

Supplementary tables

Table S1: Summary of HIV-1 subtype B Gag substitutions observed during PI-based treatment.

Gag protein	Gag position	Gag substitutions *	Reference
Matrix	12	E12K	[1]
Matrix	62	G62R	[2]
Matrix	75	L75R	[1]
Matrix	76	R76K	[3]
Matrix	79	Y79F	[4]
Matrix	81	T81A	[4]
Matrix	125	S125K	[2]
Matrix	132	Y132F	[2]
Capsid	219	H219Q	[1] [5]
p2	370	V370A, V370I, V370M	[6]
p2	373	S373Q	[7] [8]
p2	374	A374G, A374N, A374P, A374S, A374T	[7] [9]
p2	375	T375N	[7]
Nucleocapsid	390	V390D	[1]
Nucleocapsid	409	R409K	[1]
Nucleocapsid	431	A431V	[8] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19]
p1	435	G435E, G435R	[12] [20] [21]
p1	436	K436E, K436N, K436R	[9] [12] [13] [20] [22]
p1	437	I437A, I437T, I437V	[9] [12] [13] [16] [19] [20] [22] [23] [24]
p1	438	W438R	[12]
p1	440	S440C	[20]
p6	449	L449F, L449P, L449Q, L449V	[8] [9] [10] [13] [16] [19] [20] [24] [25] [26] [27] [28] [29] [30]
p6	451	S451I, S451N, S451T	[30] [31]
p6	452	R452K, R452S	[10] [13] [19] [32]
p6	453	P453L, P453T	[9] [11] [13] [16] [17] [19] [26] [27] [29] [32]
p6	459	P459I	[33]

*: Only Gag substitutions associated with FDA-approved PIs in HIV-1 subtype B are summarized. The substitution is expressed relative to the subtype B consensus sequence (<http://www.hiv.lanl.gov/>).

Table S2: Summary of Gag, protease and RT substitutions in 12 patients of the Leuven cohort. For the first sequence, PI/RTI drug resistance mutations, detected by the drug resistance interpretation algorithms HIVdb v7.0 [34] and/or Rega V9.1 [35], are colored red. The other protease and RT variants are indicated in black. Ambiguous nucleotide letters are decomposed and translated into amino acids, which are indicated by brackets. Gag substitutions and PI/RTI resistance mutations with therapy changes are mapped in Figure 1. “na” indicates that the sequence does not cover the corresponding position.

Patient ID	Subtype	Sampling day	Gag substitution	Protease variants and PI resistance mutations	RT variants and RTI resistance mutations
123	A1	2002-10-08	363L+477E	10V	49R+103N+108I+118V+173[L,S]+179I+184V+189[I,V]+215[Y,N,T,S]+225[P,H]+238[T,K]+248T+303L
		2003-12-10	363L+477Q	10V+20T	49[R,K]+103N+108I+118V+173L+179L+184M+189I+215Y+225P+238T+248T+303[L,W]
		2004-10-27	363W+477Q	10V+20T	49K+103N+108I+118[V,I]+173L+179L+184M+189I+215Y+225P+ 238T+248[T,I]+303L
343	C	2010-03-17	453P	64I+70[K,R]	40[E,D]+49[K,R]+106[V,M]+123[S,G]+126[K,R]+165[I,T]+175N+277[K,R]+281R
		2012-11-30	453PTAPPE	64[I,L]+70K	40E+49K+106V+123S+126K+165I+175[N,Y]+277K+281[R,K]
357	C	2004-07-26	363F+389N+422P+445P	74S	13[K,R]+86D+148V+154[K,R]+280[C,Y]
		2005-09-05	363L+389T+422Q+445L	74S	13K+86[D,G]+148[V,I]+154K+280C
445	C	2007-06-27	411K	60[D,E]+66I	3S+28K+32E+36A+49K+60I+65K+67D+70K+75[V,I]+101K+103N+123[N,S]+173V+177D+184M+190G+207[E,G]+211R+214L+215[T,I]+219K+277[R,K]+ 278Q+281R
		2008-01-02	411K	60E+66I	3S+28K+32E+36A+49K+60I+65R+67D+70K+75V+101K+103N+123S+173V+177D+184M

					+190G+207E+211R+214L+ 215[T,I] +219K +277R+278[Q,R]+281K
		2011-07-15	411Q	60D+66[I,M]	3S+28E+32K+36E+49[K,R]+60V+65K+ 67N + 70R +75V+101K+103K+123S+173A+177E + 184V + 190A +207E+211K+214[L,F]+215T +219K+277R+278Q+281K
		2012-04-04	411Q	60D+66I	3[N,S,D,G]+28[K,E]+32K+36E+49K+60V+65K + 67N + 70R +75V+ 101[K,E] +103K+123S+173A +177E+ 184V + 190A +207E+211K+214[L,F] +215T+ 219[K,E] +277R+278Q+281K
834	C	2003-02-12	472P+474P	20R+62V	103N +173T + 184V
		2007-01-04	472S+474L	20R+62V	103K+173A +184M
1039	C	2010-08-12	427T +452R	62[I,V] +72I+ 74S +82[I,V]	8[V,I]+28E+39E+48[T,S]+53[E,D]+67D+121[D,H] +123[S,N,D,G]+135I+142[I,V]+166[K,R]+184M +208H+214F+241V+286[A,T]+324[E,D] +334Q
		2011-02-14	427T +452R	62I+72I+ 74S +82V	8V+28E+39E+48T+53E+ 67[N,D] +121D+123S +135I+142[I,V]+166[K,R]+ 184[I,M] +208H+214F +241V+286A+324E+334[Q,H]
		2011-04-01	427T +452R	62[I,V] +72I+ 74S +82[I,V]	8V+28E+39E+48[T,S]+53[E,D]+ 67[N,D] +121D +123S+135I+142I+166[K,R]+ 184V +208[H,Y] +214F+241V+286A+324E+334na
		2011-08-08	427T +452G	62V +72I+ 74S +82I	8V+28K+39E+48T+53E+67D+121D+123S+135L +142I+166K+ 184V +208H+214F+241V+286A +324E+334Q
		2013-04-22	427P+452G	62[I,V] +72[I,M]+ 74S + 82[I,V]	8V+28E+39[E,K]+48T+53E+67D+121D+123S +135[I,T,S,L]+142I+166K+ 184V +208H+214[F,L] +241[V,L]+286A+324E+334Q
1075	C	2005-11-07	363L	10I +18H+ 33F + 43T + 48V + 54A + 62V + 74S + 82A + 89I	35T+ 41L + 44D + 67N +84[T,S]+101K+ 106M + 118I + 184V + 190A + 210W + 215Y + 219N + 227L
		2006-03-01	363W	10I +18H+ 33F + 43T + 48V + 54A + 62V + 74S + 82A + 89I	35T + 41L + 44D + 67N +84T+ 101[K,E] + 106[M,I,V] + 118I + 184[V,M] + 190A + 210W + 215Y + 219N + 227L
		2006-05-15	363L	10I +18[H,Q]+ 33F + 43T + 48V + 54A + 62V + 74S + 82A + 89I	35[T,I]+ 41L + 44D + 67N +84T+ 101[K,E] +106V+ 118I + 184[V,M] + 190A + 210W + 215Y + 219N + 227L
552	D	2002-02-18	415K+469I	13I+14K+62I+72I+93[I,L]	20[K,R]+136N+275K+294P+297A
		2003-07-28	415K+469T	13I+14K+62I+72I+93L	20[K,R]+136N+275[K,R]+294P+297A+334R+335G
		2004-03-22	415K+469T	13I+14K+ 62[I,V] +72[I,V] +93L	20[K,R]+136N+275K+294P+297A +334R+335E

		2009-02-11	415K+469T	13[I,V]+14[K,R]+62I +72[I,V]+93[I,L]	20K+136N+275K+294T+297[A,T]+334R+335E
		2011-01-24	415R+469T	13[I,V]+14[K,R]+62[I,V] +72[I,V]+93[I,L]	20K+136[N,H]+275K+294na+297na+334na+335na
27	F1	2008-07-24	453P	10V+62V+69Y	207E+276V
		2010-07-14	453L	10V+62V+69Y	207K+276[I,V]
		2011-01-12	453L	10V+62V+69[H,Y]	207[K,E]+276V
666	J	2001-06-13	138M+374A	10I+20R+35E+54I+60[S,N] +62V+82V+89M	67D+70K+101K+174[K,R]+184M+200I+288A +294T+322T
		2003-11-12	138L+374A	10I+20R+35E+54I+ 60S +62V+82V+89M	67D+70K+101K+174[K,R]+184V+200[I,T, A, V] +288[A,S]+294T+322T
		2004-11-03	374T	10I+20R+35D+54V+ 60S+62V+82A+89L	67N+70R+101E+174K+184M+200I+288A+294P +322A
407	01_AE	2009-11-02	374V+387R +451S+453P	79P	11K+184I+200A+281R+286A+304[E,A]+325L+326I
		2010-03-22	374A+387K +451G+ 453EPTAPP	79H	11[K,T]+184[I,M]+200[A,V]+281K+286[A,T] +304A+325I+326V
652	02_AG	2008-01-31	420G	20I	11R+27[T,S]+35I+106V+207G+276I+291D+292I +294T+311R
		2008-08-19	420A	20I	11[R,K,Q]+27T+35[I,T]+106[V,I]+207[E,G]+276[I,V] +291[D,E]+292[I,V]+294[T,P]+311[R,K]

Table S3: Summary of PI-resistant and PI-susceptible sequence datasets.

Subtype/ CRF	Number of PI-susceptible Gag-protease sequences *	Number of PI-resistant Gag-protease sequences #	Total
A1	185+72=257	6+3=9	266
B	1820+313=2133	434+31=465	2598
C	1728+58=1786	119+18=137	1923
D	98+20=118	1+0=1	119
F1	21+25=46	3+0=3	49
G	33+0=33	14+0=14	47
01_AE	1112+45=1157	22+2=24	1181
02_AG	55+71=126	3+4=7	133
Total	5657	660	6317

*: Number of PI-susceptible Gag-protease sequences used in this study. These sequences were amino acid sequences translated from nucleotide sequences obtained from the Los Alamos + Leuven datasets. PI-susceptible sequences were estimated to be fully susceptible to all PIs by both the HIVdb v7.0 [34] and the Rega V9.1 [35] algorithms.

#: Number of PI-resistant Gag-protease sequences used in this study. These sequences were amino acid sequences translated from nucleotide sequences obtained from the Los Alamos + Leuven datasets. PI-resistant sequences were estimated to be partially or fully resistant to at least one PI by both the HIVdb v7.0 [34] and the Rega V9.1 [35] algorithms. All the Los Alamos sequences encode the full-length Gag polyprotein.

Table S4: Prevalence in individual HIV-1 subtypes of Gag amino acid variants observed during PI therapy.

Gag amino acid variant	Subtype*	Amino acid prevalence		p-value #	Adjusted p-value
		PI-resistant dataset	PI-susceptible dataset		
12E	C	0.8%(1/119)	0.2%(4/1724)	0.286	1
12K	B	25.4%(31/122)	25.3%(158/624)	0.923	1
62G	C	1.7%(2/117)	0.2%(4/1700)	0.054	0.741
62R	B	4.1%(5/121)	4.1%(26/639)	0.599	1
76K	C	48.3%(57/118)	44.7%(769/1719)	0.999	1
76R	01_AE	9.1%(2/22)	19.1%(212/1112)	0.958	1
79F	G	14.3%(2/14)	48.5%(16/33)	0.998	1
79Y	01_AE	9.1%(2/22)	33.7%(374/1111)	0.999	1
81A	B	15.6%(19/122)	7.5%(48/639)	0.021	0.165
81A	01_AE	4.5%(1/22)	10.9%(121/1112)	0.929	1
125K	B	4.1%(5/122)	1.6%(10/636)	0.087	0.559
128A	B	0.8%(1/121)	2.8%(18/638)	0.966	1
128A	G	15.4%(2/13)	9.7%(3/31)	0.532	1
128I	B	5.8%(7/121)	0.9%(6/638)	0.002	0.024
128I	C	7.7%(9/117)	11.8%(201/1709)	0.964	1
128I	G	15.4%(2/13)	0.0%(0/31)	0.101	1
128I	01_AE	4.5%(1/22)	0.7%(8/1089)	0.172	1
130R	B	2.5%(3/122)	1.3%(8/639)	0.263	0.925
130R	G	7.1%(1/14)	0.0%(0/33)	0.313	1
132F	B	10.7%(13/122)	3.4%(22/639)	0.004	0.035
132F	G	21.4%(3/14)	0.0%(0/33)	0.035	0.925
135I	B	1.6%(2/122)	0.3%(2/657)	0.121	0.625
135I	C	0.8%(1/119)	0.2%(3/1728)	0.236	1
135M	B	0.8%(1/122)	0.0%(0/657)	0.158	0.694
135M	G	7.1%(1/14)	0.0%(0/32)	0.319	1
138M	B	10.7%(13/122)	8.4%(55/657)	0.369	1
138M	C	0.8%(1/119)	0.2%(3/1721)	0.236	1
219Q	B	28.1%(34/121)	21.2%(141/665)	0.458	1
219Q	C	9.2%(11/119)	18.3%(314/1715)	0.999	1
219Q	01_AE	22.7%(5/22)	22.8%(253/1112)	0.768	1
362I	B	17.7%(22/124)	21.9%(149/680)	0.978	1
362I	C	2.5%(3/119)	1.6%(27/1727)	0.317	1
362I	G	21.4%(3/14)	0.0%(0/33)	0.035	0.925
363F	B	0.8%(1/124)	0.0%(0/680)	0.155	0.694
364G	C	0.8%(1/119)	0.1%(1/1728)	0.126	1
370A	B	17.8%(23/129)	14.7%(106/720)	0.491	1
370I	B	3.9%(5/129)	2.4%(17/720)	0.254	0.925
370M	B	4.7%(6/129)	6.1%(44/720)	0.83	1
370V	01_AE	36.4%(8/22)	31.3%(348/1111)	0.768	1
373A	B	4.9%(7/143)	4.8%(37/766)	0.6	1
373Q	B	0.7%(1/143)	2.0%(15/766)	0.938	1
373S	C	5.9%(2/34)	2.4%(27/1114)	0.229	1

373T	B	3.5%(5/143)	4.0%(31/766)	0.719	1
374G	B	2.8%(4/142)	1.1%(8/756)	0.114	0.625
374G	C	1.5%(1/65)	0.2%(1/591)	0.191	1
374N	B	8.5%(12/142)	5.4%(41/756)	0.168	0.706
374P	B	2.8%(4/142)	5.2%(39/756)	0.938	1
374S	B	1.4%(2/142)	3.0%(23/756)	0.929	1
374T	B	13.4%(19/142)	14.2%(107/756)	0.819	1
374V	B	0.7%(1/142)	2.5%(19/756)	0.97	1
374V	C	9.2%(6/65)	14.7%(87/591)	0.955	1
375A	B	28.0%(40/143)	17.2%(131/761)	0.089	0.559
375A	C	1.8%(2/111)	2.2%(34/1513)	0.727	1
375N	B	15.4%(22/143)	19.2%(146/761)	0.973	1
375N	B	15.4%(22/143)	19.2%(146/761)	0.973	1
375S	B	6.3%(9/143)	11.4%(87/761)	0.989	1
375T	C	0.9%(1/111)	0.5%(7/1513)	0.436	1
375T	G	14.3%(2/14)	0.0%(0/22)	0.171	1
376A	B	0.7%(1/144)	0.3%(2/763)	0.407	1
376A	G	15.4%(2/13)	3.0%(1/33)	0.227	1
376M	B	3.5%(5/144)	2.1%(16/763)	0.249	0.925
376M	C	2.5%(3/118)	0.3%(5/1724)	0.012	0.251
376M	G	7.7%(1/13)	3.0%(1/33)	0.512	1
376V	B	15.3%(22/144)	18.0%(137/763)	0.945	1
376V	C	9.3%(11/118)	6.8%(118/1724)	0.282	1
376V	G	23.1%(3/13)	12.1%(4/33)	0.412	1
378V	B	1.3%(2/153)	1.0%(8/769)	0.52	1
378V	C	0.8%(1/119)	0.9%(15/1726)	0.66	1
380K	B	29.4%(45/153)	25.0%(192/769)	0.771	1
380K	01_AE	4.5%(1/22)	10.5%(117/1111)	0.922	1
380R	G	7.1%(1/14)	15.2%(5/33)	0.91	1
381G	C	22.7%(27/119)	18.1%(310/1714)	0.488	1
381G	G	28.6%(4/14)	24.2%(8/33)	0.688	1
381S	B	2.6%(4/153)	2.3%(18/770)	0.527	1
382K	B	1.3%(2/153)	1.2%(9/768)	0.575	1
382K	G	7.1%(1/14)	0.0%(0/33)	0.313	1
387K	B	4.6%(7/152)	5.5%(42/769)	0.764	1
387R	01_AE	27.3%(6/22)	20.5%(223/1090)	0.526	1
389N	B	2.7%(4/150)	4.2%(32/758)	0.884	1
389N	C	4.4%(5/114)	2.1%(35/1682)	0.12	1
389T	C	9.6%(11/114)	17.8%(300/1682)	0.998	1
389T	G	8.3%(1/12)	0.0%(0/2)	0.867	1
415R	B	0.6%(1/169)	1.7%(13/786)	0.937	1
415R	C	2.5%(3/119)	0.0%(0/1727)	<0.0001	0.012
427P	B	1.2%(2/168)	0.6%(5/784)	0.363	1
427P	C	0.8%(1/119)	0.0%(0/1725)	0.065	0.771
430R	C	2.5%(3/119)	0.1%(1/1727)	0.003	0.046
430R	G	15.4%(2/13)	0.0%(0/33)	0.093	1
431V	B	13.5%(23/170)	0.1%(1/787)	<0.0001	<0.0001
431V	C	1.7%(2/119)	0.2%(4/1728)	0.054	0.741
431V	G	23.1%(3/13)	0.0%(0/33)	0.03	0.925

431V	01_AE	18.2%(4/22)	0.7%(8/1111)	<0.0001	0.007
435E	B	0.6%(1/170)	0.0%(0/785)	0.179	0.715
436R	B	4.7%(8/170)	4.8%(38/787)	0.637	1
436R	C	3.4%(4/119)	4.1%(71/1724)	0.75	1
436R	G	15.4%(2/13)	15.2%(5/33)	0.716	1
436R	01_AE	31.8%(7/22)	27.0%(300/1112)	0.702	1
437V	B	8.9%(15/168)	1.7%(13/784)	<0.0001	<0.0001
437V	C	1.7%(2/119)	0.6%(11/1726)	0.208	1
438R	B	0.6%(1/170)	0.3%(2/787)	0.446	1
449F	B	5.6%(21/377)	0.5%(7/1352)	<0.0001	<0.0001
449I	B	1.9%(7/377)	1.0%(13/1352)	0.132	0.644
449P	B	6.9%(26/377)	8.0%(108/1352)	0.871	1
449P	C	2.5%(3/119)	3.0%(52/1727)	0.716	1
449V	B	4.8%(18/377)	0.9%(12/1352)	<0.0001	<0.0001
451G	B	3.4%(13/378)	1.3%(17/1348)	0.008	0.041
451N	B	13.0%(49/378)	14.7%(198/1348)	0.962	1
451S	C	42.0%(50/119)	38.5%(663/1721)	0.992	1
451S	G	14.3%(2/14)	6.1%(2/33)	0.395	1
451T	B	2.1%(8/378)	0.0%(0/1348)	<0.0001	<0.0001
452G	B	0.8%(3/384)	0.1%(2/1374)	0.074	0.542
452G	G	7.7%(1/13)	0.0%(0/33)	0.298	1
452K	B	1.0%(4/384)	0.9%(13/1374)	0.535	1
452S	B	3.4%(13/384)	0.3%(4/1374)	<0.0001	<0.0001
453I	B	0.8%(3/384)	0.2%(3/1399)	0.12	0.625
453I	G	15.4%(2/13)	0.0%(0/33)	0.093	1
453Ins	C	25.7%(35/136)	24.0%(413/1722)	0.3561	1
453L	B	18.5%(71/384)	7.1%(99/1399)	<0.0001	<0.0001
453L	C	3.4%(4/119)	10.4%(179/1722)	0.999	1
453T	B	4.4%(17/384)	4.7%(66/1399)	0.699	1
453T	C	21.8%(26/119)	3.1%(53/1722)	<0.0001	<0.0001
453T	01_AE	40.9%(9/22)	14.1%(157/1112)	0.026	0.386
469I	B	3.1%(12/383)	2.9%(46/1570)	0.508	1
469I	C	1.7%(2/116)	1.1%(19/1660)	0.411	1
469T	G	14.3%(2/14)	0.0%(0/33)	0.102	1
472P	01_AE	9.5%(2/21)	13.1%(121/927)	0.821	1
472S	B	0.9%(4/426)	1.6%(28/1795)	0.893	1
472S	C	1.8%(2/114)	7.6%(119/1560)	0.999	1
474L	B	0.5%(2/430)	0.3%(6/1811)	0.475	1
474P	B	0.7%(3/430)	1.7%(30/1811)	0.968	1
474P	01_AE	4.5%(1/22)	21.2%(218/1028)	0.996	1
486F	B	0.2%(1/433)	0.5%(9/1806)	0.884	1
486F	C	0.8%(1/119)	0.1%(1/1728)	0.126	1

*: We only examined the sequence datasets of subtypes B, C, G and CRF01_AE, which contained more than 10 sequences estimated to be (partially or fully) PI-resistant (see Table S3).

#: One-tailed Fisher's exact tests were performed on amino acid variants that occurred more than once in individual subtype datasets. For each HIV-1 subtype, the obtained p-values were adjusted using multiple testing corrections via the false discovery rate approach (software: Matlab 2013a, also see reference [36]). Amino acid variants are colored orange if their p-values and adjusted p-values were lower than 0.05. Amino acid variants are colored blue if their p-values were lower than 0.05 but adjusted p-values were above 0.05. Amino acid variants whose p-values are equal to 1 are not shown.

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