



Didactic Series

Archive Genotype Resistance Testing in the Setting of Regimen Switching

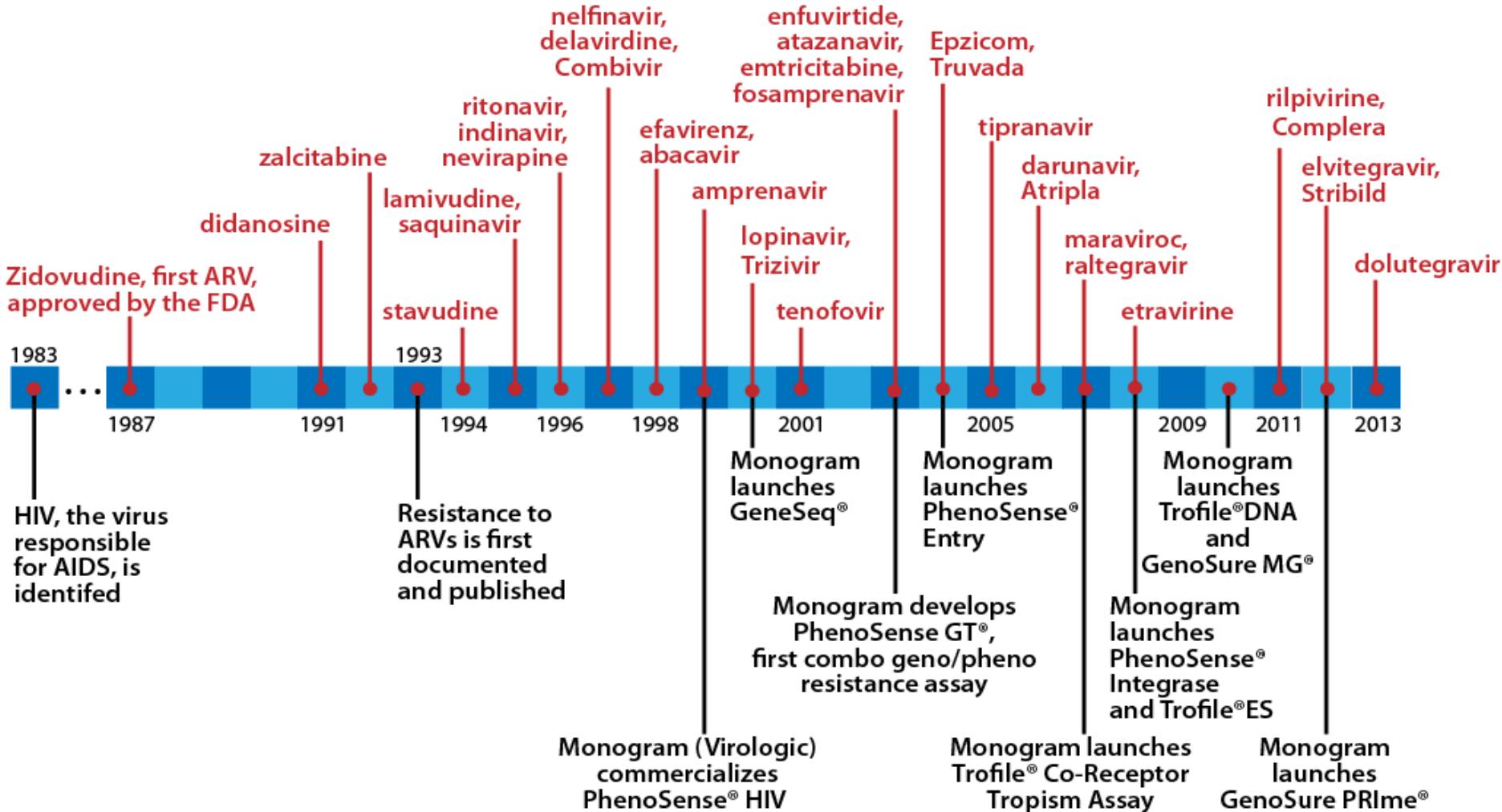
Craig Ballard, Pharm.D., AAHIVP
UCSD Medical Center Owen Clinic
June 11, 2015

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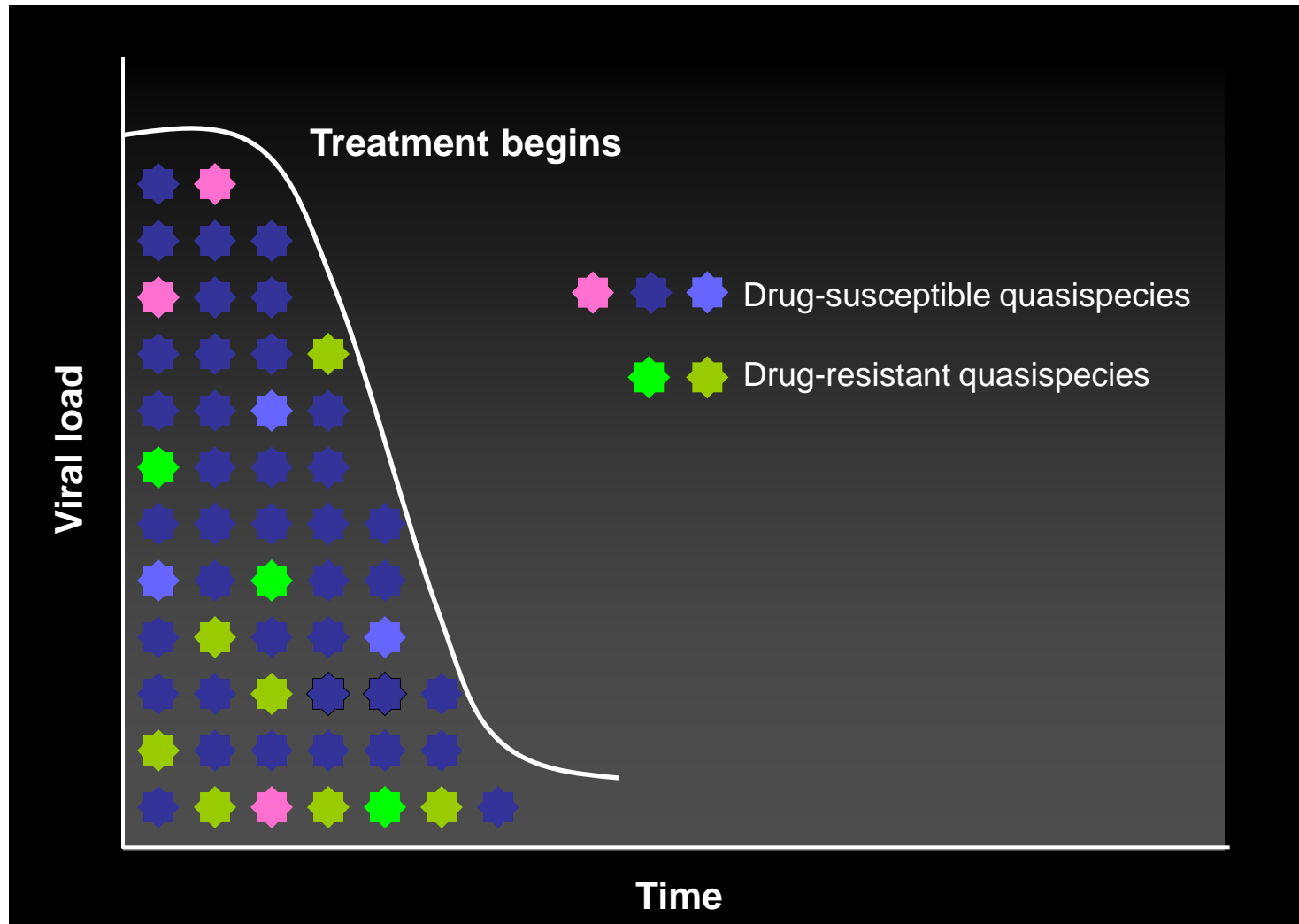
Learning Objectives

- 1) Describe differences between HIV-1 genotype archive (GenoSure Archive) resistance testing versus standard genotype resistance testing
- 2) Identify patients that may benefit from HIV-1 genotype archive resistance testing when switching antiretroviral therapy
- 3) Identify when HIV-1 genotype archive resistance testing is not appropriate

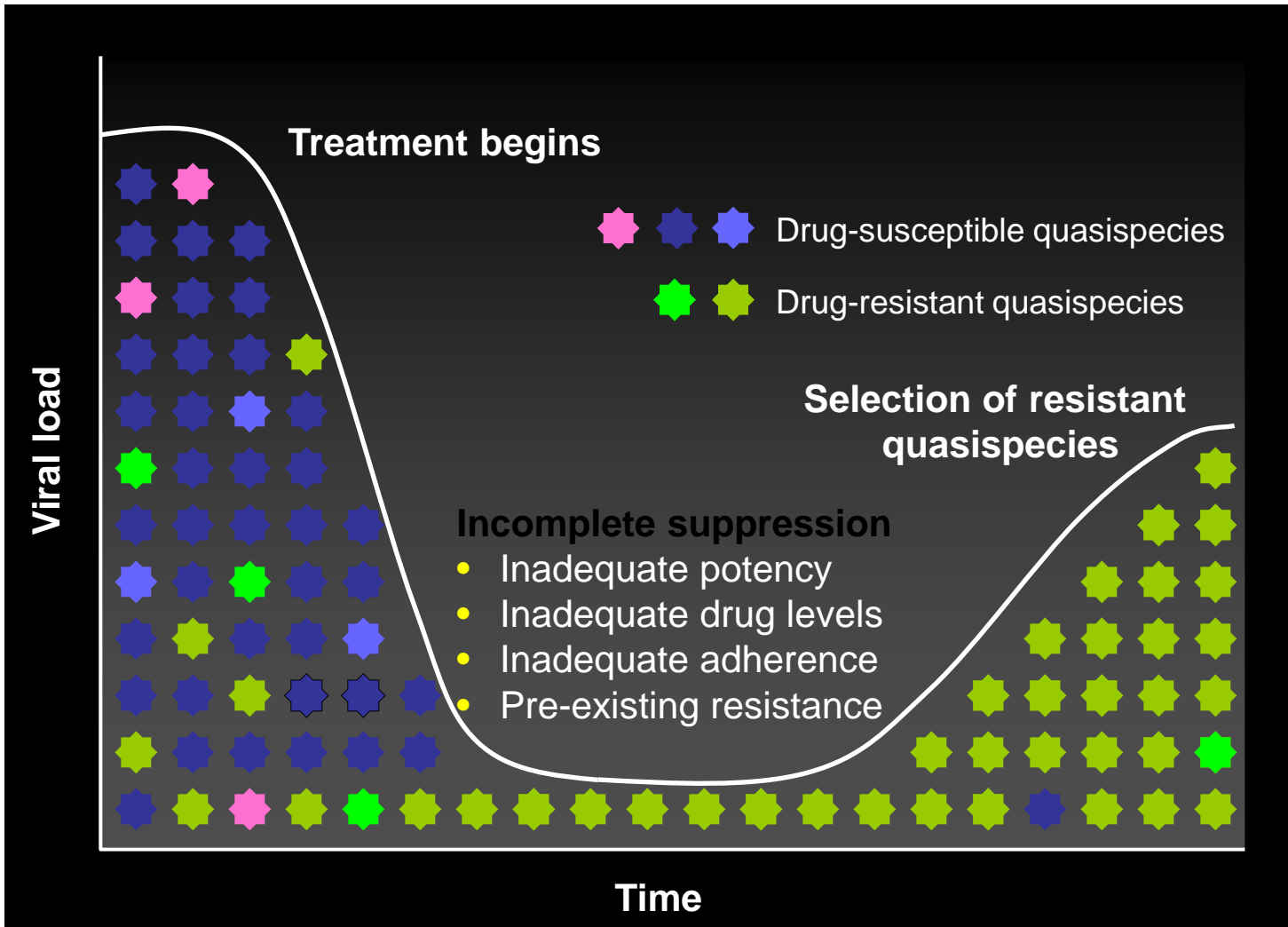
HIV Timeline



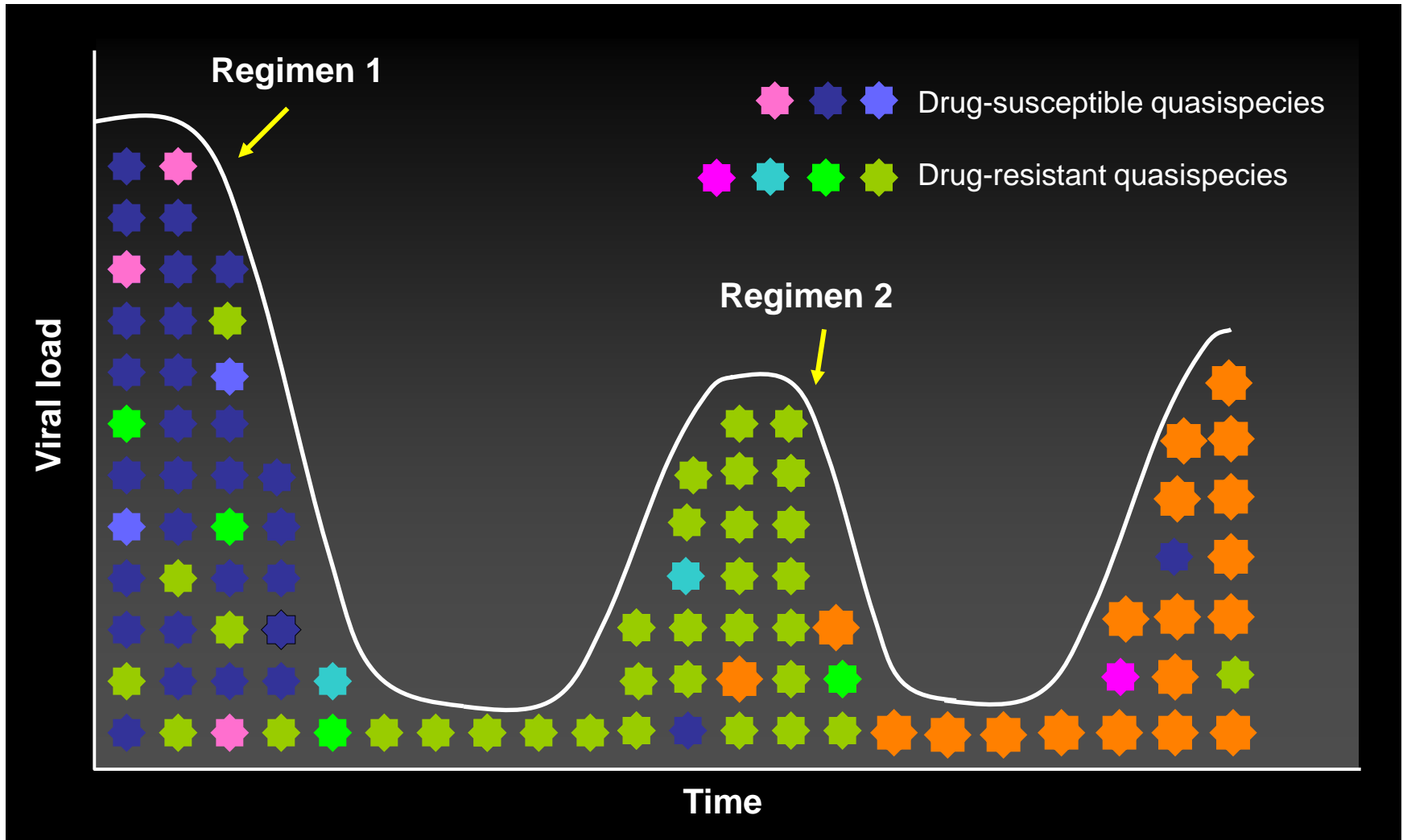
Selective Pressure of Therapy



Selective Pressure of Therapy



Successive Therapies



Patient Case (GJ)

- 57 yo African American male
- HIV-HCV coinfectd
- DM2 diagnosed 2011 last A1c 6.1
 - metformin 500 mg bid
- HTN
 - Losartan 100 mg bid
- Dyslipidemia
 - Atorvastatin 20 mg daily

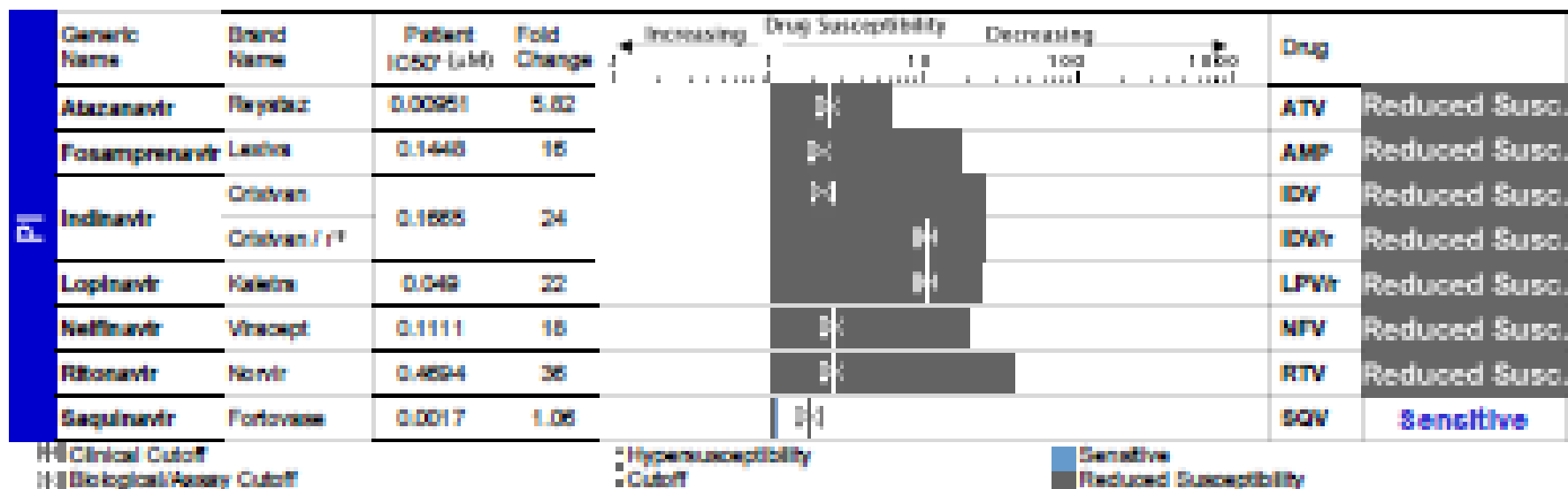
HIV History Case (GJ)

- Most recent VL and CD4 (9/14)
 - HIV RNA < 20 cpm
 - CD4 509 cells/ μ L (33%)
- ARV History
 - Zidovudine, + 2 others 1990s
 - Stavudine, lamivudine, Indinavir, ritonavir 12/2000– 8/2004
Changed d/t resistance/viral failure
 - Tenofovir+lamivudine/zidovudine (FDC) + SQV 1000 mg bid/ritonavir 100mg bid 8/2004 to 1/2009
Changed to eliminate zidovudine from regimen to treat Hep C with peg-inf/rbv (failed)
 - Tenofovir/FTC (FDC)+ RAL + SQV/r BID 1/2009 to 4/2013
Changed to qualify for SOF/DCV Hep C Clinical Trial
 - Tenofovir/FTC (FDC) + RAL 400mg + DRV 600mg/RTV 100mg BID

HCV History (GJ)

- Treatment history: Failed Interferon/ribavirin (Null Responder)
- Failed Sofosbuvir / Daclatasvir (NS5B/NS5A) Clinical Trial 12 wk ETR (Relapse post tx week 4)
- Genotype 1A
- HCV RNA (9/2014)
 - 12,000,000 IU/mL (7.08 log₁₀ IU/mL)
- Well compensated Cirrhosis
- Childs A

GJ HIV-1 Phenotype July 2004 on D4T 40mg / 3TC 150mg / IDV 800mg / RTV 200mg bid



Replication capacity cannot be reported on this sample because results did not meet assay acceptance criteria.

GJ HIV-1 Phenotype July 2004 on D4T 40mg / 3TC 150mg / IDV 800mg / RTV 200mg bid

DRUG		PHENOSENSE™				ASSESSMENT		
Generic Name	Brand Name	Patient IC50* (µM)	Fold Change	Increasing Drug Susceptibility	Decreasing	Drug		
NRTI	Abacavir	Ziagen	4.33	2.80			ABC	Sensitive
	Didanosine	Videx	6.07	1.24			ddI	Sensitive
	Emtricitabine	Emtriva®	>100	>MAX			FTC	Reduced Susc.
	Lamivudine	EpiVir	>300	>MAX			3TC	Reduced Susc.
	Stavudine	Zerit	0.42	0.88			d4T	Sensitive
	Tenofovir	Viread®	0.281	0.44			TFV	Sensitive
	Zidovudine	Retrovir	0.011	0.31			ZDV	Sensitive
	NNRTI	Delamanid	Receptor	0.0039	1.89			DLV
Efavirenz		Sustiva	0.0019	0.89			EFV	Sensitive
Nevirapine		Viramune	0.064	0.70			NVP	Sensitive

GJ HIV/Hep C gt 1A

- TDF/FTC (FDC) + RAL 400mg bid + DRV 600 mg / RTV 100 mg bid
- Antiretroviral therapy regimen changed to
- TDF/FTC/RPV (FDC) + DTG 50 mg bid
- SMV/SOF/RBV1000 started 10/10/2014 x 12-24 weeks

GJ HIV/Hep C gt 1A

HIV (TDF-FTC-RPV+DTG BID) HCV (SMV+SOF+RBV)

- HIV Viral load

Oct 2014	<20
Nov 2014	59
Dec 2014	95

- HCV Viral load

3,457,502
Not Detected
Not Detected

HIV viral load is increasing. What should we do?

Poll 1: GJ's HIV viral load is increasing after change to TDF-FTC-RPV plus DTG 50 mg bid, and Hep C viral load is not detected. What would you do next?

1. Order genotype resistance testing (PR-RT and INT)
2. Order phenotype plus genotype including INT
3. Order DNA Sequencing (archive genotype)
4. Order DNA Sequencing (archive genotype) & Trofile DNA (Tropism)
5. I would not order anything for this patient's HIV because I want to go to lunch
6. Other?

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Client: 00269 Project: 00073
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Date Collected 07-NOV-2014 16:14	Date Received 11-NOV-2014 16:54 PT	Date Reported 17-NOV-2014 20:42 PT	Mode R,W	Report Status FINAL
Referring Physician Edward Cachay			Reference Lab ID/Order #	
Comments			HIV-1 Subtype: B	

	Generic Name	Brand Name	Assessment	Drug Resistance Associated Mutations Detected	Comments
NRTI	Abacavir	Ziagen	Resistance Possible	V118V/I, M184M/V	
	Didanosine	Videx	Resistance Possible	M184M/V	
	Emtricitabine	Emtriva	Resistant	V118V/I, M184M/V	
	Lamivudine	Epivir	Resistant	V118V/I, M184M/V	
	Stavudine	Zerit	Sensitive	V118V/I	1
	Tenofovir	Viread	Sensitive	None	1
	Zidovudine	Retrovir	Sensitive	V118V/I, T369A/V	1
NNRTI	Efavirenz	Sustiva	Sensitive	V179D, T369A/V	
	Etravirine	Intelence	Sensitive	V179D, T369A/V	
	Nevirapine	Viramune	Sensitive	V179D, T369A/V	
	Rilpivirine	Edurant	Resistant	V179D, M230M/I	
INI	Dolutegravir	Tivicay	Sensitive	None	
	Elvitegravir	Elvitegravir	Sensitive	None	
	Raltegravir	Isentress	Sensitive	None	
PI	Atazanavir	Reyataz	Sensitive	L10I, M46M/L, I62I/V, V82V/F	
		Reyataz / r†	Sensitive	L10I, M46M/L, I62I/V, V82V/F	
	Darunavir	Prezista / r†	Sensitive	L10I, M46M/L, V82V/F	
	Fosamprenavir	Lexiva / r†	Resistant	L10I, M46M/L, V82V/F	
	Indinavir	Crixivan / r†	Sensitive	L10I, M46M/L, V82V/F	
	Lopinavir	Kaletra†	Resistance Possible	L10I, M46M/L, V82V/F	
	Nelfinavir	Viracept	Resistant	L10I, M46M/L, V82V/F	
	Ritonavir	Norvir	Resistant	L10I, M46M/L, V82V/F	
	Saquinavir	Invirase / r†	Sensitive	L10I, M46M/L, I62I/V	
	Tipranavir	Aptivus / r†	Sensitive	M46M/L, L63T	

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Date Collected 07-NOV-2014 16:14	Date Received 11-NOV-2014 16:54 PT	Date Reported 02-DEC-2014 13:36 PT	Mode R,W	Report Status FINAL
Referring Physician Edward Cachay			Reference Lab ID/Order #	
Comments:			HIV-1 Envelope Subtype: B	

Troptotype Result



Virus uses CCR5 co-receptors to enter the CD4+ cell.

R5

ABOUT TROPISM

TROFILE[®]DNA ---A NEW TROPISM ASSAY FROM MONOGRAM BIOSCIENCES

Trofile DNA meets the US standards for technical validation as established by the Clinical Laboratory Improvement Amendments. Trofile DNA is a single cycle pseudovirus based tropism assay that uses the complete gp160 coding region of HIV-1 to evaluate tropism. Instead of using HIV-1 RNA isolated from patient plasma, Trofile DNA uses cell associated viral DNA taken from whole blood cells infected with HIV. HIV-1 envelopes encoded by the viral DNA are tested in a cell-based viral infectivity assay in order to determine which co-receptor the HIV-1 population is capable of using: CCR5, CXCR4, or both, known as D/M(dual/mixed).

TROFILE DNA VIRAL CLASSIFICATION

Co-receptor tropism is defined as an interaction of a virus with a specific co-receptor on the target cell. To gain entry into CD4+ cells, HIV must bind to the cell surface CD4 receptor and to one of two co-receptors, CCR5 and CXCR4. Trofile DNA uses the complete gp160 coding region of the HIV-1 envelope protein ensuring that all of the determinants of tropism tested.

CCR5 Tropic (R5) HIV-1

Virus uses CCR5 to enter into CD4+ cells.

CXCR4 Tropic (X4) HIV-1

Virus uses CXCR4 to enter into CD4+ cells.

DUAL/MIXED Tropic (D/M) HIV-1

Dual-tropic viruses can use either CCR5 or CXCR4 to enter into CD4+ cells. Mixed-tropic populations contain viruses with 2 or more tropisms.

GJ HIV/Hep C gt 1A

HIV (TDF-FTC-RPV+DTG BID) HCV (SMV+SOF+RBV)

- HIV Viral load

Oct 2014 <20

Nov 2014 59

Dec 2014 95

- cART changed to TDF-FTC-RPV+DTG BID+ MVC 300mg BID

Jan 2015 25

Feb 2015 <20

Apr 2015 <20

May 2015 <20

- HCV Viral load

3,457,502

Not Detected

Not Detected

Not Detected

Not Detected

Not Detected

SVR4

New Resistance Assay Introduced October 2014



The first commercial assay designed to provide HIV-1 antiretroviral drug resistance data in virologically suppressed patients.

Suppression Management

- Definition: The ability to make adjustments to antiretroviral drug regimens in patients whose HIV-1 virus is fully suppressed
- **“The cardinal principle of regimen change is to maintain viral suppression without jeopardizing future treatment options.”**
- Often, changes are made without full knowledge of a patient’s treatment history or antiretroviral resistance history

“ Regimen switching in the setting of virologic suppression” DHHS Guidelines, 01 May 2014

Principles and Strategies of Regimen Switching

If there is uncertainty about prior resistance, it is generally not advisable to switch a suppressive ARV regimen unless the new regimen is likely to be as active against resistant virus as the suppressive regimen.

“ Regimen switching in the setting of virologic suppression” DHHS Guidelines, 01
May 2014

Principles and Strategies of Regimen Switching

Recommendations for Changing the ART Regimen in Treatment-Experienced Patients

Design of a new regimen should consider previous antiretroviral therapy exposure, previous resistance profile, drug interactions, and history of intolerance or toxic effects (Alla).

JAMA. 2014;312(4):410-425. doi:10.1001/jama.2014.8722

Principles and Strategies of Regimen Switching

Recommendations for Changing the ART Regimen in Treatment-Experienced Patients

Switching or regimen simplification in virologically suppressed individuals is generally safe if prior treatment and resistance profile are considered and full activity of the nucleoside reverse transcriptase inhibitors can be ensured for switches from a ritonavir-boosted protease inhibitor to drugs with low barriers to resistance (nonnucleoside reverse transcriptase inhibitors, unboosted protease inhibitors, or integrase strand transfer inhibitors) (Ala).

JAMA. 2014;312(4):410-425. doi:10.1001/jama.2014.8722

THE VIRAL ARCHIVE

Archived vs Plasma Virus

“The PBMC-derived HIV-1 DNA and circulating HIV-1 RNA represent two different viral compartments in the same individual.”*

HIV-1 DNA (“Archived”)

- Virus transmitted and archived at the time of acute infection
- Mutations acquired during the course of the patient’s ARV treatment

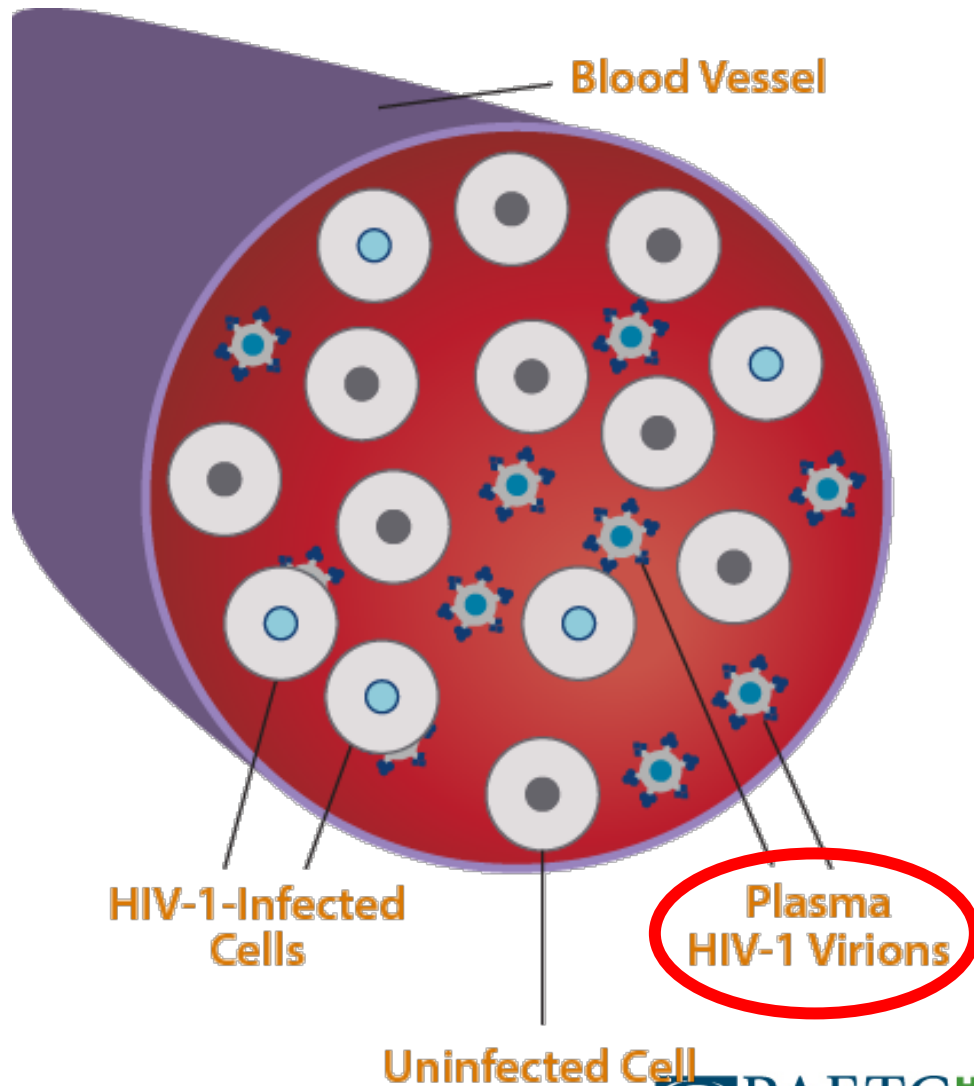
HIV-1 RNA (“Plasma”)

- The actively replicating virus from productively infected cells.
- The most current form of the virus.

* VanDamme AM, et al. *AIDS Rev.* 2011; 13:77-108.

The Viral Archive:

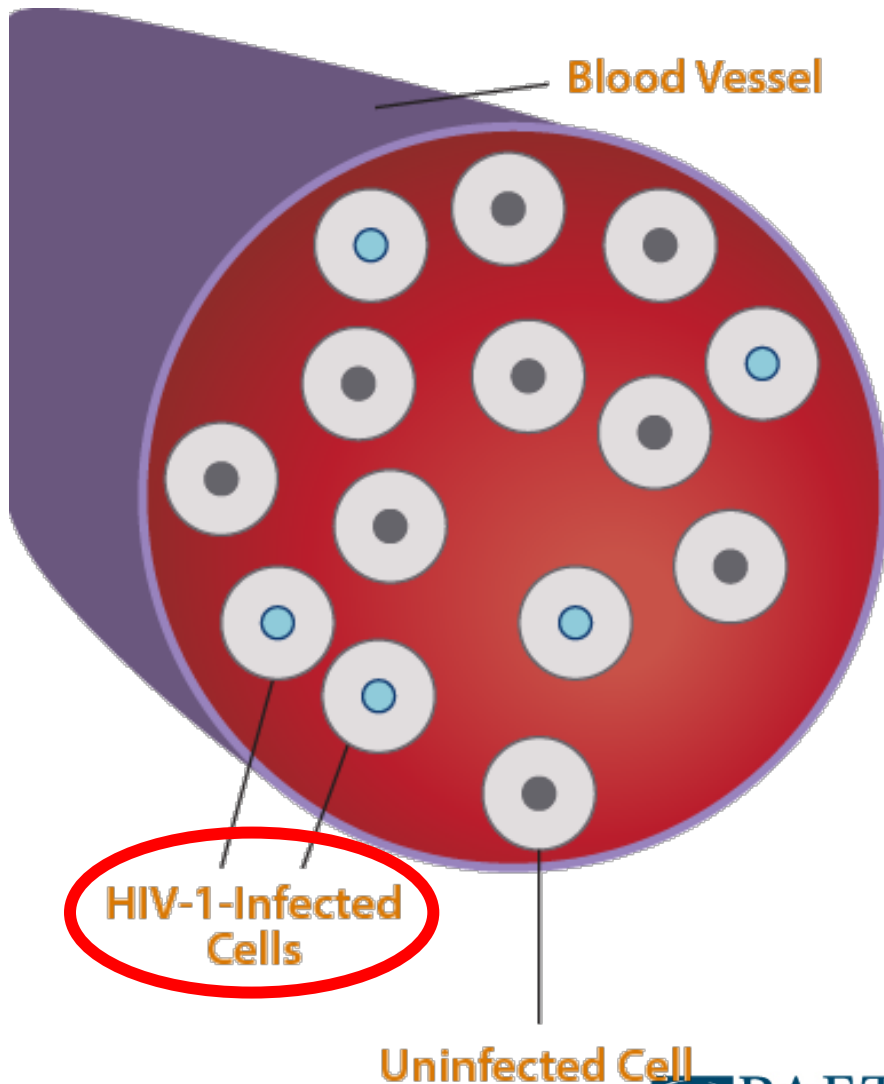
A Second Source of Viral Information



Viral loads and standard resistance assays analyze viral RNA in plasma.

The Viral Archive:

A Second Source of Viral Information



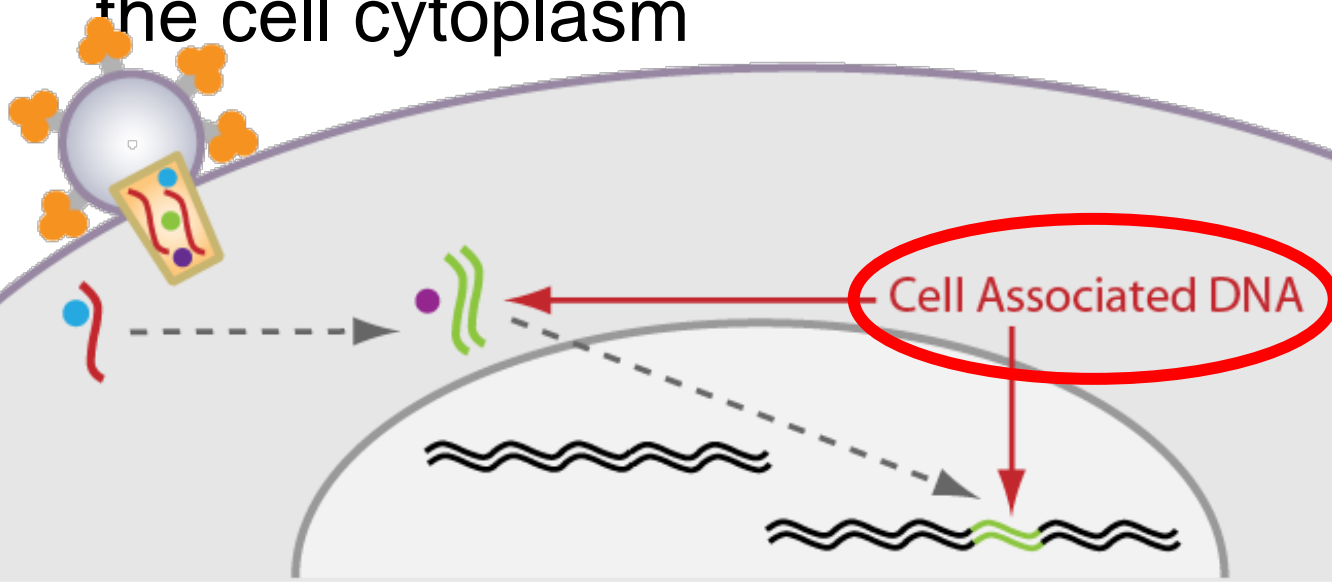
GenoSure ArchiveSM analyzes archived HIV-1 proviral DNA embedded in host cells during virus replication. This may be referred to as “cell-associated” DNA

Uninfected Cell

Cell-Associated DNA

Archived Viral DNA may be from:

1. **Integrated DNA** – Double-stranded viral DNA integrated into the host cell genome; proviral DNA
2. **Cytoplasmic DNA** – Unintegrated viral DNA in the cell cytoplasm



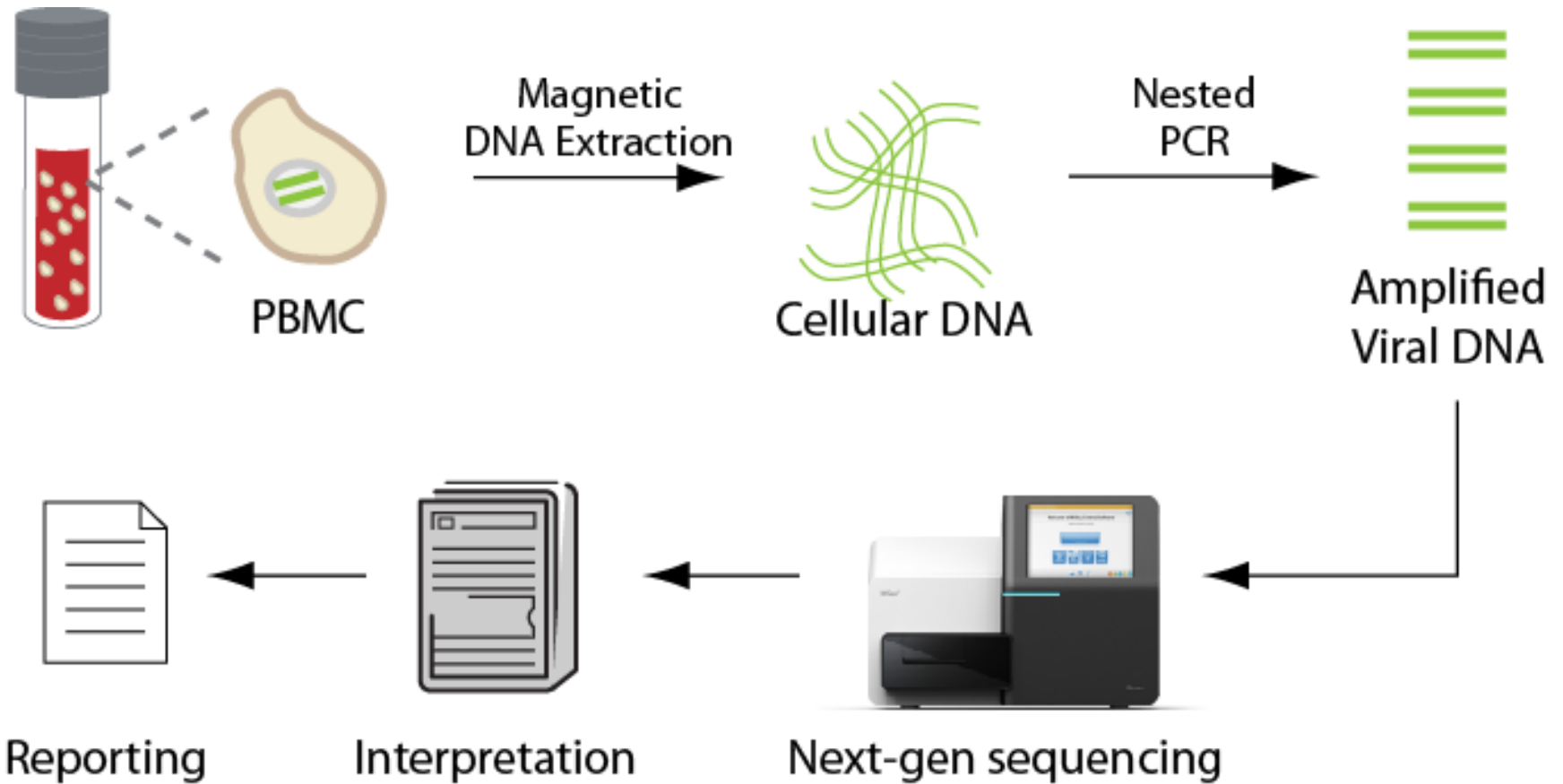
Proviral DNA Assays

- Any replicating resistant virus variant in a patient will be archived and may later appear under appropriate selective pressure
- DNA is more stable than RNA, can be easily obtained, does not require reverse transcription and can be sequenced efficiently
- The development of resistance mutations appear earlier in the plasma RNA, but persist longer in PBMC DNA

Vandamme AM, et al. European Recommendations for the Clinical Use of HIV Drug Resistance Testing: 2011 Update. AIDS Rev. 2011;13:77-108

HOW GENOSURE ARCHIVESM WORKS

GenoSure ArchiveSM Process



Sample Requirements

Important points:

- GenoSure ArchiveSM uses whole blood, not plasma, because the viral DNA is found in the cells.
- All Monogram assays must be FULLY frozen (3-4 hours) at -20°C before shipping on dry ice



RC 59 yo male

- Epzicom, tenofovir, atazanavir, ritonavir since 2007 (Recent HIV VL 683 copies)
- Stavudine, didanosine, nevirapine 1998-2001
- Trizivir, nelfinavir since 2001-2005
- Trizivir, tenofovir, atazanvir, ritonavir 2005-2007

Provider orders archive genotype to simplify regimen for patient

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Date Collected 24-MAR-2015 00:01	Date Received 26-MAR-2015 16:12 PT	Date Reported 03-APR-2015 10:03 PT	Mode R,W	Report Status FINAL
Referring Physician Charles Hicks, Attn: Dr. Craig Ballard 4168 Front Street, Third Floor, San Diego CA 92103 USA			Reference Lab ID/Order #	
Comments			HIV-1 Subtype: B	

	Generic Name	Brand Name	Assessment	Drug Resistance Associated Mutations Detected	Comments
NRTI	Abacavir	Ziagen	Sensitive	T69T/N	
	Didanosine	Videx	Sensitive	None	
	Emtricitabine	Emtriva	Sensitive	None	
	Lamivudine	Epivir	Sensitive	None	
	Stavudine	Zerit	Sensitive	T69T/N	
	Tenofovir	Viread	Sensitive	None	
	Zidovudine	Retrovir	Sensitive	None	

NNRTI	Efavirenz	Sustiva	Sensitive	None	
	Etravirine	Intelence	Sensitive	None	
	Nevirapine	Viramune	Sensitive	None	
	Rilpivirine	Edurant	Sensitive	None	

INI	Dolutegravir	Tivicay	Sensitive	None	
	Elvitegravir	Elvitegravir	Sensitive	None	
	Raltegravir	Isentress	Sensitive	None	

PI	Atazanavir	Reyataz	Sensitive	E35E/D/I/K/N, M36M/I	
		Reyataz / r†	Sensitive	E35E/D/I/K/N, M36M/I	
	Darunavir	Prezista / r†	Sensitive	E35E/D/I/K/N	
	Fosamprenavir	Lexiva / r†	Sensitive	E35E/D/I/K/N	
	Indinavir	Crixivan / r†	Sensitive	E35E/D/I/K/N, M36M/I	
	Lopinavir	Kaletra†	Sensitive	E35E/D/I/K/N	
	Nelfinavir	Viracept	Sensitive	E35E/D/I/K/N, M36M/I	
	Ritonavir	Norvir	Sensitive	E35E/D/I/K/N	
	Saquinavir	Invirase / r†	Sensitive	E35E/D/I/K/N	
	Tipranavir	Aptivus / r†	Sensitive	E35E/D/I/K/N, M36M/I	

Poll 2: Since RC's Archive genotype resistance test shows no mutations I would change the antiretroviral regimen to the following:

1. Stribild (tdf-ftc-evg-cobi)
2. Triumeq (abc-3tc-dtg) [if HLA B5701 negative]
3. Complera (tdf-ftc-rpv)
4. Any single tablet regimen will work
5. I need more antiretroviral treatment history
6. Other

Formerly ViroLogic, Inc.
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Date Collected 06/05/2007 11:31	Date Received 06/08/2007 10:24	Date Reported 06/21/2007 11:27	Mode W,X	Report Status FINAL
Referring Physician William Christopher Mathews, 200 W. Arbor Drive Owen Clinic, San Diego CA 92103 USA			Reference Lab ID	
Comments			HIV-1 Subtype: B	

DRUG	PHENOSENSE™ SUSCEPTIBILITY			Evidence of Susceptibility		Net Assessment	
	Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Pheno Sense	Gene Seq	
NRTI	Abacavir	Ziagen	(4.5 - 6.5)	4.33	Y	N	Sensitive 16
	Didanosine	Videx	(1.3 - 2.2)	1.55	P	Y	Partially Sensitive 19
	Emtricitabine	Emtriva	(3.5)	>MAX	N	N	Resistant
	Lamivudine	Epivir	(3.5)	>MAX	N	N	Resistant
	Stavudine	Zerit	(1.7)	1.80	N	Y	Resistant 3,19
	Zidovudine	Retrovir	(1.9)	5.91	N	N	Resistant 3
	Tenofovir	Viread	(1.4 - 4)	1.08	Y	Y	Sensitive 3
NRTI Mutations		D67N, K70R, M184V, K219E					

NNRTI	Delavirdine	Rescriptor	(6.2)	65	N	N	Resistant
	Efavirenz	Sustiva	(3)	34	N	N	Resistant
	Nevirapine	Viramune	(4.5)	67	N	N	Resistant
NNRTI Mutations		K103N					

PI	Atazanavir	Reyataz	(2.2)	0.82	Y	Y	Sensitive
		Reyataz / r†	(5.2)	0.82	Y	Y	Sensitive
	Darunavir	Prezista / r ‡	(10 - 90)	0.60	Y	Y	Sensitive
	Fosamprenavir	Lexiva	(2)	0.48	Y	Y	Sensitive
		Lexiva / r†	(4 - 11)	0.48	Y	Y	Sensitive
	Indinavir	Crixivan	(2.1)	0.72	Y	Y	Sensitive
		Crixivan / r†	(10)	0.72	Y	Y	Sensitive
	Lopinavir	Kaletra	(9 - 55)	0.56	Y	Y	Sensitive
	Nelfinavir	Viracept	(3.6)	1.19	Y	Y	Sensitive
	Ritonavir	Norvir	(2.5)	1.00	Y	Y	Sensitive
	Saquinavir	Invirase	(1.7)	0.79	Y	Y	Sensitive
		Invirase / r†	(2.3 - 12)	0.79	Y	Y	Sensitive
Tipranavir	Aptivus / r†	(2 - 8)	1.17	Y	Y	Sensitive	
PI Mutations		M36M/I					

> Lower Clinical Cutoff (in bold) < > Hypersusceptibility
 < > Upper Clinical Cutoff (in bold) < > Cutoff
 < > Biological Cutoff
 Sensitive Partially Sensitive Resistant
 Y Evidence of Drug Sensitivity P Evidence of Partial Drug Sensitivity
 N Evidence of Drug Resistance

RC 59 yo male

Current Treatment Epzicom, Tenofovir, Atazanavir and Ritonavir

- HIV Viral load

2/21/14 173

7/9/2014 276

10/31/2014 683

03/24/2015 10687 GenoSure Archive Resistance
Test Ordered

4/21/2015 106 Regimen changed to Complera
plus Prezcoibix

Regimen Switching in the Setting of Viral Suppression

- Cardinal principle of regimen switching
 - Maintain viral suppression without jeopardizing future options
- Virologic failure with emergence of new resistance mutations
 - Increases need for more complex, difficult-to-follow, or expensive regimens

Principles for Successful Regimen Switching

- Review ART history
 - Virologic suppression, resistance test results, and past adverse events
 - If resistance data are unavailable, resistance may often be inferred by treatment history. Consider Archive Genotype Resistance assay if patient is on antiretroviral therapy and HIV-1 viral load is suppressed to help identify archived mutations. If HIV-1 viral load is > 500 cpm then the GenoSure Archive resistance test may not yield accurate results
 - Consult with an HIV specialist for patients with a history of resistance ≥ 1 drug classes
- During first 3 months after a regimen switch
 - More intensive monitoring of tolerability, viral suppression, adherence, and laboratory changes is recommended

Monitoring After Switching Regimens

- Evaluate more closely for several months after a treatment switch
 - 1 to 2 weeks post switch: a clinic visit or phone call
 - 4 to 8 weeks post switch: viral load test (rebound viremia)
- Goal of the intensive monitoring
 - Assess medication tolerance
 - Conduct targeted laboratory testing within 3 months after the regimen switch (ie, pre-existing laboratory abnormalities or potential concerns with the new regimen)
- Absent any specific complaints, laboratory abnormalities, or evidence of viral rebound at this 3-month visit, clinical and laboratory monitoring of the patient may resume on a regularly scheduled basis

GenoSure ArchiveSM: Facilitates Regimen Changes

Increasingly common today is the need for “fine tuning” regimens while a patient’s virus remains suppressed. Reasons for this include:



- Regimen simplification
- Adverse events
- Side effects
- Concern for long-term toxicities
- Drug-drug interactions
- Regimen intolerance
- Optimization of ARVs in pregnancy

GenoSure ArchiveSM: Resistance Information At Hand

- GenoSure Archive may also provide valuable and useful information for patients who are missing historical resistance data
- “Historic resistance reports remain important in the clinical management of patients on antiretroviral therapy, though proviral DNA testing may be useful in patients where historic reports are not available¹.”



1.Booth, CL, McCormick A, Garcia-Diaz A, et al. Feasibility of testing and detection of HIV-1 drug resistance in proviral DNA. BMC Infectious Diseases. 2014; 14(Suppl 4):O25.

Summary

- **GenoSure Archive (DNA Sequencing Assay) can be a useful tool to help effectively and safely switch antiretroviral therapy to easier regimens to durably maintain viral suppression.**
 - Patient HIV-1 viral load should be < 500 cpm when sample collected
 - Do not use if HIV-1 viral load is > 500 cpm as inaccurate results can occur
 - Must collect whole blood and frozen. Do not centrifuge sample.
- **Switching antiretroviral therapy to fewer tablets and safer alternatives is attractive to both patients and providers**
 - Details of treatment history remain important
 - Pharmacovigilance will remain important!