Supplementary Appendix

Short duration response-guided treatment is effective for most individuals with recent hepatitis C infection: the ATAHC II and DARE-C I studies

Marianne Martinello[1], Margaret Hellard[2,3,4], David Shaw[5], Kathy Petoumenos[1], Tanya Applegate[1], Jason Grebely[1], Barbara Yeung[1], Laurence Maire[1], David Iser[6], Andrew Lloyd[7], Alexander Thompson[6], Joe Sasadeusz[8], Paul Haber[9,10], Gregory J Dore[1,11], Gail V Matthews[1,11]

1. The Kirby Institute, UNSW Australia, Sydney, NSW, Australia
2. Burnet Institute, Melbourne, VIC, Australia
3. Infectious Disease Unit, Alfred Hospital, Melbourne, VIC, Australia
4. Department of Epidemiology and Preventative Medicine, Monash University, Melbourne, VIC, Australia
5. Royal Adelaide Hospital, Adelaide, SA, Australia
6. St Vincent's Hospital, Melbourne, VIC, Australia
7. UNSW Australia, Sydney, NSW, Australia
8. Royal Melbourne Hospital, Melbourne, VIC, Australia
9. Royal Prince Alfred Hospital, Camperdown, NSW, Australia
10. University of Sydney, Sydney, NSW, Australia
11. St Vincent's Hospital, Sydney, NSW, Australia

Table of Contents

List of Investigators

ATAHC II

Coordinating investigators

A/Prof Gail V Matthews

Prof Greg J Dore

Site primary investigators:

A/Prof Gail V Matthews, St Vincent's Hospital, Sydney, NSW, Australia

Prof Paul Haber, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

Prof Margaret Hellard, Infectious Disease Unit, Alfred Hospital, Melbourne, VIC, Australia

Dr Phillip Read, Kirketon Road Centre, Sydney, NSW, Australia

A/Prof Joe Sasadeusz, Royal Melbourne Hospital, Melbourne, VIC, Australia

A/Prof David Shaw, Royal Adelaide Hospital, Adelaide, SA, Australia

Dr Alexander Thompson, St Vincent's Hospital, Melbourne, VIC, Australia

DARE-C I

Coordinating investigator:

A/Prof Gail V Matthews

Site primary investigator:

Prof Gregory J Dore, St Vincent's Hospital, Sydney, NSW, Australia

Prof Margaret Hellard, Infectious Disease Unit, Alfred Hospital, Melbourne, VIC, Australia

A/Prof David Shaw, Royal Adelaide Hospital, Adelaide, SA, Australia

Methods

ATAHC II

Inclusion and exclusion criteria

Adults (age ≥16 years) with recent HCV infection were eligible for study inclusion. Participants with detectable HCV RNA at screening were assessed for treatment eligibility.

Exclusion criteria for enrolment in the treatment arm included: age 16-18 years; pregnant women or male partners of pregnant women; breast feeding; systemic anti-viral, anti-neoplastic or immunomodulatory therapy ≤6 months prior to first dose of study drug; any investigational drug ≤6 weeks prior to first dose of study drug; positive anti-HAV IgM Ab or anti-HBc IgM Ab at screening; alternative aetiology of chronic liver disease; decompensated liver disease; active thyroid disease; severe retinopathy; severe seizure disorder; immunologically mediated disease, chronic pulmonary disease with functional limitation, severe cardiac disease, organ transplantation (apart from corneal, skin or hair graft), malignancy, or other severe illness (including psychiatric) which would make the participant unsuitable; and the following lab values at screening: neutrophil count <1500 cells/mm^3, platelet count <90,000 cells/mm^3, creatinine >1.5 times the upper limit of normal, haemoglobin <12 g/dL in women or <13 g/dL in men. Heavy alcohol intake and active illicit drug use were not exclusion criteria. A drug and alcohol assessment was performed at screening to determine treatment suitability.

Study assessments

In the treated arm, study visits were undertaken at baseline, day 1 and weeks 2, 4, 6, 8, 12, 16, 20 and 24, depending on treatment duration, and post-treatment weeks

12, 24, 48 and 72, until the individual completed study follow-up or the study closed (May 2015). In the untreated arm, study visits were undertaken at baseline and weeks 4, 8, 12, 24, 48, 72 and 96, until the individual completed study follow-up or the study closed (May 2015). The presence of HCV RNA was assessed at all scheduled study visits. HCV GT was assessed at screening. Adverse events were recorded on all treated participants from screening until week 12 post treatment. Questionnaires were administered at screening and every 12 weeks through follow-up to obtain information on illicit drug use, social functioning (Opiate Treatment Index Social Functioning Scale (1)) and psychological parameters (Mini-International Neuropsychiatric Interview (2) and the Depression Anxiety Stress Scale (3)). Adherence to therapy was assessed at clinical review and by self-reported questionnaire.

DARE-C I

Inclusion and exclusion criteria

Adults (age ≥18 years) with recent genotype 1 HCV infection, HCV RNA ≥10,000 IU/mL at screening and baseline and hepatitis B sAg negative were eligible for enrolment and treatment commencement.

The following additional inclusion criteria were required for HIV-positive individuals: 1. HIV >6 months duration, 2. CD4 count >200 cells/mm^3 and HIV viral load <50 copies/ml on stable combination antiretroviral therapy (cART) or 3. CD4 count ≥500 cells/mm^3 and HIV viral load <100,000 copies/mL not on cART. The following antiretroviral agents were permitted: tenofovir, lamivudine, emtricitabine, efavirenez, abacavir, raltegravir, etravirine, rilpivirine and ritonavir-boosted atazanavir.

Exclusion criteria were the same as ATAHC II and in addition included: infection with non-GT 1 HCV; injecting drug use (IDU) within the previous 4 weeks; poorly

controlled diabetes mellitus (haemoglobin A1c ≥8.5%); prior treatment with HCV protease or polymerase inhibitors; congenital QT prolongation or family history of congenital QT prolongation or sudden death; pancreatitis; haemophilia or other bleeding disorder; serious bacterial or fungal infection; and the following lab values at screening: potassium <3.5 mmol/L, calculated creatinine clearance <50mL/min.

Study assessments

Study visits were undertaken at baseline, day 1, weeks 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24, depending on treatment duration, and post-treatment weeks 12, 24, 48 and 72. The presence of HCV RNA was assessed at all scheduled study visits. HCV genotype was assessed at screening. Adverse events were collected from screening until week 12 post treatment. The same questionnaires as in ATAHC II were administered at screening and every 12 weeks through follow-up evaluation (1-3).

Study definitions for ATAHC II and DARE C I

Recent HCV infection was defined as initial detection of serum anti-HCV antibody and/or HCV RNA within six months of enrolment and either (i) documented recent HCV seroconversion (anti-HCV antibody negative result in the 24 months prior to enrolment) or (ii) acute clinical hepatitis (jaundice or alanine aminotransferase [ALT] greater than 10 times the upper limit of normal [ULN]) within the previous 12 months with the exclusion of other causes of acute hepatitis (4), with estimated duration of infection less than 18 months at screening.

The presentation of recent HCV infection at the time of diagnosis was classified as either acute clinical or asymptomatic infection. Acute clinical infection included participants with a documented clinical history of symptomatic seroconversion illness

and those without clinical symptoms but with a documented peak ALT > 400 U/L at or before the time of diagnosis. Asymptomatic infection included participants with anti-HCV Ab seroconversion but no acute clinical symptoms or documented peak ALT > 400 U/L. The estimated date of clinical infection was calculated as six weeks before onset of seroconversion illness or six weeks before the first ALT >400 U/L. The estimated date of asymptomatic infection was calculated as the midpoint between the last negative anti-HCV antibody and the first positive anti-HCV antibody. For participants who were anti-HCV antibody negative and HCV-RNA positive at screening, the estimated date of infection was six weeks before enrolment, regardless of symptom status.

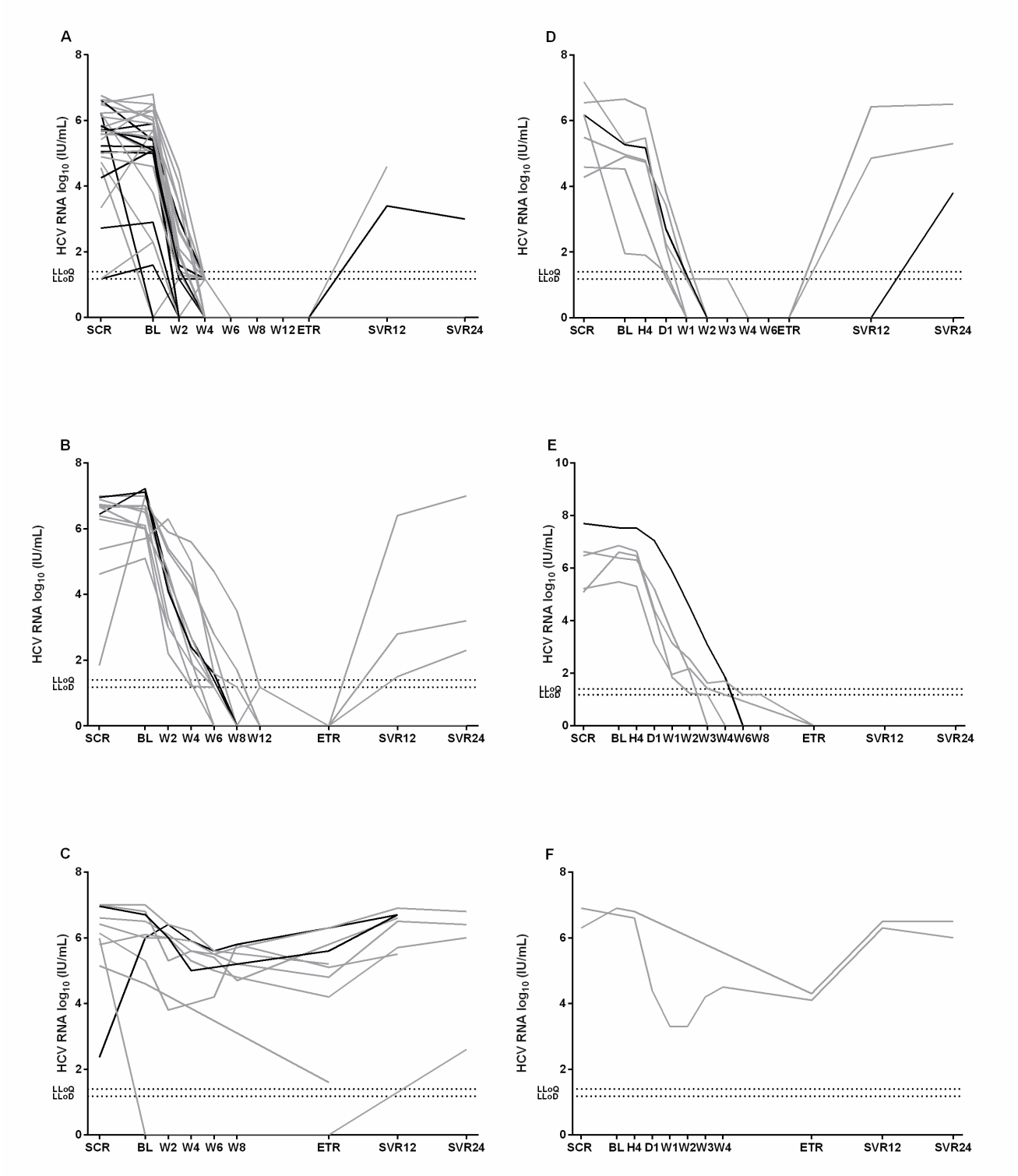
On-treatment adherence was calculated for each medication individually (PEG-IFN, RBV and telaprevir) by subtracting the number of missed doses from the total number of doses prescribed for therapy duration and dividing by the total number of doses prescribed for therapy duration. By pill count and self-reported questionnaire, compliance with each medication was individually calculated at the 80/80 and 100/100 adherence levels, defined as receipt of ≥80% or 100% of scheduled doses for ≥80% or 100% of the scheduled treatment period, respectively.

Statistical analysis

For all endpoints, means and proportions with two-sided 95% confidence intervals (CI) were determined, and were unadjusted for multiple comparisons. Continuous variables were analysed using ANOVA methods or non-parametric equivalents, as appropriate. Binary endpoints were analysed using chi-square methods or logistic regression. A Cox proportional hazards model was used to assess factors associated with time to first HCV RNA below the limit of detection and logistic

regression analyses were used to identify baseline and on-treatment predictors of HCV treatment response. Potential predictors were determined *a priori* and included participant, virological and treatment characteristics including sex, age, weight, education, employment, accommodation, social functioning, opioid substitution therapy, mental health status (depression and suicidality, based on the Mini-International Neuropsychiatric Interview(2)), ethnicity, IDU characteristics, alcohol consumption, estimated duration of HCV infection, presentation (acute clinical, asymptomatic), peak and baseline ALT, baseline HCV RNA, and HCV genotype. Social functioning was calculated using a validated scale from the Opiate Treatment Index (1) that addresses employment, residential stability, interpersonal conflict, social support, and the role of drug use in the participant's social networks. A higher value indicates poorer functioning (range: 0– 48). The multivariate model for predictors of treatment response and HCV clearance were determined using a backwards stepwise approach, considering factors that were significant at the 0.2 level in univariate analysis. The final models included only factors that remained significant at the 0.05 level. All p-values are two-sided. All analyses were performed using STATA version 13.0 (Stata Corporation, College Station, TX).

Figures



Supplementary Appendix Figure 1. Viral kinetics and outcome by study and treatment allocation. (A) ATAHC II – 8 and 16 weeks (n=29). (B) ATAHC II – 24 and 48 weeks (n=13). (C) ATAHC II – Non-response and early treatment discontinuation (n=10). (D) DARE-C I – 8 weeks (n=7). (E) DARE-C I – 12 and 24 weeks (n=5). (F) DARE-C I – Non-response and early treatment discontinuation

(n=2). Participants with HIV/HCV co-infection indicated in grey and HCV mono-infection in black.

Abbreviations: End of treatment (ETR); sustained virological response (SVR); SCR (screening); BL (baseline, hour 0); H4 (baseline, hour 4); D1 (day 1); W1-12 (week 1-12); LLoQ (lower limit of quantitation); LLoD (lower limit of detection)

Tables

Supplementary Appendix Table 1. Participant enrolment characteristics in ATAHC II by HIV co-infection

Enrolment characteristics	HCV mono-infection (n=31)	HCV/HIV co-infection (n=51)	*P*
Age (years), mean (SD)	36 (9)	41 (8)	0.010
Male, n (%)	22 (71)	51 (100)	<0.001
Weight (kg), mean (SD)	77 (17)	77 (10)	0.983
BMI (kg/m^2), mean (SD)	25 (4)	25 (3)	0.311
Caucasian ethnicity, n (%)	25 (81)	42 (82)	0.846
Higher education or qualification [a], n (%)	15 (48)	36 (71)	0.044
Full or part time employment	8 (24)	32 (63)	0.001
Prison/juvenile justice centre ever, n (%)	4 (13)	1 (2)	0.047
Social functioning score, median (IQR)	15 (9-21)	10 (6-14)	0.007
Current major depression, n (%)	8 (26)	8 (16)	0.262
Injecting drug use ever, n (%) Ever Current [b]	 26 (84) 19 (61)	 36 (71) 27 (53)	 0.220 0.209
In those reporting injecting drug use: Age at first injecting, median (IQR) Last injected within last month, n (%) Last injected between 1-6 months ago, n (%) Last injected >6 months ago, n (%)	 23 (18 – 30) 14 (54) 5 (19) 7 (27)	 29 (25-38) 16 (44) 11 (31) 9 (25)	 0.012
Drug injected most in last month, n (%) Amphetamines Heroin Other opiates	 9 (64) 2 (14) 3 (21)	 16 (100) 0 0	 0.024
Opioid substitution therapy, n (%) Ever Current	 6 (19) 6 (19)	 4 (8) 0	 0.131 0.002
Estimated duration of infection (weeks) At screening, median (IQR) At baseline, median (IQR)	 26 (11-33) 37 (27-43)	 24 (14-40) 33 (27-50)	 0.899 0.899
Acute clinical illness - jaundice +/- ALT >10x ULN Asymptomatic seroconversion	18 (58) 13 (42)	34 (67) 17 (33)	0.950
Mode of HCV acquisition, n (%) Injecting drug use Sexual exposure - same sex Sexual exposure – opposite sex Other	 25 (81) 3 (9) 2 (6) 1 (3)	 20 (39) 29 (57) 2 (4) 0	 0.002

[a] Completed higher technical qualification/TAFE/College/university degree

[b] Current injecting drug use refers to use within 6 months of screening

Supplementary Appendix Table 2. Factors associated with time to first HCV RNA below the limit of detection in ATAHC II on Cox proportional hazards analysis (n=52)

Variable	HR	95% CI	*P*
Sex			
Male	1.00	-	-
Female	3.27	0.91, 11.73	0.069
Social functioning score			
<6	1.00	-	-
7-12	0.75	0.36, 1.55	0.435
>12	1.11	0.54, 2.3	0.777
IDU ever			
No	1.00	-	-
Yes	0.85	0.44, 1.64	0.627
HIV co-infection			
No	1.00	-	-
Yes	0.65	0.34, 1.25	0.195
Presentation of acute HCV			
Asymptomatic seroconversion	1.00	-	-
Acute clinical	1.87	0.93, 3.72	0.080
Peak ALT			
≤400 U/L	1.00	-	-
>400 U/L	0.65	0.34, 1.24	0.189
HCV RNA at baseline			
<400,000 IU/mL	**1.00**	**-**	**-**
≥400,000 IU/mL	**0.34**	**0.18, 0.64**	**0.001**
HCV genotype			
GT 1	1.00	-	-
GT 2	0.94	0.13, 7.06	0.956
GT 3	1.19	0.65, 2.22	0.562
GT 4	1.56	0.21, 11.72	0.666

Supplementary Appendix Table 3. Factors associated with SVR 12 in ATAHC II on logistic regression analysis (n=52)

Variable	SVR	No SVR	OR	95% CI	*P*
Sex					
Male	35	13	1.00	-	-
Female	2	2	0.37	0.05, 2.91	0.346
Social functioning score					
<6	13	4	1.00	-	-
7-12	12	5	0.74	0.16, 3.41	0.698
>12	12	6	0.62	0.14, 2.72	0.523
Injecting drug use - ever					
No	11	6	1.00	-	-
Yes	25	9	0.66	0.19, 2.31	0.516
Injecting drug use - frequency					
Have not injected in past month	13	7	1.00	-	-
Have injected in past month	12	2	3.23	0.56, 18.71	0.191
Never injected	11	6	0.68	0.25, 3.82	0.985
HIV co-infection					
No	11	4	1.00	-	-
Yes	26	11	0.90	0.22, 3.30	0.825
Presentation of acute HCV					
Asymptomatic seroconversion	10	7	1.00	-	-
Acute clinical illness	27	8	2.36	0.68, 8.22	0.177
HCV genotype 1					
No	20	4	1.00	-	-
Yes	11	17	0.31	0.08, 1.15	0.080
HCV RNA at baseline					
<400,000 IU/mL	18	4	1.00	-	-
≥400,000 IU/mL	19	11	0.38	0.10, 1.43	0.153
Rapid virological response					
No	**10**	**12**	**1.00**	**-**	**-**
Yes	**27**	**3**	**10.80**	**2.51, 46.43**	**0.001**

Supplementary Appendix Table 4. Safety – Clinical adverse events and laboratory parameters

Clinical and lab adverse events	ATAHC II					DARE-C I
	All treated (n=52)[a]	Treatment group (weeks)				All treated (n=14)
		8 (n=13)	16 (n=16)	24 (n=11)	48 (n=2)	
Any adverse event, n (%)	347 (100)	88 (25)	117 (34)	82 (24)	19 (6)	103 (100)
Grade 3 or 4, n (%)	4 (1)	0	0	1	1	0
Serious adverse events, n (%)	3 (1)					2 (14)
Adverse events[b]						
Common (>10%), n (%)						
Fatigue	31 (60)	9 (69)	4 (25)	9 (82)	1 (50)	10 (71)
Insomnia	27(52)	6 (46)	9 (56)	6 (55)	2 (100)	6 (43)
Headache	17 (33)	5 (38)	7 (44)	3 (27)	1 (50)	6 (43)
Nausea	17 (33)	5 (38)	6 (38)	3 (27)	2 (100)	5 (36)
Arthralgia	14 (27)	7 (54)	1 (6)	5 (45)	1 (50)	0
Myalgia	14 (27)	4 (31)	3 (19)	4 (36)	1 (50)	4 (29)
Diarrhoea	13 (25)	2 (15)	4 (25)	7 (64)	0	0
Influenza-like illness	9 (17)	2 (15)	4 (25)	1 (9)	0	0
Depressed mood	8 (15)	1 (8)	3 (19)	2 (18)	2 (100)	0
Injection site erythema	8 (15)	1 (8)	0	5 (45)	1 (50)	0
Irritability	8 (15)	2 (15)	3 (19)	1 (9)	1 (50)	3 (21)
Decreased appetite	7 (13)	2 (15)	3 (19)	1 (9)	0	2 (14)
Lethargy	7 (13)	0	4 (25)	3 (27)	0	0
Injection site reaction	6 (12)	0	3 (19)	1 (9)	0	3 (21)
Pruritus	6 (12)	3 (23)	1 (6)	1 (9)	1 (50)	6 (43)
Rash	6 (12)	3 (23)	0	1 (9)	0	7 (50)
Mean on-treatment nadir Hb (g/L), SD	122 (14)	120 (14)	121 (12)	119 (12)	104 (16)	105 (17)
Decrease in Hb >30g/L, n (%)*	24 (46)	6 (46)	8 (50)	7 (64)	2 (100)	12 (86)
Decrease in Hb <100g/L, n (%)	3 (6)	1 (8)	1 (6)	0	1 (50)	5 (36)
Decrease in Hb <85 g/L, n (%)	0	0	0	0	0	2 (14)
Decrease in ANC ≤0.75, n (%)	13 (25)	1 (8)	5 (31)	4 (36)	1 (50)	2 (14)
Decrease in ANC ≤0.5, n (%)	4 (8)	1 (8)	2 (13)	1 (9)	0	1 (7)
Decrease in plt <50, n (%)	0	0	0	0	0	0

[a] Includes individuals with no allocated treatment duration due to early treatment discontinuation and virological non-response

[b] Other common adverse events in DARE-C I included abdominal pain (21%, n=3) and perianal pain/discomfort (21%, n=3)

[c] Decease in Hb >30g/L at any time between baseline and end of treatment

Abbreviations: Haemoglobin (Hb), absolute neutrophil count (ANC), platelet (plt)

Reference List

1. Darke S, Hall W, Wodak A, Heather N, Ward J. Development and validation of a multi-dimensional instrument for assessing outcome of treatment among opiate users: the Opiate Treatment Index. British journal of addiction. 1992;87(5):733-42.
2. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of clinical psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57.
3. Henry JD, Crawford JR. The short-form version of the Depression Anxiety Stress Scales (DASS-21): construct validity and normative data in a large non-clinical sample. The British journal of clinical psychology / the British Psychological Society. 2005;44(Pt 2):227-39.
4. Hajarizadeh B, Grebely J, Dore GJ. Case definitions for acute hepatitis C virus infection: a systematic review. Journal of hepatology. 2012;57(6):1349-60.
5. Jacka B, Applegate T, Krajden M, Olmstead A, Harrigan PR, Marshall BD, et al. Phylogenetic clustering of hepatitis C virus among people who inject drugs in Vancouver, Canada. Hepatology. 2014;60(5):1571-80.
6. Lamoury FM, Jacka B, Bartlett S, Bull RA, Wong A, Amin J, et al. The Influence of Hepatitis C Virus Genetic Region on Phylogenetic Clustering Analysis. PLoS One. 2015;10(7):e0131437.