Summary
Summary

Our aim was to understand the role of DNA Damage response (DDR) pathways associated with the development of breast carcinoma and how pretherapeutic samples and neoadjuvant chemotherapy (NACT) treated samples differ in their alterations. Among the total 178 primary breast carcinoma samples collected, 133 were breast carcinomas without any treatment (pretherapeutic) and the rest 45 were prior treated with NACT.

We have focused first on Homologous recombination repair (HRR) pathway genes. In HRR pathway, we have analyzed the alterations (deletion, methylation) and mRNA/protein expression of BRCA1, BRCA2, FANCC and FANCD2 which are candidate tumor suppressor genes (TSG), in primary breast lesions. Hence, alteration in any of these genes abrogate the whole pathway. The deletion frequencies were high in BRCA1 and BRCA2 loci (60-68%) followed by FANCC and FANCD2 (29-36%) in both pretherapeutic and NACT treated samples. Promoter methylation of FANCD2 and BRCA2 were higher (53-60%) followed by FANCC and BRCA1 (29-46%) in both pretherapeutic and NACT treated samples. The overall alterations (deletion/methylation) ranged from 64-78% in the genes in these samples. Significantly higher alterations in ER/PR(-) BC were found in HRR genes compared to ER/PR(+) BC in both pretherapeutic and NACT treated BC. In addition, the BRCA1 alterations were significantly high in early age of onset than the late age of NACT treated BC. Reduced mRNA expression was found in HRR genes in BC samples. The immunohistochemical analysis of these proteins showed concordance with the molecular alterations. In pretherapeutic BC, the alterations of the genes showed poor patient outcome than NACT treated BC patients. The immunohistochemical analysis showed NACT tolerant cells have low frequency of stem cell marker CD44+ cells and low expression of proliferative marker PCNA irrespective of the subtypes.

In mismatch repair (MMR) pathway, deletion, promoter methylation and mRNA/protein expression analysis of MLH1 and MSH2 genes were determined. The deletion frequencies were (37-38%) in MLH1 and (32-34%) in MSH2 gene in both pretherapeutic and NACT treated samples. The Promoter methylation frequencies were (49-62%) in MLH1 and (41-51%) in MSH2 gene in both pretherapeutic and NACT treated samples.
The overall alteration of MLH1 and MSH2 was 71% and 58% in pretherapeutic samples and 64% and 62% in NACT treated samples respectively. The overall alteration frequency of MLH1 and MSH2 gradually increased from Luminal A to TNBC in pretherapeutic samples and almost comparable in NACT treated samples. Alterations of MMR genes were variable (30-76%) in early and late age of onset BC. Reduced mRNA expression was found in both the genes in BC samples. The mRNA and protein expression analysis of these genes showed concordance with the molecular alterations. The pretherapeutic patients showed significant poor DFS (Disease free survival) with alterations of MLH1 and MSH2 where no such correlation could be drawn in case of NACT treated patients.

Altogether, 87% (103/118) of the pretherapeutic samples and 78% (32/41) of the NACT treated samples showed alteration in both HRR and MMR pathways. This coalterations were significantly associated with the nodal invasion positive status in both pretherapeutic and NACT treated samples which suggests the role of these pathways in micro and distant metastasis of the disease.

To understand the mechanism of chemo-tolerance in BC and the role of HRR/MMR genes in this, MCF-7 and MDA MB 231 cells were treated with two anthracycline anti-tumor antibiotics doxorubicin and nogalamycin. MCF-7 (IC50 0.214-0.242 µM) was found to be more sensitive to these drugs compared to MDA MB 231 (IC50 0.346-0.37 µM) as shown by cell viability assay. The drugs reduced the protein expression of PCNA (proliferation index) in the BC cells. At the lower IC50 concentration, these drugs induced increased mRNA/protein expression of the HRR/MMR genes. At the IC50/higher concentrations, differential pattern of expression was observed in the two different cell lines. To understand the mechanism of increased expression of these genes, promoter methylation analysis was done after treating the cells with doxorubicin and nogalamycin. Quantitative methylation assay showed an increased percentage of hypomethylation of the promoters of these genes after drug treatment. Neoadjuvant chemotherapy (NACT) treated primary BC samples also showed significantly higher percentage of hypomethylation than the pretherapeutic BC samples. The drugs induced
reduction in mRNA and protein expression of DNMT1 (DNA methyltransferase 1) in MCF-7 and MDA MB 231 cells.

Thus, the high alterations of HRR/MRR pathway genes in both pretherapeutic and chemotolerant NACT treated BC samples suggest their importance in breast cancer progression and chemotherapeutic tolerance. In chemotolerant BC cells, anthracycline anti-tumor drugs induced increased mRNA/protein expression of HRR/MMR genes is probably due to their promoter hypomethylation by reduced DNMT1 expression.