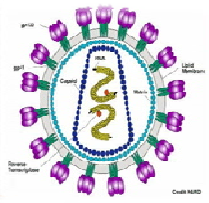
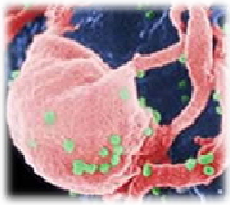
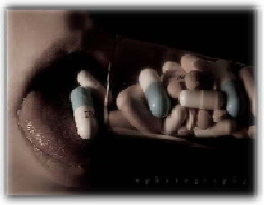
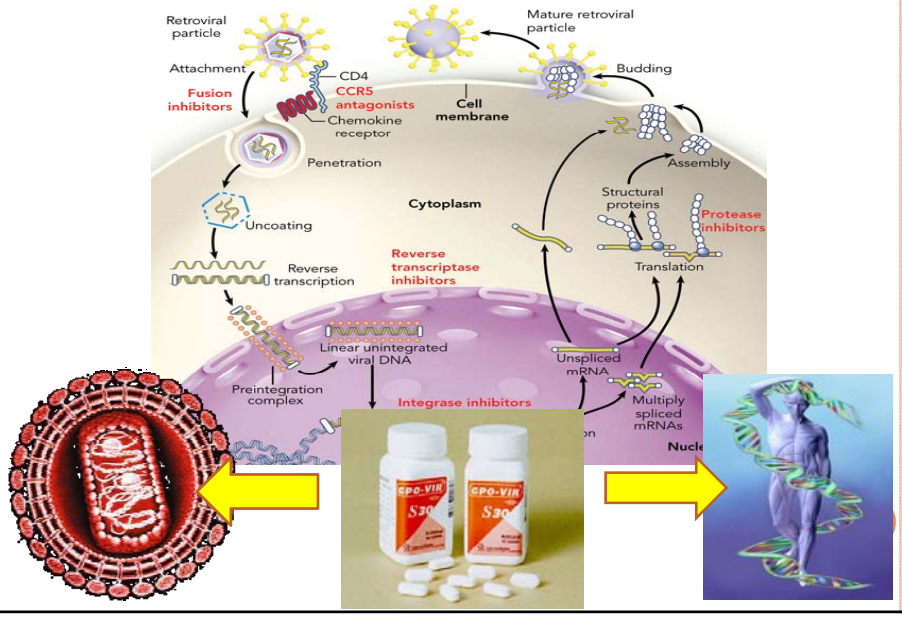
QUALITATIVE AND QUANTITATIVE HIV-1 DRUG RESISTANCE INTERPRETATION SYSTEM


Ekawat Pasomsub
Unit of Virology and Molecular Microbiology,
Department of Pathology, Ramathibodi Hospital
raeps@mahidol.ac.th

HIV infection (HIV and Host interaction)



- Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand

ตารางที่ 1 จำนวนผู้ป่วยเอดส์ จำแนกตามกลุ่มอายุ และเพศ ประเทศไทย ถึงปี พ.ศ. 2527 - 31 ตุลาคม พ.ศ. 2553

Total 369,885

กลุ่มอายุ	พ.ศ. 2527 - 2549			พ.ศ. 2550			พ.ศ. 2551			พ.ศ. 2552			พ.ศ. 2553			รวม
	ชาย	หญิง	รวม	ชาย	หญิง	รวม	ชาย	หญิง	รวม	ชาย	หญิง	รวม	ชาย	หญิง	รวม	รวม
0 - 4	6968	4917	11885	88	71	159	46	43	89	21	24	45	10	9	19	12197
5 - 9	2292	2158	4450	61	100	161	41	30	71	21	16	37	8	4	12	4731
10 - 14	575	701	1276	59	86	145	38	39	77	28	32	60	7	11	18	1576
15 - 19	1073	1366	2439	67	69	136	47	96	143	41	50	91	8	19	27	2836
20 - 24	15155	11624	26779	407	315	722	272	300	572	217	146	363	70	46	116	28552
25 - 29	51271	24612	75883	1373	1048	2421	921	857	1778	656	458	1114	155	111	266	81462
30 - 34	60183	22970	83153	2428	1720	4148	1659	1251	2910	1092	878	1970	284	194	478	92659
35 - 39	42357	14931	57288	2510	1445	3955	1894	1175	3069	1241	674	1915	343	198	541	66768
40 - 44	23222	8505	31727	1825	913	2738	1399	749	2148	1041	489	1530	267	134	401	38544
45 - 49	11732	4595	16327	991	513	1504	826	419	1245	583	292	875	156	81	237	20188
50 - 54	5612	2255	7867	510	300	810	416	241	657	368	151	519	70	38	108	9961
55 - 59	2951	1120	4071	245	131	376	167	121	288	123	75	198	55	11	66	4999
60 +	3486	1005	4491	244	109	353	174	110	284	138	69	207	60	17	77	5412
รวม	226877	100759	327636	10808	6820	17628	7900	5431	13331	5570	3354	8924	1493	873	2366	369885

In 2002

THAILAND

Thai Ministry of Public Health

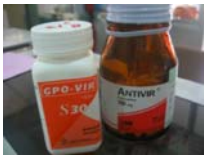
- **The National Access to Antiretroviral Program for People Living with HIV/AIDS (NAPHA)**
  - The aim of providing treatment to all Thai patients with HIV infection

The Government Pharmaceutical Organization (GPO)

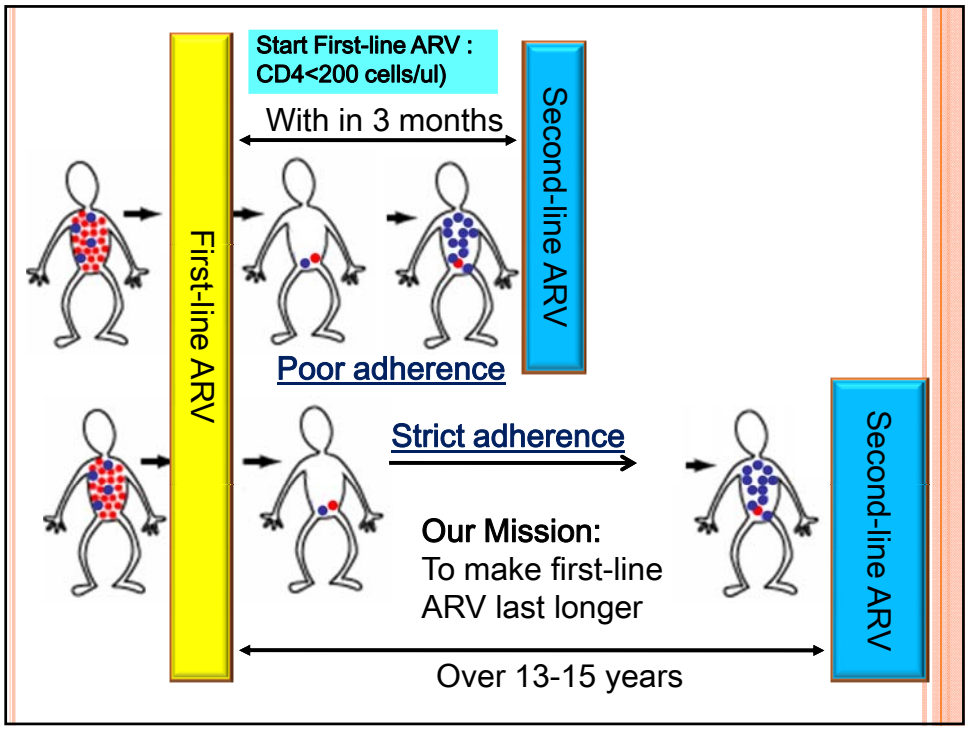
- Began producing a generic version of three anti-HIV drugs mixed together into one pill called **GPO-VIR**

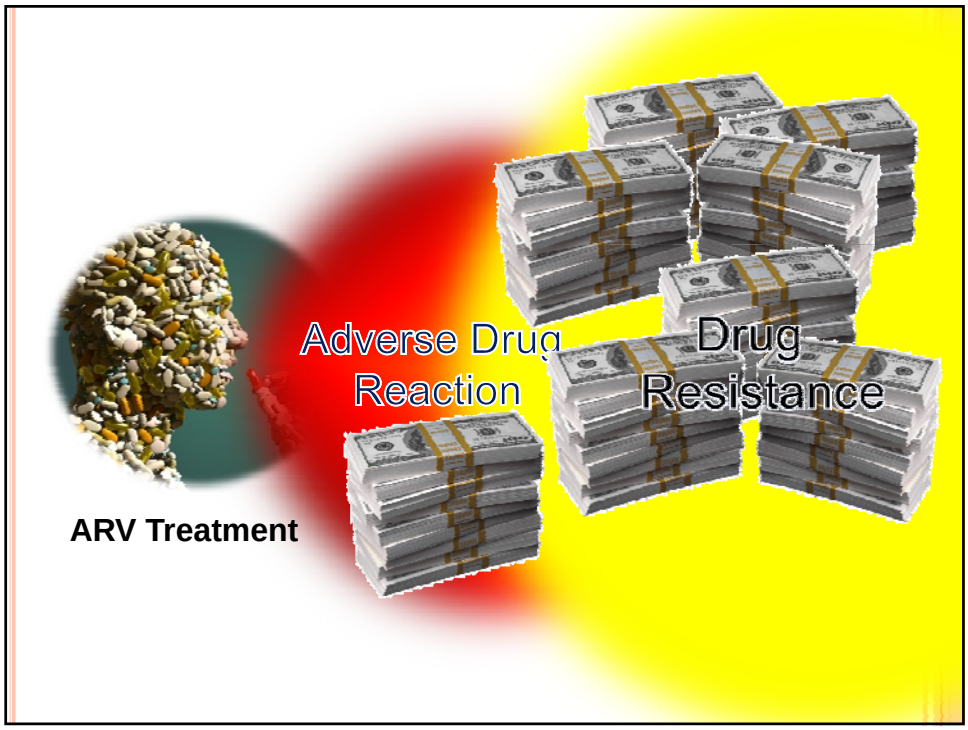
d4T
3TC } **NRTIs**

Nevirapine → **NNRTIs**



Using this combination of drugs as the first-line HAART (drug) regimen


Start First-line ARV :
CD4<200 cells/ul)
With in 3 months
First-line ARV
Second-line ARV
Poor adherence
Strict adherence
Our Mission:
To make first-line
ARV last longer
Over 13-15 years
Second-line ARV


Adverse Drug
Reaction
Drug
Resistance
ARV Treatment

Thai Universal Health Care Scheme for AIDS

B.E 2550 = 3,855,600,000 baht
B.E 2551 = 4,325,966,000 baht
B.E 2552 = 2,983,800,000 baht
B.E.2553 = 2,770,000,000 baht

Approximately, 100,000 HIV infected people in need of antiretroviral treatment

Fiscal expenditure

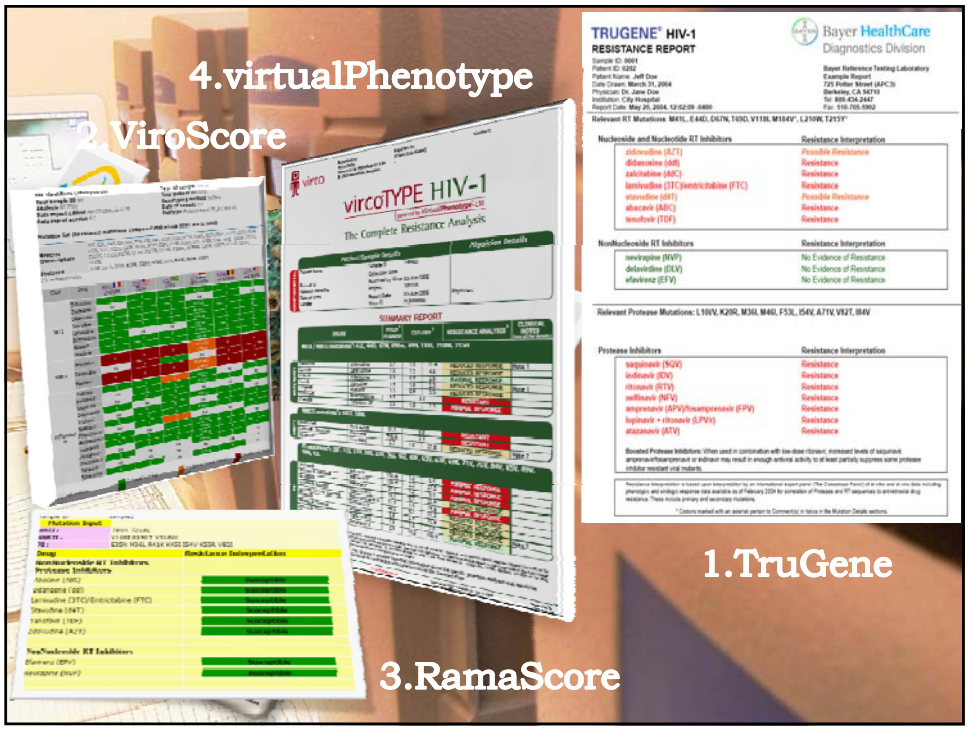
First-line ARV therapy
GPOvir 1,200 baht/patient/month = 1,440,000,000 baht
NVP 200 mg + d4T 30 mg + 3TC 150 mg

Viral load (HIV in the blood) & HIV-1 drug resistant testing

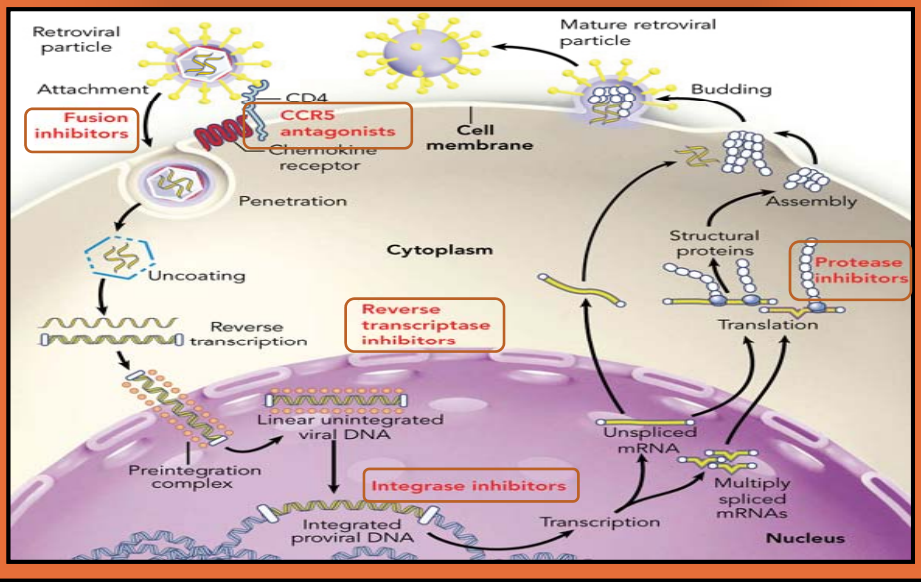
Good /Poor Adherence

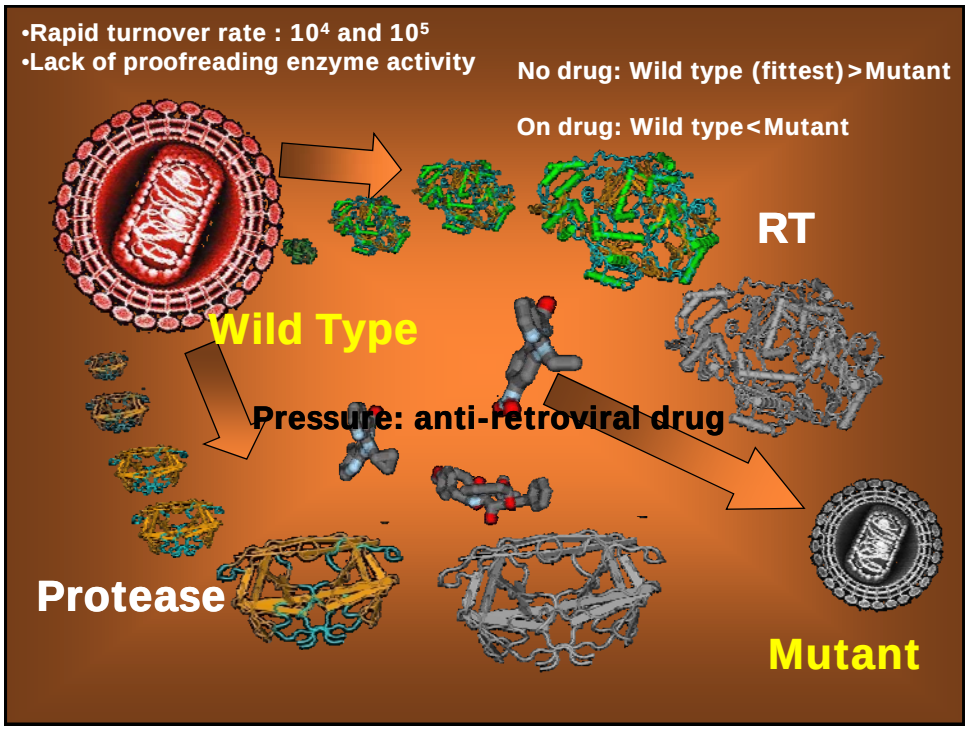
The earlier the better (a lot cheaper)

Second-line therapy (2-9 times more expensive)
NRTI + PI /patient/month = 2,880,000,000-12,960,000,000 baht



CLINICAL APPLICATIONS FOR THERAPY ON HIV INFECTION





Mutant type virus

↓

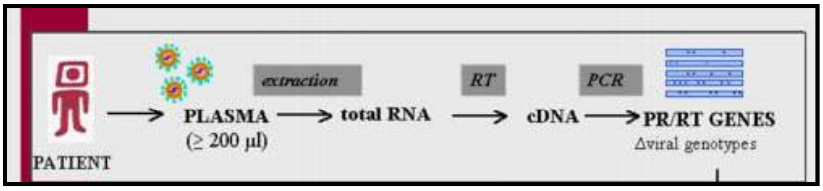
High viral load -> low CD4

=

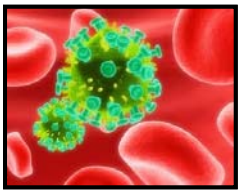
Drug resistance

11

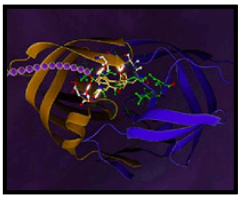
Resistant Testing



Phenotype



Genotype



Virtual Phenotype

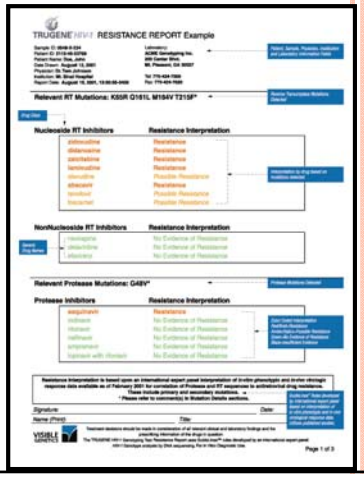
GENOTYPIC TESTING

- Investigate the nucleotide sequence of an HIV on the region that control the Protease and Reverse Transcriptase gene
- Comparing with nucleotide sequence of HIV wild-type

```
Ref – AGTGTGCAGTAGATC
Pt  - AGTGTGCAGAAGATC
```

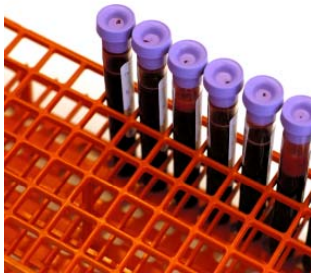
M184V

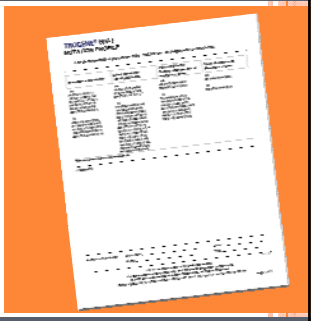
Rule-base knowledge



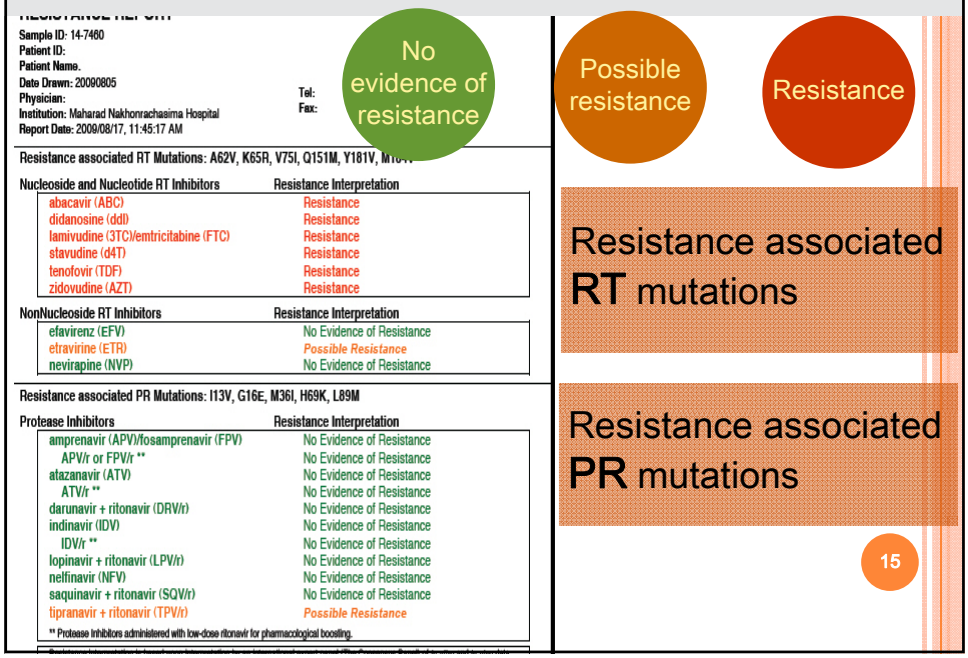
TRUGENE SYSTEM BY SIEMENS

FDA



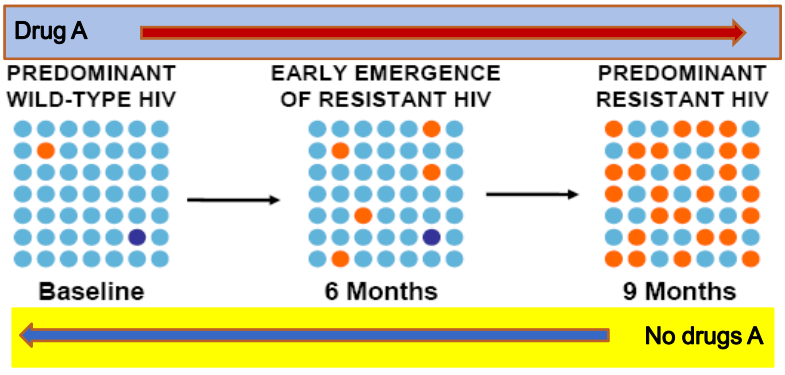


TRUGENE HIV-1 RESISTANCE REPORT

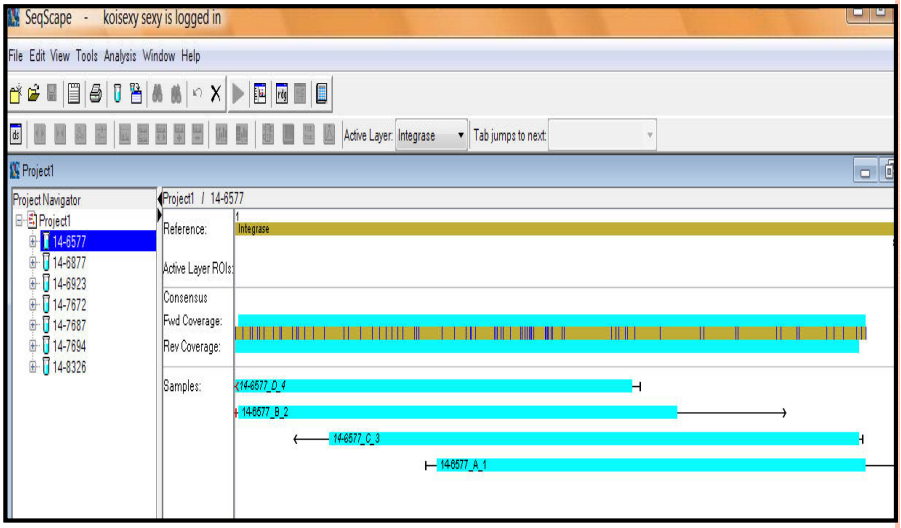


Disappearance of resistance

- When treatment interrupted for example, non-adherence
- Drug-resistant strains cannot detected by genotyping assays
- Still minority population that can re-emerge if selection pressure re-applied



DRUG RESISTANCE TESTING ON THE INTEGRASE INHIBITOR




```
>Consensus for segment Integrase for specimen 14-8326
TTTTTAGATGGGATAGATAAAGCTCAAGAAGATCATGAAAAATATCACAGCAATTGGAAAACAATGGCTAGTGAATTTAA
TTTGCCACCTATAGTAGCAAAGGAAATAGTAGCCAACTGTGATAAATGTCAGCTAAAAGGGGAAGCTATGCATGGACAAG
TGGACTGTAGTCCAGGGATATGGCAATTAGATTGCACACATCTAGAAGGGAAAGTCATCCTGGTAGCAGTCCACGTGGCC
AGTGGATATATAGAAGCAGAAGTTATCCCAGCAGAAACAGGACAGGAAACAGCATACTTTCTGCTAAARTTAGCAGGAAG
ATGGCCAGTAAAAGTAGTACACACAGACAATGGTAGCAATTTCACCAGCGCTGCAGTCAAAGCAGCCTGTTGGTGGGCCA
ATATCCACCAGGAATTTGGGATCCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTAAAGAAA
ATCATAGGGCAGATAAGAGAGCAAGCTGAACACCTTAAGACAGCAGTACAAATGGCAGTATTCATTCACAATTTTAAAAG
AAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGGATAATAGACATAATAGCAACAGACATACAAACTAAAGAATTAC
ARAARCAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAATTTGGAAAGGACCAGCAAAA
CTACTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGACAATAGTGATATAAAAGTAGTACCAAGAAGAAAAGCAAAAA
```

STANFORD UNIVERSITY
HIV DRUG RESISTANCE DATABASE
A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVdb PROGRAM

HIVdb: Genotypic Resistance Interpretation Algorithm

Date: 16-Sep-2010 10:01:10 PDT

Seq ID: Input Sequence

Summary Data

Sequence includes IN: codons: 1 - 266

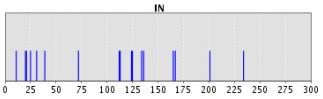
There are no insertions or deletions

Subtype and % similarity to closest reference isolate:

1. *IN: CRF01_AE (96.9%)*

Sequence Quality Assessment

Gene	QA Problem	Codons
IN	Stop Codons, Frame Shifts:	*None*
IN	B,D,H,V,N:	*None*
IN	Unusual Residues:	*None*



Blue lines indicate differences from consensus B; tall blue lines indicate sites associated with drug resistance.
Red lines indicate QA problems.

18

DRUG RESISTANCE INTERPRETATION FOR INTEGRASE INHIBITOR

Drug Resistance Interpretation: IN

IN Major Resistance Mutations:	None
IN Minor Resistance Mutations:	None
Other Mutations:	E11D, R20K, A21T, D25E, V31I, S39N, I72V, T112V, I113V, T124A, T125A, G134N, K136H, V165I, D167E, V201I, L234I

Integrase Inhibitors	
elvitegravir (EVG)	Susceptible
raltegravir (RAL)	Susceptible

IN Comments

Other

- V72I is a highly polymorphic mutation selected in vitro by pre-RAL/EVG INIs. It does not decrease susceptibility INI susceptibility (Fransen et al. 2006; Goethals et al. 2008; Low et al. 2009; da Silva et al. 2010). I72V is an unusual mutation at this position.
- V201I is a common polymorphism possibly associated with reduced susceptibility to early investigational INIs but not to current INIs (Hombrouck et al. 2008; Low et al. 2009; Miller 2009; da Silva et al. 2010).

Mutation Scoring

IN	EVG	RAL
Total:	0	0

VIROSCORE™ SUITE

VIROSCORER – NAÏVE PATIENT

Mutation list (Resistance mutations compared with strain HXB2 are in bold)

Reverse transcriptase	K11T, K30K, E40E, G45G, A62A, E79E, K82K, I94I, Q102K, V108V, F116F, D121Y, K122E, I142V, C162A, D177E, V178I, I180I, R211K, L234L, Q242Q, Q258Q, G273G, R277R, T286A, E312E, I326V, Q334L
Protease	V3I, L19L, S37N, **L63A**, K70K, **V77I**, Q92Q

(*): ambigous codon

Class	Drug	ANRS 12-09/04	DMC 01/04	GAV 11/04	CHL v5.0-11/04	REGA v6.3-09/04	STAN v4.0-10/04
NRTI	Zidovudine	S	S	S	Active	S	S
	Zalcitabine	na	S	S	na	S	na
	Didanosine	S	S	S	Active	S	S
	Stavudine	S	S	S	Active	S	S
	Lamivudine	S	S	S	Active	S	S
	Emtricitabine	S	na	na	Active	S	S
	Abacavir	S	S	S	Active	S	S
	Tenofovir	S	S	S	Active	S	S
NNRTI	Nevirapine	S	S	S	Active	S	S
	Delavirdine	na	S	S	na	S	S
	Efavirenz	S	S	S	Active	S	S
PI/Boosted PI	Indinavir	S	S	S	Active	na	S
	Indinavir/r	na	S	S	Active	S	na
	Saquinavir	na	S	S	na	na	S
	Saquinavir/r	S	na	S	Active	S	na
	Ritonavir	na	S	S	na	S	S
	Nelfinavir	S	S	S	Active	S	S
	Amprenavir	na	S	S	na	na	S
	Amprenavir/r	S	na	S	Active	S	na
	Lopinavir/r	S	S	S	Active	S	S
	Atazanavir	na	S	S	Active	S	S
	Atazanavir/r	S	na	na	Active	S	na
	Tipranavir	na	na	na	na	na	na
	Tipranavir/r	S	S	na	Active	S	na

not available | Susceptible/Active | Possible resistance/Partially active | Resistance/Inactive

VIROSCORER – FAILING NNRTIS

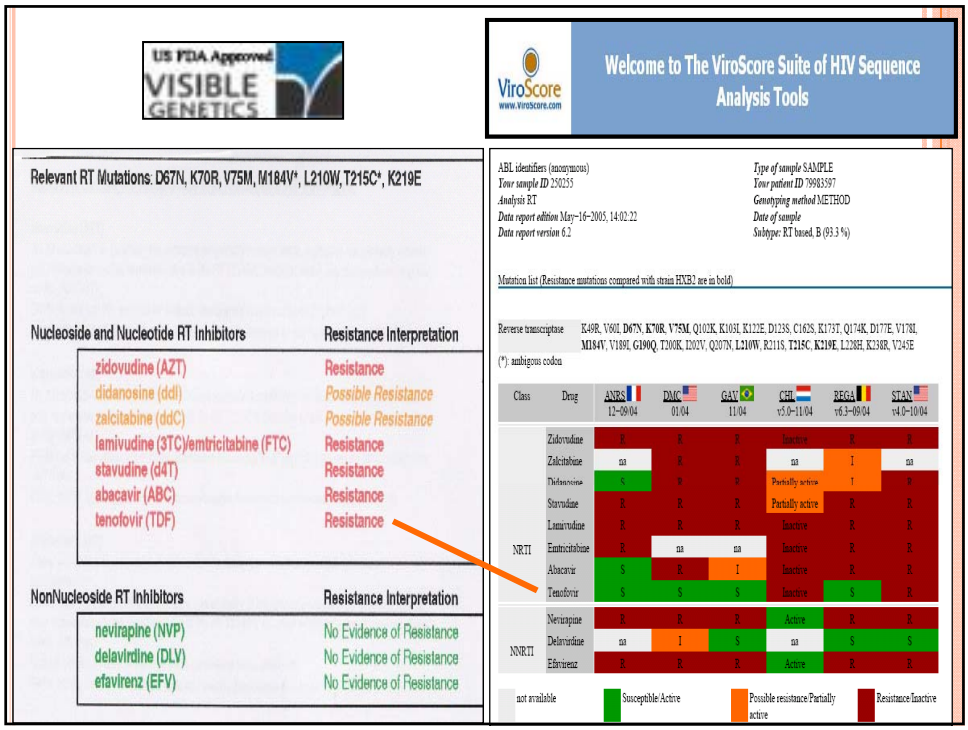
Mutation list (Resistance mutations compared with strain HXB2 are in bold)

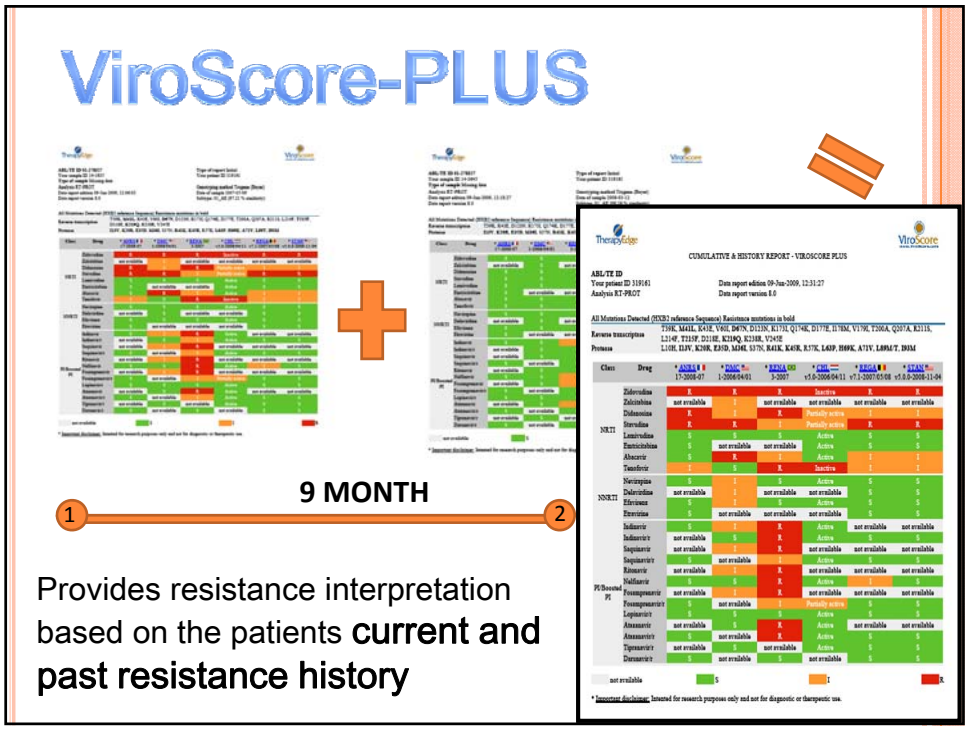
Reverse transcriptase	V35T, E36D, T39*, V60I, **A98S**, Q102K, **K103N**, I135T, C162S, V178I, D192N, R211K, F214L, **P225***, **P225H***, R277K, E297A
Protease	V3I, K14R, **I15V**, **E35D**, S37N, **L63T**, I64V

(*): ambigous codon

Class	Drug	ANRS 12-09/04	DMC 01/04	GAV 11/04	CHL v5.0-11/04	REGA v6.3-09/04	STAN v4.0-10/04
NRTI	Zidovudine	S	S	S	Active	S	S
	Zalcitabine	na	S	S	na	S	na
	Didanosine	S	S	S	Active	S	S
	Stavudine	S	S	S	Active	S	S
	Lamivudine	S	S	S	Active	S	S
	Emtricitabine	S	na	na	Active	S	S
	Abacavir	S	S	S	Active	S	S
	Tenofovir	S	S	S	Active	S	S
NNRTI	Nevirapine	R	R	R	Inactive	R	R
	Delavirdine	na	R	I	na	R	R
	Efavirenz	R	R	R	Inactive	R	R
PI/Boosted PI	Indinavir	S	S	S	Active	na	S
	Indinavir/r	na	S	S	Active	S	na
	Saquinavir	na	S	S	na	na	S
	Saquinavir/r	S	na	S	Active	S	na
	Ritonavir	na	S	S	na	S	S
	Nelfinavir	S	S	S	Active	S	S
	Amprenavir	na	S	S	na	na	S
	Amprenavir/r	S	na	S	Active	S	na
	Lopinavir/r	S	S	S	Active	S	S
	Atazanavir	na	S	S	Active	S	S
	Atazanavir/r	S	na	na	Active	S	na
	Tipranavir	na	na	na	na	na	na
	Tipranavir/r	S	S	na	Active	S	na

not available | Susceptible/Active | Possible resistance/Partially active | Resistance/Inactive


US FDA Approved
VISIBLE GENETICS
ViroScore
www.viroscore.com
Welcome to The ViroScore Suite of HIV Sequence Analysis Tools
Relevant RT Mutations: D67N, K70R, V75M, M184V*, L210W,T215C*, K219E
Nucleoside and Nucleotide RT Inhibitors
Resistance Interpretation
zidovudine (AZT) Resistance
didanosine (ddI) Possible Resistance
zalcitabine (ddC) Possible Resistance
lamivudine (3TC)/emtricitabine (FTC) Resistance
stavudine (d4T) Resistance
abacavir (ABC) Resistance
tenofovir (TDF) Resistance
NonNucleoside RT Inhibitors
Resistance Interpretation
nevirapine (NVP) No Evidence of Resistance
delavirdine (DLV) No Evidence of Resistance
efavirenz (EFV) No Evidence of Resistance
Mutation list (Resistance mutations compared with strain HXB2 are in bold)
Reverse transcriptase
K49R, V60I, D67N, K70R, V75M, Q102K, K103I, K122E, D123S, C162S, K173T, Q174K, D177E, V178I, M184V, V189I, G190Q, T200K, I202V, Q207N, L210W, R211S, T215C, K219E, L228H, K238R, V245E
(*): ambigous codon
Class
Drug
ANRS 12-09/04
DMC 01.04
GAV 11/04
GHL v5.0-11/04
REGA v6.3-09/04
STAN v4.0-10/04
NRTI
Zidovudine
Zalcitabine
Didanosine
Stavudine
Lamivudine
Emtricitabine
Abacavir
Tenofovir
NNRTI
Nevirapine
Delavirdine
Efavirenz
not available
Susceptible/Active
Possible resistance/Partially active
Resistance/Inactive


ViroScore-PLUS
9 MONTH
1
2
Provides resistance interpretation based on the patients current and past resistance history
CUMULATIVE & HISTORY REPORT - VIROSCORE PLUS

RESULT OUTPUT

RamaScore 2.3

Sample ID 142361

Date Draw 17-Nov-2010

Report Date 17/11/2553 16:44:37

Resistance associated RT mutations:

T69N, A98G, K101N, Y181

Nucleoside and Nucleotide
lamivudine/emtricitabin
abacavir
AZT
D4T
DDI
tenofovir

NonNucleoside RT Inhibitors	Resistance Interpretation	Scored Ramascore 2.3 Mut
efavirenz	Possible resistance	Y181C
etravirine	Possible resistance	A98G, Y181C
nevirapine	Resistance	Y181C

Resistance associated PR mutations:

L10I, E35D, L89M

Protease Inhibitors	Resistance Interpretation	Scored Ramascore 2.3 Mut
atazanavir	No evidence of resistance	
atazanavir/r	No evidence of resistance	
darunavir/r	No evidence of resistance	

26

COMMENT/NOTE BY EXPERTISE

Note for Rama Score:

Note No.	Text	Conditions
1	- Boosted PI-based regimens is recommended	- NNRTI resistance presents
2	- Potent boosted PI is recommended due to the weakness of new backbone	- NNRTI resistance presents and susceptible NRTI < 2 drugs
		- NNRTI resistance presents and only susceptible NRTIs (2 drugs)
4	- Please check baseline creatinine and UA and make sure HBsAg has been checked before using tenofovir	- NNRTI resistance presents and tenofovir is susceptible
5	- Consult any HIV experts or at hivrama@yahoo.com	- No NRTI is susceptible
6	- Please review patient's history of antiretroviral exposure and previous genotype results before choosing the next regimen	- all

Caution !

- Boosted PI-based regimens is recommended
- Consult any HIV experts or at hivrama@yahoo.com
- Please review patient's history of antiretroviral exposure and previous genotype results before choosing the next regimen

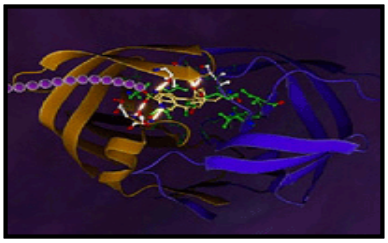
27

GENOTYPIC DRUG RESISTANCE TESTING

- In highly experienced patients, the multitude of accumulated mutations may be complex.
- "Archived" mutations.
- Mutations may interact with each other making interpretation difficult.

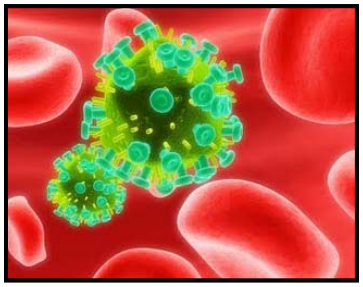
Disadvantages

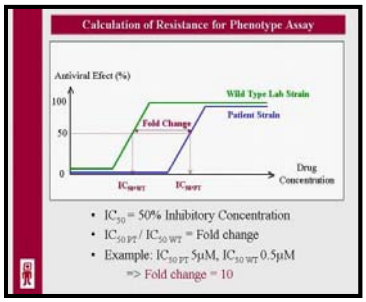
- Relevance of some mutations unclear
- Unable to detect minority variants (< 20% to 25% of viral sample)
- Complex mutational patterns may be difficult to interpret



Jing Zhang et al., PNAS, 2009; 1321-1326

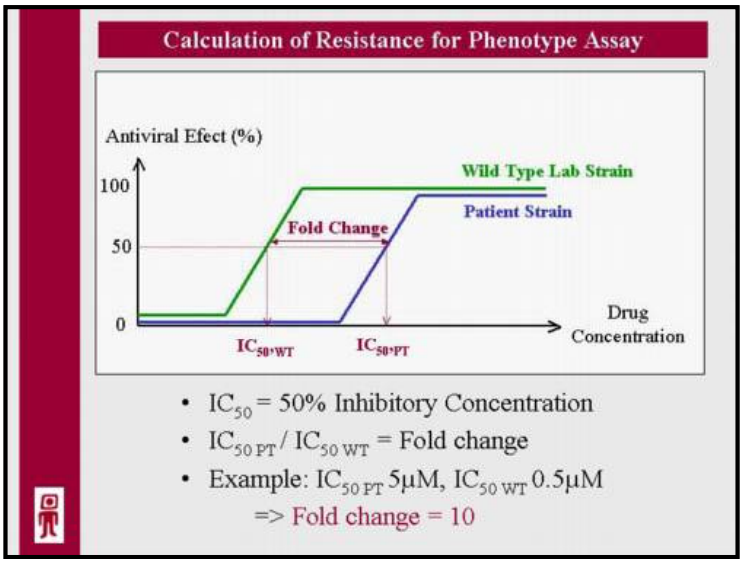
Phenotypic drug resistance testing





- Direct assay
- Determine the degree of virus replication inhibition at different drug concentrations
- Usually calculated at the 50% or 90% inhibition concentration (IC50 or IC90)

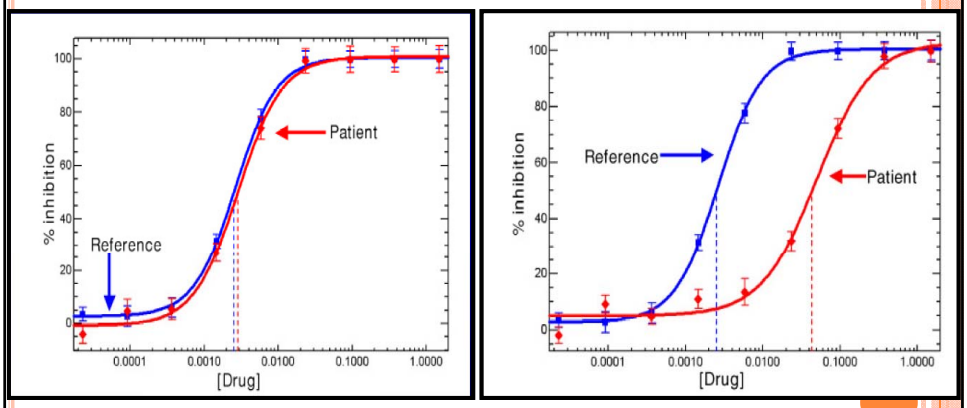
Calculation of Resistance for Phenotype Assay



INTERPRETATION

= Patient

= Reference



www.monogramhiv.com

PHENOTYPIC RESISTANCE REPORT

	Generic Name	Brand Name	Patient IC50*(μM)	Fold Change	Drug	
NRTI	Abacavir	Ziagen	4.02	2.26	ABC	Sensitive
	Didanosine	Videx *	6.13	1.39	ddI	Reduced Susc.
	Emtricitabine	Emtriva§	2.49	2.25	FTC	Sensitive
	Lamivudine	Epivir	5.57	1.83	3TC	Sensitive
	Stavudine	Zerit	0.88	1.80	d4T	Reduced Susc.
	Tenofovir	Viread *	1.602	2.43	TFV	Reduced Susc.
	Zidovudine	Retrovir	1.219	39	ZDV	Reduced Susc.
NNRTI	Delavirdine	Rescriptor	0.0139	0.63	DLV	Sensitive
	Efavirenz	Sustiva	0.0014	0.77	EFV	Sensitive
	Nevirapine	Viramune	0.101	1.10	NVP	Sensitive
PI	Atazanavir	Reyataz	0.00897	5.89	ATV	Reduced Susc.
		Reyataz / r#			ATV/r	Reduced Susc.
	Fosamprenavir	Lexiva	0.0151	1.20	AMP	Sensitive
	Indinavir	Crixivan	0.0206	2.86	IDV	Reduced Susc.

Phenotype tests are 3-4 times more expensive than genotype tests.

They take longer to get results - usually 2—4 weeks

Virtual Phenotype

- Predicts phenotypic resistance using database of genotype/phenotype combinations
- Less time consuming and labor intensive than actual phenotype
- Less costly than actual phenotype

Genotype →

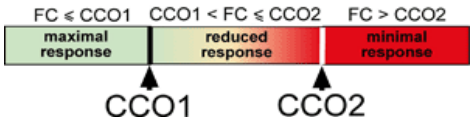
Phenotype →

→ Prediction system

Quantitative level

Report and Interpretation

	DRUGS		FOLD[1] CHANGE	CUT-OFF[2]		RESISTANCE ANALYSIS[3]	CLINICAL NOTES (see p2 for details)
	NRTI / NtRTI mutations[4]: 116Y, 151M, 184V, 215V						
NRTI/NtRTI	Retrovir®	Zidovudine	8.5	1.5	11.4	REDUCED RESPONSE	Note 1
NRTI/NtRTI	Epivir®	Lamivudine	53.0	1.2	4.6	MINIMAL RESPONSE	
NRTI/NtRTI	Videx®	Didanosine	12.2	0.9	2.6	MINIMAL RESPONSE	
NRTI/NtRTI	Zerit®	Stavudine	4.8	1.0	2.3	MINIMAL RESPONSE	Note 1
NRTI/NtRTI	Ziagen®	Abacavir	7.5	0.9	3.5	MINIMAL RESPONSE	
NRTI/NtRTI	Emtriva®	Emtricitabine	51.7	3.1		RESISTANT	
NRTI/NtRTI	Viread®	Tenofovir DF	0.9	1.0	2.3	MAXIMAL RESPONSE	
	NNRTI mutations[4]: 190A						
NNRTI	Viramune®	Nevirapine	34.9	6.0		RESISTANT	
NNRTI	Sustiva® , Stocrin®	Efavirenz	23.9	3.3		RESISTANT	
NNRTI	Intelence™	Etravirine	1.9	1.6	27.6	REDUCED RESPONSE	Note 2
	PI mutations[4]: 13V, 36I, 69K, 89M						
PI	Crixivan®	Indinavir	0.5	1.0	5.4	MAXIMAL RESPONSE	
PI	Crixivan ®; boosted	Indinavir/r	0.5	2.3	27.2	MAXIMAL RESPONSE	
PI	Viracept®	Nelfinavir	0.6	1.2	9.4	MAXIMAL RESPONSE	
PI	Invirase®; boosted	Saquinavir/r	0.4	3.1	22.6	MAXIMAL RESPONSE	
PI	Lexiva®,Telzir®; boosted	Fosamprenavir/r	0.5	1.5	19.5	MAXIMAL RESPONSE	
PI	Kaletra®	Lopinavir/r	0.6	6.1	51.2	MAXIMAL RESPONSE	
PI	Reyataz®; boosted	Atazanavir/r	0.4	2.5	32.5	MAXIMAL RESPONSE	
PI	Aptivus®; boosted	Tipranavir/r	0.8	1.5	7.0	MAXIMAL RESPONSE	Note 2
PI	Prezista™; boosted	Darunavir/r	0.4	10.0	106.9	MAXIMAL RESPONSE	



TruGene

Protease Inhibitors	Resistance Interpretation
saquinavir + ritonavir (SQV/r)	Resistance
indinavir (IDV)	Resistance
IDV/r **	Resistance
nelfinavir (NFV)	Resistance
amprenavir (APV)/fosamprenavir (FPV)	Resistance
APV/r or FPV/r **	Resistance

ViroScore

Class	Drug	ANRS 14-2006/06/07	DMC 2006/04/01	GAV 2006/06/01	CHL v5.0-2006/04/11	REGA v6.4.0-2006/04/12	STAN v4.2.2-18-09-06
PI/Boosted PI	Indinavir	R	R	R	Inactive	na	R
	Indinavir/r	na	I	R	Partially active	R	na
	Saquinavir	na	I	R	na	na	I
	Saquinavir/r	I	na	R	Partially active	I	na
	Ritonavir	na	R	R	na	R	R
	Nelfinavir	I	R	R	Inactive	R	R
	Fosamprenavir	na	R	R	na	na	R
	Fosamprenavir/r	R	na	R	Inactive	R	na

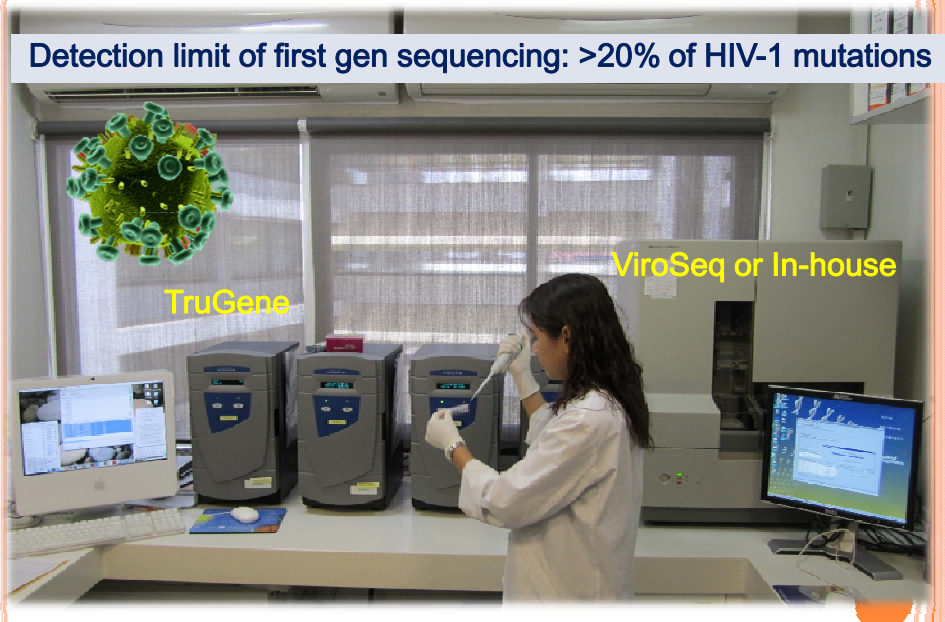
RamaScore

Protease Inhibitors	
Amprenavir (APV)/Fosamprenavir (FPV)	Resistance
APV/r or FPV/r **	Intermidiate Resistance
Atazanavir (ATV)	Susceptible
Atazanavir + Ritonavir (ATV/r)	Susceptible
Darunavir + Ritonavir (DRV/r)	Susceptible
Indinavir (IDV)	Susceptible
IDV/r	Susceptible
Lopinavir + Ritonavir (LPV/r)	Susceptible

VircoNet

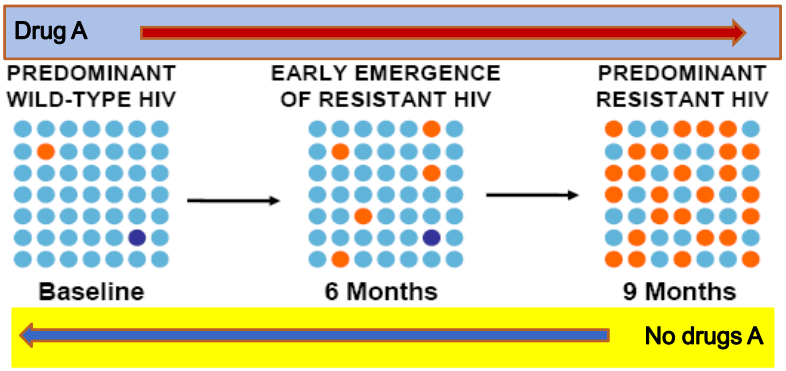
DRUGS		0.3 1 10 100 200 (>200)	FC	(95% confidence limits)	CCO 1	CCO 2	BCO
Indinavir	IDV		46.5	(39.0-55.5)	1.0	5.4	
Indinavir/r	IDV/r		46.5	(39.0-55.5)	2.3	27.2	
Nelfinavir	NFV		16.1	(14.3-18.3)	1.2	9.4	
Saquinavir/r	SQV/r		0.4	(0.3-0.6)	3.1	22.6	
Fosamprenavir/r	FPV/r		75.3	(65.0-87.4)	1.5	19.5	
Lopinavir/r	LPV/r		26.9	(19.5-37.1)	6.1	51.2	
Atazanavir/r	ATV/r		1.3	(1.0-1.6)	2.5	32.5	
Tipranavir/r	TPV/r		2.7	(2.4-3.2)	1.5	7.0	
Darunavir/r	DRV/r		8.5	(6.4-11.3)	10.0	106.9	

Detection limit of first gen sequencing: >20% of HIV-1 mutations



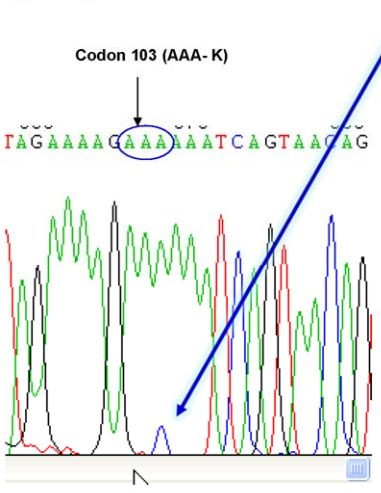
Disappearance of resistance

- When treatment interrupted for example, non-adherence
- Drug-resistant strains cannot detected by genotyping assays
- Still minority population that can re-emerge if selection pressure re-applied



Methods to Detect Low-level Resistant Viral Variants

Standard sequencing reads (AAA – K) for HIV RT codon 103, however, in an enlarged view of the trace file one can see a minor C peak suggesting a low-level variant a NNRTI resistance mutation (AAC – N).



Minor peak cannot be differentiated from noise

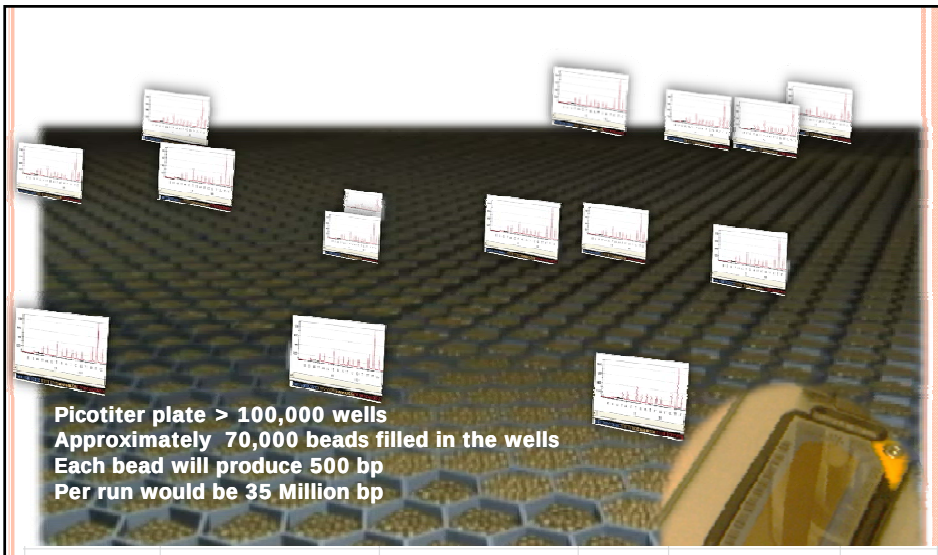
NEXT GENERATION SEQUENCING ?

- 1st generation
  - Maxim-Gilbert Sequencing Dideoxy nucleotide (dye base) sequencing
  - 4 lanes 1 dye or 4 dyes 1 lane
- 2nd generation
  - Sequencing by Ligation

50-100 bases

Massively Parallel Sequencing

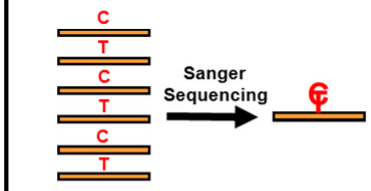
Billions bases



Price(baht)/run	Number of Bead	Number of Amplicons	X depth	Number of samples	Price
50,000	70,000	8	1,500	6	8,571
50,000	70,000	6	1,000	12	4,286
50,000	70,000	6	500	23	2,143

COMPARING TO THE BASE CALLING

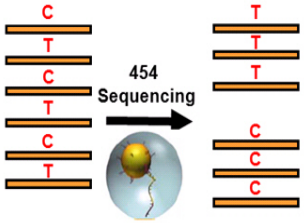
First Generation (Sanger Sequencing)



Mixture sequenced = sequenced mixture

Base calling error!

Second Generation (454)



Sequence cloned = 1 DNA = 1 bead = 1 read

No Base calling

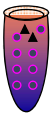
SANGER VS ULTRA DEEP TECHNOLOGY IN HIV

HIV virus

RNA Extraction

RT-PCR

Amplicons



A: >20 %

B: < 20 %

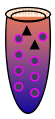
Sanger

454, Roche

Sequencing

emPCR & Sequencing

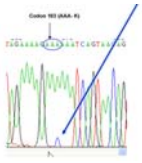
TruGene



✓ Detectable

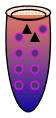
A: >20 %

B: < 20 %



× Undetectable

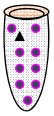
✓

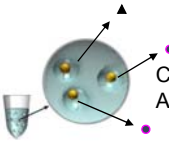


A: >20 %

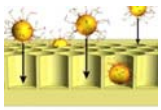
B: < 20 %

✓

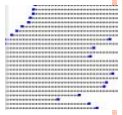




Clonal Amplification



Sequencing, individually



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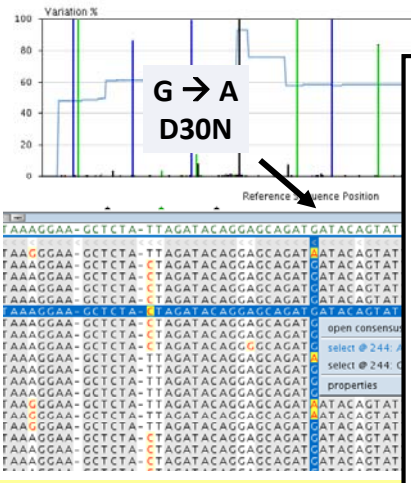
A Major Protease Inhibitor Mutation at 3.5% Prevalence



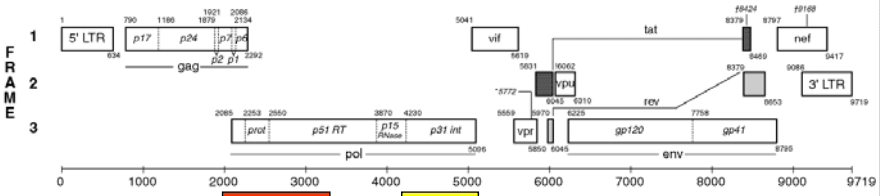
D30N causes intermediate-level resistance to the PI nelfinavir

Table 3. Participants with a specific drug-resistant HIV mutation detected, according to method(s) of detection.

	Sequencing method		
Stanford HDRM	Standard only	Both	Ultra-deep only
NNRTI			
98G	0	0	1
101E	0	1	2
103N	1	3	1
103R	0	4	3
108I	0	4	7
179D	0	4	4
181C	0	0	2
190A	0	1	1
190E	0	0	1
225H	0	0	1
227Y	0	0	1
PI			
30N	0	2	2
33F	0	1	0
46I	0	1	2
46L	0	2	0
73S	0	0	1
84V	0	1	0

HIV-1 TROPISM

DETECTING HIV DRUG RESISTANCE MUTATIONS



PR RT — **PIs and RTIs**

IN — **Integrase inhibitors**

V3 — **Coreceptor Antagonists CCR5**

gp 41 — **Fusion inhibitors**

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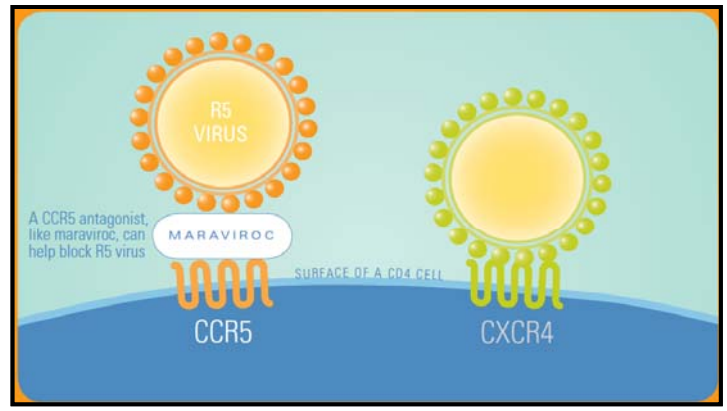


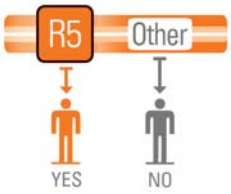
trofile

Your doctor needs to know your HIV tropism before prescribing co-receptor antagonists

Know your tropism before starting a new drug regimen

HIV-1 Tropism Screening

Am I a candidate for maraviroc?



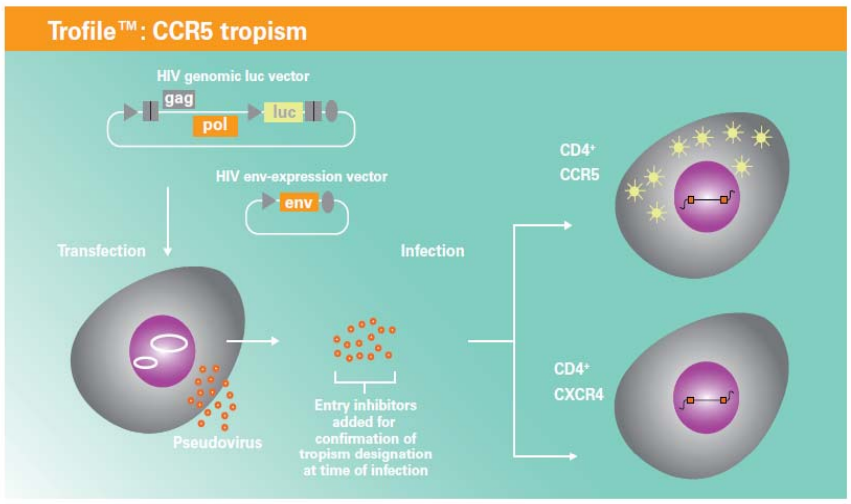


Figure 2. Schematic diagram of the assay. Replication-defective virus particles are produced by cotransfecting HEK-293 cells with the env-expression vector derived from patient's sample and an HIV-1-genomic vector in which the *env* region is deleted and replaced with a luciferase cassette. The recombinant virus particles are harvested and used to infect target cells that express CD4 and either the CCR5 or CXCR4 co-receptor. The R5 virus will be able to infect the cells expressing CCR5 but not the CXCR4-expressing cells. Conversely, an X4 virus will infect the CXCR4-expressing cells but not CCR5-expressing cells. Tropism designation can be confirmed by the addition of specific co-receptor antagonists at attempted infection.[13]

Large-Scale Application of "Deep" Sequencing Using 454 Technology to HIV Tropism Screening (http://www.natap.org/2010/CROI/croi_106.htm)

Reported by Jules Levin
17th CROI 2010 Feb 16-19 SF

Luke C Swenson1, Winnie Dong1, Theresa Mo1, Alexander Thielen2, Mark Jensen3, Doug Chapman4, Ian James5, Jayvant Heera4, Hernan Valdez4, P Richard Harrigan1 1BC Centre for Excellence in HIV/AIDS, Vancouver, Canada; 2Max-Planck Institute for Informatics, Saarbrucken, Germany; 3Fortinbras Research, Buford, GA; 4Pfizer, Inc. New York, NY; 5Pfizer Research and Development, Sandwich, UK

CONCLUSION

"Deep" sequence analysis of the HIV V3-loop gives improved prediction of virological response to MVC relative to the original Trofile assay, and similar to the currently-available Trofile assay (ESTA).

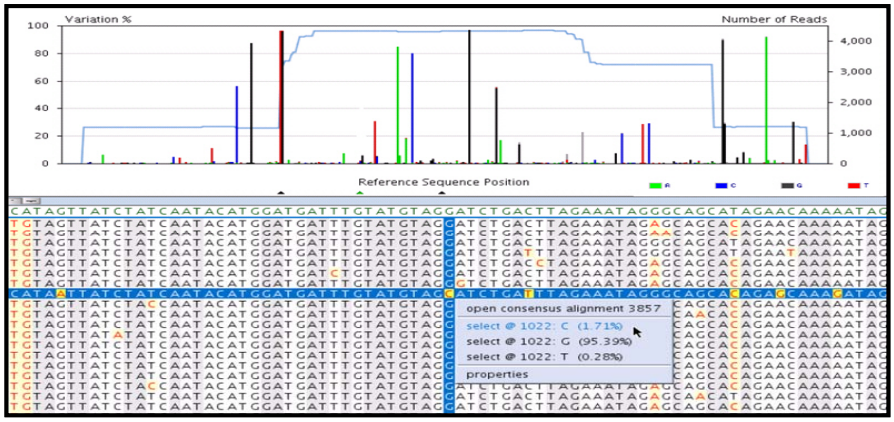
BACKGROUND

HIV tropism testing is required prior to treatment with CCR5-antagonists such as maraviroc (MVC). Currently, the recombinant phenotypic Trofile assay (Monogram Biosciences) is the most commonly used method to test for HIV tropism/coreceptor-usage.

Genotypic testing using either population-based sequencing or novel "deep" 454 sequencing of the V3 loop of HIV env may be viable alternatives, with some practical advantages over Trofile.

"Deep" sequencing can quantify minority HIV variants within an individual, including non-R5 variants.

We retrospectively tested response to a MVC-containing regimen across 4 clinical trials, with HIV tropism determined by deep sequencing. Results were compared to those obtained using the original Trofile assay, population-based V3 sequencing and, where available, the Enhanced Sensitivity Trofile Assay (ESTA).



~500 bp

Co-receptor prediction service

Geno2pheno http://coreceptor.bioinf.mpisb.mpg.de/cgi-bin/coreceptor.pl
PSSM algorithm http://ubik.microbiol.washington.edu/computing/pssm/

48

R5 or R4 Virus ?????

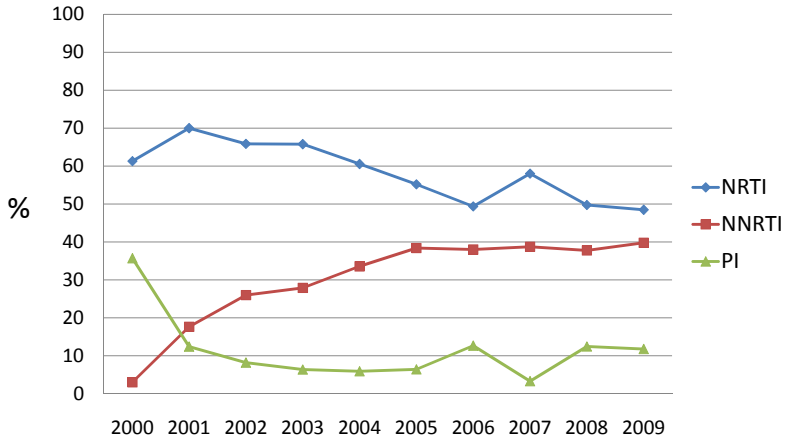
NUMBER OF SAMPLES FOR DRUG RESISTANCE MONITORING

Years	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
RT	83	88	387	406	412	504	639	2,216	2152	3411
PR	83	88	314	298	269	285	470	2,216	2152	3278
Total	83	88	387	406	412	504	639	2,216	2152	3415 *

*4 (order PR only)

Total 10,302 tests

NRTIS, NNRTIS AND PIS RESISTANCE



Use total resist each year as reference

QUALITATIVE AND QUANTITATIVE HIV-1 DRUG RESISTANCE INTERPRETATION SYSTEM

Virology and molecular microbiology Unit
Department of Pathology
Ramathibodi Hospital