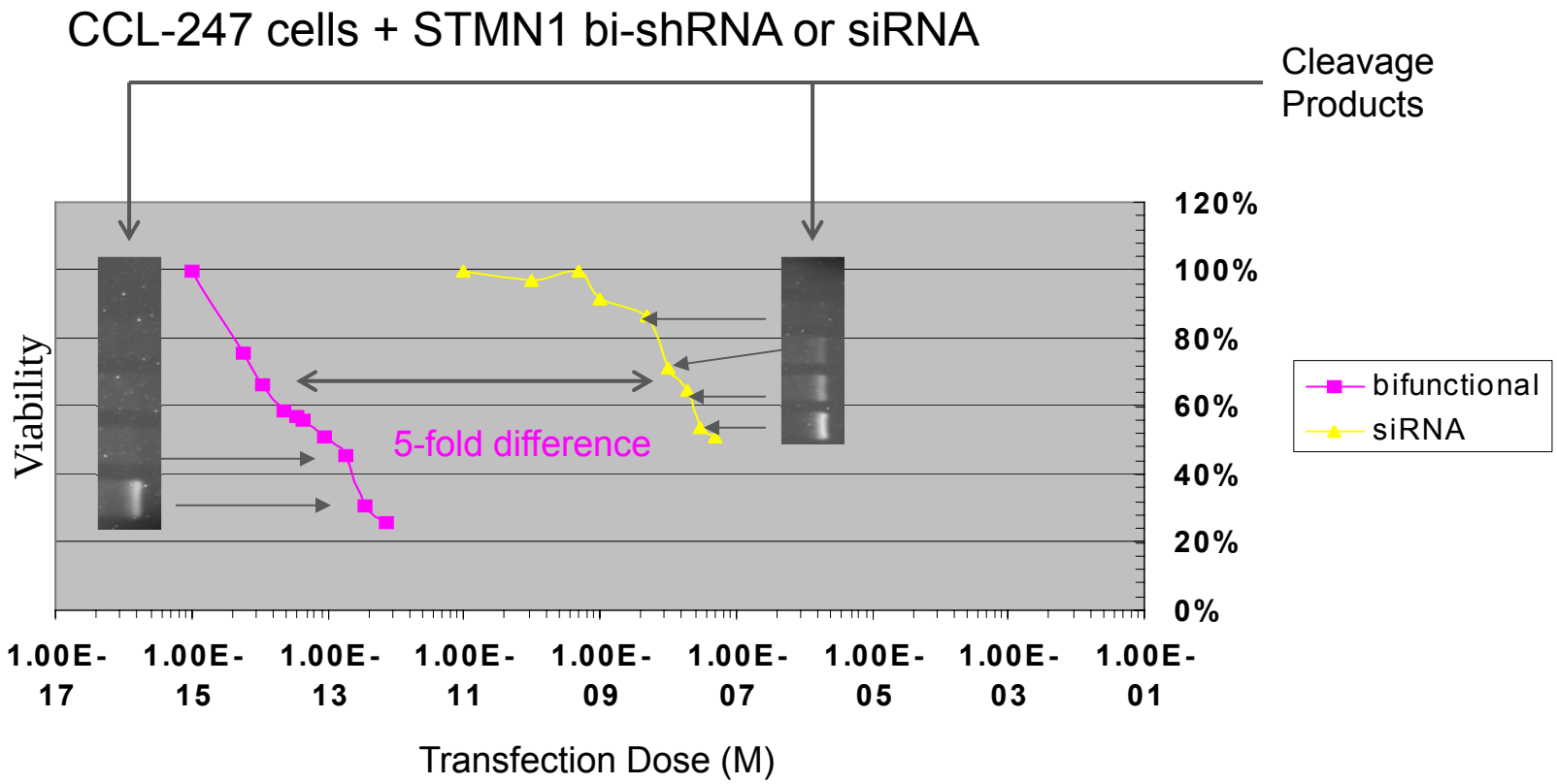
- Stathmin (STMN1) is a critical microtubule modulator highly expressed in a variety of human malignancies related to cancer survival
- Bioinfromatic analysis demonstrated STMN1 protein expansion as clinical target in melanoma (Nemunaitis et al 2007 Cancer Gene Therapy)





Pre-Clinical Development

bi-shRNA vs. siRNA to same sequence



Rao et al 2010 Cancer Gene Therapy

GMP Manufacturing (Gradalis, Inc.)

Process for making stock BIV NPs

Weigh DOTAP and Cholesterol
↓
Dissolve in Chloroform
↓
Rotory-evaporate to a thin film
↓
Rehydrate lipid film in D5W
↓
Sonicate and Extrude to create BIV NPs
↓
QC and final fill
↓
QA testing and release

Process for making stock plasmid DNA

Bacterial cell fermentation
↓
Alkaline lysis and filtration
↓
Anion exchange (AEX) chromatography
↓
Hydrophobic interaction (HIC) chromatography
↓
Tangential flow (TFF) filtration
↓
Sterile filtration and final fill
↓
QA testing and release

Process for making plasmid DNA + BIV NPs

Dilute stock BIV NPs in D5W
↓
Dilute stock plasmid in D5W
↓
Mix diluted plasmid into diluted BIV NPs
↓
QC and final fill
↓
QA testing and release

GMP Manufacturing (Gradalis, Inc.)

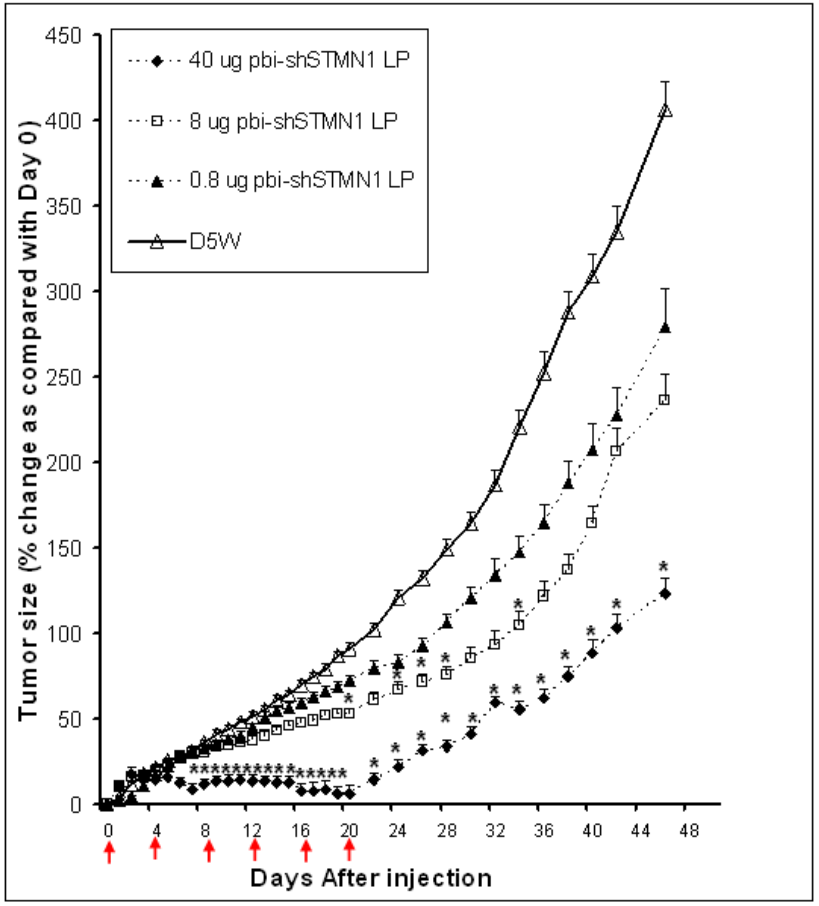
Plasmid DNA + BIV NPs Release Specifications and Characterization

Release Test	Test Method	Specification
OD400	Spectrophotometer	0.65-0.95
Particle size	ZetaSizer Nano	< 500nm
Zeta Potential	ZetaSizer Nano	> 40mV
Endotoxin	GLP Kinetic Chromogenic LAL	< 0.5 EU/ml
Sterility	21 CFR 610.12	No growth
Chloroform	Gas Chromatography	<75ppM
DNA Banding	Restriction Digest	Not I = 4220 bp Sal I = 4220 bp Not I + Sal I = 3968 & 252 bp
mRNA Expression	RT-qPCR	cT ≤ 26

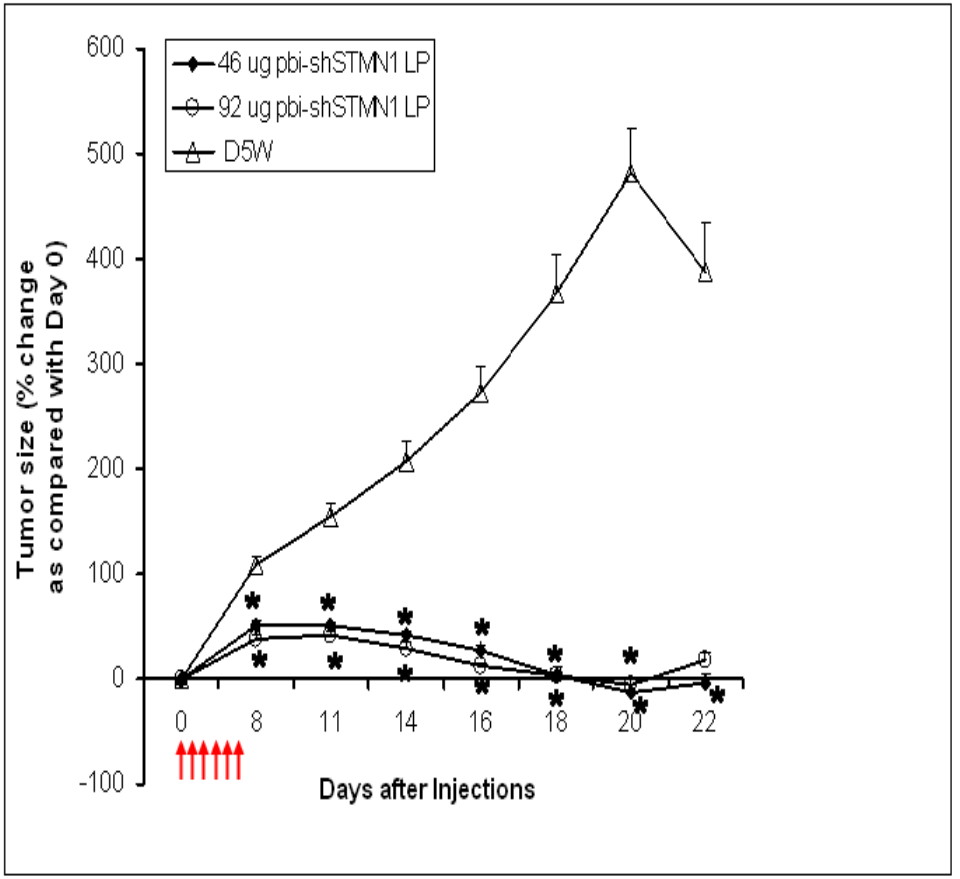
Pre-Clinical Development

pbi-shRNA™ STMN1 + BIV NPs Reduces Primary Tumorgraft Growth

melanoma



osteosarcoma



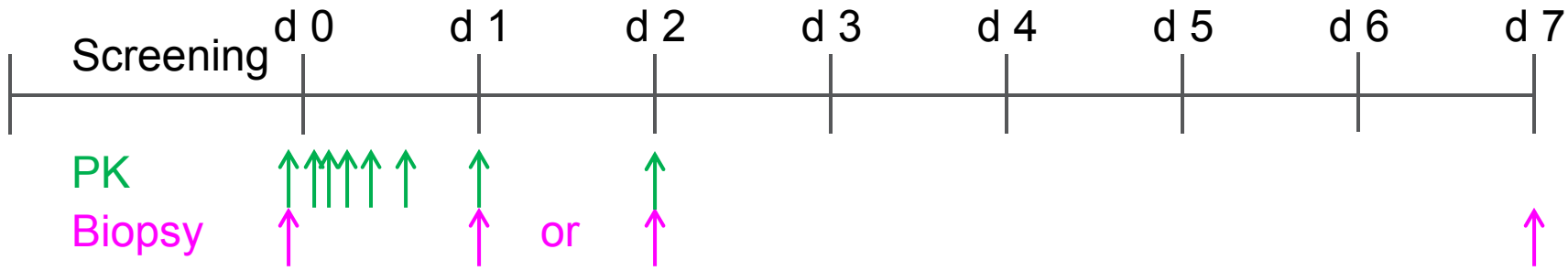
IT injections

(Phadke et al 2011 DNA and Cell Biology)

- No limitations to toxicity, pharmacokinetics in mice and biorelevant rats

pbi-shRNA STMN1 Phase I Clinical Trial (BB-IND- 14938)

Study Design



Cohort	Number of Patients	Dose (mg DNA / injection)	Dose (mg / kg / 70kg)
1	4	0.7	0.010
2	4	1.4	0.020
3	4	2.0	0.028
4	4	2.7	0.038
5	4	3.7	0.053

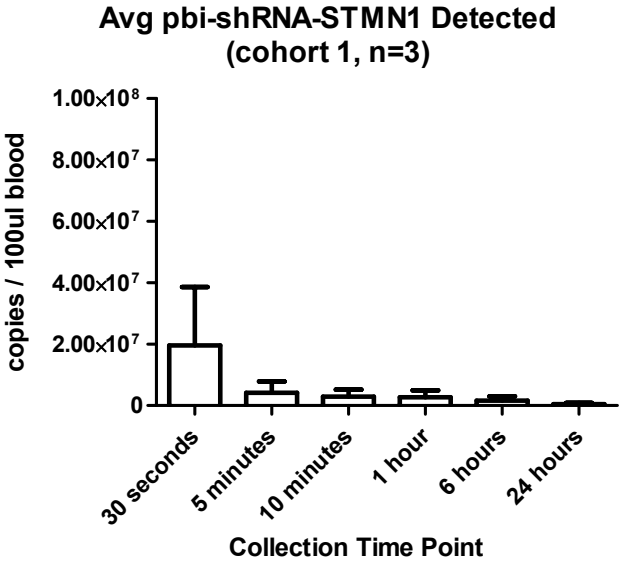
- Single IT injection, 4 patients / cohort
- Premeds – Dexamethasone, Indocin, and Acetaminophen (BB-IND 10718, 12233, 13744)
- Whole blood PK at baseline; 30 sec; 5 & 10 min; 1, 6, 24, and 48 hr
- Tumor biopsy at baseline; 24 or 48 hr; day 7

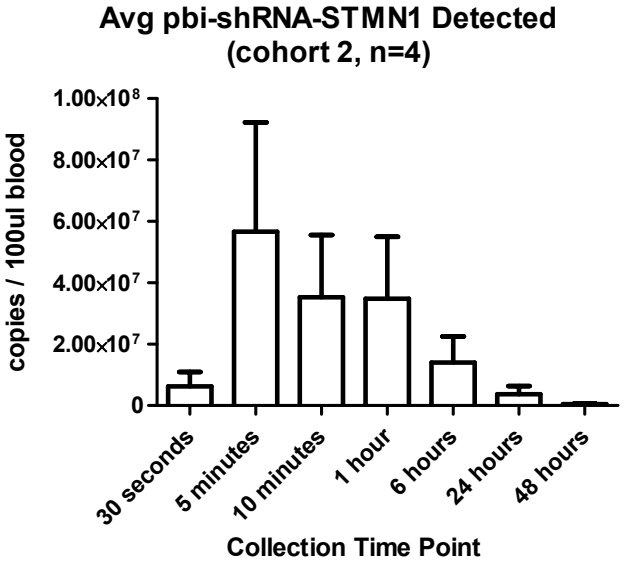
Gradalis STMN1 Phase I Clinical Trial
Patient Characteristics

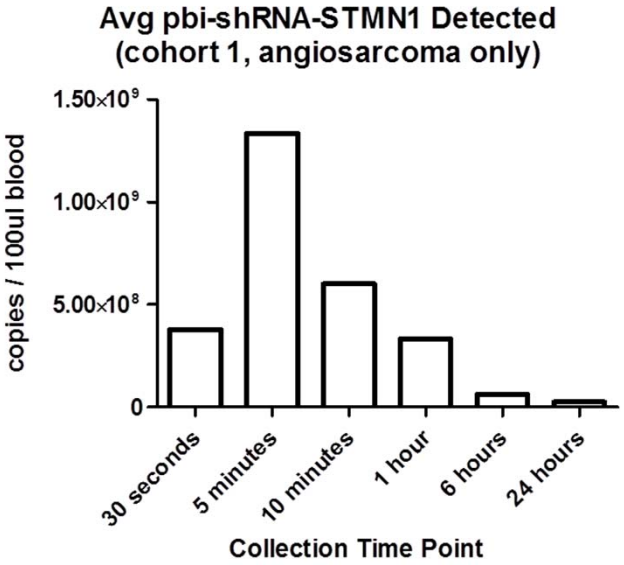
Cohort #	Patient #	Age / Sex	Cancer	Site	Dose (mg DNA)
1	1002	80 / F	Angiosarcoma	Arm	0.7
	1004	60 / F	Anal	Labia	0.7
	1005	70 / M	Colorectal	Chest	0.7
	1006	63 / F	Ovarian mets	Axillary Lymph Node	0.7
2	1007	59 / M	Melanoma	Right Axilla	1.4
	1008	49 / F	Breast	Chest	1.4
	1009	70 / M	Colorectal	Abdomen	1.4
	1010	50 / F	Breast	Chest	1.4

Gradalis STMN1 Phase I Clinical Trial

Whole blood PK Sample Analysis - Results°







ASGCT 2013 Abstract # 510

- ° No toxic efficient was observed in the 8 patients up to 30 days of observation
- ° Cleavage product demonstrated in cohorts 1 and 2 (7/7 patients, NGS) ------- ASGCT 2013 Abstract # 234

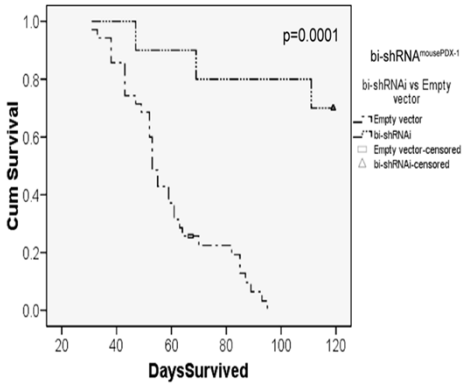
Phase I bi-shRNAi STMN1 Nanoplex Results

- Suggest single dose safety
- Demonstrate circulating plasmid
- Demonstrate cleavage product
- Justify IV administration assessment (based on post IT injection achieved plasma concentrations)

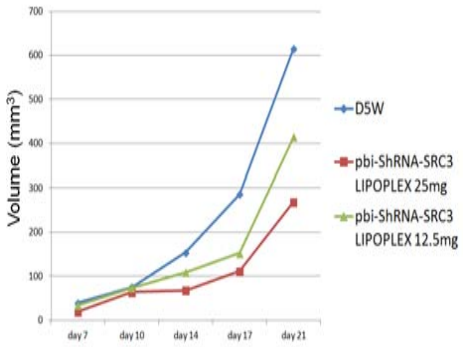
Preclinical assessment of the bi-shRNAi platform with several other targets

PDX-1	C. Brunicardi	UCLA	(ASGCT 2013 Abstract # 272, 659)
SRC-3	B. O'Malley	Baylor College	(ASGCT 2013 Abstract # 459)
AR	N. Weigel	Baylor College	
KRAS Multiplex	D. Rao	Gradalis	(ASGCT 2013 Abstract # 317)

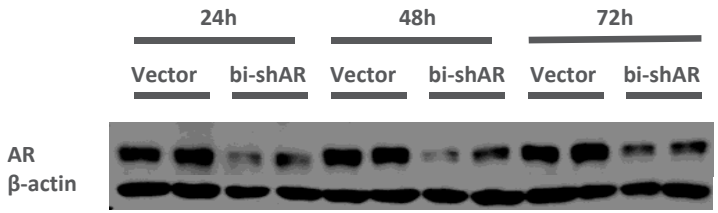
Beta TC-6 SCID survival in bi-shRNA PDX-1 BIV therapy



Bifunctional shRNA SRC3 BIV reduces tumor volume in MDA-MB-231 model

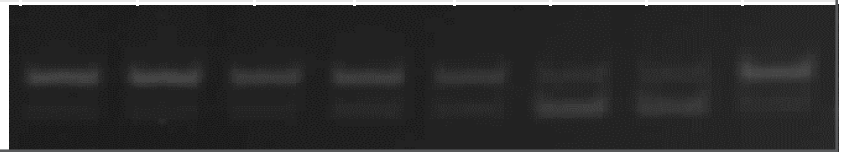


RNA Expression Real Time RT-PCR



Triple Constructs Are Very Effective in Mutant Knockdown Without Affecting wt Expression (Restriction Enzyme Digest)

	PANC1	Empty vector	G12D	Triple DVR	Triple CDV	Triple DVR	Triple CDV	G12V
Mutant	80%	84%	82%	70%	63%	9%	12%	83%
wt	20%	16%	18%	30%	37%	91%	88%	17%



Conclusion

- bi-shRNAi platform technology demonstrates clinically relevant target expression knockdown activity
- Further preclinical and clinical testing is underway