MULTIMODAL THERAPY USED ON A TREATMENT RESISTANT HEPATITIS C VIRUS (HCV) GENOTYPE 3a OVERCOMES NON-RESPONSE

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Background

50 year-old Caucasian male; HCV-G3a; Diagnosed 1996; Infected ~26 years; Twice non-responder; Utilizes multi-modal therapy targeting molecular pathways suspected of impacting treatment response.

Previous Treatments:

- 1997 Interferon-alfa-2b 3MU TIW
- 2006 PegIFNα-2a 180µg plus Ribavirin 800mg

Treated for 24 weeks and HCV-RNA was detected at end-of-treatment (EOT) both times.

Introduction

Treatment with peginterferon alfa and ribavirin produces a sustained virologic response (SVR) in ~40-50% in HCV-G1 and ~65-80% of HCV-G3 infected patients. A substantial proportion of patients, however, do not have an optimum response to current treatment regimens. Genotype 3 is considered "easy-to-treat", however those who fail Tx should not be considered easy to treat.

Alternate options are needed for patients who relapse or do not respond to therapy. Individualization of therapy and taking a multi-modal approach offers the possibility of tailoring treatment to particular patients and improve SVR rates.

HCV has evolved elaborate and broad mechanisms to disarm both host innate and adaptive immunity. These mechanisms favour viral persistence even in the face of exogenous interferon-based therapies.

Key to this strategy is an understanding is of the molecular pathways HCV circumvents and what can be done both pre-treatment and during to restore these pathways.

Method

Pre-treatment: Along with diet and exercise, the supplements below were taken to help reverse insulin resistance, reduce inflammation and lower oxidative stress plus slow down fibrosis progression.

Anti-Oxidants

- Acetyl-L-Carnitine
- Alpha-Lipoic Acid (ALA)
- Coenzyme Q10
- N-acetyl-L-cysteine (NAC)
- S-Adenosyl-L-Methionine (SAMe)
- Taurine
- Trimethylglycine (TMG) / Betaine
- Vitamins C, D3 and E

Anti-Fibrotic/Anti-Inflam

- Resveratrol
- Curcumin
- Green Tea
- Milk Thistle
- Ursodeoxycholic Acid

The following treatment approach was devised to overcome the barriers to Interferon non response.

Approaches to overcoming Interferon Resistance

SAMe	Restoration of STAT1 methylation and rescue from PIAS inhibition
Nitazoxanide (Alinia)	Increase PKR expression
Pre-Dose Ribavirin	High RBV levels before 1st IFN shot
High Dose Ribavirin	IFN sensitization by increasing ISG expression and decreasing inhibitors of IFN signaling
Reversal of IR	Decrease SOCS3, PI3K?
Vitamins D3 & B12	Low levels negatively impact SVR

The aim being restoration of the Jak/STAT signaling pathway pre-treatment and enhancement during treatment.

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HepC Nomads: http://hepcnomads.co.uk/phpBB3/index.php

Interferon Signaling Pathway

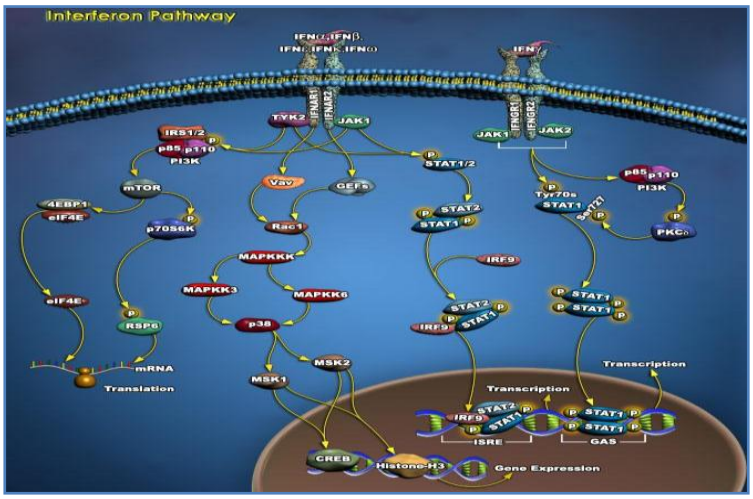

Figure 1. Interferon Signaling pathway

Negative Regulators of Interferon Signaling

Important negative regulators of the Jak-STAT pathway have been found at two levels.

First, the suppressor of cytokine signaling (SOCS) family members, SOCS1 and SOCS3, prevent phosphorylation and activation of IFNα induced STATs by inhibiting the IFNα receptor associated Jak-Kinases.

Second, downstream of STAT activation by tyrosine phosphorylation, IFNα induced gene transcription can be inhibited by protein inhibitor of activated STAT1 (PIAS1). PIAS1 inhibits binding of STAT1 dimers to the response elements in the promoters of target genes (Figure. 2A).

HCV Interference of Interferon Signaling

Binding of IFNα to its receptor on the cell surface induces the activation of the phosphotyrosine kinases Jak1 and Tyk2. STAT1 is recruited to the receptor kinase complex and activated by phosphorylation on tyrosine 701.

STAT1 then forms dimers, translocates into the nucleus, and binds to promoter elements of Interferon alpha stimulated genes (ISGs). The protein inhibitor of activated STAT1 (PIAS1) can not bind to STAT1 dimers because the STATs are methylated on an arginine (Figure 2B).

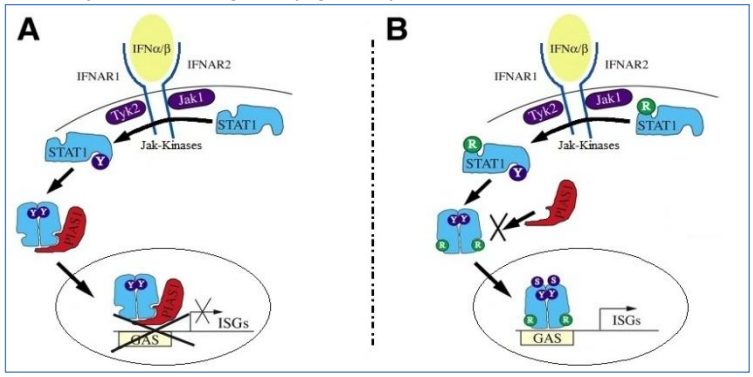

Figure 2 Model of HCV Interference with IFNα signaling.

Tyrosine phosphorylation: Y, Arginine methylation: R

HCV induces the over-expression of PP2Ac via an ER stress response pathway. PP2A inhibits PRMT1, the enzyme responsible for STAT1 methylation. The resulting hypomethylation of STAT1 facilitates the binding of PIAS1, an inhibitor of DNA binding of activated STAT1.

The binding of PIAS1 to STAT1 is regulated by methylation of STAT1 by protein arginine methyl transferase 1 (PRMT1). Arginine methylation inhibits binding of PIAS1 to STAT1, whereas demethylation of STAT1 enhances its association with PIAS1.

A number of ISGs are not upregulated when STAT1 is bound by PIAS1.

Insulin Resistance

Hepatitis C promotes insulin resistance / hyperinsulinemia, which is associated with resistance to interferon, steatosis and fibrosis progression.

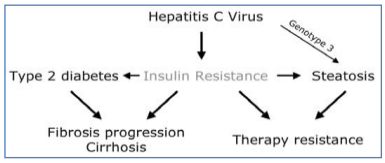

Figure 3. HCV and Insulin Resistance

Insulin resistance is a common denominator in difficult-to-treat *patient's* such as those overweight/obese, HIV-coinfected, cirrhotic, have significant steatosis and African Americans.

Elevated insulin levels (Hyperinsulinemia) suppress the antiviral effects of interferon, which negatively impacts response to therapy. Insulin inhibits interferon-stimulated phosphorylation of three JAK pathway molecules

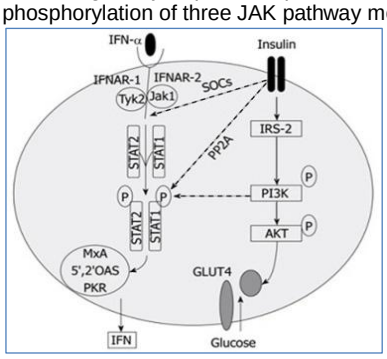

Figure 4. Interaction between insulin and the interferon signaling pathway

PKR, eIF2α and STAT phosphorylation.

Changes in eIF2a phosphorylation is mediated thru the PI3K signal transduction pathway, with reduction of STAT1 phosphorylation and subsequent inhibition of the antiviral effects of interferon.

PI3K activated by insulin seems to be responsible for the block of STAT1 translocation that avoids the antiviral effect of interferon.

Dotted Lines: Inhibition,
Continuous Lines: Activation

Oxidative Stress

Oxidative stress encourages lipid peroxidation, a process whereby free radicals break down fats by taking their electrons, producing byproducts that contribute to liver steatosis. Oxygen radicals and lipid peroxidation byproducts also stimulate release of pro-inflammatory cytokines and promote hepatic stellate cell activity leading to fibrosis progression.

HCV infection is characterized by increased markers of oxidative stress; a consequence of both increased generation of ROS and the reduction of multiple antioxidants. Oxidative stress, alcohol use, and HCV core protein have each been proposed to inhibit the cellular interferon response by interfering with the Jak-STAT signaling pathway.

Drug Dose Rationale

SAMe + TMG:

Figure 5: PP2Ac Inhibits STAT1 methylation

Increased PP2A levels inhibit the activity of PRMT1, leading to hypomethylation of STAT1.

The hypomethylated STAT1 is bound by PIAS1 and therefore STAT1 dimers, even when phosphorylated upon IFNα treatment, cannot bind anymore to the promoter of IFN target genes.

Figure 6. SAMe restores STAT1 methylation

S-Adenosyl-L-Methionine (SAMe/AdoMet) is the methyl group donor for STAT1 methylation catalyzed by PRMT1.

Trimethylglycine (TMG) / Betaine is the methyl group donor for the conversion of homocysteine to methionine, a direct precursor of SAMe.

Dose
SAMe 800-1600 mg/day, TMG ~5 g/day taken to restore STAT1 methaylation.
Vits B12 300 mcg & folic acid 800 mcg were added as SAMe and TMG co-factors.

PegInterferon alpha-2b:
Pegylated Interferon produces a biphasic viral decline that consists of a rapid first-phase decline and a subsequent slower second-phase decline.

The first-phase decline is dose dependent while the second-phase viral decline is predictive of an SVR.

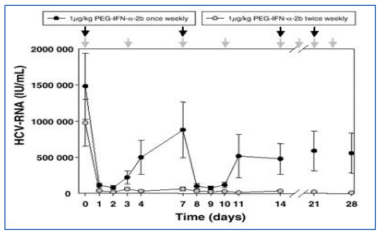

Figure 7. Using PegIFNα-2b HCV RNA Intraweek rebound

HCV RNA levels have been shown also to correlate inversely with drug levels of both pegylated interferons.

Viral kinetic data have shown that many patients receiving PegIFNα weekly have a rebound in HCV RNA between doses.

The increase of HCV RNA at the end of the PegIFNα-2b dosing interval can be reduced by using higher doses or twice weekly injections of standard doses.

Dose
To enhance the Viral Kinetics and Pharmacokinetics of PegIFNα-2b, the dose was 240 mcg 1st shot, then another 120 mcg shot 3 days later. Move the shots out to every 4 days for two weeks. Then every 5 days for two more weeks, then every 6 days, then weekly 120 mcg dosing after ~8 weeks.

Ribavirin:
The antiviral mechanism of ribavirin remains somewhat controversial. The current prevailing hypothesis is that ribavirin acts as a mutagen to increase the likelihood of lethal mutations ("error catastrophe") in the HCV genome, while other evidence supports a possible immune modulatory activity.

These two mechanisms are not mutually exclusive.

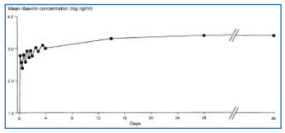

Figure 8. Ribavirin Serum concentration

Ribavirin takes 2-4 weeks to reach maximum serum concentration.

The mean half-life for multiple doses of ribavirin is ~12 days

Ribavirin down-regulates IFN-inhibitory pathways and studies have shown a higher starting dose of RBV was associated with lower relapse rates and higher rate of SVR. While the incidence of anemia rises with higher ribavirin doses. The likelihood of sustained response was also significantly associated with the magnitude of hemoglobin (≥3 g/dL) decline.

Nitazoxanide (Alinia):
Alinia was taken to lower the relapse rate as in the Alinia clinical trials the relapse rate was very low.

Nitazoxanide's mechanism of action against HCV involves induction of PKR phosphorylation which results in increased intracellular concentrations of eIF2a, which controls host cell defences against viral infection.

Dose
Predosed Nitazoxanide 500 mg BID for two weeks.
Then further 8 weeks, discontinued due to side effects.

Treatment Summary

Reduced HOMA-IR from 2.44 to 1.2; Weight from ~77 to 70kg; BMI from ~25.7 to 23.3; Waist circumference from ~93 to 87cm SAMe + TMG, Vitamins B12 & Folate pre-treatment.

Anti-inflammatory and anti-fibrotic supplements were stopped and dose of SAMe & TMG increased at least 6 weeks prior to starting Treatment. Anti-oxidants continued.

1. Pre-dosed Ribavirin for 2 weeks.
2. Pre-dosed Nitazoxanide* for 2 weeks.
3. Nitazoxanide further 8 weeks.
4. Titrated RBV dose to maintain a ≥3 g/dL drop in Hb.
5. High-dosed PegIFNα-2b tapered to 120 mcg/wk over 8 wks.
6. Supplemental Vitamin D3.
7. Duration 36 weeks (based on response).

- First in Australia given TGA approval for Nitazoxanide use in HCV therapy.

Results

HCV RNA	IU/mL	Log	Drop
Baseline	1,380,000	6.14	N/A
2 wks pre-dosing RBV + NTZ	559,000	5.75	0.39
Week 1	104	2.02	3.73
Week 2	<15 Detected	1.18	0.87

Undetected at treatment weeks 4, 12, 24 and 36 (EOT).
Also undetected at weeks 4, 12 and 24 post-EOT (SVR).

Modifying standard of care with a multimodal approach including:

- **Reversal of insulin resistance.**
- **Supplemental SAMe + TMG, Vit B12, Folate and Vit D3.**
- **Pre-dosing Ribavirin.**
- **Titrating Ribavirin to maintain ≥3g/dl drop in Hb.**
- **Addition of Nitazoxanide.**
- **Early high dose PegIFNα-2b.**

Treatment resistance was overcome in this case, likely through enhancement of interferon signaling.

Co-Author 3

National Genetics Institute

HR is the co-founder of National Genetics Institute (NGI).
He is responsible for inventing ultra-sensitive PCR tests that have played a key role in the development of therapies against viral diseases like HIV and hepatitis B and C. His molecular detection technology inventions are also used for cancer detection.
He is currently researching anti-fibrotic supplements for the reversal of fibrosis.

http://www.ngi.com

Co-Author 2

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Silvia Hinojosa-Price is a Registered Nurse and has previously been involved in treating HepC patients. While working as a HepC nurse and Diabetes Educator she noticed that patients who were Insulin Resistant frequently failed to respond to Tx. Currently, she works in dialysis and is an advocate and educator at HCVets.com educational website & forums.

http://www.hcvets.com