Tekmira

PROGRESS IN THE DEVELOPMENT OF LIPID NANOPARTICLE-RNA THERAPEUTICS

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Outline

- LNP Technology and RNA Drugs
- TKM-PLK1 Clinical Trials
- Pan-Marburg siRNA
- TKM-Ebola Phase 1 Results

Tekmira's Lipid Nanoparticle Platform

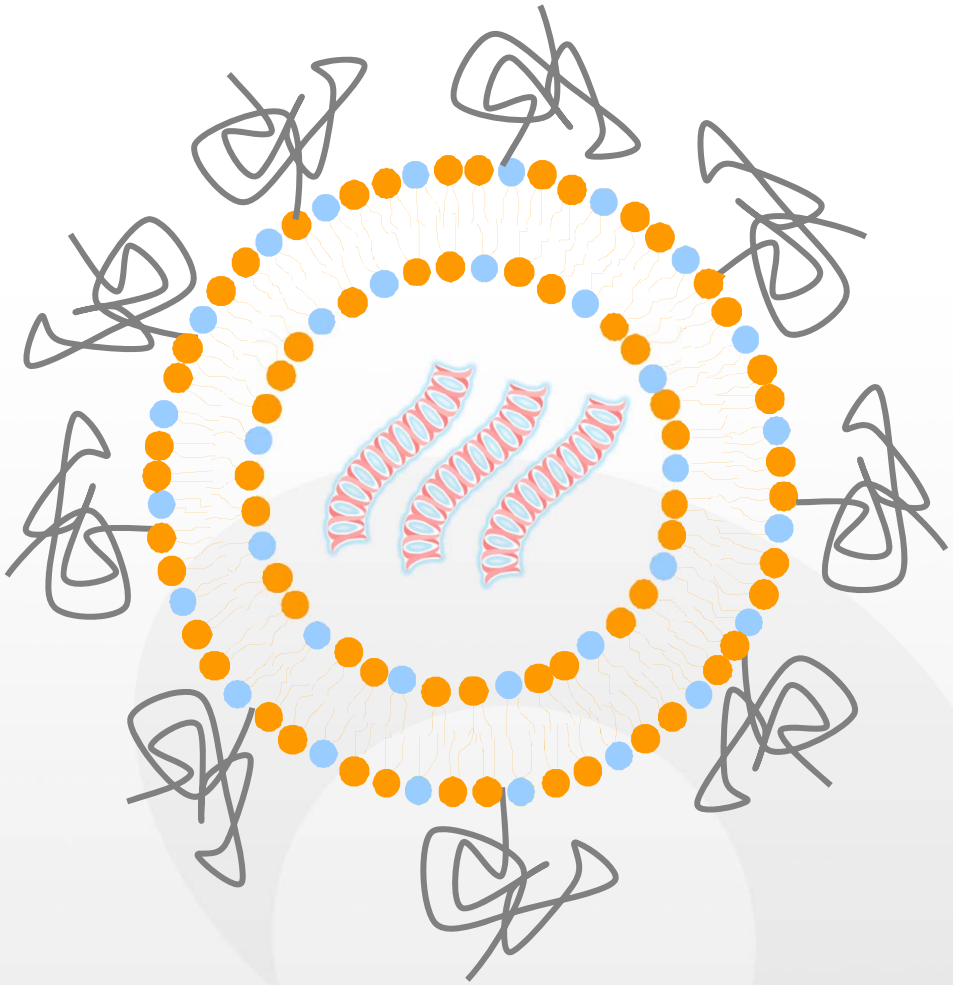
Components:

Amino Lipid

Structural Lipid

PEG - Lipid

Nucleic Acid

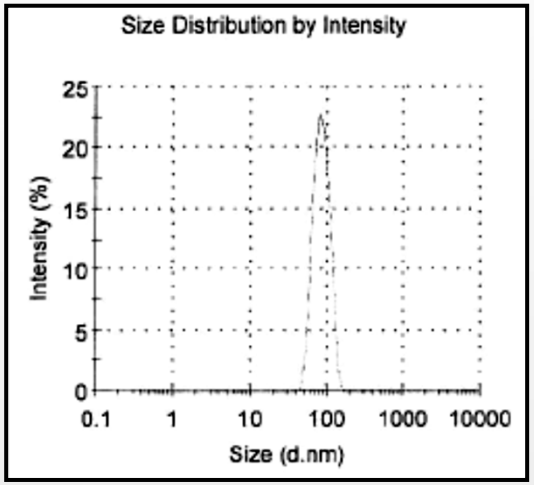


40-140 nm diameter

Jeffs et al. Pharm. Res. 2005.

cGMP Manufacturing at Tekmira

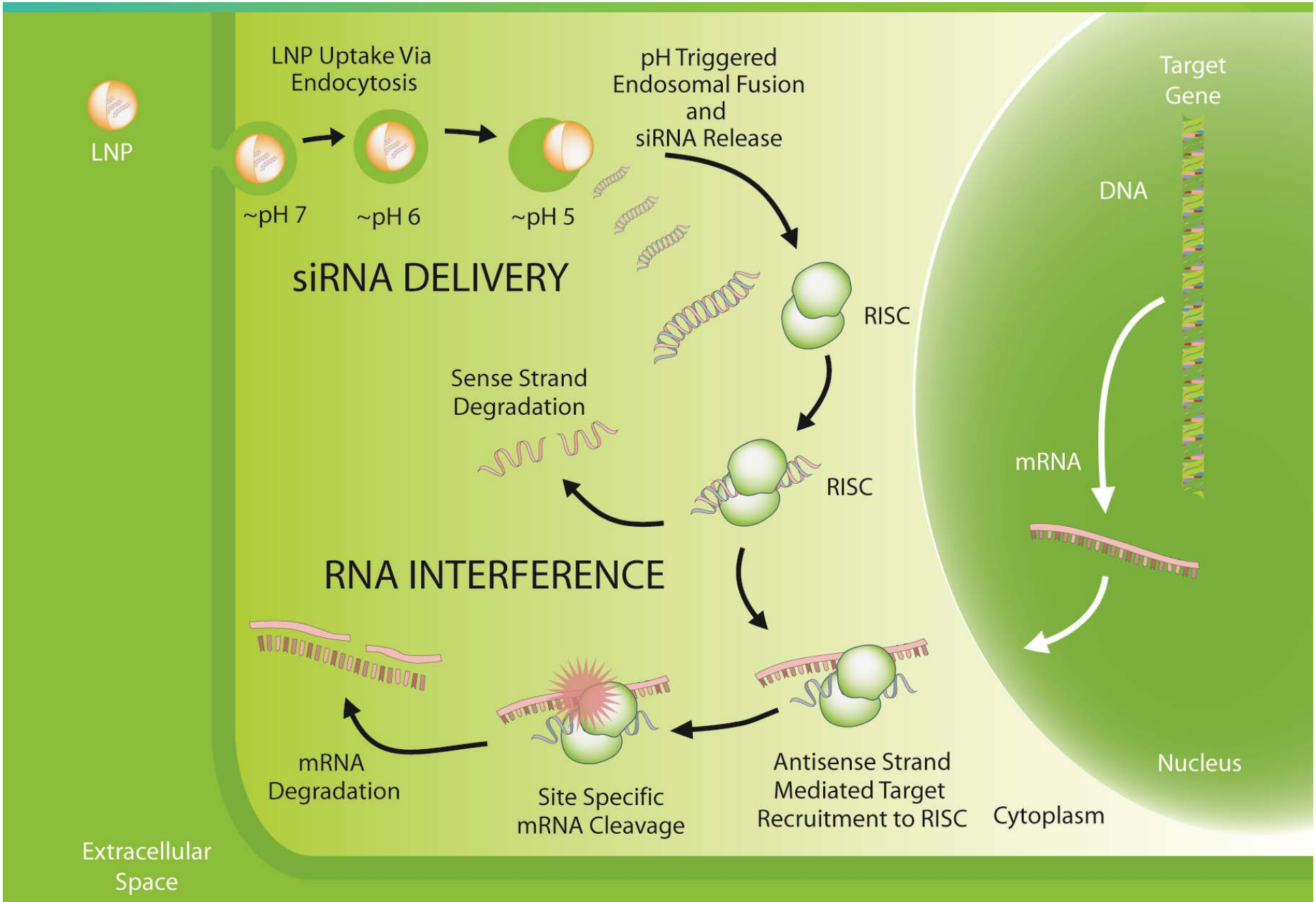
- Controlled self assembly
- Highly scalable
- Efficient
- Consistent particle size
- cGMP



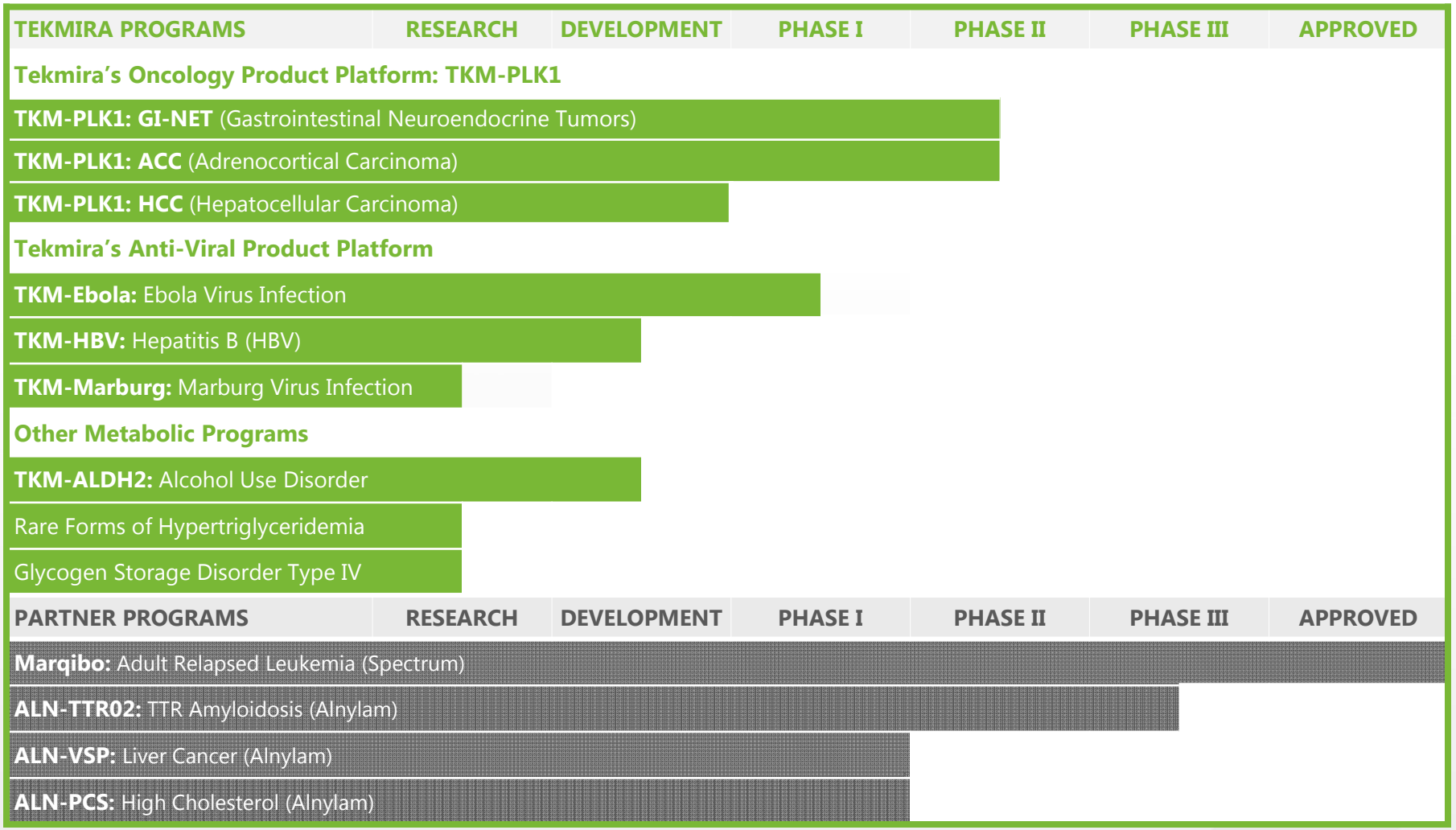
Particle Formation Skid in Tekmira cGMP Facility
(suitable for 100g+ batches)

Stability of LNP Products

- Tekmira's LNP products can be provided in wet, "ready-to-use" format, stable for 2 years at 5°C, or in lyophilized form.
- Lyophilized LNP are stable at 40°C, no cold chain required.


LNP
LNP Uptake Via Endocytosis
~pH 7
~pH 6
~pH 5
pH Triggered Endosomal Fusion and siRNA Release
siRNA DELIVERY
RISC
Sense Strand Degradation
RISC
RNA INTERFERENCE
mRNA Degradation
Site Specific mRNA Cleavage
Antisense Strand Mediated Target Recruitment to RISC
Target Gene
DNA
mRNA
Nucleus
Cytoplasm
Extracellular Space

Tekmira's Product Pipeline



Lipid Nanoparticles (LNP) – Experience in Man

Seven LNP products have entered clinical development

Representative Ongoing Clinical Activity				
Product	Company	Phase	Indication	Comments
ALN-TTR02	Alnylam	3	Amyloidosis	Potent pharmacodynamic effect demonstrated, well tolerated
TKM-Ebola	Tekmira	1	Ebola Infection	New improved lyophilized formulation
TKM-PLK1	Tekmira	2	Oncology	Promising signs of RNAi drug activity

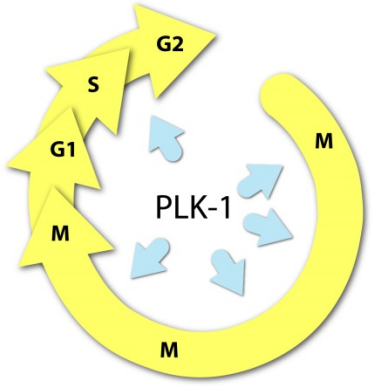
- > 200 patients treated w/ LNP-siRNA, some for >1 year
- Potent, long lasting effects after a single dose
- Growing body of clinical safety data
- Formulation improvements translate from the lab to the clinic

LNP enabled RNAi drugs are a validated therapeutic modality, capable of delivering ***clinically and commercially viable products***

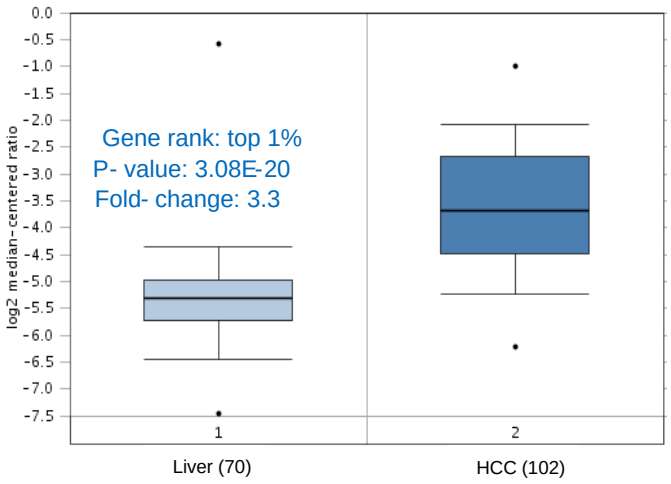
TKM-PLK1: Tekmira's Lead Oncology Therapeutic

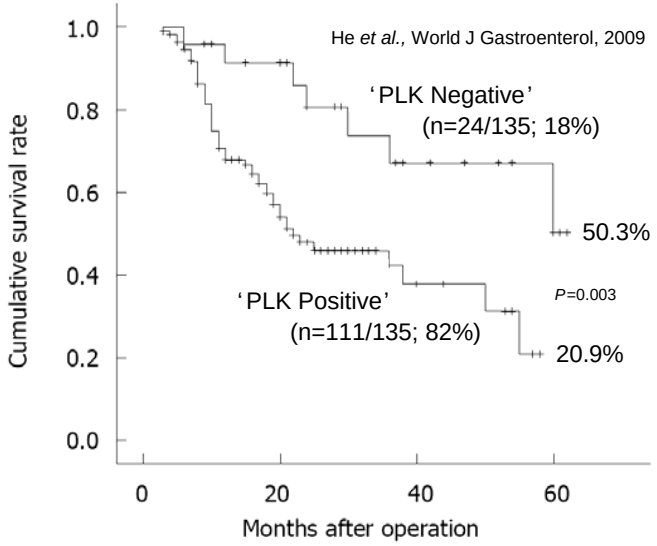
Targeting an Essential Cell Cycle Kinase

- PLK1 is a validated oncology target; a key regulator of mitosis
  - Many tumor types have increased PLK1 levels predicting poor clinical outcome
  - TKM-PLK1 is selective for PLK1 with no cross-reactivity to PLK family members or other kinases
  - TKM-PLK1 avoids bone marrow – no hematotoxicity



A KEY KINASE IN CELL DIVISION

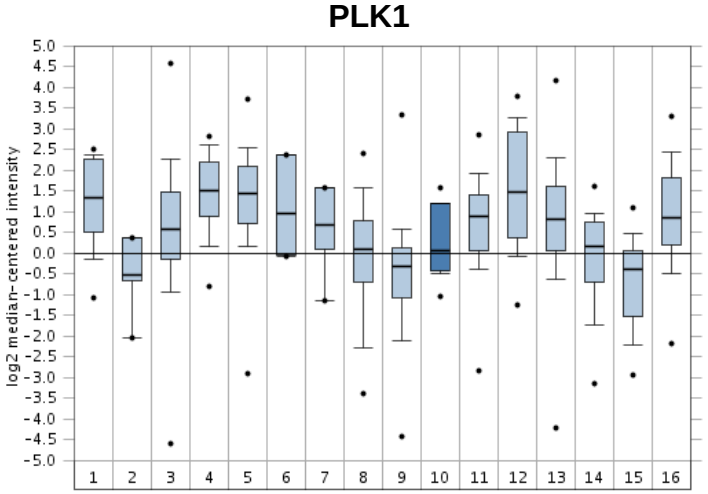


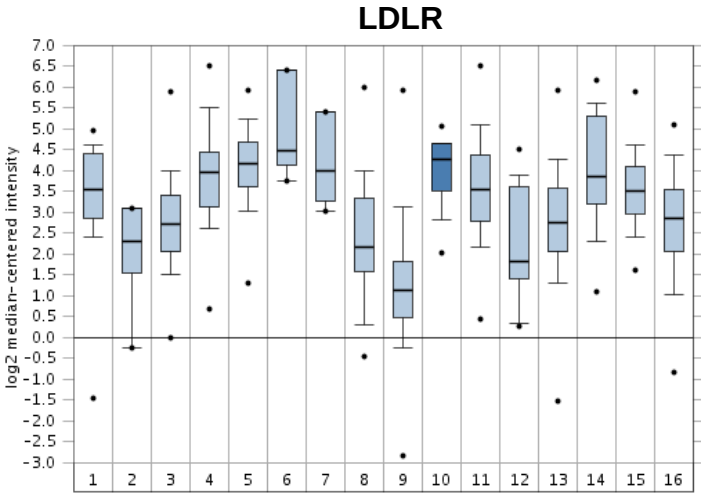


PLK1 Expression and Outcome in Hepatocellular Carcinoma

TKM-PLK1 Targets Tumor Cells

- TKM-PLK1 LNP designed for oncology applications
  - Longer plasma half life; delivery beyond the liver
  - LNP cellular uptake by autogenous targeting via LDL receptor family
- Many cancer cells overexpress LDL family receptors





Legend

1. Bladder Cancer (32)
2. Brain and CNS Cancer (4)
3. Breast Cancer (328)
4. Cervical Cancer (35)
5. Colorectal Cancer (330)
6. Esophageal Cancer (7)
7. Gastric Cancer (7)
8. Head and Neck Cancer (41)
9. Kidney Cancer (254)
10. Liver Cancer (11)
11. Lung Cancer (107)
12. Lymphoma (19)
13. Ovarian Cancer (166)
14. Pancreatic Cancer (19)
15. Prostate Cancer (59)
16. Sarcoma (49)

Cancers with higher relative PLK1 expression tend to also have increased LDLR expression

Development Plans for Oncology Platform

Development Rationale:

- ✓ **PLK1 expression levels correlate with poor outcomes**
- ✓ **Clinical benefit seen in Phase I**
- ✓ **Areas of high unmet need**
- ✓ **Accelerated regulatory opportunity**

GI-NET
(Gastrointestinal Neuroendocrine Tumors)

- 55,000 individuals in the U.S. have GI-NET
- Poor prognosis for advanced metastatic NETs; 25% of patients survive less than one year
- No approved drugs specifically for GI-NET

ACC
(Adrenocortical Carcinoma)

- Rare cancer that forms in the adrenal gland
- Poor prognosis and large % of patients not good surgical candidates
- Lack of effective systemic therapies

HCC
(Hepatocellular Carcinoma)

- One of the most common cancers worldwide; ~630,000 diagnosed each year
- Major unmet need – 19,000 deaths each year
- Only 10% five-year survival rate after liver resection

Phase I/II in GI-NET/ACC

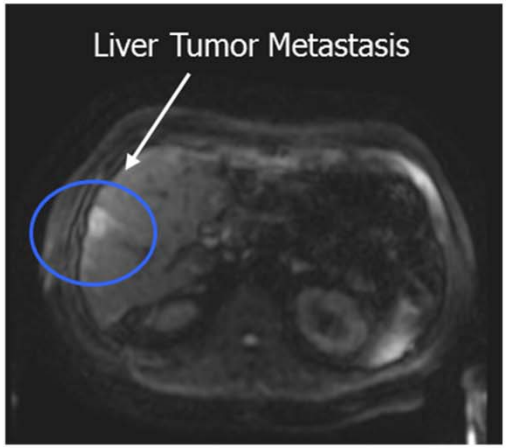
- Open-label
- 3 weekly doses/month
- 20 patients, single arm
- Multi-site
- Tumor response endpoints
- **Currently enrolling**
- **Initial data in 2H:2014**

- **Expect to initiate Phase I /II in HCC in 1H:2014**
- Safety & tolerability, and MTD endpoints
- Exploratory tumor response endpoints

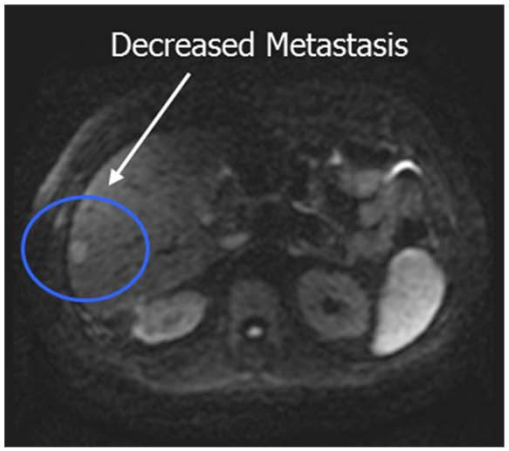
TKM-PLK1: Clinical Benefit in GI-NET Patients

64 year old male GI-NET patient:

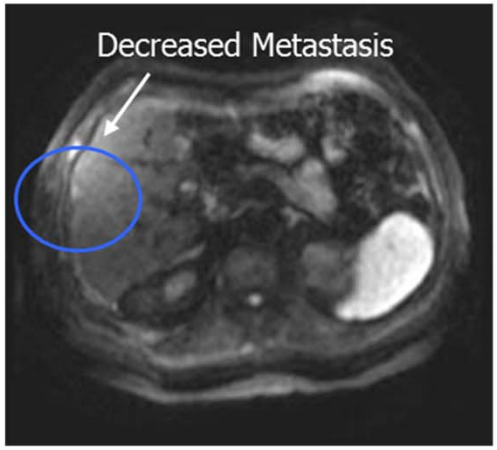
- Partial response observed ~2 months post-treatment initiation
- Patient continued to have a PR after Cycles 4, 6, 8, and 10



Baseline MRI 2/16/12
Pre-Treatment



Follow Up MRI 3/16/12
Post-Treatment, End of Cycle 1



Follow Up MRI 4/30/12
Post-Treatment, End of Cycle 2

72 year old female GI-NET patient:

- Treated for 4 cycles
- Achieved stable disease
- >50% decline in Chromogranin A levels (biomarker for NETs)

Time point	Chromogranin A	Change
Pre-dose	3350 ng/mL	
15 days post-last dose	1321 ng/mL	↓**60.6%**
78 days post-last dose	1525 ng/mL	↓**54.5%**
114 days post-last dose	1390 ng/mL	↓**58.5%**

TKM-PLK1 Phase I/II Experience in ACC (May 13 '14)

Subject	Dose (mg/kg/wk)	Best Response	Duration of treatment
002-25	0.75	SD	6 full cycles (~6 mths) [DC'd due to dental abscess]
002-35	0.75/0.6	SD	2 full cycles (~2 mths) [DC'd to pursue other Rx]
002-37	0.75	PD	1 full cycle (16 days) [DC'd due to intractable pain from disease]
002-39	0.75	PR	12 full cycles - *active subject*

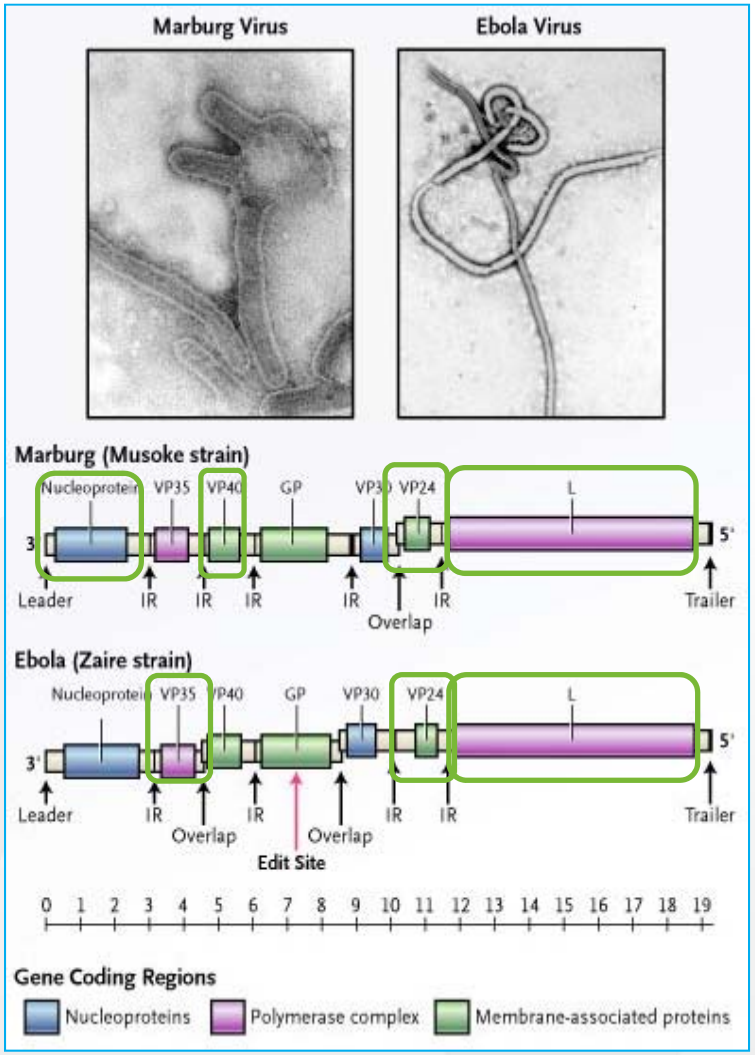
PR = Partial Response (≥ 30% decrease in lesions); SD = Stable Disease; PD = Progressive Disease; 1 cycle = ~1 month

3 (of 4 evaluated for efficacy) have shown evidence of clinical benefit.

- **PR: 1/3** – Post Cycle 8 and 10 scans in this active pt showed RESIST PR with evidence of necrosis. Target tumor with overall decrease of 42%.
- **SD: 2/3** – One pt treated for 6 cycles, the other treated for 2 cycles.
- 1 of 4 subjects was hospitalized due to intractable pain related to disease and thus discontinued after only one cycle.

Marburg and Ebola Viruses

- MARV and EBOV are the two members of the virus family Filoviridae
- Enveloped and contain a single negative-sense RNA genome encompassing ~ 19 kb
- Genome encodes for 7 proteins
- High virulence achieved through host immune response modulation
- Angola strain responsible for largest and deadliest MARV outbreak (~90% mortality)
- Zaire strain responsible for largest and deadliest EBOV outbreaks (~90% mortality)



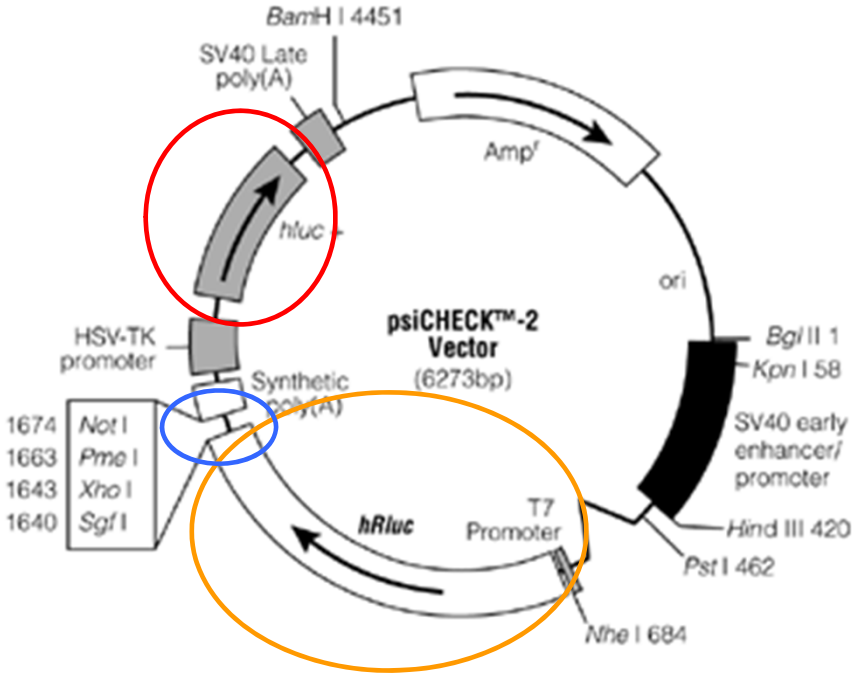
N Engl J Med 352;25 June 23, 2005

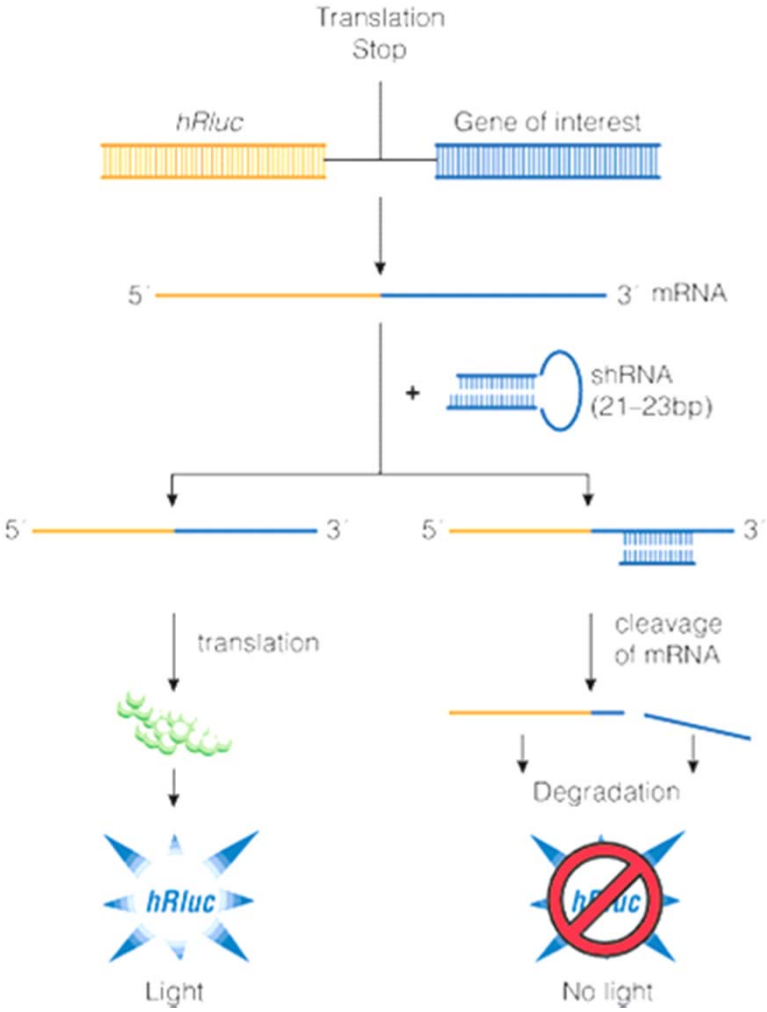
LNP Uptake and Activity in Key Cells Involved in HFV Infection

Cell Type	EBOV Infection	LNP Activity	Supporting Data
Hepatocytes	Yes	**Yes**	Rodent, NHP – PKBD, histology, RACE, QG
Macrophage	Yes	**Yes**	Human, Rodent – flow cytometry, RACE, QG
Monocytes	Yes	**Yes**	Human, Rodent – flow cytometry (CD14+ and CD14/CD16 subpopulations)
Dendritic Cells	Yes	**Yes**	Human, Rodent – flow cytometry
Sinusoidal Cells	Yes	**Yes**	Rodent – histology (Kupffer cells)
Adrenalcortical Cells	Yes	**Yes**	Rodent, NHP - PKBD, histology, RACE, QG (only whole adrenal gland analyzed)
Endothelial Cells	Yes	**+/-**	Rodent – histology

Development of Anti-HFV siRNA

Virus Free psiCHECK-2 Plasmid Based System



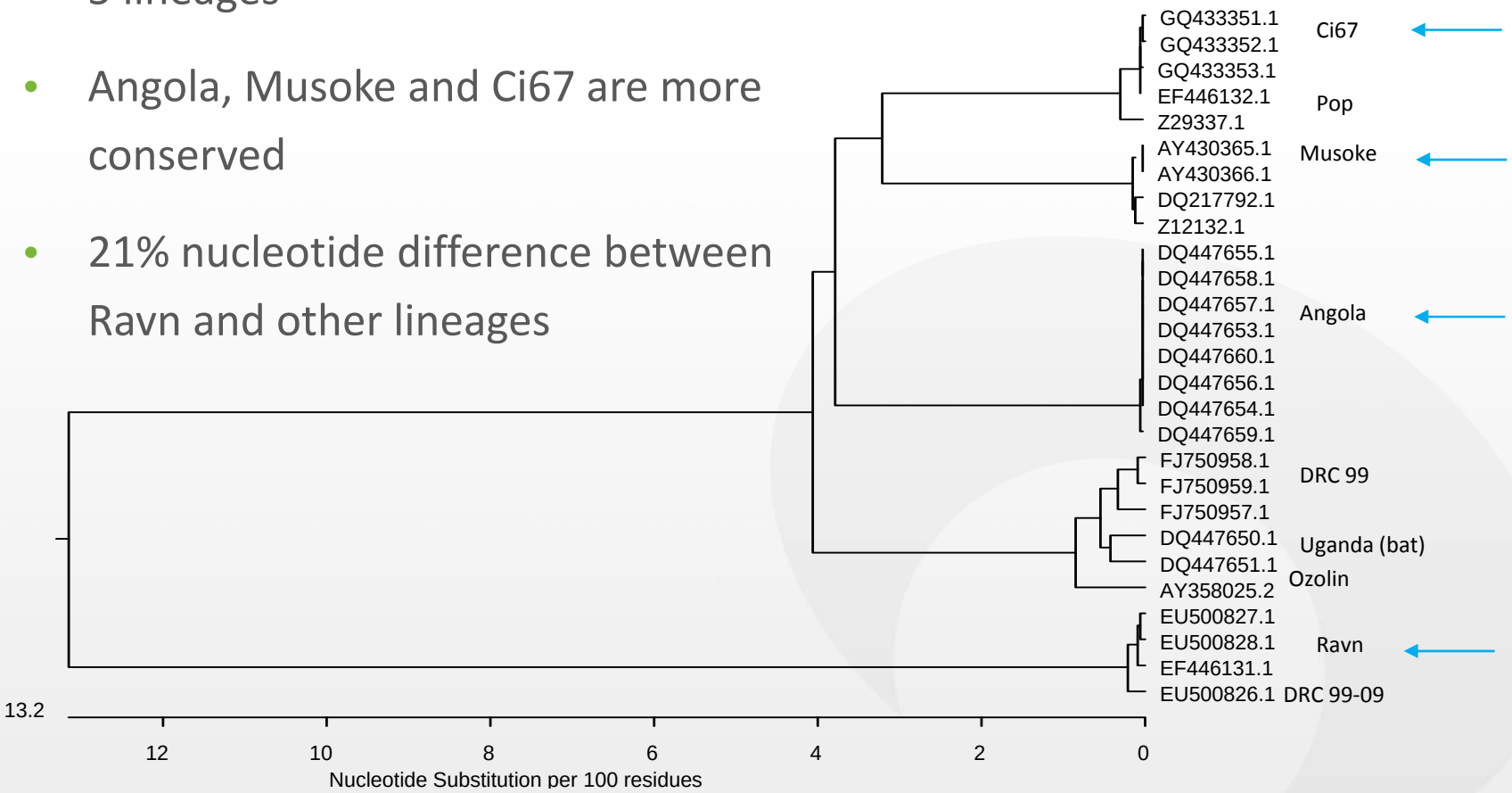


- Insertion Site for Gene of Interest
- Renilla Luciferase Reporter
- Firefly Luciferase Reporter

MARV Phylogenetics

Developing a Pan-Marburg RNAi Drug

- One species
- 5 lineages
- Angola, Musoke and Ci67 are more conserved
- 21% nucleotide difference between Ravn and other lineages



Proof-of-Concept Studies in Guinea Pigs

The Journal of Infectious Diseases

Protection against Lethal Marburg Virus Infection Mediated by Lipid Encapsulated siRNA

Raul Ursic-Bedoya, Chad E. Mire, Marjorie Robbins, Joan B. Geisbert, Adam Judge, Ian MacLachlan, Thomas W. Geisbert

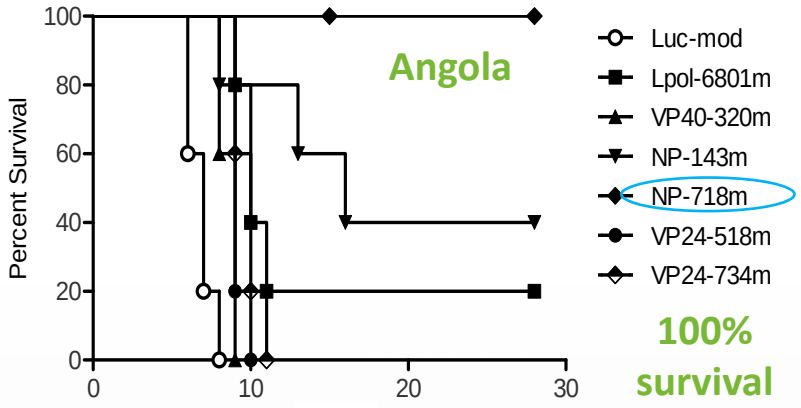
Abstract

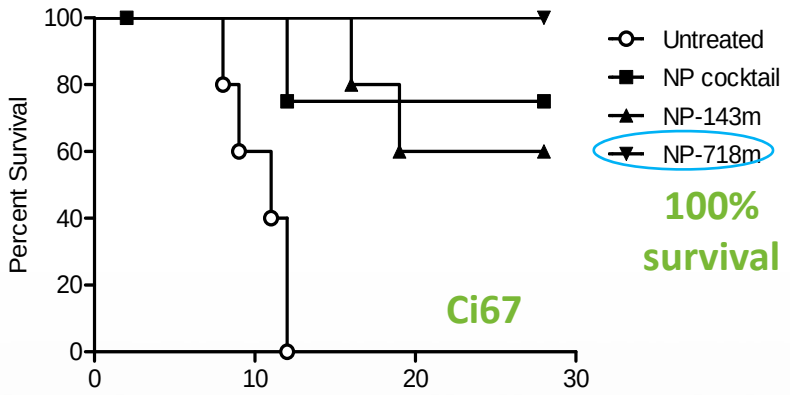
Background. Marburg virus (MARV) infection causes severe morbidity and mortality in humans and nonhuman primates. Currently, there are no licensed therapeutics available for treating MARV infection. Here, we present the in vitro development and in vivo evaluation of lipid–encapsulated siRNA (LNP) as a potential therapeutic for the treatment of MARV infection.

Conclusions. These data show protective efficacy against the most pathogenic Angola strain of MARV. Further development of the LNP technology has the potential to yield effective treatments for MARV infection.

Ursic-Bedoya et al., J Infect Dis. (2013)

TKM-Marburg Payload Development





- siRNAs targeting conserved regions screened using Dual Luciferase Reporter (DLR) assays
- Downselected siRNAs chemically modified
  - Tested in human whole blood and mouse IFIT assays for immune stimulation
  - Tested in vitro for antiviral activity
- Guinea pig studies with 3 MARV variants identified NP 718m as lead siRNA

  (Ursic-Bedoya et al., 2013, J.Inf. Dis.)

TKM-Marburg NHP Delay to Treat (DTT) Studies

Rhesus Inoculated with 1000 pfu MARV Angola

- 4 independent studies assessing LNP treatment efficacy starting 1 h, 24 h, 48 h and 72 h post-exposure with Angola

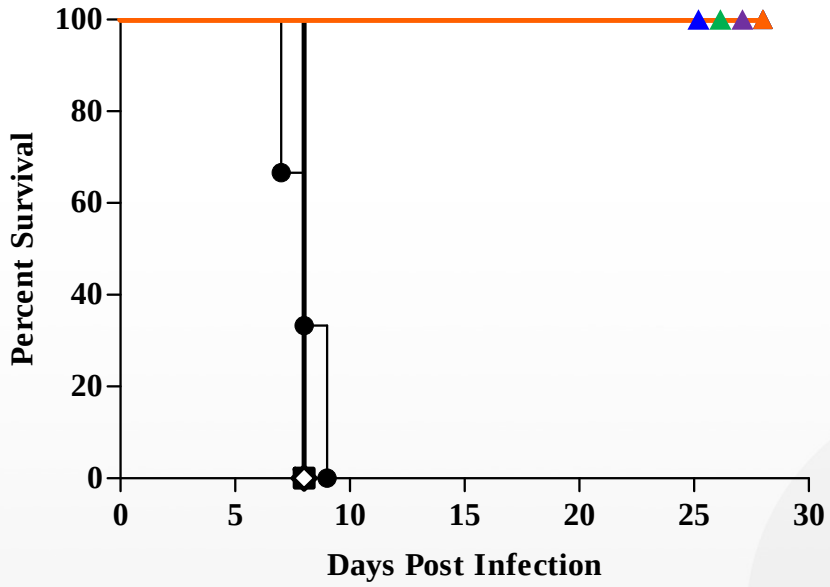
Group	Treatment	# NHPs	Daily Dosage
1	Untreated or Luc LNP*	1	0.5 mg/kg or 1.0 mg/kg**
2	MARV NP 718m	4	0.5 mg/kg or 1.0 mg/kg**

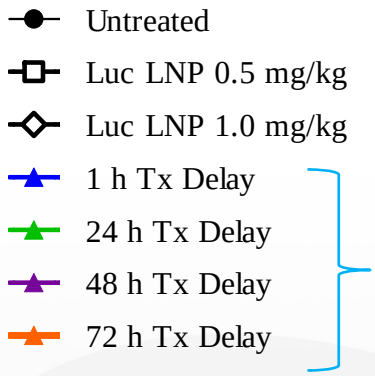
* Control Group was Untreated in 1 h & 24 h DTT studies, Luc LNP in 48 h & 72 h DTT studies
** LNP @ 1.0 mg/kg/d in 72 h DTT study

- Real World: Timing of infection not always known
  Incubation period can range from 2-21 days prior to onset of symptoms
  Goal: Efficacy when treatment is initiated upon onset of early symptoms

Anti-viral Efficacy in a Lethal Viral Challenge Model

Rhesus Inoculated with 1000 pfu MARV Angola





Survival

0% (0/3)

0% (0/1)

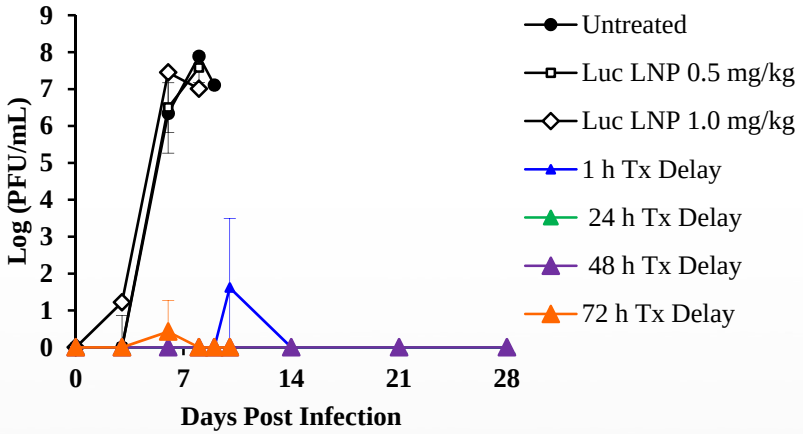
0% (0/1)

100% (4/4) for each active group

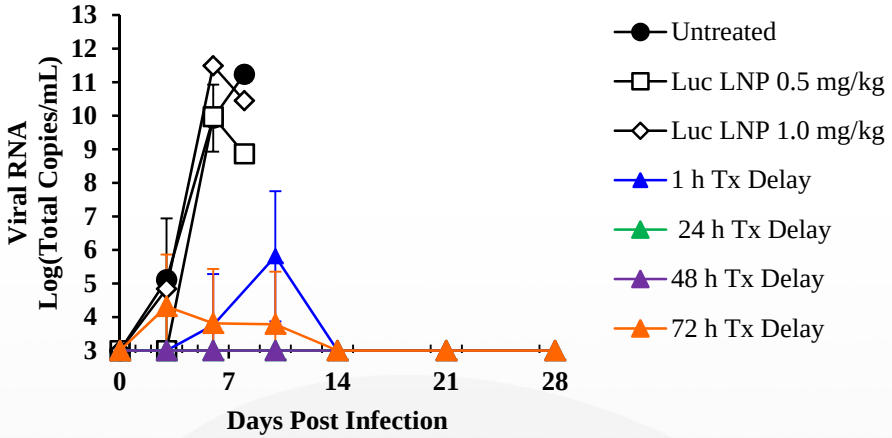
- 100% protection with up to 72 h delay in treatment
- No significant difference between Luc LNP (negative control siRNA) and Untreated controls

Viremia and Viral RNA Assessments

Plaque Assay

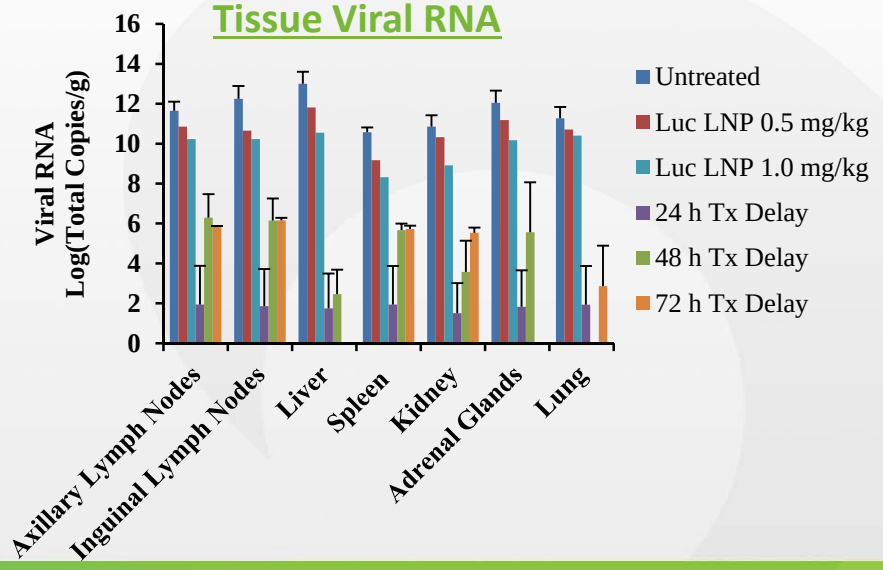


Viral RNA

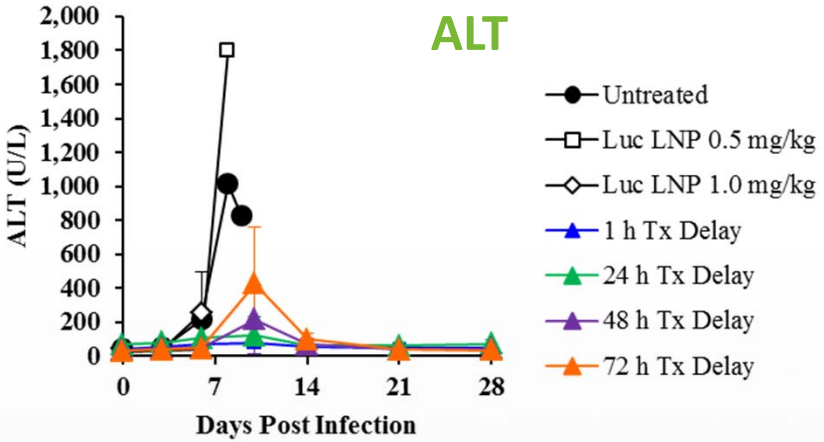


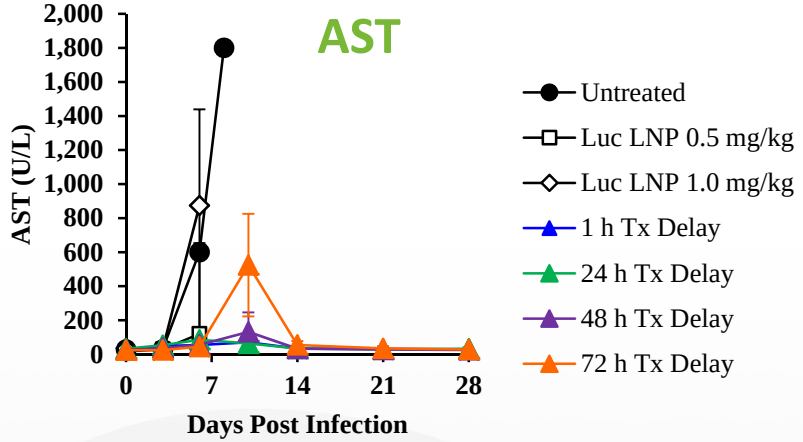
- TKM-Marburg effectively reduces serum viremia and viral RNA by 6-10 log

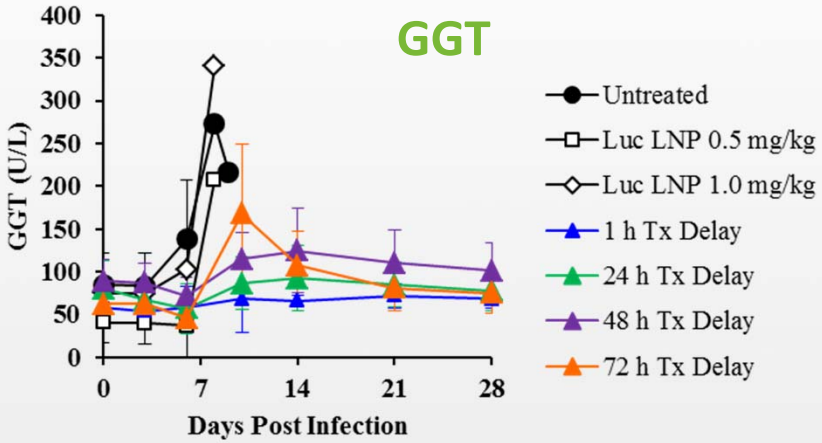
Tissue Viral RNA

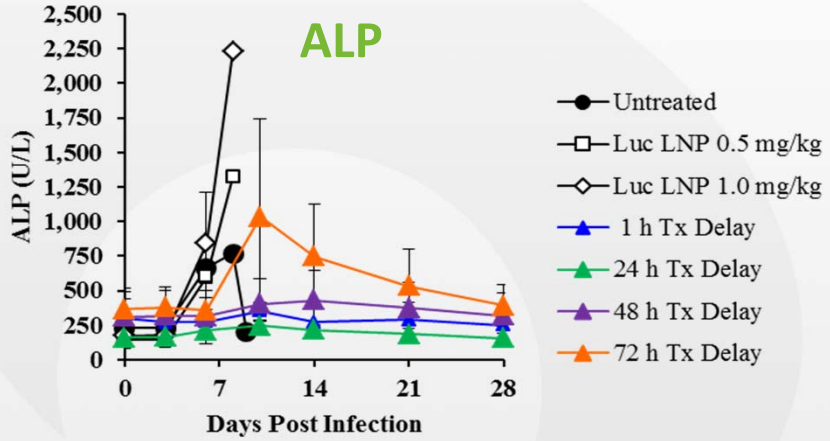


TKM-Marburg Treatment Ameliorates Disease Associated Pathology

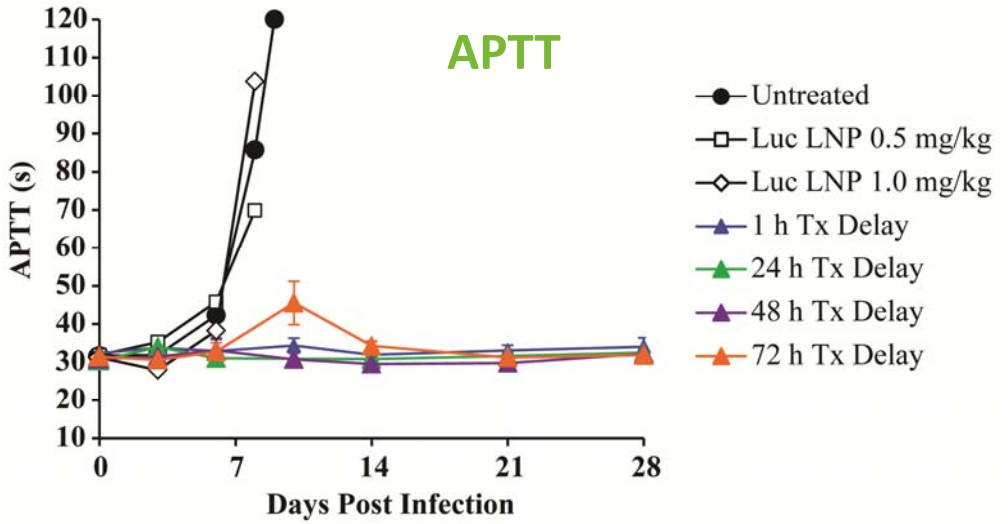


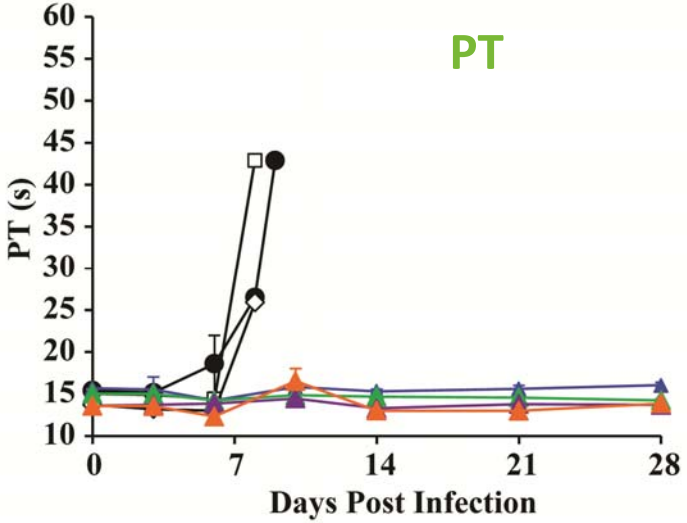






TKM-Marburg Treatment Ameliorates Disease Associated Coagulopathy





Ebola Proof-of-Concept in Non-Human Primates

ELSEVIER

THE LANCET

Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study

Thomas W Geisbert, Amy C H Lee, Marjorie Robbins*, Joan B Geisbert, Anna N Honko, Vandana Sood, Joshua C Johnson, Susan de Jong, Iran Tavakoli, Adam Judge, Lisa E Hensley, Ian MacLachlan*

Summary

Background We previously showed that small interfering RNAs (siRNAs) targeting the Zaire Ebola virus (ZEBOV) RNA polymerase L protein formulated in stable nucleic acid-lipid particles (SNALPs) completely protected guineapigs when administered shortly after a lethal ZEBOV challenge. Although rodent models of ZEBOV infection are useful for screening prospective countermeasures, they are frequently not useful for prediction of efficacy in the more stringent non-human primate models. We therefore assessed the efficacy of modified non-immunostimulatory siRNAs in a uniformly lethal non-human primate model of ZEBOV haemorrhagic fever.

Geisbert et al, Lancet 2010; 375: 1896-905

TKM-Ebola Formulation Change

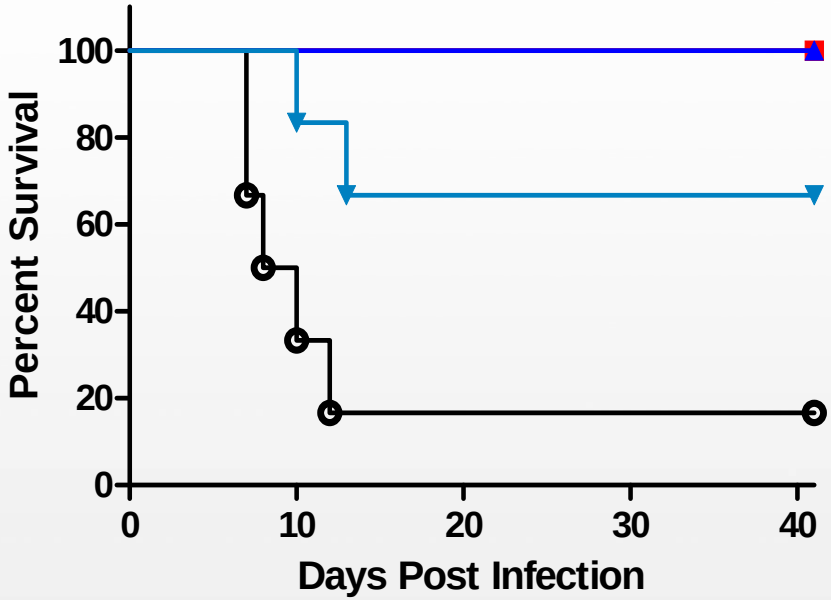
Integrating Improvements in Formulation Technology in Infectious Diseases

- TKM-Ebola product originally utilized a wet formulation (c. 2006)
  - Efficacy POC in NHP at 2 mg/kg/dose, 7 daily doses
  - 100% survival against highly lethal Kikwit strain
- Recently transitioned to a lyophilized formulation with increased potency and increased therapeutic index
  - ~9x more potent in guinea pig endogenous liver target
  - ~30x more potent in NHP endogenous liver target

TKM-Ebola Antiviral Efficacy

NHP Dose Response Study: Survival to Day 41

- Original wet '2006' LNP (TKM 100201) 100% protection at 2 mg/kg/dose
- New LNP formulation achieves 100% survival using 4-fold lower dose
- Significant survival advantage even at 10-fold lower dose



	Survival
Saline	15% (1/6)
TKM-100701 Wet 0.5 mg/kg	100% (6/6)
TKM-100802 Lyo 0.5 mg/kg	100% (6/6)
TKM-100802 Lyo 0.2 mg/kg	67% (4/6)

7 daily doses

- Challenged with Ebola virus Zaire 1995 Kikwit

TKM-EBOV-002 Phase 1 Trial: Single Ascending Dose Portion

Number of Subjects Per Cohort and Dose Level

A total of 19 subjects were enrolled across all 4 dose level cohorts.

Dose Cohort	Dose Level	Study Treatment Administered	
		TKM-100802	Placebo
1	0.075 mg/kg	3	1
2	0.15 mg/kg	3	1
2 exp.*	0.15 mg/kg	3	1
3	0.3 mg/kg	3	1
4	0.5 mg/kg	2	1

There were no discontinuations from the study due to adverse events (AEs) or any other reason.

*An additional cohort (Cohort 2 exp.) was added at the 0.15 mg/kg dose level to permit further characterization of the safety profile.

Treatment Emergent Adverse Events by Frequency

AEs Present in 10% or More Subjects Treated

System Organ Class & Preferred Term	0.075 mg/kg (N= 3)	0.15 mg/kg (N= 6)	0.30 mg/kg (N= 3)	0.50 mg/kg (N= 2)	Total (N= 14)
Subjects with at least one AE	2 (66.7%)	3 (50.0%)	1 (33.3%)	2 (100%)	8 (57.1%)
Feeling hot	2 (66.7%)	1 (16.7%)	1 (33.3%)	2 (100%)	6 (42.9%)
Erythema	1 (33.3%)	2 (33.3%)	1 (33.3%)	1 (50.0%)	5 (35.7%)
Headache	0	2 (16.7%)	0	2 (100%)	4 (28.6%)
Chest discomfort	0	2 (33.3%)	0	1 (50.0%)	3 (21.4%)
Nausea	0	2 (33.3%)	0	1 (50.0%)	3 (21.4%)
Back pain	0	1 (16.7%)	0	1 (50.0%)	2 (14.3%)
Dizziness	0	1 (16.7%)	0	1 (50.0%)	2 (14.3%)
Sinus Tachycardia	0	0	0	2 (100%)	2 (14.3%)

Adverse events frequency and severity at doses below the DLT similar to those observed with parenteral nutrition, imaging contrast agents and well tolerated parenteral therapeutics.

Adverse Events and Dose Limiting Toxicities

Most of the reported treatment related AEs were consistent with a transient inflammatory response that begins within 6 hours after infusion and dissipates in most cases by 24 hours post-dosing. This interpretation is consistent with:

- Transient elevations in some cytokines (e.g., MCP-1, IL-6, IL-1ra, IL-8) in some patients measured 2 and 6 hours post-dosing, returning to at or near baseline levels by 24 hours.

One subject treated at the 0.5 mg/kg dose level experienced DLT of AEs of nausea, emesis, sinus tachycardia and hypotension.

No evidence for histamine mediated AEs, no basis for use of H1/H2 blockers (antihistamines).

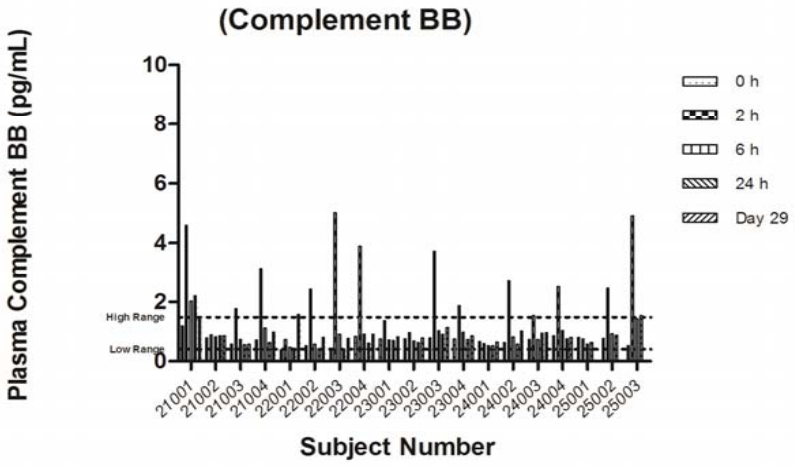
No need or basis for blanket adoption of NSAID prophylaxis.

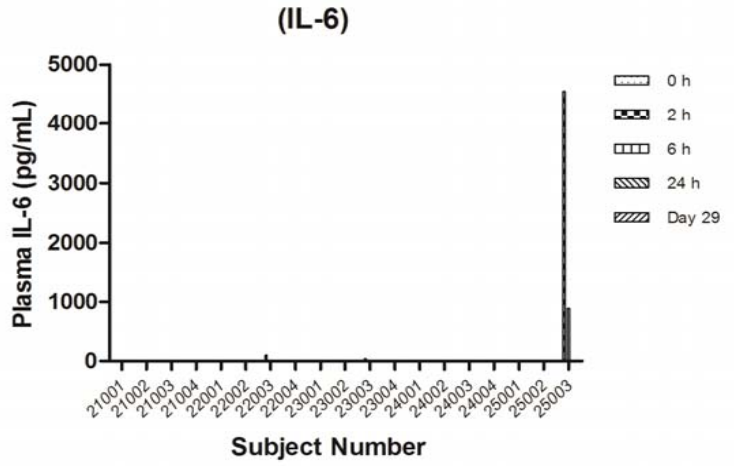
No need or basis for blanket adoption of steroid prophylaxis.

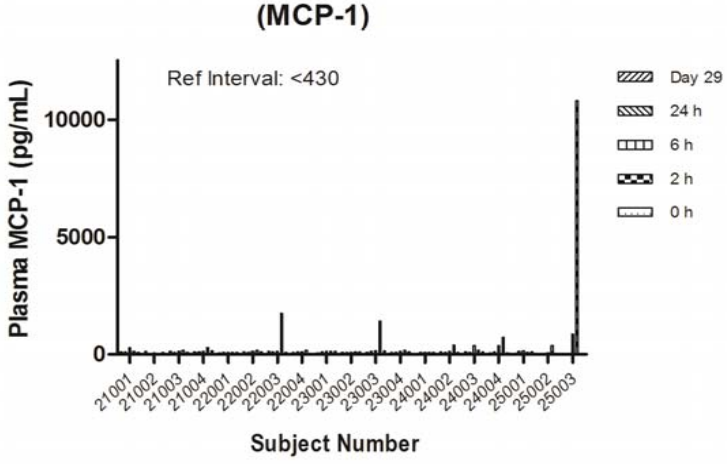
LNP Can Be Used at Effective Dose Levels Safely, Without Pre-medication

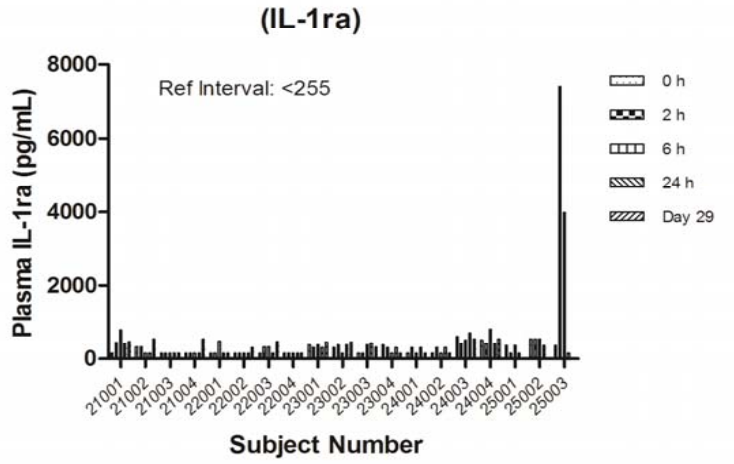
Complement and Cytokine Safety Data

Inflammatory Response Manifests as Dose Limiting Toxicity

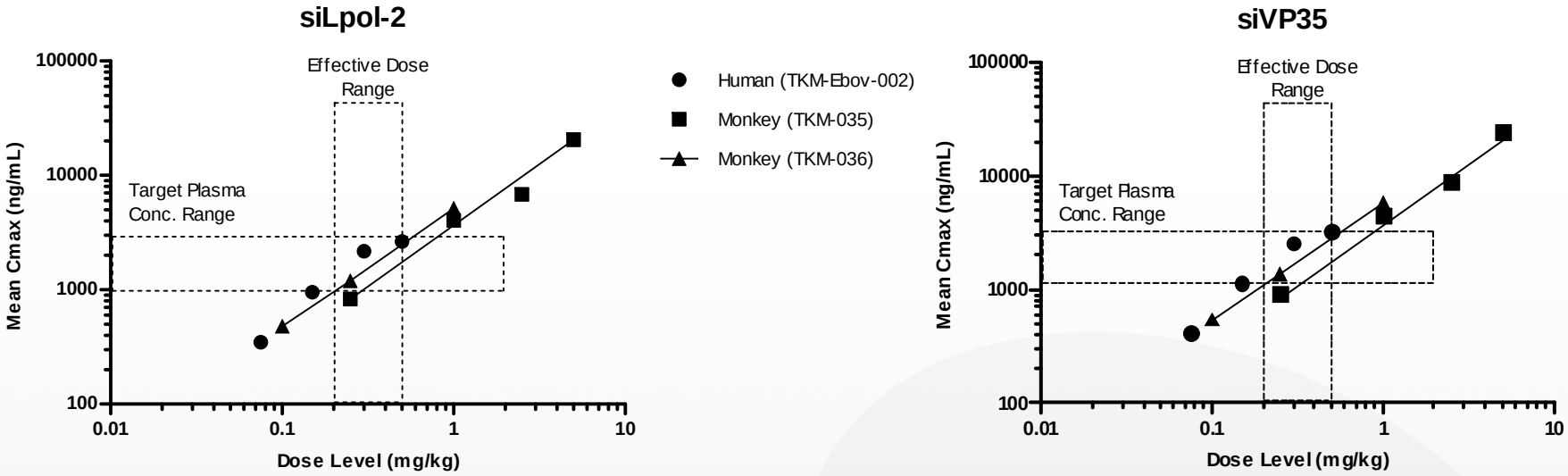








Pharmacokinetic Analysis and Determination of Dose Levels for Multiple Dose Portion of Trial



- siLpol-2 and siVP35-2 have similar plasma kinetics and retention properties within LNP.
- A dose related and proportional increase in AUC_{0-t}, AUC_{0-25}, and C_{max} was observed for both siRNA across all cohorts.
- Predicted C_{max} following dosing at the highest planned dose in the MAD phase is in the target range for efficacy.

TKM-EBOV-002: Multiple Ascending Dose Portion

Number of Subjects Per Cohort and Dose Level

A total of 12 subjects will be enrolled across 3 dose level cohorts.

Dose Cohort	Dose Level	Study Treatment Administered	
		TKM-100802	Placebo
1	0.06 mg/kg	3	1
2	0.12 mg/kg	3	1
3	0.24 mg/kg	3	1

We anticipate the C_{max} at the highest planned dose in the MAD phase (0.24 mg/kg/day) to be well within the target plasma concentration.

TKM-Ebola Single Ascending Dose Summary

- 1-hour IV administration of TKM-100802 was well-tolerated to a dose level of 0.3 mg/kg.
- The safety profile observed with escalating doses up to the MTD of 0.3 mg/kg supports initiation of the multiple ascending dose (MAD) portion of the study at a starting dose of 0.06 mg/kg/day.
- Drug exposure analysis indicates that a dose of 0.24 mg/kg/day will meet exposure targets derived from the highest dose level currently used in monkey efficacy studies (0.5 mg/kg/day).
- Interim safety data submitted to the FDA for review May 7, 2014.
- MAD portion of trial scheduled to start June.
- Pivotal Animal Efficacy studies to follow.

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