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Immune responses in DAA treated chronic hepatitis C patients with and without prior RG-101 dosing

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ABSTRACT

Background&aims: With the introduction of DAA's, the majority of treated chronic hepatitis C patients (CHC) achieve a viral cure. The exact mechanisms by which the virus is cleared after successful therapy, is still unknown. The aim was to assess the role of the immune system and miRNA levels in acquiring a sustained virological response after DAA treatment in CHC patients with and without prior RG-101 (anti-miR-122) dosing.

Methods: In this multicenter, investigator-initiated study, 29 patients with hepatitis C virus (HCV) genotype 1 (n = 11), 3 (n = 17), or 4 (n = 1) infection were treated with sofosbuvir and daclatasvir ± ribavirin. 18 patients were previously treated with RG-101. IP-10 levels were measured by ELISA. Ex vivo HCV-specific T cell responses were quantified in IFN-γ-ELISpot assays. Plasma levels of miR-122 were measured by qPCR.

Results: All patients had an SVR12. IP-10 levels rapidly declined during treatment, but were still elevated 24 weeks after treatment as compared to healthy controls (median 53.82 and 39.4 pg/mL, p = 0.02). Functional IFN-γ HCV-specific T cell responses did not change by week 12 of follow-up (77.5 versus 125 SFU/10^6 PBMC, p = 0.46). At follow-up week 12, there was no difference in plasma miR-122 levels between healthy controls and patients with and without prior RG-101 dosing.

Conclusions: Our data shows that successful treatment of CHC patients with and without prior RG-101 dosing results in reduction of broad immune activation, and normalisation of miR-122 levels (EudraCT: 2014-002808-25).

Trial registration: EudraCT: 2014-002808-25.

1. Introduction

Chronic hepatitis C virus (HCV) infection is a major public health problem affecting an estimated 170 million people worldwide(-Shepard et al., 2005). During chronic infection, plasma CXCL10 (or interferon gamma-induced protein 10, IP-10) levels are increased, reflecting a state of ongoing interferon-α signaling and prolonged immune activation(Meissner et al., 2014; Spaan et al., 2015). In addition, constant exposure to viral antigens results in so-called 'exhausted' virus-specific $CD8^+$ T cells, T cells that have lost some

Abbreviations: HCV, hepatitis C virus; IP-10, interferon gamma-induced protein; CHC, chronic hepatitis C; DAAs, direct acting antivirals; SVR, sustained virological response; miR-122, microRNA-122; RAS, resistance associated substitutions; SOF, sofosbuvir; DCV, daclatasvir; RBV, ribavirin; LLOQ, lower limit of quantification; LLOD, lower limit of detection; IQR, interquartile range.

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or all of their effector functions(Bengsch et al., 2010; Klenerman and Hill, 2005). Functional virus-specific $CD8^+$ T cells are crucial in viral clearance of acute HCV infection(Urbani et al., 2006). However, interferon-α induced clearance of chronic HCV infection is not associated with improvement of HCV-specific T cell function(Barnes et al., 2009). Current treatment regimens for chronic hepatitis C (CHC) patients include interferon-free combinations of direct acting antivirals (DAAs) which result in high sustained virological response (SVR) rates in the majority of patients. The exact mechanisms that lead to viral clearance in these patients treated with DAAs are not well known. Recent data suggests that the proliferative potential of exhausted T cells is improved after successful treatment with DAAs(Martin et al., 2014; Spaan et al., 2015). However, the extent of T cell restoration is unknown, as is the role of HCV-specific T cells in viral clearance after DAA treatment.

Inhibition of microRNA-122 (miR-122), an important host factor for replication of HCV, is an alternative therapeutic approach to clear HCV infection(Jopling et al., 2005; Lanford et al., 2010; van der Ree et al., 2017). Recently, it was shown that a single dose of RG-101, an N-acetylgalactosamine conjugated anti-miR-122 oligonucleotide, resulted in substantial viral load reduction in CHC patients, and in SVR for 76 weeks in 3 of 28 patients participating in a phase 1b study(van der Ree et al., 2017). Viral load reduction and eradication was not associated with restoration of HCV-specific T cell function(Stelma et al., 2017). Virological rebound following RG-101 dosing was associated with the emergence of resistance associated substitutions (RAS) in 5′ UTR miR-122 binding sites of the HCV genome(van der Ree et al., 2017). It is unknown if 5' UTR C3U and C2G/C3U RAS persist and if these viruses are susceptible to DAAs in vivo. Furthermore, the effect of RG-101 and DAA treatment on plasma miR-122 levels has not been established.

The primary objective of this study was to analyse the impact of DAA treatment on immune activation and functionality of HCV-specific T cells in CHC patients with and without prior RG-101 dosing. The secondary objective was to assess treatment success, and circulating miR-122 levels prior and after DAA treatment.

2. Material and methods

2.1. Study design

In this investigator-initiated, open-label, multicenter study, we included CHC patients with genotype 1, 3, or 4 infection at two hospitals in the Netherlands; Academic Medical Center Amsterdam (AMC), and University Medical Center Groningen (UMCG). Patients with HCV genotype 1 and 4 were treated with sofosbuvir (SOF) and daclatasvir (DCV) for 12 weeks, and patients with HCV genotype 3 were treated with SOF, DCV and ribavirin (RBV) for 12 or 24 weeks (patients with cirrhosis were treated for 24 weeks) (Fig. S1). All patients were followed for 24 weeks after treatment cessation.

2.2. Patients

Twenty-nine CHC patients ($n = 25$ in AMC and $n = 4$ in UMCG) were included in this study. Of these, 18 patients had a virological rebound following RG-101 dosing (van der Ree et al., 2017), and were offered retreatment as part of this study. Only males and postmenopausal females were enrolled. Eligible patients were treatment-naïve (other than previous RG-101 dosing) or had previously had a relapse after antiviral therapy other than combination of SOF + NS5A inhibitor with or without ribavirin. Patients with co-infection (hepatitis B virus or human immunodeficiency virus infection), evidence of decompensated liver disease, or a history of HCC were excluded. Before enrolment and before any study procedure, written informed consent was obtained from all patients.

2.3. Study oversight

The study was approved by the independent ethics committee at each participating site (MEC AMC + UMCG), and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. The trial was registered with EudraCT, number 2014-002808-25.

2.4. Study sampling

Plasma and heparinized peripheral blood samples were collected at various time points before (<90 days before start), during, and after treatment. PBMCs were isolated from heparinized blood using standard density gradient centrifugation and subsequently cryopreserved until the day of analysis.

2.5. Study assessments

2.5.1. Chemistry and viral assessments

Serum HCV RNA levels were measured using the Roche COBAS AmpliPrep/COBAS Taqman HCV v2.0 assay, with a reported lower limit of quantification (LLOQ) and lower limit of detection (LLOD) of 15 IU mL-1. Effective antiviral treatment was defined by undetectable HCV RNA levels 12 weeks after the cessation of antiviral therapy (SVR12). Safety assessment was based on adverse events reporting and laboratory testing of blood samples (e.g. clinical chemistry, hematology, coagulation).

2.5.2. Sequence analysis

Sequence analysis of the HCV 5′ UTR of HCV RNA was performed by 5′ rapid amplification of cDNA ends (5′ RACE System, version 2.0, Thermo Fisher Scientific, Waltham, MA, USA), followed by population-based sequencing and was done at baseline for patients with 5′UTR RAS in miR-122 binding sites at virological rebound in a previous phase 1b study(van der Ree et al., 2017).

2.5.3. Immunological assays

Plasma levels of IP-10 were measured with a DuoSet ELISA (R&D Systems, Minneapolis, MN, USA) with a lower limit of quantification of 62.5 pg mL-1. IFN-γ-ELISpot assays were performed ex vivo in duplicate at 2×10^5 PBMCs/well at the Peter Medawar Building for Pathogen Research, Oxford, UK. Thawed PBMC were rested overnight (37 °C + CO_2) and were stimulated with panels of 15 mer peptides that overlapped by 11 amino acids corresponding to HCV genotypes 1a, 1b, 3a, or 4a(Barnes et al., 2012; Kelly et al., 2015). The peptides were arranged into 10 pools, and each peptide was used at a final concentration of 3 μg mL-1. Internal controls were dimethyl sulfoxide (DMSO) (Sigma-Aldrich, UK) as a negative control and concanavalin A (Sigma-Aldrich, UK) as a positive control. Other antigens used were a pool of MHC class 1 restricted epitopes of influenza A, EBV and CMV (BEI Resources, Manassas, VA, USA), and a lysate of CMV infected cells (Virusys Corp, Taneytown, MD, USA). Spot forming units (SFU) were calculated per 10^6 PBMC and background levels (responses in matched negative control wells) were subtracted. Positive responses were defined as (i) the mean of responses to a pool minus background being greater than 48 SFU/10^6 PBMC and (ii) the mean of responses to a well exceeding 3 × background. Background subtracted data is shown. The background level of 48 SFU/10^6 PBMC per pool was determined previously in 74 healthy controls, which was the mean + 3 standard deviation (SD)(Barnes et al., 2012).