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DAkkS Deutsche Akkreditierungsstelle D-ML-13345-01-00

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Practitioner

XXXXXX

Bayreuth, 07.08.2020

Your patient:

Born: XXXXX

Blood collection date: XXXXX
Our Lab number: XXXXX
Initial findings: XXXXX,XXXXX

**Report on diagnostic findings on Circulating Tumor Cells (MAINTRAC)**

Dear XXXXX,

Many thanks for sending your examination request regarding the detection of circulating tumor cells. Follow up.

Diagnosis:
Adenocarcinoma NSCLC, Stage: IV, initial diagnosis: 01.07.2018
Histology: EGFR Exon 19 deletion
Clinical Information:
- xxxxx: Surgery (RHJ upper lobe lobectomy)
- xxxx: Complementary therapy
- xxxxxx:xxxxxx Therapy with Gefitinib
- xxxxxx: Pleural effusion RHJ - TACE Pleurodesis
- xxxxxx: near complete metabolic response
- xxxxxxxxxxx: Therapy with Afatinib
- since xxxxxx: Therapy with Osimertinib

Test methods and Results:
The automated microfluorimetric image analysis of the **epithelial cell adhesion molecule (EpCAM)**-positive cells with visual control (MAINTRAC) from **1 ml EDTA blood** resulted in following findings (detection limit is at 10 cells/ml):

| | **Number of potential tumor cells** | | | |
|---|---|---|---|---|
| Examination parameter | In the sample (1ml) | In circulation (5l) (in millions) | In addit. examination: % of EpCAM-pos. cells | Cell fragments |
| EpCAM | **100** | **0,5** | | numerous |
| Ki67 | **75** | **0,38** | **75,0%** | |

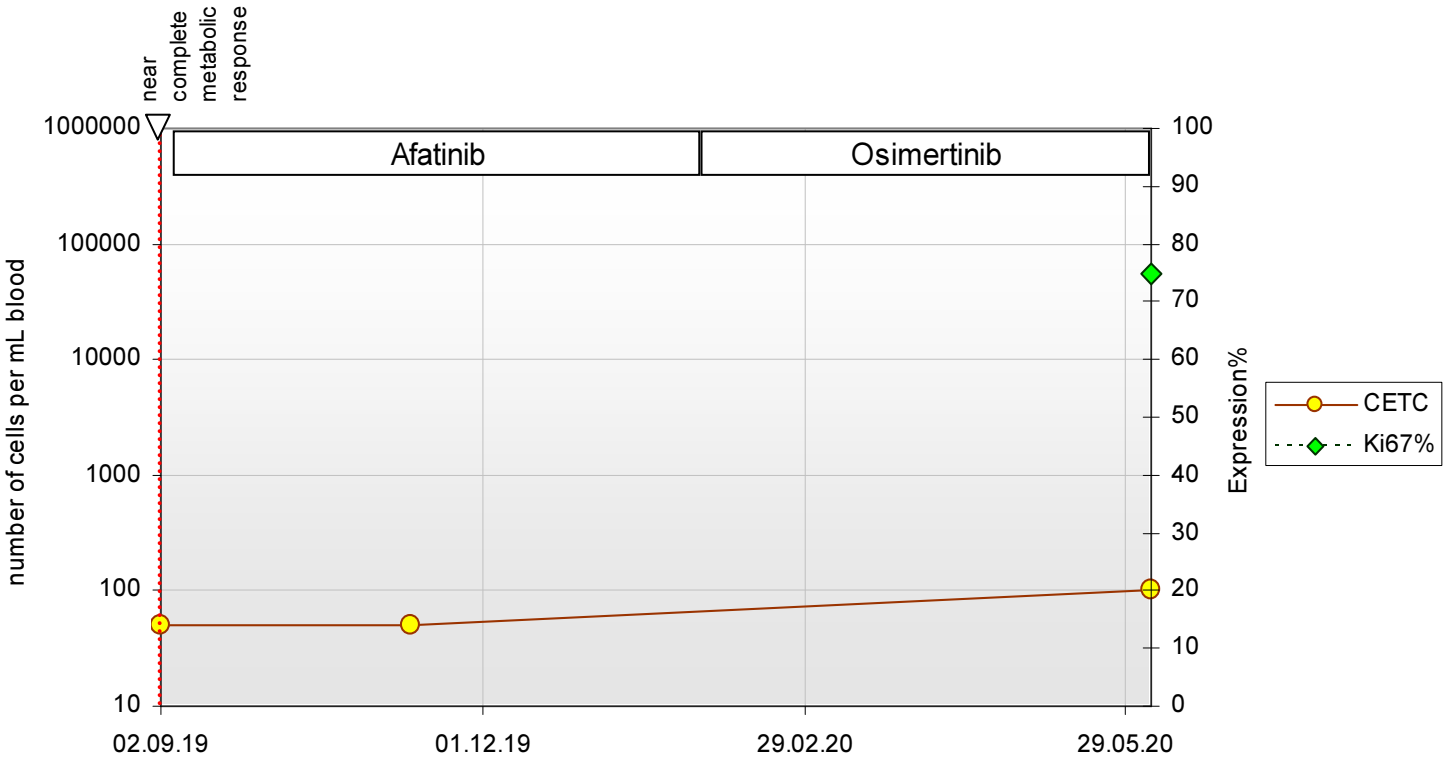
| **in-vitro-vitality reduction in relation to concentration and time (in%) with eutherapeutic concentrations of** | | | | |
|---|---|---|---|---|
| Artemesin | **70** | Curcumin | **80** | The ideal is a reduction by 100% in short-term cell culture |
| EGCG | **30** | 6-Shogaol | **40** | |
| Quercetin | **40** | Resveratrol | **75** | |
| Vitamin C | **20** | | | |

The material for examination could be thoroughly evaluated.

We detected **a minimally to slightly increased number of live, potentially malignant tumor cells circulating in the blood. In comparison to the previous findings from November 2019 the number of potential tumor cells has doubled.**
A high number of cells are in the **growth phase** of the cell cycle (**Ki67-Index 75 %**).
Additionally, numerous specific cell fragments detected.
Specific cell fragments may occur as part of an immune response and/or a cytotoxic therapy response and indicate damaged/non-viable cells.

**The results of in vitro vitality reduction** are given in the above table.

Comments:
Initially, following therapy with Gefinitib, pleural effusion and TACE - Pleurodesis, we could detect cell numbers at a minimal level.
After change of therapy to Afatinib, the number of EpCAM positive cells remained stable, however, a larger number of tumour spheres were seen in Nov 2019. These are possibly cells present which have downregulated their EpCAM expression but still are responsive to growth stimulation of epithelial grwoth factors and resume expression of EpCAM in the tumor spheres.
Now, over a period of 7 months and change of therapy to Osimertinib since February 2020, there is an increase in CTCs. The changes are still within the biological variations, only a 10-fold or a repeated increase in cell numbers is significant.




You now have a reference point, from which it can be determined, if and how the number of these cells changes in response to therapy.

With best regards,
Dr. med. Ulrich Pachmann Prof. Dr. med. Katharina Pachmann Dr. med. Joachim Fluhrer