3rd Singapore Sarcoma Consortium
Education and Research Meeting
12 - 13th Sept 2015
Academia, Outram Campus
Knowing the patient, curing the cancer


# Molecular diagnostics in GIST

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# Introduction

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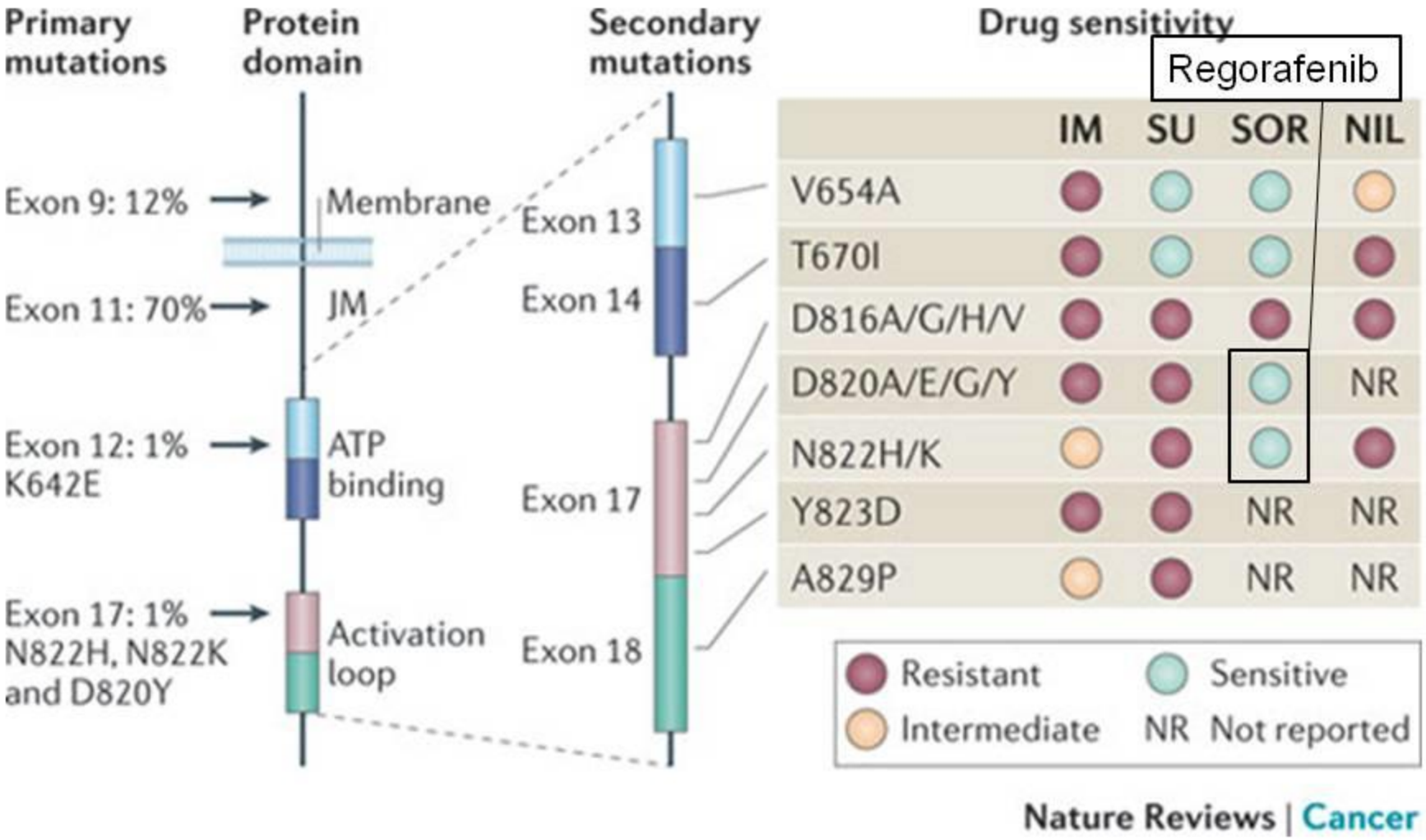
SingHealth

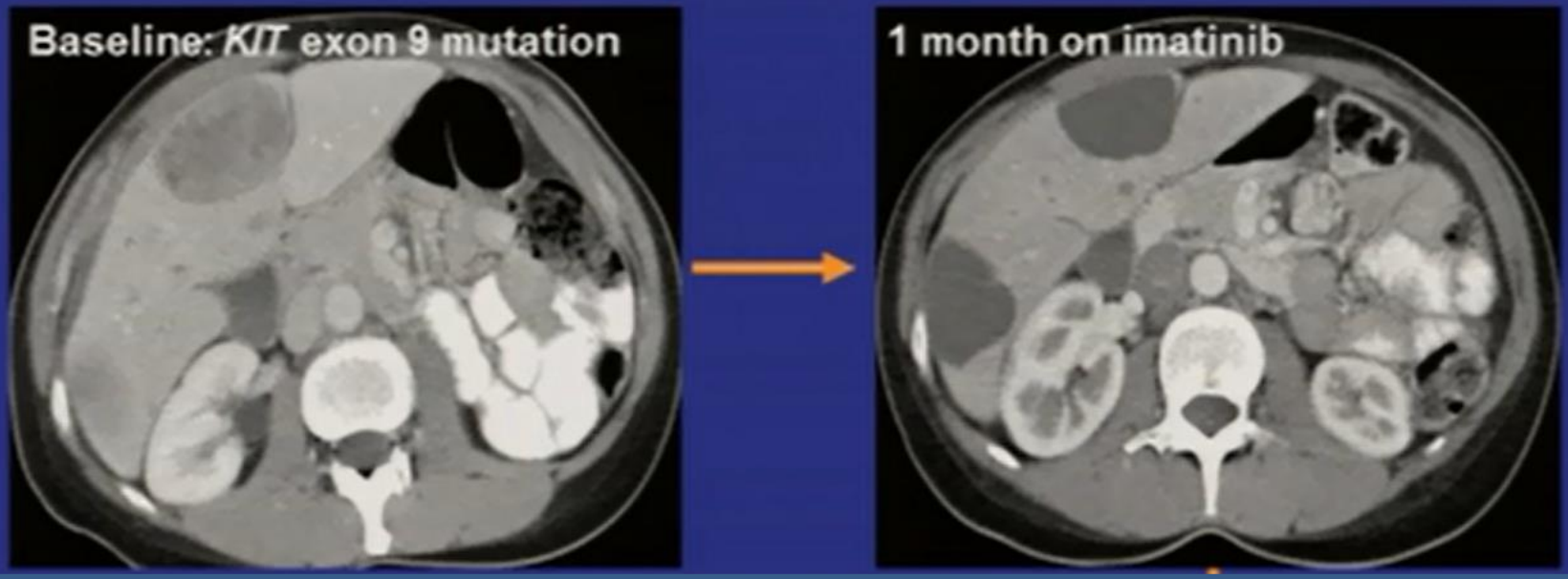
- Gastrointestinal stromal tumors (GISTs) are neoplasms of mesenchymal origin
- Characterised by one primary mutation – either in KIT or PDGFRA gene
- Following exposure to Imatinib, resistance develops under pharmacological pressure resulting in the development of one or more secondary mutations
- Ability to detect such resistance mutations will have a great impact on choice of pharmacological treatment



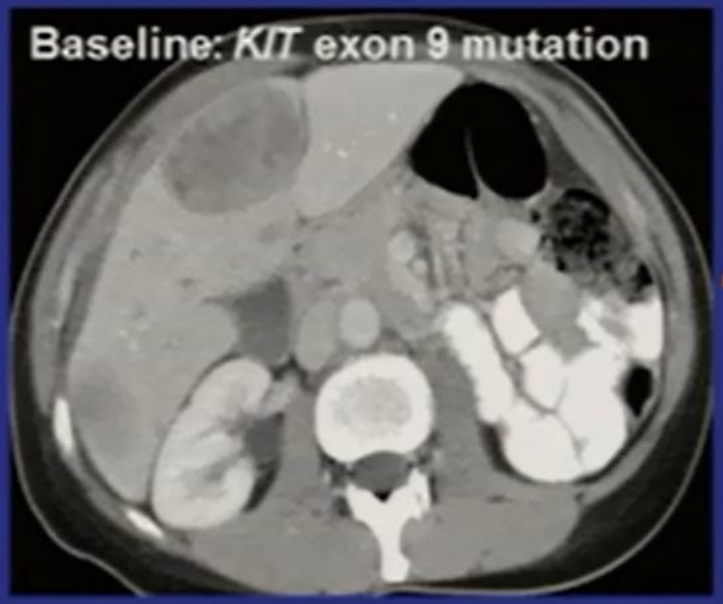

George et al. J Clin Oncol. 2012 May 21. [Epub ahead of print]

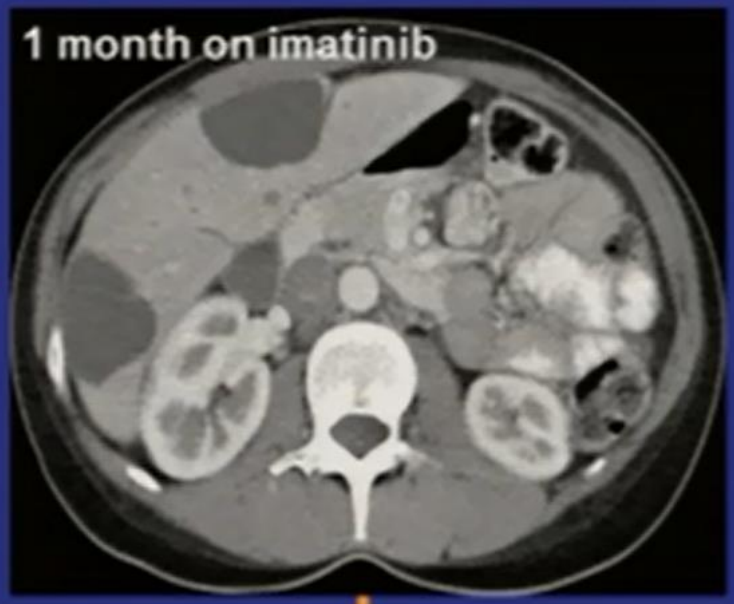
Corless, Barnett & Heinrich. *Nature Reviews Cancer* **11**, 865-878 (December 2011)

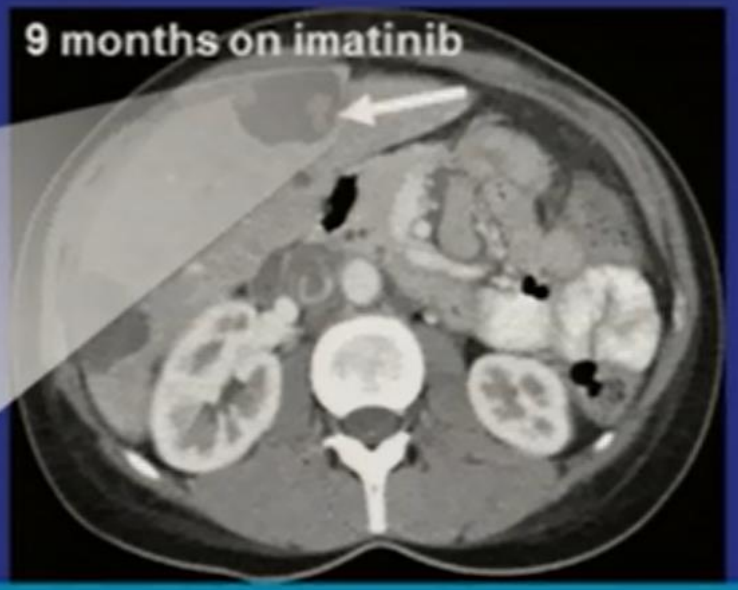
Courtesy of Dr Corless



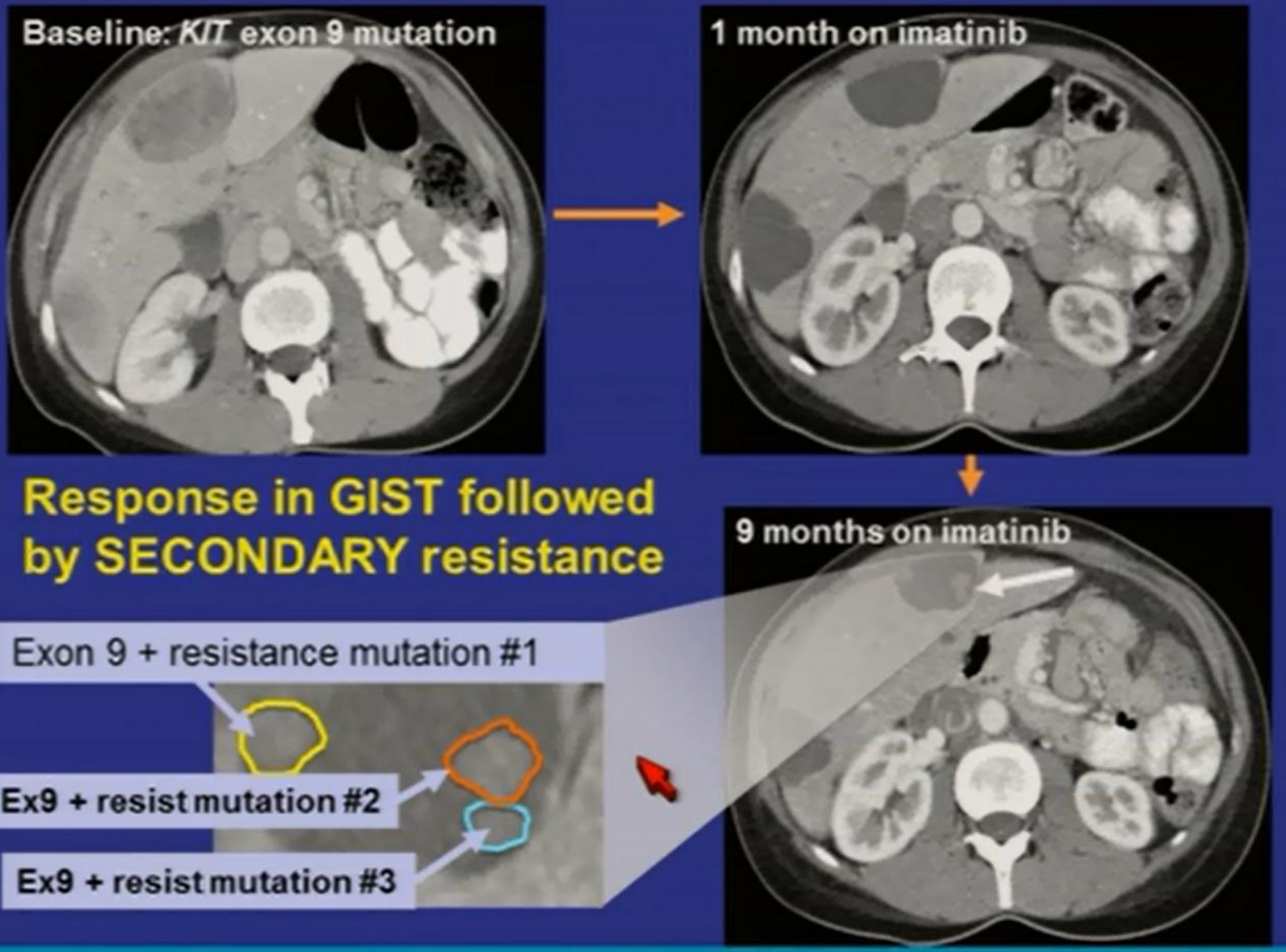

Demetri et al ASCO 2013




Demetri et al ASCO 2013




Demetri et al ASCO 2013

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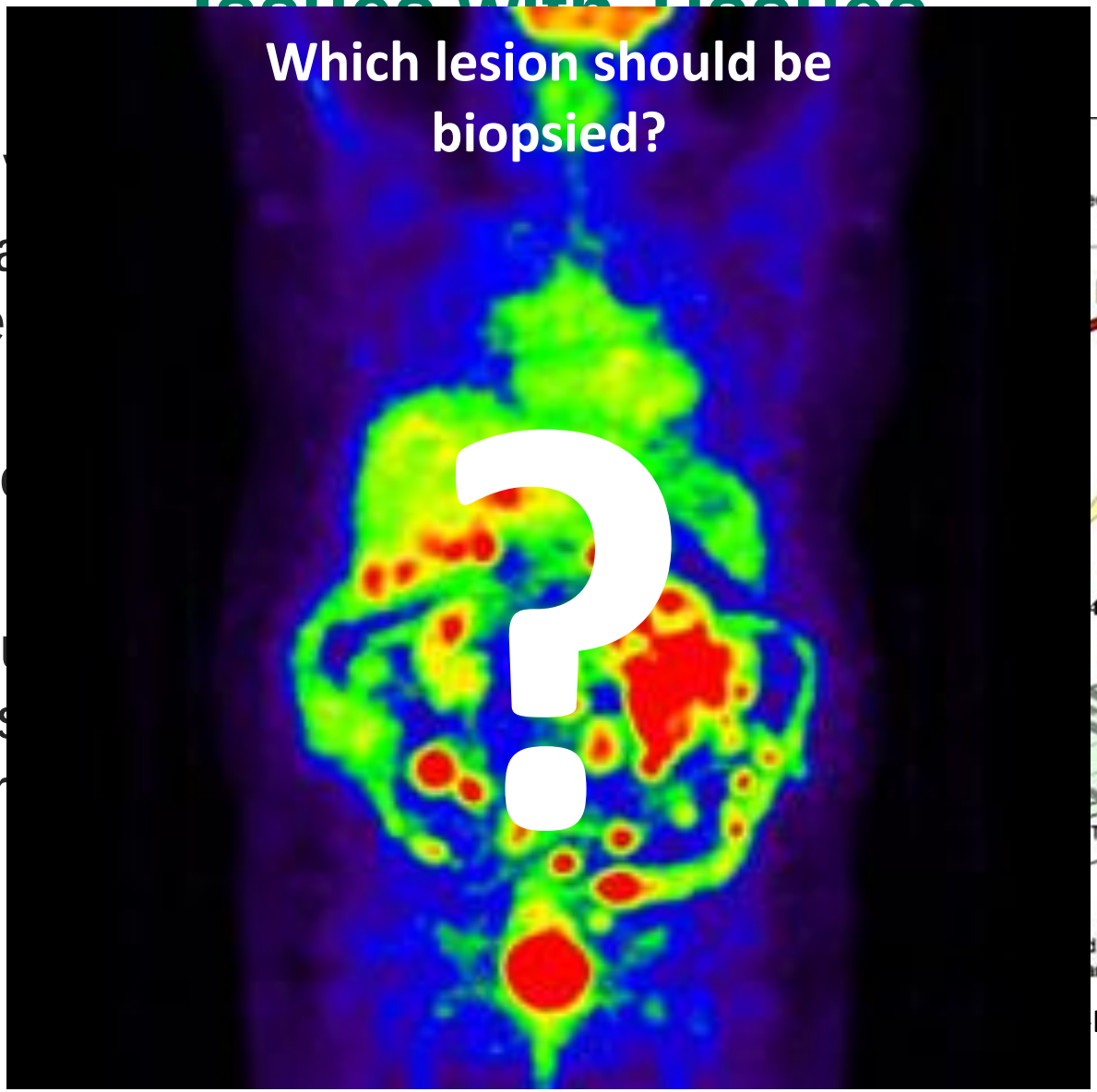
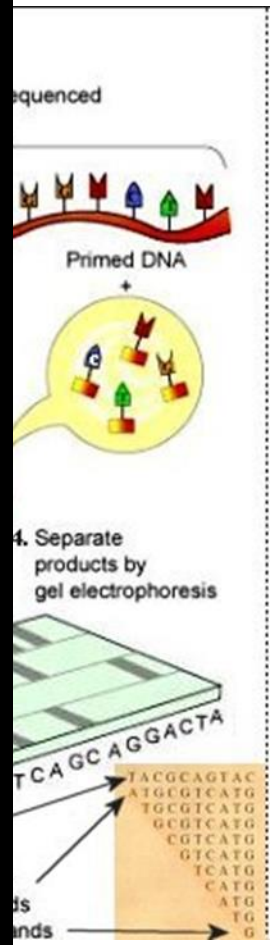
# Issues with Tissues

- Incon
- Pain a relate
- Ease of dis
- Cost
- Inaccu repres tumor




DNAsequencing.htm

# Liquid biopsies

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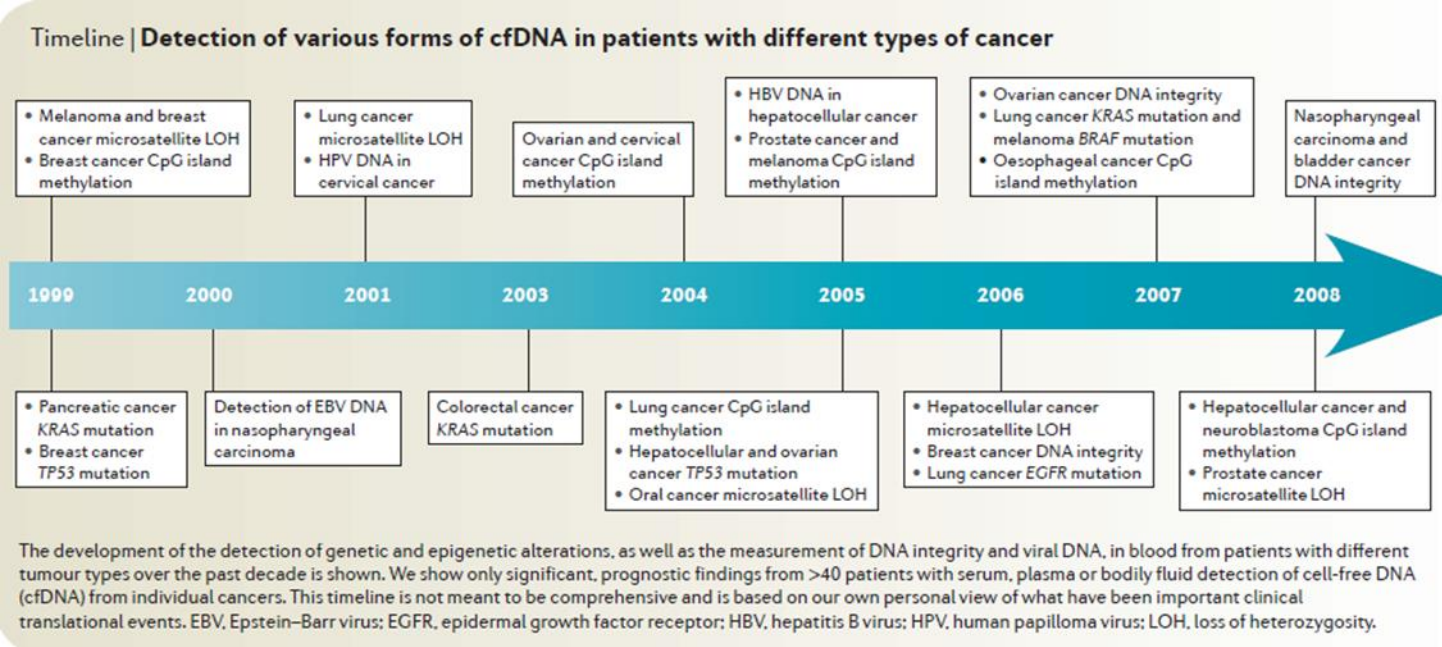
- The presence of cell free DNA in the circulation was first demonstrated in 1948[1]
- Detection of tumor related mutations in the blood in 1994 spiralled interest in this arena[2]




The development of the detection of genetic and epigenetic alterations, as well as the measurement of DNA integrity and viral DNA, in blood from patients with different tumour types over the past decade is shown. We show only significant, prognostic findings from >40 patients with serum, plasma or bodily fluid detection of cell-free DNA (cfDNA) from individual cancers. This timeline is not meant to be comprehensive and is based on our own personal view of what have been important clinical translational events. EBV, Epstein–Barr virus; EGFR, epidermal growth factor receptor; HBV, hepatitis B virus; HPV, human papilloma virus; LOH, loss of heterozygosity.

[1] Mandel P & Metais P et al C. R Acad Sci Paris 142, 241-243 (1948)
[2] Vasioukhin V et al Br J Hematol 86, 774-779 (1994)
[3] Schwrzenbach et al Nat Reviews May 2011

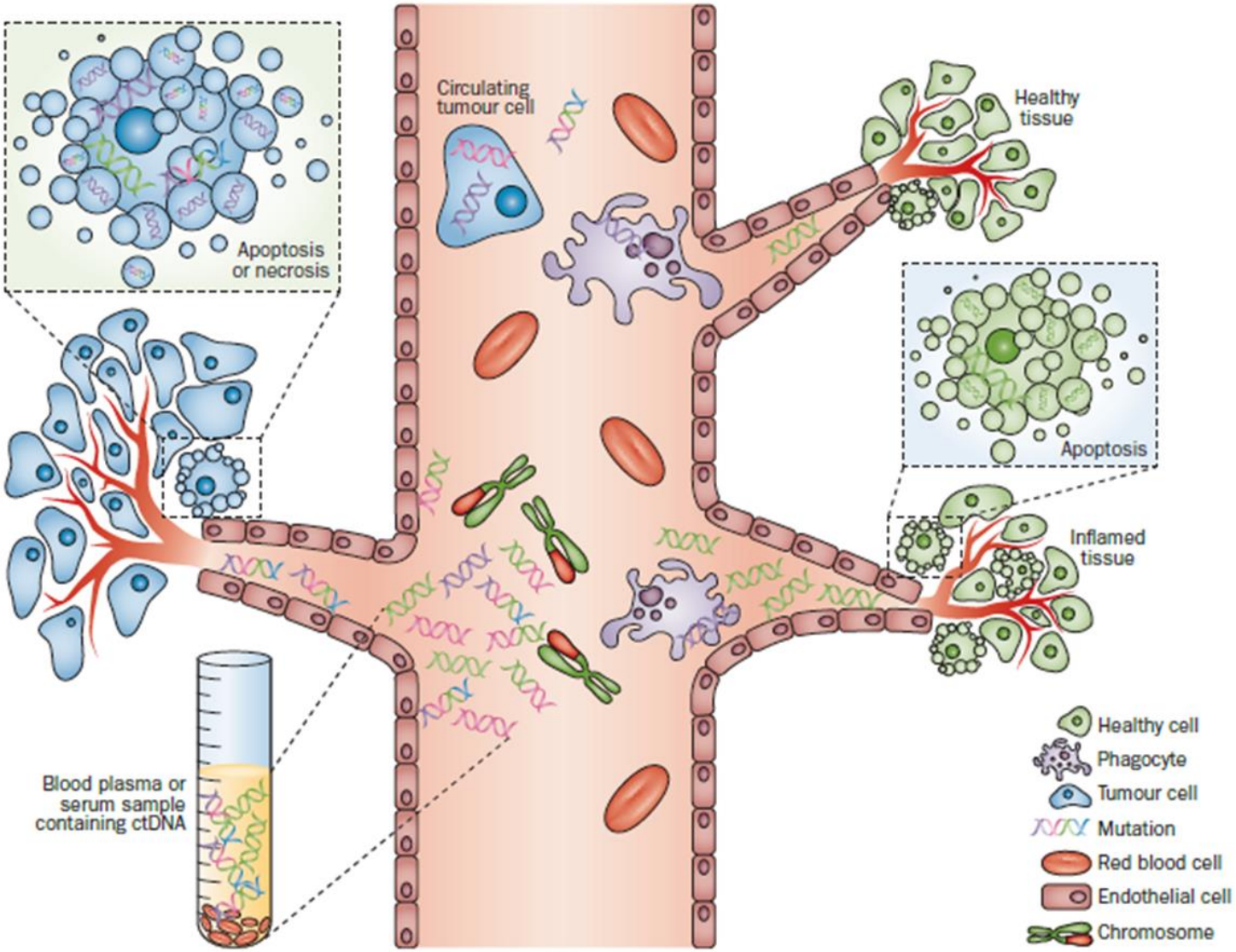



**Figure 1** | Release and extraction of cfDNA from the blood. cfDNA is released from healthy, inflamed or diseased (cancerous) tissue from cells undergoing apoptosis or necrosis. cfDNA can be extracted from a blood sample and genetic aberrations in the DNA released from cancerous tissue detected and quantified. Tumour-derived genetic alterations that can be detected in the blood include point mutations (consecutive purple, red, green and blue DNA strands), copy number fluctuations (red portion of chromosomes) and structural rearrangements (green and red DNA strands). Abbreviations: cfDNA, circulating free DNA; ctDNA, circulating tumour DNA.

# GIST and ctDNA

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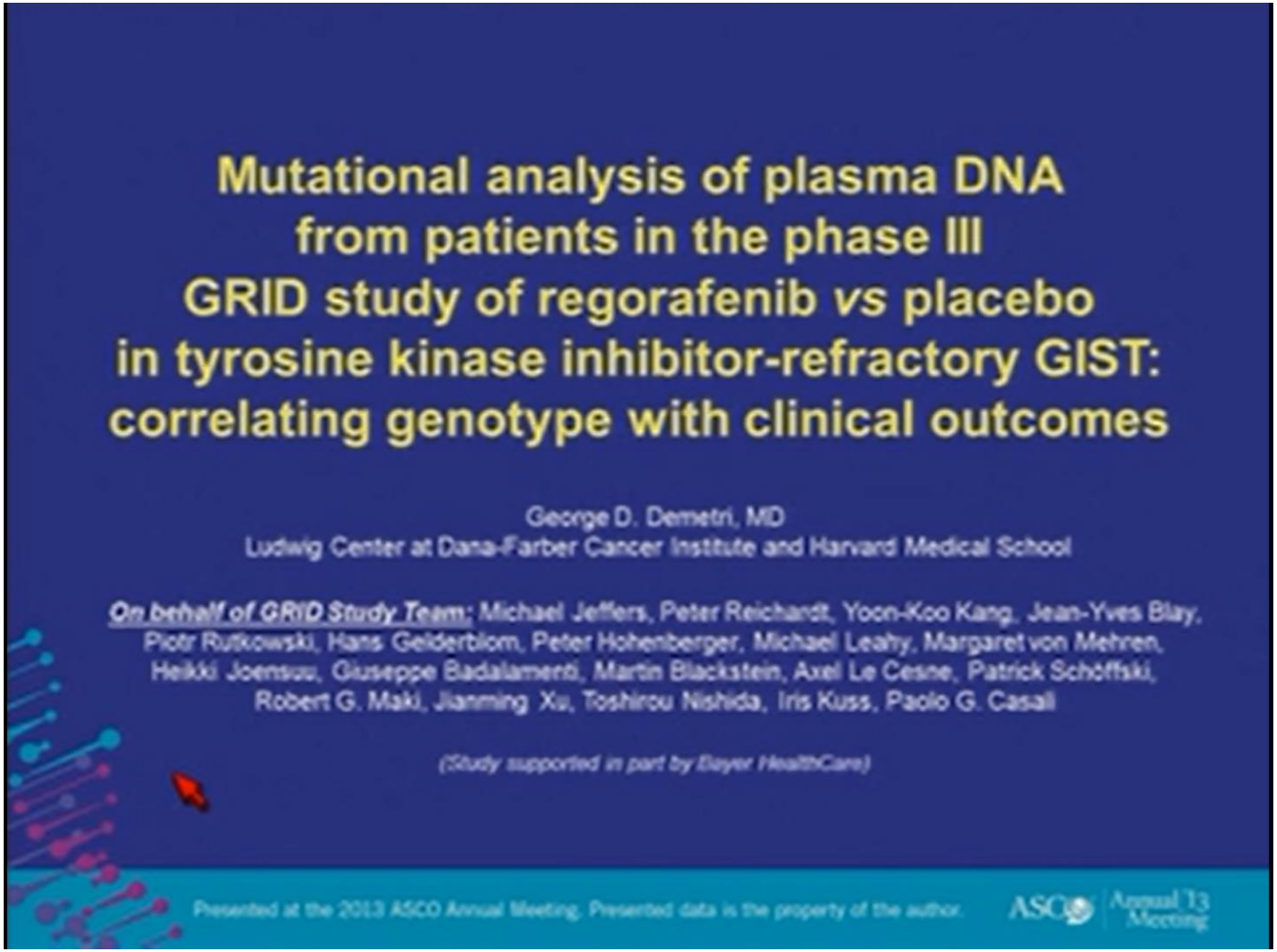
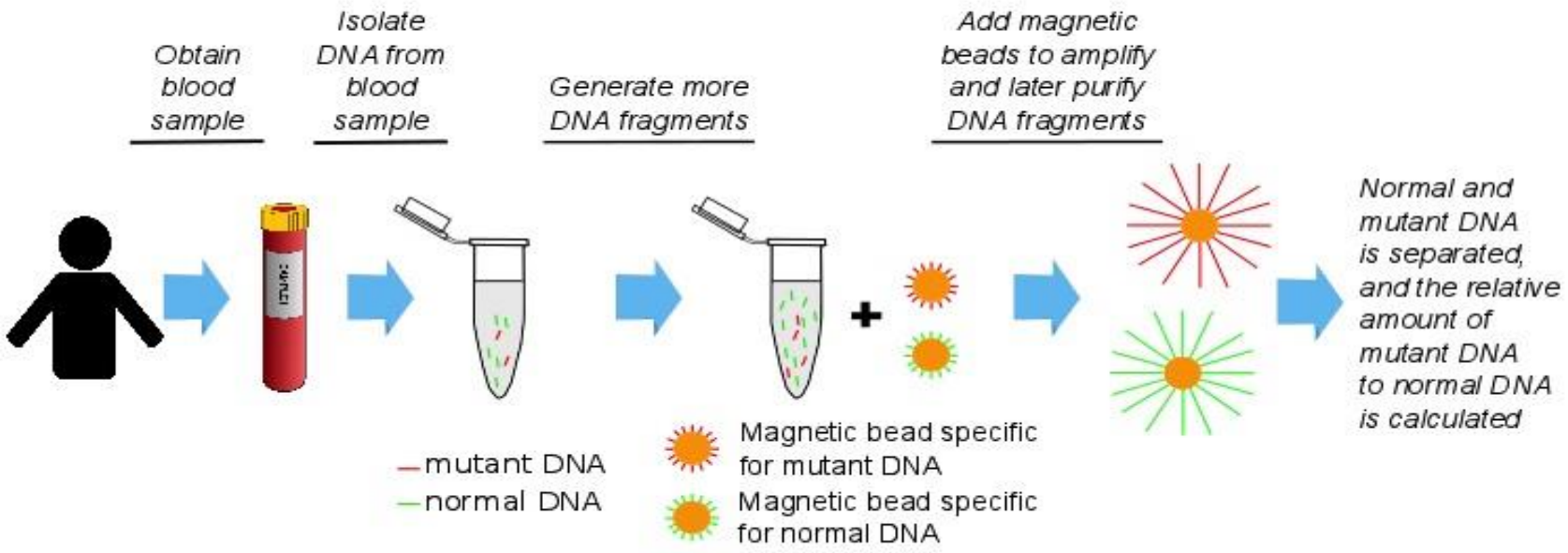


# BEAMing Technology

- Beads Emulsion Amplification and Magnetics Technology
- Able to detect **known** mutations in a plasma sample at a high sensitivity




http://sitn.hms.harvard.edu/flash/2014/fingerprinting-cancer-with-blood-blood-based-biopsies-bring-new-ease-and-precision-to-cancer-screening

# Drawbacks

- *You can't detect what you aint lookin' for*
  - Only able to detect mutations that are predetermined

- Detection of mutations that span across a large segment of the gene is challenging
  - 12% of exon 11 mutations detected in plasma vs 43% detected in tumor tissue

# Our work

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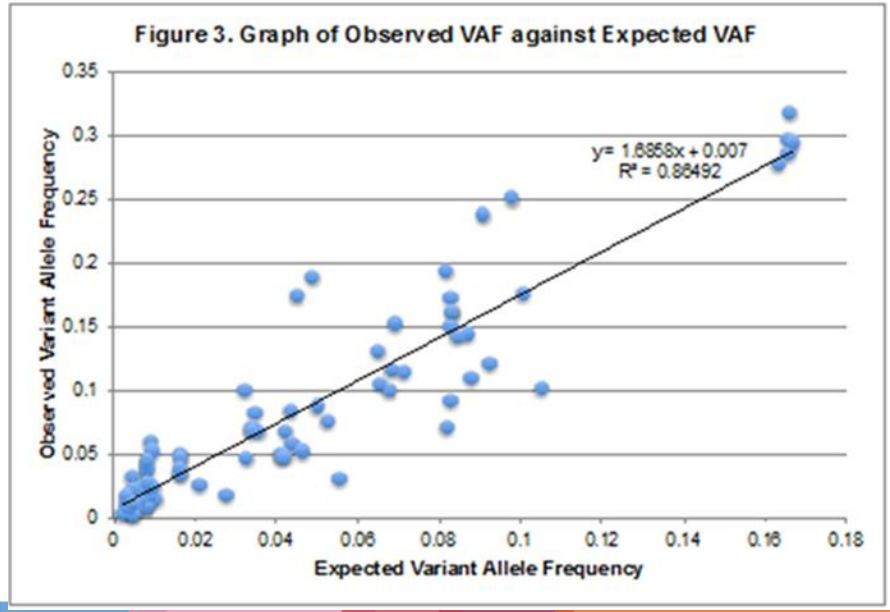
We developed an NGS based assay that was able to

- Detect mutant DNA present at 0.1% of variant allele frequency from cfDNA that is present in plasma samples of patients with metastatic GIST

- To be able to do multiplex sequencing of the primary and secondary mutations using ctDNA



# Preliminary Results

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- This assay system was piloted on 8 patients
- Primary mutations were detected at a sensitivity of 62.5%
- In a separate cohort of 3 patients, detection of primary as well as multiple secondary mutations was demonstrated

# What the future holds…

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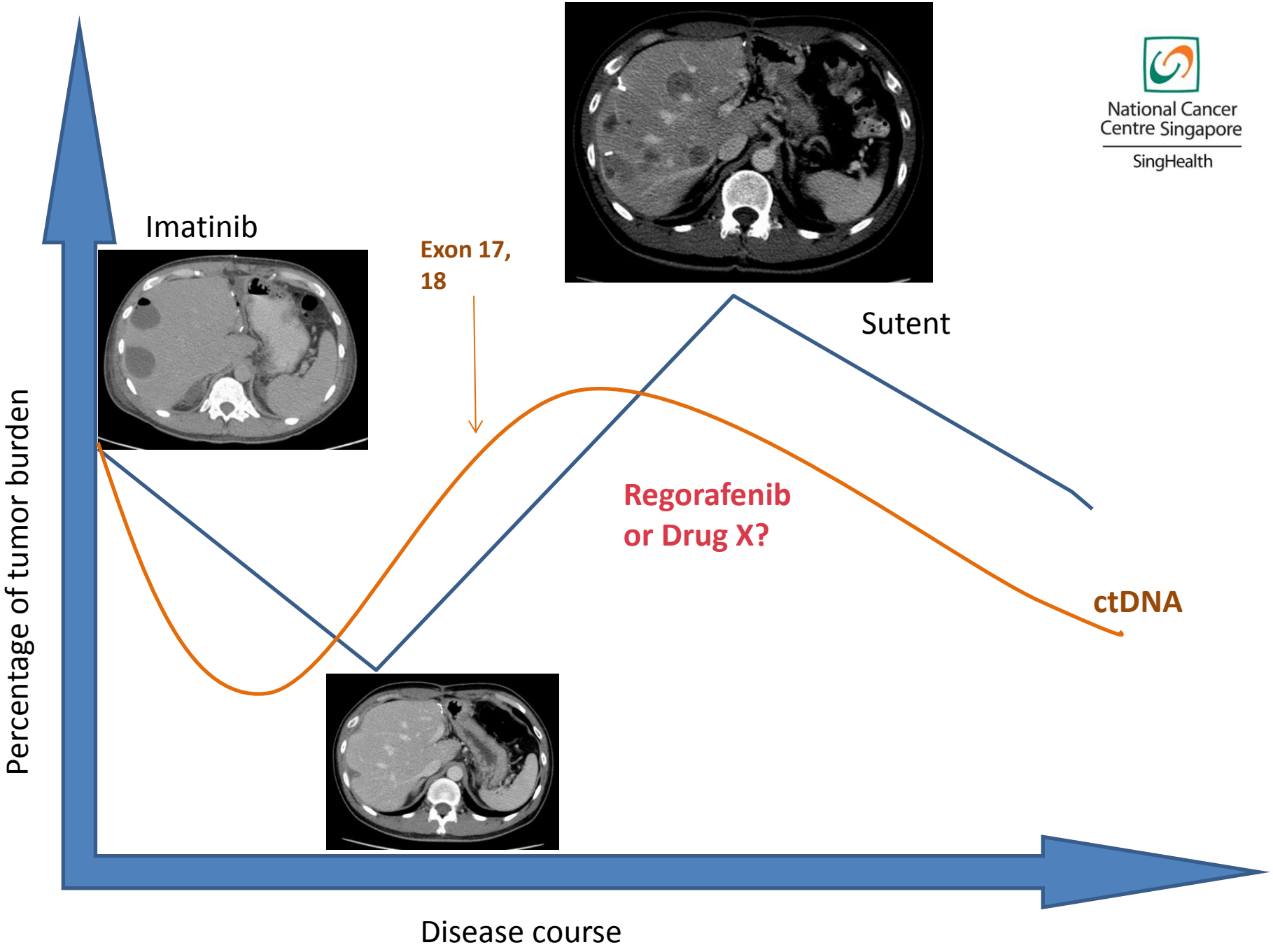
- Overcome the biological barrier of tumor heterogenity
- Ability to detect resistance at a genomic level prior to development of clinical or radiological resistance
- Identification of mutational burden at various time points in the course of disease of a patient – a potential non invasive biomarker
- To be able to select the truly high risk group for adjuvant treatment
- Can this replace imaging in the future?

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# Thank you!

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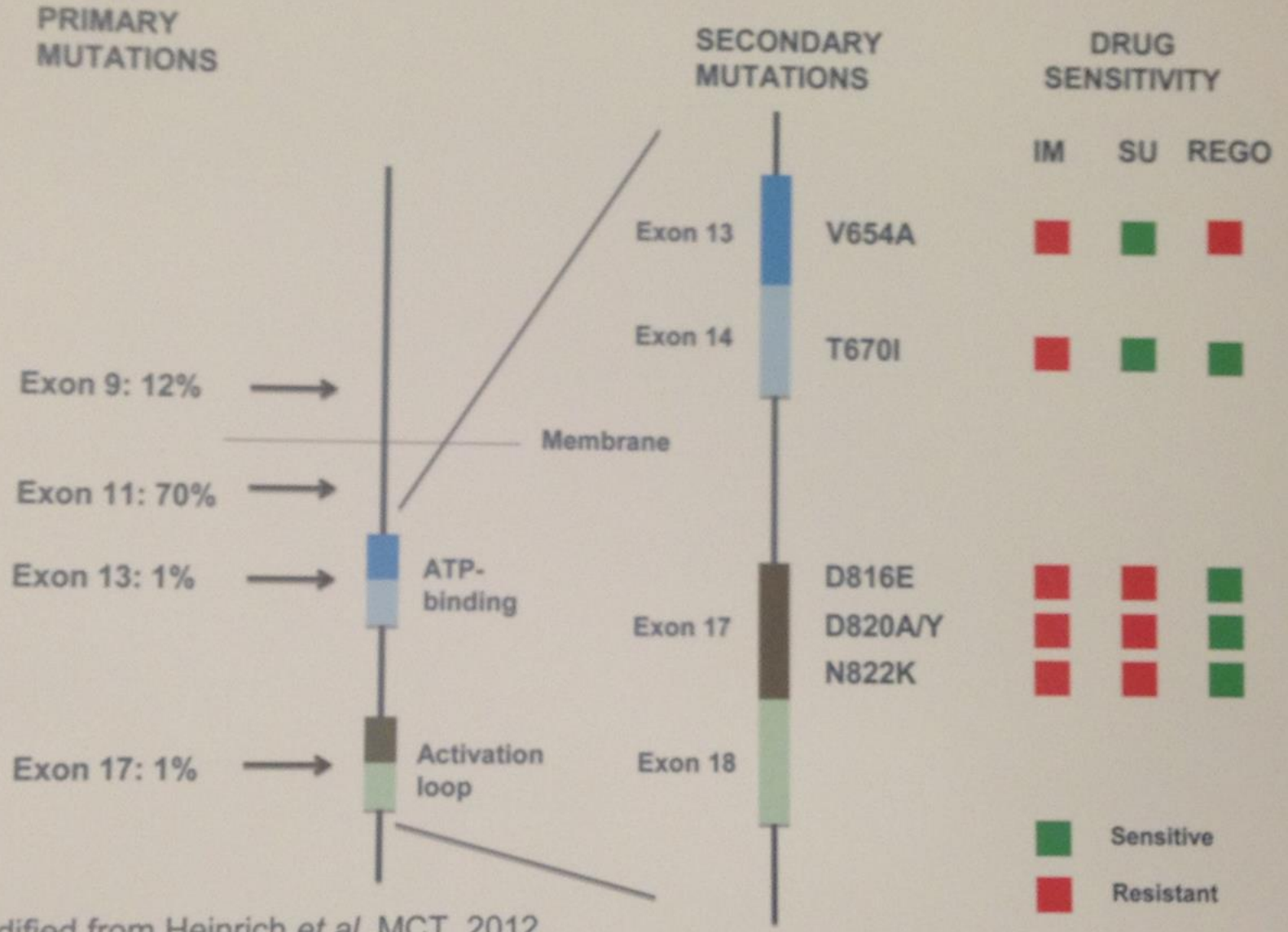
# Predicted sensitivity profile of REGO compared to IM and SU




Modified from Heinrich *et al*. MCT, 2012

Slide courtesy of Dr Richard Quek

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