CONCEPTION

The Indian Cancer Research Centre (ICRC) was inaugurated in 1952. The need for an institute such as this was first envisaged when the plans for the Tata Memorial Hospital (TMH) were drawn up as even then Dr. Khanolkar had stated that it was essential "to elucidate the nature of cancer and so to devise means, for preventing and curing it." Dr. Khanolkar's goal was to establish an institute dedicated to understanding the mechanism underlying the conversion of a normal cell to a cancer cell with the ultimate goal of translating this understanding to the prevention and cure of cancer. To achieve these goals the first departments established at the institute were the pathology section, which was part of the department of Pathology at TMH, a statistics section, which would focus on the collection and analysis of epidemiological data about the prevalence and types of cancers in India, a genetics section to examine the role of genetic variation in tumor progression, an experimental biology section to study the ultrastructure and function of cancer cells and a photography section that would document the data collected by all the sections in the institute. All of these sections began functioning in 1952. Dr. Khanolkar understood the importance of both national and international collaborative efforts and stated that he wanted the work done at ICRC to be shared with the scientific community as that was the only way in which progress could be made in the cure and prevention of cancer.

1952-1965. The Indian Cancer Research Centre (ICRC) era

The first director of the ICRC was Dr. V. R. Khanolkar. At first the institute had four major sections, the section of pathology which focused on setting up an efficient service laboratory for the hospital, including the establishment of procedures required for frozen sections and setting up a blood bank. In addition, clinical and routine biochemistry departments were also established along with a genetics and statistics unit that focused on understanding the genetics and epidemiology of tumor progression in India. A cytology unit was established to study the role of genetic lesions in tumor progression including the study of the cytology of exfoliated cells in bodily secretions. A unit dedicated to Neuropathology and the histopathology of early lesions in people suffering from leprosy was also begun along with one of the first tissue culture facilities in the country.

Dr. V. R. Khanolkar, (founder and director) Dr. Prof. E. V. Cowdry (director, Wernse Cancer Research Laboratory, Washington University School of medicine) and Dr. K.C.K.E. Raja (director general of health services, Govt. of India) in the new ICRC building

Over the next five years in addition to the existing departments, the AEC-Biology and Medical divisions were established to study the effects

of radiation on human tissues and patients. A biophysics domain was established with the purchase of an electron microscope to study the ultra-structure of tumor cells. The study of statistics of cancer in India gained pace with the addition of new collection centers and the genetics unit was now called the human variation unit. The animal house was established with the import of various inbred strains of mice to be used for carcinogenesis studies and animal models of tumor development. An experimental biology division was also established to encourage the study of mechanisms underlying malignant transformation. The activities of these sections were strengthened over the next two years along with the addition of the departments of embryology and a family planning research unit focused on contraceptive research. An enzyme chemistry unit was set up to study enzymes related to vitamin B metabolism and their relationship to tumor progression. A chemotherapy unit was added in 1961 to complement the other activities in the institute. Dr. Khanolkar retired as director of the ICRC in 1963 and Dr. K. J. Ranadive was appointed as Acting director.

1966-2002: The Cancer Research Institute (CRI) era.

The Cancer Research Building in Parel.

In 1966 the Indian Cancer Research Center and the Tata Memorial Hospital were combined to form the first comprehensive center dedicated to cancer care and research in India – the Tata Memorial Center. As part of the reorganization, the ICRC was renamed as the Cancer Research Institute. The direction of research shifted to emphasize new focus areas that were emerging in the field of cancer research. The addition of new departments for studying Immunology, Cell and Molecular Biology, Carcinogenesis and Restorative surgery complemented the existing

departments of Chemotherapy, Biochemistry, Biophysics and Epidemiology and Genetics. CRI also served as the source for the genesis of other institutes and departments. The human variation unit was later converted to the blood group reference center, which later became the Institute of Immunohaematology while the Neuropathology unit moved to JJ hospital. In 1975 a further reorganization of departments occurred with the establishment of Endocrinology, Cell and Developmental Pathology and Ultrastructure sections. The advent of the year 1981 saw further additions to the institute in terms of new approaches to research in cancer biology. These new additions included departments of genotoxicity, radiobiology, cellular chemotherapy and comparative oncology. The period of 1987-2001 saw the establishment of the departments of virology, genetic engineering, cancer genes, cellular carcinogenesis, cellular immunology, chemotherapy and stem cell biology and neurooncology. Some of these were combined with existing departments just prior to the move to the new facility at ACTREC in 2002.

Transgenic animal workshop held at CRI.

During the period of 1966-1973, the institute was headed by a Dean and the position was held by rotation by all the senior scientists, who also functioned as heads of the various departments. In 1973, Dr. A.Sreenivasan was appointed as Research Director of the institute. In 1975 Dr. B. M. Braganca took over as Research Director and during her time the Cancer Research Institute completed it's silver jubilee. In 1978, Dr. M. G. Deo took charge as Research Director and this lead to the establishment of several new facilities and departments at the institute as detailed above. He stepped down as director in 1995 and Dr. A. Bhisey took over as director of the institute. Dr. Bhisey stepped down in 2001 and Dr. S. M. Zingde took over as deputy director CRI-ACTREC and Dr. S. S. Agarwal was appointed as ACTREC director in 2001. The institute moved to the new facility at ACTREC in 2002.

2002-present: Advanced Centre for Treatment Research and Education in Cancer (ACTREC) - a new beginning.

Dr. K. M. Dinshaw, director Tata Memorial Centre, inaugurated the Advanced Center for Treatment Research and Education in Cancer (ACTREC), in 2002. ACTREC started with a basic research division, which comprised the faculty and departments at the erstwhile Cancer Research Institute and a Clinical Research Centre with a fifty bed hospital and blood bank which would focus on providing novel therapeutic strategies for the treatment of cancer. Dr. S. S. Agarwal served as director from 2001 to 2004 and was followed by Dr. R. Sarin, who served as director from 2005 to 2013. Dr. S. M. Zingde served as deputy director during the period from 2001-2013. Dr. S. V. Chiplunkar served as deputy director ACTREC and in September 2013, she took charge as the Director, ACTREC and Dr. S. Gupta took charge as deputy director CRC-ACTREC. In November 2018, Dr. S. Gupta took charge as director ACTREC, with Dr. N. Khattry as deputy director CRC-ACTREC and Dr. V. Prasanna as deputy director CRI-ACTREC.

The ACTREC Campus

This period saw a number of changes at ACTREC. Dr. Sarin suggested that all the independent scientists be given independent charge of their own laboratories, leading to the generation of a Principal Investigator based system. This period also saw the establishment of several new directions of research at the center that complemented the previously existing programs. A new focus on cancer genetics including the use of next generation sequencing technology and proteomic approaches to understanding tumor biology, were some of the new initiatives that were implemented at ACTREC. In addition to these initiatives, ACTREC invested heavily in adopting new biophysical approaches to addressing basic problems in cancer biology, including structure based drug design and the use of in silico approaches to aid in drug design. A further emphasis was the development of small animal models that represent human disease conditions and an emphasis on studying cancer stem cells and their role in chemo and radio resistance. This has gone hand in hand with an emphasis on the development of imaging technologies both in cell culture in vitro and in small animals in vivo to understand the mechanisms of tumor progression. In addition to these initiatives, the existing programs have been strengthened to focus on understanding the cell biology of the cancer cell, tumor immunology, epigenetic changes during tumor progression and the role of HPV in the development of tumors in the Indian subcontinent. In addition, ACTREC has established several programs that focus on providing services to the research community. This includes the anti-cancer drug screening facility, the establishment of several training programs for scientific assistants such as the one run by the laboratory animal facility and

the flow cytometry facility. ACTREC continues to expand these efforts and we will see more of these in the future.

RESEARCH AT ICRC, CRI and ACTREC

Cancer Epidemiology and Genetics

Cancer Epidemiology in India.

One of the questions that is encountered by a research institute such as ICRC when it is first established is the frequency of development of the disease in the country. Therefore, one of the first departments established at the ICRC was a "Statistics Division" that aimed to generate data to address the following questions: 1. What are the different types of cancers prevalent in India? 2. Does the distribution of cancer in India change with ethnicity, geographic location and habit? 3. As cancer is a genetic disease, what are the different changes in the genome that can be correlated with tumor development? 4. Is cancer a heritable disease and can we identify at risk individuals in families that show a pre-disposition to develop tumors? These questions and others like them lead to the formation of a department at ICRC that would establish a data base directed at accumulating information relevant to the questions described above. The first surveys at ICRC were directed at identifying the most prevalent tumors in the Indian subcontinent. The most common tumor types were tumors of the oral cavity and upper aero digestive tract in men and cancers of the cervix in women. This has changed over the years to include breast cancer as one of the most common tumor types in women and an increase in the frequency of lung cancer, especially in male smokers. Further, the scientists at ICRC, attempted to provide a correlation between different lifestyle parameters and the development of cancer, with an emphasis on tobacco use. Tobacco is consumed in many ways in the Indian sub-continent. People smoke cigarettes and bidis, people chew tobacco in combination with lime or in combination with betel leaf and nut and finally people even brush their teeth with a tobacco product known as 'masheri'. All of these habits showed a striking association with a particular tumor type suggesting that it was not just the use of tobacco but the route of ingestion was also important in terms of disease progression. Finally, studies from CRI demonstrated that other than tobacco users, people working in factories that manufacture tobacco products such as bidis, showed greater susceptibility to tumor development. These studies established the role played by tobacco use in the development of multiple tumor types in the Indian subcontinent and have provided the justification for the presence of warning labels on tobacco products that are now a part of our everyday life.

In addition to the lifestyle associated tumors, the ICRC began analyzing the epidemiological basis for a link between Rh factor and blood grouping and tumor progression. While this did not lead to any significant conclusions about tumor development, the ICRC laboratory developed this as one of the first such service laboratories in Mumbai. These studies were further expanded to explore the relationship between blood groups of the parent and foetus and pregnancy related problems and death of the foetus. Further, in addition to these experiments begun at CRI began focusing on identifying genetic changes that lead to tumor progression in endogamous families and in close knit communities such as the Parsis. This further led to the generation of studies that examined the roles of HLA type in the development of breast cancer in Parsi women and also in the development of a program to identify the presence of mutant genes in families/communities where a higher than normal incidence of cancer is observed, such as in families with the syndrome Xeroderma Pigmentosum, families with LiFraumeni syndrome, families where the children suffer from

childhood retinoblastoma and families with a history of breast cancer. These studies ultimately led to the establishment of a one of a kind Cancer Genetics Unit at ACTREC that now serves as a nation-wide nodal centre for the submission of samples for clinical and laboratory genetic services. In addition, the identification of novel mutations in breast cancer families in the BRCA1 tumor suppressor has generated a wonderful opportunity not just to understand the biology of disease progression but also identifies mutations unique to the Indian sub-continent permitting the identification of people who might be at risk for disease, which will permit an improvement in patient care and allow medical professionals to provide advice that might prevent disease onset.

In addition to the studies on cancer, the human genetics division at ICRC and then at CRI also focused on understanding the hereditary basis of other genetic disorders in the Indian sub-continent. The goal of these studies was to catalogue the genetic changes associated with these disorders and also to create a database of the numbers of people affected by these disorders in the city of Mumbai and throughout the rest of India. Some of the problems that were studied were color blindness and the ptc taster phenotype, which are common genetic variants that are present in the population. These genes while not associated with disease, serve as markers that can help track the inheritance of disease associated genes in families. Investigators at ICRC and CRI also studied the genetics of hemolytic anemia, a blood disease that leads to a decrease in the RBC count in affected individuals, β-thallisemia and the relationship between steroid hormone status, sex linked disorders and excessive facial hair growth. Studies such as this in the normal human population are important as they help identify causes for the progression of disease and might identify potential areas of therapeutic intervention. They also allowed the identification of individuals who are at risk and who might benefit from early treatment and care.

Further work at CRI focused on identifying changes in the chromosomes of patients with different cancer types. As a first step, studies were done to analyze the presence of the Philadelphia chromosome in patients suffering from Chronic Myeloid Leukemia (CML). The Philadelphia chromosome is an abnormal chromosome generated when a portion of chromosome 9 is fused to a portion of chromosome 22. This event results in the generation of an aberrant protein known as BCR-ABL. This protein transfers a phosphate group to other proteins and is absolutely required for the progression of the disease. While almost all patients suffering from CML contain the aberrant chromosome, the point at which the fusion occurs might vary resulting in the genesis of proteins with different properties. A polymerase chain reaction (PCR) based approach was used to identify the different types of fusions present in patients suffering from CML to determine whether the type of Philadelphia chromosome present in the individual correlated with the severity of the disease. Similar studies were performed in other leukemias and lymphomas to identify aberrant chromosomes whose appearance correlated with disease progression. The identification of aberrant chromosome fusions that are required for disease progression is important from the point of view of diagnosis as well as determining treatment regimens for the patient suffering from the disease.

With the advent of new technology, the focus at CRI shifted from examining gross changes in chromosome morphology, such as the ones identified above, to identifying smaller changes in chromosome structure (such as small expansions or deletions) using a technique called comparative genomic hybridization (CGH). This technology identified several smaller changes in chromosome structure that were not apparent with earlier technology identifying potential genes

whose gain or loss could be associated with tumor progression. Further, investigators at CRI focused on identifying changes in sequence in genes that are important for tumor progression in multiple tumor types. Several molecular genetic studies were identified in samples from patients suffering from oral cancer. These included the presence of activated oncogenes such as the small GTPase ras which stimulates cell growth and c-myc which activates the expression of genes required for cell division. In addition, it was observed that genes that prevent tumor formation, such as the tumor suppressor p53, were inactivated or deleted in oral cancer. Similar studies established the correlation between infection with oncogenic Human Papillomavirus (HPV) and cervical cancer progression and these studies along with other studies from India served as the basis for the approval of the HPV vaccine trial.

A number of studies from ACTREC have identified single nucleotide polymorphisms (SNPs) that are associated with the incidence of head and neck cancer. Further, the presence of these SNPs was correlated with environmental factors and the habits exhibited by the patients. A number of these SNPs were found in enzymes required for the scavenging of reactive oxygen species and xenobiotic enzymes that are normally required for the excretion of toxic chemicals such as carcinogens. Gain or loss of different xenobiotic enzymes leads to either disease progression or provides protection from disease. These studies when correlated with the habits of the patients provide insights into how genetic factors collaborate with the environment to either promote or inhibit tumor progression. Similar studies on enzymes required for the response to DNA damage suggest that deficiencies in these enzymes render human beings more sensitive to acquiring genetic lesions that can lead to tumor progression. These studies have been expanded to using SNP arrays to identify multiple SNP's in a given tumor and their role in the generation of a tumor.

The "OMICS" era:

The dawn of the new millennium has resulted in the generation of large quantities of data through the application of technology related to achieving a holistic understanding of all the possible alterations that occur during the conversion of a normal cell to a tumor cell. Among the first approaches at ACTREC using these new technologies focused on identifying changes in gene expression in cervical cancer that could serve as prognostic or diagnostic markers or even as targets for therapeutic intervention. Further, similar studies were performed to identify genes whose expression was affected by HER2 and IGFBP2 expression in breast cancer and to identify any potential cross talk between these two important oncogenes. An expression analysis of brain tumors identified expression and then microRNA (miRNA) signatures that are unique to different subtypes and can serve as both prognostic and diagnostic markers. Further, understanding the mechanisms by which these changes in gene expression and miRNA expression result in tumor progression can result in the identification of new targets for therapeutic intervention in tumors.

Investigators at ACTREC have been using next generation sequencing technology to identify the landscape of mutations in both head and neck squamous cell carcinomas and in brain tumors. The goal of these studies is to identify mutations that are responsible for tumor progression and to use this information both to understand the molecular pathways underlying tumor progression and to identify diagnostic/prognostic markers that might indicate the best type of therapy to be used and if possible identify targets for therapeutic intervention. ACTREC is also part of the International Cancer Genome Consortium (ICGC) whose goal is to catalogue the mutation landscape of fifty

most common human tumors and to translate this information into better patient care and diagnostics. ACTREC, in collaboration with NIBMG in West Bengal, is identifying genetic lesions in gingiva buccal carcinomas and the first report of this work was recently published in Nature Medicine. Exome sequencing and SNP arrays have been used to identify copy number variations and single nucleotide polymorphisms (SNPs) associated with cervical cancer progression. These studies have not only identified SNPs that might be unique to the Indian population they have identified changes that might be important for cervical cancer pathogenesis. Identification of SNPs unique to the Indian population is important because it permits the generation of a reference Indian genome/genomes for further studies and provides insight into the vast genetic variation in the population and how this might affect cervical cancer progression.

Most cellular functions are performed by proteins. Therefore, a change in the protein profile of a cell can result in phenotypic changes that result in an increase in tumor progression. Further, as changes in protein levels can be independent of changes in gene expression or gene structure, it is important to identify proteins whose levels are altered in tumor cells as compared to normal cells. This information is especially useful in terms of identifying markers, both in the tumor sample as well as in patient sera, which can be used for early diagnosis of disease or be used as prognostic markers. Further, these discoveries could lead to the identification of novel targets for therapeutic intervention. The science of studying the entire protein complement of a cell or tissue type is called “proteomics”. Proteomics studies have led to the identification of proteins that can be used as prognostic or diagnostic markers in oral cancer. Further, these markers can be used to distinguish between different type of oral tumors based on the site where the tumor originated and this information can be used by clinicians to design the appropriate treatment regimen for patients. Further, the identification of antibodies to these proteins in patient sera could result in the development of an easy immunological assay permitting early diagnosis/prognosis of the disease.

Other investigators at the institute have been using genomic approaches to identify alterations in “protein kinases” in tumors derived from the oral cavity and the lung. A protein kinase transfers a small phosphate group to other proteins in the cell and this serves as either an “on or off” switch for the protein that is being phosphorylated. A number of these kinases are activated by “mutation” in tumors and the mutant form of the kinase is often required for tumor growth. Identifying the kinase that is activated in a particular tumor type allows scientists to design small molecules that will inhibit the expression of these enzymes potentially resulting in death of the tumor cell. Therefore, identifying the kinase that might be active in a tumor might allow a clinician to prescribe the appropriate therapy resulting in specific killing of the tumor cell. In addition to these studies, sequencing analyses in brain tumors has led to the identification of pathways that might contribute to the pathogenesis of these very difficult to treat diseases. Further, these studies will help in the classification of these tumors resulting in better prognosis and diagnosis and hopefully better treatment outcomes.

Carcinogenesis

Carcinogenesis is the process by which a normal cell is converted into a tumor cell. Carcinogens are substances that stimulate the development of tumors in animals or humans. Three major classes of carcinogens exist: 1. Physical (such as radiation) 2. Chemical (such as constituents of tobacco smoke) and 3. Biological (such as viruses). Studying the process of carcinogenesis allows scientists

to understand the molecular events underlying this process, to identify contributing factors to the different stages of this process and finally, to identify potential areas of therapeutic intervention that could be used for the development of therapeutic agents. These studies involve identifying agents in the environment that can serve as carcinogens, identifying genetic markers that contribute to the process of carcinogenesis, studies in cell culture and animal models to demonstrate that the processes identified lead to tumor formation and finally identifying agents that can inhibit this process in the animal.

Most chemical carcinogens are mutagens that alter DNA sequence, either by either inducing changes in the order in which the nitrogen bases are present in the DNA or by cleaving the backbone of the DNA molecule resulting in deletions of DNA or chromosome fusions as described above. A simple bacterial test known as the Ames test is used to identify potential mutagens in the environment and in products such as tobacco. As a large number of the cancers in India are associated with tobacco, scientists at ICRC and later at CRI, focused on identifying the carcinogenic compounds present in the various forms of tobacco used in the Indian subcontinent. As a first step, they attempted to identify the chemical constituents of tobacco smoke (both bidi and cigarette smoke) and test the ability of these different constituents to induce mutations in the Ames test. Similarly, non-smokeless tobacco was subjected to various chemical extraction protocols and the different fractions were analyzed for their ability to serve as mutagens in the Ames test. The results from these experiments determined that all of these products contained possible mutagens that might serve as carcinogens and promote tumor formation.

The studies above were extended to studies in laboratory animals. As a first step the potential carcinogenic compounds were painted on the skin of laboratory animals to see if they developed skin cancer. This was followed by development of the hamster cheek pouch model, in which the compounds are applied to the inner lining of the cheek pouch to develop an animal model that mimicked the process of chewing. These extracts were also combined with lime as most tobacco chewers consume tobacco with lime in the Indian sub-continent. Most of these studies demonstrated that the compounds present in tobacco could induce tumor formation in these animals. Similar experiments were performed with compounds from masheri and other forms of smokeless tobacco and paan masala. It was also demonstrated that in pregnant animals, the embryos were exposed to these carcinogens demonstrating that the consumption of tobacco by pregnant mammals could have deleterious consequences for the progeny. These studies provided the experimental basis for the idea that the consumption of tobacco and tobacco products led to tumor progression, thus validating the epidemiological data described above. Further, these animal models serve as excellent pre-clinical models for the testing of potential anti-cancer drugs and for chemoprevention studies using curcumin, black tea polyphenols and anti-oxidants such as vitamins.

In addition to the studies on tobacco, similar experiments were carried out on other potential carcinogens present in the environment. Primarily experiments were focused on the ability of hydrocarbons to serve as carcinogens, the ability of the drugs Ioniside and Hydrazine Sulphate to induce tumor formation and most importantly on the ability of different food colouring agents to induce tumors in animals. Two commonly used food colouring agents, Metanil yellow and malachite green, were identified as potential carcinogens in an animal model of hepatocellular carcinoma (liver cancer). Further, the establishment of carcinogen mediated tumor development

models in animals at the centre generated a pool of very useful pre-clinical models for drug testing and to study the biology of tumor development in multiple organ types. Examples of this are the DMBA/TPA model for skin cancer and the DEN induced hepatocellular carcinoma model.

To complement the experiments used above, other scientists at ACTREC were engaged in using animal models to study biomarkers of exposure to DNA damage and to identify agents that would prevent tumor progression induced by carcinogens. These experiments involved the induction of tumors in animal models using model carcinogens followed by an experimental analysis to determine whether early damage to DNA could be detected in vivo either directly or using surrogate markers. Further, experiments were performed to identify chemo-preventive agents that could prevent tumor formation in these animals in response to carcinogen treatment. These studies have led to the identification of agents in black tea, notably the black tea polyphenols, which prevent tumor formation upon treatment with carcinogens. Similar studies on curcumin, the active agent in turmeric, have demonstrated that pre-treatment with curcumin inhibits tumor development in response to carcinogen treatment. Further, analyses of the molecular pathways that are activated by these chemo-preventive agents have identified gene products whose increased activity might help to prevent tumor progression. Therefore, these studies have successfully identified compounds, which have the ability to prevent tumor progression when ingested by humans and have identified molecular targets of these compounds whose activity is important for the chemo-preventive effect. Thus, if the chemo-preventive agents identified in these studies are not successful in clinical trials, the proteins identified as being required for these chemo-preventive effects can serve as potential targets for the identification of new compounds that might prevent the occurrence of cancer due to carcinogenic agents present in the environment.

Basic Cancer Biology

Animal model development:

The use of animals as models to study various processes has a long history in the biological sciences and cancer research is no exception. One of the first departments to be established at the ICRC was an animal house that housed several inbred strains of small rodents such as mice, rats and hamsters. These serve as models for the carcinogenesis studies described above and also as ways for people studying basic cancer biology to study the process of tumor development in an animal rather than in cell culture models in a petri dish. The advantages of animal models are that they permit a holistic examination of the process of tumor development. ICRC established breeding pairs for several inbred strains and also developed it's own cancer prone mouse strain - the ICRC mouse model. This model was initially developed as a strain of mice that were susceptible to breast cancer development at ICRC. Later experiments at CRI showed that it could also serve as a model for oesophageal cancer. Further, at CRI, the institute established breeding colonies for immunocompromised mice. This was an important development as this allowed the institute to culture human tumor tissue and cell lines by implanting them into this mouse model, thereby allowing scientists to study the growth of a human tumor in a living animal.

The late 1980's saw the development of the first transgenic animal models for research. Transgenic mice are mice that express a foreign gene that has been introduced into the mouse to examine the role of the gene in regulating cellular physiology. The 1990's saw the development of genetically

modified mice that lacked copies of a given gene. These “knock-out” mice were very useful as they provided genetic model systems to study growth, development and disease progression. One of the first transgenic animals to be generated in India was the Enhancing Factor (EF) expressing transgenic mouse generated at CRI in the late 1990’s. EF stimulates the activity of the EGF receptor and this receptor is often prematurely activated in many tumor types. The transgenic mouse line generated at CRI expressed EF in the basal cells of the skin leading to the acquisition of various phenotypes. Subsequently, work at ACTREC resulted in the development of a novel technique for generating transgenic animals to aid in the generation of animal models that model human tumors both to provide novel biological insights and as a model for drug screening. The development of a small animal imaging facility at ACTREC that employs imaging technology has led to the generation of animal models that incorporate transgenes allowing the visualization of tumor development in live animals. This is important as this means that fewer animals will be sacrificed during the course of these studies and early events in tumor development and metastasis that may not be easy to appreciate on histological examination might be visualized using these models. Recently, ACTREC has evolved a program using the genetically tractable system of the fruit fly, *D. melanogaster*, as a model system to study the role of checkpoint signaling and cell adhesion in tumor development. The advantages of this sort of system are that the pathways observed in the fly are conserved in human cells. Therefore, any information generated in the fly has direct application to human biology. Finally, fly culture is relatively simple and cheap and it is easy to perform large drug screens in the animal to identify potential lead compounds for the treatment of disease.

Cell cycle progression, the response to DNA damage and tumor progression

The process by which eukaryotic cells duplicate their genome and segregate the duplicated genome to the daughter cells is known as the “cell cycle”. Most mammalian cells will withdraw from the cell cycle and undergo a growth arrest that is either temporary, as in the case of certain cell types such as stem cells and lymphocytes, or permanent as in the case of neurons and skeletal muscle. A major feature of most tumor cells is that unlike normal cells, they keep dividing and do not withdraw from the cell cycle. Hence, they become “immortal” and this ability to live forever is often the first step in the establishment of a tumor. Thus, deregulation of cell cycle progression and cell division is one of the first “Hallmarks of Cancer”. Various studies initiated at ICRC and CRI were performed to examine the differences in the growth rate of normal and leukemic cells in culture. Further, the expression of proteins required for cell cycle progression were studied in both primary tumor samples and in cell culture models to determine which of these proteins was required for the increased cell division observed in tumors. The goal of these studies was to identify proteins that could serve as therapeutic targets in human tumors.

The presence of DNA damage in a mammalian cell prevents progression through the cell cycle and cell division allowing the cell to repair the damaged DNA so that mutations in the genome are not inherited by daughter cells. The pathways that inhibit cell division and induce DNA repair in response to DNA damage in a mammalian cell are called “checkpoint” pathways. These pathways are often inactivated in human tumors resulting in the acquisition of mutations leading to tumor progression. As a first step, results from studies on hepatic tumor samples demonstrated that the levels of proteins required for cell division such as the D and E type cyclins were elevated in poorly differentiated hepatocellular carcinoma as compared to normal liver. These studies were extended

to an animal model of hepatic carcinogenesis to examine the mechanisms by which the levels of these proteins increased during tumor progression. Similarly, gene therapy strategies were developed to inhibit the expression of one of the D type cyclins, cyclin D1, in tumor cells. These studies suggested that inhibiting the expression of this protein sensitized cells to commonly used chemo-therapeutics. These results suggest that cyclin D1 expression could serve as a target for therapeutic intervention in tumor cells that have acquired resistance to these commonly used therapeutic strategies.

DNA damage due to the presence of carcinogens in the environment leads to cells inducing a developmental program known as “apoptosis” or programmed cell death. This is a protective mechanism that prevents the inheritance of deleterious mutations that could lead to diseases such as cancer. An analysis of the expression of proteins that either promote or inhibit programmed cell death in oral tumor samples has demonstrated that alterations in the ratios of these proteins might make a tumor cell more or less susceptible to DNA damage, thereby allowing cells to proliferate resulting in the inheritance of mutations by the daughter cells, which might lead to clonal expansion and the development of a tumor. Subsequent work on the role of these protein classes, particularly the survivins and clusterins and their role in regulating tumor progression is being continued at the institute. This work, in conjunction with the work described above, aims to generate a holistic picture of how the presence of carcinogens in the environment in conjunction with the genotype of the individual results in tumor progression.

The levels of proteins required for the DNA damage response are decreased in transformed cells as compared to normal cells when grown in culture. These results suggest that loss of gene products required for the response to DNA damage can lead to tumor progression, suggesting that pathways affecting these functions are inactivated in tumor cells. However, a complete inactivation of these pathways might result in very high levels of DNA damage resulting in the death of tumor cells. To determine if this is the case gene therapy vectors were generated to induce an shRNA mediated knockdown of the checkpoint kinase ATM in tumor cells to determine if inactivation of ATM sensitized tumor cells to DNA damaging agents. Another set of experiments has focused on understanding the mechanisms by which DNA damage prevents entry into mitosis and how these pathways can be exploited for therapeutic intervention. Previous work had suggested two 14-3-3 family members inhibited the activity of the mitotic phosphatase, cdc25C, in response to DNA damage. These results were extended to demonstrate that loss of these two 14-3-3 family members results in a decrease in cell viability in response to DNA damage, which is accompanied by activation of cdc25C. These results suggest that small molecules that disrupt the 14-3-3 cdc25C complex might induce premature mitotic progression in tumor cells resulting in cell death. Further, this might sensitize tumor cells to therapeutic agents that induce DNA damage resulting in lower side effects from treatment with these agents. The role of chromatin organization in regulating resistance to cyotoxic therapies and the DNA damage response have been recently addressed in two laboratories at the center, which will lead to further understanding of these processes and an increase in the number of possible targets for therapeutic intervention.

Chromosome structure and function

Each somatic human cell contains 23 pairs of chromosomes and each chromosome has a protein component and contains DNA, which is the information storage system used by all organisms. The

total length of the DNA molecules present in a human nucleus, when placed end to end, is almost two meters. The packaging of this DNA into the µm volumes of the nucleus is accomplished by wrapping the DNA around positively charged proteins called histones. This combination of DNA and protein is known as “chromatin” and the condensation of chromatin allows the information in DNA to be stored in the nucleus of every human cell. Compact chromatin cannot be converted to RNA unless the chromatin is unpacked allowing access of enzymes required for RNA synthesis to the naked DNA. The mechanism by which this packing and unpacking of DNA, also known as “epigenetic” regulation of gene expression, is regulated is different in each cell type allowing for cell type specific expression of different genes. Each chromosome can contain regions of tightly packed and loosely packed chromatin depending on the epigenetic program being followed in that particular cell. During the process of carcinogenesis, these epigenetic programs are altered leading to differences in gene expression that drive tumor progression.

The first set of experiments to study chromosome structure and function were initiated at ICRC and used light microscopy coupled with dyes that stain DNA to examine chromosome structure in dividing (mitotic) cells. The hypothesis being tested in this case was that as cancer is a genetic disease, alterations in the structure of chromosomes might be responsible for tumor progression. Among the first studies conducted at the centre concerned the identification of the Philadelphia Chromosome in patients suffering from CML. These studies were extended to other leukemias, lymphomas and myelomas. In addition to these studies, the process of biogenesis of histones was studied in animals to understand the mechanisms by which different histones could regulate chromosome structure.

Experiments done at ACTREC have begun to identify changes in the types of histones present at different stages of liver carcinogenesis in rats as well as changes in the way the DNA is packaged in these cells. Interestingly, it appears that as the tumor progresses, the DNA is more loosely packed as opposed to that found in normal cells, suggesting that this could serve as a marker for tumor progression. Further, there are multiple histone genes in human cells and the type of histone in complex with a particular DNA sequence will determine whether the gene is expressed or not. Further, each histone can be part of either an actively expressed region or an inactive region and this depends on the activity of enzymes that add small chemical groups to the histones. These small chemical groups (such as methylation, acetylation and phosphorylation to name a few) serve as molecular switches and determine whether the DNA is expressed or not. Work at ACTREC and other institutes has determined that not only are global epigenetic changes observed during the process of carcinogenesis but that the epigenetic enzymes are often mutated in tumors as compared to normal cells suggesting that changes in the epigenetic program lead to tumor progression.

The role of hormones in tumor development

Tumors arising in organs that are dependent on hormonal stimuli for their growth and development such as the mammary glands, the ovaries and the prostrate gland often exhibit dysfunctional responses to hormones that determine their ability to grow and survive in the body. The first studies that were performed at ICRC were animal experiments that wished to determine the contribution of gonadectomy to breast cancer progression. These experiments demonstrated that in the absence of the ovary, the adrenal gland becomes hyperactive leading to the presence of a constant state of estrus in the animal, which then develop both adrenal and mammary gland tumors. Subsequently,

studies were performed to determine the contribution of carcinogen treatment of the ovary to the development of the fetus. Various birth defects were observed in the progeny. Similarly, carcinogen treatment of mouse embryos resulted in high levels of mortality in the embryos with very few pups surviving to term. The abnormalities observed were also seen in subsequent mating experiments with the female mice that had been injected with the carcinogen suggesting that the presence of the carcinogen resulted in permanent alterations in the ability of the mother to deliver normal pups.

Experiments performed at CRI led to the development of assays to measure the levels of hormones in human tumor samples as well as the circulating hormone levels in breast cancer patients and to determine whether the levels of hormones correlated with tumor progression. In addition to these studies, the levels of steroid hormone receptors in patients with breast cancer were evaluated. Studies of this sort from all over the world have lead to the classification of breast tumors into three clinical subtypes and it has recently been demonstrated that the subtype of the tumor determines the sort of treatment to be provided to the patient. Further, studies were carried out to determine the level of placental hormones secreted by various tumor types and on the role of hormone treatment in promoting/inhibiting breast cancer progression. These were complemented by basic studies on how steroid hormones regulate mammary gland development and how the pituitary and adrenal gland might affect the synthesis of the steroid hormones produced by the ovary. These studies allow researchers to compare the properties of normal cells with those exhibited by tumor cells, allowing them to identify potential sites for therapeutic intervention in the tumor that will not affect normal cells.

Migration, invasion and metastasis of tumor cells

One of the major “hallmarks” of a tumor cell is the ability to migrate out of the primary organ, survive in the bloodstream and colonize other organs and sites. This is true of all cell types in tumors of the lymphoid system and also tumors of epithelial origin. The ability of both types of cells to leave the primary site and enter the blood stream has been investigated at CRI and ACTREC.

Cell migration is a developmental process that is required for tissue organization and for the ability of lymphoid cells to leave the niche in which they are synthesized and enter the blood stream. The actin-based cytoskeleton is required for cell migration in all cell types. The rate of migration of a cell is dependent not only on the ability of the cell to regulate the dynamics of actin filament formation but also on the ability of the cell to bind to proteins present in the Extra-cellular Matrix (ECM). The ECM is made up of a number of molecules secreted by the cells that provide structural support to the cells. For a cell to migrate in a particular direction, the actin filament network provides the motive force while the cell-ECM adhesion provides the traction required for the cell to move forward. The more dynamic the actin movement and the less cell-ECM adhesion, the faster the cells will migrate. Early studies on the ability of poly morpho-nuclear lymphocytes (PMNL) from patients suffering from CML demonstrated that they underwent changes in actin morphology that were consistent with their ability to migrate for larger distances as compared to PMNLs purified from control patients. Similarly, these cells showed a decrease in adhesion to the ECM thus allowing these cells to enter the bloodstream faster than control cells. In addition, other experiments at CRI demonstrated that the leukemic cells secreted proteases resulting in

degradation of certain components in the bone marrow, thereby allowing the tumor cells to enter the circulation. These events are important in the ability of a leukemic cell to enter the blood stream, resulting in disease progression.

Most adult tumors are tumors derived from organs that are epithelial in origin (e.g. breast, cervix, lung, colon, oral mucosa, skin, prostrate etc.). In a number of these cases, the tumor cells migrate from the organ where they originated and colonize a distant organ. This process is known as metastasis and is the leading cause of death in patients suffering from solid tumors. There are several steps in the metastatic process: 1. The tumor cells detach from the neighboring cells (loss of cell-cell adhesion) and degrade the ECM by secreting proteases; 2. The cells invade through the walls of the blood vessels and enter circulation; 3. The tumor cells pass through the walls of blood vessels and invade a distant organ site; 4. The tumor cells colonize the organ forming a metastatic tumor. In addition to metastasis being the major cause of death in patients with solid tumors, metastatic tumor cells acquire resistance to most commonly used therapeutic agents. These two factors make the study of the process of metastasis very important as the understanding of the basic cellular processes contributing to metastasis are vital to the design of novel therapeutic strategies for the treatment of metastatic tumors.

One mechanism by which cells regulate the ability of a protein to perform it's function is by adding control switches to proteins in the form of a covalent addition of a functional group. One such functional group is the addition of either a single sugar to the protein or the addition of a sugar polymer to the protein. A protein modified in this fashion is said to be "glycosylated" and the process is known as "glycosylation". Most glycosylated proteins are present on the cell surface and often mediate cell-cell and cell-ECM interactions. Experiments performed by scientists at CRI and ACTREC demonstrated that a specific sugar polymer (Higher Antennary N-Linked Oligosaccharides) was increased on the cell surface of highly metastatic tumor cells (that metastasize to the lungs) versus tumor cells that did not form metastatic colonies. Decreasing the levels of the sugar on the cell surface by inhibiting the enzymes that are required for addition of the sugars caused a decrease in metastasis. Further, the receptor for these sugar moieties on the surface of lungs was identified as the protein galectin-3 and disrupting the adhesion between the sugar and this molecule leads to a decrease in invasion assays. Further, the presence of these sugars leads to an increase in adhesion to ECM components permitting better invasion and metastasis. These results have identified the role of these sugar moieties in promoting metastasis and may lead to the development of therapeutic strategies that alter cell-surface glycosylation and lead to an inhibition of metastasis.

One of the primary requirements for the formation of a metastatic tumor is the ability to invade and migrate into the surrounding tissue. One cellular event that seems to be associated with this ability is an increase in the level of the keratin pair K8 and K18. Keratins, like actin, are a component of the cytoskeleton and exist as dimers between two types, a basic and an acidic keratin. Specific keratin pairs are expressed in different tissues. Work at CRI demonstrated that the levels of the keratin pair, K8 and K18, were often increased in oral tumor samples, suggesting that keratin levels might serve as diagnostic markers for tumor progression. Later experiments demonstrated that increasing the level of K8 in cells in culture lead to an increase in transformation and tumor formation in nude mice, while the reverse experiment lead to a decrease in migration,

transformation, tumor formation and metastasis. These results suggest that modulating the levels of this keratin pair might serve as a potential strategy for inhibiting tumor growth and metastasis.

Dissolution of cell-cell contacts is required for the initiation of the metastatic cascade. Experiments done at ICRC and CRI, which examined the ultrastructure of tumor samples, had demonstrated that loss of cell-cell adhesion structures such as adherens junctions and the desmosome were a common feature of all tumor types. Subsequent work at ACTREC has focused on the formation of structures such as the desmosome, which mediates attachment between epithelial cells and the hemi-desmosome, which is required for adhesion between the base of the cell and the basement membrane, to understand how these structures form and whether disruption of these structures can lead to tumor formation. Dissolution of desmosome formation leads to an increase in invasion, migration, cellular transformation and an increase in metastasis. The loss of desmosome formation leads to alterations in cellular signal transduction pathways that are often associated with tumor progression and tumor survival. The identification of the pathways by which cells acquire metastatic properties could result in the development of new targets for drugs to inhibit metastatic disease.

Stem cell biology and Cancer Stem Cells

Multiple leukemic tumors arise from hematopoietic stem cells suggesting that these cells have properties that make them permissive for tumor cell development. Therefore, understanding the processes that regulate the development of normal stem cell populations during hematopoiesis is critical in terms of identifying altered processes in tumor cells. Therefore, work in the stem cell biology division at CRI focused on first identifying and purifying stem cells from cord blood, generating a stem cell bank and standardizing the culture of these stem cells to study stem cell development in the laboratory. These were challenging experiments at the time and resulted in the addition of valuable resources and technology at the institute and resulted in the creation of a stem cell bank that could serve as a resource for the institute in the future.

Recent data in the literature suggests that of the many cells in a tumor there are a few who have the ability to give rise to a new tumor. These tumor cells are known as “cancer stem cells” or “cancer initiating cells”. It has been postulated that these cancer stem cells are the ones that acquire resistance to both chemo and radio therapy and give rise to new tumors and metastatic tumors post therapy. Recent work at ACTREC has focused on identifying these cancer stem cells from different tumor types and understanding the mechanisms by which they become resistant to both chemotherapy and radiation therapy. Several investigators at ACTREC are trying to identify and characterize cancer stem cells in tumors of the nervous system, oral cancer and ovarian cancer. Dr. Waghmare’s group is studying the contribution of several cellular pathways that regulate stem cell proliferation and differentiation in the hair follicle in mice. These studies will be extended to stem cells in other tissues and also to cancer stem cells. His group is also attempting to purify cancer stem cells from oral tumors to understand their biology and their role in tumor progression. The groups of Dr. Ray and Dr. S. Dutt are trying to understand how cancer stem cells develop resistance to commonly used chemotherapeutic drugs and radiotherapy in ovarian cancer and leukemias respectively. Identifying the mechanisms by which these cancer stem cells become resistant to these drugs will help clinicians design better therapeutic regimes for cancer patients and will also identify new drug targets for the treatment of chemo and radio resistant tumors.

Biophysical approaches to understanding tumor progression and diagnosis

There are many differences between a normal cell and a tumor cell as well as normal versus tumor tissues that are not observable by conventional light microscopy. These differences can be observed using an electron microscope to identify changes in the shape and size of cells, determine how cells interact with neighboring cells and the ECM and changes in the shape and size of cellular organelles. Early studies initiated at ICRC and CRI were performed on the electron microscope and examined the differences between normal tissues/cells and tumor tissues/cells. These studies revealed that multiple changes were observed at the ultra-structural level such as: 1. Changes in cell-cell adhesion in metastatic tumors as alluded to above; 2. Changes in the architecture of the extra-cellular connective tissue in cases of sub-mucous fibrosis (SMF), which is thought to be a pre-cancerous lesion; 3. Changes in the ultra-structure of cellular organelles such as mitochondria in cells treated with anti-cancer agents; 4. A comparative ultra-structure of human tumors to identify changes in the organization of tumor cells isolated from different organs. The goal of these studies was to identify the changes present in tumor cells and to understand the mechanism underlying these changes and determine the contribution of these changes to tumor progression. As described above, the changes in cell-cell adhesion observed initially in tumors has translated into identifying mechanisms that underlie the conversion of a tumor cell to a metastatic tumor cell. In addition, investigators at ACTREC are currently attempting to identify pathways that regulate organelle size in both budding yeast and human cells to determine how these structures are generated and how alterations in these structures could lead to tumor progression. This is a wonderful example of how research goes from the bedside to the bench and hopefully will result in progress that allows us to go back to patients with a novel approach to treating tumor cells.

The biophysics program has been recently expanded at ACTREC with a number of investigators working in the areas listed below. The older generation of chemotherapeutic drugs mostly functioned by damaging the DNA of the tumor cell resulting in death of the tumor cell. An unfortunate side effect of these drugs was that they damaged normal cells as well leading to side effects that were often as bad for the patient as the disease. The search for chemotherapeutics that were specific to tumor cells resulted in the hypothesis that drugs inhibiting the activity of proteins required for tumor progression would kill tumor cells without damaging normal cells. The design of such drugs requires knowledge of protein structure and function and investigators at ACTREC have used various approaches to approach the goal of “intelligent drug design”, which will target a specific protein required for tumor cell growth. One of the first steps in this regard is the establishment of an X-Ray crystallography facility at ACTREC where investigators are determining structure of proteins involved in breast cancer progression and the response to DNA damage to facilitate structure based drug design. Other investigators are using protein structure and prediction software to identify putative signaling networks altered in tumor cells and performing experiments in the laboratory to determine whether these can serve as targets for therapeutics. An exciting idea emerging from this research is the that rather than the generation of small molecules that bind to the active sites of proteins and inhibit their activity, a better approach to targeting signaling networks in tumor cells might be to disrupt protein-protein interactions in cells as this might better serve to disrupt oncogenic signaling networks. Other experiments have focused on the biochemical and structural characterization of proteins that induced programmed cell death or apoptosis. Drugs that activate these proteins in tumor cells could result in tumor cell

killing and the studies performed at ACTREC will aid in drug design. Finally, ACTREC has been developing non-invasive detection and prognostic techniques based on Raman spectroscopy for the early diagnosis and classification of tumor types.

Cancer Immunology

The role of the immune system is to provide a defense against foreign substances that enter the body including infectious agents. Tumor cells often acquire a morphology or display antigens that are recognized by the immune system as foreign. Consequently, the immune system targets tumors for degradation, thus resulting in tumor cells evolving to avoid detection or killing by the immune system. Current efforts in cancer immunology focus on understanding how tumor cells evade the immune system and how the immune system can be activated to specifically kill tumor cells.

The role played by the immune system in preventing tumor spread was initiated at CRI. Some of the first studies initiated were the study of immune dysfunction in patients suffering from lymphoma and leukemia. These lead to the identification of soluble IL2R as the agent that inhibits the T-cell response in patients with Hodgkin's lymphoma. T cells derived from these patients were not responsive to IL2 or PHA. These studies also demonstrated that decreased NK and antibody mediated cell dependent cytotoxicity and lower levels of PBLs were observed in patients with non-Hodgkins lymphoma. In addition, sera from patients suffering from Hodgkin's disease inhibited the duplication of T-lymphocytes. The types of TNFα receptors present on cells isolated from patients with Non Hodgkins Lymphoma and these studies were extended to the cells role of these receptors in the pathogenesis of Hodgkins lymphoma. These studies identified various immune regulatory mechanisms that were altered in patients with these diseases. To counter this, investigators at CRI attempted to develop cytotoxic T-lymphocytes from the PBLs of NHL patients in vitro as a potential therapeutic strategy for the treatment of disease. A similar strategy was adopted to develop cytotoxic lymphocytes in vitro against CML leukocytes.

In addition to tumors of the hematopoeitic system, investigators at CRI began studying the immune response to various tumor types. These studies included identifying tumor associated antigens in cervical cancer and monitoring the changes in the ability of LAK (Lymphokine Activated Killer) cells from cervical cancer patients to kill the target cell type. Similar studies were initiated in patients suffering from oral tumors that began with classifying the tumor infiltrating lymphocytes present in oral cancer patients, establishing xenografts of oral tumors in nude mice and determining ploidy and their susceptibility to killing by cytotoxic T-cells and studying the activity of Natural Killer cells, NK, LAK cells, Antibody dependent cytotoxicity and Cytotoxic T-lymphocytes in oral tumors. Studies are ongoing to study immune dysfunction in oral cancer patients and to understand the role of TH17 and regulatory T cells in the pathogenesis of gall bladder cancer.

Experiments initiated at CRI aimed at understanding the role of a specific type of T-cell known as the γδ T cells in immune surveillance in tumors. γδ T cells express T-cell receptors that are composed of γ and δ chains. These cells are present at much lower levels in the body as opposed to cells with the αβ receptor, however they seem to be the first cells that defend the body against foreign antigens. The first set of experiments determined that this cell type recognized heat shock proteins present on tumor cells and that this results in the activation of programmed cell death in these T-cells leading to a decrease in immune function in these patients. In addition, the

rearrangement of genes required for formation of the γδ receptor could serve as a prognostic marker in T-ALL. Further mechanistic studies have discovered a role of Notch in regulation of γδ T lymphocytes and regulatory T cell function and additional studies have focused on understanding the role of bisphosphonates and γδ T lymphocytes in patients with breast cancer and bone metastasis.

Therapeutics

The goal of any cancer institute is to understand the process of tumor progression and to identify new ways of treating the disease. The identification of new drugs that inhibit tumor progression is a long and complex process. As a first step at ICRC, scientists began evaluating the ability of plant extracts to inhibit tumor progression in cells in culture as well as in animal models of tumor progression. In addition, they focused on identifying inhibitors of the hexose monophosphate pathway, the major pathway used for glucose metabolism in tumor cells, with the idea that these drugs would specifically kill tumor cells as opposed to normal cells. The chemotherapy section at CRI focused on identifying novel compounds for inhibiting the growth of tumor cells from natural sources such as plants, bacteria and marine biota. In addition, they optimized methods of synthesis for existing anti-cancer drugs such as methotrexate and performed experiments to identify new combinations of drugs that could inhibit tumor progression. The role of metabolic pathways in conferring resistance or sensitivity to anti-cancer drugs were also investigated as this would permit clinicians to use the right drug for the appropriate tumor type/subtype. The B16F10 model was used to identify drugs that could inhibit the metastasis of tumor cells. The establishment of an anti-cancer drug screening facility that served to determine whether potential anti-cancer compounds could kill tumor cells in culture as well as prevent tumor formation in animal models followed the development of this animal model.

In addition to the chemotherapeutic approaches described above, scientists at CRI and ACTREC focused on developing therapeutic strategies that utilized macromolecules as opposed to small compounds. A gene therapy protocol using the Herpes Simplex Virus Thymidine Kinase (HSV-TK) gene was developed at CRI. This enzyme converts a pro-drug known as gancyclovir to a toxic metabolite that kills the cells producing it. The toxic metabolite can also kill neighboring tumor cells that do not harbor the gene leading to a bystander effect. Investigators at CRI cloned the gene for HSV-TK into a retroviral vector, packaged the virus and introduced it into an oral cancer cell line and demonstrated that tumors formed by the cell line in mice could be killed upon administration of gancyclovir. These pre-clinical studies have suggested that these therapeutic strategies might work in patients suffering from oral cancer.

As described above the Philadelphia chromosome produces the BCR-ABL gene product that is required for progression of chronic myeloid leukemia (CML). This gene is required for tumor progression and can be inhibited by the use of tyrosine kinase inhibitors. However, in a small percentage of patients with CML, the tumor cells become resistant to tyrosine kinase inhibitors and disease progression is not halted. Therefore, there is an increasing need for alternative strategies to treat this tumor type. In addition to assaying the ability of tyrosine kinase inhibitors to prevent CML progression, investigators at CRI designed antisense oligonucleotides to inhibit the expression of this protein as an alternative to using the tyrosine kinase inhibitors. Antisense nucleotides bind to the messenger RNA of the gene product and inhibit it’s translation into the

protein resulting in an inactivation of gene expression. Other investigators at ACTREC have developed self-inactivating viral vectors that can be used for the expression of anti-sense RNA's, shRNA's and therapeutic proteins that can be used in different types of tumors.

Viruses and Cancer

Peyton Roux first postulated the link between viral infection and tumor formation in the first half of the twentieth century. Some viruses are important etiologic agents in tumor progression and this section will focus on the work done at the institute to study the contributions of viruses to cancer. One of the first studies done on breast cancer progression in mice at ICRC resulted in the identification of an agent secreted in the milk of cancer prone mice that promoted tumor formation in other mice. Electron microscope studies demonstrated that this agent was a virus called the mouse mammary tumor virus. The investigators purified the virus and showed that it had the ability to induce tumor formation in animals that did not have the virus.

Almost all patients who present at the clinic with cervical cancer in India have a Human Papillomavirus (HPV) infection. There are many different subtypes of HPV (greater than 100 at the last count) and most of them result in the formation of benign structures such as warts. However, some of these subtypes, particularly HPV16, HPV18 and HPV31, are associated with the risk of developing cervical cancer. Much work at CRI and ACTREC has resulted in the identification of HPV subtypes associated with cervical cancer and oropharyngeal cancer and in conjunction with other studies performed in India have demonstrated that the major HPV subtype associated with tumor progression in India is HPV16, followed by HPV18 and HPV31. These studies have helped establish protocols for determining the presence of HPV in tumor biopsies and along with the other studies in India have helped make the case for initiating a vaccine trial for HPV in India. In addition to these studies, investigators at ACTREC have devised a test for antibodies against HPV in the blood which could serve as a potential diagnostic tool for HPV infection resulting in the identification of individuals who might be at risk for developing HPV associated cancer.

Infectious diseases

Leprosy

In addition to the work on cancer biology, scientists at the institute have studied other diseases that are major clinical challengens in the Indian subcontinent. The first of these was leprosy, which before the advent of the vaccine was a significant clinical problem in India. At ICRC, a pathological analysis of leprosy lesions and macules was undertaken as a first step to enable clinicians to identify the different stages of the disease. This was followed by the development of a screening program to identify the different types of leprosy bacilli present in the Indian population. This resulted in the identification of the ICRC strain of the leprosy bacillus. The metabolism and growth properties of the ICRC strain were studied in vitro to enable scientists to identify potential proteins that could serve as targets for small molecules and these were complemented by the establishment of an animal model for leprosy. Finally, at CRI, a vaccine for leprosy based on the ICRC bacillus was developed that entered clinical trials where it showed great efficacy in preventing the onset of the disease.

HIV

The advent of the AIDS epidemic led to the development of a program to identify the strains of the HIV virus present in Indian patients as this sort of epidemiological data is very important in terms of designing public health strategies to prevent disease progression. The first HIV strains were isolated from sero-positive Indian patients in 1992 at CRI. This was followed by performing a molecular characterization of HIV from seropositive patients in Mumbai to determine how many different strains were present in the Indian population. The Virology division was able to identify new HIV strains from India and determine the co-prevalence of HIV1 and HIV2 in the Indian population. Finally, the virology department was able to develop an indigenous WB kit for the detection of HIV1 and HIV2 based on the clinical isolates they had cultured in the laboratory. These kits have been licensed to a company and are available in the market.

EDUCATIONAL PROGRAMMES AND DEVELOPMENT OF FACILITIES

ICRC was first recognized as a center for the education of MSc and PhD students by Mumbai University in the 1950's. In addition to the graduate program, the Masters lectures for the Biophysics course at Mumbai University were conducted at the center until the 1980's. After moving to ACTREC, the PhD program expanded and stayed with Mumbai University until 2005 at which point TMC became part of the DAE deemed university, the Homi Bhabha National Institute (HBNI). The first batch of HBNI PhD students began at ACTREC in 2006.

CRI and ACTREC have always had a comprehensive cancer biology course that covers various areas of cancer biology for the PhD students. After joining HBNI, this has been refined further and the course now consists of a common core course that is taken by all students and electives that are offered to students at ACTREC. In addition, students from other constituent institutes of HBNI also attend some parts of the core course and some electives held at ACTREC. The goal of the ACTREC PhD coursework is to train students to critically evaluate the scientific literature so that they can learn how to think about science and plan their own experiments based on the findings in the literature. In addition, to these efforts, ACTREC conducts multiple conferences and workshops every year to further education in various aspects of cancer biology. Finally, in addition to the PhD program, ACTREC conducts training programs for scientific assistants and technicians that allow them to learn new techniques and improve their skills at the bench resulting in the generation of trained manpower at the institute and other institutes in the country.

VISION AND IDEAS FOR THE FUTURE

As the cancer burden in India is increasing, research at the Tata Memorial Centre will focus on translating the findings of basic biology into better diagnostic and prognostic tools and identifying the best therapeutic strategy for tumor types common to the Indian sub-continent. As a first step, the molecular alterations in tumors are being catalogued be they changes in DNA sequence leading to the acquisition of pathogenic mutations, changes in epigenetic patterns in tumor samples, changes in miRNA levels in human tumors or changes in the levels of proteins and modifications of proteins, all of which contribute to tumor progression. A holistic understanding of the changes

present in a given tumor type will contribute to better understanding of the tumor cell and hopefully result in better strategies for the management of neoplastic disease. In addition, scientists at the institute are increasingly focusing on developing animal models that mimic human tumors. These models allow scientists to study tumor progression in an animal, including the interaction of the tumor with immune cells, which is important for tumor survival as discussed above. Further, these models can be used to test new therapeutic strategies and new anti-cancer agents before these are tested in a phase one clinical trial. Finally, the institute hopes to educate and produce the next generation of cancer researchers so that the fight against cancer can continue until the battle is won.