Effects of Sofosbuvir/Ribavirin Treatment and ITPA Phenotype on Endogenous Purines

Leah C. Jimmerson, Carolyn W. Clayton, Samantha MaWhinney, Eric G. Meissner, Zayani Sims, Shyamasundaran Kottilil and Jennifer J. Kiser
ATP reduction and anemia

- The most common adverse effect from RBV treatment is hemolytic anemia
- Several *in-vitro* and *ex-vivo* studies have shown that ATP is reduced in erythrocytes as a result of RBV treatment*
- Decreased ATP leads to oxidative stress of the cell causing membrane damage and eventual lysis

*De Franceschi, Fattovich et al. 2000, Grattagliano, Russmann et al. 2005, Hitomi, Cirulli et al. 2011, Karasawa, Saito et al. 2013*
Involvement of other purines

- Rescues the production of ATP
Anemia and ITPA activity

- Low ITPA activity has been associated with less incidence of anemia

<table>
<thead>
<tr>
<th>rs1127354</th>
<th>rs7270101</th>
<th>ITPA activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (C/C)</td>
<td>Wild type (A/A)</td>
<td>100</td>
</tr>
<tr>
<td>Wild type (C/C)</td>
<td>Heterozygosity</td>
<td>60</td>
</tr>
<tr>
<td>(C/A)</td>
<td>(A/C)</td>
<td></td>
</tr>
<tr>
<td>Heterozygosity</td>
<td>Wild type (A/A)</td>
<td>30</td>
</tr>
<tr>
<td>(C/A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type (C/C)</td>
<td>Homozygosity</td>
<td>30</td>
</tr>
<tr>
<td>(C/C)</td>
<td>(C/C)</td>
<td></td>
</tr>
<tr>
<td>Heterozygosity</td>
<td>Heterozygosity</td>
<td>10</td>
</tr>
<tr>
<td>(C/A)</td>
<td>(A/C)</td>
<td></td>
</tr>
<tr>
<td>Homozygosity</td>
<td>Wild type (A/A)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>(A/A)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

100%=WT (low ITP)  
≤60%=non-WT (high ITP)

These genotypes have a lower ITPA= increased ITP in RBCs

Thompson, Fellay et al. 2010
Objective:

Determine the effect of ribavirin+sofosbuvir treatment and ITPA activity on levels of ATP, GTP and ITP in red blood cells (RBCs)
Study design

50 subjects enrolled with all stages of liver disease; samples from 47 of the subjects obtained for this analysis

- **Sofosbuvir (SOF) 400 mg + 1000-1200 mg RBV (n=25)**
- **Sofosbuvir (SOF) 400 mg + 600 mg RBV (n=22)**

1:1 randomization

Follow up 48 weeks

Study day: 0 3 28 84 168 336

- Whole blood was collected on these days

Osinusi A et al. JAMA. 2013;310(8):804-11
Methods

Analytical quantification:
- An LC-MS/MS method was developed and validated for quantification of ATP, GTP and ITP with various ranges
- RTP measured using similar validated method*

Statistical modeling
- Data (pmol/10^6 cells) was log transformed
- Mixed effects regression used due to repeated measures
- Longitudinal outcomes: ATP, GTP and ITP over time
- Predictors: RTP concentration, ITPA status (WT vs non-WT), RTP interaction with ITPA status
  - Latter allowed RTP to vary between ITPA groups

## Patient Demographics

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>N=47</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ITPA activity (%)</strong></td>
<td>26% w/\leq 60% activity (non-WT)</td>
</tr>
<tr>
<td></td>
<td>74% w/100% activity (WT)</td>
</tr>
<tr>
<td><strong>Sex (%)</strong></td>
<td>66% M</td>
</tr>
<tr>
<td><strong>Race (%)</strong></td>
<td>81% black, 19% white/other</td>
</tr>
<tr>
<td><strong>Fibrosis stage (%)</strong></td>
<td>F0-2: 70%</td>
</tr>
<tr>
<td></td>
<td>F3-4: 30%</td>
</tr>
<tr>
<td><strong>ΔHgb ≥ 3.0 g/dL (%)</strong></td>
<td>19.1%</td>
</tr>
<tr>
<td><strong>HCV genotype (%)</strong></td>
<td>74% 1a</td>
</tr>
<tr>
<td></td>
<td>26% 1b</td>
</tr>
<tr>
<td><strong>Age, years mean (SD)</strong></td>
<td>54 (9.0)</td>
</tr>
<tr>
<td><strong>Weight (kg) mean (SD)</strong></td>
<td>90 (20.8)</td>
</tr>
</tbody>
</table>
Hypothesis 1: ATP, GTP and ITP will be decreased over time on RBV/SOF
ATP

• ATP is significantly decreased over time

103 (35.1) -35% (p<0.0001)

88.3 (24.8)

87.9 (22.9) -39% (p<0.0001)
GTP

- GTP not significantly changed per week on study.
- 0.51% change, P=0.47
ITP

- No significant decrease of ITP per week on treatment.
- -2.2% change, $p=0.08$
Hypothesis 2: The effect of RTP on ATP, GTP and ITP levels will differ by ITPA status

- Non-WT = LOW (≤60%) ITPA ACTIVITY

Recall in vitro data indicated ITP is higher in **non-WT** and can be used for this reaction if GTP is depleted.
Looking at the interaction between RTP and ITPA status allows the effect of RTP to vary for WT and non-WT.

<table>
<thead>
<tr>
<th>Subject</th>
<th>D84* % change</th>
<th>CI %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTP*ITPA WT vs non-WT</td>
<td>--</td>
<td>-18.6, -29.4</td>
<td>0.006</td>
</tr>
<tr>
<td>ITPA WT</td>
<td>89.2</td>
<td>-9.6%, -20%, 2.9%</td>
<td>0.13</td>
</tr>
<tr>
<td>ITPA non-WT</td>
<td>66.3</td>
<td>-29.4%, -39.9%, -17.1%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*considering a RTP conc of 120 pmol/10^6 cells
### GTP

<table>
<thead>
<tr>
<th>Effect</th>
<th>% Change</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTP*ITPA non-WT vs WT</td>
<td>−15.0</td>
<td>−26, -2.3</td>
<td>0.02</td>
</tr>
<tr>
<td>RTP on ITPA WT</td>
<td>16.5</td>
<td>3.3, 31</td>
<td>0.01</td>
</tr>
<tr>
<td>RTP on ITPA non-WT</td>
<td>−0.995</td>
<td>−13, 12</td>
<td>0.88</td>
</tr>
</tbody>
</table>

**Graph:**
- **Time (days):** 0, 3, 28, 84
- **p mol/10^6 cells:**
  - **ITPA WT**
  - **ITPA non-WT**

- **Legend:**
  - O: ITPA WT
  - ◆: ITPA non-WT
**ITP**

The image shows a graph with data points indicating changes in fmoles/10^6 cells over time (days) for two groups: ITPA WT and ITPA non-WT. The graph includes a table summarizing the effects of RTP on ITPA WT and non-WT, as well as baseline comparisons and interactions involving RTP and ITPA non-WT.

<table>
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<tr>
<th>Effect</th>
<th>% change</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTP on ITPA WT</td>
<td>22.7</td>
<td>−1.2, 52.4</td>
<td>0.06</td>
</tr>
<tr>
<td>RTP on ITPA non-WT</td>
<td>1.5</td>
<td>−18.4, 26.2</td>
<td>0.89</td>
</tr>
<tr>
<td>Baseline ITPA non-WT vs WT</td>
<td>49.1</td>
<td>−9.8, 147</td>
<td>0.12</td>
</tr>
<tr>
<td>RTP*ITPA non-WT vs WT</td>
<td>−17.3</td>
<td>−35.1, 5.5</td>
<td>0.13</td>
</tr>
</tbody>
</table>

- RTP effect was not significantly different between ITPA groups.
Conclusions

- **ATP**
  - -35.1% decrease at D28 and -38.6% at D84 compared to baseline
  - ITPA non-WT subjects had a larger decrease in ATP (-29.4% vs -9.6% in WT)

- **GTP**
  - No significant change over time from baseline (p=0.47)
  - GTP was higher in WT subjects when allowing RTP to vary (p=0.02) but did not affect overall change during treatment

- **ITP**
  - No significant difference over time (p=0.08) or difference of RTP effect (p=0.13) between ITPA groups
  - ITP was not significantly higher in ITPA non-WT subjects, though some had extreme values compared to WT subjects
Discussion/limitations

- ITPA non-WT subjects had a larger decrease in ATP compared to WT
  - Non-WT subjects have significantly higher RTP levels which may compete with ATP*
  - RTP uses ATP for phosphorylation so this may cause more depletion in the non-WT group
- GTP was not significantly changed over time
  - Similar effect of RTP on WT GTP levels being higher

*Jimmerson et al., J Clin Pharmacol [Accepted].
Discussion/limitations

- ITPA non-WT subjects had a larger decrease in ATP compared to WT
  - Non-WT subjects have higher RTP levels which may compete with ATP*
  - RTP uses ATP for phosphorylation so this may cause more depletion in the non-WT group
- GTP was not significantly changed over time
  - Similar effect of RTP on WT GTP levels being higher
- Little effect on ITP levels despite ITPA status
  - Low concentration of ITP in RBCs
  - Only had 12 subjects with non-WT activity
  - Larger variability in the data

*Jimmerson et al., J Clin Pharmacol [Accepted].
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