

D. STUDY DESIGN AND METHODS

To answer the two aims of this study, we plan to recruit 400 recently initiated injecting drug users between the ages of 15 and 30 years. They will be eligible if they report having injected illicit drugs at least one time in the past 6 months, and have started to inject less than five years prior to enrollment. An additional sample of 200 noninjection drug users (heroin/cocaine sniffers and crack smokers) between the ages of 15 and 30 years will also be recruited over the same study period. They will be eligible if they report having used heroin, cocaine, and/or crack in a non-injected way at least 3 times per week in the past month and have initiated noninjection drug use between 1 and 10 years prior to enrollment. The total sample will be followed longitudinally to study risk factors for HIV infection. **A cross-sectional and prospective study design will be employed to address HIV seroincidence, HIV seroprevalence, personal characteristics of initiates and NIUs, social support factors surrounding initiation and HIV, social network factors surrounding initiation and HIV, physical/sexual abuse characteristics as they relate to initiation/transition and HIV. (Aims 1, 2 and 3).** For Aim 1, HIV seroprevalence and seroincidence will be estimated through the baseline, 6-month and 12-month interviews. For Aims 2 and 3, lifetime and 6-month histories of personal characteristics such (e.g. detailed drug use and sexual risk behaviors) will be assessed for an association with current HIV serostatus. Also, while addressing these aims, we will assess psychiatric co-morbidity and social network/social support changes from a pre-initiation period to baseline and prospectively at 6 and 12 months. To address Aim 4, correlates and risk factors examined in Aim 2 will guide the steps to assess these aims utilizing baseline data. For Aim 5, the rate of transition into injection drug use by covariates (e.g. physical/sexual abuse, psychiatric condition, social networks/social support) will be estimated during follow-up at 6 and 12-month interviews.

Preliminary analyses, through the review of the ALIVE retrospective and seroincidence data, review of the REACH prevalence and seroincidence data, and informal interviews with young injectors, will guide the choice of risk behaviors to be investigated. The proposed study will attempt to identify new and validate previously reported drug and sexual risk correlates of HIV infection in a group of new injectors. Baseline results will be corroborated with data collected prospectively.

1. Population.

a) **The New Initiates Cohort:** A new cohort of 400 IDUs and 200 NIUs will be recruited for an initial interview including an HIV test. These individuals will be asked to return for a second interview and HIV test six months later to ascertain any changes in **abuse, psychiatric condition, social networks/social support**, drug injection patterns, sexual behavior and HIV infection status. Efforts will be made to **enroll 50% of the young women and 50% of the young men ≤ 25 years of age.** We expect to be able to recruit these percentages given our 50% recruitment of both men and women during our past REACH Study. We also expect our cohort to have similar racial and socioeconomic status characteristics to that of the REACH cohort. The demographic features of those enrolled for REACH were as follows: 45% with no reported an income or receive social services, 79% African American, and 55% injected 2 years or less at enrollment (conversely, 45% had injected for **3 or more years**). We expect to screen 70 to 80 young adults each month in Baltimore City and enroll 10 to 12 initiates and 5 to 6 NIUs each month.

2. Eligibility Criteria. For the recent initiates cohort, criteria will include having begun to inject illicit psychoactive drugs the first time within five years of enrollment and had at least one injection in the past 6 months. **For the NIU cohort, criteria will include having begun to sniff or smoke heroin, cocaine and/or crack between 1 and 10 years prior enrollment and have used drugs at least 3 times per week in the past month.** Injection status will be verified by presence of injection stigmata (scar tissue or "tracks") and via a series of questions aimed at identifying the plausibility of the individual's experience injecting drugs. Noninjection status will be verified by a series of questions aimed at the plausibility of an individual's experience of noninjection use, no presence of stigmata, and the striking physical appearance of a NIU. Participant ages will range from 15-30 years and will be verified by presentation of a current piece of identification with photo and birth date. **If the photo identification does not have a birth date, a legal/official document with the participants name and birth date must accompany the photo (i.e. medical papers/card, court/correctional facility papers, birth certificate, etc.)** All must agree to HIV testing, counseling and learning the results.

3. Recruitment. Recruitment will be community-based drawing from the Baltimore City area. We will distribute flyers within the community and rely on word-of-mouth spread of the information. **Street outreach recruiters**, who are trusted within the drug using community, will circulate flyers in different areas of Baltimore City. Potential sites for recruitment include: local youth dance clubs, runaway youth shelters, recreation centers, basketball courts, local health fairs, local Emergency Rooms, adolescent clinics, parental referrals from treatment centers, Baltimore Needle Exchange Program, and **other high drug activity areas** where young adults 'hang-out'. To further ensure targeting of a non-clinic seeking population, we will collaborate with other community outreach services such as UJIMA of the Baltimore Health Department (mobile street outreach services such as

HIV/STD testing, pregnancy screening, etc. targeting high risk populations) and **HERO street outreach services**. Given the fact that young injectors may be difficult to reach, because of reluctance to self-identify as an IDU, we were still able to successfully recruit 250 young injectors into REACH in under 2 years. In order to successfully **recruit 400 initiates and 200 NIUs**, increased efforts will be necessary. One potential drawback in our past recruitment strategy was our initial lack of visibility and readiness in different parts of the city. However, now we are more established in the community to meet this specific population by virtue of conducting the **REACH I, and II studies**. Nevertheless, to meet this challenge, we plan to use an RV for mobile recruitment, testing and follow-up visits. The RV will enable us to increase our enrollment in high risk areas too distant for participants to travel. To further expand our outreach services, we will extend our hours of outreach into the early evening. We will also include some weekends for outreach, primarily during summer and spring. We plan to continue to enlist the cooperation of the Community Advisory Board whose members have consisted of eight study participants, a community outreach recruiter, community leaders, and the study managers (Appendix A). This board will continue to provide feedback to the Principal Investigator, and Project Director, concerning successful and unsuccessful recruitment strategies. As with the ALIVE and REACH studies, we expect that much of our recruitment will be achieved through word-of-mouth referrals from study participants. We also learned through **our past and current REACH studies** that conducting interviewing activities at a city health department clinic across town from the primary site proved fruitful for enrollment and we plan to continue this strategy and move to other parts of the city. The van rental will be essential for such arrangement where space may not be available for HIV testing and assurance of interviewing privacy.

4. Interview. Prior to enrollment and after screening, we will disclose the purpose of the study and the study participant's rights of confidentiality, which includes showing a Certificate of Confidentiality (obtained from DHHS). Written informed consent will be obtained. After receiving pretest counseling, 10cc of blood for HIV and other viral testing, will be drawn from each participant. A trained interviewer will then administer an interview of approximately 45 minutes to all participants. Each participant will be scheduled to return in two weeks to receive post-test counseling and HIV test results. **At the result visit**, he/she will be given an appointment to return in six-months for a follow-up evaluation consisting of a blood draw and interview. **The 12-month visit will entail the same procedures as the 6-month visit.**

5. Data Collection.

a) Instrument: Our instrument will roughly follow the same format as **our preliminary study instruments** when examining previously identified HIV risk behaviors (i.e. injection and sex practices), circumstances of initiation, non-injectable drugs (i.e. crack, etc.) and demographics among initiates and NIUs (Appendix B). The portion of the instrument that which examines physical/sexual abuse and violence surrounding disclosure of HIV seropositive status, will be adapted from previous piloted instruments used by well published researches in the field. **The drug dependence disorder will be assessed using the standardized National Household Survey.** Portions of the DIS-6 will be used to assess 6-month prevalence of anxiety and depression. (Appendix C). Dr. Carl Latkin will provide the appropriate tool for examination of social networks and social support given his extensive background in this area and his current social network/HIV research projects among similar populations. He is in the process of developing a new instrument (SHORE Study) given his recent NIMH funding from which we will closely draw from.

The survey instrument will be pilot tested and we will adhere to the informed consent procedures approved by the IRB. All information will remain confidential and reported illicit behaviors will be covered under the Certificated of Confidentiality.

b) Interview Data from Baseline Visit: The baseline interview will obtain demographic characteristics, sex practices, injection practices, non-injectable drug use, physical/sexual abuse as a child, adolescent and/or young adult, psychiatric condition, social networks and all the above social and demographic characteristics as they relate to initiation. A descriptive list of 8 areas of focus containing suggested variables interest has been provided in Table 4. In addition to basic demographic information, these variables include drug use behaviors such as sharing injection equipment, location of drug use, age at initiation, frequency and type of drug including noninjection drug use behaviors. Sexual behavior correlates of the initiate and the partners thereof will include age at first sex, frequency of unprotected sex, **number and type of sex partners** and self-reported history of STD's. Social and injecting networks will include a detailed profile of injecting and **noninjecting partners**, characteristics of drug sub-network, characteristics of social support within drug sub-networks. Through more detailed network analysis, additional measures of social influence constructs will be collected. Variables regarding social support will include tangible support, emotional/esteem support and information support. Physical/sexual abuse characteristics will be measured through self-reports of abuse as a child and/or an adult. In the event of seropositive status, reports of violence or fear surrounding partner notification will also be obtained. These data are important to meet specific aims 2, 3, 4 and 5.

c) **Interview Data from 6 and 12- month Follow-up Visits:** Both 6 and 12-month visits will obtain all the above baseline information with the exception of fixed demographic characteristics. This instrument will include more detailed questions than the baseline instrument in obtaining recent history of sex and injection practices and events surrounding current abuse and psychiatric condition. A more detailed assessment of violence or fear surrounding an HIV seroconversion result during follow-up will also be included. HIV testing will also be done on those with an HIV negative baseline result. These data are also necessary to meet specific aims 1 and 2.

d) **Interviewer Training:** Interviewers will undergo extensive training and staff development during the first 6 months of the study. A portion of the interviewer training will be devoted to learning how to administer excerpts from the Diagnostic Interview Schedule (DIS-6) to assess 6-month prevalence of anxiety and depression. Interviewers will be involved in progressive development of the questionnaire to ensure flow of skip patterns and the ease to which it can be used in the field. Interviewers will have an opportunity to develop their interviewing skills through role playing and further refine their skills during the pilot testing phase. The training will be conducted and coordinated by the project director Ms. Fuller. Co-investigators Drs. Larkin and Arria will also have integral involvement in the interview training process in the areas of social networks and psychiatric condition. The project director will also ensure to the best of her ability that the interviewers chosen are culturally sensitive to young adult IDUs and possess a non-judgmental attitude while affiliated with the research study. Fortunately, seasoned interviewers who have extensive experience on the ALIVE and REACH studies will be available to work on this study if funded.

e) **Quality Control:** There will be constant efforts throughout the study period addressing issues of quality control. First, during the pilot testing of the questionnaire, efforts will concentrate on tool refinement for accuracy and efficiency. There will also be a structured cognitive testing procedure carried out during this time to ensure that the questions asked are actually measuring the variables we are interested in exploring. To assess completeness and standardization across different interviewers, frequency distributions of selected questions will be done and compared with frequency distributions of other interviewers. This will be done periodically throughout the study. In the event there are differences across interviewers, additional training and staff development will ensue.

To enhance standardization of prevention and education counseling (pre- and post-test counseling), all HIV counselors have undergone Level I and II, HIV Prevention Counseling Training through the Baltimore City Health Department/University of Maryland, Department of Mental Hygiene. Additionally, in-house quarterly HIV prevention and education counseling workshops will be conducted to further standardize the education and materials disseminated.

In an effort to deal with socially desirable responding by the study participant, Drs. Vlahov and Latkin have experience in measuring validity of self-reports using social influence constructs and scales of social desirability responding all of which have been documented in peer reviewed literature. These approaches will be carried through during this study and given proper consideration during the analysis phase of this study.

TABLE 4: Exposure Variables of Interest for Specific Aims:

Exposure Measurement	Variables	Variable Definition
1. Demographics	Age*; Sex*; Race*; Low income*; Education; Marital or live-in status	
2. Drug Use Behaviors	Following*	Using a fellow injector's equipment directly after he/she has injected
	Location of drug use: 1. Shooting gallery* 2. Outdoors* 3. Private home	1. Sharing injection equipment in a clandestine location with many anonymous partners 2. Injecting outdoors without ability to clean injecting equipment 3. Injecting in a private home with ability to clean injecting equipment
	Age at first injecting drug use	Age and calendar year of first injection

Exposure Measurement	Variables	Variable Definition
	Frequency of injecting*	Average number of daily, weekly, monthly injections
	Type of drug injected*	Various routes of administration and frequency of injecting cocaine, heroin, amphetamines, other drugs or combinations; Transitions of drug use leading up to and at first injection
	Noninjection drug dependence	Drug use patterns preceding first injection
3. Sexual Behavior Correlates (with both injecting and with other partners)	Age at first sex	Age and calendar year of first sexual encounter
	Frequency of unprotected sex*, sober and 'high'	Number of anal, oral, or vaginal sex without barrier protection, such as condoms; if post-injection number of unprotected acts by length of injecting career and type of drug used when 'high'
	Number of sex partners*	Number of sex partners with whom one has unprotected sex; distinguish if regular, occasional, trading sex partner , IDU, or known to be HIV+
	Self-reported STD history*	Lifetime history and detailed 6 month history of STDs (Syphilis serology as corroboration)
4. Social & Injecting Networks a. Profile Injecting Partners	Partners' length of injecting career*	Number of years IDU partner(s) have injected
	Age and sex of partner(s)*	Partner demographics
	Length of partner/initiate injecting relationship	Number months/years initiate and partner(s) have been injecting together; Did initiate seek out a 'teacher' or 'doctor' at first injection?
	Number of persons present at first injection	Group versus individual initiation process
b. Drug sub-network	Access to drugs	Able to buy drugs on the street; family use; selling; renting out home as SG or stash house
	Residence	Defined by Baltimore City zipcodes/census tracts
	Occasions to inject	Number of opportunities to use or witness injecting before actually starting to inject
	Means of getting drugs or getting money for drugs	i.e. Trading sex or illegal means
	Frequency of trading sex for drugs or money that involve anal sex	Number of unprotected anal sex encounters in the context of trading sex

Exposure Measurement	Variables	Variable Definition
c. Social support of drug subnetworks	Peer influence	overlap of friends and drug users subnetworks, overlap of friends and material support in the social network, and closeness to peers
	Perceived peer norms	cohesiveness of drug network, peer discussion of risk behaviors, and perceived peer risk behaviors and peers' self-reported risk behaviors.
5. Measures of Social Influence Constructs	Additional social network characteristics	Number of individuals listed in each domain, the centrality of the domain, the overlap of domains, and the quality and history of the relationships
	Social integration	network density and multiplicity, size of social network, and homelessness
	Social Control	Number of family and significant others in social network, duration of close relationship, marital status, cohabitation, and employment
6. Social Support	Tangible support	Number and proportion of individuals in the social network who give material and physical support and by income
	Emotional or esteem support	number and proportion of intimates and individuals who give positive feedback in the social network, closeness to network members, and individuals' positive experience of disclosure of HIV status
	Informational support	health advice given by persons in social networks
7. Physical/sexual abuse	Physical abuse as a child	duration and magnitude of physical child abuse
	Sexual abuse as a child	duration and magnitude of sexual child abuse
	Physical abuse as an adult	duration and magnitude of adult physical abuse
	Sexual abuse as an adult	duration and magnitude of adult sexual abuse
	Physical abuse following HIV disclosure	duration and magnitude of emotional and physical abuse
	Fear of violence as a result of disclosure	number of young women reporting fear if they chose to disclose HIV seropositivity
8. Psychiatric condition	Past 6 months drug dependence disorder	National Household Survey
	Past 6 months of anxiety	DSM-IV diagnosis
	Past 6 months of depression	DSM-IV diagnosis

* Previously reported correlates

6. Counseling and Referrals. Each new injector that gives consent to be screened for HIV antibody will receive the required pre- and post-test counseling by a trained counselor. From the information obtained at each visit, participants will be counseled and as appropriate, referred for additional evaluation and care. Efforts will be made to make direct referrals to the Hopkins or other HIV clinics and drug treatment services. In REACH, all HIV seropositive participants were directly referred to the ALIVE Study in addition to other HIV clinics. In our previous experience with this population, referrals have been received well by participants along with the respective clinical services. Finally, immediately after each interview is completed, all participants will receive risk reduction counseling. We feel that this is ethically imperative to minimize HIV transmission, even though this will probably result in a cohort that is different from the general population from which they were selected; however as participants receive such counseling, relative differences between seroconverters and persistent seronegatives should remain meaningful.

7. Retention. To assure retention in the cohort, methods to facilitate a high return rate throughout the follow-up period will include mailed reminders, tracing through contacts, travel expense vouchers for public transportation to clinic visits, and minimal remuneration for time spent in the study. In an effort to deal with the occasional transient nature of our population, a seasoned recruiter/tracker who has worked in this capacity for over 5 years on the ALIVE and REACH studies will be hired to track participants over the 5 year study period. Modest remuneration of injecting drug users has been found to be essential to maintain high rates of participation in cohort studies.

8. Laboratory Assays.

1. Phlebotomy and Sample Collection: At each visit, a phlebotomist trained in biosafety procedures will draw 10 cc of whole blood. The blood specimen will be transported daily to the serology laboratory at the Johns Hopkins School of Hygiene and Public Health where it will be centrifuged and aliquotted. Residual sera and plasma will be stored locally in a biological repository at -70° C for future studies.

The following assays will be performed from blood collected by venipuncture. 1) HIV antibody positives will be determined by enzyme linked immunosorbant assay, (ELISA) (Ortho Diagnostics, Raritan, NJ); 2) confirmation of an HIV positive test will be done with Western Blot (WB) (Ortho Diagnostics). Indeterminants on WB will undergo a redraw and retest.

Although not budgeted, we are interested in doing Hepatitis C viral (HCV) assays on all sera obtained. Antibody to HCV will be detected using the ELISA. Upon repeated reactive specimens that also demonstrated an optical density less than four times the cutoff, a first-generation recombinant immunoblot assay HCV Test System (Chiron Corp., Emeryville, Calif., and Ortho Diagnostics). Additional sera will be tested with a second-generation ELISA because potentially up to 95% of the repeatedly reactive specimens may also be positive by the recombinant immunoblot assay. Specimens repeatedly reactive by the second-generation ELISA will be considered positive without additional testing.

9. Data Management. The study managers have extensive prior experience designing and entering demographic, behavioral, laboratory, and medical history forms from studies. Specific tracking and locator information will be directly entered twice from coded forms into the Microsoft ACCESS database program on a microcomputer. After the questionnaire has been double checked by the interviewer for completion and consistency, it will be reviewed by the project director and sent to an off-site data entry service, Socio Corp, Inc., to ensure efficient data entry. This firm uses programs designed to verify data for range and logical checks to identify and eliminate data entry errors. Upon timely receipt of the questionnaires from Socio, inconsistent values will be checked by interviewers and laboratory personnel for verification and corrections will be made if necessary. Our current arrangement with Socio has been quite efficient for the daily coordination of the research study.

After cleaning the data, files will be merged for data analysis on minicomputers using SAS and STATA. Exploratory data analysis, such as contingency checks and tests for outliers for further quality control, will be performed. If necessary, the Johns Hopkins University mainframe computers are equipped with sufficient memory for more sophisticated statistical modeling.

10. Statistical Analysis.

a) Aim 1: HIV prevalence and incidence are the primary outcomes of interest in this study. **Crude HIV prevalence will be ascertained at baseline visits.** Those seronegative at baseline will be followed for ascertainment of HIV seroconversion at 6 and 12-month visits using person-time techniques to account for differential lengths of follow-up. In an effort to increase power

when calculating HIV seroincidence, participants from our recent initiate studies will be added in order to increase the sample size.

b) Aim 2 and 3: After cleaning the data, exploratory analysis will be performed. This process will include a detailed description of initiation into injecting drug use by person, place and time. Cross-sectionally, univariate analysis will include cross-tabulations of frequency distributions of exposure variables by HIV serostatus. Means, percentages, proportions and standard deviations will be calculated. Standardized cutpoints in the data will guide recoding of continuous variables. Where possible, external standards will be used for recoding the data. For example, the frequency of drug injection will be divided into levels previously reported in the REACH study so that direct comparisons of results can be made. The influence of outliers will be assessed and medians used if required. The need for transformation of data will be evaluated. Differences between two means will be tested using the t-tests or rank tests and the categorical data will be analyzed using Chi-square tests and/or with 95% confidence intervals to guide interpretation.

Shown in Figure 1, known HIV risk behaviors such as injection and sex practices among young initiates as they relate to HIV serostatus is an example of our first step of many different variables and outcomes that can be examined. The second step, according to Figure 1, will be the examination of our new variables (**i.e. abuse, psychiatric condition, social network/social support, circumstances of initiation**) by HIV serostatus. It is important to note that while HIV serostatus among our initiates **and NIUs** is one of our primary outcomes, Figure 1 also shows other outcomes of interests such as specific HIV risk behaviors. These steps of interests will be done using bivariate analysis, generation of 2x2 and 2xK tables and the examination of crude associations using Mantel-Haenszel Chi-Square test. As a primary measure of association, we will consider the odds of HIV infection by risk factor of particular interest. Statistical significance of the association will be determined at the 95% confidence limit. The level of correlation or collinearity between behavioral exposures will be determined by Pearson or Spearman rank correlation coefficients. It will be important to determine how correlated are behaviors such as social network, social support, patterns of abuse, psychiatric condition, and circumstances of initiation. Stratification will be conducted to check for confounding and examine effect modification. **For example, the association of recent sexual/physical abuse and HIV infection will be stratified by number of sexual partners.**

To simultaneously adjust for potential confounders such as duration of drug use, we will use Mantel-Haenszel techniques as well as multiple logistic regression methods and multivariate logistic regression analysis. Exposure variables that are statistically significant at the 0.05 level in bivariate analysis will be entered into the models. However, we will not restrict ourselves to the use of p-values, using epidemiological judgment to examine associations that are biological. For instance, the log odds of HIV infection will be evaluated for probabilities of sexual and drug use practices simultaneously. Variables representing levels of combined drug and sexual exposures will be developed. For example, those who practice no sharing and never have unprotected sex will be compared to those who practice only one of the mentioned behaviors and those who practice both behaviors.

c) Aim 4: If effect modification exists, additive interaction terms will be created and entered into the models, and the best fit models will be determined by the log-likelihood ratio and the amount of deviance from the saturated model. The Wald statistic will be used for hypothesis testing. Adjusted odds ratios and 95% confidence intervals will be reported. Interaction will also be tested on a multiplicative scale when assessing risk factors for HIV seroincidence.

d) Aim 5: To estimate rate of transition into an injecting career, Kaplan-Meier survival analysis **will be utilized** so that stratified estimates by different covariates can be obtained. In order to adjust for confounding covariates and potentially differential follow-up, Cox proportional hazards model will be employed. Again, through linking participants from recent previous studies, we gain power to detect significant differences assuming an approximate 30% transition rate estimated in recent literature.

Given the additional prospective nature of the design, an assessment of the causal pathway between the reported behavior to the acquisition of HIV infection and the exposures that are correlated with HIV infection at baseline can be evaluated among HIV seroconverters at the 6 and 12-month follow-up visits. Nested case-control and incidence studies can be performed and thus risk factors can be disentangled from behavioral correlates of HIV infection. Through similar steps as mentioned above with the exception of univariate and bivariate stratified person-time techniques, simultaneous adjustment for potential confounders will be done using person-time analysis and Poisson regression to obtain incidence rates and risk of HIV seroconversion. For example, in

Figure 1, we may begin to unveil the role of changes in social network characteristics as a risk for HIV seroconversion.

d) Social Network Analysis: Again repeating the previously mentioned steps, where appropriate, repeated analysis of covariance models will be employed. As many outcome variables will be dichotomous or polytomous, log-linear/logistic models will be used for these analyses. Logistic regression models will be used to describe how odds ratio estimates of association change as a function of independent variables and mediating variables. For previous studies we have used multiple logistic regression models to analyze the independent effect of network characteristics on risk behaviors. Multiple measures of networks and psychological scale data will allow us to employ causal modeling with LISREL. LISCOMP will be used for latent variable analysis with categorical data. (98) For those analyses that use the individual as the unit of analysis, multivariate regression models will be employed to estimate standard errors based on the network as the unit of analysis. Ordinal measures of change in HIV risk behaviors will be utilized under conditions of invariance under monotonic transformation and distribution free characteristics. Several recent modifications of traditional ordinal methods such as the Mann-Whitney-Wilcoxon statistic and Wilcoxon signed rank test (for matched pairs) will be used to broaden their inferential aspects and enhance their utility. (99,100)

In addition to software described above, the network data will be analyzed with standard statistical packages of SPSS and SAS and with the UCINET IV network analysis program design by Borgatti, Everett, and Freeman. (101) UCINET is a menu-driven network program for PCs with unlimited technical support. Most of UCINET's programs accept asymmetrical data. From raw data matrices the program has the capacity to measure network attributes, connections, centrality, subgroups, positions, stochastic models, and density tables.

Additional analysis will include the examination of sub-groups with the network programs, including true cliques, n-cliques, and k-plexes. Each subnetwork will be categorized on the standard network properties of centrality, density, and distance. One important social network measure is path length, the number of steps between two individuals along observed links between them. To examine bridges between subnetworks we will assess the betweenness measure of centrality. (101) Another network property that will be examined is homophily (Farraro & Sunshine, 1964). The property of homophily will allow us to examine the tendency of network members to associate with similar others. Also analyzed will be multiplexity, that is, the extent to which individuals are connected by a single tie (e.g., sex only) or multiple ties (e.g., sex and work together and are neighbors). It is anticipated that multiplex ties are more likely to “convert” to sexual ties than uniplex ties. Other social network measures include reach, flow, centrality (e.g., betweenness, closeness, flow, Bonacich power), position, structural equivalence, paths, and walks. (102,103,104)

10. Power. To address the first three specific aims of this study, we are taking into account information on rates of HIV seroprevalence among participants of the REACH Study (Table 5). Rates are based on the most recent year with complete data - 1996. As the rate of HIV seroprevalence was 14.0% in 1996 for those enrolled in the within one year of their first injection, we anticipate a similar seroprevalence for those initiating in 1996 through 2001. In order to calculate the minimum detectable odds ratio, with a fixed sample size of 400 new injectors, we are estimating a minimum of 56 (14%) prevalent HIV infections. Cases will be considered the HIV positive individuals and controls will be 344 HIV negative individuals giving a case-control ratio of one to six. With an alpha level set at 0.05, we will have greater than 80% power to detect a minimal odds ratio of 2.50 considering an exposure among the controls of 0.20, 0.30, and 0.50 (Table 5).

TABLE 5: Power Estimates for HIV-1 Prevalence in Baltimore, MD
alpha = 0.05 case-control ratio = 1:6

Exp	Odds Ratio						
	2.00	2.25	2.50	2.75	3.00	3.25	3.50
.20	.597	.733	.833	.899	.941	.967	.981
.30	.659	.792	.881	.935	.965	.982	.991
.50	.644	.775	.865	.922	.956	.975	.986
.70	.473	.593	.692	.771	.831	.876	.909

* In the REACH cohort, exposures of 0.22 = > 100 lifetime sex partners, 0.50 = injected 5 /day; 0.60 = trading anal/oral/penile or vaginal sex.

Using the same format above and exposure prevalence in recent literature (.20-.40 = snort heroin, .28-.88 = snort cocaine, .40 = smoke crack, .39 = heroin and cocaine), **we estimate 69% power (exposure=0.40)** to detect an odds ratio of 2.25 and 79% power (exposure=0.20) to detect an odds ratio of 2.7. These are conservative estimates given the fact that our sample size will be increased once we have linked this data with data from our recent initiate studies (REACH I, II).

Further analysis will be conducted on predictors of seroconversion among the seronegatives during the follow-up periods. We estimate 80% (approximately 275) of the 344 seronegatives will remain in the study of whom 4.5% (approximately 12 participants) will seroconvert to HIV. This number will be too small to generate statistical power for analysis of predictors of seroconversion. However, assuming that a considerable number of new initiates not previous participants in our recent preliminary initiate study (REACH) will also be eligible to enroll in this proposed study and similar variables will be assessed, database linking may provide the power necessary to predict risk factors of seroconversion.

11. Strengths of the Study. New injectors are an **important public health issue**. With the largest increases in HIV infection occurring among young adults and adolescents, and since the rate of new HIV infection hovers at about 4% per 100 person years, it is vitally important to better document risks of infection among IDUs. The study design will allow us to determine the importance of HIV transmission risks at the first injection and in the first three years of injection. **By comparing the new cohort results with the aging ALIVE cohort results and the past REACH studies**, we can begin to document changes in the HIV epidemic in IDUs over time.

We will study the *new correlates and risk factors of HIV infection* independently and the interrelationship of each new correlate and risk factor proposed. During follow-up, we can begin to disentangle the relative importance of these exposures and further elucidate various causal pathways leading to HIV infection.

The HIV status of the participant will be unknown at the time of interview. Since the baseline data and examination for stigmata will be completed prior to HIV testing and counseling, *both the interviewers and the participant will be masked* to HIV serostatus. While at baseline some will already know their HIV status due to prior testing, we will ask about this and include in our analysis to account for this. In the prospective component, this is even less of an issue as follow-up questionnaire of HIV seronegatives will be performed at time of follow-up venipuncture with results done two weeks later, thus assuming that both interviewers and participants are blinded to the results.

Both ALIVE and REACH studies have established *strong links with Baltimore City community groups and outreach organizations* that target young IDUs. These resources will be invaluable to the recruitment process and will help to ensure that there is community support of the research project.

This study is similar to our previous initiate study *giving strength to the prospective component* of the study design. Additionally, this study is a 5-year study, unlike the preliminary 3-year REACH **studies**, which can further increase numbers due to more intensive recruitment and opportunities for follow-up. In addition to combining data for seroconversion analysis and transitional rate analysis, results from this study will add to the current literature and may guide the development of youth specific interventions to prevent new infections among IDUs and NIUs approaching transition into and injecting career.

12. Limitations of the Study.

Selection - Using community wide recruitment of volunteers into this study, it might be possible that individuals who enroll into the study, might differ from those who do not, and more importantly, that subgroups who are the subject of certain analyses (e.g., men vs women) might differ from their respective populations. An example might be the recruitment of a different subset of men than women due to study location or other characteristic. If this occurred, epidemiologic associations might be biased. **If, in fact, such bias occurred due to study location, our mobile recruitment will enable us to enroll participants from all areas of Baltimore City tending towards non-differential bias.** Otherwise, without a defined frame for this otherwise hidden population, few efficient methodological alternatives exist. (19) To offset this limitation, we will compare the characteristic of our sample with relevant information from other data sets (e.g., jail intakes, other local community cohorts) to explore this issue. In addition, inferences will be drawn with caution.

Validity of Self-reports - Distortion of self-reports might occur for a variety of reasons, intentional and unintentional as we have described in detail earlier. (19) Invalid reports can be minimized by study design whereby responses are not tied to perceived desirable or undesirable outcomes (e.g., **report of injection drug use to get remuneration will be minimized by masking this criteria through enrollment of NIUs**). Also, validity of self reports can be measured using social influence constructs or scales of social desirability responding.

There may be some limitations with regard to *identifying the most recent, young injectors*. Because the mean age of initiation into injection has been typically reported as 20 years old, we have limited the age range of our population to fall between 15 and 30 years. This age range will ensure the inclusion of all recent young adult injectors. In the event that possibly among the 25 to 30 year old injectors, an injection career may exceed 5 years, such an injector will be referred to the ALIVE or HIVNET Studies where they may be eligible to be enrolled in a seronegative study. Using this technique, participants will not be penalized for longer injection careers. This strategy will discourage individuals from misrepresenting the duration of their injector status. Injection stigmata of the initiate will be verified by a phlebotomist. In the case of the youngest and most recent injectors, stigmata may not be apparent. In this situation our **recruiters** and carefully trained interviewers will carefully probe the individual on their past injecting behavior. In a case in which the validity of new injection status is in doubt, the individual will be excluded from further participation our study. **For example, if during the interview, it is absolutely clear that the individual has injected drugs for a period exceeding 5 years or has not injected in the past 6 months, he/she will be thanked for his/her time and the interview will be terminated.**

Misclassification Bias - Exposure status in both prevalence and cohort studies is determined by self-reported risk behaviors, and may produce exposure misclassification. This source of bias can be limited by incorporating questions that use appropriate language, and that are ordered in a manner to aid recall.

Decreased Power - We may limited power for interactions, particularly without a sizeable number of past participants of the REACH study enrolled in this proposed study. So although this study may not be able to rule out many plausible associations due to limited precision and statistical power, it will be able to distinguish major differences of public health significance.

Response Bias - As we study people at each visit, we are ethically obligated to provide risk reduction counseling which can impact on future self-reports (imprecision management) therefore responses can be biased. However, the thrust of prospective analysis is to compare seronegatives who seroconvert to persistent seronegatives, all of whom receive the same level of counseling. So, relative differences should be unbiased and meaningful, although absolute levels of risk behavior might be dampened.

13. Time line. Start date: October 1, 1998.

- 1.) During the first 6 months of the study, staff will be hired and staff development will be implemented. The questionnaire will be developed and piloted with the assistance of the Community Advisory Board.
- 2.) During the second 6 months through mid-year 4, recruitment will take place in addition to the respective 6-month and 12-month follow-up visits.
- 3.) From year 2 though the end of year 4, 6-month visits will take place.
- 4.) From mid-year 2 through mid-year 5, 12-month follow-up visits will take place.
- 5.) During the last 6 months of the study, analysis and manuscript generation will take place in addition to taking the appropriate measures for study closure.

End date: September 30, 2003.