


**Bioengineering: An Interface with Biology and Medicine**  
**Prof. Sanjeeva Srivastava**  
**Department of Biosciences and Bioengineering**  
**Indian Institute of Technology – Bombay**

**Lecture - 15**  
**Clinician's Perspective-II**

Welcome to the MOOC NPTEL course on bioengineering an interface with biology and medicine. In the last few lectures, we have discussed about gene cloning, polymerase chain reaction and various DNA tools. What is so interesting to learn that currently the revolutions in gene and genomic technologies have started paying directly in the clinics, lot of patients are getting benefitted because of these genomic technologies.


In order to illustrate some successful examples and to give you message that how biotechnology engineering technologies have started making its impact in the clinics today we have invited a distinguish guest Dr. Nikhil Phadke to interact with you.

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**Dr. Nikhil Phadke**  
PhD (Molecular, Cellular and Developmental Biology), *Founder & Chief Scientific Office*

- Dr. Nikhil Phadke obtained his PhD from the Molecular and Cellular Biology, University of Michigan, Ann Arbor, MI, USA
- He is currently the founder and head, Genepath DX.
- He and his team is involved in the development and offering of >250 molecular assays in state of the art lab in Pune
- He has years of experience in multidisciplinary collaborative research, including clinical molecular genetic diagnostics, development of approved diagnostic tests and platforms, etc.
- His collaborations include leading clinicians including oncologists and hemato-oncologists (Dr. Vijay Ramanan, Dr. Shona Nag, Dr. Pranjali Gadgil)



Dr. Nikhil Phadke obtained his PhD from the Molecular and Cellular Biology, University of Michigan, Ann Arbor in USA. He is currently the founder and head of a company GenePath DX in Pune. He and his team is involved in development of over 250 molecular assays in a state of the art lab in GenePath in Pune.

He has years of experience in multidisciplinary collaborative research including clinical molecular genetic diagnostics, development of approved diagnostic tests and platforms. He

has various collaborators and therefore his projects involved in different areas of oncology and hemato-oncology as well as infectious diseases.

I definitely felt very impressed with Dr. Nikhil Phadke's work and realize that whatever the latest advancement we see happening in US clinics where patients can directly see the benefit of looking at the gene mutation, the sequencing analysis, those things Dr. Nikhil has brought very quickly back here and those have been started making revolution in patient care.

Today, it is great pleasure to have Dr. Nikhil Phadke with us who is going to talk about clinical and commercial applications of molecular genetic diagnostics, the HandyLab and GenePath DX story. Welcome Nikhil.

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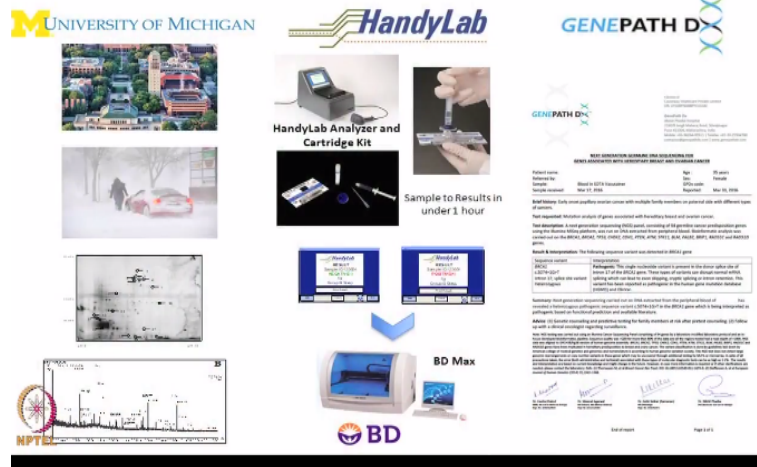


Our story actually starts in a class very similar to this. Two of my colleagues who were the cofounders of the company one is from IIT Mumbai and one is from IIT Chennai which is it is really a personal story. I have been teaching classes in medical schools and international research and I am told that these are some of the brightest students in the country. So I am sure you are all going to be superbly successful at whatever you do.

It would be nice if you could also enjoy yourself by being while being successful and make a positive impact on society while doing that, so if all these things happen then it is absolutely fantastic. I will talk a little bit about myself. I trained at University of Michigan, Ann Arbor. I worked in the medical school there and in the science and engineering schools.

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## Nikhil Phadke



And Ann Arbor always has those pretty pictures up there in the brochures but really most of the time it is just full of snow. So we have two seasons, winter and construction anyway. So after working there for slaving day and night in the lab, I got together with a bunch of my friends, many of whom were from IIT and we set up a company known as HandyLab and we built a molecular diagnostic platform.

Molecular diagnostic platform that integrated engineering principles, science principles and medical principles all together. So really a melting of minds of different areas of specialization and after we were successful with that I came back to India and started a company known as GenePath, which is focused on doing pretty much the same thing that we did in the US but catering to the needs of Indian patients and Indian doctors okay. I come from a medical background.

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## Long family history in the medical field



Phadke Hospital  
Jangli Maharaj Road  
Deccan Gymkhana

Established 1945



Dr. Manohar Phadke, MD (Paed)



Dr. Sumati Phadke, MD (ObG)



Dr. Manohar Wagle, MD (Paed)



Dr. Jaywanti Wagle, MD



Dr. Anuradha Naralkar, MD (Paed)



Dr. Dileep Phadke, Mch (Paed Surg)



There is 4 generations of doctors in my family on both sides and we have a hospital that was started in 1945 in Pune and my lab is actually based out of there. So there was a motivation to get into medicine but not actually do day-to-day medicine the way they had been doing it. I was also interested in engineering, so nothing better to combine all those areas into one.

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## Doctoral Research at the University of Michigan

- **Genomics, Proteomics and Bioinformatics** (temporal and spatial protein expression patterns) in the laboratories of Dr. Janine Maddock (Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor) and Dr. Philip Andrews (Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor)



### Complete genome sequence of *Caulobacter crescentus*

William C. Hnisman<sup>1\*</sup>, Tamara V. Feldblyum<sup>2\*</sup>, Michael T. Laub<sup>3</sup>, Ian T. Paulsen<sup>4</sup>, Karen E. Nelson<sup>5</sup>, Jonathan Eisen<sup>6</sup>, John F. Heidelberg<sup>7</sup>, M. R. K. Alley<sup>8</sup>, Neriko Onk<sup>9</sup>, Janine R. Maddock<sup>10</sup>, Isabel Pollock<sup>11</sup>, William C. Nelson<sup>12</sup>, Austin Rowland<sup>13</sup>, Craig Stephens<sup>14</sup>, William D. Pridmore<sup>15</sup>, Bert Ely<sup>16</sup>, Robert I. Dobson<sup>17</sup>, Robert J. Dodson<sup>18</sup>, A. Scott Durkin<sup>19</sup>, Michelle L. Gouin<sup>20</sup>, Daniel H. Haft<sup>21</sup>, James F. Klotzel<sup>22</sup>, John Smith<sup>23</sup>, M. B. Craven<sup>24</sup>, Hoda Khoury<sup>25</sup>, Iyoni Shetty<sup>26</sup>, Kristi Berry<sup>27</sup>, Teresa Ulsterback<sup>28</sup>, Kevin Tran<sup>29</sup>, Alex Wolf<sup>30</sup>, Jessica Vamatheswaran<sup>31</sup>, Maria Froelicher<sup>32</sup>, Owen White<sup>33</sup>, Steven L. Salzberg<sup>34</sup>, J. Craig Ventor<sup>35</sup>, Lucy Shapiro<sup>36</sup>, and Claire M. Fraser<sup>37</sup>

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Shotgun sequencing precursor to Celera's Human Genome Carried out at TIGR

My doctoral research was at the University of Michigan and I worked on genome sequencing before the Human Genome Project was completed and if you look at the people on this, so there is Craig Ventor there, that is me there, this is one of the projects that we did before the Human Genome Project was completed and we took almost 2 years to finish the sequencing of this genome.

It was a lot of fun but we were totally clueless at that point in time but the two years is an important part to remember. Anyway, throughout my PhD work, I did a lot of work on genomics, bioinformatics and proteomics.

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### Doctoral Research at the University of Michigan

The collage features three scientific papers and a bar chart. The papers are: 'Analysis of the outer membrane proteome of Clostridium clostridioforme by two-dimensional electrophoresis and mass spectrometry', 'Two-dimensional electrophoresis and peptide mass fingerprinting of bacterial outer membrane proteins', and 'Proteomic Analysis of the Spore Coats of Bacillus subtilis and Bacillus anthracis'. The bar chart is titled 'Citations in Each Year' and shows citation counts from 2002 to 2012.

Year	Citations
2002	10
2003	25
2004	35
2005	38
2006	35
2007	25
2008	20
2009	15
2010	12
2011	15
2012	18

Proteomic analysis of hard to dissect samples and using unannotated/poorly annotated genomes.

And the nice thing is that even after all these years many people are still quoting our research. It remains relevant even after all these years.

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### Driving Motivations and Subsequent Career

- Interest in pursuing inter-disciplinary state of the art technology development (i.e. the geek factor)
- Desire to be able to positively impact individual patient healthcare (i.e the bleeding heart)

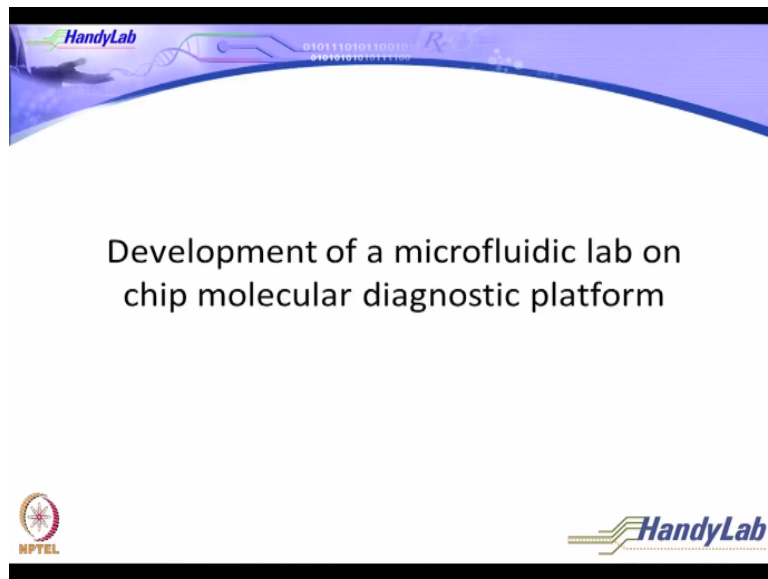
But after I finished my PhD a question that I had to ask myself and a question that you guys will be asking yourself at some point. What is it that really drives you and what is it that makes you really happy because you are going to spend more hours in a day doing that than not doing it. So find out what really drives you okay. If it is photography that is great, if it is

making boatloads of money that is great whatever it is but find out what it is and then go after that and if you need to take some time off to figure that out, do it.

So mine were I was really interested in interdisciplinary state of the art work, so I did not want to do just medicine, just science, just engineering. I liked all that stuff coming together. I am a geek basically and I also wanted to be able to impact individual patients and there is no joy like seeing a patient come up to you and say you know what sir I had no idea what was wrong with my kid but you guys have figured it out.

There is nothing like it, I think that is an amazing sort of motivation to keep you going through the day and the night. So these were my two motivations, they do not have to be your motivation. I am just saying figure out what yours is whatever it is is fine but take the time to find out.

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So the first thing we did was HandyLab. So HandyLab was developing a microfluidic lab-on-a-chip diagnostic platform. So lot of the stuff Sanjeeva has been teaching you about how you work with DNA, etc. These things are done in a lab and there are lots of people in the lab with lots of expensive equipment. If you look at a lab from the top, it is basically unit operations of fluid moving from one place to another.

So you take some liquid a blood sample, it goes into the labs. Somebody might heat it up or cool it down or centrifuge it, stick it on the machine you measure the pH or you measure the

conductivity or you measure the optical properties you combine it with something else. These are all unit operations.

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What we did was automate all those unit operations on a microfluidic cartridge that is a network of tubes and pipes inside a thin little chip. Unfortunately, I did not bring it with me today and the idea was to have a diagnosis in a completely automated manner at micro scale in a very low time.

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So one of the first test that we developed in the US was a test for Group B Strep. Now Group B Strep is an infection that infects pregnant women around the time of delivery and it can cause meningitis in the child and the child can die. The thing with that diseases that it can be



treated with a simple single dose of penicillin up to four hours before the child is born but the older test took 72 hours to do by growing it on a culture microbiological culture.

So you had to go in between 38 weeks, 37 to 38 weeks to get a swab, 72 hours later they would tell you if it is not and then they would give you antibiotics. Now you can lose the infection in that amount of time or gain a fresh infection. So that is a little bit of a problem. So we develop this test that gives a diagnosis in 45 minutes. It takes the sample, extracts the DNA from it, mixes it with all the reagents which are inside that cartridge, optics everything, built-in and you get a result positive or negative in 45 minutes okay.

So that to actually pull off something like that we needed lots of diverse areas of expertise to come together.

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**HandyLab Platform**

- Microfluidic cartridge design and manufacture
- On chip thermo-pneumatic propulsion, and control
- Semiconductor heating
- On chip thermal cycling
- On chip lyophilized reagent storage
- On chip sample preparation
- Fluorescence optical detection
- Integrated control system and data analysis

The slide features several images: a microfluidic cartridge, a green cartridge, a fluorescence image, a graph of temperature cycles, a microfluidic chip, a vial, and a bar chart showing data analysis results. The NPTEL logo is visible in the bottom left corner.

So one is microfluidic cartridges, designing them and manufacturing them. They have to be something that can be built for individual patients not something that only works at 1 million dollar lab. Patients have to be able to get it. All the stuff that we moved on the chips was through thermo-pneumatic propulsion and control. So that means we would heat up areas of the chip, air pockets above that would expand and push liquid.

By firing many of these different types of pumps on the chip, we could mix liquids together. We had gates and valves, so phase transition materials that are solid at one temperature but when you heat it they melt and then you push an air pocket through, so you can open a



channel that was closed and the reverse when you have a closed channel or you can close a channel by forcing in wax from the side.

So wax that is solid at normal temperature but liquid when you heat it, so wax gates, valves, etc. So this is like I do not know if you can see it on this particular video here but yeah can you see that that is the heating profile of the chip. So we are heating different areas of the chip, it is almost like one of those predator kind of movies but this is a thermal image of the chip and we are pumping different areas, mixing liquids together.

This is thermal cycling for PCR. Here is some liquids mixing, right there you can see liquids mixing on the chip. So basically what is happening in a big lab is now happening in an automated manner out there.

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**Multidisciplinary team & IP creation**

Chemistry, Genetics, Biochemistry, Business, Molecular Biology, Physics, Electrical Engineering, Chemical Engineering, Mechanical Engineering, Industrial Operations

**United States Patent**  
 Patent No.: US 8,479,586 B2  
 Date of Patent: Jun. 15, 2013

**United States Patent**  
 Patent No.: US 8,842,863 B2  
 Date of Patent: Oct. 7, 2014

**United States Patent**  
 Patent No.: US 9,440,208 B2  
 Date of Patent: May 19, 2017

**United States Patent Application Publication**  
 Pub. No.: US 2007/0210100 A1  
 Pub. Date: Nov. 8, 2007

**NPTL**

As I said again I keep stressing interdisciplinary, so people from physics, people from electrical engineering, mechanical engineering, chemical engineering, molecular biology, business people, genetics all working very tightly together to build all this technology. There was a huge number of patents. I have just shown 4 out here. Large number of patents that were generated along the way.

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**History of success in molecular diagnostics with HandyLab**

1/3/2000 HandyLab

HandyLab Analyzer and Cartridge Kit

BD Max 11/20/2009 BD Acquires

**Affinity Bead based DNA Cleanup**

Raw Clinical Samples

Other Debris (May include inhibitors)  
 ODS caps (Chairs or Samples)  
 Human Epithelial Cells  
 Other Bacteria

2 min Heat & path release all DNA

Released DNA + Other Heat Stable Components

Non-DNA

5-10 min incubation

DNA Affinity Magnetic Beads

Magnetic Separation

DNA + bead Complexes Separated, Contaminants Washed Away

Wash & release

Inhibitor free PCR ready DNA

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**Core Technology**

UM Microfluidics License  
 Micro PCR  
 Microfab Cost Model  
 Injection Molding Cartridge  
 Sample Prep & Lyophilization  
 SNP Demo

Rts10 Started

Confidential

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Home » GenomeWeb Daily News

**Qiagen Licenses HandyLab Extraction Technology**

April 17, 2009  
 By a GenomeWeb staff reporter

NEW YORK (GenomeWeb News) – HandyLab said this week that Qiagen has licensed the firm's extraction technology for use in the sample preparation part of its human papillomavirus assays.

Ann Arbor, Mich.-based HandyLab said its extraction technology would be used with the HPV molecular diagnostic tests on Qiagen's QIAensemble and QIAampophony random access platforms. Financial and other terms of the license were not disclosed.

HandyLab is a privately-held molecular diagnostics company developing products for the decentralization of nucleic acid testing.

From HandyLab

12

And we took nearly 10 years to build this, 9 years to build this. So hard work, there is no substitute for hard work but at the end of the day you can make to be polite a shitload of money. So it is well worth doing and if you are interested in this kind of work, there is a huge reward at the end of it. The technology that we created was globally valuable. So Qiagen which is one of the world's largest nucleic acid sample prep companies actually licensed our patents for their current generation of products.

So the idea is that people who are sitting here are going to make great things in lives in their lives and you know these are examples of things that you can do to be world leaders not just the best in India, you will be world leaders with the kind of work we do. So Becton Dickinson bought over our product after we took it for an FDA and then now market rate globally under the BD MAX brand name.

So having done that I wanted to continue doing that but to do something that was more relevant to our patients here in the context of Indian Healthcare and in India unlike in the United States patients pay out of their own pocket and so they cannot afford the same kind of things that they afford in US.

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## GenePath Dx Mission

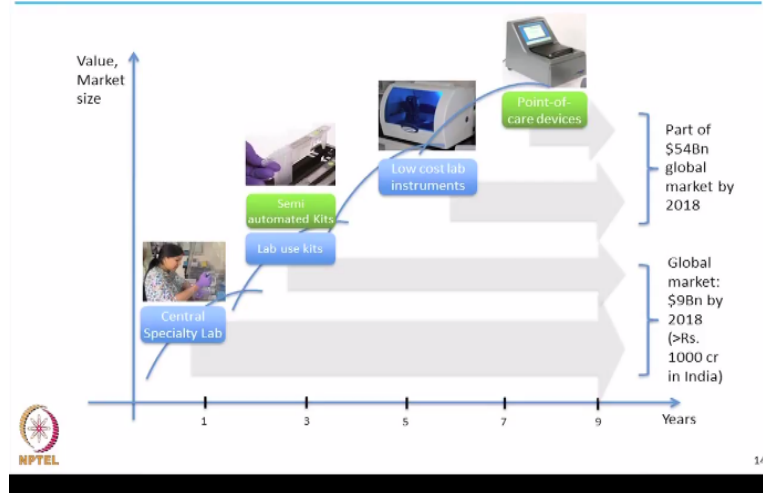
Our mission is to provide access to **high-quality, reliable, relevant, actionable**, validated diagnostics that are **rapid** and **affordable**, by developing and deploying improved and next generation diagnostic services, devices and platforms for human healthcare



So we wanted the same high quality, it had to be reliable but they needed to be relevant to the problems here and they had to be affordable and so we wanted to start by offering services and then go on to building devices and platforms. So go back to building what we did similar to the US but do it in the context of India.

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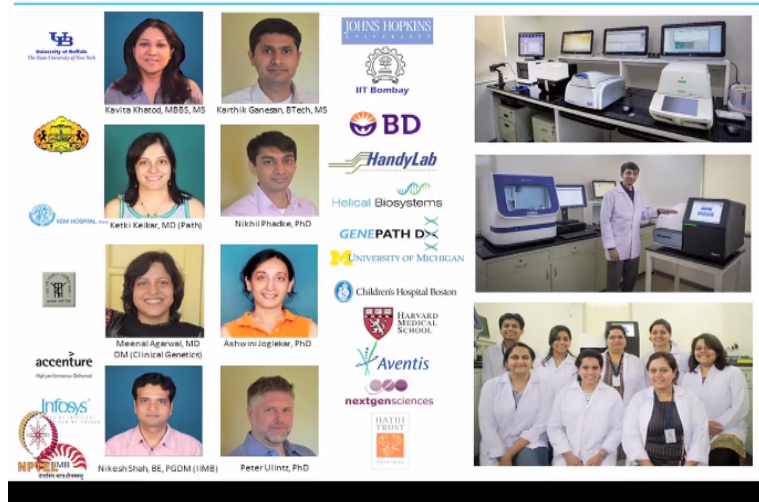
## We plan to become a full spectrum provider



So our company in Pune is now in the service mode and we have just embarked into building devices okay. So service mode is there, it is a big market. We do this, we have over 350 molecular diagnostic tests in our lab right now but we are also starting to build products.

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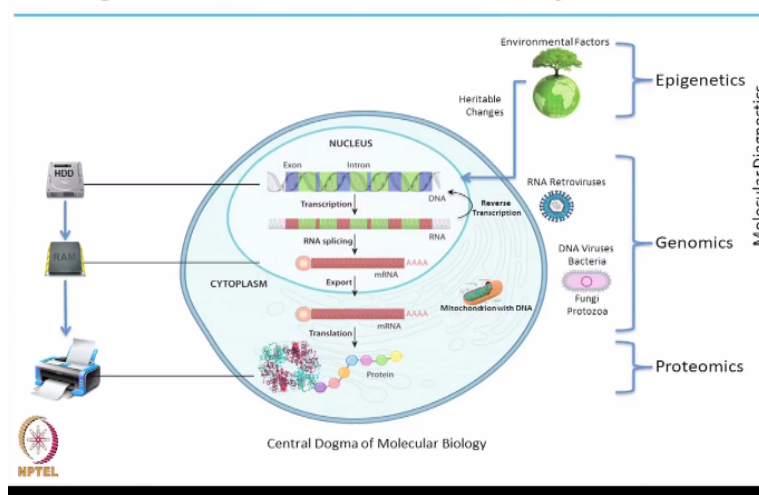
## Multidisciplinary team and state of the art lab



Keep stressing multidisciplinary but here is our team core team. We have MD DM people, we have PhD's. Karthik is from ITB and from Hopkins, I am from the University of Michigan, Meenal and Ketki are MD path and clinical geneticist, Kavita is a mixture of scientists and a clinician, Peter is a bioinformatics person. So we all work very, very closely. We also have PhD students in our lab.

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## Biological Information Flow And Analysis



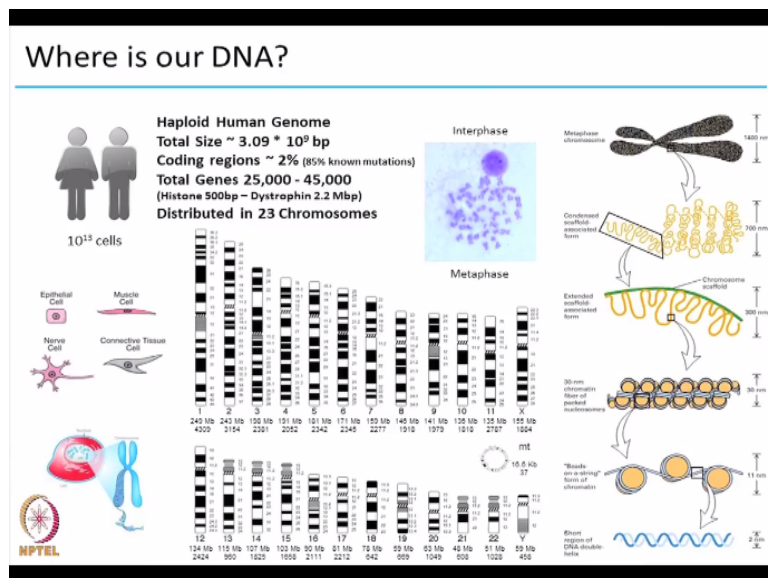
So now to get to some examples and a little background, so Sanjeeva has been teaching you about the basis of you know DNA and the basics of molecular biology. I will just go through it really quick. In human information systems, information usually flows in a direction, usually there are some exceptions. Information goes from DNA to RNA to protein. So DNA is like a hard drive for your system like you buy a new computer, all the information that you need is on the hard drive.

But when you want to use a program like I am using PowerPoint right now, the information that is required is taken up into the RAM. Similarly, in biological system the information that is required at a given point in time is taken up into RNA and finally the effector molecules like the outputs in a computer system like you have a printer or a display, similarly the actual work is done by protein molecules.

So information flows from DNA to RNA to protein. There are some exceptions; retro viruses like HIV change the game. They go from RNA back to DNA and mess up the system. We also have interaction with the environment through processes known as epigenetics and epigenetics actually reversibly writes through the DNA and modulates how the DNA is converted into RNA.

So there is more and more understanding. This is a very exciting area of research right now, so just keep that in mind.

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So where is this DNA and what is this DNA basically? So it is like an encyclopedia for each one of us okay. It is like an encyclopedia with all the information you need in your life essentially how your body is going to react to its environment and this information is a very long message. It is 10 to the 6 base pairs or 10 to the 6 letter of an alphabet and the alphabet has got four types of letters A, T, G and C unlike English which has 26.

Now this big long messages actually divided into 23 volumes from your father and 23 volumes from your mother. So think of it as an encyclopedia that is broken up into many volumes, 23 volumes from your father, 23 from your mother totally 46. Now each one of these volumes is a chromosome, so you heard of the word chromosome. The volumes are the encyclopedia of the chromosome.

Within every chromosome or within every volume, there are paragraphs that make sense and the rest of the paragraph that do not make sense. The parts of the paragraph that make sense are the genes. So we have about 25,000 to 45,000 genes in our system, so that many paragraphs in there. They are distributed amongst the 23 chromosomes and you have 2 copies of each of these chromosomes, one from your father, one from your mother except for the sex chromosomes.

Males have one X and one Y, females have two X's. The interesting thing is that in this entire encyclopedia only about 2% of it has known function today. This 2% is known as the exome and 85% of all mutations in our disease sort of the diseases that we know and understand are located in that 2%. So that 2% becomes very, very important to study. So every time a cell divides this entire encyclopedia gets replicated okay.

Six billion bases are getting replicated every time you make a copy of a cell. Mistakes will happen, we have machines to correct those mistakes but sometimes those machines fail and you have the mistakes carried over.

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Where can things go wrong? What do we need to detect?	Analogy (using plain English)
Presence and quantification of foreign nucleic acids (e.g. infectious disease agents like viruses, bacteria, fungi, protozoa)	☹️ THE DOG BIT THE CAT THE CAT ATE THE RAT ☹️ THE DOG BIT THE CAT THE CAT ATE THE RAT
Single nucleotide polymorphisms (SNPs) (e.g. Sickle Cell Anemia)	COW EGG PIG ASS THA DOG BIT THE CAT THE DOG BIT THE BAT
Insertions / Deletions (InDels) & Nucleotide Repeat Polymorphisms – can cause frameshifts (e.g. Fragile X, Huntingtons Disease)	THE EDO GBI TTH ECA T THD OGB ITT HEC AT
Copy number variations (CNVs) e.g. (Deletion, Duplications such as <i>Her2</i> amplification in some Breast Cancers)	THE DOG BIT THE CAT THE DOG BIT THE CAT THE DOG DOG BIT THE CAT OR THE BIT THE CAT
Inversions (e.g. Factor VIII Haemophilia)	THE GOD BIT THE CAT TAC EHT TIB GOD EHT
Translocations (e.g. chronic myelogenous leukemia CML: Philadelphia chromosome 22 & 9 translocation causes <i>BCR-ABL</i> fusion)	THE DOG BIT THE CAT ATE THE RAT THE CAT ATE THE DOG BIT
Loss of Heterozygosity (LoH) (e.g. retinoblatoma) / Uniparental Disomy	THE DOG BIT THE CAT THE DOG BIT THE CAT THE DOG BIT THE CAT

So what are the things that can go wrong? One is that so I have an English sentence, the dog bit the cat, the cat ate the rat. There is two copies of it, one from the father, one from the mother. This is how the sentence should normally look. You can one have an infection, you can have something coming from outside cow, pig, ass whatever that is not supposed to be in there, if it comes and messes up the meaning of the sentence.

So that is foreign DNA or foreign nucleic acids coming in. You can have spelling mistakes which have single nucleotide polymorphisms SNPs. This is when a single alphabet or a single letter in the alphabet changes. So in the top case you can actually understand the meaning of the sentence, so it does not really matter so much but in the lower one because the C has changed to a B, the entire meaning of the sentence has changed.

So this SNPs can be bad, they can be harmless depending on where they happen that is important. You can have insertions and deletions that is addition of a letter into the message, these are almost always bad. Conditions like Fragile X and Huntington's disease they are really nasty diseases, they are often caused by insertions and deletions and this is because when you say added the C there, the complete meaning of the sentence changes.

Similarly, when you remove something the meaning of the sentence changes, you cannot recognize it anymore. You can have copy number variations where entire messages or parts of the message are replicated. You can have inversions where the message is flipped over. You can have translocations which is like buying a cheap pirated book on the street, you are reading chapter 9. Suddenly it goes to chapter 22.

The sentence in itself makes sense but overall the story is making no sense. So that sentence makes sense makes a protein that does not really work well, hell breaks loose okay. Loss of heterozygosity is very complicated, I will skip it further. So there is a lot of mistakes that can happen, you need to use the right tools to figure out what the mistake is. Somebody will want to use the most complicated tool available and charge a huge (()) (17:51) but that does not make sense.

So the right diagnostician has to understand the context of the disease, the molecular and the clinical background and pick the right test that is going to be relevant to the patient in a clinical relevant time.



**(Refer Slide Time: 18:04)**

**The PCR Process**

The slide illustrates the PCR process through several components: a molecular diagram of Taq polymerase extending a primer on a DNA strand; a graph showing the temperature profile (95°C denaturation, 55°C annealing, 72°C extension) over time; a diagram of the PCR cycle (denaturation, annealing, extension); a photograph of a thermal cycler machine; a photograph of a person working in a laboratory; a photograph of a PCR block; and a photograph of a gel electrophoresis image showing PCR products.

So the most all of our technology is based on two very major things. One is PCR, the polymerase chain reaction. Do you all know PCR? Have you all done it in this class? Briefly, so the polymerase chain reaction was invented by a total slacker known as Kary Mullis who got the Nobel Prize and everybody is still scratching their heads about why he got the Nobel and how he managed to pull off something so brilliant.

But it is basically a system for photocopying DNA, it is like a xerox machine, you take one molecule of DNA it becomes 2, it becomes 4, it becomes 8, 16 and in a very short amount of time half an hour 45 minutes you can make a billion copies of DNA from a single copy in a test tube okay and that is really the basis of almost everything that we do in the lab. The Watson and base Crick pairing that he talked about where A pairs with T, G pairs with C is exploited by the PCR process.

Again this class is too short a time to explain and introduce PCR but it is the cornerstone of everything we do.

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## Real-time / Quantitative PCR (TaqMan)

1. **Polymerization:** A fluorescent reporter (F) dye and a quencher (Q) are attached to the 5' and 3' ends of a TaqMan probe respectively.

2. **Strand displacement:** When the probe is intact, the reporter dye emission is quenched.

3. **Cleavage:** During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.

4. **Polymerization completed:** Once separated from the quencher, the reporter dye emits its characteristic fluorescence.

Fluorescence level

Cycles

Target present

No target

- 5' exonuclease assay
- Use of a probe improves specificity
- Ability to multiplex using 2-6 different fluorescent dyes

You can couple PCR with a fluorescence event and monitor it in real-time. So when there is more DNA you get this S-shaped curve. The more to the left this is the more starting material you had, so you can use that to detect whether infections were present, how much of it was present. If you had a mutation in a gene, what ratio of mutant to non-mutant, so this is probably one of the most important techniques that we use in the lab day-to-day.

(Refer Slide Time: 19:30)

## Traditional Capillary/ Sanger Sequencing

Nucleotides	dA dT dG dC, ddA ddT ddG ddC
Polymerase	
Sequence	X-X-X-T-T-G-S-T-A-T
Complementary Template	Y-Y-Y-A-A-C-W-A-T-A
Primer	X-X-X
Synthesized labeled DNA fragments	X-X-X-T X-X-X-T-T X-X-X-T-T-G X-X-X-T-T-G-C X-X-X-T-T-G-G X-X-X-T-T-G-C-T X-X-X-T-T-G-C-T-A X-X-X-T-T-G-C-T-A-T

Sequencing Reaction

Chromatogram

Capillary Electrophoretic Separation

Shorter fragments      Longer fragments

The other one is capillary sequencing. In capillary sequencing again was a Nobel Prize winning effort for Maxam and Gilbert as well as for Sanger and it is a way to read DNA base by base one by one.

(Refer Slide Time: 19:43)

## From capillary sequencing to next generation sequencing

**Sequencing Reaction**

**Capillary Electrophoretic Separation**

Traditional Gold Standard Sanger Sequencing Used For Human Genome Project ~ 3000 scientists, multiple centers, 6 countries, over 13 years, cost US\$ ~ 3B

**Cost per Genome**

NGS-fueled sequencing capacity growth greater than Moore's Law. Possible to sequence an entire genome under 3 days for cost under US\$ 5000

Adapted from Metzker ML, Nature Reviews Genetics, 2010

Now Sanger sequencing has been the cornerstone of our sequencing abilities for a long, long time and the Human Genome Project which everybody must have heard of hopefully was completed in 2002, it took 3,000 scientists, 13 years and a cost of 3 billion dollars to sequence the first human genome. Many countries across the world they were all working day and night these machines running by Sanger sequencing.

Today, it is possible to sequence a single genome in under 3 days for 5,000 dollars. This has put Moore's law completely to shape and this is because of a series of technologies known as Next Generation Sequencing.

**(Refer Slide Time: 20:17)**

### What is Next (2<sup>nd</sup>) Generation Sequencing (NGS)?

Fragmentation of target DNA

↓

Addition of priming Adaptor Molecules

↓

Clonal Amplification of fragments

↓

Sequencing (by synthesis / ligation)

↓

Raw data capture (optical / ion)

↓

Conversion/deconvolution Assembly to nucleotide base sequence

Adapted from Metzker ML, Nature Reviews Genetics, 2010


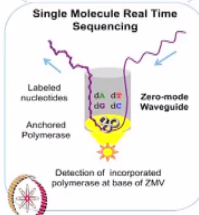
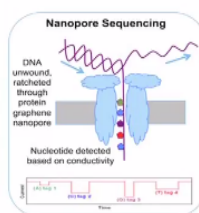
Again explaining Next Gen Sequencing is beyond the context of this class but it is a parallel process. So basically what you do is you take all your DNA, you fragment it into small

pieces, each small piece you clonally amplify and you sequence it individually. So our sequencers in the lab today can do 25 million sequencing reactions in parallel on a chip of this size.

And the newer models that are coming can be 10 or 100 times more powerful than that. So you break your DNA, you add adapter molecules to barcode them, you clonally amplify them, you sequence them in real-time, capture the data and then you put it back all together like a giant jigsaw puzzle. So because of the increase in computational power this has been possible but just think about it, 17 years ago what took 13 years to do, today can be done in under 3 days okay.

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### Single molecule (3<sup>rd</sup> gen) sequencing



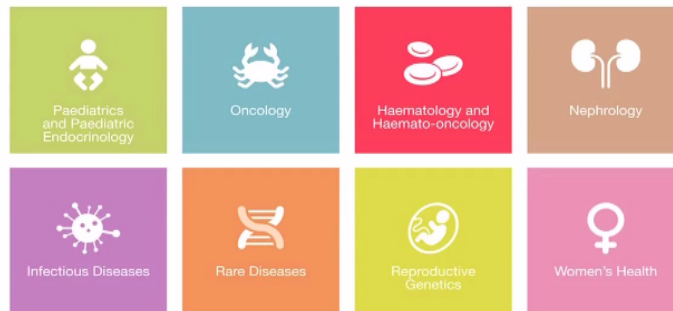
- True single molecule sequencing; long reads without clonal pre-amplification
- Improved sequencing of complex or repetitive genomic regions
- Differentiation between *cis* and *trans* elements
- Epigenetic modification detection
- Further decreased costs

This is just mind blowing as it just boggles the mind and even that is only the start. Now we have this single molecule sequences and we have some of these in our lab which can take a single molecule of DNA, thread it through a nanopore and read out the sequence in matter of seconds. So they are going to further change this.

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## Large Test Portfolio Across Therapeutic Areas

Over 250 molecular diagnostic tests



<http://www.genepathdx.com>

So we use all of this, now this is over 350 tests that we have but we use this for many, many different areas to actually manage patients and I am going to give you very few examples of where this technology is used.

**(Refer Slide Time: 21:32)**

## Some Examples of Personalized Treatment Following Genetic Testing



So one very common use is in hereditary breast and ovarian cancer okay. There are I told you there are about 25,000 to 40,000 genes and every time the DNA gets a cell divides the entire DNA gets copied over. Now there are some machines that prevent errors and BRCA1 and BRCA2 are two examples of those machines that prevent errors. So when there are errors, they fix it.

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## BRCA1 and BRCA2 in Hereditary Breast and Ovarian Cancer

- *BRCA1* & *BRCA2* are tumor suppressor genes - involved in maintaining genomic stability, DNA repair, and pre-mitotic checkpoints. Both show autosomal dominant transmission. Point mutations, small deletions, frameshifting InDels, splice mutations and large genomic rearrangements seen.
- *BRCA1* 17q21. 24 exons (22 coding) 1863 amino acids; ~300 different mutations reported
- *BRCA2* 13q12-13 26 exons 3418 amino acids; ~500 different mutations reported.
- Presence of mutations in either of these genes can increase lifetime chance of being affected to as high as 55-85%.
- Nearly 20 – 30% of breast cancers seem to follow a hereditary pattern, but only about 10% can be attributed to particular genes (mostly *BRCA1/2* which account for 5 – 8%).



about 80% of the people who test for *BRCA1/2* have negative or uninformative (Variants of Unknown Significance – VUS) results.

Identification of Breast and Ovarian Cancer Cluster Regions in the *BRCA* genes in a study of 30,000+ patients. Rebbeck et al./JAMA. 2015;313(13):1347-1361

Unfortunately, sometimes these error correction mechanisms also fail and so if you have a mutation or an error in one of these genes you have as high as an 85% chance of getting cancer okay. It is a very, very high chance and nearly 20 to 30% of all breast cancers and ovarian cancer seem to follow that kind of pattern. So what are the advantages of genetically testing for this?

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## What are the advantages of predictive testing?

- Modify breast cancer surveillance options and age of initial screening
  - Breast mammography & MRI,
  - Pelvic examination & CA-125 for ovarian cancer,
  - Colonoscopy for colon; PSA for prostate.
- Suggest specific risk-reduction measures
  - Consider prophylactic salpingo-oophorectomy after childbearing,
  - Prophylactic bilateral mastectomy,
  - Non-surgical preventive options like chemoprevention e.g. anti-estrogen therapy especially for *BRCA2* mutation carriers
- Offer treatment guidance (e.g. avoidance of radiation-based treatment methods for individuals with a *TP53* mutation).
- Provide customized, gene-specific, treatment options (e.g., PARP-inhibitors and Platinum salts in *BRCA*-mutation carriers).
- Clarify self and familial cancer risks (e.g. secondary breast and ovarian)



identification of other at-risk family members; planning family.

One is that if you know that you have this mutation, you can modify your surveillance options. So you can go in for improve surveillance and this is not only affecting women, men can also get prostate cancer, they can get breast cancer themselves which is far, far more dangerous in men than in women. So we can have specific risk reduction measures. So we can have salpingo-oophorectomies, we can have bilateral mastectomies and advanced surveillance.

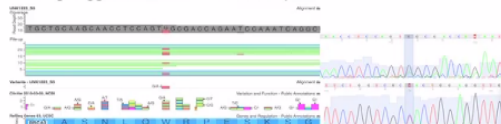
Depending on the mutation they have to decide whether they need to get radiation or not get radiation. Once they have the cancer, we can change the therapy that they use, so if you have a BRCA mutation, we can use platinum based salts, we can use PARP-inhibitors which are very new exciting drugs.

Some of our patients who have been using them were in sort of almost terminal stages and now they have got a fresh lease of life and we can figure the risk to other family members also.

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### Genetic testing in Oncology: HBOC family example 1

Case of papillary serous ovarian carcinoma at age 65; in remission after chemotherapy; two subsequent relapses thereafter. Strong family history of ovarian and breast cancer. Genetic testing suggested after second relapse.



**BRCA2 exon 25 Pathogenic terminator mutation (SNP) detected c.9380G>A [p.Trp3127Ter] (Heterozygous)**

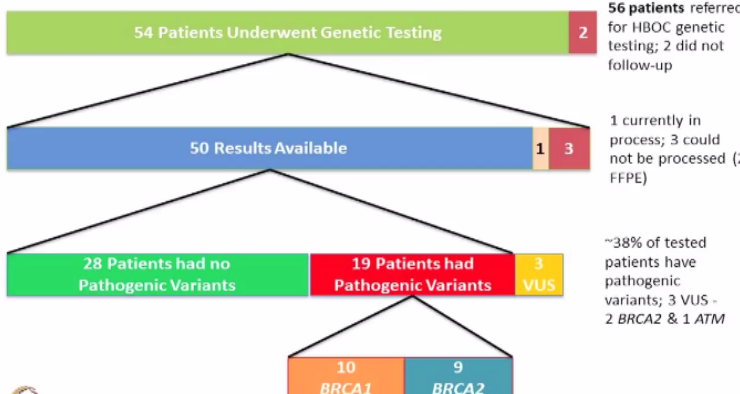
**Modification of treatment – PARP inhibitor Olaparib and Platinum compounds.**

- Targeted sequencing for the *BRCA2* c.9380G>A [p.Trp3127Ter] mutation for three family members.
- Mutation detected in all 3 by NGS and bidirectional capillary sequencing in three family members
- Various option suggested for each by oncologist based on age and reproductive history

I would not get into details of individual patients because I think we are running out on time.

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### Genetic testing in oncology: HBOC testing results summary



56 patients referred for HBOC genetic testing; 2 did not follow-up	2
54 Patients Underwent Genetic Testing	
50 Results Available	1 3
28 Patients had no Pathogenic Variants	
19 Patients had Pathogenic Variants	3 VUS
10 BRCA1	9 BRCA2

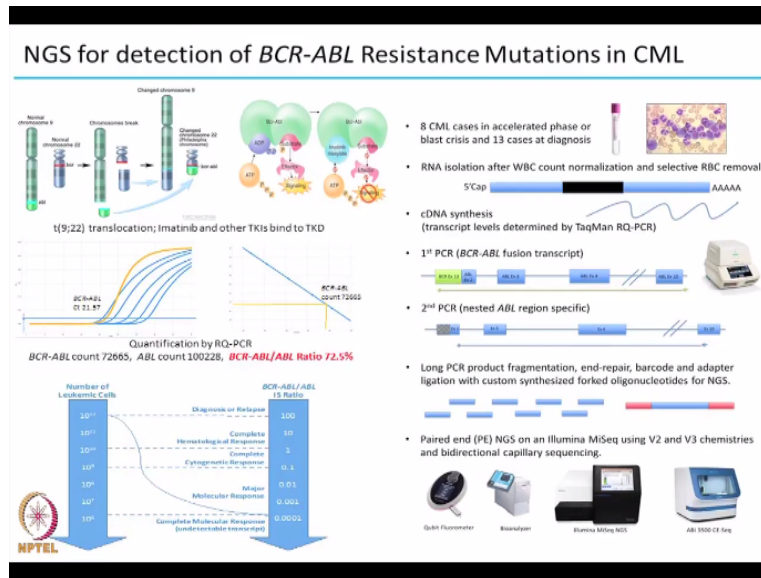
~38% of tested patients have pathogenic variants; 3 VUS - 2 *BRCA2* & 1 *ATM*

Many of the patients diagnosed with mutations, who we have data for, had a change in clinical management (surgical intervention, chemo plan and/or surveillance & family testing).



But just the summary is basically of all the patients we have tested nearly 40% of them had a change in clinical management after they did genetic testing. So you do the testing and what you would have done by the old method of medicine is no longer done, they would have some new treatment options.

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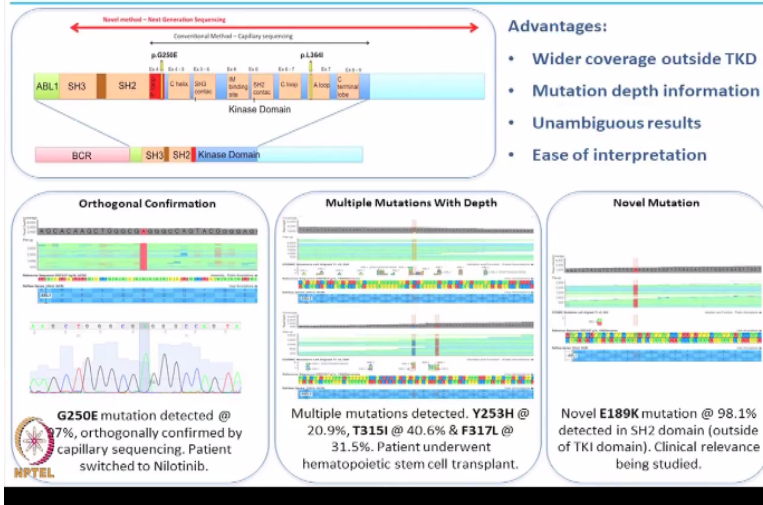


Another very nice example, use of genetic testing is in CML which is chronic myeloid leukemia. Chronic myeloid leukemia up to 15-20 years ago used to kill over 90% of the people who got it. Today, thanks to a drug known as imatinib, 98% of people have a normal healthy life. It is absolutely like magic but to prescribe imatinib, you have to show that your chromosome 9 and 22 have fused and we have a genetic test to do that.

We test the patients; find out if they have that fusion, if they have that fusion they get imatinib, every 3 months we monitor them to make sure they are responding correctly to that drug. As I said 98% of patients do very well but there are 2% of patients who do not do well.

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## NGS Examples for *BCR-ABL* Resistance Mutations



In that case, we sequence that translocated gene, we find different mutations in them and based on those mutations we switch them to other drugs. So some patients are switched to imatinib, some patients switched to dasatinib, some patients have multiple mutations and for those you need a hematopoietic stem cell transplant but this genetic information is actually being used for personalized medicine on a day-to-day basis and that is pretty, pretty remarkable.

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## Summary of *BCR-ABL* mutations detected by NGS

Case history	<i>BCR-ABL</i> / <i>ABL</i> %	NGS Result (depth)	Interpretation
Case of CML in accelerated phase	53.03%	p.G250E (96.5%)	Reduced sensitivity to Imatinib (11.7 fold dose increase). Sensitive to Nilotinib and Dasatinib.
Case of CML for 11 years; in accelerated phase.	44.48%	p.G250E (97%)	Reduced sensitivity to Imatinib (11.7 fold dose increase). Patient switched to Tasigna (Nilotinib) after test.
Case of CML in accelerated phase	72.5%	p.G250E (94%)	Reduced sensitivity to Imatinib (11.7 fold dose increase). Patient switched to Dasatinib after test.
Case of CML with myelofibrosis	74.21%	p.L366I (77.8%)	Rare mutation; resistant to Imatinib. Limited data for sensitivity to other TKIs. Dasatinib has been tried before.
Known case of CML (with complex translocation between chromosomes 9, 22 and 8) on Imatinib for 4 years	74.6%	p.T315I (95.7%)	Mutation causes resistance to Imatinib, Nilotinib and Dasatinib; sensitive to third line TKI - Ponatinib.
Case of CML in accelerated phase	92.88%	p.F359V (86.8%)	Resistance to Imatinib and Nilotinib. Suggested Dasatinib.
Case of CML in accelerated phase	78.64%	p.M244V (65.7%)	Reduced response to Imatinib.
Case of CML in accelerated phase	59.87%	p.M351T (97.8%)	Reduced response to Imatinib.
Case of CML in accelerated phase	72.33%	p.E189 (95%)	Novel mutation in SH2 domain likely pathogenic, but clinical relevance unclear.
Suspected case of CML in accelerated phase	1% p.T210 major (variants also tested; FISH recommended)	p.Y253H (20.9%), p.T315I (40.6%), p.F317L (31.5%)	Resistance to multiple TKIs. Hematopoietic stem-cell transplant carried out.
Case of CML in accelerated phase	95.85%	p.M244V (77.4%)	Reduced sensitivity to Imatinib.
Case of CML in accelerated phase	70.97%	p.Y253H (10%)	Clinical impact of low level mutation being studied.
Case of CML in accelerated phase	72.33%	p.E189K (98.1%)	Novel mutation in SH2 domain. Pathogenicity being evaluated.

Treatment modified in 13 out of 18 (72%) cases after mutation testing.

So I give you an example from solid tumors and example from leukemia blood cancer.

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### Genetic testing in Endocrinology (Neonatal Diabetes)

**Neonatal Diabetes (very high sugar at 1 month)**  
**Initially managed with 2 insulin shots per day**

ATP-sensitive potassium channel ( $K_{ATP}$ ) consists of two subunits - SUR1 (*ABCC8*) and Kir6.2 (*KCNJ11*).

Many mutations in these genes are implicated in NDM  
*KCNJ11* located 11p15.1 – size 4000bp  
*ABCC8* located 11p15.1 – size 84,000bp

**Detected two missense mutations in *ABCC8* gene G111R and R826W**

**Switched to oral sulfonylurea (glibenclamide)**

**Loss of function/inactivating G111R**  
 (likely inherited congenital hyperinsulinism (inherited from asymptomatic father))

**Gain of function R826W**  
 transient neonatal diabetes (inherited from asymptomatic mother)

**ABCC8 gene product (SUR1)**

This is also used in endocrine system, so here is an example of a 2-month old baby, very high sugar at one month, initially being managed with two insulin shots a day which is very, very painful for a you know family of a small child. We went ahead and sequenced and found that there were two missense mutations in this *ABCC8* gene. We knew how this gene behaved, so the child could be switched to an oral sulfonylurea.

The child stopped the insulin dosage and now is being orally treated, doing absolutely fine. So this is an example of this in action.

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### Genetic testing in Endocrinology (Monogenic Obesity)

Fig ref: Gregory S. Barsh, I. Sadaf Farooqi and Stephen O'Rahilly, *Nature* 404, 644-651 (6 April 2000)

- MC4R primarily expressed in the brain; reduced MC4R levels result in increased appetite and decreased satiety.
- The leptin gene product plays a significant role in regulation of human appetite, and mutations in the protein can result in a limited or non-functional protein that can cause early onset obesity and other endocrine disturbances.

**Example 1: Profoundly obese child (weight 28.7 kg at 2.5 years) from a consanguineous marriage in a family with a history of obesity.**

**Example 2: 1.8 yr old patient from a consanguineous family with excessive weight gain (18kg) and hyperphagia with minimal levels of plasma leptin.**

Deletion (frameshift) results in chain termination at AA residue 21 of MC4R Protein

Deletion (frameshift) results in chain termination at AA residue 48 of Leptin Protein

**Genetic testing revealed the presence of a protein terminating deletion in the *MC4R* gene in example 1 and a novel protein terminating deletion in the *LEP* gene in example 2. The patient with the *LEP* mutation can benefit from treatment with recombinant Leptin.**

Another example in endocrine, two obese children profoundly obese 28.7 kilos at two-and-a-half years, another one 18 kilos at 1.8 years went in and sequenced the *MC4R* and leptin genes. One patient has an *MC4R* mutation. The other one had a leptin mutation. Recombinant

leptin is now available, so the child who had leptin mutation we could actually start that child on recombinant leptin and that child is doing fine. So there is an example of that.

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**Genetic testing in Infectious Diseases**

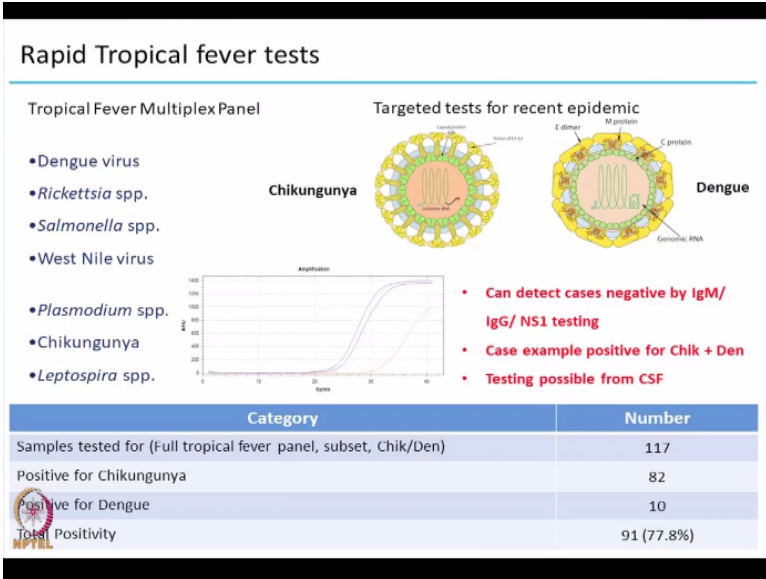
**16S rRNA, 18S rRNA and Metagenomic Testing for Unknown Bacteria and Fungi**

Case history	Sample	PCR	Pathogen detected
Case of recurrent Breast abscesses, Caseating granulomas. Differential MTB vs. Granulomatous mastitis. Suspected Actinomycete co-pathogen?	Bacterial culture	16S rRNA V3/V4 region	<i>Streptomyces somnensis</i> (known to cause deep tissue infection; responds to amikacin, imipenem and Rifampicin).
Unidentified ocular infection in 48 year old female. Started on antibacterials, antifungals and antivirals.	Vitreous tap fluid	16S rRNA V3/V4 & 18S rRNA ITS regions	<i>Stenotrophomonas maltophilia</i> (Known ocular pathogen. Resistant to most beta-lactams and aminoglycosides; variable susceptibility to fluoroquinolones. Subsequently confirmed by culture).
Endophthalmitis in 50 year old male. Primary immunodeficiency disorder (? Chronic Granulomatous disease) in 3 year old.	Aqueous tap Gastric lavage	Entire 16S rRNA gene 18S rRNA ITS region	<i>Sphingomonas sp</i> (sensitive to common antibiotics). <i>Candida albicans</i> (commonly detected fungal agent in esophageal and stomach samples, which can cause esophagitis, gastritis and gastric ulcers in immunocompromised individuals).

Completely switching tracks, we also do a lot of testing for infectious diseases using the same kind of DNA techniques. Here is an example of a patient who had an eye infection okay. They were worried that this person would go blind, so they started the patient on antibacterials, antifungals and antiviral.

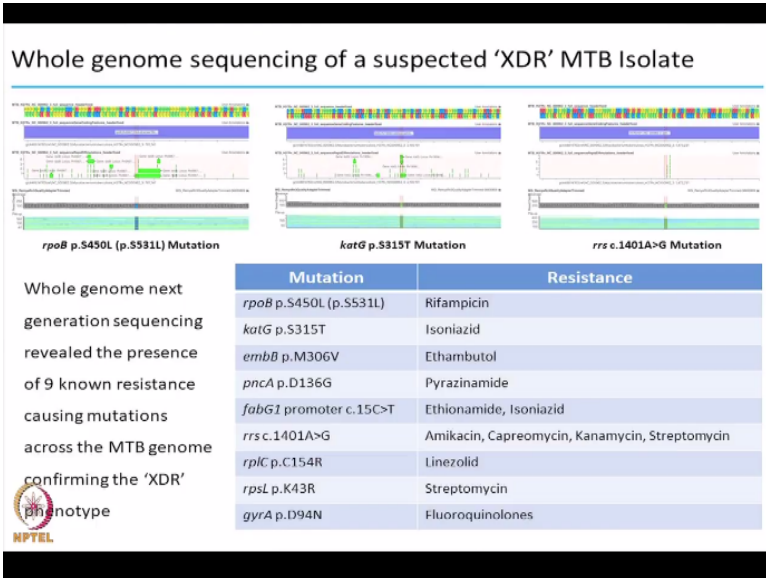
This is like throwing the kitchen sink at the patient. In about 12 hours we showed that it was not a fungus, it was not a virus but it was a bacterium and within 48 hours we showed it was *Stenotrophomonas maltophilia*. This means that the drugs that it has a different drug reaction profile, so they had to switch the drug, patient got fine but they would have missed that because they only pick that up after 5 days by conventional cultural methods. So these genetic tools are really, really important.

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Recently, there has been a spate of Chikungunya and Dengue in the state. In Pune, it is an insane epidemic and again conventional testing was missing in huge amount, using DNA and RNA based testing we were able to show almost 70 almost 80% of our patients were positive and this can be diagnosed instantly like within a day after you get the infection. One of our patients was even Chikungunya and Dengue positive, so that is great.

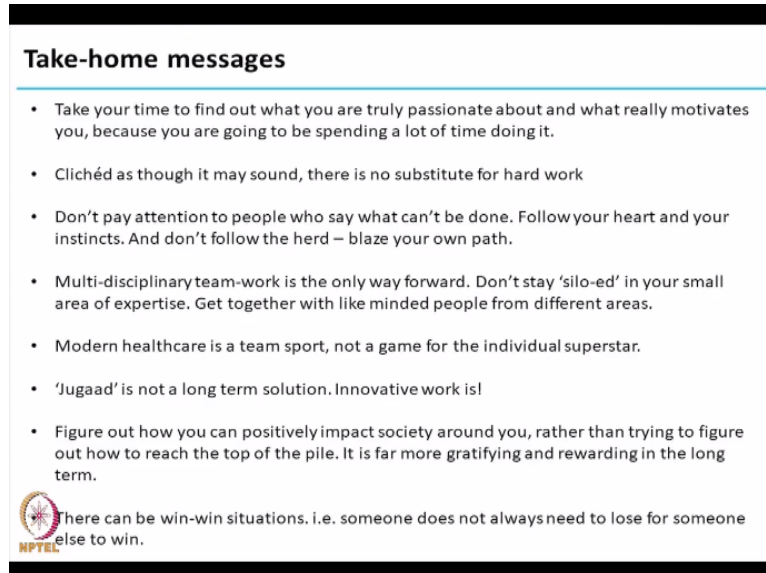
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And then at the start I told you that when I started my career, we sequenced the genome of *Caulobacter crescentus* as part of my PhD thesis and that took us nearly two years to do. Recently, we had a patient from Hinduja Hospital in Bombay with extended drug extensively drug-resistant tuberculosis and they said can you do anything for us and said sure we can try and sequence the genome.


So for about 12,000 rupees we sequence the entire genome of that patient, we found 9 mutations that are corresponding to the XDR phenotype and we did this in under two days okay.

**(Refer Slide Time: 27:25)**



**Take-home messages**

- Take your time to find out what you are truly passionate about and what really motivates you, because you are going to be spending a lot of time doing it.
- Clichéd as though it may sound, there is no substitute for hard work
- Don't pay attention to people who say what can't be done. Follow your heart and your instincts. And don't follow the herd – blaze your own path.
- Multi-disciplinary team-work is the only way forward. Don't stay 'silo-ed' in your small area of expertise. Get together with like minded people from different areas.
- Modern healthcare is a team sport, not a game for the individual superstar.
- 'Jugaad' is not a long term solution. Innovative work is!
- Figure out how you can positively impact society around you, rather than trying to figure out how to reach the top of the pile. It is far more gratifying and rewarding in the long term.

 There can be win-win situations. i.e. someone does not always need to lose for someone else to win.

So what took millions of dollars when I was a student today can be done in days or like 12,000 rupees is like 200 dollars. So it is just incredible. So I just snapshots of examples I can go on for hours but I did not want to waste your time. I have some take-home messages for you guys before I go away. Take your time to find out what you are truly passionate about and what really motivates you because you are going to be spending a lot of time doing it and clichéd as though it might sound.

There is absolutely no substitute for hard work and do not pay attention to people around you who say you cannot do something you can almost always do something. When we started building that chip, everyone said sample prep is not possible, we succeeded in doing it. Follow your heart and your instincts and do not follow the herd you know blaze your own paths.

Multidisciplinary teamwork is the only way to go forward. Get do not stay side out in your small areas, get together with smart like-minded people from other areas no matter what they are. Modern healthcare is really a team sport and doctors do not know this. They think they are all superstars and they want to score the goal themselves but you need a strong team, you have to all work together and Jugaad is not a long-term solution.



Everybody in India loves to do Jugaad but it just takes us back. You have to innovate and do real hard work. Jugaad might take you 3 places ahead in the queue but the entire line gets pulled backwards so that does not help and really figure out how you can positively impact society around you rather than trying to reach out how you can reach the top of the pile because in a long-run it is much more gratifying and evolving.

And there can be win-win solutions in my experience you know people always think that you have to screw somebody over to come up on top, you do not, everybody can win and be really successful, so I have learned that in my journey. So that is it really. Thank you for your time. Thanks.

**(Refer Slide Time: 29:12)**

## Thank you!

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