# Clonal evolution in primary breast cancers under sequential epirubicin and docetaxel monotherapy

**Andreas Venizelos**[1,2], Christina Engebrethsen[1,2], Wei Deng[1,2], Jürgen Geisler[2,§], Stephanie Geisler[2,§], Gjertrud T. Iversen[1,2], Turid Aas[3], Hildegunn S. Aase[4], Manouchehr Seyedzadeh[4,‡‡], Eli Sihn Steinskog[2], Ola Myklebost[5,6,‡], Sigve. Nakken[5,6,7], Daniel Vodak[5,6], Eivind Hovig[5,6,8], Leonardo A. Meza-Zepeda[5,9], Per E. Lønning[1,2], Stian Knappskog[1,2,*], Hans P. Eikesdal[1,2,*]

[1] K.G.Jebsen Center for Genome Directed Cancer Therapy, Department of Clinical Science, University of Bergen, Bergen, Norway [2] Department of Oncology, Haukeland University Hospital, Bergen, Norway [3] Department of Surgery, Haukeland University Hospital, Bergen, Norway [4] Department of Radiology, Haukeland University Hospital, Bergen, Norway [5] Department of Tumor Biology, Institute of Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway [6] Norwegian Cancer Genomics Consortium, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway [7] Centre for Cancer Cell Reprogramming, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway. [8] Centre for Bioinformatics, Department of Informatics, University of Oslo, Oslo, Norway. [9] Genomics Core Facility, Department of Core Facilities, Institute of Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway.

## Abstract

**Background:** Subclonal evolution during primary breast cancer treatment is largely unexplored. We aimed at assessing the dynamic changes in subclonal composition of treatment-naïve breast cancers during neoadjuvant chemotherapy.

**Methods:** We performed whole exome sequencing of tumor biopsies collected before, at therapy switch, and after treatment with sequential epirubicin and docetaxel monotherapy in 51 patients with primary breast cancer, included in a neoadjuvant phase II trial.

**Results:** There was a profound and differential redistribution of subclones during epirubicin and docetaxel treatment, both in tumors with and without a clinical response to either treatment. While truncal mutations and main subclones persisted, smaller subclones frequently appeared or disappeared. Reassessment of raw data, beyond formal mutation calling, indicated that the majority of subclones seemingly appearing during treatment were in fact present in pretreatment breast cancers, below conventional detection limits. Likewise, subclones which seemingly disappeared were still present, below detection limits, in most cases where tumor tissue remained. Tumor mutational burden (TMB) dropped during neoadjuvant therapy, and copy number analysis demonstrated specific genomic regions to be systematically lost or gained for each of the two chemotherapeutics.

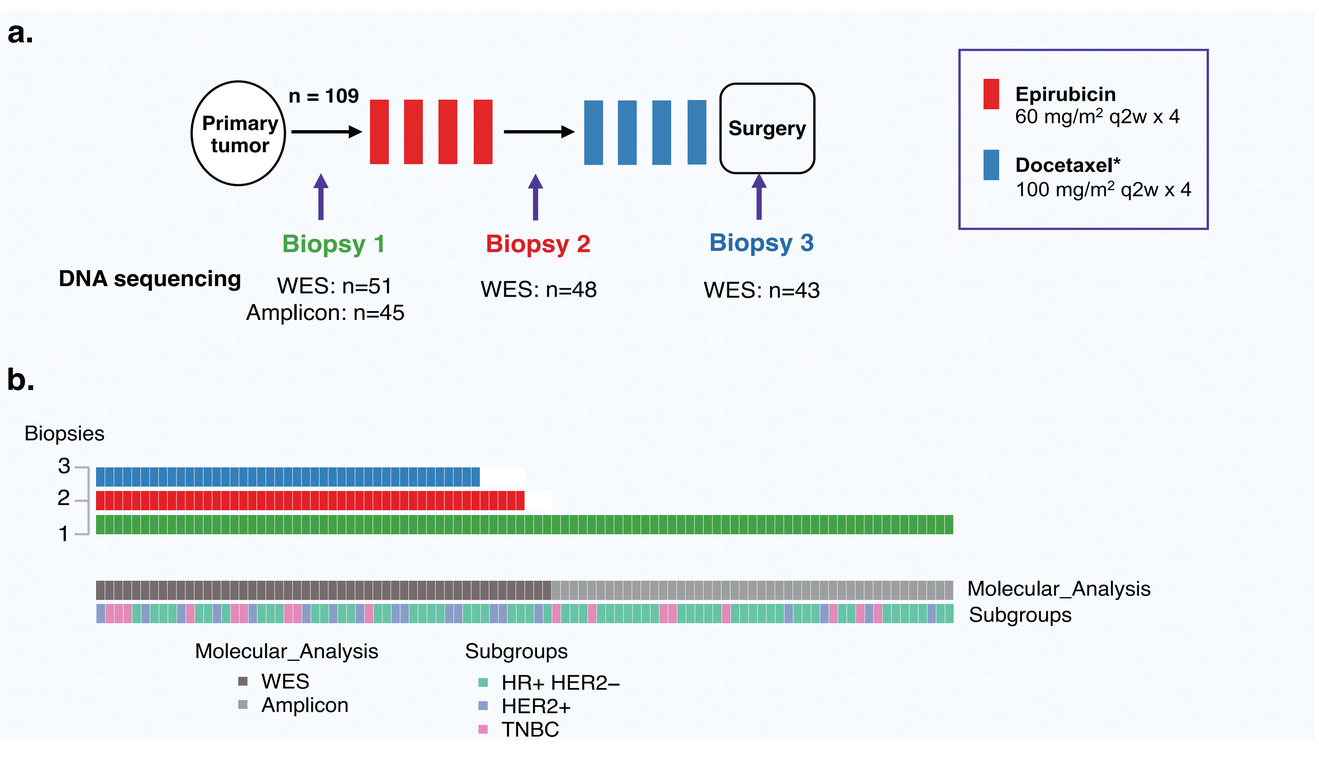
## Aim

- The identification of factors predicting outcome to individual compounds (monotherapy) [2], contrasting combination regimens for which factors predicting sensitivity to individual compounds may not be identified.
- To assess the dynamic changes in subclonal composition in 109 patients with large, treatment-naïve primary breast cancers.

## Background

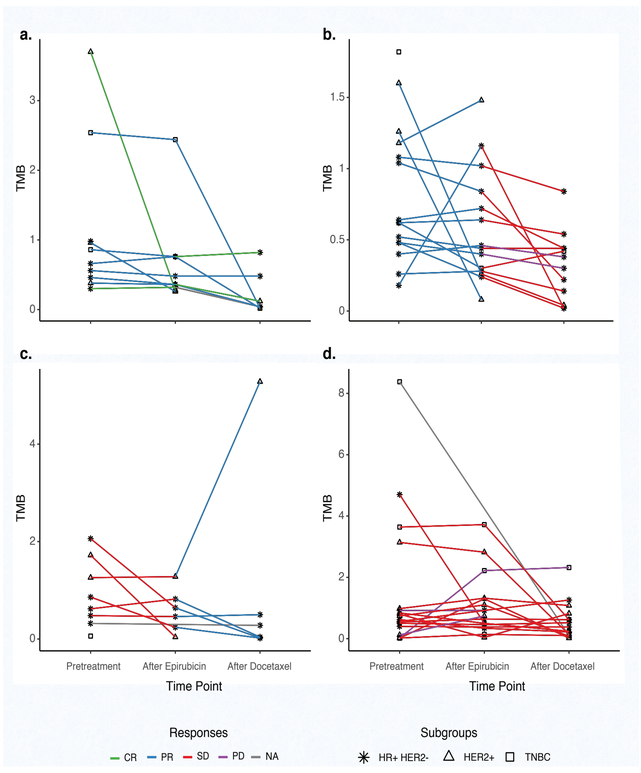
Conventional chemotherapy still plays the main role in the treatment of multiple cancer forms and resistance to chemotherapy remains the main reason for treatment failure and death among cancer patients. However, the molecular mechanisms underlying resistance to chemotherapy in vivo remains poorly understood. Taking breast cancer as an example, there have been significant improvements in cancer therapy over the last decades, although, resistance to chemotherapy remains the main obstacle to cure among patients. In metastatic disease, responses are in general of short duration (months), leading to multi-resistance and death within a time-frame of a few years only [1]. A long-term goal is to identify biomarkers that may be applicable in testing for drug sensitivity prior to commencement of therapy for individual patients, leading to early administration of optimal treatment and sparing patients from side-effects of inefficient treatment.

## Material & Methods




## Results

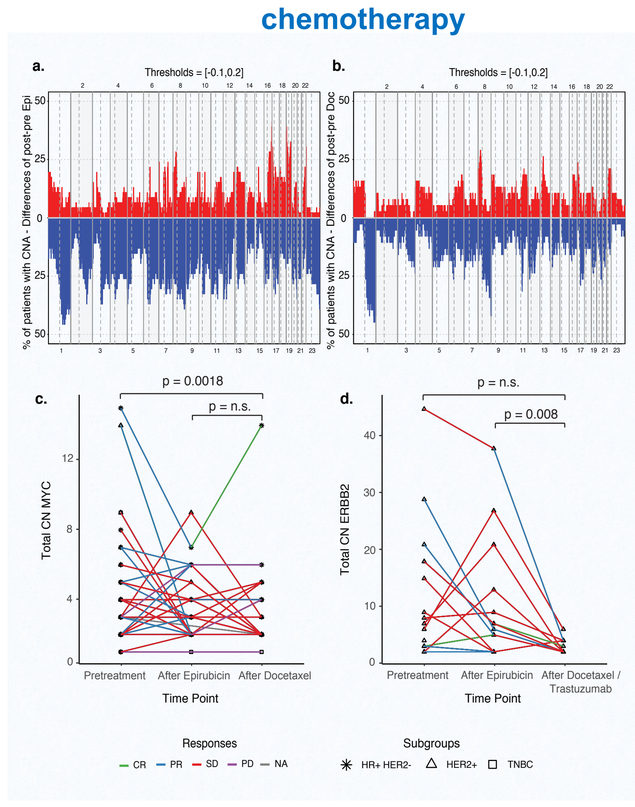
### 1. Tumor Mutational Burden during chemotherapy stratified based on response groups




TMB was significantly decreased among responders to Epirubicin treatment. ($p=0.043$)

A significant decrease in TMB was observed amomg non-responders to Docetaxel. ($p=6x10^{-3}$)

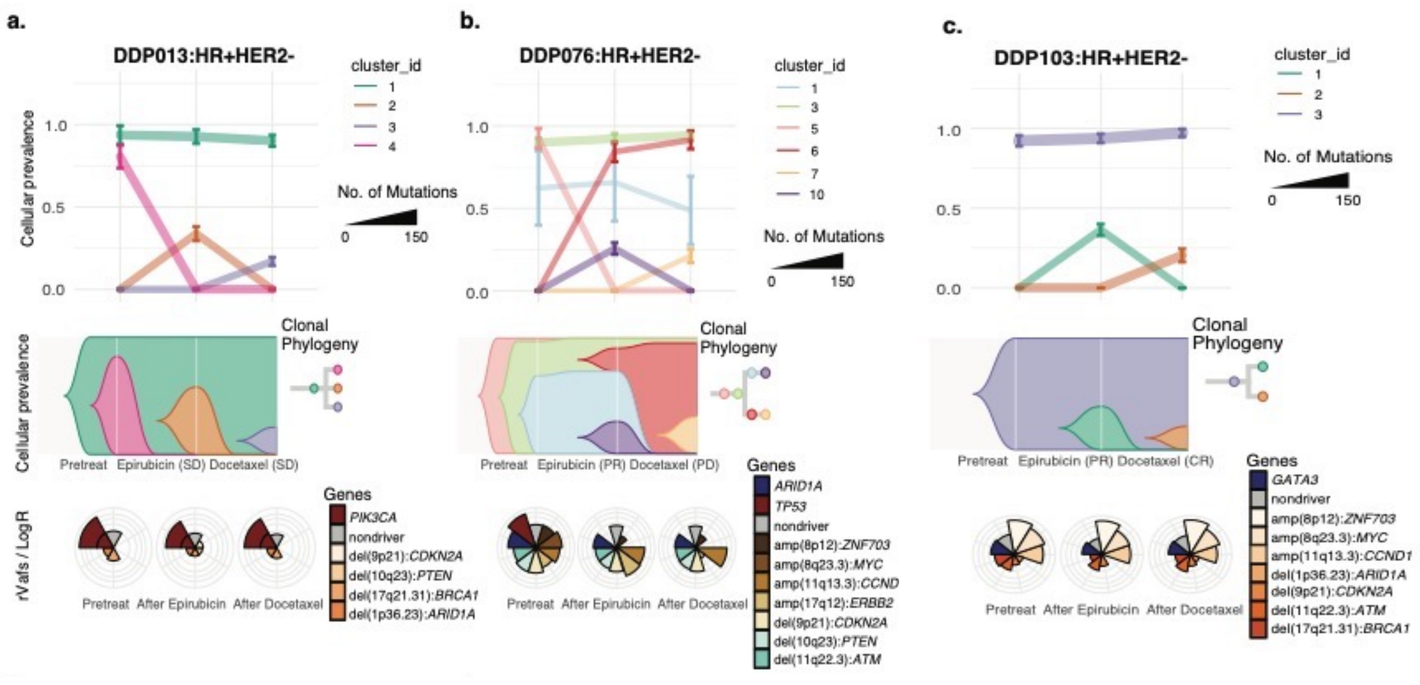
### 2. Differences in Copy Number Alterations during chemotherapy




**After Epirubicin**
Increase in copy number gains for chromosomes 16 and 18 and an increase in copy number losses for chromosomes 1 and 6.

**After Docetaxel**
Increase in copy number gains in chromosome 8p and an increase in copy number losses in chromosomes 1 and 8q arm.
A significant increase in copy number losses for the *MYC* gene and a significant decrease in copy numbers of the *ERBB2* gene.

### 3. Subclonal redistribution during chemotherapy




During chemotherapy subclones are shrinking while other acquire new mutations and form new subclones.

Early truncal sublones are predominantly persisted during chemotherapy while smaller sublcones are more dynamic.

## Conclusions

Both epirubicin and docetaxel monotherapy to causing profound redistribution of smaller subclones in primary breast cancer, while early truncal mutations and the main subclones generally persisted through treatment.

## Acknowledgements

We gratefully acknowledge the contribution made by the patients included in the dose-dense trial. Furthermore, the trial could not have been conducted without the laboratory facilities provided by Trond Mohn to the Mohn Cancer Research Laboratory, the skilful expertise of colleagues in the Departments of Oncology, Surgery and Radiology at Haukeland University Hospital, and the technical assistance from H.B. Helle, C. Eriksen, B. Leirvaag, N.K. Duong and D. Ekse. The authors would like to express their gratitude to the late Dr. Gun Anker for her clinical support in the early years of the trial, and the Norwegian Cancer Genomics Consortium for exome sequencing and IT-infrastructure.

## References

1. EBCTCG. Lancet, 2005. 365(9472): 1687-717.
2. Lonning PE. Lancet Oncol., 2003 4(3): 177-85.

## Contact information

Andreas Venizelos, PhD e-mail: andreas.venizelos@uib.no
Stian Knappskog, PhD e-mail: stian.knappskog@uib.no