A New Algorithm For Calculating Cumulative CTC Changes During Treatment

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RATIONALE

Chemotherapy is slowly being supplemented by a new generation of drugs that recognize specific targets in or on cancer cells and has proven to be more effective with markedly fewer side effects. However, alternative oncogenic signaling pathways take over during the course of the disease, inevitably leading to drug resistance. As a consequence renewed tumor analysis is required to redefine the optimal treatment regimen.

Circulating tumor cells (CTCs) represent a "liquid biopsy" that can be used to tailor treatment for individual patients. CTCs are however rare and can only be obtained for further characterization in a small fraction of patient. CTC number often fluctuates during treatment, creating a pattern of peaks and troughs which may preclude the possibility to accurately assess significant differences in any patient at any time.

USE OF THE ΔAUC AS PREDICTOR OF TREATMENT EFFICACY

To investigate the algorithm potential to detect significant response-related changes of CTCs, an interim analysis was conducted on 34 breast cancer patients (12 early breast and 22 MBC) treated with standard chemotherapy and tested for M30-positive (apoptotic) CTCs and 29 MBC treated with denosumab (compassionate use) and tested for RANK-positive CTCs.

Methods

We have developed integrated assays to monitor both total and target-positive CTC changes under treatment whose mechanism is well known.
We then expressed the observed variations by a parameter named ΔAUC.
The detected numbers of target-negative and target-positive CTCs were separately plotted in relation to time and the Area under the Curve (AUC) of longitudinal graphs was calculated (by the trapezoidal rule), following a procedure which is commonly adopted to evaluate cumulative changes of serological tumor markers.

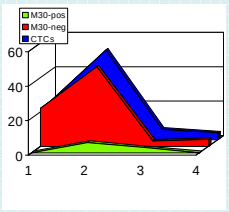
THE ΔAUC

The difference between target-negative and target-positive CTC concentration-time Area was calculated in all patients according to the following formula:

ΔAUC= target-negative CTC AUC - target-positive CTC AUC

Relative numbers were obtained in this way:
Positive ΔAUC value is expression of extra target-negative CTC over the follow-up period;
Negative ΔAUC value is expression of targeted-positive CTC over the follow-up period;
ΔAUC = 0 derives from balanced numbers of the two subset of CTCs.

Monitoring apoptosis in breast cancer during standard chemotherapy

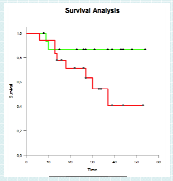


The figure shows a representative MBC case.
During treatment the total CTC number initially increased, then the number decreased (blue area). The area under the M30- CTC curve (red area) was greater than M30+ CTC curve (green area).
The ΔAUC value was >0

We found Progressive Disease (PD) at the re-evaluation by imaging

Notably, a peak of M30-negative (live) CTCs is often observed at the progression

Breast cancer according to ΔAUC = M30-negCTC AUC – M30-posCTC AUC (IOV cohort)

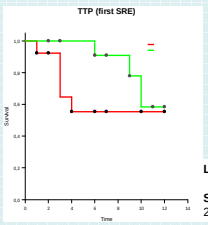


11 non-M breast pts and 23 MBC were monitored by serial CTCs and M30-positive CTCs assessment.

The patients were stratified according to:
ΔAUC < 0 (green line) 16 pts
ΔAUC > 0 (red line) 18 pts

We found a weak association
between ΔAUC > 0 and poor outcome
(Log-Rank test, P = 0,056)

Breast cancer according to ΔAUC = RANK-negCTC AUC – RANK-posCTC AUC (Campus cohort)

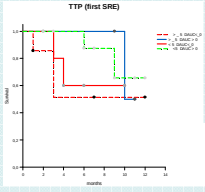


Log-Rank Test:

Statistic	DF	P Value
2,207	1	0,137

A 6 months delay in the first Skeletal Related Event (SRE) was observed in the denosumab group for MBC patients that retain RANK expression on CTCs (ΔAUC < 0, green line)

Breast cancer according to ΔAUC = RANK-negCTC AUC – RANK-posCTC AUC (Campus cohort)/2



Log-Rank Test:

Statistic	DF	P Value
2,505	3	0,474

Noteworthy, a ΔAUC < 0 seems associated to a delay of the first SRE also in the MBC patients with CTC numbers ≥5 cells (blu line)

CONCLUSIONS

- The algorithm ΔAUC seems to have the potential to detect significant response-related changes of CTC number;
- The use of specific CTC assays may be indicated for monitoring treatments with well-known mechanism of action

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