Mutation analysis in cell-free DNA from cancer patients

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CRIG's digital PCR mini-symposium, May 15, 2018

advantages of liquid biopsies

suitable technology

Biogazelle is an expert digital PCR service provider

https://bgzlle.com/2jUZZhd

> 10,000 successful dPCR analyses

experts in custom dPCR assay design and data analysis

high quality standard in GCLP-compliant environment

using Bio-Rad's QX200 ddPCR System

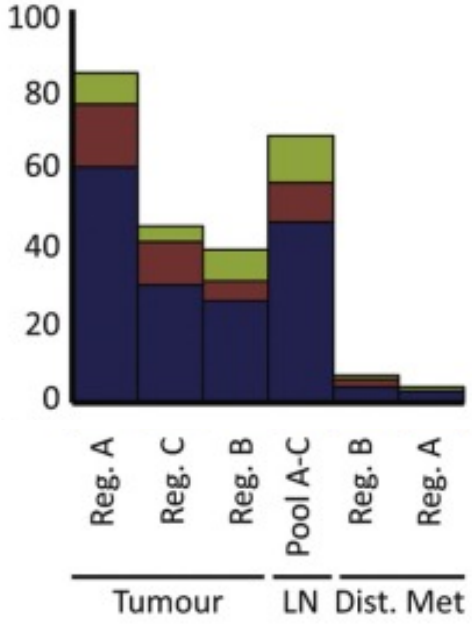
reference center for Bio-Rad's ddPCR system in Europe

Mutation analysis in cell-free DNA from cancer patients

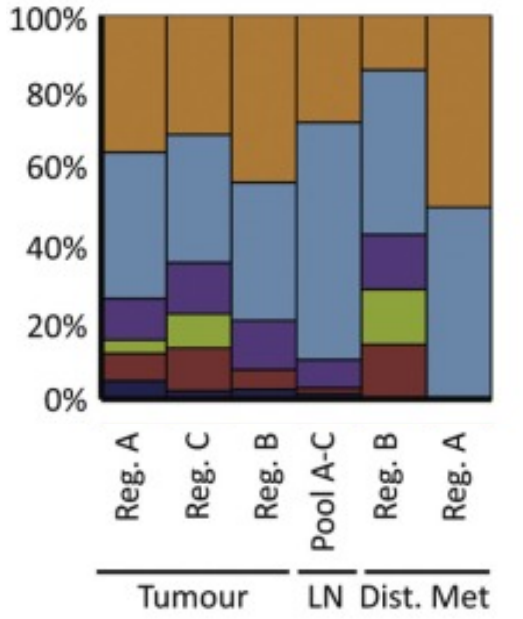
- advantages of liquid biopsies
- suitable technology
- case study

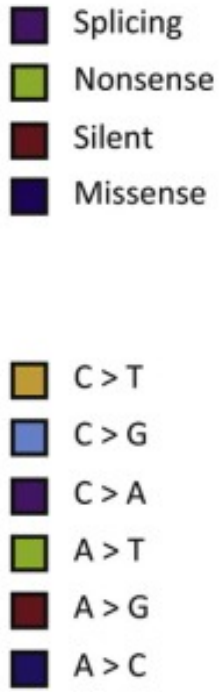
Difference in mutational landscapes between primary tumors and distant metastases

mutation count



nucleotide changes





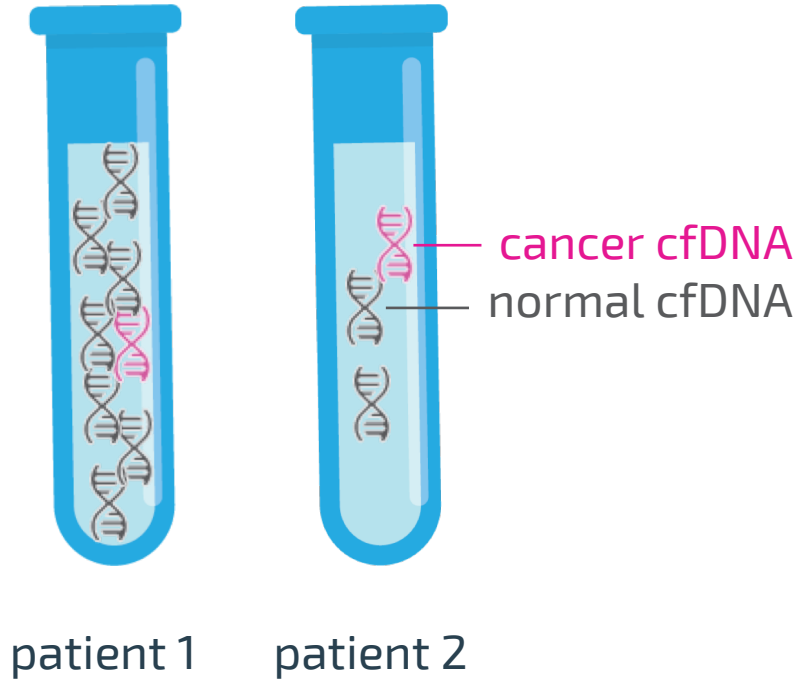
Thomsen M, *et al.*, Molecular Oncology, 2016

Liquid biopsies are the cornerstone of precision medicine

https://bgzlle.com/2KiGLNQ

Screening for mutations in cell-free DNA comes with specific analytical challenges

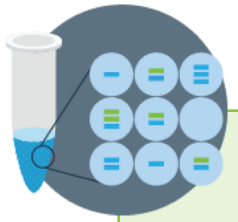
- only a **small fraction** of the cell-free DNA collected from plasma, comes from the tumor cells
- the **yield** of cell-free DNA from plasma is **highly variable**



Mutation analysis in cell-free DNA from cancer patients

- suitable technology

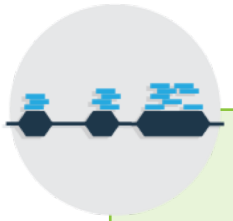
Both NGS and dPCR offer advantages for mutation analysis of cfDNA



digital PCR

(+) superior sensitivity and specificity

(-) not suited for detecting new mutations



next generation sequencing

(+) unbiased analysis

(-) error rate (0.1–1%)

(-) cost

Mutation analysis in cell-free DNA from cancer patients

- case study

Case study: mutation analysis in cell-free DNA from cancer patients

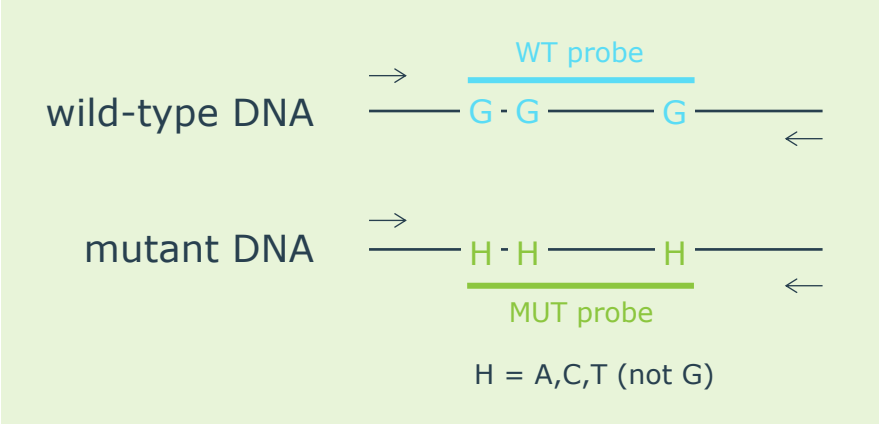
- **Clinical trial** for Servier compound S95005 in metastatic colorectal cancer
- Can the presence or absence of mutations in cell-free DNA **predict therapy response or resistance**?
  - 30 mutations in 3 genes of interest

Case study: mutation analysis in cell-free DNA from cancer patients

1. Experiment design
2. Method validation
3. Summary of results

The experimental set-up at a glance

- 204 patients enrolled
- 30 mutations in 3 genes of interest
- intended sensitivity of 2%
- covered by 13 dPCR assays
  - 8 mutation detection assays
  - **5 multiplex screening assays ➔**

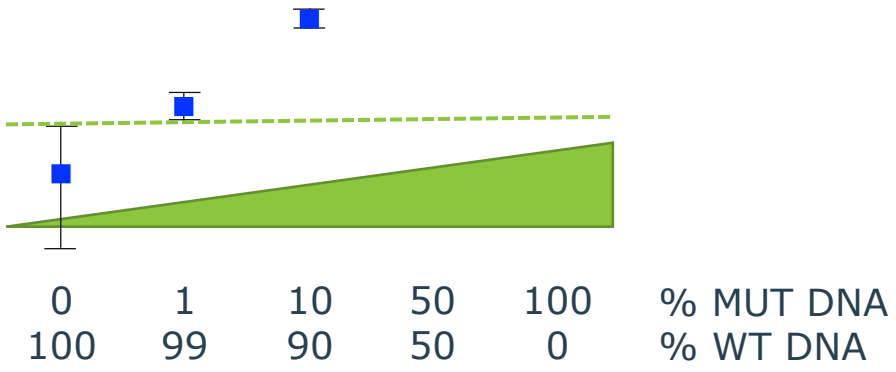


Case study: mutation analysis in cell-free DNA from cancer patients

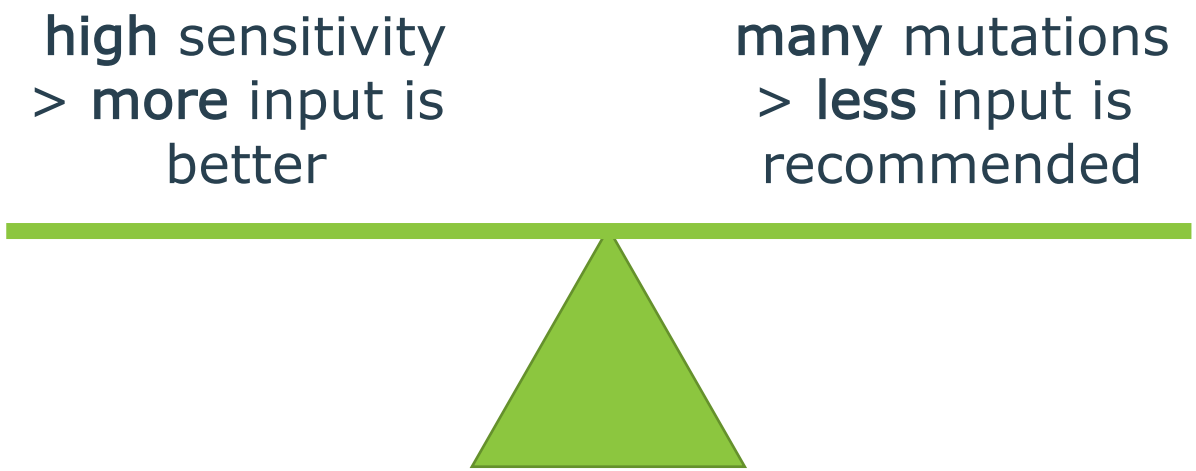
2. Method validation
   - validation of dPCR mutation detection assays
     - optimal annealing temperature (not further discussed)
     - limit of detection
   - determination of optimal cfDNA input in dPCR reaction

Method validation: determination of limit of detection

- limit of detection = lowest mutant concentration that can be reliably distinguished from the mutation-negative control
- depends amongst others on
  - assay specificity
  - sample input amount and quality
  - measurement uncertainty



Method validation: determination of optimal cfDNA input



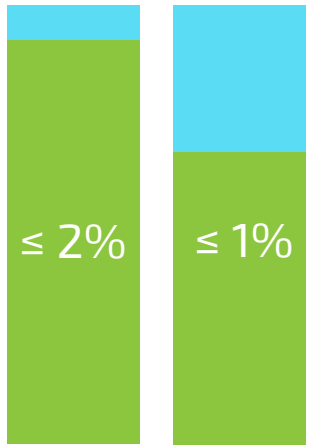
- 3 priority levels for the assays (top, medium, low)
- start with ¼ of cfDNA for at least the 4 top priority assays; dilute cfDNA if possible to screen more mutations

Case study: mutation analysis in cell-free DNA from cancer patients

3. Summary of results

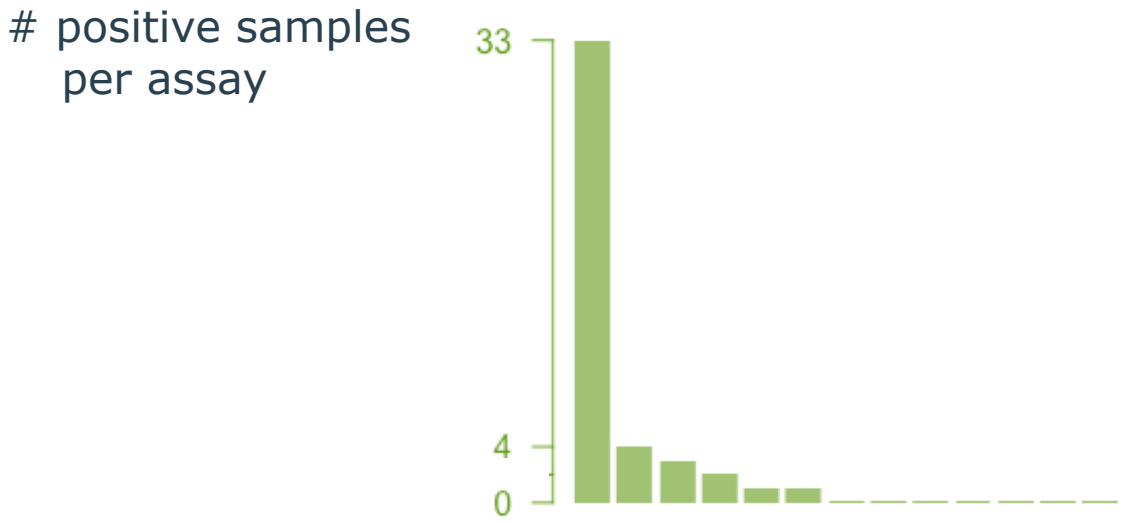
For the majority of dPCR reactions, the desired sensitivity was obtained

- 691/744 reactions (92.5%) contained sufficient DNA to reach the target sensitivity of 2%
- for 65% of reactions, detection sensitivity was ≤ 1%



Most patients were mutation-positive for one particular assay

- for 42 patients, at least 1 test was positive
- in total, 6 different tests were found to be positive



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Mutation analysis in cell-free DNA from cancer patients

- powerful method for accurate and precise measurements
- liquid biopsies: low input, rare variants, small differences
- careful assay design and validation
- inclusion of positive and negative controls
- advanced (statistical) data-analysis

The leading company for innovative RNA and DNA based solutions