A Single Dose of RG-101, a GalNAc-Conjugated Oligonucleotide Targeting miR-122, Results in Undetectable HCV RNA Levels in Chronic Hepatitis C Patients at Week 28 of Follow-up

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No conflict of interest.
NS5A inhibitors

miR-122 inhibitors

Adapted from Manns et al, Nature Reviews Drug Discovery;12;595–610;2013
Hepatitis C Virus and miR-122

**Introduction**

**miR-122**
- Highly conserved liver-specific miRNA
- Key regulator of cholesterol and fatty-acid synthesis
- Important host factor for hepatitis C virus replication

**miR-122 and HCV RNA**
- 5’ UTR contains two highly conserved miR-122 binding sites (S1 and S2)
- miR-122 binding promotes HCV RNA stability and accumulation, and protects the HCV genome from degradation

2. Esau et al, *Cell Metab* 2006
5. Lanford et al, *Science* 2010
7. Sedano et al, *Cell Host Microbe* 2014
RG-101 Targets miR-122

**RG-101**

- Oligonucleotide inhibitor of miR-122 that is linked to GalNAc carbohydrate
- GalNAc binds the asialoglycoprotein receptor expressed by hepatocytes
- Increased potency (~20-fold) compared to non-conjugated oligonucleotide
Study Objectives

Primary
- Safety and tolerability of administration of a single subcutaneous dose of RG-101 to patients with a chronic hepatitis C virus infection

Secondary
- Pharmacokinetic profile of RG-101
- Antiviral effect of RG-101

Exploratory
- Cytokines/chemokines kinetics
- Viral sequencing of 5’UTR HCV RNA
Key Inclusion/Exclusion Criteria

**Inclusion criteria**
- Male or female, 18-65 years old
- Chronic HCV infection, genotype 1, 3 or 4
- Treatment-naive or relapsed after IFN based therapy

**Exclusion criteria**
- HBV or HIV co-infection
- Cirrhosis Child-Pugh B or C
- Other causes of liver disease
- History of hepatocellular carcinoma
Study Design

**RG-101**
Single s.c. dose

**Main study**

- **Randomized**
  - n=32

  - 2 mg/kg
    - n=16
  - 4 mg/kg
    - n=16

**Extended follow-up**

- **Extended FU**
  - n=10
  - n=12

**Study visit every 4 weeks**

- **W0**
- **W1**
- **W2**
- **W3**
- **W4**
- **W5**
- **W8**
- **W28**

**Exclusion**
- Placebo dosed patients
- Viral rebounders (> 1 log increase in HCV RNA from nadir)

**Inclusion**
Patients with HCV RNA:
- > 2 log decrease from baseline AND
- < 1 log increase from nadir

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## Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>2 mg/kg RG-101 (n=14)</th>
<th>4 mg/kg RG-101 (n=14)</th>
<th>Placebo (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) - mean ± SD</td>
<td>49 ± 8</td>
<td>52 ± 8</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Male - no.</td>
<td>13</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Weight (kg) - mean ± SD</td>
<td>84 ± 16</td>
<td>84 ± 25</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>Log_{10} HCV RNA level - mean ± SD</td>
<td>6.2 ± 0.5</td>
<td>6.2 ± 0.4</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>Treatment naive - no</td>
<td>8</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Ethnicity – no.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• White</td>
<td>13</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>• Asian</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>• Other</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HCV genotype – no.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 1</td>
<td>9</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>• 3</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>• 4</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Stage of fibrosis(^a) – no.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• F0-F1</td>
<td>10</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>• F2-F3</td>
<td>4</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>• F4</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

\(\text{a. Stage of fibrosis was determined by liver elastography (Fibroscan)}\)
### Adverse Events

> 10% of incidence in patients dosed with RG-101

<table>
<thead>
<tr>
<th></th>
<th>Main study</th>
<th>Extended Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n=4)</td>
<td>2 mg/kg RG-101 (n=14)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>8</td>
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<tr>
<td>Injection site reaction</td>
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<td>4</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Emotional disorder</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Back pain</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Headache</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Skin irritation</td>
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<td>1</td>
</tr>
<tr>
<td>Muscle spasms</td>
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<td>0</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

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Safety

Overall
• Single injection of RG-101 was well tolerated in 28 HCV patients
• AE’s were mostly mild and transient; no SAE’s or study discontinuations
• One patient had severe intrahepatic cholestasis related to alcohol abuse after a single dose of 4 mg/kg RG-101

Laboratory values
• No clinically significant changes in hematological and renal laboratory values
• Decrease in mean ALT and AST levels to a normal range

Cytokine/chemokine levels
• Decline in circulating IP-10 levels
• Other cytokines and chemokines did not differ between patients dosed with RG-101 and placebo

1. See poster #2263, F. Stelma et al.
Pharmacodynamics
Markers of miR-122 inhibition

- Alkaline phosphatase levels increased in patients dosed with RG-101 (direct effect of miR-122 inhibition)
- Cholesterol levels decreased in patients dosed with RG-101 (indirect effect of miR-122 inhibition)

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Substantial Decrease in HCV RNA Levels

Week 4

Change in HCV RNA from baseline +/- SEM (log10 IU/mL)

Placebo 2 mg/kg 4 mg/kg

Mean change from baseline: 0.0  - 4.1  - 4.8

P < 0.0001

P < 0.0001

ns
Undetectable HCV RNA Levels at Week 28

Main study

Extended follow-up

2 mg/kg RG-101

4 mg/kg RG-101

HCV RNA TND (n=3)

HCV RNA TND at week 28:
- GT 1 (n=1)
- GT 3 (n=2)
- GT 4 (n=3)
Sequencing of HCV RNA 5’ UTR

Samples

- Baseline and at time of viral rebound

Method

- RNA isolation from plasma
- 5’ RACE cDNA synthesis (HCV RNA > 20,000 IU/mL)
- Population sequencing

Figure adapted from Machlin et al, PNAS. 2011, PMID: 21220300
Sequencing Results

- No baseline mutation in binding sites (S1, S2 and additional base pair interactions)
- In 11/19 patients with viral rebound “matched” sequence available
  - In 4/11 patients no mutation in miR-122 binding sites
  - In 5/11 patients C3U mutation (GT 1)
  - In 2/11 patients C2G+C3U mutation and selection of A1G+U4A polymorphisms (GT 3 and 4)
Conclusions

Safety

- Single dose RG-101 was safe and well tolerated in HCV patients
- AE’s were generally mild and transient; no SAE and discontinuations

Antiviral effect

- Viral load reductions observed in HCV genotype 1, 3 and 4 patients
- 6 patients had undetectable HCV RNA levels 28 weeks after a single dose RG-101
- Mutation(s) in miR-122 binding site in patients with virological rebound ≤ week 16

Ongoing studies

- Replicon assay to assess viral fitness of mutants
- T- and NK-cell analyses
- Plasma miRNA profiling
- Phase II study to combine RG-101 with DAA’s
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