HIV stands for Human Immunodeficiency Virus and is the cause of AIDS (Acquired Immunodeficiency Syndrome). Though there are 2 types of HIV viruses. HIV-1 & HIV-2, the predominant virus globally is HIV-1. There are several subtypes of HIV-1 virus of which subtype C is predominant in India whereas subtype B is more common in the developed world.

PATHOGENESIS OF HIV INFECTION

To understand the action of antiretroviral drugs (ARVs) and thus determine drug resistance, it is necessary to understand the pathogenesis of HIV. HIV virus infects the CD4 cells with help of envelope proteins gp 120 and gp 41 followed by binding to chemokine receptors [chemokine receptor 5 (CCR 5) or CXC chemokine receptor 4 (CXCR 4)] that leads to fusion of cell and viral membrane (1). The contents of the HIV particle are then released into the cell. Once inside the cell, the HIV enzyme reverse transcriptase converts viral RNA into DNA. This DNA is transported to the CD4 cell nucleus where it is spliced into the human DNA by HIV enzyme integrase. This integrated HIV DNA also called as provirus forms messenger RNA that is transported out of the nucleus to form new HIV proteins and new copies of HIV RNA. The enzyme protease chops long strands of protein into smaller cells. These form new viral particles which are then released from the cell (2).

ANTIRETROVIRAL DRUGS

Antiretroviral drugs are groups of drugs that act on various HIV enzymes to inhibit HIV replication. They do not destroy the virus and thus are not curative. However as they prevent HIV viral replication, continued treatment helps to suppress the virus multiplication and thus helps to keep the disease under control. However, the virus remains in check till the drug is continued. With non-compliance or underdosing, the HIV virus mutates and leads to drug resistance. The first antiretroviral drug rolled out in 1994 and monotherapy was issued for treatment of HIV. However it was found that the virus again started multiplying within 3-6 months of therapy. In 1994 with advent of newer antiretroviral therapies, dual drug therapy was recommended. Though the virus was suppressed to a greater extent, the viral load again started increasing within 6-24 months of therapy. In 1997, with advent of highly active antiretroviral therapy (HAART) and use of triple drug therapy, it was found that the virus was highly suppressed and remained suppressed even after 24 months of therapy. This formed the basis of triple drug antiretroviral therapy (ART) for treatment of HIV infection.

The common ARVs used for treatment of HIV infection belong to the following groups:

1. Nucleoside Reverse Transcriptase Inhibitors (NRTIs)
2. Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
3. Protease Inhibitors (PIs).

There are newer groups of HIV drugs that are now available and usually used for chronically HIV infected and those who have had treatment failure. Those groups of drugs are:
1. Second generation NNRTI – Etravirine (TMC 125)
2. Entry Inhibitors – Enfuvirtide, Maraviroc
3. Integrase Inhibitors – Raltegravir.

**TREATMENT FAILURE**

The commonly used triple drug ART combination consists of 2NRTI + 1NNRTI or 2NRTI + 1PI. Currently ART is lifelong therapy. Strict adherence, dosage adjustment as per weight and body surface area are important to keep the virus controlled. However, treatment failure can still occur. Common causes of treatment failure are:

a) Non-adherence
b) Impaired drug absorption
c) Drug interactions
d) Altered drug pharmacokinetics
e) Inadequate regimen potency

All these can lead to mutations of the virus which can confer drug resistance to the virus.

**Non-adherence**: Starting ART is never an emergency. Starting ART when child/caregiver is not ready can result in poor adherence and ART resistance. Taking ART on time every day is not an easy task (it means not BD dose but strictly 12 hourly doses). ART should only be started when the patient/parent understands what is HIV infection, what are the benefits and side effects of ART, the importance of taking ART on time and can effectively administer the chosen ART regime. Every visit to the ART clinic should be a chance to reinforce the good adherence policy to caretaker and child and check for missed doses or incorrect dosing. An adherence of 95% is needed to keep the virus under check i.e., if the child is on an ART regime that consists of 12 hourly doses, ideally he should take 60 doses in a month. To keep the virus suppressed, he must take at least 95% of the doses i.e. he cannot afford to miss more than 3 doses in a month. Thus ensuring adherence is absolutely necessary to prevent virological failure.

Factors that are associated with reduced adherence are intolerance, mental illness, active substance abuse, comorbidities such as TB, asymptomatic status of patient when treatment begins, poverty, poor understanding of regimen and inadequate pharmacy service. Thus, these issues have to be tackled also to ensure resistance.

**Impaired drug absorption**: Lot of ARVs have poor absorption in relation to food for e.g., among the protease inhibitors; atazanavir + ritonavir, darunavir absorption increases with food. Nelfinavir & Tiprinavir needs fatty food for absorption. Saquinavir should be taken within 2 hours of a meal. Among the NRTI, food decrease absorption of Didanosine (ddI) and thus it needs to be taken either half an hour before a meal or 2 hours after a meal. Among the NNRTIs, efavirenz (EFV) is taken on an empty stomach or with a low fat diet. Concurrent meal increases
peak level by 40-50% which may increase side effects. Thus, proper attention to food and drug absorption is to be made to prevent poor absorption of the medicine.

**Drug Interactions:** Very often patients with HIV have co-infection with TB. Rifampicin can interact with PIs as well as NNRTIs and can reduce their drug availability considerably. Thus a patient with HIV-TB should either be on EFV based regimen (EFV dose should also be increased by 15%) or rifampicin should be substituted by Rifabutin to prevent these drug interactions. However EFV cannot be given in a child less than 10kg weight or less than 3 years of age.

Antifungals especially fluconazole also have interactions with ART and simultaneous treatment can lead to underdosing. This in turn can lead to improper drug levels in the system and higher chance of drug resistance. Other drugs that can interact with PIs or NNRTIs are antiarrhythmics, statins, newer generation antihistamines, benzodiazepines, proton pump inhibitors, cisapride and anticonvulsants such as Phenytoin, Carbamazepine and Phenobarbital.

**Drug Pharmacokinetics:** Children tend to metabolize ARVs at a faster rate and hence need to be dosed as per body weight or as per body surface area. Just reducing adult dose by 75% or 50% does not ensure adequate drug levels in the child. Thus, in children ARV is to be given as per body weight or surface area else underdosing is a problem.

**Inadequate regimen potency:** Preferred regimens for initial therapy for treatment as per DHHS (3) and IAS-USA (4) are

- Zidovudine + Lamivudine or Abacavir + Lamivudine
  - and Efavirenz or Lopinavir / Ritonavir

Initial regimen for resource poor settings as per WHO guidelines 2006 are (5)

- Zidovudine + Lamivudine or Stavudine + Lamivudine
  - and Nevirapine or Efavirenz

An ideal regimen should achieve an undetectable viral load by 16-24 weeks of therapy. Regimens with 3 NRTI or unboosted PI do not have such a response (6). Failure to achieve these goals despite of good adherence and adequate systemic blood levels suggest lack of antiretroviral potency and can lead to drug resistance.

**TYPES OF HIV TREATMENT FAILURE:**
Treatment failure can be
- Virologic failure
- Immunologic failure
- Clinical failure.
**Virologic failure**: The goal of therapy is a sustained viral load of less than 50 copies/ml. Also in treatment naïve patients decrease of 0.7-1 log copies/ml at 1 week, 1.5-2 log copies/ml at 4 weeks and < 50 copies/ml at 16-24 weeks is an optimal virologic response. Failure to achieve the goal of viral load < 50 copies/ml at 24 weeks and inability to maintain sustained undetectable viral load leads to acquisition of resistance mutations. Levels more than 200 copies/ml or sustained viral load > 50 copies/ml usually indicate virologic failure. Viral load > 10,000 copies/ml is associated with clinical progression and rapid CD4 decline and if sustained may promote resistance.

**Immunologic failure**: This is arbitrarily defined as failure of the CD4 count to increase 25-50/cumm in the first year of HAART. CD4 count usually increases by 50-120 cells/cumm during the first 3 months, thereafter increasing by 2-7 cells/cumm/month. Return of CD4 cell count to pre-therapy baseline or below after initial immune recovery, without any other concomitant infection to explain transient CD4 cell decrease or a greater than 50% fall from on therapy CD4 cells peak level are also suggestive of immunological failure and drug resistance.

**Clinical failure**: This is defined as the occurrence or reoccurrence of an AIDS defining opportunistic complication after 3 months of HAART. Immune reconstitution syndrome (IRIS) does not qualify as a clinical failure and should be excluded. It also includes progressive neuro-developmental deterioration or growth failure despite adequate nutritional support. Pulmonary TB is not indicative of treatment failure.

Virological failure is the initial treatment failure to occur followed by immunological failure and lastly by clinical failure. Treatment failure is an indication for change of therapy. In most cases, the usual regimen is switched from an NNRTI based to a PI based HAART regimen or from a PI based regimen to either another PI based or an NNRTI based regimen. In each instance, the second regimen should include > 2 nucleosides based on resistance testing.

**RESISTANCE MUTATIONS AND TREATMENT ADJUSTMENT**

With virologic failure, the first reverse transcriptase mutation to appear with most regimens containing Lamivudine (3TC) or Emtricitabine (FTC) is M184V. This results in high level resistance to 3TC and FTC but resultant increase in activity to Zidovudine (AZT), Stavudine (d4T) and Tenofovir (TDF). For this reason, it is common practice to continue 3TC or FTC despite resistance, but in such cases these drugs should not be counted as active components of an antiviral regimen. Patients failing boosted PI-based regimens frequently have no resistance mutation. With unboosted PIs, resistance mutations are present and treatment would depend on resistance test results. The favored PIs for salvage are ritonavir boosted PI such as Atazanavir, Lopinavir and Darunavir. Among NNRTI based regimen, resistance to Efavirenz (EFV) or Nevirapine (NVP) usually results in cross-resistance to all currently available members of the class. Thus, there is no apparent benefit of continuation of NNRTIs in a failing regimen which may even lead to cross-resistance to second-generation NNRTIs such as etavirine.

For patients with multi class resistance, rescue therapy is needed. New antiretrovirals such as enfuvirtide (T20), Darunavir and Tiprinavir have shown efficacy against resistant strains.
Also 3 newer class of drugs; CCR5 inhibitors (maraviroc), integrase inhibitors and maturation inhibitors offer great promise to patients who have substantial resistance to other drugs. However efficacy and safety of these newer antiretrovirals in children has still not been established.

**RESISTANCE TESTING**

As the ART rollout continues, drug resistance monitoring will become increasingly important. Moreover person to person transmission of drug resistant viruses will occur indicating that testing for drug resistance even before initiating therapy may be needed for treatment naïve patients. There are 2 general types of resistance assays used in clinical practice: genotypic assays (i.e., HIV-1 gene sequencing to detect mutations that confer HIV-1 drug resistance) and phenotypic assays which are more like antibacterial sensitivity results which measure the ability of HIV to replicate at different concentrations of tested drugs. Both require a viral load > 500-1000 copies/ml to be effectively produced.

**Genotypic Assays:** Genotype analysis identifies mutations associated with phenotypic resistance. Genotypic testing can be performed by commercial assay kits or in house protocols. Assays vary in cost, number of mutations tested and method of reporting and interpreting results. The methodology involves amplification of the reverse transcriptase (RT) and protease (Pr) gene by Real Time PCR, DNA sequencing of amplicons generated for the dominant species and reporting of mutations for each gene using a letter-number-letter standard, in which the first letter indicates the amino acid at the designated codon with wild-type virus, the number is the codon position, and the second letter indicates the amino acid substituted in the mutation. Thus the RT mutation K103N indicates that asparagines (N) has replaced lysine (K) on codon 103. Table 1 shows the amino acids and corresponding letters used to describe mutations in genotype analyses.

**Table 1 – Letter Designation for Amino Acids**

<table>
<thead>
<tr>
<th>A</th>
<th>Alanine</th>
<th>I</th>
<th>Isoleucine</th>
<th>R</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Cytosine</td>
<td>K</td>
<td>Lysine</td>
<td>S</td>
<td>Serine</td>
</tr>
<tr>
<td>D</td>
<td>Aspartic Acid</td>
<td>L</td>
<td>Leucine</td>
<td>T</td>
<td>Threonine</td>
</tr>
<tr>
<td>E</td>
<td>Glutamic Acid</td>
<td>M</td>
<td>Methionine</td>
<td>V</td>
<td>Valine</td>
</tr>
<tr>
<td>F</td>
<td>Phenylalanine</td>
<td>N</td>
<td>Aspargine</td>
<td>W</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>G</td>
<td>Glycine</td>
<td>P</td>
<td>Proline</td>
<td>Y</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>H</td>
<td>Histidine</td>
<td>Q</td>
<td>Glutamine</td>
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</table>

Results of genotypic tests use lists of predefined drug resistance mutations or classifications by computerized, rules based algorithms to characterize virus as “susceptible”, “possibly resistant”, or “resistant” to each antiretroviral drug. Currently genotypic algorithms are based on data that were obtained using subtype B viruses. More in vivo and in vitro resistance data are needed for non-subtype B HIV. Genotype assays do not reliably detect minority species and consequently are more useful in determining which agents are unlikely to be effective than to determine which drugs are likely to be effective.
**Phenotypic Assays**:- This test involves insertion of the RT & Pr genes from the patient’s strain into a backbone laboratory clone. Replication is monitored at various drug concentrations and compared with a reference wild-type virus. Standard phenotypic testing using recombinant virus assays is performed by few laboratories only.

<table>
<thead>
<tr>
<th>Genotypic Assay</th>
<th>Phenotypic Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Less expensive</td>
<td>• More expensive</td>
</tr>
<tr>
<td>• Early results (1-2 weeks)</td>
<td>• Longer time to result</td>
</tr>
<tr>
<td>• Well standardized</td>
<td>• Clinical thresholds not available for all drugs</td>
</tr>
<tr>
<td>• More sensitive to detect multidrug resistance</td>
<td>• Detects resistance only to single drug</td>
</tr>
<tr>
<td>• Interpretation requires expertise</td>
<td>• Interpretation straight forward</td>
</tr>
<tr>
<td>• Algorithms may be incomplete for new drugs</td>
<td>• Reproducibility is good</td>
</tr>
<tr>
<td>• Limited data on non-subtype B virus</td>
<td></td>
</tr>
</tbody>
</table>

**Common Resistance mutations**:- Thymidine analog mutations (TAMS) reduce susceptibility to all NRTIs. Most frequent TAMS are 41L, 210W, 215Y. TAMS cause hyper susceptibility to NNRTIs. M184V mutation leads to resistance to lamivudine and emtricitabine but increase activity of Zidovudine (AZT), Stavudine (d4T) and Tenofovir (TDF). Thus, 3TC & FTC need a single 184V mutation for resistance whereas other NRTIs require multiple mutations for resistance. TDF and Abacavir (ABC) resistance depends on number of TAMS and the TAM pathway. Among the NNRTIs, 181C mutation is commonly seen with NVP and 103N mutation is commonly seen with EFV leading to cross-resistance. Resistance to PIs requires multiple mutations and is commonly seen with unboosted PIs.

**WHEN TO CHANGE REGIMEN**

Switching to second line regimen should be only after establishing good adherence and child has clinical or immunological failure. In case of change of regimen because of toxicity and intolerance, agents with different side effect profile should be chosen. Do not add a drug to a failing regimen and consider change of at least 2 drugs with one in the NRTI group.

**CONCLUSION**
With access to antiretroviral drugs increasing rapidly, HIV drug resistance is on the rise and drug resistance monitoring will become increasingly important to ensure potent antiretroviral regimen and optimum viral suppression and continued benefit of ART.

References:

