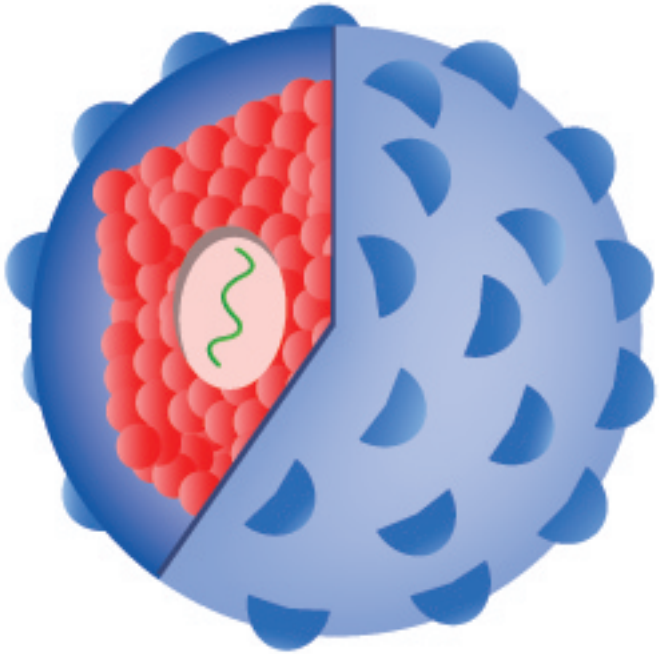

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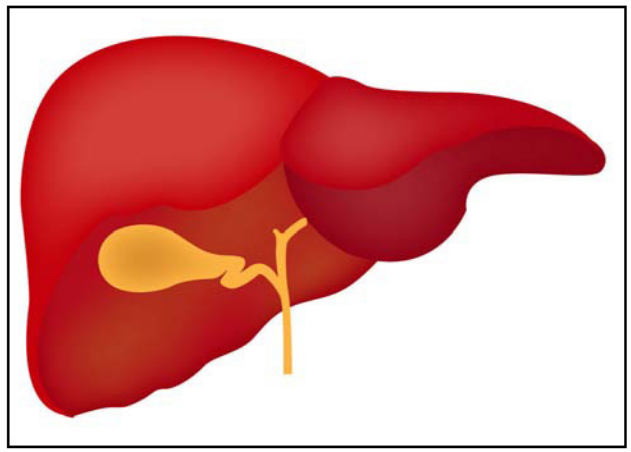
UPTAKE

A TREATMENT FOR HEPATITIS C

By
Jenny Zhou
Leanna Morinishi
Samira Daswani

Introduction

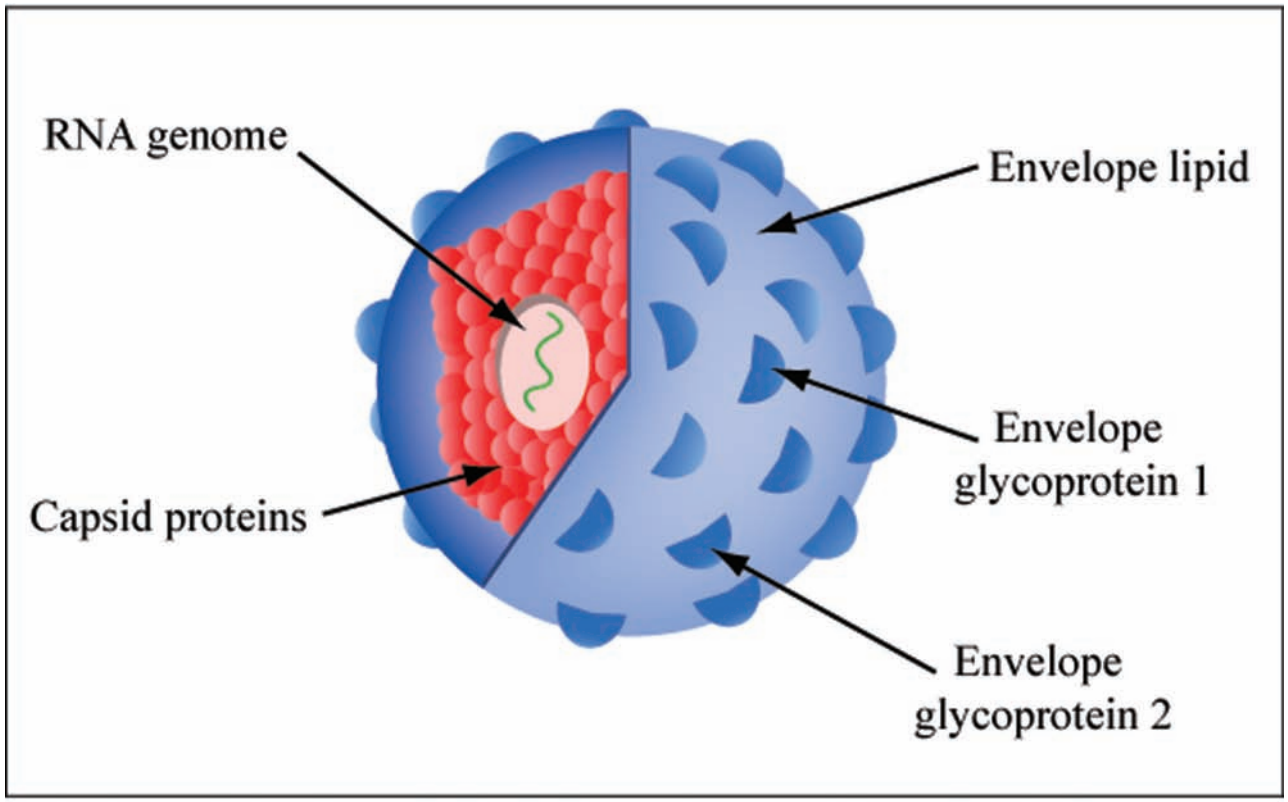
- What does the liver do?
- Hepatitis C : Infection of the liver causing cirrhosis.



Importance of disease

- WHO labeled it a "silent" epidemic.
- Affects at least 4 million people in the U.S. and 175 million people worldwide.
- HCV infects 4 times more people in the US than HIV
- Leading cause for liver cancer

What is HCV?



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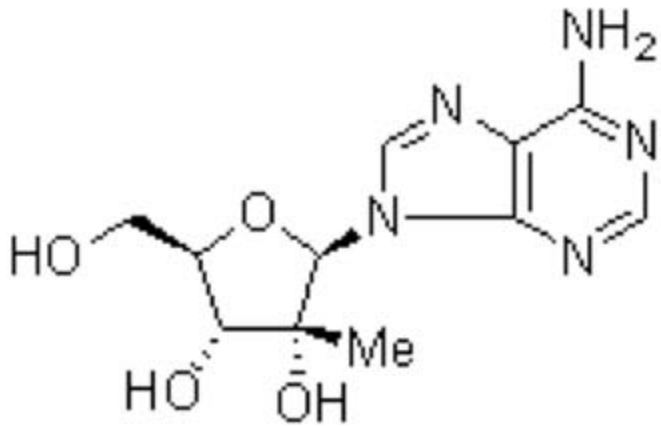
http://www.the-scientist.com/article/display/24827/

Chimp study

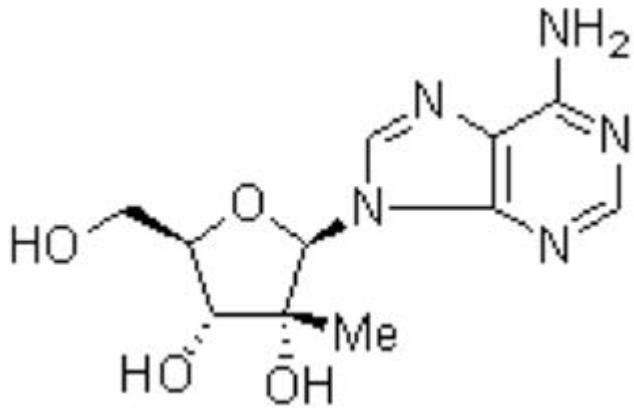
- Overview
- Nucleoside analog with robust HCV antiviral effect
- 2'C- 7- Deaza Methyladenosine
- Specifically inhibits viral RNA- dependent RNA- polymerase
- Dosage (.2-2 mg per kg/day intravenous)
- Treatment length (7 days)
- Results (significant decrease in viral load)



Why we chose this study?

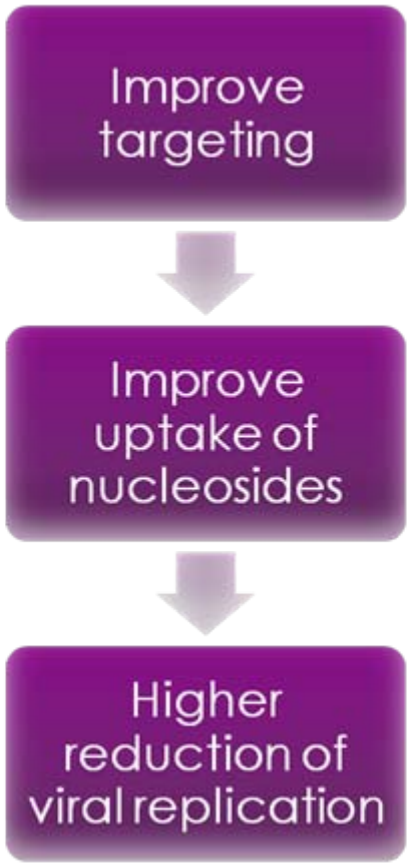
- Robust drop in viral load using non-toxic doses of nucleosides.
- Nucleoside specific to viral RNA polymerase
- Method tested in vivo in human-like species

Image courtesy of Ori2uru on Flickr.

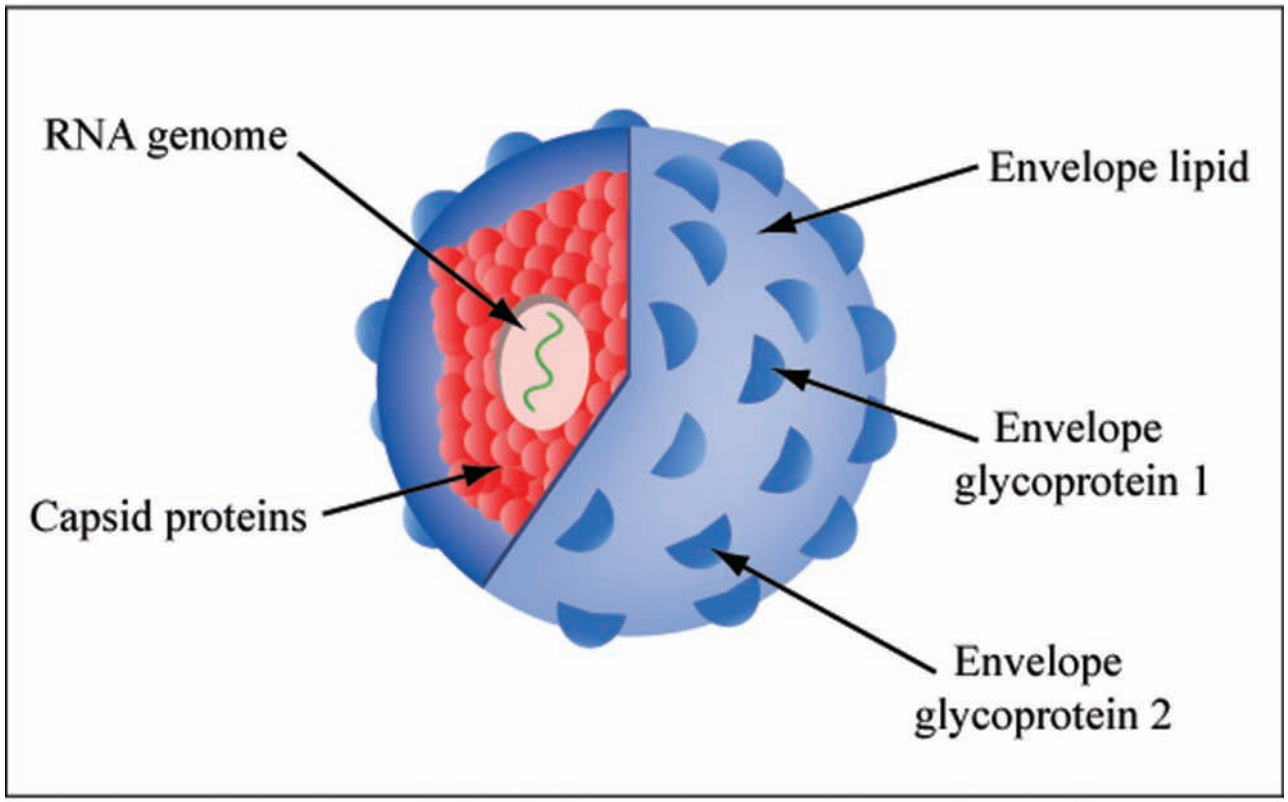


Improve nucleoside therapy

- What was wrong with the treatment?
  Low nucleosides uptake



HCV targeting mechanism:

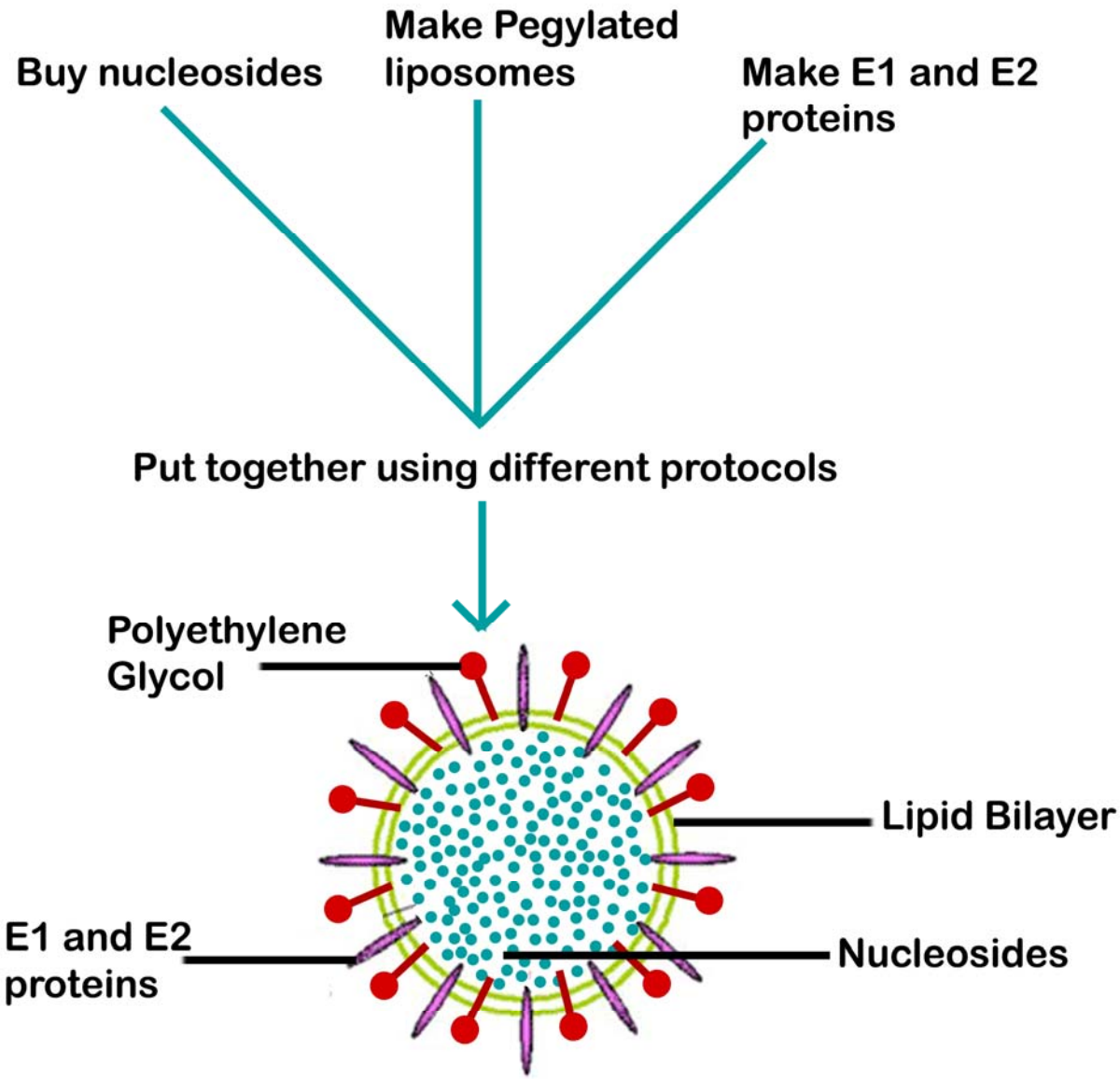


http://www.the-scientist.com/article/display/24827/

Virus targets the liver:

Image removed due to copyright restrictions.
Figure 2, "Current model for hepatitis C virus (HCV) entry."
In Moradpour, D., F. Penin, and C. M. Rice. "Replication of hepatitis C virus."
Nature Reviews Microbiology 5 (June 2007): 453-463.
DOI:10.1038/nrmicro1645

Systems diagram:



NETL:
Nucleoside
Encapsulated
Targeted
Liposomes

Figures by MIT OpenCourseWare.

Collected NETL are injected either systemically or specifically into hepatic portal artery

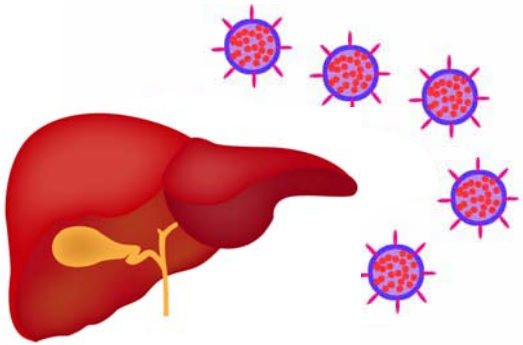
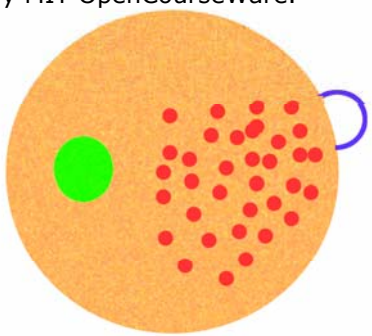
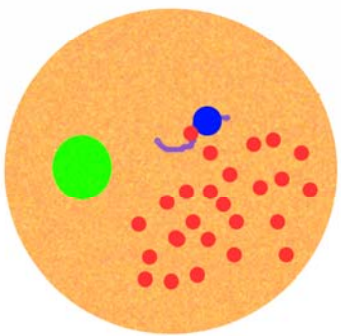


Figure by MIT OpenCourseWare.

E1 and E2 target NETL to the liver.

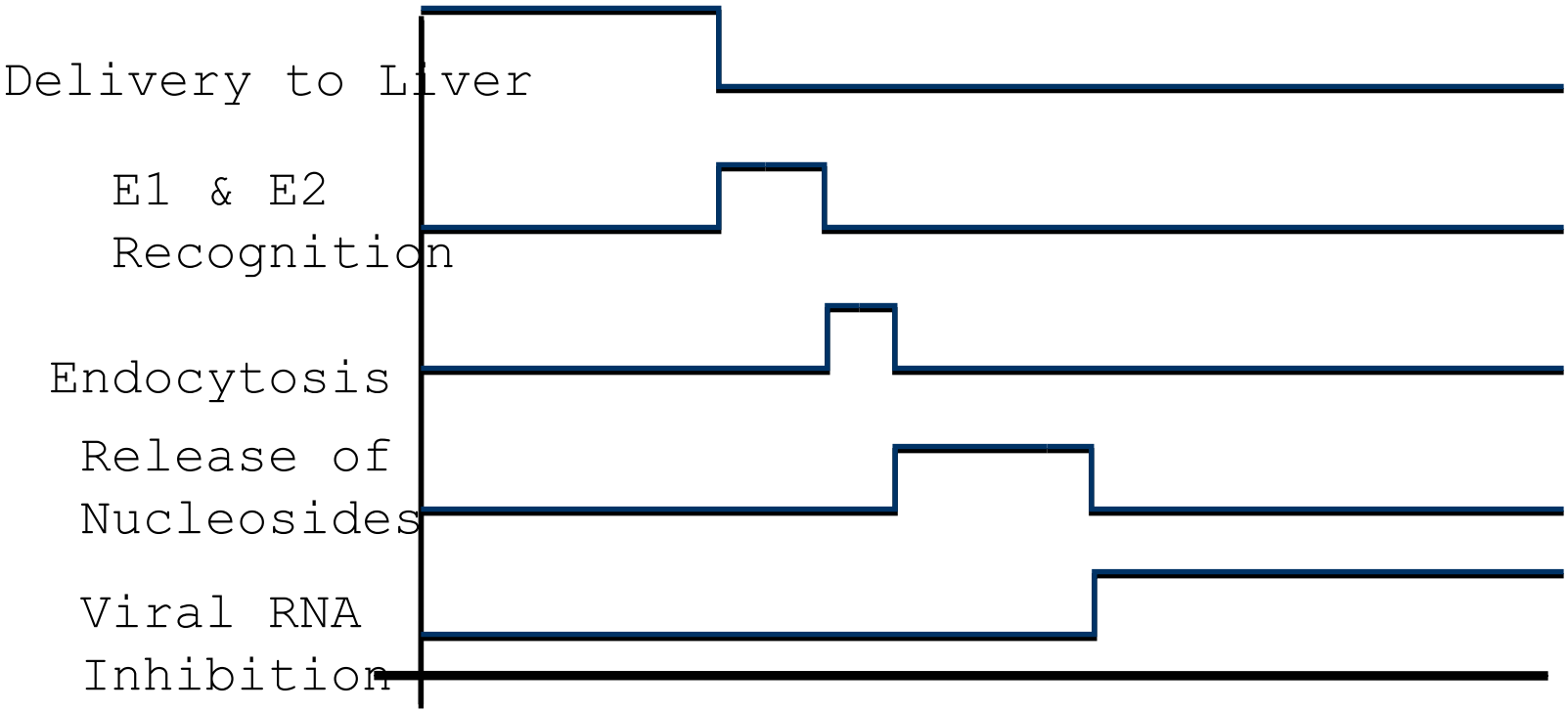


NETL endocytose into the liver cell, releasing the nucleosides.

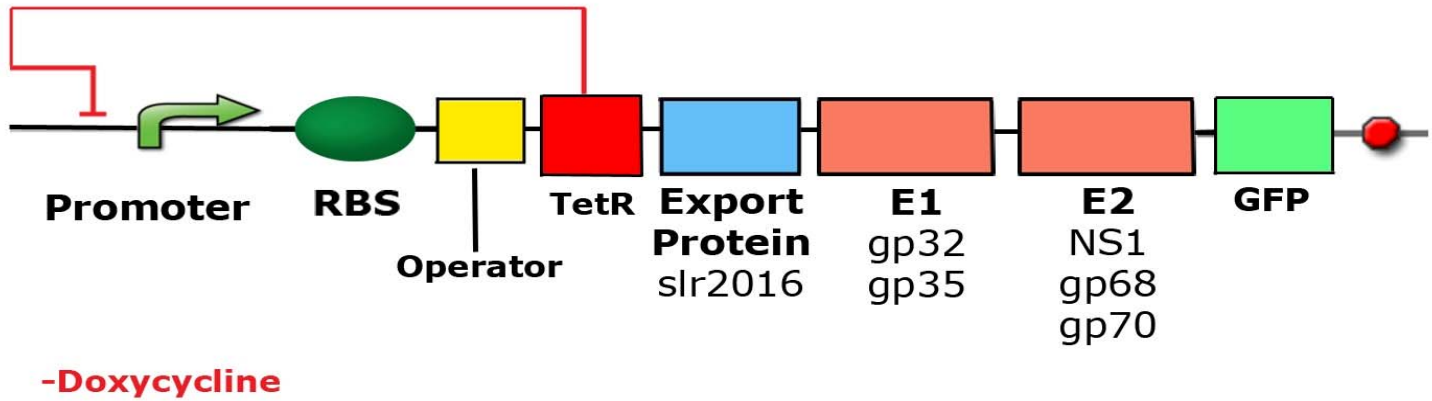


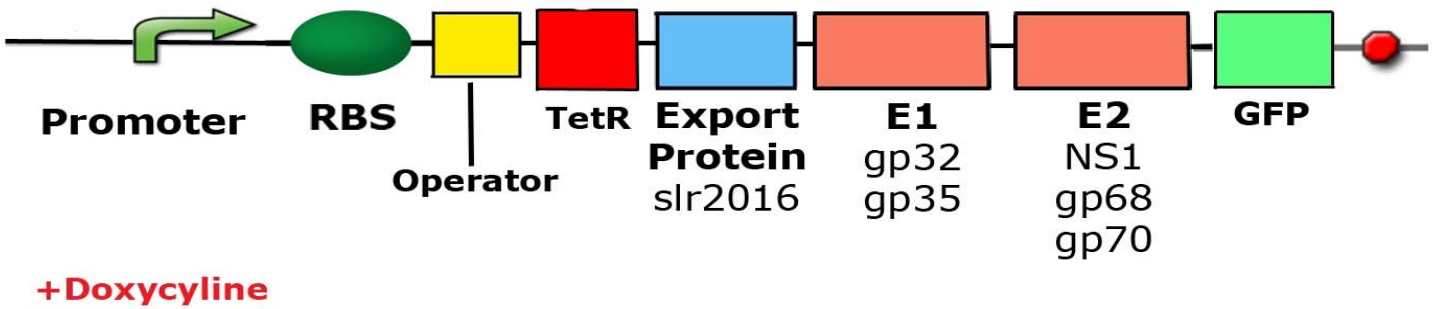
Nucleosides stop production of viruses RNA-dependent RNA polymerase preventing viral replication

Timing diagram – In Vivo



Device level diagram – E1 and E2 production





Device Diagram – Production of Liposomes

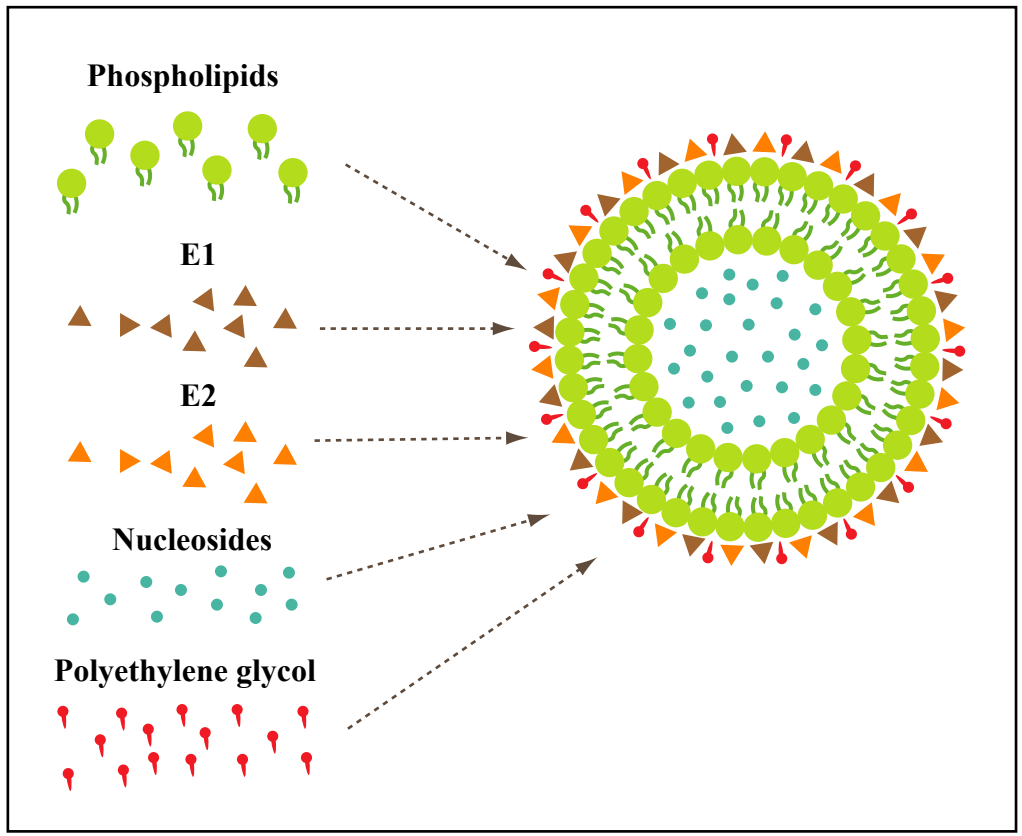


Figure by MIT OpenCourseWare.

Debug Plan

- **In Vitro:**
- Use GFP to ensure production of E1 and E2
- Validation of nucleosides – infect liver culture cells, and then use NETLs to see if they stop viral replication.

- **In Vivo:**
- Taqman assay on infected human and chimp

Projected Treatment Plan (All hypothetical)

- Will be dependent on the body mass index of human.
- For our model we picked a human with average weight ~ 60 Kg
- Human being who does not drink alcohol, with a low fat diet.

How many nucleosides would be needed?

- Use baseline of **0.2 mg/kg** weight of infected person (as seen in chimp study)
- In known dosages nucleosides are non-toxic
- Estimated number of nucleosides needed per liver cell for an average adult liver **(~10^{11} cells) = ~10^{8} nucleosides per cell**

Cost of production (R and D)

Part	Cost
Nucleosides (12mg of nucleoside per day)	$360 per 12mg dosage
• **Liposomes** (3.6 X 10^{14} Liposomes per do	
Phosopholipids	$100 per gram of Phosphatidylcholine
Sphingomyelin	$150 per gram
Cholesterol	$100 per vial
E1 and E2 glycoproteins (138 nucleotides)	$200
Liver Cell Culture	$794 for 3-6 X 10^6cells

Patient would pay …

- Estimated cost for **one-time dosage** for ~60 kg person
- Approximately **$800/day** for drug
- If 30 doses a year ~$24000
- Systemic injection vs. Hepatic Artery Infusion

Estimated length of viral load reduction

- Chimp study – injected 0.2mg/kg for 7 days the viral load dropped below the LOQ for 12 days and overall reduction for 35 days

- NETL improvement: If cell uptake increases then we can expect 2 results:
- Dosage period < 7 days with viral load reduction longer
- Dosage period = 7 days with longer time where viral load is less than LOQ

Unknowns

- How the body will react to a high influx of liposomes
- Unforeseen complications of therapy
- Number of dosages needed in vivo to stop replication
- Total cost of treatment

???

Safety and Security

- **Safety:**
- The methylated nucleosides are not toxic in the known dosages.

- **Security:**
- Design should not be available to the general public, due to potential for targeting infectious agents to the liver.

Competing technologies

- **Lipidoids**
  - Lipid like structure used for delivering RNAi therapeutics to specific organs

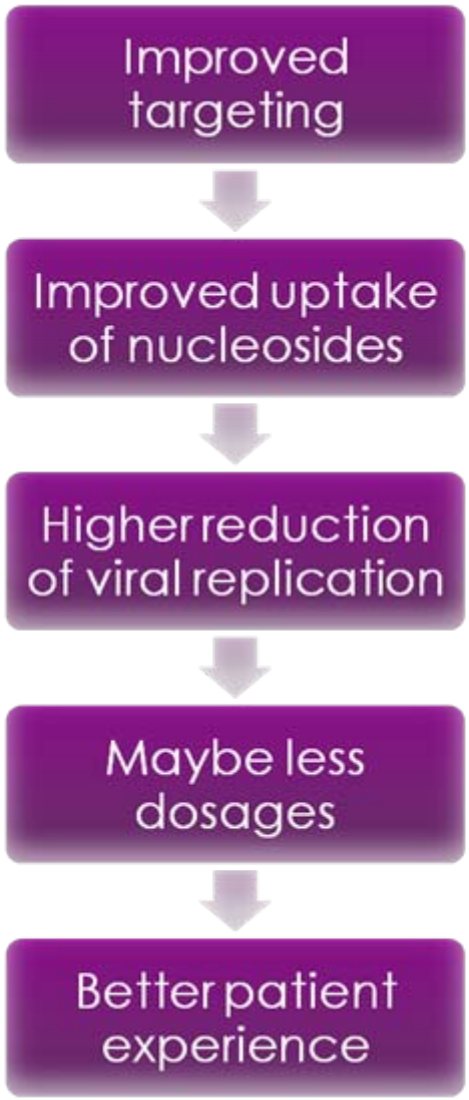
NETLs can also…

- Deliver other drugs to the liver
  - PEG-IFN-α and ribavirin
    - ~ 48 injections / year
  - New novel IFN treatments
  - Drug cocktails often more effective
    - Protease inhibitors and polymerase inhibitors

Current treatment

- **Combination therapy of pegylated interferon and ribavarin** costs ~ $30,000 / year
- With high toxicity and serious side effects

Impact of NETLs



What else we looked at:

- Using HCV capsid and lipid bilayer
- Using a different viral vector (adenovirus)
- Biosynthesis of nucleosides

Summary

- What is hepatitis C?
- Chimp study
- NETLs : Nucleoside Encapsulated Targeted Liposomes
- Improve targeting = Improved uptake of nucleoside = Higher reduction of viral replication

A big thank you to…

- Natalie Kuldell
- Rebecca Adams
- Drew Endy
- Lee Gherke
- Andrew Webb
- Brett Pellock
- Kevin Solomon
- Carmen Ng

Any
Questions?

References

- Slide 5 'Chimp Study' : Carroll et al. (March 2009). *Robust Antiviral Efficacy upon Administration of a Nucleoside Analog to Hepatitis C Virus-Infected Chimpanzees.* http://aac.asm.org/cgi/content/abstract/53/3/926.
- Slide 14 'Device Diagram – Production of Liposomes': Vladimir Torchilin and Volkmar Weissig. *Liposomes: A Practical Approach*
- Slide 23 'Competing technologies' http://www.nature.com/mt/journal/v17/n5/abs/mt200936a.html
- Slide 24 'NetIs can also…': Dorey. *Competition intensifies around hepatitis C.* Nature biotechnology
- Slide 25 'Current treatment' http://hab.hrsa.gov/tools/coinfection/barriers.html
- Hepatitis C virus image (all slide): http://www.duke.edu/web/gromlab/hcv.jpg

Extra Slides (possible questions)

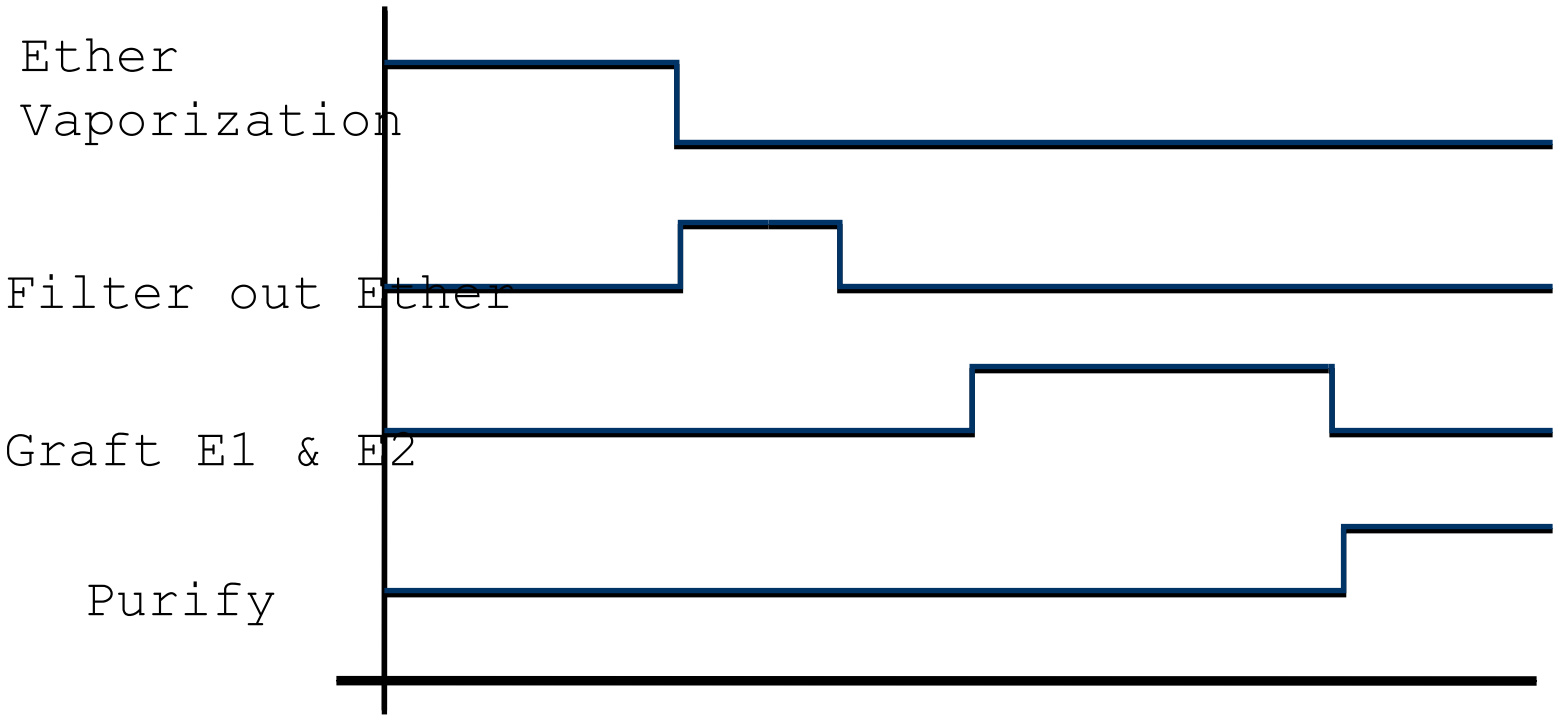
Why we rejected the biosynthetic pathway to make nucleosides?

- No known natural methylase that would methylate the 2'C of the nucleoside
- Purification of the nucleoside from an organism is difficult without chemically changing it

Why pegylated liposomes…

- Stealth liposomes
- Reduced inflammatory response from immune system
- To increase bioavailability of drugs
- They are passively targeted to inflammed tissue

Timing Diagram for protocols



How calculations were done for cost?

Patient cost: cost of nucleosides + liposomes – Liver culture + $100 for equipment

Company selling the nucleosides

- Carbosynth

Different protocols used maybe for debugging

- Equipment and reagents:
- Membrane Filters
- PEG with a molecular weight of 1-10 kDa
- Succinic anhydride, chloroform
- DPPE, DCC
- Ethanol, diethyl ether
- Method:
- 1. To prepare PEG-succinate, mix 0.75 mmol of PEG with 0.75 mmol of succinic anhydride in 20 ml distilled chloroform and react overnight.
- 2. Dissolve 0.75 mmol of DPPE and 0.85 mmol of DCC in 20 ml chloroform containing 0.75 mmol of PEG-succinate
- 3. React overnight at 50 C
- 4. Evaporate the chloroform, redissolve the residue in 30 ml of ethanol, and filter.
- 5. Re-precipitate the product with diethyl ether and dry in vacuum.
- 6. Disperse the product in 30 ml of distilled water and filter through a 0.2 microm membrane filter.
- 7. Freeze-dry the filtrate to obtain white powder of PEG-PE.

20.020 Introduction to Biological Engineering Design

Spring 2009