Division of

Cancer Biology and Diagnosis

1986 Annual Report
Volume II

October 1, 1985-
September 30, 1986

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

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DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

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EXTRAMURAL RESEARCH PROGRAM ANALYSIS

Analysis of the DCBD Extramural Research Program includes both grants and contracts. The use of the research contract has been completely eliminated except for one contract in the Cancer Diagnosis Program which is a long-term study to evaluate the use of the hemoccult test as a screen for the presence of colon cancer. The current extramural contract portfolio of the Division is small, and is focused mostly on biological resources important to both basic and clinical cancer research. The major emphasis of the Division favors investigator-initiated grant mechanisms, which include the Traditional Research Grant (R01), the New Investigator Grant (R23), the Conference Grant (R13), the Program Project Grant (P01), Small Business Innovative Research (SBIR) Grants (R43 and R44), the Academic Enhancement Research Award (R15) and the Outstanding Investigator Grant (OIG or R35). Two new award mechanisms have been added to the portfolio of the NCI as well as throughout the NIH. The FIRST Award or R29 is intended to provide new investigators with more substantial funding than the R23 above, as well as more time and greater budget flexibility from year to year. It is intended to replace the R23 completely in the next few years. In addition, the first MERIT Awards or R37s have been implemented by DCBD. They are intended to provide greater budget flexibility from year to year, and up to ten years of support without additional peer review. The major difference between the R37 and the R35 is that the R37 is applied to a specific research proposal rather than to the consolidation of all cancer-related research under one funding instrument. The Division continues to achieve most of its program objectives using the mix of investigator-initiated grant mechanisms noted above, since the two largest program areas of Tumor Biology and Immunology focus strictly on basic research where progress is completely dependent upon the creative ideas of individual scientists. The smaller program area of Cancer Diagnosis, however, uses both investigator-initiated grant mechanisms and program initiatives in the form of RFAs to stimulate studies that link basic research findings in Tumor Biology and Immunology to the clinic. In addition, the Cancer Diagnosis Program places greater emphasis on the use of SBIR grants, which are intended to promote basic information into the commercial arena.

The budget of the Division reflects a number of trends. The Immunology Program has been relatively stable both in the total budget as well as in the dollars devoted to more specific scientific areas of research. The Diagnosis Program continues to grow largely because of its greater use of program initiatives and a sympathetic response of the NCI Executive Committee in approving important grant applications for funding which are outside priority score paylines. The Tumor Biology (TB) Program continues to grow at an unexplainably rapid rate, especially considering the fact that many non-competing and competing grants have been cut below recommended budget levels in order to meet the legislative restrictions and limits set by Congress on the NCI's total budget and on the number of grants which must be funded. The TB Program grew nearly 12% over the previous year. At the current rate of funding of approved grants, grant portfolios should decrease for all programs unless the number of investigator-initiated applications submitted for review are increasing at a much faster rate than in previous years. This appears to be the case for Tumor Biology which is the largest program in the NCI by a considerable margin. Overall, the research proposals from the biomedical community continue to be increasingly creative and innovative, the science supported by the Division is of the highest quality and the rate of discovery is progressing rapidly and effectively as new technologies such as DNA recombinant biology, monoclonal antibody technology, computer technology and anti-sense RNA technology are used more widely.

Description and Introduction

The Tumor Biology Program supports a broad spectrum of basic biological research to determine the cellular and molecular factors which distinguish cancer cells from normal healthy cells and tissues and which facilitate progression of cancer cells to stages of greater malignacy. The supposition is that knowledge of these properties and processes will help us learn how to manipulate or reverse the biological signals responsible for the aberrant behavior of cancer cells. Ultimately, this should result in more effective methods for the diagnosis, treatment and management of cancer victims.

Within the Tumor Biology Program, there are three major areas and one minor area of investigation, all of which correspond to different theories of how to control the development and progression of neoplastic disease. The first is understanding the basic biochemical, molecular and genetic mechanisms involved in growth control; whether these involve particular extracellular signals which initiate the process of cell division or intracellular signals which more directly control DNA replication and metabolism. Oncogene studies are the hallmark of research on growth control and it is from these kinds of inquiries that we can expect progress in the development of new, more specific hormonal and drug therapies. The second is studying molecular and genetic factors which are responsible for the development of tumor cell heterogeneity. Although it is clear that most cancers begin from a single aberrant cell, it is equally clear that nearly all well developed tumors contain heterogeneous populations of cells which vary not only in their phenotypic and genotypic properties but more importantly in their resistance to different therapies. Learning to control or inhibit the further diversification of tumor cell populations may be the significant advance which is required to increase the effectiveness of different therapeutic regimens and to extend the period of patient survival without recurrence of neoplastic disease. The third is studying the changes that occur at the molecular and cellular levels which lead to cancer cell invasion and metastasis. A prerequisite to malignancy is the invasive behavior of cancer cells, or the ability of tumors to invade surrounding tissues, escape normal host defense mechanisms and become established at multiple secondary metastatic sites of growth. Theoretically, if the invasive properties of malignant tumors can be controlled and these tumors confined to particular sites, metastasis, the major killer in cancer patients, can be prevented. Treatment of tumors confined to a single site has the highest probability of success. A fourth but minor objective is to develop detailed biological and biochemical information about the processes which induce cancer cell differentiation. Although it is clear that most cancers represent permanent genetic changes of normal cells, there are a few cancers which appear to respond to external stimuli by undergoing differentiation. If the genetic program of an actively growing cancer could be changed to one of terminal differentiation, then the malignant tumor could be rendered harmless. Although the above emphases of the the Tumor Biology Program in the areas of growth, cell heterogeneity, invasion and metastasis and differentiation are stated in simple terms, they provide a purposeful way of viewing the role of basic biological research in the ultimate goal of curing cancer.

The kinds of information developed in the Tumor Biology Program provide a foundation for and relate directly or indirectly to nearly every other program area within the National Cancer Institute. The importance of basic tumor biology research to the National Cancer Plan is reflected by the large $89 million commitment of the NCI to this program area in FY 1986. (See Budget Table.) Complete listings and summaries of all grants supported by the Tumor Biology Program are included in the attached Appendices.

Scientific progress in the area of tumor biology is developing at a remarkable pace. There is no doubt that DNA recombinant technology continues to be the centerpiece of basic cancer biology research. This technology combined with other methods for introducing foreign genes into cells, especially by transfection and microinjection, has introduced experimental approaches which are yielding insightful results explaining the nature of the changes in normal cells which lead to uncontrolled growth and spread of cancer cells. In addition, newer technologies involving transgenic mice and anti-sense messenger RNA are likely to yield even more powerful experimental approaches for delving into previously intransigent areas of cancer research. The following report selectively reviews areas in which progress has been exceptional and in which there is considerable promise for future research.

Regulation of Growth in Normal and Tumor Cells

The composition of the environment surrounding normal cells has a profound influence on their rate of growth and ability to survive. Tumor cells, in contrast, are frequently oblivious to control by external influences and molecules. It is now clear that this control is imposed by a series of intracellular signaling mechanisms that mediate the action of specific endocrine hormones or growth factors and that expression of defects in the control of cellular proliferation is required for carcinogenesis. Unlike the quick responses seen when cells are induced to differentiate by specific agents, the time lapse between exposure to mitogenic agents and cell proliferation requires up to 24 hours, thus it is necessity to consider a number of second messenger systems transmitting information through various terminals. At present three known effector systems seem to be implicated, 1) cyclic nucleotide metabolism, 2) tyrosine kinase activity and 3) the metabolism of phosphoinositides. All three systems employ the activation of protein kinases and thus may be components of a complex biological system acting in series or in parallel. The evidence for phosphorylation as a major mechanism in the biology of cell regulation is very clear. The widely distributed cAMP-dependent, serine/threonine protein kinases have been described in many reports over the years but are not included as a discussion topic here. During the past year more attention has focused on the tyrosine kinase and the phosphoinositol pathways as they occupy a prominent portion of

The pp60^{src} Kinase

When chicken embryo fibroblasts become transformed by Rous sarcoma virus (abbreviated RSV) they undergo morphological, biochemical and physiological alterations that together are called the "transformed phenotype." As many as 4% of the cell's proteins are quantitatively or qualitatively altered by RSV transformation (Weber, 1984). All the known manifestations of transformation are dependent on the expression of a single gene called the "src" gene and the only protein known so far to be coded by this gene is a

60,000 dalton polypeptide known as "pp60^{src}", which acts as a tyrosine-specific protein kinase (Brugge and Erikson, 1977). Eventually other proteins may be implicated here, but for now the focus is this enzyme, pp60^{v-src} (for the viral enzyme), and how it causes transformation. RSV is currently the best understood oncogenic agent with respect to both the molecular and cellular biology of malignant transformation and the current approaches are aimed at understanding the mechanism whereby phosphorylation of tyrosine in cellular proteins can induce this transformation. Unfortunately this process has not yet provided the key to growth control as had been hoped.

The first step has quite naturally been to try to identify the proteins that act as kinase substrates in the virus infected cell. pp60^{v-src} easily phosphorylates a large number of substrates *in vitro*, many of which are likely not relevant to transformation. Within the cell, the first substrate identified was pp60^{v-src} itself, phosphorylated at tyrosine in the carboxy-terminal half of the enzyme where the active site is located, and three sites on the amino-terminal side. Evidence indicates that this occurs by autophosphorylation since intrinsic kinase activity of this class of enzymes is a widespread feature. A serine in the amino-terminal end of pp60^{v-src} is also phosphorylated via another kinase system. A large number of other cellular proteins have been identified as tyrosine kinase substrates, including vinculin, a cytoskeletal protein component of the sites of attachment for cells with their substratum. A major cellular protein of 36,000 molecular weight (called the 36K protein as is the custom for proteins with unknown function), is one of the predominant phosphotyrosine-containing proteins in RSV-transformed cells although it also contains phosphoserine. Unfortunately no physiological function has yet been identified for this protein, however its localization to the terminal web structure underlying the cell's microvilli suggests a structural function in membrane organization (Hunter and Cooper, 1984). Some recent information suggests that 36K may be a member of a family of calcium-binding proteins (Gordon, 1986). Among several other apparent substrates of pp60^{v-src} are enolase, phosphoglycerate mutase and lactate dehydrogenase. These three glycolytic enzymes are not thought to be important in the regulation of glycolysis so the physiological significance of their tyrosine phosphorylation is unknown. Two other proteins of 50,000 and 81,000 molecular weight that complex with the viral *src* protein are also its substrates. It is important to emphasize that no change in the function of any protein as a result of tyrosine phosphorylation has been demonstrated.

pp60^{v-src} binds to the membranes of RSV-infected cells with great strength, characteristic of an integral membrane protein. It has been demonstrated that this attachment is facilitated by covalent binding of the fourteen carbon fatty acid, myristic acid, to the amino-terminal glycine residue of the kinase. Mutations in the *src* gene which eliminate this glycine abolish the myristylation and eliminate the membrane binding; the *src* protein can not induce morphological transformation of fibroblasts when no longer bound to the membrane. On the other hand, all the substrates listed above were shown to be extensively phosphorylated (Kamps el al., 1986). This leads to the intriguing conclusion that transforming activity requires myristylation but tyrosine kinase activity does not. It would also seem to suggest that the *src*-kinase substrate responsible for transformation must be membrane bound. In one further step, Soric and Gordon (1985), demonstrated that one of the major *src* substrates, the 36K, is itself myristylated in normal cells, but in RSV-infected transformed cells about half the 36K molecules are without the

myristic acid attachment. Since these fatty acyl groups act as lipophilic handles to anchor proteins in their proper membrane location, movement of both the kinase and its substrates may also be involved in the complicated regulation of transformation.

Attention has recently been focused on the 40S ribosomal protein called S6. Following expression of the src gene in RSV-transformed cells, phosphorylation of S6 increases towards maximum levels apparently through the increased activity of an S6 kinase; however, at least in vitro, this phosphorylation occurs solely at serine residues. Phosphorylation of S6 previously has been suggested to play a role in cell proliferation, moving quiescent cells into the cell cycle. The possibility thus exists that S6 kinase activity is altered by tyrosine phosphorylation, suggesting one direct mechanism of influencing cell growth (Blenis and Erikson, 1986).

Several years ago there was much interest in the ability of $pp60^{src}$ to phosphorylate inositol lipids. In the complex pathway involved, phosphoinositol (PI) is phosphorylated to phosphatidylinositol-4-phosphate (PIP), then to phosphatidylinositol-4,5-diphosphate (PIP_2) and then the critical step occurs -- the hydrolysis of PIP_2 to diacylglycerol (DAG) and inositol triphosphate (IP_3) by phospholipase C. IP_3 mediates the mobilization of calcium from intracellular stores, an action which might disrupt normal growth control circuits. DAG is a well known and potent activator of protein kinase C as described in a later section of this report. $pp60^{src}$ was initially implicated in the phosphorylation of PI and PIP in vitro, however it now is clear that other cellular enzymes are responsible for PI and PIP kinase activity in RSV-transformed chick fibroblasts and $pp60^{src}$ itself is not the phosphorylating enzyme. However, the src kinase activity must somehow induce an increase in these relevant lipid kinases (Sugimoto and Erikson, 1986).

The normal cellular protein counterpart to v-src is c-src, present in low levels (1000 to 10,000 molecules per cell) in uninfected chick fibroblasts and a wide variety of mammalian cells including high concentrations in neurons and platelets. It is nearly identical to the viral src protein. This tyrosine protein kinase does not transform cells, further, overproduction of $pp60^{c-src}$ does not result in transformation. The enzyme protein undergoes self-phosphorylation at only one major site, a carboxy-terminal tyrosine that is missing from $pp60^{v-src}$ (Resh and Erickson, 1985). The specific activity of the $pp60^{c-src}$ as a kinase is vastly reduced compared to the viral kinase, for instance the major substrate 36K has neither detectable phosphotyrosine nor phosphoserine in normal cells. The relationship of phosphorylation with activity is unknown. It was also recently shown that single point mutations in the c-src protein, of glutamate to glycine at position 378, or isoleucine to phenylalanine at position 441 turned c-src into a highly transforming enzyme with much higher levels of tyrosine kinase activity (Levy et al., 1986). Such information seems to further confuse the picture of the distinction between v-src and c-src.

The EGF Receptor

Epidermal growth factor (EGF), is the best characterized growth regulator of epithelial cell populations at both the cellular and the sub-cellular levels. In pursuit of answers to the question - what is the mechanism by which a growth factor is able to regulate cell proliferation? - much effort has been spent

studying all aspects of EGF, its cellular receptor, intracellular processing of the EGF:receptor complex and associated influences and responses.

It was demonstrated years ago (Ushiro and Cohen, 1980) that when EGF was added to a membrane preparation from a specific cell line rich in EGF receptors, cyclic nucleotide-independent protein kinase activity specific for tyrosine residues was dramatically increased. These cells, the A-431 epidermoid carcinoma cell line, were used to isolate and characterize the 170,000 dalton receptor which is a tyrosine specific protein kinase and cloning of the EGF receptor was completed in 1984 (Ullrich et al., 1984). A high level of homology has been demonstrated between the sequence of this human EGF receptor and the product of the transforming gene of the avian erythroblastosis virus, the oncogene called v-*erb*. Despite the obvious interest in the relationship of the EGF receptor and oncogene protein products, so far it has not provided any special clues to how growth factors modulate growth. The signals in this process are presumed to begin with the stimulation of the tyrosine kinase activity and the phosphorylation of physiological substrates, relevant to mitogenesis. However these have been difficult to find.

As is seen with *src*-kinase, EGF receptor in A-431 cells autophosphorylates several of its own tyrosyl residues following the binding of EGF. In normal human fibroblasts, EGF-treatment causes the appearance of only a small amount of phosphotyrosine (Decker, 1985). The *in vivo* phosphorylation of the EGF-receptor is of a complex nature. As is described in the next section, the receptor is also a substrate for the serine/threonine-specific protein kinase called kinase C. In fact, phosphorylation of threonine at position 654 inhibits EGF binding to its receptor and the subsequent responses imposing a negative control step in the system (Lin et al., 1985). Other substrates of the receptor tyrosine kinase observed in the A-431 cells include proteins of molecular weight 81,000 and 42,000, as well as the ubiquitous 36K protein best known as the *src*-kinase substrate described above. Normal cells, stimulated with EGF in contrast, do not show increased phosphorylation of 36K. One prominent substrate of intense current interest and potential importance is a 35,000 dalton protein (called 35K), the tyrosine phosphorylation of which is totally dependent on EGF. This protein is different than the 36K just mentioned but it too contains both phosphoserine and phosphotyrosine. 35K was first purified from A-431 cells in 1985 (Sawyer and Cohen, 1985). A short time later the cloning and partial sequencing from human cells of a 37 thousand dalton protein (37) with activity as a phospholipase A_2 inhibitor, called lipocortin, was reported (Wallner et al., 1986). Recent information now suggests that 35K and lipocortin are the *same* protein (Pepinsky and Sinclair, 1986). Lipocortin is an anti-inflammatory agent whose action mimics that of steroids; by inhibiting phospholipase A_2 it prevents biosynthesis of prostaglandins and leukotrienes. There is also evidence that steroids induce the production of lipocortin. The relevance of a major substrate of EGF-receptor kinase functioning as a phospholipase inhibitor remains to be established.

As with the *src*-kinase system, much interest has been focused on the stimulation of the phosphoinositol (PI) pathway by EGF action as one mechanism to mediate the hormone effect. Again, this involves the phosphorylation of PI and the generation of diacylglycerol (DAG) and inosital triphosphate (IP_3). DAG is well known as a potent activator of protein kinase C and IP_3 as an inducer of calcium release (as described earlier). This lipid phosphorylation step is likely not the result of activity of the EGF-receptor kinase but of activation of an associated phospholipid kinase.

Much less detailed information is currently available but evidence indicates that the receptor for platelet-derived growth factor and for insulin are also tyrosine kinases (Daniel et al., 1985). This further suggests the importance of continuing research on all these systems to add new pieces to this intriguing puzzle that suggests a common mechanism for growth control.

The Phosphoinositide Pathway

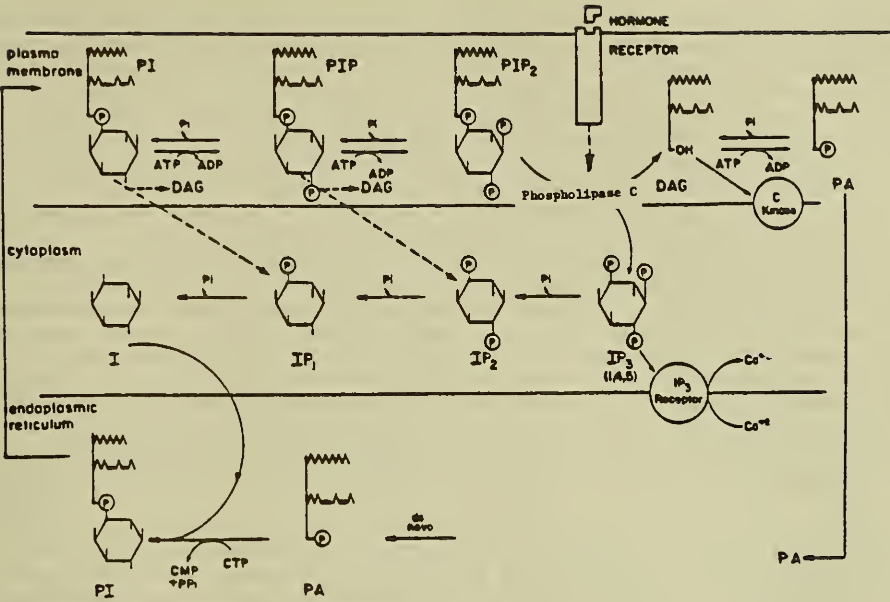
This pathway has been the focus of considerable attention the past several years as the evidence mounted that a large number and variety of hormones activated the system and that a key step in the pathway generates two molecules both of which have potential as intracellular messengers. They are diacylglycerol (DAG) and inositol triphosphate (IP_3). This pathway is unique in the way it is anchored within the cell membrane, both substrates and enzymes being integrated membrane proteins. DAG, once regarded as just an ordinary intermediate of glycerolipid synthesis and degradation, is a potent activator of the important enzyme protein kinase C. This novel phospholipid and calcium-dependent, cAMP independent serine/threonine kinase was first described and named by Nishizuka and co-workers in 1977 (Nishizuka, 1983).

Activation of kinase C by DAG, a natural and transient activator, or by the structurally related phorbol diesters, well-known as tumor promoters, causes the phosphorylation of a variety of proteins and, depending on the cell type, elicits pleiotropic effects on differentiation, secretion, cell growth and oncogenesis (Vandenbark and Niedel, 1984; Bell, 1986). Protein kinase C has been demonstrated to be the cell receptor for the phorbol diesters and DAG interacts with the enzyme at the same site. Activity of the enzyme requires formation of a highly ordered complex of monomeric kinase C, phospholipid, DAG and calcium and each cofactor modulates the others. In A-431 cells, phorbol diester treatment increases both serine and threonine phosphorylation of the EGF receptor which attenuates its activity and has been suggested as a feedback mechanism to modulate the system (Hunter, 1984). This phosphorylation is presumed to be caused directly by kinase C.

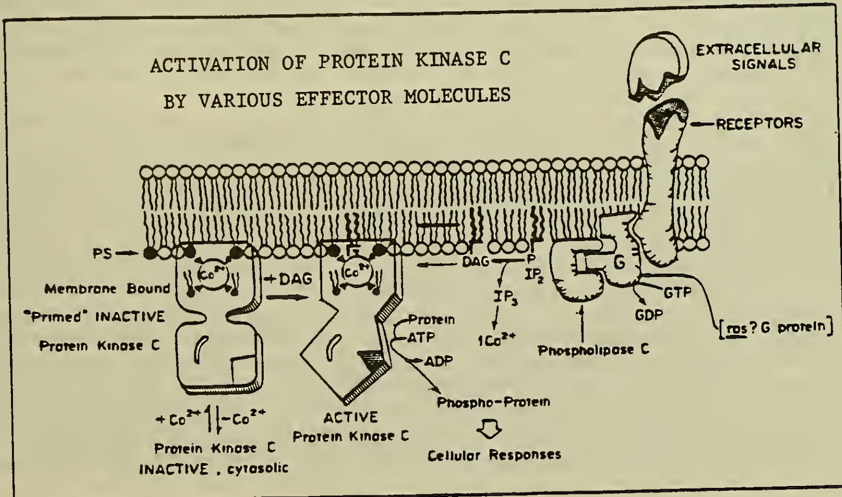
Inositol triphosphate (IP_3), the other potential intracellular messenger, has been briefly described earlier in this report. It is released into the cell's cytoplasm and there functions to elevate intracellular calcium which also plays a role in regulation of cell growth.

The enzyme which catalyzes the cleavage of PIP_2 into DAG and IP_3 is also the subject of renewed interest among researchers because it is a critical point in this pathway and also subject to control mechanisms. The enzyme is phospholipase C. Since it can hydrolyze all three phosphoinositides, its activity toward PIP_2 is dependent on the accessibility to that substrate as well as the prevailing level of calcium. To make things even more complex, some models propose that guanine nucleotide triphosphate (GTP) binding proteins, called G proteins, transmit transmembrane signals from hormone receptors that are responsible for activiting phospholipase C (Cockcroft and Gomperts, 1985). The protein products of the ras oncogene family are GTP binding proteins and may someday be implicated in this maze of regulatory pathways. (See the next Section.)

THE PHOSPHOINOSITIDE PATHWAY



Phospholipase C activity generates DAG and IP_3.



Oncogenes and Cancer

In the past several years nearly 40 distinct oncogenes of viral and cellular origin have been identified. Thirty of these have originated from the normal eukaryotic cell genome and ten of these are found exclusively in the genomes of DNA viruses, but all oncogenes can be classified into general groups on the basis of cellular location and biological activity (Weinberg, 1985; Bishop, 1985; Pimentel, 1985; see Table from 1985 Annual Report). The most encouraging aspects of oncogene research are that these genes are limited in number (i.e., few new oncogenes have been discovered in the last year) and that of the known biological activities of oncogenes, they would be expected to exert significant influences on growth control. Furthermore, the general groupings of oncogenes suggest a small number of mechanisms of action for oncogene-encoded proteins. Only one oncogene has been associated with a growth factor activity (i.e., c-sis which codes for PDGF); there are a large number which appear to be membrane receptors for growth factors (e.g., c-erbB, c-neu, c-fms), a large number which are membrane-associated and exhibit tyrosine specific phosphorylating activity (e.g., c-src, c-abl) and a large number which appear to be in the nucleus and may modulate the activity of the cell's transcriptional machinery (e.g., c-mos, c-myb, c-myc, c-fos). An oncogene of ubiquitous significance is c-ras, but it appears to be in a class of its own and the search for a biological function for c-ras has not yet been successful. The most major distinction between the v-onc's of retroviruses and c-onc's from which they were derived is their size; v-onc's contain no introns or intervening sequences. The expression of different oncogenes depends on the tissue, and stage of development and differentiation. When the transcriptional activity of different oncogenes is measured in normal human tissues, some oncogenes are almost universally active in different cells and different stages of differentiation (e.g., c-abl, c-mys, c-ras), some are active only in certain cells at certain stages of differentiation (e.g., c-myb), and some are apparently inactive in most cells (e.g., c-fes, c-sis). The universal presence of oncogenes in vertebrates and probably all metazoa, as well as their high conservation in evolution, indicates that their protein products are of immense biological importance. Four basic types of mechanisms may be postulated which upset the delicate balance of expression of these important genes in human cancer: increased transcriptional activity of oncogenes, amplification of oncogenes, translocation of oncogenes, and mutation of oncogenes. The next major stage of oncogene research will concentrate on the mechanisms that disturb oncogene expression and the biological activities and cellular sites of action of oncogene-encoded proteins.

Last year the discovery of genes in yeast with sequence homology to the ras oncogenes raised considerable hope that the sophisticated genetic technology available for analysis of yeast physiology could be marshalled to understand the role of the ras gene in mammalian tumorigenesis. It was found that in yeast genes homologous to c-ras were involved in stimulating adenylate cyclase causing a rise in cyclic AMP concentrations which in turn stimulated cell growth. Since c-AMP has long been known as a key "second messenger" in mammalian cells which influence a number of metabolic processes, a role was postulated for c-ras which involved it in controlling c-AMP metabolism. Unfortunately, it is clear from recent experiments with the protein p21, the protein product of c-ras, that the c-ras gene in mammalian cells does not inhibit or stimulate adenylate cyclase (Bourne, 1985; Bechner et al., 1985). Thus, the original question of the function of mammalian ras continues to remain unanswered. The known biological activities of ras p21 proteins include GTP binding and GTPase activities. In

fact, last year several laboratories reported that mutations leading to ras transforming ability correlated well with decreased GTPase activities. However, new results with a different mutation clearly show that the efficient transforming properties of ras occur by mechanisms not involving reduced in vitro GTPase activity (Lacal et al., 1986). The idea that mutations in ras are necessary to activate transforming activity has been open to considerable controversy because the majority of spontaneous tumors do not carry the mutations (Duesberg, 1985) and because there are results which clearly show that unmutated ras can transform 3T3 cells. Another hypothesis is that ras genes are activated by truncation of the 5'exon (Cichutek and Duesberg, 1986). The newest results in the search for a function for ras may be inching closer to its real role in cell proliferation. Transformation by three growth factor receptor-like oncogenes depends on c-ras proteins, while transformation by two cytoplasmic oncogenes appears to be independent of c-ras protein (Smith et al., 1986), implying that c-ras proteins are somehow involved in the transfer of proliferation signals from cell surface growth factor receptors to the cytoplasm. Also, it has been suggested that the ras p21 protein may act as a regulatory element in intermediates of phosphatidylinosital turnover cycle which can act as a second messenger to mediate diverse responses in cells (Fleishman et al., 1986). Now that it has been shown that the original model proposing that these intermediates are directly regulated by oncogenes coding for tyrosine kinases is no longer viable a new model involving the same intermediates has been revived with c-ras as a important regulatory element.

The normal expectation is that, because v-onc genes impose a mitotic response on virally infected cells, c-onc genes from which they were derived also encode mitogenic signals. This does not appear to be the situation for c-src, the first protooncogene to be discovered. It has been clearly shown that the c-src pp60 protein is highly elevated in both neurones and astrocytes (Brugge et al., 1985), both of which are highly differentiated, non-dividing cell types. The levels of pp60 activity in neurons are augmented even further by an unknown post-translational modification. Past experiments have shown that c-src alone is unable to transform cells to neoplastic growth, regardless of its level of expression. Thus, there is a tremendous discrepancy between the v-src protein product, which has the ability to transform cells and cause tumors, compared to the c-src gene product which cannot do either. One hypothesis is that there are differences in v-src which confer upon it a novel but abnormal substrate specificity.

The idea that changes in substrate specificty are involved with the transforming activity of cellular oncogenes has been implied by two separate observations demonstrating that some transforming gene products represent hybrid proteins. In one case the presence of fused abl and bcr gene transcripts was observed in chronic myelogenous leukemia (CML) (Shtivelman et al., 1985) and in a second case a new oncogene in human colon carcinoma was discovered which contains gene sequences of both tropomyosin and an unknown tyrosine protein kinase (Martin-Zanca et al., 1986). The first situation involves one of the most well-known chromosome translocations specifically associated with a particular type of cancer, the 9:22 translocation which produces the Phildadelphia chromosome in CML. The hybrid transcript is referred to as bcr-abl, the bcr standing for the breakpoint cluster region on chromosome 22 within which all chromosome 22 breakpoints are found, c-abl being located on chromosome 9. The bcr region represents a 5.8-kb region but, on the other hand, the chromosome 9 breakpoints are scattered over a wide 50-kb region. Although the primary

bcr-abl transcripts from each CML patient can differ widely in size, in the final processed transcript the abl segment always begins at precisely the same nucleotide. Thus in CML, the abl protein product has an N-terminal bcr substitution. Interestingly, the v-abl product also differs from the normal c-abl by an N-terminal substitution. It seems likely that the substrate specificity of c-abl is altered by the replacement of the N-terminus by bcr sequences and that phosphorylation of the wrong substrate leads to transformation. The second situation which involves gene sequences from tropomyosin and a protein tyrosine kinase can be interpreted in the same way. In this case the new oncogene, onc-D, might become transforming because the tropomyosin part of the hybrid protein directs the phosphorylation activity to a new location, again allowing phosphorylation of an illegitimate substrate. This second case is interesting because it implies that translocation and/or rearrangements of chromosomes occur in solid tumors to produce cancer, possibly in the same way as they do in leukemias and lymphomas. These new findings raise the important question of whether hybrid proteins as the products of oncogenes are more commonly involved in the initiation of human cancers than originally believed.

Of the major mechanisms cited earlier which can alter the action of oncogenes and result in cell transformation, only translocation has been consistently associated with specific human neoplasms. The 9:22 translocation involving c-abl is clearly associated with CML and translocations involving c-myc oncogene are regularly associated with B-cell lymphomas. It has not been possible to demonstrate any consistent, predictable relationship between loss of growth control and increased oncogene transcription or mutation in primary human tumors. If the results with N-myc can be generalized, they would suggest that gene amplification and increased expression of cellular oncogenes is involved more in tumor progression rather than initiation. Translocation can either produce hybrid proteins with different substrate specificities or result in the expression of a normal cellular oncogene at the wrong time and place as it comes under the influence of different enhancer and promotor controlling DNA sequences. Much more information is required before any suitable models explaining oncogene activation and action can be formulated.

Genetic Suppression of Tumor Formation

Since the early 1970's, when methods were developed for preparing reproducible fusions between mammalian cells in culture, the hypothesis that tumor-inducing genes existed was pursued vigorously using somatic cell hybridization genetics. If fusions between normal (N) and tumor (T) cells resulted in a tumorigenic phenotype, the result was interpreted as evidence of dominance of a tumor-inducing gene; but if a non-tumorigenic phenotype was observed, the result was interpreted as evidence of a recessive tumor-inducing gene. Careful analysis of the karyotypes of interspecies hybrids (e.g., mouse x human) where the chromosomes of one species usually are selectively lost before a stable hybrid cell emerges, demonstrated that hybrids were non-tumorigenic when first tested and that the gradual appearance of tumor-forming ability correlated with progressive chromosome loss. The conclusion was that tumorigenicity is a recessive genetic trait. This simplistic one-gene hypothesis was not questioned until recently when the discovery of oncogenes changed the views of many on the origin and progression of cancer. Recent results using a Chinese hamster embryo fibroblast model system clearly demonstrated that traits associated with transformation and tumor formation segregate independently in fusions between

transformed and non-transformed cells (Smith and Sager, 1985) and that the malignant phenotype can be stably suppressed in cell hybrids from fusions of normal and tumor cells (Sager and Craig, 1985). One explanation for the recessive nature of tumorigenicity and suppression of the malignant phenotype in hybrid cells is the presence of suppressor genes which inhibit the expression of genes involved in transformation. While a few years ago it was postulated that tumor suppressor genes or anti-oncogenes may exist, the acceptance of this concept has become more universal as evidenced by the recent lengthy discussions and reviews of the topic (Sager, 1985; Sager, 1986; Knudson, 1985; Stanbridge, 1985).

Although the tumor suppressor gene hypothesis is consistent with results from in vitro somatic cell hybridization studies between normal and tumor cells, the best physical evidence for the existence of tumor suppressor genes or anti-oncogenes is based on genetic analyses of two cancers, retinoblastoma and Wilms' tumor (see 1985 Annual Report). It has been clearly shown that individuals predisposed to these tumors inherit only one normal allele on one chromosome (represented genomically as +/- or +/o). While this one normal allele is adequate to protect against the occurrence of cancer, any event which creates a homozygous condition (represented genomically as -/- or -/o or o/o) leads to cancer. This is the clearest evidence available which suggests that in humans there is a particular class of genes which are different than oncogenes. They do not induce cancer as a result of their activation but rather their inactivation. Important new data, which may have broad implications, has been obtained for both Wilms' tumor and retinoblastoma. It has been shown using molecular probes that two other embryonal tumors, hepatoblastoma and rhabdomyosarcoma, which are clinically associated with Wilms' tumor, also involve a mutant allele which maps in the same region of chromosome 11 (Koufos et al., 1985). In addition, it has been shown by similar analysis that their is involvement of the same or a similar gene locus on chromosome 13 for survivors of retinoblastoma that develop osteosarcomas (Hansen et al., 1985). The number of genetic loci with suppressor-like activity may be much more limited than previously believed. It would appear that a more reasonable hypothesis at this time is that these loci serve a broad function in that they can be responsible for the formation of tumors in different tissue types depending when they are expressed relative to the general state of differentiation and development of the host. The fact that different tumor types seem to result because of the same pathogenetic mechanism implies that tumors which can be clinically associated on some heritable basis are likely to involve the same chromosome suppressor gene loci. It is intriguing to note that using the methods of somatic cell hybridization techniques, two separate laboratories have obtained evidence that chromosome 11 is involved in the suppression of tumorigenicity (Sager, 1985; Sager, 1986; Marx, 1986). While there is no evidence that inherited cancers and tumor suppression in cell hybrids involve the same gene, experiments such as the one designed by Klinger (see Anti-sense RNA section) offer new hope that this question can be answered.

Although the data are not conclusive, it would appear that what has been termed anti-oncogenes or tumor suppressor genes have a considerably broader function in differentiation and are fewer in number than oncogenes. Clearly, the one-gene hypothesis of the 1970's has been disproved and displaced by more complex, multigene theories of the initiation and progression of cancer. If this new hypothesis on the role of suppressor genes is true, it will be of interest to find out whether a given suppressor gene product acts to control the expression of many different genes. It is still somewhat confusing, however,

that somatic cell hybrid studies have never turned up the dominant effects of oncogenes, unless there is an anti-oncogene controlling the expression of every oncogene. But this possibiliity seems unlikely because oncogenes represent so many different aspects of the growth process.

Anti-Sense RNA - Potential and Promise

Within the last two years there has been a flourish of interest in using anti-sense RNA for genetic analysis of eukaryotic systems. Ordinarily, when double-stranded DNA is transcribed into a single-stranded RNA molecule, only one strand of the DNA, the coding or sense strand, is read by the enzyme RNA polymerase. The promotor region of the DNA molecule tells the RNA polymerase which strand is the sense strand. Using DNA recombinant technology and restriction enzymes, it becomes feasible to prepare constructs in which a promotor sequence is inserted in such a way that the RNA polymerase transcribes what is normally the non-coding or anti-sense strand of the DNA molecule. DNA constructions designed to transcribe the anti-sense DNA strand will result in anti-sense transcripts with sequence complementarity to normal RNA that can presumably anneal with this RNA and disrupt normal processing and translation. Model systems using anti-sense RNA can provide additional methodology for genetic analysis in eukaryotic systems which are not as readily amenable to mutational analysis as prokaryotic systems. The attractiveness of using anti-sense RNA to inhibit gene functions is the exquisite specificity inherent in complementary nucleotide sequences. Although considerable technical difficulties remain to be solved, the fact that some prokaryotes actually use anti-sense RNA as a normal physiological process for regulating translation lends much optimism to this developing technology.

Recently, the feasibility of using anti-sense inhibition has been tested in both transient and stable DNA-mediated transformation systems (Izant and Weintraub, 1985). In a first experiment, it was shown that when the nuclei of a strain of mouse L cells deficient in the enzyme thymidine kinase (TK), were co-injected with DNA constructs containing anti-sense and sense herpes simplex virus TK genes in a ratio of 200:1, respectively, that TK activity was completely suppressed. Controls clearly showed that this suppression was sequence specific and only required anti-sense transcripts complimentary to as little as 52 bases of the 5' untranslated target gene m-RNA. Parallel results were obtained using DNA constructs stably transfected into cells rather than transiently injected into nuclei. An equally important variation of this experimental protocol involved the demonstration that conditional expression of the anti-sense TK RNA could be achieved using a promotor responsive to hormonal induction. This is a critical study because a major obstacle to overcome in using anti-sense RNA analysis is the ability to create conditional expression. Clearly, constitutive expression of anti-sense RNA in a crucial gene function would have lethal consequences. While the feasibility of inhibiting exogenous gene function using herpes simplex virus TK as the model gene was promising, it was also critical to show that an endogenous gene function could be controlled in the same way. Using anti-sense RNA to actin, it was clearly shown that actin cables in BSC-1 kidney epithelial cells are disrupted. The conclusions from these studies were that anti-sense RNA technology may provide a general methodology for genetic analysis, that the method of delivery can range from microinjection to calcium phosphate-mediated stable transfection, that inhibition can be achieved with as little as 52 base pairs of sequence homology, that conditional suppression of gene function can be achieved using the proper promotor, and that both exogenous and

endogenous gene functions can be studied. What remains puzzling is the different ratios of anti-sense RNA to sense RNA required to achieve significant inhibition. These range from ratios of 200:1 to 1:1 depending upon the system. While this may be due to differences in the stability of anti-sense RNAs, it emphasizes the importance of understanding the details of the molecular mechanism of anti-sense mechanism of anti-sense inhibition.

An illustration of the potential importance of this methodology to the understanding of cancer biology is demonstrated in an experimental strategy recently proposed by Harold Klinger (unpublished, CA 16720) at the Albert Einstein College of Medicine. He suggests ways to identify genes involved in the suppression of the tumorigenic phenotype, a topic more thoroughly discussed in another section of this report. The background leading up to this possibility is important to review because it demonstrates how the accumulation of information over years can be blended with other parallel scientific achievements and the development of new technologies to design what may be breakthrough experiments. To summarize, studies in somatic hybridization genetics have clearly shown that when normal cells (N) are hybridized with cancer cells (C), the tumorigenic phenotype is a recessive, not a dominant, characteristic. Furthermore, precise karyotypic analysis of NC hybrids has shown that human chromosome 11 contains genes which suppress tumorigenesis. Those hybrids which are tumorigenic consistently express high levels of the c-*fos* oncogene. Recently, the Laurence Livermore National Laboratory has purified human chromosome 11 using a highly sophisticated flow-sorting system and constructed from the purified chromosome 11 preparation a specific DNA library. Dr. Klinger's strategy will make use of anti-sense RNA technology to identify suppressor genes *without ever knowing either their map position on a chromosome or the nature of their protein products*:

(1) Vectors will be constructed using the strong promotor region of Rous Sarcoma Virus and DNA fragments from the human chromosome 11 library.

(2) These DNA constructs will be used individually to transfect a well-defined NC hybrid cell line in which the tumorigenic phenotype is suppressed. Because the orientation of the inserts in the library will be random after introduction into the NC genome, some of the putative suppressor inserts will be transcribed in an anti-sense configuration.

(3) Any insert transcribed in an anti-sense fashion will restore tumorigenicity by inhibiting normal suppressor gene function. Thus, suppressor gene inserts will be identified by assaying for restoration of tumorigenicity.

(4) Steps 1 through 3 involve considerable work because tumorgenicity assays are time-consuming and cumbersome. However, by using the fact that c-*fos* expression always increases significantly when tumorigenicity appears, a simple *in vitro* preselection system is possible to envision. A DNA construct could be made which contains the c-*fos* gene, its regulatory sequences, and the gene which codes for dehydrofolate reductase (DF). This would ensure that any increase in c-*fos* expression would be accompanied by an increase in the levels of DF. A new suppressed NC hybrid cell

line could be created by stably transfecting the original hybrid cell line with this c-*fos*-DF construct. The new hybrid, NC-c-*fos*-DF would be transfected with the inserts from the chromosome 11 library as discussed in steps 1 and 2 above. On the assumption that restoration of NC-c-*fos*-DF tumorgenicity would be accompanied by increased expression of c-*fos*-DF, the *in vitro* prescreening system would expect tumorigenic hybrid cells to exhibit increased resistance to methotrexate. Thousands of hybrid clones could be tested for increased resistance to methotrexate before actually testing for tumorigenicity by an *in vivo* assay.

Although this is a risky venture, the use of anti-sense RNA technology in identifying tumorigenic suppressor genes offers the possibility of identifying critical genes involved in tumorigenesis which heretofore have not been accessible by any other experimental approaches.

While anti-sense RNA technology may prove very important for basic research in cancer biology, it may also become equally important to cancer therapy. Dr. Paul Ts'o and his associates at The Johns Hopkins University are currently studying the potential of non-ionic deoxyoligonucleotide analogs as probes for basic research studies as well as for use as drugs in therapeutic studies (CA 42762). The only difference between these analogs and deoxyoligonucleotides is that their backbone consists of non-ionic methylphosphonate linkages (Murakame et al., 1985) instead of negatively charged phosphodiester linkages. It is a well-known fact that negatively charged molecules will not penetrate cellular membranes because of charge repulsion. Currently, methylphosphonate oligomers can be synthesized in any desired sequence up to the 12 nucleotides in length using solid phase chemical techniques. Raising the capability of the synthetic procedures to produce olignucleotides up to 15 units long statistically decreases the likelihood that any other specific sequence exactly like it exists in the genome, and represent a potentially very specific reagent. Preliminary studies have already shown that methylphosphonate oligomers with anti-sense configurations complementary to globin messenger RNA can inhibit translation of messenger RNA at very low concentrations (CA 42762). These analogs are totally resistant to nucleases, are taken up by mammalian cells, form stable sequence-specific double-stranded hybrids with complementary single-stranded regions of RNA, and can selectively and specifically inhibit the function of a target messenger RNA. It is possible that once a nucleotide sequence is known for an RNA(s) contributing to the cancer phenotype (e.g., oncogenes) that an anti-sense methylphosphonate oligomer can be custom synthesized to either reverse or eliminate the neoplastic condition. Anti-sense oligomers known to inhibit the activity of each oncogene could be made readily available as standard therapeutic agents.

While much of the above discussion is speculative at this time, there is considerable optimism that anti-sense RNA technology will have important conceptual as well as practical consequences to cancer biology. While the implications of this technology to therapy remain to be shown, conceptually it represents a completely new approach to cancer treatment.

Cancer Progression/Tumor Cell Heterogeneity

It is generally agreed that tumors arise through the accumulation of several changes affecting the control of cell growth. It is also accepted that

the majority of tumors initially arise from a single aberrant cell that has undergone the requisite number of stepwise changes, which may vary depending upon the cell type and the cell location. Although a few still believe that cancer is a developmental disorder that occurs by disturbing the normal homeostatic relations among and between cells, tissues and organs (Rubin, 1985), the bulk of the scientific evidence available, especially the current wave of research on oncogenes, supports the view that both the initiation of the cancerous state and the progression of cancers in their life history to states of increased aggressiveness and malignancy are a result of sequential genetic changes (Nowell, 1986). In the clinical sense, the increasing appearance within the tumor of genetically altered subpopulations of cells with new characteristics, not the initiation of cancer, poses the greatest threat to a patient's survival. The continued acquisition by the neoplastic cells of an increased capacity to invade surrounding tissues and eventually metastasize to distant sites of growth remains the aspect of progression that is of greatest clinical significance. This is still the fundamental definition of malignancy, and metastases resistant to conventional therapeutics are usually what cause the death of cancer victims. One of the greatest challenges to basic cancer research is to understand and learn how to control the biological mechanisms involved in progression which generate heterogeneous subpopulations of tumor cells with increasingly malignant properties. How many processes are involved? Are there host factors which provide particularly suitable selective pressures for malignant subpopulations?

Four years ago the concept of tumor cell heterogeneity was merely an interesting topic of discussion. Although it was recognized as the major obstacle to understanding and treating cancer, there were few experimental approaches available for studying the mechanisms of tumor progression. It is now generally accepted that new cell subpopulations of a tumor contain traits which are inheritable. Thus, any mechanism involved in generating entirely new subpopulations must occur through genetic changes or through stably inherited epigenetic changes.

Although the list of possibilities is growing, the major genetic and epigenetic changes which are potentially capable of generating new subpopulations of cells in tumors are likely to remain in the following categories: (1) point mutation or deletion of the DNA; (2) chromosome translocation; (3) errors in chromosome segregation during mitosis; (4) gene amplification; (5) chromosome duplication; (6) cell fusion and (7) DNA methylation. All of these processes will result in either changes in the expression of existing genes or alterations in gene dosage. Most of these processes require cell division to occur. Host factors such as tissue environment and immune surveillance, which might provide sélective pressures for the choice of process or more likely the choice of new cell type, are also likely to be important. Clearly, tumor cell hetergeneity is a result of both intercellular and intracellular mechanisms which contribute to the adaptive response of tumors.

In general, advanced cancers show more extensive chromosomal aberrations than do early stages of neoplasia. The most convincing information relating to sequential changes in karyotypic properties has been derived largely from individual cases of leukemia and lymphoma. There is no doubt that many of the chromosome translocations observed in hematopoietic neoplasias occur in a nonrandom fashion, very close to the map points of known oncogenes. For example, the Philadelphia chromosome, which involves a translocation between chromosomes

9 and 22 involving the c-*abl* oncogene, is consistently observed in chronic granulocytic leukemias (see section on oncogenes). But it is also clear that the terminal accelerated phases of the disease involve subpopulations of cells with additional karyotypic changes. It is probably the careful cataloging of these additional changes which will help reveal the location and identity of genes involved in invasion and metastasis. Chromosomal reports on human non-hematopoietic tumors (i.e., the more common solid tumors) are considerably more fragmentary because it is not as easy to obtain serial samples which can be used to relate changes in karyotype to more aggressive tumor behavior. Nevertheless, there are some consistencies in chromosomal abnormalities of solid tumors (Wolman, 1983), and recently it has been shown that advanced cases of melanoma involve an extra dosage of chromosome 7 with an associated increase in expression of the receptor for epidermal growth factor (Koprowski et al, 1985) suggesting involvement of c-*erb* B oncogene. However, the pathogenesis of human melanomas may involve other chromosomes as well, since polymorphic restriction fragment analysis of melanoma cell lines from 21 different patients showed somatic genetic changes which led to homozygosity or hemizygosity (i.e., loss of one chromosome of diploid pair) (Dracopoli et al., 1985). In mice, thymic lymphoma induced by a carcinogen appear to result because there is somatic loss of the normal N-*ras* allele, creating a situation where only the mutated form of N-*ras* is expressed (Guerro et al., 1985). All of the evidence to date suggests that processes such as chromosome translocations, errors in chromosome segregation, and chromosome duplication, are important genetic consequences leading to increased malignant behavior of tumor cells during tumor progression. In addition to the obvious need for more karyotypic data on all types of tumors, it is of general interest to determine if continuous changes impart greater and greater selective advantage for growth. Increased escape from normal growth controls may improve the probability of selecting for additional genetic changes responsible for increased malignancy or there may be an inherent genomic instability of cancer cells that promotes the continuous production of cells with more malignant characteristics. Some of the recent results correlating the *ras* oncogene with increased metastatic potential may support the former possibility (Muschel et al., 1985; Bondy et al., 1985; Greig et al., 1985), but results with gene amplification support the later possibility (see below).

The idea that cell fusion is one mechanism responsible for the generation of tumor diversity has existed for a long time. Basically, this would represent another way in which tumor cells could alter their complement of chromosomes and stabilize at a new karyotype as a result of some exchange of genetic material. There are only a *few* examples which support cell fusion as an event which can occur between the interactions of tumor cells with tumor cells or tumor cells with normal cells. Although these are convincing because they involved traceable genetic markers, there are more reasons to believe that in the majority of cases cell fusion does not play a significant role in tumor progression. First, the great difficulty in creating somatic cell hybrids *in vitro* without introducing tremendously artificial conditions such as Sendai virus or ethylene glycol, argues against somatic cell hybridizations *in vivo*. Second, it has been shown that cancer cells can progress to stages of greater malignancy without incurring significant alterations in karyotype. At this juncture, cell fusion phenomenan do not appear to be the most important area of research to emphasize.

Gene amplification appears to be a significant genetic event in cancer cells and also appears to correlate well in some cases with the prognosis of neoplastic disease (e.g., N-*myc* amplification in neuroblastomas). In last

year's annual report, gene amplification was documented to occur for many different oncogenes in different forms of cancer. This list has continued to grow. Furthermore, it is a well known fact that amplification is the major process by which tumor cells develop resistance to therapeutic drugs. There seems to be considerable support for the hypothesis that gene amplification, which is essentially a process that increases a given gene dosage in a cell, plays a significant role in the adaptive response and progression of tumors. Recently, using a Chinese hamster embryonic fibroblast model system, it was discovered that tumorigenic cells compared to non-tumorigenic cells underwent amplification of the dehydrofolate reductase gene at an accelerated and directed rate. The conclusion from this study was that unregulated gene amplification is a pathological process which occurs readily in neoplastic cells but rarely in normal cells and contributes to the rapid evolution and progression of cancer (Sager et al., 1985). This would imply that there is a special property of cancer cells which can be studied and used to advantage in improving therapy. A more theoretical consideration suggests that loss of proper control of the number and timing of multiple initiation sites for replication on single chromosomes can lead to unusual recombinations and amplifications within the eukaryotic genome (Schimke et al., 1986). The implication is that a relaxation or loss of replication control may result more frequently in all kinds of genetic alterations, including gene amplification, and represent the major general defect in cancer cells which allows them to progress to states of greater malignancy. Thus, studying the mechanisms responsible for replication control and gene amplification may provide key insights into how tumors generate new subpopulations of cells. Preliminary results with amplification of oncogenes suggest that the increased dosage of an oncogene contributes to the more malignant phenotype.

The idea that tumor progression and heterogeneity are due entirely to inherited genetic mutations in the tumor cell population may be too simplistic. Not all neoplastic cells show a higher rate of mutational frequency than normal cells (Elmore et al., 1983) and the rate of metastatic variation can be as much as two orders of magnitude higher in the same population of cells (Rubin, 1985). Although there is a tendency for tumor cell populations to increase in their growth rate with time and to show cumulative evidence of further escape from local growth mechanisms, it is important to recognize that there may be considerable variability in the time period and sequence in which these various aspects of tumor progression become apparent (Nowell, 1986). In some instances, the properties of a far-advanced malignancy may be established while the neoplasm is still microscopic in size; in other cases, well-differentiated, slow growing tumors may persist for years before undergoing a relatively abrupt shift to more aggressive behavior. In addition to the genetic theories of tumor progression, there is considerable evidence accumulating that changes in DNA methylation, which are known to be implicated in eukaryotic gene control, may play a central role in the generation of heterogeneity and phenotypic instability in cancer (Jones, 1986). DNA methylation patterns are often tissue specific and, since the methyltransferase enzyme keys its activity on the presence of CpG doublets in the DNA molecule, methylation patterns are somatically inheritable. Thus, any changes in DNA methylation, although epigenetic and not genetic in nature, satisfy the requirement of producing heritable changes in tumor cells which could account for the appearance of more malignant cell populations. Unlike mutation, changes in the control of DNA methylation might be expected to alter the patterns of expression of many genes rather than just the gene which has been mutated. But this also implies that changes in methylation might induce many new or exaggerated properties in a tumor cell population very quickly or

almost simultaneously, shortening the time required for selection of a malignant phenotype by the slower process of stepwise genetic mutations. In any case, the large body of evidence suggests that methylation levels and patterns are deranged in tumor cells, more often leading to undermethylation which leads to increased gene expression (Riggs and Jones, 1983).

Recent research studies on DNA methylation implicate a role for it signaling which DNA sequences are to be constantly available for transcription versus sequences which would be available on a more selective basis (Bird, 1986). Studies also suggest a potential role for it in the enhancement of the metastatic capacity of human tumor cell lines (Ormerod et al., 1986) as well as in the mechanisms responsible for tumor progression (Lileplo et al., 1985). Because methylation patterns are copied less rigorously as cells divide more rapidly, (or as more cells undergo cell division), it has been speculated that changes in the timing of DNA synthesis, DNA methylation and DNA packaging upset the normal coordination of these processes and result in slower than normal methyation at some sites and higher than normal methylation at other sites (Jones, 1986). This idea that a disruption in the timing and coordination of methylation is important to tumor progression parallels the hypothesis that genetic variability in tumors is a result of disruptions in the number and timing of replication initiation sites (Schimke, 1986).

On the basis of current information, it is possible to speculate that DNA amplification and DNA methylation are two of the processes most likely to be contributing to tumor progression. The major question that needs to be answered is whether the generation of heterogeneous cell populations in tumors occurs because of a statistical relationship between the number of cell divisions and the probability of selecting for a more malignant phenotype or whether this phenomenon is a result of an inherent genetic instability of cancer cells that operates by some defined mechanism. In the first hypothesis, it should be possible to demonstrate that malignant cells appear more often as more cells within a tumor become less influenced by local growth controls. It would also predict the cumulative abnormal involvement of many different oncogenes. But this possibility would not offer as much hope for developing specific therapeutic interventions which would inhibit the appearance of new cell populations. The second hypothesis would suggest that there are biochemically defined reasons why tumor cell populations in particular become more heterogeneous and malignant, offering the possibility that tumor progression can be specifically altered or inhibited.

The Transforming Growth Factor Beta

Transforming growth factor beta (TGF-beta) was described simultaneously by two different groups just five years ago (Goustin, et al, 1986). It received its name because it stimulates the growth of fibroblasts and causes them to behave as if they have been transformed. Now its name has turned out to be a misnomer since the action of TGF-beta clearly is inhibitory to growth of many types of cells (Marx, 1986). The idea of molecules with growth suppressing activity operating within normal cells has been around for some time but is now gaining new respect as a model to explain transformation. TGF-beta was recently shown to have considerable homology with a growth inhibitor (GI) purified from medium conditioned by growth of monkey kidney epithelial cells. This factor is now referred to as GI/TGF-beta. Certain cells seem especially

sensitive to GI/TGF-beta, for example, it inhibits growth of human mammary carcinoma cells carried as tumors in nude mice. Use of a cDNA probe for TGF-beta should facilitate definitive chemical and biological characterization of the molecule produced by kidney cells.

Other demonstrations have also been made of TGF-beta's inhibition of growth of other cells such as lymphocytes and epithelial cells. Further, the growth of cultured prokeratinocytes is inhibited by TGF-beta in a serum-free medium while a squamous carcinoma cell line grows very well in the same medium.

TGF-beta has been purified to homogeneity from bovine kidney, human placenta, human platelets and virally-transformed rat cells and the gene has been cloned. The TGF-beta protein and its specific cell receptors can be detected in many tissues and cultured cells, however, these cells fail to exhibit the phenotype induced by adding exogenous TGF-beta. Evidence now suggests that TGF-beta as produced in cells or released from cultured cells exists as a high molecular weight form, through an association with a binding protein. This larger precursor molecule is inactive but might quickly be cleaved into an active form by some important regulatory step.

TGF-beta is mitogenic for a variety of fibroblastic cell types. This activity is apparently conveyed by the expression of an oncogene, c-*sis*, and the appearance of platelet-derived growth factor (PDGF) activity in the activated cells. The c-*sis* protein is identical with one of the two peptide chains of PDGF. It is inherently simpler to study forces that actively stimulate cells than to study forces that suppress cell division or prevent development of cancer. TGF-beta thus seems to be an ideal candidate for investigations to help explain how cells do or do not grow.

The Mechanisms of Angiogenesis

Solid tumors are angiogenesis-dependent, thus the study of how and why endothelial cells are directed to proliferate and to form new capillary blood vessels is a continuing high priority of cancer biology. The rate of endothelial cell proliferation is usually measured in years yet they can quickly respond to angiogenic factors and sprout new capillaries (Folkman, 1984).

A resurgence of interest followed the recent reports (Fett et al., 1985; Strydom et al., 1985; Kurachi et al., 1985) of the purification of "angiogenin" from human colon cancer cells. This factor is angiogenic *in vivo* but is not mitogenic for endothelial cells *in vitro*. It has been sequenced and has 35% homology to pancreatic ribonuclease. Previously angiogenic protein factors had been purified from rat chondrosarcoma (Shing et al., 1984) and bovine cartilage (Sullivan and Klagsbrun, 1985) by the clever use of heparin affinity chromatography. It is now clear that there is a whole class of related endothelial cell growth factors (ECGF) that share a strong affinity for heparin including fibroblast growth factors, retinal-derived growth factor and a factor derived from a human hepatoma cell line (Folkman, 1986). They stimulate angiogenesis *in vivo* and endothelial cell proliferation *in vitro*. There seem to be no inherent functional differences between the factors found in normal tissue and those that are tumor-derived. There is speculation that endothelial cell mitogen expression may be under tight

regulation in normal cells but continously expressed in tumors, as such they may have potential as diagnostic markers of active tumor growth. Detection of ECGF's, for example in urine, could become a simple routine procedure because of their avid heparin-binding activity. A recent report (Schreiber et al., 1986) indicates that tumor growth factor alpha promotes angiogenesis in a hamster cheek pouch assay. This factor is likely an important autocrine growth regulator for the tumor cells that produce it but it could also play a role in malignancy-associated neovascularization.

There is also considerable interest in the developing program of angiogenesis inhibition. In 1983, Folkman and associates (Folkman et al., 1983) published the exciting observation that administration of cortisone to tumor-bearing mice while feeding them heparin caused regression of certain types of tumors (B-16 melanoma, Lewis lung carcinoma, reticulum cell carcinoma, bladder carcinoma) but not others. Parallel *in vitro* experiments showed that the steroid-heparin combination prevented capillary growth. In the past three years a new class of "angiostatic" steroids without glucocorticoid or mineralocorticoid activity have been identified. Further, a collaborative effort has been initiated between the Folkman group and Peter Albersheim (CA42538) to isolate and purify the active anti-angiogenic fragment(s) of heparin. Consistent biological activity is seen in hexasaccharides of heparin and further chemical characterization is proceeding. Interestingly, certain heparin fragments that have been generated appear to have growth-*enhancing* activity toward capillaries. It may eventualy be possible to assign angiogenesis promoting and angiogenesis-inhibiting activities to discrete domains of the heparin molecule. The combined use of angiostatic steroids and heparin fragments as an adjunct to more standard therapy seems to have future application in the clinical treatment of malignancy.

Conclusions

Although research progress in the Tumor Biology Program continues to move at a rapid pace on many different fronts, it is becoming increasingly apparent that both tumor formation and tumor progression involve very complex and interwoven metabolic pathways and physiological controls. These affect nearly every aspect of normal cell behavior ranging from cell growth to cell differentiation and tissue development. There are not likely to be simplistic answers, but there are, at this time, a few facts which may help determine the future directions of basic research as it relates to practical aspects of diagnosis, prevention, and treatment of cancer. There is very little doubt that cancer is a disease which begins and progresses through a series of permanent genetic or epigenetic alterations of a few cells. Most common forms of human cancer (e.g., breast, lung, bowel), at the time of clinical presentation, have undergone extensive and variable somatic genetic changes and selection within the individual host environment and that process is a continuing one. This suggests that learning to prevent any further genetic changes may be a critical step in the success of therapy, but it also explains why it has been difficult to find consistent alterations in common cancers that permit the design of specific therapy. It also offers little hope of using differentiating agents to reverse the aggressive growth and metastatic patterns of advanced neoplastic cells. Nevertheless, differentiating agents still may have a use in stablizing further progression. Continued emphasis on studying the particular genes (i.e., oncogenes, anti-oncogenes) associated with various characteristics of human malignant growth is warranted because of the possibility of finding altered gene products

that might be susceptible to specific immunological or chemotherapeutic attack. The information available on the genetics of the more common solid tumors compared to the less common leukemias and lymphomas is disturbingly sparse. Although technically very difficult there is a clear need to pursue the genetics of solid tumors with considerable energy. Some of the results in this report indicate that translocations producing hybrid proteins may be a critical event in solid tumors, but there is no cytogenetic confirmation available as is the case in tumors of the hematopoietic system. An understanding of the genetics of solid tumors will continue to be limited by our ability to obtain and study sufficient numbers of human tumors under controlled conditions. Perhaps a recent report on the growth of human tumors in vitro represents a breakthrough in this area (Freeman and Hoffman, 1986) but much more needs to be done.

Widely polarized views continue to exist on the question of immune responses to tumors, but it could be that oncogene research is pointing in those directions which are most likely to be fruitful for immunotherapy (Klein and Klein, 1985). It is clear that for immune rejection to be most efficient, it must react to some form of foreign protein or foreign body in the host. Thus, it would seem logical that specific immune recognition and rejection of foreign tumor cells would be most successful when cancer is caused by DNA tumor viruses because the oncogenes of these viruses have no normal counterpart in the cell. However, in those cases where the cellular oncogene product is fundamentally the same as the normal gene product, although expressed in an unregulated fashion, immune rejection is not likely to be successful and no perturbation of the immune system would seem to be indicated. In those situations where tumor cell surface proteins are altered by mutation (i.e., ras) or truncation (i.e., erb-B) and in those cases where there are hybrid proteins (i.e., abl, onc D) with potentially new three dimentional conformations, monoclonal antibody therapy might be successful. In any case, it is clear that every tumor is going to be different and that the selection of a therapeutic combination, whether biological, immunological, chemical or physical, will depend upon an accurate and thorough diagnosis which clearly identifies all of the properties of each subpopulation within a tumor that are important in the design of effective therapeutic measures. These answers are likely to come from the continued emphasis on areas such as oncogene research.

As we continue to find further evidence for cancer as a genetic disease, the emphasis on cancer research is likely to continue to skew in that direction.

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FISCAL YEAR 1986 EST.

TUMOR BIOLOGY PROGRAM
SUMMARY BY SUB CATEGORY (DOLLARS IN THOUSANDS)

	NON-COMPETING		COMPETING		TOTAL	
	No.	Amount	No.	Amount	No.	Amount
A. Cell Surface	67	$9,724	25	$3,591	92	$13,315
B. Enzymes	25	3,774	16	2,036	41	5,810
C. Peptide Hormones	12	1,724	7	957	19	2,681
D. Steroids	20	2,965	4	518	24	3,483
E. Membraneous Organelles	8	1,528	1	141	9	1,669
F. Ribosomes & Polyribosomes	3	563	0	0	3	563
G. M-RNA	10	1,082	3	440	13	1,522
H. T-RNA	3	245	1	108	4	353
I. DNA	7	951	4	588	11	1,539
J. Growth Factors	41	5,692	18	2,235	59	7,927
K. Nucleus	10	1,091	3	303	13	1,394
L. Contractile Elements	18	2,284	0	0	18	2,284
M. Development & Differentiation	42	5,446	16	2,116	58	7,562
N. Cell Growth, Cell Division	22	3,030	11	1,245	33	4,275
O. Metastasis	3	321	11	1,279	14	1,600
P. Somatic Cell Genetics	4	651	5	464	9	1,115
Q. Inheritance of Neoplasms	1	136	0	0	1	136
R. Plasmids, Viruses	1	164	1	110	2	274
S. In Vivo & In Vitro Tumor Lines	7	849	2	235	9	1,084
W. Difficult to Classify	5	778	2	192	7	970
X. Oncogenes	39	5,123	14	1,764	53	6,887
SUB TOTAL	348	48,121	144	18,322	492	66,443
V. Program Projects	14	11,345	4	3,294	18	14,639
U. Small Business Grants	0	0	5	279	5	279
T. Conferences	1	25	5	120	6	145
Y. Outstanding Investigators	6	4,445	7	3,713	13	8,158
SUB TOTAL	21	15,815	21	7,406	42	23,221
TOTAL	369	$63,936	165	$25,728	534	$89,664

R01 CA08759 Structure, Biosynthesis and Function of Glycoproteins
Kornfeld Washington University

R01 CA12306 Role of Cell Surface in Initiation of Cell Division
Cunningham University of California, Irvine

R01 CA12790 Membrane Transport Adaptations in Human Blood Cells
Lichtman University of Rochester

R01 CA13402 Surface Membranes in Normal and Cancer Cells
Atkinson Yeshiva University

R01 CA13605 Chemistry of Intercellular Adhesion
Steinberg Princeton University

R01 CA14464 Intercellular Communication and Cancer
Loewenstein University of Miami

R01 CA14551 Penetration of Macromolecules into Mammalian Cells
Ryser Boston University

R01 CA14609 Molecular Basis of Cellular Adhesiveness
Grinnell University of Texas Health Science Center, Dallas

R01 CA15483 Biochemistry of Mucopolysaccharides
Davidson Pennsylvania State University Hershey Medical Center

R01 CA16740 Proteases in Growth Control and Malignant Transformation
Quigley Health Science Center at Brooklyn

R01 CA16777 The Biosynthesis of Cell Envelope Glycoproteins
Schutzbach University of Alabama at Birmingham

R01 CA17007 Cell Surface Structure and Cell Transformations
Hynes Massachusetts Institute of Technology

R01 CA18470 Antigenicity and Tumorigenicity of Somatic Cell Hybrids
Knowles Wistar Institute of Anatomy and Biology

R01 CA18801 Glycolipid Metabolism and Tumorigenesis
Morre Purdue University, West Lafayette

R01 CA19130 The Surface Membranes of Normal and Cancer Cells
Warren Wistar Institute of Anatomy and Biology

R01 CA19144 Membrane Changes Caused by Tumor Virus Transformation
Buck Wistar Institute of Anatomy and Biology

R01 CA20026 Glycolipids of Normal and Transformed Cells
Hakomori Fred Hutchinson Cancer Research Center

R01 CA20421 Mutants Altered in Glycosylation of Soluble & Membrane Proteins
Krag Johns Hopkins University

R01 CA20424 Murine Ascites Tumor Cell Glycoproteins
Goldstein University of Michigan at Ann Arbor

R01 CA21246 The Roles of Laminin and Entactin in Cell Adhesion
Chung University of Pittsburgh

R01 CA21463 Metastasis Cell Migration/Adhesion Fibronectin Peptide
Furcht University of Minnesota of Minneapolis-St. Paul

R01 CA21722 Membrane Pathology in Carcinogenesis
Scott Mayo Foundation

R01 CA21923 Oligosaccharide Structure and Function in Recognition
Baenziger Washington University

R01 CA22202 Studies of Fibrin Deposition in Cancer
Rickles University of Connecticut Health Center

R01 CA22451 Contact Behavior of Developing and Transformed Cells
Trinkaus Yale University

R01 CA22659 Studies of Proteins Involved in Cell Interaction
Chen Dana-Farber Cancer Institute

R01 CA22729 Hormonal Regulation of Membrane Phenotype
Gelehrter University of Michigan at Ann Arbor

R01 CA23016 Animal Cell Surface Heparin Sulfate and Transformation
Keller University of Health Sciences/Chicago Medical School

R01 CA23540 Collagen and Its Relationship to Tumors
Smith Boston University

R01 CA23753 Proteases and the Malignant Phenotype
Rifkin New York University

R01 CA23907 Fibronectin and Other Glycoproteins Defining Malignancy
Hakomori Fred Hutchinson Cancer Research Center

R01 CA25730 Adhesion and Surface Membrane in Mammary Carcinomas
Steiner University of Kentucky

R01 CA26122 Effects of Hormones on Cell Membrane Properties
Baumann Roswell Park Memorial Institute

R01 CA26294 Glycoproteins of Normal/Malignant Human Blood Cells
Gahmberg University of Helsinki

R01 CA27117 A Study of Membrane Bound ATPases of Human Tumor
Knowles University of California, San Diego

R01 CA27460 Alpha-Fetoprotein: Structure and Function
Ruoslahti La Jolla Cancer Research Foundation

R01 CA27755 Fibronectin: Proteoglycan Binding in Adhesion Sites
Culp Case Western Reserve University

R01 CA28287 Driving Forces for Nutrient Transport in Tumor Cells
Smith University of Texas Health Science Center, San Antonio

R01 CA28471 Biology of Solid Tumor Growth and Immune Rejection
Dvorak Beth Israel Hospital

R01 CA28548 Developing Immunological Probes for Gap Junctions
Johnson University of Minnesota

R01 CA29571 Malignant Cell Variants of Lymphosarcoma
Nicolson University of Texas System Cancer Center

R01 CA29995 Role of Laminin in Metastasis and Migration of Tumor Cells
Furcht University of Minnesota of Minneapolis-St. Paul

R01 CA30117 Regulation of Cells by Matrix and Hormones
Reid Yeshiva University

R01 CA30129 Quantitative Approach to Junctional Defects in Cancer
Atkinson University of Minnesota of Minneapolis-St. Paul

R01 CA30289 Epithelial Cells—Extracellular Matrix Interactions
Vlodavsky Hadassah University Hospital

R01 CA31277 Cancer-Associated Glycoproteins and Galactosyltransferases
Isselbacher Massachusetts General Hospital

R01 CA31761 Membrane Sialoglycoconjugates and Sialyltransferases
Taub Columbia University

R01 CA32311 Cytoskeleton-Membrane Interaction—Antisera Induced CHA
Damsky Wistar Institute of Anatomy and Biology

R01 CA32516 Mechanisms of Biological Prevention of Leukemogenesis
Moore Sloan-Kettering Institute for Cancer Research

R01 CA32829 Defective Adhesion in Transformed Cells
Izzard State University of New York at Albany

R01 CA32927 Anion Transport in Ehrlich Carcinoma Cells
Levinson University of Texas Health Science Center, San Antonio

R01 CA32949 Biosynthesis and Secretion of HCG by Human Trophoblasts
Ruddon University of Michigan at Ann Arbor

R01 CA33208 Prostate Cell Surface Phenotype and Tumor Behavior
Ware Duke University

R01 CA33238 Sherblom — Sialylation of a Tumor Cell Glycoprotein
University of Maine at Orono

R23 CA33751 Basu — II-Glycolipid Biosynthesis in Mouse T- and B-Lymphomas
University of Notre Dame

R01 CA34014 Fukuda — Endo-Beta Galactosidase and Cell Surface Glycoconjugate
La Jolla Cancer Research Foundation

R01 CA34691 Segel — A Specific Abnormality in Chronic Leukemic Lymphocytes
University of Rochester

R01 CA35377 Pierce — Asparagine-Linked Carbohydrates
University of Miami

R01 CA36069 Wells — Cell Contact in Regulation of Embryonal Carcinoma
University of Colorado Health Sciences Center

R01 CA36132 Varani — Endogenous Laminin Expression and Metastasis
University of Michigan at Ann Arbor

R01 CA36248 Skubitz — Granulocyte Membrane Structure and Function
University of Minnesota of Minneapolis-St. Paul

R01 CA36434 Stanley — Glycosylation Defects of Lectin-Resistant Tumor Cells
Yeshiva University

R23 CA37191 Sekiguchi — Molecular Heterogeneity of Fibronectins
Fred Hutchinson Cancer Research Center

R01 CA37626 Cummings — Surface Oligosaccharides in Embryonal Carcinoma Cells
University of Georgia

R01 CA37662 Briles — Substrate-Specific Adhesive Variants of Metastatic Cells
University of Texas System Cancer Center

R01 CA37785 McKeown-Longo — Regulation of Fibronectin Matrix Assembly
State University of New York at Albany

R01 CA37853 Glick — Structures of Neurectoderm Tumor Glycoproteins
Children's Hospital of Philadelphia

R23 CA38006 Maher — New Focal Contact Proteins and Transformation
University of California, San Diego

R01 CA38701 Varki — Sulfated Oligosaccharides of Normal and Malignant Cells
University of California

R01 CA38773 Doyle — Proteins of the Hepatoma Cell Plasma Membrane
State University of New York at Buffalo

R01 CA38801 Carter — Structure and Function of the Carbohydrate Units of GP140
Pacific Northwest Research Foundation

R01 CA38817 Fibroblast Tumor Cell Interactions in Tumor Invasion
Biswas Tufts University

R01 CA38849 Pteridine Metabolism and Transport in Malignant Cells
Webber Scripps Clinic and Research Foundation

R01 CA39037 Basement Membranes of Cultured Cells - A Model System
Keller University of Health Sciences/Chicago Medical School

R01 CA39077 Molecular Ultrastructure Underlying Cell Adhesion
Chen Georgetown University

R01 CA39919 Analysis of Extracellular Matrix of Transforming Cells
Hawkes University of California, San Francisco

R01 CA40059 Extracellular Components in Blood Stromal Cell Interaction
Sorrell West Virginia University

R01 CA40225 The Desmoplastic Response to Tumor Invasion
Barsky University of California Los Angeles

R01 CA40422 Basement Membrane Degradation by Tumor Cells
Jones University of Southern California

R01 CA40475 Cell-Substratum Interactions
Barnes Oregon State University

R01 CA40624 Pathogenesis of Tumor Stroma Generation
Dvorak Beth Israel Hospital

R01 CA41359 Biosyntehsis of Basal Lamina by Human Malignant Cells
Peters University of Michigan at Ann Arbor

R01 CA41407 The Human Glucocorticoid Receptors, Its Gene and Actions
Thompson University of Texas Medical Branch Galveston

R01 CA41524 Tumor Basement Membrane Degrading Enzymes
Nakajima University of Texas

R01 CA41642 Plasmalogens and Related Ether Lipids in Cancer Cells
Snyder Oak Ridge Associated Universities

R01 CA41701 Tumor Cell Receptors for Heparan Sulfate
Biswas Tufts University

R01 CA41756 Cancer Cell-Derived Plasminogen Activators
Harvey New York State Department of Health

R01 CA42032 Cytoskeleton-Membrane Interactions
Damsky University of California, San Francisco

R01 CA42576 Cell Communication and Clonogenicity of Melanoma Cells
Schroder Boston University

R01 CA42650 Ivatt — Role of Cell Surface Glycoproteins in Embryogenesis
University of Texas System Cancer Center

R01 CA42714 Hixson — Molecular Determinants of Multicellular Organization
Rhode Island Hospital

R01 CA42804 Look — Mechanisms of Gene Regulation in Myelopoiesis
St. Jude Children's Research Hospital

R01 CA42877 Lotan — Tumor Cell Surface & Growth Modulation by Retinoic Acid
University of Texas

R23 CA43009 Varki — Metastasis of Human Lung Adenocarcinoma
University of California San Diego

R23 CA44328 Swanson — Pinocytic Regulation in Normal and Transformed Cells
Harvard Medical School

ENZYMES (B)

R01 CA04679 Lerner — Biology of Normal and Malignant Melanocytes
Yale University

R01 CA10916 Weinhouse — Metabolism of Normal and Neoplastic Tissue
Temple University

R01 CA11655 Silber — Studies of Leukocyte Metabolism
New York University

R01 CA14030 Rudolph — Regulation of Metabolism by Purine Interconversions
Rice University

R01 CA14764 Basu — Glycolipid Metabolism in Tumor and Transformed Cells
University of Notre Dame

R01 CA14881 Shiman — Regulation of Tyrosine Synthesis in Hepatoma Cells
Pennsylvania State University/Hershey Medical Center

R01 CA18138 Pegg — Mammalian Polyamine Metabolism
Pennsylvania State University/Hershey Medical Center

R01 CA25005 Greengard — The Regulation of Mammalian Enzyme Synthesis
Mount Sinai School of Medicine

R01 CA25617 Dabbous — The Collagenolytic System of Invasive Tumors
University of Tennessee Center for Health Sciences

R01 CA26546 Canellakis — The Regulation of Polyamine Biosynthesis
Yale University

R01 CA27674 Evans — Control of Pyrimidine Biosynthesis in Mammalian Cells
Wayne State University

R01 CA27808 Tischler — Neuroendocrine Gene Expression in Neoplastic Progression
Tufts University

R01 CA28376 Silber — Nucleotide Metabolism in Chronic Lymphocytic Leukemia
New York University

R01 CA28725 Schuster — Asparagine Biosynthesis in Normal and Tumor Cells
University of Nebraska, Lincoln

R01 CA28781 Adair — Biosynthesis of Dolichyl Phosphate
University of South Florida

R01 CA29048 Coffino — Mechanisms of Ornithine Decarboxylase Regulation
University of California, San Francisco

R01 CA29307 Baker — Control of Protease Action on Human Cells
University of Kansas, Lawrence

R01 CA29331 Glode — Regulation of Cystathionase Expression
University of Colorado Health Sciences Center

R01 CA30645 Stanley — Glycosylation Mutants of Animal Cells
Yeshiva University

R01 CA32369 Da Costa — Folate Binders in Hematopoiesis and Cell Replication
Health Science Center at Brooklyn

R01 CA32961 Schuster — Specific Inhibition of Tumor Cell Metabolism
University of Nebraska, Lincoln

R23 CA34025 Senger — Secreted Phosphoproteins Associated with Tumorigenicity
Beth Israel Hospital

R01 CA34517 Clinton — Mechanism of Transformation by Tyrosine Kinases
Louisiana State University Medical Center, New Orleans

R01 CA34746 Tam — Inhibitors of Tyrosine-Specific Protein Kinase
Rockefeller University

R01 CA35680 Niedel — Maturation of Human Myeloid Leukemia
Duke University

R01 CA36481 Sloane — Cathepsin B-Like Cysteine Proteinases and Tumor Invasion
Wayne State University

R01 CA36777 Kuo — Phospholipid-Ca2+ and Phosphoproteins in Leukemic Cells
Emory University

R01 CA38270 Back — Biochemical and Structural Studies of Kinin-Forming Systems
State University of New York at Buffalo

R01 CA38821 Casnellie — Properties of a Lymphoma Cell Tyrosine Protein Kinase
University of Rochester

R01 CA38992 Molecular Analysis of NA^{+}/K^{+}-ATPase
Levenson Yale University

R01 CA39445 Multienzyme Complex for DNA Synthesis in Mammalian Cells
Reddy University of Virginia

R01 CA40010 Glucocorticoid Inhibition of Plasminogen Activator
Littlefield Yale University

R23 CA40495 HMG COA Reductase Regulation in Human Leukemia
Harwood University of Florida

R01 CA40758 Factors Involved in Malignant Behavior of Human Tumors
Ossowski Rockefeller University

R01 CA40929 Regulation of Neural Development by Protein Kinases
Wagner Dana-Farber Cancer Institute

R01 CA41210 Studies of Methionine Dependence in Tumors
Sakai University of Alabama at Birmingham

R01 CA41521 Regulation of Synthesis of Glycolipid Tumor Antigens
Holmes Pacific Northwest Research Foundation

R01 CA41560 Studies on the Myristylation of the 36 KD Protein
Gordon University of Colorado

R01 CA41756 Cancer Cell-Derived PA of Unusually Large Size
Harvey New York State Department of Health

R01 CA41991 Regulation of Folylpolyglutamate Synthesis
Shane University of California, Berkeley

R23 CA42426 Regulation of cAMP Levels in Xenopus Oocytes
Sadler University of Colorado Health Sciences Center

PEPTIDE HORMONES (C)

R01 CA07535 Control of Pituitary Gland and Pituitary Tumor Hormones
MacLeod University of Virginia, Charlottesville

R01 CA11685 Tumor Cell Synthesis and Secretion of Peptide Hormones
Orth Vanderbilt University

R01 CA16417 Pituitary Hormones in Normal and Neoplastic Growth
Ramachandran University of California, San Francisco

R01 CA22394 Hormonal Control of Cell Proliferation
Thompson University of South Carolina at Columbia

R01 CA23185 Regulation of Alpha and Beta Subunits of TSH
Kourides Sloan-Kettering Institute for Cancer Research

R01 CA23248 Hymer — Prolactin Cell Function in Breast Cancer
Pennsylvania State University, University Park

R01 CA23357 Hussa — HCGB From Cervical Cancer: Peptide Heterogeneity
Medical College of Wisconsin

R01 CA24604 Surks — Triiodothyronine Receptors and Nonthyroidal Diseases
Montefiore Medical Center, Bronx, New York

R01 CA28218 Biswas — Hormone Production by Pituitary Tumor Cells
Harvard University

R01 CA30388 Golde — Humoral Regulation of Normal and Malignant Hemopoiesis
University of California, Los Angeles

R01 CA33030 Rosenfeld — Neuroendocrine Peptide Switching Events in Cancer
University of California, San Diego

R01 CA33320 Segre — Cancer-Hypercalcemia: New Secreted Bone-Resorbing Factor
Massachusetts General Hospital

R01 CA33852 Sorenson — Secretion and Proliferation in Small Cell Carcinoma
Dartmouth College

R01 CA36399 McGrath — Lineages in Mammary Cell Transformation
Michigan Cancer Foundation

R01 CA36526 Kourides — Expression of Human Chorionic Gonadotropin Genes
Sloan-Kettering Institute for Cancer Research

R01 CA37370 Goodman — Ectopic Hormone Synthesis in Pheochromocytoma Cells
New England Medical Center Hospital

R01 CA38228 Login — $45CA2^{+}$ Flux in Normal and Neoplastic Pituitary Cells
University of Virginia, Charlottesville

R01 CA38651 Singh — Gastrointestinal Hormones in Gastrointestinal Cancer
University of Texas Medical Branch, Galveston

R01 CA40629 Ascoli — Gonadotropin Actions in Leydig Tumor Cells
Population Council

R01 CA42306 Moody — Neurotensin and Small Cell Lung Cancer
George Washington University

R01 CA42951 Lloyd — Studies of Normal and Neoplastic Human Pituitary Tissue
University of Michigan

STEROIDS (D)

R01 CA02758 Kandutsch — Steroid Metabolism in Tumors and Normal Tissues
Jackson Laboratory

R01 CA08315 Steroid Induced Changes in Cultured Malignant Cells
Melnykovych University of Kansas, College of Health Sci. and Hosp.

R01 CA13410 Mechanism of Hormone Action on Target Cells in Culture
Sonnenschein Tufts University

R01 CA13533 Ectopic Placental Proteins in Human Cancer
Sussman Stanford University

R01 CA15062 Studies of Normal and Neoplastic Prostate
Ahmed University of Minnesota

R01 CA15648 Steroid Dynamics in Human Endometrial Cancer
Gurpide Mount Sinai School of Medicine

R01 CA18110 Prolactin Synthesis in Normal and Neoplastic Tissue
Gorski University of Wisconsin, Madison

R01 CA20535 Gene Regulation by Steroid Receptor Proteins
Yamamoto University of Calfornia, San Francisco

R01 CA24347 Hormonal Control of Proliferation of Malignant Thymocytes
Thompson University of South Carolina at Columbia

R01 CA25365 Hormonal Regulation of Cultured Endometrial Cells
Gerschenson University of Colorado Health Sciences Center

R01 CA26617 Estrogen Mediated Pituitary Tumor Cell Growth
Sirbasku University of Texas Health Sciences Center, Houston

R01 CA29497 An Adrenal Tumor: Cytochrome P-450 and Steroidogenesis
Hall Worcester Foundation for Experimental Biology

R01 CA29808 Molecular Mechanism of Desensitization
Iyengar Baylor College of Medicine

R01 CA30253 A Study of Tropic Hormone Action in Carcinoma Cells
Mason University of Texas Health Science Center, Dallas

R01 CA32226 Steroid Resistance in Human Leukemic Cells
Harmon U.S. Uniformed Services Univ. of Hlth. Sci.

R01 CA36146 Studies of Regulation in E. Coli Extended to Lymphomas
Bourgeois-Cohn Salk Institute for Biological Studies

R01 CA36370 Tumor-Associated DNA Movement
Selsing Brandeis University

R01 CA37614 Prostatic Androgen Receptors: Form and Function
Muldoon Medical College of Georgia

R01 CA38769 Glucocorticoid Receptors in Childhood Leukemia
Costlow St. Jude Children's Research Hospital

R01 CA39371 Young — Prostaglandin E Promotes Tumor Dissemination — Ball State University

R01 CA39657 Dilley — Hormonal Control of GCDFP-15 Secretion In Vitro — Washington University

R01 CA40104 Haslam — Role of Stroma in Mammary Gland Cell Proliferation — Michigan State University

R01 CA42091 Harrison — Physiology of Pituitary Cell Glucocorticoid Binding — University of Arkansas, Little Rock

R01 CA42755 Distelhorst — Glucocorticoid Receptor Defects in Human Leukemia Cells — Case Western Reserve University

MEMBRANEOUS ORGANELLES (E)

R01 CA06576 Novikoff — Biochemical Cytology of Normal and Malignant Tissues — Yeshiva University

R01 CA08964 Racker — Energy Metabolism in Normal and Tumor Cells — Cornell University, Ithaca

R01 CA10951 Pedersen — Control of Enzymatic Phosphate Transfer in Mitochondria — Johns Hopkins University

R01 CA12858 Stahl — Lysosome Biogenesis: Normal and Tumor Cells — Washington University

R01 CA27809 Sauer — Pathways of Energy Metabolism in Malignancy In Vitro — Mary Imogene Bassett Hospital

R01 CA28677 Coleman — Transport in Cholesterol-Rich Tumor Mitochondria — New York University

R01 CA32946 Fiskum — Transport-Regulated Calcium Metabolism in Tumor Cells — George Washington University

R01 CA37197 Polet — Lysosomes in Growth Control of Human Neoplastic Cells — University of Illinois at Chicago

R01 CA38891 Lane — Integration of Metabolic Pathways in Tumor Mitochondria — Johns Hopkins University

RIBOSOMES AND POLYRIBOSOMES (F)

R01 CA04186 Rich — Molecular Structure of Nucleic Acids and Proteins — Massachusetts Institute of Technology

R01 CA16608 Hardesty — Translation Control in Reticulocytes and Leukemic Cells — University of Texas, Austin

R01 CA21663 Henshaw
Intermediary Metabolism in Animals and in Man
University of Rochester

M-RNA (G)

R01 CA12550 Martin
RNA Synthesis and Transport in Mammalian Cells
University of Chicago

R01 CA16006 Darnell
RNA and Growth Control in Animal Cells
Rockefeller University

R01 CA20124 Saunders
Messenger RNA of Normal and Malignant Human Cells
University of Texas System Cancer Center

R01 CA23226 Fausto
Gene Expression in Regenerating and Neoplastic Livers
Brown University

R01 CA25078 Jacob
Poly(A) Polymerase and mRNA Processing
Pennsylvania State University Hershey Medical Center

R01 CA27607 Lee
Coordinated Gene Expression in Mammaliam Cells
University of Southern California

R01 CA30151 Ledford
Regulation of Albumin Synthesis by Amino Acids
Medical University of South Carolina

R01 CA31810 Rottman
Control of mRNA Processing in Normal and Transformed Cells
Case Western Reserve University

R01 CA31894 Jacob
Control of RNA Synthesis by Carcinogens and Hormones
Pennsylvania State University Hershey Medical Center

R01 CA33953 Volloch
Regulation of mRNA Turnover in Differentiating Leukemia Cells
Boston Biomedical Research Institute

R01 CA36207 Weber
Translation and Stability of Human Heat Shock mRNAs
University of South Florida

R01 CA39066 Linney
Embryonal Carcinoma Growth and Differentiation
Duke University

R01 CA39294 Rapaport
The Cytological and Physiological Mode of AP4A Action
Boston University

R01 CA39931 Peterson
Phenotypic Variation and Neoplastic Progression
John Muir Memorial Hospital, California

T-RNA (H)

R01 CA13591 Randerath
Chemical Studies on Tumor Nucleic Acids
Baylor College of Medicine

R01 CA20683 Control Mechanisms in Human Tumor Cells—Small RNAs
Eliceiri St. Louis University

R23 CA37836 Tumor Specific Differences in tRNA Methyltransferases
Reinhart Philadelphia College of Pharmacy-Science

R23 CA38015 Base-Pairing of Cytoplasmic LMW RNAs in Regulation
Maxwell North Carolina State University, Raleigh

DNA (I)

R01 CA15044 Pathogenetic Determinants of Human CNS Tumors
Manuelidis Yale University

R01 CA23262 Terminal Transferase in Normal and Leukemic Lymphoid Cells
Bollum Henry M. Jackson Foundation

R37 CA24158 Rates of DNA Synthesis in Normal and Transformed Cells
Collins Virginia Commonwealth University

R01 CA26391 Molecular Pathology of Leukemia and Lymphoma
Coleman University of Kentucky

R01 CA34462 Role of Double Minutes and HSR Markers in Tumor Cells
George University of Pennsylvania

R01 CA34784 The Molecular Genetics of DHFR Gene Expression
Melera Sloan-Kettering Institute for Cancer Research

R01 CA39365 Isolation of Amplified Genes from Human Tumors
Roninson University of Illinois at Chicago

R01 CA40621 Gene Expression in the DHFR Locus
Crouse Emory University

R01 CA42047 Amplified Genes in Differentiation-Resistant HL-60 Cells
Gallagher Montefiore Medical Center

R23 CA42702 Molecular Isolation of DNA Polymerase-Alpha Gene
Liu University of Washington

R01 CA43309 New Approaches to Analysis of Biopolymers
Varshavsky Massachusetts Institute of Technology

R01 CA43460 Gene Amplification in Tumors of the Nervous System
Vogelstein Johns Hopkins University

GROWTH FACTORS (J)

R01 CA11176 Factors Required for Mammalian Cell Division
Holley Salk Institute for Biological Studies

R01 CA15744 Spontaneous Transformation and Progression in Cell Lines
Rubin — University of California, Berkeley

R01 CA18608 Interferon-Induced Alterations in Cells
Tamm — Rockefeller University

R01 CA21566 Anti-Tumor Invasion Factors Derived From Cartilage
Kuettner — Rush University

R01 CA23043 Peptide Transforming Factors from Transformed Cells
Ozanne — University of Texas Health Science Center, Dallas

R01 CA24071 Studies of the Receptor for Epidermal Growth Factor
Carpenter — Vanderbilt University

R01 CA25820 Growth Factor Receptors on Normal and Neoplastic Cells
Schlessinger — Weizmann Institute of Science

R01 CA27466 Endothelial Colony-Stimulating Activity
Quesenberry — University of Virginia, Charlottesville

R01 CA28110 Nerve Growth Factor and Granulopoiesis
Young — University of Florida

R01 CA29101 Characterization of a Liver Specific Growth Promotor
La Brecque — University of Iowa

R01 CA29895 Anti-Proliferative Effects of Interferon
Baglioni — State University of New York at Albany

R01 CA30101 Structure and Function of Platelet-Derived Growth Factor
Antoniades — Center for Blood Research

R01 CA30479 Mononuclear Phagocyte-Derived Growth Factor
Gillespie — University of North Carolina, Chapel Hill

R01 CA31615 Growth Factors in Normal and Neoplastic Hematopoiesis
Adamson — University of Washington

R01 CA33209 Leech Antimetastatic Factors: Isolation and Action
Gasic — Pennsylvania Hospital

R01 CA33643 Mechanisms of Regulation of Cell Proliferation
Getz — Mayo Foundation

R01 CA34162 Growth Factors and Cellular Transformation
Scher — Children's Hospital of Philadelphia

R01 CA34472 Ca Growth Control in Neoplastic and Nonneoplastic Cells
Tucker — Johns Hopkins University

R23 CA34590 Biochemistry of a Melanoma Growth Activity
Richmond — Emory University

R01 CA34610 Receptors and Targets for Transforming Growth Factor Beta
Massague University of Massachusetts Medical School

R01 CA34738 Malignancy-Associated Hypercalcemia
Strewler University of California

R01 CA34809 Tumor Promotion and Epidermal Growth Factor Receptors
King University of Illinois at Chicago

R01 CA35373 Hepatopoietins, Liver Regeneration and Carcinogenesis
Michalopoulos Duke University

R01 CA36306 Monokines Which Regulate the Production of CSA
Bagby Oregon Health Sciences University

R01 CA36544 Synthetic Transforming Growth Factors
Tam Rockefeller University

R01 CA36740 Regulation of Myelopoiesis: Suppressor Factor Synergism
Broxmeyer Indiana University-Purdue University at Indianapolis

R01 CA37392 Cartilage and Chondrosarcoma-Derived Growth Factors
Klagsbrun Children's Hospital (Boston)

R01 CA37393 Growth and Migration of Capillary Endothelial Cells
Zetter Children's Hospital Medical, Boston

R01 CA37395 Angiogenesis: A Control Point in Animal and Human Tumors
Folkman Children's Hospital (Boston)

R01 CA37754 Metabolism of the EGF-Receptor
Decker Rockefeller University

R01 CA38684 Autostimulation in Human Hemopoietic Neoplasia
Schrader Walter and Eliza Hall Institute of Medical Research

R01 CA38784 Platelet-Derived Growth Factor in Human Malignancies
Pantazis Center for Blood Research

R01 CA38808 Role of Platelet-Derived Growth Factor in Cell Growth
Huang St. Louis University

R01 CA38939 PDGF Modulated Nuclear Protein
Scher Children's Hospital of Philadelphia

R01 CA38981 Somatomedin Actions in Normal and Transformed Cells
Furlanetto Children's Hospital of Philadelphia

R01 CA39053 Growth Regulation of Polyamine Synthesis
Morris University of Washington

R01 CA39099 Cytoplasmic Factors in Cellular Growth
Bucher Boston University

R01 CA39181 Cellular Actions of Transforming Growth Factors
Magun Oregon Health Sciences University

R01 CA39235 Tyrosine Phosphorylation and the Control of Cell Growth
Frackelton Roger Williams General Hospital

R01 CA39666 The Receptor for PDGF
Hood California Institute of Technology

R01 CA40162 Pancreatic Cancer and Epidermal Growth Factor Receptor
Korc University of Arizona

R01 CA40163 Mechanism of Action of a Human Granulopoietin
Gasson University of California, Los Angeles

R01 CA40124 Functional Characterization of the Isolated PDGF Receptor
Owen Harvard School of Public Health

R23 CA40566 Hematopoietic Regulation and the Marrow Microenvironment
Gualtieri University of Alabama at Birmingham

R01 CA40573 Molecular Studies of Transforming Growth and Factors
Donoghue University of California, San Diego

R01 CA40597 Hemopoietic Cell Regulation in Leukemia
Hines Trudeau Institute, Inc.

R01 CA40614 Tumor Necrosis Factor: Tumor Sensitivity Assay
Rubin New York Blood Center

R23 CA41390 Cell Growth Control Through Membrane Gangliosides
Bremer Rush-Presbyterian-St. Luke's Medical Center

R01 CA41527 Nerve Growth Factor and the Physiology of Neural Crest
Bothwell University of Washington

R01 CA41547 Nonfunctional EGF Receptor in ras-Transformed Cells
Chua Pennsylvania State University

R01 CA41562 Tumor Derived Mediators in Bone Marrow Cell Growth
Lee University of Washington

R01 CA42409 Fibroblast Growth Factor and Neoplastic Transformation
Shipley Oregon Health Sciences University

R01 CA42538 Angiogenesis-Inhibitor-Active Heparin Hexasaccharides
Albersheim University of Georgia

R01 CA42636 PDGF Initiation of Cellular Proliferation
Pledger Vanderbilt University

R01 CA42749 Mechanism of Chemical Carcinogenesis In Vitro
Moses Vanderbilt University

R01 CA42750 Moses — Growth Factors and Receptors in Chemical Transformation
Vanderbilt University

R01 CA42869 Cate — Biosynthesis of the Growth Factor GRP in Lung Cancer
Dartmouth College

R23 CA43320 Fava — Molecular Mechanisms of Growth Factor Action
Vanderbilt University

R01 CA43787 Das — Functional Activation of the EGF-Receptor
University of Pennsylvania

NUCLEUS (K)

R01 CA12226 Paik — Metabolism of NC-Methylarginines and Neoplasia
Temple University

R01 CA12877 Langan — Function of H1 Histone Phosphorylation
University of Colorado Health Sciences Center

R01 CA14054 Klein — Malignant Behavior and Cellular Antigen Expression
Karolinska Institute

R01 CA17782 Reeck — Tumor-Enriched Nonhistone Chromatin Proteins
Kansas State University

R01 CA28679 Biedler — Chromosomal Organization of Dihydrofolate Reductase Gene
Sloan-Kettering Institute for Cancer Research

R01 CA29476 Trent — Clonal Karyotypic Evolution in Human Solid Tumors
University of Arizona

R01 CA34003 Rosenberg — GTP Enhancement of cAMP Binding in Hepatomas
Albany Medical College

R01 CA34783 Rao — Monoclonal Antibodies to Mitotic Cells
University of Texas System Cancer Center

R01 CA36468 Testa — High Resolution Chromosome Analysis of Acute Leukemia
University of Maryland at Baltimore

R01 CA37193 Mears — Human Leukemia/Lymphoma Specific Changes in Chromatin
Columbia University

R01 CA40189 Richter — Nuclear Targeting of Protein
Worcester Foundation for Experimental Biology

R01 CA40593 Davidson — Expression of Chromosome 21 Genes During Hematopoiesis
University of Kentucky

R01 CA42969 Chiu — Hepatoma-Associated Nonhistone Proteins
University of Vermont

R01 CA05493 Leukopoietic Mechanisms
De Bruyn University of Chicago

R01 CA15544 Effect of Microtubular Proteins on Cell Surfaces
Berlin University of Connecticut Health Center

R01 CA16707 Tubulin Associations and Effects of Anticancer Drugs
Timasheff Brandeis University

R01 CA31760 Intermediate Filaments in Normal and Transformed Cells
Goldman Northwestern University

R01 CA33265 Tropomyosin Subunits: Normal and Transformed Cells
Warren University of Miami

R01 CA34282 Biochemical Mechanisms of Cellular Invasion
Rifkin New York University

R01 CA34709 Lymphoma Metastasis/Role of Endothelial Cell Recognition
Butcher Stanford University

R01 CA34763 Mutant B-Actin Gene Structure and Function in Neoplasia
Leavitt Linus Pauling Institute of Science and Medicine

R01 CA36498 Cytoskeleton-Associated Proteins of Lung Carcinomas
Bernal Dana-Farber Cancer Institute

R01 CA37233 The Centrosphere in Normal and Transformed Cells
Albrecht Northwestern University

R01 CA38729 Dynamics of Cancer Cell Traffic in the Lymphatic System
Weiss New York State Department of Health

R23 CA39510 Matrix-Mediated Tumor Cell Migration and Metastasis
McCarthy University of Minnesota, Minneapolis-St. Paul

R01 CA39755 Mechanism of Segregation of Modified Tubulin In Vivo
Bulinski University of California, Los Angeles

R01 CA40599 Tropomyosin in Normal and Transformed Cells
Helfman Cold Spring Habor Laboratory

R01 CA41424 Studies of Mitosis in Normal and Neoplastic Cells
Brinkley University of Alabama at Birmingham

R23 CA42023 Analysis of Novel Mammalian Cytoskeletal Polypeptides
Price Scripps Clinic and Research Foundation

R01 CA42742 Tropomyosins in Normal and Transformed Cells
Matsumura Rutgers University

R23 CA42773 Regulation of Tubulin Biosynthesis
Lau Roswell Park Memorial Institute

DEVELOPMENT AND DIFFERENTIATION (M)

R01 CA02662 Investigations on Teratocarcinogenesis
Stevens Jackson Laboratory

R01 CA13047 Control Mechanisms of Differentiation and Malignancy
Friend Mount Sinai School of Medicine

R01 CA15619 Normal and Malignant Hematopoietic Cell Replication
Cline University of California, Los Angeles

R01 CA16368 Control of Differentiation of Erythroleukemic Cells
Skoultchi Yeshiva University

R01 CA16720 Gene Regulation and Interaction--Normal and Malignant Cells
Klinger Yeshiva University

R01 CA16754 Hybridization DNA Function Mutation in Cell Culture
Littlefield Johns Hopkins University

R01 CA17575 Erythroid Differentiation in Friend Leukemia Cells
Housman Massachusetts Institute of Technology

R01 CA18194 Conversion of Embryonic Cells Into Transformed Cells
Holtzer University of Pennsylvania

R01 CA18375 Hemopoietic Stem Cells and Induced Differentiation
Goldwasser University of Chicago

R01 CA19492 Terminal Transferase in Mammalian Hemopoietic Tissue
Coleman University of Kentucky

R01 CA22294 Quantitative Studies on Granulocyte Differentiation
Kinkade Emory University

R01 CA22556 Differentiation of Granulocytes and Macrophages
Metcalf Walter and Eliza Hall Institute of Medical Research

R01 CA23097 Embryo-Derived Teratocarcinoma
Damjanov Hahnemann University

R01 CA25098 Alpha-Fetoprotein Regulation in Fetal and Cancer Liver
Chiu University of Vermont and State Agriculture College

R01 CA25512 Modulators of Granulopoiesis from Human Cell Lines
Brennan University of Rochester

R01 CA26038 Differentiation and Proliferation of Myeloid Cells
Koeffler University of California, Los Angeles

R01 CA26656 Cell Culture Analysis of Human Epithelial Neoplasia
Rheinwald Dana-Farber Cancer Institute

R01 CA28050 Regulation of Alpha-Fetoprotein Gene Expression
Tilghman Institute for Cancer Research

R01 CA28656 Differentiation of Capillary Endothelial Cells
Auerbach University of Wisconsin, Madison

R01 CA29894 Human Teratocarcinoma-Derived Cell Lines
Andrews Wistar Institute of Anatomy and Biology

R01 CA31271 Differentiation and Stroma-Induction in Neural Tumors
Rubinstein University of Virginia, Charlottesville

R01 CA31945 K-562: A Human Pluripotent Leukemia Stem Cell Line
Lozzio University of Tennessee, Knoxville

R01 CA32186 REC-DNA Analysis of Human Hematopoietic Differentiation
Salser University of California, Los Angeles

R01 CA33000 Glycoproteins in Differentiation and Oncogenesis
Fukuda La Jolla Cancer Research Foundation

R01 CA33434 Molecular Determinants of Tumorigenicity
Zimmer University of Kentucky

R01 CA33664 The In Vitro and In Vivo Regulation of Hemopoiesis
Cronkite Associated Universities-Brookhaven National Laboratory

R01 CA33895 Glycoproteins in Normal and Leukemic Cell Differentiation
Fukuda La Jolla Cancer Research Foundation

R01 CA33946 Teratocarcinoma Cytoskeletal Proteins
Oshima La Jolla Cancer Research Foundation

R01 CA34759 Molecular Basis of Oncogenesis and Differentiation
Tereba St. Jude Children's Research Hospital

R01 CA34891 Molecular Basis of Differentiation and Neoplasia
Roeder Rockefeller University

R01 CA35367 Embryonic Control of Neuroblastoma and Melanoma
Pierce University of Colorado Health Sciences Center

R01 CA35533 Epigenetic Regulation of the Chondrosarcoma
Miller University of Colorado Health Sciences Center

R01 CA37675 Teratocarcinoma Stem Cell Adhesion
Grabel Wesleyan University

R23 CA37727 Induction of Leukemic Cell Maturation
Tsiftsoglou Beth Israel Hospital, Boston

R01 CA37874 Scher — Effect of Proteases in Erythroid Cell Differentiation
Mount Sinai School of Medicine

R23 CA37887 Garvin — Malignant Potential of the Components of Wilm's Tumors
Medical University of South Carolina

R01 CA38138 Vielkind — Fish Melanoma: A Model for Cell Development and Cancer
British Columbia Cancer Foundation

R01 CA38189 Lee — Bone-Bone Marrow Interaction
University of Washington

R23 CA38972 Luikart — Glycosaminoglycans and Differentiation of Human Leukemia
University of Minnesota, Minneapolis-St. Paul

R01 CA39017 Medina — Cell Lineages in Normal and Preneoplastic Mammary Growth
Baylor College of Medicine

R01 CA39036 Gudas — Retinoic Acid--Role in Differentiation and Carcinogenesis
Dana-Farber Cancer Institute

R01 CA39192 Cole — DNA Rearrangements and the myc Oncogene
Princeton University

R23 CA39436 Wright — Differentiation of Cervical Epithelial Cells
Harvard University

R01 CA39667 Liu — Mechanism of cAMP Action—Neuroblastoma Differentiation
Rutgers University

R01 CA39924 Hanratty — The Controlled Initiation of Neoplasm in Drosophila
University of Pittsburgh

R01 CA40165 Cook — A-System Amino Acid Transport and Protein Kinase C
Oak Ridge National Laboratory

R01 CA40438 Yen — Pre-Commitment Regulation of Cell Differentiation
University of Iowa

R23 CA40545 Bender — Characterization of Bone Marrow Derived Granulocytes
University of New Mexico School of Medicine

R01 CA40575 Van Zant — Hemopoietic Stem Cell Proliferation and Formation
Texas Tech University Health Sciences Center

R01 CA41425 Fuller — Endocrine Regulation of Melanoma Cell Differentiation
University of Oklahoma Health Sciences Center

R01 CA41520 Biedler — Human Neuroblastoma Cell Transdifferentiation
Sloan-Kettering Institute for Cancer Research

R01 CA41556 Reiss — Growth Control of Malignant and Normal Keratinocytes
Yale University

R01 CA41829 Stein — Retinoid Regulation of Gene Expression
University of Texas Health Sciences Center, Houston

R01 CA42302 Oshima — Developmental Control of Human Cytokeratin Expression
La Jolla Cancer Research Foundation

R01 CA42520 Elias — $MN^{+}2$-Phospholipid Stim PK and Leukemic Differentiation
University of New Mexico, Albuquerque

R01 CA42664 Miller — In Vivo and In Vitro Differentiation of Leukemic Cells
University of Alabama at Birmingham

R01 CA43225 Cayre — Cloning Genes in Differentiation of HL-60 Variant Cells
Sloan-Kettering Institute for Cancer Research

R01 CA43503 Sidell — Effects of Retinoic Acid on Human Neuroblastoma Cells
University of California, Los Angeles

R01 CA43810 Glass — Hemin Transport Into Differentiating Leukemic Cells
Louisiana State University Medical Center, Shreveport

R01 CA44162 Damjanov — Activation of Primordial Germ Cells to Form Teratomas
Thomas Jefferson University

CELL GROWTH, CELL DIVISION (N)

R01 CA06663 Lieberman — Mechanisms of Control of Mammalian Cell Multiplication
University of Pittsburgh

R01 CA16463 Surks — Thyroid Hormone Effects on Cell Regulation
Montefiore Medical Center

R01 CA17323 Munck — Glucocorticoid-Resistant Leukemic Lymphocytes
Dartmouth College

R01 CA22042 Stiles — Regulation of Gene Expression by PDGF
Dana-Farber Cancer Institute

R01 CA25898 Baserga — Analysis of G1 in Mammalian Cells
Temple University

R01 CA27544 Rao — Purification and Characterization of Mitotic Factors
University of Texas System Cancer Center

R01 CA28240 Scott — Pathology in Cell Cycle Control of Differentiation
Mayo Foundation

R01 CA32094 Taetle — Humoral Control of Leukemic Blast Proliferation
University of California, San Diego

R01 CA32742 Pedersen — Glucose Catabolism in Neoplastic Tissues
Johns Hopkins University

R01 CA33505 Cell Cycle Specific Control of Cellular Differentiation
Yen University of Iowa

R01 CA33764 Analysis of Melanoma Growth and Regression
Amoss Texas Agri. and Mech. University College Station

R01 CA34460 Cell Cycle Control—The Role of Monovalent Cation Fluxes
Adelberg Yale University

R01 CA36063 Malignant Tumor Metastasis: Role of Vitamin K Metabolism
Fasco New York State Department of Health

R01 CA36464 Myelopoietic Regulation By Lactoferrin and Transferrin
Broxmeyer Indiana University-Purdue University at Indianapolis

R01 CA36487 The Role of Calcium in Cell Growth Regulation
Dedman University of Texas Health Science Center, Houston

R01 CA36535 Regulation of Cellular Growth in Multicellular Spheroids
Freyer University of California-Los Alamos National Lab

R01 CA36784 Calcium and Cell Cycle Control in Human Fibroblasts
Tupper Syracuse University at Syracuse

R01 CA36913 Biology and Therapy of Poorly Nourished Tumor Cells
Tannock Ontario Cancer Institute

R01 CA37391 Anticoagulants, Vitamin K, and Tumor Cell Growth
Hauschka Children's Hospital, Boston

R01 CA37673 Regulation of Expression of the Thymidine Kinase Gene
Deininger Louisiana State University Medical Center, New Orleans

R01 CA37789 Polyamine Metabolism and Colon Cancer
Luk Johns Hopkins University

R01 CA39256 Cloning a Mitogen-Regulated Protein
Nilsen-Hamilton Iowa State University of Science and Technology

R01 CA39481 Neoplastic Modulation of Proteoglycan Metabolism
Iozzo University of Pennsylvania

R01 CA39604 Cell Interactions During Malignant Transformation
Bertram University of Hawaii at Manoa

R01 CA39712 Regulation of Adenylate Cyclase Upon Transformation
Manning University of Pennsylvania

R01 CA40332 Studies on Mammalian Cell Proliferation
Soprano Temple University

R23 CA41740 Cellular Uptake and Interaction of Iron and Gallium
Chitambar Medical College of Wisconsin

R23 CA42153 Grimwood — Biology of Basal Cell Carcinoma
Ohio State University

R01 CA42275 McNeil — Ionic Signaling During Quiescent Fibroblast Activation
Harvard University

R01 CA42713 Pledger — Regulation of the Mammalian Cell Cycle
Vanderbilt University

R01 CA42717 Petryshyn — Regulation of Growth and Differentiation by Interferon
Massachusetts Institute of Technology

R01 CA43299 Wu — Molecular Signals of Human Monocyte Maturation
New York Medical College

R01 CA44059 Silverman — Regulation of 2-5A-Dependent RNASE Levels by Interferon
Henry M. Jackson Foundation

METASTASIS (0)

R01 CA33834 Kramer — Tumor Cell Invasion of Microvessel Subendothelial Matrix
University of California, San Francisco

R01 CA36547 Bell — The Role of Cell Movement and Contact in Tumor Invasion
University of Oklahoma, Norman

R01 CA38931 Kupchik — Characteristics Associated with Invasive Human Tumors
Boston University

R01 CA39853 Frost — Mechanism(s) of Tumor Progression and Metastasis
University of Texas System Cancer Center

R01 CA40206 Schaeffer — Role of Cytoplasm in Transfer of Malignant Phenotype
University of Vermont & State Agricultural College

R01 CA40351 Kimura — Properties of Emerging Metastatic Tumor Cell Variants
University of Florida

R01 CA41233 Kerbel — New Approach to Study Human Cancer Metastasis
Mount Sinai Hospital, Toronto

R01 CA42346 Nicolson — Pathogenesis of Human Metastatic Melanoma
University of Texas System Cancer Center

R01 CA42365 Wu — The Molecular Basis of Metastasis
Beckman Research Institute/City of Hope

R01 CA42475 Hendrix — Biological Modulation of Human Melanoma Cell Invasion
University of Arizona

R01 CA42486 Hart — Role of Cell-Surface Sialylation in Tumor Metastasis
Johns Hopkins University

R23 CA42614 Regulation of Hylauronic Acid During Tumor Invasion
Knudson — Rush-Presbyterian-St. Luke's Medical Center

R01 CA42857 Metastatic Potential of Human Colorectal Carcinoma
Jessup — University of Texas

R01 CA42898 Development of Antimetastatic Therapy and Models
Bernacki — Roswell Park Memorial Institute

SOMATIC CELL GENETICS (P)

R01 CA08416 Cytoskeletal Architecture and Gene Expression
Penman — Massachusetts Institute of Technology

R01 CA19401 Genetic Analysis of Human Malignancy
Stanbridge — University of California, Irvine

R01 CA31472 Mechanism of Gene Regulation in Somatic Cell Hybrids
Papaconstantinou — University of Texas Medical Branch, Galveston

R01 CA31995 Analysis of Cellular Oncogene Proto-*myb*
Sheiness — Louisiana State Univ. Med. Ctr., New Orleans

R01 CA33108 Characterization of Lymphoid and Myeloid Transforming Genes
Lane — Dana-Farber Cancer Institute

R01 CA42900 Molecular Basis of G Protein Mutations in S49 Lymphoma
Harris — University of Texas Health Sciences Center

R01 CA43271 Single Gene Effects on Murine Tumor Development
Barker — Jackson Laboratory

R01 CA43331 Genetics of Human Trophoblastic Disease
Surti — Magee-Women's Hospital

R23 CA44176 Recessive Tumor Genes in Neuroectodermal Malignancies
Dracopoli — Massachusetts Institute of Technology

INHERITANCE OF NEOPLASMS (Q)

R01 CA33093 Recombinant Inbred Mouse Strains and Cancer
Taylor — Jackson Laboratory

PLASMIDS, VIRUSES (R)

R01 CA11526 Tumor-Inducing Substance of Agrobacterium Tumefaciens
Kado — University of California, Davis

R01 CA44051 Molecular Genetics of Agrobacterium Plasmids
Farrand University of Illinois, Urbana-Champaign

IN VIVO AND IN VITRO TUMOR LINES (S)

R01 CA17229 Keloids: An In Vitro Model of Tumor Growth Regulation
Russell Meharry Medical College

R01 CA29078 Cellular Origins of Hepatic Preneoplasias
Iannaccone Northwestern University

R01 CA32134 Pathobiology of B16 Melanoma Metastasis
Stackpole New York Medical College

R01 CA32722 Research in Surgical Oncology: Colon Cancer
Aust University of Texas Health Sciences Center, San Antonio

R01 CA33305 Genetic Basis for Spontaneous Cancer and Aging
Rodriguez University of Texas System Cancer Center

R23 CA37238 Regulation of Pituitary Hyperplasia and Neoplasia
Lloyd University of Michigan at Ann Arbor

R01 CA37778 SL12 T-Lymphoma: A New Model for Gene Control in Tumors
MacLeod University of California, San Diego

R01 CA38110 Pathobiology of Metastasis in a New Melanoma Model
Berkelhammer AMC Cancer Research Center

R01 CA38889 Human Mammary Cells: Modulation of Differentiated State
Bartley University of California

CONFERENCES (T)

R13 CA02809 Cold Spring Harbor Symposia on Quantitative Biology
Watson Cold Spring Harbor Laboratory

R13 CA30245 1986 Annual Symposium on Fundamental Cancer Research
LeMaistre University of Texas System Cancer Center

R13 GM35709 Dicennial Review Conference
Porter Tissue Culture Association

R13 AM36420 Gordon Conference on Peptides
Habener Gordon Research Conferences

R13 GM37299 Gordon Research Conference on Molecular Genetics
Capecchi Gordon Research Conferences

R13 CA41014 XIV International Cancer Congress
Mirand New York State Department of Health

SMALL BUSINESS GRANTS (U)

R43 CA39874 Groffen — DNA Probe Assay
Oncogene Science, Inc.

R43 CA40855 Anderson — Cancer Protein Data Base
Proteus Technologies, Inc.

R43 CA40865 Iype — Production of Modulators of Cell Proliferation
Biological Research Faculty & Facility

R43 CA41937 Whitford — Cell Culture Instrument for Cell Cycle Analyses
Theta Cell Systems

R43 CA43347 Piltch — GH3-Derived Growth Factor for T-Cell Progenitor Cells
Upstate Biotechnology, Inc.

PROGRAM PROJECTS (V)

P01 CA10893 Busch — Cancer Research Center
Baylor College of Medicine

P01 CA21901 Roseman — Studies of Normal and Malignant Cell Membranes
Johns Hopkins University

P01 CA22376 Feigelson — Control of Gene Expression: Normal and Neoplastic
Columbia University

P01 CA22427 Pardee — Molecular Analysis of Malignant Transformation
Dana-Farber Cancer Institute

P01 CA23076 Mueller — Regulatory Mechanisms in Tumor Biology
University of Wisconsin, Madison

P01 CA26712 Hynes — Molecular Analyses of Cellular Proteins and Their Genes
Massachusetts Institute of Technology

P01 CA28896 Ruoslahti — Cell-Matrix Interactions in Neoplasia and Development
La Jolla Cancer Research Foundation

P01 CA29545 Carter — Interferon, Differentiation and Oncogenesis
Hahnemann University

P01 CA31768 Rifkind — Leukemia Cell Systems: Induction of Differentiation
Sloan-Kettering Institute for Cancer Research

P01 CA32737 Golde — A Program in Medical Oncology
University of California, Los Angeles

P01 CA34936 Strong — A Mutational Model for Childhood Cancer
University of Texas System Cancer Center

P01 CA36761 Morre — Membrane Regulation—Expression of Cell Transformation
Purdue University, West Lafayette

P01 CA37589 Sato — Cell Culture Factors and Their Relation to Cancer Biology
W. Alton Jones Cell Science Center

P01 CA40035 Mundy — Effects of Tumors on the Skeleton
University of Texas Health Sciences Center

P01 CA40042 Parsons — Oncogenes and Mitogens: Intracellular Mechanisms
University of Virginia, Charlottesville

P01 CA42063 Sharp — Regulation of Gene Expression by Oncogenes
Massachusetts Institute of Technology

P01 CA42762 Ts'o — Oligonucleotide Analogs as Antiviral/Anticancer Agents
Johns Hopkins University

DIFFICULT-TO-CLASSIFY (W)

R01 CA22062 Nimberg — A Bone Resorptive Protein from Cancer Ascites Fluid
Boston University

R01 CA25298 Clark — Biology of Human Cutaneous Malignant Melanoma
University of Pennsylvania

R01 CA38645 Bockman — The Effect of Gallium on Bone
Sloan-Kettering Institute for Cancer Research

R01 CA39040 Massover — Biochemical Pathology of Iron Storage in Liver Cancer
University of Medicine and Dentistry of New Jersey

R01 CA40330 Subjeck — Stress Proteins, Drug Tolerance and Cellular Deprivation
Roswell Park Memorial Institute

R01 CA40474 Barker — Computer Study of Sequences of Amino Acids in Proteins
National Biomedical Research Foundation

R01 CA43174 Furmanski — Cell Fusion and the Generation of Tumor Diversity
AMC Cancer Research Center

ONCOGENES/TRANSFECTION (X)

R01 CA14054 Klein — Malignant Behavior and Cellular Antigen Expression
Karolinska Institute

R01 CA26663 Weintraub — Cell Transformation by RSV
Fred Hutchinson Cancer Research Center

R01 CA28946 Cooper — Transfection by Endogenous Human Transforming Genes
Dana-Farber Cancer Institute

R01 CA33021 Expression of the Human C-K-ras Gene
Perucho State University of New York, Stony Brook

R01 CA35911 Expression and Structure of Invertebrate Oncogenes
Wadsworth Worcester Foundation for Experimental Biology Inc.

R01 CA36246 Chromosome Translocated Oncogenes and Neoplasia
Marcu State University of New York, Stony Brook

R01 CA36327 Isolation of Transforming Genes in Murine Thymomas
Pellicer New York University

R01 CA36355 Expression of Oncogenes and IgA Genes in Transformed Cells
Sonenshein Boston University

R01 CA36827 Oncogenes in Physiologic and Pathologic States
Slamon University of California, Los Angeles

R01 CA36928 Structure-Function Studies of Altered Oncogenic Proteins
Buchanan Massachusetts Institute of Technology

R01 CA37165 c-myc Rearrangements in Human Hematopoietic Neoplasias
Dalla-Favera New York University

R01 CA37222 Properties of Cellular and Viral SRC Genes
Parker Columbia University

R01 CA37351 Inhibition of Human Oncogene Expression
Friedman U.S. Uniformed Services University of Health Sciences

R01 CA37702 The Function of the ras Oncogene Homolog in Yeast
Tatchell University of Pennsylvania

R01 CA37866 Analysis of Human Oncogene Polymorphisms
Krontiris Tufts University

R01 CA37907 Role of Oncogenes in the Pathogenesis of Melanoma
Albino Sloan-Kettering Institute for Cancer Research

R01 CA38047 DNA Rearrangements in MoMULV Induced Thymomas
Tsichlis Fox Chase Cancer Center

R01 CA38571 c-myc Oncogene Regulation in Normal and Malignant Cells
Calame University of California, Los Angeles

R01 CA38635 onc Gene Introduction and Expression in Mice
Brinster University of Pennsylvania

R01 CA38783 The Role of c-myc and Other Oncogenes in Carcinogenesis
Lee University of California, San Francisco

R01 CA38876 Structural and Genetic Analysis of ras p21 Function
Goldfarb Columbia University

R01 CA38901 Oncogene Regulation in Malignant Cells
Klinger Albert Einstein College of Medicine

R01 CA39186 Genetics of Hematopoietic Cancers
Sakaguchi University of Texas Health Science Center, San Antonio

R01 CA39408 Oncogene Expression in Human Bladder Cancer
Viola State University of New York, Stony Brook

R01 CA39550 Regulation and Function of the c-myc Oncogene
Cole Princeton University

R23 CA39803 Expression of Oncogene Proteins in Colorectal Tumors
Gallick University of Texas System Cancer Center

R01 CA39811 Transformation by Human ras Oncogenes
Feramisco Cold Spring Harbor Laboratory

R01 CA39849 Function of Yeast Oncogene Homologues
Broach Princeton University

R01 CA40002 Activation of p53 Gene in Tumor Cells
Rotter Weizmann Institute of Science

R01 CA40099 Functional Analysis of the p53 Cellular Tumor Antigen
Oren Weizmann Institute of Science

R23 CA40242 Role of Oncogenes in Erythroid Differentiation
Lachman Yeshiva University

R01 CA 40364 Expression of Translocated c-myc in B-Cell Tumors
Hayday Yale University

R01 CA40402 The Transforming Potential of Cellular Oncogenes
Sawicki The Wistar Institute of Anatomy and Biology

R01 CA40512 Identification of Cell Cycle-Specific Proteins
Franza Cold Spring Harbor Laboratory

R23 CA40533 Molecular Study of Transforming Genes in Murine Lymphomas
Newcomb New York University Medical Center

R01 CA40540 Neoplastic Transformation by ras Oncogenes
Corces Johns Hopkins University

R01 CA40572 Detection and Analysis of onc Genes in Defined Media
Scangos Johns Hopkins University

R01 CA40582 Effect of myc-Gene Expression on Growth in Lung Cancer
Sorenson Dartmouth College

R01 CA40602 Oncogene Collaborations in Cell Transformation
Ruley Massachusetts Institute of Technology

R01 CA40620 Molecular Studies of Variation in ras Oncogene Proteins
Furth — Sloan-Kettering Institute for Cancer Research

R01 CA40636 Deregulation of Oncogene Expression in Human Tumors
Astrin — Institute for Cancer Research

R01 CA41518 Role of the c-myc Locus in T-Cell Leukemogenesis
Fleissner — Sloan-Kettering Institute for Cancer Research

R01 CA41996 Structural and Functional Analysis of Yeast ras Proteins
Tamanoi — University of Chicago

R01 CA42121 Tumors of the Human Central Nervous System
Goldthwait — Case Western Reserve University

R01 CA42335 Structure and Function of myc Family Genes
Alt — Columbia University

R01 CA42573 Interaction of Oncogenes with Avian Erythroid Cells
Hayman — State University of New York, Stony Brook

R01 CA42795 Reversion of ras Oncogene Mediated Cell Transformation
Axelrod — Rutgers University

R01 CA42810 PA-1 Human Teratocarcinoma Cells as A Model System
Tainsky — University of Texas System Cancer Center

R23 CA42835 Cooperative Effects of Viral and Cellular Oncogenes
Taparowsky — Purdue Research Foundation

R01 CA42836 Proto-Oncogenes and Cellular Proliferation
Leof — Vanderbilt University

R01 CA42978 Biological Activity of Human ras/H Oncogenes
Der — La Jolla Cancer Research Foundation

R01 CA43054 Structure and Function of c-abl Proto-Oncogene
Wang — University of California

R01 CA44311 Transformation and Epithelial Cell Polarity
Matlin — Harvard Medical School

OUTSTANDING INVESTIGATOR GRANTS (Y)

R35 CA39782 Gene Structure, Arrangement, Dynamics, and Expression
Cantor — Columbia University

R35 CA39814 Genomic Changes in Cancer: Mechanisms and Consequences
Sager — Dana-Farber Cancer Institute

R35 CA39825 Sex Hormones and Cancer
Siiteri — University of California, San Francisco

R35 CA39829	Genetics of Cell Proliferation	
Wigler		Cold Spring Harbor Laboratory
R35 CA39860	Genetics of Human Hematopoietic Neoplasias	
Croce		The Wistar Institute of Anatomy and Biology
R35 CA40029	Terminal Differentiation of Epidermal and Adipose Cells	
Green		Harvard Medical School
R35 CA42505	Glycolipids in Differentiation and Oncogenesis	
Hakomori		Fred Hutchinson Cancer Research Center
R35 CA42507	Molecular Mechanisms of Cell Adhesion	
Ruoslahti		La Jolla Cancer Research Foundation
R35 CA42556	Molecular Genetics of Cancer	
Weissman		Yale University
R35 CA42560	Development vs. Neoplastic Proliferation of Stem Cells	
Mintz		Institute for Cancer Research
R35 CA42567	Molecular Basis of Cell Growth and Transformation	
Roeder		Rockefeller University
R35 CA42572	Transforming Growth Factors in Neoplastic Transformation	
Moses		Vanderbilt University
R35 CA42580	Biochemistry of the Cancer Cell	
Erikson		Harvard University

IMMUNOLOGY PROGRAM

The Immunology Program of the National Cancer Institute supports research that contributes to an understanding of the role of the immune system in the development, growth and spread of tumors. The specific areas of investigation supported by the Program include:

- The synthesis and structure of myeloma proteins in animals and man.

- The synthesis, structure, and function of antibodies capable of reacting with tumor cells, agents which induce tumors, and agents used in the treatment of tumors.

- The synthesis, structure, and function of humoral factors other than antibody which participate in, activate and/or regulate the immune response to tumors. This would include complement, interferon, lymphokines, lymphoid cell growth factors, helper factors, suppressor factors, etc., as they are involved in immune responses to tumors.

- The immunobiology of lymphocytes which participate in antitumor responses including their development, heterogeneity, interactions, and functions.

- The immunobiology of monocytes and macrophages which participate in antitumor responses including their development, heterogeneity, interactions, and functions.

- The identification, isolation, and characterization of cell surface determinants of lymphocytes and macrophages which are involved in the responses of these cells to tumors.

- The identification, isolation, and characterization of cell surface determinants on tumor cells which serve as target antigens for the immune response.

- The immunobiology of malignancies of the immune system (lymphomas and leukemias) including studies of immunologic markers for the classification and characterization of neoplastic cells and their normal counterparts.

- Immunobiology of sarcomas, carcinomas, and melanomas including studies of immunologic markers for the classification and characterization of tumor cells and their normal counterparts.

- Immune surveillance against the development of tumors of various origins by all immune mechanisms (e.g. T cell-mediated immunity, macrophage reactivity, natural killer cell activity).

- Immunopathology studies on the host-tumor interaction.

- Immune status of tumor-bearing animals and man including studies on immunostimulation, immunosuppression, and the effects of disease course on immune function.

- Bone marrow transplantation (BMT) in man and animals as a treatment for cancer when the emphasis is on understanding how BMT affects or is affected by the immune system.

- Immunotherapy in animal models including studies on specific and non-specific stimulation of the immune system using natural and synthetic agents when the emphasis is on understanding how the therapy affects or is affected by the immune system.

- Immunotherapy including preclinical and clinical protocols where the main emphasis is upon the study of immune parameters, immune mechanisms, and other immunologic concerns rather than upon a therapeutic result. Included are studies on specific and nonspecific stimulation of the immune system using natural and synthetic agents.

The Immunology Program supports a broad spectrum of research in the areas of basic immunology and tumor immunology. The number of grants funded in each category with their costs is identified in Table 1 of this report. Since the Immunology Program funded 406 grants and expended approximately 66 million dollars during FY 1986, this report serves to highlight selected areas of research and should not be considered comprehensive.

Basic research supported by the Immunology Program has led to an increased understanding of the molecular mechanism by which cells of the immune system function to control the growth and spread of cancer. Rapid advances in biotechnology, especially the development of monoclonal antibodies and the availability of large quantities of genetically engineered lymphokines, are contributing to the rapid translation of basic research findings to clinical applications. Recent progress in the therapy of metastatic cancer by systemic

administration of lymphokine-activated killer (LAK) cells and interleukin-2 (IL-2) has provided a new approach to the immunologic control of cancer (Rosenberg et al., 1985). This new development was made possible by previous extensive research on lymphokines and cell-mediated cytotoxicity against tumor cells. This report will focus on recent developments supported by the Immunology Program, NCI, in the fields of IL-2, molecular mechanisms of cell-mediated cytotoxicity, and the potential use of lymphoid cells for adoptive immunotherapy of cancer.

Some noteworthy items will be mentioned at the end of this report, to identify interesting research findings which may have an impact on future research directions. These areas will not be covered in depth in this report, but may form the basis for research which will be covered in subsequent reports.

INTERLEUKIN-2

Interleukin-2 (IL-2; previously called T-cell growth factor, TCGF) was discovered by Robert Gallo and collaborators at NCI ten years ago (Morgan et al., 1976). IL-2 is produced by T lymphocytes after antigen or mitogen stimulation, and is required for the proliferation of activated T cells, natural killer (NK) cells and other cytotoxic effector cells. IL-2 also enhances the cytotoxic function of these cells. In addition, it plays some role in the proliferation and function of B cells. Since IL-2 also induces or enhances the production of a variety of other cytokines such as B cell growth factors, colony stimulating factors and interferon, it appears to have a central role in the regulation of the immune system as well as hematopoietic cell growth and differentiation. This field has been reviewed by Kendall Smith (CA 17643; Smith, 1980; 1984a).

Dr. Smith and collaborators isolated IL-2 from tonsilar lymphocytes and from a human T-cell leukemia line (JURKAT) which overproduces IL-2. As a result of their work, IL-2 was identified as a slightly basic, glycosylated 15 kd polypeptide. In collaboration with Gerald Crabtree (CA 39612) and Stephen Oroszlan (NCI), they sequenced peptide fragments of IL-2, cloned the gene and determined the genomic sequences coding for IL-2 (Holbrook et al., 1984; Clark et al., 1984; Copeland et al., 1984).

Dr. Smith and colleagues also played a leading role in the characterization of the IL-2 receptor (IL-2R; also called Tac antigen). They purified the high-affinity receptor with the help of a monoclonal antibody to the IL-2R and identified the receptor as a 60 kd protein (Smith et al., 1983). They followed up this work by investigating the functional consequences of the IL-2R interaction and its relevance in the immune system, and found that expression of IL-2R occurs only after appropriate immune stimuli (Cantrell and Smith, 1983; Lowenthal et al., 1985). According to their model, transient expression of IL-2R is the key mechanism whereby the responsiveness of T cells is regulated (Smith, 1984b). In agreement with this model, it was shown by Ellis Reinherz (CA 40134), Martin Hemler (CA 42368) and their collaborators that activation of T lymphocytes with antigen or mitogen results in a transient increase of IL-2R (Hemler et al., 1984; Meuer et al., 1984). These experiments, together

with the findings of Roland Mertelsmann (CA 20194, CA 33873, CA 34995) and colleagues, showing that IL-2 regulates the expression of its own receptors (Welte et al., 1984), are consistent with the model proposed by Dr. Smith and his collaborators.

Recently Dr. Crabtree and colleagues initiated a series of studies on the activation of IL-2 and IL-2R genes in a malignant lymphoma by retroviral insertion (Chen et al., 1985). The gene encoding the human IL-2R consists of 8 exons spanning over 25 kb on chromosome 10. Exons 2 and 4 were derived from a gene duplication event and show an unexpected homology to the recognition domain of human complement factor B. Dr. Crabtree and collaborators have discovered that a Gibbon Ape leukemic cell line (MLA-144) contains two copies of the Gibbon Ape leukemia virus (GALV) adjacent to its IL-2 gene. Introducing hybrid genes, they found that a sequence upstream of the start-site of transcription is essential for the activation of the IL-2 gene by phytohemagglutinin-A (PHA) stimulation (Siebenlist et al., 1986).

Two classes of IL-2R have been identified according to their binding affinity to IL-2. Gabrielle Reem (CA 33653) and collaborators have recently shown that IL-2 induces the expression of both high and low-affinity receptors on thymocytes and T cells in vivo in the absence of other mitogens, and it augments the expression of both classes of receptors on thymocytes stimulated with concanavalin-A (Con-A; Reem et al., 1985). According to their earlier findings, IL-2 is required for the optimum expression of its own receptor on activated lymphocytes and, also, for the maximum synthesis of gamma-interferon (gIFN) in vitro. Immunosuppressive drugs that inhibit IL-2 synthesis, such as dexamethasone, also inhibit the expression of IL-2R and suppress the synthesis of gIFN. Anti-Tac monoclonal antibody, which binds to the IL-2R, down-regulates the expression of receptor and inhibits the synthesis of IFN (Reem and Yeh, 1984). These findings may be of clinical importance since gIFN is the major macrophage activating factor, and this class of drugs may, therefore, inhibit one of the major defense mechanisms against infections and cancer (Reem and Yeh, 1985).

Although IL-2 was originally identified as a T-cell growth factor, it has been shown by Michael Hoffman (CA 17673) and his group that B cells express functional IL-2R (Mittler et al., 1985). Antibody against the receptor inhibits proliferation of B cells and also suppresses secretion of immunoglobulin (Ig). The receptor-specific antibody precipitates a 65 kd protein which resembles the IL-2R obtained from T cells. Similar results were obtained by Robert Stout and collaborators (CA 38408) in B cells which were activated with anti-Ig and T-cell factors (Prakash et al., 1985). Shu Man Fu (CA 34546) and colleagues have demonstrated the presence of IL-2R on activated human B cells (Jung et al., 1984). Dr. Mertelsmann and colleagues suggest that IL-2 stimulates B cells via a low affinity interaction with a receptor different from the Tac receptor identified on T cells, and that the active site on the IL-2 molecule for B cells differs from that for T cell targets (Ralph et al., 1984).

Richard Ford (CA 31479, CA 36243) and colleagues have studied growth factor-mediated tumor cell proliferaton in hairy cell leukemia (HCL), a chronic B cell leukemia in which the neoplastic cells express not only the antigens

characteristic of normal B cell lineage, but also express the Tac antigen (Ford et al., 1985). Their findings suggest that these tumor cells may represent the neoplastic counterpart of a subset of normal B cells that express this putative receptor for IL-2. Interestingly, recombinant IL-2 produced only a marginal proliferative response and could not support leukemic cell growth *in vitro*, even though these cells obtained from six of seven HCL patients expressed Tac antigen. B cell growth factor (BCGF), however, did stimulate *in vitro* growth and supported the establishment of continuous lines in four of the seven HCL cases.

James Griffin (CA 36167) and colleagues have demonstrated that gIFN induces surface expression of IL-2R by normal human monocytes and by the monocytic and promyelocytic cell lines U937 and HL60 (Herrmann et al., 1985). These results suggest the possible involvement of an IL-2R system on human monocytic cells in monocyte/T cell interaction. Thus, not only T and B cells but also normal human peripheral blood monocytes and leukemic cell lines capable of monocytic differentiation express IL-2R when exposed to the T-cell lymphokine gIFN. These findings reveal a previously unknown function of gIFN. The biological consequences of IL-2R expression by normal monocytes have not yet been determined, but these results implicate the IL-2/IL-2R system in the control of macrophage differentiation and/or function. It is also possible that macrophage IL-2R expression adsorbs IL-2 at inflammatory sites, and thus could serve as a negative feedback loop in T cell/macrophage interaction.

The clinical significance of IL-2, preceding its use in the development of LAK cells, has been reviewed by Drs. Mertelsmann and Welte (1984). They studied patients with hemophilia A and found that levels of IL-2 in the supernatants of lymphocyte cell cultures were significantly lower in the hemophiliac population than in controls. They also studied the ability of various factors to induce IL-2 production in peripheral blood lymphocytes from patients with common varied immunodeficiency (CVI). The values of IL-2 production were significantly lower in CVI patients than in controls. With the addition of IL-2, the proliferative response improved in the majority of patients (Kruger et al., 1984). Their data and those of others have shown that CVI patients generally are deficient in IL-2 production which can be partially corrected by IL-2 administration. They have also studied IL-2 production and lymphocyte response to IL-2 in patients following bone marrow transplantation. They found low IL-2 production and low proliferative response in the majority of patients. Again, the proliferative response could be partially restored by IL-2. In addition, total absence of endogenous IL-2 production was found in certain forms of congenital immune defects such as Nezelof's syndrome (Flomenberg et al., 1983a).

MOLECULAR MECHANISMS OF CELL-MEDIATED CYTOTOXICITY

Cytotoxic cells of the immune system make a major contribution to the body's defense against cancer. In this part of the report, we will focus on NCI Immunology Program-supported research involving cytotoxic T lymphocytes (CTL), natural killer (NK) cells and macrophages. Although not completely elucidated, the mechanisms of target cell lysis by different types of effector

cells show many similarities. This topic was reviewed by Giorgio Trinchieri (CA 20833, CA 32898, CA 40256) and Bice Perussia (CA 37155) in 1984.

Cytotoxic T Lymphocytes

Cytotoxic T lymphocytes (CTL) constitute a major arm of the immune system in the defense against virus infections, allogeneic cells (grafts) and malignant cells. The interactions between CTL and target cells proceed through the following steps: recognition and binding of target cells by CTL --> activation of the lytic mechanism and initiation of cell killing --> completion of target lysis. When CTL are confronted with a mixture of cells, some ex- expressing the appropriate antigen (target cells) and others not (bystander cells), only the target cells are lysed. As shown by Herman Eisen (CA 15472, CA 28900), Susumo Tonegawa (CA 28900) and their collaborators, T-cell receptors (TcR) expressed on the surface of CTL appear to be responsible for the specificity of binding of CTL to target cells (Kranz et al., 1984). Binding of a specific ligand to the TcR on CTL activates the cytolytic mechanism leading to the lysis of target cells. Similarly, antibodies to the clonally unique V-region determinants (idiotypes) of TcR were shown to render diverse cells, regardless of their own surface antigens, susceptible to lysis by the corresponding clone of CTL. To extend these findings, Dr. Eisen and collaborators have recently developed a general method for targeting cells for destruction by any CTL, without regard to its TcR idiotype and specificity for antigen (Liu et al., 1985). They used heteroantibody duplexes, formed by covalent attachment of an antibody to the T3 complex, which is associated with TcR on mature T cells, and a second antibody specific for antigen on the target cell. In the presence of the heteroantibody duplex, the target B lymphoma cells were lysed by a cloned human CTL line of unrelated specificity, but not by a non-cytotoxic clone of helper ($T4^+$) T cells. The lysis was specifically blocked by free anti-T3 or anti-target antibody. Their results suggested that anti-T3-containing heteroantibody complexes may be capable of targeting tumor cells for destruction *in vivo* by the body's CTL. How the anti-T3 component of the heteroantibody duplex activates the cytotolytic mechanism of CTL is unclear. However, as previously demonstrated by Stuart Schlossman (CA 25369), Ellis Reinherz (CA 40134), Ian Trowbridge (CA 17733) and their collaborators, the T3 molecular complex and TcR are closely associated on the surface of human CTL (Meuer et al., 1983; Brenner et al., 1985). A particularly attractive feature of the anti-T3-containing heteroantibody complex is that it can activate all CTL subsets, i.e., $T4^+$ and $T8^+$ cells with cytolytic activity. Moreover, by varying the specificity of the second component of the duplex, a wide variety of cells can serve as surrogate targets.

Timothy Springer (CA 31798), Steven Burakoff (CA 34129) and collaborators have studied the role of leukocyte function antigens (LFA) in the interactions between CTL and target cells. LFA-1 is a 95 kd plus 177 kd membrane complex that is expressed on all leukocytes. The involvement of LFA-1 in CTL-target cell interactions has been suggested by the ability of anti-LFA-1 monoclonal antibodies to inhibit CTL-mediated cytolysis. A direct assessment of the contribution of LFA-1 to CTL-mediated killing has become possible by studies on inherited deficiencies in LFA-1 expression. The involvement of LFA-1 in

CTL-mediated cytolysis of lymphoid target cells was investigated using LFA-1-deficient CTL and target cells. The results demonstrated a direct involvement of LFA-1 in CTL-mediated cytolysis of tumor cells (Anderson et al., 1985; Krensky et al., 1985). In addition to LFA-1, LFA-3 was also shown to serve a role in CTL-target cell interactions (Barbosa et al., 1986).

Douglas Faller (CA 37169) and his colleagues have studied the effects of murine retrovirus infection on the recognition of target cells by CTL. They have shown that the MuLV env gene encodes the dominant antigen recognized by MuLV-specific CTL on infected cells. Mice immunized with syngeneic cells expressing the gag gene generate CTL which are capable of lysing MuLV-infected cells. Further studies have suggested that the epitope recognized on the env gene product by BALB/c CTL is different from that recognized by CTL obtained from C57/Bl mice (Flyer et al., 1985).

The emergence of CTL from precursor cells in response to foreign antigens involves a complex series of events including cellular proliferation and differentiation. As Harvey Cantor (CA 26695) and Edward Boyse (CA 39827) demonstrated earlier (Cantor and Boyse, 1975), the induction process requires that CTL precursors interact with cell-bound antigen and with antigen-activated helper T cells or their soluble factors. John Russell (CA 28533) and his colleagues have studied the effects of various biological regulatory substances on the activity of CTL. They suggested that IL-2 may be the principal regulatory factor acting on CTL (Howe et al., 1985). In contrast, gIFN had little effect on the expression of lytic activity, either alone or in combination with IL-2. Dr. Russell has also shown that phorbol esters stimulate the lytic apparatus of CTL. The stimulation is rapid and is specific for the CTL, rather than for the target cells (Russell, 1986).

Zanvil Cohn (CA 30178), Carl Nathan (CA 22090), Eckhard Podack (CA 39201) and their collaborators have recently purified cytotoxic granules from CTL (Young et al., 1986). The purified granules induced rapid Ca^{++}-dependent membrane depolarization and formation of ion channels in target cells. The channels showed poor ion-selectivity and were permeable to all mono- and divalent ions. Ultrastructural examination revealed ring-like structures of 150-200 A in dimension. Of special interest is the analogy of lymphocyte pore-forming proteins to the ninth component of complement which had been shown earlier to polymerize spontaneously, forming transmembrane pores of similar size. Because of their function to perforate target cell membranes, the proteins of transmembrane tubular structures were named "perforins." Dr. Podack and collaborators have succeeded in isolating and characterizing perforins which were shown to form cytolytic transmembrane complexes in a Ca^{++}-dependent reaction (Podack and Konigsberg, 1984). Cytotoxic granules and perforins play similar role in the mechanism of action of NK cells (discussed below).

The possibility that specific proteases are involved in the cytolytic activity of CTL has been suggested by Dr. Eisen and his collaborators from their studies on the effects of protease inhibitors on CTL-mediated cytotoxicity (Pasternack and Eisen, 1985). They have shown that CTL clones possess considerable trypsin-like esterase activity which can be blocked by serine-esterase inhibitors. Using a specific affinity-labeling reagent, they demonstrated that

the esterase activity resides in a 28 kd protein. A wide variety of non-cytotoxic lymphocytes had about 300-fold less serine esterase activity and contained proportionately less 28 kd protein than CTL. Moreover, the esterase activity increased 20 to 50-fold in CTL and the 28 kd protein became more prominent four days after these cells had been stimulated in vitro to generate active CTL. Whether the serine esterase acts selectively like components of complement and blood coagulation cascades is currently under investigation.

Nearly twenty years ago, a cytolytic factor named lymphotoxin (LT), produced by CTL, was identified (Ruddle and Waksman, 1968). The LT-field has been recently reviewed by Nancy Ruddle (CA 16885, CA 29606; 1985). LT is a lymphokine released by activated lymphocytes that kills bystander cells such as fibroblasts and lymphocytes. Its production by mouse T cells is antigen-specific and restricted by MHC genes. It is also produced by human $T4^+$ and $T8^+$ cells and some B lymphoblastoid lines. Dr. Ruddle and colleagues have isolated LT-producing T-cell lines and showed that LT is rapidly internalized into target cells (Schmid et al., 1985). Their results have indicated that LT is capable of directing the digestion of target cell DNA into discretely-sized fragments (Schmid et al., 1986). Dr. Ruddle and colleagues have recently purified LT from class I MHC-restricted CTL and class II restricted cytolytic helpers (Ruddle et al., 1986) and cloned the gene for murine LT (Li et al., 1986).

Carl Ware (CA 35638) and colleagues have also been characterizing cytotoxic lymphokines produced by cloned CTL. They found that clones of human CTL can produce a cytotoxin that is biochemically and antigenically different from LT (Green et al., 1986). Recently it was found that LT has substantial homology with tumor necrosis factor (TNF) which will be discussed later.

Natural Killer Cells

Natural killer (NK) cell activity is mediated by a small, heterogeneous population of peripheral blood mononuclear cells which are classified morphologically as large granular lymphocytes (LGL). These cells lyse transformed target cells in vitro without prior sensitization. An interesting example of target cell specificity of NK cells was reported by Darcy Wilson (CA 14489, CA 15822) and collaborators, who have shown that human neuroblastoma cell lines are susceptible to lysis by NK cells but not by CTL (Main et al., 1985). Cloning of murine NK cells showed that the target specificity of individual clones was indistinguishable.

A series of NK cell clones have been characterized by Dr. Reinherz and his collaborators with respect to the expression TcR alpha and beta genes (Ritz et al., 1985). They found that clones expressing the T3 and T11 antigens also contain the alpha and beta TcR transcripts and express disulfide-linked TcR heterodimers. In contrast, T3-negative clones express only truncated beta transcripts without alpha and gamma transcripts and display no detectable cell surface TcR. Because of the importance of the T11 structure in triggering early T3-negative lymphocytes, Dr. Reinherz and colleagues suggested that the T11 molecule may be important in the activation of NK cells and may serve as the receptor for target antigen. In support of this hypothesis, they have recently shown that antigen-independent triggering of effector cells can induce these cells to express their cytotoxic function in the absence of the T3-TcR complex (Siciliano et al., 1985).

In addition to T11, additional molecules such as LFAs (discussed above under CTL) are also involved in the enhancement of NK cell-mediated cytolysis. Dr. Schlossman and his collaborators suggested that LFA-1 may act as a nonspecific activation factor in NK-target cell interaction (Schmidt et al., 1985a). They have also studied the role of proteoglycans in NK cell-mediated cytotoxicity and found that they are specifically endocytosed with the granules of human NK cells upon contact with sensitive targets, suggesting that they may be involved in the cytotoxic mechanism (MacDermott et al., 1985). Release of proteoglycans is closely correlated with cytolytic activity. Monoclonal antibodies directed against T3 function as cytolytic stimuli and, at the same time, initiate the release of proteoglycans (Schmidt et al., 1986). The mechanism of action of proteoglycans in the cytotoxic process is not known.

Dorothea Zucker-Franklin (CA 34378) and colleagues have shown earlier that aryl sulfatase, which is translocated from specific granules to the cell membrane on contact between effectors and target, participates in cytotoxicity. Subsequently, they have demonstrated that human NK cells carry diverse enzymes on their surface, some having serine esterase activity (Zucker-Franklin et al., 1984). In addition, there are other less well-characterized enzymes which may play a role in cytotoxicity. Douglas Redelman (CA 38396), Dorothy Hudig (CA 38942) and collaborators have shown that cell surface thiols, methylation and complement-like components are involved in the early events, while proteases participate in later Ca^{++}-dependent events in NK cell-mediated cytolysis (Redelman and Hudig, 1985; Hudig et al., 1985). Jacki Kornbluth (CA 37837) has shown that tunicamycin-treated NK and CTL effector cells reversibly lose killer function. Her experiments suggest that carbohydrate determinants are important in the interaction of NK and target cells (Kornbluth, 1985).

Gunther Dennert (CA 39623) and collaborators have demonstrated that lysis of target cells by NK cells involves secretion of cytolytic tubular components (Dennert, 1985; Dennert et al., 1985). The Golgi apparatus (GA) and the microtubule organizing center were shown to reorient in NK cells following binding to their targets (Kupfer et al., 1985, 1986). However, binding per se is not sufficient to affect GA reorientation. It is suggested that target cells emit a signal which is needed for GA reorientation to commence, a process which is dependent on Ca^{++}. The GA-derived cytotoxic granules collect close to the NK-target contact surface (Dennert et al., 1985). Subsequently, these granules were purified and were found to be able to lyse target cells. As in similar studies on CTL, perforins were isolated from the cytolytic granules of NK cells. Insertion of perforins into erythrocyte ghosts causes the release of molecules smaller than 250 kd, suggesting that the overall dimension of lesions induced by NK granules and complement are similar (Criado et al., 1985).

According to a model proposed by Benjamin Bonavida (CA 35791) and Susan Wright (CA 37199), target cells stimulate NK cells to release NK cytolytic factors (NKCF) following binding (Wright et al., 1985; Bonavida and Wright, 1986). The released NKCF is recognized by specific receptors on the target cells, followed by internalization and processing, and ultimately results in cell death. The release of NKCF is mediated by a serine protease

and a protein kinase. Since NKCF has not yet been purified to homogeneity, its relationship to other cytotoxic lymphokines has not been clarified. However, Drs. Bonavida and Wright and their collaborators have found that the target cell specificity of NKCF is different from that of TNF and LT, and were unable to detect TNF activity in supernatants containing NKCF. This suggests that NKCF consists of a number of cytoxic factors, one of which is identical to LT. Thomas Oeltman (CA 33005), James Forbes (CA 33589) and co-workers have detected similar NKCF-like soluble factors in NK cells (Chambers et al., 1984).

Relatively little is known about the factors that regulate NK cell lytic activity. In earlier studies, Drs. Reinherz and Schlossman and co-workers demonstrated that NK cells were capable of proliferating in response to T-cell conditioned media which subsequently led to the clonal propagation of NK cells. These and other studies have suggested that IL-2 has a critical role in the regulation of NK cells *in vivo* (Trinchieri et al., 1984). However, further analysis of NK cell regulation has been difficult because NK cells are heterogeneous and they represent only a minor population of peripheral blood lymphoctyes. The development of human cloned NK cell lines was a major step forward in this field (Hercend et al., 1983). The availability of these clones and pure recombinant IL-2, as well as monoclonal antibodies specific for the IL-2R, provided the opportunity to study the function of this lymphokine in the activation and proliferation of NK cells (Schmidt et al., 1985b). Studies have shown that IL-2 is necessary and sufficient to induce the proliferation of NK cells. However, in comparison to T cells, NK clones needed 10-fold higher concentration of IL-2 for maximal proliferation. Lower density of IL-2 receptors on the NK cells could explain the difference in dose-response relationships. Interestingly, expression of receptors for IL-2 was confined to the G2/M phase of the cell cycle in NK cells, but after activation with anti-T11 antibodies, expression of IL-2R increased and became independent of the cell cycle. From these studies, it is evident that despite their heterogeneity, NK cells are distinct from CTL not only in regard to phenotype and target specificities, but also with respect to specific activation and proliferation stimuli.

Macrophages

The maturation of macrophages in the bone marrow depends on the presence of specific growth factors. This field has been reviewed by Richard Stanley (CA 26504; Stanley et al., 1984; Stanley, 1985). One of these growth factors, called colony stimulating factor-1 (CSF-1) regulates the survival and differentiation of macrophages and their precursors (Jubinski and Stanley, 1985). Dr. Stanley and collaborators isolated cDNA clones encoding human CSF-1 (Kawasaki et al., 1985). CSF-1 is encoded by a single-copy gene, but its expression results in the synthesis of several mRNA species ranging in size from 1.5 to 4.5 kb. Precursor cells possess low levels of CSF-1 receptor which, as a result of CSF-1 binding, is rapidly internalized. An increase in receptor levels occurs prior to the differentiation of precursor cells into adherent proliferating mononuclear macrophages.

Dr. Stanley, in collaboration with Charles Sherr and his group, has recently shown that the CSF-1 receptor is structurally and functionally related to the c-*fms* proto-oncogene product (Sherr et al., 1985; Sherr and Stanley, 1986;

Rettenmier et al., 1986). The c-fms gene is located on the long arm of human chromosome 5 at band q33-34 (Roussel et al., 1983). Interstitial deletions in this region are associated with a variety of hematopoietic disorders including refractory anemia, myelodysplastic syndromes and therapy-related acute myelogenous leukemia. It is suggested that critical mutations or rearrangements in c-fms might predispose to certain forms of myelogenous leukemia. In addition, inappropriate expression of the c-fms gene in fibroblasts and expression of the CSF-1 gene in macrophages could elicit autocrine proliferative responses. Since macrophages function as accessory cells in inflammatory reactions, processing and presenting antigens and producing cytokines, alterations in the CSF-1 and c-fms genes may have broad immunological implications in addition to their suggested role in tumor formation.

Developmental changes induced by growth factors result in the acquisition of endocytic competence and surface expression of complement and Fc receptors in macrophage precursors. After leaving the marrow, these cells enter the circulation as blood monocytes and reach various tissues of the body where they become quiescent resident macrophages. By appropriate stimulation, profound alterations (e.g. increase in size, lytic enzymes, chemotaxis) can be induced in the resident cells, converting them to inflammatory macrophages. The cell biology of this process was reviewed by Dolph Adams (CA 16784, CA 29589) and Thomas Hamilton (CA 39621) in 1984.

The elegant studies of Dr. Cohn and collaborators during the past two decades have shown that macrophage cell surface components mediate the development of inflammatory macrophages (Nathan and Cohn, 1986). When inflammatory macrophages are exposed to various macrophage activating factors (MAF) and other signals, they become "activated" to present antigens to T lymphocytes, to destroy microorganisms, and to kill tumor cells. Mainly as the result of work of Robert Schreiber (CA 34120), Judith Pace (CA 37187), Stephen Russell (CA 38779), Amnon Altman (CA 35299), and collaborators, the major MAF has been recently identified as gIFN (Schreiber et al., 1983; Pace et al., 1983). Yum Yip (CA 37385), with Jan Vilcek and collaborators, recently produced monoclonal antibodies to gIFN and cloned a novel variant of gIFN cDNA (Nishi et al., 1985).

As a result of initial studies done by Dr. Schreiber and his collaborators, it was suggested that gIFN is recognized by specific receptors present on the surface of macrophages (Celada et al., 1984, 1986). The current status of research on interferon receptors has been recently reviewed by Sohan Gupta (CA 29991) and collaborators (Gupta et al., 1985). These investigators have demonstrated that alpha-IFN and beta-IFN bind to common receptors, whereas gIFN interacts with distinctly different receptors on human cells (Rajiuddin and Gupta, 1985). Cross-linking of IFN receptors yielded 300 kd and 150 kd protein bands. The 150 kd protein is a component of the 300 kd complex, suggesting that the receptor either consists of subunits or is associated with a macromolecule in the plasma membrane through non-covalent interactions (Sarkar and Gupta, 1986; Gupta and Rajiuddin, 1986).

Regarding the mechanism of signal transduction mediated by receptors for gIFN on macrophages, Drs. Adams and Hamilton and their collaborators have recently shown that gIFN significantly enhances the activity of protein kinase C (PK-C) (Hamilton et al., 1985). The time course of PK-C activation was found to be

identical to that required for the induction of other functions by gIFN in macrophages. Howard Johnson (CA 38587) and his collaborators have shown that ionophore A23187 was able to substitute for gIFN as the priming signal (Johnson and Torres, 1986).

The identity of gIFN with MAF raises some important conceptual questions since macrophages are one of the major producers of interferons (reviewed by Lucas and Epstein, 1985; CA 27903). Although this issue is still debated (see Adams and Hamilton, 1984), it is possible that the targets and producers of gIFN constitute different subpopulations of macrophages.

Activated macrophages selectively bind to tumor cells in a process that requires expenditure of metabolic energy. Contact with target cells triggers a respiratory burst and activates a membrane-associated oxidase that results in the ad hoc generation of cytotoxic active oxygen radicals (reviewed by Leigh et al., 1985). Dr. Nathan and his collaborators have shown a good correlation between cytolytic activity and generation of active oxygen (Lepay et al., 1985a,b). Interestingly, some human tumor cell lines are resistant to oxidative cytotoxicity (O'Donnell-Tormey et al., 1985). This resistance was not due to increased activities in catalase or other free radical-trapping agents. Studies have recently shown that pretreatment of macrophages with gIFN significantly enhanced H_2O_2-releasing activity (Murray et al., 1985), suggesting that gIFN is the primary factor responsible for the activation of macrophage oxidative metabolism. Also, Dr. Nathan and his colleagues have demonstrated increased secretion of H_2O_2 in gIFN-treated cancer patients (Nathan et al., 1985).

Tumor necrosis factor (TNF) appears to be one of the most effective cytotoxic factors produced by macrophages (reviewed by Flick and Gifford, 1985). The native TNF is a trimer composed of 17 kd subunits and is toxic against a large number of malignant cell lines. The toxic action of TNF is markedly increased by gIFN. B16 melanoma-carrying mice, as well as nude mice carrying a wide variety of human tumors, were cured by the simultaneous administration of TNF and gIFN. Cloning and sequencing of the LT and TNF genes have shown considerable (approx. 50%) sequence homology. These genes are closely linked on the human chromosome 6. Therefore, it was suggested that LT be called beta-TNF to distinguish it from the macrophage-produced alpha-TNF. However, the new nomenclature has not yet been generally accepted (Ruddle, 1986). The gene of cachectin has also been cloned from macrophages and was found to be identical with that of alpha-TNF.

Dr. Yip and collaborators used recombinant TNF for studies of TNF receptors on sensitive (L929) and resistant (FS-4) cells (Tsujimoto et al., 1985). Labeled TNF was found to bind specifically to high affinity receptors on both cells. Incubation of the labeled hormone at 37°C resulted in a rapid internalization and degradation of TNF. These results showed that the mechanism of resistance to TNF is not related to the absence of receptors and/or endocytosis of the ligand-receptor complex. The same group has recently investigated the effects of gIFN on TNF receptors (Tsujimoto et al., 1986), and found that pretreatment of several human tumor cell lines with gIFN increased the specific binding of labeled TNF. The increased binding was due to an increase in the number of TNF receptors with a significant change in binding

affinity; gIFN was the most effective type of interferon. The investigators hypothesized that the enhancing effect of gIFN on TNF action could be due to receptor modulation.

George Gifford (CA 34573) and his collaborators have compared macrophage-mediated cytolysis of murine fibrosarcoma cells to the level of secreted TNF and found a good correlation, suggesting that TNF is responsible for LPS-induced macrophage-mediated lysis of tumor cells (Gifford et al., 1986). Normal fibroblasts were resistant to macrophage-mediated cytolysis. They suggested that suppression of TNF production by a fibroblast-derived factor is responsible for the resistance. They have also demonstrated the need for the continued presence of LPS for TNF production by peritoneal macrophages and have shown that priming by gIFN and activation by LPS results in an increased production of TNF. Priming could also be demonstrated *in vivo*: administration of gIFN into mice resulted in increased yields of TNF following challenge with LPS. Concerning the *in vivo* effects, TNF was detected in the sera of normal mice after injection with LPS; however, treatment with *Corynebacterium parvum* increased the level by 200-fold (Flick and Gifford, 1986). No TNF could be detected in untreated mice.

Recently, Dr. Gifford and his collaborators have examined various parameters for TNF production by LPS-stimulated pulmonary macrophages. Because tobacco smoke is the major etiologic factor in the development of lung cancer, and because smoking had been known to alter the function of alveolar macrophages, they examined the effect of exposure to tobacco smoke on tumoricidal activity of macrophages. A direct cytotoxicity assay of lung macrophages from smoke-exposed animals demonstrated a marked impairment in cytotoxicity. Also, 5-to-10-fold lower levels of TNF and IFN production were observed in LPS-stimulated macrophages (Flick et al., 1985). These results suggest that exposure to cigarette smoke may impair pulmonary macrophage-mediated defense against tumor development.

ADOPTIVE CELLULAR IMMUNOTHERAPY

The recent success in the treatment of cancer using lymphokine-activated killer (LAK) cells has rekindled interest in the potential of adoptive cellular immunotherapy. The basic research described above on the role and mechanism of action of soluble immune mediators, especially the lymphokine IL-2, is rapidly facilitating progress in studies of cellular approaches to cancer therapy. Many laboratories supported by the Immunology Program are focusing efforts on research to determine which immune cell types are most effective in transferring anti-tumor immunity. Animal model systems are necessary to develop the principles upon which new concepts in treatment of human cancer will be developed. With our current understanding of the immune response to tumors, it appears that adoptive immunotherapy will be most successful when the tumor burden is small and when there is no adverse host immune response against passively transferred immune cells. Research indicates that different cell types may be effective in different model systems and that multiple cell subsets may be involved in an interactive fashion in causing and maintaining

the regression of malignancy. The availability of large numbers of cloned and expanded immune cells, now made possible by the availablity of large quantities of IL-2 and other lymphokines, will greatly aid in resolving the functional and phenotypic differences observed in different model systems. NK cells, natural cytotoxic (NC) cells, CTL and anomalous killer (AK) cells are cell populations responsive to IL-2, and their relationship to LAK cells is currently being investigated.

Adoptive Immunotherapy Models

In studies of the immune response to murine immunogenic tumors, Robert North (CA 16642, CA 27794) and colleagues have established that the progressive growth of transplantable immunogenic tumors can evoke the generation of an underlying state of T cell-mediated concomitant anti-tumor immunity which is down-regulated by tumor-specific suppressor T cells after the tumor grows beyond a certain size. In recent reviews, Dr. North concluded that attempts to cause regression of these tumors by immunotherapy may actually be aimed at an inappropriate stimulation of an already suppressed immune response (North, 1984, 1985). Mice bearing a progressive immunogenic tumor generate Lyt-1^-2^+ T cells which are capable, on passive transfer, of causing regression of a smaller tumor in gamma-irradiated recipients. At later stages of tumor growth, suppressor T cells are generated which are capable of inhibiting the ability of passively transferred immune T cells to cause tumor regression (North and Bursuker, 1984; Bursuker and North, 1984). These suppressor T cells display the Lyt-1^+2^-, L3T4^+ phenotype of helper T cells (North and Dye, 1985). Bursuker and North (1985) demonstrated that suppressor T cells are responsible for the decay of concomitant immunity, since infusion of suppressor T cells from donor mice bearing a large tumor can prevent recipient mice from generating concomitant immunity to a tumor transplant. These tumor-induced Lyt-1^+2^- suppressor T cells are functionally distinct from the Lyt-1^-2^+ suppressor T cells that have been previously shown to suppress a tumor-specific delayed-type hypersensitivity (DTH) reaction in tumor-immune recipients, and have recently been shown to function in this system by suppressing the generation of cytolytic T cells (Mills and North, 1985).

Philip Furmanski (CA 33939) has developed a unique model system in which acute Friend virus-induced erythroleukemia in mice undergoes predictable, immunologically mediated spontaneous regression. This particular variant of the Friend murine leukemia virus, RFV, induces a leukemia initially identical to that induced by conventional strains of virus, but which spontanteously regresses in over half the leukemic mice. Fifty percent of the regressor mice are permanently cured; in the remainder, the leukemia recurs spontaneously. Normal T cell and macrophage functions are essential for regression to occur. Dr. Furmanksi and colleagues have shown that leukemia regression can be efficiently induced in progressor leukemic mice by transfer of *in vitro* cultured T cells that are specifically reactive to virus/leukemia cell antigens. This immunotherapy is effective even in fully leukemic animals and requires no concurrent or prior adjunctive treatments, such as irradiation or cytotoxic drugs. Helper Lyt-1^+ cells are implicated in causing permanent disease cures, while cytotoxic Lyt-2^+ cells cause temporary leukemia remission (Johnson et al., 1986). This passive immunotherapy approach is dependent upon obtaining

large numbers of specifically-sensitized T lymphocytes that are capable of eliminating tumors *in vivo*. In this model system, successful therapy is achieved without added treatment of recipients with irradiation or cytotoxic drugs, occurs without supplementation with exogenous lymphokines, and results in a high proportion of permanent cures, even when treatment is initiated in fully leukemic animals.

Philip Greenberg (CA 33084), Martin Cheever (CA 43081) and colleagues have developed a murine tumor model system (Friend virus-induced leukemia, FBL-3) in which adoptively transferred immune T cells can reproducibly mediate an *in vivo* therapeutic effect against disseminated leukemia (reviewed by Cheever et al., 1984a). Recent studies have demonstrated that non-cytolytic T cells can promote the complete eradication of disseminated FBL-3 leukemia without the participation of cytotoxic T cells. Since chemotherapy is known to immunosuppress the host in addition to reducing tumor size, models of adoptive chemoimmunotherapy (ACIT), utilizing both chemotherapy and infusion of immune lymphocytes, have been developed by Drs. Greenberg and Cheever. Several important characteristics of this syngeneic ACIT model system have been determined: the effector cells are immunologically specific, H-2 restricted T cells; they exhibit a dose-response curve in prolonging survival and curing mice; and, they must be capable of proliferating after transfer into the host. It was originally assumed that tumor eradication in this model reflected killing of tumor cells by donor cytolytic T cells. However, therapy with immune donor T cell subsets revealed that transfer of the non-cytolytic Lyt-$1^{+}2^{-}$ subset could cure mice of disseminated FBL leukemia, whereas transfer of potentially cytolytic Lyt-$1^{-}2^{+}$ T cells was ineffective (Greenberg et al., 1985). To evaluate the relative contributions of host and donor T cells to tumor eradication, Drs. Greenberg and Cheever (1984a) used donor cells obtained from congenic mice for adoptive transfer, so that host and donor T cells could be readily distinguished by the expression of either Thy 1.1 or Thy 1.2 antigen. Their results demonstrated that the majority of immunologically competent T cells present in hosts cured by ACIT were of host origin. These host T cells, however, did not make a substantial contribution to the expression of the anti-tumor response and presumably had little role in either tumor eradication or long term maintenance of tumor immunity, which was mediated by T cells of donor origin.

The beneficial use of chemotherapy combined with adoptive transfer of immune cells indicates that, although many drugs currently used in cancer chemotherapy cause severe depression of bone marrow and lymphoid cell functions, under defined conditions some of these otherwise immunosuppressive drugs can potentiate immune responsiveness. Sheldon Dray (CA 30088) and Margalit Mokyr (CA 35761) have studied the potential of low-dose chemotherapy to enhance the tumor-immune response in tumor-bearing mice, and have demonstrated that a low dose of cyclophosphamide (CY) was curative even at a stage of tumor growth when anti-tumor immunity was suppressed. Potent anti-tumor immunity developed shortly after CY therapy and contributed to the eradication of a large tumor. Cured mice were resistant to subsequent tumor challenge (Mokyr et al., 1985). Similar results have been demonstrated using low dose of Melphalan (L-phenylalanine mustard, L-PAM; Dray et al., 1985). These chemotherapeutic agents in low doses overcome the suppressive anti-tumor immune potential of tumor-bearer spleen cells by eliminating the inhibitory activity of splenic macrophages and

metastatic tumor cells. Dr. Mokyr and colleagues demonstrated that the phenotype of immunopotentiating T cells following low-dose CY therapy express the Lyt-1, Lyt-2, and L3T4 phenotypes. Cell depletion and mixing experiments indicated that T cells simultaneously expressing Lyt-2 and L3T4 antigens are required for the exertion of the CY-induced immunopotentiating activity (Mokyr and Ye, 1985). Drs. Mokyr and Dray and colleagues have recently demonstrated that, although the bifunctional form of CY is more effective than the monofunctional form, cross-linking of CY is not an essential property for the immunomodulatory effect or for its direct antitumor effect (Mokyr et al., 1986). These findings shed light on the mechanism of action of cyclophosphamide in ACIT studies.

Dr. Greenberg and colleagues had previously demonstrated the capacity of exogenous IL-2 to induce the growth in vivo of antigen-activated T lymphocytes which had been cultured long-term with IL-2. In vitro, such T cells are exquisitely dependent upon exogenous IL-2 for proliferation and survival. Daily adminstration of IL-2 in vivo, beginning on the day of cell transfer, induced these IL-2-dependent long-term cultured T lymphocytes to proliferate. The magnitude of the in vivo growth was proportional to the dose of IL-2 administered (Cheever et al., 1984b). The ability of exogenous IL-2 to induce donor T cell growth in vivo correlated with its ability to augment the antitumor efficacy of specifically immune donor T cells in this ACIT model of disseminated leukemia. Since unfractionated immune cells contain non-cytolytic T cells that can produce endogenous IL-2 in response to tumor and that can function in tumor therapy, administration of exogenous IL-2 may not be useful for increasing the number and augmenting the therapeutic efficacy of such immune cells. However, CTL which had been activated in vitro and expressed IL-2 receptors could be augmented by administration of exogenous IL-2. Thus in a setting in which helper function is absent, in vivo administration of exogenous IL-2 significantly augmented the anti-tumor activity of immune CTL (Greenberg and Cheever, 1984b). Dr. Greenberg and colleagues have demonstrated that complete eradication of disseminated leukemia can be effected by purified subsets of T cells mediating DTH reactions, which may constitutively produce IL-2 as well as by purified CTL, which are dependent on the availability of IL-2. The administration of exogenous IL-2 as an immunoadjuvant to augment weak anti-tumor responses in vivo may prove to be ineffective, since in this setting helper function is presumably intact and the amount of IL-2 available may, therefore, not be the limiting factor. However, under conditions in which the availability of IL-2 is limiting -- such as following infusion of CTL generated by in vitro culture or effector cells expanded by longterm culture in the presence of IL-2, or possibily following treatment of hosts with chemotherapeutic agents that preferentially deplete helper cells rather than CTL precursors -- the administration of IL-2 may prove to significantly augment in vivo anti-tumor responses.

Immune T cells have been grown in vitro using two related but distinct approaches that allow for the derivation of IL-2-dependent or antigen-driven T cell lines (reviewed by Fathman and Fitch, 1983; CA 19266). IL-2-dependent T cell lines can be generated by activating T cells to express IL-2 receptors by specific antigen stimulation, then inducing proliferation of these antigen-activated T cells by repeated supplementation of the culture medium with IL-2. Alternatively, antigen-driven T cell lines can be generated by intermittent specific stimulation of immune T cells with antigen, without the addition of

exogenous IL-2. Thus, to circumvent the difficulty with IL-2-dependent T cell lines, which are dependent upon exogenous IL-2 and die rapidly without repeated supplementation with IL-2, Drs. Greenberg and Cheever have developed and studied antigen-driven long-term cultured T cell lines (Cheever et al., 1984b). These lines contain Lyt-2^+ and L3T4^+ T cells and were induced to express IL-2R by exposure to antigen. In distinction to IL-2-dependent T cell lines, they produced IL-2 and proliferated _in vitro_ in response to specific stimulation by irradiated tumor cells. These cells could be kept _in vitro_ for more than one month without antigen stimulation and retained the ability to subsequently respond specifically to antigen, thus providing an _in vitro_ equivalent of T cell memory. The results of studies using these cells in an ACIT system demonstrated that tumor-specific antigen-driven long-term cultured T cells could proliferate rapidly _in vivo_, distribute widely to eradicate disseminated FBL-3 leukemia, survive long-term and provide specific immunologic memory long after adoptive transfer (Cheever et al., 1986). This system may provide an alternative to the need to continue to administer exogenous IL-2 for adoptive immunotherapy.

It is likely that different T cell subsets were responsible for mediating cytotoxicity and for producing IL-2. These studies were done with uncloned T cells and it is as yet unknown whether the functional subset(s) of T helper cells required for tumor eradication will survive long-term culture. In these recent studies, tumor-specific donor CTL may have contributed significantly to therapeutic outcome; however, it is highly unlikely that this subset, which presumably lacks the capacity to secrete IL-2 and proliferate in response to tumor stimulation, could mediate tumor elimination alone without IL-2 supplied either by collaborating T helper cells or by exogenous administration. It is still unknown whether disseminated syngeneic tumor can be eradicated by individual T cell clones or whether the progeny of a single cell can produce the requisite lymphokines in the proper proportions to mediate the potentially complex set of interactions between donor and host cells required for tumor eradication. Also, the limited reactivity of a single clone to a single antigenic moiety might prove inadequate for therapy due to either tumor heterogeneity or antigenic modulation. These issues remain to be resolved.

Potential Application to Bone Marrow Transplantation (BMT)

Robert Truitt (CA 39854) and colleagues have been studying the use of cloned CTL in an adoptive immunotherapy model to provide a controlled graft vs. leukemia (GVL) effect after allogeneic BMT (Truitt et al., 1982). BMT is now an established form of treatment for leukemias and lymphomas that are resistant to standard chemotherapy. However, leukemia relapse after transplant remains a significant problem. Newly developed methods to deplete reactive T cells from donor bone marrow have increased the potential of BMT by expanding the pool of available marrow donors which can be considered for use. There is concern, however, that elimination of immune T cells from donor marrow may lead to an increase in relapse rate after this treatment. As previously discussed in this report, the availability of large quantities of purified IL-2 has made feasible preparation of large numbers of cultured lymphocytes for adoptive transfer into tumor-bearing recipients. Dr. Truitt and colleagues

are attempting to prepare immune cells for adoptive immunotherapy which would be directed against a normal histocompatibility or differentiation antigen that is expressed on tumor cells, necessitating the use of allogeneic rather than syngeneic autologous CTL. Allogeneic CTL effector cells have the advantage of being easier to obtain and capable of recognizing a variety of antigens on tumor cells. The difficulties with possible rejection or suppression by host immune cells would not be a problem if this type of adoptive immunotherapy is done in the setting of bone marrow transplantation, in which the tumor-bearing recipient is immunosuppressed.

Dr. Truitt and colleagues examined the nature of the cells responsible for the GVL effect and compared them to cytolytic cells detected in vitro. They found that Lyt-$1^{+}2^{+}$ lymphocytes that were cytotoxic for cultured AKR leukemia cells in vitro could be detected in the spleens of alloimmunized H-2-compatible mice after expansion of the cells in IL-2-containing culture medium. The frequency of leukemia-reactive CTL in the spleen showed a direct correlation with the GVL efficacy of the cells in vivo. Alloimmunization was essential for the induction of the GVL-reactive cell population (Truitt et al., 1983). These populations consisted of heterogeneous cytotoxic specificities: some CTL were leukemia-specific; some lysed only non-leukemic AKR target cells; and a third group mediated killing of both leukemic and non-leukemic target cells. The cytotoxic lymphocytes appeared to be H-2-restricted and specific for non-H-2 antigens shared by the AKR leukemia and the alloimmunizing cells. Studies of intravenous injection of an IL-2-dependent CTL clone indicated that restricted and undesirable tissue distribution, rather that impaired viabililty or loss of antigen specificity, is the major obstacle to successful use of cultured CTL for adoptive immunotherapy of disseminated cancer (LeFever et al., 1984). Further studies in collaboration with Ann LeFever (CA 40430) demonstrated that an effective anti-leukemia reaction was achieved only in tissues where effector and target proximity was maintained, suggesting that cloned CTL, specific for a normal cell surface antigen with limited host tissue distribution and present on tumor cells, could be used for adoptive immunotherapy only if CTL and tumor cell proximity can be attained (LeFever et al., 1985).

Role of IL-2 and IL-2 Receptor (IL-2R) in Effector Cell Activation

Roland Mertelsmann (CA 33873), along with Bo Dupont (CA 22507) and colleagues, has studied the various effects of IL-2 in vitro and in vivo and has demonstrated that IL-2-dependent natural killer (NK) cell lines could be developed from patients with primary T-cell immunodeficiencies (Flomenberg et al., 1983b). All of these polyclonal lines contained a cell population expressing the Leu-5 (sheep red blood cell receptor), 3A1, and OKT10 antigens, but lacking the pan T cell antigens Leu-1 and Leu-4 as well as the markers of T cell subsets, Leu-2a and Leu-3a. OKM1 was only weakly expressed and the Leu-7 antigen, present on all peripheral blood NK cells, was absent from all cultured cells studied, despite the fact that this antigen could be detected on fresh PBL from these patients. Thus, although the PBL of these patients express all the NK markers of normal LGL, the resultant cell lines demonstrated that, in culture, expression of Leu-5, OKT10, and 3A1 increases, whereas

expression of Leu-7 and OKM1 decreases. In addition, one line contained a population of Leu-5^+, $3A1^+$, $OKT10^+$, Leu-$2a^+$, Leu-1^-, and Leu-4^- cells. Three of the lines also contained populations with classic T cell (Leu-1^+ and Leu-4^+) phenotypes. The resultant lines were enriched in NK activity compared with the PBL from which they were derived and their growth was strictly dependent on IL-2-conditioned medium. Highly purified IL-2, lacking any other detectable protein contaminants or lymphokine activities, was capable of supporting the growth of the Leu-5^+, $3A1^+$ "null" cell populations from these lines without alteration in their functional activity or phenotype. Thus, studies of in vitro expanded cell lines from patients with severe disorders of T cell function and thymic involution indicate that this "null" cell population does not require thymic maturation to develop an effector function, and this cell population can be maintained in vitro in the presence of IL-2. These findings are analogous to data obtained from studies of NK cells in nude mice, and suggest a lineage relationship between T cells and LGL clones which express IL-2R.

Dr. Mertelsmann and colleagues have also demonstrated that precursors of cytototoxic lymphoid cells could be obtained from mice treated with cyclophosphamide and expanded in culture by alloantigens in the presence of purified human IL-2. In whole spleen cell cultures, IL-2 generated effector cells which were Thy-1.2^+, Lyt-2.2^+, and were capable of lysing both H-2 compatible and incompatible targets. The activity of these effector cells was not restricted to classical NK cell-sensitive targets. Depletion of T cells from spleen cell populations before culture with IL-2 resulted in generation of effector cells which were enriched in cells capable of lysing NK cell-sensitive targets. While low doses of purified IL-2 restore specific CTL responses in vitro, these investigators found that high concentrations of IL-2 generate non-specific cytotoxic T cell effectors. These effector cells are Thy-1.2^+ and lyse target cells of various H-2 types and, more importantly, target cells described as classically insensitive to NK cells. Non-T cells also responsive to IL-2 can develop into cytotoxic NK-type killer cells. Some precursors of these NK-type cytotoxic cells are Lyt-2.2^+. It appears that cytotoxic T cells can be driven by lymphokines to differentiate into cells with NK-like characteristics. Since NK cells do bear very low density Thy-1 antigen, it is not surprising that some of these precursor cells are directly responsive to IL-2. These studies demonstrate that IL-2 stimulates a varied population of normal lymphoid cells (Thy-1^- and Thy-1^+) which differentiate into cytotoxic effector cells having both T-like and NK-like characteristics (Merluzzi et al., 1984). These investigators have recently demonstrated in vitro and in vivo that primary explants of spontaneous mammary tumors from mice are susceptible to lysis by recombinant human IL-2-stimulated syngeneic T lymphocytes (Merluzzi et al., 1985a).

Paul Sondel (CA 32685) and colleagues have previously shown that the addition of crude IL-2 to a sensitization culture of peripheral blood lymphoctes (PBL) with irradiated HLA-identical leukemia cells following six days of activation can expand a weak response to minor histocompatibility antigens (Sondel et al., 1983). In studies adding purified recombinant IL-2 to these sensitization cultures at their initiation, it became clear that highly purified recombinant IL-2 by itself could activate a strong proliferative response by fresh PBL (Lifson et al., 1986). Dr. Sondel and colleagues have evaluated the immunologic specificity of these IL-2-activated LAK cells, which can destroy

autologous and allogeneic leukemia in vitro, and found that these cells were able to mediate potent destruction of nonmalignant cells, including Epstein-Barr Virus (EBV)-transformed lymphoblastoid cell lines. Furthermore, mitogen-activated lymphocytes, as well as resting cultured lymphocytes, could be destroyed. In a sensitive eight-hour chromium-release assay, it was possible to demonstrate that even fresh PBL are destroyed by these LAK cells (Sondel et al., 1986). Research is continuing to investigate the recognition mechanism involved in the destruction of normal PBL target cells, as compared to destruction of neoplastic cells. The IL-2 proliferative response appears to be dependent on interactions between different subpopulations of PBL, but probably does not simply reflect augmentation by IL-2 of antigen-driven or autoreactive processes. Both T and non-T cells proliferate in response to IL-2. T cell populations reconstituted from panned Leu-3^+ and Leu-2^+ subsets did not proliferate as well as unseparated T cells, probably reflecting additional depletion during the panning and sorting procedures of small numbers of contaminating non-T cells. These non-T cells may be the primary targets for IL-2 in this system, exerting their secondary proliferation-inducing effects on Leu-2^+ and Leu-3^+ cells through either cell-cell contact or secreted soluble factors. The observed proliferation of T cells may involve interactions of IL-2 with the IL-2R; the response of non-T cells may be explainable on the same basis.

Bijay Mukherji (CA 40361) and colleagues have very recently reported their studies of the generation and regulation of LAK cells derived from PBL from cancer patients. Although LAK precursors were M1 negative, the presence of a minimal number of monocytes was essential for the generation of LAK cells. The induction of LAK activity could be inhibited by the presence of anti-IL-2R and anti-T3 monoclonal antibodies, while anti-T4 and anti-T8 antibodies had no effect. When added at the effector phase, however, monoclonal antibody to T3, T4 and T8 inhibited LAK activity. Monoclonal antibody to T11, IL-2R, Ia and Class 1 MHC had no effect. Complementmediated lysis of $OKT4^+$ or $OKT8^+$ cells also diminished LAK activity. As the LAK activity of a culture waned, a population of cells emerged that was able to suppress the generation of LAK cells in fresh PBL, but did not suppress LAK activity when added at the effector phase. LAK suppressors were found in both the $T4^+$ as well as the $T8^+$ fractions of the suppressor population (Nashed and Mukherji, 1986).

Relationship of LAK Cells to Other Lymphoid Cells

Many laboratories are investigating the cell of origin of the LAK cell to better understand the mechanism of action of this effector cell and to further determine the relationship of this form of cellular immune response to those previously described. Dr. Mertelsmann and colleagues found that, although there is little or no IL-2 production after BMT in patients and in animal systems, the recipients of either syngeneic or allogeneic marrow do express IL-2R, and if IL-2 is supplied exogenously, T cell proliferation and LAK cell generation can be induced in vitro. Although classical CTL responses are not inducible for at least one month after transplant in the murine system, there is a clear LAK cell response as early as one week after transplant (Merluzzi et al., 1985b). Since LAK cells have been shown to be effective in reducing

metastases, they may play a role in enhancing a graft vs. leukemia effect in bone marrow transplant recipients treated for leukemia; however, LAK cells may also have a deleterious effect in enhancing graft vs. host disease. The role of LAK cells following BMT should be carefully studied to evaluate the benefits and risks of IL-2 for maintenance therapy after transplant.

Arthur Bankhurst (CA 24873) and colleagues have investigated the role of fetal bovine serum (FBS) or human serum which is present as a culture supplement in the generation of human LAK and NK cell activity *in vitro*. Their studies demonstrated that FBS markedly inhibited generation of LAK cell activity, but not NK cell activity, relative to human serum, and their results suggested that human serum is preferable to FBS in studies concerning human LAK cell generation (Imir et al., 1985).

Sidney Golub (CA 34442) and colleagues are studying the relationship of LAK cell precursors to NK cells, investigating the possibility that LAK cells are simply IL-2-expanded NK cells, since these cells share a number of properties. These investigators have described a "NK-like" or "anomalous killer" cell, derived by activation in mixed lymphocyte culture (MLC), that could kill a variety of target cell types, including NK-resistant targets, and could be derived from Fc-receptor positive or negative precursors (Gray et al., 1983/84). However, LAK cells can be generated from cell populations that are naturally NK deficient, such as thymocytes. Furthermore, some LAK precursors are resistant to the lysosomotropic drug L-leucine-O-methylester (LeuOMe), which eliminates mature NK cells (Shau and Golub, 1985a). Their results indicate that NK activity can be regenerated from LeuOMe-treated cells by incubation with either IL-2 or MLC, but not by incubation with IFN. The cells responsible for the regeneration of NK activity appear to be large agranular lymphocytes which are resistant to treatment with this agent but have the same buoyant density as NK cells. Dr. Golub and colleagues propose that the direct precursor of NK cells is the large agranular lymphocyte and that the signal for generation of NK activity from this precursor is IL-2. The signals involved in the development of IL-2-generated cytotoxicity appear to be quite complex. These investigators have found that the development of this type of lymphokine-activated killing can be inhibited with anti-Tac antibodies, although FACS-sorted Tac-negative cells can make a cytotoxic response to IL-2. Cyclosporin A inhibits the acquisition of IL-2R, but has no effect on the IL-2-induced cytotoxic response (Gray et al., 1985). The development of cytotoxic cells in IL-2 can be inhibited greatly by the addition of antibodies to the transferrin receptor during the period of IL-2 culture, but these antibodies have no effect on mature cytotoxic effector cells. These investigators propose that the transferrin receptor, which appears during stimulation of cell division by IL-2, is the next key stage after the IL-2R in the development of the cytotoxic cells. These signals then lead to an IFN-responsive stage of development among the mature cytotoxic effectors. Their results demonstrate that IL-2 can generate cytotoxic cells from NK-inactive cells (Shau et al., 1986). This work has recently been reviewed (Shau and Golub, 1985b), and much work remains to be done to evaluate the similarity or identity of the target structures in recognition systems for both cell types. Target specificity, cell regulation, and precursor phenotype all link NK cells to LAK cells.

Giorgio Trinchieri (CA 20833, CA 40256), Bice Perussia (CA 37155) and colleagues have also studied the effect of IL-2 on NK cytotoxicity and proliferation. The availability of their monoclonal anti-Fc receptor antibody B73.1 has enabled them to separate pure populations of NK cells, to define their surface phenotype and ability to produce various types of lymphokines, and to study the ability of purified NK cell preparations to be activated or induced to proliferate by IFN, phorbol diesters and IL-2 (London et al., 1985). Trinchieri et al. (1984) demonstrated that recombinant IL-2 induces a rapid and potent enhancement of the spontaneous cytotoxicity of human lymphocytes. The IL-2-induced cytotoxic effector cells have surface markers of NK cells and are generated from the same PBL subset mediating spontaneous NK activity. Unlike activated proliferating T or NK cells, resting PBL reveal only a very limited number of high affinity IL-2 receptors as demonstrated by either IL-2 binding or by reactivity with anti-Tac antibody. It is unknown which type of surface IL-2R allows resting mature peripheral blood NK cells to respond rapidly and efficiently to IL-2 with enhanced cytotoxic activity and IFN production. Like IFN, IL-2 induces increased cytotoxicity against a large panel of NK sensitive or relatively resistant target cells. Recombinant IL-2 induces gIFN in lymphocyte cultures and, in short term cultures, NK cells appear to be the major producer cells, whereas T cells are unable to produce gIFN in response to recombinant IL-2. The kinetics of enhancement of cytotoxicity and production of gIFN, and the inability of anti-gIFN monoclonal antibodies to suppress the IL-2-dependent NK enhancement suggest that the effect of recombinant IL-2 on NK cells is independent of gIFN. The enhancement of NK cell activity by IL-2 precedes any proliferative response of the lymphocytes. By contrast, proliferation is observed in longer cultures in cells with both NK or T cell markers. In this study, anti-Tac antibody was unable to reduce the IL-2 mediated enhancement of NK activity in short term cultures although even much higher dilutions of anti-Tac antibody have been reported to block generation of LAK cells in longer cultures, as well as blocking IL-2-dependent human T-cell proliferation. IL-2-induced proliferation of NK cells is completely blocked by anti-Tac antibody. Thus, Dr. Trinchieri indicates that a role for the IL-2R recognized by anti-Tac antibody in NK cell enhancement cannot be excluded. These investigators are currently analyzing the binding of IL-2 to NK cells in an effort to define these receptors and determine their relationship to Tac antigens.

Osias Stutman (CA 15988, CA 17818) and colleagues have been studying various effector cells mediating natural cell-mediated cytotoxicity (NCMC), a term which has been used generically to refer to the lysis of tumor target cells _in vitro_ by a family of effector cell types. In mice, at least two distinct NCMC effector systems have been identified: NK and natural cytotoxic (NC) cells. NK and NC cells differ in strain distribution of activity and expression of cell surface antigens. While NC and NK share many characteristics, sufficient differences exist to demonstrate that their activities are the function of distinct, although perhaps overlapping, effector cell populations (reviewed by Lattime et al., 1985; 1986). Dr. Stutman and colleagues have determined that the generation of culture-activated killer (AK) cells is IL-2-dependent, and requires self-Ia recognition (Lattime and Stutman, 1985). Following three days of culture with FBS, murine spleen cell cultures contain at least two cytotoxic populations, one expressing an NK cell phenotype and target specificity (Qa-5$^+$, Thy-1$^+$, Lyt-2$^+$) and the second sharing the characteristics of the NC

cell (Qa-5$^-$, Thy-1$^-$, Lyt-2$^-$). Generation of the NK-type AK effector cell requires the presence of a pre-AK cytotoxic cell (Qa-5$^+$, Lyt-2$^-$) and is dependent on the generation of IL-2 during the culture period, while the NC-type AK effector cell is independent of IL-2 production. IL-2 production in the cultures requires syngeneic Ia recognition by an Lyt-1$^+$, L3T4$^+$ T cell. These investigators propose a role for IL-2 in the *in vivo* regulation of NCMC. The levels of IL-2 produced in the syngeneic response are consistently lower than those found following allogeneic or mitogenic stimulation, but are sufficient to allow the development of activated NK cells *in vitro* which manifest the same target specificity as that found in freshly harvested NK cell preparations. Taken with the demonstrations that increased IL-2 levels result in the generation of effector cells with significantly increased target repertoire (i.e., as in the development of LAK cells), and that cloned cytotoxic T cells lose their target specificity following culture in high concentrations of IL-2, the generation of these low levels of IL-2 may represent one mechanism for the *in vivo* regulation of NCMC. It appears that the various reports of AK cells and their target specificities may be dependent on the relative amounts of IL-2 and, perhaps, of other lymphokines present and/or generated in the various culture systems used. These investigators have previously demonstrated that co-culture of Ia antigen-expressing macrophages (most probably dendritic cells) with syngeneic Lyt-1$^+$ T cells results in the production of low levels of IL-2 and other lymphokine activities. IL-2 production required Ia expression on the macrophage population and was independent of any exogenous antigenic or mitogenic stimuation. These findings have led to the hypothesis that such a syngeneic cell-cell interaction might be a mechanism for the production of IL-2 *in vivo* and thereby may play a role in normal T cell maturation, as well as stimulation of NK and NC cell activities. Dr. Stutman and colleagues proposed that IL-2 stimulated by this mechanism *in vivo* may be sufficient to induce the differentiation or activation of NK cell function which they have observed *in vitro*. Thus, the role of IL-2 in expanding and maintaining long-term cell lines remains to be further exploited, and further studies are necessary to investigate the role of endogenously produced IL-2 in regulation of the *in vivo* immune response.

ADDITIONAL NOTEWORTHY FINDINGS

Several recently reported observations are worthy of mention, although the long range significance of these findings, including their diagnostic and/or therapeutic applications, are unclear at this time.

- It has been known that antigenic stimulation of a T lymphocyte population induces both antigen-specific T cell response and proliferation of T cells with specificities unrelated to the stimulating antigen. For such a phenomenon to occur, there must be a mechanism whereby antigen/MHC interaction with TcR/T3 on one antigen-specific T cell activates other resting lymphocytes regardless of their antigen/MHC specificity. Recently, Reinherz and his colleagues have found a T_H cell-derived lymphokine which may have a key role in this phenomenon

(Milanese et al., 1986). They have shown that a novel 10-12 kd lymphokine is secreted from T_H cells after crosslinking their TcR-T3 complex. This lymphokine, named interleukin-4A (IL-4A), stimulates resting lymphocytes by binding to a surface component of the alternative T11 pathway and by subsequently inducing receptors for IL-2. Therefore, it appears that IL-4A is a mediator involved in amplifying the T cell immune response.

o Functional correlates are evolving from studies of the transferrin receptor (TR). There is a correlation between the state of cell activation and expression of the TR. Ian Trowbridge (CA 34787) and his colleagues (CA 25893) have used monoclonal antibodies against murine TR to eliminate antigen-activated T cells, a method superior to others thus far developed for producing specifically unreactive T cells (Rammensee et al., 1985). In earlier studies Thomas Hamilton, Dolph Adams and co-workers (CA 29859) discovered that expression of the TR may be a useful marker of the response stage of macrophage functional activation and concomitant membrane changes (Hamilton et al., 1984). More recently they have shown that the number of TR's in thioglycollate-elicited murine peritoneal macrophages is markedly depressed after exposure to murine gIFN (Weiel et al., 1985). The inappropriate expression of TR on carcinogen-altered hepatocytes appears to give competitive advantage to those cells in iron metabolism and may serve as a cellular marker for likely development of malignancy (Allison et al., 1986; CA 40040).

CONFERENCE SUPPORT

The Immunology Program provided funds for partial support of the following conferences in FY 1986:

"Conference on Membrane-Mediated Cytotoxicity"
March 9-16, 1986 Colorado

"Third International Workshop for Natural Killer Cells"
July 3-6, 1986 Canada

"Sixth International Congress of Immunology"
July 6-11, 1986 Canada

"Eleventh Congress of the Transplantation Society"
August 3-8, 1986 Finland

"Third International Conference on Human Leukocyte Differention Antigens"
September 21-25, 1986 England

FISCAL YEAR 1986

IMMUNOLOGY PROGRAM

SUMMARY OF GRANTS BY SUBCATEGORY

(Includes P01, R01, R15, R23, R35, R43, U01, R13 Grants)

Dollars in Thousands

Subcategory	No. of Grants	Total Costs Awarded
Myeloma Proteins	9	$ 1,562
Cell Surface Antigens	49	8,358
Cell Surface Determinants of Lymphocytes & Macrophages	47	7,951
Humoral Factors Other Than Antibody	42	5,883
Tumor-Related Antibodies	15	2,023
Immunobiology of Sarcomas, Carcinomas & Melanomas	10	2,105
Host/Tumor Immunopathology	8	1,235
Effects of Disease on Immune Function	15	1,620
Immunotherapy: Mechanisms Rather Than Therapeutic Result	10	1,211
Lymphocytes	92	17,128
Monocytes & Macrophages	30	5,380
Malignancies of the Immune System (Lymphoma/Leukemia)	19	3,075
Immune Surveillance	31	3,690
Immunotherapy in Animal Models	9	1,262
Bone Marrow Transplantation	15	3,352
Conference Grants	5	32
	406	65,817

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Antigens of Malignant Melanoma and Other Human Tumors
Sloan-Kettering Institute for Cancer Research

R01 CA23770 Haughton
Antigen-Induced Lymphoma
University of North Carolina, Chapel Hill

P01 CA25874 Koprowski
Human Melanoma and Tumor Specific Monoclonal Antibodies
Wistar Institute of Anatomy and Biology

R01 CA27416 Mohanakumar
Characterization of New Human IA and Leukemia Antigen
Virginia Commonwealth University

R01 CA28420 Reisfeld
Molecular Profile of Human Melanoma Antigens
Scripps Clinic and Research Foundation

R01 CA28564 Carey
Human Squamous Cell Carcinoma: Culture and Serology
University of Michigan at Ann Arbor

R01 CA29964 Haughton
Immunocytomas
University of North Carolina, Chapel Hill

R01 CA30647 Irie
Human Monoclonal Antibodies to OFA-I
University of California, Davis

R01 CA31828 Ricardo
Immune Response to Syngeneic Leukemic B-Cell Antigens
Wake Forest University

R01 CA32632 Klock
Glycoconjugate Structure and Function in Leukocytes
Medical Research Institute of San Francisco

R01 CA33529 Cullen
Processing of IA Molecules in B Cells and Macrophages
Washington University

R01 CA34232 Beisel
Expression of H-2 Antigens on SJL/J Tumors
Johns Hopkins University

R01 CA34342 Paque
Selecting Expressed Tumor Immune RNA with Hybridomas
University of Texas

R01 CA34368 Ostrand-Rosenberg
MHC Antigen Expression on Teratocarcinoma Cells
University of Maryland, Baltimore County

R01 CA34778 Walker
Analysis of Non-Small Cell Lung Carcinoma Antigens
Scripps Clinic and Research Foundation

R01 CA35592 Underhill
Structure and Function of Cell Surface Hyaluronate
Georgetown University

R01 CA35929 Carey
Monoclonal Antibodies to Human Squamous Cancer Antigens
University of Michigan at Ann Arbor

R01 CA37099 Goodenow
Immunogenetics of Unique Tumor-Specific MHC Antigens
University of California, Berkeley

R01 CA37156 Schreiber — Immunobiology of Unique Tumor-Specific Antigens
University of Chicago

R01 CA37169 Faller — Tumor-Specific CTL Recognition of Transfected Cells
Dana-Farber Cancer Institute

R01 CA37303 Pan — Structure-Function Studies of SV40 TSTA and H-2
Wistar Institute of Anatomy and Biology

R01 CA37645 Humphreys — Function and Structure of Leukemic Cell II
University of Massachusetts Medical School

R01 CA37868 Ball — Monoclonal Antibodies to Lung Small Cell Carcinoma
Dartmouth College

R01 CA39054 Callahan — Cell Surface Antigens of Murine Tumors
Colorado State University

R01 CA39559 Ferrone — Class II HLA Antigens in Melanoma
New York Medical College

R01 CA39612 Crabtree — IL-2 Receptor in the Pathogenesis of Human Lymphoma
Stanford University

R01 CA40041 Allison — Surface Antigens of Murine T Lymphocytes
University of California, Berkeley

R01 CA40134 Reinherz — TI A and B V-Gene Usage in Human T-Cell Malignancies
Dana-Farber Cancer Institute

R01 CA40216 Nadler — Characterization of Human B-Cell Activation Antigens
Dana-Farber Cancer Institute

R23 CA40311 Imam — Characterization of Mammary Carcinoma-Associated Antigen
University of Southern California

R01 CA40524 Coggin — Characterization of Oncofetal Antigens
University of South Alabama

P01 CA41085 Edgington — Extrinsic Protease Pathways in Neoplasia
Scripps Clinic and Research Foundation

R01 CA41366 Nakamura — Immunobiology of the Hemopoietic Histocompatibility
Research Foundation of SUNY at Buffalo

R01 CA41993 Webb — Immunobiology of MIS Responses
Scripps Clinic and Research Foundation

R01 CA42046 Posnett — T-Cell Leukemia and the Human T-Cell Antigen Receptor
Cornell University Medical College

R01 CA42049 Marchalonis — Immunoglobulin-Related T-Lymphocyte Surface Receptors
Medical University of South Carolina

R01 CA42246 Human Macrophage Activation Antigens
Todd — University of Michigan at Ann Arbor

R01 CA42361 Immunosuppressive Neuroblastoma Tumor Gangliosides
Ladisch — University of California Los Angeles

R01 CA42368 New Growth-Regulated Fibroblast/Lymphocyte Antigens
Hemler — Dana-Farber Cancer Institute

R01 CA42104 MHC Coded Alloantigens on Lung Tumors
Martin — University of Southern California School of Medicine

R35 CA42504 Reactions of Cytotoxic T Lymphocytes with Target Cells
Eisen — Massachusetts Institute of Technology

R01 CA42738 Autoimmunity to Testicular Germ Cell Oncofetal Antigens
Anderson — Brigham and Women's Hospital

R01 CA42923 Human Mammary Epithelial Antigens
Dion — Institute for Medical Research

R23 CA43488 Antigen Processing by Cloned Antigen Presenting Cells
Cohen — University of Kentucky

R01 CA43489 Biochemistry and Function of LY-5/T200 Molecules
Ewald — Montana State University

R01 CA44276 Cell Surface Antigens of Sarcomas
De Leo — University of Pittsburgh

CELL SURFACE DETERMINANTS OF LYMPHOCYTES AND MACROPHAGES (CS)

R01 CA04681 Genetic Studies with Mammalian Cells
Herzenberg — Stanford University

R01 CA12851 Embryonic and Viral Induced Tumor Cell Membrane Antigens
Sanders — University of Texas, Austin

R01 CA18640 Behavior of Weak Transplantation Antigens
Silvers — University of Pennsylvania

R01 CA18659 Chemical, Genetic and Cellular Aspects of Immunogenicity
Gill — University of Pittsburgh

P01 CA21112 Clinical and Basic Studies of Plasma Cell Dyscrasias
Osserman — Columbia University

R01 CA21651 Teratocarcinoma and Embryonal Tumors: Surface Antigens
Artzt — Sloan-Kettering Institute for Cancer Research

P01 CA22507 Immunogenetics of the Major Histocompatibility Complex
Dupont — Sloan-Kettering Institute for Cancer Research

R01 CA24473 David
Genetics and Functions of (H-2 Linked) I Region
Mayo Foundation

R01 CA25532 Schwarting
Glycolipids of Murine and Human Lymphocytes
Eunice Kennedy Shriver Center Mental Retardation

R01 CA25613 Ross
Membrane Components of the Leukocyte Complement System
University of North Carolina, Chapel Hill

R01 CA25893 Hyman
Cell Surface Molecules: Hematopoietic Differentiation
Salk Institute for Biological Studies

R01 CA28533 Russell
Mechanisms of Tumor Destruction by Immune Effectors
Washington University

R01 CA29194 Rajan
Somatic Cell Genetics of Cell Surface Antigens
Yeshiva University

R01 CA29548 Hansen
Differentiation Antigens on Human Lymphocytes
Pacific Northwest Research Foundation

R01 CA29679 Sibley
Genetic Analysis of Membrane Immunoglobulin
University of Washington

R01 CA30147 Gottlieb
Genetic Markers, Leukemogenesis and Thymic Function
University of Texas, Austin

R01 CA30654 Morgan
Regulation of Immune Responses by FC Portion of Antibody
Scripps Clinic and Research Foundation

R01 CA31798 Springer
Lymphocyte Function-Associated Antigens
Dana-Farber Cancer Institute

R01 CA31799 Springer
Chemistry of Tumoricidal Macrophage Surface Antigens
Dana-Farber Cancer Institute

R01 CA34787 Trowbridge
Human Cell Surface Antigens: Transferrin Receptors
Salk Institute for Biological Studies

R01 CA34900 Wang
Chemical Analysis of Human and Murine T-Cell Antigens
Medical University of South Carolina

R01 CA35638 Ware
Molecular Pathway of Human T-Cell-Mediated Cytotoxicity
University of California

R01 CA35977 Suzuki
FC-Gamma Receptor-Mediated Regulation of Macrophage
Univ of Kansas College Health Science and Hospital

R01 CA36137 Oettgen
RADLV Leukemia Antigens Recognized by Cytotoxic T Cells
Sloan-Kettering Institute for Cancer Research

R01 CA36167 Griffin
Surface Antigens of Human Myeloid Progenitor Cells
Dana-Farber Cancer Institute

R01 CA36700 Freed — Structural Studies of the Products of the H-2 Complex
National Jewish Hospital and Research Center

R01 CA37026 Hall — Killer Cell Surface Antigens—Biochemistry and Function
Virginia Commonwealth University

R01 CA37155 Perussia — Receptors for Immunoglobulin G on Leukocytes
Wistar Institute of Anatomy and Biology

R23 CA37199 Wright — Mechanisms of NK Resistance in Tumor Cell Variants
University of California, Los Angeles

R01 CA37827 Kornbluth — Analysis of Human NK Function
University of Pennsylvania

R23 CA38055 Fleit — FC Receptor Expression During Myeloid Differentiation
State University of New York at Stony Brook

R01 CA38396 Redelman — The Molecular Basis of T-Cell-Mediated Cytolysis
University of Nevada, Reno

R01 CA38500 Le Grue — Membrane Antigens that Mediate Metastatic Phenotype
University of Texas System Cancer Center

R01 CA38882 Black — Immune Effects of Shed Tumor Cell Membrane Vesicles
Boston University

R01 CA39003 Chesnut — Presentation of Integral Membrane Antigens to TH-Cells
National Jewish Hospital and Research Center

R01 CA39064 Todd — Cell Surface Antigens on Human Macrophages
University of Michigan at Ann Arbor

R01 CA39069 McDevitt — Cloning H-2I and HLA-D Region Coding Sequences
Stanford University

R23 CA39264 Greenstein — Immunobiology of the T-Cell Response to Class I MHC AG
Dana-Farber Cancer Institute

R01 CA40040 Allison — Surface Antigens of Rat Hepatocellular Carcinomas
University of California, Berkeley

R01 CA40044 Metzgar — Immunological Studies of Membrane Antigens
Duke University

R01 CA40205 Curtis — Molecular Techniques in Human Cancer Immunotherapy
Wistar Institute of Anatomy and Biology

R01 CA40294 Sato — Antibodies to Hormone Receptors for Cancer Therapy
W. Alton Jones Cell Science Center

R01 CA40552 Pollack — Function/Regulation of HLA Antigens on Cultured Cells
Baylor College of Medicine

R01 CA41619 Ritz
Characterization of Functional Structures on NK Cells
Dana-Farber Cancer Institute

R35 CA42509 Herzenberg
Genetics of Immunoglobulins and Lymphocyte Molecules
Stanford University

R01 CA42571 St. John
Homing Receptor Gene Expression in Lymphoid Tumors
Fred Hutchinson Cancer Research Center

R23 CA43184 Howell
Characterization of Acute Myelogenous Leukemia Stem Cell
Dartmouth College

HUMORAL FACTORS OTHER THAN ANTIBODY (HF)

R01 CA12779 Nowell
Leukocyte Regulatory Mechanisms
University of Pennsylvania

R01 CA15129 Oh
A Serum Immunosuppressive Factor in Cancer
Boston University

R01 CA17643 Smith
Regulation of T-Cell Proliferation
Dartmouth College

R01 CA17673 Hoffmann
Regulation of the Humoral Immune Response by B Cells
Sloan-Kettering Institute for Cancer Research

R01 CA22126 Daynes
Ultraviolet Light Radiation and Immunoregulation
University of Utah

R01 CA24974 Goldstein
Chemical and Immunological Characteristics of Thymosin
George Washington University

R01 CA26143 Lint
Control of Complement-Mediated Tumor Cell Cytolysis
Rush University

R01 CA26504 Stanley
Regulation of Granulocyte and Macrophage Production
Yeshiva University

R01 CA27903 Epstein
The Biology of the Antitumor Actions of Interferons
University of California, San Francisco

R01 CA29991 Gupta
Interferon Action: Studies on Interferon Receptor System
Sloan-Kettering Institute for Cancer Research

R01 CA30015 Mortensen
Acute Phase Reactants: Induction and Host Resistance
Ohio State University

R01 CA30515 Sidell
Immunological Aspects of Retinoids in Human Cancer
University of California, Los Angeles

R01 CA32319 Cohen
Immunologic Control of Tumor Cell Migration
University of Connecticut Health Center

R23 CA33903 Lane — Tumor Produced Macrophage Chemokinetic Factor
Medical College of Ohio at Toledo

R01 CA34141 Godfrey — Isolation of Macrophage Agglutination Factor
New York Medical College

R01 CA34344 Michael — Tumorigenesis: Immunoendocrine Systems Interactions
State University of New York at Albany

R01 CA34573 Shands — Studies of Cytolytic Factors from Macrophages
University of Florida

R01 CA34616 Stone — Lymphokine Antagonists from Tumor Cell Lines
University of Denver

R01 CA34805 Patek — Natural Cytotoxic Activity and Tumorigenesis
Salk Institute for Biological Studies

R01 CA35152 Fan — Cytolytic T-Lymphocyte Helper Factor
University of Minnesota of Minneapolis-St. Paul

R01 CA35761 Mokyr — Mechanism of Melphalan-Mediated Tumor Eradication
University of Illinois at Chicago

R01 CA37003 Johnson — Role of Complement in IgM-Dependent Leukemia Suppression
Johns Hopkins University

R01 CA37187 Pace — Role of Type I Interferons in Macrophage Activation
University of Florida

R01 CA37385 Yip — Structure-Function of Interferon-Gamma and Its Receptors
New York University

R01 CA37670 Vitetta — A B-Cell Growth Factor Produced by a Neoplastic B Cell
University of Texas Health Science Center, Dallas

R23 CA37683 Gootenberg — Variants of T-Cell Growth Factor From Malignant Cells
Georgetown University

R01 CA37834 Swierkosz — Functional Analysis of Nonspecific T-Cell Suppression
St. Louis University

R01 CA37932 Trucco — Human Lymphokine that Blocks Growth of IA+ Target Cells
Wistar Institute of Anatomy and Biology

R01 CA37943 Fulton — Cyclooxygenase Products and Mammary Cancer
Michigan Cancer Foundation

R01 CA38587 Johnson — Regulatory and Antitumor Effects of Gamma Interferon
University of Florida

R01 CA38779 Russell — Gamma Interferon Receptor of Tumorolytic Macrophages
University of Florida

R01 CA39605 Rothenberg — Analysis of T-Cell Growth Control by Gene Transfer
California Institute of Technology

R35 CA39723 Cohen — Activation and Regulation of Normal and Neoplastic Cells
University of Connecticut Health Center

R01 CA39888 Salvin — Lymphokines, Cellular Immunity, Delayed Hypersensitivity
University of Pittsburgh

R01 CA39925 Walker — Mechanism of Gamma Interferon Regulation of IA Antigens
Indiana University-Purdue University at Indianapolis

R01 CA41583 Mayer — Lymphokine Regulation of Human B-Cell Maturation
Mount Sinai School of Medicine

R01 CA41997 O'Dorisio — Vasoactive Intestinal Peptide in Diagnosis of Leukemia
Children's Hospital, Columbus

R01 CA42199 McKean — Biochemical Characterization of T-Cell Activation
Mayo Foundation

R01 CA42496 Rovera — Suppression of Normal and Leukemic Hemopoiesis
Wistar Institute of Anatomy and Biology

R43 CA42699 Pickering — T-Cell Recognition of Lung Cancers in Man
T Cell Sciences, Inc.

R01 CA43059 Schreiber — Molecular Regulation of Macrophage Cytocidal Activity
Washington University

R01 CA43119 Lernhardt — BCGF: Cloning, Expression and Role in B-Cell Neoplasia
La Jolla Cancer Research Foundation

R01 CA43386 Murasko — Characterization of Interferon: Immunoregulation
Medical College of Pennsylvania

R01 CA44323 Dray — Proteinase Inhibitors Produced by Lymphocytes
University of Illinois at Chicago

TUMOR RELATED ANTIBODIES (HI)

R01 CA15064 Chu — Immunochemical Studies on Carcinogenic Myocotoxin
University of Wisconsin, Madison

R01 CA20105 Wohlgemuth — Information from Immunological Reaction Tables
University of Maine at Orono

R01 CA25253 Bankert — Immunoregulatory Network Probed by Cell Hybridization
Roswell Park Memorial Institute

R01 CA26695 Cantor — Antigen-Specific T-Cell Clones: Generation and Analysis
Dana-Farber Cancer Institute

R01 CA28149 Vitetta — Immunotherapy of a B-Cell Leukemia (BCLI)
University of Texas Health Science Center, Dallas

R01 CA32497 Cannon — Structure and Genetics of Antibody Variable Regions
University of Massachusetts Medical School

R01 CA35525 Vogel — Molecular Mechanisms of Induced Immune Cytolysis
Georgetown University

R23 CA35692 Leonard — Immunotoxin Internalization and Processing
University of California

R01 CA35791 Bonavida — Role of NK Cytotoxic Factor NKCF in NK Cytotoxicity
University of California, Los Angeles

R01 CA37063 Anderson — Chemical Modification of Immunotoxin
University of New Mexico, Albuquerque

R01 CA37959 Ferrone — Monoclonal Antibodies to Human Melanoma Antigens
New York Medical College

R23 CA38750 Smith — Synthetic Peptides to Induce Anti-Tumor Immunity
University of Florida

R01 CA39562 Roeder — Regulation of Immunoglobulin and Oncogene Transcription
Rockefeller University

R35 CA39838 Scharff — Somatic Cell Genetics of Ig Genes
Albert Einstein College of Medicine

R01 CA40427 Alt — Ig Class-Switching and Somatic Mutations
Columbia University

IMMUNOBIOLOGY OF SARCOMAS, CARCINOMAS, AND MELANOMAS (IB)

P01 CA14489 D'Angio — Children's Cancer Research: The Biology of Neuroblastoma
Children's Hospital of Philadelphia

R01 CA19754 Cohn — Immunoselection and Cancer: A Problem in Evolution
Salk Institute for Biological Studies

R01 CA31336 Stackpole — Immunobiology of B16 Melanoma Metastasis
New York Medical College

R01 CA31564 Yates — Glycoconjugates and Glioma Resistance to Cytolysis
Ohio State University

R01 CA31918 Fanger — ADCC of Small Cell Carcinoma of the Lung
Dartmouth College

R01 CA34549 Ponzio — Role of Natural Cytotoxic Cells in Lymphomas
University of Medicine & Dentistry of New Jersey

R01 CA40266 Gooding — Tumor Cell Recognition by Activated Macrophages
Emory University

R01 CA42139 Callahan — Host-Induced Changes in MHC Antigens on Tumor Cells
Colorado State University

R01 CA42276 Mercurio — Glycoconjugates and Macrophage-Tumor Cell Interactions
New England Deaconess Hospital

R01 CA43187 Cook — Oncogenes and Tumor Cell/Immune Response Interactions
National Jewish Hospital & Research Center/NAC

HOST-TUMOR IMMUNOPATHOLOGY (IP)

R01 CA28332 Lord — In Situ Antitumor Immunity and Effects of Radiation
University of Rochester

R01 CA30196 Purtilo — Immunopathology of X-Linked Lymphoproliferative Syndrome
University of Nebraska Medical Center

R01 CA31837 Prehn — Mechanisms of Carcinogenesis
Institute for Medical Research, Santa Clara County

R01 CA33119 Warnke — Human Tumor-Host Relationships In Vivo
Stanford University

R23 CA36109 Tempelis — The Role of the Thymic Epithelium in Leukemogenesis
Mount Sinai Medical Center (Milwaukee)

R01 CA36243 Ford — Immunopathologic Studies of Hodgkin's Disease
University of Texas System Cancer Center

R01 CA37343 Lennon — Autoimmune Paraneoplastic Syndromes
Mayo Foundation

R01 CA41525 Frost — Mutage/UV Induced Immune Variants in Metastasis Theory
MD Anderson Hospital & Tumor Institute

EFFECTS OF DISEASE ON IMMUNE FUNCTION (IS)

R01 CA16885 Ruddle — Propagation of Thymus-Derived Lymphocyte Lines
Yale University

R01 CA18234 Roszman — Immunobiology of Primary Intracranial Tumors
University of Kentucky

R01 CA20543 Rossen — Antigen-Antibody Complexes in Cancer Patients' Sera
Baylor College of Medicine

R01 CA26169 Bose — Immunosuppression by Avian Acute Leukemia Viruses
University of Texas, Austin

R01 CA29200 Guerry
Autologous Immunity to Human Melanoma
University of Pennsylvania

R01 CA30461 Mukherji
Clonal Analysis of Cellular Immune Response in Melanoma
University of Connecticut Health Center

R01 CA31547 Olsen
Immunosuppressive Properties of Retrovirus Protein
Ohio State University

R01 CA33653 Reem
Mechanism of Immune Interferon Synthesis in Thymocytes
New York University

R23 CA36896 Gewirtz
Regulation of Human Megakaryocytopoiesis
Temple University

R01 CA36915 Greenberg
Cellular Modulation of Hemopoiesis
Stanford University

R01 CA37949 Gudewicz
Cancer Chemotherapy and Macrophage Activation
Albany Medical College of Union University

R23 CA39016 Yamamoto
Lymphokines in FELV Immunosuppression and Leukemia
University of California, Davis

R01 CA39068 Dunnick
DNA Sequencing Involved in the Heavy Chain Switch
University of Michigan, Ann Arbor

R01 CA39632 Lotzova
NK Cell Role in Resistance to Leukemia
University of Texas System Cancer Center

R01 CA42195 Spellman
Dermatological Changes Following UV Irradiation
University of New Mexico Albuquerque

IMMUNOTHERAPY: MECHANISMS RATHER THAN THERAPEUTIC RESULTS (IT)

R01 CA29039 Baso
Molecular Targeting of Cytotoxic Ricin A Chain
Dana-Farber Cancer Institute

R01 CA30070 Anderson
Carcinoma Associated Antigens and Immunoglobulins
Northwestern University

R01 CA30088 Dray
Synergy of Tumor Chemotherapy and Host Immunity
University of Illinois at Chicago

R01 CA33084 Greenberg
Mechanisms of Murine Tumor Eradication by Immunotherapy
University of Washington

R01 CA34358 Bystryn
Immunogenicity of a Polyvalent Melanoma Antigen Vaccine
New York University Medical Center

R23 CA39700 Berger
Mechanism(s) of Photo-Immunoregulation
Columbia University

R01 CA39854 Truitt — Clonal CVL/GVH Reactions and Adoptive Immunotherapy
Medical College of Wisconsin

R01 CA40168 Rutzky — Anti-Idiotype Antibodies: Antigens for Human Tumors
University of Texas

R01 CA41089 Graham — Prostate Cancer Effect of NK & Macrophage on Metastases
Emory University

R23 CA42537 Nagarkatti — Immune Mechanisms in Nitrosourea-Induced Tumor Rejection
Virgina Tech

LYMPHOCYTES (LB)

R01 CA04946 Bosma — Severe Combined Immunodeficiency
Institute for Cancer Research

P01 CA12800 Fahey — Immune Functions and Cancer
University of California, Los Angeles

R01 CA14462 Thorbecke — Properties of Lymphoid Tumor Cells In Vivo and In Vitro
New York University

P01 CA15822 Nowell — Immunobiology of Normal and Neoplastic Lymphocytes
University of Pennsylvania

P01 CA16673 Cooper — Cell Differentiation Studies in Cancer Immunobiology
University of Alabama in Birmingham

R01 CA17733 Trowbridge — Lymphocyte Antigens: Structure, Function and Synthesis
Salk Institute for Biological Studies

R01 CA20531 Yunis — Genetic Analysis of Normal and Malignant Lymphocytes
Dana-Farber Cancer Institute

R01 CA20819 Van Epps — Phagocytic Cells: Regulation, Dysfunction and Disease
University of New Mexico, Albuquerque

R01 CA20833 Trinchieri — Cell-Mediated Cytotoxicity in Humans
Wistar Institute of Anatomy and Biology

R01 CA22786 Bankert — Receptor Dynamics and Normal/Tumor Cell Function
Roswell Park Memorial Institute

R01 CA24436 Wofsy — Lymphocyte Receptor Function
University of California, Berkeley

R01 CA24442 Sercarz — Chemical Basis for Receptor Recognition of Lysozymes
University of California, Los Angeles

R01 CA24607 Engleman — Suppressor T Cells of Mixed Leukocyte Reaction in Man
Stanford University

R01 CA25054 Mullen — Cellular Mechanisms Regulating Antibody Production
University of Missouri, Columbia

R01 CA25250 Klein — Target Sites, Genetic Control & Role in Tumor Resistance
Caroline Institute

R01 CA25369 Schlossman — Human Leukemia Antigens: Isolation and Characterization
Dana-Farber Cancer Institute

R01 CA25583 Lopez — Cell-Mediated Immunity in Mammary Tumor Models
University of Miami

R01 CA25612 Plate — Immunologic Effects on Tumor Growth and Rejection
Rush University

P01 CA25803 Bevan — Control of Normal and Abnormal Cell Development
Scripps Clinic and Research Foundation

R01 CA26297 McKean — Primary Structure of MHC I Region Associated Antigens
Mayo Foundation

R01 CA28139 Feldman — Immunobiology of Tumor Metastases
Weizmann Institute of Science

R01 CA28708 Rohrer — Immunoregulation of Myeloma Cell Differentiation
University of South Alabama

P01 CA28900 Eisen — Control of Antigen-Specific T-Cell Responses
Massachusetts Institute of Technology

R01 CA28936 Haynes — Immunoregulation in Autoimmunity and Malignant Disease
Duke University

P01 CA29606 Janeway — Immunoregulation: T Cells and Their Products
Yale University

R01 CA30266 Gooding — Cytotoxic T-Cell Recognition of the SV40 Tumor Antigen
Emory University

R01 CA30280 Weisbart — T-Lymphocyte Regulated Tumor Cell Killing by Neutrophils
University of California Los Angeles

R01 CA31534 Tucker — Isotype Switching in a Neoplastic B Cell Model, BCL1
University of Texas Health Science Center, Dallas

R01 CA31536 Spitzer — Investigation of Human Mitogen Induced T-Cell Colonies
University of Texas System Cancer Center

R01 CA31685 LeBien — Differentiative Programs of Lymphoid Progenitor Cells
University of Minnesota of Minneapolis-St. Paul

R01 CA32277 Lynch — FC Receptor-Bearing T Lymphocytes in Murine Myeloma
University of Iowa

R01 CA32685 The Immunobiology of Human Antileukemic Lymphocytes
Sondel University of Wisconsin, Madison

R01 CA33005 Molecular Mechanism of Natural Cell-Mediated Cytolysis
Oeltmann Vanderbilt University

R01 CA33589 Mechanism of NK Mediated Cytolysis
Forbes Vanderbilt University

R01 CA34105 Immunoregulation Via Idiotype Networks
Brown St. Jude Children's Research Hospital

R01 CA34106 T-Cell Glycoproteins Controlled by Immune Response Genes
Hayes University of Wisconsin Madison

R01 CA34112 Molecular Mechanisms in Cellular Immunology
Callewaert Oakland University

R01 CA34127 Antibody Variable Genes: Development and Diversity
Gearhart Johns Hopkins University

R01 CA34129 Regulation of Human Cytolytic T Lymphocytes
Burakoff Dana-Farber Cancer Institute

R01 CA34442 In Vitro Induction of NK Cytotoxicity
Golub University of California, Los Angeles

R01 CA34461 Regulation of Natural Killer Cells
Welsh University of Masschusetts Medical School

R01 CA34546 Immunological Defects in Human Immunodeficient States
Fu Oklahoma Medical Research Foundation

R01 CA34817 Regulation of Activity in Cloned Anti-Tumor Lymphocytes
Russell Washington University

R01 CA35457 Natural Killer Cell Heterogeneity and Differentiation
Spellman University of New Mexico, Albuquerque

R01 CA35654 Monoclonal T-Lymphocyte Factor Regulation of Myeloma
Rohrer University of South Alabama

R01 CA35704 Mechanisms of Antigen Processing of Hemoglobin
Kazim University of New Mexico

R01 CA35730 Requirements for B-Cell IA-Alloantigen Presentation
Kubo National Jewish Hospital and Research Center

R01 CA36302 T-Cell Help in B-Cell Activation, Division, and Maturation
Corley Duke University

R23 CA36403 Isotype Suppressor T Cells With FC Receptors in Myeloma
Hoover University of Pennsylvania

R01 CA37006 Regulatory Interactions of NK Cells with B Cells
Pollack University of Washington

R01 CA37344 Molecular Analysis—B-Lymphocyte Activation
Raschke La Jolla Cancer Research Foundation

R01 CA37372 Tyrosine Protein Kinases and Lymphocyte Activation
Geahlen Purdue University, West Lafayette

R01 CA37374 Immunochemical Genetics of Murine Alloantigens
Michaelson Center for Blood Research

R01 CA37438 Mechanisms of B-Cell Activated Feedback Regulation
Calkins Thomas Jefferson University

R23 CA37955 Biophysical Basis of Cell Killing by CTL
Sung Columbia University

R01 CA38336 Ontogeny, Regulation and Characterization of NK/K Cells
Kim University of Health Sciences, Chicago Medical School

R01 CA38349 Significance of IR-Genes in T-Cell Mediated Suppression
Araneo University of Utah

R01 CA38350 T-Cell Influences on B-Cell Maturation
Bottomly Yale University

R01 CA38351 Ontogenetic Development of Lymphocytes
Hammerling Sloan-Kettering Institute for Cancer Research

R01 CA38352 Molecules Mediating the Attachment of Lymphocytes
Pierschbacher La Jolla Cancer Research Foundation

R01 CA38353 Modification of Regulatory T-Lymphocyte Function
Polmar Washington University

R23 CA38899 Transformed T-Cell Lines for Study of T-Cell Maturation
Jung Oklahoma Medical Research Foundation

R01 CA38942 Proteinases and Lethal Mechanism of Natural Killer Cells
Hudig University of Nevada, Reno

R01 CA39000 Mechanisms of B-Cell Transformation and Lymphomogenesis
Ewert Wistar Institute of Anatomy and Biology

R01 CA39078 Analysis of Neonatal H-2 Tolerance
Streilein University of Miami

R23 CA39345 Characterization of Activated Natural Killer Cells
Golightly State University of New York, Stony Brook

R01 CA39429 Immunobiology of Transformation and Tumorigenesis
Collins Salk Institute for Biological Studies

R01 CA39692 O'Leary — Mechanisms of Lymphocyte Mitogenesis
University of Minnesota of Minneapolis-St. Paul

R35 CA39790 Dorf — Cellular Pathways Involved in Immunoregulation
Harvard Medical School

R35 CA39827 Boyse — Normal and Abnormal Cell Surface Genetics
Sloan-Kettering Institute for Cancer Research

R01 CA39890 Lindquist — Effector Lymphocyte-Target Cell Interaction
University of Connecticut Health Center

R01 CA39891 Mastro — Protein Kinases, Ca^{2+}, and PI in Stimulated Lymphocytes
Pennsylvania State University Park

R01 CA40256 Trinchieri — Regulation of Hematopoiesis by NK Cells
Wistar Institute of Anatomy and Biology

R01 CA40272 Gallatin — Lymphocyte Homing in Normal and AIDS Affected Model
Fred Hutchinson Cancer Research Center

R23 CA40430 LeFever — Kinetic Analysis of Cytolytic Reactions
Medical College of Wisconsin

R01 CA41165 Hoover — Tumor Suppressive T Cells with FC Receptors in Myeloma
University of Pennsylvania

R01 CA41363 Scott — Mechanism of Specific B-Lymphocyte Tolerance
University of Rochester

R01 CA41448 Frelinger — Neoplastic and Normal T-Cell Differentiation Antigens
University of Rochester

R23 CA41451 Campbell — Cytomegalovirus-Induced Suppression of Tumor Immunity
Eastern Virginia Medical School

R23 CA41539 Hochman — Regulatory T Cells
Tufts University

R01 CA41679 Keitman — In Vitro Analogs of the Thymic Microenvironment
University of Texas Health Sciences Center

R01 CA41987 Koren — Human Natural Killer Cells: Regulation and Heterogeneity
U.S. Environmental Protection Agency

R01 CA42093 Fitzgerald-Bocarsly — Natural Kill of HSV-1 Infected Targets: Basic Biology
University of Medicine & Dentistry of New Jersey

R01 CA42176 Henderson — Immunobiology of Cytotoxic Lymphocytes
Tulane University of Louisiana

R01 CA42443 Bear — Suppressor T-Cell Regulation of Tumor Immunity
Virginia Commonwealth University

R01 CA42471 Rao — Role of myc and fos Oncogenes in Lymphocyte Growth
Dana-Farber Cancer Institute

R35 CA42551 Weissman — Normal and Neoplastic Lymphocyte Maturation
Stanford University

R01 CA42735 Clement — Ontogeny and Functions of Human Helper T Cell Subsets
University of California Los Angeles

R01 CA42739 Phipps — Cell Modulation of B-Cell Tolerance and Triggering
University of Rochester

R01 CA42890 Dawson — Natural Killer Cell-Target Cell Recognition
Duke University

R01 CA43062 Mescher — Avidity Analysis of CTL Interaction with Antigen
Medical Biology Institute

R01 CA43641 Greene — Manipulation of Regulatory T-Cell Specificities
University of Pennsylvania

R23 CA43490 Nagarkatti — Regulatory T Helper Cell Networks
Virginia Tech

R01 CA44322 Flomenberg — Generation and Regulation of Autocytotoxic Cells
Sloan-Kettering Institute for Cancer Research

MONOCYTES AND MACROPHAGES (MB)

R01 CA14113 Shin — Macrophage Activation for Tumor Cell Cytotoxicity
Johns Hopkins University

P01 CA14723 Benacerraf — Study of Experimental Cancer Immunology
Harvard University

R01 CA16784 Adams — Tumoricidal Effects of Macrophages: Pathologic Study
Duke University

R01 CA18672 Fishman — The Role of Macrophage Subclasses in Tumor Immunity
St. Jude Children's Research Hospital

R01 CA20822 Colvin — Cell Interaction and the Clotting System
Massachusetts General Hospital

R01 CA21225 Remold-O'Donnell — Surface Protein GP160 in Macrophage Activation
Center for Blood Research

R01 CA22105 Tomasi — Analysis of Mononuclear Phagocyte Differentiation
University of New Mexico, Albuquerque

R01 CA24067 Anderson — Fc Receptor Structure and Function
University of Rochester

R01 CA25052 Immune Response in Vitro H-2 (IR) Locus Function
Niederhuber University of Michigan at Ann Arbor

R01 CA26824 Mononuclear Phagocytes in Human Ovarian Carcinoma
Mantovani Mario Negri Institute Pharmacologiche

R01 CA27523 Macrophages and Tumor Growth
Evans Jackson Laboratory

P01 CA29589 Macrophage Activation: Development and Regulation
Adams Duke University

P01 CA30198 Human Mononuclear Leukocytes in Cancer
Cohn Rockefeller University

R01 CA31199 Macrophage-Medicated Injury Causing Tumor Regression
Russell University of Florida

R01 CA32551 Hemopoietic Stem Cell Differentiation to Macrophages
Stanley Yeshiva University

R01 CA32898 Differentiation and Function of Human Monocytes
Trinchieri Wistar Institute of Anatomy and Biology

R01 CA33225 Regulation of Myeloid Progenitro Cell Differentiation
Pelus Sloan-Kettering Institute for Cancer Research

R01 CA35893 A Mechanism by Which Macrophages Injure Cancer Cells
Granger Duke University

R01 CA35961 Macrophage Resistance Versus Viruses and Tumors
Morahan Medical College of Pennsylvania

R23 CA37954 Immune Functions of the Macrophage MAN/GLCNAC Receptor
Sung Oklahoma Medical Research Foundation

R01 CA38354 Development and Function of Pulmonary Macrophages
Kim University of Health Sciences/Chicago Medical School

R01 CA38407 MHC Class II Antigens in Leukemia Cellular Differentiation
Russo Cornell University Medical Center

R01 CA38408 Effector and Suppressor Mechanisms in Tumor Immunity
Stout East Tennessee State University

R01 CA39070 Regulation of IA Gene Expression
Woodward University of Kentucky

R01 CA39093 Lymphocyte-Released Cell Toxin
Granger University of California, Irvine

R01 CA39621 Induced Macrophage Tumoricidal Activation
Hamilton Duke University

R01 CA40477 Chapes — Mechanism of Macrophage Recognition of Tumor Cells
Kansas State University

R01 CA40550 Liu — Molecules Involved in Macrophage Response to Lymphokine
Brigham & Women's Hospital

R01 CA41103 Buckley — Immunobiology of Human Tissue Macrophages
Yale University

R01 CA41456 Tenen — Molecular Biology of Human Myeloid Differentiation
Beth Israel Hospital Boston

R23 CA43476 Davis — Study of Fluid Pinocytosis in Stimulated Neutrophils
Dartmouth College

R01 CA43610 Nathan — Biochemical Bases of Cytoxicity by Phagocytes and Lymphocytes
Cornell University Medical Center

MALIGNANCIES OF THE IMMUNE SYSTEM (LYMPHOMA/LEUKEMIA) (MI)

R01 CA15472 Eisen — Immunity and Myeloma Tumors
Massachusetts Institute of Technology

R01 CA25097 Kersey — Differentiation of Immune System: Cell Surface Antigens
University of Minnesota of Minneapolis-St. Paul

R01 CA31479 Ford — Proliferation and Differentiation in Human Lymphoma
University of Texas System Cancer Center

R01 CA31789 Datta — Genetic-Viral-Immunologic Studies
Tufts University

R01 CA32577 Wheelock — Studies on Tumor Dormancy and Emergence
Hahnemann University

R01 CA32826 Macher — Glycosphingolipids in Oncogenesis and Differentiation
University of California, San Francisco

R01 CA34654 Manson — Immune Response and Progressive Tumor Growth
Wistar Institute of Anatomy and Biology

R01 CA36776 Hardwick — Lymphoblastoid Receptors for Epstein-Barr Virus
University of South Alabama

R01 CA37097 Briggs — Nuclear Antigen Markers in Human Blood Cells
Vanderbilt University

R01 CA38325 Newburger — White Blood Cell Oxidase in Leukemia and Normal Cells
University of Massachusetts Medical School

R01 CA38663 Lerman — The Immunology of Increased Aggressiveness of SJL Tumors
Wayne State University

R01 CA39492 Monoclonal Antibodies Against Leukemia
Bernstein — Fred Hutchinson Cancer Research Center

R01 CA41047 Characterization of the Human C1Q Receptor
Ghebrehiwet — SUNY at Stony Brook

R01 CA41099 Biology of H-40, An IgH-Linked Histocompatibility Gene
Forman — University of Texas Health Sciences Center

R01 CA41305 The Function of Quiescent Cells in Pediatric Leukemia
Andreeff — Sloan-Kettering Institute for Cancer Research

R01 CA41420 Study of Viral Sequences Adjacent to HLA Class I Genes
Meruelo — New York University

P01 CA42771 Immunoregulatory Molecules on Neoplastic B Cells
Frelinger — University of North Carolina

R23 CA42902 Erythropoiesis and Resistance to Leukemia in CBA/N Mice
Worthington — Texas Tech University Health Sciences Center

R23 CA43058 Immunobiology of Cutaneous T-Cell Lymphoma
Edelson — Yale University

IMMUNE SURVEILLANCE (SR)

R01 CA03367 Natural Resistance to Lymphoma and Marrow Transplantation
Trentin — Baylor College of Medicine

R01 CA15988 Immune Surveillance and Cancer
Stutman — Sloan-Kettering Institute for Cancer Research

R01 CA20408 Immunodeficiency and Tumorigenesis
Shultz — Jackson Laboratory

R01 CA20816 Pathogenesis of Autoimmunity
Gershwin — University of California, Davis

R01 CA22677 Manipulation of Tumor-Specific Immunity
Schreiber — University of Chicago

R01 CA24433 Antigen Recognition by Cytotoxic Killer Cells
Sears — University of California, Santa Barbara

R01 CA24873 Immunosuppression in Cancer Patients
Bankhurst — University of New Mexico, Albuquerque

R01 CA25917 Cellular and Genetic Aspects of Antitumor Immunity
Daynes — University of Utah

R01 CA26344 Autologous Lymphocyte Reactions and Immune Surveillance
Weksler — Cornell University Medical Center

R01 CA27599 Williams	Genetic Control of Resistance and Immunity to P815 Northwestern University
R01 CA28231 Carlson	H-2 Associated Natural Resistance Jackson Laboratory
R01 CA28834 Dvorak	Basophil/Mast Cell Function in the Control of Cancer Beth Israel Hospital
R01 CA29355 Blank	T-Cell Nonresponsiveness in Gross Virus-Infected Mice University of Pennsylvania
R01 CA30187 Bloom	Regulatory Mechanisms in Human Natural Cytotoxicity University of California, Los Angeles
R01 CA32553 Pollack	Specific Anti-Tumor Activity by Armed Lymphoid Cells University of Washington
R01 CA33065 Daynes	Immunobiology of UVL-Induced Tumors University of Utah
R23 CA34302 Roberts	Regulation of UV-Tumor Immunity by Cloned TS-Cell Lines University of Utah
R01 CA36860 Green	Cellular Immunity to Endogenous AKR Leukemia Viruses Dartmouth College
R01 CA36921 Bennett	Immunogenetics of Hybrid Resistance University of Texas Health Science Center, Dallas
R01 CA36922 Bennett	Immunobiology of Hybrid Resistance University of Texas Health Science Center, Dallas
R01 CA37205 Targan	Mechanism of the NK Lethal Hit in Programmed Tumor Cells University of California, Los Angeles
R01 CA37570 Hiramoto	CNS Effects of IFN Production and NK Cell Activity University of Alabama at Birmingham
R01 CA38017 Ching	Natural Killer Cells and Cancer in Hawaiians University of Hawaii at Manoa
R01 CA39201 Podack	Molecular Mechanism of Lymphocyte Mediated Tumor Lysis New York Medical College
R01 CA39536 Ghosh	Immune Mechanism in the Generation of Tumor Variants Roswell Park Memorial Institute
R01 CA39623 Dennert	Target Cell Lysis by Cytolytic Effector Cells University of Southern California
R01 CA41268 Biron	Natural Killer Cell Growth and Development University of Massachusetts Medical School

R15 CA41658 Activation and Control of Macrophage Antiviral Activity
Le Blanc University of Alabama in University

R15 CA41691 Tumor-Induced Suppression of Alveolar Macrophages
Young Ball State University

R01 CA41972 Natural Killer Cell Target Structure and Its Receptor
Yogeeswaron Tufts University

R23 CA42936 UV-Induced Suppression of the Response to Alloantigens
Ullrich University of Texas System Cancer Center

IMMUNOTHERAPY IN ANIMAL MODELS (TA)

R01 CA11898 Brain Tumors: Immunological and Biological Studies
Bigner Duke University

R01 CA16642 Immunological Basis of Tumor Regression
North Trudeau Institute

R01 CA17818 Tumor Immunity and Tumor-Host Interactions
Stutman Sloan-Kettering Institute for Cancer Research

R01 CA27794 Therapeutic Manipulation of Antitumor Immunity
North Trudeau Institute

R01 CA33939 Lymphoid Cell Treatment of Leukemia
Furmanski AMC Cancer Research Center

R01 CA35299 T Cells and Their Lymphokines in Cancer Immunotherapy
Altman Scripps Clinic and Research Foundation

R01 CA37184 Antitumor Properties of Acute Inflammation
Lichtenstein University of California, Los Angeles

R01 CA38415 Evaluation of Human Melanoma Antigen as a Vaccine
Brown Oncogen

R23 CA40555 Adoptive Immunotherapy in the Mouse and Human
Eberlein Brigham and Women's Hospital

BONE MARROW TRANSPLANTATION (TT)

R01 CA28701 Chronic Graft-Versus-Host Disease in Radiation Chimeras
Beschorner Johns Hopkins University

R01 CA31787 Irradiation and Marrow Transplantation Large Animals
Thomas Fred Hutchinson Cancer Research Center

R01 CA32757 Mechanisms of Minor H Antigen GVHD
Hamilton University of Washington

P01 CA35048 Beutler | Bone Marrow Transplantation in Leukemia
Scripps Clinic and Research Foundation

R01 CA35971 Parkman | Control of Graft Versus Host Disease
Children's Hospital of Los Angeles

R01 CA36725 Vallera | Immunotoxins in Human Bone Marrow Transplantation
University of Minnesota of Minneapolis-St. Paul

R01 CA37706 Dennert | NK Cells and Bone Marrow Rejection
University of Southern California, Los Angeles

R01 CA38355 Sprent | Lymphocyte Function in Normal and Chimeric Mice
Scripps Clinic and Research Foundation

R01 CA38804 Cramer | Pathogenesis of Chronic Graft-Versus-Host Disease
University of Pittsburgh

R01 CA38951 Korngold | Etiology and Pathogenesis of Murine Graft-Versus-Host Disease
Wistar Institute of Anatomy and Biology

P01 CA39542 Burakoff | Cellular Studies of Bone Marrow Transplantation
Children's Hospital Boston

R01 CA39889 Hamilton | Marrow Transplantation: Immune Dysfunction in GVH
University of Washington

R01 CA40358 Murphy | Cellular Pathology of Cutaenous Graft-Versus-Host Disease
Brigham and Women's Hospital

R01 CA42148 Miller | T-Cell Repopulation After Human Marrow Transplantation
Boston University

R01 CA43081 Cheever | Therapeutic Potential of LAK Cells
University of Washington

CONTRACT RESEARCH SUMMARY

Title: Resource Bank and Distribution Center for Cell Lines Useful in Research in Tumor Immunology

Principal Investigator: Dr. Patrick R. McClintock
Performing Organization: American Type Culture Collection
City and State: Rockville, MD

Contract Number: N01-CB-15533
Starting Date: 9/28/81 Expiration Date: 9/27/86

Goal: To provide an efficient system for the acquisition, cataloging, storage and maintenance of cell lines which are capable of long term growth in vitro and are useful in tumor immunology research. To offer to recipients expert advice on culture and characteristics of all lines shipped.

Approach: The cell lines in the bank are listed in a catalog, which is updated annually. New acquisitions are also announced in quarterly newsletters. The cell lines in the bank include, but are not limited to: B and T cell lines; lines useful in the study of macrophage/monocyte development; myelomas and their variants; cell lines useful in the study of immune effector mechanisms; and hybridomas. Lines are shipped for a fee upon request. These lines are screened for contamination with bacteria, fungi and mycoplasma; detailed characterizations are performed. Relevant lines are actively sought and persons wishing to donate lines are encouraged to contact the principal investigator or the project officer.

Progress: Cell line shipments are averaging three hundred sixty per month. Ten cultures account for 52% of the shipments from the bank. The most popular line is CTLL-2 (TIB-214), a mouse T-cell line which is IL-2 dependent and thus can be used to assay for IL-2 (T cell growth factor). $P388D_1$(IL-1) (TIB-63) is a mouse monocyte-macrophage line which produces large quantitites of IL-1 as well as lysozyme and shows high effector activity in an antibody-dependent cell-mediated cytotoxic system. NS-1 (TIB-18) is a popular myeloma line used as a fusion partner to make hybridomas. Together these three cell lines account for 22% of the shipments from the TIB. Also very popular are: J774 A.1 (TIB-67), a mouse monocyte-macrophage line with properties similar to TIB-63 and GK1.5 (TIB-207), a rat-mouse hybridoma which recognizes a novel murine T cell surface antigen similar to the human Leu-3/T4 molecule but distinct from Lyt-1. Together these two cell lines account for another 12% of TIB shipments.

Significance to Cancer Research: The cell lines of the Tumor Immunology Bank are utilized by hundreds of laboratories as key tools in studies on tumor-specific antigens, antibody structure, immune-related cell functions, and cell fusions, as well as numerous other cancer-related projects.

Project Officer: Judith M. Whalen
Program: Immunology Program
FY 86 Funds: $ 0 B

Description and Introduction

The Cancer Diagnosis Research Program supports research designed to develop improved ability to identify populations at high risk, to detect cancer at earlier stages, to make more accurate diagnoses, to stage tumors more precisely for prognostic and therapeutic decisions and to monitor more effectively the changes during and following therapy. Identification of populations at high risk should result in more targeted screening programs; the development of better tests for detection and diagnosis should result in earlier detection and identification of tumors, before they become seriously invasive and/or metastatic. Improvements in detection and diagnosis are also critical for the physician in choosing the most effective therapeutic approaches.

The emphasis in diagnosis related research is on the transfer of conceptual and technological developments from basic research to the clinic. Some of the questions addressed include: Can altered metabolic products be used as tumor markers? Can more sensitive detection methods be developed using such technologies as monoclonal antibodies and recombinant DNA? Can detection of inherited genetic polymorphisms be used to identify individuals predisposed to cancer? How can advances in engineering technology be translated into more effective diagnostic instrumentation? Can detection of genetic alterations or altered cellular products or structures improve classification, staging and/or prediction of outcome?

Although a given research project may concentrate on a particular type of tumor or organ site, the techniques developed are often more generally applicable. Because of this, the Program has been organized into five broad disciplinary categories: Biochemistry, Immunology, Cytology, Pathology and Genetics. Biochemical diagnosis includes studies of hormones, enzymes, other proteins and metabolic products which are found in the circulation, in other biological fluids or associated with cells and which can be used to characterize tumors. Studies to develop immunological assays for identification and characterization of tumor cells and for quantitating and characterizing the host immune response are included in Immunology. Cytology research stresses the development and improvement of automated techniques for cytological evaluation. Pathology research emphasizes studies to improve methods of tumor classification, including staging and correlations with prognosis. Genetics research includes applications of the newest advances in cytogenetics and molecular genetics to cancer diagnosis. A budget summary of the Program by category is provided in the adjoining table.

The Diagnosis Research Program has continued to emphasize the application of genetic approaches to cancer diagnosis. This research area is rapidly expanding as additional studies continue to show that specific genetic alterations are involved in tumorigenesis and as technological advances facilitate the identification of these alterations in tumor tissue. These alterations are now being assessed for their value as prognostic indicators. The program also continues to support development and application of sophisticated analytical instrumentation and improved immunological techniques for cancer diagnosis. The following report describes recent developments in genetics, instrumentation and immunology as they are currently being applied to cancer diagnosis.

Progress in the Application of Genetic Technologies to Diagnosis

The recent explosion of information about genetic alterations and novel protein expression in tumors has fueled the development of new diagnostic approaches. Previous Diagnosis Program annual reports have focused on the basic technologies which improved the ability to answer questions about tumorigenesis and to begin to apply some of this knowledge to cancer diagnosis. This report will demonstrate that these applications are rapidly proving to be of value to the clinician by providing prognostic information which can be important in the choice of a treatment plan. In addition, the search for new applications of basic information is continuing to provide promising approaches to improved diagnosis.

The original observation that cell lines derived from human neuroblastomas contained multiple copies of DNA sequences related to the myc oncogene (Schwab, et al., 1983; Kohl, et al., 1983) led to numerous investigations of the new N-myc oncogene in neuroblastoma tumor tissue. A significant correlation was demonstrated between N-myc amplification and stage of disease (Brodeur, et al., 1984; CA39771). However, not every advanced stage neuroblastoma contained amplified N-myc. It was important, therefore, to determine whether N-myc amplification provided prognostic information in addition to the traditional staging based on standard clinical and pathological criteria. Seeger et al. (1985; Seeger CA22794, Brodeur CA39771) studied a series of eighty-nine patients with untreated primary neuroblastomas. The patients were treated according to standard protocols based on stage of disease at diagnosis and the outcome was analysed for correlation of progression-free survival with various parameters. A significant correlation was found between N-myc amplification and poor outcome. This correlation was independent of age at diagnosis which has been generally considered to be an important prognostic variable. There are other prognostic factors that have been identified as clinically important. Histopathological characteristics of the primary tumor can be used to identify subsets of patients in a given stage with varying prognoses (Shimada, et al., 1984). Flow-cytometrically measured tumor cell DNA content can predict outcome in Stage IV disease (Look, et al., 1984). The amount of serum neuron-specific enolase (an enzyme) can predict outcome for Stage III disease diagnosed prior to two years of age and for Stage IV disease diagnosed prior to one year (Zeltzer, et al., 1983; Zeltzer et al., 1985). Serum ferritin can indicate prognosis in Stage III patients (Hann, et al., 1985; Seeger CA22794). Correlations between these other prognostic factors and N-myc amplification have yet to be established but improving survival will undoubtedly depend on the development of more effective treatment strategies for those groups that can be identified as having a poorer prognosis at the time of diagnosis.

The clinical importance of the ability to define subsets of morphologically similar tumors has been shown in the case of another childhood tumor, Ewing's sarcoma. A tumor originally diagnosed as neuroblastoma, based on pathologic examination of the tumor tissue, did not present or behave like a classical neuroblastoma. Cytogenetic examination of a cell line derived from the tumor revealed an unusual chromosomal translocation, t(11;22). A cell line from another unusual neuroblastoma also revealed a t(11;22) translocation. Both cell lines had biochemical characteristics that distinguished them from other neuroblastomas. Based on these cytogenetic and biochemical observations,

the two cases were reclassified as neuroepithelioma, a peripheral neuronal tumor. When the pattern of oncogene expression was examined, the neuroepithelioma showed increased expression of the c-myc and c-myb oncogenes, a pattern similar to Ewing's sarcoma (a non-neuronal tumor). Neuroblastoma, as discussed above, often demonstrates increased expression of N-myc but not c-myc; c-myb can also be overexpressed in neuroblastoma. Neuroepithelioma normally has a rather poor prognosis. Based on the similarity of the pattern of oncogene expression to Ewing's sarcoma, some neuroepithelioma patients were treated according to the Ewing's protocol. The response was significantly improved over that obtained in patients on the standard neuroepithelioma protocol (Israel, et al., 1986).

Analysis of the neuroepithelioma case history demonstrates that some histopathologically indistinguishable tumors can be genetically heterogeneous, that patterns of oncogene expression may be useful for identifying genetically similar tumor groups and that genetically similar tumors may respond in the same manner to a given therapeutic intervention.

The ability to genetically characterize tumors has led to the observation that some apparently disparate tumor types involve genetic alterations in the same region of a particular chromosome. Cavenee and his colleagues (Koufos, et al., 1985; CA38583) have demonstrated that patients with the embryonal tumors hepatoblastoma, rhabdomyosarcoma and Wilm's tumor all demonstrate the loss of heterozygosity (the presence of genetic components from both parents) in the same region of chromosome 11 in their tumor tissue although their normal tissues contain a heterozygous chromosome 11. Patients who survive the hereditary form of retinoblastoma develop osteosarcomas at a significantly higher frequency than either the general population or survivors of the nonhereditary form of retinoblastoma. Analyses similar to those described above for the embryonal tumors were performed on osteosarcoma tissues and demonstrated a loss of heterozygosity at the retinoblastoma gene locus of chromosome 13 (Hansen, et al., 1985). Similar losses were detected when samples of tumor tissue from non-heritable cases of osteosarcoma were examined. Analysis of other related groups of tumors could lead to the identification of additional genetic loci associated with the development of specific tumors.

Traditionally, histopathologic evaluation has been the standard for diagnosis. Over the years, improvements in staining techniques and the addition of immunohistochemical approaches have increased the information to be gleaned from tissue sections. The discovery of the Philadelphia chromosome (Ph^1) and its usefulness in the diagnosis of chronic myelogenous leukemia (CML) indicated that additional criteria beyond those provided by morphology could be of diagnostic value. The Ph^1 is now a critical component in the diagnosis of CML and a recent retrospective study of Ph^1 negative cases of CML indicated that most of these cases were not actually CML and should be reclassified (Pugh, et al., 1985; Rowley CA16910).

The Ph^1 provided even more information when improved chromosome banding techniques indicated that it did not always represent the same chromosome translocation. Rather, it appears that a variety of simple and complex chromosomal interactions can take place to produce the small abnormal chromosome identified as Ph^1 (reviewed by Sandberg, et al., 1986). The molecular genetic

analysis of these various translocations has indicated one consistent alteration, the translocation of the c-abl oncogene at band 9q34 with resulting activation of c-abl expression. In most cases, the c-abl is translocated to a region on chromosome 22 called the breakpoint cluster region or bcr. The c-abl oncogene is thereby brought under the control of the bcr promoters and enhancers and aberrant bcr-abl m-RNA transcripts are produced. The protein product of these transcripts is a chimeric 210 Kd bcr-abl protein ($P210^{c-abl}$) which has tyrosine kinase activity. Collins (CA40728) has recently noted that the extent of bcr-abl transcription in CML blast crisis cells was increased over that in chronic phase cells. Further studies will be required to determine whether the extent of transcription of the rearranged bcr-abl gene provides prognostic information, e.g. whether changes in the level of expression can predict disease progression or whether the course of disease following bone marrow transplantation in CML patients correlates with bcr-abl transcription. Other groups are addressing similar questions by assaying both the $P210^{c-abl}$ protein product and the m-RNA transcripts (Witte CA40957).

Molecular analysis of gene rearrangements is playing an increasingly important role in the diagnosis of malignancy in non-Hodgkin's lymphomas (reviewed by Cleary and Sklar, 1985; Sklar CA38621, Cleary CA42971). The normal maturation of B-lymphocytes involves rearrangement of components of the immunoglobulin (Ig) genes. It has been demonstrated that the development of B-cell neoplasia is a clonal process reflecting genetic alterations in a progenitor cell which permit proliferation of that cell and its clonal descendents. The specific genetic alterations, Ig gene rearrangements, can be detected by restriction enzyme treatment and Southern blot analysis at a sensitivity of about one malignant cell in 100 (1%), requiring as little as 1 mg of tissue. It would be extremely rare for any benign process to result in a population of clonally altered cells equivalent to 1% of the cells in a lymph node; therefore, detection of clonal gene rearrangements would indicate a malignant event. And yet one malignant cell in 100 could be missed in histologic sections. But conventional histology is still required since the molecular analysis does not provide information about the subtype of the lymphoma and the observed gene rearrangements have been randomly detected in all subtypes of B-cell lymphomas. Subtyping is important for predicting prognosis but the molecular analysis can be an extremely valuable adjunct in the primary diagnosis of malignancy.

Genetic techniques have proven to be valuable in other diagnostic situations as well. The sensitivity of the molecular analysis has allowed the evaluation of lymphoma patients who demonstrate two different histologic types either in simultaneously or sequentially obtained biopsies (Siegelman, et al., 1985). Ig gene analyses of a number of cases have indicated that histologically distinct tissue samples contained cells of different clonal origin. In two of seven cases, the Ig gene patterns indicated progression from a single clone, revealing populations of cells which showed rearrangements in addition to those originally observed.

The sensitivity of the molecular technique should permit increased use of aspiration biopsies for multiple sampling and more thorough staging at the time of initial diagnosis (Hu, et al., 1986). This may also provide a less invasive method for confirming relapse.

T cell lymphomas are now also yielding to molecular analysis based on rearrangements of the T cell receptor genes. These analyses are at a much earlier stage of development and assessment of clinical value is very preliminary.

Most of the molecular genetic analyses described depend on the use of radioactive probes and detection of the bound radioactivity by exposure of x-ray film. Many of the techniques involve the use of ^{32}P which has a very short half-life limiting the shelf life of the reagents. In order for these molecular approaches to be more generally applied, a variety of technical improvements would be desirable. Scientists at the Cetus Corporation are developing a process to produce copies of any segment of human chromosomal DNA without having to first insert it into a microorganism (the usual "cloning" technique). The method involves the in vitro use of DNA synthesizing enzymes (polymerases) and is reported to be able to make about one million copies of the desired sequence in two hours (Science News, 1986). This method, if successful, would obviate the complicated process of inserting the desired sequence into the appropriate organism and reisolating and purifying the sequence following its replication.

Non-radioactive labels have generally lacked the sensitivity to detect unique sequences present in a large quantity of DNA. Two recent reports have described non-radioactive procedures for localization of genes on chromosomes by <u>in situ</u> hybridization (Landegent, et al., 1985; Pinkel, Straume and Gray, 1986). Both techniques involve the use of labels for detection that do not require the weeks of autoradiographic development time before the slides can be read. Landegent, et al. chemically modify the nucleic acid probe and detect hybridized probe using an antibody to the added chemical tag. The antibody is detected by the standard immunoperoxidase technique. Pinkel and his colleagues have adapted the avidin-biotin method by using biotinylated DNA probes and successive treatments with fluorescein-labeled avidin and biotinylated anti-avidin antibody to detect specific human chromosomes in interphase as well as metaphase cells. During interphase the chromosomes are not condensed and cannot be identified morphologically. Both these techniques require further refinement before they will be generally applicable to cytogenetic studies but they promise more rapid and perhaps more accurate diagnostic ability. Of course, these techniques do not yet address the requirement for radioactive probes in the Southern and Northern techniques for detection of gene rearrangements and expression, but some of these alternate detection methods may well be adapted for these techniques as well.

Another technical advance that will permit large scale retrospective studies is a method for extracting DNA from formalin-fixed, paraffin-embedded pathologic specimens. Jones and his colleagues (Dubeau, et al., 1986) have reported quantitative and qualitative Southern blot analyses using such extracted DNA. The importance of genetic alterations such as gene amplification, deletions, methylation patterns, etc. can now be studied retrospectively and other studies may not require fresh surgical specimens.

But rapid progress in the evaluation of genetic alteration does not depend solely on improvements in the detection methods. In the cases of CML and lymphoma, cytogenetic analysis and an understanding of lymphocyte biology,

respectively, guided investigators in their search for specific genetic changes. Consistent chromosomal aberrations found in other hematopoietic tumors have also provided clues and nucleic acid probes directed to these chromosomes can be used to determine whether consistent molecular alterations have occurred. However, the solid tumors, which comprise the great majority of cancers, have not yielded as easily as the hematopoietic tumors to molecular genetic investigation. The alterations in the epithelial tissue in one organ may differ from those in another but not in ways that suggest unique genetic changes. Therefore, most analyses to determine whether abnormal expression of oncogenes can be detected have depended on screening tumor samples with panels of oncogene probes. The discovery of N-*myc* in neuroblastoma was the result of this type of search. Hereditary forms of cancer have been studied for clues to specific chromosome involvement on the grounds that the same chromosomes may be involved in the non-hereditary form of the disease as in the hereditary form. This approach was fruitful in the cases of retinoblastoma and Wilm's tumor. However, solid tumors are generally more difficult to study because of the multiple cell types found in tumor samples. Often when these tumors are cultured prior to study, there appears to be loss of the three-dimensional structure of the *in vivo* tumor, of differentiated function and of the multiple cell types seen in the original explant. In many of the culture systems described, there clearly is selection of a small population of the original cells. The cells that are studied, then, may not be the critical cell population responsible for the tumor pathogenesis.

A variety of techniques have been developed to address the problems of selective culture of solid tumor tissue. Extracellular matrix has been used as a support for short term culture of gynecologic tumors (Crickard, Crickard and Yoonessi, 1984); a complex medium has been developed for growth of breast cancer tissue (Smith, et al., 1985); and recently, Freeman and Hoffman (1986) reported using a collagen substrate to support growth of a variety of human solid tumors. The results reported by Freeman and Hoffman indicate that structural differences observed in histologic sections of the tumor are maintained in culture and that multiplication of tumor cells continues in primary culture for several months. They were able to successfully culture explants from 65 of 89 tumors representing over 17 types of human tumors. This appears to be a system that could allow significant new information to be gained from solid tumors but many questions remain to be answered concerning maintenance of various differentiated functions, tumorigenicity and cytogenetic stability.

The advances in tumor tissue culture should permit more in depth study of solid tumor biology and cytogenetics. It is hoped that new insights will result in new diagnostic approaches and ultimately in more effective therapy. It should now be possible to determine whether there are primary chromosome alterations associated with tumors of particular organs or tissue types. If so, these observations can guide future molecular analyses because the number of DNA sequences to be tested can be decreased.

Applications of Modern Instrumentation Technology to Cancer Diagnosis

The diagnosis of cancer has depended mainly on routine techniques of tissue pathology, the observation of characteristics of tissues or of cell

preparations using conventional light microscopy and various specialized microscopic techniques such as fluorescence or electron microscopy. Specialized stains have been developed which augment recognition of the specific cellular characteristics required to confirm the diagnosis. These methods have proven highly successful, but require considerable effort on the part of highly trained specialists. The Diagnosis Program has emphasized the application of advances in modern technology to the diagnostic process. Two areas of programmatic importance have been digital imaging and flow cytometry. Currently a number of developments are contributing to advances in these two areas. Development during the past decade in computer hardware and in other integrated circuit elements have made possible instruments whose level of sophistication goes well beyond that envisioned just a few years ago. Simultaneously important developments in biology have created major new opportunities in cancer diagnosis. Current and projected research in the areas of automated microscopy and flow cytometry promise to capitalize on these advances to vastly improve cancer detection, diagnosis and monitoring of treatment.

Goals currently being pursued in automated imaging range from attempts to automate routine diagnosis of cervical smears to development of sophisticated instruments that can record interactions on the molecular level. When these instruments are combined with expert systems, software that allows the computer to mimic the decision-making thought processes of experts, the potential exists for fully automated cancer diagnosis. It should be noted that this goal is well beyond five years in the future, but a clear trend is that direction is already obvious. Production of large quantities of monoclonal antibodies and the development of nucleic acid probes to detect specific DNA and RNA sequences have made identification of biological processes possible at previously undetectable levels.

The development of automated cytology instrumentation has been driven by the availability of technology. From the development of the earliest automated microscopes through the development of flow cytometry to the multiparameter flow cytometers and high speed laser scanning microscopes under development today, each generation represented the limit of available technology. Fundamental to all of these developments was the amazing progress in computer technology because these technologies ultimately depend on computerized analysis of huge quantities of data. The change of scale from a roomful of electronics with processing speed of a thousand operations per second to a microcomputer that operates at a million operations per second makes the development of laboratory equipment feasible. Increases in memory capacity have paralleled or exceeded increases in processing speed. The current generation of personal computers operates at speeds of 1-2 million operations per second, can store anywhere from a quarter million to a million pieces of information in primary memory and is capable of rapidly storing and retrieving 10-30 times as much information on hard disk systems. The next generation of personal computers can be expected to have 5-10 times the processing speed and 10-20 times the memory capacity of current instruments. Display capacities and graphics capabilities have also improved at a phenomenal rate. New machines can be expected to improve resolution by a factor of 1000. The current gap between microcomputer, minicomputer, mainframe and supercomputer is rapidly diminishing. A further increase in capability can be expected from advances in digital electronics, and utilization of optical fiber technology. The development of

new hardware architectures is also important, especially the parallel processor approach. In applications such as image processing a large number of repetitive calculations must be made in order to acquire the data required for the next processing step. Bartels and his colleagues (CA38548) have utilized multiple processors operating in parallel to complete a series of computations and then to reallocate the available processors to new tasks as sufficient data become available. The Heidelberg POLYP parallel processor computer has been successfully adapted to imaging applications and programmed to operate 30 processors simultaneously.

Digital image methodology has been under development for 50 years and the usefulness of DNA content (ploidy patterns) in diagnosis known for 30 years. However, only recently has the instrumentation become available to allow the development of relatively inexpensive general purpose diagnostic instruments with the sophistication to overcome the complex problems of pattern recognition which have limited clinical applications to simple morphometric and DNA content measurements. Two examples of useful laboratory diagnostic instruments are the CAS 100 (Bacus CA36657) which was supported under the Small Business Innovation Research Program (SBIR) and the Micro TICAS system (Bibbo CA37352; Puls, 1985) currently in operation at the University of Chicago in prototype form. The CAS 100 is a complete package with all hardware and software for diagnostic ploidy analysis and the more versatile CAS 100-RS supports a full range of morphometric measurements for research applications. The micro TICAS video-based microphotometer/computer system is designed for rapid and inexpensive assessment of DNA ploidy patterns, but can also be expanded, with appropriate software, to a variety of research applications. The micro TICAS system is an implementation of the TICAS system (Wied CA42517; Wied, 1968) which initially operated on a mainframe computer and which now operates with much greater sophistication on a DEC VAX 11/730 minicomputer (Bibbo, 1985).

A considerable and convincing body of data has accumulated which supports the value of ploidy pattern in diagnosis and prognosis. Differentiation of reversible atypia from true neoplasia by morphologic criteria alone has been difficult. Fu (CA34870; Fu, 1985a,b) has demonstrated that dysplasias with diploid or polyploid patterns are preneoplastic and reversible while aneuploid patterns represent true neoplasms. Ploidy has also been shown to be important in defining neoplasms of a number of sites including endometrium, ovary, larynx, bladder, prostate, testes, esophagus, stomach, and colon. While ploidy can be measured with flow cytometric techniques, digital photometry has the added advantage that cell structure and form can also be observed.

Rosenthal (CA31718; Rosenthal, 1984a, 1984b) has applied high resolution digital image analysis to the diagnosis of cervical neoplasia and prediction of outcome. Using eight morphologic features 80% of the cases could be categorized correctly. One outcome of these studies is the finding that cells classified as moderate dysplasia, and similar on visual inspection, could be separated by digital imaging into groups based on their parent lesion. These results may allow classification of early lesions into prognostic categories and allow less drastic therapy for the majority of nonprogressive cases of cervical intraepithelial neoplasia. Hall and Fu (CA 34870; Hall, 1985) have reviewed applications of quantitative microscopy to tumor pathology and have detailed a number of site specific applications.

Computerized interactive morphometry has been proposed as a tool to achieve reproducible classification of non-Hodgkin's lymphomas. A classification algorithm based on objective size and shape criteria and the Working Formulation of Non-Hodgkin's Lymphomas for Clinical Usage (NCI 1985) classified 38 cases of non-Hodgkin's lymphoma and 6 cases of atypical hyperplasia correctly in all but two cases (Marchevsky, 1985). The two cases were subsequently reclassified by the original pathologists upon re-review of the slides.

Koss (CA15803; Koss, 1983) has analyzed images of voided urine sediment using a hierarchic classification system to detect and diagnose bladder cancer. Results were similar for 15 patient samples evaluated both by visual and by computer classification. The success of the computer analysis related, in part, to the creation of a category of "unsuitable for diagnosis" to exclude unsuitable or degenerate cells.

Digital imaging fluorescence microscopy systems utilize sensitive light detection devices such as photomultipliers or more recently, low light level video cameras to detect the light emitted in an area of interest on the microscope slide. The resulting signal can be used to form an image on a TV monitor and also provides specific quantitative information. While the ability to form images in fluorescence microscopy is subject to the same limits of resolution that apply to other forms of light microscopy, the limit of fluorescence detection is defined only by the sensitivity of the detector under the conditions of the measurement and by the intensity of the emitted light. In theory a single fluorescently labeled molecule can be detected. In practice detection of as few as 1,000 molecules has been achieved (Smith, 1985). Signals can be stored electronically using digital computers and data storage devices and recalled as needed for comparison with other data or for analysis. Barrows (1984) has described a sensitive system which utilizes a silicon intensifier target (SIT) video camera interfaced to a video digitizing system and digital computer in order to quantitate fluorescent images at the microscopic level. A similar system (Benson, 1985) has been described which uses a computer controlled image processor to provide precise light exposure times and image recording. A high speed fluorescence microscope, currently under construction at the University of Arizona, is expected to collect data at a rate approaching that of flow cytometric systems (Bartels CA38548). Detection of acridine orange by a simple microfluorometer has already proven useful in detecting abnormal bladder cells in subjects exposed to chemical carcinogens (Hemstreet, 1983, CA35898).

A unique application of fluorescence techniques was developed by Balchum (CA25582) in which pulmonary endoscopy is combined with digital imaging to allow detection of tumor. The imaging fluorescence bronchoscopy methodology uses detection of fluorescence from hematoporphyrin derivatives to localize bronchial cancer areas as small as 1 x 2 mm (Balchum, 1984). The system illuminates the examination area with diffuse violet light from a krypton laser conducted to the bronchus through an optical fiber in the bronchoscope. Emitted red light is collected through a magnifying lens and conducted to an image intensifier through a second optical fiber. The resulting image can then be subjected to digital image processing and background subtraction to improve contrast. Fluorescing dyes, hematoporphyrin derivative (HPD) or dihematoporphyrin ether (DHE), are retained longer in tumor (5+ days) than in

normal tissue, so that by 24 hours after administration most fluorescence defines tumor areas. The ability to detect tumors which are at an early stage and not visualizable on X-ray may provide diagnosis at a time when treatment can be effective.

The application of expert systems to digital imaging may lead to substantial improvements in cancer diagnosis. Expert systems, a branch of the rapidly growing field of artificial intelligence, use internal rules to mimic the thought process of human experts and to provide useful advice in specialized areas. While the utility of such systems is limited by the skill of the programmer in capturing and implementing the operative rules, the success of the expert systems developed so far is indisputable. Recently, the International Academy of Cytology announced "The First International Conference on Artificial Intelligence Systems (Expert Systems) as Diagnostic Consultants for the Cytologic and Histologic Diagnosis of Cancer." Several Diagnosis Program grantees (Bartels CA38548; Koss CA32345; Rosenthal CA37349 and Wied CA42517) represented the United States on the Program Committee and have been involved in developing approaches to the application of expert systems to cytology automation.

Flow cytometry is a method for rapidly obtaining information about a wide variety of cellular characteristics and for sorting cells with selected characteristics. Whole cells, nuclei or other cell constituents are carried entrained in a sheath of flowing liquid, past one or more sensors. A typical flow cytometer consists of a light source, either conventional or laser, photodetectors and the tubing which passes through the sample measurement chamber. The detector measures the intensity of scattered or fluorescent light and provides a profile which can be related to cellular characteristics such as size, shape, nucleic acid content, surface morphology, density and many others (Muirhead, 1985). In addition to characterizing cells, various techniques are available for selecting and sorting cells with specific characteristics for subsequent microscopic examination or additional studies.

The 1984 Diagnosis Program Annual Report provided a detailed discussion of applications of flow cytometry. However, a number of applications relevant to cancer diagnosis were not discussed, these and advances in other areas will be emphasized in this report.

Genetic applications of flow cytometry at both the chromosome and molecular levels show promise as adjuncts to conventional methodologies. Cytogenetic analysis is becoming increasingly important in cancer diagnosis, particularly as techniques applicable to solid tumors become more sophisticated. Flow karyotyping methodology has now advanced to the point where most (23/24) human chromosomes can be distinguished, albeit with overlap of chromosomes 9-12 (van den Engh, 1985). A joint project at the Los Alamos and Lawrence Livermore National Laboratories, supported by the Department of Energy, will utilize sorting techniques to sort human chromosomes in order to establish a recombinant human DNA library for each chromosome (Muirhead, 1985). The availability of the resulting DNA probes should advance cancer diagnosis. A group in the Netherlands (Collard, 1985) has reported a method for spot hybridization of flow sorted chromosomes with labeled DNA probes for localization of single-copy genes. Using this technique they were able to localize the c-*myb* oncogene to 6q21-6q23. They conclude that spot hybridization provides a rapid and simple

method to assign cloned genes to chromosomes or to identify individual chromosomes using specific, labeled DNA probes. The use of monoclonal antibody probes to detect specific chromosomes by immunofluorescent techniques can be expected to improve studies of chromatin structure and gene expression even further. The use of cDNA probes to detect genetic sequences flow cytometrically is currently limited by technical problems, but can be expected to provide major advances in several areas. It can be anticipated that the major progress in development of flow cytogenetics and the promising applications of flow techniques to molecular biology will provide important tools for studies related to cancer diagnosis.

The use of flow cytometry to measure cellular cytokinetics has recently begun to contribute not only to fundamental studies of cell growth and division, but also to cancer diagnosis and prognosis. The stages of cell growth and division can be represented by five characteristic phases. The G_0 phase represents cells which are not actively growing. Once growth begins the cells enter the G_1 phase in which cells grow and accumulate metabolic constitutents necessary for division. The next phase, the S phase, represents DNA synthesis. A second growth phase, G_2, involves increased accumulation of RNA and proteins and is followed by mitosis or cell division, the M phase. Measurements by flow cytometry of DNA, RNA and protein content of cells allows categorization of proliferating cells into G_1, S and G_2 + M phases (Darzynkiewicz, 1984; CA28704) A method for simultaneous measurement of DNA, RNA and protein has been described (Crissman, 1986) which promises to provide a means to recognize and classify tumor cells as well as to provide information on cell metabolism related to cell cycle, transformation or sensitivity to chemotherapeutic agents.

Incorporation of tritiated thymidine has been the method of choice for detection of cells which are actively synthesizing DNA. The combined flow cytometric analysis of DNA and incorporation of bromodeoxyuridine detected either by changes in DNA staining by several fluorochromes (Latt, 1974) or by specific fluorescently labeled monoclonal antibodies (Dolbeare, 1983), provides an alternative to tritiated thymidine for rapid cytokinetic analysis. The immunofluorescent technique has recently been shown to be useful for solid tumors grown and labeled in vivo as well as for cytokinetic analysis of cells grown in vitro (Pallavincini, 1985). Flow cytometry appears promising as a predictor of recurrence and survival in breast cancer patients. The thymidine labeling index (TLI) has been related to prognosis in at least three independent studies (Tubiani, 1984; Silvestrini, 1984; Meyer CA42154). Meyer has also used flow cytometry to measure the fraction of cells in S-phase and the steroid receptor content and has shown that both provide prognostic information. Currently the comparability of TLI and S-phase fraction are being evaluated (McGuire, 1985; Meyer, CA42154; Barlogie CA28771).

A number of approaches to the flow cytometric analysis of steroid receptors are under development (Oxenhandler, 1984; Van, 1984, CA28771; Benz, 1985; Raemaekers, 1984) as are assays for glucocorticoid receptor (Van, 1985, CA28771) and insulin receptor (Maron, 1984).

Immunologic markers of hemopoietic tumors have been measured by combined fluorescent labeled monoclonal antibody and flow cytometric techniques and

used to classify cells, to establish prognosis and to select appropriate therapy (Muirhead, 1985). The First International Workshop on Human Leukocyte Differentiation Antigens has published a list of phenotypic characteristics of leukocyte subpopulations and the appropriate antibodies for their detection (J. Immunol. 134:659-660, 1985). One approach to diagnosis of B-cell malignancies is detection by flow cytometry of kappa or lamda immunoglobulin on the cell surface using fluorescent labeled antibody (Weinberg, 1984; Barlogie, 1985, CA28771). These techniques have been demonstrated to detect B-cell lymphoma even in cases where conventional cell suspension studies could not differentiate lymphoma cells from normal cells and may allow early diagnosis or detection of recurrence. Barlogie has demonstrated the prognostic significance of RNA content and cytoplasmic immunoglobulin in patients with multiple myeloma (Barlogie, 1985). Ryan (1984, 1986, CA39569) has used multiparameter flow cytometry to detect Common Acute Lymphoblastic Leukemia Antigen (CALLA) positive cells in what promises to provide an extremely sensitive indicator of early leukemic relapse in Acute Lymphoblastic Leukemia. One CALLA positive cell in 100,000 cells could be detected. Recently two subpopulations of CALLA positive cells have been identified both in normal adult and in pediatric bone marrow cells. It was hypothesized that the CALLA positive subpopulations represent a maturation sequence of that early B-cell differentiation (Ryan, 1986). The delineation of B-cell maturation may lead to improved understanding of normal and neoplastic B-cell differentiation.

While flow cytometric techniques have applicability to the diagnosis and classification of solid tumors analogous to those described for hemopoietic cells, there are a number of technical difficulties which must be overcome before they can be generally applied. A number of monoclonal antibodies to surface antigens have been described which are potentially useful for detection of tumor cells. Many of these antibodies have been valuable in immunohistochemical staining. The major difficulty in the application of these reagents to the flow cytometric analysis of solid tumors results from the loss of surface markers during the cell dispersion process. Recently, however, some promising results have been obtained with monoclonal antibody reagents to surface and intracellular markers. A monoclonal antibody to a specific tumor antigen (TA-4) was used to evaluate cervical cytologic specimens with promising results (Suehiro, 1986). Anti CEA antibodies were applied to the evaluation of a colon carcinoma cell line (Noguchi, 1985) and allowed definition of a subpopulation with high CEA levels which when subsequently grown on chick embryonic skin formed significantly more acinar structures at 3 and 6 days than did cells from an unsorted population. A technique utilizing an anti-cytokeratin monoclonal antibody has been recently described to identify and sort columnar epithelial cells of adenocarcinoma and normal endometrium from fibroblasts and other non-epithelial cells (Oud, 1985). Other intermediate filament monoclonal antibodies would allow identification and selection of muscle, neural and glial derived normal and tumor cells and should refine measurements of these cell populations. While applications to solid tumors are still rare, the classification of solid tumors promises to become an important application of flow cytometry to tumor detection, diagnosis, and prognosis.

The application of flow cytometry to detection, diagnosis, prognosis and monitoring of therapy is proceeding rapidly. The chief difficulties now appear to relate to the definition of cellular subpopulations which predominate in

specific diseases and their relevance to prediction of outcome. The development of fluorescent labeled markers promises to greatly expand the ability to define cellular traits and particularly to assist in the classification of hemopoietic tumor cells. Cell cycle studies may provide important prognostic information and identification of highly proliferative cells.

Immunodiagnosis

Immunological techniques have been applied to the diagnosis of cancer in a variety of ways, including the detection of tumor-associated antigens in body fluids, the detection of cell surface antigens and their use in classification of histologic subtypes, the determination of prognosis, and the identification of malignant cells. The range of applications has been greatly expanded since the development of the hybridoma technique (Kohler, 1975) allowed the production of large quantities of monoclonal antibodies. Polyclonal and monoclonal antibodies have been developed for markers associated with almost all human malignancies, a variety of membrane receptors, oncogene products and intermediate filaments all of which are potentially useful in cancer diagnosis.

Monoclonal antibodies can be developed which are capable of binding to any specific antigenic determinant (epitope). The resulting antibody is specific only for the exact form of the antigen used for its development (Teillaud, 1983; Milstein, 1986; Jemmerson, 1986). Changes in chemical structure, even as minor as substitution of one amino acid for another without any change in conformation, may greatly affect antibody binding. In addition, changes in conformation such as those caused by denaturation of a protein or cleaving of the antigenic portion from a macromolecule may also render the antibody useless. These factors have greatly complicated the development of useful antibody reagents, requiring a great deal of trial and error in the assessment of specific reagents and leading to considerable variability in sensitivity and specificity of reagents developed to detect the same marker.

Another important consideration in the development of diagnostic antibody reagents is that antibodies themselves can trigger an immune response. Injection of murine monoclonal antibodies into humans for diagnostic imaging or treatment may initiate an immune response which can interfere with the function of the injected reagent and in some cases may even lead to potentially serious immune reactions. The development of human monoclonal antibodies hopefully would obviate these problems. Antibodies themselves can be used as antigens to develop new antibodies (anti-idiotype antibodies) which carry the structure of the original antigen. Anti-idiotype antibodies may be important in the development of vaccines and as a source of large amounts of pure antigen for the development of specific reagents for cancer diagnosis or therapy (Marx, 1985).

The production of human-human monoclonal antibodies (HMAb) was first reported in 1980 (Olsson, 1980; Croce, 1980). However, a number of technical difficulties have prevented the broad application of these techniques. Perhaps the major obstacle has been the lack of suitable malignant fusion partners with population doubling times and cloning efficiencies that are comparable to the murine myeloma cell lines. Other problems include low antibody secretion

rates and difficulties with producing adequately antigen primed B-lymphocytes. Recently significant improvements have allowed development of a number of HMAb hybridoma systems and routine production of HMAb in many laboratories (Olsson, 1985a, CA35227).

Development of malignant fusion partners has progressed rapidly in the past few years. True myeloma or B-lymphoma lines appear to be the most promising. An alternative approach utilizes Epstein-Barr virus (EBV) transformation of B-lymphoblastoid lines to produce fusion partners. Both of these approaches have led to lines with limited fusibility and cloning efficiency when compared to mouse lines. Hybridomas produced using EBV transformed human lines also exhibit variable, unstable, and low Ig secretion. Despite these limitations there are now more than 10 useful human cell lines which are promising as fusion partners (Olsson, 1985b, CA 35227), and new lines continue to be developed. A new B-lymphoblastoid line which generates hybrids at a rate comparable to that of murine systems (Heitzmann, 1986, CA 36310) has been reported to produce stable hybridomas which secrete high levels of new human Ig. Olsson (1985b, CA35227) has used 5-azacytidine treatment and selection of promising lines to further improve a useful human B-lymphoma line (RH-L4) and has created a subline which has a very high fusion efficiency, short doubling time, and high Ig secretion. A new approach utilizing short term passage of a B-lymphoma line through the peritoneal cavity or spleen of newborn mice appears promising as a method to produce altered phenotypes with desired characteristics (Honsik, 1986, CA 35227). A continuing barrier to the development of I_g producing hybridomas has been the lack of effective in vitro cell priming systems. Progress in cell priming systems has now been made and an efficient system developed (Ness, 1984; Olsson, 1985b) which will generate IgM secreting hybridomas. Improvements in B-lymphocyte culture techniques or new approaches such as DNA recombinant technology may eventually allow insertion and activation of specific genes for production of appropriate IgG antibodies (Olsson, 1985b, CA35227). An alternative approach for producing tumor reactive human monoclonal antibodies is to use B-lymphocytes isolated from regional draining lymph nodes of cancer patients, already primed for Ig production, as fusion partners with a high fusion efficiency lymphoblastoid B-cell line, UC729-6 (Glassy, 1985). While this approach does not allow selection of specific priming antigen, it does provide a means for priming B-lymphocytes in vivo and may result in hybridomas which secrete important site specific human monoclonal antibodies.

Murine monoclonal and polyclonal antibodies have provided important biological reagents useful both in cancer biology and in diagnosis. The number of new monoclonal antibodies and the variability in specificity even for different antibodies to the same epitope (antigenic site) make it difficult to summarize progress in the area of antibody development. Nevertheless, it is apparent that progress is being made and that the range of potential choices available for applications in various organ sites is rapidly expanding.

Transitional cell carcinoma of the bladder is a complex disease which can appear in several forms (papillary, sessile or in situ) and is highly variable in terms of its prognosis and response to treatment. A number of murine monoclonal antibodies have been developed which appear to be reactive to cell surface antigens which are not present on normal bladder or to blood group antigens which may be linked to invasiveness (Fradet, 1984; Juhl, 1986; McCabe, 1984; Messing, 1984; Summerhayes, 1985; Limas, 1985, CA33239).

A large number of monoclonal antibodies have been developed which react with carcinoma of the breast. A monoclonal antibody to human milk fat globule has been labeled with I-125 and used to image breast tumors in athymic nude mice (Ceriani, 1983, CA39937). A promising new monoclonal antibody (3E1-2) appears reactive to human breast but not to most other human tumors (except some lung, kidney and uterine carcinomas) when tested by immunohistochemical techniques against a panel of tissue sections (Stacker, 1985). Another murine monoclonal DF3 has defined a high molecular weight glycoprotein antigen in human carcinomas and in human milk (Sekine, 1985; Kufe CA38879). Estrogen receptors have been related both to prognosis and choice of therapy in breast cancer. A polyclonal antibody to estrogen receptor was used in an immunocytochemical assay to identify estrogen positive and estrogen negative cells in breast tissue sections and the results correlated with prognosis (Raam CA37944). Monoclonal assays to estrogen receptors have been developed (King, 1984) and will be widely applied to the immunohistochemical evaluation of receptor distribution in tissue (McCarty, 1984, CA 39635).

Colorectal carcinoma ranks high among causes of cancer related deaths in the United States. Radiolabeled antibodies against carcinoembryonic antigen (CEA) and other markers of colorectal cancer are promising for localizing relatively small tumors (Goldenberg CA39841). Otherwise, CEA has not proven useful for early detection, even though it is very useful for detecting recurrence (Steele, 1985; Zamcheck CA 04486). The use of immunohistochemical techniques has allowed detection and localization of CEA within tissue sections (DeLellis, 1984). A large number of antigens have been identified as specific cell surface markers for colorectal cancer, primarily glycolipids and glycoproteins. Recently brush-border membrane enzymes have been detected in colon cell lines by immunohistochemical techniques and may prove useful in detection of colorectal cancer (Quaroni, 1986). Another potentially important antigen, Large External Antigen (LEA), has been shown to be present on the cell surface of more than 90% of cell lines tested and not present on human non-colorectal and non-human cell lines, including other cancers (Bleday, 1986).

Tong (1984) has reported immunohistochemical characterization of two new monoclonal antibodies which react with small cell carcinoma of the lung (SCLC). One, SCLC 2051, predominantly reacted with SCLC, but only rarely (2/34) with other lung tumors. SCLC 5023 reacted with tumor tissue from SCLC in all cases tested, but also reacted with a variety of other lung tumors. Neither reacted with a variety of other tumors or with normal tissues. Another new marker of SCLC, MOC-1, reacted with all cases of SCLC, 4/9 cases of adenocarcinoma of the lung and no cases of squamous cell cancer (Postmus, 1986).

Ovarian neoplasms are the fifth most common cause of cancer related death among American women. Perhaps the most promising monoclonal antibody to ovarian antigens is that of Bast (1981). Recently this antibody, CA-125, was studied in patients with gynecologic cancer, pelvic inflammatory disease and in pregnant women. CA 125 was found to be elevated in 67% of ovarian cancer patients, 32% of patients with active cervical or endometrial cancer, 24% of pregnant woman and 33% of patients with inflammatory disease (Halila, 1986). These results suggest caution in the use of CA-125 in the diagnosis of ovarian cancer. Other reported ovarian cancer-specific monoclonal antibodies include NB/70K (Knauf, 1984), B72.3 (Thor, 1986) and OV-TL 3 (Poels, 1986). While it is yet too early to evaluate these markers, they appear promising.

Recently monoclonal antibodies have been developed to oncogene products. While relatively little has been done in this area so far, it can be anticipated that they will become increasingly important in reagents for cancer diagnosis. Niman (1985, CA 38160) has developed antisera to a number of synthetic peptides predicted from nucleic acid sequences of oncogenes and found that these urinary oncogene products appeared to be increased in both neoplasia and pregnancy. Thor (1984), using an immunohistochemical assay has measured differences in the expression of the ras oncogene product, p21, in colon carcinomas as compared to normal colon, to benign tumors or to dysplastic colon lesions. Expression of p21 has similarly been measured in prostatic carcinoma where it correlated with nuclear anaplasia and inversely with glandular differentiation (Viola, 1986) and in bladder where increases were demonstrated in all high grade lesions studied (Viola, 1985). Immunoprecipitation followed by Western blotting was used to evaluate p21 in lung tumors (Kurzrock, 1986) and elevated levels were detected in 9/11 squamous cell carcinoma of the lung vs 1/12 in other histologic types. Similarly, p21 was elevated in early stage but not late stage colon tumors and reduced levels were detected in metastases compared to primaries (Gallick, 1985).

Discussion

The emphasis on molecular genetic and cytogenetic research is reflected by an increase of over 200% in the active grants in this program area. The other program areas have remained stable with the exception of immunodiagnosis which decreased to about half its previous size, primarily reflecting reduced efforts toward the development of new monoclonal antibodies. A change in the method of tabulating projects in FY86 to include only those which received funding in the fiscal year would tend to underestimate increases and overestimate decreases. In previous years all grants active in the fiscal year were included even if they terminated and were not renewed. Overall, the program remained stable in FY86.

Progress in molecular genetics has resulted in an explosion of new clinical diagnostic approaches. While most of these remain experimental, their impact is beginning to be felt both in terms of defining prognostic categories and in influencing the choice of treatment protocols. The importance of the patterns of oncogene expression is being evaluated to determine whether such changes in the activation of specific oncogenes and/or in the resulting protein products may be related to prognosis. It will then be important to determine whether genetically similar tumors respond to therapy in a predictable manner. Advances in technology continue to simplify the assay procedures and to expand the range of molecular information that can be obtained. New techniques for culture of solid tumors promise to expand the knowledge of the molecular genetics of these tumors. The Diagnosis Program has published a Program Announcement in this area entitled "Solid Tumor Cytogenetics and Cancer Diagnosis" which is intended to stimulate collaborations between cytogeneticists and researchers with expertise in cell culture in order to improve the ability to study chromosomes in solid tumors. These studies are expected to result in new diagnostic approaches.

Developments in electronics and computer science, particularly new high speed and high data-storage-capacity hardware and the continued development of sophisticated software, have had a tremendous impact on instrumentation for cancer detection and diagnosis. New automated microscopy systems based on commercially available microcomputers are now available. High speed microscopes and imaging flow cytometers have brought these two technologies closer together in terms of capabilities. Application of molecular techniques, monoclonal antibody technology and fluorescent labeling techniques have greatly expanded the potential utility of these instruments. Continued research in these areas should result in the increased application of automated systems in cancer diagnosis.

Progress in hybridoma technology, particularly development of suitable human B-lymphocyte fusion partners and *in vitro* cell priming systems, has been impressive. A tremendous range of monoclonal antibodies has been developed and many new antibodies are in the development and testing phase. The development of monoclonal antibody reagents can be expected to expand the range of cancer diagnostics and their application through a variety of technologies.

Advances in each of the areas described in this report affect advances in the other areas. For instance the application of fluorescently labeled molecular probes and monoclonal antibodies to oncogenes and their products has advanced the capabilities of flow cytometry and digital fluorescence microscopy. Flow cytometric cytogenetic and cytokinetic analyses have facilitated experiments in molecular genetics and new monoclonal reagents have expanded capabilities in both molecular genetics and automated diagnostic systems. It is expected that the increased interest in diagnosis research will continue to encourage productive interactions and innovative research in these areas.

TABLE

CANCER DIAGNOSIS BRANCH

ALL PROJECTS FUNDED DURING FISCAL YEAR 1986

Number	Category	Grants		Contracts	
		Number	Current Funding (in Thousands)	Number	Current Funding (in Thousands)
1.	Biochemistry	22	$ 2,871	0	$ 0
2.	Immunodiagnosis	26	4,185	0	0
3.	Cytology	20	3,580	0	0
4.	Pathology	15	1,432	0	0
5.	Genetics	32	4,596	0	0
6.	Contracts Resource/Research	0	0	6	3,779
7.	Small Business Innovative Research	8	382	0	0
	TOTALS	123	$ 17,046	6	$ 3,779

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Seeger University of California, LA

R01 CA30687 Progesterone-Specific Protein in Endometrial Secretions
Richardson Massachusetts General Hospital

R01 CA33767 Bombesin-Like Peptides in Oat Cell Carcinoma
Moody George Washington University

R01 CA34881 Biochemical and Clinical Application of Acid Phosphatase 5
Lam University of Texas Health Science Center, San Antonio

R01 CA35329 Systematic Study of Three Types of Glycosyltransferases
Matta Roswell Park Memorial Institute

R01 CA36882 Surgical Application of a New Marker for Colon Cancer
McSherry Beth Israel Hospital, New York

R01 CA37056 Non-invasive Approach for Detection of Lung Cancer
O'Neil IIT Research Institute

R01 CA37200 Metabolism of Carcinoembryonic Antigen
Thomas Mallory`Institute of Pathology Foundation

R01 CA38797 Studies on CA Antigen and Related Glycoproteins
Bhavanandan Pennsylvania State University

R01 CA38878 Diagnostic Assay for I, i Antigens in Breast Carcinoma
Dube Evanston Hospital

R01 CA39233 A Study of Cancer Associated Colonic Mucin
Boland University of Michigan

R01 CA39431 Thioesterase II a New Breast Cancer Marker
Smith Children's Hospital Medical Center, Oakland

R01 CA39535 EGF Receptor Regulation in Human Tumor Specimens
Hendler University of Texas Hlth. Sci. Ctr., Dallas

R01 CA39641 Serum Tyrosyl Kinases in Human Neoplasia
Clinton Louisiana State University Medical Center

R01 CA39765 Hemoquant Detection of Colorectal Neoplasia
Ahlquist Mayo Foundation

R01 CA40193 Monoclonal Antibody for Trophoblastic Disease Diagnosis
Hatch University of Alabama at Birmingham

R01 CA40406 O'Brien — Tumor Antigen CA 125 in Ovarian Cancer Patients
University of Arkansas

R01 CA40423 Tapper — Determination of a New Growth Factor in Breast Milk
Children's Orthopedic Hospital, Seattle

R23 CA40936 Tracy — Protein Markers of Renal Cell Carcinoma
University of Vermont

R01 CA42737 Silverstone — Abnormal Terminal Transferase Biosynthesis in Remission
State University of New York, Upstate Medical Center

R01 CA43305 Schultz — Phenotypic Characterization of Transformation
Loyola University

R01 CA44131 Cole — O-Glycosylation of hCG and Cancer
Yale University

IMMUNODIAGNOSIS

R01 CA18404 Baylin — Neuroendocrine Differentiation in Human Tumors
Johns Hopkins University

R01 CA22595 Jackson — Detection of Medullary Thyroid Cancer in Families
Henry Ford Hospital

R01 CA29639 Nelp — Tumor Imaging with Radiolabelled Monoclonal Antibody
University of Washington

R01 CA30019 Gupta — Purification of Tumor Antigens of Defined Specificities
University of California, LA

R01 CA33239 Limas — Tissue Blood Group Antigens in Urothelial Neoplasia
University of Minnesota of Minneapolis - St. Paul

R01 CA34635 Dunsford — Preneoplastic Markers Detected by Monoclonal Antibodies
University of Texas Health Science Center, Houston

R01 CA35354 Brown — Monoclonal Antibodies to Human Sarcoma Membrane Antigens
University of Illinois at Chicago

R01 CA36310 Heitzmann — Cell Lines & Methods for Human Hybridomas
Salk Institute for Biological Studies

R01 CA36320 Volsky — New Approach to Produce Human Monoclonal Antibodies
University of Nebraska Medical Center

R01 CA36422 Teng — Heteromyelomas for Human Monoclonal Antibody Production
Stanford University

R01 CA36450 Taylor — Isolation of a Hodgkin-Related Antibody
University of Southern California

R01 CA36903 Radioimmunodetection of Pancreatic Tumors
Cheng University of Iowa

R01 CA36934 Immunocytochemical Studies of Prostatic Acid Phosphatase
Lam University of Texas Health Sciences Center, San Antonio

R01 CA37411 Immunological Heterogeneity of CEA
Primus University of Medicine & Dentistry of NJ

R01 CA38160 Sequence Specific Hybridomas to Growth Factors
Niman Scripps Clinic and Research Foundation

R01 CA38687 Human Myeloma Analogues for Making Monoclonal Antibodies
Posner Roger Williams General Hospital

R01 CA38879 Immunodiagnostic Approaches to Human Breast Cancer
Kufe Dana-Farber Cancer Institute

R35 CA39841 Radioimmunodetection of Cancer
Goldenberg Center for Molecular Medicine and Immunology

R01 CA39932 Circulating Tumor Components
Ceriani John Muir Memorial Hospital

R01 CA40186 Clinical Utility of a Neuroblastoma Monoclonal Antibody
Yu University of California, LA

R23 CA40357 Circulating Malignant Cells in Non-Hodgkin's Lymphoma
Weinberg Brigham & Women's Hospital

R01 CA40608 Development and Characterization of Myeloma Cell Lines
Epstein University of Southern CA

R01 CA41166 Specificity of Antitransforming Gene Product Antibody
Sklar University of Michigan at Ann Arbor

R01 CA41386 Immunologic Probes for Study of Progesterone Receptors in Breast CA
Edwards University of Colorado Health Science Center

R01 CA41549 Evaluation of MoAbs Against a Proteinase Tumor Marker
Perras University of Miami

R01 CA43368 Radiolabeled Antibody Localization of B-Cell Lymphoma
Buchsbaum University of Michigan, Ann Arbor

CYTOLOGY

R01 CA28771 Cytology Automation
Barlogie University of Texas System Cancer Center, Houston

R01 CA28921 Schlegel — Merocyanine Dyes as Leukemia Specific Probes
Pennsylvania State University

R01 CA30582 Wheeless — Multidimensional Slit-Scan Prescreening System
University of Rochester

R01 CA32345 Koss — Computer Image Analysis of Cells in Urothelial Cancer
Montefiore Medical Center, New York

R01 CA33148 Wheeless — Multidimensional Slit-Scan Detection of Bladder Cancer
University of Rochester

R01 CA34870 Fu — Nuclear DNA and Morphometric Studies of Gynecologic Cancer
University of California, LA

R01 CA35898 Hemstreet — Quantitative Probes in Cancer Prevention & Diagnosis
University of Oklahoma Health Sciences Center

R01 CA37349 Rosenthal — Improved Cancer Diagnosis by Morphometric Analysis
University of California, LA

R01 CA37352 Bibbo — Tumor Diagnosis by Rapid DNA Ploidy Pattern Analysis
University of Chicago

P01 CA38548 Bartels — Fast Digital Microscope Designs for Tumor Diagnosis
University of Arizona

R01 CA38807 Sklarew — Cytokinetics of Breast Cancer Subpopulations by Imaging
New York University School of Medicine

R23 CA39010 Benson — Assessment of Human Prostate Tumor Cell Heterogeneity
Columbia University, New York

R01 CA39022 Braylan — Flow Cytometric Analysis of DNA in Human Lymphoma Cells
University of Florida

R23 CA39569 Ryan — Biology of Calla + Cells in Lymphoblastic Leukemia
University of Rochester

R01 CA40526 Gamel — Malignant Potential of Uveal Melanoma
University of Louisville School of Medicine

R01 CA42154 Meyer — Clonal Heterogeneity of Breast Carcinoma
Saint Luke's Hospital, Missouri

R35 CA42517 Wied — Computer-Based Expert System for Cervical Cytology
University of Chicago

R01 CA42734 Sieber — Lipophilic Probes for Tumor Cell Surfaces
Medical College of Wisconsin, Milwaukee

R01 CA42891 Smith — Minimal Residual Disease in Lymphoblastic Leukemia & Lymphoma
University of Texas Health Science Center, Dallas

R01 CA43161 Parallel Blind Clinical Study: MDSS Prescreening System
Wheeless University of Rochester

PATHOLOGY

R01 CA14264 Pathology of Cell Differentiation in Leukemia
Bainton University of California, San Francisco

R01 CA22101 Study of Head and Neck Cancer by Serial Section
Kirchner Yale University

R01 CA25582 Diagnostic Imaging Fluorescence Bronchoscopy
Balchum University of Southern California

R01 CA26422 Clinico-Biologic Correlation in Lymphoma and Leukemia
Winberg City of Hope National Medical Center

P01 CA28853 Comparative Study of Primary and Metastatic Human Tumors
Holyoke Roswell Park Memorial Institute

R01 CA36245 Intermediate Filament Proteins as Tumor Markers
Trojanowski University of Pennsylvania

R01 CA36250 Cytoskeletal Hybridoma Antibodies as Diagnostic Reagents
Gown University of Washington

R01 CA36902 Macromolecular Transport in Neoplastic Capillary Beds
Jain Carnegie-Mellon University

R01 CA37083 Monoclonals for Immunodiagnosis of Processed Tissues
Rouse Stanford University

R01 CA37194 Monoclonal Antibodies in Classification of Tumors
Battifora City of Hope National Medical Center

R01 CA37944 Immunohistochemical Classification of Human Breast Tumors
Raam Tufts University

R01 CA38193 Project on the TNM Classification of Malignant Tomours
Veronesi International Union Against Cancer, Geneva, Switzerland

R01 CA38727 Histopathology, Enzymatic Analysis and Prognosis
Pretlow Case Western Reserve University

R01 CA39353 Endocrine-Paracrine (APUD) Cells and Prostate Pathology
Di Sant'Agnese University of Rochester

R01 CA39635 Immunohistochemical Receptor Localization in Gynecologic Lesions
McCarty Duke University

R01 CA16910 Rowley	Chromosome Aberration in Myeloproliferative Diseases University of Chicago
R01 CA31024 Yunis	Fine Chromosomal Defects in Leukemia & Myelodysplasia University of Minnesota
R01 CA33314 Yunis	Fine Chromosomal Defects in Non-Hodgkin's Lymphoma University of Minnesota
R01 CA34775 Chaganti	Mapping Chromosomes and Genes in Relation to Leukemia Sloan-Kettering Institute for Cancer Research
R01 CA35040 Samaan	Early Detection of Medullary Thyroid Carcinoma University of Texas System Cancer Center, Houston
R01 CA37866 Krontiris	Analysis of Human Oncogene Polymorphisms Tufts University School of Medicine
R01 CA38569 Gale	Diagnosis of Leukemia (CML) Oncogene (C-ABL) Expression University of California, LA
R01 CA38579 Perucho	Mutant Ras Oncogenes in Human Tumors State University of New York, Stony Brook
R01 CA38583 Cavenee	Recombinant DNA Technology to Pediatric Cancer University of Cincinnati
R01 CA38621 Sklar	Genetic Studies of Human B Cell Cancer Stanford University
R01 CA38685 Rowley	Detecting Inherited Cancer Predisposition Via Oncogenes University of Rochester
R23 CA39237 Spindel	Bombesin-like Peptides: Structure and Physiology Joslin Diabetes Center
R01 CA39771 Brodeur	Molecular Genetic Analysis of Human Neuroblastoma Washington University
R01 CA39926 Emanuel	Cytogenetic and Molecular Studies of Human Chromosome 22 Children's Hospital of Philadelphia
R01 CA40640 Iglehart	Expression of Ras Oncogenes in Human Tumors Duke University
R01 CA40725 Rudders	Clonal Evolution of Human Malignant Lymphomas New England Medical Center Hosp. Inc., Boston
R01 CA40728 Collins	c-abl Oncogene Expression in CML Fred Hutchinson Cancer Research Center, Seattle

R01 CA40842 Housman — Identification of a Melanoma Gene by Genetic Linkage
Massachusetts Institute of Technology

R01 CA40957 Witte — Molecular Diagnosis of Chronic Myelogenous Leukemia
University of California, L.A.

R01 CA40969 Salser — Recombinant DNA Diagnostic Probes for Small Cell Cancers
University of California, L.A.

P01 CA41124 Hecht — Cancer Cytogenetics and Fragile Sites
Southwest Biomedical Research Institute, Scottsdale, AZ

R01 CA41372 Augenlicht — Gene Expression in Human Colon Cancer
Montefiore Medical Center, New York

R01 CA41546 Gemmill — Molecular Analysis of the Common Fragile Site at 3p14
Southwest Biomedical Research Institute, Scottsdale, AZ

R01 CA41644 Le Beau — Chromosomal Fragile Site Studies in Neoplastic Diseases
University of Chicago

R01 CA41759 Gilbert — Chromosome Instability and Tumor Develpment
Mount Sinai School of Medicine, New York

R01 CA41763 Patil — Cytogenetics & Predispostion to Cancer
University of Iowa

R23 CA42142 Krawisz — Development and Progression of Human Colonic Neoplasms
University of Vermont

R35 CA42232 Nowell — Studies of Neoplastic and Normal Leukocytes
University of Pennsylvania

R35 CA42557 Rowley — Chromosome Abnormalities and Human Leukemia and Lymphoma
University of Chicago

R01 CA42595 Millan — Cancer-Related Placental-Type Alkaline Phosphatases
La Jolla Cancer Research Foundation

R01 CA42971 Cleary — Molecular Pathology of Human Lymphoid Malignancies
Stanford University

R01 CA43222 Glover — Role of Fragile Sites in Chromosome Breakage and Cancer
University of Michigan

SBIR

R43 CA39866 Heisterkamp — Immunologic Assay for the Philadelphia Translocation
Oncogene Science, Inc., Mineola, NY

R43 CA41717 Reitz — A New Serum Marker for Endocrine Cancer
Endocrine Metabolic Center, Oakland, CA

R43 CA41818 Garber — Low-Cost Interactive Videodiscs: Cancer Pathology Data
United States Video Corporation, Vienna, VA

R43 CA41958 Reynolds — Peptide Substrates and Inhibitors of Protein Kinase C
Oncogene Science, Inc., Mineola, NY

R43 CA42051 Taub — Measuring DNA Probe Hybridizations Without Radioactivity
Digene, Rockville, Md.

R43 CA42670 Dunkirk — Immobilization of Biomolecules
Bio-Metric Systems, Inc., Eden Prairie, MN

R43 CA42700 Bacus — Quantitation of Estrogen Receptor Concentration in Cells
Cell Analysis Systems, Inc., Chicago, ILL

R43 CA43325 Hurd — High-Resolution, High-Capacity Isoelectric Focusing
Focus Research, Hamden, CT

CONTRACT RESEARCH SUMMARY

Title: Human Tumor Cell Line Bank for Diagnostic Studies

Principal Investigator: Dr. Robert J. Hay
Performing Organization: American Type Culture Collection
City and State: Rockville, MD

Contract Number: N01-CB-14351
Starting Date: 9/29/81 Expiration Date: 9/28/86

Goal: The objectives of the program are to acquire, characterize, catalog, store and distribute a variety of cell lines having special utility for research in tumor diagnosis. Well characterized lines derived from solid tumors as well as some from related normal tissues will be included. Information concerning properties and utility of these lines will be provided to all interested investigators.

Approach: Cell lines selected in consultation with the Government Project Officer (GPO) and advisors will be expanded from token holdings or new submissions to produce seed and distribution stocks. These will be characterized using published ATCC procedures. Initially, standard tests for the absence of microbial contamination will be applied and species verification will be accomplished by assay for the isoenzymes of glucose-6-phosphate dehydrogenase, lactic dehydrogenase and nucleoside phosphorylase. Distribution will begin following satisfactory completion of these tests. Lines in the existing bank are being characterized further with regard to isoenzyme profiles, karyology, surface antigens, etc. as time and funds permit.

Progress: The lines selected from the former cell bank by the advisory committee were recharacterized and catalogued for distribution. In addition, 15 human breast cancer lines were transferred to the ATCC from the EG & G Mason Institute and token stocks of a variety of other human cell lines (965) were transferred from the Naval Biosciences Laboratory (NBL). Fourteen of the former 127 and of the latter were initially selected from the NBL lines for examination and possible addition to the bank. Twenty of the 127 NBL lines and the 14 EG & G Mason lines were characterized and found suitable for inclusion in the Bank. Many of the NBL lines were eliminated because they were stromal in nature. In general, fibroblast-like lines have not been included unless a tumor line from the same patient is also available. To date, the Human Tumor Cell Line Bank consists of 126 lines. These are shipped for a fee upon request along with directions on reconstitution and culture. An average of 244 cell lines per month have been shipped during the past 12 month period.

Significance to Cancer Research: This resource provides well characterized cell lines to cancer researchers. Cell lines have proven useful in both basic and applied research studies related to cancer diagnosis.

Project Officer: Roger L. Aamodt, Ph.D.
Program: Cancer Diagnosis
Technical Review Group: Special Review Group
FY 86 Funds: $0.00 A

CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer: Patient Resource

Principal Investigator: Dr. Theodore Maycroft
Performing Organization: Butterworth Hospital
City and State: Grand Rapids, MI

Contract Number: N01-CB-23927
Starting Date: 9/15/82 Expiration Date: 9/14/86

Goal: To develop a specimen resource for blood from breast cancer patients and benign disease patients to be used in a search for and verification of new breast cancer markers.

Approach: Prior to surgery, thirty milliliters of blood are collected from breast disease patients scheduled for biopsy and/or primary surgery for breast lesions. Another specimen is collected, when feasible, from the same patient 5-10 weeks postmastectomy. Annual follow-up collections and clinical data updates are made on patients diagnosed as having malignant disease. Patients diagnosed as having benign breast disease are asked to complete annual questionnaires for the two years folowing biopsy. Serum specimens are stored at -70°C , then shipped to the NCI Breast Cancer Serum Bank at The Mayo Foundation. Appropriate clinical data is sent to a central data center, Information Management Services (IMS).

Progress: Surgeons who perform 95% of all breast biopsies in any of the three participating institutions have agreed to participate in the study. From the inception of the program until March 31, 1986, 5,295 patients have been entered in the project, 1,191 of whom had malignant breast disease. A total of 2,021 annual follow-up collections have been made on malignant disease patients. A total of 5,004 follow-up questionnaires have been completed on benign disease patients and forwarded to IMS. Approximately 82,459 vials containing serum specimens were received and processed by the central storage facility at Mayo Clinic as of March 28, 1986.

Significance to Cancer Research: This resource supports The Breast Cancer Serum Bank which is maintained by the Cancer Diagnosis Program to evaluate potential biological markers for breast cancer diagnosis, detection, prognosis, and monitoring of therapy.

Project Officers: Roger L. Aamodt, Ph.D. and Judith M. Whalen, M.P.A.
Program: Cancer Diagnosis
Technical Reveiw Group: _Ad Hoc_ Cancer Resources and Repositories
FY 86 Funds: $91,867 A

CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer: Patient Resource

Principal Investigator: Dr. Ralph D. Reynolds
Performing Organization: Cancer Research Center
City and State: Columbia, MO

Contract Number: N01-23925
Starting Date: 9/01/82 Expiration Date: 8/31/86

Goal: To serve as a specimen resource for serum from breast cancer patients and controls to be used in a search for and verification of new breast cancer markers.

Approach: Since late 1977, blood samples have been drawn from volunteer Breast Cancer Demonstration Detection Project and Women's Cancer Control participants and from patients scheduled for breast surgery at two local hospitals. In addition, post-mastectomy (30 to 100 days) and annual samples are drawn from women with confirmed diagnosis of breast cancer. After obtaining appropriate consent, 30 ml of blood is collected and processed into 10-13 one ml aliquots of serum.

The serum is stored at -70°C and frozen samples shipped to the Mayo Clinic for storage and dissemination. Associated clinical data is sent to and retained by the central data center, Information Management Services (IMS). Clinical histories are updated at one year for patients with benign biopsies and annually for two years from normal participants.

Progress: By March 31, 1986, 13,848 samples of blood had been drawn on 7,764 different participants. This included 6,735 control women, 451 pre-op benign, 281 pre-op malignant and 554 other malignant. As of March 31, 1986, Mayo Clinic reported that they have in storage 147,823 vials submitted from Columbia. In addition, clinical update histories have been submitted to IMS on 7,443 participants. Because of special efforts on the part of the serum markers personnel and a cooperative effort with the Women's Cancer Control Program, we have only had to designate 72 participants as lost to followup.

Significance to Cancer Research: This resource supports The Breast Cancer Serum Bank which is maintained by the Cancer Diagnosis Program to evaluate potential biological markers for breast cancer diagnosis, detection, prognisis, and monitoring of therapy.

Project Officers: Roger L. Aamodt, Ph.D. and Judith M. Whalen, M.P.A.
Program: Cancer Diagnosis
Technical Review Group: _Ad Hoc_ Cancer Resources and Repositories
FY 86 Funds: $64,510 A

CONTRACT RESEARCH SUMMARY

Title: Biomedical Computing Software Services in Support of the Diagnosis Program

Principal Investigator: Ms. Marlene Dunsmore
Performing Organization: Information Management Services, Inc.
City and State: Silver Spring, MD

Contract Number: N01-CB-51010
Starting Date: 6/15/85 Expiration Date: 6/14/89

Goal: To provide computer related support services to the Diagnosis Program for studies designed to evaluate promising new serum markers for cancer diagnosis.

Approach: Computer support for the Diagnosis Program includes a) maintaining and updating clinical data files and specimen inventories from the two breast cancer serum collection centers, b) processing requests and assembling serum panels for shipments from the serum banks to researchers, and c) preparation of data files and execution of computer programs for complex statistical analysis of the clinical data and the data resulting from panel assays. The resulting data file is useful for testing ideas, identifying groups of subjects suitable for more detailed study, and for preparing reports to the medical community and the general public. The contractor utilizes sophisticated data handling and analytic techniques. The current system relies heavily on the NIH/DCRT IBM/370 and DEC-10 computer systems.

Progress: Biomedical computing support for the Diagnosis Program has continued in an exemplary manner. New tasks related to the Diagnosis Serum Bank are being successfully carried out. Responsibility for composing serum panels has been transferred from the Project Officer to the contractor and is being carried out successfully. Analysis of serum assay results has also been completed in a timely and satisfactory manner. Work related to the Breast Cancer Serum Bank has continued without any diminution of quality. To date nearly 23,000 forms reporting background and clinical data have been received and added to the file. The maintenance of various data bases and analysis of assay results constitutes the main workload. Data from two breast cancer serum collection centers is checked for consistency, verified and stored. Clinical data for two serum Banks is maintained and the composition of coded serum panels determined by the Contractor. Analysis of results using sophisticated statisitical techniques is being accomplished for all Breast Cancer Serum Bank and Diagnosis Serum Bank users.

Significance to Cancer Research: This resource supports two serum banks which are maintained by the Cancer Diagnosis Program to evaluate potential biological markers for cancer diagnosis, detection, prognosis, and monitoring of therapy.

Project Officers: Roger L. Aamodt, Ph.D. and Judith M. Whalen, M.P.A.
Program: Cancer Diagnosis
Technical Review Group: Intramural and Administrative Support Contract Review Subcommittee B
FY 86 Funds: $183,181 A

CONTRACT RESEARCH SUMMARY

Title: Maintenance of the NCI Serum Diagnostic Bank

Principal Investigator: Dr. Eugene DiMagno
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: N01-CB-51002
Starting Date: 9/01/85 Expiration Date: 8/31/89

Goal: To collect, maintain and distribute sera from patients with cancer, benign diseases and from healthy controls, in order to evaluate serum assays with potential as biological markers of cancer in a search for and verification of clinically useful assays.

Approach: Serum samples are collected, frozen and made available for studies to validate biochemical and immunodiagnostic tests for cancer, and as a central storage facility. Serum collected by two breast cancer collection centers under separate contracts are received, stored and distributed as a separate task.

Progress: Task 1: Diagnosis Serum Bank A bank of sera from patients with malignant disease, benign diseases and sera from healthy individuals has been established and is being maintained. A computerized clinical data and inventory system has also been developed. Sera are stored in 51 freezers at -70°C with adequate continuous temperature monitoring and quality control. The Project Officer makes sera available in response to requests from investigators. The serum is provided as coded panels to evaluate immunodiagnostic, hormonal and enzymatic tests for cancer. Panels are designed for determination of the sensitivity and specificity of specific tumor markers and for comparison of these values with those of other tumor markers. Current inventory also includes blood collected under previous contracts. During the past year, April 1985 through March 1986, 14 panels were shipped. The collection included 111,511 specimens on February 28, 1986. There are, in addition, a total of 177,487 vials from other investigators, 119,007 from University of Minnesota, 41,975 from Philadelphia Geriatrics 13,917 from Memorial Sloan-Kettering and 2,588 from Emory University. Task 2: Breast Cancer Serum Bank Storage and inventory methods have been refined and are working well. Samples are shipped from the collection centers to the central bank on a regular basis. As each shipment is received, samples are catalogued and systematically stored in one of 36 freezers. As of February 28, 1986 the collection included 236,423 vials, 6,131 from the Wilmington, DE collection center, 82,459 from Grand Rapids, MI and 147,823 from Columbia, MO. Since June 19, 1979, 31 coded serum panels have been shipped to individual investigators for evaluation of new breast cancer markers.

Significance to Cancer Research: This resource supports two serum banks maintained by the Cancer Diagnosis Program to evaluate potential biological markers for cancer diagnosis, detection, prognosis, and monitoring of therapy.

Project Officers: Roger L. Aamodt, Ph.D. and Judith Whalen M.P.A.
Program: Cancer Diagnosis
Technical Review Group: Cancer Resources & Repositories Contracts Review Committee
FY 85 Funds: $326,738 A

CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool to Detect Early Cancer of Bowel

Principal Investigator: Dr. Victor A. Gilbertsen
Performing Organization: University of Minnesota Health Sciences Center
City and State: Minneapolis, MN

Contract Number: N01-CB-53862
Starting Date: 6/30/75
Expiration Date: 2/15/86

Goal: To demonstrate significant reduction in colorectal cancer mortality between the screened and the control groups. The test groups will be screened employing the Hemoccult (R) form of the guaiac test for occult blood in the stool. A diagnostic protocol will be followed to locate the source of bleeding if the test is positive.

Approach: Forty-five thousand participants between 50 and 80 years of age, residing in the state of Minnesota, with no prior history of colorectal cancer were randomized into three groups (two experimental, one control) by age, sex and geographic region of the state. Test groups submitted slides annually or biennially; the control group did not submit slides. Guaiac slides were completed and returned to the University by mail to be developed. Participants submitting slides positive for blood were evaluated at the University of Minnesota hospitals and clinics using the specified diagnostic protocol; this included a complete history and physical examination, upper G.I. series x-ray (and gastroscopy if indicated), proctoscopy and colonoscopy.

Progress: The initial screening phase of the study was completed in December, 1982. Follow-up procedures to determine vital status and to monitor the incidence of disease among the participants are continuing. Contact has been maintained with over 95% of the participants. The Policy and Data Monitoring Group, established in 1984 to assess the data, recommended that screening be resumed; this appeared to be necessary to increase the statistical power of the study. Resumption of screening will begin under a replacement contract (cf. N01-CB-61005) and is scheduled to continue for five years. During this period, follow-up will also continue and all deaths will be recorded. All cancer deaths receive particular attention to determine colorectal involvement; deaths shown to involve colorectal malignancy are carefully evaluated by the Death Review Committee to determine whether the individual died from or with colorectal cancer.

Significance to Cancer Research: Colorectal cancer accounts for close to 70,000 deaths per year in the U.S. and a variety of approaches are necessary to reduce this number. This study will determine whether screening for occult blood in the stool will reduce mortality from colon cancer.

Project Officers: Sheila E. Taube, Ph.D, Ihor Masnyk, Ph.D. & Philip Prorok, Ph.D.
Program: Cancer Diagnosis
Technical Review Group: Ad Hoc Techical Review Group
FY 86 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool to Detect Early Cancer of Bowel

Principal Investigator: Dr. Victor A. Gilbertsen
Performing Organization: University of Minnesota Health Sciences Center
City and State: Minneapolis, MN

Contract Number: N01-CB-61005
Starting Date: 2/16/86
Expiration Date: 12/31/88

Goal: To demonstrate significant reduction in colorectal cancer mortality between the screened and control groups. Test groups will be screened employing the Hemoccult (R) form of the guaiac test for occult blood in the stool. A diagnostic protocol will be followed to locate the source of bleeding if the test is positive.

Approach: This contract represents a continuation of an ongoing study (cf. N01-CB-61005) in which forty-five thousand participants between 50 and 80 years of age, residing in the state of Minnesota, with no prior history of colorectal cancer were randomized into three groups by age, sex and geographic region of the state. Test groups submit Hemoccult slides annually or biennially; the control group does not submit slides. Completed slides are returned to the University by mail to be developed. Participants whose slides are positive are evaluated at the University of Minnesota hospitals using the specified diagnostic protocol. This protocol now includes a complete history and physical examination, colonoscopy and other follow-up tests, as needed to determine the source of bleeding. All participants are asked to complete a short annual questionnaire concerning their health status.

Progress: The initial five years of screening were completed in December, 1982. Contact has been maintained with 98% of the participants to determine vital status and to monitor the incidence of disease. The Policy and Data Monitoring Group, established in 1984 to assess the data and conduct of the study, recommended that screening be resumed to increase the statistical power of the study. The proposal for resumption of screening was reviewed in September, 1985, and the screening program resumed in February, 1986. The initial response from participants has been very good and most submitting positive slides have agreed to have the diagnostic follow-up at the University hospitals. Contact with all participants is continuing through the annual questionnaire. Records of all deaths from cancer are examined to determine colorectal involvement and the Deaths Review Committee evaluates those involving colorectal malignancy to determine whether the individual died from or with colorectal cancer.

Significance to Cancer Research: Colorectal cancer accounts for close to 70,000 deaths per year in the U.S. and a variety of approaches are necessary to reduce this number. This study will determine whether screening for occult blood in the stool will reduce mortality from colon cancer.

Project Officers: Sheila E. Taube, Ph.D, Ihor Masnyk, Ph.D. & Philip Prorok, Ph.D.
Program: Cancer Diagnosis
Technical Review Group: Ad Hoc Contracts Technical Review Group
FY 86 Funds: $1,556,363

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