



# **TKM-Ebola – A Lipid Nanoparticle siRNA Therapeutic for Ebola Virus Infection**

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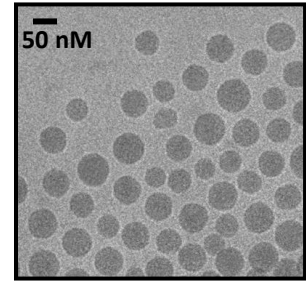
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# Outline

- LNP Technology & Clinical Translation
- TKM-Ebola-Guinea Animal Efficacy
- Manufacturing Capabilities
- RAPIDE-TKM Phase 2 Clinical Trial

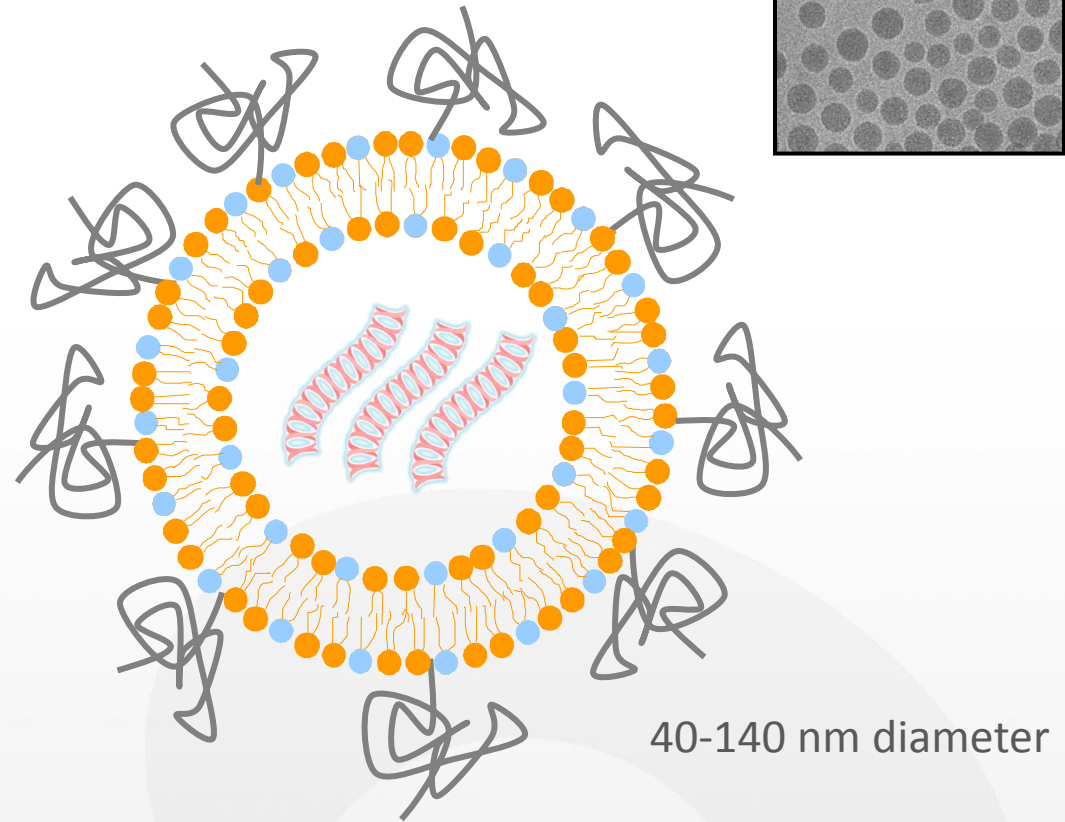


# Tekmira's Lipid Nanoparticle Platform



## Components:

- Amino Lipid 
- Structural Lipid 
- PEG - Lipid 
- Nucleic Acid 



*The modular nature of the siRNA-LNP platform allows for changes to siRNA payload while maintaining LNP delivery functionality*

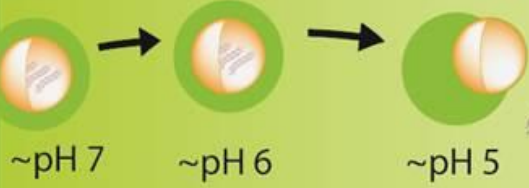
***Can accommodate viral diversity / viral mutation***

*Jefferies et al. Pharm. Res. 2005.*



LNP

LNP Uptake Via Endocytosis



pH Triggered Endosomal Fusion and siRNA Release

### siRNA DELIVERY

Sense Strand Degradation

### RNA INTERFERENCE



mRNA Degradation

Site Specific mRNA Cleavage

Antisense Strand Mediated Target Recruitment to RISC

Target Gene

DNA

mRNA

Nucleus

Cytoplasm

Extracellular Space

# Accumulated LNP Experience in Man

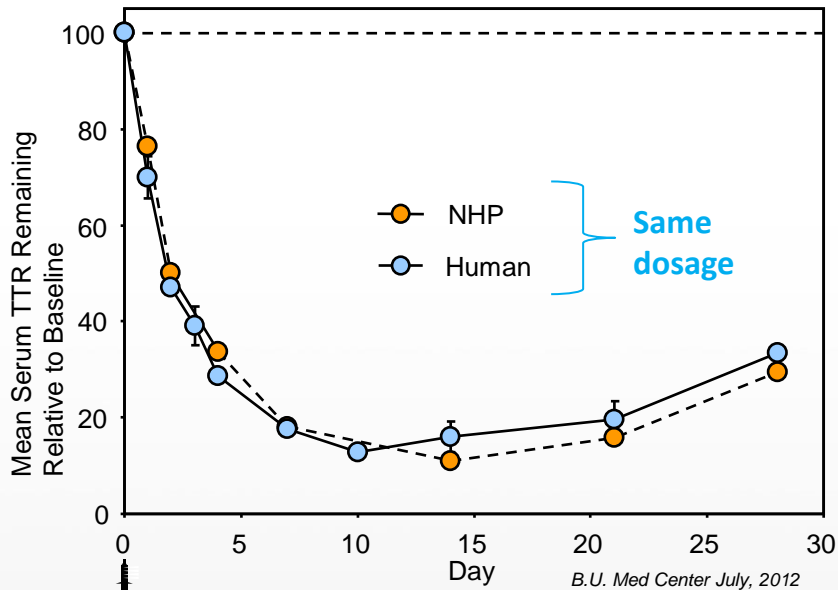
*Eight LNP products have entered clinical development since 2008*

Representative Ongoing Clinical Activity				
Product	Company	Phase	Indication	Comments
ALN-TTR02	Alnylam	3	Amyloidosis	Potent pharmacodynamic effect demonstrated, well tolerated
TKM-PLK1	Tekmira	2	Oncology	Promising signs of RNAi drug activity
TKM-Ebola	Tekmira	1	Ebola Infection	Lyophilized formulation
TKM-Ebola-Guinea	Tekmira	2	Ebola Infection	Trial initiated recently in Sierra Leone

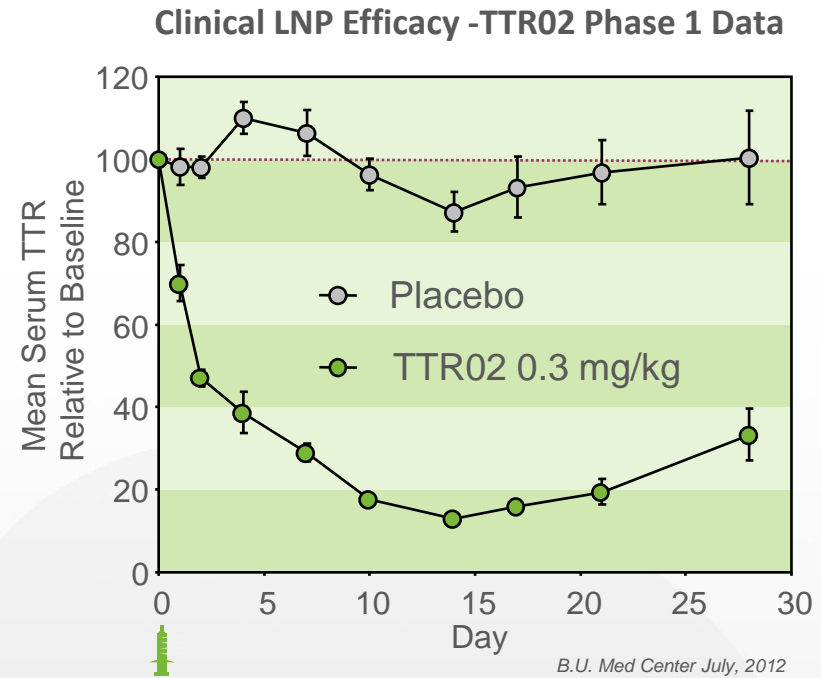
- > 250 patients treated w/ siRNA-LNP, some with repeat-dosing for >1 year
- Potent, long lasting effects after a single dose
- Continually growing body of clinical safety data since 2008
- Formulation improvements translate from the lab to the clinic

*LNP enabled RNAi drugs have established clinical validation*

# Proven Efficacy Translation from NHP to Human



Single LNP Dose  
0.3 mg/kg



- Alnylam partnered program developing siRNA-LNP therapeutic against transthyretin amyloidosis (endogenous gene target)
- Currently in Phase 3 clinical trial

# Anti-Ebola Efficacy in Non-Human Primates

*Proof-of-Concept published 5 years ago*



## 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*

# Recent *Ebolavirus Zaire* Outbreak in West Africa

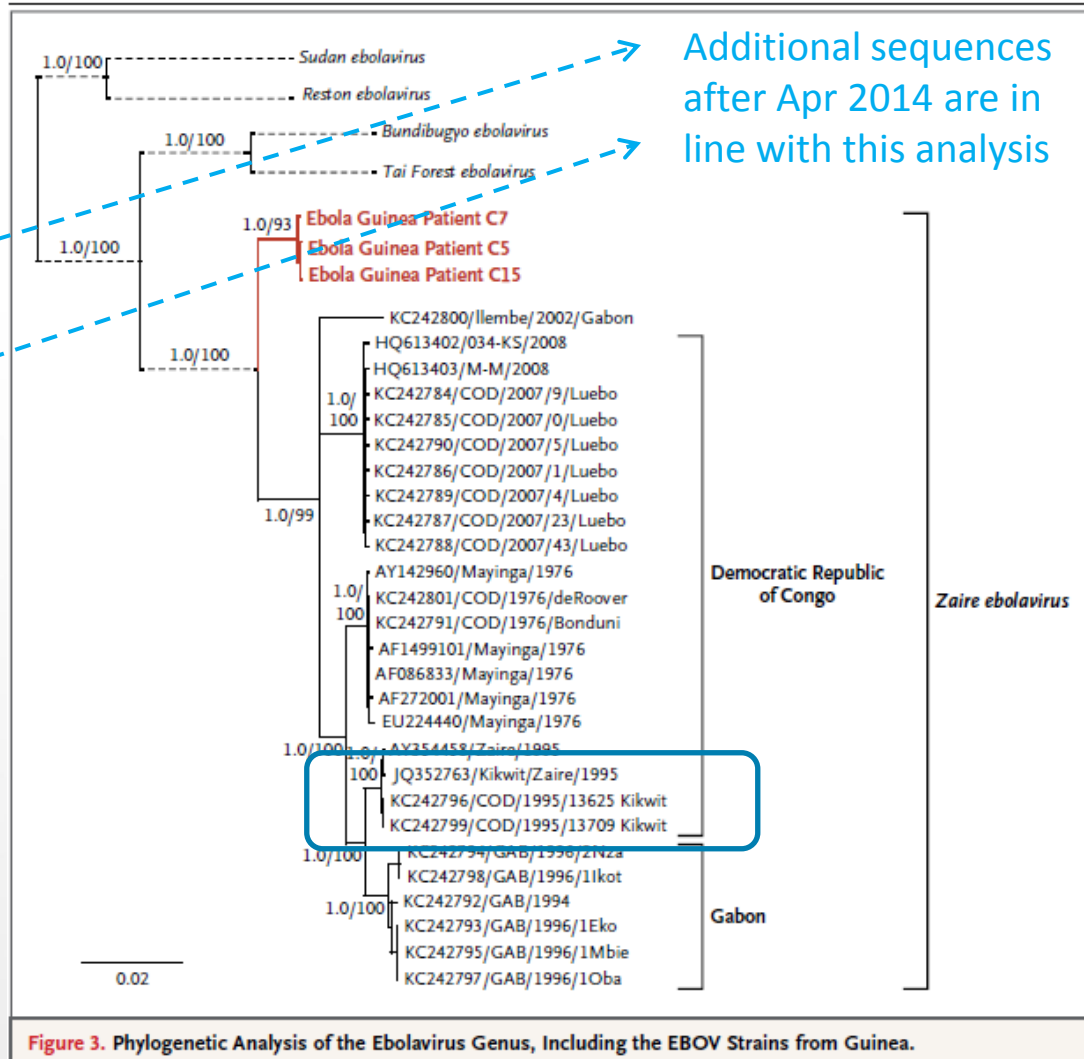
## *Makona* Variant is Divergent from Central African Outbreaks



**Figure 1.** Map of Guinea Showing Initial Locations of the Outbreak of Ebola Virus Disease.

The area of the outbreak is highlighted in red. The main road between the outbreak area and Conakry, the capital of Guinea, is also shown. The map was modified from a United Nations map.

- Phylogenetic analysis suggests West African EBOV variant forms a single clade distinct from outbreaks in central Africa
- 2014 DRC outbreak aligns with previous Central African EBOV variants



# Alignment of siRNA Target Sequences against EBOV

## VP35

Year	Outbreak	3886	3906
1976	Mayinga, DRC	GCAACTCATTGGACATCAT	
1976	Mayinga, DRC	GCAACTCATTGGACATCAT	
1995	Kikwit, DRC	GCAACTCATTGGACATCAT	
1996	Gabon	GCAACTCATTGGACATCAT	
2007	Luebo, DRC	GCAACTCATTGGACATCAT	
2014	DRC	GCAACTCATTGGACATCAT	
2014	Guinea	GCAATTCATTGGACATTAT	
2014	Sierra Leone	GCAATTCATTGGACATTAT	
2014	Liberia	GCAATTCATTGGACATTAT	
	siVP35-2	GCAACTCATTGGACATCAT	
	siVP35-3	GCAATTCATTGGACATTAT	

- TKM-Ebola (siVP35-2, siLpol-2) targets EBOV variants from Central Africa

- Mismatches against EBOV Makona
  - VP35-2, 2 mismatches at AS3, AS15
  - Lpol-2, 1 mismatch at AS6

## L-pol

		17397	17417
1976	Mayinga, DRC	GTACGAAGCTGTATATAAA	
1976	Mayinga, DRC	GTACGAAGCTGTATATAAA	
1995	Kikwit, DRC	GTACGAAGCTGTATATAAA	
1996	Gabon	GTACGAAGCTGTATATAAA	
2007	Luebo, DRC	GTACGAAGCTGTATATAAA	
2014	DRC	GTACGAAGCTGTATATAAA	
2014	Guinea	GTACGAAGCTGTACATAAA	
2014	Sierra Leone	GTACGAAGCTGTACATAAA	
2014	Liberia	GTACGAAGCTGTACATAAA	
	siLpol-2	GTACGAAGCTGTATATAAA	
	siLpol-3	GTACGAAGCTGTACATAAA	

- TKM-Ebola-Guinea (siVP35-3, siLpol-3) targets West African EBOV variant Makona

- All publicly available viral sequences from current West African outbreak are targeted by TKM-Ebola-Guinea*

# How Do the Mismatches Affect Product Efficacy?

- Relating number and position of siRNA target mismatches to their effects on activity is an imprecise science
- TKM-Ebola containing siLpol-2 and siVP35-2 was predicted to retain *some* activity against EBOV Makona
- Adjusted, perfectly matched siRNAs in TKM-Ebola-Guinea are anticipated to have greater activity against 2014 outbreak virus
- The nature of the siRNA-LNP technology allows for a rapid response to emerging viral variants

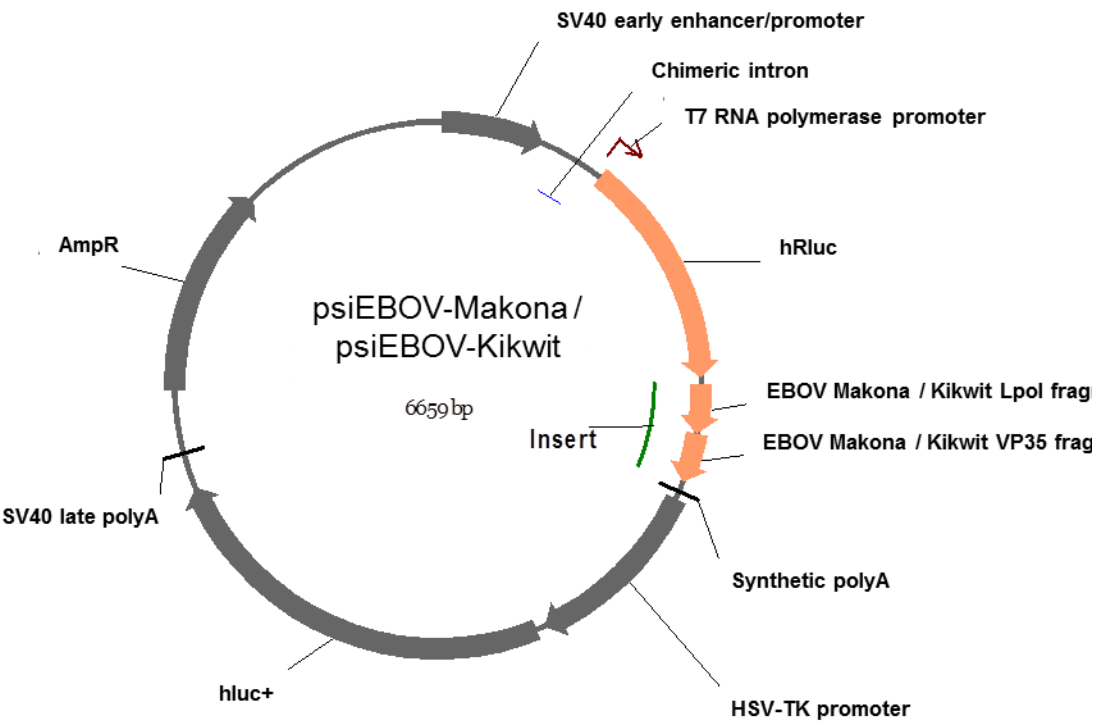
# Assessing TKM-Ebola Anti-Viral Activity

- Challenges:
  - Direct anti-viral efficacy data was unobtainable at the time:
    - 2014 outbreak strain (Makona) animal models *were* not established
    - Virus *was* unavailable for direct assessments of antiviral activity
- Interim Solution:
  - Focus on assessments of siRNA function (mRNA cleavage) in virus-free systems

# Predicting siRNA Activity in a Virus-free Model

## Plasmid-Based Dual Luciferase Reporter (DLR) System

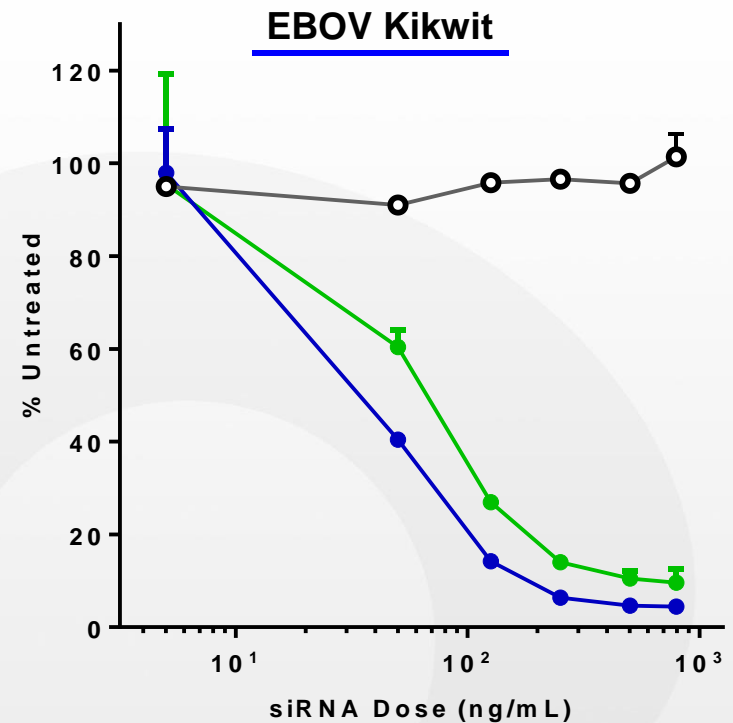
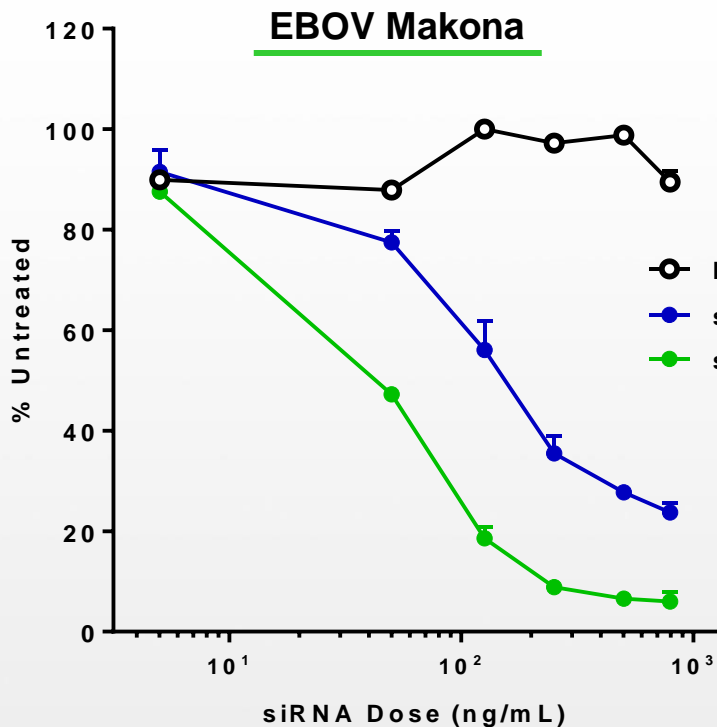
Example: 201+201 nt of EBOV Lpol and VP35 sequences inserted into DLR plasmid



- Chimeric mRNA transcription is driven from SV40 promoter
- Fuses Renilla luciferase transcript with the Makona (or Kikwit) EBOV fragments
- Tests ability of siRNA to mediate RNAi activity against target sequence (mRNA cleavage)
- Measured by detecting light generated by Renilla luciferase protein, translated from the intact chimeric mRNA
- Results validated with multiple cell lines, reporter constructs and transfection systems

# DLR Prediction of TKM-Ebola-Guinea Activity

- TKM-Ebola siRNA cocktail siEbola-2 retains some activity against EBOV Makona sequence
- TKM-Ebola-Guinea revised siRNA cocktail siEbola-3 shows strong activity against Makona and Central African (Kikwit) sequences

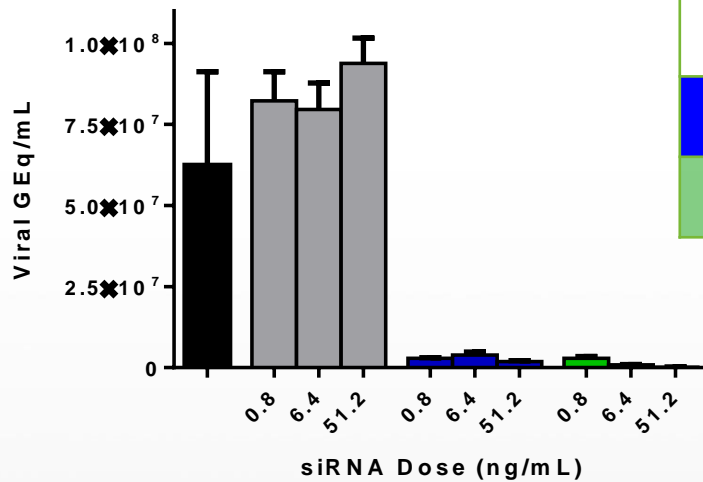


HepG2 transfection using siRNA-LNP; mean  $\pm$  SEM of two biological replicates (with technical triplicates)

# Confirming TKM-Ebola-Guinea Anti-Viral Activity

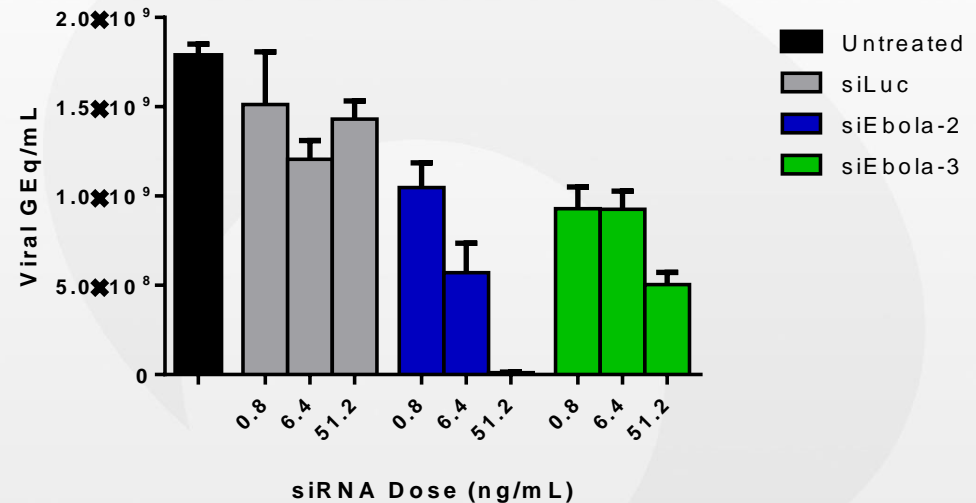
## HepG2 Cells Infected with EBOV Makona or Kikwit

EBOV Makona



Drug Product	Drug Substance	EBOV Target Endemic Region
TKM-Ebola	siEbola-2	Central Africa
TKM-Ebola-Guinea	siEbola-3	West Africa

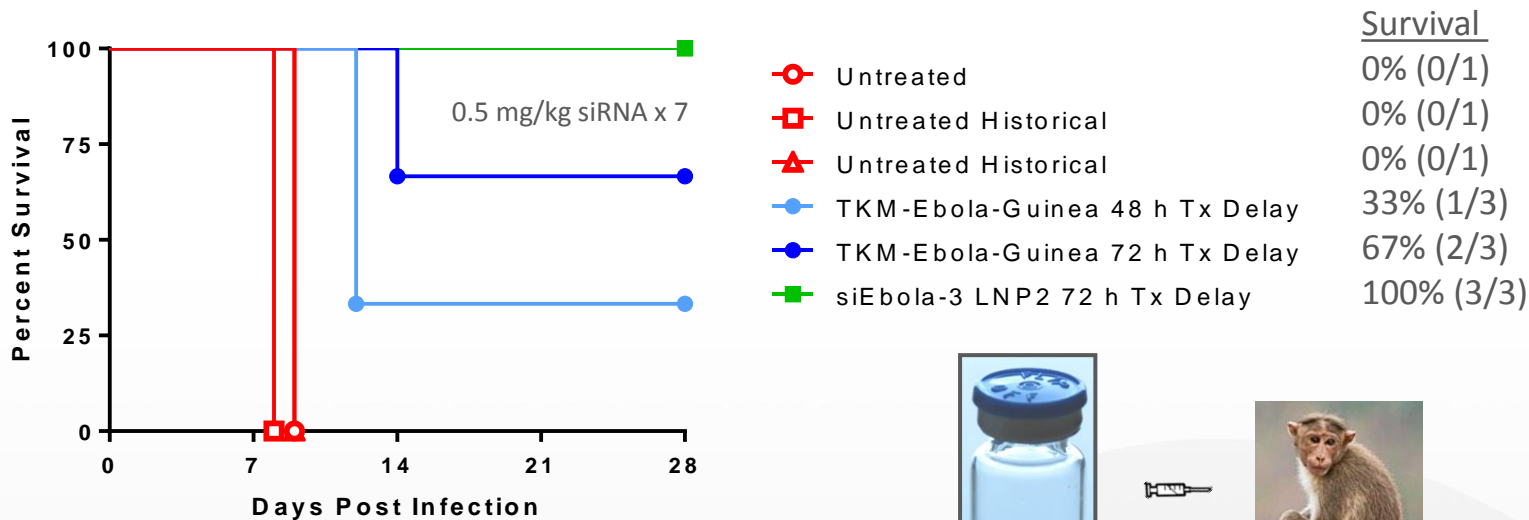
EBOV Kikwit



- TKM-Ebola siRNA cocktail is active against EBOV Makona
- New TKM-Ebola-Guinea siRNA cocktail inhibits EBOV Makona and Kikwit viral replication *in vitro*

# TKM-Ebola-Guinea Efficacy Against New Strain

*Rhesus challenged with 1000 pfu of EBOV Makona 2014*



X 7  
daily  
doses



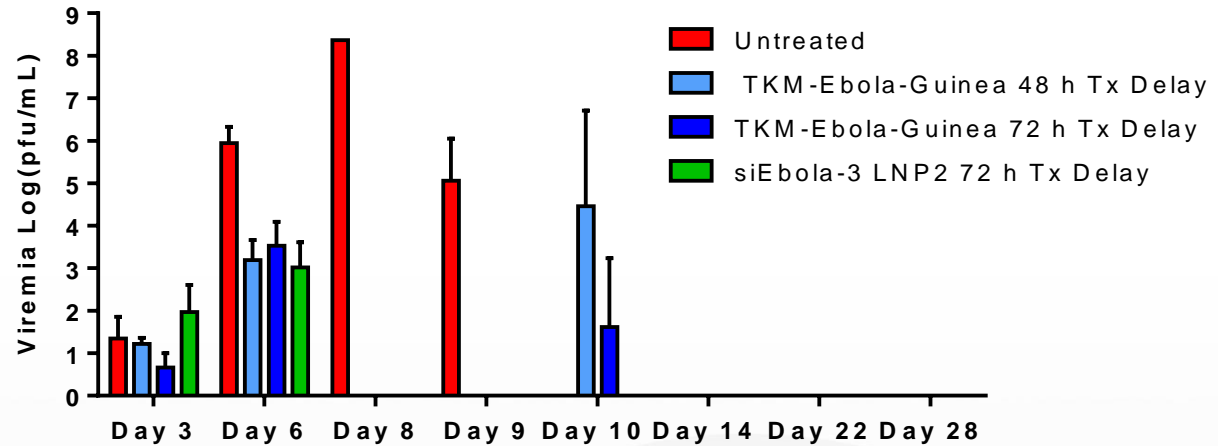
Rhesus  
Macaque

- TKM-Ebola-Guinea Tx enhances survival against a lethal EBOV Makona challenge
- Efficacy at 48 h and 72 h delay to treat comparable to TKM-Ebola against EBOV Kikwit
- 'LNP2' (different lipids, same siRNA) confers additional protection, 100% at 72 h

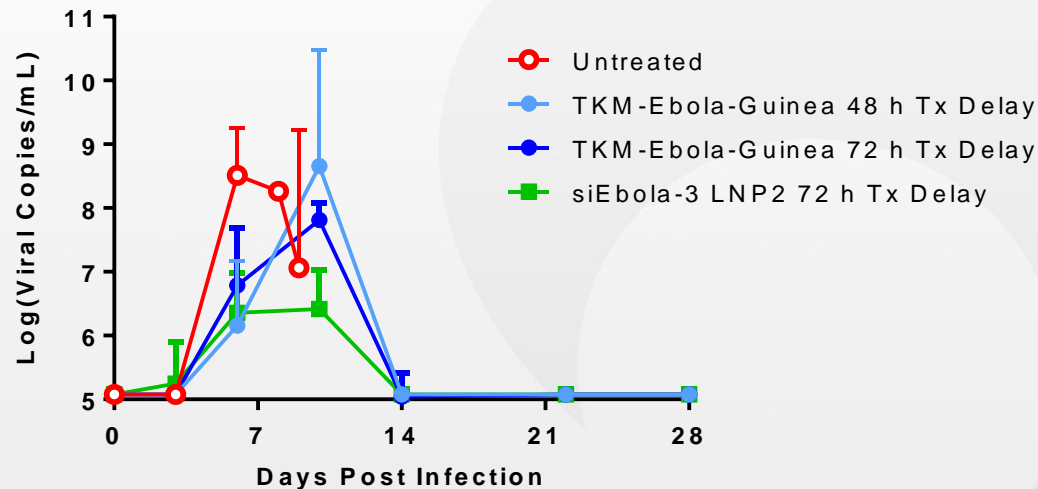
# Control of Viral Replication

*siRNA-LNP treatments reduced viral load*

Infectious  
Viral Particles



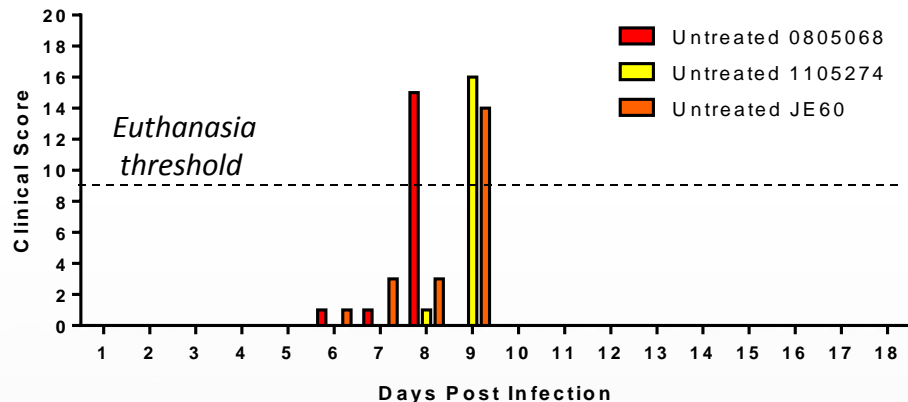
Viral RNA



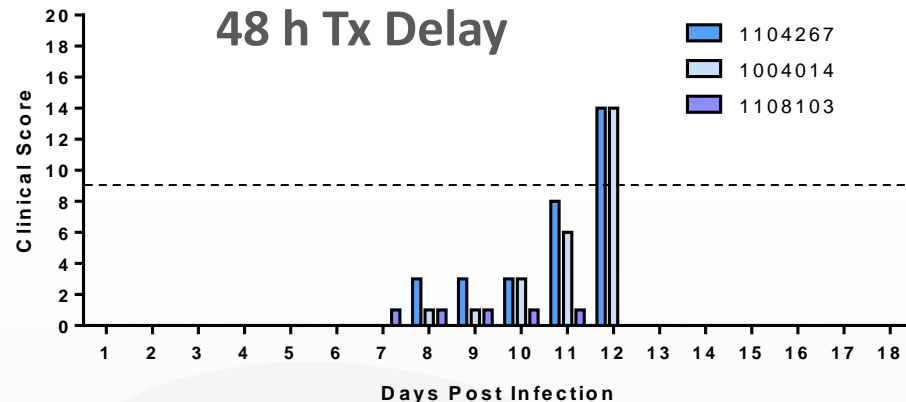
# Treatment Ameliorates Disease Symptoms

## Clinical scoring

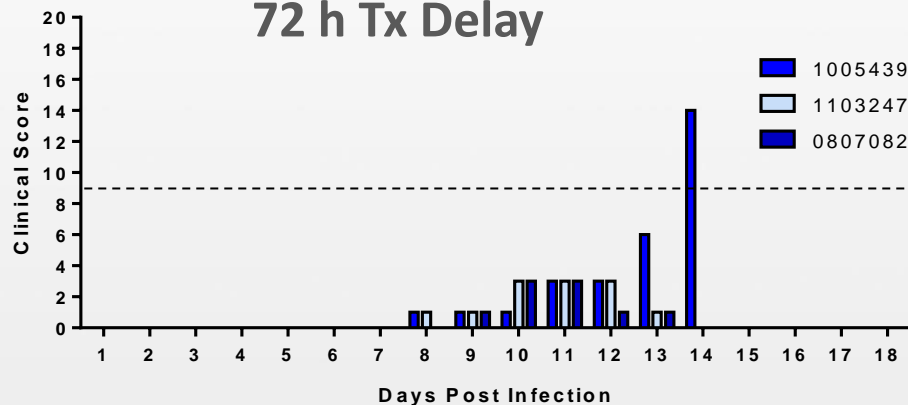
### Untreated



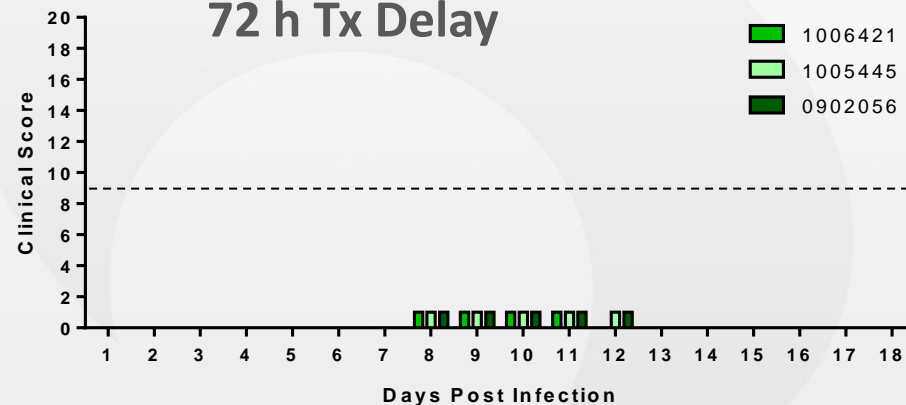
### TKM-Ebola-Guinea 48 h Tx Delay



### TKM-Ebola-Guinea 72 h Tx Delay



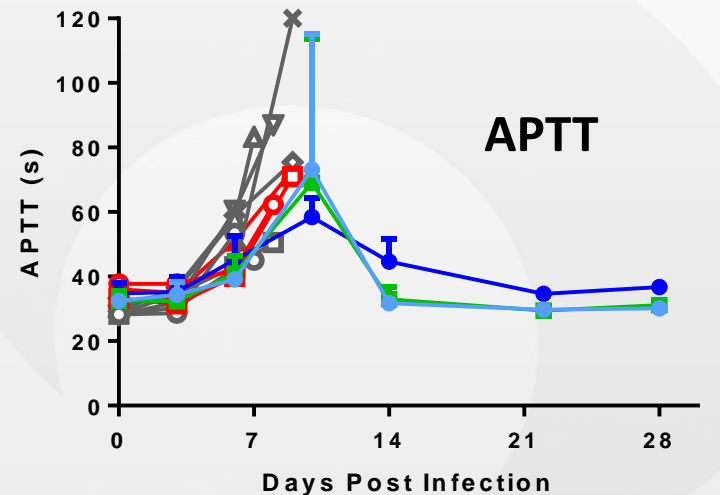
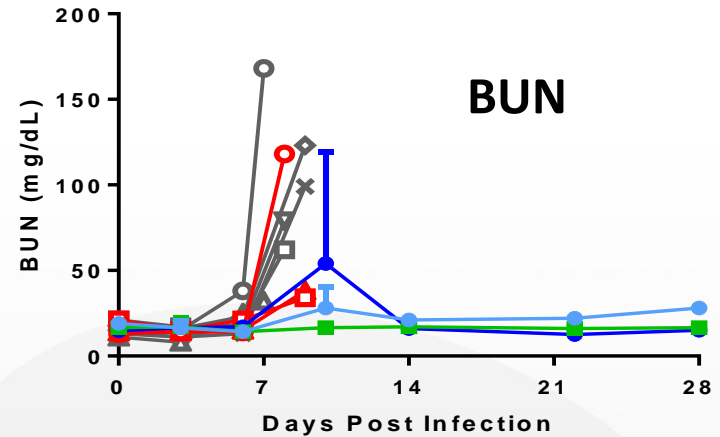
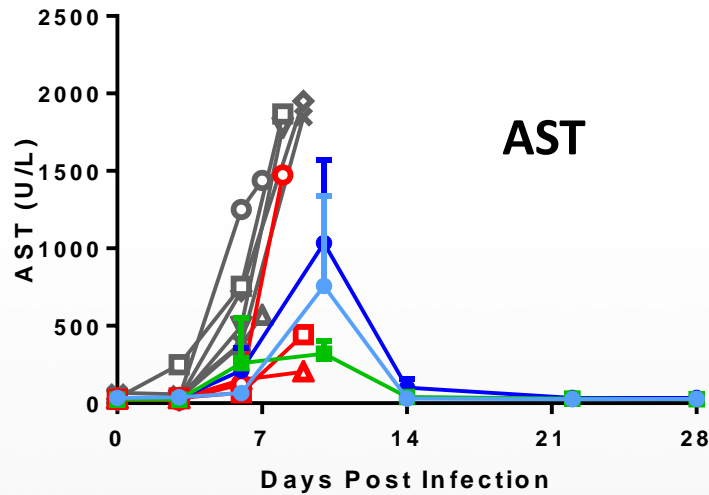
### siEbola-3 LNP2 72 h Tx Delay



# EBOV Makona Disease Presentation

*Infected NHP disease manifestation differs from EBOV Kikwit infection*

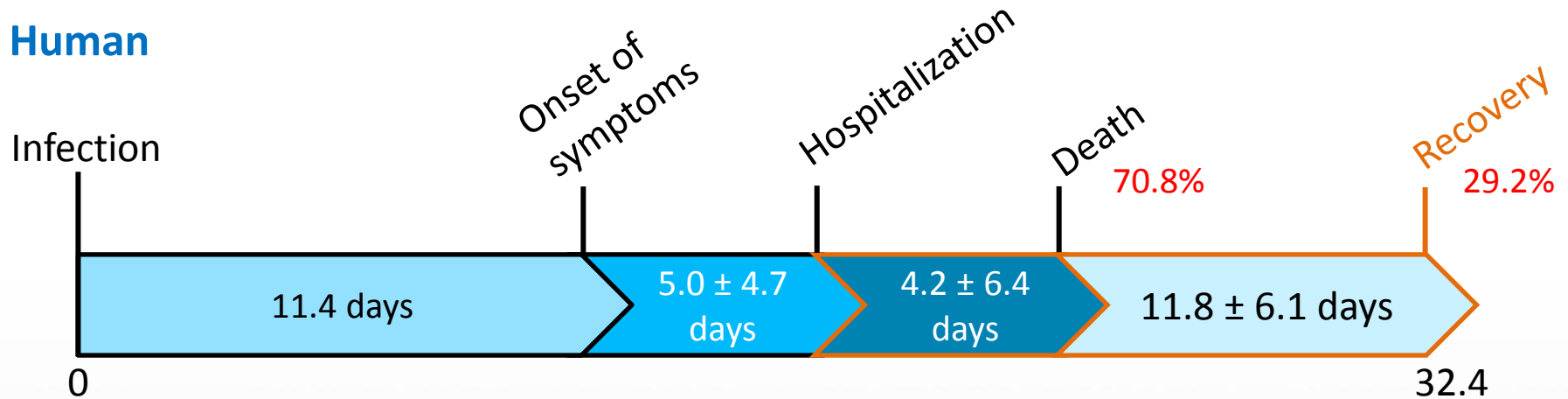
— EBOV Kikwit Untreated      — TKM-Ebola-Guinea 48 h Tx Delay      — siEbola-3 LNP2 72 h Tx Delay  
— EBOV Makona Untreated      — TKM-Ebola-Guinea 72 h Tx Delay



- siRNA-LNP treatments confer moderate protection
- Milder liver and kidney dysfunction observed in EBOV Makona infection compared to historical Kikwit infection
- Diarrhea more frequently observed

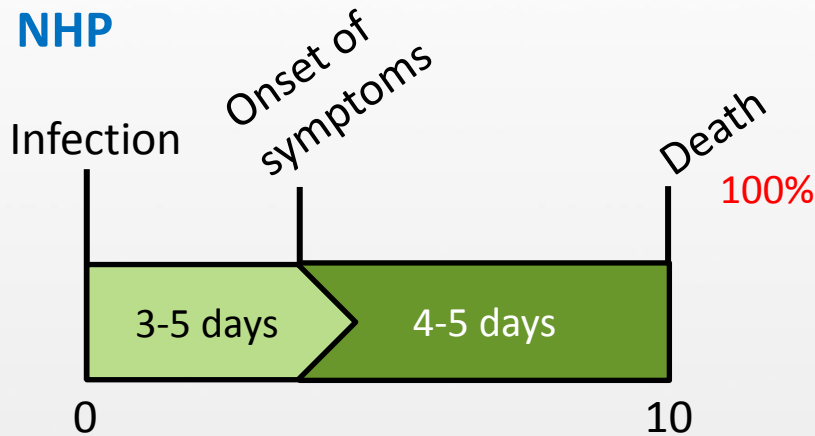
# NHP and Human Infection Time Course

## Human



WHO Ebola Response Team, NEJM, 2014, 371(16):1481

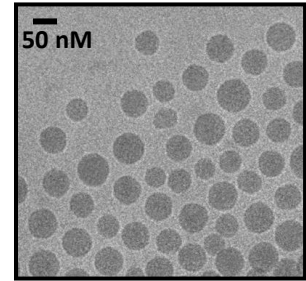
## NHP






Geisbert et al., Lancet, 2003, 362:1953  
Accumulating EBOV Makona data

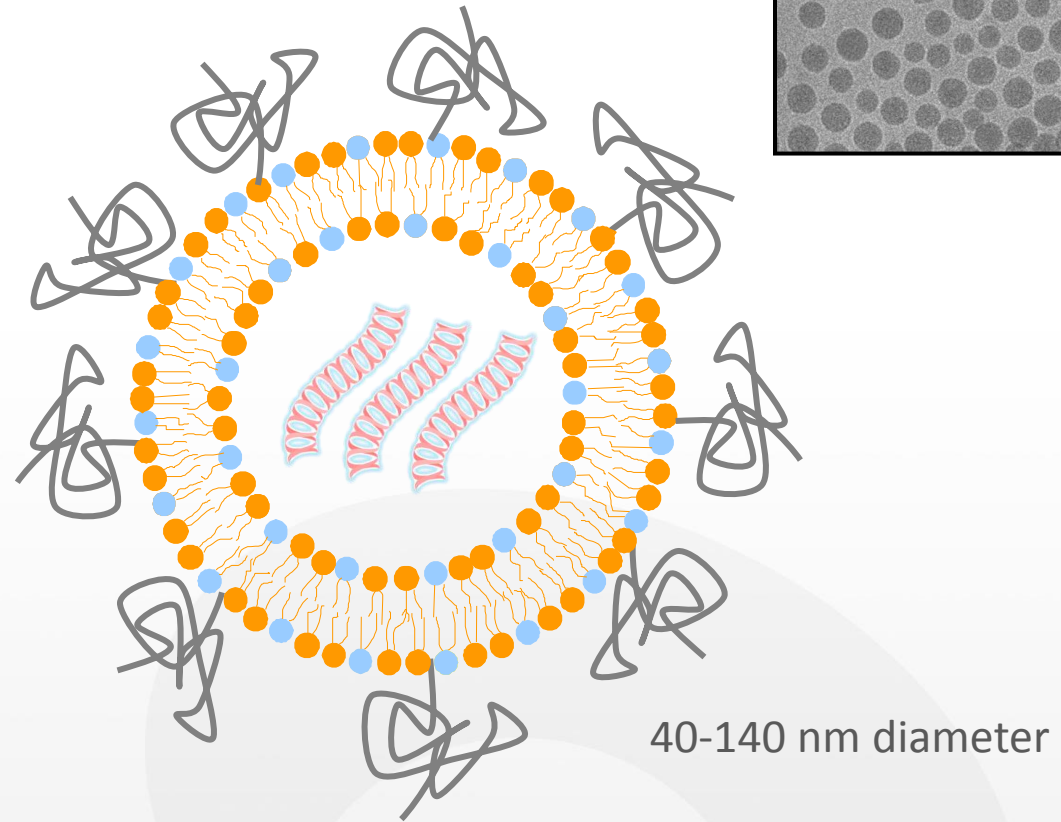
- Human time course data based on analysis of 3343 confirmed and 667 probable EVD cases from Guinea, Sierra Leone, Liberia and Nigeria
- Infection time course is accelerated in this lethal NHP model
- Animals also not provided supportive care

# Tekmira's Lipid Nanoparticle Platform



## Components:

- Amino Lipid 
- Structural Lipid 
- PEG - Lipid 
- Nucleic Acid 



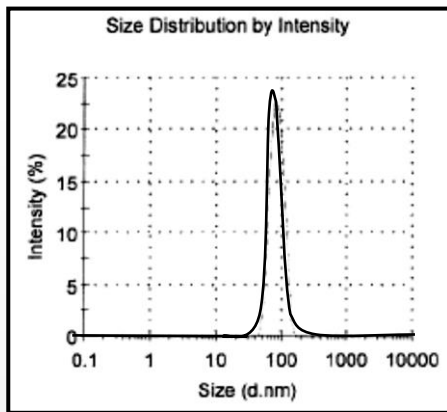
*The modular nature of the siRNA-LNP platform allows for changes to siRNA payload while maintaining LNP delivery functionality*

***Can accommodate viral diversity / viral mutation***

*Jefferies et al. Pharm. Res. 2005.*

# Established cGMP Manufacturing Expertise

- Controlled self assembly
- Highly scalable to kgs
- Efficient
- Consistent particle size



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

*Rapid response to viral target sequence changes*

*Once sequence is known,*

*cGMP clinical product manufactured in as little as 8-10 wks*

# TKM-Ebola-Guinea Therapeutic Agent

## *Manufacture and Supply Timeline*

- **Currently:** TKM-Ebola-Guinea ('ready-to-use' wet presentation)
  - ~100 treatment courses prepared
  - Supplying RAPIDE-TKM Ph 2 clinical trial in Sierra Leone
- **Longer Term Supply:** Lyophilized TKM-Ebola-Guinea
  - Plans and funding (U.S. DoD MCS-BDTX) for ~500 treatment courses
  - Capacity for ~500 treatment courses per month (could be scaled up further)

# TKM-Ebola Emergency Use

- To date, a small number of subjects have received TKM-Ebola under compassionate use protocols
  - Almost all with confirmed Ebola infections
- Challenges:
  - Patients treated with several concomitant medications
  - All infected patients received at least one other anti-EBOV Rx
  - Limited sample collection for safety assessments and PK
  - Pathology of EVD may mask or even suggest drug related AEs (coagulopathy, LFTs, thrombocytopenia, leukopenia, fever, hypotension)

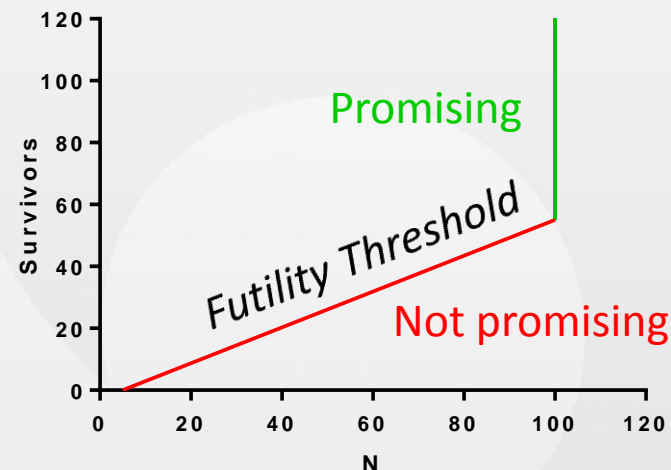
*Well controlled clinical trials are required to accumulate decision making data*

# TKM-Ebola-Guinea Phase 2 Trial

## RAPIDE-TKM in Sierra Leone

- Rapid Assessment of Potential Interventions & Drugs for Ebola (RAPIDE) – TKM
- Open label, single arm with comparison to concurrent observational cohort for Ebolavirus Disease in an outbreak setting in West Africa
  - Dosing at 0.3 mg/kg x 7 doses
- Primary outcome: Mortality at Day 14 after admission
  - Will be assessed in patients who survive the first 48 hours as well as in all patients
- Max number of patients: 100
- Futility study design allows early stopping if number of survivors falls below threshold based on sequential analysis of outcomes
- Trial began Mar 11, results are expected in second half 2015

### Conceptual Schematic



# Summary and Conclusions

- Tekmira's siRNA-LNP technology platform has broad utility in a number of disease indications

*Accumulated clinical safety, efficacy and GMP manufacturing expertise*

- Modular nature of the technology allows for rapid adaptation to emerging viral variants to ensure full retention of activity

*TKM-Ebola retains some activity against EBOV Makona, but  
TKM-Ebola-Guinea allows maximal antiviral activity*

- TKM-Ebola-Guinea is currently being evaluated for efficacy in a Ph 2 clinical trial led by the University of Oxford on behalf of ISARIC

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Anna Honko  
Joshua Johnson

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