

The J. David Gladstone Institutes has been approved for a reconsideration of the total cost commitments as stated in a letter dated 16 November 2004 from Joseph Ellis, acting director of the NIH Office of Policy for Extramural Research Administration. Please find a copy of the letter attached and a new checklist reflecting the revised F&A rates. In addition, effective January 1 2005, fringe benefit costs will be transferred from F&A costs to direct costs as the result of the revised F&A structure. Accordingly, we request additional funds for the fringe charges at the rate specified in the attached budget and justification.

1. Has there been a change in the other support of key personnel since the last reporting period?

No.

2. Will there be, in the next budget period, a significant change in the level of effort for the PI or other personnel designated on the Notice of Grant Award from what was approved for this project?

We request decreasing Principal Investigator Robert Grant's effort from 16% to 10%. At the end of FY3 recruitment and enrollment activities will be suspended. Staff effort will be diverted to retention with a goal of exiting all individuals from the cohort during the first six months of the year. Following the completion of clinical activities personal effort will be further diverted to laboratory analysis. Closure of enrollment will reduce coordination and oversight responsibilities of the Project Manager and PI.

3. Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25 percent of the current year's total budget?

No.

Completion of study accrual. Since the start of R01 AI056988, 138 couples have enrolled in the study of the 150 proposed. We expect to enroll the remainder prior to the end of FY3. Prior to the award of this grant 32 couples had already been enrolled in pilot and feasibility versions of this protocol. Of the 170 couples enrolled 55% have completed the study. On-time study completion has consistently run at about 70% of the enrolled cohort. However, more than half of those individuals who exit late do so only for logistical reasons. Ultimately we expect retention to be ~85% of individuals or higher. With the closure of enrollment Positive Partners has become the largest prospective study of HIV superinfection among seroconcordant couples.

Table 1: Accrual and Retention Status of the Positive Partners Cohort						
	Enrolled/in follow up	%	Completed	%	Total	%
Individuals enrolled with partner	151	45.1%	184	54.9%	335	100.0%
High-risk singles	6	15.4%	33	84.6%	39	100.0%
Total	157	42.0%	217	58.0%	374	100.0%

No evidence of systemic superinfection in highly exposed seroconcordant couples. Of these, baseline screening for divergent viruses has been completed in 132 couples using phylogenetic analysis of population-based *pol* sequences obtained from viral RNA in plasma or DNA obtained from PBMCs (using an adaptation of the Bayer TRUGENE assay). One hundred eighteen couples with divergent viruses at baseline were followed over time to look for evidence of superinfection. Despite extensive sexual exposure to genetically distinguishable viral variants, no evidence of systemic superinfection was detected in the 174 individuals who have so far exited and been assessed virologically.

Antiretroviral treatment (ART) account for some, but not all, of the absence of systemic superinfection observed in the cohort. In the absence of mechanisms blocking HIV superinfection we would expect reinfection to occur as commonly as primary infection given the same exposure. In Table 2 we show the total exposure burden reported by 35 couples during observation and retrospectively during their entire relationship.

Table 2: Summary of Sexual Exposure to Highly Divergent HIV Variants				
	<i>N</i>	<i>Mean</i>	<i>Person-years</i>	<i>Total UI Exposures</i>
Prospective data				
UI with enrollment partner	54	90.69		4897
Years observed	54	1.044	56.4	
UI with other partners	25	49.28		1424
Years observed	25	1.156	28.9	
Totals			56.4	6317
Retrospective data				
Total UI with enrollment partner before enrolling	67	215.04		14,408
Years observed	67	2.64	176.9	
Grand Totals			233.3	20,725

While under observation individuals reported 6,317 episodes of unprotected anal intercourse with their enrollment partner and outside partners; combined with our estimate of exposures with enrollment partner prior to enrollment this cohort experienced 20,725 episodes of unprotected intercourse.

Based on published infectivity statistics¹ these exposures could have led to over 21 new infections had the partnerships been serodiscordant (Table 3).

Eliminating exposures from partners who had viral loads of <1,500 reduced the number of expected

Table 3: Expected Incidence of Superinfection in 54 Individuals in the Absence of Mechanisms Blocking Superinfection			
Type of contact	Risk	Number of Contacts	Expected superinfections
Prospective data			
URAI	0.82%	2395	19.64
UIAI	0.06%	2395	1.44
URVI	0.09%	120	0.11
UIVI	0.01%	120	0.01
Total:			21.20
Retrospective data			
URAI	0.82%	6819	55.92
UIAI	0.06%	6819	4.09
URVI	0.09%	335	0.30
UIVI	0.01%	335	0.03
Total:			81.54

superinfections to 9.5; additionally eliminating all individuals who were on ART and thus possibly protected by chemoprophylactic effects further reduced the expected superinfections during observation to 2.85 (Table 4).

When adjusted for infectivity of partner and, in addition, ART chemoprophylaxis expected retrospective exposures dropped from over 80 to about 23 and 2.5 respectively (table 5). Adjusting for the protective effects ART may have against superinfection we would still expect to have seen more than five superinfections among this small cohort of 35 couples. No evidence of superinfection was found suggesting that in addition to protective effects of ART, if any, additional mechanisms may be blocking HIV superinfection. These data are being submitted for peer-reviewed publication.

Table 4: Expected Incidence of Superinfection in 54 Individuals with Partners VL >1500 copies/ml Expected in the Absence of Mechanisms Blocking Superinfection during 56.4 Person-years of Observation			
Type of contact	Risk	Number of Contacts	Expected superinfections
Individuals on or off ART			
URAI	0.82%	1030	8.45
UIAI	0.06%	804	0.48
URVI	0.09%	120	0.11
UIVI	0.01%	80	0.01
Total:			9.05
Individuals not on ART (at one or more timepoints)			
URAI	0.82%	312	2.56
UIAI	0.06%	297	0.18
URVI	0.09%	120	0.11
UIVI	0.01%	80	0.01
Total:			2.85

Evidence of frequent sequentially expressed dual infection (SEDI) in recent seroconverters. In contrast with the lack of evidence of superinfection in chronically infected couples, work has continued to assess the risk of systemic superinfection in untreated recent seroconverters. We screened a sample of 104 individuals from a San Francisco cohort in close collaboration with Rick Hecht, a UCSF

Table 5: Estimates of Superinfections Expected at Enrollment Based on Past Exposure	
Estimate of Retrospective Superinfection Expected in 54 Individuals with Partners VL > 1500 copies/ml	
Total Number of Expected Superinfections during 56.4 years of Observation	9.05
Mean years observed per participant	1.044
Total Number of Expected Superinfections during a year	8.67
Total Number of Years Observed Retrospectively	2.64
Total Number of Expected Superinfections during 2.64 years of Retrospective Observation	22.88
Estimate of Retrospective Superinfection Expected in 54 Individuals with Partners VL > 1500 copies/ml and Not on ART*	
Total Number of Expected Superinfections during 56.4 years of Observation	2.85
Mean years observed per participant	3
Total Number of Expected Superinfections during a year	0.95
Total Number of Years Observed Retrospectively	2.64
Total Number of Expected Superinfections during 2.64 years of Retrospective Observation	2.51

*=> one time point

investigator funded by AIEDRP. Using sensitive heteroduplex tracking assays – verified to detect minor variants circulating at >1.5-3.0% of population – we have analyzed multiple timepoints among five cases of apparent superinfection. Further confirmatory clonal analysis has been completed in all five cases. This analysis revealed that in one

case that initially appeared to be dual infection at baseline the second virus could not be detected and appears between 10 and thirteen weeks post infection. A SEDI this early in primary infection has not yet been reported in the literature. In three cases, a virus that appeared during the first year of infection could not be detected at baseline, and in a fourth case the second virus appeared during the third year of infection (based on infection dates estimated by less-sensitive ELISA). In all four of these cases the original infecting virus did not persist at detectable levels during follow-up after the emergence of the second virus. For this reason, initial dual infection could not be ruled out and sequential *acquisition* of a second virus could not be confirmed, particularly as potential source partners could not be identified.

Three cases of apparent transient SEDI have been identified in this sample. In one case HLA typing suggested that specimen processing error was responsible for the aberrant virus. Two other cases will be assessed by confirmatory clonal analysis. Based upon these findings, durable systemic SEDI occurs at an incidence of 2-5% during the first three years of infection. While sexual exposure data are incomplete in the San Francisco AIEDRP cohort, existing data suggest that as few as 50% of newly infected individuals experience ongoing exposure during the first three years of HIV infection. Thus, we estimate that SEDI may occur among as many as 8% of recently infected individuals who are sexually exposed to HIV variants. Our screening protocol, which only included population sequencing at two timepoints, was not designed to identify transient SEDI that can be detected at as few as one timepoint like the two cases currently being analyzed. It appears likely that transient SEDI during recent infection is possibly very common. We have proposed a new study to more rigorously follow and screen recently infection Options patients to look for evidence of common multiple primary infections, transient SEDIs, localized superinfection and their behavioral, immunological, and clinical correlates.

Analysis of neutralizing antibodies, tropism shifts, and viral replication capacity.

Based upon the hypothesis that frequent SEDI during early infection may occur due to patterns of early immunological response or viral interference, three of the confirmed SEDIs were analyzed in collaboration with colleagues at Monogram Biosciences, using separate funding from institutional sources. In two of three cases, neutralizing antibodies effective against the initially infecting virus were not effective against the virus that predominated later during infection. In none of the three cases were initial neutralizing responses effective against the virus that emerged and became predominant during follow-up. In one of the three cases, an R5 virus was replaced by a X4/R5 dual tropic virus. In at least one case, the replacement virus had a significantly higher replication capacity than the initial virus. None of the replacement viruses had a lower replication capacity than the initially predominant strain.

Five SEDIs in recently infected individuals were analyzed for neutralization titers against autologous virus and those of highly-exposed recently and chronically infected individuals in the Positive Partners cohort. As expected, responses to autologous virus were weaker than responses to other viruses. Importantly, autologous and control responses were considerably stronger among highly exposed recently infected individuals without superinfection than those with superinfection. All differences were statistically significant. Taken together, these data provide some support for the hypothesis that slowly deepening and broadening neutralizing responses are protective against SEDI or systemic superinfection and that early development of neutralizing

responses may help to close the window of opportunity for superinfection in recently infected individuals. These data were presented at the Conference on Retroviruses and Opportunistic Infections (CROI) in 2006.

We are continuing to collaborate with Monogram to analyze highly exposed recently infected individuals and their partners from the cohort who show no evidence of superinfection. We hypothesize that these exposed individuals will consistently have broader neutralizing responses than SEDI cases, and that there will be heightened responses to partner's viruses.

Evidence of frequent recombination in SEDIs may be a mechanism of viral interference and escape. Intratype-B recombinant envelope sequences were identified in 4 of 5 SEDI cases that were studied by clonal analysis. The cytoplasmic tail region was identified as a "hot spot" for recombination in these viruses, which is expected to alter infectivity and fusion phenotypes that may drive viral fitness during transmission and systemic viral spread. The recombinants were transient appearing during the period when the superinfecting virus appeared and overgrew the primary infection virus. Neutralization titers in one patient were not effective against the primary virus, the recombinant, or the second virus and never developed against the primary virus during observation. Neutralizing responses to the recombinant and second virus appeared within five and after ten weeks of their appearance respectively and peaked at 50 weeks post appearance. After that time the recombinant virus was reduced to a minor variant of the dominant second virus. The ability of the second strains to overgrow the initial virus population has implications for the development of a broadly protective vaccine. Data regarding evidence of recombination in *env* and development of neutralizing antibodies to competing viruses over time were presented at the Keystone conference in 2006.

Analysis of neutralization responses to partner's virus in highly exposed seroconcordant couples. We have begun testing the hypothesis that highly exposed individuals may develop neutralizing responses to a regular partner's virus which may circumvent systemic superinfection among chronically infected individuals. Among the 54 couples already assessed in Positive Partners, we identified the 8 most highly exposed due to frequency of recent unprotected intercourse or duration of exposure. These couples are currently being assessed for neutralizing antibodies to index and partner viruses. We are planning to submit this analysis as an abstract to CROI for presentation early in 2007.

Evidence of viral heterogeneity in treated and virally suppressed individuals. We previously hypothesized that successful antiretroviral treatment might create an opportunity for superinfection due to decreased immune response and increased target cell populations. In addition, we also hypothesized that limited or localized superinfection may be more common than systemic superinfection and that mechanisms blocking systemic superinfection may be informative to vaccine research. To date we have identified eight cases of apparent SEDI in cellular DNA among individuals who have been virally suppressed on ART. Clonal analysis has been completed in six of these cases confirming that at least one viral lineage was replaced by a second lineage during ART induced viral suppression. Although the partner was also assessed in three cases neither of the viruses was linked to the partner suggesting an earlier superinfection event or multiple primary infections. Clonal analysis of the two remaining cases and participant follow-up are underway determine if cellular viral heterogeneity

may have clinical consequences, including whether or how this diversity may be expressed during treatment breakthrough or STI. Screening of viral DNA for evidence of multiple lineages is still underway. At this point, it appears that the prevalence of DNA SEDI that cannot be linked to a partner virus may be between 10% and 25%. Further analysis of this phenomena will be informative for interpreting apparent superinfection cases and understanding how clinical outcomes may be driven by one virus in cases of multiple infections while additional viruses are controlled and appear to have little clinical impact.

Plans for the coming year:

1. Retention activities to maximize the number of enrolled couples who exit the study and who are evaluable for evidence of superinfection.
2. A program of immunological studies in collaboration with Douglas Nixon to determine if slowly broadening CTL responses can account for lowered susceptibility to SEDI, and if adaptive CTL responses occur frequently during early infection when SEDI occurs. In addition, a research plan has been developed to test the hypothesis that alloimmunization may occur in highly-exposed partnerships. Currently work is underway to select partner pairs in which evidence for CTL response to escape mutations in a partners virus may develop over time. Separate funding is being requested to support this work.
3. Continued collaboration with Monogram to fully characterize cases of SEDI or possible superinfection in terms of activities of neutralizing antibodies, viral tropism, and replication capacity.
4. Additional analysis to look for evidence of localized superinfection in GALT derived from rectal biopsies of highly exposed individuals who were enrolled in Positive Partners with a potential superinfection source partner.

Project-related presentations that cite R01 AI056988-02:

Grant RM, McConnell JJ, Marcus JL, Kreis C, Spotts G, Liegler T, Brennan R, and Hecht FM. Higher frequency of apparent HIV-1 superinfection – sequentially expressed dual infection (SEDI) – in recent infection compared to chronic infection. 12th Conference of Retroviruses and Opportunistic Infections, Boston, 2005.

Liu Y, Chappay C, Wrin T, Stawiski E, Hecht R, Petropoulos C, McConnell J, and Grant R. Frequent envelope recombination in patients with sequentially expressed dual infection by distinct HIV-1 subtype B strains. AIDS Vaccine Conference, Montreal, 2005.

Grant RM. Superinfection: Real? Or really overblown? Center for AIDS Prevention Studies Town Hall Meeting, San Francisco, 2005.

McConnell J and Grant RM. Is all barebacking created equal? A community forum for HIV-positive men. Magnet Men's Health Clinic, San Francisco, 2005.

Grant RM. Current research on HIV superinfection, levels of viral load and infectivity, serosorting, strategic positioning, risks of oral sex, STDs: What do we know? Fifth Grantee Meeting, Special Projects of National Significance and the University of

California, San Francisco's Enhancing Prevention with Positives Evaluation Center, Washington, 2005.

Marcus JL, Krek J, Bragg L, Grant RM, and McConnell J. When Harry met Larry: Serosorting and HIV-1 epidemic spread among San Francisco MSM. Society for the Scientific Study of Sexuality Western Region Conference, San Francisco, 2005.

McConnell JJ, Bragg L, Krek J, Marcus JL, Grant RM. Effective sexual health counseling strategies for HIV-positive individuals in light of new data on HIV superinfection and serosorting. Gay Men's Health Summit, Salt Lake City, 2005.

References cited

1. Vittinghoff, E. et al. Per-contact risk of human immunodeficiency virus transmission between male sexual partners. *Am J Epidemiol* **150**, 306-11. (1999).