

Scenario C – HIV Genotyping of Drug Resistant Strains

Background:

HIV is a virus that kills hundreds of people throughout the world each day. One of the main problems with the fight against HIV is that the virus is constantly mutating into newer, and sometimes stronger, forms that make detection and treatment very difficult. The high natural mutation rate of HIV also results in increased resistance to anti-HIV drugs used to help the patient fight the disease. Just as antibiotic use leads to resistant strains of bacteria, anti-HIV drugs such as protease and reverse transcriptase inhibitors can be overcome by new, mutated, strains of the virus that form during the infection. The high mutation rate of HIV makes this drug-resistant strain formation occur at a much higher rate than most viruses and bacteria.

Your team is a research group that has decided to study the HIV mutations occurring during the use of two different common drug cocktails prescribed to HIV infected patients. The two drug cocktail treatments you decide to research are commonly prescribed combinations of different reverse transcriptase inhibitors. The two treatments are:

Combination #1 – zidovudine (AZT) + efavirenz (sustiva) + didanosine (videx)

Combination #2 – lamivudine (epivir) + efavirenz (sustiva) + didanosine (videx)

Looking at the two treatments, you can see that the variable factor in the experiment is whether AZT or epivir is taken. In the experiment you will compare the mutations that occur during each form of treatment as well as look into which specific types of mutations lead to drug resistance. All other variables are controlled as much as possible. Variables that were controlled included the infection stage of the patient, the age of the patient, and whether they were a male or female. Also, their symptoms were all matched as much as possible. You have enrolled 20 patients in the study. None of the patients have taken any of these drugs – individually or as a cocktail – before the study began.

The study has two parts to it. In part one, you did a phenotypic study to determine whether drug resistance of the HIV changes as a result of taking the drug cocktail. This requires isolation of the HIV from the patients' blood, identifying the strain, and doing an in vitro tissue culture study on the resistance to anti-reverse transcriptase drugs.

In part two, you need to perform a genotyping study that looks at the change in the HIV genome after taking the drug cocktail (i.e., the mutation(s)). You decide to do this genotyping study by using an HIV Genome GeneChip microarray*. The full microarray looks at 347 exons of the HIV genome from two genes that code for the protease enzyme and the reverse transcriptase enzymes (both vital for replication of HIV).

*Note: This array is based on the real GeneChip microarray known as the HIV PRT Genome Microarray produced by Affymetrix ©.

This microarray basically resequences each exon, allowing you to look for most mutations such as point mutations (single mutations of one base pair in the DNA). To narrow it down, you decide to look at segments of the two most commonly mutated exons within the reverse transcriptase gene – exon 74 and 184. Notice that you will not look at the entire exon, but rather a 20 base pair segment of each exon known as “hotspots” for mutations, where most mutations in the exon are commonly found.

This second part of the experiment requires you to have isolated HIV from the patient prior to the start of drug treatment. This provides you with an original “baseline” sequence of the HIV at these two codons. Then, the patients take the drug cocktail for approximately one year, at which time you isolate the HIV in the patients blood stream and repeat the resequencing, looking for mutations in the two exons.

Results:

In the study, patients #1-10 received Combination #1: AZT + sustiva + videx and patients #11-20 received Combination #2: epivir + sustiva + videx

Baseline Study

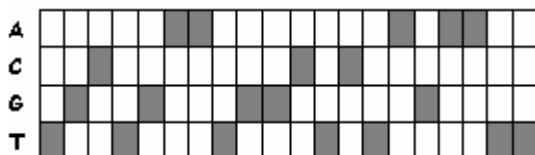
*Phenotype study: Each person was tested to determine their HIV strain and that strain’s resistance to reverse transcriptase drugs. Their results are below. The scale of the resistance is from 1 to 20 where 1 is “low resistance”, 10 is “mid level resistance”, and 20 is “high resistance”.

<u>Resistance level</u>	<u>Patients</u>
1	1 -5, 8, 12-16, & 19
2	6-7, 11, 17-18, & 20
3	9 & 10

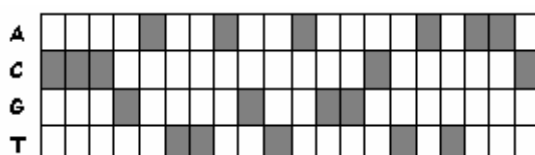
*Note: no patients showed a resistance level beyond 3.

*Genotype study: The baseline HIV sequence study found that none of the patients showed mutations in either exon 74 or 184 at the start. They all had the same sequence at these two exons. (This does not mean they have the exact same strains, but that all the strains they were infected with have no differences at these two exons). Remember, the researchers did not look at the entire exon, but rather the area of the exon known to have the most mutations (called “hotspots”). Here are the sequences of the two (non-mutated) segments you are studying:

Exon 74



Exon 184



Final Results

*Phenotype study: At the end of the study, you isolated HIV from each person's blood stream and once again tested each for its resistance to reverse transcriptase. The final results for each patient are below.

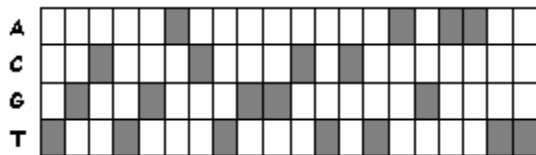
<u>Patient</u>	<u>Final HIV Resistance Level</u>	<u>Patient</u>	<u>Final HIV Resistance Level</u>
#1	2	#11	4
#2	1	#12	1
#3	2	#13	10
#4	1	#14	10
#5	1	#15	2
#6	2	#16	1
#7	4	#17	20
#8	1	#18	10
#9	15	#19	1
#10	3	#20	2

*Genotype study: After a year of taking the drug cocktail medication, you isolated HIV from the patients and resequenced exons 74 and 184. Here are the results for each patient:

Exon 74

Exon 184

Patient #1



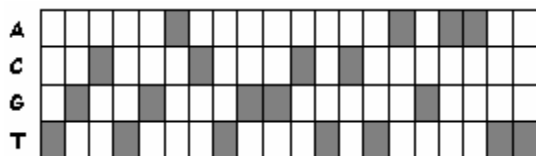
No Mutation

Patient #2

No Mutation

No Mutation

Patient #3



No Mutation

Patient #4

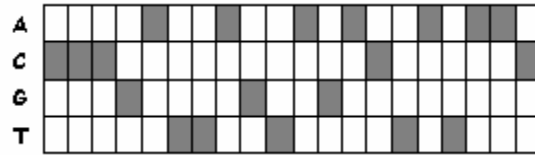
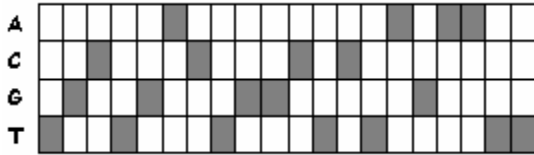
No Mutation

No Mutation

Exon 74

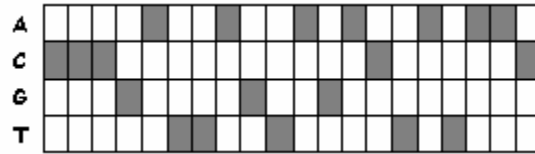
Exon 184

Patient #13

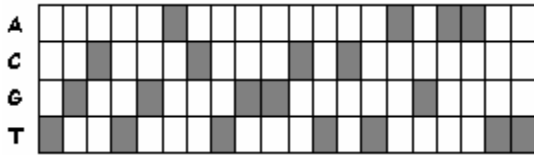


Patient #14

No Mutation



Patient #15



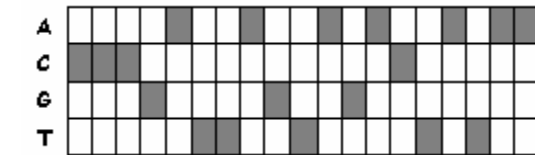
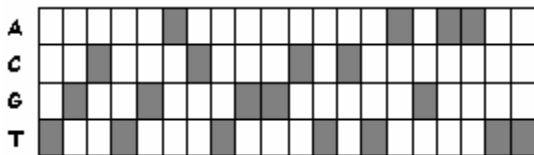
No Mutation

Patient #16

No Mutation

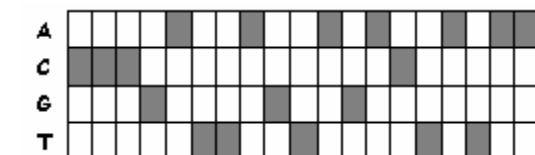
No Mutation

Patient #17



Patient #18

No Mutation



Exon 74

Exon 184

Patient #19

No Mutation

No Mutation

Patient #20

No Mutation

No Mutation

*Notice – for simplicities sake, only those exons that show mutation(s) are shown

Directions:

Now that you have the data, you need to make sense of it. What is happening here? Which drug combinations lead to the proliferation of which resistant strains? Which drugs lead to more mutations? What type of mutations occurred? Which drugs lead to which mutations and, therefore lead to greater resistance to the drugs? How much of a change in resistance occurred?

There is a lot to take a look at! It is suggested that you build some sort of table to help organize and analyze the data. Maybe include columns for changes in resistance, type of mutation, etc. Good luck!