LifeStrands
Empowering lives with Genomics

Molecular Pathology: A Rapid Evolution of Technology

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Molecular Diagnostics and Personalised Medicine

Molecular Diagnostics - broad term describing a class of diagnostic tests that assess a person's health at a molecular level

Involves detection and measurement of specific genetic sequences in DNA and RNA, or the proteins they express

Personalised medicine - Tailor made treatment plans for smaller groups and individual patients of specific cancer types, according to the patients' unique genetic make up

Newsweek
June 25, 2001

"…pharmacogenetics promises to target treatment to a patient's genetic profile…"

The Next New Thing

A revolution in genetic research is targeting treatments to patients' unique characteristics. It can mean the difference between life and death. By Sharon Begley

Made-to-Order Medicine

JILL WAS ONLY 2 WHEN the diagnosis came: acute lymphoblastic leukemia (ALL). This rare childhood cancer, the doctors assured her parents, is highly curable with a cocktail of four chemotherapy drugs. But from the very beginning the chemo made Jill acutely ill: her white-cell, red-cell and platelet counts plummeted, and even with biweekly transfusions "her counts kept going lower and lower," says Dr. Mary Relling of St. Jude Children's Research Hospital in Memphis, where Jill was treated. Doctors didn't know whether the leukemia was knocking out her blood production—or whether the chemo itself was. But they had a way to find



- Jill, 2 yrs old, with Acute Lymphoblastic Leukaemia
- ALL- *usually* highly curable with chemotherapy
- But Jill did **not** respond and in fact **deteriorated** with treatment
- WBC, RBC and platelet count *decreased* with treatment
- Doctors don't know whether it was her ALL or chemotherapy at fault
- Further tests - found Jill belonged to a mere 0.3 % population with *TPMT* gene mutation, prevents adequate drug metabolism
- Chemotherapy dose subsequently adjusted - **Jill treated successfully**



Next Frontiers

The Next New Thing

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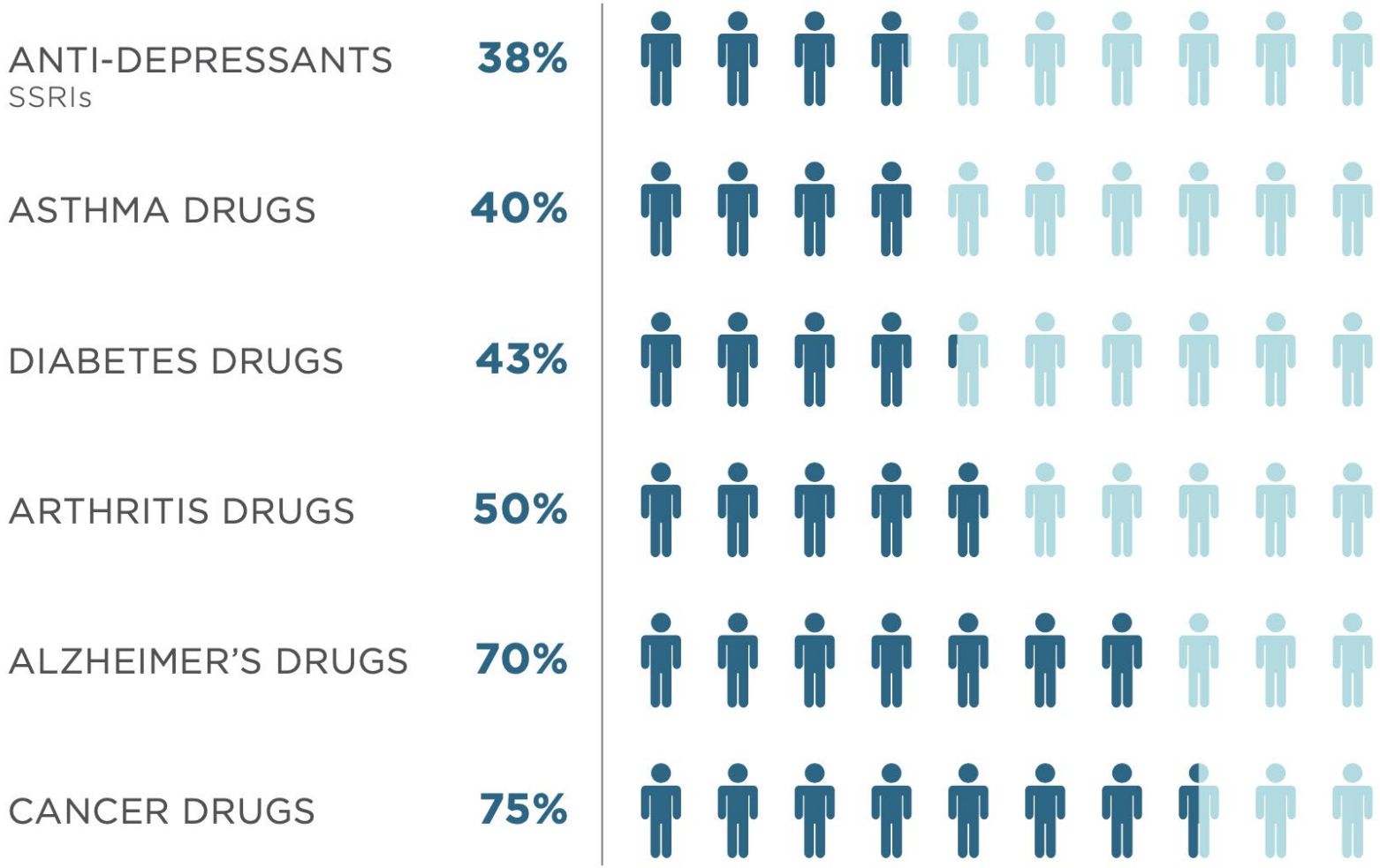
JILL WAS ONLY 2 WHEN the diagnosis came: acute lymphoblastic leukemia (ALL). This rare childhood cancer, the doctors assured her parents, is highly curable with a cocktail of four chemotherapy drugs. But from the very beginning the chemo made Jill acutely ill: her white-cell, red-cell and platelet counts plummeted, and even with biweekly transfusions "her counts kept going lower and lower," says Dr. Mary Relling of St. Jude Children's Research Hospital in Memphis, where Jill was treated. Doctors didn't know whether the leukemia was knocking out her blood production—or whether the chemo itself was. But they had a way to find out. Researchers at St. Jude and at the Mayo Clinic in Rochester, Minn., had recently discovered that patients with a single mistake in a gene called TPMT fail to produce the enzyme that metabolizes the chemo drug, 6-mercaptopurine. As a result, the drug builds up in the body to toxic levels. Jill belonged to the 0.3 percent of the population—one person in 300—that carries two copies of the misspelled TPMT

SPECIAL TREATMENT: Herceptin, a drug developed by Dr. Dennis Slamon, targets a receptor found in only 30 percent of breast cancers

One size does not fit all

- Percentage of the patient population for which a particular drug in a class is ineffective, on average

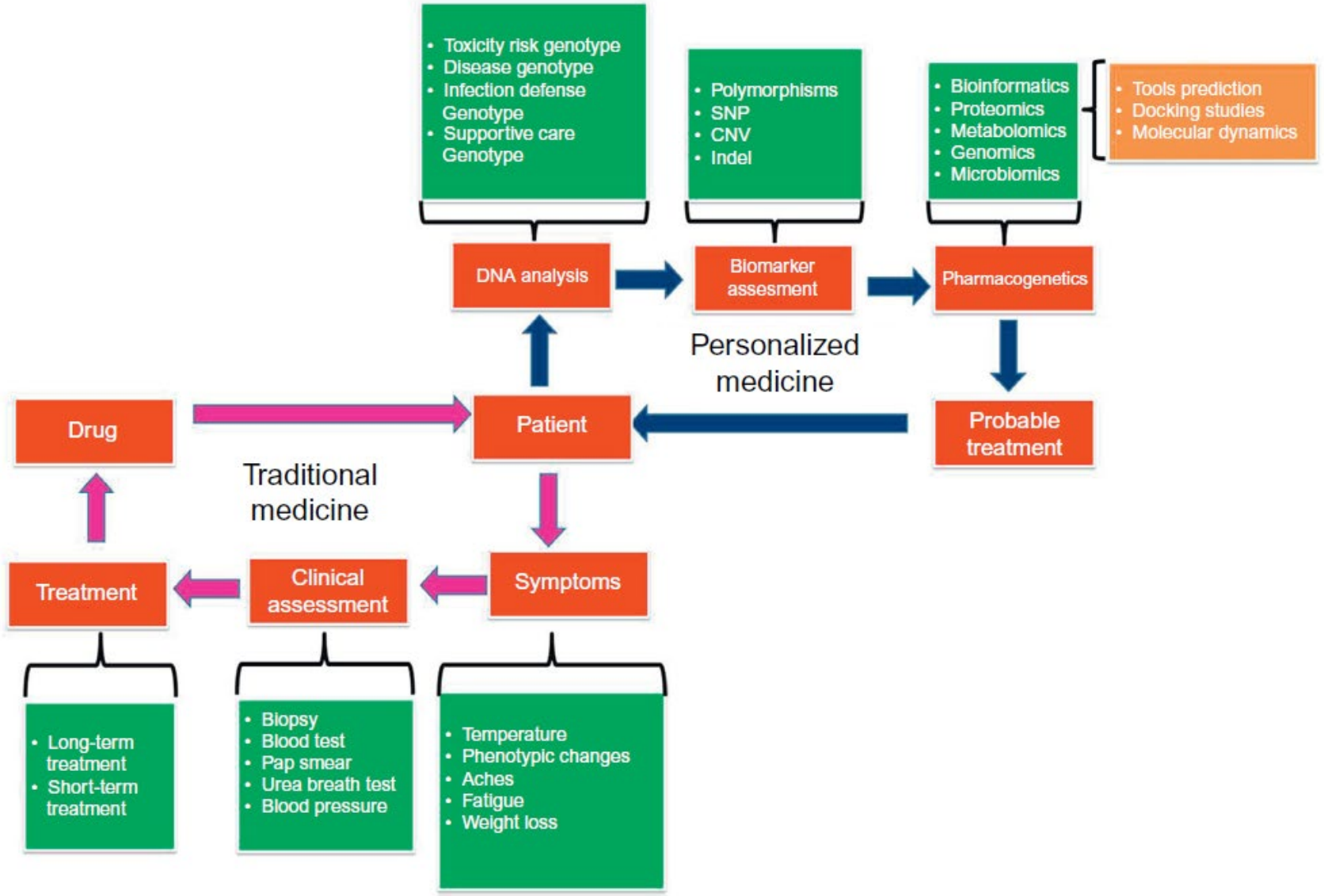


Source: Brian B. Spear, Margo Heath-Chiozzi, Jeffrey Huff, "Clinical Trends in Molecular Medicine," Volume 7, Issue 5, 1 May 2001, pages 201-204.

Out with the old, in with the new…

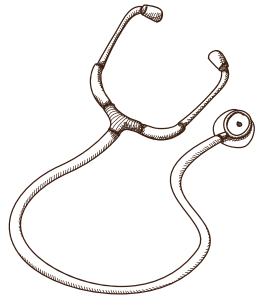
- The ***old*** paradigm of **“empirical medicine”** is slowly disappearing
- There is **wide variability in drug response** seen in many chronic diseases & solid cancers
- The ***new*** paradigm of **“personalised medicine”** breaks the cycle of “trial & error medicine” by linking observations with actions & therapy which result in a more **predictable response**

Traditional versus personalised medicine



Taken from: P.Sneha, C. George Priya Doss. Molecular Dynamics: New Frontier in Personalized Medicine. Ch 7. p190.

Why is precision medicine better?

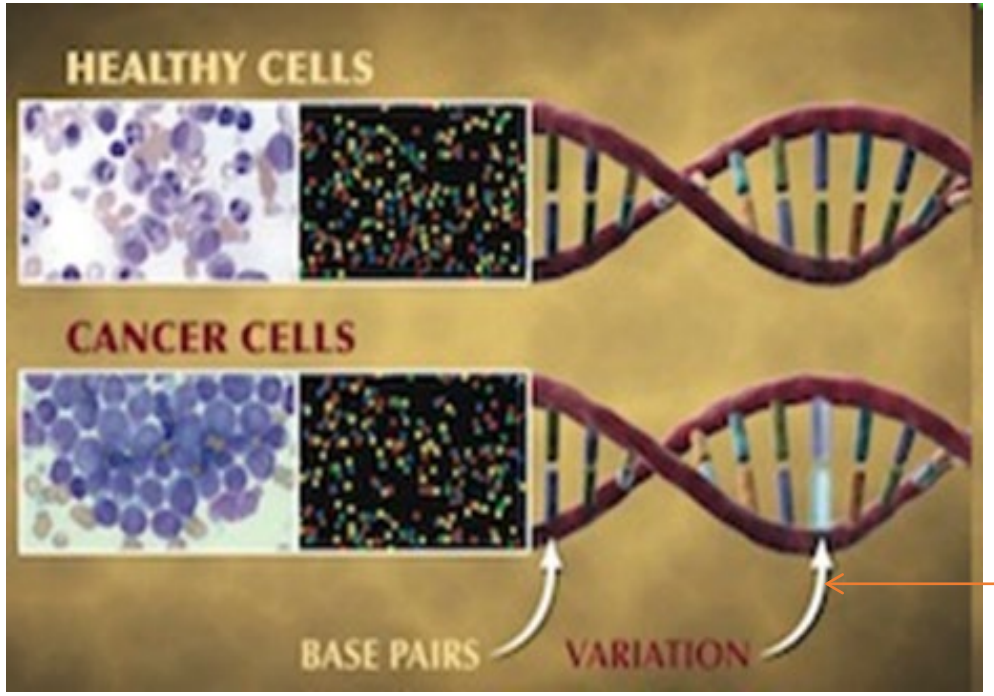


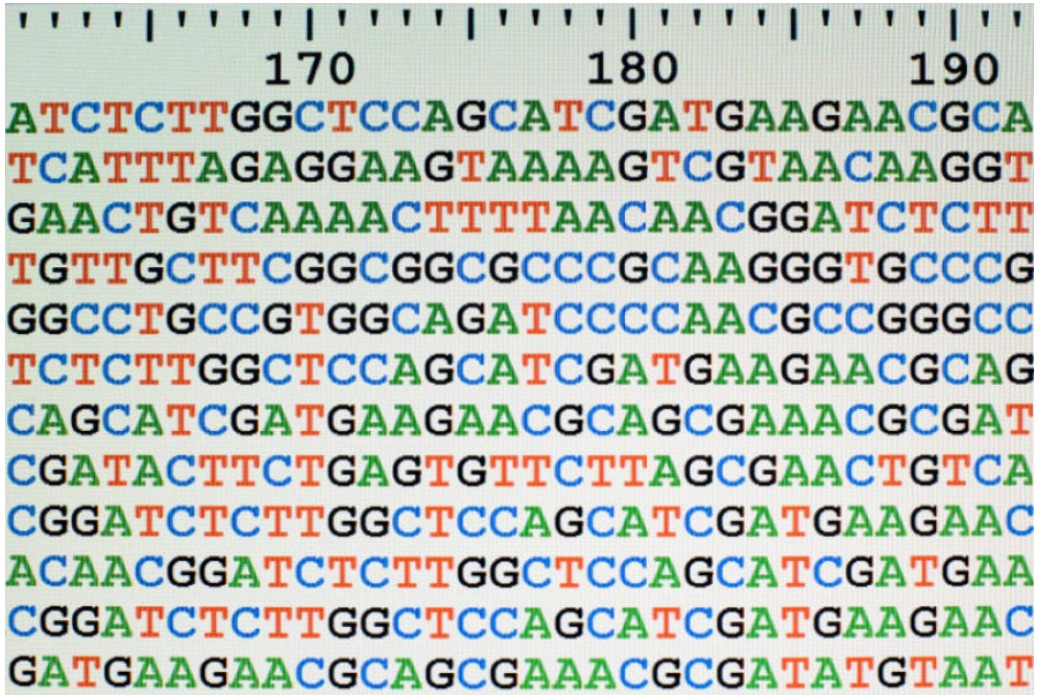
- More effective treatment
- Faster treatment
- Less side effects
- Cost effective
- Better prognosis, and so -

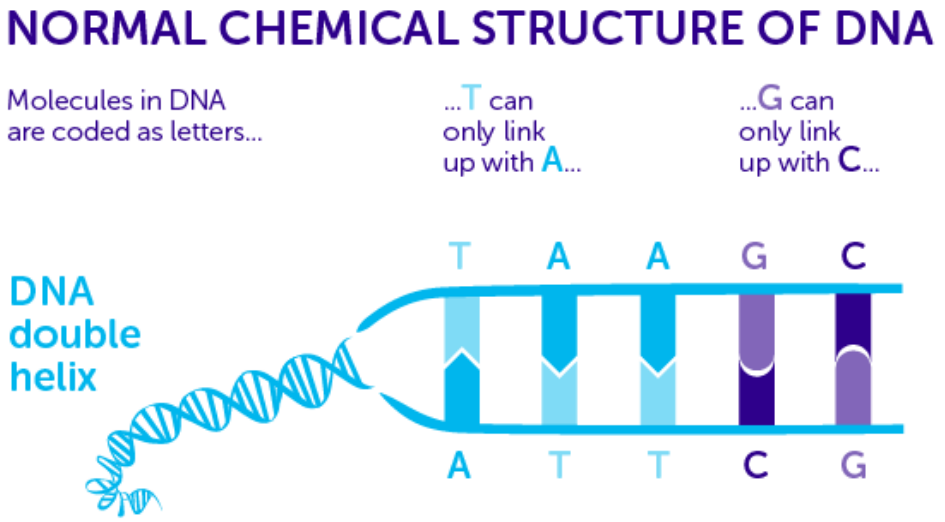
Fast, reliable and affordable genomic/molecular analysis for cancer patients is NOT optional anymore

Composition of the Human Genome

Human genome	**3,000,000,000 bp**
Chromosome	150,000,000 bp
Gene (exons and introns)	50,000 bp
Coding sequence (exons)	3,000 bp
Unit of genetic code	3 bp
Mutation/Polymorphism	1 bp

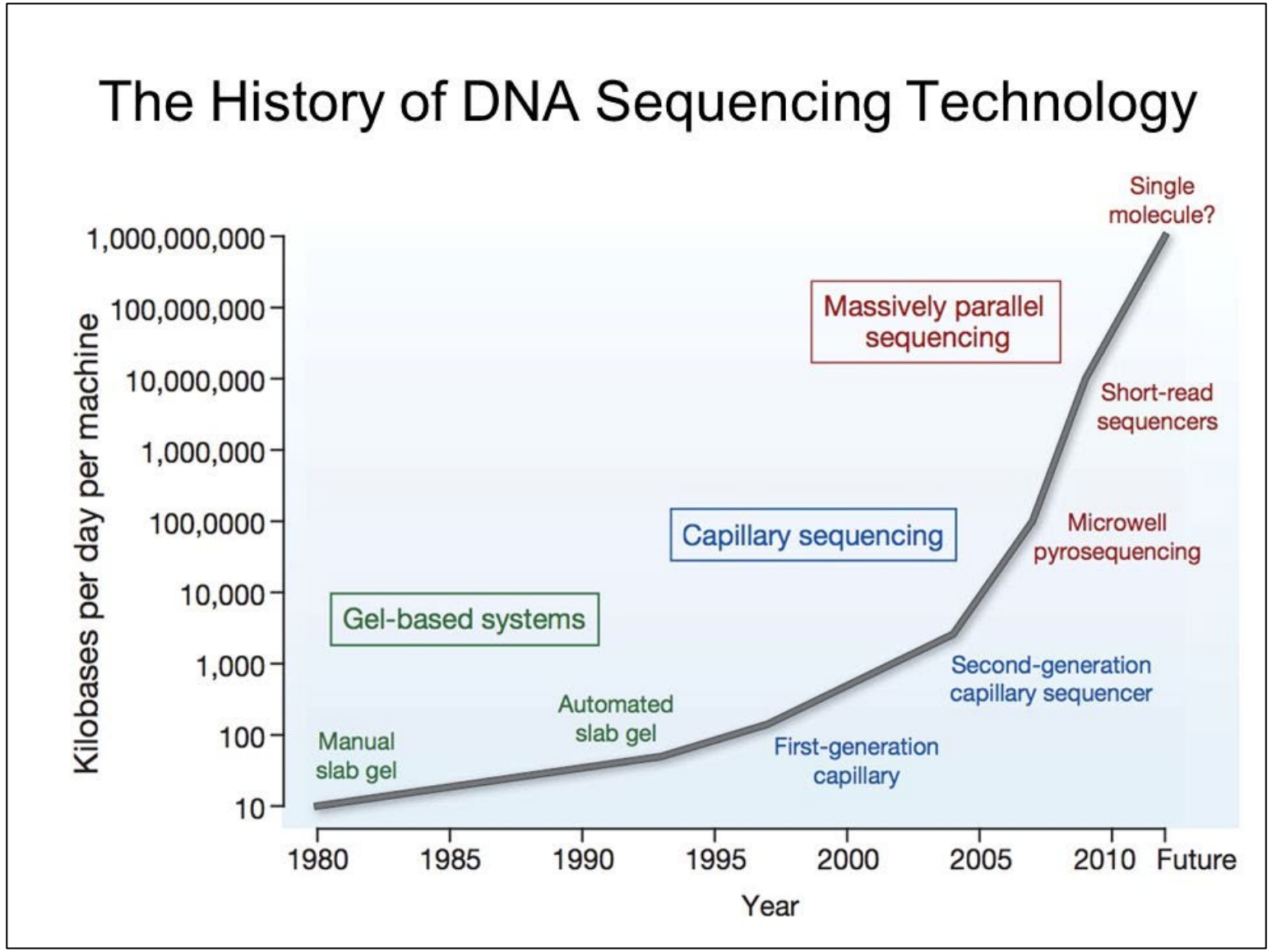






DNA sequencing

- DNA sequencing is a process of determining the precise order of nucleotides within DNA molecule.
- It includes any method or technology that is used to determine the order of the four bases-Adenine (A); Guanine (G); Cytosine (C); Thymine (T)



Gupta AK et al.: Next Generation Sequencing and its Applications. https://doi.org/10.1016/B978-0-12-416002-6.00019-5

Human Genome Project (1993 - 2003)



WANTED

20 Volunteers

to participate in the

Human Genome Project

a very large international scientific research effort.

The goal is to decode the human hereditary information (*human blueprint*) that determines all individual traits inherited from parents. The outcome of the project will have tremendous impact on future progress of medical science and lead to improved diagnosis and treatment of hereditary diseases.

Volunteers will receive information about the project from the Clinical Genetics Service at Roswell Park, and sign a consent form before participating.

No personal information will be maintained or transferred.

Volunteers will provide a one-time donation of a small blood specimen. A small monetary reimbursement will be provided to the participants for their time and effort.

Individuals must be at least 18 years of age.
Persons who have undergone chemotherapy are not eligible.

ROSWELL PARK CANCER INSTITUTE

For more information please contact the
Clinical Genetics Service
845-5720 (9:00 am - 3:00 pm)
March 24 - 26, 1997

Landmark global scientific effort (20 Universities from 6 countries)

Took 13 years and $2.7 billion (USD)

First sequence of human genome was generated

3 billion base pairs (A,T,G,C) and >20,000 genes discovered

Sequencing the Human Genome

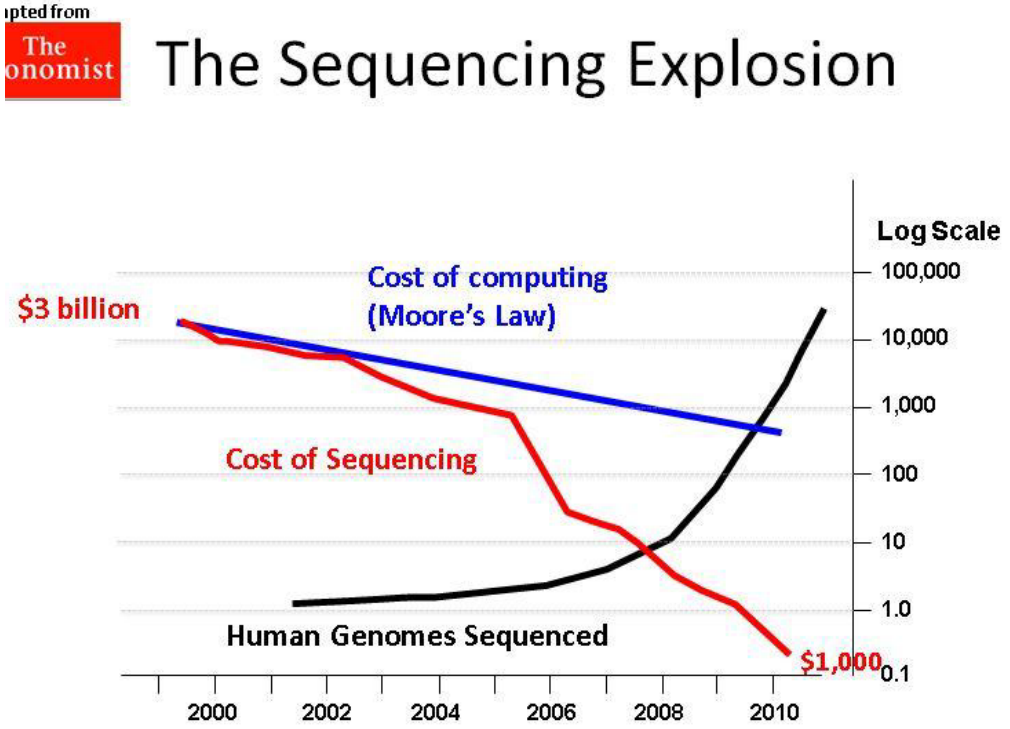
• The human genome project : Started in 1990 by the NIH & the U.S. Department of Energy:

- Sequence the 3 billion bases of the human genome

- Discover the 20,000 – 25,000 human genes

• Lasted 13 years- Cost 3 billion $ (1$/base)

• Presently – WES < $1000



This led to a Genomic Revolution



- Multiple new technological advances from 2008 – present

- The advent of Next Generation Sequencing (NGS) made a huge positive impact on data generation capacity



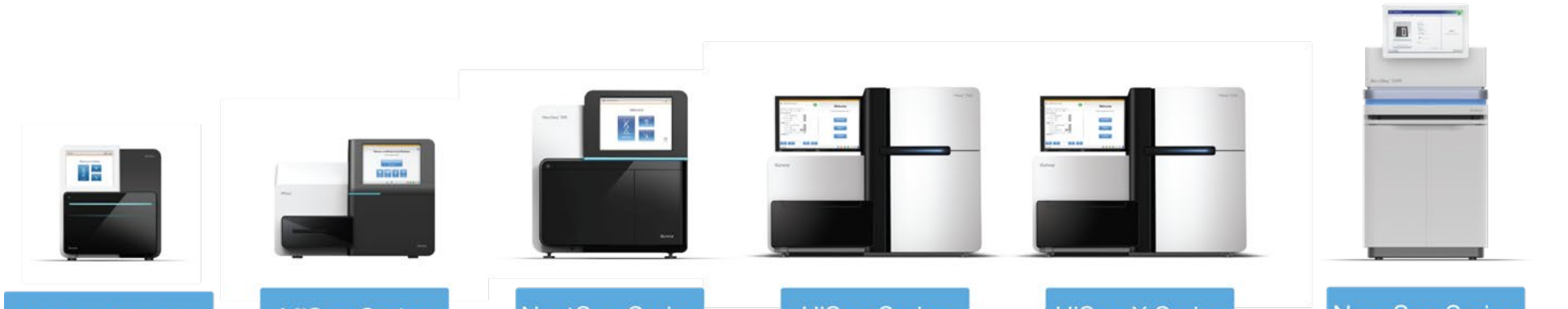


Advantages of NGS platform

- Tiny amount of biopsy tissue or cytology sample,
- Only 10-50ng DNA/RNA
- Accurate
- Sensitive and Specific
- Fast results

Next Generation Sequencing (NGS)

Technologies that share the ability to massively sequence millions of DNA templates in parallel



MiniSeq System
Power and simplicity for targeted sequencing.

MiSeq Series
Small genome and targeted sequencing.

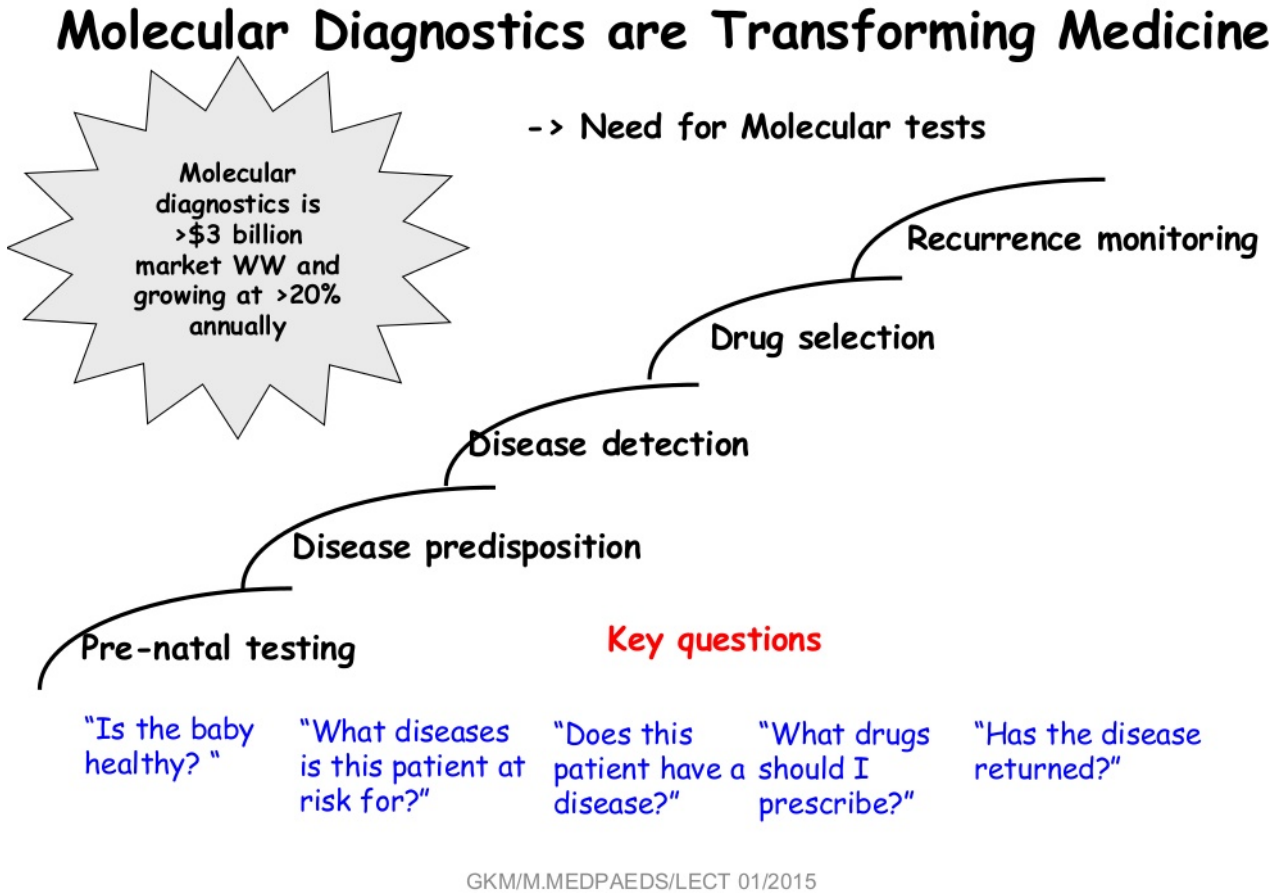
NextSeq Series
Everyday genome, exome transcriptome sequencing, and more.

HiSeq Series
Production-scale genome, exome, transcriptome sequencing, and more.

HiSeq X Series
Population- and production-scale human whole-genome sequencing.

NovaSeq Series
Population- and production-scale genome, exome, transcriptome sequencing, and more.

Significance of molecular diagnostics – A paradigm shift in the way we treat diseases



Advent of Personalised Medicine

- Facilitated by next generation sequencing
- Ability to analyse large parts of gene/entire gene/large parts of genome/whole exome/and whole genome
- At a fraction of the cost compared to earlier technologies with quick turnaround times
- Many samples can be analysed simultaneously in the same tube
- Accuracy/sensitivity/specificity allows clinical use
- There is continuous discovery of new biomarkers/therapeutic targets which are gradually being transitioned into clinical use

Molecular Diagnostics Examples

There are hundreds of examples of molecular diagnostics across this continuum.

	Risk Assessment	Screening	Diagnosis	Staging and Prognosis	Therapy Selection	Monitoring
Description	Diagnostic tests to complement traditional risk factors	Applied to high-risk patient to identify disease early	Use for definitive diagnosis and general cancer typing	Assess severity and/or risk of recurrence Inform adjuvent chemo decision	Used to predict efficacy or safety response to specific treatments	Recurrence monitoring Monotoring for treatment efficacy
Molecular Diagnostic Example	CF carrier testing BRCA1 testing	MRSA HPV	TB CT/NG	OncoType Dx MammaPrint	BRAF KRAS Her2	BCR-ABL HIV viral load

https://dx.advamed.org/sites/dx.advamed.org/files/resource/advameddx_dxinsights_pdf.pdf

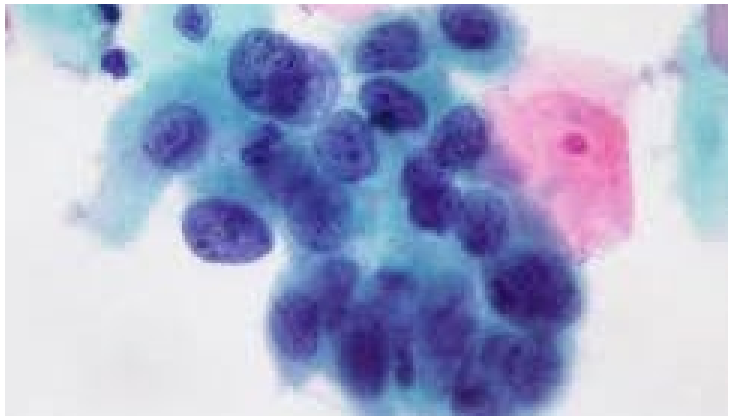
Biomarkers in Oncology with FDA approved therapy

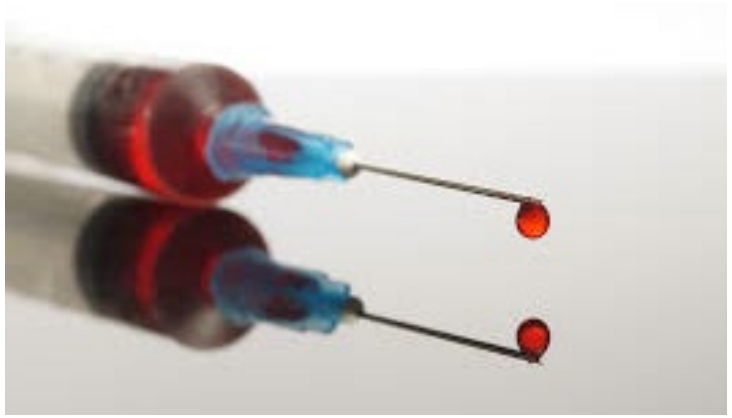
Tumour-agnostic	Tumour mutation burden score (TMB), Microsatellite Instability (MSI) status, Fusions in NTRK1, NTRK2, NTRK3
Lung	EGFR, BRAF, KRAS (G12C), ALK, ROS1, RET, MET , TMB, MSI, NTRK1, NTRK2, NTRK3
Colorectal	RAS (wild-type), BRAF, TMB, MSI, NTRK1, NTRK2, NTRK3
Melanoma	BRAF, TMB, MSI, NTRK1, NTRK2, NTRK3
Ovarian or Fallopian Tube	BRCA1, BRCA2, HRD, TMB, MSI, NTRK1, NTRK2, NTRK3
Breast	BRCA1, BRCA2, HER2, PIK3CA, TMB, MSI, NTRK1, NTRK2, NTRK3
Oesophageal	HER2, TMB, MSI, NTRK1, NTRK2, NTRK3
Gastric	HER2, TMB, MSI, NTRK1, NTRK2, NTRK3
Urinary Bladder	FGFR2, FGFR3, TMB, MSI, NTRK1, NTRK2, NTRK3
Pancreas	BRCA1, BRCA2, TMB, MSI, NTRK1, NTRK2, NTRK3
Prostate	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L, TMB, MSI, NTRK1, NTRK2, NTRK3
Thyroid	RET, TMB, MSI, NTRK1, NTRK2, NTRK3
Cholangiocarcinoma	FGFR2, TMB, MSI, NTRK1, NTRK2, NTRK3
GIST	KIT, TMB, MSI, NTRK1, NTRK2, NTRK3
Sarcoma	Various Fusions, TMB, MSI, NTRK1, NTRK2, NTRK3
Head & Neck (SCC)	TMB, MSI, NTRK1, NTRK2, NTRK3
Brain	TMB, MSI, NTRK1, NTRK2, NTRK3
Cervical Cancer	TMB, MSI, NTRK1, NTRK2, NTRK3
Uterine/Endometrial	TMB, MSI, NTRK1, NTRK2, NTRK3



Tumour material utilized for sequencing





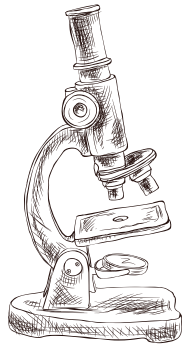




- FFPE
- Cytology
- Blood
- Bone marrow

Challenges with Molecular Testing using Tissue

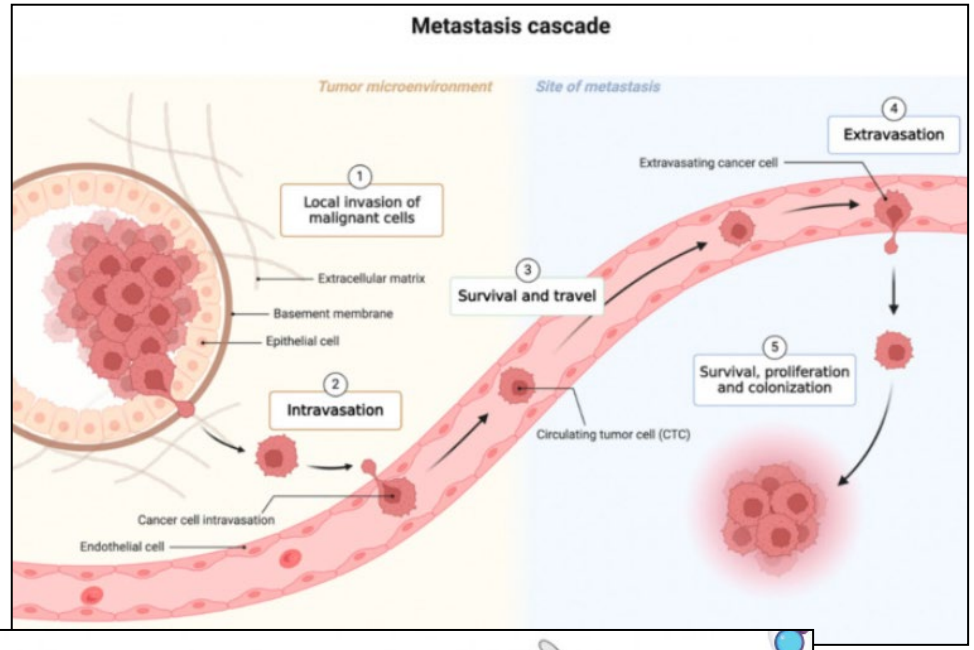
- Advanced disease patients may not be eligible for repeated biopsies
- Patient's performance status, tumour location, disease extent
- Risk of complications with repeat biopsies
- FNA cytology specimens are the only potential source of tissue for molecular testing in many cases – may not be adequate for accurate testing

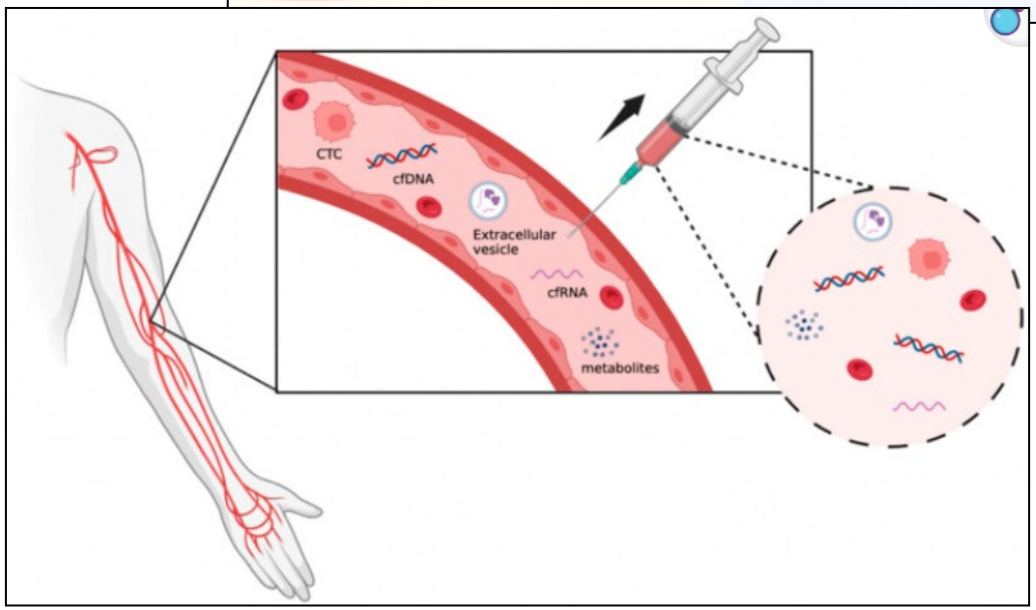


Fintelmann, F.J.; Troschel, F.M.; Kuklinski, M.W.; McDermott, S.; Petranovic, M.; Digumarthy, S.R.; Sharma, A.; Troschel, A.S.; Price, M.C.; Hariri, L.P.; et al. Safety and Success of Repeat Lung Needle Biopsies in Patients with Epidermal Growth Factor Receptor-Mutant Lung Cancer. Oncologist 2019, 24, 1570–1576.

Biology behind Liquid Biopsy

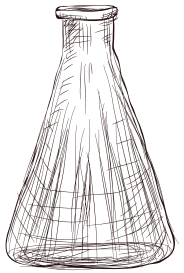
- Components of liquid biopsy are released into body fluids through processes such as apoptosis, necrosis, and secretion
- Circulating cell free DNA/ circulating tumour DNA (cfDNA/ctDNA)
- Circulating tumour cells (CTCs)
- micro-RNA (miRNA)
- Tumour educated platelets (TEPs)
- Extracellular vesicles (EVs)

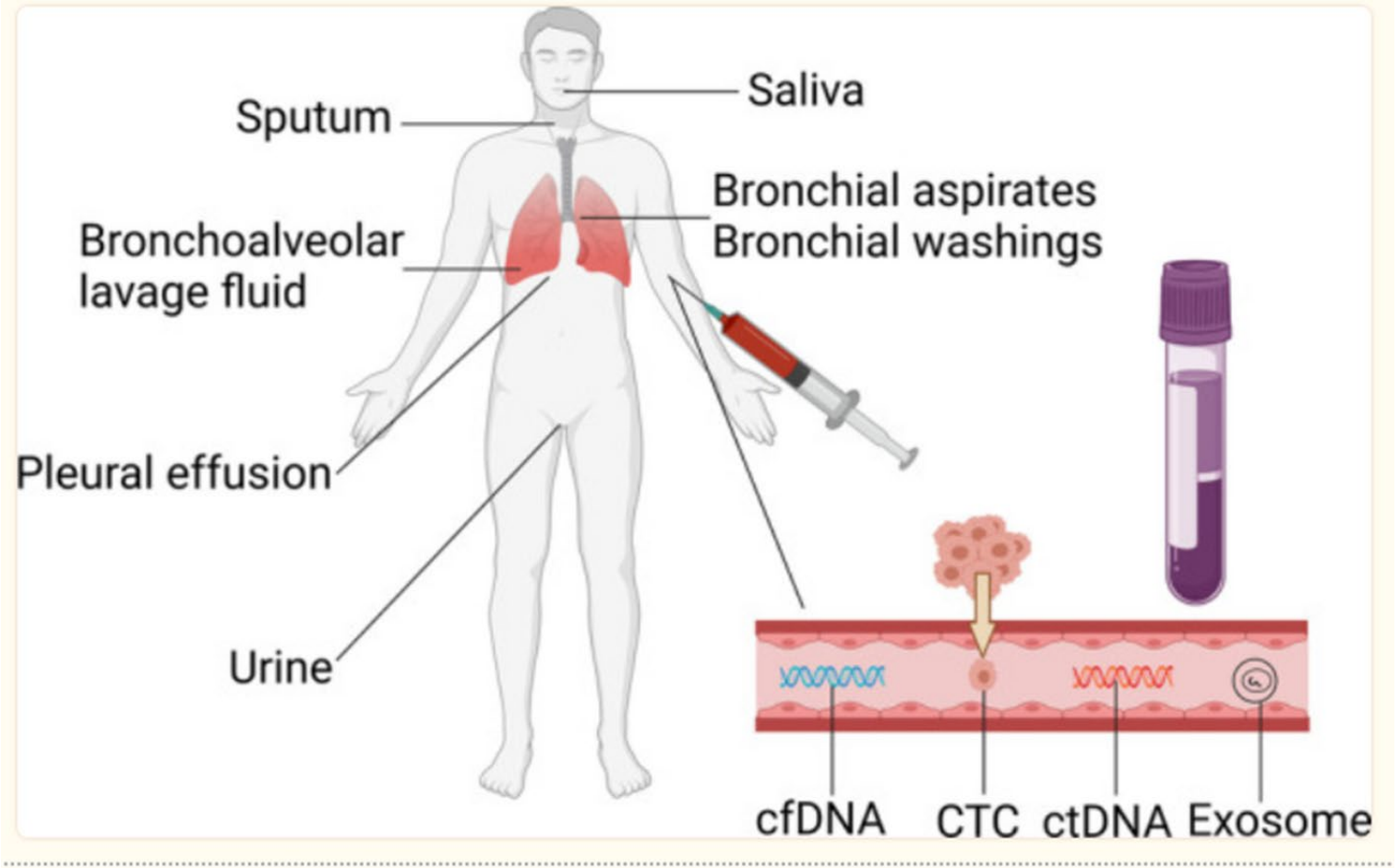




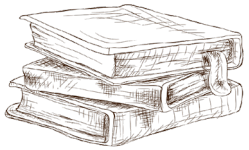
Ho-Yin Ho: Int J Mol Sci. 2024 Aug 6;25(16):8594. doi: 10.3390/ijms25168594

Liquid Biopsy Methods for Lung Cancer

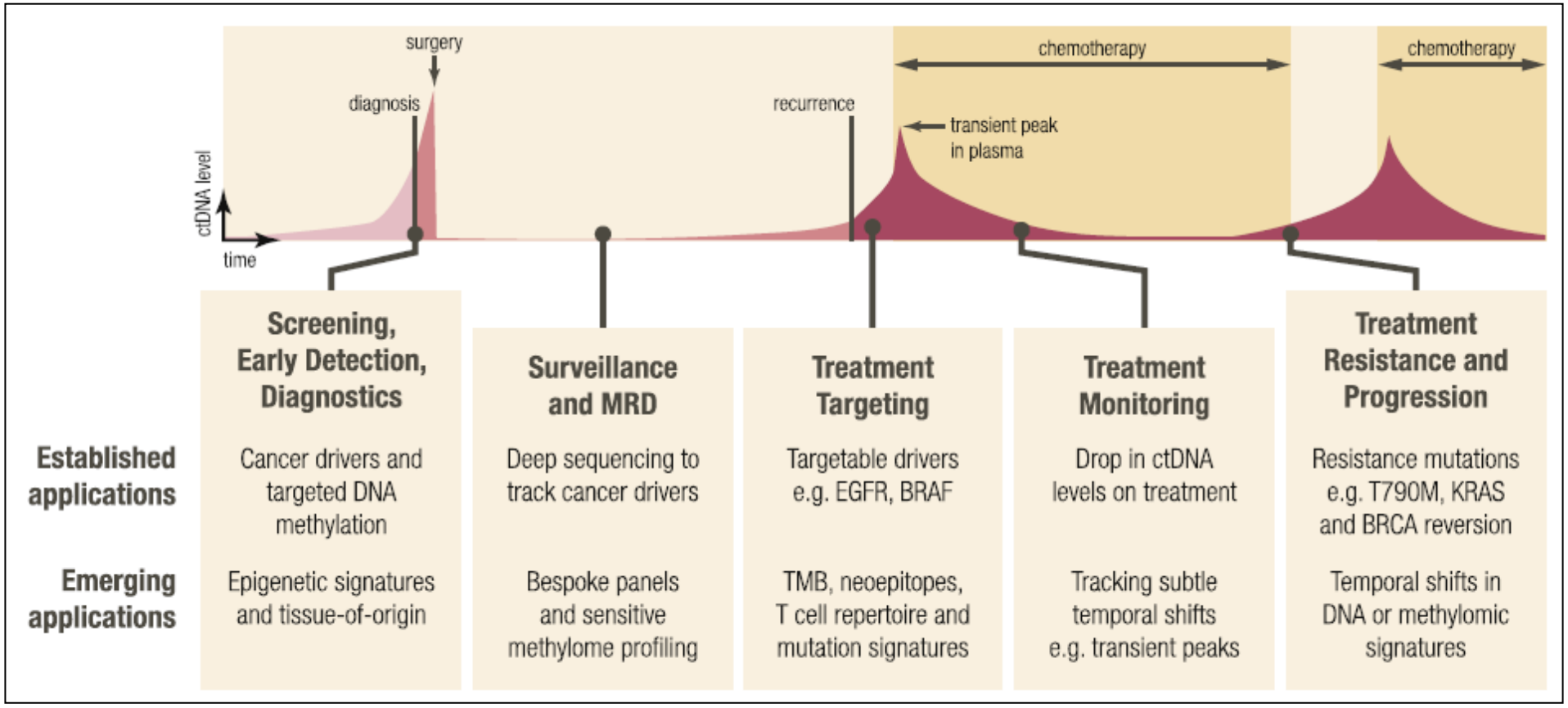




Clin Epigenetics. 2022; 14: 118.doi: 10.1186/s13148-022-01337-0

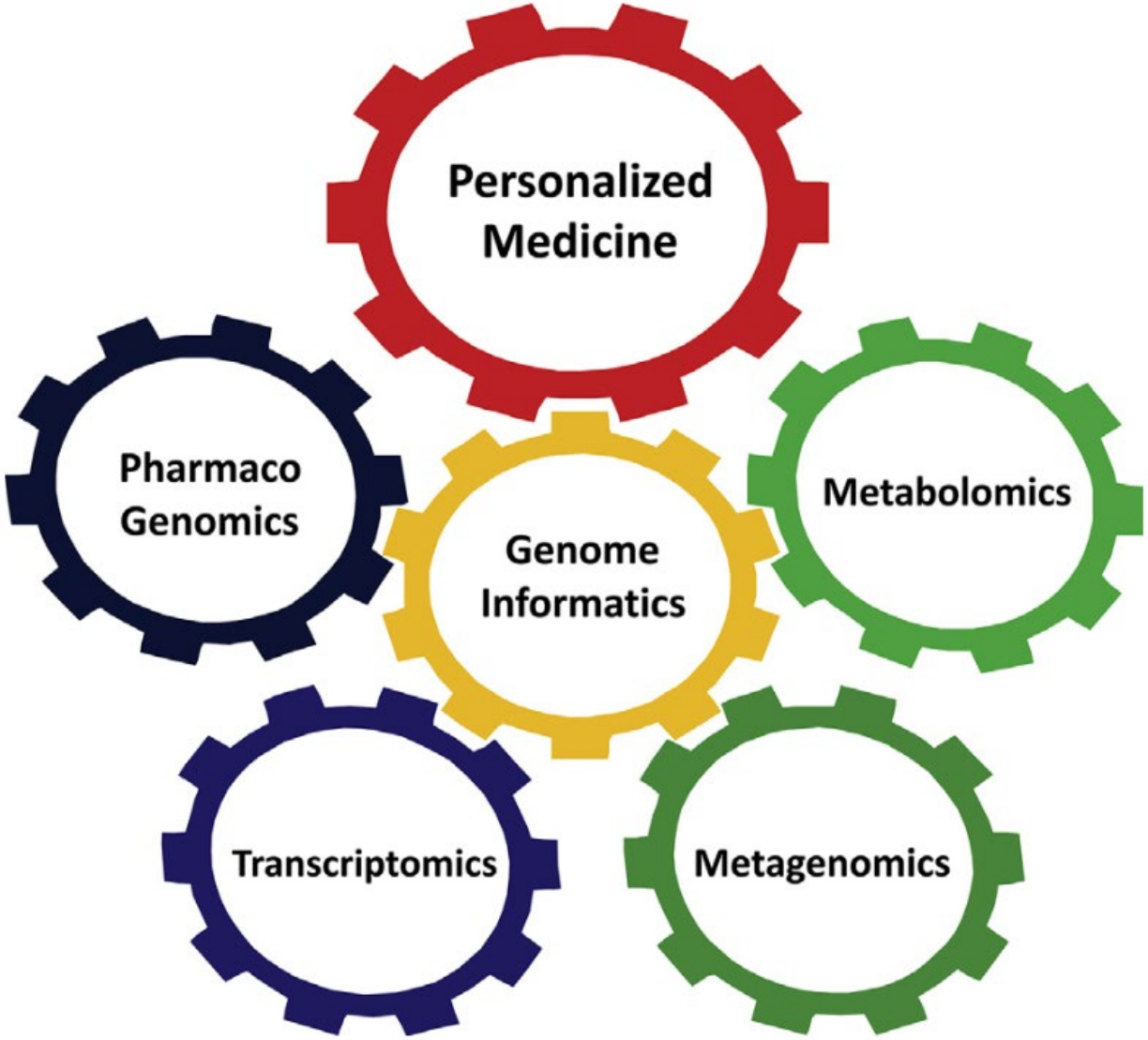


LBx Applications using ctDNA



E.Y. Zhao, S.V. Bratman / Clinical Oncology 32 (2020) 626–631

Personalised medicine – interplay between various omics



Patrinos, Danielson and Ansorge.
https://doi.org/10.1016/B978-0-12-802971-8.00001-8

Pharmacogenetics/genomics



Proteins (encoded by genes) dictate most of the functions that control drug disposition



PGx studies the variable response to drugs due to genetic variation in the genes that metabolise the drugs or drug targets



Genetic testing before drug exposure may predict variable response, avoid adverse/toxic reaction and indicate lack of efficacy

Schutzbank TE. Pharmacogenetics. Amp.2019

Goals for PGx

- Right Drug



- Right Dose

Anesthetic/Analgesic Drug Dosing Chart

Feline Drug Protocol

Weight Lbs.	Drug #1 ml (IM)	Drug #2 ml (IM)	Drug #3 ml (IM)
2.0	0.01	0.05	0.03
2.5	0.02	0.06	0.04
3.0	0.02	0.07	0.04
3.5	0.02	0.08	0.05
4.0	0.03	0.09	0.06
4.5	0.03	0.10	0.07
5.0	0.03	0.11	0.07



- Right Patient



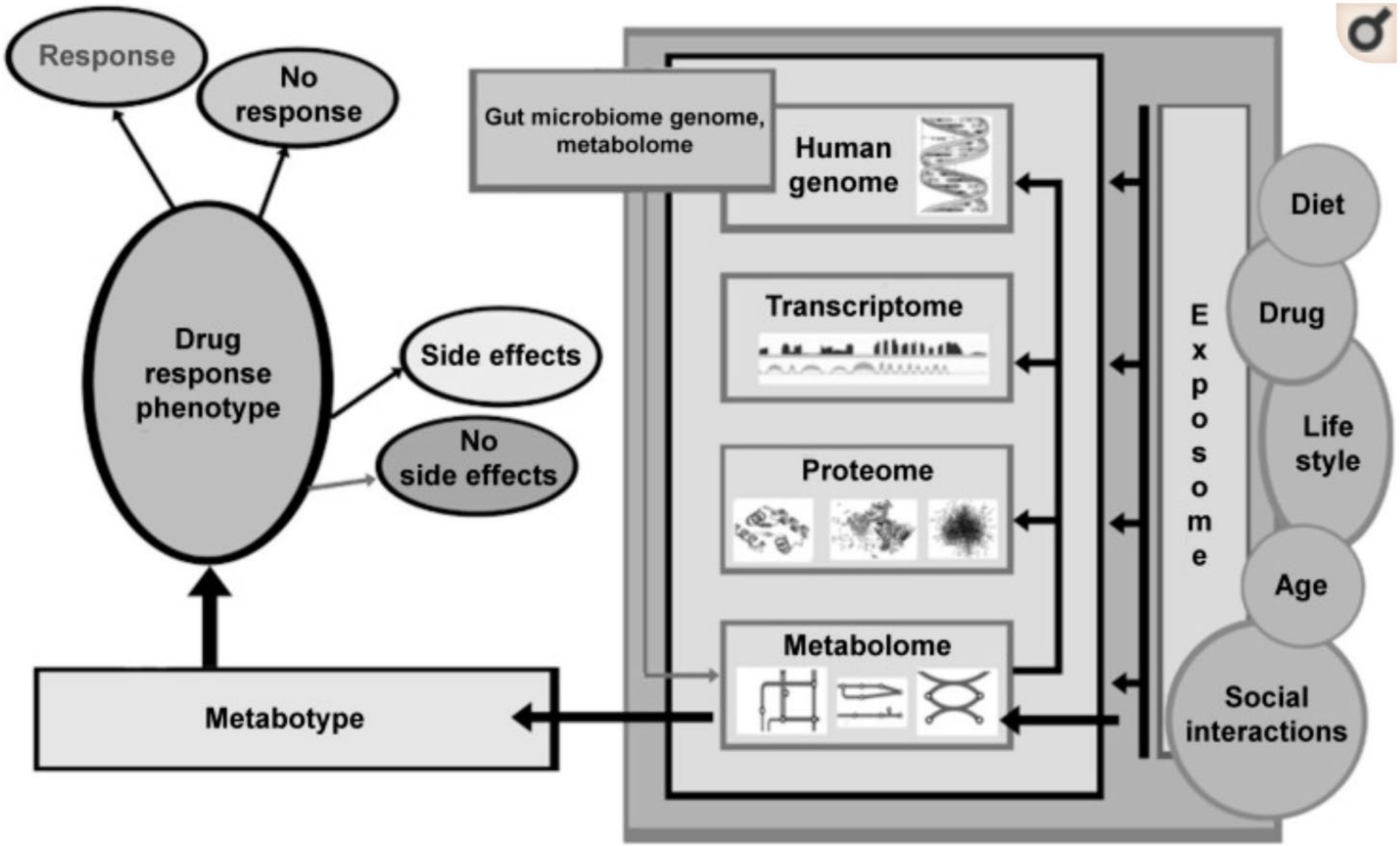
Taken from Schutzbank TE. Pharmacogenetics. Amp.2019

Metabolomics

- Comprehensive analysis of metabolites and low molecular weight molecules in a biological specimen
- Person's metabolic state provides a close representation of that individual's overall health
- This metabolic state reflects what's encoded by the genome and modified by diet, environmental factors and the gut microbiome
- Emerging technology which holds promise for precision medicine – offers detailed characterization of metabolic phenotypes
- Goes well beyond the scope of standard clinical chemistry techniques and capable of precise analyses of hundreds of thousands of metabolites



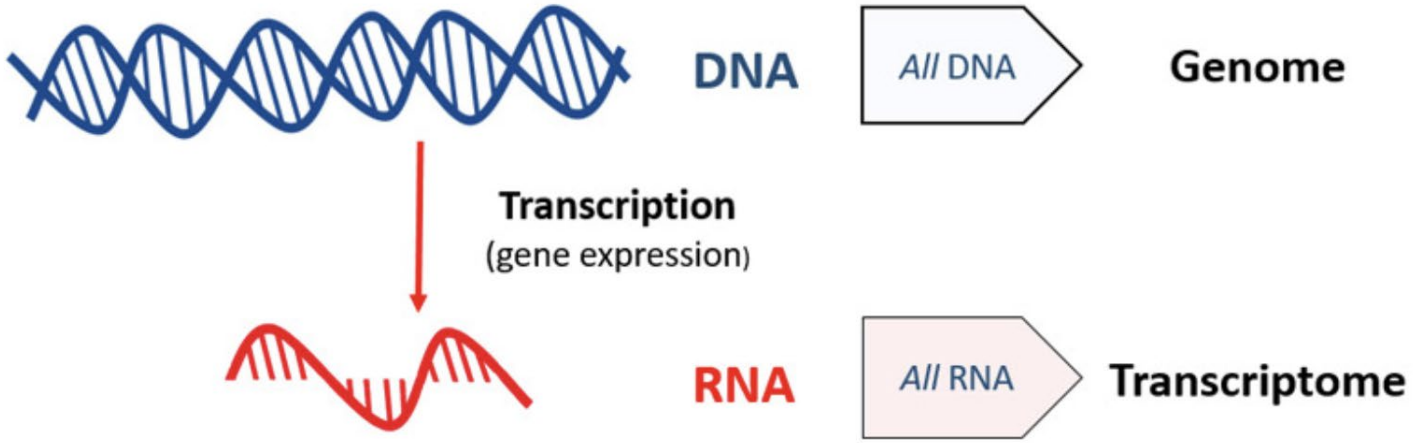
Beger RD. Metabolomics. 2016;12(10):149.



Taken from Beger RD. Metabolomics. 2016;12(10):149.

Transcriptomics

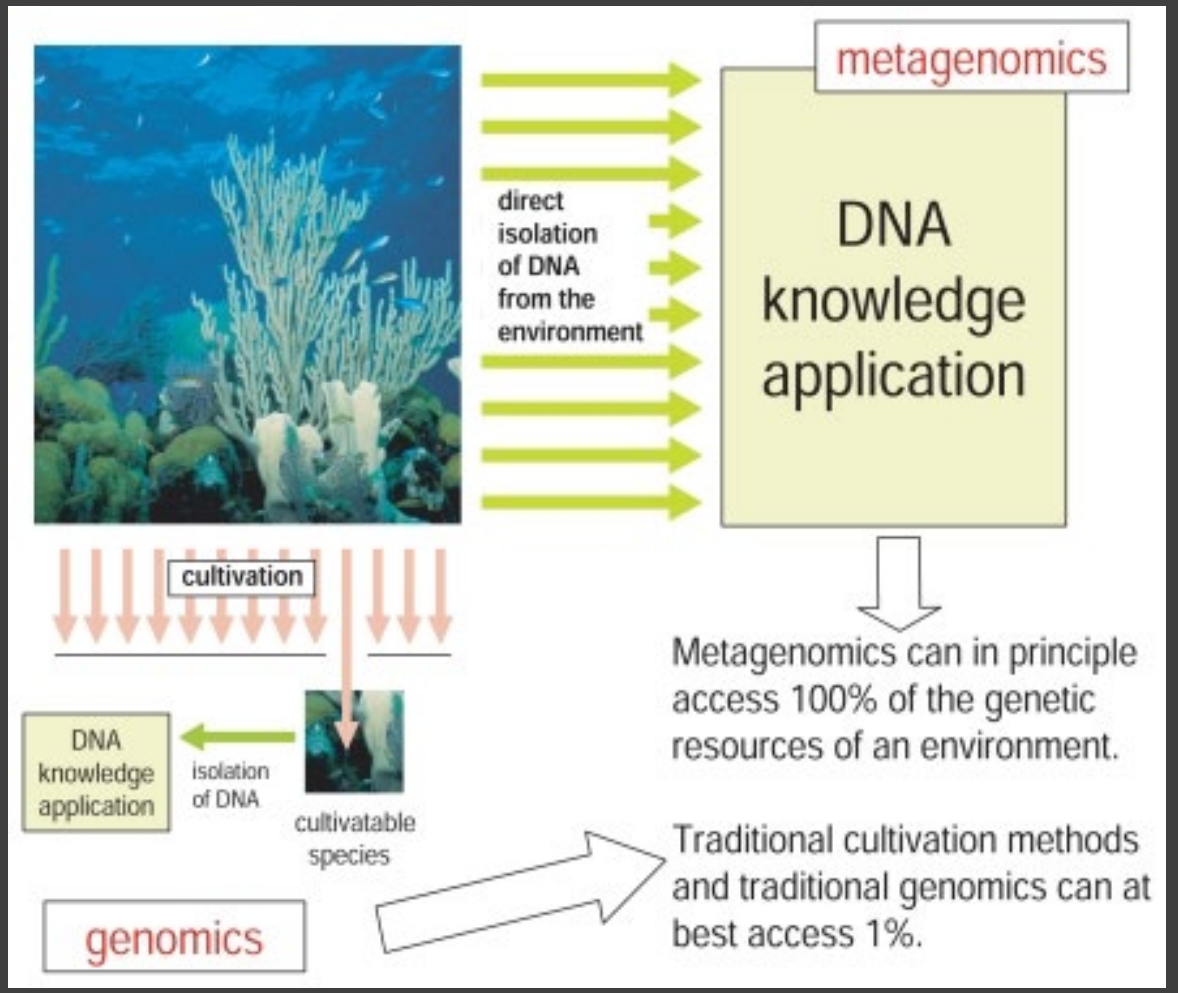
- Study of transcriptome – all the RNA molecular within a cell
- Major part of this “omics” focuses on messenger (m) RNA molecular only – reflective of genes actively expressed as protein products in a cell or a tissue at a given time or given situation
- Also includes the study of the non-coding RNAs (over 95% of the RNAs in a cell is not translated into a protein)



http://laundregen564s17.weebly.com/transcriptomics.html

Metagenomics (Environmental genomics)

- Analyses of DNA acquired from environmental samples with an aim to study the community of microorganisms present, without the necessity of obtaining pure cultures
- Does not rely on cultivated clonal cultures which is used for traditional microbiology and microbial genomics
- Genomics is not enough as most microbes cannot be cultured



National Research Council (US) Committee on Metagenomics: Challenges and Functional Applications. The New Science of Metagenomics: Revealing the Secrets of Our Microbial Planet. Washington (DC): National Academies Press (US); 2007. 1, Why Metagenomics? Available from: https://www.ncbi.nlm.nih.gov/books/NBK54011/

Proteomics

- Analysis of the entire protein complement (proteome) of a cell, tissue, or organism under a specific, defined set of conditions
- The proteome is dynamic as protein changes in response to many conditions
- Proteomics is used to investigate:

  - when and where proteins are expressed;

  - rates of protein production, degradation, and steady-state abundance;

  - how proteins are modified (for example, post-translational modifications

    (PTMs) such as phosphorylation);

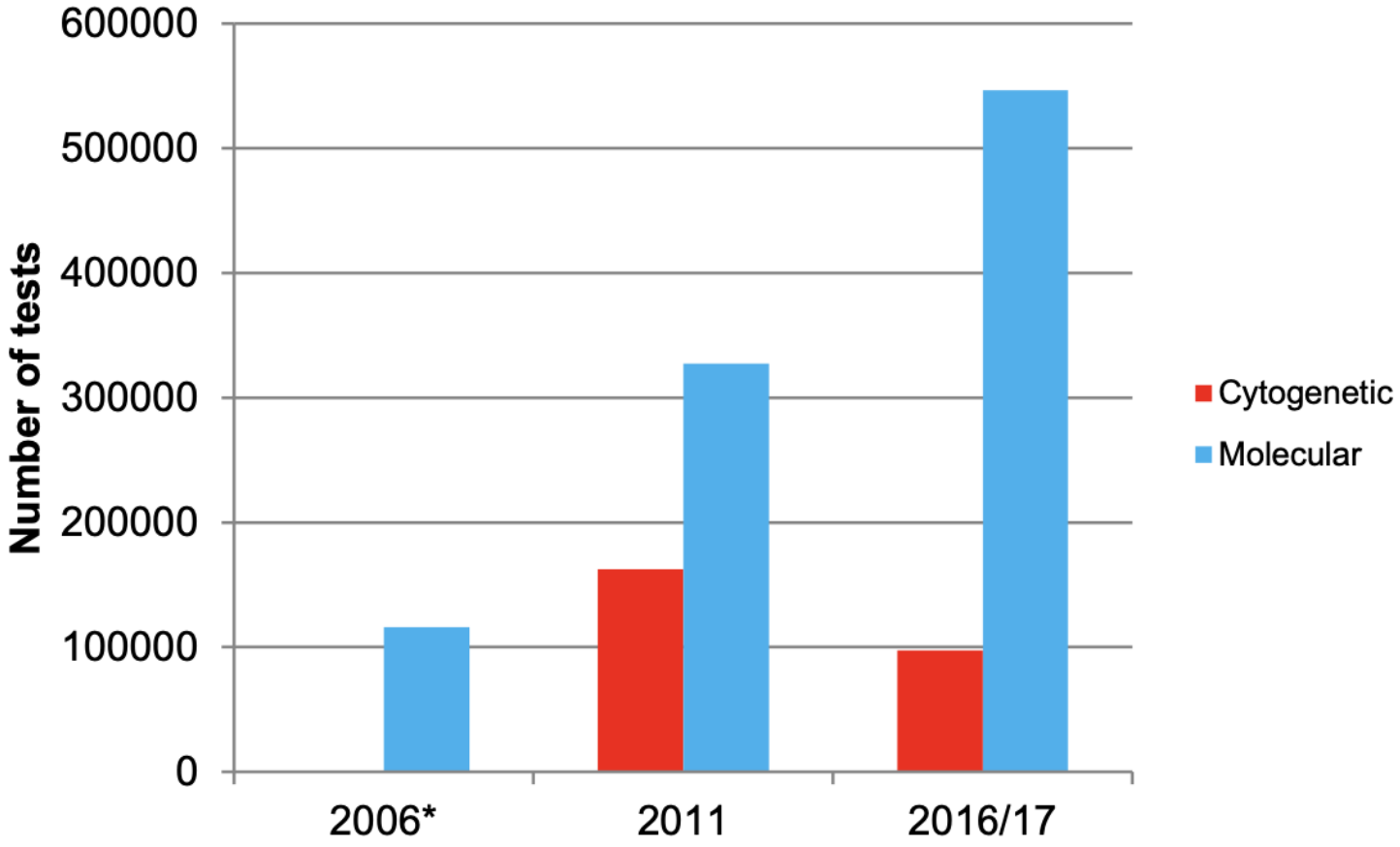
  - the movement of proteins between subcellular compartments;

  - the involvement of proteins in metabolic pathways;

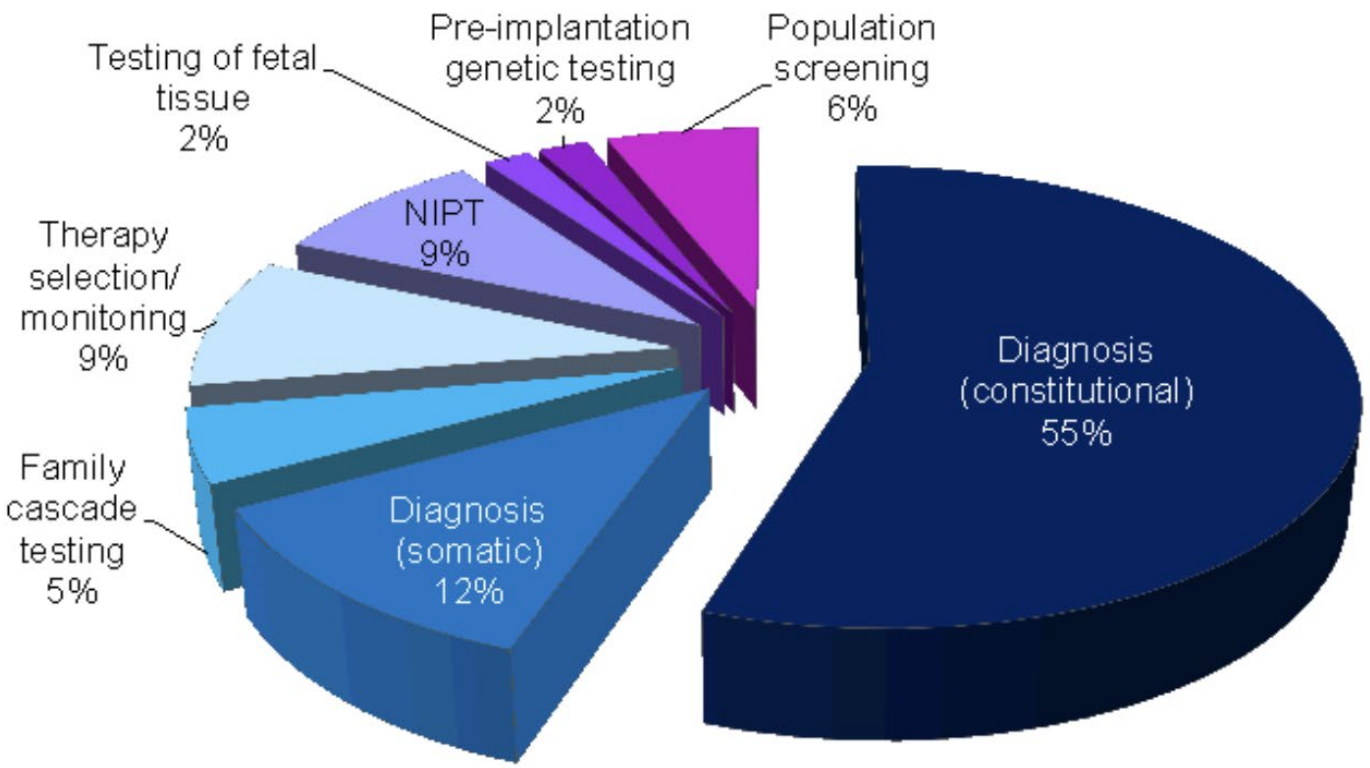
  - how proteins interact with one another.
- Most common way of high throughput analysis – mass spectrometry
- Several limitations to the field

Test Volumes - Australia

Molecular – All genetic/genomic tests
Cytogenetic – FISH and Microscopy based Karyotyping



Test Indications

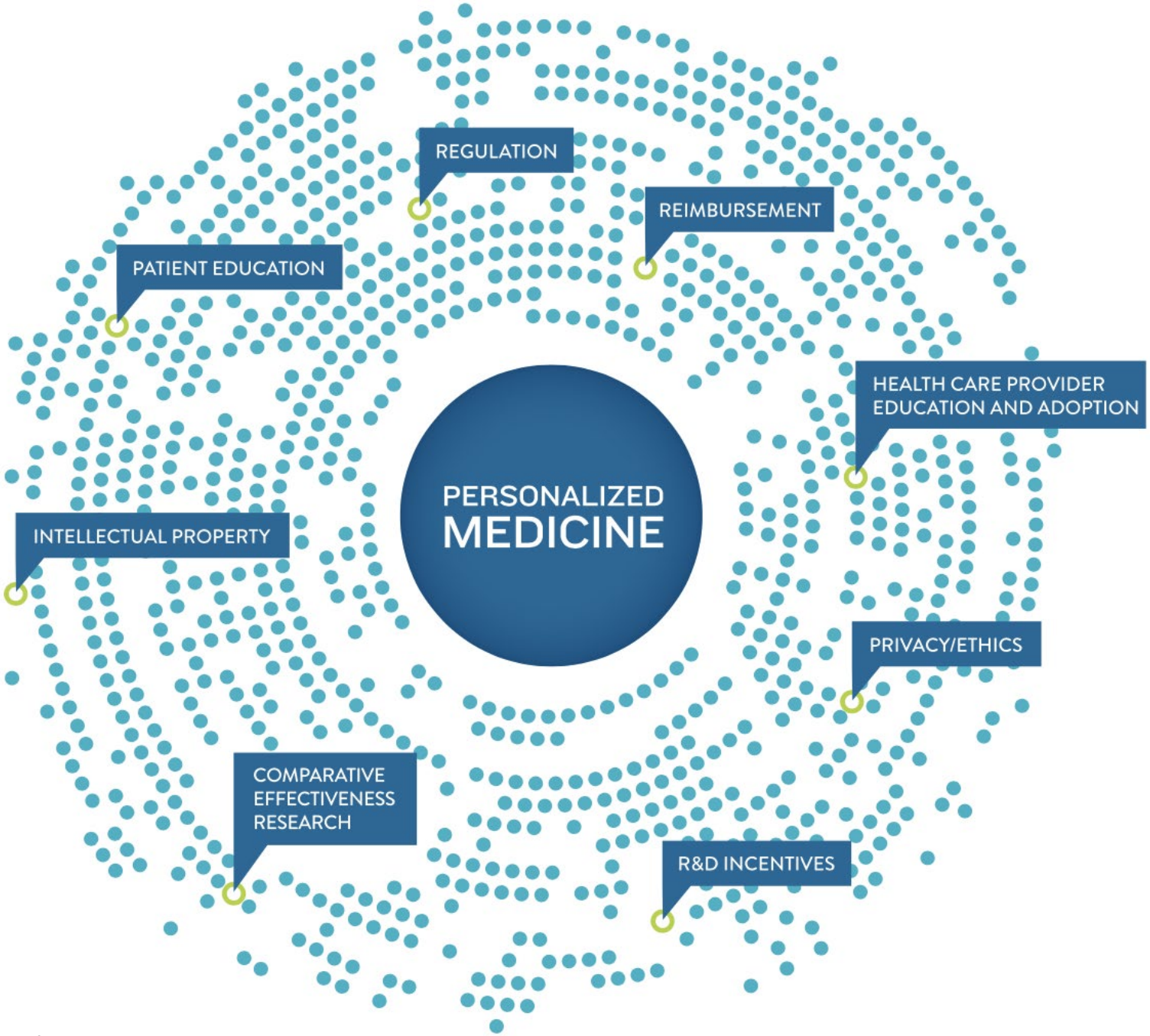


18 April, 2019 | RCPA The Royal College of Pathologists of Australasia

Taken from – RCPA. Know Pathology Know Healthcare.
FROM SCIENCE FICTION TO SCIENCE FACT: THE RISE AND RISE OF GENETIC TESTING

Test choice


Turnaround times
Cost
Sample requirements
Sensitivity and specificity
Positive/negative predictive value
Types of mutation detected
Genotyping vs mutation screening



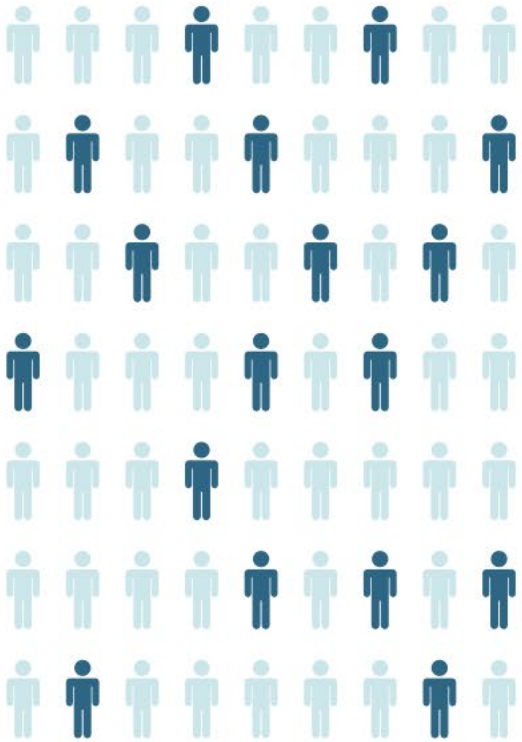
Issues with an impact on personalized medicine

The implementation of personalized medicine requires a confluence of multiple factors. Full implementation of personalized medicine can only be achieved when all sectors converge toward the centre.

Taken from pmc@personalizedmedicinecoalition.org

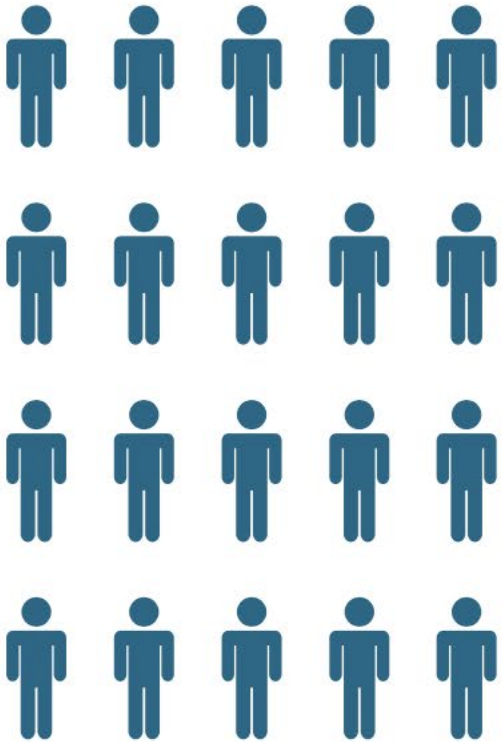
THE RIGHT PERSON

Finding the right people to benefit from genomic medicine can improve disease management and lower health care costs.



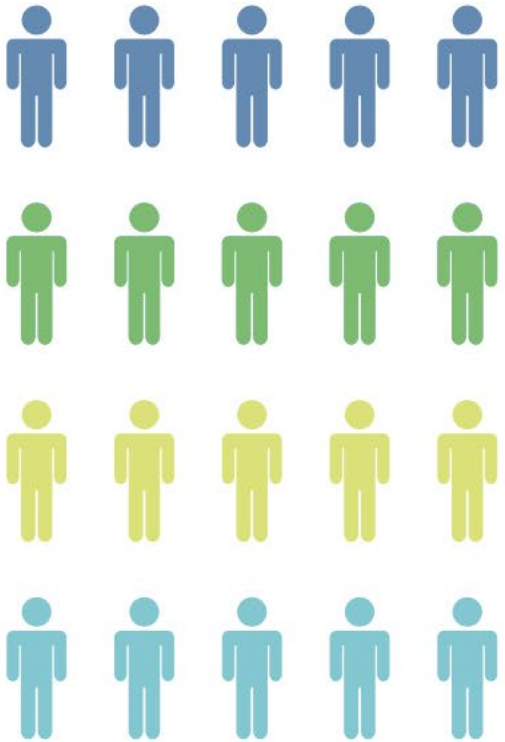
THE RIGHT TEST

Getting the wrong test can misinform medical decisions and increase health care costs.



THE RIGHT INTERPRETATION

Delivers the full value of genetic information and enables physicians to make appropriate management decisions.



Taken from pmc@personalizedmedicinecoalition.org

THANK YOU!