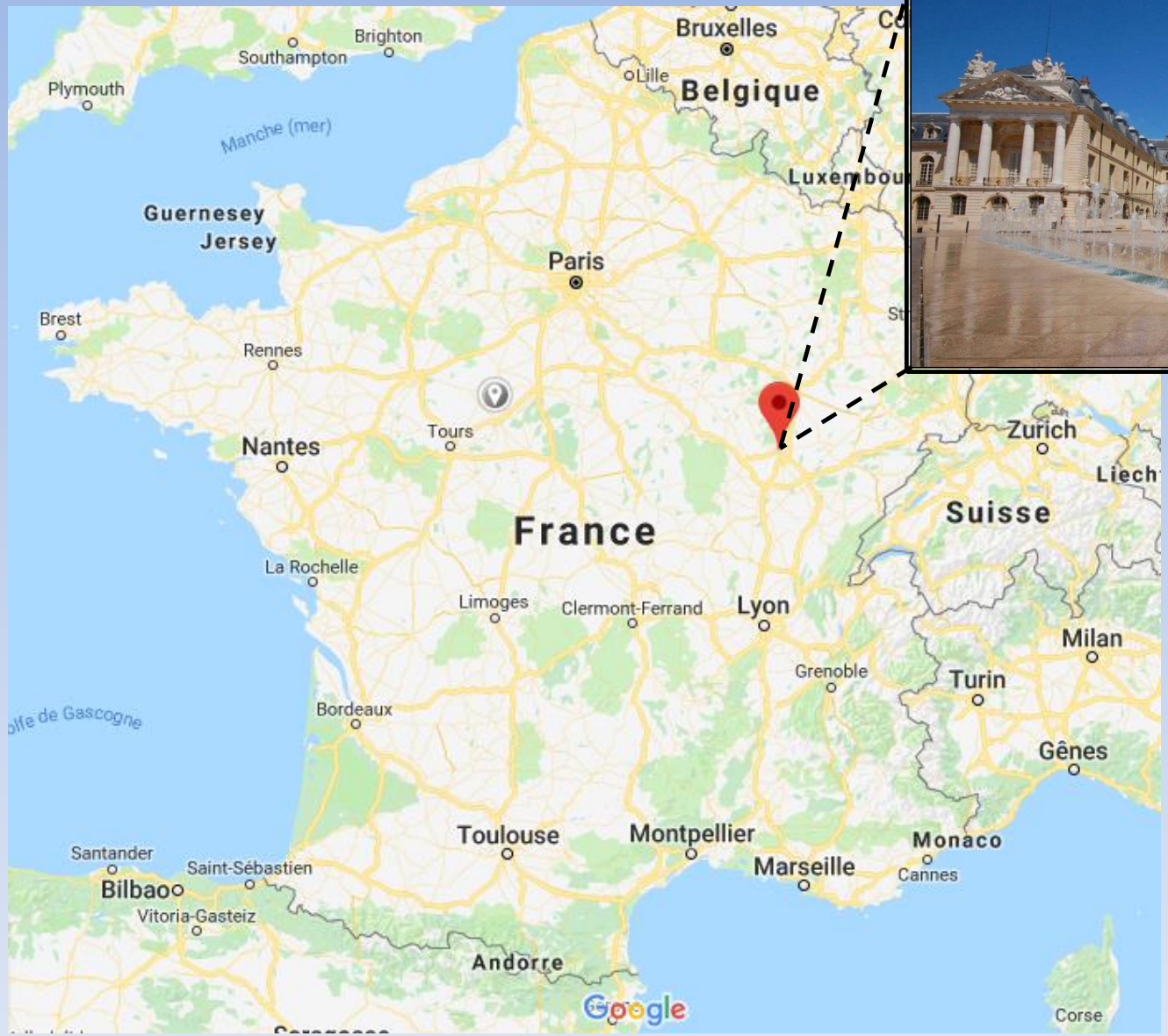
Implementation of Digital PCR in a Molecular Diagnostic Laboratory: Evaluation of Minimal Residual Disease and metastatic cancer progression

Benjamin Tournier, PhD
Plateform of Somatic Oncology
INSERM LNC-UMR 1231
University Hospital of Dijon, France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Turin
Bordeaux
olfe de Gascogne
Gênes
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Corse


Louis Latour
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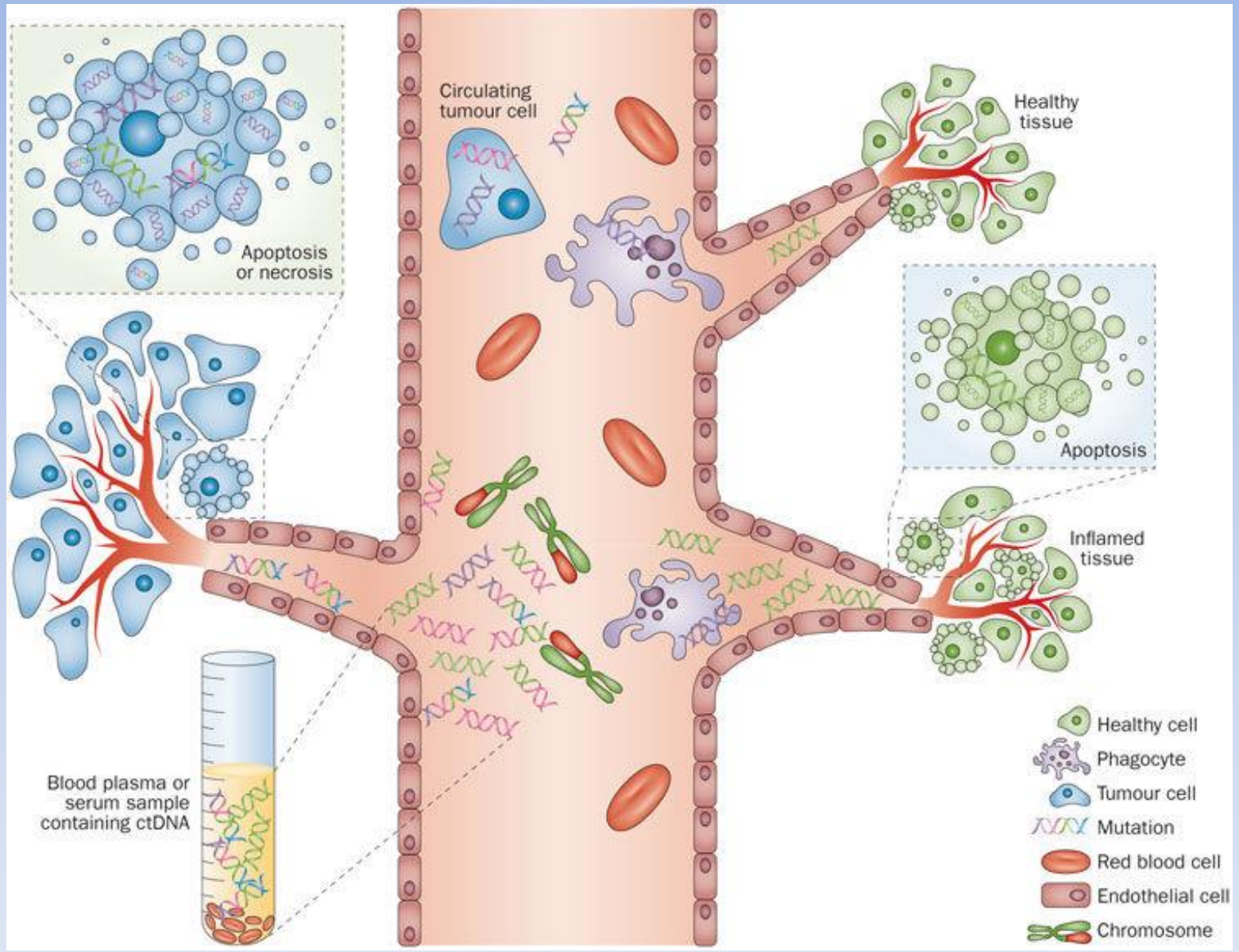
1747
MAILLE
Dijon Originale


Edmond Fallot
MOUTARDE DE DIJON

The liquid biopsy in oncology



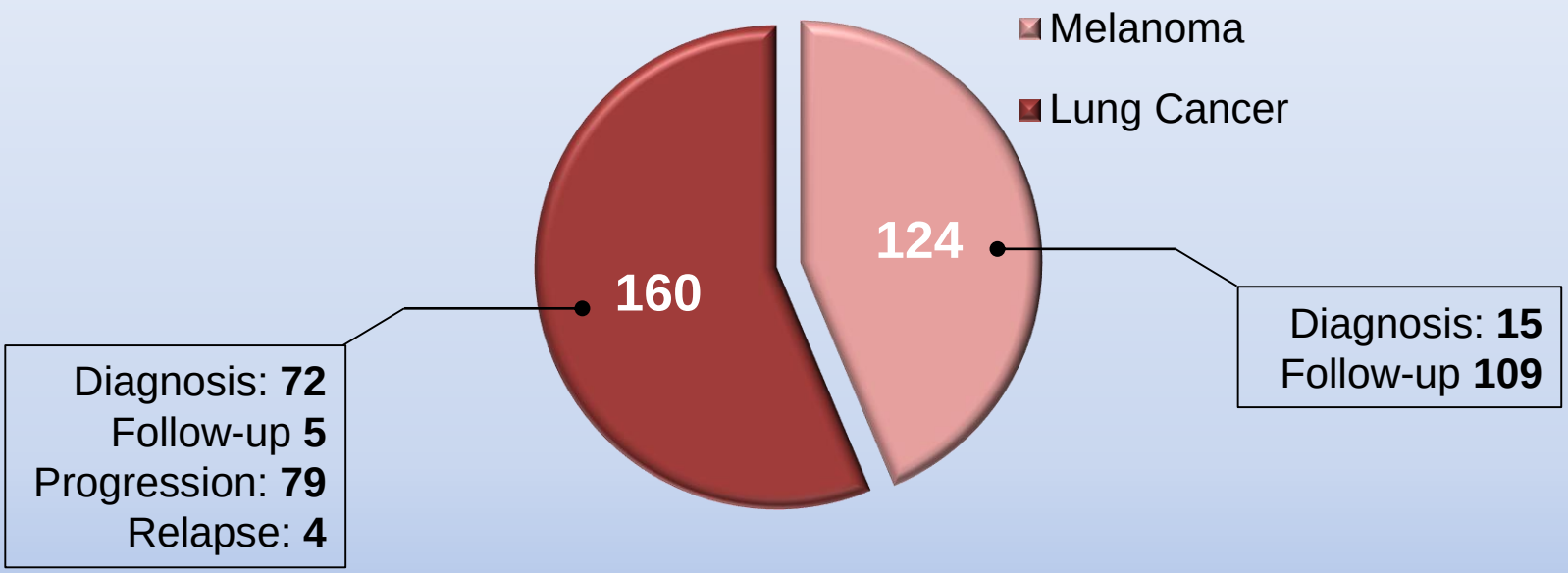
Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. *Nat Rev Clin Oncol.* 2013

1. Setting up of the liquid biopsy process at Dijon Hospital

Setting up of the liquid biopsy process at Dijon Hospital

- Global activity of the platform >2000 somatic molecular analyses in solid tumours / year
- Liquid biopsy process set up in 2016
- For metastatic melanoma and lung cancer patients

Number of liquid biopsies performed in 2017



Setting up of the liquid biopsy process at Dijon Hospital

The liquid biopsy workflow: The blood sampling

K2 EDTA tubes
(BD Vaccutainer®)

or

« Cell-free DNA collection tube » (Roche®)

Used for local blood sampling

Used for external blood sampling

- Cell-Free DNA BCT® (Streck)
- PAXgene® Blood DNA Tube (PreAnalitiX, QIAGEN/BD)
- LBgard® Blood Tubes (Biomatrica)
- cf-DNA/cf-RNA Preservative Tubes (NORGEN Biotek corp.)

- *Warton K, Yuwono NL, Cowley MJ, McCabe MJ, So A, Ford CE, Mol Diagn Ther. 2017* → EDTA, Streck and PAXgene tubes
- *Zhao Y, Li Y, Chen P, Li S, Luo J, Xia H, J Clin Lab Anal. 2018* → EDTA, Roche and Streck tubes

Setting up of the liquid biopsy process at Dijon Hospital

The liquid biopsy workflow: The pre-analytical steps

-80°C → + 4°C 1 night

RT 1 hour

16 000g
6 min.

up to 4 hours for **K2 EDTA tubes**

up to 7 days for **Cell-free DNA collection tubes**

cfDNA extraction
+ Molecular analyses
+ Storage -20°C

Setting up of the liquid biopsy process at Dijon Hospital

<u>The liquid biopsy workflow:</u> The cfDNA extraction method

- Automated Extraction: QIAsymphony, DSP Circulating DNA Kit

4 mL plasma
60 µL elution

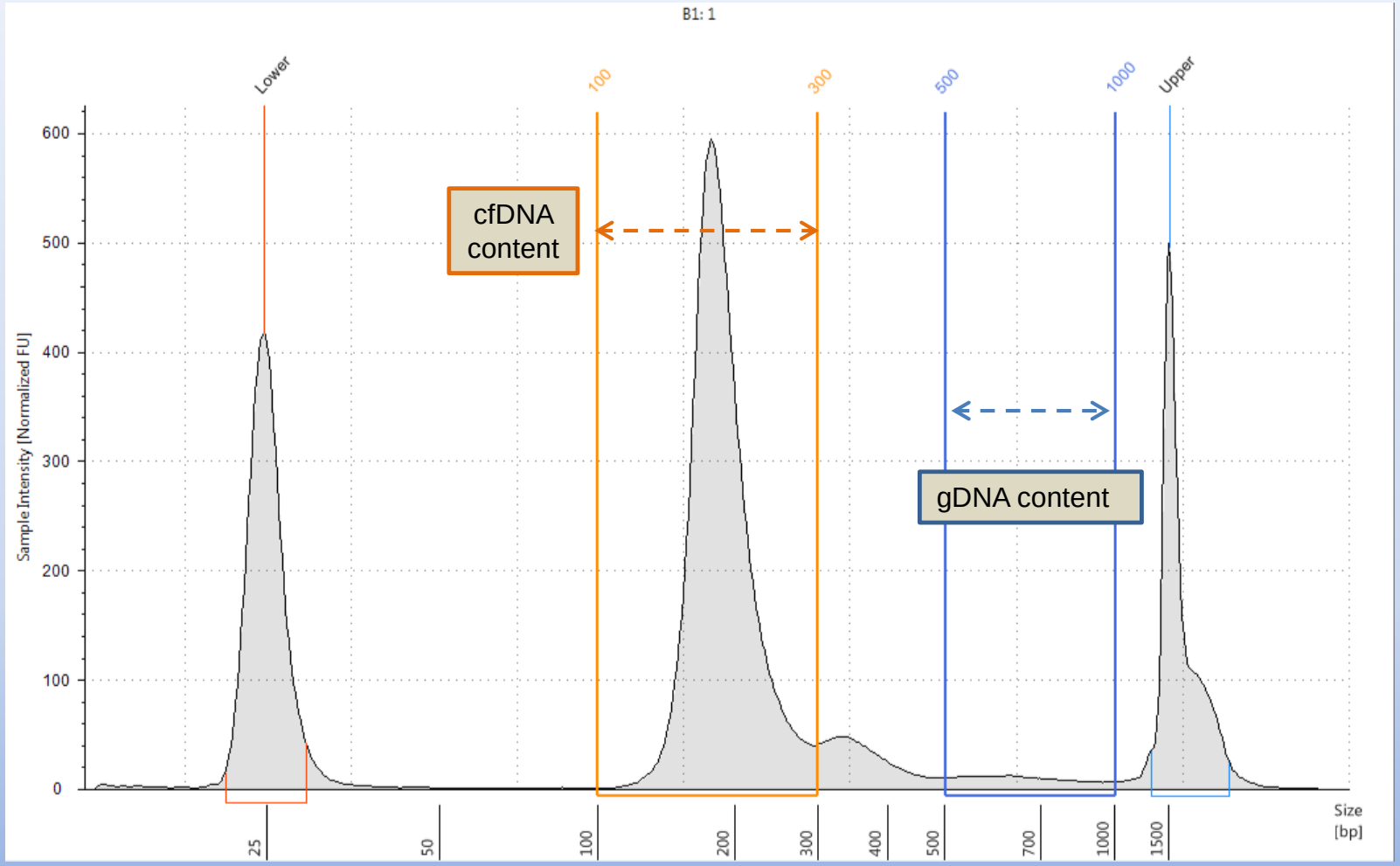
- Manual Extraction: Macherey-Nagel, NucleoSnap® DNA plasma

Up to 5 mL
plasma
45 µL elution

Setting up of the liquid biopsy process at Dijon Hospital

The liquid biopsy workflow: The cfDNA quantification/qualification

- Qubit ds DNA high Sensivity
- TapeStation 4200, D1000 High Sensivity



Setting up of the liquid biopsy process at Dijon Hospital

Conclusion of critical parameters:

- Plasma minimal volume of 4 mL
- Transport time
- Double centrifugation of plasma (even 3 centrifugation)
- Gentle thawing

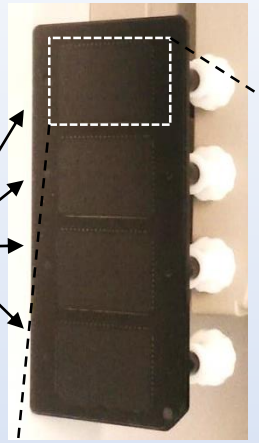
1. Setting up of the liquid biopsy process at Dijon Hospital

2. NAICA digital PCR (Stilla Technologies®)

NAICA digital PCR (Stilla Technologies®) Principle and Workflow

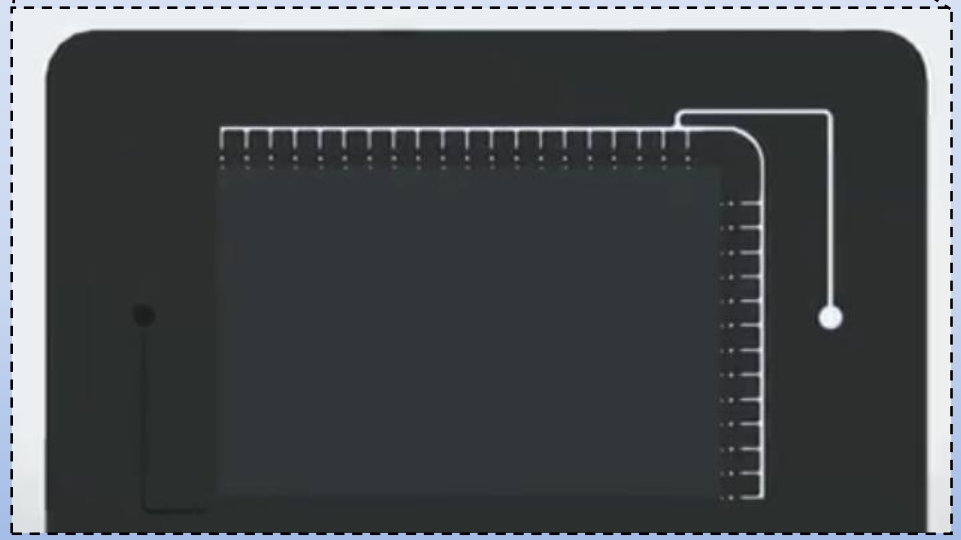
The “Sapphire” chip

4 chambers



Oil loading

Loading of PCR reagents + 14µL of cfDNA



Zoom of a Sapphire chamber during repartition of the reaction mix

Principle and Workflow

The Naica dPCR system: 3 instruments

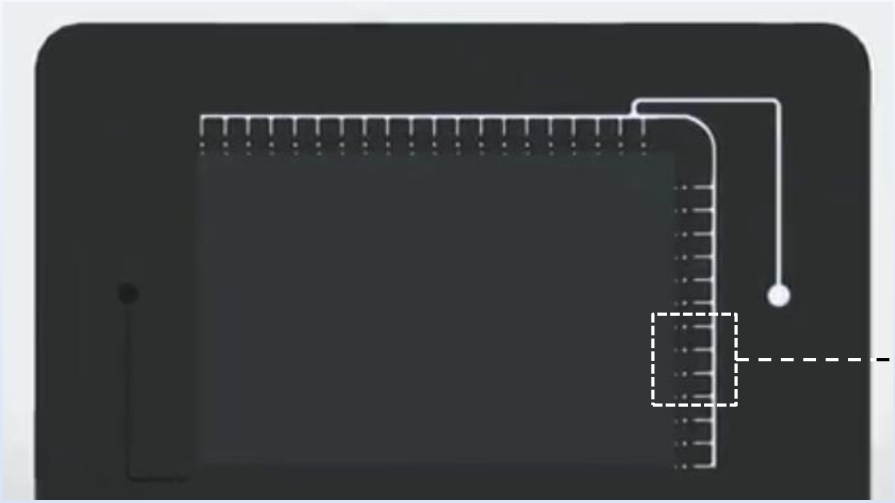
Air pump instrument

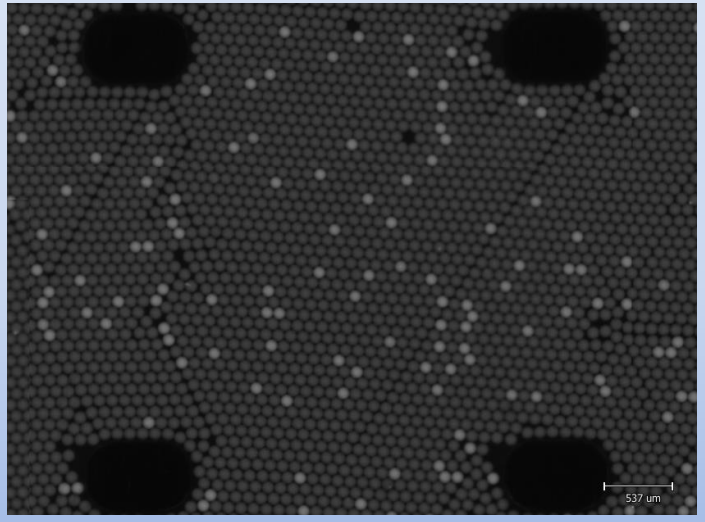
Naica Geode:
- Droplet generator
- Thermocycler

Naica Prism3:
Fluorescence scanner

Principle and Workflow

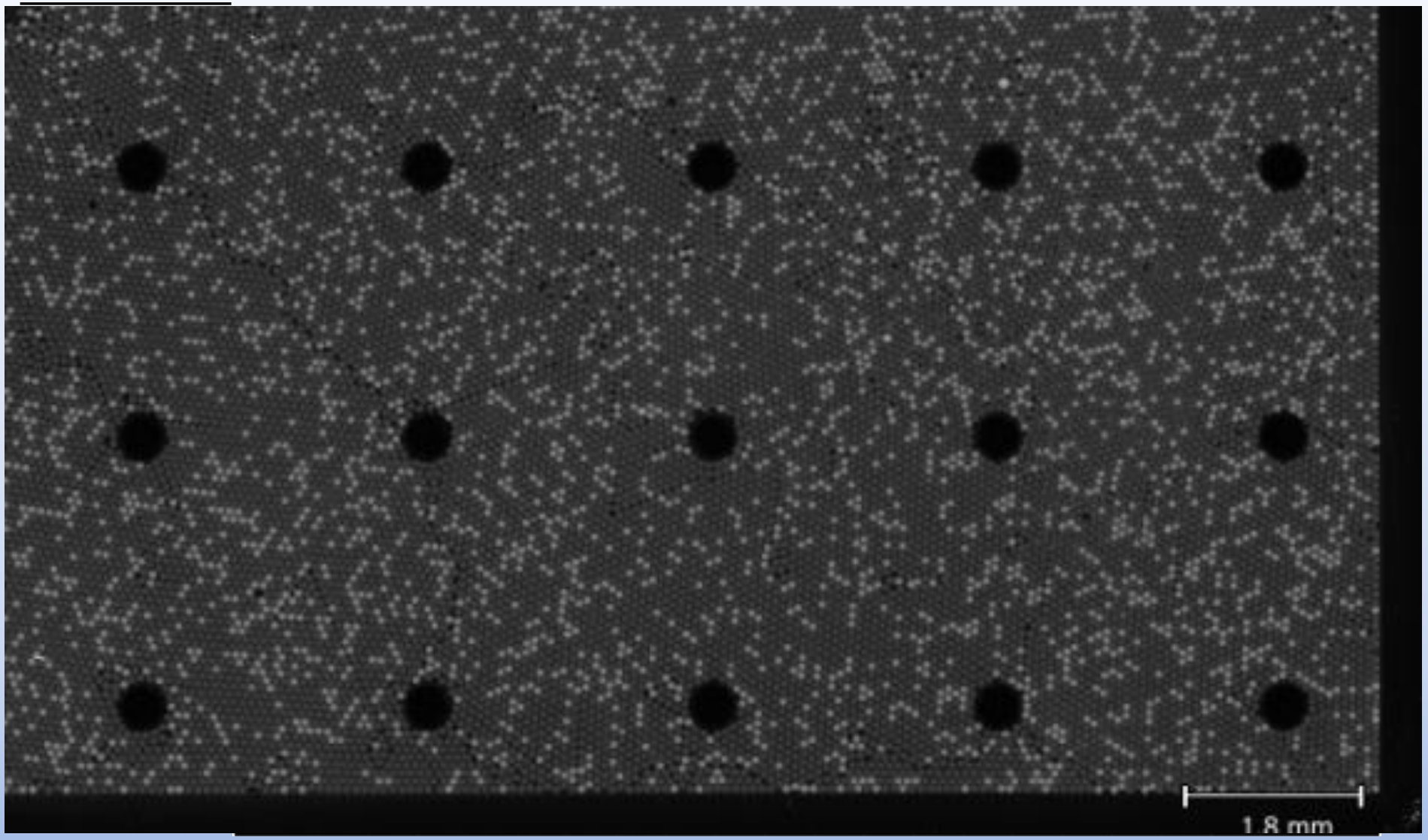
The “Sapphire” chip: Generation of droplets & Size calibration





NAICA digital PCR (Stilla Technologies®) Demonstration

Visualization:

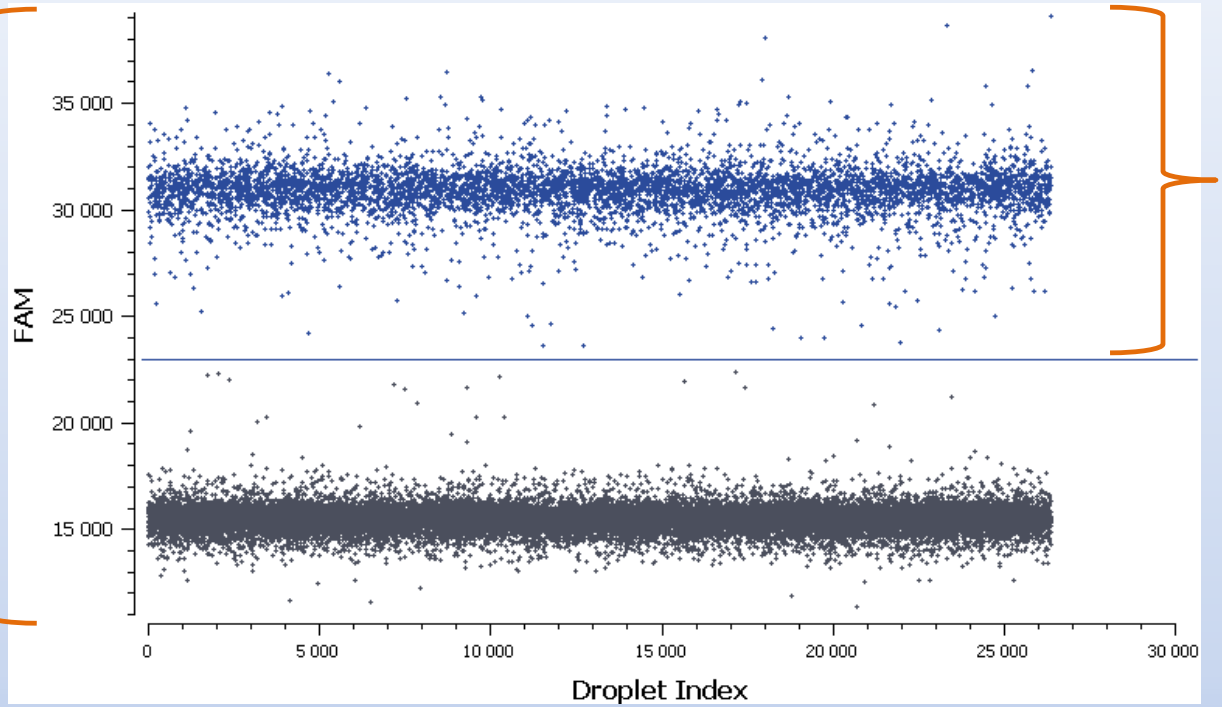


BLUE channel (495-515nm)

NAICA digital PCR (Stilla Technologies®) Demonstration

1D graphical representation:

Total number of droplets: 26 363

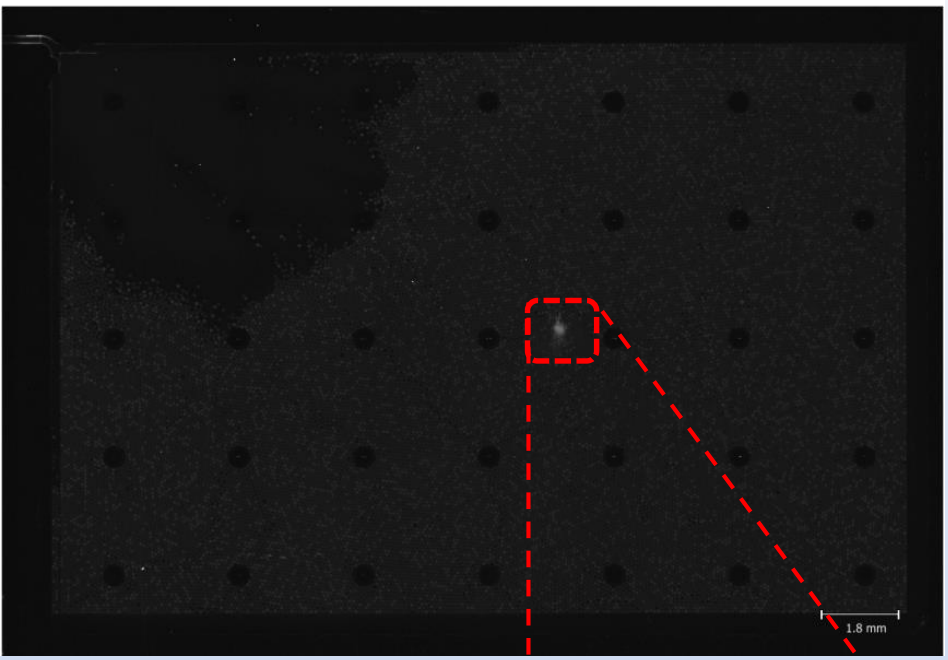


Number of FAM positive droplets: 5 948

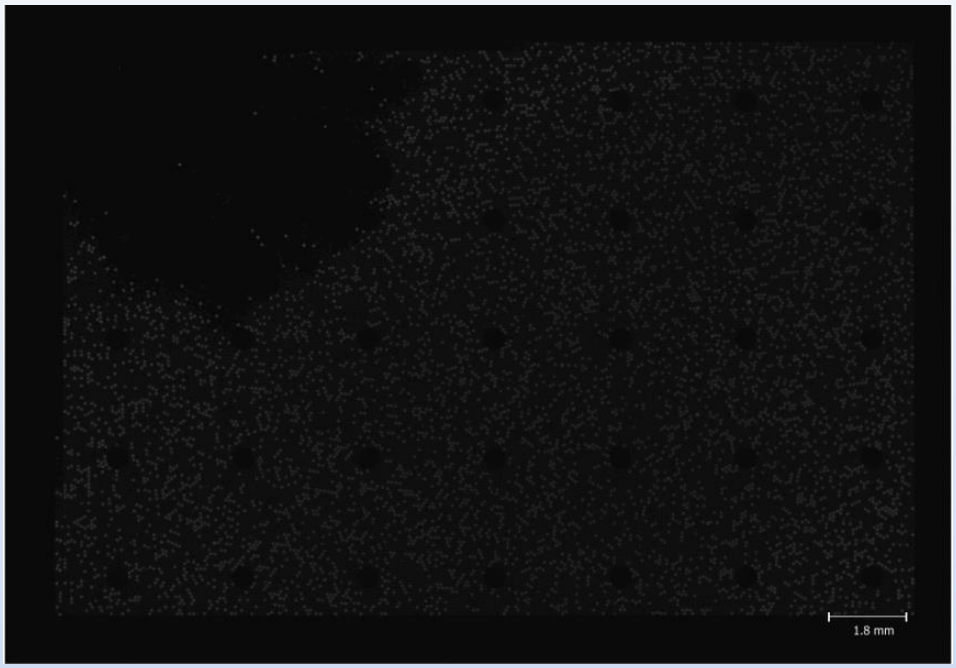
BLUE channel (495-515nm)

NAICA digital PCR (Stilla Technologies®) Demonstration

Visualization:



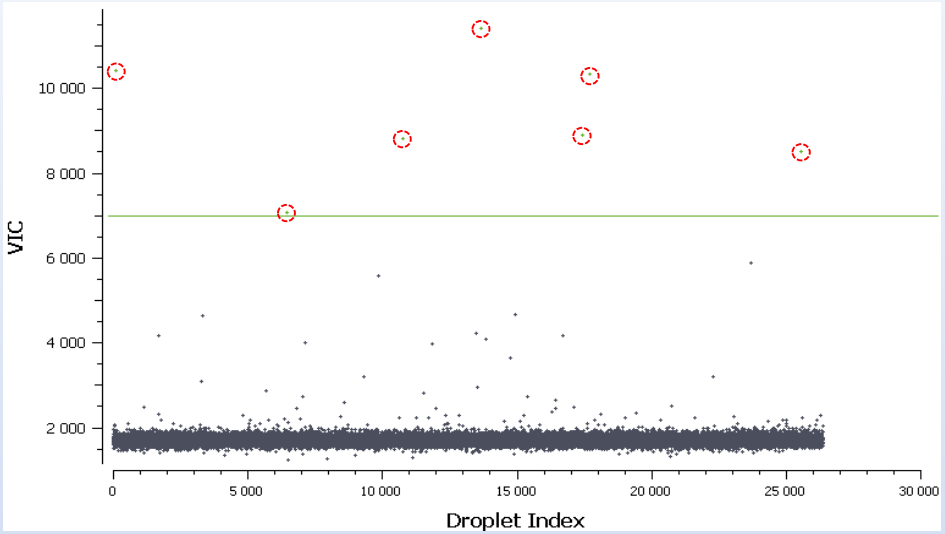
GREEN channel (560-610nm)



RED channel (655-720nm)

NAICA digital PCR (Stilla Technologies®) Demonstration

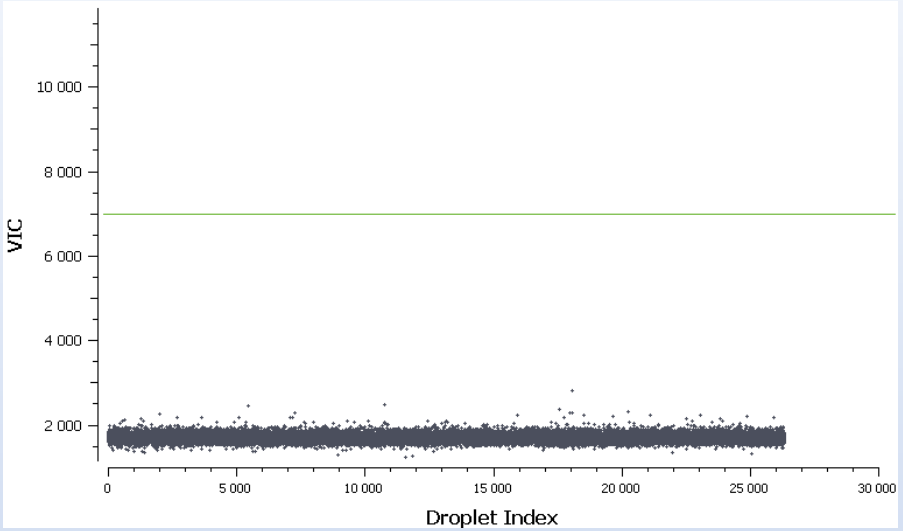
1D graphical representation:



GREEN channel (560-610nm)

Before correction

7 VIC positive droplets…

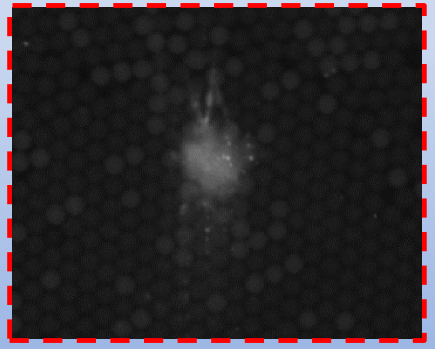


GREEN channel (560-610nm)

After correction

No VIC positive droplets

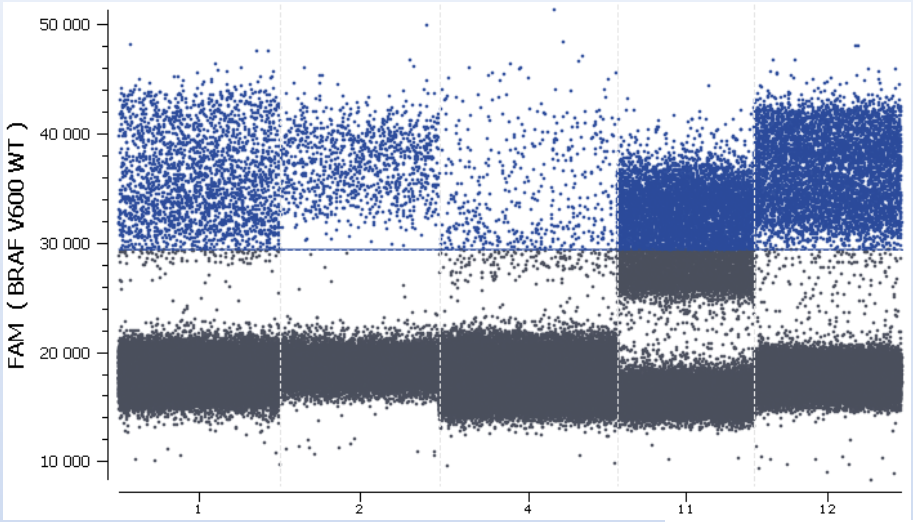
… which are actually artefacts



NAICA digital PCR (Stilla Technologies®) Demonstration

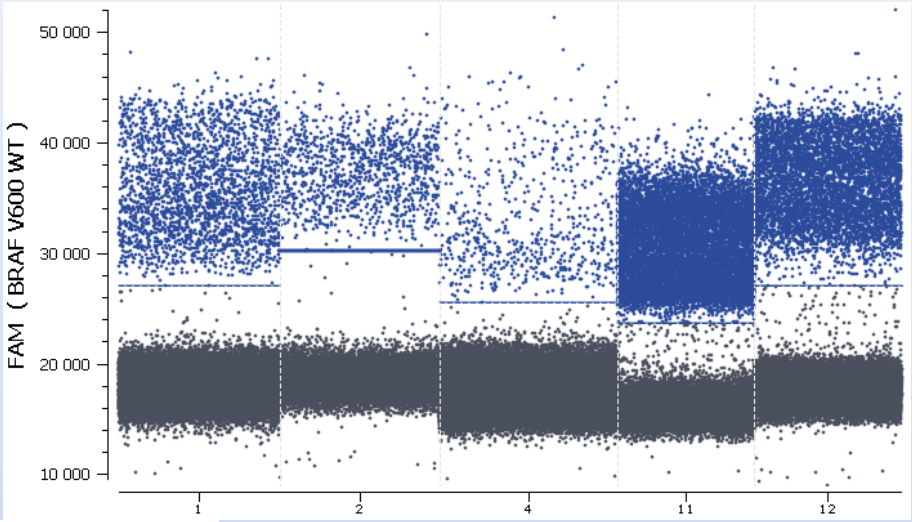
1D graphical representation:

Nature of samples influences the fluorescence intensities…



BLUE channel (495-515nm)

Adaptation of fluorescence threshold



BLUE channel (495-515nm)

1. Setting up of the liquid biopsy process at Dijon Hospital

2. NAICA digital PCR (Stilla Technologies®)

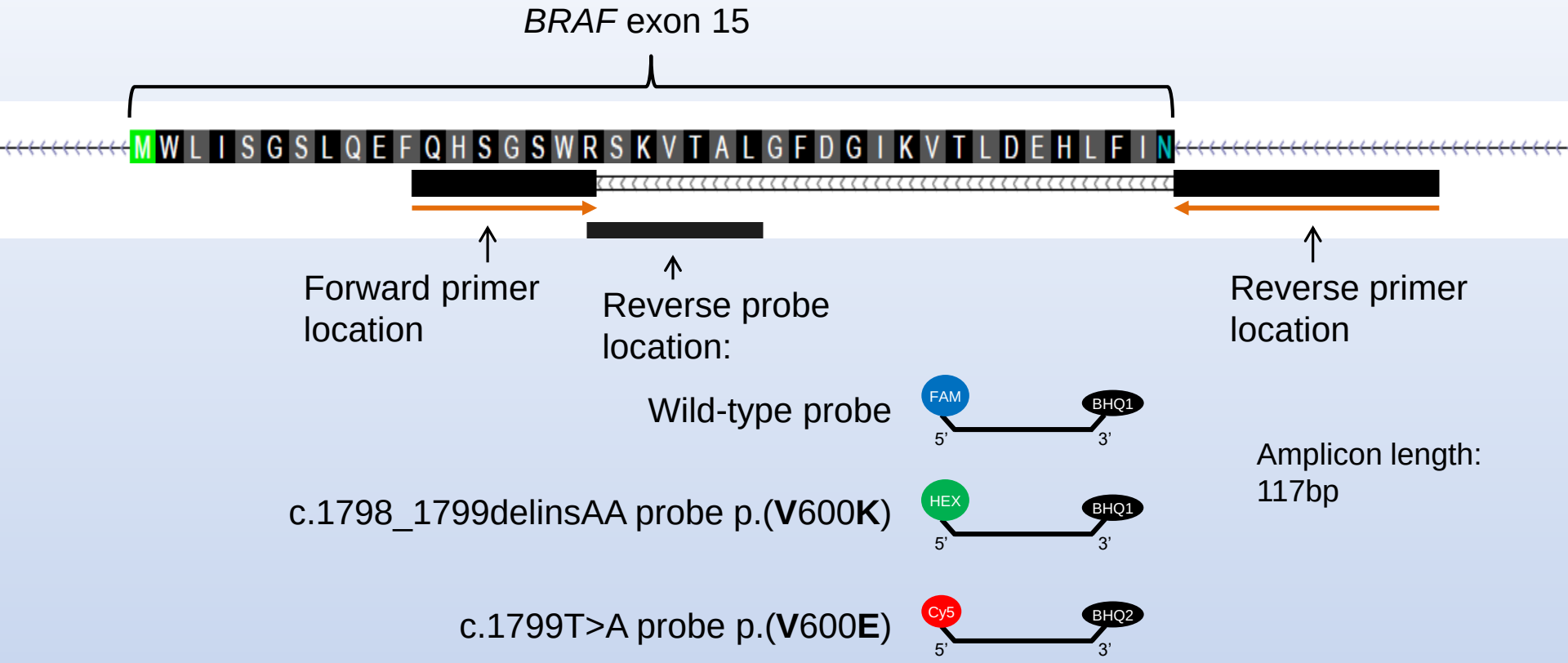
3. Example of applications

3.1 *BRAF* testing in Melanoma

Molecular test set up for Melanoma

Testing of *BRAF* V600K/E mutations

Design:



Amplicon length: 117bp

Adapted from Punnoose et al., Clin Cancer Res, 2012

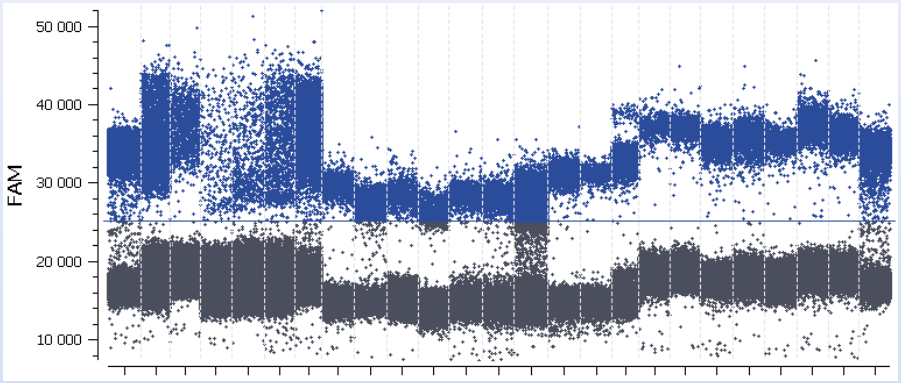
Molecular test set up for Melanoma

Testing of *BRAF* V600K/E mutations

Validation:

1. classical PCR optimization
2. qPCR testing: evaluation of the PCR efficiency
3. Digital PCR testing: evaluation of the noise and determination of the limit of detection (LOD)

Analysis of 25 *BRAF* wild-type cell-free DNAs:



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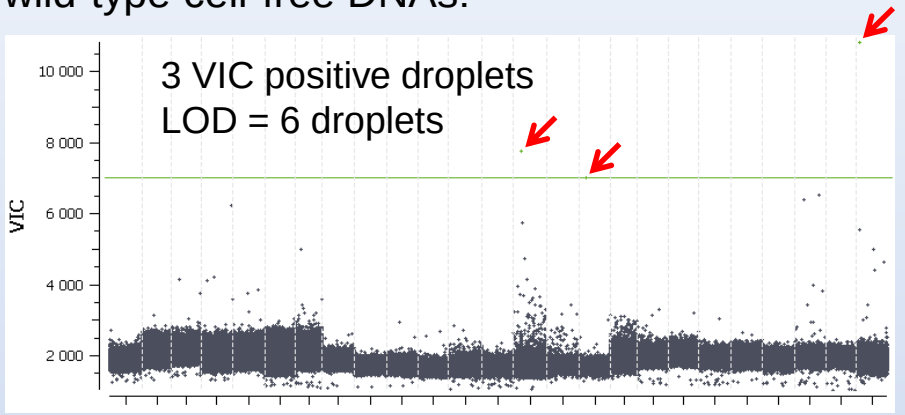
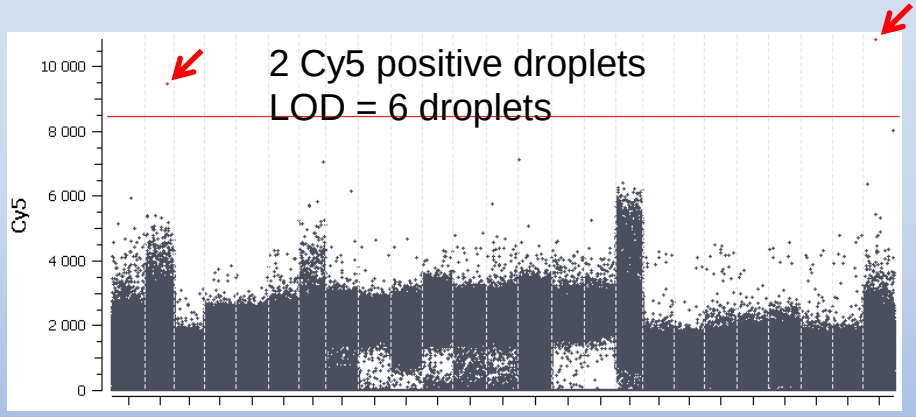


GREEN channel (560-610nm)



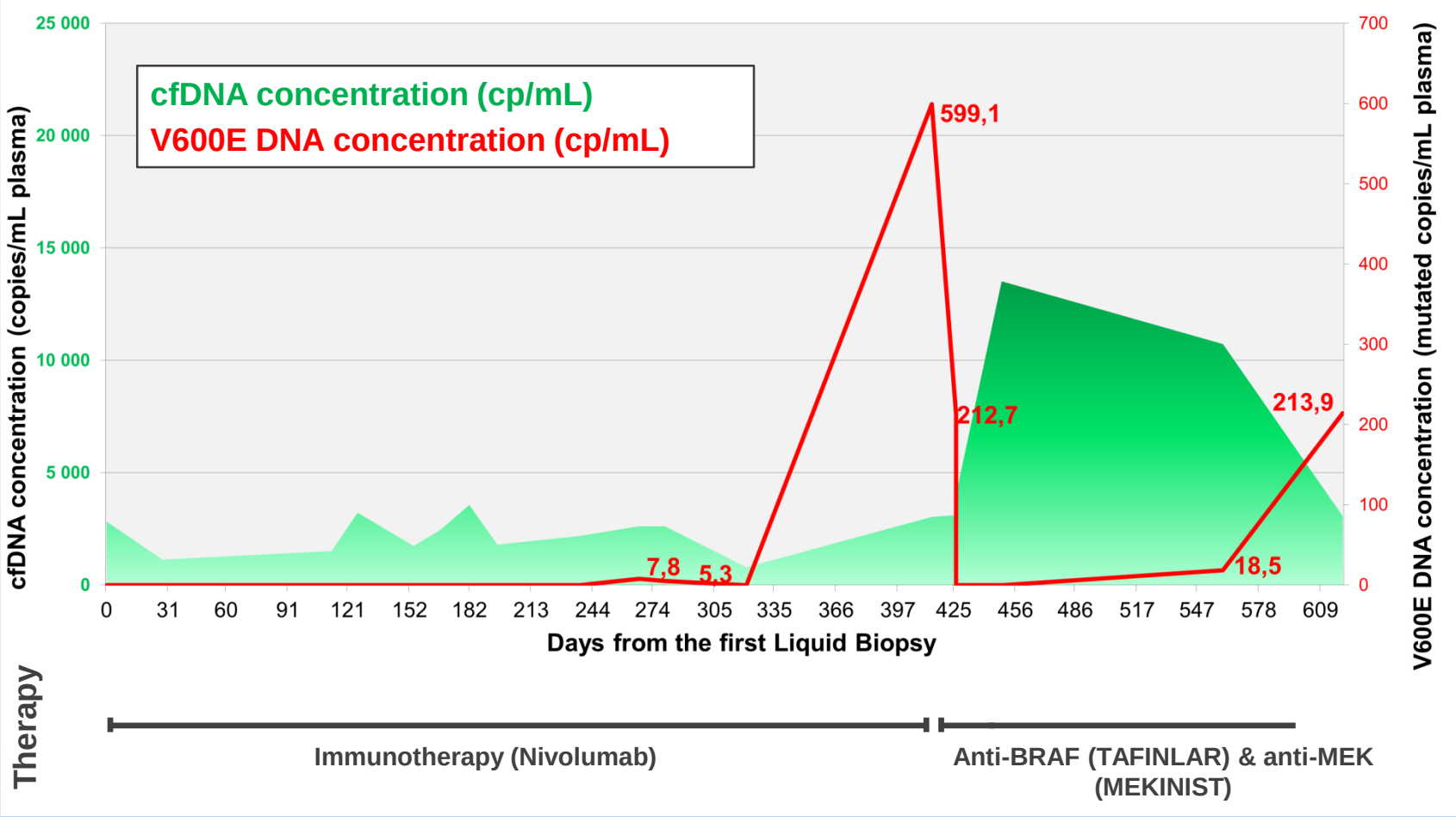
RED channel (655-720nm)

Calculations:

1. Corrected mean number of wrong positive droplets (µ corr.)
2. Limit of blank (LOB)
3. Limit of detection (LOD)

Example for the MRD checking in a melanoma patient

- Forty-seven year old man diagnosed for an invasive SSM melanoma in 2008
- Detection of the **BRAF p.(V600E)** mutation in a tissue biopsy at diagnosis
- Treatment: Anti-BRAF (TAFINLAR) during 10 months, then radiotherapy, then immunotherapy (NIVOLUMAB)



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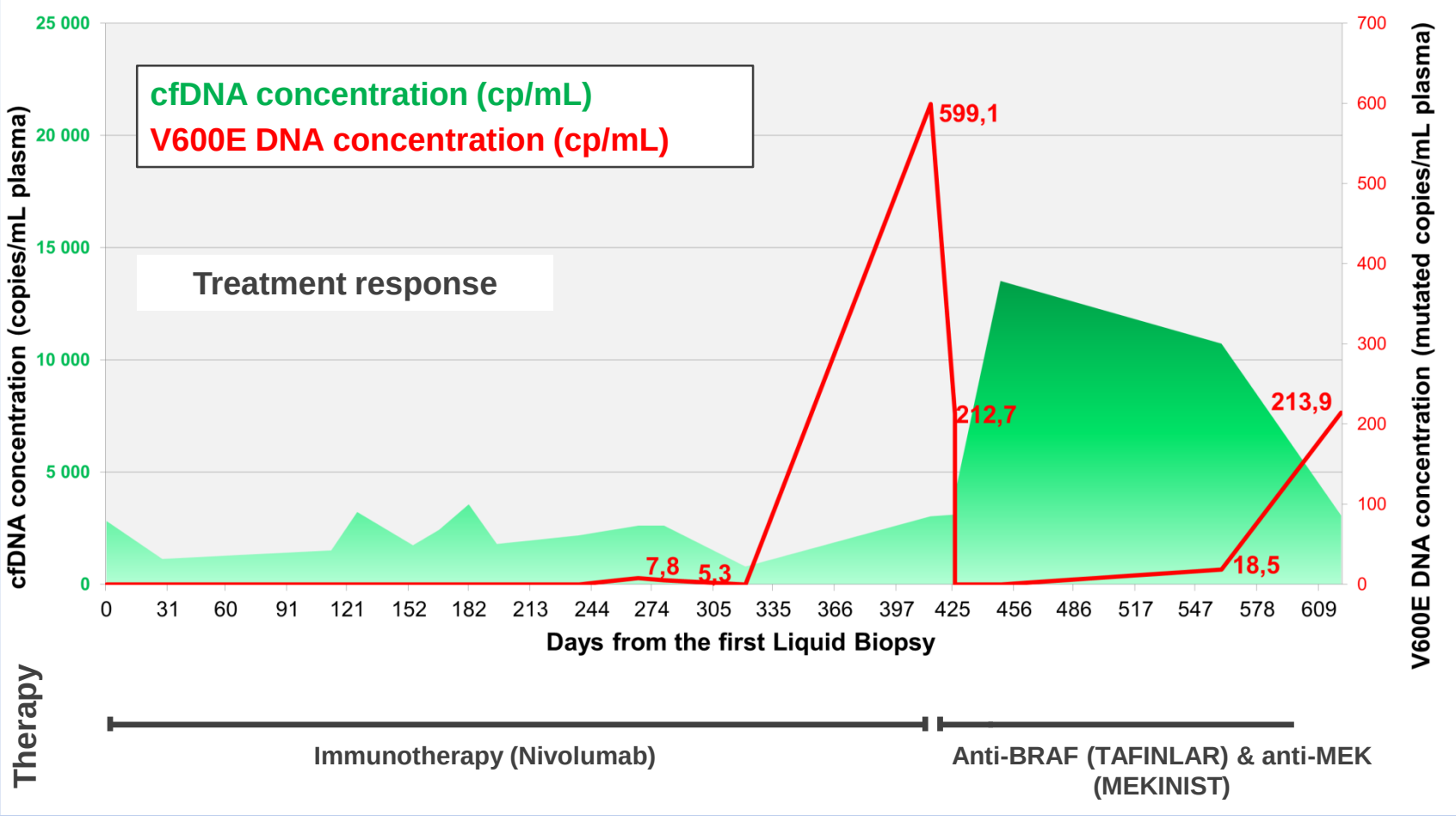

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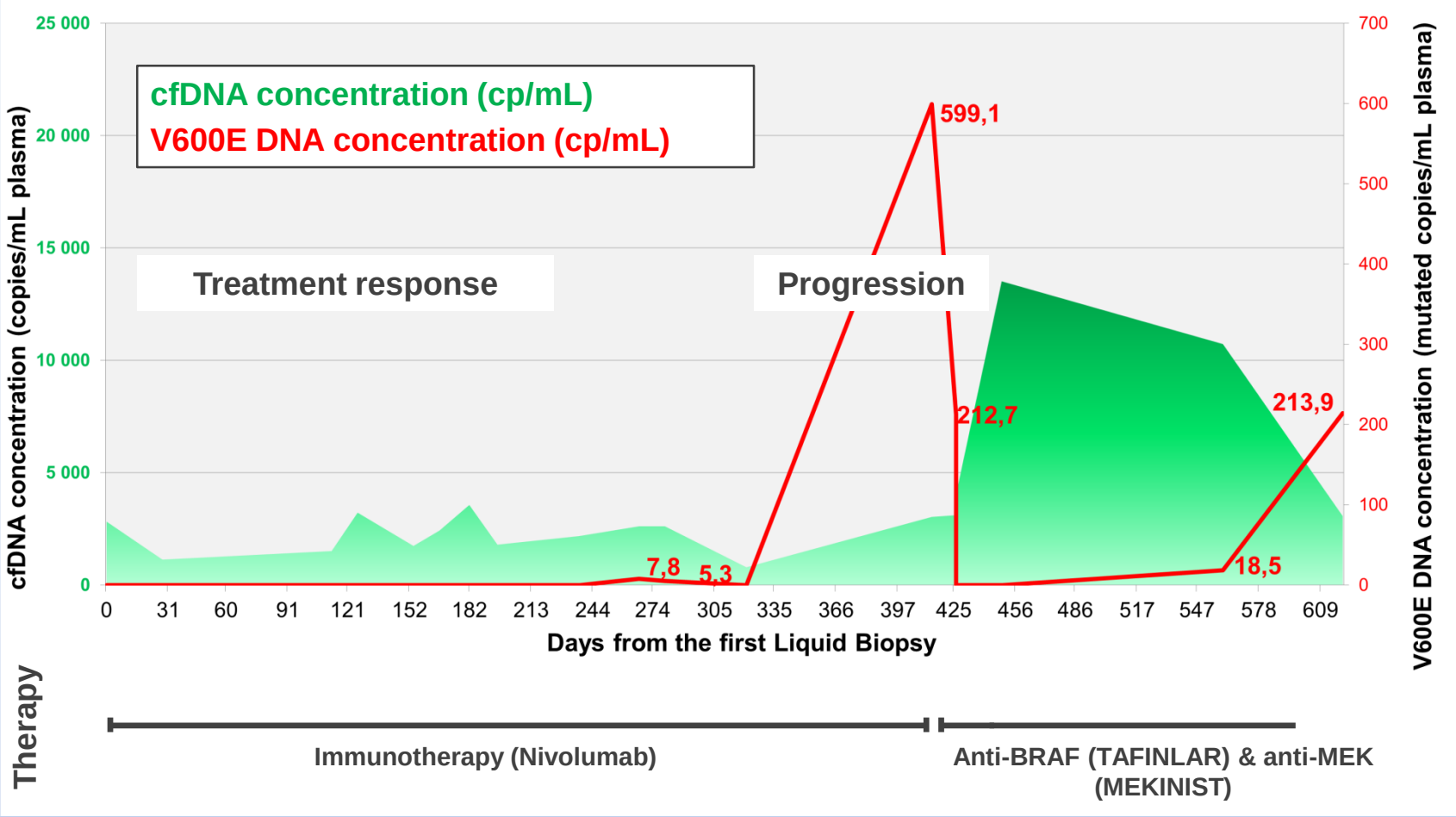

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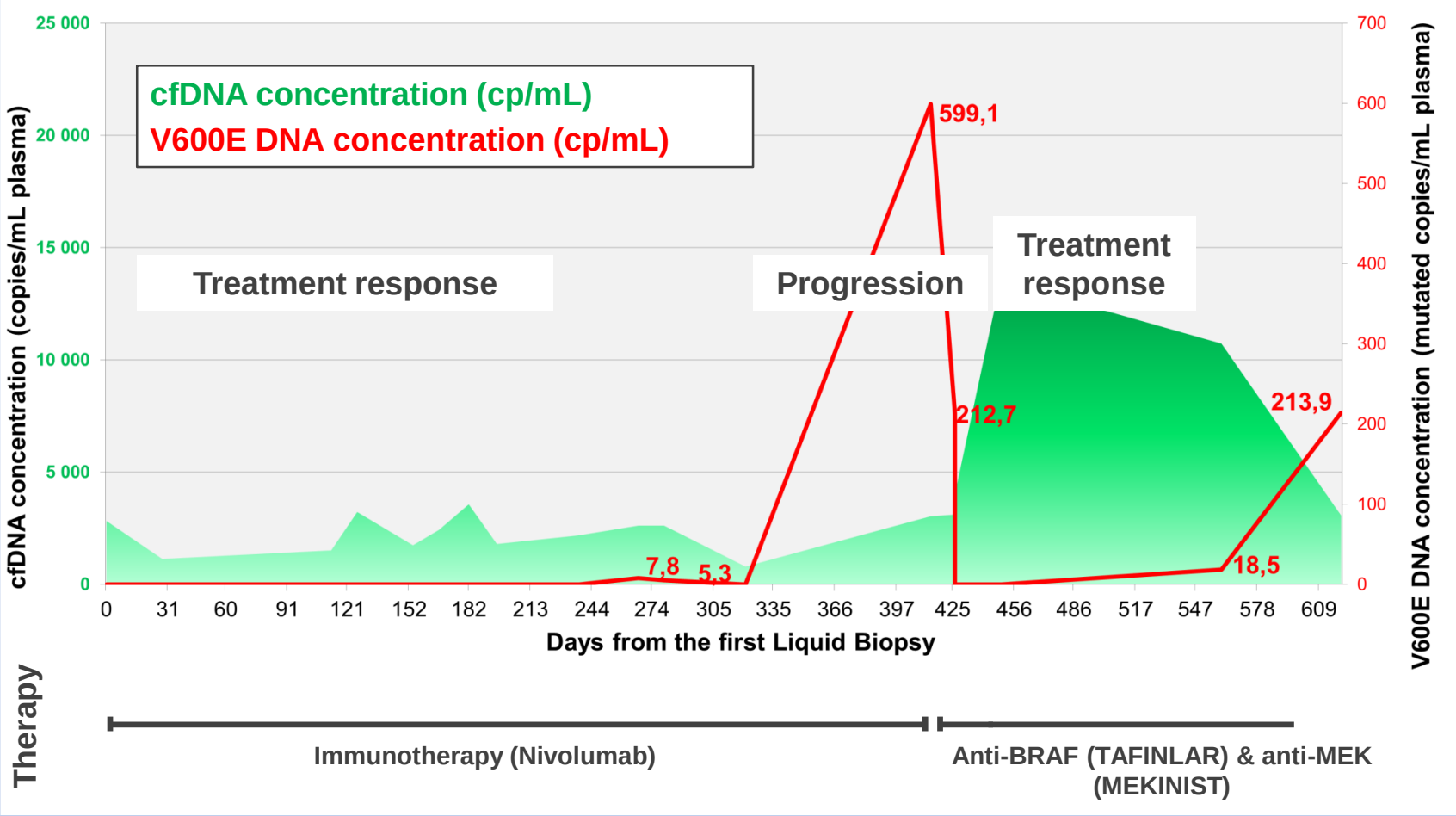

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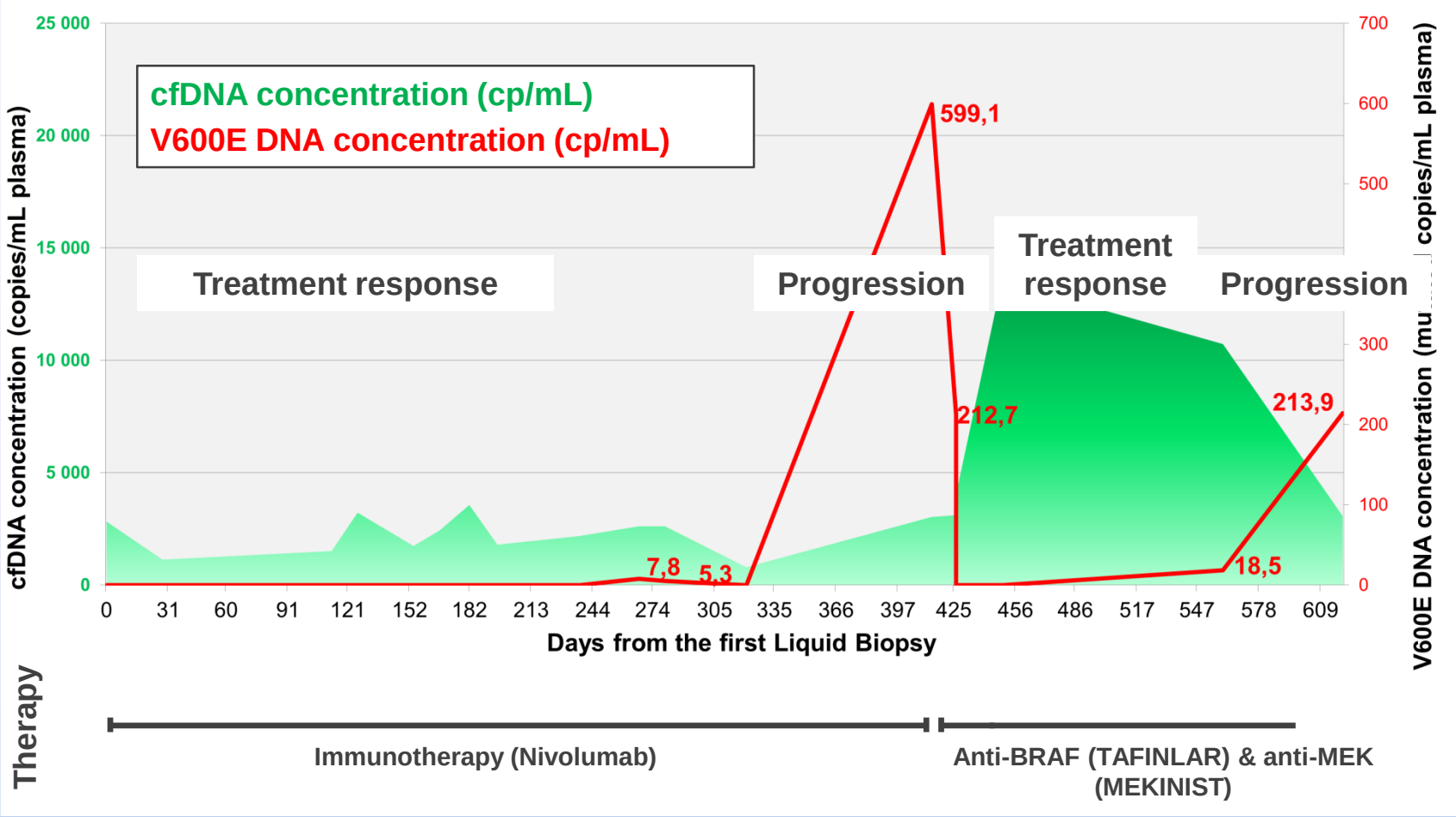

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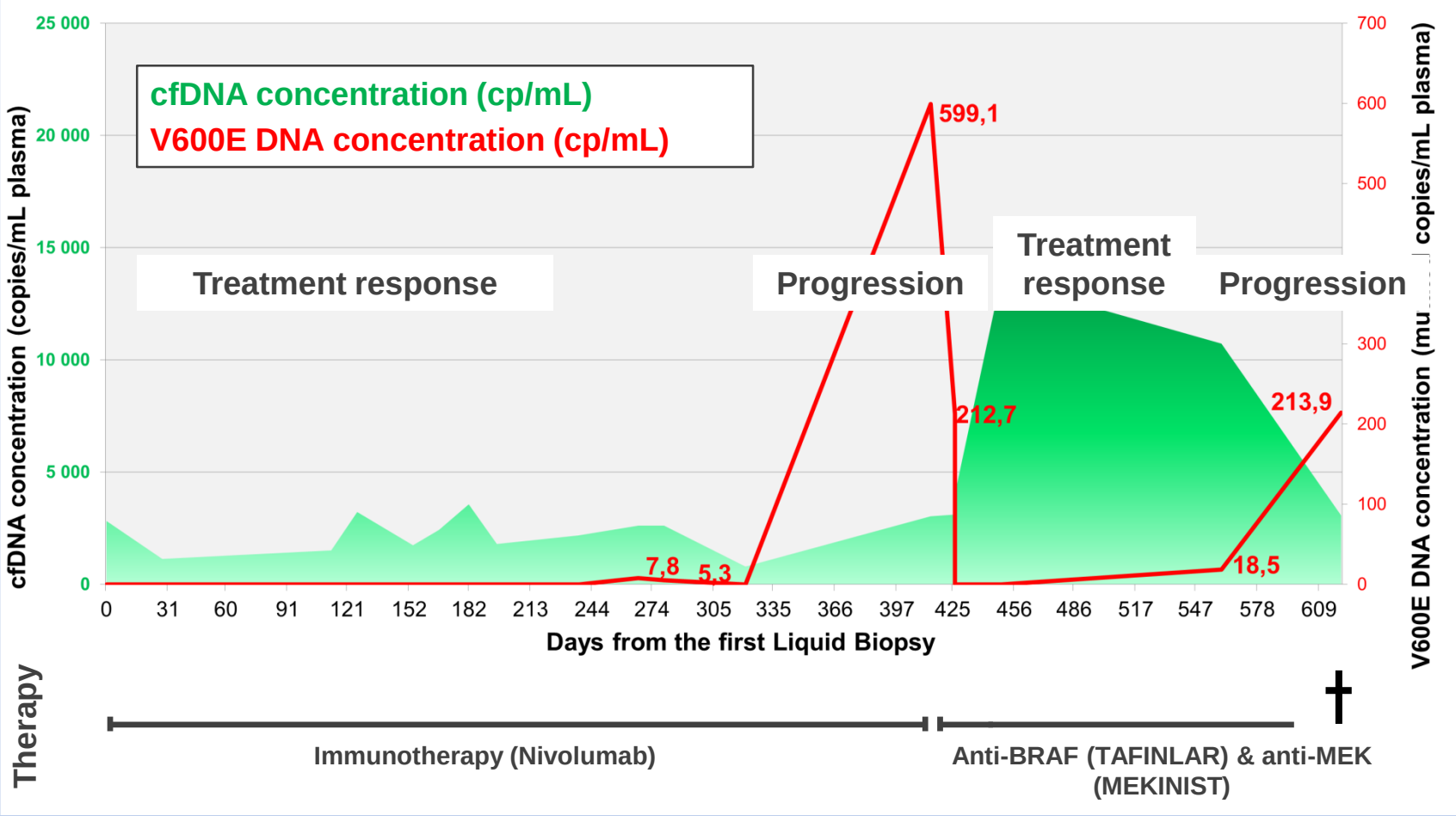


1. Setting up of the liquid biopsy process at Dijon Hospital

2. NAICA digital PCR (Stilla Technologies®)

3. Example of applications

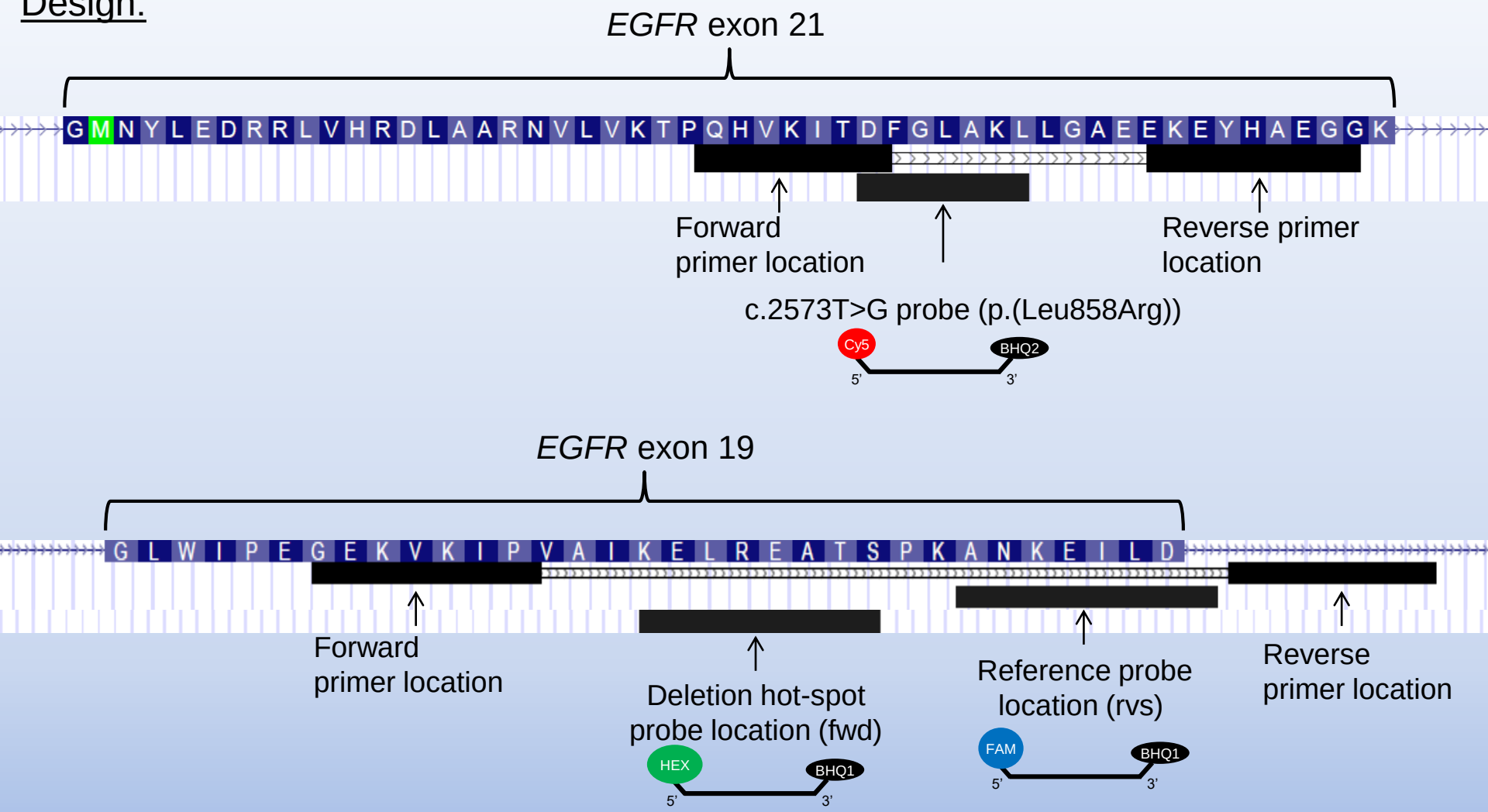
3.1 *BRAF* testing in Melanoma

3.2 *EGFR* testing in NSCLC

Molecular test set up in Lung Cancer

Testing of *EGFR* deletions of exon 19 + L858R mutation

Design:



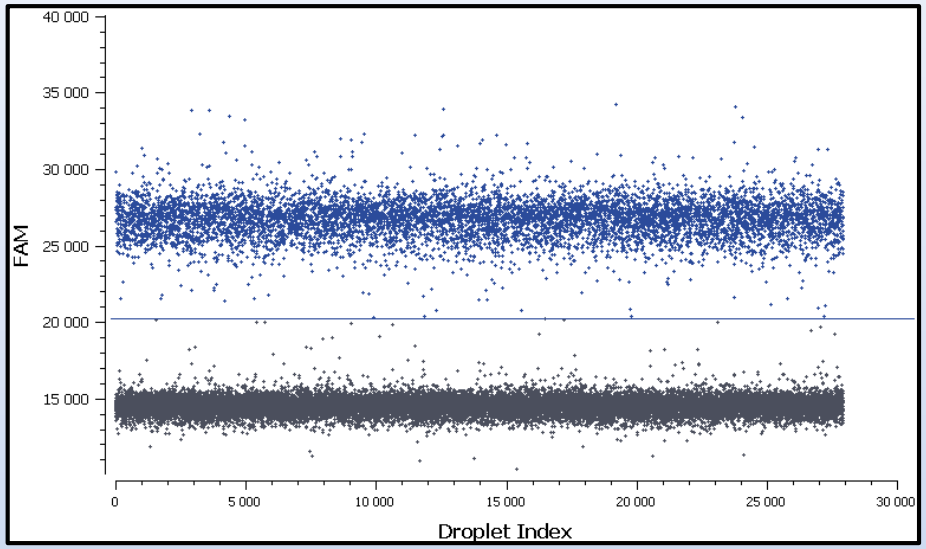
Adapted from Oxnard et al., Clin Cancer Res, 2014 and Punnoose et al., Clin Cancer Res, 2012

Molecular test set up in Lung Cancer

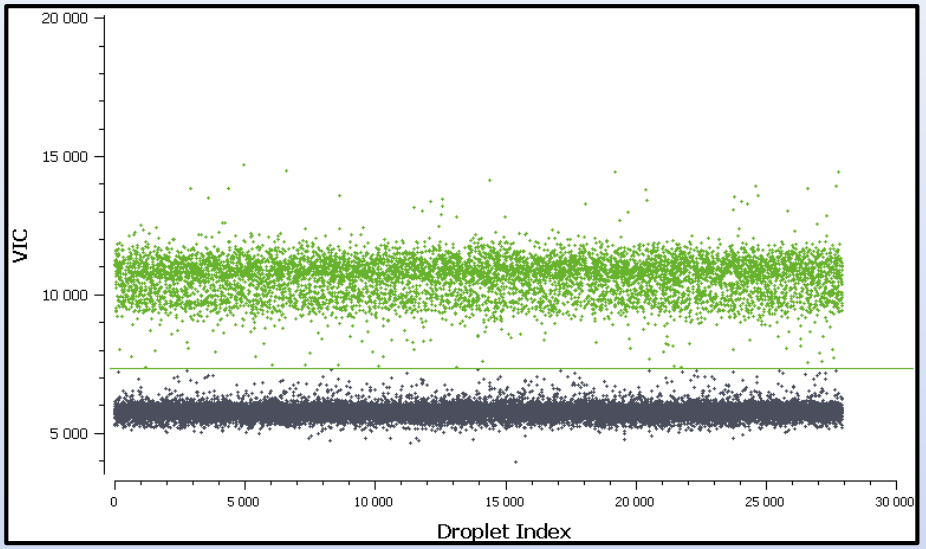
Testing of *EGFR* deletions of exon 19 + L858R mutation

1D graphical representation:

Sample: cell-free DNA from patient with *EGFR* Del19 lung ADK



BLUE channel (495-515nm)



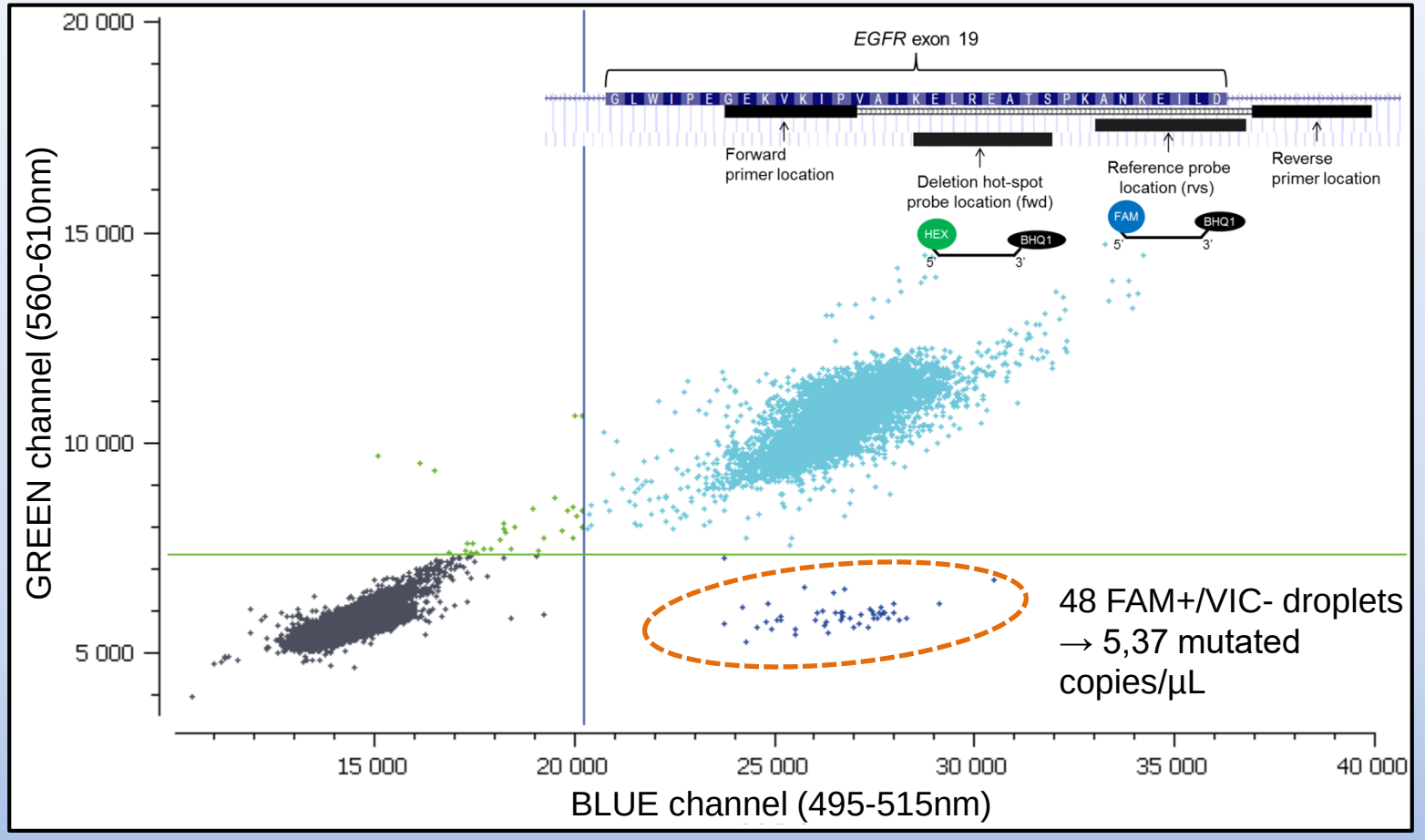
GREEN channel (560-610nm)

Molecular test set up in Lung Cancer

Testing of *EGFR* deletions of exon 19 + L858R mutation

2D graphical representation:

Sample: cell-free DNA from patient with *EGFR* Del19 lung ADK

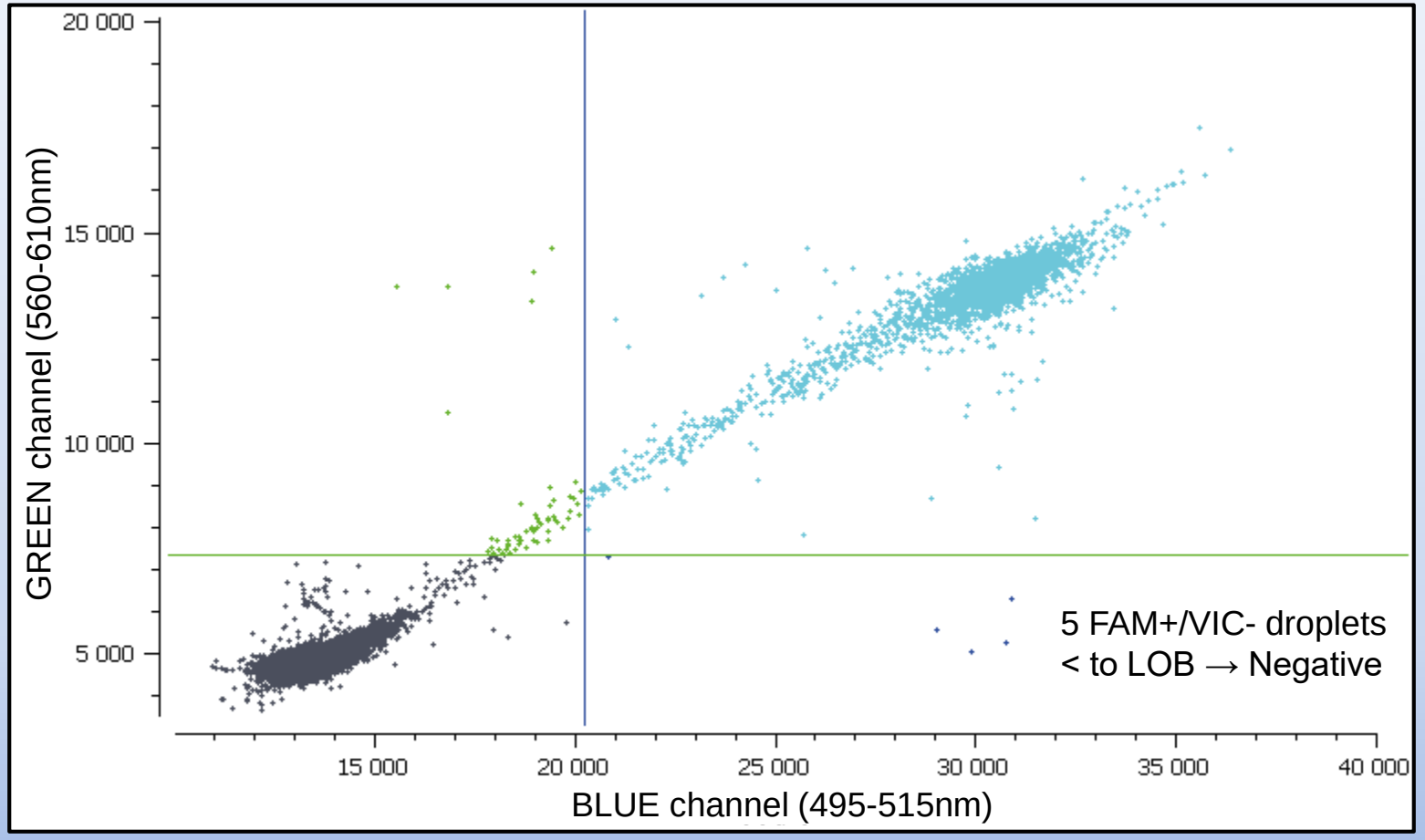


Molecular test set up in Lung Cancer

Testing of *EGFR* deletions of exon 19 + L858R mutation

2D graphical representation:

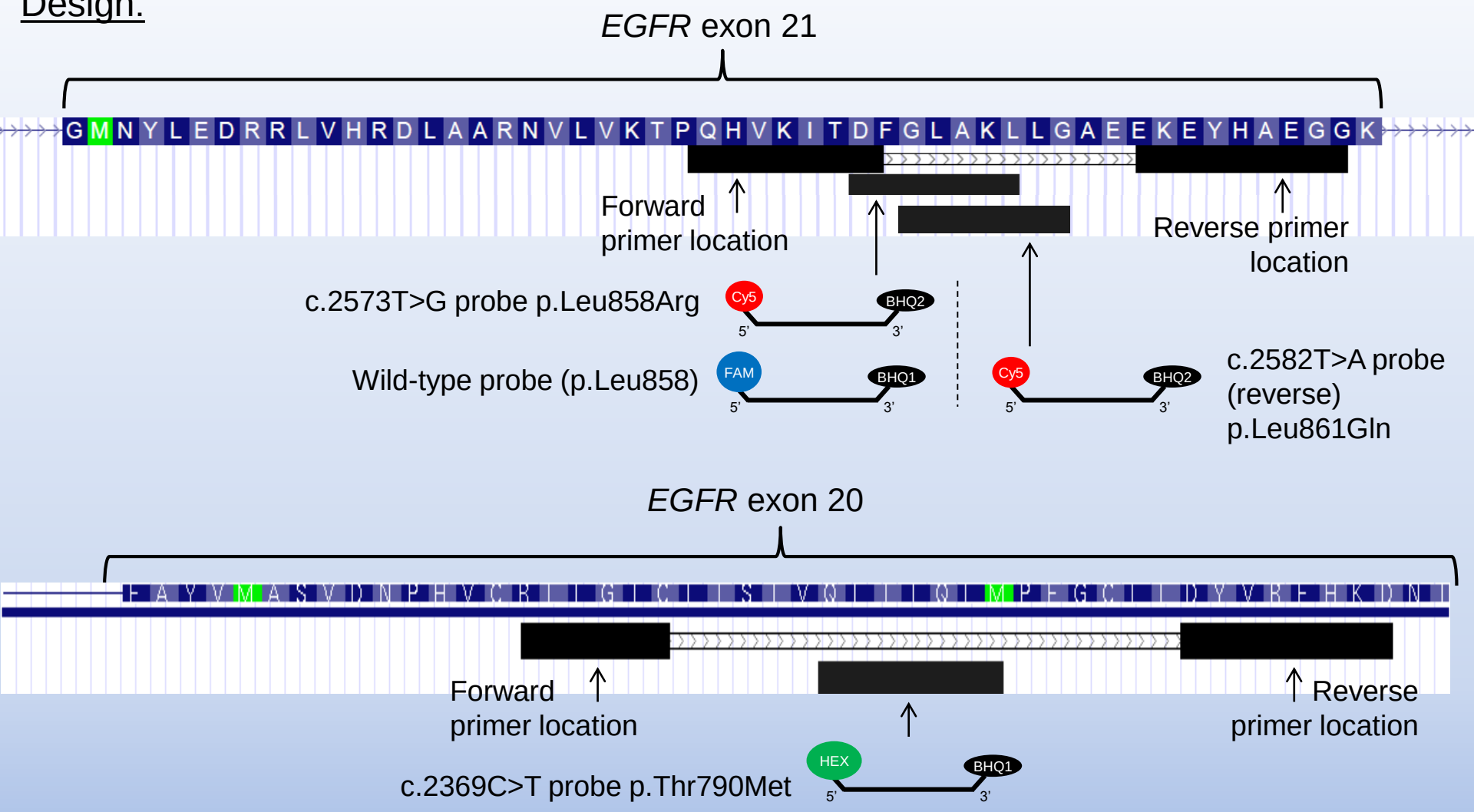
Sample: Human genomic DNA, *EGFR* wild-type



Molecular test set up in Lung Cancer

Testing of *EGFR* L858R/L861Q/T790M mutations

Design:

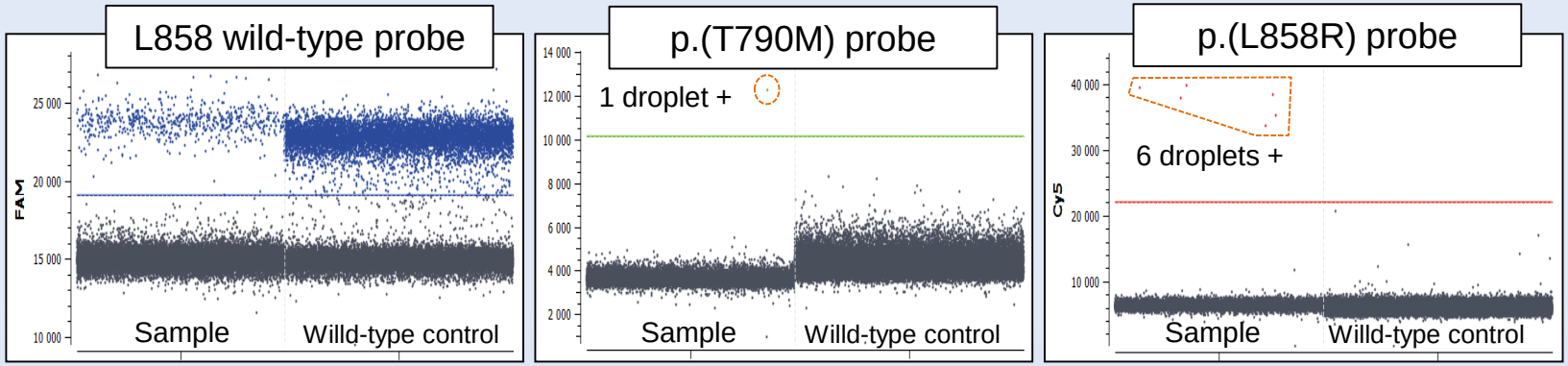


Adapted from Punnoose et al., Clin Cancer Res, 2012

Example for the metastatic cancer progression evaluation in lung cancer

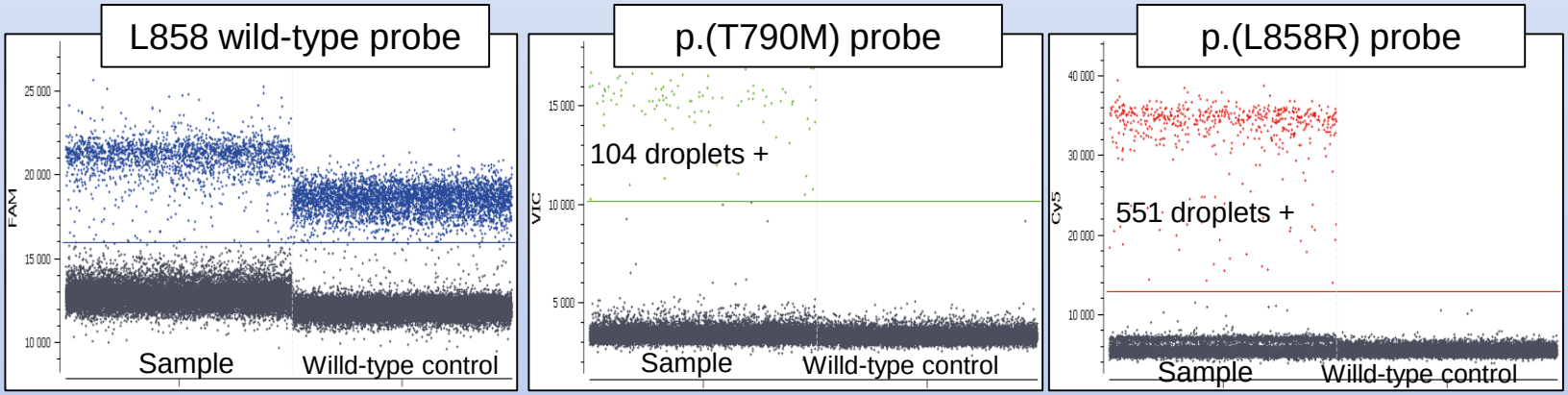
- 1. Forty-one year old man at diagnosed for a lung metastatic adenocarcinoma in 2014
- 2. Detection of the *EGFR* p.(Leu858Arg) mutation on a tissue biopsy
- 3. Treated by an anti-EGFR 2nd generation (GIOTRIF)

27/02/2017
First liquid biopsy:



08/03/2017: Biopsy from a vertebral metastasis: EGFR L858 & T790 wild type but very little tissue sample…

24/07/2017
Second liquid biopsy:



1. Setting up of the liquid biopsy process at Dijon Hospital

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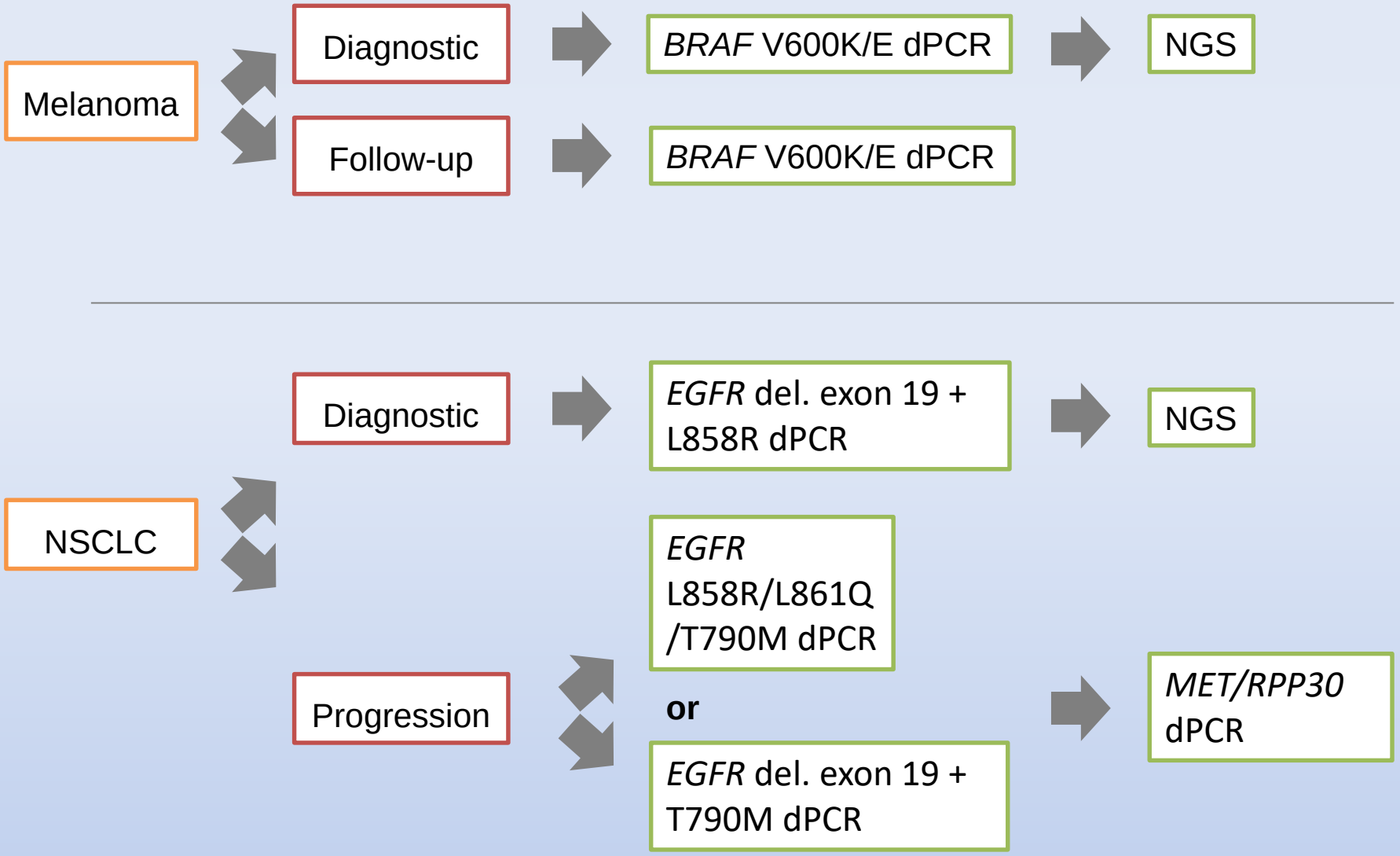
4. Conclusions

Conclusion


Melanoma
Diagnostic
Follow-up
BRAF V600K/E dPCR
BRAF V600K/E dPCR
NGS
NSCLC
Diagnostic
Progression
EGFR del. exon 19 + L858R dPCR
EGFR L858R/L861Q /T790M dPCR
or
EGFR del. exon 19 + T790M dPCR
NGS
MET/RPP30 dPCR

Conclusion

NAICA Digital PCR:

- Fast workflow → possibility to give results in less than 1 day (from blood sampling to dPCR)
- Possibility to multiplex (3 fluorescence channels) → adapted to **Diagnosis**
- Very sensitive and reliable system → adapted to **Follow-up**

Plateform of somatic oncology

Pathology unit:
Pr. Laurent Martin , MD PhD
Dr. Marie-Hélène Aubriot-Lorton, MD

Molecular Unit:
Dr. Bernard Aral, MD PhD
Dr. Caroline Chapusot, PhD
Dr. Benjamin Tournier, PhD
Technical staff:
Manon Aubry
Isabelle Choux
Lyse-Marie Dubois
Celia Pioche
Alicia Remond

Biobank:
Dr. Céline Schaeffer, PhD
Laetitia Barbier

Clinicians

Lung Oncology:
Dr. Anne-Lyse Fanton
Dr. Pascal Foucher
Dr. Ayoube Zouak

Dermato-oncology:
Dr. Bertille Bonniaud
Dr. Sophie Dalac
Dr. Géraldine Jeudy

Thanks for your attention

Research team: Genetic and epigenetic Innovation in Oncology
Pr. Mary Callanan, PhD

CHU Centre Hospitalier Universitaire Dijon Bourgogne

Inserm Institut national de la santé et de la recherche médicale

UBFC UNIVERSITÉ BOURGOGNE FRANCHE-COMTÉ