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Assessment of circulating tumor cells and circulating cell-free DNA in patients with metastatic melanoma

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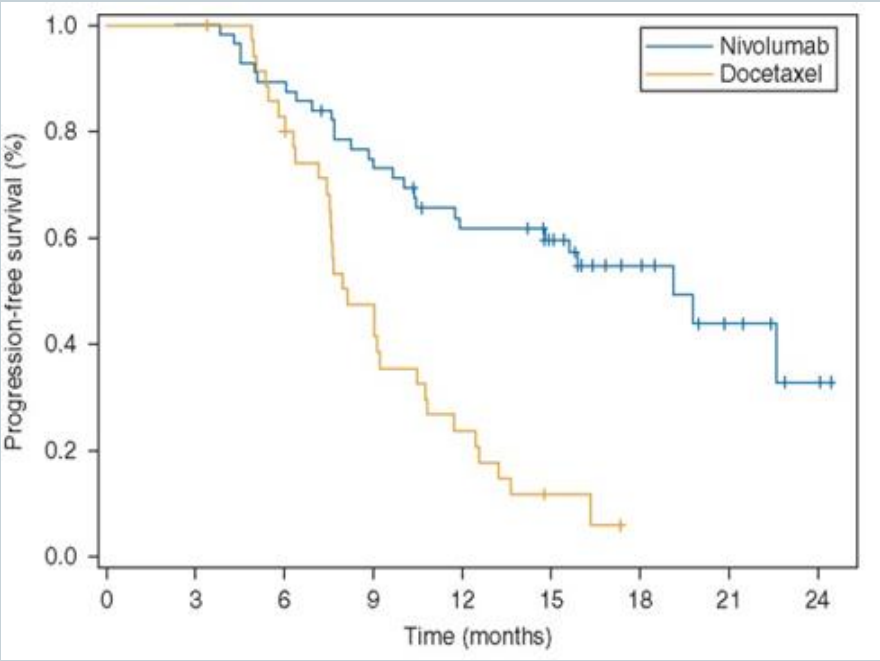
Background (1)

- Incidence of melanoma increase (14325 new cases in France in 2015)
- New therapies (antiBraf-antiMek and immunotherapies) : Improvement of overall survival (OS) and progression-free survival (PFS)
- Long follow-up (clinical and radiological)



- Liquid biopsy
- Patient with BRAF mutation (40%) :

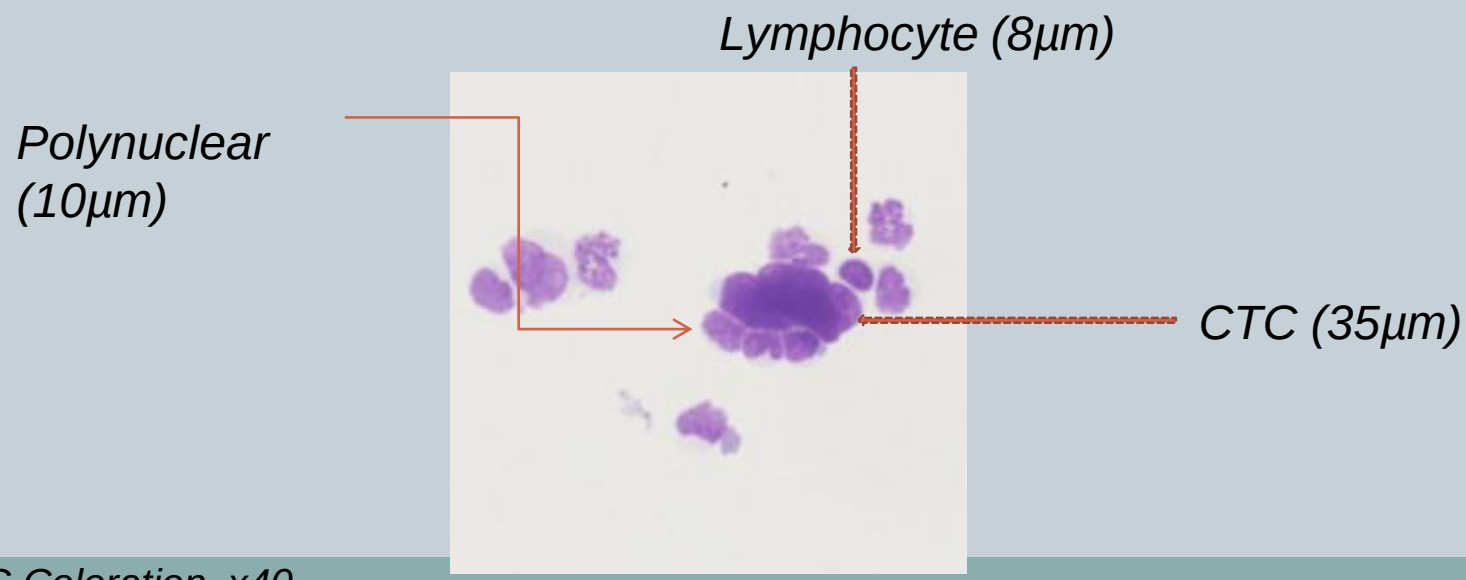
[ADNlc]* (mutated copies/mL)

(1) *Progression-free survival for melanoma patients treated with docetaxel (yellow curves) versus nivolumab (blue curves) in the CheckMate-057 trial. Assessing treatment efficacy in the subset of responders in a randomized clinical trial. E.L.Korn and all, Ann Oncol. 2017 Jul; 28(7): 1640–1647*

*[cfDAN] = concentration of cell-free DNA

Background (2)

- Circulating tumor cells = CTCs
  - Malignant cells from primitive cancer/recurrence/metastasis
  - Epithelial-mesenchymal transition
  - In blood stream with other hematologic cells
  - Isolated or in clusters
- Could we use CTCs to improve patients' monitoring ?



MGG Coloration, x40

Objectives

1. To evaluate the feasability of the ScreenCell® method for the detection of CTCs, in a pathology lab.

2. To describe if kinetics of CTCs count and cfDNA levels were correlated to treatment efficiency assessed by CT scan

*CTC = Circulating tumor cells

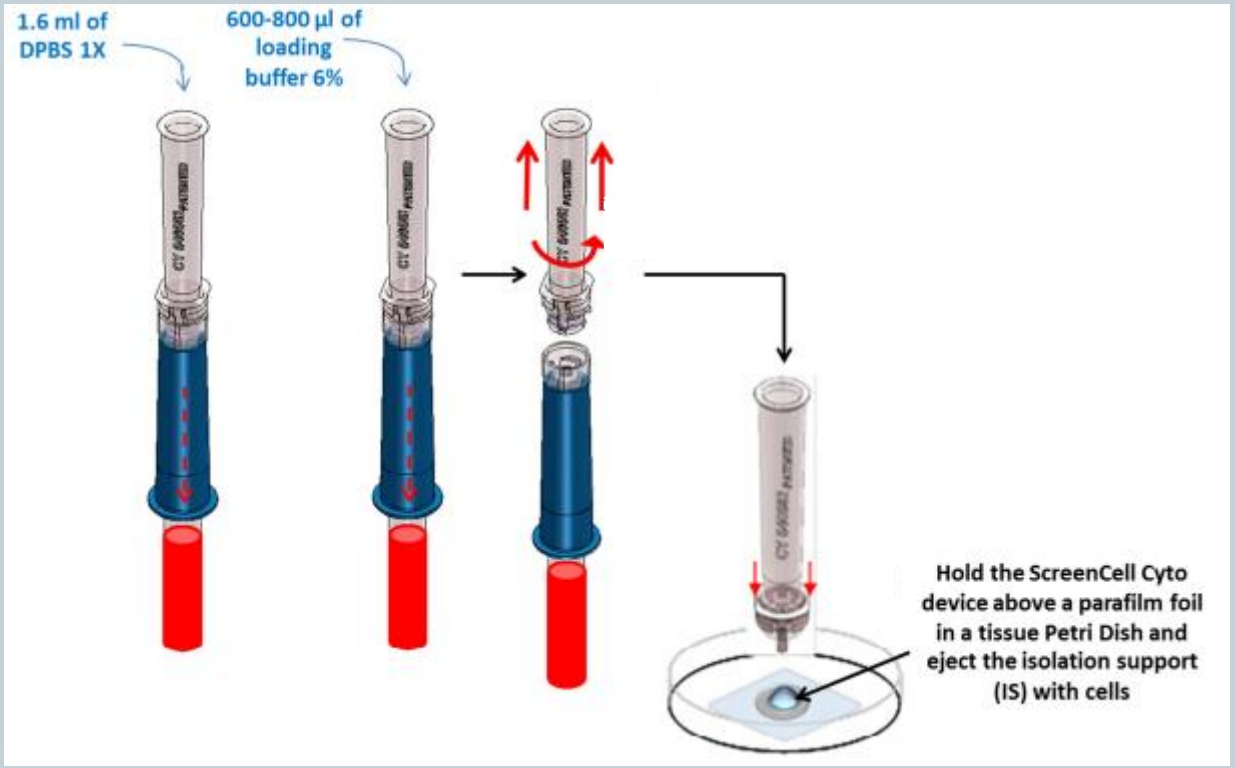
Method (1)
Inclusion criteria

- 5 patients:
  - stage IV Melanoma (With or without BRAF mutation)
  - no treatment before inclusion

- Usual recommended follow-up (clinical and radiological)
- Blood tests every 3 months during a year:
  - [cfDNA]
  - CTC count on slides (2 observers, one with two counts) more filters

- One healthy patient (control)

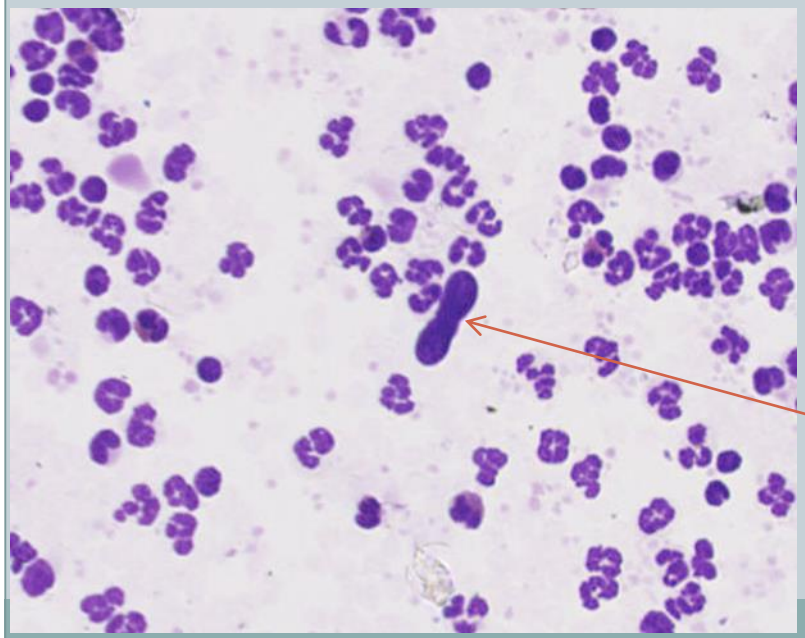
Method (2)
ScreenCell® technical



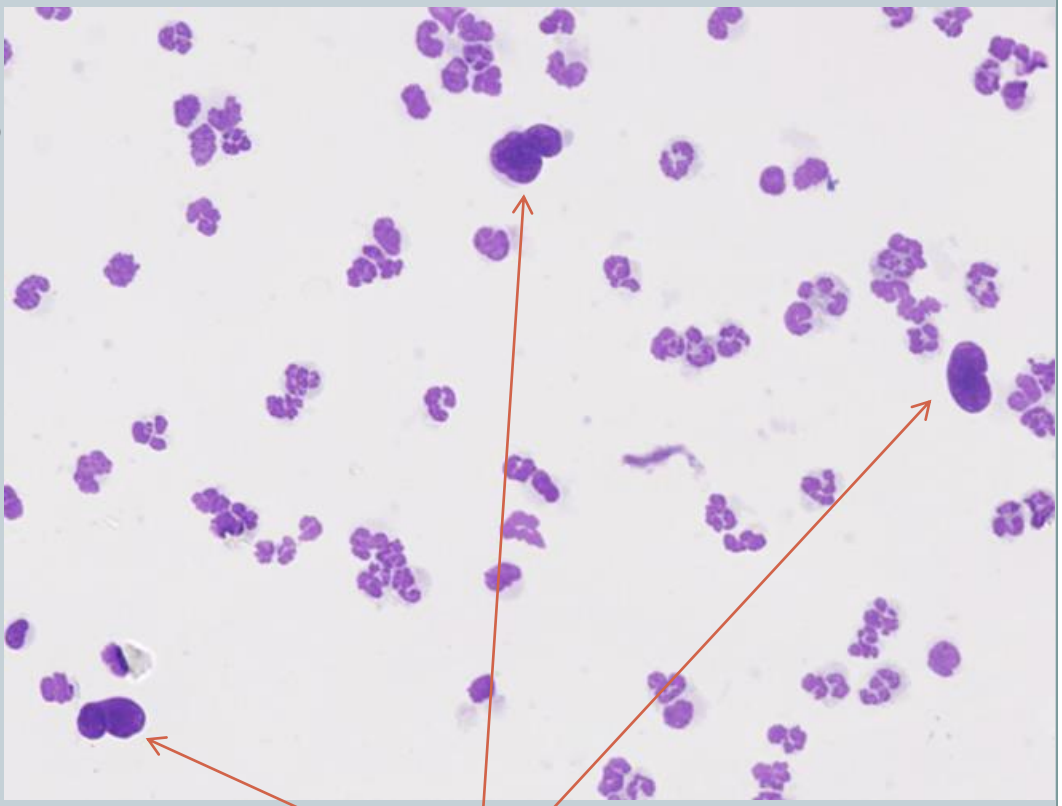
Blood => filter => cytospin => slide

Method (3)
Malignancy criteria for isolated CTCs

- Whole cell
- Nuclear size ≥20 μm
- High nucleo-cytoplasmic ratio ≥0,75
- Irregular nuclear membrane
- Dense, hyperchromatic nucleus, but not totally opaque



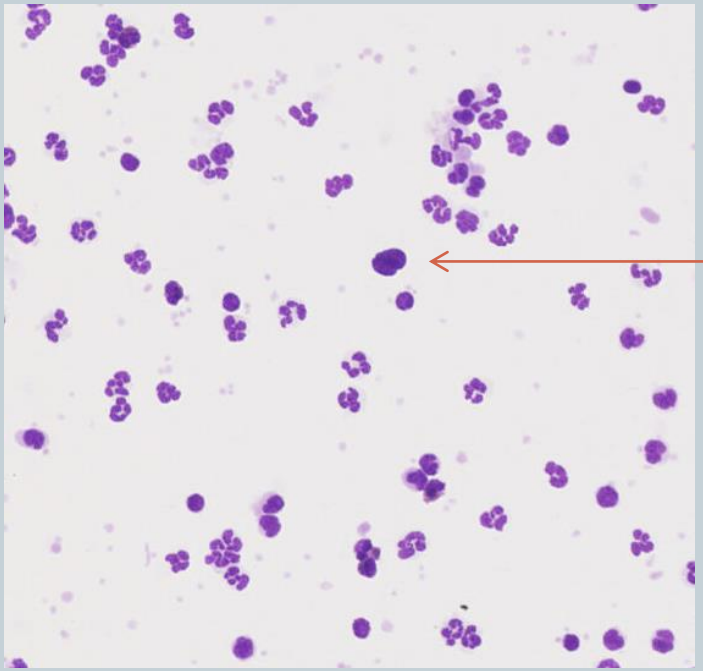
MGG, x20



MGG, x30

Method (4)
Cells of uncertain malignancy = UMC

- 1 malignancy criteria missing or difficult to assess

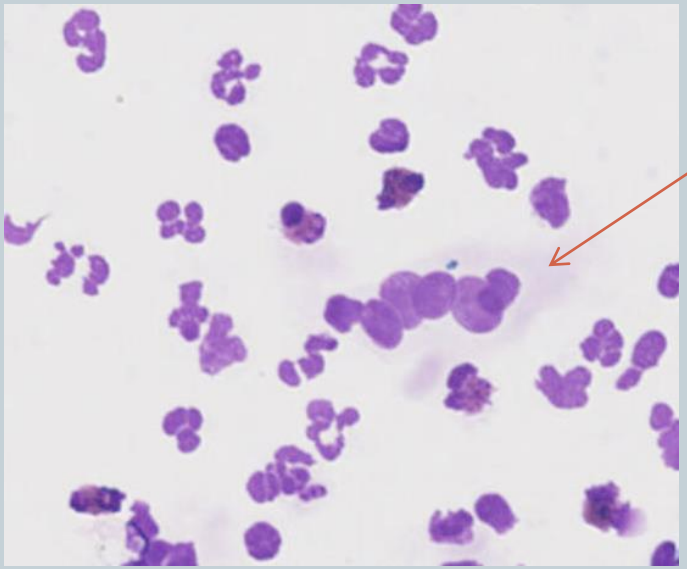


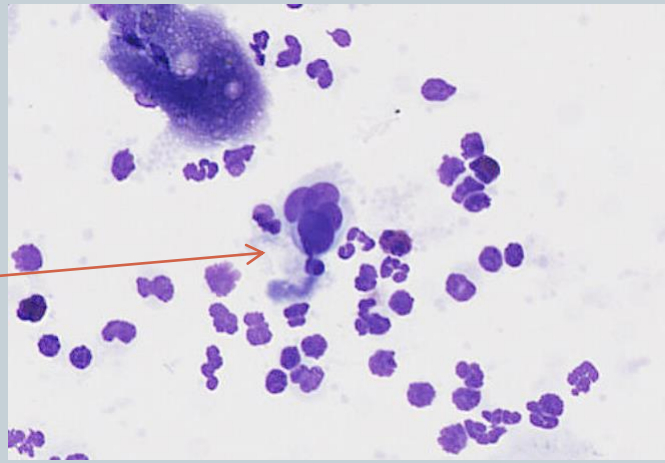
UMC (15µm)

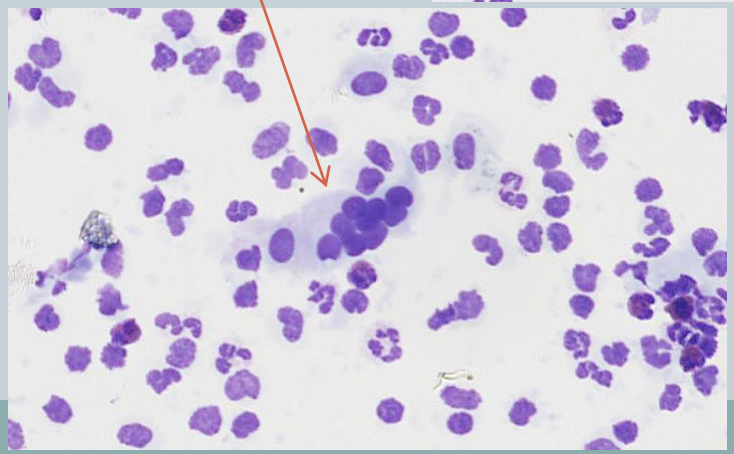
MGG, x20

Method (5)
Clusters of CTCs

- 2 to several dozen
- Nucleo-cytoplasmic ratio smaller than in isolated CTCs
- **anisokaryosis**







Clusters

MGG, x30

Outcomes

- CTC
  - Cut-off + : 5 CTC / filtration (2)
  - Significative variation: no literature data

- [cfDNA]
  - Cut-off + : 10 ng/mL (3)
  - Significative variation: ± 10 ng/mL

- Radiological evaluation (WHO criteria)
  - Complete response (RC) ↓
  - Partial response (RP) ↘
  - Disease stabilisation (SD) →
  - Disease progression (PD) ↗

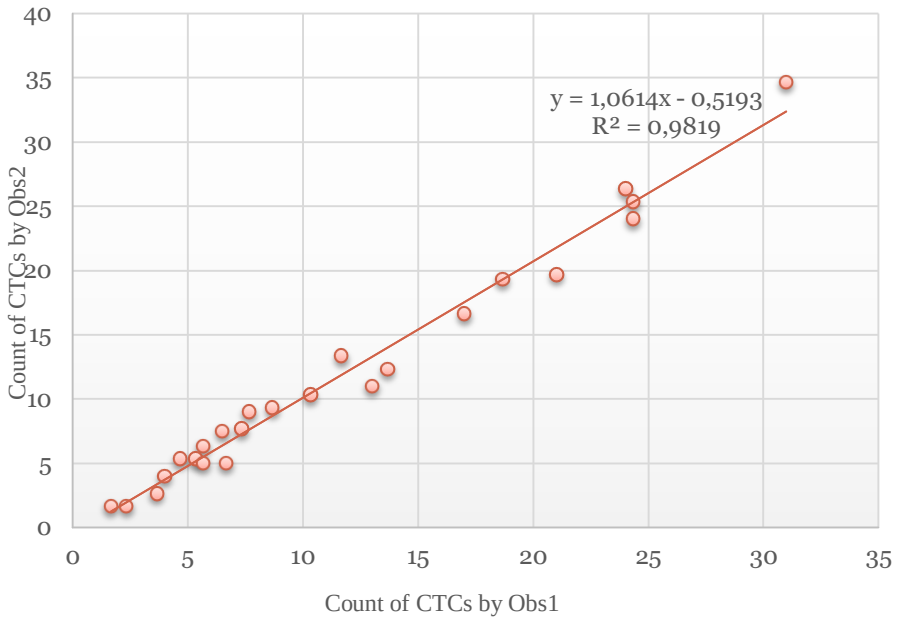
(2) *Circulating Tumor Cells: A Novel Prognostic Factor for Newly Diagnosed Metastatic Breast Cancer ; Massimo Cristofanilli, and al, Journal of Clinical Oncology 2005 23:7, 1420-1430*
(3) *DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells ; Jahr S and al, cancer res 2001 Feb 15;61(4):1659-65.*

Results (1)
Demographic and clinical data

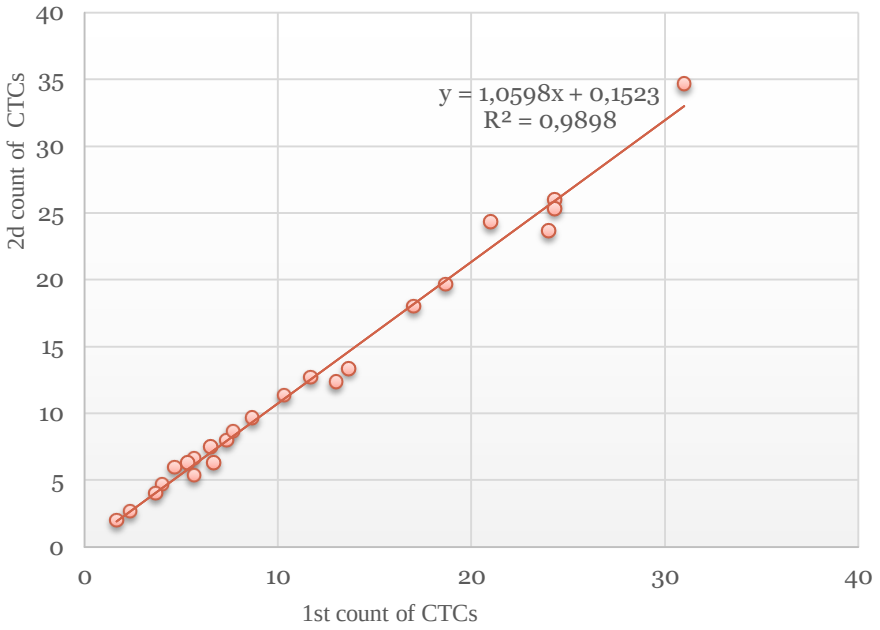
Clinical features	n
Sex	
Men	1
Women	4
Age	
< 65 years	2
≥ 65ans	3
Stage IV at diagnosis	1
Mutation BRAF V600E/V600K	1
Previous adjuvan treatment (interferon)	
Yes	2
No	3
Types of melanoma	
Superficial spreading	1
Nodular	2
Desmoplastic	1
Without primitive	1
Location of metastases	
Ganglion	3
Cutaneous	3
Hepatic	2
Lung	3
Brain	2
Spleen	1
Adrenal	1
Tissue expression of immunochemistry markers	
MelanA	4
HMB45	4
PS100	5
Treatment implemented at inclusion	
Tafinlar + Mekinist	1
Nivolumab	3
Pembrolizumab	1

Results (2)
CTCs count: observers' correlations

Inter-observer correlation



Intra-observer correlation

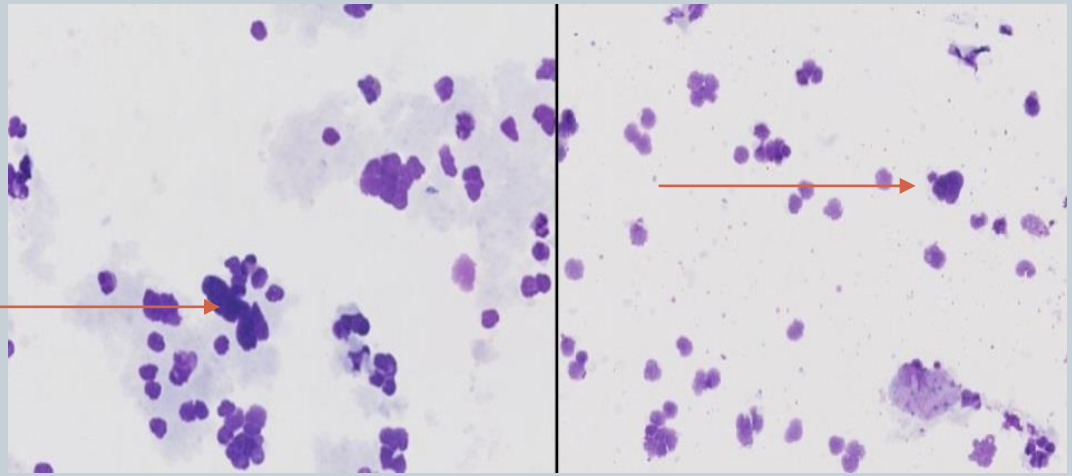


- R^2=98,19%
- Slope: 0,93 (standard error : 0,03) with CI95% **[0,87 ; 0,98]**
- Orderly: 0,69 (standard error : 0,39) with CI95% [-0,12 ; 1,51]

- R^2 = 98,98%
- Sloape: 0,93 (standard error : 0,02) with CI95% **[0,89 ; 0,98]**
- Orderly: -0,02 (standard error : 0,31) with CI95% [-0,66 ; 0,61]

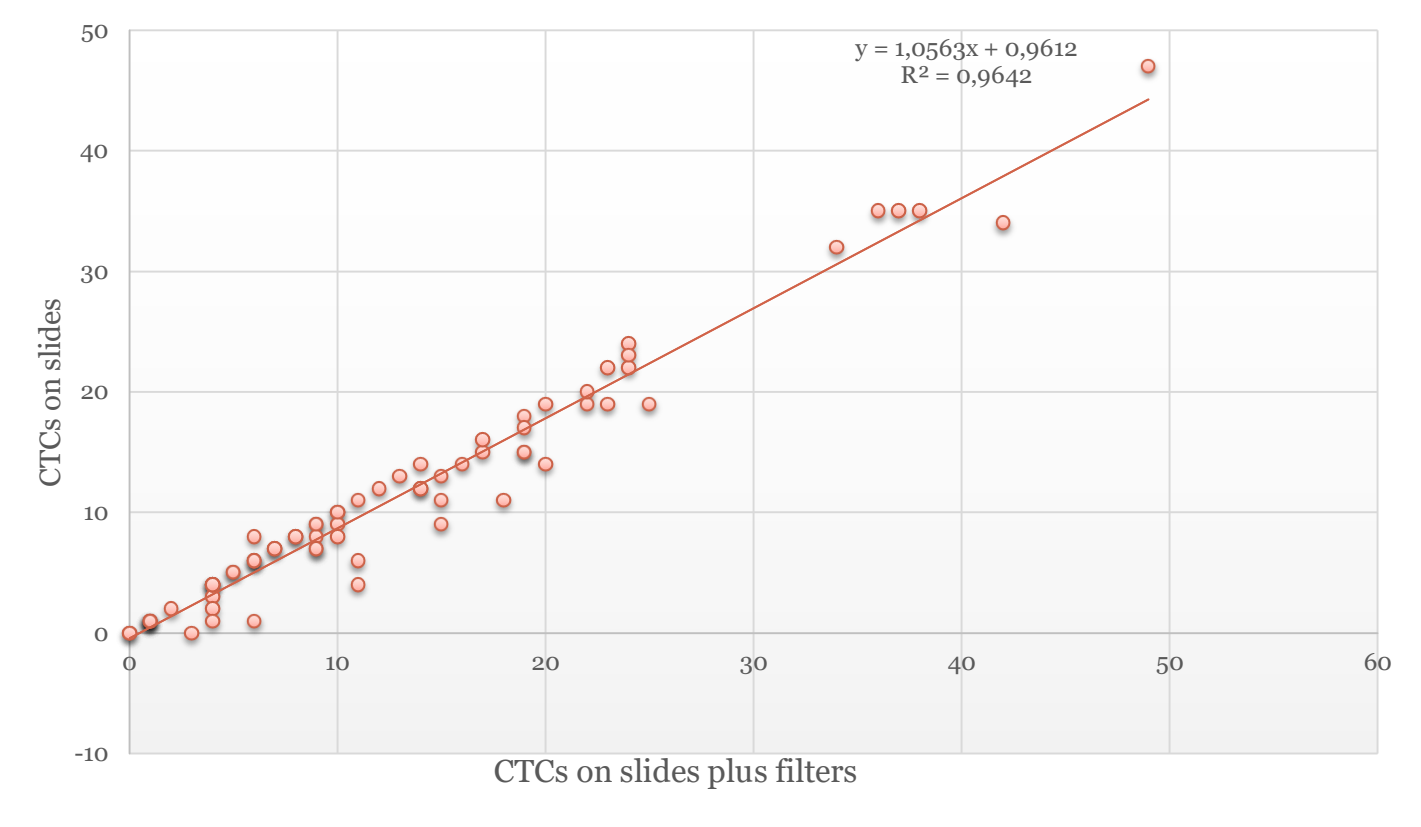
Results (3)
Malignancy assesment

- Easy evaluation of cytological criteria for malignancy
  - Only 0,7 « cell of uncertain malignancy » ± 0,6 / filtration
- Healthy control
  - 2 « CTCs »
  - Not specific



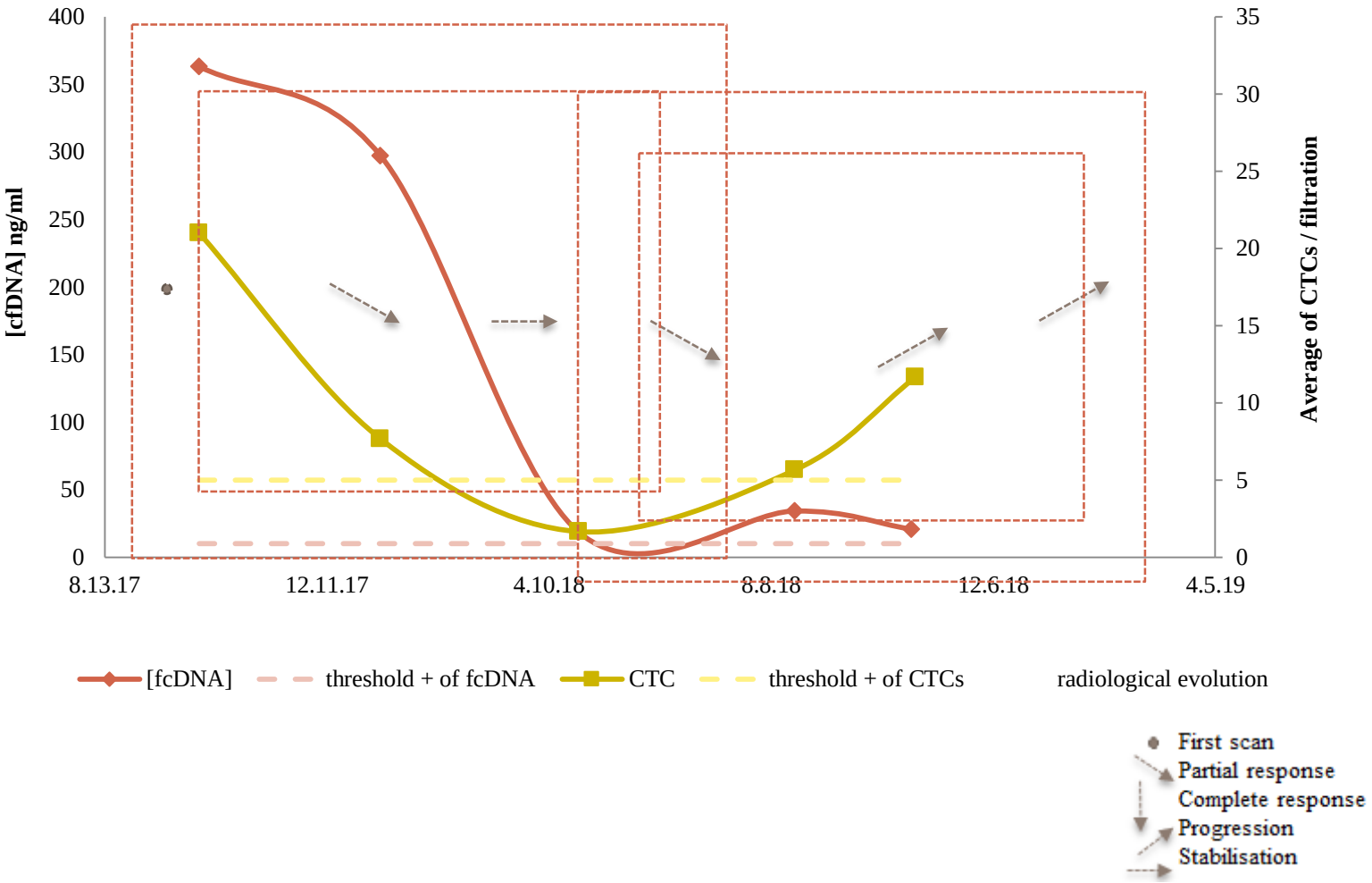
Coloration MGG (x20)

Results (4)
correlation of CTCs count on slides alone or on slides + filters

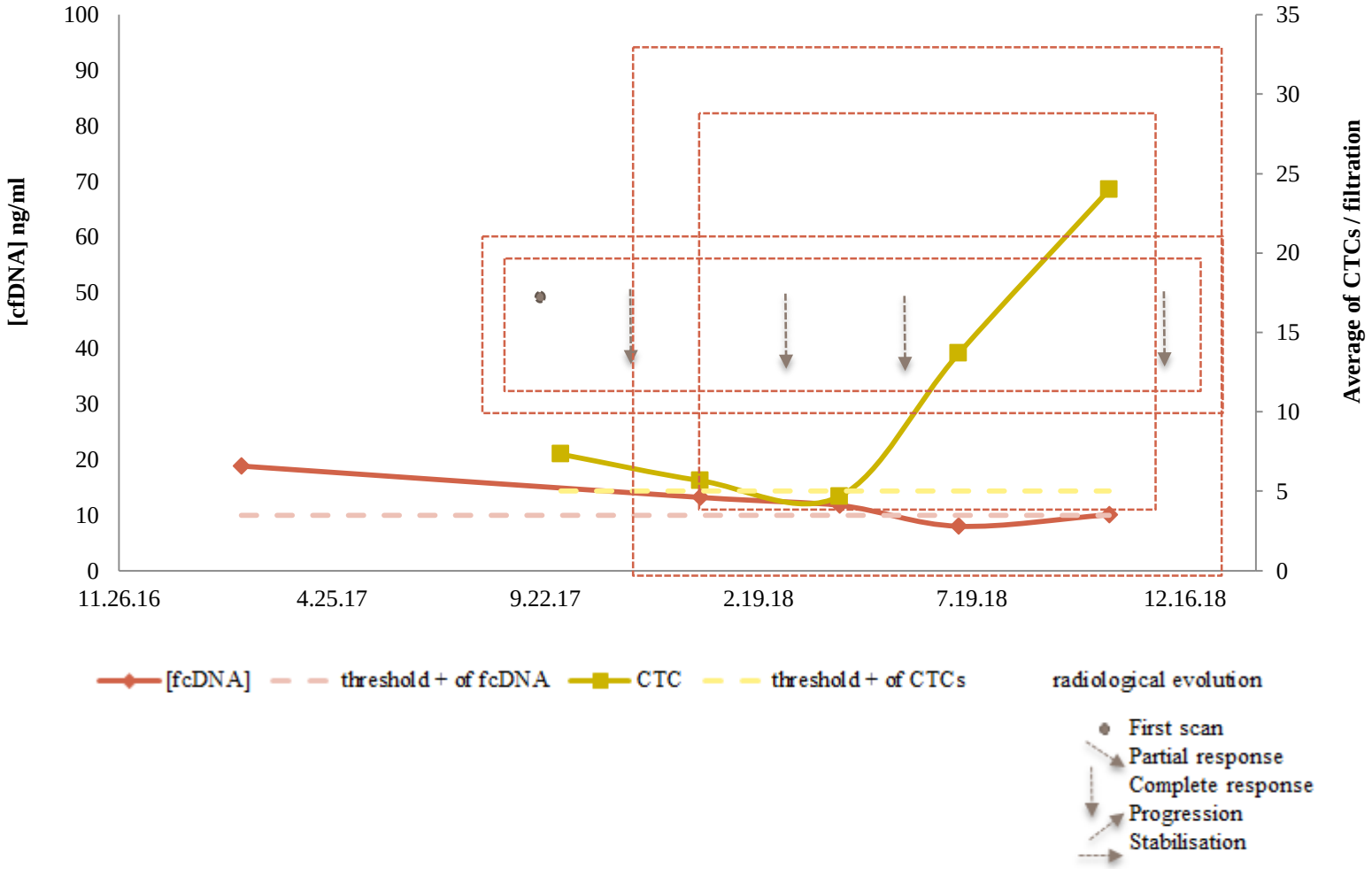


Adequacy coefficient : R^2 = 96,42%
Slope: 0,91 (standard error : 0,02) with CI95% **[0,87 ; 0,95]**
Orderly: -0,46 (standard error : 0,36) with CI95% [-1,18 ; 0,26]

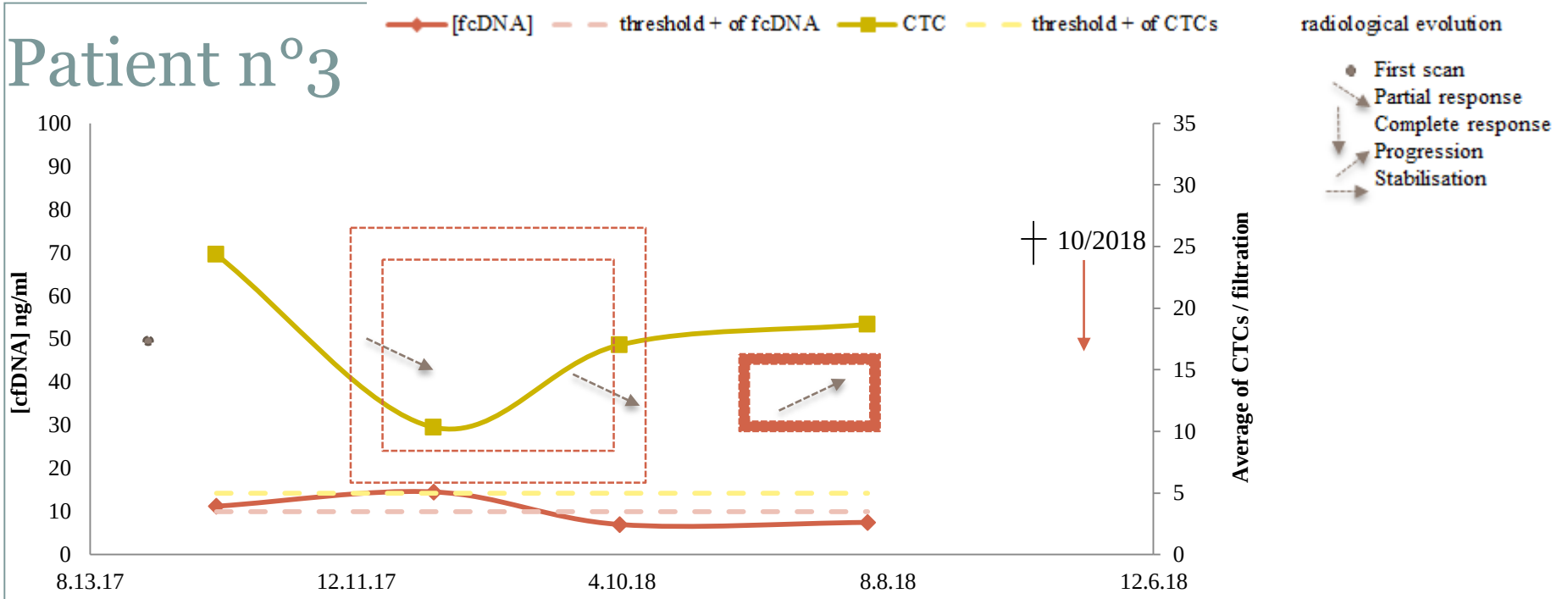
Patient n°1 (BRAF-mutated)



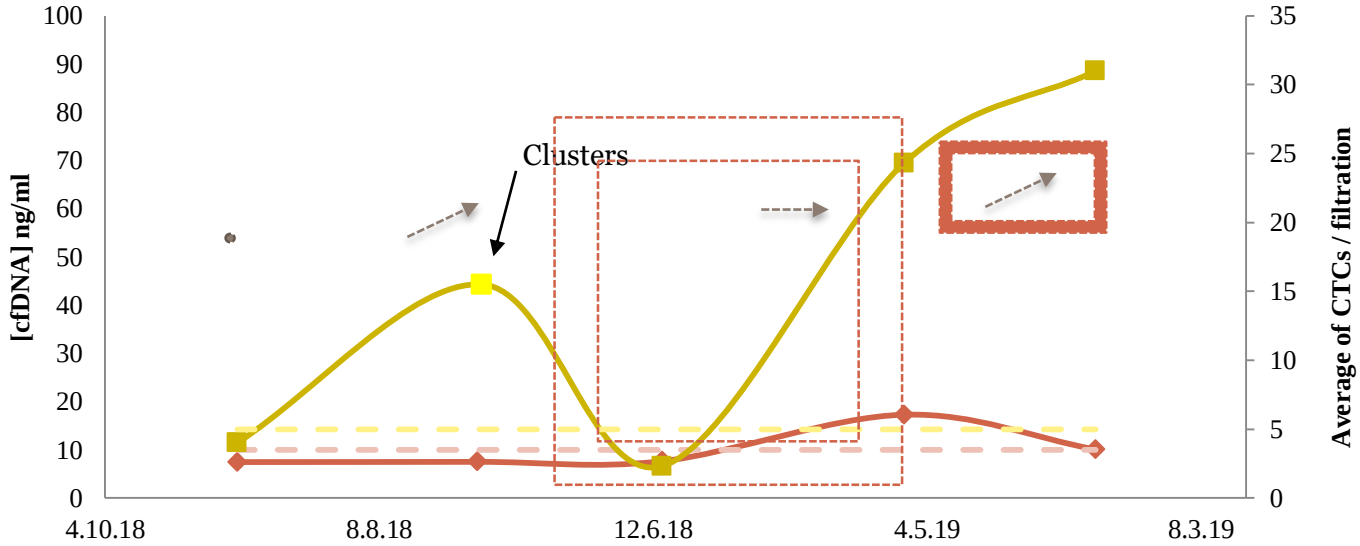
Patient n°2


[cfDNA] ng/ml
Average of CTCs / filtration
100
90
80
70
60
50
40
30
20
10
0
35
30
25
20
15
10
5
0
11.26.16
4.25.17
9.22.17
2.19.18
7.19.18
12.16.18
[fcDNA]
threshold + of fcDNA
CTC
threshold + of CTCs
radiological evolution
First scan
Partial response
Complete response
Progression
Stabilisation

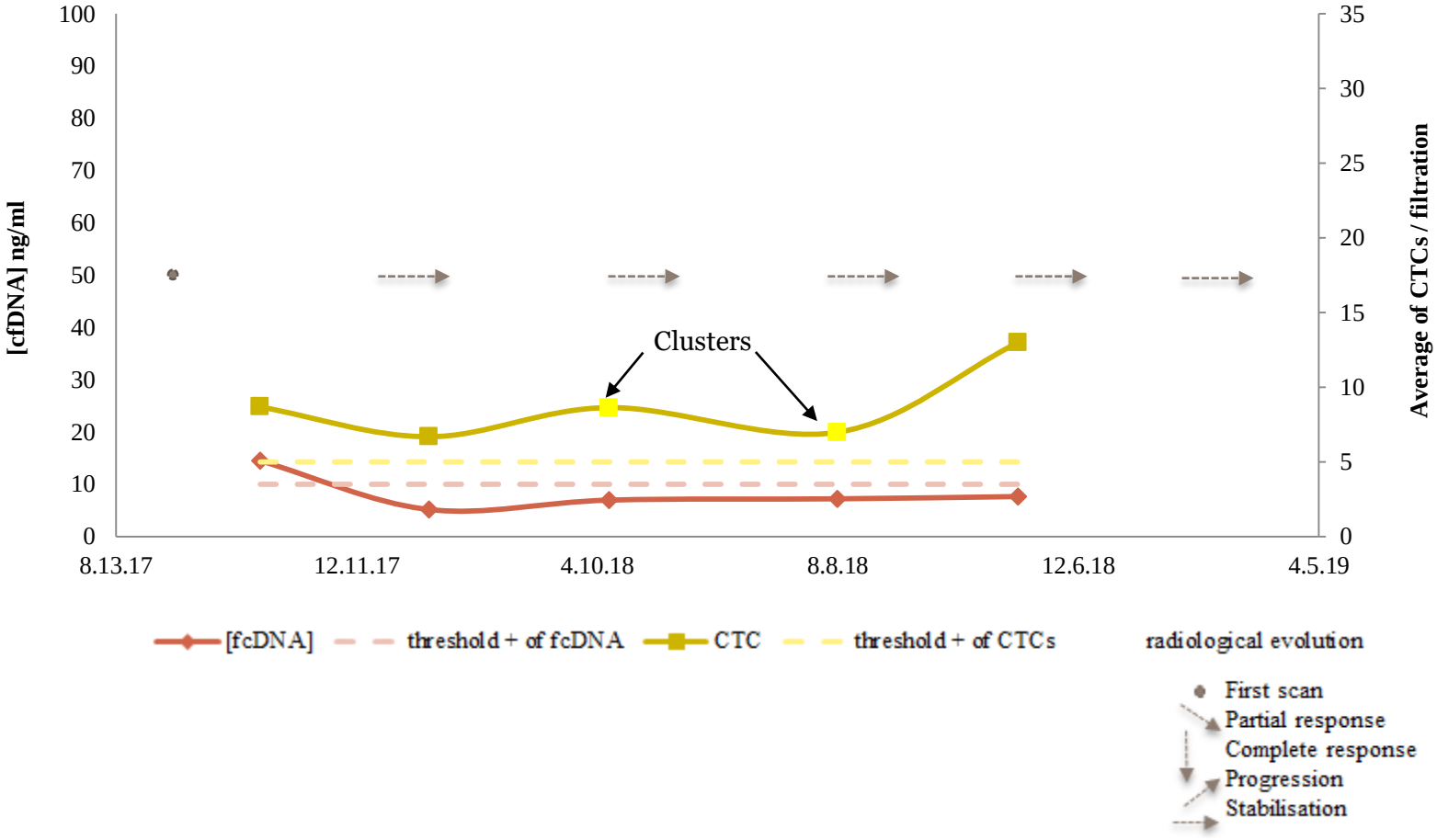
Patient n°3



Patient n°4



Patient 5


100
90
80
70
60
50
40
30
20
10
0
[cfDNA] ng/ml
35
30
25
20
15
10
5
0
Average of CTCs / filtration
Clusters
8.13.17
12.11.17
4.10.18
8.8.18
12.6.18
4.5.19
[fcDNA]
threshold + of fcDNA
CTC
threshold + of CTCs
radiological evolution
First scan
Partial response
Complete response
Progression
Stabilisation

Conclusions

- The ScreenCell® method:
  - **+** : minimally invasive – simple – reliable – low-price
  - - : no reproductibility (population's characteristics)
  - **-** : time-consuming (count on filters necessary)

- Preliminary study
  - Evolution of CTC's and radiological assesment correlated for no-responder patients
  - CTCs earlier than radiological lesion
  - positive and variable in responder patients
  - [cfDNA] informative for the patient with BRAF mutation

Perspectives

Further studies :

⇒ CTCs = melanocytic cells ? ⟶ Immunocytochemistry

⇒ CTCs count on filters = necessary ?

⇒ malignant CTCs = metastatic capacity ?

- Pre-apoptotic
- Agressive

Thank you for your attention

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We thank Dr Wechsler for her support in this work