STATISTICAL ANALYSIS PLAN

A precision randomized trial to evaluate the impact of tailored hepatitis C virus (HCV) treatment adherence support on HCV treatment outcomes in HIV/HCV co-infected and HCV mono-infected people who inject drugs (PWID) in India

NCT04652804

Supporting Treatment Outcomes among PWID (The STOP-C Study)

Funded by:

The National Institute of Allergy and Infectious Diseases

R01-AI-145555-01

Industry Support Provided by:

Abbott Diagnostics

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DAIDS-ES Document Number:	**DAIDS-ES ID 38666**
Version Number:	**1.0**
Version Day Month Year:	**5 January 2024**

SIGNATURE PAGE

I agree to conduct the analysis in accordance with the relevant, current protocol.

I agree to personally conduct or supervise the analysis.

Co-Principal Investigator: Shruti H. Mehta, PhD MPH / Professor

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1. Primary outcome

The primary outcome is sustained virologic response (SVR) defined as HCV RNA < lower limit of quantification (LLOQ) 10 – 60 weeks after completion of treatment (cure). The primary analysis will be intention to treat and will be stratified by the risk of treatment failure (minimal and elevated).

The primary analysis will compare the proportion achieving SVR across level of support (low, medium, high intensity) within the two strata of risk (minimal and elevated) independently. Using log-binomial models (or their extensions, i.e., Poisson regression with robust variance estimation) including adjustment for site as a fixed effect, in the elevated risk stratum, we will compare the proportion achieving SVR in those who received the low and moderate intensity interventions (in separate models) to those who received the high intensity intervention resulting in a relative risk (RR). Analysis will be repeated for the minimal risk stratum except comparing those who receive the high and moderate intensity interventions to the low intensity intervention.

Primary comparisons will be intention to treat. That is, the study population for this analysis will include everyone who was randomized. Individuals with missing data on the outcome of interest either due to loss-to-follow-up or death will be treated as failures.

1a. Per protocol analysis. We will conduct a per protocol analysis which will exclude individuals who died prior to SVR assessment, who are alive but lost to follow-up (i.e., do not complete the SVR visit) and those who prematurely discontinued treatment. The study population for this analysis will include everyone who was randomized, completed a treatment course and completed the SVR evaluation visit.

1b. Sensitivity analyses.

- Regression models will be repeated considering site as a random effect (instead of as a fixed effect).[1]

- Regression models will be repeated without adjustment for site.

- To account for covariates that are differentially distributed by arm or prognostic for the outcome:

    - We will repeat the regression models described above adjusting for any baseline demographic and behavioral factors that are significantly different by study arm within strata of risk.
    - We will repeat the regression models described above including a covariate for the prognostic score.
    - We will incorporate the covariates included in the prognostic score through application of the standardized estimator of Moore and van der Laan[2,3] in the stratified analyses (minimal and elevated). This will allow us to estimate the average treatment effect (ATE) within strata contrasting the proportion of the population that would achieve SVR in the groups of interest.

- To account for missing data/non-adherence related to the primary outcome.
    - Of interest is estimating the most beneficial effect of the higher intensity intervention as if everyone fully complied and there were no losses to follow-up or nonadherence to the intervention. Therefore, missing data will result from persons who do not

complete the SVR visit within the window including both those who are lost to follow-up before and after treatment completion. We will conduct an additional per protocol analysis which will exclude individuals who died prior to SVR assessment and who are alive but lost to follow-up (i.e., do not complete the SVR visit) but account for the informative loss to follow-up using inverse probability of censoring weights. Further, we will include the randomization to the different intervention arms as an instrumental variable. An instrumental variable analysis estimates the "complier" average treatment effect. Therefore, by using the randomization as an instrumental variable for the treatment arms, we would be able to estimate the complier treatment effect for those that fully comply with the intervention.

- A second approach will use fully conditional multiple imputation of the SVR outcome based on only two variables: treatment completion and adherence. In this analysis, undetermined SVR due to death will not be imputed; deaths will be treated as SVR failures.
- Assessing the relationship of the treatment arms with loss to follow-up is also of interest in and of itself. This is because as an implementation strategy, policy makers would need to know the potential for loss to a program that they are implementing. Therefore, a time to lost to follow-up analysis will be examined using standard survival methods and accounting for prognostic score.

1c. Non-inferiority analyses. While the overall trial is designed to assess superiority, if there is a failure to identify that one support intervention is superior to another in the minimal risk stratum, sensitivity analyses will examine non-inferiority within this stratum. Non-inferiority is of interest in this case because it is possible that the additional interventions confer no benefit beyond what is offered by the low intensity intervention among those at minimal risk of failure – this has important implications for costs and programs. The non-inferiority margin is 5% which is also clinically meaningful.

1d. Subgroup analyses. Subgroup analyses will explore whether the effect of the intervention in either risk stratum varies by the prognostic score itself as well as by other covariates of interest including age, employment status, HIV status and treatment, drug use, injection drug use, alcohol use, homelessness, depressive symptoms, medication for opioid use disorder (MOUD), distance to study site, readiness for HCV treatment and study site. Interactions will be included in regression models that also adjust for site.

1e. Exploratory analyses. Using viral sequence analysis of baseline and SVR samples, we will reclassify persons with evidence of reinfection (vs. treatment failure) as having achieved SVR. The primary outcome and sensitivity analyses by arm within strata described above will be repeated after this reclassification. We will also conduct exploratory analyses to explore correlates of HCV treatment failure and reinfection overall and by HIV status.

2. Secondary outcomes

Secondary outcomes are 1) treatment completion defined as completing the prescribed course of treatment (12/24 weeks); 2) adherence; 3) reinfection defined as testing positive for HCV Core Ag after achieving SVR; and 4) HIV viral suppression defined as HIV RNA<LLOQ.

2a. Treatment completion.

Those who discontinue treatment prior to the end of their prescribed course will be considered as failures for this analysis. In the elevated risk stratum, using log binomial regression models (or their extensions, i.e., Poisson regression with robust variance estimation), including adjustment for site as a fixed effect, the proportion completing treatment in those who received the low and moderate intensity interventions will be compared to those who received the high intensity intervention (in separate models) resulting in a relative risk (RR). For those at minimal risk of failure, analyses will be similar except comparing the high and medium intensity interventions to the low intensity interventions.

2a.1.Sensitivity analyses.

- Regression models will be repeated considering site as a random effect (instead of as a fixed effect).[1]

- Regression models will be repeated without adjustment for site.

- To account for covariates that are differentially distributed by arm or prognostic for the outcome:
    - We will repeat the regression models described above adjusting for any baseline demographic and behavioral factors that are significantly different by study arm within strata of risk.
    - We will repeat the regression models described above including a covariate for the prognostic score.
    - We will incorporate the covariates included in the prognostic score through application of the standardized estimator of Moore and van der Laan[2,3] in the stratified analyses (minimal and elevated). This will allow us to estimate the average treatment effect (ATE) within strata contrasting the proportion of the population that would achieve SVR in the groups of interest.

2a.2. Non-inferiority analyses. While the overall trial is designed to assess superiority, if there is a failure to identify that one support intervention is superior to another in the minimal risk stratum, sensitivity analyses will examine non-inferiority within this stratum. Non-inferiority is of interest in this case because it is possible that the additional interventions confer no benefit beyond what is offered by the low intensity intervention among those at minimal risk of failure – this has important implications for costs and programs. The non-inferiority margin is 5% which is also clinically meaningful.

2a.3. Subgroup analyses. Subgroup analyses will explore whether the effect of the intervention in either risk stratum varies by the prognostic score itself as well as by other covariates of interest including age, employment status, HIV status and treatment, drug use, injection drug use, alcohol use, homelessness, depressive symptoms, medication for opioid use disorder (MOUD), distance to study site, readiness for HCV treatment and study site. Interactions will be included in regression models that also adjust for site.

2b. Adherence.
Adherence will be defined both using both self-reported data and objective data on refills completed and pill counts. It will be analyzed as both a continuous outcome (e.g., percentage of doses reported as taken; percentage of doses taken according to refills completed/pill counts) and a dichotomous variable (e.g., >90% of doses reported as taken; >90% of doses taken according to refills completed/pill counts). For the dichotomous adherence outcome, analyses will be conducted as they are specified for the primary outcome using log binomial regression models or

their extensions (Poisson regression with robust variance estimation) with adjustment for site as a fixed effect. For the continuous variable, the analysis will compare the percentage value of adherence across level of support (low, medium, high intensity) within the two strata of risk. Using a linear regression model, in the elevated risk stratum, the difference in adherence level for those who received the low and moderate intensity interventions will be compared to those who received the high intensity intervention with adjustment for site. For those at minimal risk of failure, analyses will be similar except comparing the high and medium intensity interventions to the low intensity interventions. In this analysis, adherence will be calculated based on the data from the surveys at 4, 8 and 12 weeks as well as all medication dispensation records. In the primary intent to treat approach, all those randomized will be included regardless of whether treatment was completed or on treatment visits were missed.

2b.1. Per protocol analysis. We will conduct a per protocol analysis which will exclude individuals who died or were lost to follow-up before the end of treatment or who discontinued treatment prematurely.

2b.2. Sensitivity analyses.

- Regression models will be repeated considering site as a random effect (instead of as a fixed effect).[1]

- Regression models will be repeated without adjustment for site.

- To account for covariates that are differentially distributed by arm or prognostic for the outcome:
    - We will repeat the regression models described above adjusting for any baseline demographic and behavioral factors that are different by study arm within strata of risk.
    - We will repeat the regression models described above including a covariate for the prognostic score.
    - We will incorporate the covariates included in the prognostic score through application of the standardized estimator of Moore and van der Laan[2,3] in the stratified analyses (minimal and elevated). This will allow us to estimate the average treatment effect (ATE) within strata contrasting the proportion of the population that would achieve SVR in the groups of interest.

- To account for missing data
    - We will use fully conditional specification multiple imputation of the adherence outcome based on available reports and treatment completion data.

2b.3. Subgroup analyses. Subgroup analyses will explore whether the effect of the intervention in either risk stratum varies by the prognostic score itself as well as by other covariates of interest including age, employment status, HIV status and treatment, drug use, injection drug use, alcohol use, homelessness, depressive symptoms, medication for opioid use disorder (MOUD), distance to study site, readiness for HCV treatment and study site. Interactions will be included in regression models that also adjust for site.

2c. Reinfection.

In the primary analysis, reinfection will be defined as testing positive for HCV Core Ag after achieving SVR. This may be at any of the post SVR follow-up visits. To evaluate factors independently associated with reinfection, all clients who achieve SVR and have at least one

subsequent HCV Core Ag assessment will be included. Reinfection rate per 100 person years will be calculated. Using survival analysis, factors associated with reinfection will be assessed. These including ongoing substance use, needle sharing, MOUD, SSP use, HIV status and frequency of visits to the ICC. We will also evaluate whether the risk stratum or intervention had impact on reinfection outcomes as well as the re-infection rate over time elapsed since treatment completion. In order to account for missing data due to loss-to-follow-up, we will use inverse-weighting methods.[4,5]

2c.1. Sensitivity analyses. Analyses will be repeated using a reclassified reinfection outcome that incorporates sequence analysis (to reclassify samples at SVR as treatment failures vs. reinfection).

2d. HIV viral suppression.
Annual assessments of HIV viral load among HIV/HCV coinfected participants in the 7 ICCs are expected as part of other ICC activities and standard of care at Indian government ART centers. All available viral load measurements for HIV/HCV co-infected PWID enrolled in the trial will be used. Viral suppression will be defined as HIV RNA less than LLOQ. We will compare viral suppression across two groups defined by primary outcome status, which will be cured vs. not cured using logistic regression with generalized estimating equations (GEE) to account for repeated measures within individuals. Because these groups will be defined by the primary outcome and no longer balanced by randomization, analysis will be adjusted for confounding factors including demographics, substance use, needle sharing, MOUD, SSP use, social support and frequency of visits to the ICC. Analyses will be combined across all groups but we will include a covariate for the classification of risk of treatment failure (minimal or elevated) and intervention received. In order to account for missing data due to loss-to-follow-up, we will use inverse-weighting methods.[4,5]

2d.1. Sensitivity analyses. We will vary the threshold for HIV viral suppression (<150 copies/ml vs. 400 copies/ml vs. 1000 copies/ml) and will also incorporate the revised definition of SVR vs. treatment failure using sequence data.

3. Exploratory analysis

3a. Impact of interventions on medication for opioid use disorder (MOUD) utilization.
Daily MOUD utilization data is available in the electronic health record. We will explore the impact of the interventions within strata by arm on new MOUD initiation and MOUD retention using methods of survival analysis, logistic regression with GEE and growth mixture models. Analyses will be adjusted for site. Additional sensitivity analyses will be similar to those reported in the primary and secondary outcome analyses and will include accounting for the prognostic score.

3b. Impact of interventions on quality of life.
Quality of life data is captured at the SVR visit and at semi-annual post-treatment follow-up visits. Using logistic and linear regression with GEE, we will explore the impact of interventions within strata on quality of life after treatment.

3c. Impact of interventions on mortality.
Using Poisson regression, we will explore the impact of interventions within strata on all-cause mortality.

3d. Effect of delays in treatment completion on SVR.
Additional exploratory analyses will evaluate the impact of non-adherence and delays in completing treatment on SVR. Initial analyses will incorporate data from all three arms. Two variables will be calculated: 1) the percentage of doses completed (as described above); and 2) days to treatment completion. While the scheduled course of treatment will be 84 days for most, participants will be instructed to take all of the doses prescribed (even if doses are missed and the total time for treatment completion extends beyond 84 days). In separate models, log-binomial models (or their extensions, i.e., Poisson regression with robust variance estimation) will be used to estimate the association between 1) estimated adherence and SVR; and 2) days to treatment completion and SVR. These analyses will be combined across risk strata and treatment arm but these variables will be included as covariates. Additional analysis will be conducted exclusively among participants assigned to Arm 3 (high intensity support) where weekly adherence data will be collected using biometric data.

3e. Estimation of heterogeneity in treatment effect and estimation of average treatment effect.
Additional analyses will be completed to estimate heterogeneity in treatment effect and the average treatment effect for the primary (SVR) and secondary outcomes defined above.

The average treatment effect will be determined in order to estimate the effect of the different support strategies in the overall population. Under the unbalanced randomization scenario that is planned, a prognostic score is intentionally created. This score lies between the causal pathway from the individual characteristics to the treatment assignment, thus introducing an “informed confounding”, i.e., the causal relationship between treatment assignment and outcome is confounded by the prognostic score (i.e., individual’s propensity for treatment failure). To estimate the unbiased, average treatment effect, given that there is information on how the prognostic score affects study arm allocation, the targeted randomization can be used to reweight the sample back to the initial sample as if the randomization allocation was equivalent to all levels of the prognostic score. That is, by using inverse probability of treatment weights, the relationship between the prognostic score and treatment assignment is removed to estimate the average treatment effect to provide answers about whether the high and moderate intensity interventions resulted in superior outcomes in the overall population (i.e., is high intensity support a superior intervention to standard of care in all PWID regardless of propensity for treatment failure?).

Further analyses will seek to use information from the prognostic score and the observed data to identify the optimal threshold for assigning treatment support. That is, by assessing heterogeneity in treatment effect by level of prognostic score, we will potentially be able to identify a range of scores to assist in the decision of whether low, moderate, or high level of support would be most efficacious for individuals based upon the characteristics that determine their prognostic score. First, within the two strata defined by the prognostic score, there remains variability in the prognostic score. Therefore, we will evaluate the impact of the interaction between the prognostic score and the intensity of support received (low, medium and high intensity) on SVR and treatment completion rates in the full sample. The goal of this analysis will be to evaluate the heterogeneity of treatment *effect within strata.*

Of additional interest is identifying the heterogeneous treatment effect of the intensity of support received on SVR and secondary outcomes across the *entire distribution of* prognostic score (note that the prognostic score will be continuous). Therefore, by using the weighted (by inverse probability of treatment weights) study sample, the interaction between the prognostic score and intensity of support received on SVR and treatment completion rates can be examined across the

entire study population. Specifically, a model will include the indicator variables for level of support, the main effect of the prognostic score, and the interaction term. The model will allow for non-linear relationship between prognostic score and outcomes and depict the estimated treatment effect graphically to determine the optimal cut-points of the prognostic score to assign low, medium and high intensity treatment support to maximize SVR.

3f. Effect of heterogeneity of intervention effect by sex assigned at birth.
It is of interest to examine whether the impact of the intervention varies by sex assigned at birth. We anticipate that power will be limited in these analyses since in many of the field sites, the number of participants who were assigned female sex at birth is negligible, consistent with the epidemiology of injection drug use in India. However, we will conduct stratified analyses to explore whether the effect of the level of treatment support varies by sex assigned at birth within strata of treatment nonadherence risk. We will also conduct the analyses of the average treatment effect stratified by sex assigned at birth.

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