H-906

Clonal Analysis of the gp120 V3 Loop from Clinical Isolates Displaying Phenotypic Resistance to Vicriviroc

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Abstract

Background: **Vicriviroc (VCV) is a potent allosteric inhibitor of the interaction between HIV-1 gp120 and CCR5. Reduced susceptibility to VCV is associated with mutations in gp120 that permit the virus to utilize the inhibitor-bound form of CCR5. To identify regions of gp120 that contribute to the resistance phenotype, viral envelopes were cloned from 5 VCV-treated subjects in whom decreased susceptibility to VCV was observed.**

Methods: **Full length gp160 clones (12/time point) were generated from plasma samples at Day 1 and time of discontinuation (TOD). Susceptibility to VCV and maraviroc (MVC) were measured by the PhenoSense Entry® assay. For selected envelopes, reciprocal amino acid substitutions were introduced in the V3 loop of Day 1 and TOD clones and susceptibility to inhibitor assessed.**

Results: **The gp160 clones from Day 1 plasma samples were fully susceptible to VCV while the majority of the TOD clones exhibited reduced susceptibility to VCV. Changes in susceptibility were manifested as reductions in maximum percent inhibition (MPI); changes in IC_{50} values rarely mirrored changes in MPI. Phenotypically resistant clones had multiple amino acid changes throughout gp120 including the V3 loop. However, no single amino acid substitution was invariably associated with phenotypic resistance. For 3 subjects' reciprocal exchanges of the V3 loop mutations were made between Day 1 and TOD clones. Introduction of the V3 loop from the Day 1 clones into the corresponding phenotypically resistant envelope, partially or completely restored susceptibility to VCV and MCV. In contrast, the reciprocal exchange failed to recapitulate phenotypic resistance.**

Conclusion: **Decreased susceptibility to VCV was associated with multiple sequence changes in gp120. Although mutations were frequently observed in the V3 loop, these mutations alone were insufficient to confer reduced susceptibility to VCV.**

Introduction

Human immunodeficiency virus type-1 (HIV-1) can gain entry into CD4 positive cells using one of two chemokine coreceptors, CCR5 and CXCR4. Depending on which coreceptor is used, the virus is phenotypically designated as being R5 (CCR5-tropic) or X4 (CXCR4-tropic); dual-tropic viruses can utilize both coreceptors.

The CCR5 antagonist vicriviroc (VCV) binds to the CCR5 receptor with high affinity and blocks entry of R5-tropic viruses through allosteric interactions. HIV-1 can evade the inhibitory effects of VCV in two ways: through a change in coreceptor usage from CCR5 to CXCR4 or through alterations in the envelope protein, gp160, that allow the virus to utilize the inhibitor-bound form of CCR5. Due to the allosteric nature of VCV inhibition, development of phenotypic resistance is manifested as a decrease in the maximal level of suppression (also termed maximal percent inhibition (MPI)) of viral infection observed.

In order to better understand the genotypic changes associated with reduced susceptibility to VCV, sequence and phenotypic analysis was performed on envelope clones from five subjects enrolled in the P03672 Phase II trial. In addition, site directed mutagenesis studies were performed on selected clones from representative Baseline and TOD clones from three subjects.

Methods

Study Design: P03672 was a placebo-controlled, double-blind study of 20 and 30 mg VCV given once daily in adults failing standard three-drug antiretroviral therapy (ART); 79 subjects received VCV and 35 received placebo. Tropism and susceptibility to VCV and MVC were assessed at Baseline, Week 8, Week 20, and at the TOD using the Trofile™ and PhenoSense Entry® assays respectively, performed by Monogram Biosciences (South San Francisco, CA).

Clonal Analysis: For subjects with reduced susceptibility to VCV, clonal analysis of the gp160 envelope was performed on HIV-1 from Baseline and TOD plasma samples. Individual clones were sequenced and evaluated for VCV and MCV susceptibility. The dose ranges for VCV and MVC in the PhenoSense Entry® assay were 667–0.003 nM and 3000–0.1 nM, respectively.

Site Directed Mutagenesis: Clonal analysis and gp120 domain exchanges were performed by Monogram Biosciences. Alignment of gp160 sequences was performed using CLUSTAL 2.0.8 multiple sequence alignment software. Generation of consensus V3 loop sequences was performed using Vector NTI version 9.0 software.

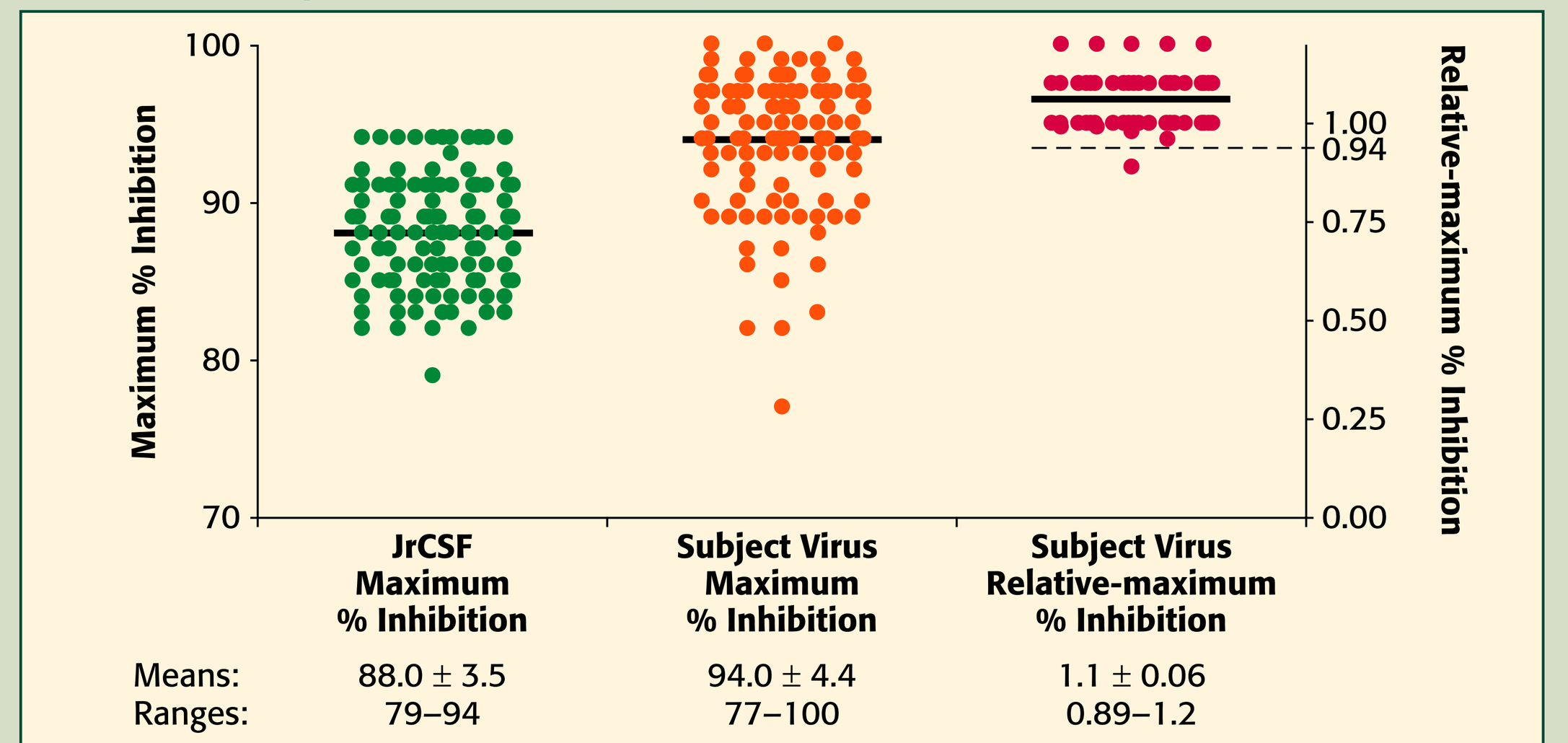
Results

Susceptibility Measurements

The MPI values from the PhenoSense Entry® assay for the viral swarms from baseline plasma samples, and the control virus JrCSF, exhibited a range of values (**Figure 1**). However, the relative-MPI (R-MPI), which is the ratio of the MPI values for the subject and control virus evaluated in parallel, controls for day to day fluctuations in the assay.

Phenotypic resistance to VCV was defined as a R-MPI value that was two standard deviations lower than the mean baseline value for all subjects collected prior to study initiation. For the samples from P03672 this value was 0.94.

Figure 1. Variability of Maximal Percent Inhibition for Baseline Samples from Subjects in P03672 and Assay Reference Virus (JrCSF)



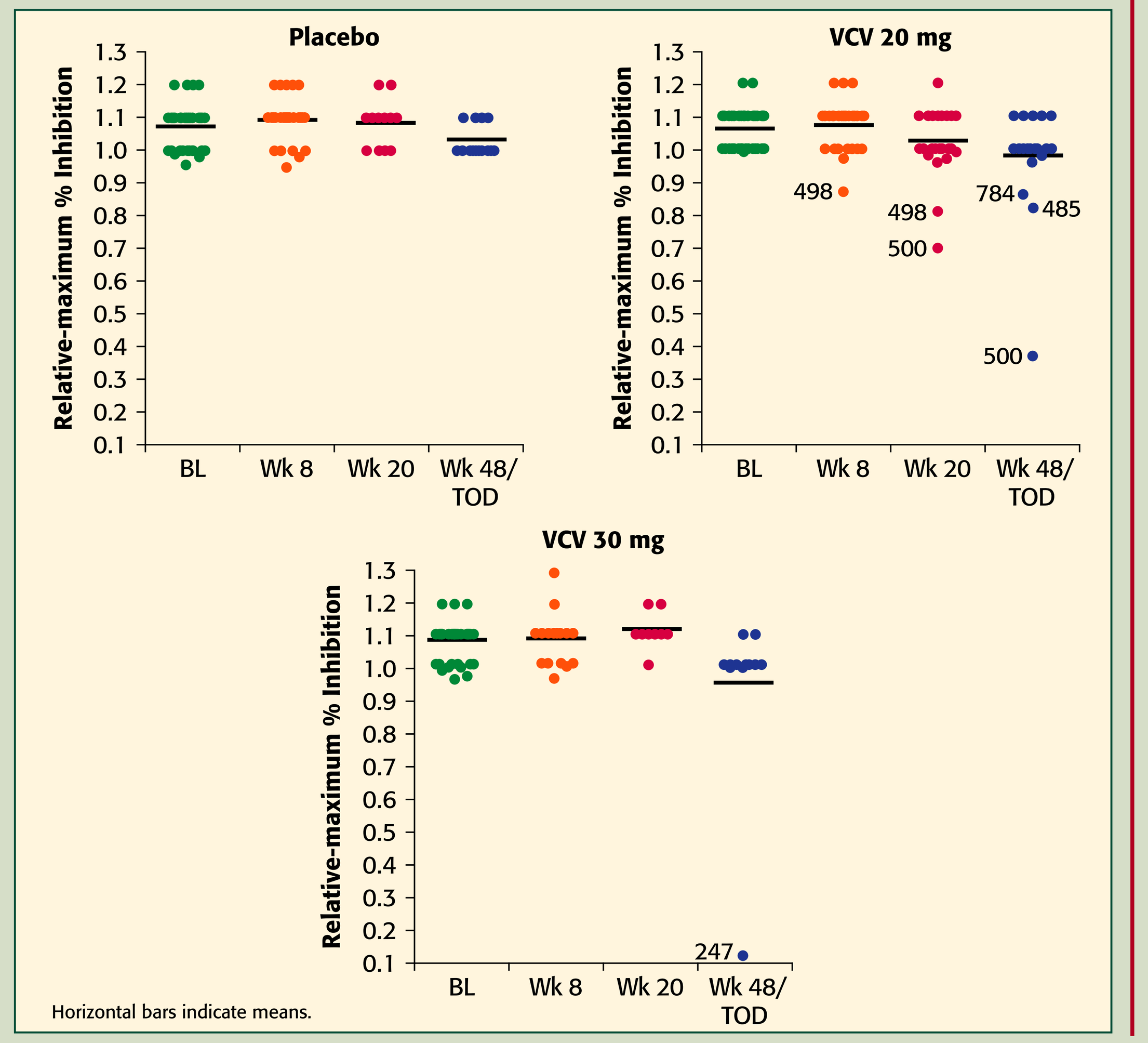
	JrCSF Maximum % Inhibition	Subject Virus Maximum % Inhibition	Subject Virus Relative-maximum % Inhibition
Means:	88.0 ± 3.5	94.0 ± 4.4	1.1 ± 0.06
Ranges:	79–94	77–100	0.89–1.2

Horizontal bars indicate means; also shown is 0.94 cut-off value for R-MPI.

Identification of Subjects With Virus With Reduced Susceptibility to VCV

- Of the 79 subjects treated with VCV, virus with reduced susceptibility to VCV was recovered from five subjects – four (#498, #500, #784, #485) and one (#247) from the 20 and 30 mg dose groups, respectively (**Figure 2** and **Table 1**).
- All five subjects with virus with reduced susceptibility to VCV were virologic failures: of the 114 subjects enrolled there were an additional 8, 10 and 23 virologic failures in the 20 mg, 30 mg and placebo arms, respectively.
- Four of the five subjects did not have any fully active drugs in their background regimen and the remaining subject had one active drug.
- Reduced susceptibility was manifested as reductions in MPI values (**Figure 3**). The IC_{50} values for the virus swarm collected at TOD were lower than the values for the Baseline (BL) samples for three subjects. For the remaining two subjects it was not possible to calculate an IC_{50} as the MPI values were below 50%.
- The viral swarm from three subjects also had detectable X4-tropic virus (time to the detection of X4-tropism varied from 8 to 48 weeks) and were considered dual/mixed.

Figure 2. Subjects from P03672 With HIV-1 That Exhibited Reduced Susceptibility to Vicriviroc



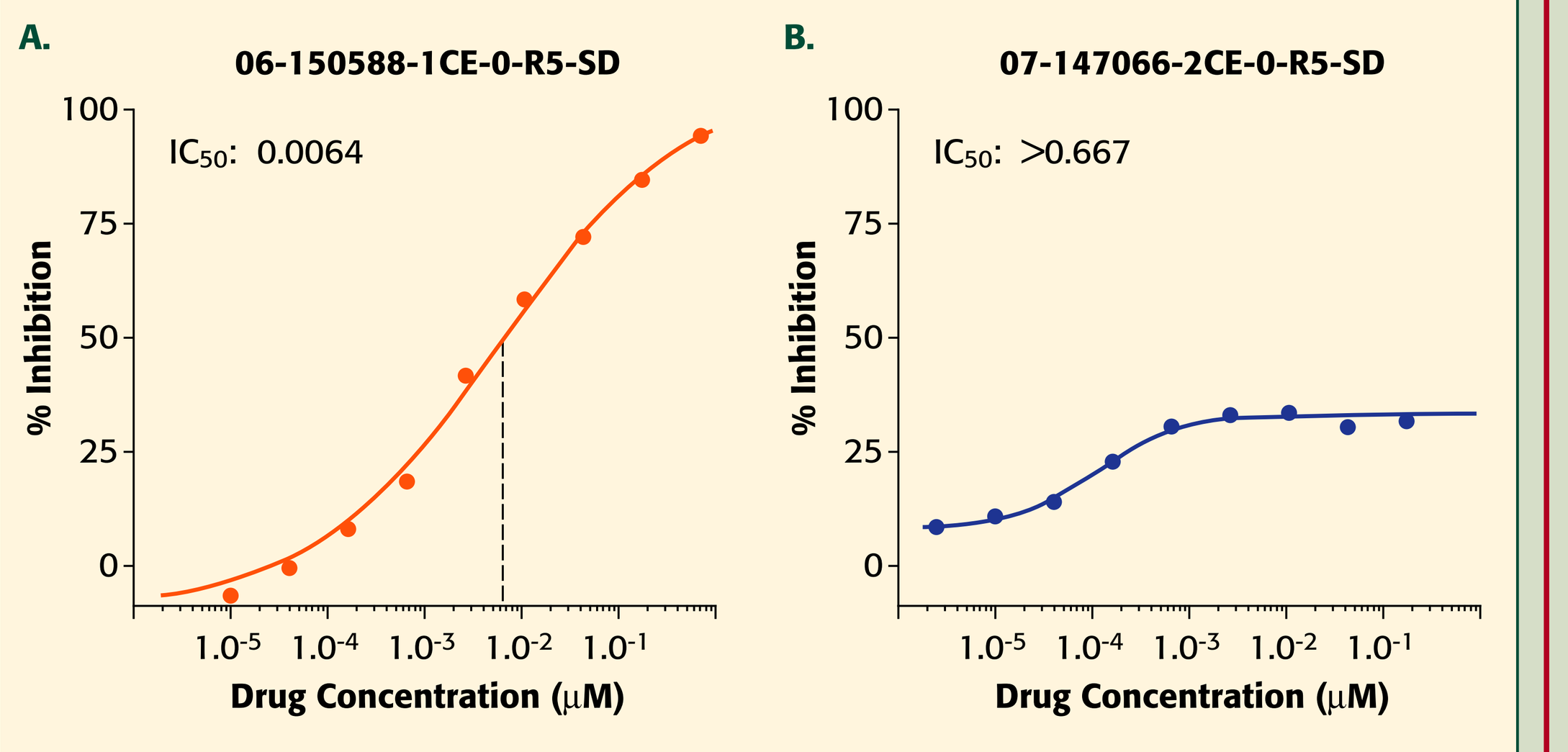
Horizontal bars indicate means.

Table 1. Clinical Data for the Five Subjects from the P03672 Clinical Trial That Developed Phenotypic Resistance to Vicriviroc

Subject #/ HIV Subtype/ VCV Dose (mg)	OSS[1]	Time Point (week)	CD4 Count (µl)	Viral Load (copies/ml)	MPI (%)[2]	Relative MPI[3]	IC_{50} (nM)[2]	Fold Change IC_{50}[4]	Tropism[5]	Luciferase Activity (RLU)[5] CCR5 Cells	CXCR4 Cells
247/B/30	0	0	231	42,800	95	1	1.58	0.97	R5	1,666,563	108
		8	211	638	98	1.1	1.2	0.11	DM	109,656	614,660
		20	155	110,000	100	1.2	1.1	0.07	DM	211,295	524,874
		48	82	69,300	100	1.1	0.85	0.16	DM	214,450	1,280,863
		TOD	NA[6]	106,000	0	0	>667[7]	>[7]	DM	352,627	111,396
485/F1/20	0	0	64	51,900	89	1	26	1.73	R5	1,195,106	123
		8	181	79,799	82	0.97	15.6	0.87	R5	452,323	62
		20	154	12,500	88	0.97	6.3	0.86	R5	642,489	102
		48	113	5,200	75	0.82	2.9	0.55	DM	1,072,793	519
498/F/20	1	0	NA	988,000	89	1	9.8	0.76	R5	307,669	80
		8	75	180,000	77	0.87	5.5	0.44	R5	210,493	72
		20	82	264,000	75	0.81	4.9	0.71	R5	88,152	61
500/F/20	0	0	60	226,000	94	1.1	6.4	0.68	R5	731,734	109
		8	252	248,000	88	1	6.0	0.39	R5	1,235,998	444
		20	238	275,000	59	0.7	3.4	0.22	DM	271,169	12,583
		48	225	60,700	33	0.37	>667	>	DM	510,408	38,724
784/B/20	0	0	267	1,110	93	1.1	4.9	0.38	R5	321,747	74
		8	291	50	94	1.1	10.7	0.57	R5	99,196	70
		20	324	55	93	1	4.8	0.76	R5	370,321	65
		48	245	1,530	81	0.86	2.9	0.92	R5	268,457	79

Abbreviations: DM, dual-mixed; MPI, maximum percent inhibition; OSS, overall susceptibility score; TOD, time of discontinuation.
1. Overall susceptibility score determined at Baseline.
2. Determined for the viral swarm using PhenoSense Entry® assay.
3. Relative MPI: MPI of subject/MPI of virus control evaluated in parallel.
4. Fold change-IC_{50}: IC_{50} of subject/IC_{50} of virus control evaluated in parallel.
5. Determined for the viral swarm using Trofile™ assay.
6. Not available.
7. The MPI value was too low to allow calculation of IC_{50}/fold change IC_{50}.

Figure 3. PhenoSense Entry® Result for Baseline (A) and Week 48 (B) Samples from Subject 500 Tested Against Vicriviroc



Clonal Analysis of Phenotypically Resistant Virus

- For each of the five subjects, 12 full length gp160 clones were generated from Baseline and TOD plasma samples and subjected to susceptibility testing (**Figure 4**) and DNA sequencing (**Tables 2–5**).
- Alignment of V3 loop and full length gp160 sequences did not identify a consistent pattern of mutations that was associated with phenotypic resistance. The previously characterized K305R mutation (1) was seen in a number of resistant clones from Subjects 485, 498 and 784; however it was also detected in susceptible clones generated from the baseline sample in Subject 485.

Figure 4. Relative MPI Values for Both Viral Swarm and Clones from Subjects With HIV-1 That Exhibited Reduced Susceptibility to Vicriviroc

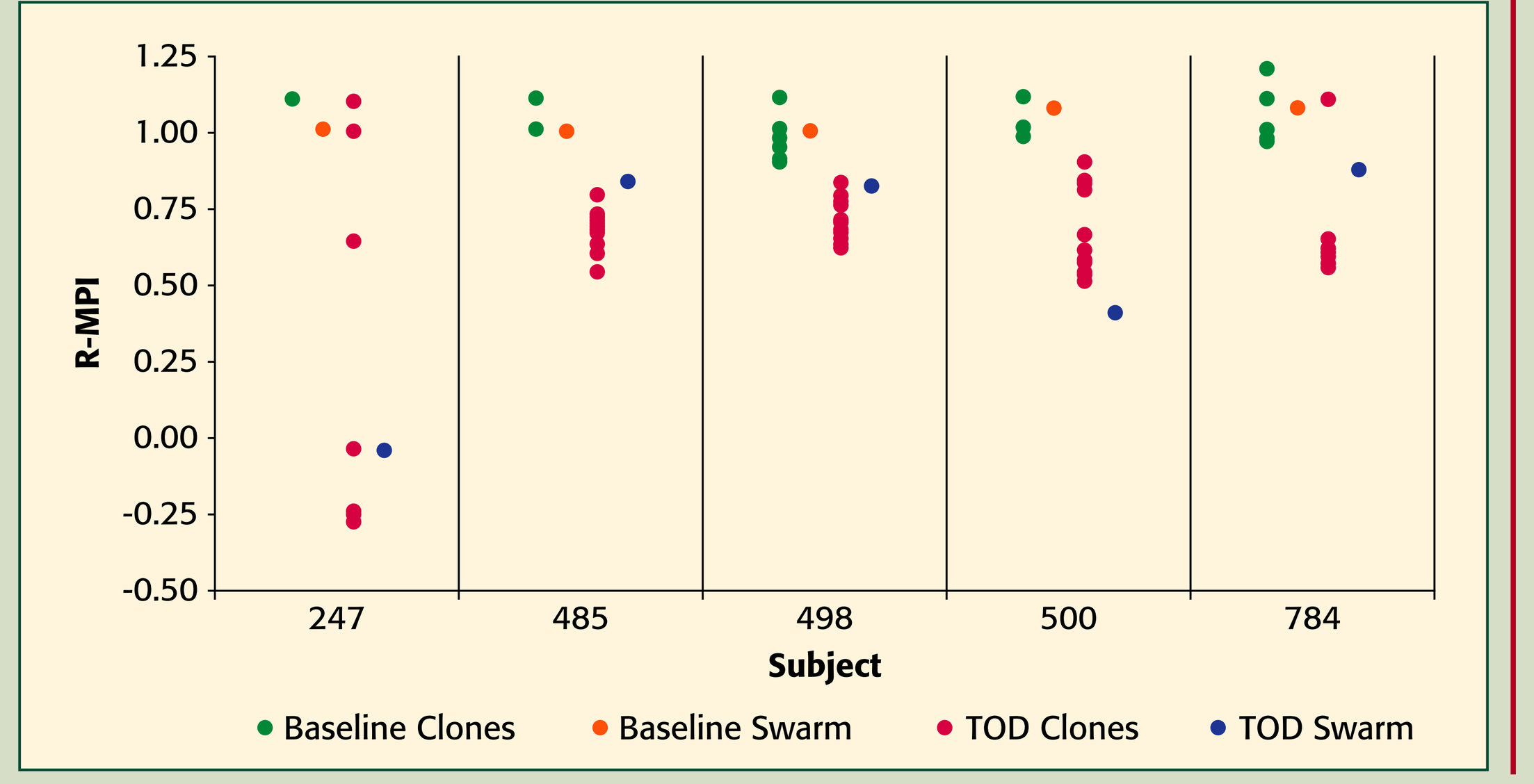


Table 2. Susceptibility of env Clones Generated from Subject 247 on Day 1 and Week 53 to Vicriviroc

Clone ID	Time Point (week)	MPI (%)	R-MPI[1]	RLUs in CCR5 Cells	IC_{50} (nM)	Fold Change IC_{50}[2]	Consensus V3 Loop Sequence from Baseline CTRPNNNTRKGI–––HIGPGRAFYTTGDIIGDIRQAHC
015	0	95	1.1	1,016,682	2.0	0.54	............–––......................
017	0	95	1.1	1,251,558	1.8	0.51	............–––......................
022	0	95	1.1	1,075,187	1.9	0.52	............–––......................
026	0	99	1.1	1,504,855	1.9	0.53	............–––......................
027	0	94	1.1	2,047,916	2.4	0.67	............–––......................
041	0	93	1.1	1,286,186	2.8	0.76	............–––......................
010	0	98	1.1	46,424	1.6	0.43	............–––.L..G......E.....K...
014	0	98	1.1	18,804	8.0	0.23	............–––.L..G......E.....K...
024	0	93	1.1	1,507,262	2.9	0.8	............–––.L..G......E.....K...
033	0	99	1.1	37,672	9.0	0.24	............–––.L..G......E.....K...
039	0	100	1.1	138,874	1.0	0.27	............–––.L..G......E.....K...
048	0	100	1.1	34,825	1.1	0.32	............–––.L..G......E.....K...
012	53	97	1	2,000	3.0	0.09	..........S.RIG.....PL....G..........
029	53	99	1.1	36,562	2.0	0.07	..........S.RIG.....PL....G..........
022	53	99	1.1	15,108	2.0	0.06	..........S.RIG.....PL....G.V........
033	53	–[3]	–[3]	393	–[3]	–[3]	..........S.RIG.....PL....G.V........
008	53	–	–	91	–	–	..........S.RIG.....PL....G.V.....V..
019	53	–	–	1,216	–	–	..........S.RIG.....PL....G...V......
004	53	59	0.64	260,468	2.4	0.72	............–––.L..E.....G..........
006	53	-22.3	-0.24	852,942	>667[4]	>[4]	............–––.L..AE.....G..........
010	53	-25.8	-0.28	1,056,859	>667	>	............–––.L..AE.....G..........
014	53	-22.9	-0.25	1,204,976	>667	>	............–––.L..AE.....G..........
024	53	-26	-0.28	1,006,076	>667	>	............–––.L..AE.....G..........
036	53	-3.85	-0.04	1,221,084	>667	>	............–––.L..AE.....G..........

Abbreviations: MPI, maximum percent inhibition; R-MPI, relative maximum percent inhibition; RLU, relative light unit.
1. R-MPI: MPI of subject/MPI of virus control evaluated in parallel.
2. Fold change-IC_{50}: IC_{50} of subject/IC_{50} of virus control evaluated in parallel.
3. The RLU values were too low to allow for calculation of MPI and IC_{50}/fold change IC_{50}.
4. The MPI value was too low to allow calculation of IC_{50}/fold change IC_{50}.

Table 3. Susceptibility of env Clones Generated from Subject 485 on Day 1 and Week 48 to Vicriviroc

Clone ID	Time Point (week)	MPI (%)	R-MPI[1]	RLUs in CCR5 Cells	IC_{50} (nM)	Fold Change IC_{50}[2]	Consensus V3 Loop Sequence from Baseline CTRPNNNTRKSIHIGPGSAFYATGEIIGDIRQAHC
013	0	98	1.1	1,025,893	1.6	0.46	....
014	0	98	1.1	1,027,439	1.6	0.47	....
033	0	98	1.1	682,707	1.0	0.3	....
003	0	94	1	2,102,401	3.0	0.9	.........R..............D..........
009	0	93	1	2,197,500	2.6	0.76	.........R..............D..........
024	0	93	1	1,128,183	2.4	0.71	.........R..............D..........
038	0	95	1	1,598,386	2.3	0.68	.........R..............D..........
046	0	95	1	1,839,015	5.2	1.53	.........R..............D..........
027	0	95	1	1,695,849	4.1	1.23	.........R.........................
040	0	100	1.1	1,320,304	6.0	0.19	..............................M.....
045	0	100	1.1	1,219,365	7.0	0.22	..............................M.....
048	0	100	1.1	1,198,968	7.0	0.2	..............................M.....
011	48	58	0.67	709,412	2.0	0.25	.........R..P...........D..........
018	48	64	0.73	1,101,743	2.1	0.26	.........R..P...........D..........
028	48	52	0.6	1,305,172	4.9	0.61	.........R..D...........D..........
032	48	62	0.72	1,456,123	1.8	0.23	.........R..P...........D..........
038	48	60	0.69	782,364	1.4	0.18	.........R..P...........D..........
041	48	59	0.68	1,489,817	5.6	0.69	.........R..P...........D..........
002	48	61	0.7	1,641,373	3.2	0.4	.........R..D...........D..........
013	48	64	0.73	1,559,228	2.6	0.33	.........R..D...........D..........
017	48	47	0.54	1,152,772	>667[3]	>[3]	.........R..D...........D..........
026	48	55	0.63	950,090	2.3	0.28	.........R..P...........D..........
036	48	62	0.71	1,508,120	3.0	0.36	.........R..D...........D..........
037	48	69	0.79	2,075,684	9.6	1.2	....S....R..P...........D..........

Abbreviations: MPI, maximum percent inhibition; R-MPI, relative maximum percent inhibition; RLU, relative light unit.
1. R-MPI: MPI of subject/MPI of virus control evaluated in parallel.
2. Fold change-IC_{50}: IC_{50} of subject/IC_{50} of virus control evaluated in parallel.
3. The MPI value was too low to allow calculation of IC_{50}/fold change IC_{50}.

Table 4. Susceptibility of env Clones Generated from Subject 498 on Day 1 and Week 20 to Vicriviroc

Clone ID	Time Point (week)	MPI (%)	R-MPI[1]	RLUs in CCR5 Cells	IC_{50} (nM)	Fold Change IC_{50}[2]	Consensus V3 Loop Sequence from Baseline CTRPNNNTRKSIHIGPGKAFYATGDIIGDIRQAHC
010	0	78	0.89	1,071,863	9.2	2.33	....
011	0	82	0.94	1,443,053	18.7	1.59	....
016	0	91	1	1,011,790	12.8	0.64	....
017	0	87	1	62,830	5.1	2.06	....
018	0	92	1.1	728,631	16.5	1.04	....
021	0	84	0.97	268,724	8.4	1.11	....
023	0	93	1.1	762,315	8.9	1.35	....
024	0	84	0.97	611,362	10.8	1.15	....
025	0	82	0.94	772,644	7.5	0.93	....
027	0	78	0.9	1,356,684	12.2	1.53	....
028	0	79	0.9	848,644	16.0	1.99	....
045	0	78	0.9	1,637,943	18.2	2.27	....
019	20	71	0.83	264,614	2.5	0.14	................................R...
025	20	67	0.77	757,803	4.1	0.49	................................R...
026	20	59	0.68	592,527	3.3	0.3	................................R...
032	20	62	0.71	856,748	2.9	0.31	................................R...
033	20	61	0.7	347,615	1.1	0.37	................................R...
001	20	53	0.62	494,214	1.0	0.29	................................K...
020	20	58	0.67	271,029	3.0	0.18	................................K...
006	20	68	0.79	596,828	2.4	0.13	....
008	20	58	0.67	460,202	2.6	0.36	....
039	20	56	0.65	616,799	1.5	0.51	....
031	20	55	0.63	652,320	2.4	0.32	.........R..........................
045	20	66	0.76	645,209	4.0	0.4	.........R.......................Y.

Abbreviations: MPI, maximum percent inhibition; R-MPI, relative maximum percent inhibition; RLU, relative light unit.
1. R-MPI: MPI of subject/MPI of virus control evaluated in parallel.
2. Fold change-IC_{50}: IC_{50} of subject/IC_{50} of virus control evaluated in parallel.

Table 5. Susceptibility of env Clones Generated from Subject 500 on Day 1 and Week 48 to Vicriviroc

Clone ID	Time Point (week)	MPI (%)	R-MPI[1]	RLUs in CCR5 Cells	IC_{50} (nM)	Fold Change IC_{50}[2]	Consensus V3 Loop Sequence from Baseline CTRPNNNTRKSISMGPGRAFYATGAIIGNIRQAHC
009	0	92	1	1,458,055	6.4	1.78	........................G..........
010	0	97	1.1	1,090,639	1.9	0.52	........................G..........
011	0	96	1.1	738,160	4.2	1.17	........................G..........
026	0	94	1.1	1,472,633	5.2	1.43	........................G..........
012	0	95	1.1	235,190	2.3	0.62	............H...G.V................
048	0	94	1.1	255,083	2.2	0.61	............H...G.V................
004	0	93	1	849,978	1.5	0.42	..........G.HI..G.V................
029	0	92	1	1,654,792	3.1	0.87	..........G.HI..G.V................
030	0	100	1.1	932,332	5.0	0.14	..........G.HI..G.V.........R......
016	0	87	0.98	1,717,246	17.1	4.74	....................F...E..........
032	0	90	1	1,079,669	3.8	1.04	.I..................F...E..........
024	0	97	1.1	162,773	3.0	0.82	........................E..........
014	48	72	0.81	513,096	7.0	0.2	..........G.YR..G.V................
040	48	48	0.54	343,998	>667[3]	>[3]	........L.G.YI..G..................
003	48	52	0.58	617,695	2.2	0.61	........L.G.YI..G......V...........
008	48	47	0.53	1,035,097	>667	>	........L.G.YI..G......V...........
020	48	45	0.51	1,403,025	>667	>	........L.G.YI..G......V...........
047	48	59	0.66	301,666	7.0	0.21	........L.G.YI..G......V...........
016	48	51	0.57	399,669	1.8	0.5	........L.G.YI..G......V...........
007	48	54	0.61	326,409	1.0	0.27	........L.G.YI..G..................
037	48	74	0.83	570,908	9.0	0.25	..........G.YR..G.V................
027	48	80	0.9	406,997	8.0	0.22	..........G.YR..G.V................
028	48	80	0.9	570,106	9.0	0.26	..........G.YR..G.V................
045	48	75	0.84	649,885	9.0	0.24	..........G.YR..G.V................

Abbreviations: MPI, maximum percent inhibition; R-MPI, relative maximum percent inhibition; RLU, relative light unit.
1. R-MPI: MPI of subject/MPI of virus control evaluated in parallel.
2. Fold change-IC_{50}: IC_{50} of subject/IC_{50} of virus control evaluated in parallel.
3. The MPI value was too low to allow calculation of IC_{50}/fold change IC_{50}.

Table 6. Susceptibility of env Clones Generated from Subject 784 on Day 1 and Week 48 to Vicriviroc

Clone ID	Time Point (week)	MPI (%)	R-MPI[1]	RLUs in CCR5 Cells	IC_{50} (nM)	Fold Change IC_{50}[2]	Consensus V3 Loop Sequence from Baseline CTRPGNNTRKSIPIGPGRAFYATGDIIGDIRKAHC
012	0	95	1.1	331,311	3.5	0.26	....
014	0	99	1.1	676,813	3.2	0.23	....
018	0	89	1	567,959	4.0	0.4	....
022	0	97	1.1	320,378	3.3	0.14	....
031	0	84	0.97	404,876	1.1	0.4	....
034	0	100	1.2	423,656	1.7	0.21	....
040	0	94	1.1	793,923	2.4	0.29	....
043	0	87	1	1,012,674	6.6	0.8	....
048	0	83	0.96	772,373	3.8	0.46	....
002	0	90	1	444,119	1.9	0.62	..............................V.....
015	0	91	1.1	467,851	5.1	0.43	..............................V.....
016	0	94	1.1	430,063	2.1	0.49	...........................V..V.....
003	48	99	1.1	194,555	1.6	0.19	.........R..........I...............
024	48	99	1.1	206,653	1.3	0.16	.........R..........I...............
004	48	49	0.57	1,055,403	>667[3]	>[3]	.........R..........I...............
020	48	50	0.57	1,025,600	>667	>	.........R..........I...............
013	48	52	0.6	713,477	1.3	0.16	.........R..........I...............
014	48	53	0.61	993,246	1.4	0.17	.........R..........I...............
038	48	56	0.64	1,050,146	3.6	0.44	.........R..........I...............
001	48	52	0.6	519,756	2.3	0.28	.........R..........I..E............
021	48	53	0.61	640,100	1.1	0.14	.........R..........I..E............
026	48	51	0.59	486,335	3.4	0.42	.........R..........I..E............
034	48	49	0.57	550,890	>667	>	.........R..........I..E............
045	48	48	0.56	289,093	>667	>	.........R..........I..E............

Abbreviations: MPI, maximum percent inhibition; R-MPI, relative maximum percent inhibition; RLU, relative light unit.
1. R-MPI: MPI of subject/MPI of virus control evaluated in parallel.
2. Fold change-IC_{50}: IC_{50} of subject/IC_{50} of virus control evaluated in parallel.
3. The MPI value was too low to allow calculation of IC_{50}/fold change IC_{50}.

Role of the V3 Loop in Mediating Reduced Susceptibility to VCV

- For select clones from three subjects, reciprocal exchanges of the V3 loop region were made between susceptible and phenotypically resistant clones. In some instances introduction of the V3 loop from the susceptible clone into the phenotypically resistant clone partially or fully restored susceptibility to VCV and MCV (**Table 7**). In contrast, introduction of the V3 loop from the phenotypically resistant clone into the susceptible clone had no impact on susceptibility to either drug.

Table 7. Impact of V3 Loop Exchanges on Susceptibility (MPI and R-MPI) to Vicriviroc and Maraviroc

Subject #	Clone ID/ Domain Swap	Time Point (week)	Vicriviroc MPI (SD)[1]	Vicriviroc R-MPI[2] (SD)	Maraviroc MPI (SD)	Maraviroc R-MPI (SD)	V3 Loop Sequences
485	024	0	96 (1.7)	1.0 (0)	100 (0.6)	1.0 (0.01)	CTRPNNNTRRSIHIGPGSAFYATGDIIGDIRQAHC
	002	48	78 (2.0)	0.84 (0.01)	84 (4.6)	0.84 (0.05)	............D......................
	011	48	71 (6.1)	0.77 (0.05)	76 (4.2)	0.76 (0.04)	............P......................
	002 with V3 from 024[3]	–	87 (2.6)	0.93 (0.01)	86 (3.6)	0.86 (0.04)	....
	011 with V3 from 024	–	69 (1.0)	0.74 (0.01)	68 (2.6)	0.68 (0.02)	....
	024 with V3 from 002	–	100 (0)	1.1 (0)	100 (0)	1.0 (0)	............D......................
	024 with V3 from 011	–	99 (1.1)	1.1 (0)	100 (0)	1.0 (0)	............P......................
498	023	0	95 (1.1)	1.0 (0)	99 (0)	0.99 (0)	CTRPNNNTRKSIHIGPGKAFYATGDIIGDIRQAHC
	031	48	64 (1.7)	0.69 (0.01)	68 (1.5)	0.68 (0.02)	.........R.........................
	033	48	72 (2.0)	0.78 (0)	74 (2.9)	0.74 (0.03)	................................R...
	031 with V3 from 023	–	74 (2.6)	0.79 (0.01)	79 (2.6)	0.79 (0.03)	....
	033 with V3 from 023	–	74 (1.7)	0.79 (0.02)	75 (2.6)	0.75 (0.03)	....
	023 with V3 from 031	–	94 (1.1)	1.0 (0)	98 (0)	0.98 (0)	.........R.........................
	023 with V3 from 033	–	96 (0.6)	1.0 (0.06)	99 (0)	0.99 (0)	................................R...
784	014	0	100 (0.6)	1.1 (0)	100 (0)	1.0 (0)	CTRPGNNTRKSIPIGPGRAFYATGDIIGDIRKAHC
	020	48	65 (4.6)	0.70 (0.03)	74 (5.6)	0.74 (0.06)	.........R..........I..............
	014 with V3 from 020	–	96 (0)	1.05 (0.06)	99 (0)	0.99 (0)	.........R..........I..............
	020 with V3 from 014	–	92 (0.6)	0.99 (0.01)	96 (0.6)	0.96 (0.01)	....

Abbreviations: MPI, maximum percent inhibition; NT, not tested; SD, standard deviation.
1. Each measurement was performed in triplicate.
2. R-MPI: MPI of subject/MPI of virus control evaluated in parallel.
3. Clone with backbone from Week 48 clone (02) and V3 loop from Day 1 clone (024).

Impact of Continued VCV Therapy on Two Subjects With Phenotypically Resistant Virus

- After the end of the P03672 trial, Subjects 485 and 500 elected to enroll in an open label extension (despite already being classed as virologic failures) and received 30 mg VCV QD for an additional 12 months (**Table 9**). Neither subject had a fully active drug in their OBT during the course of therapy.
- Subject 485 maintained stable CD4 counts over the 12 months; viral load was stable out to nine months and at 12 months it increased >three-fold compared with Baseline.
- For Subject 500 the viral loads at Baseline and at 12 months were similar; CD4 counts remained stable over the first nine months but at 12 months they were two-fold lower than at Baseline.
- Both subjects had DM virus at start of study, in Subject 485 the virus was exclusively R5-tropic at Month 12. Virus from both subjects exhibited further decreases in susceptibility to the VCV over the course of therapy and both subjects were considered virologic failures at 12 months.

Table 9. Clinical Data for Two Subjects from the P03672 Clinical Trial That Rolled Over into the Open Label Extension of P03672

Subject #	OSS[1] Base-line	OSS[1] TOD	Time Point (month)	CD4 Count (µl)	Viral Load (copies/ml)	MPI (%)[2]	Relative MPI[3]	IC_{50} (nM)[2]	Fold Change IC_{50}[4]	Tropism[5]	Luciferase Activity (RLU)[5] CCR5 Cells	CXCR4 Cells
485	0	0	0	113	5,200	75	0.82	2.9	0.55	DM	1,072,793	519
			3	121	3,090	–[6]	–	–	–	–	–	–
			6	108	8,550	34	0.37	>0.667[7]	>[7]	DM	491,183	7,750
			9	107	3,610	–	–	–	–	–	–	–
			12	108	18,000	38	0.38	>0.667	>	R5	345,296	182
500	0	0	0	225	60,700	33	0.37	>0.667	>	DM	510,408	38,724
			3	261	103,000	–	–	–	–	–	–	–
			6	228	55,000	47	0.49	>0.667	>	DM	180,956	68,787
			9	211	163,000	–	–	–	–	–	–	–
			12	109	75,500	0	0	>0.667	>	DM	151,990	13,301

Abbreviations: DM, dual-mixed; MPI, maximum percent inhibition; OSS, overall susceptibility score.
1. Overall susceptibility score determined at Baseline and time of discontinuation.
2. Determined for the viral swarm using PhenoSense Entry® assay.
3. Relative MPI: MPI of subject/MPI of virus control evaluated in parallel.
4. Fold change-IC_{50}: IC_{50} of subject/IC_{50} of virus control evaluated in parallel.
5. Determined for the viral swarm using Trofile™ assay.
6. Not tested.
7. The MPI value was too low to allow calculation of IC_{50}/fold change IC_{50}.

Conclusions

- **In Study P03672, five VCV-treated subjects had HIV-1 that exhibited reduced susceptibility to VCV following therapy. All five subjects had inadequate optimized background therapy at Baseline with zero or one active drug in the OBT.**
- **Susceptibility testing confirmed that all subjects had VCV susceptible R5 virus populations at Baseline; reduced susceptibility to VCV was detected by Week 8 in one subject, Week 20 in a second subject and Week 48 in three additional subjects.**
- **Comparative sequence analysis of gp160 clones from Baseline and TOD samples identified multiple amino acid changes throughout gp160 with no consistent pattern of changes among resistant clones from individual subjects.**
- **Introduction of the V3 loop domain from phenotypically resistant clones into susceptible baseline clones failed to recapitulate the resistant phenotype.**
- **These findings demonstrate that mutations in the V3 loop are not solely responsible for changes in susceptibility to VCV and MCV in these subjects.**

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