

# F13E

Poster # 397T

# ABSTRACT

## GENOTYPIC AND PHENOTYPIC ANALYSIS OF IN VITRO GENERATED HIV-1 ESCAPE ISOLATES TO THE CCR5 ANTAGONIST SCH-C.

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**Background:** SCH-C represents a new class of CCR5 antagonists that has potent antiviral activity against HIV-1 infection. This molecule binds to CCR5 and blocks the entry of HIV-1 isolates that uses this receptor for infection.

**Method:** To investigate the potential mechanism of viral resistance to this CCR5 antagonist we generated escape variants by infection of peripheral blood mononuclear cells (PBMCs) with different HIV-1 strains followed by continuous passaging in the presence of increasing increments of SCH-C.

**Results:** After 19 weeks of passage, variants with reduced sensitivity to the compound emerged. Characterization of these viruses showed no switch to other chemokine receptors including CXCR4. Further analyses of these adapted variants showed high level of cross-resistance with receptor ligands RANTES, MIP1 $\alpha$  and a neutralizing CCR5 monoclonal antibody, 2D7. Genotypic analyses of the viral isolates showed a marked degree of sequence variation in gp120 amino acid residues in viruses treated with the compound. To determine the stability of the resistant mutants in the absence of selection, drug was removed and the cultures were passaged in the absence of drug selection. Drug resistant viruses were still detected following 14 weeks of passage in the absence of drug, however by 20 weeks the cultures became sensitive to SCH-C.

**Conclusion:** From these analyses we have determined that escape viruses to SCH-C continue to use CCR5 as the co-receptor in HIV-1 infection. Additionally, these variants showed high levels of cross-resistance to other receptor ligands and to CCR5 monoclonal antibody 2D7. Genetic analyses showed some sequence diversification in the envelope gene. Further work will be required to determine the effect amino acid substitutions on viral co-receptor usage, viral fitness and drug potency. Finally, removal of selective pressure resulted in gradual reversal to a drug sensitive phenotype suggesting that the mutant virus maybe less fit compared with the wild type.

# INTRODUCTION

Highly active antiretroviral therapy or HAART, consists of a combination of drugs targeting the viral reverse transcriptase and protease proteins . Initiation of HAART typically results in a dramatic reduction in viral load, improved CD4 cell counts and a significant decrease in opportunistic infection. Despite the success of HAART, the genetic variability of HIV often leads to the emergence of drug resistant viruses. Additionally, current antiviral agents may produce toxic side effects in some individuals, which further limit their effectiveness. Recent development of novel classes of antiviral agents, targeting a different part of the viral life cycle, namely virus fusion and entry, have generated new optimism.

SCH-C represents one of the new class of CCR5 antagonists that has potent antiviral activity against HIV-1 infection. This molecule binds to CCR5 and blocks the entry of HIV-1 isolates that use this receptor for infection. This compound is presently undergoing clinical trials as a potential therapy for HIV-1 infection. However the issue of viral resistance to SCH-C is an area of intense interest because of its novel mechanism of action. HIV-1 transmission involve strains that uses CCR5 for entry (R5 viruses) , however these R5 viruses can undergo a phenotypic switch to X4 viruses that use CXCR4 as the co-receptor. These X4 strains are associated with rapid disease progression.

To investigate the potential mechanism of viral resistance to this CCR5 antagonist we generated escape variants by serial passaging of HIV-1 in peripheral blood mononuclear cells (PBMCs) in the presence of increasing increments of SCH-C. Here we report the phenotypic and genetic characterization of these escape variants.

# SCH-C:

## Physical and Biological Properties

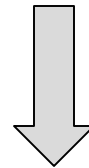
- **Amorphous solid - MW 557.5**
- **Ki - 2 nM**
- **Specific CCR5 antagonist:**
  - Inhibits Ca Flux, chemotaxis and GTP $\gamma$ S binding
- **Antiviral activity(IC<sub>50</sub>) - 4 nM**
- **Synergistic with other antivirals**
- **Favorable PK and ancillary pharmacology profiles**



# METHOD

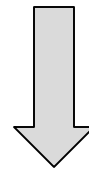
Generation of Escape Variants to SCH-C

Infect PBMC or PM-1 with the virus



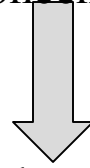
1 week

Add SCH-C (10nM)

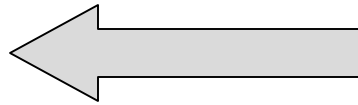


Passage weekly with fresh cells

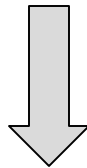
Increase SCH-C concentration gradually



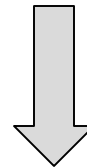
Collect  
Supernatants



Genotypic and Phenotypic Analysis

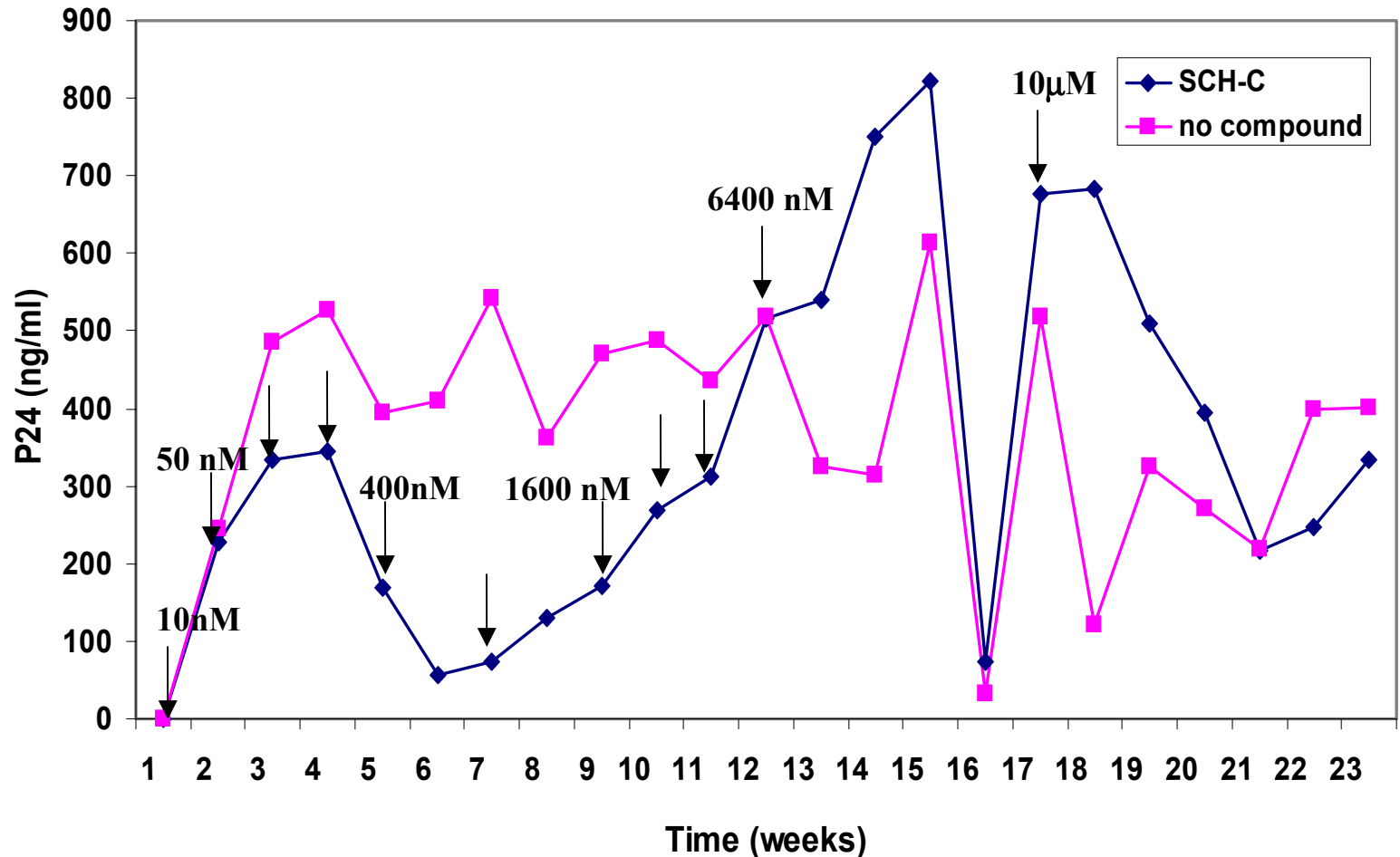


PCR amplification of  
viral DNA or RNA and  
sequencing



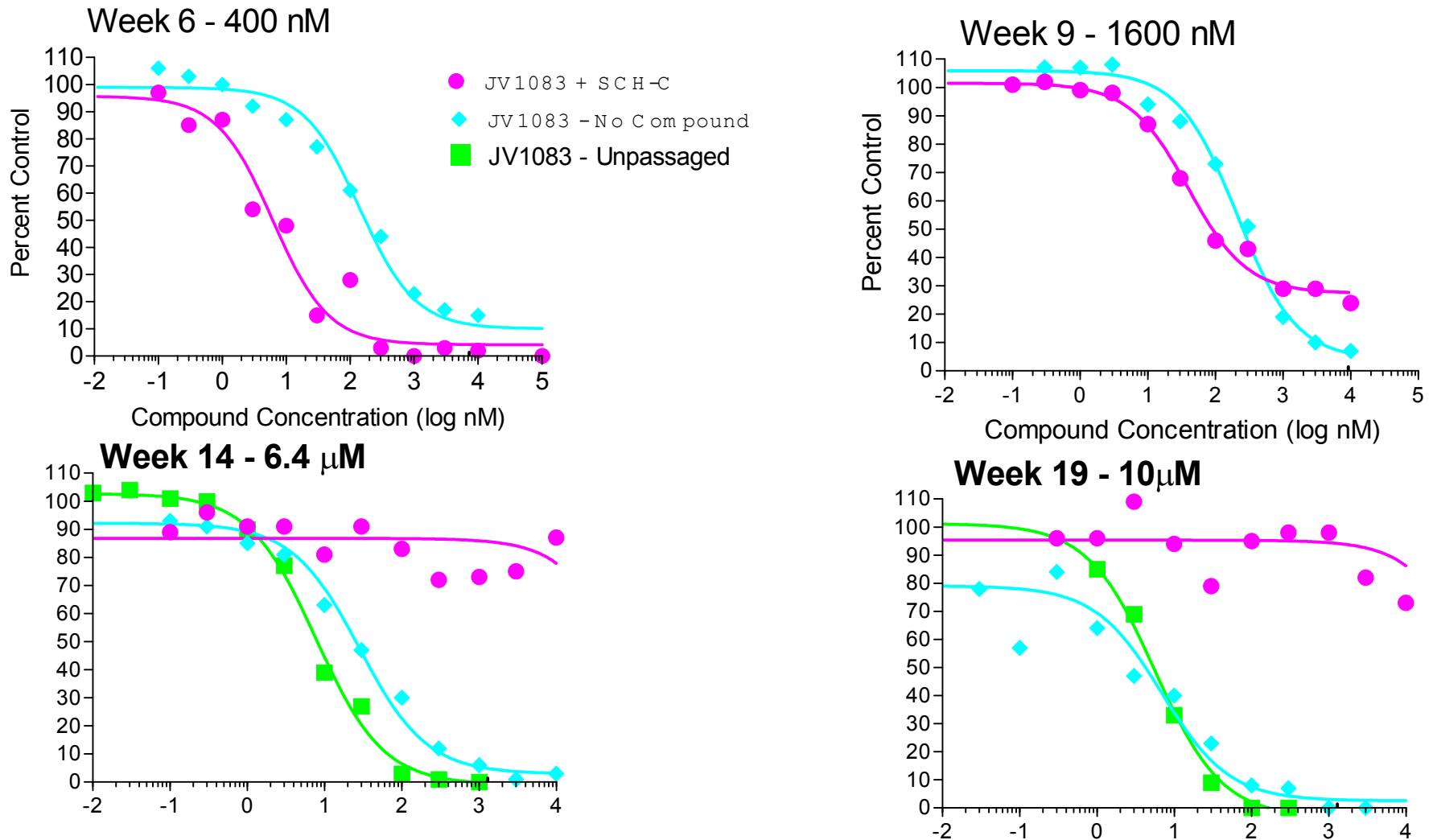
Infection of target cells  
and p24 antigen ELISA

# HIV-1 Replication in the Presence of CCR5 Antagonist SCH-C



**Figure 1: Replication of HIV-1 (JV1083) in the absence or presence of increasing concentration of SCH-C over time. HIV-1 replication was quantitated by the measurement of the extracellular p24 antigen by ELISA. Arrows indicate time points where compound concentration was increased.**

# Emergence of Resistance to SCH-C



**Figure 2: Viral supernatants from cultures passaged with and without inhibitor were tested for sensitivity to SCH-C by infection of PBMCs. Viral resistance began to emerge by week 9 and the cultures were totally resistant by week 14. Virus passaged without SCH-C remained sensitive.**

# Resistance to SCH-C Can Occur in the Absence of Co-receptor Switching

Virus	MT-2 Syncytia Formation	Growth in U87 cells expressing*			
		CXCR4	CCR2	CCR3	CCR5
Parental	–	–	–	–	+
JV1083-no drug	–	–	–	–	+
JV1083+SCH-C	–	–	–	–	+
LAI (X4)	+	+	–	–	–

\* – p24 values < 120 pg/ml; + p24 values > 200 pg/ml

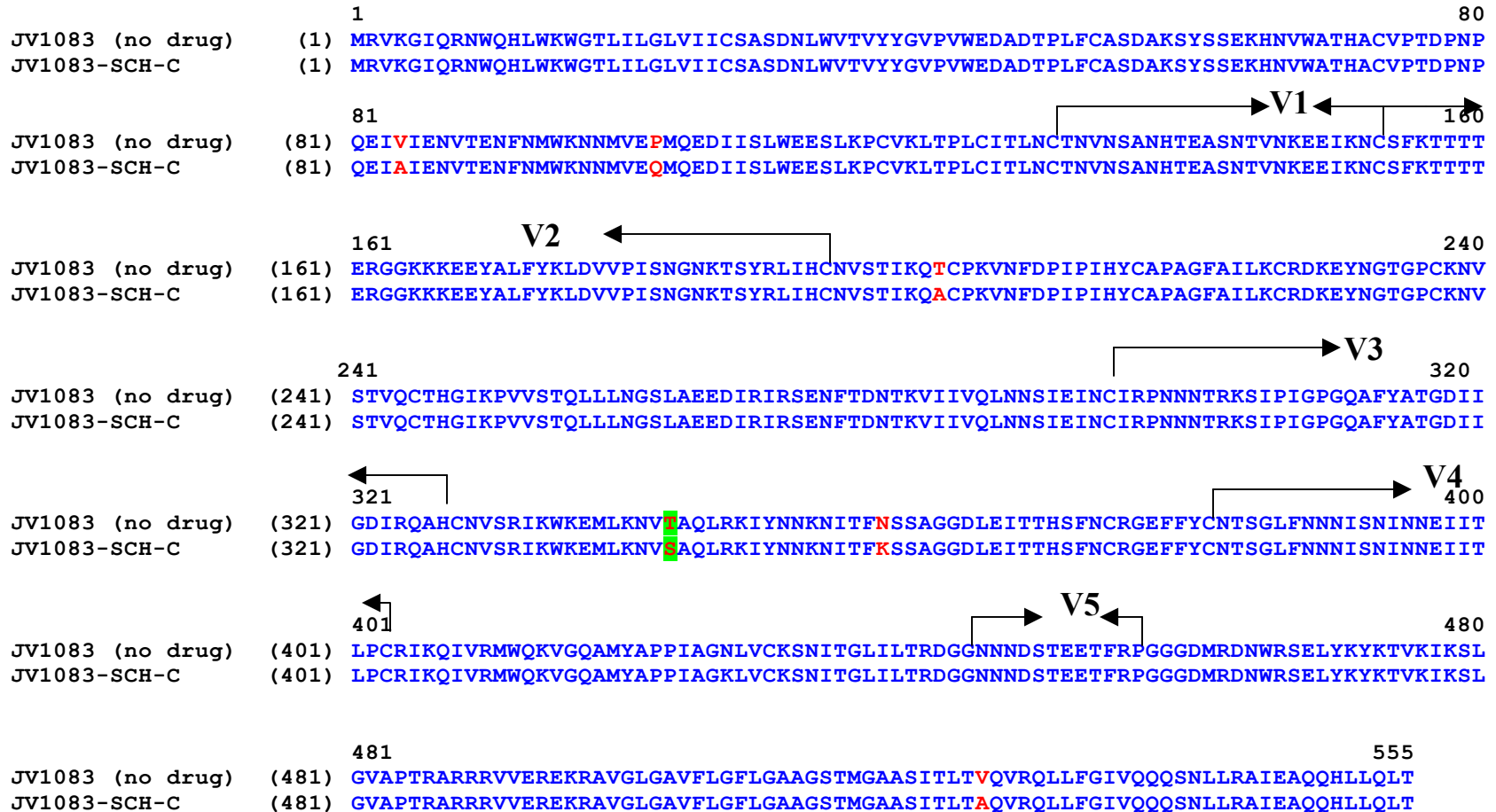
U87 cells stably expressing CCR2, CCR3, CXCR4 or CCR5 were infected with JV1083 passaged in the absence or presence of SCH-C. The resistant virus only replicated in CCR5 expressing cells demonstrating that resistance can emerge without co-receptor switching.

# SCH-C Escapes Variants are Cross-Resistant to other CCR5 Ligands

Virus	IC <sub>50</sub>			
	SCH-C (nM)	RANTES (ng/ml)	MIP-1 $\alpha$ (ng/ml)	2D7 ( $\mu$ g/ml)
JV1083-unpassaged	40	43	72	6.1
JV1083-No Drug (Week 21)	99	63	13	>10
JV1083+SCH-C (week 21)	>3000	>1000	>1000	>10

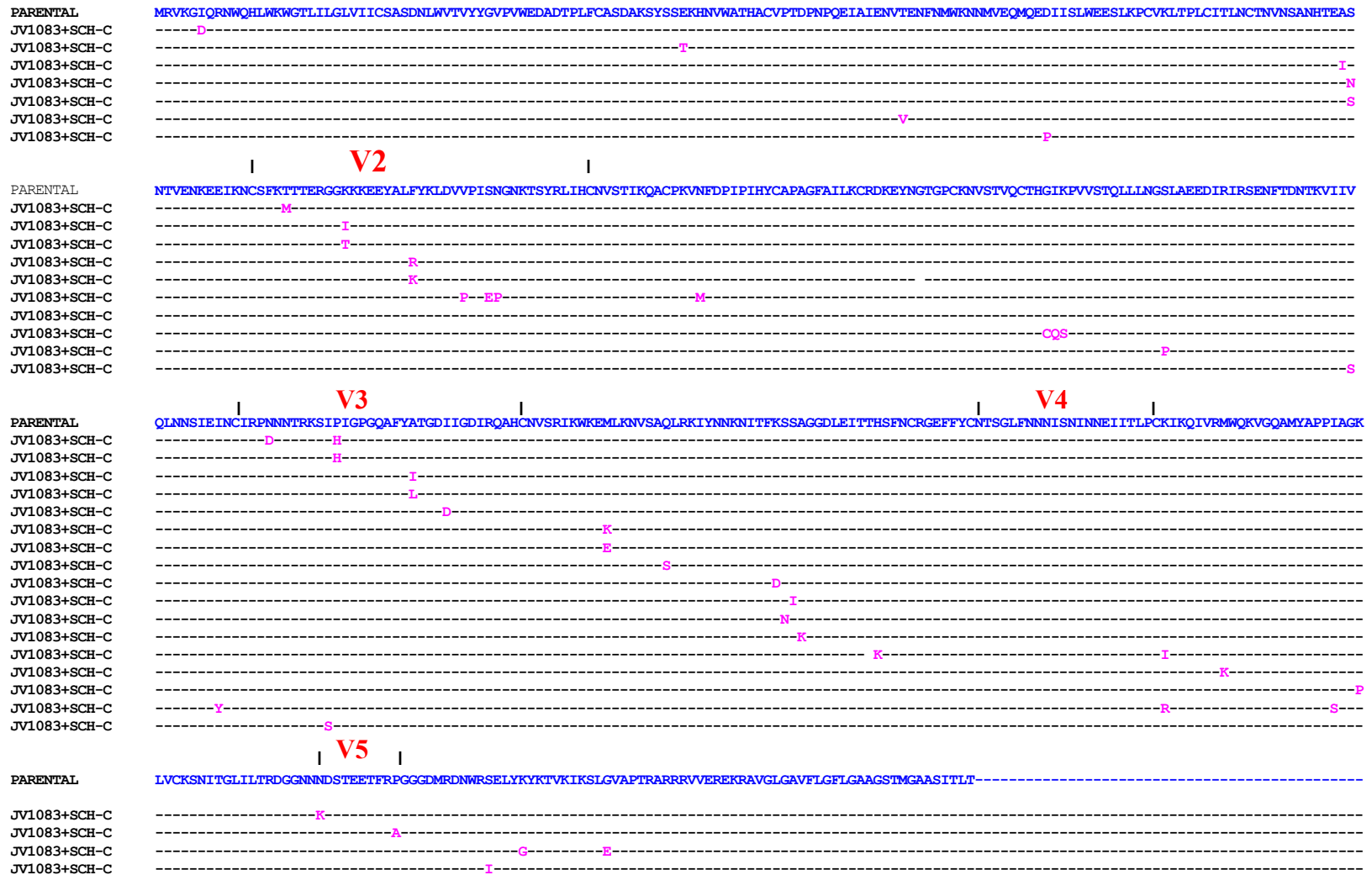
The parental and passaged viruses were tested for their sensitivity to SCH-C, RANTES, MIP1 $\alpha$  and a monoclonal antibody 2D7. While the parental and no drug viruses were sensitive to SCH-C, RANTES and MIP1 $\alpha$ , the escape virus was cross-resistant to both of these chemokines.

# Genetic variation in gp120 sequences from JV1083 virus passaged in the presence and absence of SCH-C



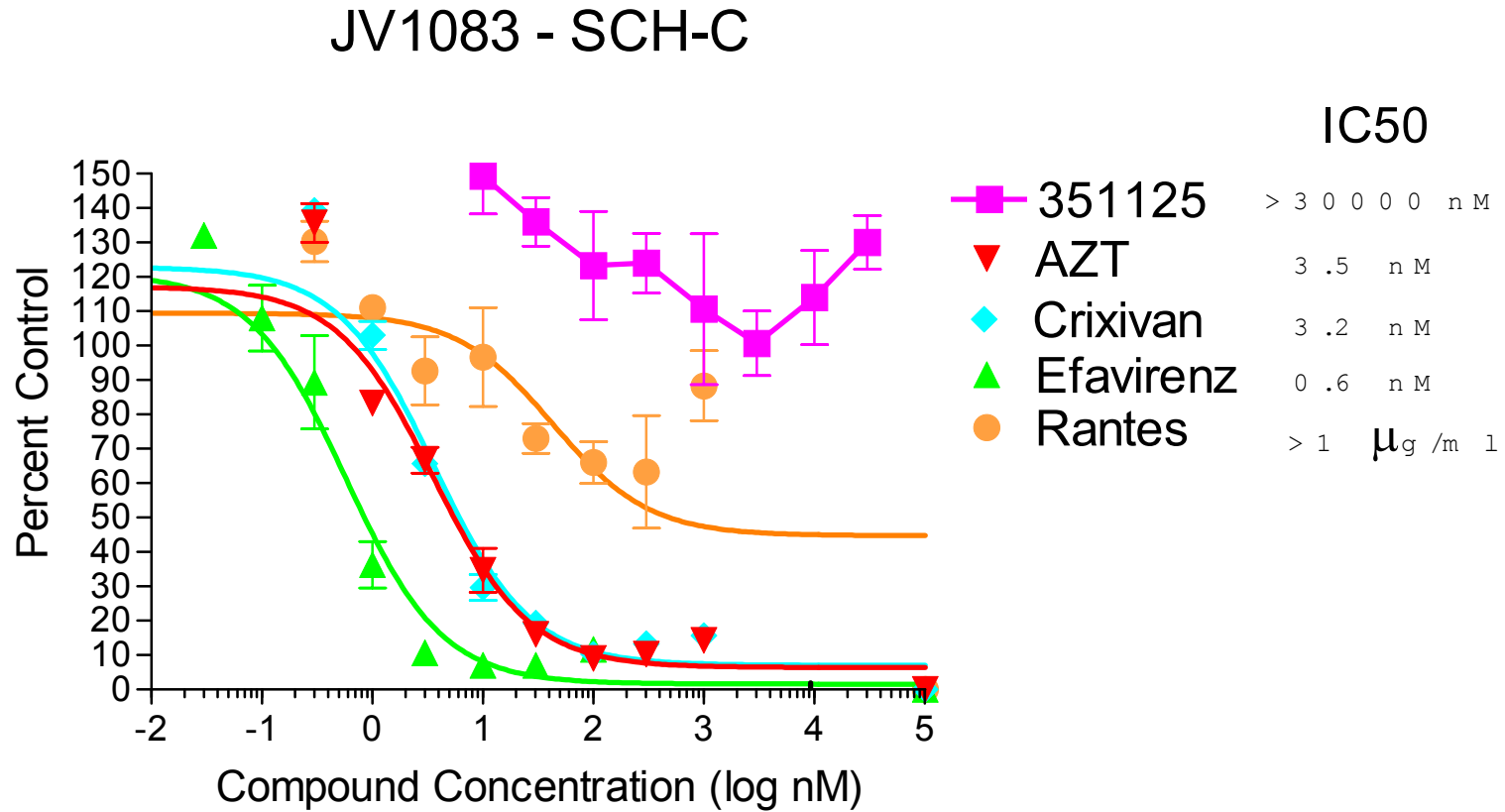
**Figure 3: Amino acid sequence alignment of the gp120 sequences consensus from JV1083 clones passaged in the absence or presence of SCH-C. Non-identical amino acids are shown in red.**

# Genetic Variation in gp120 sequences from JV1083 virus passaged in the presence of SCH-C



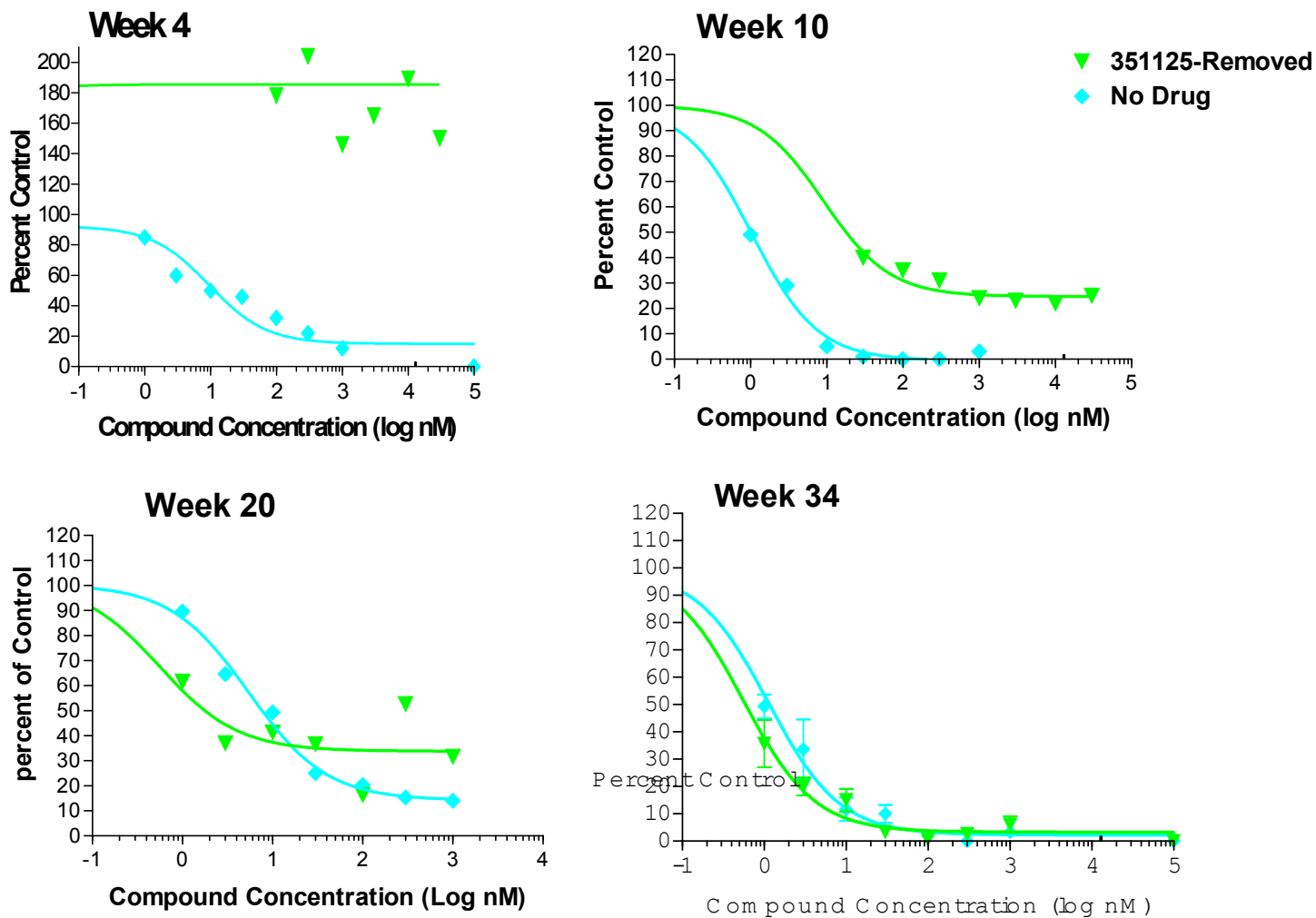
**Figure 4: Amino acid sequence alignment of gp120 clones from JV1083 virus passaged in the presence of SCH-C. Non-identical amino acids are shown in pink.**

# SCH-C Resistant Virus is Still Sensitive to RT and Protease Inhibitors



**Figure 5:** Effect of RT and protease inhibitors on the SCH-C escape mutant. Resistant isolate was tested for sensitivity to AZT, Crixivan and Efavirenz and RANTES. As expected, the SCH-C resistant virus was sensitive to the RT and protease inhibitors.

# Resistant Cultures Revert to a Sensitive Phenotype After Removal of SCH-C



**Figure 6:** To determine the stability of the resistant phenotype, SCH-C resistant cultures were passaged as usual except in the absence of SCH-C selection. Following 10 weeks of passage, the cultures began to revert to the sensitive phenotype, and by week 34 were completely sensitive to the compound.

# SUMMARY

- **Escape variants to SCH-C emerge following 14-19 weeks of continuous culture.**
- **Resistant viruses maintain CCR5 use with no evidence of coreceptor switch to CXCR4, CCR2 or CCR3.**
- **Resistant variants are cross-resistant to other CCR5 ligands.**
- **Resistant variants are sensitive to protease and RT inhibitors.**
- **Marked genetic variations are noted in gp120 sequences from the escape variants.**
- **Resistant cultures revert to a sensitive phenotype after the removal of the compound.**
- **Cloning, expression and characterization of functional envelopes from escapes, revertant and control viruses is ongoing.**

# Acknowledgements

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## SPRI

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