

Predictors of Early Virological Response of Viral Hepatitis C to Combination Therapy with Pegylated Interferon Plus Ribavirin

Ahmed Faisal^{1*}, Ashraf Anas Zytoon², Abdel-Naser Gad Allah³, Alaa Dawood³

¹Infectious and Endemic Diseases Unit, Internal Medicine Department, Suez Canal University, Egypt

²Radiology Department, Faculty of Medicine, Menoufiya University, Egypt

³Internal Medicine Department, Menoufiya University, Egypt

*Corresponding author: ahmedf65@yahoo.com

Received August 24, 2013; Revised September 09, 2013; Accepted September 15, 2013

Abstract A combination therapy with pegylated interferon (PEG-IFN) plus ribavirin (RBV) has made it possible to achieve a sustained virological response (SVR) of 50% in refractory cases with genotype 1b and high levels of plasma HCV RNA. Several factors including virus mutation and host factors such as age, gender, fibrosis of the liver, lipid metabolism, innate immunity, and single nucleotide polymorphism (SNPs) are reported to be correlated to therapeutic effects. However, it is difficult to determine which factor is the most important predictor for an individual patient. Data mining analysis is useful for combining all these together to predict the therapeutic effects, It is important to analyze blood tests and to predict therapeutic effects prior to initiating treatment. Our aim is to determine the independent contribution of factors including age, gender, viral load, liver fibrosis, hepatitis activity index score, and the homeostasis model assessment of insulin resistance (HOMA-IR) score in predicting response to therapy in chronic hepatitis C (CHC). Multivariate analysis of factors predicting rapid (RVR) and sustained (SVR) virological response in 280 consecutive, treatment-naïve CHC patients treated with peginterferon alpha and ribavirin in a prospective multicentre study.

Keywords: predictors of early virological, viral hepatitis C, combination therapy, pegylated interferon

Cite This Article: Ahmed Faisal, Ashraf Anas Zytoon, Abdel-Naser Gad Allah, and Alaa Dawood, "Predictors of Early Virological Response of Viral Hepatitis C to Combination Therapy with Pegylated Interferon Plus Ribavirin." *American Journal of Clinical Medicine Research* 1, no. 4 (2013): 54-60. doi: 10.12691/ajcmr-1-4-3.

1. Introduction

Hepatitis C virus (HCV) is one of the most important Flaviviridae infections in humans and is the second most common cause of viral hepatitis [1]. The World Health Organization (WHO) estimates that about 3% of the world's population has been infected with HCV and that there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and/or liver cancer [2]. Egypt contains the highest prevalence of hepatitis C in the world and a high morbidity and mortality from chronic liver disease, cirrhosis, and hepatocellular carcinoma. Approximately 12% of blood donors are seropositive for HCV antibodies [3]. A community-based study reported positive HCV RNA in sera of 9.8% of 1,126 representative Egyptian citizens [4].

Chronic HCV infection frequently results in liver cirrhosis and is associated with an elevated risk of developing hepatocellular carcinoma [5]. Although symptoms may be mild for decades, 20% of persistently infected individuals may eventually develop serious liver disease including cirrhosis and liver cancer [2]. The only

effective treatment is based on interferon alpha (IFN- α). Treatment with either IFN- α alone or in combination with ribavirin leads to a sustained virological response (SVR) in 20% to 56% of patients with chronic hepatitis C [6,7]. The combination of interferon and ribavirin is the preferred treatment and achieves a better response than interferon or ribavirin alone [8]. The more rapidly HCV RNA becomes negative during treatment, the higher the rate of SVR [9].

However, nonresponse to this therapy remains common and is associated with several factors such as HCV genotype, duration of a person's HCV infection and HCV viral load in addition to host factors such as sex, HLA type and cytokine polymorphisms [10,11]. Patient age, grade of liver inflammation and ethnicity have also been shown to influence response to therapy [12,13]. The strongest predictors for a SVR to treatment is the HCV genotype, with HCV genotype 1 (HCV-1) being the least sensitive to IFN- α based therapy [14,15].

Several studies are available on the response rates to combinatorial IFN- α /ribavirin treatment of hepatitis C in Pakistan [16,17], however, these do not describe positive and negative predictors for the SVR rates. The aim of this study was to determine the efficacy and safety of a 24

weeks or 48 weeks treatment with IFN- α plus ribavirin in patients with chronic hepatitis C genotypes none-1 and 1 respectively and to identify factors that impaired response to antiviral therapy. We focused our study on naïve patients that had not previously received antiviral treatment and who presented with HCV genotypes non-1 and 1. Several baseline and on-treatment variables affect the likelihood of achieving SVR [18]. Older age, advanced stage of fibrosis, African-American ethnicity and HCV-related factors, including HCV genotype 1 and high viral load at baseline, predict poor response to anti-viral therapy. Furthermore, metabolic factors, such as high body mass index (BMI), presence and severity of liver steatosis and increasing homeostasis model assessment of insulin resistance (HOMA-IR) score have been reported as negative predictors of response [19,20]. On the other hand, early on-treatment kinetics of HCV RNA, e.g. undetectable HCV RNA at week 4, has a high positive predictive value of SVR [21,22].

2. Patients and Subjects

2.1. Patients Demographics

This study included fifty five consecutive chronic hepatitis C (CHC) patients. Their age ranged from 18-60 years eligible for combined therapy of of Pegylated interferon (PEG-IFN) and Ribavirin. All patients undergone a percutaneous liver biopsy at the beginning of treatment in the Gastroenterology and Hepatology Unit of Suez Canal University Hospital and Al Menoufyia university hospital between November 2010 and January 2012. All patients were subjected to

1. clinical evaluation; including demographic data, present history of smoking, alcohol consumption, presence of chronic diseases (DM, hypertension...), and past history of dental intervention, surgery or blood transfusion.

2. laboratory investigations; including, ALT, AST, Hemoglobin (Hb), Albumin, Bilirubin (Total and Direct), Alpha fetoprotein (AFP), Prothrombin time (PT), Anti-schistosomal antibody test.

3. Quantitative HCV RNA. After 12 weeks of treatment with combined PEG-IFN/Ribavirin therapy, to detect the early virologic response (EVR) was done. EVR is considered if the HCV RNA level is undetectable or if a greater than 2-log-fold reduction in HCV RNA level is present.

So, patients were divided into 2 groups:

- 1) Responders: those are patients whose PCR results showed EVR.

- 2) Non- responders: those are patients whose PCR results didn't show EVR.

The viral load result of respective patient was collected from patients. Samples were enrolled in the study after getting informed consent from each patient.

2.2. Methods

The serological and biochemical tests were done at Clinical Pathology department of Suez Canal University Hospital. Data were obtained from the patients' sheets. Quantitative HCV RNA was done using Real Time PCR technique in an API PRISM® 7000 thermocycler (applied

biosystems, Foster city, CA) at the Oncology Diagnostic Unit of Suez canal University Hospital, by the unit's staff

Liver histology: For all patients, conventional liver histology was performed on formalin-fixed liver biopsy by pathologists at pathology department of Suez Canal University Hospital and Al Menoufyia university hospital. The liver fibrosis was staged on a 0-6 scale as follows: F0 = no fibrosis; F1 = Fibrous expansion of some portal areas; F2 = Fibrous expansion of most portal areas; F3 = Fibrous expansion of most portal areas with occasional portal to portal bridging; F4 = Fibrous expansion of most portal areas with marked bridging; F5 = Marked bridging with occasional nodules (incomplete cirrhosis); F6= Cirrhosis. The fibrosis stage can be classified to low stage fibrosis (stage 0-3), and high stage fibrosis (stage 4-6).The pathology report of all study subjects were obtained from the department. The histological activity index (HAI) can be classified as minimal (grade 0-3), mild (grade 4-8), moderate (grade 9-12) and severe (grade 13-18).

Estimation of IL-6 level plasma level was done by using IMMULITE® 1000 IL-6 (Siemens, Immulite, cat no. 06604071). Assessing insulin resistance: Insulin resistance was done by using the Homeostatic Model Assessment (HOMA). It is the product of the fasting values of glucose (G0) (expressed as mmol/L) and insulin (I0) (expressed as μ U/mL) divided by a constant: $10 \times G0 / 22.5$. Patients with HOMA-IR values > 2 were considered insulin-resistant. Plasma samples were tested for insulin hormone level using AccuBind™ ELISA Microwells (Cat# 2425-300, Monobind Inc, Lake Forest, USA).

Detection of IL-6 -174 G $>$ C (rs1800795) polymorphism: Genomic DNA was extracted from patients' and controls' peripheral blood leucocytes of EDTA anticoagulated blood using AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, USA). The integrity of the extracted DNA was assessed by performing DNA gel electrophoresis at 1% concentration of agarose in 1x Tris Acetate EDTA (TAE) running buffer. The 1000 Spectrophotometer (NanoDrop Technologies Inc., Washington, USA). Genomic DNA samples extracted from blood samples extracted DNA samples were subjected to DNA quantitation using the NanoDrop® (ND)- were amplified by PCR technique with IL-6 gene promoter specific primers [19].

3. Results

As shown in Table 1, 77% of patients responded to combined PEG INF and Ribavirin therapy while 23% of patients didn't respond, the response rate was nearly similar in male patients (76%) and female patients (78%). The difference was statistically not significant. As shown in Table 2, there is significantly higher viral load in non-responders than responders (P value = 0.04). The anti-schistosomal antibody was positive in high percentage of non-responders (64%), but this was statistically not significant. Most patients have mild degree histological activity (79% of total patients). The minimal HAI reported 100% response rate. The mild HAI reported 76% response rate. The moderate grade of HAI reported the lowest response rate of 50%. There were no cases with severe grade of HAI. The difference between responders and non-responders was statistically not significant. The

difference in other laboratory and pathological data (mean bilirubin, mean albumin, α fetoprotein, ALT and AST) was statistically not significant.

Table 3 shows that the CC genotype reported the higher response rate (80%) than CG genotype (50%) and GG genotype (57%). There is no statistically significant difference in response to treatment between all genotypes of IL-6 -174 polymorphism, IL-6 level was significantly higher in the responders patients than non-responders patients (P value = 0.01), the mean stage of fibrosis was significantly higher in non-responders than in responders (P value = 0.03). Patients with low stage fibrosis (stage 0-3) tend to have higher response rate (80%) than patients with high stage fibrosis (stage 4-6) (response rate 50%). This difference was statistically not significant, the mean fasting insulin level was higher in non-responders than in responders. But this difference was statistically not significant, there is no statistically significant difference in insulin resistance (HOMA-IR) between responders and non-responders.

Table 3 shows that IL-6 level was significantly higher in the responders patients than non-responders patients (P value = 0.01). the mean stage of fibrosis was significantly higher in non-responders than in responders (P value = 0.03). Patients with low stage fibrosis (stage 0-3) tend to have higher response rate (80%) than patients with high stage fibrosis (stage 4-6) (response rate 50%). This difference was statistically not significant. The mean fasting insulin level was higher in non-responders than in responders. But this difference was statistically not

significant. There is no statistically significant difference in insulin resistance (HOMA-IR) between responders and non-responders.

In receiving operating characteristic curve (Figure 1), IL-6 level > 2.15 pg/ml is significantly associated with EVR (p-value = 0.04) with 81.1% sensitivity and 72.7% specificity (95% CI: 0.521-0.889). The fibrosis stage at a cut off > 2/6 is not associated with EVR with 32.4% sensitivity and 45.5% specificity (95% CI: 0.153 – 0.488), this was near to statistical significance (P value = 0.07). The viral load (measured by PCR) at a cut off > 336,500 Iu/ml is also not associated with EVR with 56.8% sensitivity and 45.5% specificity (95% CI: 0.389 – 0.754) but this was statistically not significant.

Table 4 shows that by multivariate logistic regression analysis, IL-6 level is significantly an independent predictor of EVR (P value = 0.03, OR: 1.865, with 95% CI: 1.048-3.318) while the fibrosis stage is near to be significant predictor of EVR (P value = 0.08, OR: 0.514, with 95% CI: 0.245-1.08).

Table 1. Distribution of patients according to response to therapy

	Responders N (%)	Non-responders N (%)	
Patients (n=48)	37(77%)	11(23%)	
Gender			p-value
Male	26/34 (76%)	8/34 (24%)	NS
female	11/14(78%)	3/14 (22%)	

Table 2. some demographic and laboratory data among responder and non-responder patients

Laboratory data Mean \pm SD	Responders (n=37)	Non-responders (n=11)	P value
α -fetoprotein (ng/ml)	2.3 \pm 1.3	3.2 \pm 1.2	NS
Viral load (PCR)	450,701 \pm 426,559	1498,406 \pm 3522,681	0.04
AST (IU/L)	36.0 \pm 28.0	34.0 \pm 12.0	NS
ALT (IU/L)	36.0 \pm 23.0	35.0 \pm 18.0	NS
Albumin (gm/dl)	4.0 \pm 0.43	4.0 \pm 0.41	NS
Bilirubin (mg/dl)	0.74 \pm 0.25	0.65 \pm 0.22	NS
Histological activity index (HAI)	6.1 \pm 1.9	5.5 \pm 1.8	NS
Minimal (0-3) (n=5)	5 (100%)	0 (0%)	
Mild (4-8) (n=38)	29 (76%)	9 (24%)	
Moderate (9-12) (n=4)	2 (50%)	2 (50%)	
Severe (13-18) (n=0)	0 (0%)	0 (0%)	
anti-schistosomal antibody			NS
Positive n (%)	21 (55%)	7 (64%)	
Negative n (%)	17 (45%)	4 (36%)	

*Statistically significant difference (p-value < 0.05)

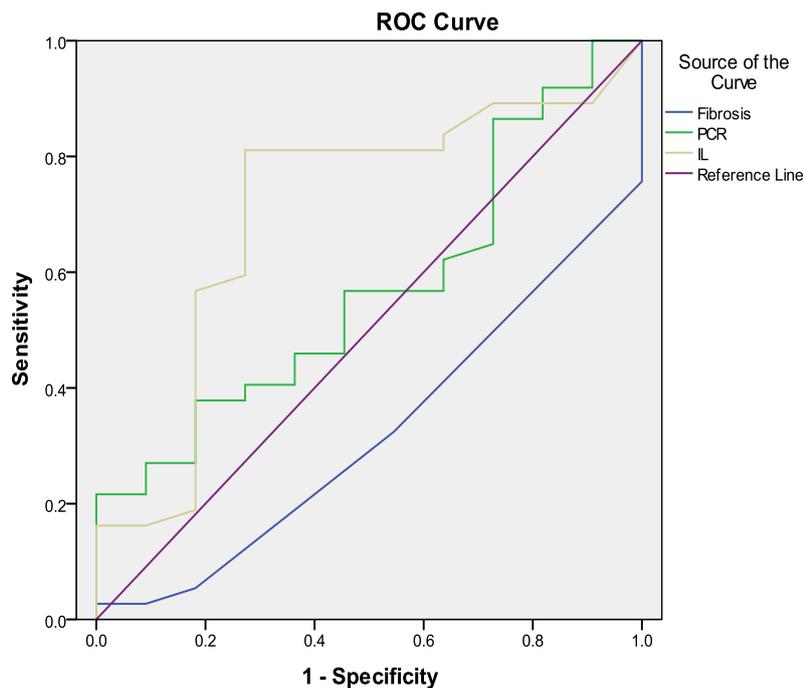
Table 3. relation between the IL-6 -174 promoter polymorphism, IL-6, Fibrosis stage, fasting IL, and homa -IR and the response to treatment

IL-6-174 polymorphism	Responders (n= 37)	Non-responders (n= 11)	P-value
CC (n=26) N (%)	21(80%)	5 (20%)	NS
CG (n=6) N (%)	3(50%)	3 (50%)	
GG (n=7) N (%)	4(57%)	3 (43%)	
	Responders (n-37)	Non-responders(n=11)	
IL-6 level (pg/ml)	3.8 \pm 2.5	2.0 \pm 1.7	0.01*
Stage of fibrosis			0.03*
Mean \pm SD	2.1 \pm 1.1	2.8 \pm 1.0	
Low stage fibrosis (stage 0-3) (n=44)	35/44 (80%)	9/44 (20%)	
High stage fibrosis (stage 4-6) (n=4)	2/4(50%)	2/4 (50%)	NS
Fasting insulin level (μ U/ml)M/SD	10.8 \pm 9.4	13 \pm 12.9	NS
HOMA-IR M/SD	4.0 \pm 3.6	3.6 \pm 3.1	NS

*Statistically significant difference (p-value < 0.05)

Multivariate logistic regression for independent predictors of the response to antiviral therapy in chronic

hepatitis C patients treated with peg-interferon plus Ribavirin. (Method: by backward stepwise method)



Diagonal segments are produced by ties.

Figure 1. Roc for fibrosis stage, IL-6 level and PCR

In this receiving operating characteristic curve, IL-6 level > 2.15 pg/ml is significantly associated with EVR (p-value = 0.04) with 81.1% sensitivity and 72.7% specificity (95% CI: 0.521-0.889). The fibrosis stage at a cut off > 2/6 is not associated with EVR with 32.4% sensitivity and 45.5% specificity (95% CI: 0.153 – 0.488),

this was near to statistical significance (P value = 0.07). The viral load (measured by PCR) at a cut off > 336,500 Iu/ml is also not associated with EVR with 56.8% sensitivity and 45.5% specificity (95% CI: 0.389 – 0.754) but this was statistically not significant.

Table 4. logistic regression of the fibrosis stage and IL-6

Variable	B	Std error	Wald	significance	Expected (B)	95% CI of expected (B)	
						Lower	upper
Fibrosis	- 0.666	0.379	3.087	0.08	0.514	0.245	1.08
IL-6	0.623	0.492	4.492	0.03	1.865	1.048	3.318
Constant	1.127	1.109	1.033	0.31	3.087		

4. Discussion

There are a series of viral, host and treatment characteristics that influence the likelihood of HCV treatment success and are useful when assessing the benefits and risks of therapy [23].

In this study, 48 patients did PCR after 12 week of treatment with combined PEG IFN and Ribavirin. 77% of patients develop EVR, and 23% of patients didn't develop EVR. This finding is comparable with the finding of [24] who found the response rate is 65% for genotype 4. It is higher than the finding of [25]. They found that for genotype 1, the response rate was 48%, and 61% for all genotypes. Also [26] found that the response rate for genotype 1 is 56% or 44% according to whether PEG-IFN α -2a or PEG-IFN α -2b is used respectively.

In this study low basal viral load is significantly associated with EVR. This finding is in agreement with previous studies [27,28] who demonstrated that low pretreatment viral load is significantly associated with

SVR. We found also that basal HCV RNA < 336,500 UI/ml is associated with EVR, but this failed to reach the statistically significance. It is demonstrated that HCV RNA < 400,000 IU/mL (OR 2.74; 95% CI: 1.03-7.27) is an independent predictor of rapid virological response [29]. Another study by Berg et al.,(30) showed that Patients with a high viral load >800,000 IU/ml are less sensitive to the treatment than patients with a low viral load <800,000 IU/ml.

A possible explanation of this finding is that several HCV viral proteins (E2, NS5A, and core protein) have been shown to inhibit IFN- α activation of signals and antiviral proteins [24].

In this study we found that mean level of α fetoprotein was higher in non-responders (3.2 \pm 1.2 ng/ml) than responders (2.3 \pm 1.3 ng/ml), but this difference was statistically not significant. This finding is in agreement with the finding of [31] who found that α fetoprotein level was significantly lower in patients who had SVR than non-responder patients.

In this study we found that there are a high percentage of non-responders who have positive anti-schistosomal

antibody test. This difference was statistically not significant. This finding is in agreement with the finding of [32] who reported that CHC patients with schistosoma co-infection responded poorly to interferon therapy and had a higher relapse rate than HCV patients not having concomitant schistosomiasis. Previous studies showed that schistosomiasis upregulated thymus-dependent type 2 response (Th-2) while downregulating CD4+ Th-1 responses, leading to persistence of concomitant viral infections. Moreover, there is a significant decrease in core-specific CD8+ T Cell interferon gamma, IL-4, and IL-10 responses [33].

We noted in this study that there is no significant difference in AST and ALT level between responders and non-responders. The mean AST level in responders is 36.0 ± 28.0 U/l. This finding is in agreement with the finding of [34] who demonstrated that serum AST levels < 40 U/l is correlated independently with complete response. Pockros et al. [35] demonstrated that there is no significant difference in ALT level between responders and non-responders.

In this study there is no significant difference in serum albumin between responders and non-responders. In contrast, it was reported that serum albumin < 3.9 g/dl is significantly associated with a non-virological response [34].

We noted in this study that both responders and non-responders had low total bilirubin serum level with no statistically significant difference. Hosogaya et al., [36] reported that low total bilirubin level is significantly associated with SVR.

A possible explanation of this finding is that the patients with co-infections have higher HCV-RNA titers, more advanced liver disease, more hepatic complications, and a greater mortality rate than those infected with only HCV [37]. This favors the persistence of HCV and non-response to interferon therapy. patients with minimal HAI reported 100% response rate, those with mild HAI reported 76% response rate and patients with moderate grade of HAI reported the lowest response rate 50%. This finding is in agreement with the finding of others [38,39,40,41]. They reported that patients with advanced liver necroinflammatory activity and fibrosis have poor SVR.

This finding may be explained by that the severity of hepatic inflammation is a major factor driving progression of chronic hepatitis C to cirrhosis. The more advanced liver disease is more associated with poor response to IFN therapy [35].

In this study, regarding IL-6 -174 promoter polymorphism, we reported that CC genotype showed higher response rate (80%) than GG genotype (57%), and CG genotype (50%). This finding is in agreement with another study [42] founded that CG and GG genotypes are significantly associated with lower rate of SVR

This finding may be explained by that we found that IL-6 was expressed in high level in CC genotype and the correlation between IL-6 level and response was significant. As we discussed, IL-6 can overcome HCV core-induced inhibition of STAT 3 activation and phosphorylation, improving the response rate. CHC patients who achieved EVR have significantly higher IL-6 level than those who didn't. IL-6 level > 2.15 pg/ml (OR: 1.8; 95% CI: 1.048- 3.318) is significantly associated with

EVR and can be considered as an independent predictor of EVR (p-value = 0.04 with 81.1% sensitivity and 72.7% specificity).

The association between IL-6 level and response to treatment may be explained by that IL-6 has been shown to activate STAT3 by phosphorylation in hepatic stellate cells and promote their survival and proliferation. Activation of STAT3 is followed by induction of a wide variety of antiviral and proapoptotic genes that may contribute to the antiviral and antitumor activities of IFN- α in human livers [43].

We found in this study the mean stage of fibrosis is significantly higher in non-responders than responder patients, and fibrosis stage $\leq 2/6$ is associated with EVR (OR: 0.514; 95% C.I 0.245-1.080). This association was statistically near to significance (P value =0.079, with sensitivity= 32.4% and specificity= 45.5%).

Another researcher [44,45] found that patients with established cirrhosis are more resistant to IFN- α therapy than those who have fibrosis, whereas patients with fibrosis are less responsive to IFN- α therapy than those without fibrosis.

This also agrees with the findings of other group [46] who found that the mean fibrosis was lower between responders (1.41 ± 0.88 vs. 2.16 ± 1.39 ; P = .0001). The low fibrosis stage ($\leq 2/6$) is significantly associated with EVR and can be used as independent predictor of EVR (OR, 1.36; 95% CI, 1.01-1.84; P = .029). This finding may be explained by that changes in intrahepatic inflammatory response and mediators during fibrosis progression may affect combined PEG-IFN and Ribavirin response [43].

In this study, we reported that mean insulin resistance (measured by HOMA-IR) is high in both responders (4.0 ± 3.6) and non-responders (3.6 ± 3.1) with no statistically significant difference. This finding is in agreement with a study of Fattovich et al., [29] who reported that the mean HOMA-IR score was (2.9 ± 3.0) with no statistically significant difference between responders and non-responders. Another study [47] showed also that there is no significant difference in HOMA-IR between responders and non-responders.

We reported in this study that the mean fasting insulin level is higher in non-responders than responders, but the difference was statistically not significant. This finding is in agreement with the finding of others [48], who found that Hyperinsulinaemia is associated with low SVR.

This finding may be explained by that HCV core protein, has been proposed to cause IR in hepatocytes by reducing the level or activity of molecules involved in insulin signaling, particularly IRS-1 (insulin receptor substrate-1) and IRS-2 [49]. This can increase insulin level and insulin resistance in CHC patients. In addition, activation of SOCS3 by the viral core protein inhibits IFN- α -induced signaling and antiviral activity [50].

5. Conclusion

In this study, serum the IL-6 level is significantly higher in responder patients and can be used as an independent predictor to response to therapy. The low fibrosis stage and low viral load are significantly associated with early response to therapy.

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