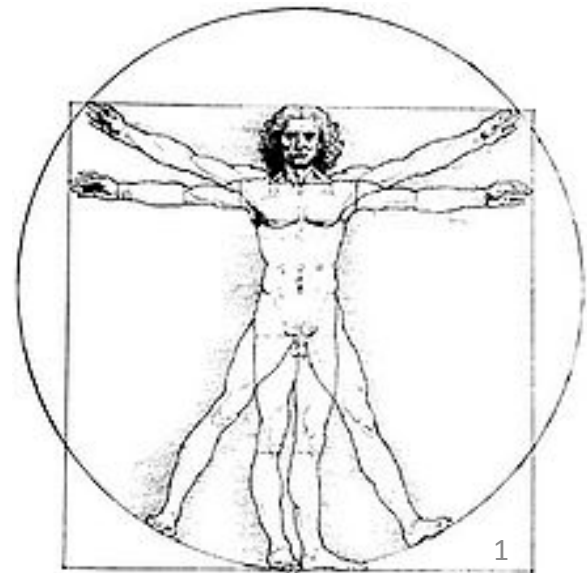


Cost-effectiveness Analysis: Next Generation Sequencing versus Conventional Technologies

KF Wong, MD

8 May 2018



Choice of Tests

- referral reasons
- type of referral
- sensitivity and limitations
- turnaround time
- cost
- local policy and healthcare funding

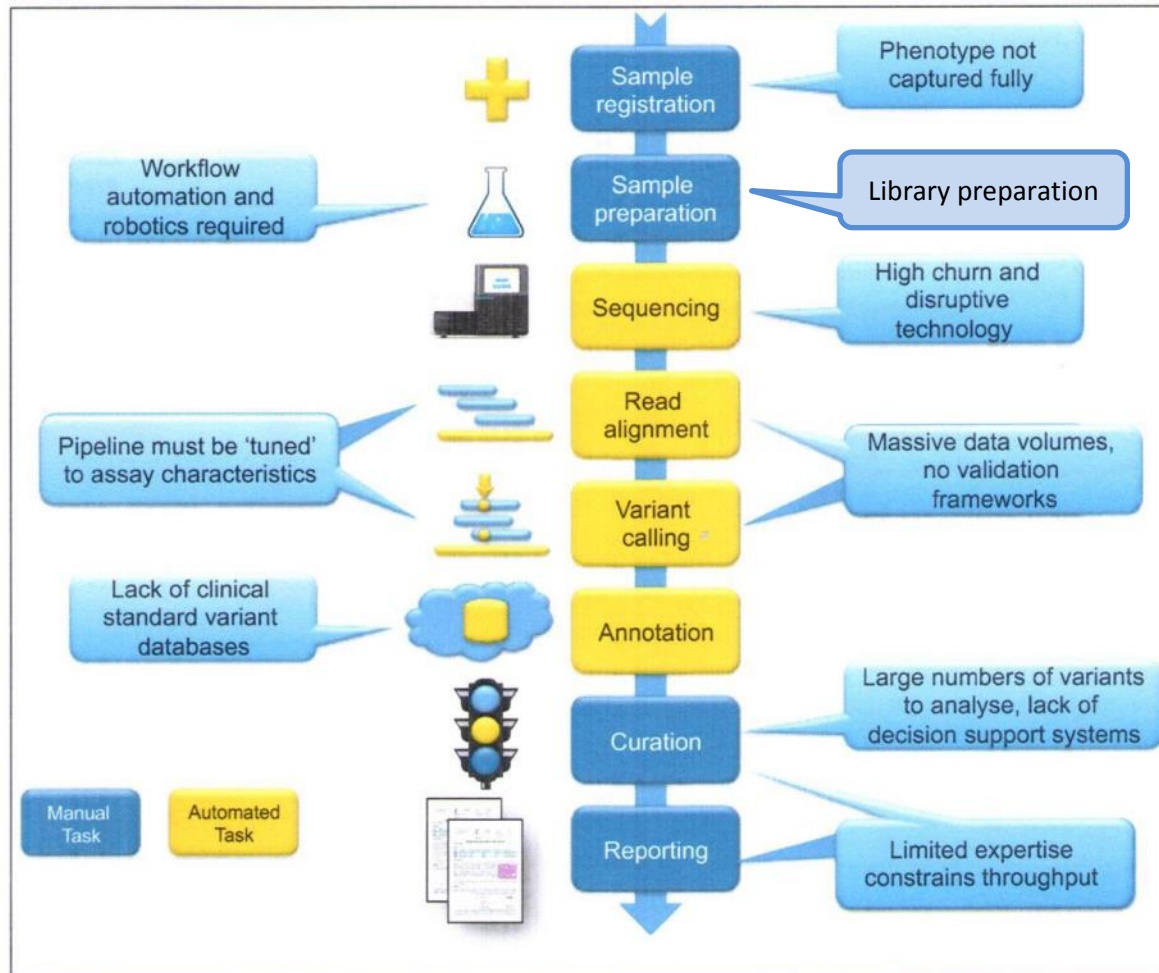
What is Next Generation Sequencing?

- simultaneous (parallel), unselective sequencing of huge numbers of DNA sequences in a short time with a high depth using a relatively little sample in affordable cost
- conventional technologies: gene-by-gene, exon-by-exon
- gene panel: targeted gene sequencing for analyzing specific mutations (scalability, speed, and resolution)
- whole exome sequencing (WES): analysis of protein-coding exons of genes (~1.5% of the genome)
- whole genome sequencing (WGS): analysis of complete set of DNA sequence of an organism's genome

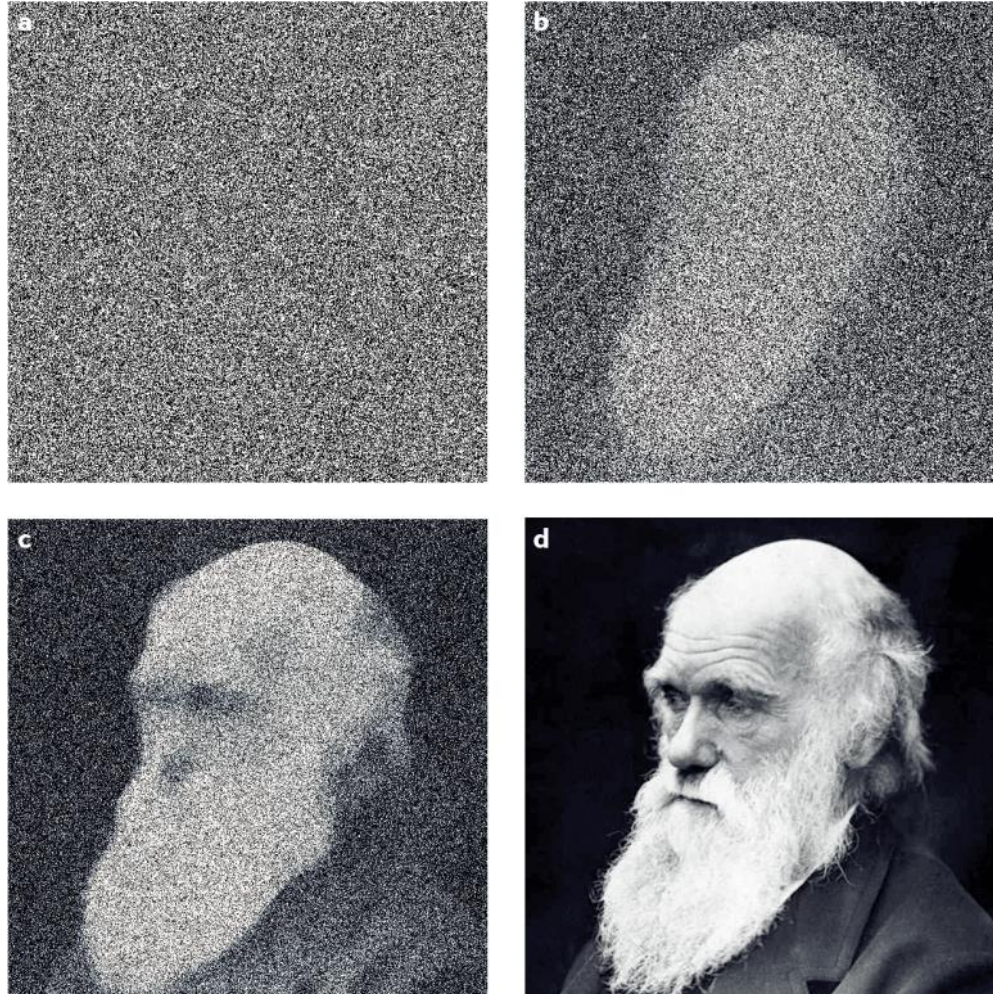
*genome (基因組) = entire genetic makeup of an organism
(3.2 billion letters, 20000 genes, genes = 1.5% of genome)*

Next Generation Sequencing

a single but multi-step technology



Next Generation Sequencing the signal-to-noise problem

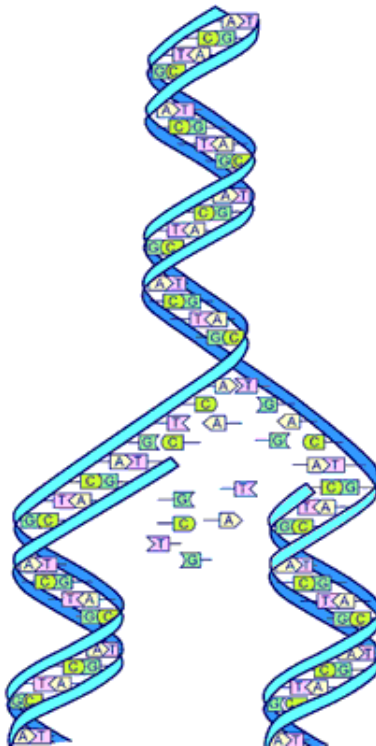


Salk JJ, et al. Nat Rev Genet 2018 Mar 26

Next Generation Sequencing

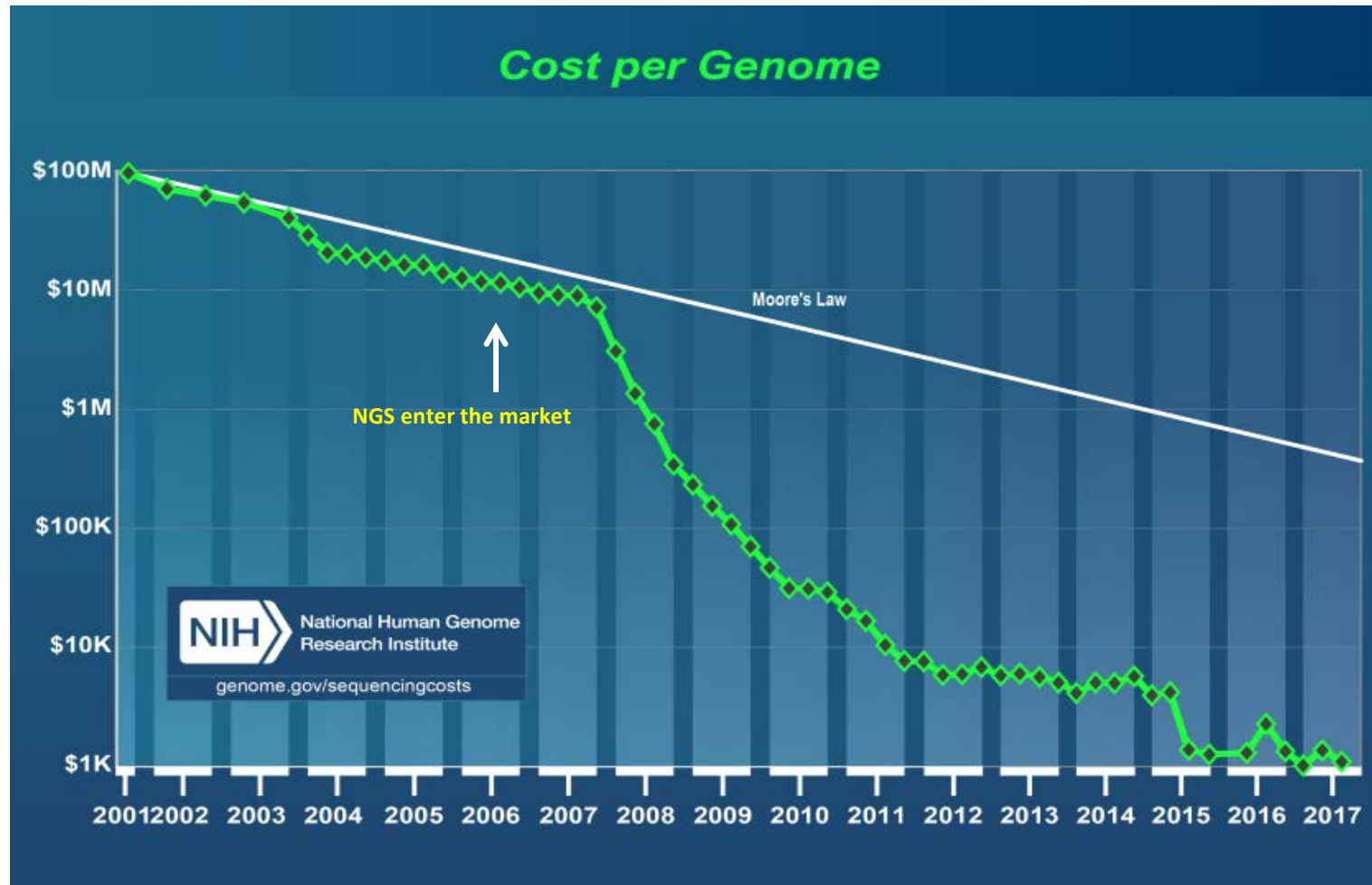
diagnosis is the foundation

- cost reduction and scale of genomic tests increased by many orders of magnitude
- diagnosis, prognosis, monitoring, prediction (risk assessment) and therapy selection
- for individual patient and family members



Next Generation Sequencing

the declining cost of WG sequencing



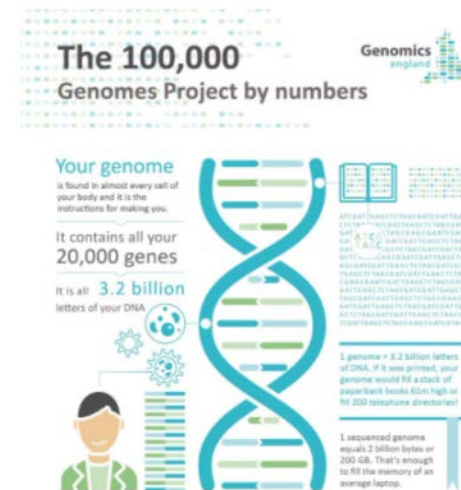
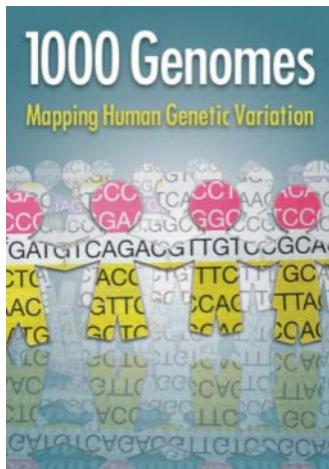
Next Generation Sequencing

the declining cost of WG sequencing

- 2003: US\$53 million
- end-2007: US\$7.1 million
- late-2015: <US\$1500
- now: the \$1000 barrier
- ↓cost of sequencing per base by 100k fold
- global network of laboratories:
 - WGS: \$1105 – \$16420 (Oct 2017)
 - WES: \$534 – \$7637
 - *gene panels*: \$400 – \$5800
 - depending on the number of samples, sequencing coverage and depth, number of reads, platform used, laboratory procedures (overheads & skill mix)
- economies of scale (for high volume service)

The rapid advancement of Next Generation Sequencing

- Human Genome Project started in 1990 & completed in 2003
 - 2013: small number of human genomes sequenced
 - 2015: 65,000 human genomes sequenced
 - 2017: >500,000 human genomes sequenced
- (Many national population genome projects have launched.)



Illumina Promises To Sequence Human Genome For \$100 – But Not Quite Yet, Forbes Jan 9, 2017

Next generation sequencing

a must in modern era of clinical practice

Cancer	Current practice	Near future
non-small cell lung CA (new cases)	EGFR & ALK	EGFR, ALK, ROS1 (CAP recommended) BRAF, RET, KRAS, MET & NTRK (many requiring FISH)
non-small cell lung CA (relapsed cases)	EGFR T790M, ALK & MET amplification (plasma cfDNA) NGS is the way out (digital PCR only an intermediate step)	
colorectal CA	KRAS & NRAS	BRAF, PIK3CA & PTEN
breast CA	NGS has a higher diagnostic and sequencing sensitivity than Sanger sequencing	
haemic malignancies	cytogenetics, FISH, single-gene diagnostics (replaced by NGS?)	

Higher speed, higher throughput.

Less cost, less labour-intensive. More informative, more treatment options.

Next Generation Sequencing for acute myeloid leukaemia

- genetic heterogeneity of AML (importance of NGS)
- 70% AML: at least one mutation by targeted NGS
- clinically actionable genetic information
- e.g. *ASXL1*, *TET2*, *RUNX1*, *DNMT3A*, *IDH1/2* and *TP53*

Hussaini MO, et al. Cancer Genom Proteom 2018;15:121

- clinical utility of NGS in AML with complex karyotype or cryptic aberration (negative for gene mutations)

Ma ESK, et al. Cancer Genet 2017;218:15

- NGS improves risk stratification with distinct prognoses for guiding therapeutic decisions

Lin P, et al. Cancer Med 2017;6:349

Next Generation Sequencing

Is it cost-effective?

- considerable variability in the costing: multiple platforms, panel designs, analysis pipelines and practices for result review and reporting
- key drivers of costs: sequencing instruments, reagent kits, computing infrastructure, data analysis/reporting, technical/bioinformatics personnel

Sabatini LM, et al. J Mol Diagn 2016;18:319

- multiplex samples up to a full batch using the DNA barcode technology → substantial cost reduction

Next Generation Sequencing

Is it cost-effective?

- **cancer:** broad molecular profiling of oncogenes, identification of tumour hotspot mutations, potential targetable (drug-able) genetic changes
- FFPE tissue and plasma DNA (in relapsed cases)
- saving/gain in QALY for metastatic melanoma (34 cancer-related genes) vs single-site mutation test, lower cost for treatment-related morbidity

Li Y, et al. Mol Diagn Ther 2015;19:169

- **neurodevelopment disorder:** WGS economically viable if >3 single genes (with >1000 loci, WES or WGS the preferred diagnostic modality) → diagnostic yield 73%

Williams M, et al. Abstract 260, ACMGG 2013

- useful in acutely ill children, 40% in nonacute cases with trio sequenced and diagnosis made 77 months earlier

Soden SE, et al. Sci Transl Med 2014;6:265

Next Generation Sequencing

Is it cost-effective?

- diagnosis of malignancy does not rely on molecular diagnostics to the same degree as inherited disease, but
- 5-10% of all cancers with a strong genetic component
 - BRCA1 and BRCA2 in breast cancers
 - mismatch repair genes in colonic cancers
 - genetic heterogeneity (>1 gene for 1 disease)
- conventional technologies detection: one-gene-at-a-time testing strategy (iterative, labour intensive & time-consuming), often only on hotspots, the problem of running out of sample
- cost-effective by reduced cancer-related morbidity/mortality
- WHO classification: mutation status (recurrence and targeted therapy) as a part of disease name of many cancers, eg chronic myeloid leukaemia, BCR-ABL1-positive

Next Generation Sequencing

Is it cost-effective?

- NGS panel: Lynch Syndrome genes and other genes associated with highly penetrant CRCP syndromes
- increased 0.157 year of life and 0.128 QALY (US\$4650)
- an incremental cost-effectiveness ratio of US\$36500 per QALY compared with standard care and a 99% probability of cost-effectiveness at a threshold of \$100000 per QALY
- addition of genes with low colorectal cancer penetrance → an incremental cost-effectiveness ratio of \$77300 per QALY

Gallego CJ et al. J Clin Oncol 2015;33:2084

Next Generation Sequencing

Is it cost-effective?

- **syndromic children:** multisystem disease, higher hospitalisation rate, longer admission
- 2-3% of births with a genetically determined abnormality, 7% of population has a rare medical condition with a significant genetic component

Joseph L, et al. J Mol Diagn 2016;18:605

- diagnostic odyssey (conventional): clinical assessment, multiple investigations (invasive/costly)
- NGS → highest diagnostic yield: genetically heterogeneous disorder or overlapping diseases
- WES: future reanalysis in undiagnosed conditions

Tan TY, et al. JAMA Pediatr July 21, 2017

differentiating among the overlapping diseases is important because the clinical course of each syndrome differs

Diagnostic Odyssey

phenotype-driven iterative algorithm

- diagnostic consultation
 - non-genetic specialist consultation
 - genetic specialist consultation
 - hospitalisation for procedures (18%)
 - pathology tests requested
 - single-gene test at a time, biopsy, metabolic study, cytogenomic analysis, re-biopsy
 - anaesthesia for diagnostic procedures
- (Total cost = AUD\$554,342 for 44 patients)

astronomical cost if one added the non-genetic testing to the entire diagnostic testing equation, not to mention the protracted and painful diagnostic odyssey which can affect the patient, family and clinician

Next Generation Sequencing

Is it cost-effective?

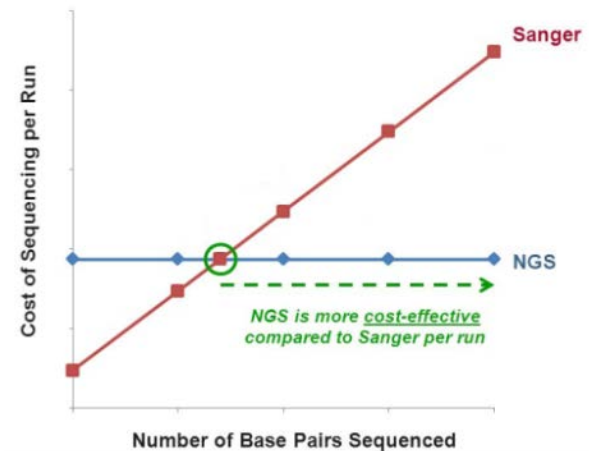
Association for Molecular Pathology report

- targeted gene panel: advanced non-small cell lung cancer
- targeted gene panel: sensorineural hearing loss
- exome sequencing: children with neurodevelopmental disorders of unknown genetic aetiology
- value: reducing healthcare costs/identifying care pathways

Joseph L, et al. J Mol Diagn 2016;18:605

The advantage of Next Generation Sequencing

- higher diagnostic yield:
 - standard karyotyping (5-15%)
 - chromosomal microarray (15-20%)
- single gene test: variable yield (0-64%)
 - phenotype specificity
 - availability of complementary diagnostics
- standard karyotyping
 - requiring culture, lack of sensitivity
- fluorescence in situ hybridization
 - targeted approach, lack of sensitivity
- Sanger/PCR-based single-gene assay
 - false positivity due to sample contamination



About 50% patients had ≥ 4 genetic tests (CMA and single gene sequencing) & in some cases (over 10 tests), the combination of genetic tests was even more expensive than WES itself.

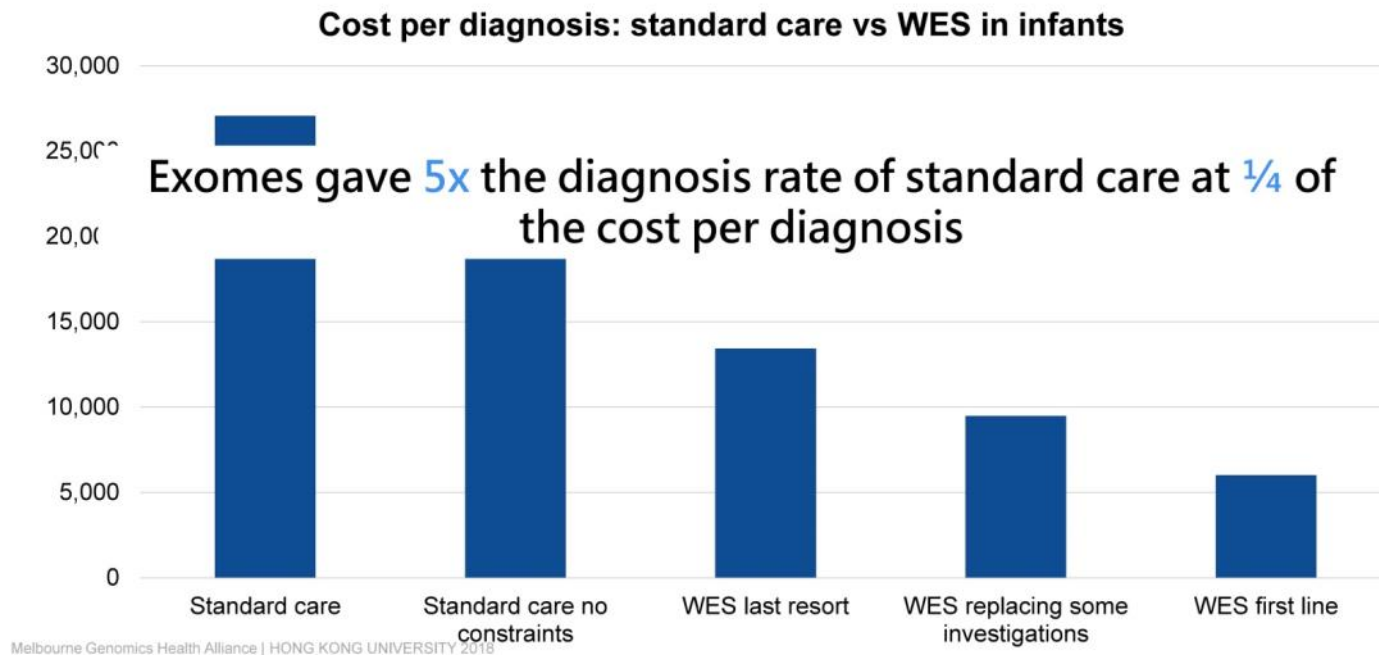
The advantages of **Next Generation Sequencing**

- for limited sample: insufficient for multiple analyses, improve diagnostic yield and time to result
- informed therapy decisions for tumour types
- cost increase for targeted therapy offset by improved outcomes (morbidity and progression-free survival)

Diagnostic Odyssey

How much does it cost?

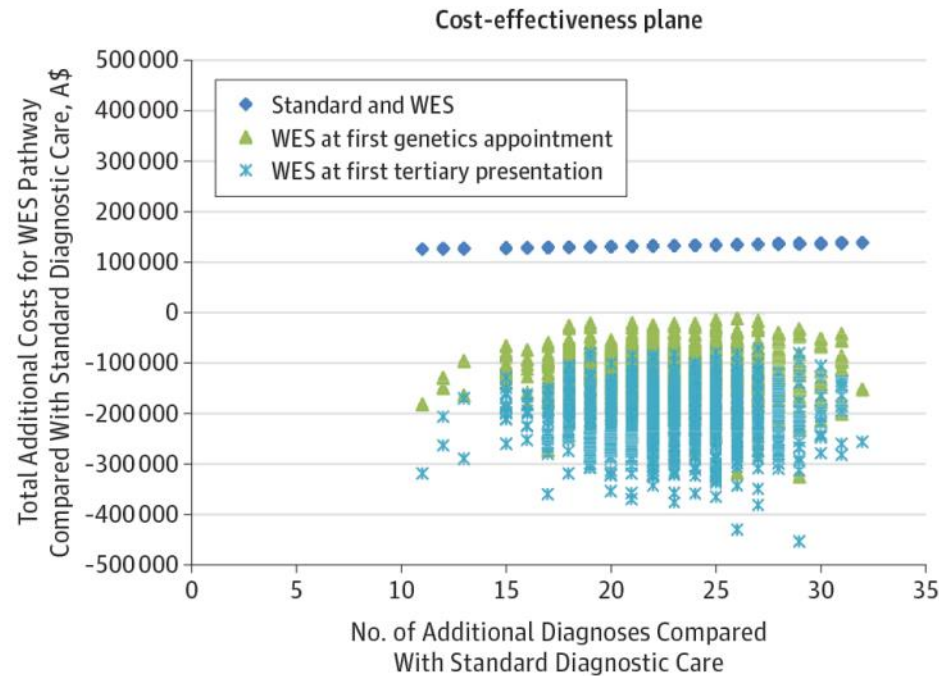
- whole exome sequencing
 - 5x diagnostic rate of standard care
 - cost-effective when ordered early



Next Generation Sequencing

Is it cost-effective?

Figure 2. Cost-effectiveness Plane for Standard Diagnostic Pathway Compared With WES Combined With Standard Diagnostic Pathway, WES at First Genetics Appointment, and WES at Initial Tertiary Presentation



Incremental cost saving of A\$9020 per additional diagnosis compared with standard diagnostic pathway.

Tan TY, et al. JAMA Pediatr July 21, 2017

Next Generation Sequencing

Is it cost-effective?

- diagnosis reached in 52% (23/44) by singleton WES
- clinical management was altered in 26% (6 of 23)
- duration of (original) diagnostic odyssey was 6 years
- each child had a mean of 19 tests, 4 clinical genetics and 4 non-genetics specialist consultations
- 59% (26) taken a procedure under GA for diagnosis

Tan TY, et al. JAMA pediatr July 21, 2017

Next Generation Sequencing

(>cost-effectiveness in conventional sense)

- minimize diagnostic consultation
- minimize hospitalisation and LOS
- reduce pathology tests requested
- reduce procedure-related morbidity
- reduce anxiety of patient and family

Adding WES after a protracted diagnostic odyssey is most expensive.

Next Generation Sequencing

(>cost-effectiveness in conventional sense)

- analysis for negative cases
- early family engagement
- laboratory commitment
(repeated sequential testing)
- future re-analysis of difficult cases

Next Generation Sequencing

What about the local situation?

Next Generation Sequencing

cost comparison of BRCA1/BRCA2 testing between NGS and Sanger sequencing

- WLU (bioinformatics pipeline/SO interpretation)
- reagent cost and manpower (WLU)
- equipment investment and maintenance
- computer hard/software and storage service
- assumptions:
 - interpretation/reporting by pathologist not included
 - testing of large rearrangement by MLPA not counted

Bioinformatics is used to piece together the DNA fragments by mapping with the individual reads to the human reference genome.

Next Generation Sequencing

a local project

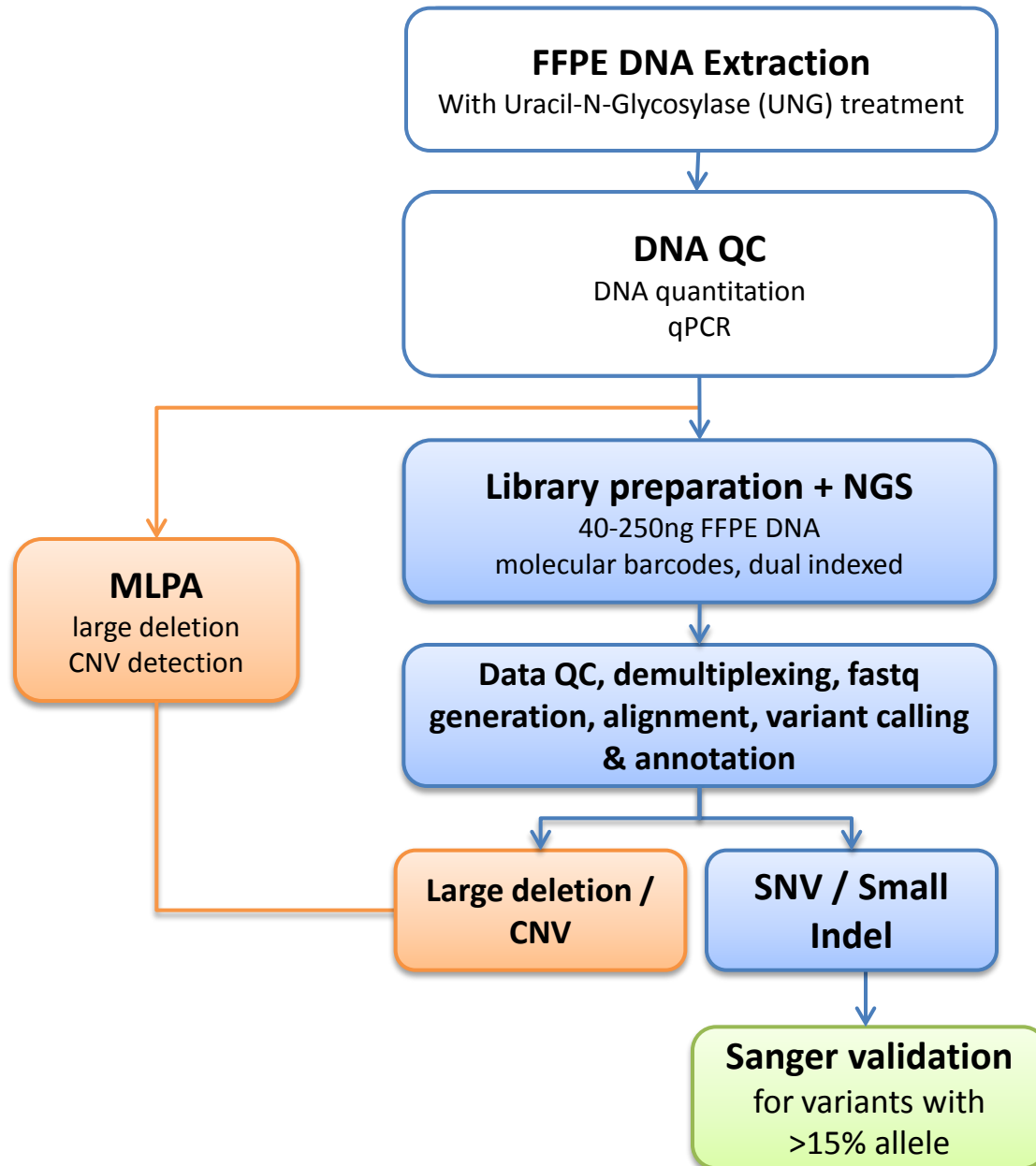
- BRCA 1 and 2 mutation study by NGS in ovarian cancers
- 6th most common cancer among Hong Kong women
- 500 new cases/year; overall 5-year survival 40%
- 7th leading cause of female cancer-related death
- new targeted therapy: poly (ADP-ribose) polymerase
- effective in recurrent ovarian cancer with mutated BRCA
- BRCA mutations occurring across a spectrum of sites:
 - BRCA1 (22 coding exons) and BRCA2 (26 coding exons):
conventional sequencing: labour-intensive & technically challenging

Next Generation Sequencing

a local project

- NGS for targeted sequencing of all exons and flanking introns of BRCA1 and BRCA2 genes on FFPE tissue, supplemented by multiplex ligation-dependent probe amplification for large deletions and duplications
- cell lines with known BRCA mutations, inter-laboratory sample exchange, and EQAP materials
- test development: protocols for sample preparation, library preparation, bioinformatics pipeline, variant curation, quality assurance and clinically applicable reporting system
- with Clinical Oncology Department, QEH and Chinese University of Hong Kong, City University of Hong Kong, and supported by Astra Zeneca

Experimental Design



Next Generation Sequencing

cost comparison of *BRCA1/BRCA2* testing

	NGS	Sanger sequencing	Remarks
1. Test development			
Reagent cost	\$738,334	\$571,820	
Manpower	1 SO, 1 Bioinformatician, 1 MLT	1 SO, 2 MLT	
Manpower (WLU) per validation project	Mapping WLU for NGS test is not available (probably less hands-on time for MLT)	141,333	Sanger sequencing WLU estimation: Reference to KRAS sequencing (265 WLU for 3 amplicons). = $265/3 \times 80$ amplicons x 20 samples
Analysis - Bioinformatics	3-4 months for pipeline development and validation	NA	
Analysis - Interpretation (Scientists)	2-3 hr/sample	20 hr/sample	Sanger: 5 min per sequence, 3 sequences / amplicon (control F, sample F+R), 80 amplicons per sample

an amateurish estimation without the help of professionals

Next Generation Sequencing

cost comparison of *BRCA1/BRCA2* testing

	NGS	Sanger sequencing	Remarks
2. Testing cost			
Reagent cost per test	\$3,485	\$28,591	
Manpower (WLU) per test	Mapping WLU for NGS test is not available (probably less hands-on time for MLT)	7,067	Sanger sequencing WLU estimation: Reference to KRAS sequencing (265 WLU for 3 amplicons). = $265/3 \times 80$ amplicons
Analysis - Bioinformatics	2 days per run (8 samples per run)	NA	
Analysis - Interpretation (Scientists)	2-3 days per 8 samples	20 days per 8 samples	

Next Generation Sequencing

cost comparison of *BRCA1/BRCA2* testing

	NGS	Sanger sequencing	Remarks
3. Equipment			
Sequencing instrument	\$2,800,000 (NextSeq) or \$1,000,000 (MiSeq)	\$1,200,000 (ABI 3500)	
Annual maintenance cost	\$330,000 (NextSeq) or \$160,000 (MiSeq)	\$130,000	
Computing & storage server	>\$1,000,000, varies depending on data volume	NA	
Software	Variable if commercial software is required	\$55,000 (two licenses)	Mutation surveyor or equivalent
Database	Variable if subscription to any annotation database is required	NA	

Next Generation Sequencing

All that glitters is not gold!

- for unexplained global developmental delay and intellectual disability, chromosomal microarray followed by karyotyping (perhaps cost-effective)
- but the addition of reflex NGS would become cost effective if the NGS became more efficient in detecting additional pathogenic variants or if the cost of NGS testing became lower

Li Y, et al. Mol Diagn Ther (2018) 22:129–138

- *However, one should also note that exomes are cost-effective, particularly when ordered early (5X diagnostic rate at ¼ of the cost of standard care).*
- *WES: not to replace a clinical geneticist evaluation.*

Next Generation Sequencing

All that glitters is not gold!

- negative results with NGS:
 - lack of understanding
 - technical limitations
 - low coverage regions
 - regulatory or deep intronic regions
 - multiple pseudogenes
 - Homologous and repetitive regions
 - patient selection bias
- reanalysis: newly discovered genes associated with human diseases

Next Generation Sequencing

Is all that glitters really not gold?

- 83% of sequenced samples with ≥ 1 mutation; 37% gene-targeted therapy
- no need to undertake orthogonal assay: high sensitivity and specificity of NGS, high concordance between NGS outcome and Sanger sequencing
- limiting factors: availability and effectiveness/specificity of molecular inhibitors, heterogeneity of disease, access of targeted therapy, regions which sequence poorly or map erroneously due to high GC content
- NGS is the more cost-effective way to reach a diagnosis and can guide appropriate management by reducing the time of diagnosis/cost of testing
- despite the cost of NGS, an accurate molecular diagnosis can lead to more efficient and appropriate use of healthcare resources (an experienced pathologist in morphology and molecular diagnostic is still much needed)
- it can help stop the repeated clinic visits and testing associated with the diagnostic odyssey, which can increase costs and reduce effectiveness

The cost of treatment is significantly higher than the cost of NGS.

Next Generation Sequencing

incorporating genomics into clinical management

- size of eligible population (specific to the intended use of the test), current mix of tests and treatment or intervention, anticipated result after test introduction
- who is paying? self or government? inflation changes
- cost of drug therapy, drug administration and associated adverse events, costs of NGS testing
 - average monthly cost of cancer therapy increased by 39% in 10-year period of 2004 to 2014, targeted therapy accounted for almost 50% of the spending when adjusted for inflation
 - for cancers, likelihood of finding a mutation for which there is an expensive therapy, possibly off-label, or in a clinical trial
 - for inherited diseases, variants of currently unknown significance triggering a cascade of other medical procedures

Next Generation Sequencing

is only as good as we ourselves

- experienced pathologist (morphology)
- bioinformatics support (personnel)
- IT and software engineer support
- clinician input into variant curation
- pre- and post-test genetic counselling
- *biggest challenge is what to do with all the information from a CGP test.*

The main disadvantage of NGS in the clinical setting is putting in place the required infrastructure. (computer capacity and storage) , and the personnel training and expertise development is also very important!

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