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Randomized controlled trial of interferon gamma versus amantadine in combination with interferon alpha and ribavirin for hepatitis C genotype 3 non-responders and relapsers

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Abstract

Objectives: To evaluate the efficacy and safety of triple combination regimens comprising of interferon alpha-2b (IFN-alpha) and ribavirin plus either IFN-gamma or amantadine in genotype 3 patients, responders or relapsers to interferon plus ribavirin combination.

Methods: Patients were randomized to receive IFN-alpha 3MU thrice a week, ribavirin 800-1200 mg per day with either IFN-gamma 2 MU thrice a week or amantadine 100 mg twice daily. Treatment was continued for 48 weeks in patients showing complete or partial (2 log reduction) early virological response (EVR) at 12 weeks and negative PCR at 24 weeks.

Results: Total enrollments were 44; 25 were previously non-responders out of them 12 were in the IFN-gamma arm. Nineteen were relapsers, out of them 10 received IFN-Gamma. Overall EVR with triple regimens was 61.4% (27/44). The EVR for IFN-gamma arm was 72.7% (16/22) and for amantadine arm 50% (11/22) ($p=0.089$). Sustained virological response (SVR) was 50% (11/22) in the gamma arm and 27.3% (6/22) in the amantadine arm ($p=0.122$). This figure was 60% (6/10) and 44% (5/9) for relapsers ($p=0.845$), and 41.6% (5/12) and 7.7% (1/13) for non-responders ($p=0.046$). Treatment was well tolerated by most of the patients in both arms.

Conclusions: About one third of patients responded to the triple regimens. However IFN-gamma was a better option. Its combination with pegylated interferon and ribavirin needs further evaluation. (Trial Registration: ClinicalTrials.gov Identifier NCT00538811).

Keywords: Interferon gamma, Interferon alpha, Ribavirin, Hepatitis C, Non responders, Relapsers (JPMA 62: 338; 2012).

Introduction

The current standard treatment for chronic hepatitis C virus (HCV) infection is combination of pegylated interferon (PEG-IFN) and ribavirin. The treatment is given for a period of six months for genotype 2 and 3 and one year for genotype 1 and 4.¹ This combination gives a sustained virological response (SVR) of 54%-56%; 42-52% of patients with genotype 1 and 76-84% of those with genotypes 2 and 3.² Comparing PEG-IFN with standard interferon, there is much better response by using PEG-IFN for the treatment of genotype 1. For genotype 3, the response rate with standard interferon and ribavirin is equally good, cost effective^{3,4} and recommended by the different societies.^{5,6}

Current standard therapy for chronic hepatitis C with interferon alpha (standard or pegylated) and ribavirin is still less than ideal and the problem of the non-responding patients to the combination therapy remains. SVR remains a difficult goal to achieve in many patients. After a relapse from standard interferon plus ribavirin therapy, retreatment with PEG-IFN plus ribavirin for one year may increase the response rate to 40-50%. However, patients who have failed to respond to combination of standard interferon and ribavirin, re-treatment with PEG-IFN plus ribavirin therapy has a response rate of only 10%.² Different strategy is, therefore, needed for patients who are considered as non-responders to interferon and ribavirin therapy. Due to the limitations of antiviral drugs, there is a need to explore other avenues to enhance the immune system's ability to fight HCV.

In the context of discovery of IL-28 B gene polymorphism coding for lambda interferon and its importance as an independent predictor of response to interferon alpha based therapy,^{7,8} there is a resurgence of interest and enthusiasm about the role of interferon lambda and gamma in clearing the virus. Interferon gamma (IFN-gamma) inhibits HCV virion production by an effect on viral RNA and protein synthesis, enhancement of immune lysis of HCV infected cells, and inhibition of hepatic fibrosis by an effect on TGF-beta.⁹ IFN- alpha and IFN-gamma induce distinct patterns of gene expression¹⁰ and differential actions of IFN-alpha and IFN-gamma has its implications in the context of therapeutic intervention.

Considering the antiviral effects of IFN-gamma and expected favourable effects if used in combination with IFN-alpha, we started an investigator driven study to evaluate the efficacy of a regimen combining IFN-gamma with IFN-alpha plus ribavirin in patients who had not responded to the combination of last two drugs.

Patients and Methods

Trial design: This was a single centred investigator initiated, open label, parallel randomized controlled trial. The

patients were divided into two arms namely experimental (IFN-gamma) and active comparator (amantadine). Randomization was done by opaque sealed envelope method. Non-responders and relapsers to previous treatment with standard interferon and ribavirin were separately randomized. Primary end point was the efficacy in terms of sustained virological response (SVR) defined as undetectable HCV RNA 24 weeks after treatment discontinuation (week 72). Secondary end points were normalization of ALT at week 72, and tolerance and safety. No changes were made in methods after the trial commencement.

Participants: The study was conducted between July 2008 and December 2010. The patients for this were recruited at the Aga Khan University Hospital, Karachi, Pakistan. The target patients were adult males and females patients infected with HCV genotype 3, ranging in age from 18-70 years, who had previously received standard interferon alpha 2a or 2b 3MU thrice a week in combination with ribavirin (800-1200 mg) for 24 weeks and had not shown a response as depicted by disappearance of HCV RNA from serum done in the last month of therapy (non-responders) or who relapsed at six months post-treatment (relapsers).

Other inclusion criteria were Hb \geq 10 g/dL (females) and \geq 11 g/dL (males), Platelets count \geq 100 x 10⁹/L, neutrophil count \geq 1.5 x 10⁹/L, at least one abnormal ALT values in the last year, normal TSH, non-pregnant adult females and absence of drug or alcohol abuse. Exclusion criteria were antiviral treatment in the last three months, hepatitis B or HIV co-infection, severe renal dysfunction or creatinine clearance less than 50 ml/min, pregnant or breast feeding women, suspected hypersensitivity to Interferon alpha, gamma or ribavirin, decompensated liver cirrhosis, history or any evidence of other concomitant causes of chronic liver disease, active malignant disease, any known pre-existing medical condition that could interfere with subject's participation or completion of study.

Interventions: Experimental arm received IFN-alpha 2b 3 MU thrice a week, ribavirin 800-1200 mg per day, and IFN-gamma 200 MU thrice a week. Patients of less than 70 kg of weight received 800 mg of ribavirin, while 70 kg or above received 1200 mg daily. Active Comparator arm received amantadine 100 mg twice a day in place of IFN-gamma. Duration of therapy was 48 weeks.

Baseline evaluation: The following base line evaluation was conducted for each patient: written informed consent, medical history, physical examination including height and weight, serum HCV RNA (quantitative) to measure the viral load (RT-PCR assay: Cobas Amplicor Monitor v2.0, Roche Diagnostics). HCV genotype determined by Innolipa kits, abdominal ultrasound, and alpha-fetoprotein levels. A liver biopsy was done prior to therapy. Hepatic inflammation

and fibrosis were assessed by METAVIR scoring system. For METAVIR system, stage of fibrosis was assessed on a scale of 0-IV and activity was graded on a scale of 0-3. Once patient became PCR negative with the above mentioned quantitative assay, reverse transcription polymerase chain reaction assay with a sensitivity of 100 copies per millimeters was used in the follow up visits.

Follow-up evaluation: Patients were followed up in the clinic at week 2 and 4 and then at monthly intervals until the end of treatment (48 weeks). Patients were able to contact the investigators during the course of the treatment to report any adverse events or side effects and for their queries if any. Following evaluation was performed at each visit. a. physical examination b. assessment of any adverse event. c. Concurrent medication d. Verbal check of compliance e. CBC and biochemistry. Determination of serum HCV RNA was performed at 0, 12, 24 and 48 weeks, and then 12 and 24 weeks post treatment (72 weeks). PCR at baseline and 12 weeks was quantitative.

Outcomes: Patients were evaluated for response to therapy which was defined as clearance or 2 log reduction of HCV RNA levels at 12 weeks (early virological response or EVR) and negativity of serum HCV RNA by PCR at 24 weeks. Patients who were considered as responders continued the study till the end of treatment (48 weeks). Non-responders at 24 weeks were withdrawn from the study. Patients who successfully completed 48 weeks of the study were seen at 12 weeks and 24 weeks after treatment discontinuation and were evaluated for the sustained response. No changes to trial outcomes were made after the trial commencement.

Randomization: Simple randomization was done using sealed envelope method. Non-responders and relapsers

to previous treatment with standard interferon and ribavirin were separately randomized. Randomization, enrollment of participants and assignment of participants to interventions was done by the principal investigator. It was an open label study.

Safety and ethical issues: Treatment was aimed to be interrupted if subject's health or wellbeing was considered threatened by continuation of the treatment, occurrence of serious adverse events or any unmanageable factors, and withdrawal of consent by the subject. Adverse events were graded as mild, moderate, severe and life threatening. Therapy was permanently discontinued for life-threatening events. Doses of both interferons were reduced to half if the neutrophil count fell between 0.75 and 0.5 x 10⁶/L and/or platelets fell below 60 x 10⁹/L. If neutrophil count fell below 0.5 x 10⁶/L or platelets below 30 x 10⁹/L therapy was stopped. Ribavirin dose was lowered to half if haemoglobin levels fell below 8.5 g/dl and stopped if level fell below 7.5 g/dl.

Ethical Review Committee of the Institute approved the study. Written informed consent was obtained from all the patients before enrolling the patients in the study after explaining the study objectives and methodology.

Statistical methods: Data was analyzed by the Statistical Package for Social Sciences SPSS 16.0 for Windows (SPSS inc. Chicago, IL). Means with standard deviations were calculated for continuous variables, and compared through Wilcoxon rank-sum test. Proportions were calculated for categorical variables, and compared using the Chi square test with Yate's correction. The statistical significance level of two-sided tests was set at P=0.05.

Results

Participant flow: The total number of patients

Table-1: Baseline characteristics of study patients.

	Gamma interferon arm (n=22)	Amantadine arm (n=22)	P value
Age (years)	44.95±10.1	42.32 ±8.5	0.451
Male sex	15 (68.2%)	13 (59.1%)	0.754
Body mass index, kg/m ²	28.57 ±6.3	25.72 ±3.9	0.163
Haemoglobin, g/dL	14.0 ±1.76	13.06 ±2.0	0.144
Total leukocyte count (10 ⁹ /L)	6.84 ±1.59	6.44 ±1.81	0.386
Platelet count (10 ⁹ /L)	232.6±89.8	193.2±97.5	0.156
Bilirubin (mg/dl)	0.70 ±0.36	0.88 ±0.54	0.275
Alanine aminotransferase (IU/L)	88.82 ±65.4	70.10 ±41.9	0.406
Gamma glutamyltransferase (IU/L)	51.29 ±31.9	59.00 ±42.23	0.723
Alkaline phosphatase (IU/L)	112.14 ±58.7	103.05 ±55.6	0.715
Fasting blood sugar (mg/dL)	100.00 ±32.9	98.33 ±7.5	0.309
HCV RNA titer (IU/mL)	606691.2 ±872128.9	947214.6 ±1266694.8	0.346
Liver biopsy Grade			
0-1	5	8	0.509
3-Feb	17	14	
Liver biopsy stage			
0-2	15	5	1
4-Mar	7	7	

Values are means ± standard deviation or n(%).

Table-2: Comparison between patients who achieved sustained virological response (SVR) with those who did not achieve SVR.

	Patients with SVR	Patients without SVR	P value
Baseline Characteristics			
Mean age (years)	42.81 ±9.7	44.07 ±9.2	0.715
Male sex n(%)	13 (76.5%)	15 (55.6%)	0.279
Body mass index	26.85 ±4.4	27.28 ±5.7	0.754
Haemoglobin (g/dl)	13.90 ±1.9	13.32 ±1.8	0.481
Total leukocyte count (10 ⁹ /L)	6.82 ±1.5	6.52 ±1.8	0.552
Platelet count (10 ⁹ /L)	222.2±125.9	206.3±71.3	0.885
Bilirubin (mg/dl)	0.84 ±0.4	0.75 ±0.5	0.23
Mean ALT (U/L)	77.35 ±58.9	81.64 ±54.5	0.682
Gamma GT (U/L)	47.20 ±23.9	59.79 ±42.7	0.567
ALP (U/L)	105.00 ±48.8	109.44 ±62.1	> 0.999
Fasting blood sugar (mg/dl)	110.75 ±35.5	94.00 ±24.6	0.174
Diabetic	2	7	0.494
Mean HCV RNA titer (IU/mL)	958279.6 ±1409331.2	673982.8 ±862324.1	0.594
Baseline PCR <500000	12	17	0.847
Liver biopsy Grade: 0-1	5	8	> 0.999
2-3	12	19	
Liver biopsy stage: 0-II	11	19	0.952
III-IV	6	8	
Responses			
Early Virological Response	17	10	< 0.0001*
Early biochemical response	15	22	0.863
End of treatment virological response	17	1	< 0.0001*
End of treatment biochemical response	16	2	0.0001*
Received IFN-Gamma	11	11	0.216
“Non-responders” who Received IFN-gamma	5	7	0.046*
“Relapsers” who Received IFN-gamma	6	4	0.669

Values are in numbers or means ± standard deviation. *Significant p values.

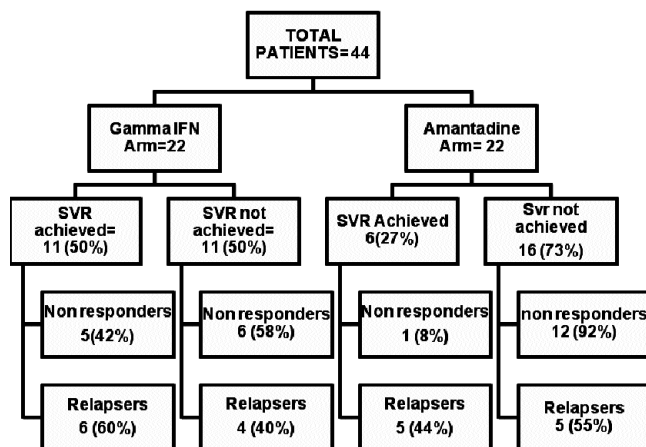


Figure: Response to the therapy. Terms "non responders" and "relapsers" refer to the subgroups based on response to previous therapies.

included in this study were 44 out of which 28 (63.6%) were male and 16 (36.4%) female; mean age 43.6± 9.3years (range 28-60). Twenty two were in the experimental arm and 22 in the active comparator group, Out of the 44 patients,25 were previously non-responders and 12 of them were in the IFN-gamma arm. Nineteen were relapsers; 10 received IFN-gamma.

Baseline data: Out of the total recruited patients 9 (22.3%) were diabetic, 8 (20 %) hypertensive, 12 (31.6%) had hepatomegaly and 8 (20.5%) had splenomegaly. Liver biopsy revealed no fatty change in 16 (57.1%), mild fatty change in 8 (28.6%), moderate fatty change in 4 (14.3%) patients. F3 or F4 fibrosis was seen in 14 (34%). Baseline characteristics are summarized in the Table-1.

Virological response: By intention-to-treat analysis, the overall early virological response (EVR) with triple regimens was 61.4% (27/44). The EVR for IFN-gamma arm was 72.7% (16/22) and for amantadine arm 50% (11/22) (p = 0.089). In the subgroup analysis, this figure was 90% (9/10) and 55% (5/9) for relapsers (p=0.119), and 46.1% (7/12) and 8% (6/13) for non-responders in both arms respectively (p=0.543).

The overall end of treatment response (ETR) was 40.9% (18/44). The ETR was 50% (11/22) and 31.8% (7/22) for IFN-gamma and amantadine arms respectively (p= 0.220). In the subgroup analysis, this figure was 60% (6/10) and 55% (5/9) for relapsers (p=0.845), and 41.6% (5/12) and 15.3% (2/13) for non-responders to previous treatments in both arms respectively (p=0.144).

Sustained virological response (SVR) with both triple regimens was seen in 38.6% (17/44). SVR was 50% (11/22)

in IFN-gamma arm and 27.27% (6/22) in amantadine arm ($p=0.122$). In the subgroup analysis, this figure was 60% (6/10) and 44% (5/9) for relapsers ($p=0.845$), and 41.6% (5/12) and 7.69% (1/13) for non-responders in both arms respectively ($p=0.046$).

Biochemical response: The normalization of ALT at week 12 was seen in 84.1% (37/44) patients. It was 86.3% (19/22) and 81.1% (18/22) for IFN-gamma and amantadine arms respectively ($p=0.680$). In the subgroup analysis, this figure was 100% (10/10) and 77.7% (7/9) for relapsers ($p=0.115$), and 75% (9/12) and 84.6% (11/13) for non-responders to previous treatments in both arms respectively ($p=0.548$).

Normalization of ALT at the end of treatment was seen in 41% (18/44) patients. It was 50% (11/22) and 31.8% (7/22) for IFN-gamma and amantadine arms respectively ($p=0.220$). In the subgroup analysis, this figure was 70% (7/10) and 55% (5/9) for relapsers ($p=0.515$), and 33.3% (4/12) and 15.3% (2/13) for non-responders to previous treatments in both arms respectively ($p=0.294$).

The sustained normalization of ALT at six months post treatment was seen in 38.6% (17/44) patients. It was 50% (11/22) and 27.3% (6/22) for IFN-gamma and amantadine arms respectively ($p=0.122$). In the subgroup analysis, this figure was 60% (6/10) and 55% (5/9) for relapsers ($p=0.845$), and 41.6% (5/12) and 7.6% (1/13) for non-responders to previous treatments in both arms respectively ($p=0.047$).

Assessment of adverse events: Treatment was well tolerated in both arms. The 80/80/80 rule i.e. 80% of the drug taken for 80% of the scheduled time was well followed. Mild adverse events included fever, headache, body ache, joint pain, weakness, disturbed sleep, retrosternal burning, decreased appetite, hairloss, weight loss, abdominal discomfort, nausea, vomiting, burning sensation in hands and feet, disturbed sleep and bloating. Treatment was stopped in two patients due to severe adverse events including severe neutropenia in one patient and arthralgia involving knee joint in the other. Both these patients belonged to the active comparator (amantadine) group. Dose reduction was done in one patient in gamma arm because of complaints of hand tremors and jerky movements.

Discussion

This is the first study to examine IFN-gamma in combination with IFN-alpha and ribavirin for difficult to treat hepatitis C patients. Previously, in a small pilot study of IFN-gamma monotherapy given for four weeks, in the doses of 100-400 microgram thrice weekly, the drug was well tolerated. It did not show any effect on the serum HCV RNA levels.¹¹ However, another study of monotherapy given for 24

weeks showed improvement in liver fibrosis.¹² There was successful eradication of virus with a combination of IFN-gamma, interferon alfacon-1, and ribavirin in a nonresponder HCV patient to pegylated interferon therapy.¹³

Combining IFN-gamma with IFN-alpha appears logical. When administered simultaneously, IFN-alpha together with IFN-gamma results in dramatic enhancement of antiviral activity against hepatitis C.¹⁴ There is a distinct pattern of gene expression by two interferons.¹⁰ The synergistic effect is likely to be due to differential cell surface receptors and signaling pathways employed.¹⁵ IFN-gamma enhances the production of IFN-alpha from immature plasmacytoid dendritic cells.¹⁶ Moreover, IFN-alpha can inhibit production of IL-12, a potent activator of STAT4 and IFN-gamma production¹⁷ which again stresses the importance of IFN-gamma supplementation in anti-HCV regimens.

Direct anti-HCV effect of IFN-gamma in cell culture is, at least in part, mediated through the Ras-MAPK signaling pathway, which possibly involves a direct or indirect modulation of NS5A protein phosphorylation.¹⁸ The mechanism of action of IFN-gamma in inhibiting the HCV infection may involve the down regulation of Claudin-1 expression and HCV receptors' distribution.¹⁹ Ribavirin has shown to enhance IFN-gamma levels in patients with chronic hepatitis C treated with interferon-alpha.²⁰ So immune responses may further be enhanced with better efficacy by combining IFN-gamma with IFN-alpha plus ribavirin for treatment of chronic hepatitis C. IFN-gamma has potent anti-fibrotic effects on stellate cells. Even if the combination does not clear HCV, it would slow or prevent fibrosis.^{12,21}

In our study we used standard interferon instead of pegylated interferon for the retreatment. In our country the cost of pegylated interferon based therapy is exorbitant and out of the reach of an ordinary person. Moreover, pegylated interferon with ribavirin is not an effective choice for retreatment of failures of standard interferon plus ribavirin therapy.² In the comparator group, we used amantadine. Role of amantadine in combination with interferon and ribavirin for the treatment of hepatitis C remains controversial. Previous studies related to retreatment of difficult to treat patients have shown contradictory results; some studies favour^{22,23} while others refute its role.^{24,25} We considered it unethical to retreat patients with dual combination of standard interferon with ribavirin again and compare it with a triple regimen.

The strength of this study is that first time IFN-gamma was used in combination with IFN-alpha plus ribavirin to treat difficult to treat hepatitis C patients. The combination was found to be safe and effective especially in non-responders to previous treatment. There are certain weaknesses in this trial which include using standard

interferon instead pegylated interferon, small sample size of the study and including genotype 3 only. This was an investigator initiated trial, with limited financial support. As IFN-gamma is not registered in our country, we were granted special permission by the Ministry of Health to import this drug once for this study. We did not include genotype 1, as preferred interferon for this genotype even for naïve patients is pegylated interferon. We included in this study all the patients regardless of degree of fibrosis as the goals of therapy are to prevent complications and death from HCV infection; regardless of the stage of fibrosis.²⁶ Moreover patients with advanced fibrosis need prompt intervention.^{27,28}

Conclusion

In conclusion, this single centre randomized study indicates that half of the patients with HCV genotype 3 infection who were retreated with a combination of IFN-gamma with interferon alpha plus ribavirin achieved a sustained virological response. This response rate was better than comparator triple regimen which included amantadine. Whether our strategy of including IFN-gamma for retreatment is indeed effective, needs further evaluation by combining it with pegylated interferon plus ribavirin in a bigger study recruiting all genotypes. It may be worthwhile evaluating IFN-gamma in combination with the standard of care for patients who had unfavourable IL-28 B genotype polymorphisms

Conflict of interests and Acknowledgements

The authors do not have any financial relationships to disclose related to this study. The study includes the off-label use of the drug. The study was approved by the ERB of the Institute and permission was taken from the Ministry of Health to import and use interferon gamma for hepatitis C patients. The cost of the drugs and the PCR based investigations were funded by the Genetech Biopharm Pakistan. No limitations on publication were imposed by the sponsor. The company does not market gamma interferon in Pakistan.

References

1. No author listed. National Institutes of Health Consensus Development Conference Panel Statement: management of hepatitis C. *Hepatology* 1997; 26: 2S-10S.
2. Diensl JL, McHutchison JG. American Gastroenterological Association: medical position statement on the management of hepatitis C. *Gastroenterology* 2006; 130: 225-30
3. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958-65.
4. Jamall IS, Yusuf S, Azhar M, Jamall S. Is pegylated interferon superior to interferon, with ribavirin, in chronic hepatitis C genotypes 2/3? *World J Gastroenterol* 2008; 14: 6627-31.
5. NIH Consensus Statement on Management of Hepatitis C: 2002. *NIH Consensus State Sci Statements* 2002; 19: 1-46.

6. Hamid S, Umar M, Alam A, Siddiqui A, Qureshi H, Butt J; Pakistan Society of Gastroenterology. PSG consensus statement on management of hepatitis C virus infection - 2003. *J Pak Med Assoc* 2004; 54: 146-50.
7. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399-401.
8. Imazeki F, Yokosuka O, Omata M. Impact of IL-28B SNPs on control of hepatitis C virus infection: a genome-wide association study. *Expert Rev Anti Infect Ther* 2010; 8: 497-9.
9. Gattoni A, Parlato A, Vangieri B, Bresciani M, Derna R. Interferon-gamma: biologic functions and HCV therapy (type I/II) (2 of 2 parts). *ClinTer* 2006; 157: 457-68.
10. He XS, Nanda S, Ji X, Calderon-Rodriguez GM, Greenberg HB, Liang TJ. Differential transcriptional responses to interferon-alpha and interferon-gamma in primary human hepatocytes. *J Interferon Cytokine Res* 2010; 30: 311-20.
11. Soza A, Heller T, Ghany M, Lutchman G, Jake Liang T, Germain J, et al. Pilot study of interferon gamma for chronic hepatitis C. *J Hepatol* 2005; 43: 67-71.
12. Muir AJ, Sylvestre PB, Rockey DC. Interferon gamma-1b for the treatment of fibrosis in chronic hepatitis C infection. *J Viral Hepat* 2006; 13: 322-8.
13. Balan V, Rosati MJ, Anderson MH, Rakela J. Successful treatment with novel triple drug combination consisting of interferon-gamma, interferon alfacon-1, and ribavirin in a nonresponder HCV patient to pegylated interferon therapy. *Dig Dis Sci* 2006; 51: 956-9.
14. Okuse C, Rinaudo JA, Farrar K, Wells F, Korba BE. Enhancement of anti-viral activity against hepatitis C in vitro by interferon combination therapy. *Antiviral Res* 2005; 65: 23-34.
15. Larkin J, Jin L, Farmen M, Venable D, Huang Y, Tan SL, et al. Synergistic antiviral activity of human interferon combinations in the hepatitis C virus replicon system. *J Interferon Cytokine Res* 2003; 23: 247-57.
16. Wang T, Blatt LM, Seiwerd SD. Immunomodulatory activities of IFN-gamma1b in combination with type I IFN: implications for the use of IFN-gamma1b in the treatment of chronic HCV infections. *J Interferon Cytokine Res* 2006; 26: 473-83.
17. Byrnes AA, Li DY, Park K, Thompson D, Mocilnikar C, Mohan P, et al. Modulation of the IL-12/IFN-gamma axis by IFN-alpha therapy for hepatitis C. *J Leukoc Biol* 2007; 81: 825-34.
18. Huang Y, Chen XC, Konduri M, Fomina N, Lu J, Jin L, et al. Mechanistic link between the anti-HCV effect of interferon gamma and control of viral replication by a Ras-MAPK signaling cascade. *Hepatology* 2006; 43: 81-90.
19. Wei X, Jia ZS, Lian JQ, Zhang Y, Li J, Ma L, et al. Inhibition of hepatitis C virus infection by interferon-gamma through downregulating claudin-1. *J Interferon Cytokine Res* 2009; 29: 171-8.
20. Fang SH, Lai MY, Hwang LH, Yang PM, Chen PJ, Chiang BL, et al. Ribavirin enhances interferon-gamma levels in patients with chronic hepatitis C treated with interferon-alpha. *J Biomed Sci* 2001; 8: 484-91.
21. Horras CJ, Lamb CL, Mitchell KA. Regulation of hepatocyte fate by interferon-?. *Cytokine Growth Factor Rev* 2011; 22: 35-43.
22. Oguz D, Cicek B, Filik L, Odemis B, Kilic M, Altintas E, et al. Effect of interferon and ribavirin combined with amantadine in interferon and ribavirin non-responder patients with chronic hepatitis C (genotype 1). *World J Gastroenterol* 2005; 11: 580-3.
23. Adinolfi LE, Utili R, Tonziello A, Ruggiero G. Effects of alpha interferon induction plus ribavirin with or without amantadine in the treatment of interferon non-responsive chronic hepatitis C: a randomized trial. *Gut* 2003; 52: 701-5.
24. Langlet P, D'Heygere F, Henrion J, Adler M, Delwaide J, Van Vlierberghe H, et al. Clinical trial: a randomized trial of pegylated-interferon-alpha-2a plus ribavirin with or without amantadine in treatment-naïve or relapsing chronic hepatitis C patients. *Aliment Pharmacol Ther* 2009; 30: 352-63.
25. Wenger C, Bischoff T, Gonvers JJ, Renner EL, Mullhaupt B. Interferon and ribavirin with or without amantadine for interferon non-responders with chronic hepatitis C. A randomized, controlled pilot study. *Swiss Med Wkly* 2007; 137: 418-23.
26. Rosen HR. Clinical practice. Chronic hepatitis C infection. *N Engl J Med* 2011; 364: 2429-38.
27. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; 55: 245-64.
28. Ghany MG, Strader DB, Thomas DL, Seeff LB; American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335-74.