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From Principles to Practice – Understanding the Pharmacogenomics of HIV

Despite the increasing, diverse efforts by the international healthcare community, the HIV epidemic continues to pose a major challenge to humanity. According to the World Health Organization, 33.3 million people were living with HIV worldwide in 2009 and 2.6 million people were newly infected with disease that year alone[1]. Since 1995 however, the use of highly active antiretroviral therapy (HAART), a combination of substances directed against various steps in the HIV (human immunodeficiency virus) virus life cycle, has led to significant decreases in the morbidity and mortality associated with HIV infections[2]. These improvements result largely from new assays, genetic advances, and recombinant DNA technologies that have provided new knowledge about HIV virology, pathology, and resistance mechanisms[2]. With the help of genotypic and phenotypic assays that have been developed to asses HIV antiviral resistance, scientists are increasingly more capable of predicting which antiviral substances are more effective in controlling viral replication in a given patient[2]. Being able to predict drug response, absorption, metabolism and elimination is a continually evolving field of research that is empowering the international healthcare community in the global fight against HIV. After introducing the biology of HIV and the field of pharmacogenomics,

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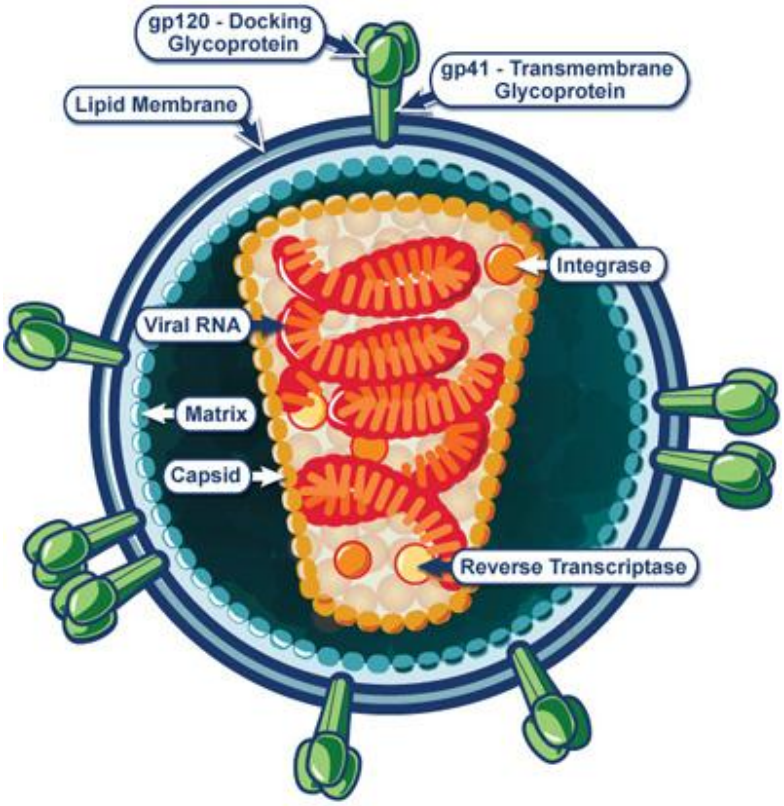
[1] "Universal Access to HIV/AIDS Prevention, Treatment and Care." *HIV/AIDS*. World Health Organization, 2011. Web. 05 Dec. 2011. <http://www.who.int/hiv/topics/universalaccess/en/index.html>.
[2] W. Luke. "Pharmacogenomics of HIV." *Current Opinions on Molecular Therapeutics* June (2004).

this paper will discuss the intersection of these topics and how the genetics driven revolution in personalized medicine is serving HIV patients worldwide.

Understanding the incredibly complex biology of the HIV virus is essential for building effective diagnostics and drugs. HIV belongs to a class of viruses known as retroviruses because it contains ribonucleic acid (RNA) as its genetic material. After the HIV virus infects a cell, it uses an enzyme called reverse transcriptase to convert its RNA into DNA so that it can replicate itself using the host cell's own replication machinery[3]. The figure on the left from the National Institute of Allergy and Infectious Diseases shows the structure of the HIV virus. The HIV replication cycle begins with fusion of the virus to the host cell surface, which begins the influx of viral proteins into the host cell. After viral DNA is formed by reverse transcription and integrated into the host DNA, new viral RNA is used as genomic RNA to make viral proteins, which travel



[3] "Biology of HIV." *HIV/AIDS*. National Institute of Allergy and Infectious Diseases, 28 Apr. 2009. Web. 05 Dec. 2011. <http://www.niaid.nih.gov/topics/hivaids/understanding/biology/Pages/biology.aspx>.

to the cell surface to form a new HIV virus[4]. This vicious cycle rapidly produces several billion new viruses every day in persons infected with HIV and is further complicated by the ability of reverse transcriptase to mutate, causing new strains of HIV to develop in infected individuals. HIV targets the immune system directly by infecting CD4+ lymphocytes, which also leads to the systematic degradation of the immune system because CD4+ cells are pivotal in helping immune responses[5]. The constant process of evolution and replication in the HIV virus creates incredible stress for the immune system and has been one of the reasons why HIV has been especially difficult for medical researchers to combat[6].

Drugs against HIV are called antiretroviral drugs because HIV is considered a retrovirus as previously described and the drugs fall into three classes consistent with the underlying biology of the virus: 1) nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs)[7]. Reverse transcriptase inhibitors directly inhibit the reproductive capacity of the HIV virus because of the mandatory role the reverse transcriptase enzyme plays in viral reproduction. NRTIs contain faulty versions of the nucleotides used by reverse transcriptase to convert RNA to DNA, causing improper build of the new DNA so that HIV's genetic material cannot be incorporated into the healthy genetic

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[4] "Steps in the HIV Replication Cycle." *HIV Replication Cycle*. National Institute of Allergy and Infectious Diseases, 8 July 2008. Web. 5 Dec. 2011. <http://www.niaid.nih.gov/TOPICS/HIVAIDS/UNDERSTANDING/BIOLOGY/Pages/hivReplicationCycle.aspx>

[5] Blum, Ronald A. "HIV Resistance Testing in the USA – a Model for the Application of Pharmacogenomics in the Clinical Setting." *Pharmacogenomics* 06 (2005). *Future Medicine*. Mar. 2005. Web. 06 Dec. 2011. <http://www.futuremedicine.com/doi/full/10.1517/14622416.6.2.169>

[6] "HIV Evolves to Evade the Immune System." *HIV/AIDS*. National Institute of Allergy and Infectious Diseases, 30 Mar. 2009. Web. <http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/Pages/evolves.aspx>

[7] Pirmohamed, M. "The Pharmacogenomics of HIV Therapy." *The Pharmacogenomics Journal* 1 (2001): 243-53. Web. <http://www.nature.com/tpj/journal/v1/n4/full/6500069a.html>

material of the host cell[8]. On the other hand, NNRTIs work by attaching themselves to reverse transcriptase and prevent the enzyme from converting RNA to DNA, similarly disabling the incorporation of viral genetic material in the host cell[9]. Lastly, protease inhibitors function by blocking the protein-cutting enzyme HIV protease[10]. After viral RNA is translated into a polypeptide sequence, the sequence is assembled into a long chain that includes the proteins like reverse transcriptase and protease. Before these enzymes become functional, they must be cut from the longer polypeptide chain by protease, making protease inhibitors effective therapeutics because they interfere with this process[11]. According to *Shafer et al 2000*, human immunodeficiency virus reverse transcriptase and protease enzymes are the molecular targets of 15 licensed antiretroviral drugs[12].

Before further discussion of how HIV treatment based on genomic information is possible, it is important to understand the principles behind drug resistance for HIV. The two important factors that contribute to the development of drug-resistant strains for HIV are the high rates of viral replication and the diversity of the virus population as a result of mutations[5]. Beginning with the viral load, estimates for total HIV-1 (one of two major types of HIV) production are around 10.3 billion virions per day with an average

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[8] "Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)." *HIV - AIDS - Treatment - Drugs*. AIDSmeds, 5 May 2008. Web. 09 Dec. 2011. <http://www.aidsmeds.com/archive/NRTIs_1082.shtml>.

[9] "Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)." *HIV - AIDS - Treatment - Drugs*. AIDSmeds, 05 May 2008. Web. 09 Dec. 2011. <http://www.aidsmeds.com/archive/NNRTIs_1612.shtml>

[10] "Protease Inhibitors (PIs)." *HIV - AIDS - Treatment - Drugs*. AIDSmeds, 05 May 2008. Web. 09 Dec. 2011. <http://www.aidsmeds.com/archive/PIs_1068.shtml>

[11] "HIV Protease." *Biology*. Davidson. Web. 08 Dec. 2011. <http://www.bio.davidson.edu/courses/HIVcellsalive/hiv4.htm>

[12] Shafer, Robert W. "Human Immunodeficiency Virus Reverse Transcriptase and Protease Sequence Database: an Expanded Data Model Integrating Natural Language Text and Sequence Analysis Programs." *Nucleic Acids Research* 29 (2001). Oxford University Press, 1 Jan. 2001. Web. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC29795/?tool=pubmed>.

virus life cycle of 1.2 days each[13]. Furthermore, *Perelson et al 1996* found that it takes approximately 2.6 days from the time a virus particle is released until infection of another cell and release of a new generation of virion, which is an alarmingly short time period[7]. The diversity of the virus population comes from mutations previously discussed. The reverse transcriptase enzyme, for example, converts viral RNA to DNA with approximately five mutations per genome per replication cycle, and each mutation enriches the diversity of the virus population[14]. Because of these two factors, HIV infected patients have a mix of different virus types and a mutation in one type increases that particular form of the HIV virus because the mutated virus is not as susceptible to antiretroviral therapy[5]. At times a particular subspecies of the HIV virus may persist because of a particularly advantageous mutation or less antiretroviral levels at the site of replication[5]. Another reason for antiretroviral drug resistance is incomplete viral suppression from poor medication compliance[15]. Poor compliance can have many causes, including the public health crisis of stock-outs where patient treatment is interrupted by availability issues[16]. Finally, to contextualize the issue of antiretroviral resistance, approximately 76% of patients surviving with HIV are resistant to one or more antiretroviral drugs[17].

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[13] Ho, David D. "HIV-1 Dynamics in Vivo: Virion Clearance Rate, Infected Cell Life-Span, and Viral Generation Time." *Science* 271 (1996). 29 Jan. 1996. Web. 07 Dec. 2011. <http://www.sciencemag.org/content/271/5255/1582.long>.

[14] Kunkel, T. A. "Specificity and Mechanism of Error-prone Replication by Human Immunodeficiency Virus-1 Reverse Transcriptase." *The Journal of Biological Chemistry* 264 (1989)

[15] Volberding, PA. "HIV Therapy in 2003: Consensus and Controversy." *AIDS* April (2003)

[16] Yazdanpanah, Yazdan. "Impact of Drug Stock-Outs on Death and Retention to Care among HIV-Infected Patients on Combination Antiretroviral Therapy in Abidjan, Côte D'Ivoire." *Open Access*. Plus One. Web. <http://www.plosone.org/article/info:doi/10.1371/journal.pone.0013414>.

[17] Richman, DD. "The Prevalence of Antiretroviral Drug Resistance in the United States."*Lippincott Williams & Wilkins, Inc.* 18 (2004). *Ovid SP*. 2 July 2004. Web

The recent revolution in genomics and personalized medicine, however poses uniquely beneficial implications in areas related to HIV that are difficult to navigate. Pharmacogenomics can be defined as the strategy of personalizing therapies based on an individual's genetic make-up and is becoming an increasingly important field of medicine because 60-80% of variability in drug response is considered to be genetic[5]. According to Blum and French in their 2005 paper called *HIV resistance testing in the USA – a model for the application of pharmacogenomics in the clinical setting,* pharmacogenomics is likely to play an expanded role in 1) drug selection 2) patient-specific immune boosting 3) drug dosing 4) progression prognosis and 5) pharmacogenomics can be used to change an individual's treatment based on mutation analysis of that individual's virus[5]. Before exploring the applications of pharmacogenomics on HIV, it is important to understand the underlying biological principles. Genetic variation in the human genome occurs predominantly as single nucleotide polymorphisms (SNPs) where single nucleotides differ between humans. An individual's DNA contains as many as ten million SNPs, which are responsible for the diversity of human phenotypes, as well as susceptibility to drugs and diseases[18]. Scientists approach the problem of identifying, cataloging and characterizing SNPs from two different angles – a genomic approach and a functional approach[19]. The genomic approach involves scientists comparing the genomes of numerous individuals to study differences and recording their results in forums such as the dbSNP short genetic variation database hosted by the National Center for Biotechnology Information

[18] Twyman, RM. "SNP Discovery and Typing Technologies for Pharmacogenomics." *Current Topics in Medicinal Chemistry* 4.13 (2004): 1423-31.
[19] "Making SNPs Make Sense." *Learn Genetics*. University of Utah. Web. 09 Dec. 2011. <http://learn.genetics.utah.edu/content/health/pharma/snips/>

(NCBI)[2013]. This method of SNP characterization requires a large amount of computer-powered data analysis unlike the functional approach where scientists focus on select genes known to be associated with a particular process or disease, and then they examine them across a population[13]. In pharmacogenomics, such genetic variation with respect to drug response is studied and used to guide patient care. The primary bioinformatics resource for pharmacogenomics is the PharmGKB database developed by Stanford University. PharmGKB curates knowledge about the impact of genetic variation on drug response and focuses on clinical interpretation of variants, drug dosing guidelines, genetic tests, and other information that is practically applicable for actors in the health sector[21]. Although pharmacogenomics supports the promise of more effective drug therapy for patients, there are some important barriers to progress that currently exist. Aside from finding gene variations that affect drug response being a time-consuming and complicated process as alluded to above in the context of finding SNPs, there is the issue of limited drug alternatives because only one or two approved drugs may be available for treatment of a particular condition. Another health policy concern is that drug companies prefer the more economically favorable approach of producing "one size fits all" drugs rather than spending billions of dollars to bring modified alternatives that serve only small portions of the population[22]. Even without expanding the numbers of drugs available, however, pharmacogenomics is still incredibly impactful because doses and preferences for existing drugs can be employed for patient care.

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[20] "DbSNP Home Page." *DbSNP Short Genetic Variations*. NCBI. Web. 8 Dec. 2011. <http://www.ncbi.nlm.nih.gov/projects/SNP/>

[21] "PharmGKB Tutorial." *PharmGKB*. Stanford University. Web. 08 Dec. 2011. <http://www.pharmgkb.org/tutorial/PharmGKB_tutorial_2011.pdf>.

[22] "Pharmacogenomics: Medicine and the New Genetics." *Human Genome Project Information*. Oak Ridge National Laboratory, 10 Sept. 2011. Web. 09 Dec. 2011. <http://www.ornl.gov/sci/techresources/Human_Genome/medicine/pharma.shtml>

There is incredible potential at the intersection of HIV and the rapidly growing field of pharmacogenomics. In fact, some major innovations have already occurred in resistance testing technologies and in HIV treatment selection based on genomics. Genotyping and phenotyping are two established methods for identifying antiretroviral resistance in patients on therapy, which *Blum et al 2005* discusses in detail. In HIV genotyping, a discrete sequence of reverse transcriptase and protease genes in extracted RNA specimens is amplified to generate cDNA amplicon, which then undergoes sequencing. A software system called OpenGene then aligns the sequences, reports mutations, and produces an interpretive report. The interpretation does not provide any insight though with regards to the degree of resistance to a drug because the output is either 1) no evidence of resistance 2) possible resistance 3) resistance or 4) insufficient evidence. Bayer Healthcare Systems produces both OpenGene and the TRUGENE HIV-1 GENOTYPING TEST discussed here. On the other hand, HIV phenotyping is a procedure that measures the susceptibility of a patient's virus to antiretroviral drugs *in vitro* compared with a control virus. PHENOSCRIPT by Specialty Laboratories gives a rapid assessment of an individual's likelihood to respond to any of 15 FDA-approved drugs based on a comparison between the ability of a drug to inhibit the replication of that individual's virus and a control virus. This produces a Patient Resistance Index (PRI) that measures the difference in viral replication between the patient's virus and the reference virus. Using cut-off values for the PRI, the results of testing are reported categorically as likely, possible, or unlikely[5]. The table below adapted from *Blum et al 2005* comparatively analyzes the advantages and disadvantages of the phenotypic and genotypic assays being discussed.

	Advantages	Disadvantages
Genotypic Assays	Rapid turnaround time (days versus weeks), reduced cost, proven utility in predicting short-term virologic outcome, can identify emerging mutations before onset of phenotypic drug resistance, and can detect contamination between specimens	Measurement of drug resistance is indirect – identified mutations are subjectively translated into conclusions on viral resistance, Only identifies mutations in predominant viral quasispecies (> 25% of viral population), Unclear relevance of certain mutations, Drug resistance does not correlate with mutations in all cases, Interpretation can be difficult when multiple mutations are present
Phenotypic Assays	Directly measure drug susceptibility: determines IC50 or IC90 (concentration of drug required to inhibit viral replication by 50 or 90%), can measure susceptibility to any drug, determine presence of cross-resistance and multi-drug resistance, measure overall effects of mutations, can assess non-B-clade HIV-1 strains	Non-standardized cut off values, only identifies mutations in predominant viral quasispecies (> 25% of viral population), long-term drug response cannot be determined, fails to account for interaction between drugs in combination therapy, highly complex testing platform with longer turnaround time, increased cost

HIV drug resistance databases also play a key role by providing advanced information to clinicians. Important databases often used in HIV genetic research are the Los Alamos HIV Drug Resistance database and the Stanford HIV RT and Protease Sequence Database[5]. The former collects all sequences and focuses on annotation and data analysis. The HIV Database at Stanford collects sequences associated with the development of viral resistance against antiretroviral drugs and focuses its analyses only on those sequences[5]. According to *Shafer et al 2000*, the database contains a compilation of nearly all published HIV reverse transcriptase and protease sequences,

which are linked to data about the source of the sequence sample and the antiretroviral drug treatment history of the individual from the isolate was obtained[12] [23]. Another group developed the HIVbase software solution that helps researchers effectively manage DNA/amino acid sequences and related genetic/clinical data using storage and query capabilities[24] [5]. Storing genotypic resistance data and linking to other clinical information is an important tool for successful disease management[5]. Examples of such systems that identify mutation patterns associated with resistance are Virodec (Roche Diagnostics) and ViroScore (ABL)[5]. Virodec HIV is an online application engineered to upload, analyze, interpret, deliver, and store genetic sequence data from genotyping assays. ViroScore is an HIV resistance sequence management system with a sequence database that is used by analysis tools/algorithms for resistance interpretation[5]. Despite these advanced technologies, interpretation is still the limitation with all resistance testing, and the systematic approaches developed to predict phenotypes based on mutational patterns are complicated by the complex mutation patterns for resistance[5].

HIV drug resistance testing represents one of the first, widely accepted, used and reimbursed scenarios for personalized medicine where treatment selection and practice is guided based on genotypic data from the individual patient[5]. Given the constantly mutating nature of the HIV virus, constant analysis and data management is required for determining the correct treatment based on scientific interpretations[5]. Today, drug resistance genotyping represents the major application of pharmacogenomic data in the

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[23] Shafer, Robert. "Human Immunodeficiency Virus Reverse Transcriptase and Protease Sequence Database." *Nucleic Acids Research* 31.1 (2003): 298-303. Web. 08 Dec. 2011. <http://nar.oxfordjournals.org/content/31/1/298.long>

[24] Salemi, Marco. "HIVbase: a PC/Windows-based Software Offering Storage and Querying Power for Locally Held HIV-1 Genetic, Experimental and Clinical Data." *Oxford Journal of Bioinformatics* 20.3 (2004): 436-38. 24 Jan. 2004. Web. <http://bioinformatics.oxfordjournals.org/content/20/3/436.short>

USA and its application in the battle against HIV helps answer critical questions that other methods are unable to address[5]. In the years to come, pharmacogenomics is likely to play an expanded role in HIV management by improving drug selection, patient specific-immune boosting, drug dosing, and progression prognosis[5]. As the technologies considered in this paper develop further and become more advanced with time, clinicians will be further empowered to serve the needs of patients infected with HIV. Management of HIV drug resistance through mutation analysis of that individual's virus represents a new, general paradigm in the treatment of infectious diseases, that of pharmacogenomics[5]. While the HIV epidemic continues to pose a major challenge to humanity, the development of new medical technologies and techniques has improved the global horizon for eliminating suffering from the virus.

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