Understanding HIV-1 Drug Resistance Testing

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OBJECTIVES

- Develop/apply real time, quality assured, economic molecular virology assays to enhance patient care (collaborate 41 DGH's in UK)
- Support clinical trials and audits (same setting)
- Contribute to international studies improving understanding of patient management (MRC FORTE, ERA and PERA, EuroSIDA, SPREAD, INITIO and more)
- Collaborative grants from industry to improve and assure molecular technologies for patient care
- Development of low technology molecular approaches to support patient care in the developing world
- Teaching to help all groups to understand new technologies

Understanding Genetics

- Human genome or genetic code carries all information about the individual in every cell nucleus
- It is in the form of a chemical package called DNA
- The information is packaged in genes that are collected in chromosomes
- There are 23 chromosomes in man carrying this information in bundles

Understanding Genetics

- There are about 30-80,000 genes in man carrying all the genetic information
- This represents about 10% of the total DNA in cells
- The other 90% contains non-coding, archived or other DNA
- By June 26th 2000 scientists understood the rough chemical sequence of the human genome but not what it all coded for in man
- (about 8000 genes have been identified)

Understanding Genetics

- In each cell there are 2 complete sets of the human genome (double stranded DNA)
- Except in eggs and sperm (which carry 1 set each)
- Each cell contains 30-80,000 genes (discrete units of genetic information) on the same 23 chromosomes
- When we breed we pass on one set each and swapping of bits of maternal and paternal chromosomes (recombination) takes place

Understanding Genetics

- Imagine the human genome as a book:
  - 23 chapters called CHROMOSOMES
  - Each chapter has several thousand stories called GENES
  - Each story is made of paragraphs called EXONS (10%)
  - Each paragraph is interrupted by “advertisements” called INTRONS (90%)
  - Each paragraph in the stories is made of words called CODONS
  - Each word is written in letters called BASES

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Understanding Genetics

• One billion words in the book, if you read the genome at one word (CODON) per second for 8 hours per day it would take a century.
• This is a gigantic document, in a microscopic cell.
• The genome like a book is written in linear, one dimensional and one directional form, and is defined by a code that trans literates a small alphabet of signs into a large lexicon of meanings through their order and grouping.

Understanding Genetics

• The chemistry used 4 letters A,G,C,T.
• These are chemical bases adenine, guanine, cytosine and thymine and in different sequences they form the code.
• Almost everything in the body is made of protein or needs proteins to make it
• Proteins are made of chemicals (building blocks) called amino acids (21)
• The sequence of these bases in groups of 3 spell out the code for different amino acids
• Thus a template to build proteins is carried in the DNA.

Understanding Genetics

• The DNA (book) can REPLICATE (photocopy itself)
• This is due to the unique property of of the 4 bases: A likes to bind only with T and G only with C.

Understanding Genetics

• REPLICATION

Understanding Genetics

• The making of proteins is more complex and involves translating the genetic code
• First the code is transcribed by copying the base pairing as a chemical called RNA - it is very similar to DNA but has a base called uracil (U) instead of thymine (T).
• RNA also carries a linear code (but is much shorter) and this so called messenger RNA can leave the nucleus after transcribing a small section (one gene)

Understanding Genetics

• The messenger RNA carries the code to protein factories in the cytoplasm of the cell (RIBOSOMES)
• The messenger RNA is translated 3 bases at a time (one codon) and the amino acid is transferred by transfer RNA according to the codon present on the messenger RNA to build proteins
Understanding Genetics

- **Nucleus**
- **DNA**: -ACA - CAC - CAA - CCG - GCG-
- **Cyto**
- **mRNA**: UGU - GUG - GUU - GGC - CGC-
- **Ribosome**
- **tRNA**: ACA | CAC | CAA | GGC | GCG
- ----Thr----His-----Gln-----Gln----Ala--

Introduction

- 1981 - first reported cases of AIDS
- 1982/3 - defined epidemiology of epidemic
- 1984 - first serology tests used in UK
- 1985 - full genome sequenced
- 1986 - first antiretroviral drug (AZT)
- 1987 - heterosexual spread identified
- 1987/8 - PCR technology

- 1989 - antiretroviral resistance described
- 1990 - first viral load assay for clinical care
- 1991 - first resistance assay in clinical care
- 1993/4 - commercial viral load assays
- 1994 - dynamics of HIV-1 replication defined
- 1995 - viral load as a study endpoint
- 1996 - PCR-based commercial resistance assays devised from existing technology
Genetics of HIV (1)

- 9.2 Kb genome (9200 bases)
- 3 open reading frames
- Replication involves reverse transcription RNA - DNA
- Prone to base errors (at least 1 per replication cycle)
- Rapid turnover (one generation = 1.5 days)
- Thus process is a route of HIV EVOLUTION

Genetics of HIV (2)

A mutational event may be (virus outcome):
- Neutral (no influence on biology)
- Disadvantage (poor virus survival)
- Advantage (improved virus survival)

Viral Diversity (1)

Intra-host
Frequent rounds of replication and expanding genetic diversity
Thus the longer the infection the greater the diversity of virus in the host
(Quasispecies)
After 1-2 years equilibrium achieved

Geographical
A broader genetic diversity of viruses exists around the world (subtypes)
Subtype B commonest in developed world
Subtype A,C,D,E,F,G,H,I,J and O are found in different geographical areas

Distribution of HIV in the Body
The Polymerase Reaction (PCR)  
( Kary Mullis, 1985)  
- Process of automated and specific DNA amplification  
- Possible because of technical/computer advances  
- Allows minute and undetectable quantities of specific microbial DNA to be specifically primed, amplified and biochemically detected  
- Requires a heat-resistant polymerase enzyme (Taq P)  
- Automated primer synthesis  
- A thermal cycler to allow the reaction in one tube in 2 hours

Applications of PCR  
- Diagnostic assays  
- Quantitative viral load assays  
- Resistance assays  - phenotyping  - genotyping

Resistance Introduction (1)  
- Drug resistance testing has supported therapy of infectious disease for years  
- It has been widely used in bacteriology to guide antibiotic selection  
- In virology its use has been limited by lack of appropriate technologies
Drug Resistance & HIV - Background
- High frequency error-prone replication
- Allows Darwinian evolution of HIV
- Specifically in RT and protease genes
- Process selects viruses day by day with mutations in RT and protease that will function in presence of drugs
- These viruses are archived in the host and may remain many years
- Represents an important cause of treatment failure

A Number of Facts Hold True for HIV and Resistance
- Rapid replication = HIV evolution
- Evolution obeys Darwinian rules
- HIV-1 responds rapidly to environmental pressures
- Single mutations occur daily in untreated patients
- Double mutations less frequently

Causes of Drug Failure
- Evolution of drug resistant viruses
- Pharmacological ‘resistance’
  - absorption
  - distribution
  - metabolism
  - activation
  - interactions
  - excretion
- Adherence to therapy

Basic Needs
- Collect EDTA blood
- Store plasma within 6+ hours -70C
- Viral >1000 c/ml
- Geographical origin
- Store blood at every therapeutic cross-roads
- Test at failure or within 2 weeks of stopping Rx

Measures
- Phenotyping (capacity for HIV-1 growth in the presence of individual drugs – BIOLOGICAL)
- Genotyping (sequence changes known to be associated with resistance – MOLECULAR)

Methods - Phenotyping
- PBMC co-culture, VIRCO, Virologic
- These 2 approaches use PCR
- Viruses from plasma
- RT-PCR amplified
- ‘Shotgunned’ into recombinant viruses
- Recombinant viruses cultured with lab. CD4 cells in presence/absence of drug
Methods - Genotyping

- Sequencing
  - ‘OpenGene’ system
  - ABI automated sequencing
  - ‘GeneChip’ technology

- PMA
  - LiPA HIV-1 RT
  - Chiron PMA
  - PMA- UCL/MRC

Comparison of Genotyping and Phenotyping

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<th>Advantages</th>
<th>Limitations/Inconveniences</th>
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<tr>
<td>Genotypes</td>
<td>Available in laboratories</td>
<td>Slower (weeks)</td>
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<td>Rapid (days)</td>
<td>Technologically demanding:</td>
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<td>– P3 lab necessary</td>
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<td>Less sensitive</td>
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<td>No analysis of sensitivity to a combination of drugs</td>
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<td>Phenotype</td>
<td>Direct measure</td>
<td>Restricted availability</td>
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<td>Familiar to physicians</td>
<td>Broad (toxic)</td>
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<td></td>
<td>Clear clinical relevance</td>
<td>Technologically demanding: P3 lab necessary</td>
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‘Virtual Phenotype’

- Use sequence data from patient
- Compare it to a large database of existing sequences with known phenotype (i.e., biological response to drugs)
- Derive an estimate of sensitivity to drugs by computer analysis

Limitations:
- Size of comparative database
- The appearance of new drugs
- Computer capacity - analysis done as groups and may revert to ‘rules’

Guidelines
(IAS-USA, Euroguidelines, BHIVA)

- PHI: If early Rx local policy then minimum delay. Recommend - modify or real-time testing
- Chronic (Naive): Consider/Recommend - response to 1st therapy is important
- 2nd+ Rx: Recommend
- Salvage: Recommend
- Pregnancy: Recommend in mums with detectable VL
- Paediatrics: Recommend testing babies if Rxed mum
- PEP: Recommend in kids with failure
- Evidence - In Vitro

- Growth of viruses in the presence of drug in multiple rounds of culture result in mutational changes in the RT/protease genes.
- The resulting daughter viruses have decreased drug sensitivity
- Sequencing of these viruses revealed mutation sites in genome

Clinical Virologist (‘Expert’) Report

- Results are complex
- New data is continuously becoming available
- Clinical virologist (‘expert’) should review
- Opinion should be documented
- Opinion/advice/consultation/discussion should be available to physicians
Unresolved Problems

- Sensitivity of assays
- Specificity of assays
- QC/QA
- Primer/probe fidelity and diverse viruses
- Applications/role of resistance for clinical management
- Further evidence with RCT’s to define exact role for resistance testing