

## COHORT PROFILE

# Cohort Profile: The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC<sup>3</sup>) Study

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The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC<sup>3</sup>) Study is an international multi-cohort project of pooled biological and behavioural data from nine prospective cohorts of people who inject drugs (PWID). InC<sup>3</sup> brings together researchers from Australia, Canada, USA and the Netherlands with expertise in epidemiology, biostatistics, clinical and behavioural sciences, virology and immunology to investigate research questions relevant to hepatitis C virus (HCV) and HIV outcomes. InC<sup>3</sup> was established to: (i) create a merged multi-cohort study of pooled data from well-characterized cohorts of PWID with prospective data on HIV and HCV infections, with a particular focus on HCV; (ii) facilitate new studies not possible within individual cohorts; and (iii) bring together researchers across disciplines to answer a broad range of research questions. Study cohorts identify acute HCV cases through follow-up of high-risk HCV antibody-negative PWID or through clinical referral networks. To date, data from 1986 to 2010 have been received from all contributing cohorts, with 821 HCV-infected and 1216 HCV-uninfected participants (overall,  $n = 2037$ ). Data collected include demographics, host genetics, HCV ribonucleic acid testing, alanine aminotransferase testing, HIV/hepatitis B virus testing, HCV therapy, loss to follow-up and mortality. Potential collaborators should contact the InC<sup>3</sup> PI Dr Kimberley Page (kPage@psg.ucsf.edu) for further information.

## Why was the cohort set up?

HIV and hepatitis C virus (HCV) infections represent major public health problems among people who inject drugs (PWID). In 2007, it was estimated that there were 16 million PWID worldwide, with approximately 3 million (19%) living with HIV.<sup>1</sup> Although similar data are not available for HCV, given an HCV prevalence of 65%,<sup>2,3</sup> it is estimated that 10 million active PWID have been exposed to HCV and 8 million are living with chronic infection. HIV/HCV co-infection is also common among PWID in many parts of the world, resulting in significant comorbidity.<sup>4</sup>

Despite an expanded understanding of HIV and HCV pathogenesis and rapidly improving treatments, there are still no protective vaccines and disparities in access to effective interventions persist.<sup>5–10</sup> Although major improvements have been made in the understanding of how to prevent and treat HIV among PWID, this has not been the case for HCV. Compared with HIV, HCV is significantly more infectious through percutaneous exposure, raising distinct challenges for prevention and treatment of HCV among PWID.<sup>5,7,8</sup> Studying incident and acute HCV infection among this marginalized patient population is informative for several reasons. Understanding the factors associated with incident infections provides information about exposures and factors that drive transmission, informing the development of preventive strategies. Ongoing surveillance of acute HCV infection provides vital statistics on changes in disease burden, enabling assessment of prevention policies.<sup>11,12</sup> In addition, preventive strategies that may reduce transmission of HCV among PWID (such as needle exchange programmes) are also likely to have a positive impact on HIV transmission as well. Studying acute HCV infection can provide valuable insights into HCV pathogenesis, which are critical for vaccine design. Finally, there may be benefits for early identification and treatment, given that treatment during the early stages of HCV infection is more effective than in chronic infection.<sup>13,14</sup> Assessing the dynamics of incident HIV and HCV across geographic regions, in particular declines in HIV infection, may yield valuable information relative to prevention of these different epidemics.

Our knowledge of the epidemiology of acute HIV and HCV infections in PWID remains limited for two reasons: (i) the majority of those with recent HCV infection is asymptomatic, whereas those with primary HIV are often misdiagnosed with a viral syndrome or do not seek medical attention; and (ii) the identification and follow-up of PWID at risk of these infections are challenging outside well-defined cohort studies.

In the past 2 decades, numerous prospective studies of acute HCV among PWID have been conducted worldwide, including in Australia,<sup>15–18</sup> Canada,<sup>19</sup> the Netherlands<sup>20</sup> and the USA.<sup>21–24</sup> Each has contributed

significantly to the understanding of acute HCV infections, and offered unprecedented information on complex diagnostic, preventive and treatment strategies. However, many important questions remain understudied and unanswered, with small study populations representing a major limiting factor.

The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC<sup>3</sup>) Study is a merged international multi-cohort project of pooled behavioural and biological data from nine prospective cohorts evaluating HIV and HCV infection risk and outcomes among PWID (Table 1). The InC<sup>3</sup> Study was established to: (i) create a merged multi-cohort study of pooled data from well-characterized cohorts of PWID with prospective data on HIV and HCV infections, with a particular focus on acute HCV; (ii) facilitate new in-depth studies not formerly possible within individual research centres; and (iii) bring together researchers across many disciplines to answer a broad range of research questions. The unique breadth of data on acute HCV infection are complemented with data on prevalent and incident HIV infections, enabling a variety of study outcomes to be evaluated relative to both pathogens. InC<sup>3</sup> brings together a consortium of HIV and HCV researchers with strong expertise in epidemiology, biostatistics, clinical sciences, behavioural sciences, virology and immunology from nine cohorts in Australia, Canada, the Netherlands and the USA.

This collaboration dates back to March 2009, when the feasibility of a multinational study of acute HCV infection among PWID was explored by organizing a half-day meeting held at the 13th International Symposium on Viral Hepatitis and Liver Disease in Washington, DC, USA. This meeting provided an overview of previous 'merged' collaborative studies of incident HIV infection, potential available cohorts for inclusion (including study designs and available data/samples), an overview of important unresolved research questions in the field of acute HCV (epidemiology, clinical sciences, immunology and virology) and discussions around potential funding opportunities.

The first data merge, consisting of behavioural, clinical and virological data on 522 participants with incident HCV infection from eight cohort studies, was conducted between August and November 2009. The first analysis from this initial data merge investigated factors associated with spontaneous clearance of acute HCV infection.<sup>25</sup> Subsequent funding from the Canadian Institutes of Health Research and the University of New South Wales provided further support for ongoing organizational activities contributing to the groups' formation and structure.

In 2011, the InC<sup>3</sup> Study Group was awarded a 5-year grant (2011–2016) from the National Institute of Drug Abuse (R01DA031056) to conduct scientific studies and analyses regarding epidemiology, behavioural, clinical and biological factors

**Table 1** InC<sup>3</sup> cohort profiles

| Study name                                                          | Study short title | Study co-ordinating centre                     | City, country              | Main study recruitment methodology                        | Total participants enrolled | Participants enrolled in InC <sup>3</sup> | InC <sup>3</sup> participants with incident HCV infection <i>n</i> (%) <sup>a</sup> |
|---------------------------------------------------------------------|-------------------|------------------------------------------------|----------------------------|-----------------------------------------------------------|-----------------------------|-------------------------------------------|-------------------------------------------------------------------------------------|
| Australian trial in acute hepatitis C                               | ATAHC             | University of New South Wales                  | Sydney, Australia          | Clinical referral                                         | 163                         | 156                                       | 156 (100)                                                                           |
| Amsterdam cohort study among drug users                             | ACS               | Public Health Service of Amsterdam             | Amsterdam, the Netherlands | Community-based outreach                                  | 1,657                       | 165                                       | 47 (28)                                                                             |
| Boston acute HCV study: transmission, immunity and outcomes network | BAHSTION          | Harvard Medical School                         | Boston, USA                | Clinical referral and prison surveillance                 | 233                         | 63                                        | 63 (100)                                                                            |
| Baltimore before and after acute study of hepatitis                 | BBAASH            | Johns Hopkins University                       | Baltimore, USA             | Community-based outreach                                  | 480                         | 300                                       | 129 (43)                                                                            |
| St. Luc cohort, HEP-PCO                                             | HEPCO             | Centre Hospitalier de l'Université de Montréal | Montreal, Canada           | Community-based outreach                                  | 1,188                       | 266                                       | 97 (36)                                                                             |
| Hepatitis C incidence and transmission study—community              | HITS-c            | University of New South Wales                  | Sydney, Australia          | Respondent-driven sampling and targeted outreach sampling | 121                         | 99                                        | 11 (11)                                                                             |
| Hepatitis C incidence and transmission study—prisons                | HITS-p            | University of New South Wales                  | Sydney, Australia          | Prison surveillance and prison-based outreach             | 500                         | 500                                       | 149 (30)                                                                            |
| Networks 2                                                          | N2                | Burnet Institute                               | Melbourne, Australia       | Social network sampling                                   | 243                         | 77                                        | 25 (32)                                                                             |
| UFO study                                                           | UFO               | University of California San Francisco         | San Francisco, USA         | Community-based outreach                                  | 615                         | 414                                       | 144 (35)                                                                            |

<sup>a</sup>Percentage of participants enrolled in InC<sup>3</sup>

associated with HIV and HCV infections in PWID. This supports and cultivates a significant and innovative scientific agenda, as well as provides the infrastructure required to support its execution, including administration and governance, creation of an InC<sup>3</sup> Data Coordinating Center at the University of California San Francisco, research exchange and training for junior scientists and selected laboratory procedures for harmonization of cohort data. The behavioural, clinical and virological data and access to stored specimens from this merged study have the potential to provide invaluable insights into the control of HIV and HCV infections, inform vaccine development and improve clinical management of these infections.

The research programme plans to conduct studies of risk, incidence and the natural history of HIV and HCV infection from a unique pooled data set, compiled from the nine prospective observational cohorts studying incident HCV in PWID.<sup>15,16,18–23</sup>

## Who is in the cohort?

The geographic scope of the sample is shown in Figure 1. InC<sup>3</sup> has wide geographical representation, including four cohorts of PWID from Australia,<sup>15–18</sup> one in Canada,<sup>19</sup> one in the Netherlands<sup>20</sup> and three in the USA.<sup>21–24</sup> Study cohorts identify acute HCV cases (and some cohorts, HIV cases) through follow-up of high-risk HCV antibody-negative PWID (seven cohorts) or through clinical referral networks (two cohorts). The study methodologies for the cohorts involved in the InC<sup>3</sup> Study are shown in Table 2.

The first merger for the InC<sup>3</sup> Study was focused on data for an analysis of spontaneous clearance of acute HCV infection in PWID and thus only included those with acute HCV infection. Criteria for inclusion in the first pooled InC<sup>3</sup> data set included a history of injecting drug use, documented HCV antibody seroconversion within a 2-year window period (recent HCV infection) and  $\geq 2$  HCV ribonucleic acid (RNA) tests after HCV seroconversion. Data were pooled on 522 participants from eight participating cohorts for the first data merge.

The InC<sup>3</sup> Study has been expanded for the second data merge and includes participants who remain seronegative to HCV antibodies (not exposed) in addition to participants with acute HCV infection to facilitate investigation into the epidemiology of acute HCV infection. The inclusion of individuals without HCV infection provides an opportunity to assess factors associated with incident HCV infection. Data from all nine participating cohorts were included. Criteria for inclusion in the second pooled InC<sup>3</sup> data set included either

- (i) HCV-infected participants with HCV antibody seroconversion ( $\geq 2$  HCV antibody or RNA tests); or

- (ii) HCV-uninfected participants with no HCV antibody seroconversion ( $\geq 2$  HCV antibody tests).

Participants with HIV infection were included if they met the criteria outlined previously. Documented HCV antibody seroconversion is defined as either: (i) an anti-HCV-negative test followed by either an anti-HCV-positive test or an HCV RNA positive test within an interval of 2 years or less; or (ii) evidence of seroconversion illness (jaundice or symptomatic HCV illness). To date, data from 1986 to 2010 have been received from all nine contributing cohorts, including a completed data set ( $N=2037$ ) with 821 HCV-infected participants and 1216 HCV-uninfected participants. The participant characteristics among the overall study population as stratified by HCV seroconversion (exposure to HCV) are shown in Table 3.

## How often have they been followed up?

Participant follow-up for InC<sup>3</sup> cohorts is shown in Tables 2 and 4. Behavioural and clinical data are collected as per individual cohort follow-up schedules (Table 2). Sensitive qualitative HCV RNA testing (limit of detection:  $<50$  IU/ml) has been performed in all cohorts. Blood samples are systematically collected and stored in all cohorts, enabling laboratory testing on historical samples.

Overall, the median follow-up of InC<sup>3</sup> participants was 2.0 years, and cohort-specific median follow-up ranged from 1.1 to 13 years. Table 4 shows cohort-specific follow-up characteristics. Participant retention over the first 2 years of follow-up varied. For example, at 6 months, retention ranged from 49 to 97%. Similar patterns are observed at 12, 18 and 24 months of follow-up. Twelve-month retention ranged from 0 to 87%. The number of behavioural assessments per participant was similar between cohorts (median visits: 2–7), with the exception of Amsterdam Cohort Studies (median visits: 19). The median number of phlebotomy visits was variable across cohorts. Several sites had median phlebotomy visits between two and eight, and three sites had more visits (median visits: 12, 17 and 20, respectively).

## What has been measured?

The preparation of data for the first merger of data for the InC<sup>3</sup> Study was developed based on the HIV Cohorts Data Exchange Protocol for data sharing. The centralized data processing for the second set of analyses from the InC<sup>3</sup> Study occurs only once. Each cohort gathers and computerizes its own data; subsequently, it is merged in a database in San Francisco at the University of California, San Francisco. A standardized operating procedure for formatting of tables and fields for data submission has been developed and



**Figure 1** Geographical locations of the nine participating cohorts in the InC<sup>3</sup> study. This includes the following cohorts: (1) Australian Trial in Acute Hepatitis C (ATAHC), Sydney, Australia; (2) Amsterdam Cohort Studies (ACS), Amsterdam, the Netherlands; (3) Boston Acute HCV Study: Transmission, Immunity and Outcomes Network (BAHSTION), Boston, USA; (4) Baltimore Before and After Acute Study of Hepatitis (BBAASH), Baltimore, USA; (5) St. Luc Cohort, HEPCO (for the acute HCV subcohort), Montreal, Canada; (6) Hepatitis C Incidence and Transmission Study–community (HITS-c) (HCV VPS/HITS-c), Sydney, Australia; (7) Hepatitis C Incidence and Transmission Study (HITS) in prison (HITS-p), Sydney, Australia; (8) Networks 2 (N2), Melbourne, Australia; and (9) UFO STUDY, San Francisco, USA

implemented as per the first data merger and revised with the second merger.

As shown in Table 2, data collected include baseline and longitudinal information on demographics (date of birth, gender, ethnicity, injecting behaviours), host genetics (e.g. interleukin-28B testing), HCV RNA testing (qualitative and quantitative HCV RNA, HCV genotype, assays used), alanine transaminase testing, HIV/hepatitis B virus testing, HCV therapy, loss to follow-up and mortality. Data are sent with password protection to the co-ordinating centre at the University of California San Francisco and are converted and stored in an ORACLE database. After submission, data are cleaned at the co-ordinating centre and questions about potential errors and any discrepant information are sent to the sites for clarification. The co-ordinating centre then works with the sites to address queries and ensure high data quality. The data are then subsequently merged for pooled analyses.

## What has it found? Key findings and publications

A better understanding of the host and viral factors associated with spontaneous clearance of acute HCV infection is central to guiding vaccine design and therapeutic management. However, little is known about factors influencing spontaneous clearance during acute HCV, given the small numbers of reported

cases and heterogeneity in design and methods between studies. Using data from the InC<sup>3</sup> Study's first data merge, factors associated with spontaneous clearance during acute HCV infection were evaluated.<sup>26</sup> Among 517 with incident HCV infection (173 were female), spontaneous clearance was observed in 26% [95% confidence interval (CI) 22%, 31%] during the first 2 years of infection. In adjusted logistic regression analysis (Table 5), female sex (adjusted odds ratio 2.66, 95% CI 1.69–4.18;  $P < 0.001$ ) and favourable CC *IL28B* genotype (vs CT/TT: adjusted odds ratio 2.03, 95% CI 1.30–3.19;  $P = 0.002$ ) were independently associated with spontaneous clearance. These data suggest that delayed therapeutic intervention during acute HCV infection could be recommended for female individuals with favourable *IL28B* genotypes to allow time for spontaneous clearance.

To guide future vaccine development, in a literature review by Grebely *et al.*,<sup>27</sup> investigators from the InC<sup>3</sup> Study assessed data from studies of HCV clearance, reinfection and persistence along with insights from studies of PWID. The review concluded that data from chimpanzee and human studies of primary HCV infection, viral clearance and HCV reinfection indicate that previous HCV infection is unlikely to provide substantial levels of sterilizing immunity. However, those PWID who spontaneously clear viraemia appear to have some partial protection against reinfection on recurrent exposure. Therefore, a vaccine designed to enhance spontaneous clearance of primary HCV may be effective.

**Table 2** InC<sup>3</sup> cohort research methods

|                                           | ACS                   | ATAHC                 | BAHSTION            | BBAASH  | HEPCO    | HITS-c                | HITS-p                | N2       | UFO                  |
|-------------------------------------------|-----------------------|-----------------------|---------------------|---------|----------|-----------------------|-----------------------|----------|----------------------|
| Participants                              | 183                   | 163                   | 63                  | 300     | 266      | 99                    | 500                   | 77       | 415                  |
| Person-years of follow-up                 | 1711                  | 211                   | 93                  | 787     | 707      | 127                   | 917                   | 236      | 835                  |
| Year of first participant enrolment       | 1984                  | 2004                  | 1997                | 1996    | 2004     | 2008                  | 2005                  | 2005     | 2000                 |
| Year of last participant enrolment        | Current               | 2007                  | Current             | Current | Current  | Current               | Current               | Current  | Current              |
| Year of last participant visit            | Current               | 2008                  | Current             | Current | Current  | Current               | Current               | Current  | Current              |
| Lifetime history of injection drug use, % | 100                   | 77                    | 97                  | 100     | 100      | 100                   | 100                   | 100      | 100                  |
| Treatment offered to participants         | Yes                   | Yes                   | Yes                 | Ref.    | Yes      | Ref.                  | Ref.                  | Ref.     | Ref.                 |
| Behavioural survey frequency              | 4 months <sup>a</sup> | 3 months              | None                | None    | 3 months | 6 months              | 6 months              | 3 months | 3 months             |
| Blood sample collection frequency         |                       |                       |                     |         |          |                       |                       |          |                      |
| Serum/plasma                              | 4 months <sup>a</sup> | 3 months <sup>b</sup> | Varied <sup>c</sup> | 1 month | 3 months | 6 months <sup>d</sup> | 6 months <sup>e</sup> | 3 months | 1 month <sup>f</sup> |
| Peripheral blood mononuclear cells        | 4 months <sup>g</sup> | 3 months              | Varied <sup>c</sup> | 1 month | 3 months | 6 months <sup>d</sup> | 6 months <sup>e</sup> | 3 months | 1 month <sup>f</sup> |
| HCV RNA testing frequency                 | 12 months             | 3 months <sup>b</sup> | Varied <sup>c</sup> | 1 month | 3 months | 6 months <sup>d</sup> | 6 months <sup>e</sup> | 3 months | 1 month <sup>f</sup> |
| HCV RNA assay                             |                       |                       |                     |         |          |                       |                       |          |                      |
| Qualitative                               | TMA                   | TMA                   | Cobas               | Cobas   | Cobas    | TMA                   | TMA                   | Cobas    | TMA                  |
| Quantitative                              | Cobas/bDNA            | bDNA                  | Cobas/bDNA          | Taqman  | Cobas    | Taqman/bDNA           | Taqman/bDNA           | None     | Cobas/bDNA           |
| HCV genotyping                            | Yes                   | Yes                   | Yes                 | Yes     | Yes      | Yes                   | Yes                   | Yes      | Yes                  |
| HCV serotyping                            | No                    | No                    | Yes                 | No      | Yes      | No                    | No                    | No       | Yes                  |
| HIV testing                               | Yes                   | Yes                   | No                  | Yes     | Yes      | Yes                   | No                    | Yes      | Yes                  |
| Hepatitis B testing                       | Yes                   | Yes                   | No                  | Some    | Some     | Yes                   | No                    | Yes      | Yes                  |
| Alanine transaminase (ALT)                | Yes                   | Yes                   | Yes                 | Yes     | Yes      | Yes                   | No                    | Yes      | Yes                  |
| Host genetics                             |                       |                       |                     |         |          |                       |                       |          |                      |
| IL-28B                                    | Yes                   | Yes                   | Yes                 | Yes     | Yes      | Yes                   | Yes                   | Yes      | Yes                  |
| HLA                                       | Yes                   | Yes                   | Yes                 | Yes     | No       | No                    | Yes                   | Limited  | Yes                  |
| IL-18                                     | No                    | No                    | No                  | Yes     | No       | No                    | No                    | No       | No                   |

<sup>a</sup>Six months beginning in 2003.<sup>b</sup>Four-week interval during treatment.<sup>c</sup>Collection based on clinical follow-up.<sup>d</sup>Incident cases have follow-up testing at baseline, 2, 4, 6, 8, 12 and 24 months until October 2010 (baseline, 1 month and 3 months until October 2010 and later).<sup>e</sup>Incident cases have follow-up testing at baseline, 2, 4, 6, 8, 12 and 24 months. Participants enrolled with HCV antibody have follow-up at baseline, 3 months and 6 months.<sup>f</sup>Collection from HIV – participants only.<sup>g</sup>Between 1989 and 1994; for HIV + and a random sample of HIV – participants in 1995 and later.

Cobas, COBAS AMPLICOR HCV Test v2.0 (Roche Diagnostics, Mannheim, Germany); Taqman, COBAS TaqMan HCV Test (Roche Diagnostics, Mannheim, Germany), TMA, VERSANT HCV RNA Qualitative Assay (TMA) (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), bDNA, VERSANT HCV RNA 3.0 Assay (bDNA) (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

**Table 3** InC<sup>3</sup> cohort participant characteristics

| Characteristic                                                             | Total ( <i>n</i> = 2037) | Without HCV infection ( <i>n</i> = 1216) | With HCV infection ( <i>n</i> = 821) |
|----------------------------------------------------------------------------|--------------------------|------------------------------------------|--------------------------------------|
| Male sex, <i>n</i> (%)                                                     | 1289 (63)                | 770 (63)                                 | 519 (63)                             |
| Age at enrolment, median (IQR)                                             | 26 (22–31)               | 26 (22–31)                               | 26 (22–32)                           |
| Race, <i>n</i> (%)                                                         |                          |                                          |                                      |
| Caucasian                                                                  | 1505 (74)                | 849 (70)                                 | 656 (80)                             |
| Black                                                                      | 56 (3)                   | 29 (2)                                   | 27 (3)                               |
| Aboriginal/native American                                                 | 142 (7)                  | 87 (7)                                   | 55 (7)                               |
| Other                                                                      | 187 (9)                  | 122 (10)                                 | 65 (8)                               |
| Unknown                                                                    | 147 (7)                  | 129 (11)                                 | 18 (2)                               |
| Grade 12 education or greater, <i>n</i> (%)                                | 782 (55)                 | 455 (52)                                 | 327 (59)                             |
| Unstable housing at enrolment, <i>n</i> (%) <sup>a</sup>                   | 429 (43)                 | 253 (45)                                 | 176 (41)                             |
| Prison ever, <i>n</i> (%)                                                  | 1175 (73)                | 779 (81)                                 | 396 (61)                             |
| HIV infection at enrolment, <i>n</i> (%)                                   | 51 (3)                   | 11 (1)                                   | 40 (6)                               |
| History of injecting drug use, <i>n</i> (%)                                | 2004 (98)                | 1216 (100)                               | 788 (96)                             |
| Recent injecting drug use at enrolment, <i>n</i> (%)                       | 903 (77)                 | 553 (80)                                 | 350 (74)                             |
| HBV surface antigen positive at enrolment, <i>n</i> (%) <sup>b</sup>       | 27 (2)                   | 7 (1)                                    | 20 (4)                               |
| HCV seronegative for >2 visits during follow-up, <i>n</i> (%) <sup>c</sup> | 1490 (79)                | 1155 (95)                                | 335 (50)                             |
| HCV seroconversion during follow-up, <i>n</i> (%)                          | 614 (92)                 | –                                        | 614 (92)                             |
| Mode of HCV acquisition, <i>n</i> (%)                                      |                          |                                          |                                      |
| Injecting drug use                                                         | 785 (95)                 | –                                        | 785 (95)                             |
| Sexual—same sex                                                            | 24 (3)                   | –                                        | 24 (3)                               |
| Sexual—opposite sex                                                        | 6 (1)                    | –                                        | 6 (1)                                |
| Other                                                                      | 5 (1)                    | –                                        | 5 (1)                                |
| HCV genotype <sup>d</sup> , <i>n</i> (%)                                   |                          |                                          |                                      |
| Genotype 1                                                                 | 350 (43)                 | –                                        | 350 (43)                             |
| Genotype 2                                                                 | 43 (5)                   | –                                        | 43 (5)                               |
| Genotype 3                                                                 | 227 (28)                 | –                                        | 227 (28)                             |
| Genotype 4                                                                 | 7 (1)                    | –                                        | 7 (1)                                |
| Genotype 6                                                                 | 4 (0)                    | –                                        | 4 (0)                                |
| Mixed                                                                      | 14 (2)                   | –                                        | 14 (2)                               |
| Unknown                                                                    | 176 (21)                 | –                                        | 176 (21)                             |
| HCV treatment during follow-up, <i>n</i> (%) <sup>e</sup>                  | 159 (30)                 | –                                        | 159 (30)                             |

<sup>a</sup>Incarcerated participants and those with unknown housing excluded (*n* = 869).

<sup>b</sup>Percentage of persons with a known test result.

<sup>c</sup>ATAHC and BAHSTION participants recruited after seroconversion and not included (*n* = 219).

<sup>d</sup>First identified genotype.

<sup>e</sup>Treatment status not reported by BAHSTION and UFO.

## This early work from InC<sup>3</sup> provides important information on HCV pathogenesis to guide vaccine development.

A major question that has been explored in studies of HCV reinfection after spontaneous clearance is whether previous HCV infection reduces the chance of subsequent reinfection and/or increases the likelihood of

clearing any subsequent infection.<sup>16,20,22,28–31</sup> Studies investigating the potential for protection against HCV reinfection among PWID had similar experimental designs but produced conflicting results. In a study led by Vickerman *et al.*,<sup>32</sup> it was demonstrated quantitatively, using mathematical modelling, that studies using long HCV RNA testing intervals underestimate the incidence of HCV reinfection and probability of spontaneous HCV clearance after reinfection. These findings provide empirical support for the hypothesis that long HCV RNA

**Table 4** InC<sup>3</sup> cohort follow-up characteristics by cohort

|                                                | ACS       | ATAHC     | BAHSTION | BBAASH    | HEPCO    | HITS-c  | HITS-p   | N2      | UFO                   |
|------------------------------------------------|-----------|-----------|----------|-----------|----------|---------|----------|---------|-----------------------|
| Enrolled at baseline, <i>n</i> (%)             | 165       | 156       | 63       | 300       | 266      | 99      | 500      | 77      | 411                   |
| Enrolled at 6 months, <i>n</i> (%)             | 160 (97)  | 130 (83)  | 31 (49)  | 244 (81)  | 242 (91) | 85 (86) | 331 (66) | 72 (94) | 312 (76)              |
| Enrolled at 1 year, <i>n</i> (%)               | 159 (96)  | 103 (66)  | 26 (41)  | 220 (73)  | 208 (78) | 67 (68) | 295 (59) | 63 (82) | 238 (58)              |
| Enrolled at 18 months, <i>n</i> (%)            | 150 (91)  | 57 (37)   | 21 (33)  | 191 (64)  | 184 (69) | 37 (37) | 258 (52) | 56 (73) | 186 (45)              |
| Enrolled at 24 months, <i>n</i> (%)            | 143 (87)  | 31 (20)   | 18 (29)  | 160 (53)  | 161 (61) | 0 (0)   | 221 (44) | 44 (57) | 128 (31)              |
| Number of behavioural interviews, median (IQR) | 19 (9–32) | 5 (3–6)   | –        | –         | 5 (3–8)  | 5 (4–6) | 2 (1–3)  | 7 (4–9) | 4 (2–6)               |
| Number of phlebotomy visits, median (IQR)      | 20 (9–32) | 12 (8–16) | 6 (4–9)  | 13 (4–23) | 8 (4–14) | 5 (4–6) | 3 (2–5)  | 6 (4–9) | 4 (2–7)               |
| Deaths, <i>n</i> (%) <sup>a</sup>              | 29 (18)   | 5 (3)     | 2 (6)    | –         | 7 (3)    | 0 (0)   | 2 (2)    | –       | 16 (n/a) <sup>b</sup> |

<sup>a</sup>Percentage of participants with known survival status.<sup>b</sup>Denominator unknown owing to high false-negative rate.

testing intervals may account for lower rates of reinfection in PWID with spontaneous clearance when compared with rates of primary infection.<sup>16,20,22,28–31</sup> The mechanism behind this finding is simply that broad HCV testing intervals miss HCV reinfection events with spontaneous clearance, particularly if the duration of reinfection is less than the HCV RNA testing interval. The results of this study have important implications for HCV vaccine design because they suggest that although absolute protection against primary reinfection (sterilizing immunity) is probably overestimated, the rate of spontaneous clearance of reinfection (partial protective immunity against persistent HCV reinfection) is also underestimated.

A complete list of publications for the InC<sup>3</sup> Study is located at the following web address: <https://inc3.epi-ucsf.org/publications.aspx?Cat=1&SubCat=7>. Further data from subsequent mergers will improve the ability to perform novel analyses of HCV epidemiology not possible in other studies. These analyses include, but are not limited to, examining temporal trends in HIV and HCV incidence (including associated factors), estimating the rate and determinants of spontaneous clearance of acute HCV infection, estimating the rate and predictors of HCV reinfection and assessing factors associated with treatment response for acute HCV infection.

## What are the main strengths and weaknesses?

The nine cohorts comprising InC<sup>3</sup> have a great deal in common, including systematic follow-up with regular serological testing for HCV and HIV, HCV RNA testing and methodically collected demographic, behavioural and clinical information. InC<sup>3</sup> offers an unprecedented opportunity to overcome the limitations of current studies and address questions previously unanswered owing to a lack of statistical power, limited observation time, heterogeneous populations or other constraints.

The InC<sup>3</sup> Study will significantly enrich the study of HIV and HCV risk and outcomes in the following way: (i) as these outcomes are best examined by prospective studies that require time and extensive funding resources, this project is extremely efficient in using existing prospective studies and data that have already been collected; (ii) by merging data, InC<sup>3</sup> will have enough power to study rare outcomes that may offer significant insights into disease prevention and pathogenesis; (iii) there are opportunities for researchers to explore new methods to examine cross-study comparisons; (iv) InC<sup>3</sup> will result in new collaborative explorations and promote long-term potential for the project; and (v) the InC<sup>3</sup> team will encourage further research, generate new hypotheses, encourage training of postgraduates and postdoctoral

**Table 5** Factors associated with spontaneous clearance of HCV infection

| Characteristic                                      | Odds ratio | 95% CI    | P      | Overall | Adjusted odds ratio | 95% CI    | P      |
|-----------------------------------------------------|------------|-----------|--------|---------|---------------------|-----------|--------|
| Age (years)                                         |            |           |        | –       |                     |           |        |
| <25                                                 | 1.00       | –         | –      | –       | –                   | –         | –      |
| 25–29                                               | 0.94       | 0.57–1.56 | 0.818  |         | –                   | –         | –      |
| 30–34                                               | 1.01       | 0.55–1.85 | 0.984  | 0.971   | –                   | –         | –      |
| ≥35                                                 | 0.88       | 0.49–1.56 | 0.653  | –       | –                   | –         | –      |
| Female sex (vs male)                                | 2.32       | 1.55–3.49 | <0.001 | –       | 2.66                | 1.69–4.18 | <0.001 |
| Ethnicity                                           |            |           |        |         |                     |           |        |
| Caucasian                                           | 1.00       | –         | –      | –       | –                   | –         | –      |
| Indigenous                                          | 2.06       | 1.00–4.26 | 0.050  | –       | –                   | –         | –      |
| Asian                                               | 0.93       | 0.25–3.51 | 0.920  | 0.139   | –                   | –         | –      |
| Black                                               | 0.28       | 0.04–2.22 | 0.228  | –       | –                   | –         | –      |
| Recent injecting drug use (vs none)                 | 0.52       | 0.30–0.90 | 0.020  | –       | –                   | –         | –      |
| HIV infection (vs none)                             | 1.06       | 0.50–2.26 | 0.882  | –       | –                   | –         | –      |
| HCV genotype                                        |            |           |        |         |                     |           |        |
| Genotypes 2/3                                       | 1.00       | –         | –      | –       | –                   | –         | –      |
| Genotype 1                                          | 1.49       | 0.94–2.35 | 0.091  | 0.237   | –                   | –         | –      |
| Other genotypes                                     | 1.20       | 0.42–3.47 | 0.735  | –       | –                   | –         | –      |
| IL28B favourable genotype (rs12979860 CC vs. CT/TT) | 1.89       | 1.23–2.91 | 0.004  | –       | 2.03                | 1.30–3.19 | 0.002  |

fellows and support scientists' collaborations in new areas within the fields of virology and immunology.

However, many of the challenges facing the InC<sup>3</sup> Study relate to strengths enumerated previously. Challenges for merging pooled data across multiple studies present both structural and contextual challenges. Participating cohorts bring a range of data types and structures that present issues surrounding both inconsistent measurement and biological data testing protocols. Further, 'retrofitting' data from cohorts presents special challenges with respect to handling and testing synchronization of events. Nevertheless, these challenges provide opportunities to use our team's diverse collective expertise to develop new methodologies to provide solutions. Internationally, behaviours and exposures can vary for many reasons, such as types of drugs and routes of administration. Although some differences between sites may impose limitations, we also foresee these differences as opportunities to explore population-related factors, temporal features and access to care.

## Can I get hold of the data? Where can I find out more?

We encourage the participation of other interested investigators and graduate students to collaborate with the InC<sup>3</sup> Study. As the InC<sup>3</sup> Study evolves, it is

anticipated that new sites may be invited to participate to expand the geographic and population representation in the collaboration. Potential collaborators should contact the InC<sup>3</sup> principal investigator Dr Kimberly Page (KPage@psg.ucsf.edu) for further information. Readers who wish to find out more should visit the InC<sup>3</sup> website at <https://InC3.epi-ucsf.org>.

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### KEY MESSAGES

- Spontaneous clearance of HCV occurs in ~26% of individuals with acute infection and associated factors include female sex and favourable *interleukin-28B* genotype.
- Data from chimpanzee and human studies of primary HCV infection, viral clearance and HCV re-infection indicate that previous HCV infection is unlikely to provide substantial levels of acquired sterilizing immunity, but characterization of the course of primary HCV infection and reinfection suggests that some protection against persistent HCV reinfection is developed through previous HCV infection.
- Studies using long HCV RNA testing intervals underestimate the incidence of HCV reinfection and the probability of spontaneous HCV clearance after reinfection, thus underestimating the potential for partial protective immunity against persistent HCV reinfection, which has important implications for HCV vaccine design.

## References

- Mathers BM, Degenhardt L, Phillips B *et al.* Global epidemiology of injecting drug use and HIV among people who inject drugs: a systematic review. *Lancet* 2008;**372**: 1733–45.
- Hagan H, Pouget ER, Des Jarlais DC, Lelutiu-Weinberger C. Meta-regression of hepatitis C virus infection in relation to time since onset of illicit drug injection: the influence of time and place. *Am J Epidemiol* 2008;**168**: 1099–109.
- Nelson PK, Mathers BM, Cowie B *et al.* Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet* 2011;**378**: 571–83.
- Aceijas C, Rhodes T. Global estimates of prevalence of HCV infection among injecting drug users. *Int J Drug Policy* 2007;**18**:352–58.
- Grebely J, Tyndall MW. Management of HCV and HIV infections among people who inject drugs. *Curr Opin HIV AIDS* 2011;**6**:501–07.
- Mathers BM, Degenhardt L, Ali H *et al.* HIV prevention, treatment, and care services for people who inject drugs: a systematic review of global, regional, and national coverage. *Lancet* 2010;**375**:1014–28.
- Grebely J, Dore GJ. Prevention of hepatitis C virus in injecting drug users: a narrow window of opportunity. *J Infect Dis* 2011;**203**:571–74.
- Page-Shafer K, Hahn JA, Lum PJ. Preventing hepatitis C virus infection in injection drug users: risk reduction is not enough. *AIDS* 2007;**21**:1967–69.
- Hagan H, Latka MH, Campbell JV *et al.* Eligibility for treatment of hepatitis C virus infection among young injection drug users in 3 US cities. *Clin Infect Dis* 2006;**42**:669–72.
- Sacks-Davis R, Horyniak D, Grebely J, Hellard M. Behavioural interventions for preventing hepatitis C infection in people who inject drugs: a global systematic review. *Int J Drug Policy* 2012;**23**:176–84.
- Hahn JA, Page-Shafer K, Lum PJ *et al.* Hepatitis C virus seroconversion among young injection drug users: relationships and risks. *J Infect Dis* 2002;**186**:1558–64.
- Hagan H, Pouget ER, Des Jarlais DC. A systematic review and meta-analysis of interventions to prevent hepatitis C virus infection in people who inject drugs. *J Infect Dis* 2011;**204**:74–83.
- Corey KE, Mendez-Navarro J, Gorospe EC, Zheng H, Chung RT. Early treatment improves outcomes in acute hepatitis C virus infection: a meta-analysis. *J Viral Hepat* 2010;**17**:201–07.
- Grebely J, Matthews GV, Dore GJ. Treatment of acute HCV infection. *Nat Rev Gastroenterol Hepatol* 2011;**8**:265–74.
- Dore GJ, Hellard M, Matthews GV *et al.* Effective treatment of injecting drug users with recently acquired hepatitis C virus infection. *Gastroenterology* 2010;**138**: 123–35.e1–22.
- Aitken CK, Lewis J, Tracy SL *et al.* High incidence of hepatitis C virus reinfection in a cohort of injecting drug users. *Hepatology* 2008;**48**:1746–52.
- Park JN, White B, Bates A, Enriquez J, Liao L, Maher L. Motivators and barriers influencing willingness to participate in candidate HCV vaccine trials: perspectives of people who inject drugs. *Drug Alcohol Depend* 2012;**123**: 35–40.
- Teutsch S, Luciani F, Scheuer N *et al.* Incidence of primary hepatitis C infection and risk factors for

- transmission in an Australian prisoner cohort. *BMC Public Health* 2010;**10**:633.
- <sup>19</sup> Bruneau J, Daniel M, Kestens Y, Abrahamowicz M, Zang G. Availability of body art facilities and body art piercing do not predict hepatitis C acquisition among injection drug users in Montreal, Canada: results from a cohort study. *Int J Drug Policy* 2010;**21**:477–84.
  - <sup>20</sup> van de Laar TJ, Molenkamp R, van den Berg C *et al*. Frequent HCV reinfection and superinfection in a cohort of injecting drug users in Amsterdam. *J Hepatol* 2009;**51**: 667–74.
  - <sup>21</sup> Cox AL, Page K, Bruneau J *et al*. Rare birds in North America: acute hepatitis C cohorts. *Gastroenterology* 2009; **136**:26–31.
  - <sup>22</sup> Page K, Hahn JA, Evans J *et al*. Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. *J Infect Dis* 2009;**200**:1216–26.
  - <sup>23</sup> Cox AL, Netski DM, Mosbrugger T *et al*. Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. *Clin Infect Dis* 2005;**40**:951–58.
  - <sup>24</sup> McGovern BH, Wurcel A, Kim AY *et al*. Acute hepatitis C virus infection in incarcerated injection drug users. *Clin Infect Dis* 2006;**42**:1663–70.
  - <sup>25</sup> Grebely J, Dore GJ, Schim van der Loeff M *et al*. Factors associated with spontaneous clearance during acute hepatitis C virus infection [Poster Abstract 1063]. *J Hepatol* 2010;**52**:S411.
  - <sup>26</sup> Grebely J, Dore GJ, Schim van der Loeff M *et al*. Female sex and variations in IL28B are independently associated with spontaneous clearance of acute HCV infection. *J Hepatol* 2012; (in press).
  - <sup>27</sup> Grebely J, Prins M, Hellard M *et al*. Hepatitis C virus clearance, reinfection, and persistence, with insights from studies of injecting drug users: towards a vaccine. *Lancet Infect Dis* 2012;**12**:408–14.
  - <sup>28</sup> Mehta SH, Cox A, Hoover DR *et al*. Protection against persistence of hepatitis C. *Lancet* 2002;**359**:1478–83.
  - <sup>29</sup> Micallef JM, Macdonald V, Jauncey M *et al*. High incidence of hepatitis C virus reinfection within a cohort of injecting drug users. *J Viral Hepat* 2007;**14**:413–18.
  - <sup>30</sup> Osburn WO, Fisher BE, Dowd KA *et al*. Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. *Gastroenterology* 2010;**138**:315–24.
  - <sup>31</sup> Grebely J, Conway B, Raffa JD, Lai C, Krajden M, Tyndall MW. Hepatitis C virus reinfection in injection drug users. *Hepatology* 2006;**44**:1139–45.
  - <sup>32</sup> Vickerman P, Grebely J, Dore GJ *et al*. The more you look the more you find—Modelling the effects of hepatitis C virus testing interval on reinfection incidence and clearance among injecting drug users. *J Infect Dis* 2012;**205**: 1342–50.