Abstract #017

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

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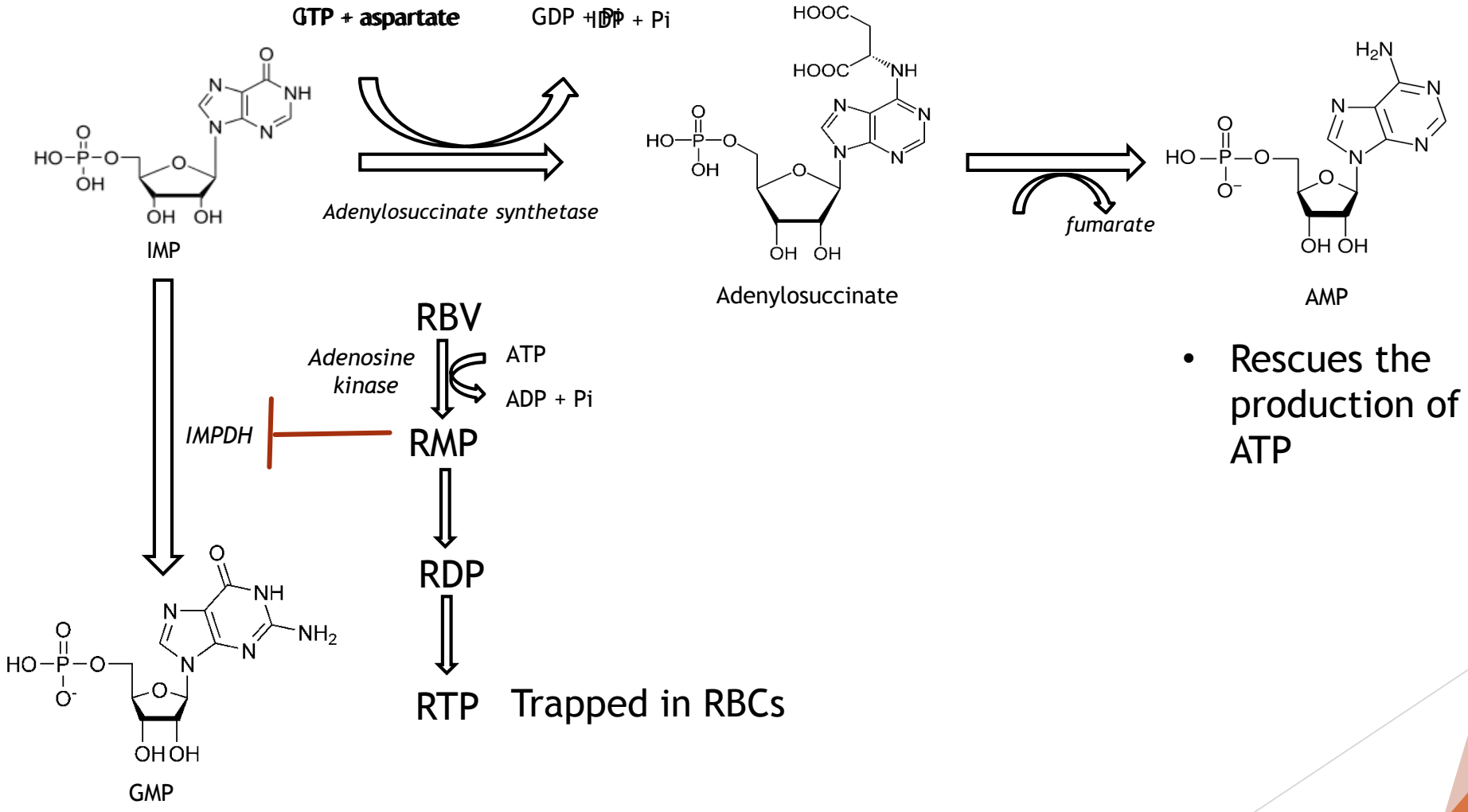
ATP reduction and anemia

- 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

Hitomi, Cirulli et al. 2011

Anemia and ITPA activity

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

rs1127354	rs7270101	ITPA activity (%)
Wild type (C/C)	Wild type (A/A)	100
Wild type (C/C)	Heterozygosity (A/C)	60
Heterozygosity (C/A)	Wild type (A/A)	30
Wild type (C/C)	Homozygosity (C/C)	30
Heterozygosity (C/A)	Heterozygosity (A/C)	10
Homozygosity (A/A)	Wild type (A/A)	<5

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

These genotypes have a lower ITPA= increased ITP in RBCs

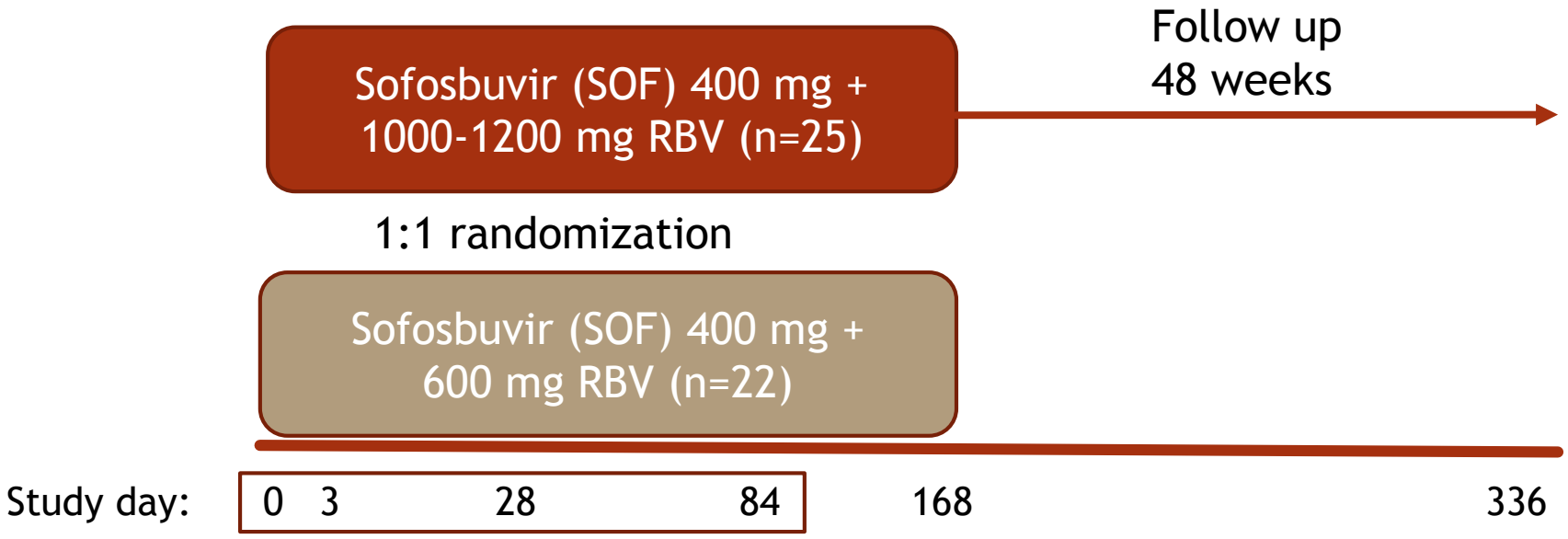
Thompson, Fellay et al. 2010

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



- 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

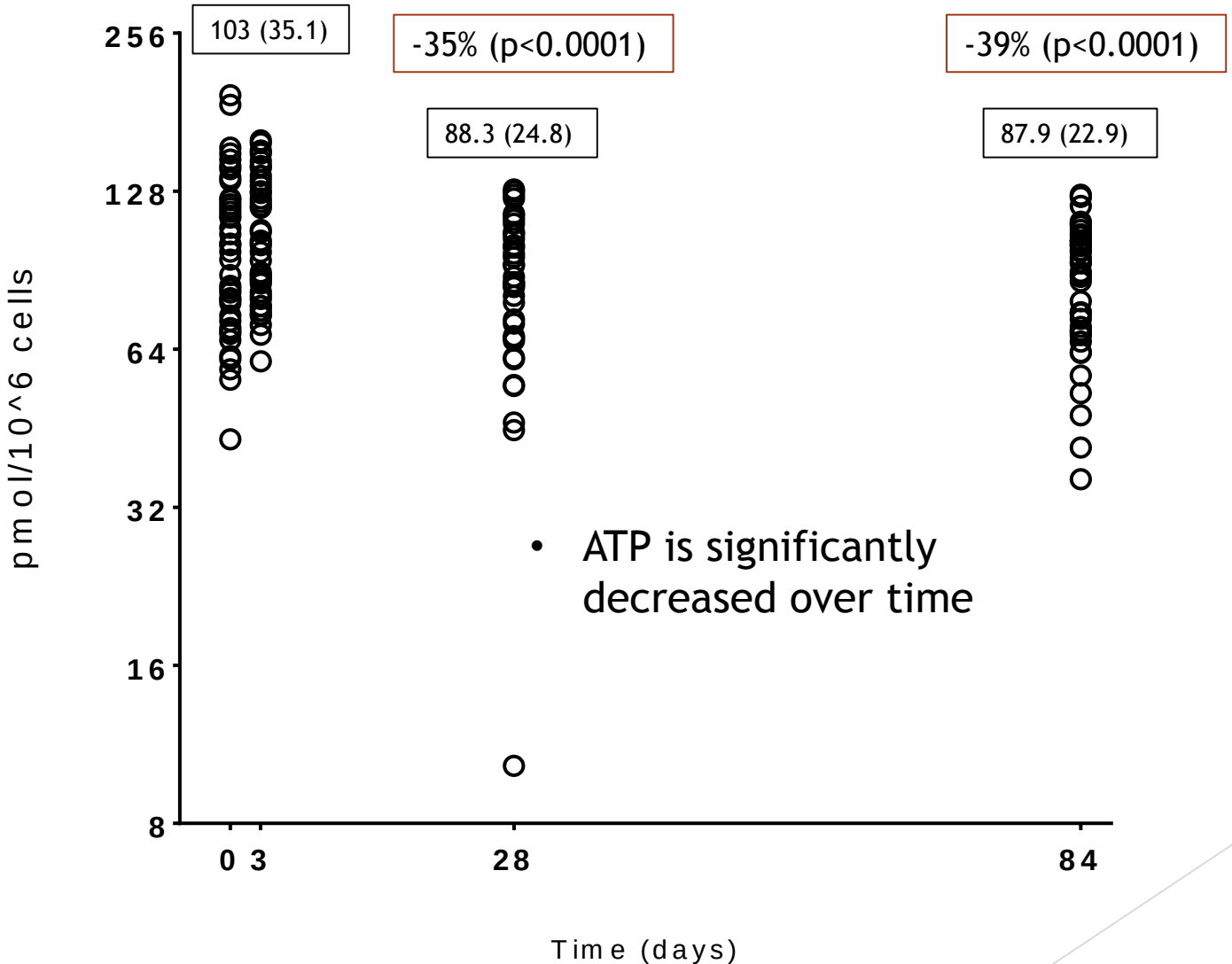
*Jimmerson, L. C., 2015. J Chromatogr B Analyt Technol Biomed Life Sci **978-979**: 163-172.

Patient Demographics

Clinical Data	N=47
ITPA activity (%)	26% w/≤ 60% activity (non-WT) 74% w/100% activity (WT)
Sex (%)	66% M
Race (%)	81% black, 19% white/other
Fibrosis stage (%)	F0-2: 70% F3-4: 30%
ΔHgb ≥ 3.0 g/dL (%, D0-D84)	19.1%
HCV genotype (%)	74% 1a 26% 1b
Age, years mean (SD)	54 (9.0)
Weight (kg) mean (SD)	90 (20.8)

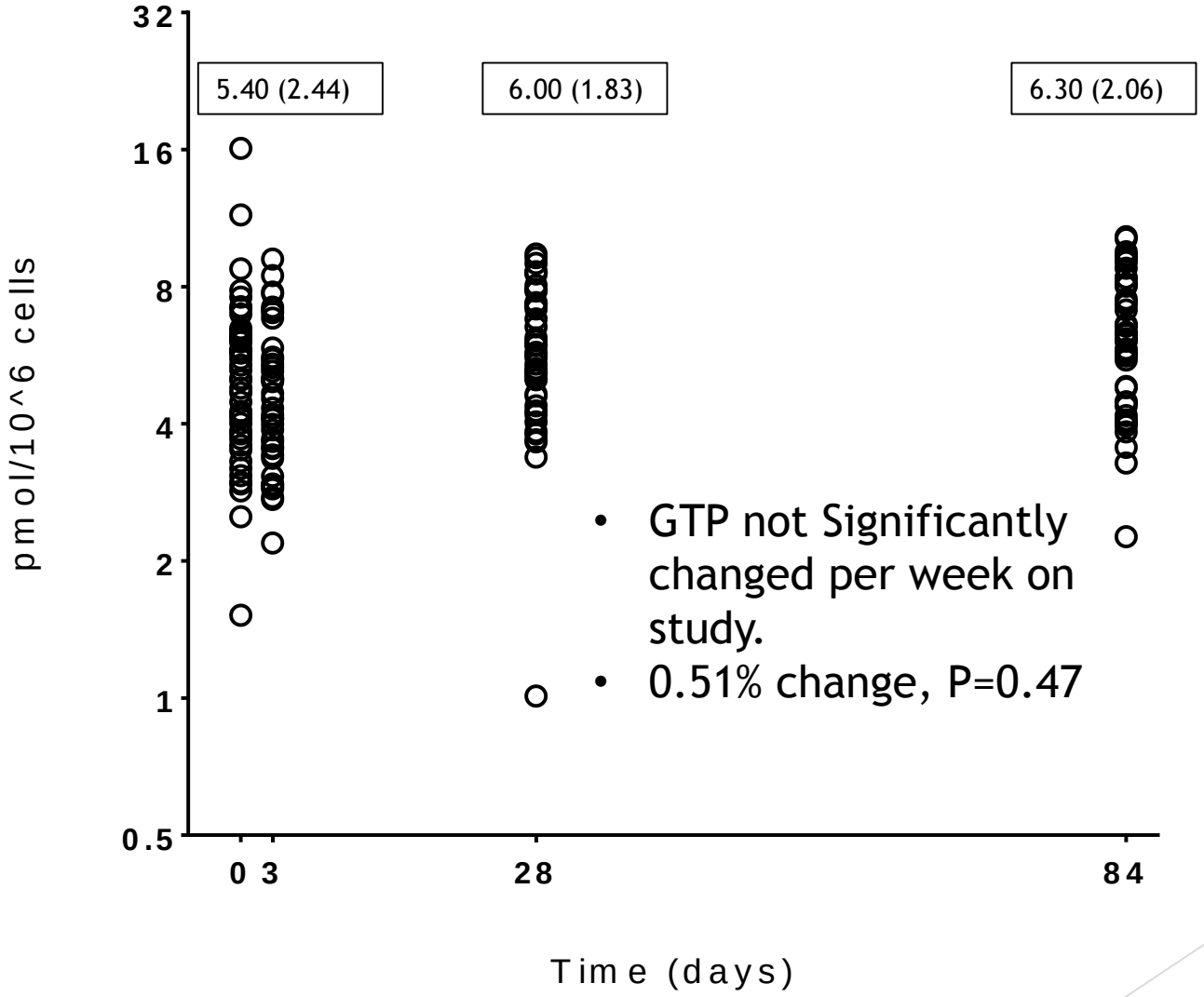
Hypothesis 1: ATP, GTP and ITP will be decreased over time on RBV/SOF

ATP



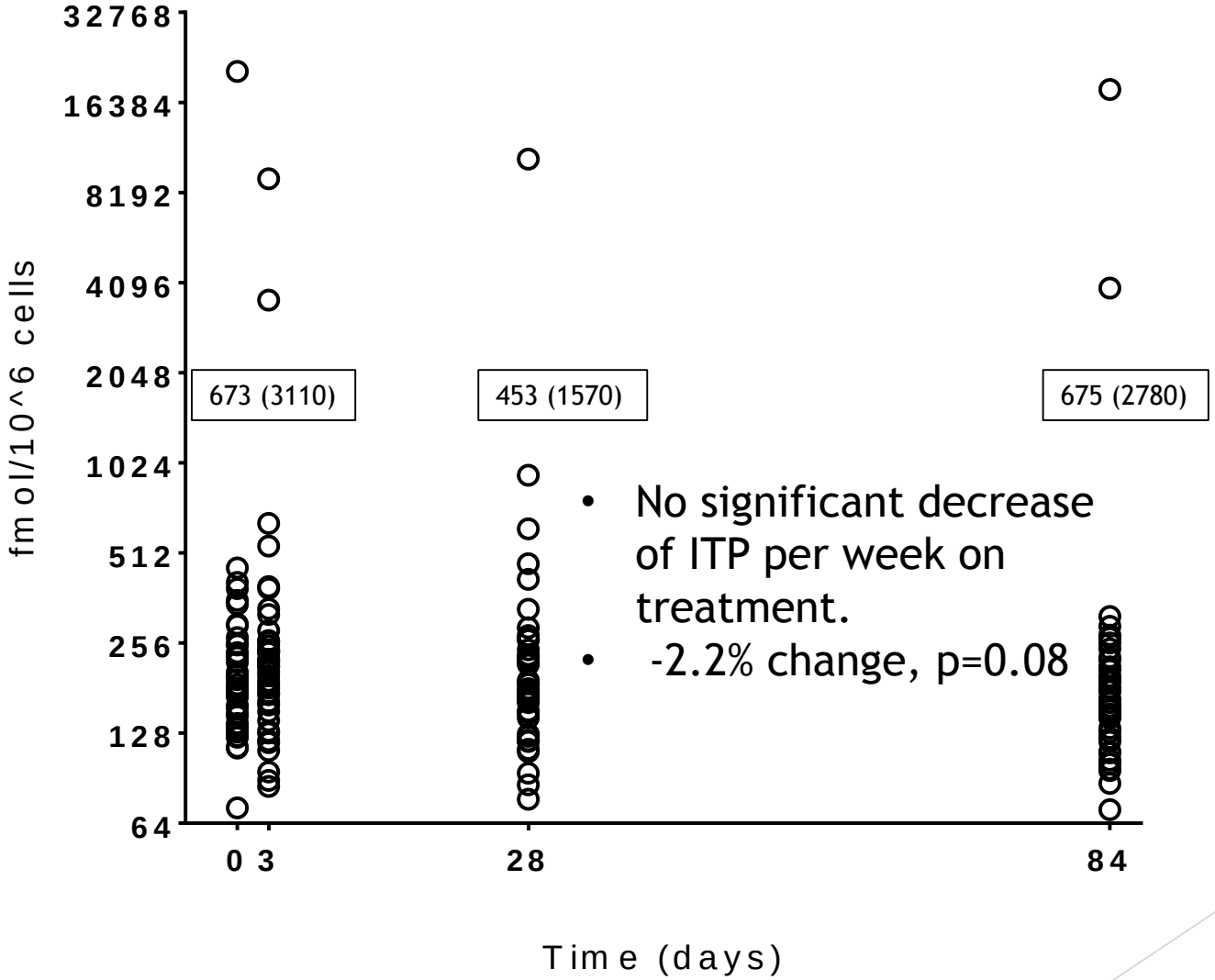
- ATP is significantly decreased over time

GTP



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

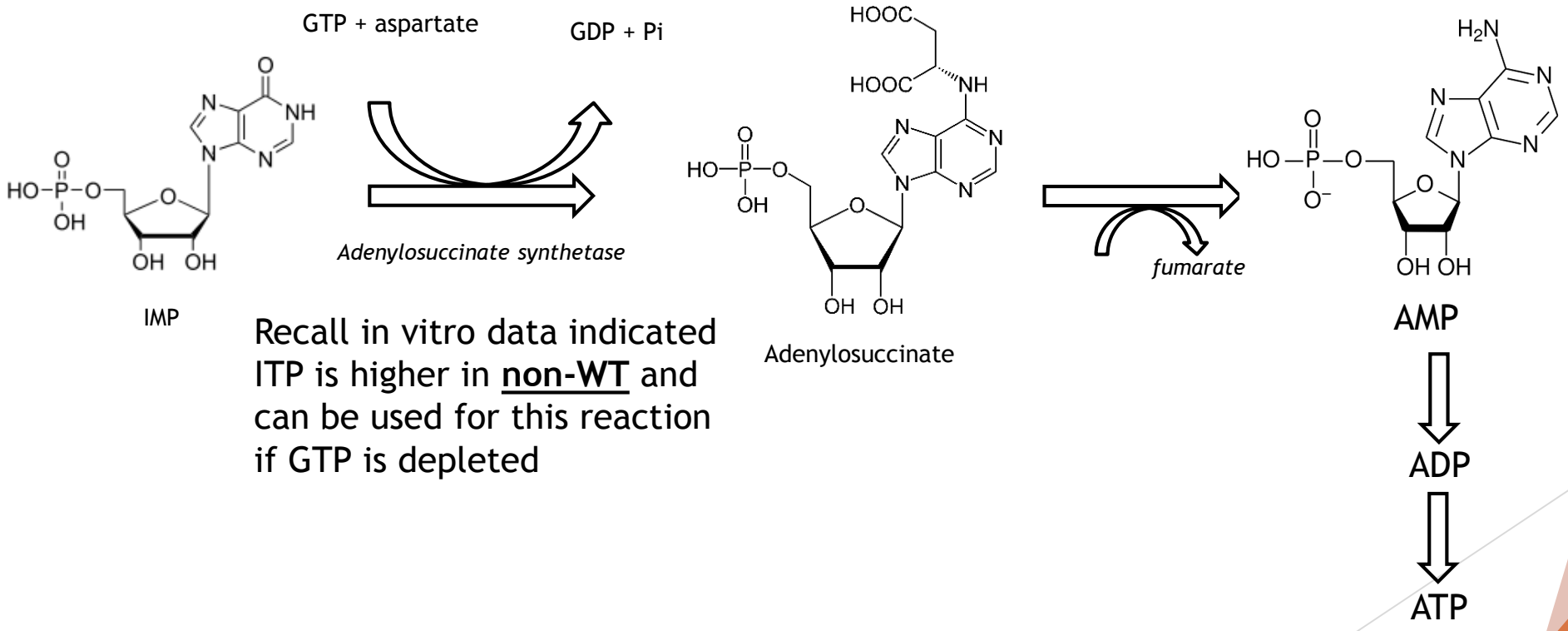
ITP



- 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

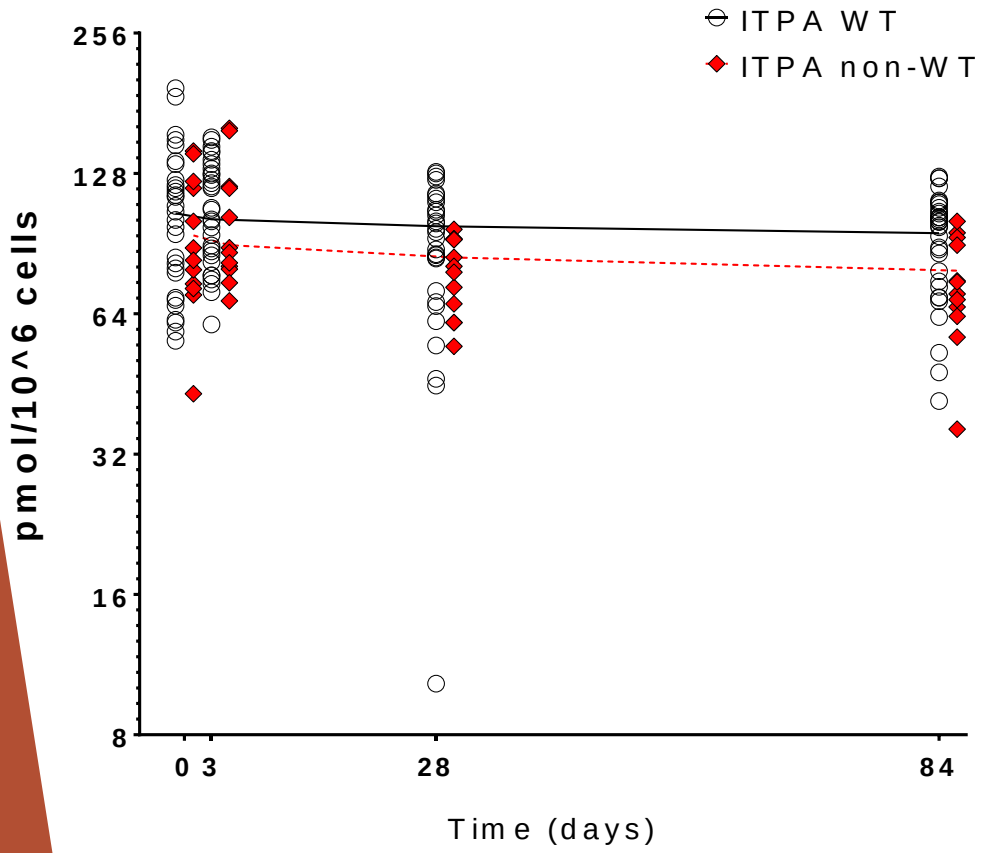
- Non-WT= LOW (≤60%) ITPA ACTIVITY



Recall in vitro data indicated ITP is higher in **non-WT** and can be used for this reaction if GTP is depleted

ATP

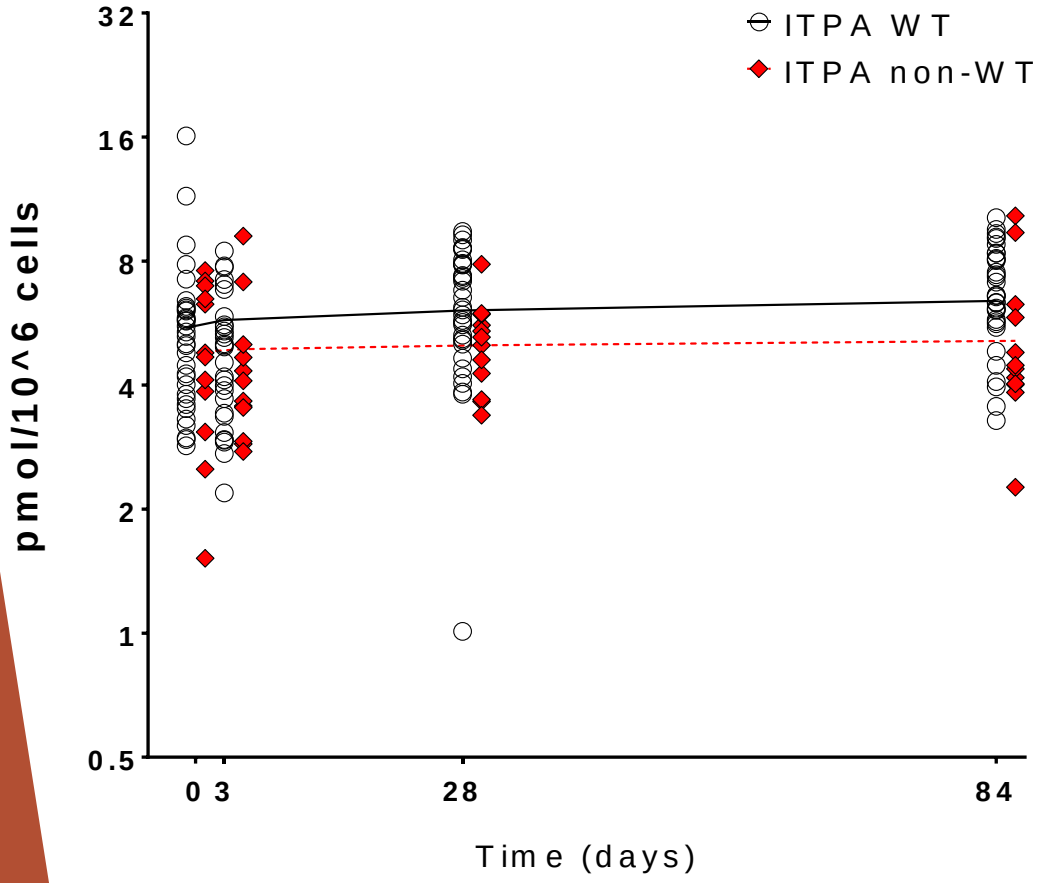
- Looking at the interaction between RTP and ITPA status allows the effect of RTP to vary for WT and non-WT



Subject	D84*	% change	CI %	P-value
RTP*ITPA WT vs non-WT	--	−18.6	−29.4, -6.1	0.006
ITPA WT	89.2	-9.6%	-20%, 2.9%	0.13
ITPA non-WT	66.3	-29.4%	-39.9%,-17.1%	<0.001

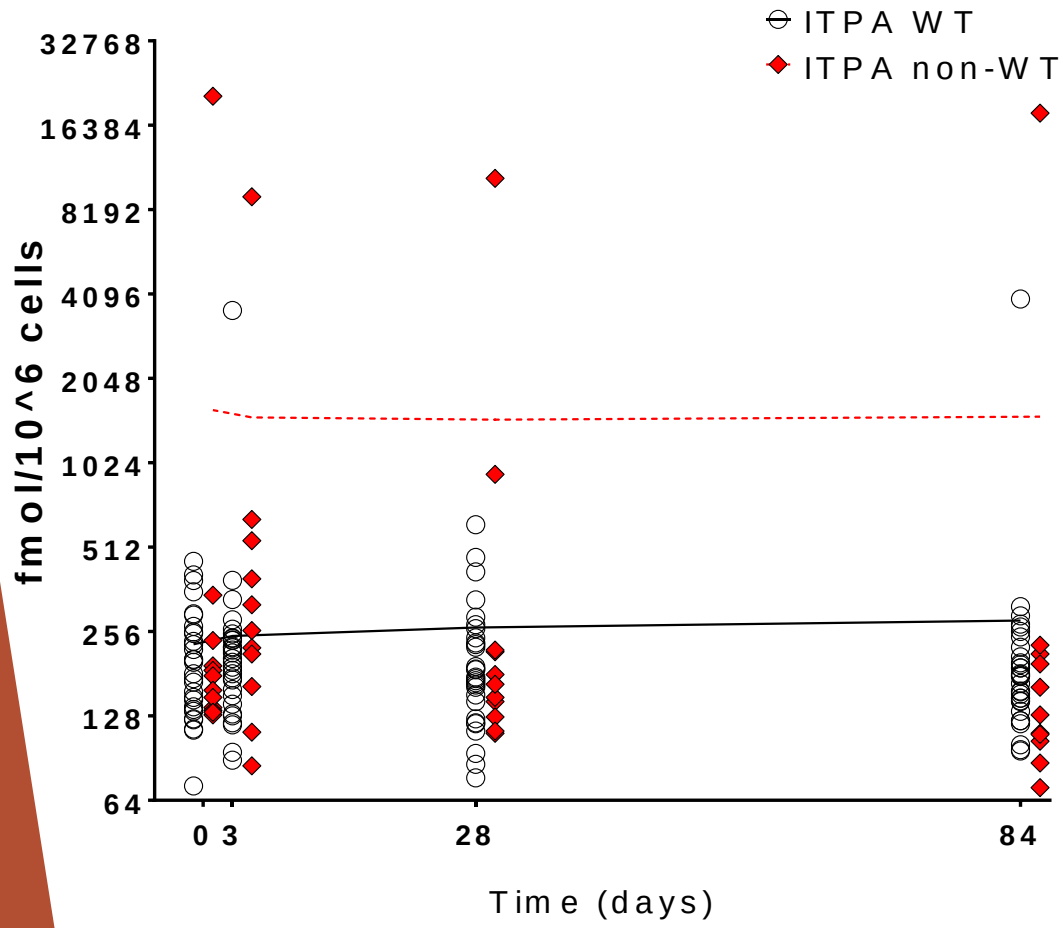
*considering a RTP conc of 120 pmol/10^6 cells

GTP



Effect	% Change	95% CI	P value
RTP*ITPA non-WT vs WT	−15.0	−26, -2.3	0.02
RTP on ITPA WT	16.5	3.3, 31	0.01
RTP on ITPA non-WT	−0.995	−13, 12	0.88

ITP



Effect	% change	95% CI	P value
RTP on ITPA WT	22.7	−1.2, 52.4	0.06
RTP on ITPA non-WT	1.5	−18.4, 26.2	0.89
Baseline ITPA non-WT vs WT	49.1	−9.8, 147	0.12
RTP*ITPA non-WT vs WT	−17.3	−35.1, 5.5	0.13

- RTP effect was not significantly different between ITPA groups

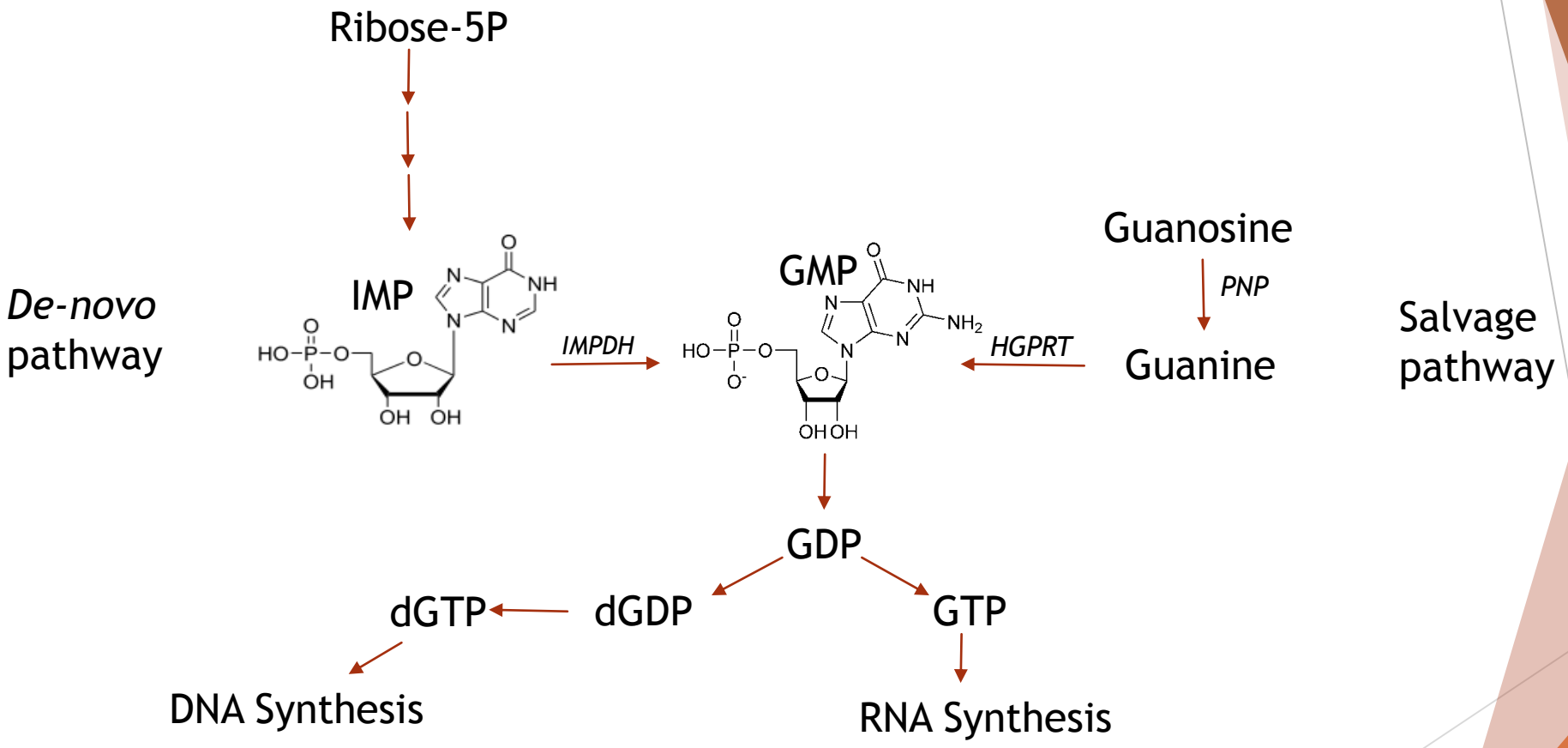
Conclusions

- 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].


Ribose-5P
De-novo pathway
IMP
IMPDH
GMP
HGPRT
Guanosine
PNP
Guanine
Salvage pathway
GDP
dGTP
dGDP
GTP
DNA Synthesis
RNA Synthesis

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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