# Non-invasive treatment follow-up using cfDNA in responders and non-responders following ICB treatment - an ultra-sensitive multiplex mutation detection method using flow cytometer readout

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## Background and Introduction

This study aims at investigating the utility of the ultra-sensitive superRCA method for detecting cfDNA mutations (EGFR and KRAS) in peripheral blood from metastatic NSCLC patients, for estimation of tumor burden and early detection of responders and non-responders after ICB immunotherapy treatment. This is an ongoing study and presented today is the subset of data from the initial pilot cohort of 12 patients, comprising of 51 samples. Average of 3ml plasma and 50ng ctDNA input for mutation assessment.

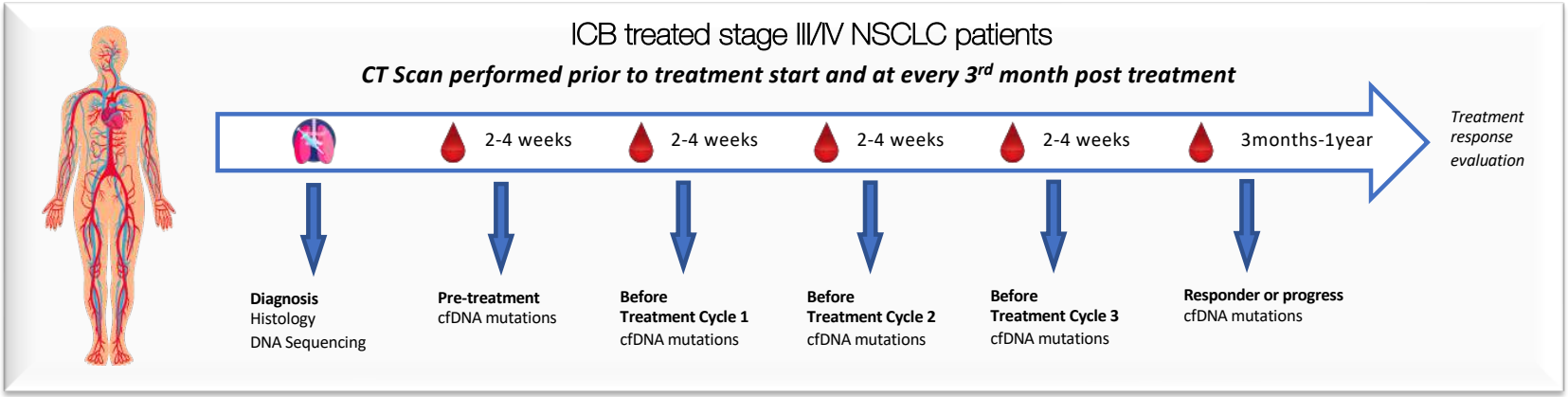


**Figure 1**: Study design; treatment, sampling and analysis of metastatic Stage III and IV NSCLC patients at Sahlgrenska University hospital

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## Material and Methods

Patients in this retrospective study have all been diagnosed with stage III and IV metastatic NSCLC. Sequencing was done primarily on the primary tumour and CT scans were performed on these patients every 3rd month. Response evaluation was done at 9 month**.** Longitudinal blood samples were taken before treatment start and before each cycle of treatment. cfDNA were extracted from 3,4-4,2ml (avg. 3,3ml) blood plasma and 15-280ng (avg. 50ng) DNA was analysed using a multiplex superRCA mutation assay (Rarity Bioscience), a novel and ultra-sensitive technique for mutation detection using a flow cytometer for readout.

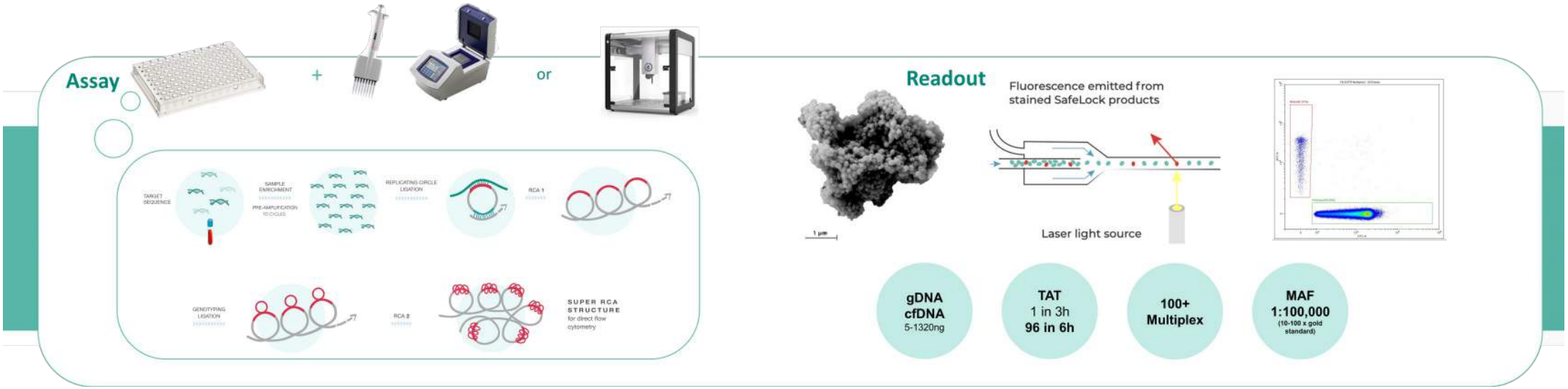


**Figure 2**: superRCA technology, workflow and read-out

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## Results

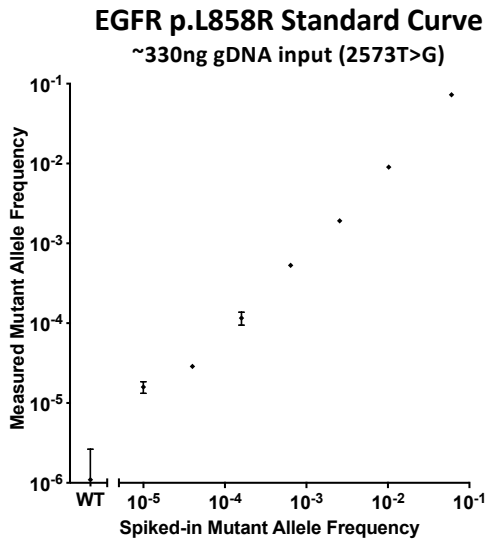


**Figure 3**: 4-fold dilution curve of superRCA EGFr p.L858R mutation assay using positive genomic DNA samples spiked into wild-type genomic DNA, total 330ng assay input. Mean and SD from 2 replicates.

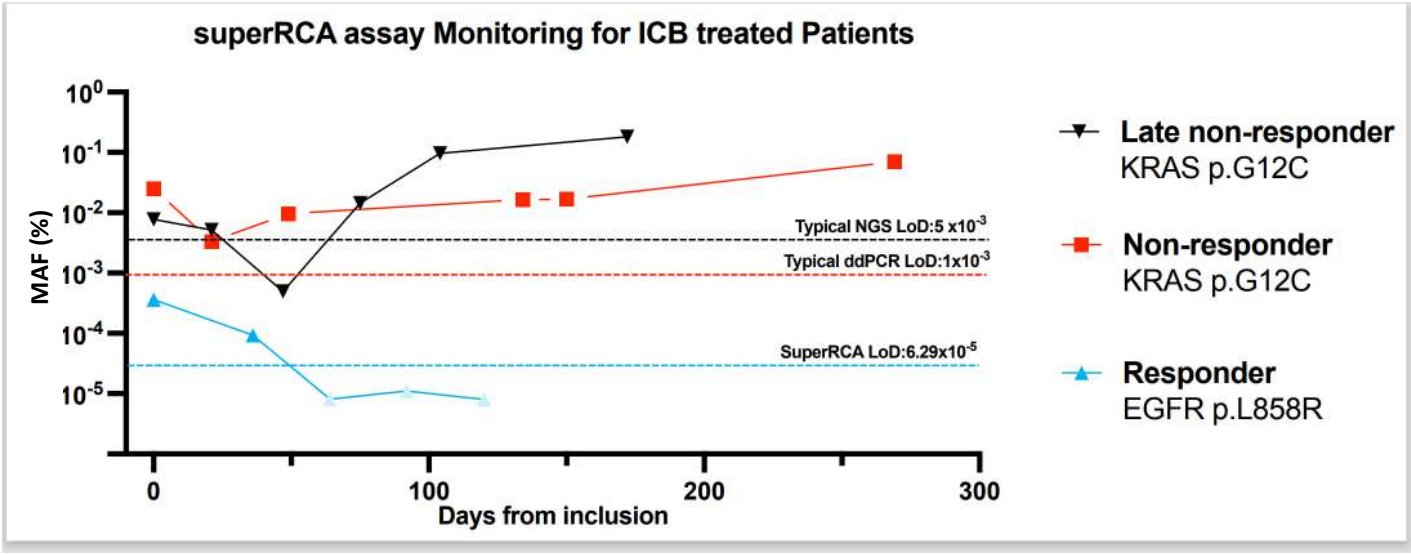


**Figure 4**: Example data from the pilot cohort. ***Non-responder***; Patient with clinical progress at 9 months. After initial molecular response to treatment, lowering target mutation cfDNA, it then increases in good agreement with clinical progress at 9 months. ***Responder:*** Patient with clinically stable disease at 12 months. superRCA enabled to pick up the initially low levels of target mutant cfDNA and then follow the molecular response to treatment until mutant molecules in plasma disappears at later time points. ***Late non-responders****:* Patient with clinical progress at 6 months. After initial molecular response to treatment, where superRCA can monitor all data points and lowest level of mutant molecules in plasma, disease relapses and shows progress in good agreement with CT scan at 6 months.

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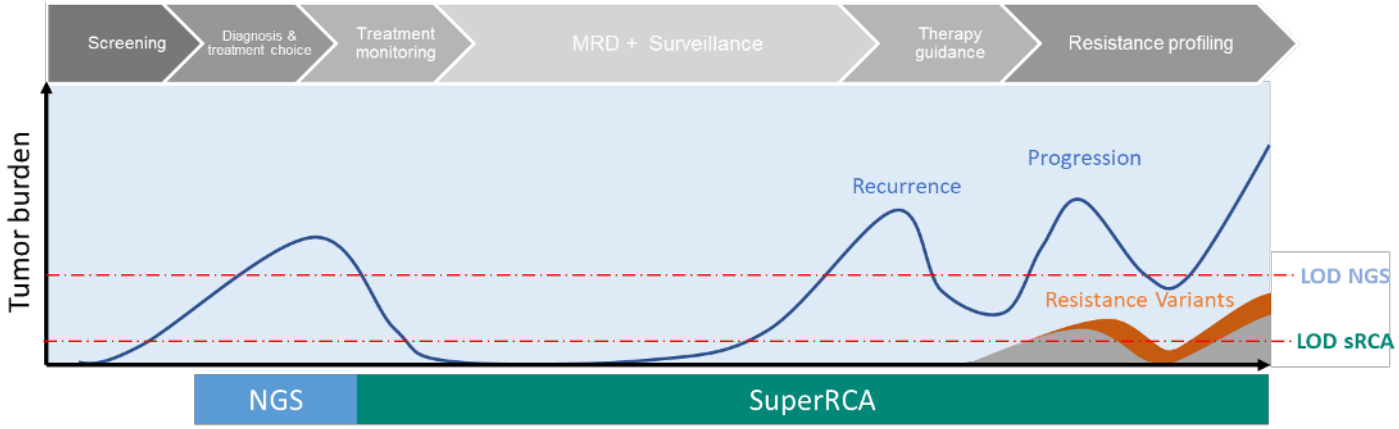
## Conclusion - Discussion

The preliminary data suggests that cfDNA mutation analysis in blood plasma liquid biopsies correlates with clinical response follow-ups in ICB responders and non-responders. It also show an initial systemic molecular remission response in patients that only later appear to become non-responders. For the responders, the sensitivity of the assay can pick up early time points and then follow subsequent molecular remission. These data indicate that superRCA cfDNA mutation assays can detect several ctDNA mutations in the same blood plasma sample at high sensitivity using flow cytometry.

The high sensitivity of the assay and non-invasive nature of the sampling would allow for more frequent monitoring of systemic disease progress in patients during treatment and presumably also earlier response detection and therapy switch guidance. In both responders and late non-responders the superRCA assay may also present possibilities to avoid unnecessary adverse side-effects due to over- or mis- treatment. Combined with sequencing at diagnosis it presents a compelling case for inclusion in the clinical setting and agrees especially well with a tumor informed approach.





## Acknowledgements

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