Paths of chromosomal instability and copy number alteration in circulating tumor cells of progressing early-stage breast cancer patients

M. Silvestri[1],C. Reduzzi[2], M. Vismara[1], M. Valenti[1], S. Folli[3], M. Cristofanilli[2], G. Pruneri[1], S. Di Cosimo[1], V. Cappelletti[1]

[1]Department of Advanced Diagnostics, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy; [2]Department of Medicine, Division of Hematology-Oncology, Weill Cornell Medicine, New York, USA; [3]Department of Surgery, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy

Fondazione IRCCS Istituto Nazionale dei Tumori

Sistema Socio Sanitario Regione Lombardia

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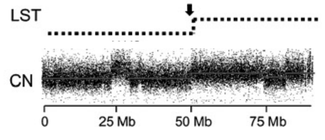
Background

Sequencing of circulating tumor cells (CTC) allows capturing genetic diversity by copy number alterations (CNA) and measuring chromosomal instability (CIN) at single-cell level during cancer treatment and progression.

Among the different methods to evaluate CIN, Large-scale state transtions (LST) represents a surrogate defined as the number of consecutive chromosomal breakages that generate gains or losses of greater than or equal to 10Mb.



Large-scale state transitions (LST) genome wide

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Breakpoints between adjacent regions of at least 10 Mb

LST score = sum

T. Popova *et al.*, Cancer Research, 2012.

Methods

We analyzed baseline and end-of-treatment primary tumor samples of a cohort of 31 triple negative breast cancer patients receiving neoadjuvant anthracycline/taxane and prospectively followed with periodical blood draws for CTCs analysis from initial diagnosis to eventual relapse. We used large scale state transition (LST) as a surrogate measure of CIN, and analysed CNAs in 35 CTCs by low-pass WGS and by Targeted NGS in primary tumor samples.

Goals

- **To investigate the role of chromosomal instability computed in circulating tumor cells as LST measure, in the evaluation of tumor progression.**
- **To evaluate the association between the amount of copy number aberrations and chromosomal instability level.**

Results

Mean LSTs varied according to the time of collection and the phase of treatment. Globally LSTs dropped during chemotherapy suggesting either a hit of CTCs with higher CIN or the selection of CTCs with a threshold compatible with cell viability for seeding. Notably, LSTs increased after chemotherapy with no differences between CTCs LST values after treatment.

CIN was not associated with relapse as CTCs of recurrent patients were evenly distributed according to the value of LST. However, CTCs characterized high-LST had significantly more genomic alterations than those with lower LSTs (502 vs 186 P<0.001, Fisher test). The genomic alterations of CTCs which we previously reported to be more similar to residual rather than primary tumor ***(Silvestri et. al. Sci. Rep. 2022)***, were overall characterized by more gains than losses. In high-LST CTCs numbers of gains and losses were similar (270 vs 232), whereas CTCs with low LST had less gains than losses (72 vs 114).

Table 1. Clinical patients characteristics

ID	Initial stage	NAC	pCR	Relapse	Relapse time (months)	n CTC	LST high	LST low (n)
p1	cT2N1	AP-E	No	No		1	0 (0%)	1 (100%)
p2	cT2N0	AP-CMF	No	No		1	1 (100%)	0 (0%)
p3	cT2N1	AC-CBDCA/P	Yes	No		3	2 (66.6%)	1 (33.3%)
p4	cT2N0	AP-CMF	Yes	No		8	3 (37.5%)	5 (62.5%)
p5	cT1N2	AP-CMF	Yes	Yes	74.1	1	0 (0%)	1 (100%)
p6	cT2N1	AP-CMF	No	Yes	3.9	3	2 (66.6%)	1 (33.3%)
p7	cT2N1	AP-E	No	Yes	11.8	3	2 (66.6%)	1 (33.3%)
p8	cT3N1	CBDCA/P/imm	No	Yes	9.06	3	2 (66.6%)	1 (33.3%)
p9	cT3N1	AC-P	No	Yes	5.4	4	3 (75%)	1 (25%)
p10	cT2N1	AP-CMF	No	Yes	19.5	8	7 (87.5%)	1 (12.5%)

Fig. 1. LST measurements in CTCs from early breast cancer patients and correlation with CNAs distribution. LST score were evaluated in CTCs collected at baseline (BL), during treatment (DT), end of treatment (EOT), follow-up (FU) and at progression (PD). Barplots report CTCs grouped by timeline on x-axis and LST score on y-axis (A). The amount of CNAs detected in CTCs were associated with LST status. The heatmap reports CTCs on the columns and top 50 altered genes *(Silvestri et. al. Sci. Rep. 2022)* on the rows. Red and blue colors refer to GAIN and LOSS CNA status (B).

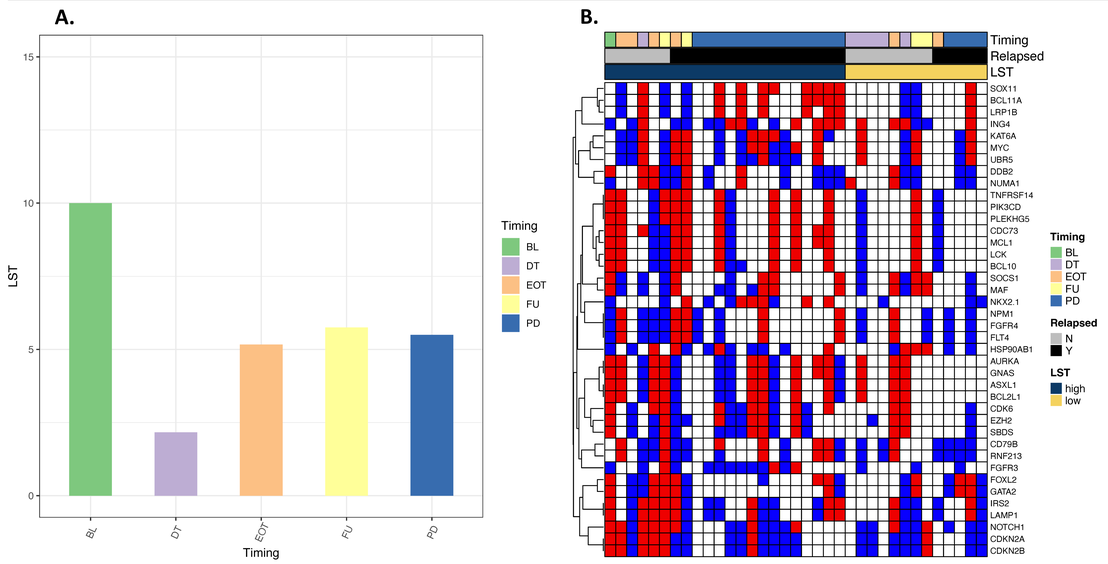
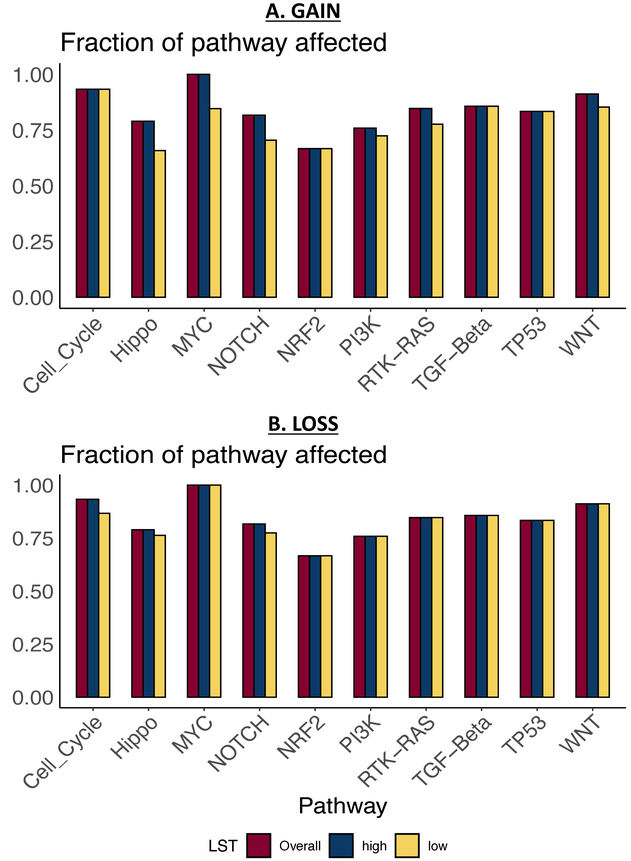


Fig. 2. Comparison between 10 canonical cancer pathway (Vega F.S. et. al., Cell 2018) enriched in LST high and low CTCs. Fraction of pathway affected and fraction of samples affected by CNA events in terms of GAIN (A) and LOSS (B) were reported as yellow and blue bar for LST high and low groups, respectively. "Fraction of pathway" and "fraction of samples" affected refer to the number of altered genes present in a specific pathway and to the number of cases showing the altered pathway, respectively.



Conclusions

- **Early-stage triple negative breast cancer patients have CTCs featuring high levels of genomic instability at initial diagnosis. CIN and CNA are reduced by chemotherapy. CTCs of progressive cases recover increased levels of CINs and CNA during follow up and at the time of progression, yet relapses are unexpectedly less instable than baseline.**
- **Levels of CIN are associated with numbers and type of CNA with low-LST CTCs being associated with less CNA events mostly represented by gene loss.**

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Disclosure and Contact

The presenting author declares no conflict of interest.

marco.silvestri@istitutotumori.mi.it