EPILOGUE
Carcinoma of breast has emerged as the leading cancer among females at Ahmedabad and Bombay. In spite of advances in diagnosis and treatment for breast cancers, the overall survival rates after detection have not improved significantly. Research so far done has indicated that genomic constitution of a female largely decides her chances of afflicting breast cancer during lifetime. Differential interindividual response to carcinogen exposure has been attributed to interindividual genomic variants. Such variants have been described as genetic polymorphism or heteromorphism. If such elements can be identified, they can help in identifying women at higher risk for breast cancer. If so, they can be monitored closely and the disease associated morbidity and mortality can be reduced effectively. It therefore infers that, studying genetic heteromorphism is important.

Recent advancements in molecular biology can provide vital help in this direction. However, in spite of its precision, the molecular approach is not feasible at population level, because of the enormous costs involved. Heteromorphism in the human genome can be identified at the cellular level with the help of cytogenetic techniques. Advent of banding techniques have permitted precise identification of subchromosomal regions. This facilitates accurate identification of genetic heteromorphism at chromosomal level. Certain chromosomal heteromorphisms have
been correlated with an increased risk for human health problems. In particular for C-band heteromorphism, it has been hypothesized that the carriers are predisposed to cancer. Subsequent to that, a number of reports have recorded very high prevalence of C-band heteromorphism among patients with different malignancies. Certain premalignant conditions have also revealed higher incidence of C-band heteromorphism. Previously, we have reported carriers of C-band heteromorphism to be more common among patients with leukemia or oral premalignant/malignant conditions. Therefore, for the present study, C-band heteromorphism was chosen as a parameter to ascertain its prevalence among breast cancer patients.

C-bands of chromosome #1, #9 and #16 are large enough and hence, permit exact length measurements and easy detection of localization variants. Hence, for the present study C-bands of these three chromosomes were considered. Following two types of variabilities were considered to constitute heteromorphism: (1) length difference between the homologues of all the chromosomes were calculated. The difference was converted into percentage of the size of smaller C-band. An arbitrary cut off value of 25.00 % was decided and carriers of 25.00 % or more difference were considered heteromorphic for the size of C-band. (2) Inversions of C-band positive regions involving centromere were considered for localization variants. When a part of C-band was inverted to p arm, it was
known as **partial inversion**. Whereas, in case of whole C-band inverted to p arm, it was termed a **complete inversion** of C-band.

The present study aimed at evaluation of prevalence of chromosomal heteromorphism among breast cancer patients, which is largely a female specific cancer. Considering the clinical conditions, females were classified into three groups, viz. (1) Primary breast cancer patients (ICD.9:174) (2) Patients having benign breast diseases and (3) Controls. Since, genetic heteromorphism are transmitted in Mendelian fashion and are decided at the time of zygote formation, their occurrence is identical in all the tissues of same person. For these reasons, any somatic tissue can be utilized for studying chromosomal heteromorphism, however, in view of convenience short-term cultures of PBL were used for the work envisaged.

**PART-I** of the thesis details the prevalence of C-band heteromorphism among the individuals studied. The data obtained for size and localization variations in C-bands of chromosome #1, #9 and #16 were compared with the same obtained for the control group. The results obtained indicated:

1) Significantly higher prevalence of C-band heteromorphics was observed among breast cancer patients compared to the controls. This supported the hypothesis that there is an
association between the presence of C-band heteromorphism and occurrence of malignant disease.

ii) Increased incidence of C-band heteromorphism among patients having benign breast diseases indicated presence of genetic heteromorphism in this group of patients as well. Benign breast diseases are considered to elevate the risk of breast cancer. Whether, C-band heteromorphic benign breast disease patients have higher risk of developing breast cancer than their non-heteromorphic counterparts, remains to be confirmed.

iii) Higher incidence of C-band heteromorphism among premenopausal breast cancer patients compared to postmenopausal breast cancer patients, strengthen the view that genetic factors play more decisive role in patients afflicting the disease in early years of life.

As detailed in PART - I, we have observed significant number of breast cancer patients carrying C-band heteromorphism. It was therefore, important to ascertain how this genetic variant can render the individual more prone to cancer. It has been proposed that C-band heteromorphism causes genomic instability and thereby increase the risk of developing cancer. Variants of C-bands represent structural alterations in genome and constitute potent mutations. It therefore, can be presumed that carriers of such mutations might respond differentially to a mutagen treatment. In
order to test this presumption, an attempt was made to quantitate genomic damage inflicted in the lymphocytes by an in vitro treatment with a known mutagen. Chromosome aberration (CA) is a time-tested parameter, known for its validity and precision in the quantification of genomic damage. Hence, PART-II of the study aimed at examining Mitomycin-C (MMC) induced frequency of lymphocytic CA among breast cancer patients.

Following the in vitro MMC treatment, significantly higher frequency of lymphocytic CA were observed among the breast cancer patients carrying C-band heteromorphism, compared to their non-heteromorphic counterparts. This suggested that PBLs of C-band heteromorphic breast cancer patients were hyper-sensitive to in vitro mutagen treatment. Considering the fact that anticancer chemo and/or radiotherapies often damage the genome of normal tissues, C-band heteromorphic breast cancer patients being treated with these therapies, carry a higher risk of developing secondary neoplasia as possible side effects. It is suggested that such patients should be treated with caution and be monitored more closely during follow ups. Present findings also reinforce the opinion that in vitro response of somatic cells to DNA damaging agents may help in understanding altered susceptibility to external carcinogens/mutagens.

The important aspect in better management of a cancer
patient is to detect the disease in its earlier stages. Since breast cancer spreads with minimum clinical evidence, by the time of its detection, most breast tumours have already metastatised. For these reasons, having a preclinical marker is highly advantageous in management of a breast cancer patient.

Cytogenetic parameters like frequencies of sister chromatid exchange (SCE) and chromosome aberrations (CA) have been normally used as cytological manifestation of DNA breakage and repair processes. However, during recent years they have been studied in relation to various types of cancers and in some precancerous conditions. Review of literature reveals reports regarding elevations in the frequencies of lymphocytic CA and SCE in different malignant diseases. Some authors have even suggested application of lymphocytic SCE levels as a preclinical marker of malignancy. Since such a marker is acutely required in present group of patients, we have studied frequencies of lymphocytic SCE and CA in PART-III to evaluate their possible utility. The results obtained suggested that:

1) Although, mean spontaneous CA and SCE frequencies were elevated in lymphocytes of patients with breast cancer or benign breast disease, considerable overlapping values existed between patients and controls. Hence, their application as preclinical marker of breast cancer is not
feasible.

ii) To find out possible reasons for elevated SCEs and CAs in PBL, a second blood sample from some of the breast cancer patients was obtained after surgical removal of the tumour. When pre-operative and post-operative values of CA and SCE were compared in the same patient, SCE/cell values were found to reduce in 12 out of 16 patients and CA/cell values were reduced in 13 out of 16 patients in their post-operative samples. This clearly indicated that the higher genomic damage observed among pre-operative blood samples of breast cancer patients, can be attributed to the presence of malignant tumour in the body.

iii) It has been suggested that certain tumours produce and release clastogenic substances, which inflict chromosomal damage in the somatic tissues. To evaluate the validity of this assumption, CHO cells were grown in medium containing sera obtained from the blood of primary breast cancer patients and controls. SCE frequencies were examined as the end point. The results indicated elevated SCE frequencies in CHO cells grown in medium containing sera obtained from the breast cancer patients. The observations supported the former contention. This observation also partly explains increased risk of second malignancy among cancer patients.

In conclusion, it can be suggested from the results of present work, that analysis of somatic cells is a worthwhile
approach for detecting individuals at higher risk for breast cancer. In this context, chromosomal heteromorphisms have so far been not considered seriously. However, along with other conventional markers, C-band heteromorphism can be used as a marker for genetic predisposition to breast cancer. Higher sensitivity to MMC induced CA frequencies in C-band heteromorphic breast cancer patients indicate hypersensitivity to mutagens in these patients. However, more experimental efforts will be needed to decipher the exact mechanisms involved. Through precise identification of environmental risk factors and the genetic constitution in the form of predisposing markers, like the one discussed here, it will be possible to provide proper counseling to the first degree relative of the breast cancer patients and to asymptomatic carriers of C-band heteromorphism. The development of breast cancer depends on a dynamic interplay between genetic and environmental factors. Intervention at any stage will help at least to reduce the risk. All these may help in reducing the disease burden in long run. The ultimate dream after all is not only to cure the disease but to prevent it.