

The Development of Immune Escape Mutations within Immunodominant Gag epitopes is Associated with Increased Viral Replication During Structured Treatment Interruption

Becky Schweighardt¹, Fatema Legrand¹, Duncan A. Meiklejohn¹, Ann Erickson¹, Gerald Spotts², Brinda Emu¹, Douglas F. Nixon¹ and Frederick M. Hecht²

¹Gladstone Institute of Virology and Immunology, UCSF, San Francisco, CA; ²Positive Health Program, San Francisco General Hospital, San Francisco, CA

Abstract

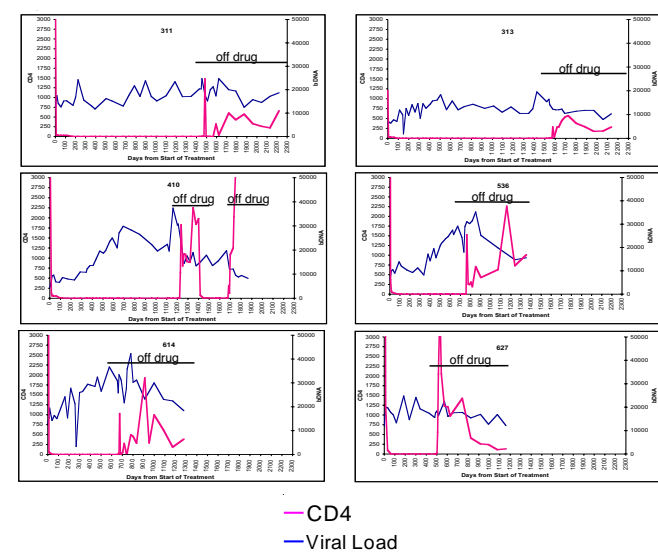
The goal of this study is to investigate the effectiveness of structured treatment interruption (STI) in patients who initiated HAART within six months of seroconversion. The objective is to evaluate patient outcome after 24 continuous weeks off treatment. At the current time, we have evaluated six subjects who have completed the protocol. The viral load of five of these subjects peaked within 2-4 weeks off treatment, but then quickly declined, in some cases to < 50 copies/ml. The initial decline of viremia coincided with the development of high levels of Gag-specific CD8 T cells of an effector phenotype. In all 5 subjects achieving low levels of viremia during early treatment interruption, there was a rebound in viremia within 24 weeks. Notably, viral mutations within the targeted immunodominant Gag epitopes were detected at the time of viral rebound. One STI subject was unable to control viremia to significant levels during STI. This subject had a very narrowly-directed immune response against two Gag epitopes, elicited by CD8 T cells of a central memory phenotype. We did not detect mutations within these Gag epitopes during STI, however, we did detect multiple mutations in other regions of Gag typically targeted by individuals with the same haplotype. We suspect that this patient was unable to control viremia due to the large number of immune escape mutations acquired before STI therapy. Taken together, these data indicate that STI can augment effective HIV specific immune responses in infected individuals who initiate HAART during primary infection, and that evasion of these immune responses by CTL escape may account for the inability to permanently constrain viral replication.

Fig. 1 Characteristics of the Study Participants

Number of STI Cycles	Patient	Age	ELISA at Onset of Symptoms	Days from Onset of Symptoms	Western Blot	CD4+ T cells (cells/mm ³)	HIV-1 RNA Load (copies/ml)	Initial Therapy	Days on Therapy Before First Treatment Interruption	HLA Haplotype
1	OP-311	40	Pos	60	Pos	704	63540	AZT,3TC,NFV	1452	A01, A23, B6
1	OP-313	38	Pos	141	Pos	375	20180	AZT,3TC,NFV	1547	A11, A29, B1
2	OP-410	43	Pos	82	Pos	270	79470	AZT,3TC,NFV	1189	A02, A48, B1
1	OP-536	61	Pos	53	Pos	385	25502	AZT,3TC,NFV,EFV	734	A02, A24, B1
1	OP-414	32	Neg	11	Neg	780	180005	ABC,AZT,3TC,NFV	661	A02, A31, B1
1	OP-427	35	Neg	12	Neg	720	>500000	AZT,3TC,NFV,EFV	487	A03, A33, B0

White Americans except for OP-410 who was Hispanic. Ind, indeterminate; NA, not available/applicable; neg, negative; pos, positive.

Fig. 2 Viral Loads and CD4 T cell Counts On and Off Antiviral Therapy



To qualify for this study, participants must have initiated HAART within 6 months of HIV seroconversion, and have maintained viral loads below 50 copies/ml for 16 weeks or longer prior to entering the protocol. HIV-1 RNA was measured using the Bayer (Emeryville, CA) branched-chain DNA (bDNA) test version 3.0 (lower limit of detection 75 copies/ml). CD4 T-cell counts were calculated using standard flow-cytometry methods.

Fig. 3A Viral Load During Treatment Interruption

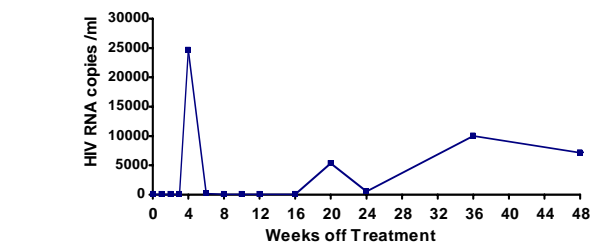


Fig. 3B IFN-γ Response Broadened and Increased in Magnitude During STI

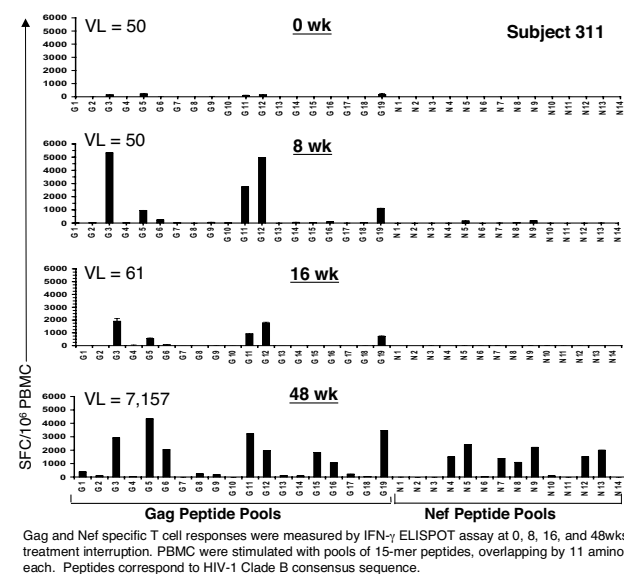


Fig. 3C Viral Mutations Developed within Targeted Gag Epitopes During STI

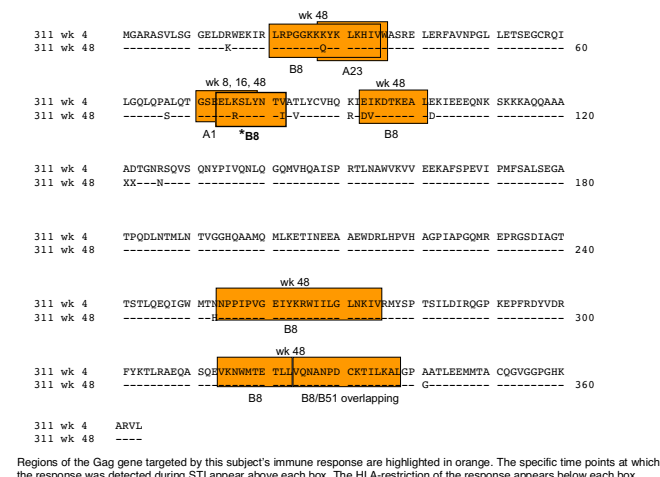


Fig. 3D Gag Sequence of Subject 311 is Similar to HIV-1 Consensus B Sequence

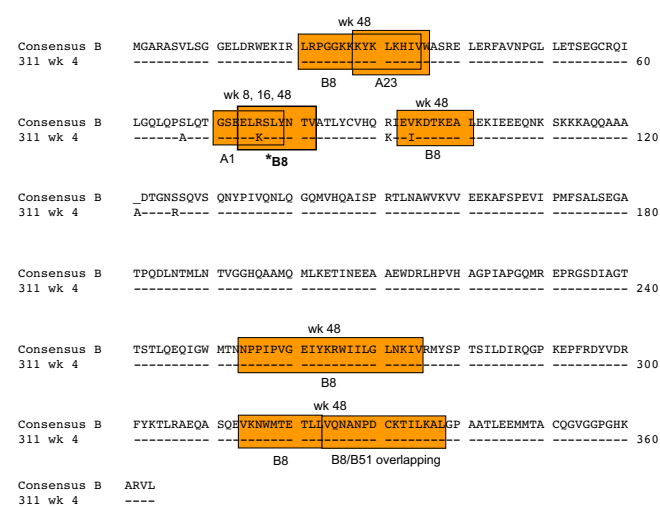


Fig. 4A Viral Load During Treatment Interruption

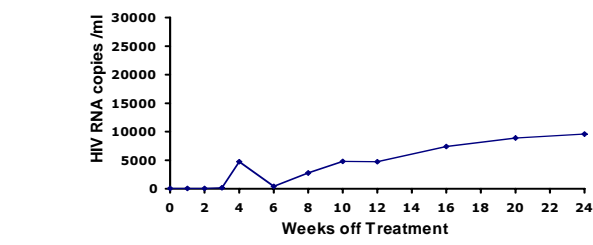


Fig. 4B IFN-γ Response Broadened and Increased in Magnitude During STI

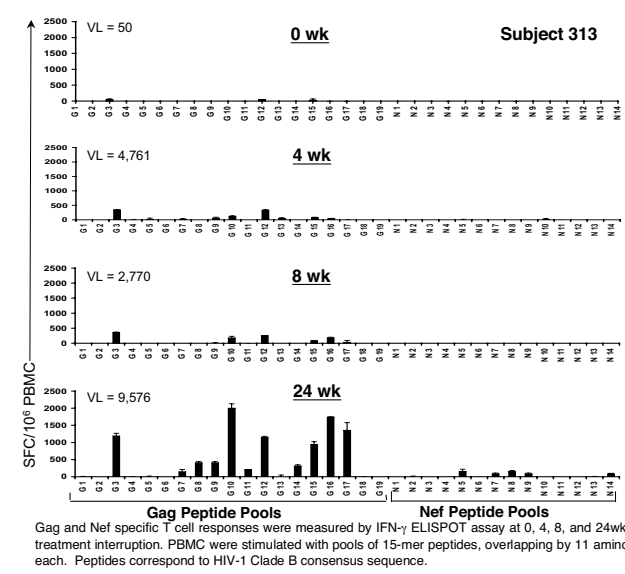


Fig. 4C Viral Mutation Developed within an Immunodominant Gag Epitope During STI

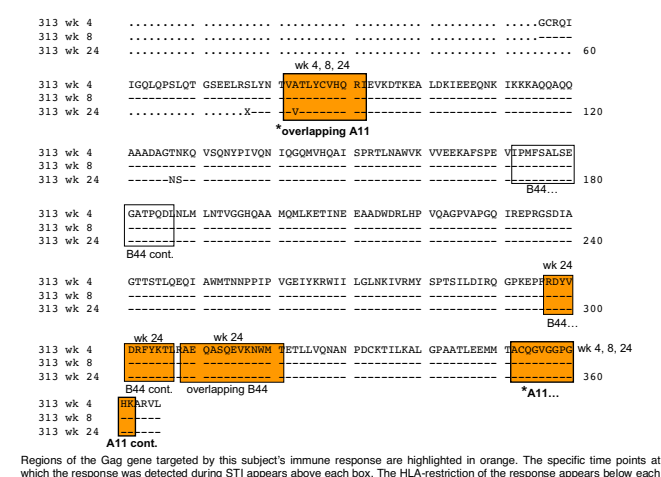


Fig. 4D Gag Sequence of Subject 313 is Similar to HIV-1 Consensus B Sequence

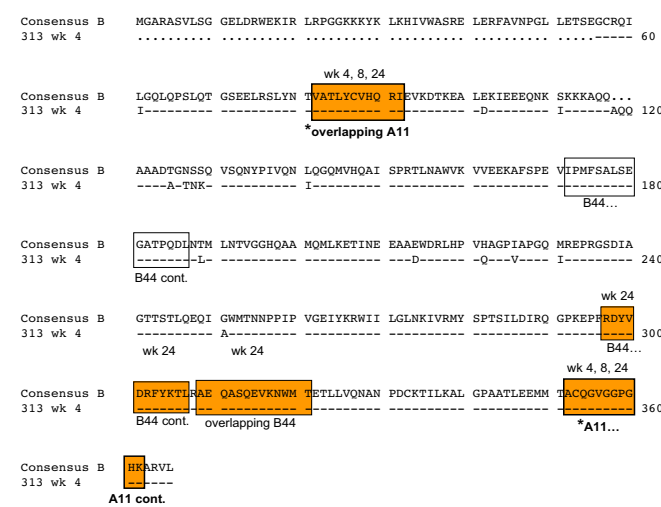


Fig. 5A Viral Load after Treatment Interruption

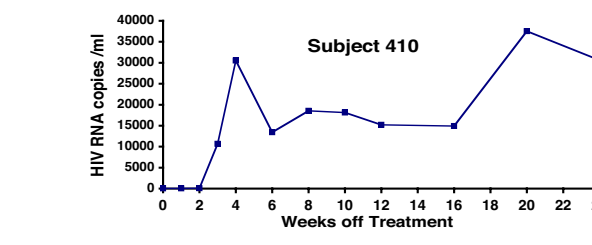


Fig. 5B IFN-γ Response Increases in Magnitude, But Not Breadth During STI

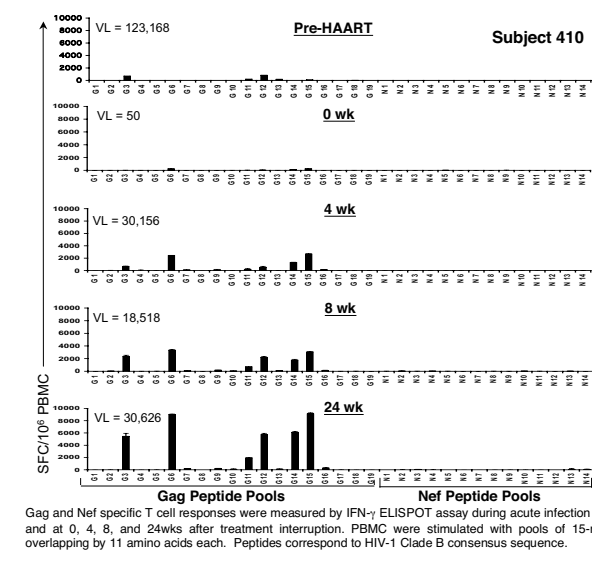


Fig. 5C No Viral Mutations Detected in Gag during STI, Despite Robust Immune Response and High Viral Replication

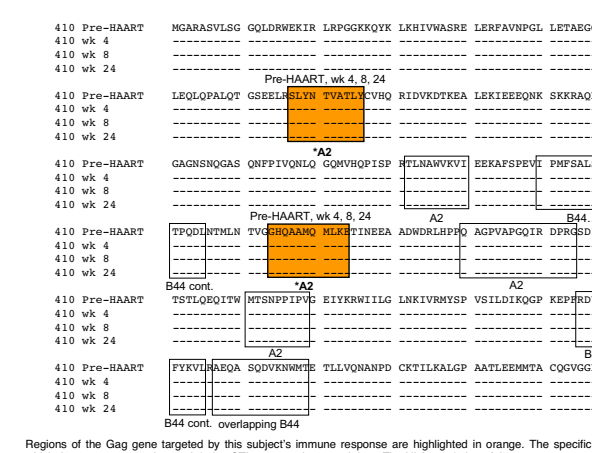


Fig. 5D Gag Sequence of Subject 410 is Highly Divergent from HIV-1 Consensus B

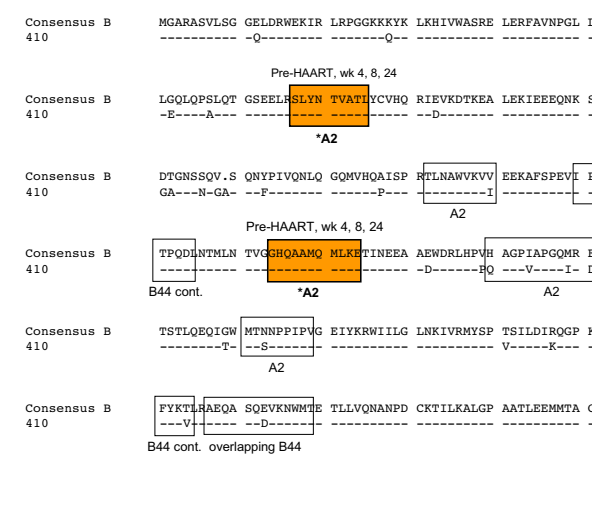
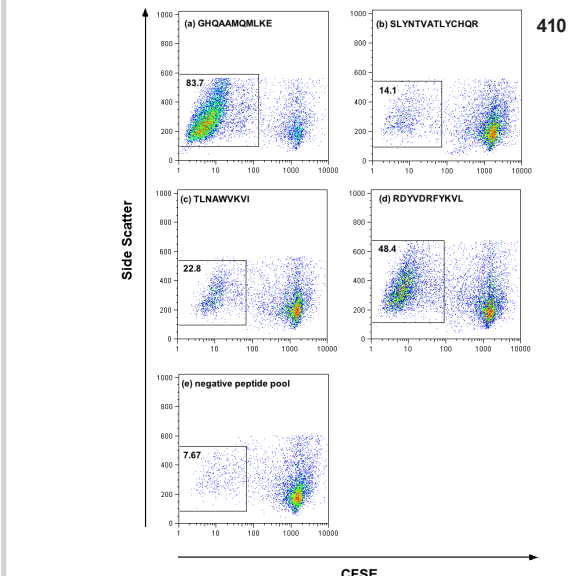
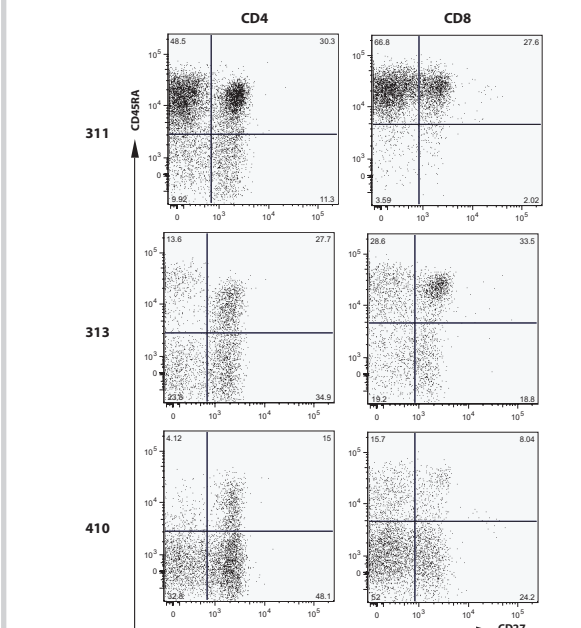


Fig. 6 High Levels of HIV-Specific CD8 T Cell Proliferation Detected in Subject 410



PBMC were stained with CFSE, stimulated with peptide, and then cultured for 7 days. Cells were then fixed, permeabilized, and stained with fluorescently labeled monoclonal antibodies directed against CD3, CD4, and CD8. Samples were acquired on a FACS Calibur™ (Becton Dickinson), and the resulting data were analyzed using FlowJo Software™ (Tree Star, Inc.). Boxed regions represent the population of CD8 T cells that underwent proliferation in response to peptide stimulation. The number in the corner of each boxed region refers to the percentage of proliferative cells.

Fig. 7 Higher Percentages of Naive and Effector T Cells Detected in Viral Controllers



PBMC were stained with fluorescently conjugated monoclonal antibodies directed against CD3, CD8, CD45RA, and CD27. Samples were acquired on a FACS Calibur™ (Becton Dickinson), and the resulting data were analyzed using FlowJo Software™ (Tree Star, Inc.). The numbers in the corners of each FACS plot represent the percentage of cells in each quadrant. Characterization of cellular phenotypes is based on the following receptor staining: Effector (CD45RA⁺ CD27⁻), Central Memory (CD45RA⁻ CD27⁺), Effector Memory (CD45RA⁺ CD27⁺), Naive (CD45RA⁻ CD27⁻).

Conclusions

- STI induces HIV-specific immune responses within infected individuals who initiate HAART during early infection
- Viral control during STI is associated with high numbers of Gag-specific CD8 T cells of an effector phenotype
- Viral mutations within targeted immunodominant Gag epitopes were detected at time of viral rebound in subjects with early viral control
- Virologic failure occurred in the presence of a robust, narrowly-directed CD8 response with a memory phenotype
- The presence of multiple CTL escape mutations at the onset of STI may predict virologic failure during STI
- Highly static, consensus-divergent sequence may predict rapid disease progression in HIV-1 infection