

Proposal for a Thesis
in the Field of Biology
in Partial Fulfillment of Requirements for
the Master of Liberal Arts Degree

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I.

Tentative Title

“Determining cisplatin efficacy in a mouse model of human lung cancer.”

II.

The Research Problem

Lung cancer is the leading cause of cancer related mortality worldwide (Greenlee *et al.*, 2001). A five-year survival rate of only ten percent illustrates this poor prognosis (Loprevite *et al.*, 2001). The high fatality rate is due to advanced stages of the cancer at diagnosis, the aggressive nature of the disease, and the poor response to current therapies. The high prevalence rates and ineffectiveness of current treatments substantiates more research in novel therapeutics.

Cisplatin, taxol, and doxorubicin are examples of agents routinely used to treat many cancers. These chemotherapeutic agents are known to trigger cell death or arrest. However, we know little about why these agents are only effective in some tumors and not in others. Historically, human lung tumors respond inconsistently to chemotherapy and chemoresistance often contributes to the failure of existing forms of therapy (Langendijk *et al.*, 2000). Failure of traditional therapy justifies more research in understanding the DNA damage response to classical and novel therapeutics.

Traditional assays that test the efficacy of chemotherapeutic agents evaluate the response of human tumor cell lines and xenografts to the test drug of interest. However, these preclinical tests neglect the normal *in vivo* tissue microenvironment, which most likely plays a role in susceptibility to various drugs (Bearss *et al.*, 2000; Song, *et al.*, 2000). Indeed, *in vitro* assays do not always predict the efficacy of a particular drug in treating a specific cancer in the clinical setting (Waldman *et al.*, 1997). Cancer researchers need better preclinical models that more accurately predict the response of human cancer to chemotherapy. To model human cancer, genetically engineered mice are available that recapitulate the disease. In contrast to cell culture assays, the mouse more accurately models *in vivo* tumorigenesis (Van Dyke & Jacks, 2002). The

tissue microenvironment is inherent in the mouse model. Consequently, the mouse tumor response should more closely mimic the human tumor response to therapy.

In addition to the lung microenvironment, the stage of tumor development (early versus late) may also be critical to drug sensitivity. Preliminary *in vitro* experiments in our laboratory show that wild type (WT) P53, a tumor suppressor, is an important component in the response of mouse lung cancer cell lines to cisplatin (Sweet-Cordero & You, personal communication).

Little is known why cisplatin efficacy is variable in humans. Therefore, we propose an *in vivo* test to determine the efficacy of cisplatin in treating a mouse model of human lung cancer. In particular, we want to examine whether mouse lung adenomas are sensitive to cisplatin. Adenomas are early stage tumors and likely WT for p53. We will confirm this by real time PCR of p53 in these tumors. Our hypothesis is that cisplatin is effective in treating mouse lung tumors. This effect is mediated by increasing cell cycle arrest or inducing apoptosis. We will evaluate efficacy by observing the difference in tumor number, tumor cell death, and cell proliferation between untreated and cisplatin treated lung cancer prone mice. We expect lesions will be cisplatin sensitive, as they should have p53 function.

Decreases in tumor number, increases in cell death, or decreases in cell proliferation will signify cisplatin efficacy. If results are as expected, we can study the *in vivo* DNA damage response mechanism at the molecular level in tumors. For example, we can look at p53 expression and downstream targets of p53. Such studies will reveal known genes that mediate cisplatin sensitivity and provide new targets for therapy. Alternatively, if we observe no difference in lung tumor phenotype between cisplatin treated and untreated mice, we would reject our hypothesis of cisplatin efficacy. Drug resistant tumors would provide important material to analyze gene expression, which will provide insight into how lung tumors resist the DNA damaging effects of cisplatin. Certainly, results of tumor sensitivity or resistance will be valuable as human lung tumor responses to cisplatin are diverse.

Finally, this proposed drug trial will be an important first step towards a long-term goal to determine if this mouse is useful as a model to test chemotherapeutic agents. If the model is

useful, the potential for cost effective, efficient drug screening in mice is very promising.

Furthermore, the study of these tumors will help us understand the phenomena of chemosensitivity and in turn uncover new targets for therapy. In the future, drugs directed against these new targets used alone or in combination with traditional therapies such as cisplatin, will potentially treat lung cancer patients more effectively.

III.

Definition of Terms

“Angiogenesis”: is the process by which new blood vessels form from existing vessels. Blood supply limits tumor growth.

“Apoptosis”: programmed cell death.

“Cell cycle”: phases that facilitate DNA replication and cell division.

“Cell cycle checkpoints”: “regulatory pathways that control cell cycle arrest in response to DNA damage, allowing time to repair.” (Zhou & Elledge, 2000).

“Chemotherapy”: chemical compounds given orally or intravenously to treat various cancers.

“Chemoresistance”: when cells destroyed by a particular drug no longer respond to treatment with that drug. Resistance may be acquired or ineffective at outset.

“Cisplatin”: a chemotherapeutic that binds to and damages DNA, explaining its cytotoxicity

“*ex in vivo*”: cell lines cultured *in vitro* implanted into a recipient mouse to analyze *in vivo*.

“DNA adduct”: complex formed when a compound like cisplatin chemically binds to DNA

“DNA repair”: DNA damage occurs in many ways, and cellular mechanisms exist to repair this damage.

“Genetic mutation”: a permanent change in a gene, defective or damaged DNA.

“Growth factors”: proteins that stimulate cell proliferation or are necessary for cell survival.

“*in vitro*”: experiments on cultured cells.

“*in vivo*”: experiments on intact organisms.

“*K-ras*”: a protooncogene that promotes cell growth and survival. *K-ras* alternates between an inactive and active state serving as a molecular switch. Mutations of *K-ras* can result in oncogenic *K-ras* that remains in the active state.

“Preclinical model”: an assay that mimics cancer. The model is used to test drug cytotoxicity prior to human drug testing.

“Stroma”: cells that produce connective tissue and extra cellular matrix components.

“Tissue microenvironment”: extra cellular and cellular surroundings.

“Transgenic mice”: mice permanently altered to carry foreign genes.

IV.

Background of the Problem

Lung Cancer

Lung cancer arises due to numerous genetic lesions in lung cells caused by smoking or other environmental carcinogens such as radon, air pollution, and asbestos (Ross & Rosen, 2002). The World Health Organization (WHO) classifies lung cancer into two categories, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is more common than SCLC, and generally grows and metastasizes more slowly. According to the cell type in which the cancer develops, the WHO further classifies NSCLC into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Adenocarcinoma is the most prevalent in the United States (Greenlee *et al.*, 2001). Treatment of NSCLC is similar based on the extent of disease at the time of diagnosis. Early stages of lung cancer are treated with surgery and radiation therapy. For patients with unresectable metastatic disease, the treatment of choice is cisplatin combination chemotherapy (<http://www.cancer.gov>, 2002).

Lung cancer is the leading cause of cancer related mortality worldwide (Greenlee *et al.*, 2001). A five-year survival rate of only ten percent illustrates this poor prognosis (Loprevite *et al.*, 2001). The high fatality rate is due to advanced stages of the cancer at diagnosis, the aggres-

sive nature of the disease, and the poor response to current therapies. The high prevalence rates and ineffectiveness of current treatments justify more research in novel therapeutics.

Current Assays to Evaluate Drug Efficacy

Traditional assays used to test the efficacy of therapeutic drugs involve testing human tumor cell lines *in vitro* with drugs and observing evidence of cytotoxicity. Unfortunately, drugs effective in killing tumor cell lines *in vitro* have failed in the *in vivo* clinical setting. That cells lines respond and tumors do not raises the possibility that there are specific *in vivo* mechanisms of resistance to these agents. Alternative assays utilize xenograft models to test drugs. In these models, researchers transplant human cell lines or tumors into the flank of immuno-compromised mice and then treat the mice with various therapies (Perdomo *et al.*, 1998). However, it is dangerous to draw too many conclusions from these studies as this xenograft model also has limitations as discussed later.

Traditional assays have focused on the tumor's cell of origin and less on the microenvironment that supports tumor cell's growth. In addition to genetic mutations in the tumor cell, the tissue microenvironment can promote or inhibit the tumorigenic process (Song *et al.*, 2000). Although poorly understood, these extrinsic effectors include immune cells, hormones, tissue stroma, growth factors, extracellular matrix, and angiogenesis.

Tissue culture assays neglect this host tissue microenvironment, which most likely has a symbiotic relationship with the tumor (Hann & Balmain, 2001; Van Dyke & Jacks, 2002). In addition, these *in vitro* assays neglect the pharmacokinetic and pharmacodynamic properties of the test drug (Hurley, 2002). In xenograft models, the pharmacokinetic and pharmacodynamic properties of the drug are considered. However, the tumor does not arise in the appropriate location, for example the lung. Therefore, the complex cellular communication with the lung microenvironment is surely deficient in this model (Van Dyke & Jacks, 2002). Moreover, nude mice also have deficient immune function, which is itself involved in tumorigenesis. Therefore, we might expect *in vitro* and xenograft data to fail to predict the clinical response.

Mouse Models of Human Cancer: A Better Alternative?

In contrast to tissue culture assays, genetically engineered cancer prone mice are available that model various human cancers. We refer to these as mouse models of human cancer. Mice are easy to manipulate genetically, they are easy to house and breed, and tumor material is fully accessible for study. Additionally, inbred strains enable scientists to define the genetic background of the mouse model and hold it constant to control experimental variability. Thus, these cancer prone mice may represent better models than the conventional assays to test the efficacy of drugs.

First, mouse models maintain the intact cellular environment of the tumor in its host and consequently should more accurately model tumorigenesis *in vivo* (Van Dyke & Jacks, 2002). Secondly, the mouse model accounts for the pharmacokinetic and pharmacodynamic characteristics of a drug. Therefore, the mouse model preclinical test should be more predictive in its assessment of a tumor's response to a drug, potentially allowing the correlation between mouse and human responses to individual chemotherapies. Importantly, if the mouse genetically models its human counterpart, the mouse should mimic the human response to therapy.

As summarized by Hann & Balmain (2001), the ideal mouse model should have high penetrance where all mice get the appropriate phenotype. The cancer should be short in latency so analysis of tumor material is prompt. Variability of tumor development between mice should be limited to facilitate statistical evaluation. Finally, the cancer is similar to the human counterpart in histology and molecularly. Transgenic techniques and chemical induction have created lung cancer in the mouse. Although tumors tend to represent adenomas or early adenocarcinoma, they share some of the histological features of NSCLC (Malkinson, 1998). Furthermore, limited molecular genetic analysis has revealed additional similarities between human and mouse NSCLC. However, we know little about the resemblance between mouse and human lung cancer therapeutically.

The *K-ras Latent Mouse*; A Promising Preclinical Model for Lung Cancer

Recently we created a *K-ras* mutant mouse model of human lung cancer (Johnson *et al.*, 2001). *K-ras* is a gene that normally functions as a molecular switch, and transmits extra cellular

signals from growth factor receptors to a cascade of protein kinases, leading to alteration in transcription factors and cell cycle proteins in the nucleus (Bos, 1988). However, any one of many single mutations can give rise to a highly oncogenic protein. Indeed, *K-ras* mutations occur in a variety of human tumors, predominantly epithelial in origin. In NSCLC *K-ras* is mutated in twenty to fifty percent of cases and is thought to be an important initiating event (Rodenhuis *et al.*, 1988).

Our *K-ras* model mimics sporadic human lung cancer, as a recombination event spontaneously activates the *K-ras* oncogene *in vivo* and results in multifocal lung tumors (figure 1). As a result, we refer to this mouse as the *K-ras Latent mouse*. The model generates sporadic *K-ras* mutations in the lung similar to how *K-ras* mutations are found in human lung cancer. This mouse model is novel in a number of respects.

K-ras is expressed under its endogenous promoter as it would be naturally, ensuring physiologic regulation. This more closely mimics an *in vivo* situation, with normal levels of *K-ras* expressed in the lung. As mentioned above, a recombination event generates the *K-ras* oncogene.

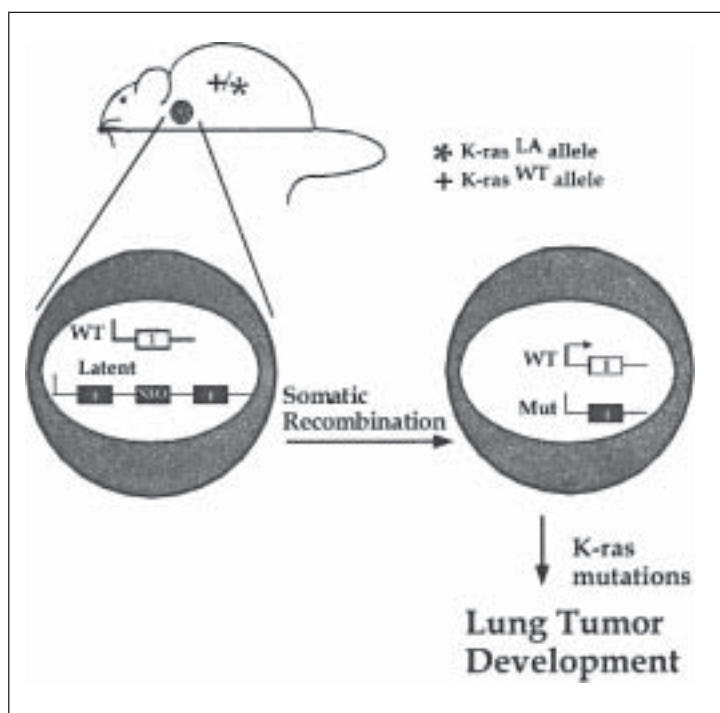


Figure 1. *K-ras* Latent Mouse Strategy. Pathway of Lung Tumor Development

This event occurs at some stochastic frequency and is a unique feature of this model as the initiating event occurs in a discrete cell. Therefore, the discrete mutated cell should more precisely model the clonal origin of lung cancer. Not all cells will carry a mutant *K-ras* allele thus wild-type cells should surround the single cell carrying the mutation. Accordingly, tumors grow in an environment of genetically

normal cells and stroma, recapitulating the microenvironment that supports tumor cell's growth (Tuveson & Jacks, 2002).

In contrast, other mouse models are germ line transgenic or knockout mice in which the activating mutation occurs in every cell of the mouse. Over expression of transgenes in mice leading to artificially increased levels of Ras (Barrington *et al.*, 1998; Bearss *et al.*, 2000) are not necessarily good preclinical models as differing levels of Ras effect the cellular response (Tuveson, personal communication).

The K-*ras* latent mice are well-characterized (Johnson *et al.*, 2001). In many ways, these mice meet the standards of an ideal model. All mutant mice develop a range of multifocal tumors with short latency. Time course experiments show that tumor number and size progressively increase with time. The tumors exhibit significant morphologic heterogeneity similar to human NSCLC although the tumors tend to represent adenomas or early adenocarcinoma. For these reasons outlined above, we believe this model is superior to traditional mouse models and represents a promising preclinical model for human lung cancer.

Little is known about the affect of a single ras mutation on regulation of cell cycle and response to DNA damage. Therefore, the K-*ras* latent mouse provides a unique opportunity to study the effects of single-copy K-*ras* activation *in vivo*.

However, the similarity between human and mouse systems is unclear and it is important to establish whether mouse models are better preclinical models. Little validation of mouse models has occurred to date (Van Dyke & Jacks, 2002). The K-*ras* latent mouse mimics human lung cancer histologically and molecularly. Therefore, the mouse cancer should have similar response mechanisms to therapy. If the mice respond to traditional therapy, further preclinical drug trials of new compounds may predict the outcome of human trials with greater accuracy (Van Dyke & Jacks, 2002).

A caveat to our experimental system is that we will treat early lung tumors. In contrast, humans present with late stage tumors. We are choosing to examine early tumors in the mouse as it a biological model to study genetically simple tumors. Although our experiments will not

mimic human treatment exactly, they will probe chemosensitivity mechanistically allowing conclusions to be made regarding cisplatin efficacy.

Pre-Clinical Trial: Determining Cisplatin Efficacy in the *K-ras* Latent Mouse

We propose to use the *K-ras* latent mouse model of lung cancer described above to evaluate the response of the lung tumors to a traditional chemotherapeutic cisplatin. We will compare the difference in tumor number, tumor cell death, and cell proliferation between cisplatin treated and untreated mice to see if tumors are sensitive. Decreases in tumor number, increases in cell death, or decreases in cell proliferation will signify cisplatin efficacy. Knowledge of how our model responds to a traditional lung cancer therapy will validate the *K-ras latent* model and provide a baseline from which to compare novel therapies. We are very interested in looking at the DNA damage response mechanism in *K-ras* Latent lung tumors.

Many chemotherapies including cisplatin induce the DNA damage response. This response includes an array of pathways. In reaction to DNA damage, these pathways cause cells to arrest or stop proliferating (decrease in S-phase), thus providing time for repair to occur. If repair does not occur, the damage may induce apoptosis (measured by TUNEL). The integrity of pathways mediating this response is crucial. Interestingly, the tumor suppressor gene P53 mediates apoptosis and cell arrest in response to DNA damage and its inactivation correlates with chemo resistance *in vitro*. Nevertheless, we know little about the *in vivo* DNA damage response to various chemotherapies.

Results of cisplatin sensitivity or resistance in our model will be critical for determining how tumors respond to DNA damage. For example, tumor cells may induce certain pathways of the DNA damage response causing cell death or arrest. Alternatively, tumor cells may have developed mechanisms that circumvent the DNA damage response. Specific components of this response mechanism will be discussed later.

Cisplatin Therapy in the Lung Cancer Clinic

Cisplatin is relatively inexpensive and often used in combination with other drugs to treat advanced lung cancer (Monnet *et al.*, 2002). Cisplatin regimens are suitable for patients in good health with unresectable, locally advanced, and metastatic NSCLC. Cisplatin-based chemotherapy either simultaneous with or followed by radiation has been proven to prolong survival, and ameliorate tumor symptoms (Manegold, 2001).

Crystallography of cisplatin demonstrates that cisplatin binds DNA and facilitates the binding of a tertiary protein. This complex, or DNA adduct, causes the DNA duplex to bend (Takahara *et al.*, 1995). This adduct in turn may mask the cisplatin-DNA lesion from the cell's repair machinery hence inducing cell death or arrest. Alternatively, proteins involved in DNA replication, recombination, transcription, and repair that normally assemble on DNA may be sequestered (Hurley, 2002). The disruption of these proteins critical for normal cell growth may explain how the drug functions in killing cells.

However, one third of lung tumors are resistant to cisplatin, and many tumors attain resistance during the course of therapy. Interestingly, of multiple variables considered in one study by van de Vaart *et al.* (2000) such as tumor stage and patient age, cisplatin DNA adduct staining was the only independent prognostic factor associated with increased survival (van de Vaart *et al.*, 2000). Therefore, a primary step for cisplatin efficacy is for cisplatin to gain access to the DNA and form adducts. Then how the cell responds to the resultant DNA damage depends on secondary events. These secondary events may involve certain pathways like p53, for example, which will be discussed later.

It is important to understand the mechanism of how tumors respond to therapy. Thus far, no special combination of therapy has emerged as a significantly better treatment for NSCLC. We realize that several genetic abnormalities underlying NSCLC contribute to the acquired resistance to certain cytotoxic drugs. Therefore, identification of mediators that lead to chemoresistance may uncover new targets for chemotherapy.

Acquired Cisplatin Resistance is Common in Human Tumors

Decreased accrual of cisplatin and increased levels of certain macromolecules in the cell are proposed mechanisms of cisplatin resistance. Indeed, tumors may acquire mutations that enable them to block cisplatin entry or degrade the cisplatin itself (Zamble & Lippard, 1995). Furthermore, if cisplatin enters the cell metallothionein and glutathione, molecules that normally detoxify heavy metal ions and oxygen byproducts respectively, can bind and eliminate cisplatin from the cell. Over expression of metallothionein and glutathione due to cell mutation or some sort of induction by cisplatin, may also assist the tumor in becoming resistant (Arai *et al.*, 2000; Matsumoto *et al.*, 1997). Thus, these protective mechanisms for evading DNA damage could facilitate acquired cisplatin resistance.

Since cell proliferation and death govern tumor formation, traditional drugs like cisplatin target tumors with various modes of action that in effect induce cell death or activate cell cycle checkpoints causing cells to arrest (Bearss *et al.*, 2000). It seems that cell arrest or death is necessary for drug function, and disturbances in the integrity of these pathways may contribute to resistance. Just as various mutations of tumor suppressors, oncogenes, or cell cycle proteins distinguish certain tumors, some mutations alone or in conjunction with other mutations also determine sensitivity to chemotherapy (Bearss *et al.*, 2000).

Increased expression of proteins that enhance DNA repair may also contribute to cisplatin resistance. Normally, DNA damage triggers apoptosis explaining the cytotoxicity of cisplatin. In contrast, extra efficient repair would mend DNA lesions and thereby evade induction of apoptosis. In accord with this hypothesis, Tsai *et al.* (2000) found that increased Her-2 expression found in NSCLC correlated with cisplatin resistance and high nucleotide excision repair (NER) activity. These studies were *in vitro* experiments so whether these mechanisms play an important role *in vivo* remains to be determined.

P53 May Play a Role in Chemosensitivity

The lung environment or the stage of tumor development (early versus late) may explain differences in cisplatin sensitivity. Preliminary *in vitro* experiments have shown that lung cell lines from *K-ras* latent mice respond to cisplatin therapy by inducing p53 and apoptosis (Sweet-Cordero & You, personal communication). However, p53 deficient cell lines derived from compound p53null/*K-ras* latent mice resist cisplatin treatment *in vitro*. Interestingly, *K-ras* latent lungs were examined at approximately six months of age when advanced stage adenocarcinoma predominate over early stage adenomas. Real-time PCR analyses of these late stage tumors show that many have lost p53 in the course of tumorigenesis. We suspect that loss of p53 may represent an important step in the transition from adenoma to adenocarcinoma and may make the tumors resistant to therapy.

The tumor suppressor p53 accumulates after DNA damage and responds by inducing cell death or arrest (Attardi & Jacks, 1999). Various experiments implicate p53 in chemosensitivity (Lowe *et al.*, 1994; Schmitt *et al.*, 1999). Interestingly, cisplatin is ninety percent successful in the treatment of early stage testicular cancer (Zamble *et al.*, 1998). Coincidentally, p53 mutations are extremely rare in this cancer.

This prompted Zamble *et al.*, (1998) to investigate whether the DNA damage response was p53 dependent in testicular cancers. Zamble *et al.* (1998) showed that cisplatin induced apoptosis in WT but not p53 deficient testicular cell lines. However, colony-forming assays that have been argued to more closely mimic the *in vivo* tumor growth, demonstrated that p53 status had no effect on overall cell viability. The authors concluded that WT p53 was not necessary for cisplatin cytotoxicity and that cells responded with p53 dependent and independent mechanisms. What this tells us about lung cancer and the role of p53 in the *in vivo* DNA damage response is unclear. These results are from a different context to the lung environment that may be vital to the cisplatin response.

In contrast to the rarity of p53 mutations in testicular cancer, sixty percent of NSCLC have p53 mutations. Consequently, Perdomo *et al.* (1998) investigated whether p53 status affected lung tumor growth and chemosensitivity. By injecting human NSCLC lines into nude (immune compromised) mice to model lung tumorigenesis *ex in vivo*, they observed that p53 deficient

xenografts were less sensitive to cisplatin compared to WT xenografts. Unlike p53 deficient tumors, WT tumors regressed, had increased apoptosis, and decreases in S-phase following cisplatin treatment. The authors concluded that p53 was important for cisplatin response in the lung *in vivo*, although as explained earlier, xenografts do not model the *in vivo* microenvironment optimally.

Recent experiments performed by Bearss *et al.* (2000) examined the effects of p53 on the DNA damage response pathways *in vivo*. As expected, a transgenic mouse model of salivary tumors revealed that p53 deficiency correlated with chemoresistance to doxorubicin, a known p53 dependent DNA damaging agent. In contrast, p53 deficient tumors did respond to taxol, a p53 independent agent that disrupts mitosis and does not damage DNA. These results implicate p53 in the response to the DNA damaging agents doxorubicin. Unfortunately, cisplatin was not tested in this model. In addition, the salivary tumor response observed in these experiments may differ to a lung tumor response. Therefore, the importance of p53 in lung tumors to cisplatin sensitivity remains ambiguous.

The experiments above suggest that p53 induction is important in the DNA damage response; however, an *in vivo* study of human lung tumors by Rusch *et al.* (1995) found that mutation, rather than absence of p53, correlated with chemoresistance. Interpretation of p53 immunohistochemistry (IHC) used by Rusch *et al.* is complicated in general. WT p53 expression is not detectable by IHC whereas WT induction and point mutant expression is detectable due to increased protein levels. Point mutant p53 has a longer half-life than WT p53, hence it is IHC positive, and may have gain of function properties. Consequently, the role of WT p53 in lung tumor chemosensitivity remains to be determined.

Tumor Microenvironment Also May Contribute to Chemosensitivity

In addition to the intrinsic mutations mentioned above, disturbances extrinsic to the cell may aid chemoresistance. These disturbances relate to the tissue microenvironment such as growth factors, growth factor receptors, and tissue stroma. For example, investigators have found increased levels of various growth factors in lung cancer. These growth factors possibly assist cell survival and result in resistance to therapy.

Song *et al.* (2000) demonstrated the importance of the tumor microenvironment in chemosensitivity. *In vitro* experiments showed that taxol, doxorubicin, and 5-fluorouracil resistant cultured tumors lose their resistance with tissue culture passage. Song *et al.* (2000) also observed that histocultures were more drug resistant than monolayer cultures of the same tumor. Cultured three-dimensional tumor pieces (histoculture) mimic *in vivo* tumorigenesis more closely than monolayers as they include multiple tumor associated cell types. Furthermore, media conditioned by these cultures induced resistance on chemosensitive cells. Analysis of this conditioned media allowed Song *et al.* (2000) to identify fibroblast growth factor (FGF) as the resistance-inducing agent, highlighting the importance of extra cellular factors in drug resistance.

Indeed, cellular context and extra cellular effects may explain the contradictory role of p53 in chemosensitivity outlined above. We hope our model will overcome the caveats of other models discussed above and maintain an intact tumor microenvironment. In addition, cisplatin treated and untreated tumor material collected after the trial will be invaluable in the study of various components of the DNA damage response mechanism.

Expectations of Cisplatin Trial

From the literature cited above, it is expected that early *K-ras* latent lung tumors will be sensitive to cisplatin as they have functional p53. We will evaluate efficacy by observing the tumor response in young, thirty-day-old mice. Specifically, we will assess the tumor response by measuring the difference in tumor number and volume between untreated and cisplatin treated *K-ras* latent mice. This is a rather crude measure, and we expect tumor growth rate differences only if the tumors have dramatic response to cisplatin. To detect more subtle differences, we will also study the DNA damage response by examining the amount of apoptosis and cell proliferation (S-phase) in the tumors by immunohistochemistry (IHC). These IHC markers will be more sensitive and will measure any cellular responses to cisplatin, if they exist.

As mentioned previously, p53 is thought to be an important mediator of the cell's response to chemotherapy. DNA damaging agents like cisplatin can induce increases in p53 levels

that mediate several cellular responses including cell cycle arrest and apoptosis (programmed cell death). P53 arrest is mediated by p21. P53 mediated apoptosis is less well-understood and downstream effectors like Bax may be involved albeit in a tissue specific manner. Therefore, we will also use IHC to determine whether p53 induction occurs in response to DNA damage. If induction occurs, we will examine whether p53 is transcriptionally active by looking at downstream targets p21, Bax, and mdm2.

We predict that lung lesions will respond to treatment. These *K-ras* latent mice will be thirty days old and should only have a single *K-ras* mutation and intact p53 function at this early stage of tumorigenesis. This model will be useful to assess whether Ras activation alone is adequate to provide resistance to cisplatin or whether a secondary genetic or epigenetic event in the process of tumor development is required.

If cisplatin treated mouse tumors are less numerous or are smaller in volume than their littermate controls we will conclude that these *K-ras* latent tumors are sensitive to cisplatin. In addition, increases in apoptosis or decreases in S-phase in the treated tumors compared to untreated controls would indicate some cisplatin efficacy. If cisplatin treated tumors respond at the gross or cellular level, we can accept our hypothesis and study the tumor response mechanism at the cellular and molecular level by examining p53 levels and induction of downstream targets of p53.

If lung tumors do not respond to cisplatin we can reject our hypothesis. We would conclude that the *K-ras* latent tumors are fundamentally resistant to cisplatin given that secondary mutations are unlikely. P53 is differentially induced by unknown mechanisms in different tissues in response to radiation (Kemp et al., 2001). The failure to respond could be due to inhibition of p53 induction or inhibition of downstream effectors. The resistance could also be due to the tissue microenvironment.

As P53 induction occurs in *K-ras* latent lung tumors cell lines *in vitro*, failure to induce P53 *in vivo* could be due to extracellular growth factors. If P53 is not induced, we could look at Mdm2 levels, which inhibit p53. Interestingly, extracellular growth factors FGF, PDGF have been shown to increase Mdm2. Indeed, Ras itself may also activate Mdm2 (Ries et al., 2000). If p53 is

induced but no detectable difference in TUNEL or BrdU is observed we can use Real time PCR to look at levels of downstream targets p21 and Bax.

Knowledge of the mechanism of response to cisplatin will help us understand how some human tumors respond to therapy. New information could justify genetically identifying patient tumors to predict the response to cisplatin before starting a course of therapy and possibly identify new gene targets for therapy.

As mentioned above, *in vitro* assays do not always predict the efficacy of a particular drug and the *in vivo* response remains to be determined. Therefore, we may observe no difference in lung tumor phenotype between cisplatin treated and untreated mice. In this case, we would reject our hypothesis of cisplatin efficacy. Nevertheless, tumor material resistant to cisplatin will be valuable in the study of lung cancer chemoresistance.

It will be important to test that cisplatin is functioning in the lung. To do this we can analyze the cisplatin treated tumors for adducts (van de Vaart *et al.*, 2000). If cisplatin is causing DNA damage, adducts will be found. Alternatively, the tumor cells may be evading cisplatin induced adducts by increasing metallothionein or glutathione expression, which give the tumors resistance to cisplatin therapy. Interestingly, preliminary evidence demonstrates that metallothionein is increased in gene expression profiles (Sweet-Cordero & You, personal communication). We can detect over expression of metallothionein or glutathione by IHC. Finding DNA adducts in *K-ras* resistant lung tumors imply other mechanisms of resistance. These mechanisms might involve disruption of the DNA damage response pathways such as DNA repair, cell-cycle arrest, and apoptosis.

Fortunately, drug resistant tumors would provide important material to analyze gene expression, which will provide insight into how lung tumors resist the DNA damaging effects of cisplatin. Inaccessibility of human tissue and genetic variation hinder tumor analysis in human samples. In contrast, tumor material is fully accessible for study in the mouse model. Certainly, results of tumor sensitivity or resistance will be valuable as human lung tumor responses to cisplatin are diverse (Joseph *et al.*, 2000).

Does the K-ras Latent Model Mimic Human Lung Cancer?

There is a critical need to test the resemblance of mouse to human tumors in their response to therapy (Hann & Balmain, 2001). As mentioned above, it must be determined how the *K-ras* latent mouse lung tumors respond to classical therapy like cisplatin. Validation of the *K-ras* latent mouse as a preclinical model will facilitate the testing of other compounds, as results of this trial will give us a baseline from which to compare new treatments. Thus, the proposed experiments represent an initial step in validating this mouse as a model for lung cancer. If mouse and human responses are similar, the potential for cost effective, efficient drug screening in mice is very promising. Researchers can establish drug efficacy in the *K-ras* latent mouse before conducting costly human trials. Finally, drug treated lung tumors will be an invaluable resource to study the mechanisms of chemoresistance versus sensitivity. Tumor analyses may aid the discovery of new targets for therapy and compounds directed against these new targets used alone or in combination with traditional therapies such as cisplatin, may provide a better prognosis for lung cancer patients.

V.

Research Methods

The purpose of this study is to examine the efficacy of cisplatin in mice pre-disposed to the development of lung cancer maintained at the Massachusetts Institute of Technology (MIT). Mice are housed in an environmentally controlled room with a 12hr light/dark cycle according to the Committee of Animal Care (CAC) guidelines. Mice eat and drink *ad libitum*. The Division of Comparative Medicine monitors mouse health.

K-ras latent *129 svj* mice will be bred to wild-type *c57Bl6* mice to obtain (129svjxC57Bl6) F1 *K-ras* latent mice for the drug study. Heterozygous offspring will be genotyped by PCR from DNA tail preparations (Johnson *et al.*, 2001). Therefore, the groups of mice will be fifty percent 129svj and C57Bl6 and have identical genetic backgrounds to control experimental variability due to genetic modifiers. These mice are well characterized. At the time of weaning, 4 weeks of age, these mice have approximately 60 (+/- 10) tumors (Johnson *et al.*, 2001).

Groups of 25 mice will be used for this study. Normal power calculations based on the known variance of lung tumor numbers in these mice, illustrate that this number will enable us to detect a twenty percent difference between treated and control mice (significance level 0.05, power 0.8, one tailed test). There will be one cisplatin group and one control group that receive drug vehicle alone. The intervention will begin at 4 weeks of age. Cisplatin, supplied by Dr. Stephen Lippard (MIT), will be administered intraperitoneally (9 μ g/g of body weight) with a 26 gauge tuberculin needle once weekly for two weeks, followed by two weeks rest, for a total of three cycles. Cisplatin has been used safely in previous mouse studies and levels of toxicity are known. We will prepare the cisplatin fresh in phosphate buffered saline (PBS). We expect no morbidity during this treatment study. If we encounter any unexpected morbidity, we will immediately sacrifice the mouse. We will assess mice daily for activity, hunched posture, ruffled fur, and dehydration.

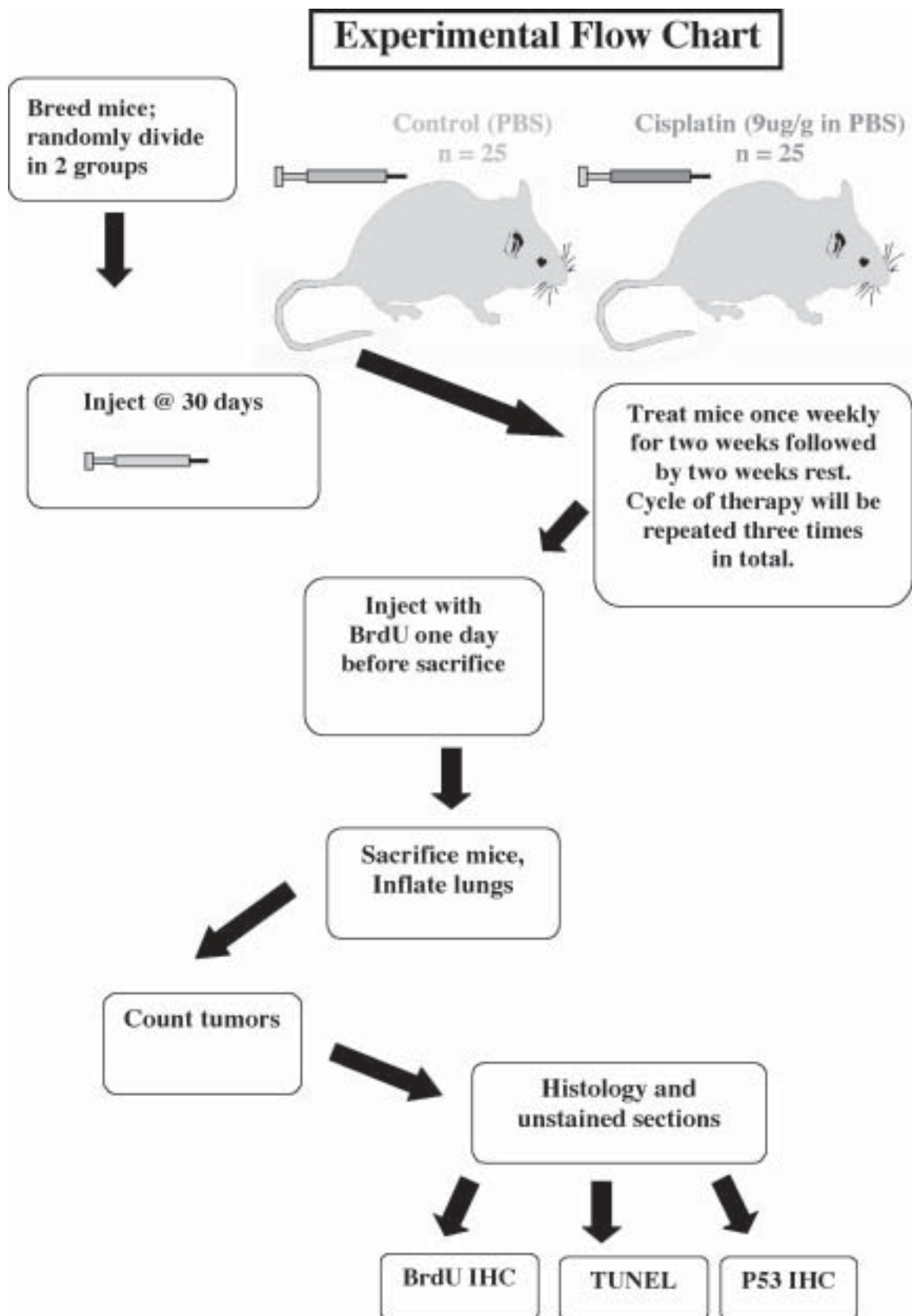
Following treatment, we will sacrifice mice by carbon dioxide asphyxiation, and remove the lungs for further studies following inflation in formalin. In addition, a mixture of BrdU (5-bromo-2'-deoxyuridine; Sigma) and FdU (5-fluoro-2'-deoxyuridine; Sigma) will be injected intraperitoneally (30 μ g and 3 μ g/gm body weight, respectively) one hour prior to sacrifice. After suitable fixation time (at least sixteen hours), the number of tumor nodules visible on the lung surface will be counted under a dissecting scope, the average size and range also noted. Following tumor counting, the lungs will be processed and embedded in paraffin, sectioned at 4 μ m and stained with hematoxylin and eosin for tumor pathology.

In addition, unstained paraffin sections will be prepared for TUNEL, BrdU, and p53 IHC. The TUNEL method labels apoptotic cells *in situ* and will be stained according to Gavrieli *et al.* (1992). Briefly, paraffin sections are blocked in 3% H₂O₂, processed in proteinase K, and incubated in rTdT (recombinant terminal deoxynucleotidyl transferase; GIBCO) and biotin-16-dUTP (biotin-16-uridine-5'-triphosphate; GIBCO). P53 and BrdU IHC labels cells with high p53 expression and cells in S-phase of the cell cycle respectively. IHC be performed using the Vectastain™ ABC kit (Vector Laboratories) following pressure cooker antigen retrieval in Trilogy™ (Cell Marque) for fifteen minutes. Paraffin sections will be incubated in anti-p53

primary antibody CM5 (Novocastra Laboratories Ltd.; 1/500 dilution) and Anti BrdU (BD Biosciences; 1/200 dilution) overnight at four degrees Celsius.

I will count tumor numbers and IHC positive cells blindly. For IHC a minimum of two hundred nuclei will be counted in randomly selected tumor fields under a light microscope (Perdomo *et al.*, 1998). The Student's t-test (independent samples with assumed equal variances) will be used to see if there are any significant differences in the mean tumor number of IHC positive cells between control and cisplatin treated groups. I am assuming that the population is normally distributed. Dr. Stephen Lippard will perform any adduct analysis of tumor DNA.

	Logic	Experiment	Control and Why
1.	Drug trial: to determine if <i>K-ras</i> latent mouse lung tumors are cisplatin sensitive.	Treat 25 <i>K-ras</i> latent F1 mice intraperitoneally with cisplatin (9ug/g body weight) once weekly for 2 weeks, followed by two weeks rest, for three cycles.	25 <i>K-ras</i> latent F1 mice treated with drug vehicle, PBS alone. Any differences are due to cisplatin injection not PBS.
2.	Examine differences between control and drug group to see if cisplatin reduces tumor number.	Count surface lung tumors of cisplatin treated mice following inflation.	Count surface lung tumors of untreated control mice following inflation.
3.	TUNEL IHC to detect any differences for apoptosis in treated versus untreated mice.	Cut paraffin sections of treated tumors and stain with TUNEL. Measure the percentage of positive cells.	Cut paraffin sections of untreated tumors and stain with TUNEL. Measure the percentage of positive cells. This will determine the background level of apoptosis. IHC: positive control (irradiated E13.5 mouse embryo).
4.	BrdU IHC to determine differences in S-phase in treated versus untreated mice. This is a measure of cell proliferation.	Cut paraffin sections of treated tumors and stain with anti-BrdU. Measure the percentage of positive cells.	Cut paraffin sections of untreated tumors and stain with anti-BrdU. Measure the percentage of positive cells. This will determine normal proliferation levels. IHC negative control (tissue not injected with BrdU).
5.	P53 IHC: P53 induction may occur in response to cisplatin therapy.	Cut paraffin sections of treated tumors and stain with anti-CM5. Measure the percentage of positive cells.	Stain untreated paraffin sections with anti-CM5. Measure the percentage of positive cells. This will determine normal p53 levels. IHC: positive control (irradiated E13.5 mouse embryo); negative control (p53 ^{-/-} lung tumor).



VI.

Research Limitations

A surface lung tumor count estimates the total lung tumor number and will only detect gross differences between treated and untreated groups. In addition, we are only observing early stage tumors. Advanced stages may differ in cisplatin response as p53 function may be compromised.

The mouse genome differs to the human genome by twenty five percent http://www.ensembl.org/Mus_musculus/. Therefore, knowledge obtained from mouse models may not always be applicable to humans due to species-specific differences. Genes vary in their role between mice and humans. In addition, mouse and human cells are distinct in that fewer genetic mutations are required to transform mouse cells than human cells *in vitro* (Hahn & Weinberg, 2002). Consequently, one would expect *in vivo* tumor development to differ between mice and humans. For that reason, information gained from this study and future studies may not always be transferable to the human setting.

Furthermore, the lung cancer histology in the latent mouse somewhat differs to its human counterpart. Mouse tumors tend to represent earlier lesions. In contrast, human lung cancer is normally diagnosed at advanced stages. However, molecular mechanisms are similar regarding genetic mutations and chromosomal loss. It is probably more important that the mechanism of the disease is comparable even if tumor histology is different as treatments are based on the molecular alterations (Macleod & Jacks, 1999).

Finally, complicated interactions determine cancer properties. Many genes products expressed in cancer cells are involved in apoptosis, repair, metabolism, and detoxification of drugs. Different combinations would affect individual responses. Therefore modeling a complex disease like cancer in a mouse may be an over simplification.

VII.

Tentative Schedule

Submission of proposal to research advisor	May 1, 2003
Draft I Proposal returned for revision	June 1, 2003
Draft II Proposal returned for revision	July 1, 2003
Submission of final proposal	August 1, 2003
Proposal accepted by research advisor	September 30, 2003
Thesis director agrees to serve	October 30, 2003
Data collection completed	April 30, 2004
First draft completed	July 30, 2004
First draft returned by thesis director	August 15, 2004
Revised draft completed	September 1, 2004
Revised draft returned by thesis director	September 15, 2004
Final text submitted to thesis director and research advisor	October 1, 2004
Final text approved	October 15, 2004
Bound copies delivered to Extension	October 30, 2003
Graduation	November, 2004

VIII.

Bibliography

Works Annotated

Arai, T., Yasuda, Y., Takaya, T., Hayakawa, K., Toshima, S., Shibuya, C., Kashiki, Y., Yoshimi, N., & Shibayama, M. (2000). Immunohistochemical expression of glutathione transferase-pi in untreated primary non-small-cell lung cancer. *Cancer Detect Prev*, 24(3), 252-257.

- IHC of human NSCLC revealed a positive correlation between glutathione expression and cisplatin and etoposide resistance.
- Results suggest that a patients response to therapy could be predicted by determining tumor glutathione expression.

Attardi, L. D., & Jacks, T. (1999). The role of p53 in tumour suppression: lessons from mouse models. *Cell Mol Life Sci*, 55(1), 48-63.

- Description of how the p53 knockout mouse has been valuable in the study of p53.
- The mouse model has verified p53 as a tumor suppressor, and shown the importance of p53 in cell arrest and apoptosis.

Barrington, R. E., Subler, M. A., Rands, E., Omer, C. A., Miller, P. J., Hundley, J. E., Koester, S. K., Troyer, D. A., Bearss, D. J., Conner, M. W., Gibbs, J. B., Hamilton, K., Koblan, K. S., Mosser, S. D., O'Neill, T. J., Schaber, M. D., Senderak, E. T., Windle, J. J., Oliff, A., & Kohl, N. E. (1998). A farnesyltransferase inhibitor induces tumor regression in transgenic mice harboring multiple oncogenic mutations by mediating alterations in both cell cycle control and apoptosis. *Mol Cell Biol*, 18(1), 85-92.

- Preclinical trial evaluating farnesyltransferase inhibitor (FTI) in a transgenic *ras* mouse model.
- FTI induced high levels of apoptosis that was partially p53 dependent. Therefore, FTIs may be important chemotherapeutics used alone or in combination with traditional therapies.

Bearss, D. J., Subler, M. A., Hundley, J. E., Troyer, D. A., Salinas, R. A., & Windle, J. J. (2000). Genetic determinants of response to chemotherapy in transgenic mouse mammary and salivary tumors. *Oncogene*, 19(8), 1114-1122.

- Tumor response to doxorubicin and taxol was observed in transgenic mouse models of cancer with defined genetic alterations in p53, *ras*, or *myc*.
- Cell cycle arrest played a bigger *in vivo* role than apoptosis in reducing tumor growth. Experiments addressed two drugs with distinct mechanisms of action, p53 dependent and independent. As expected, P53 was important to doxorubicin's not taxol's response.

Bos, J. L. (1988). The *ras* gene family and human carcinogenesis. *Mutat Res*, 195(3), 255-271.

- Outline of the various point mutations of the *ras* gene family that convert them to oncogenes.
- Describes the prevalence of *ras* mutation in many human cancers in early and late lesions.

Gavrieli, Y., Sherman, Y., & Ben-Sasson, S. A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol*, 119(3), 493-501.

- First method to describe *in situ* visualization of apoptosis in histology sections.

- Greenlee, R. T., Hill-Harmon, M. B., Murray, T., & Thun, M. (2001). Cancer statistics, 2001. *CA Cancer J Clin*, 51(1), 15-36.
- lung cancer statistics for 2001
- Hahn, W.C., & Weinberg, R. A. (2002). Modeling the Molecular Circuitry of Cancer. *Nature Reviews Cancer*, 2, 331-341.
- Excellent review of the differences between humans and mice concerning signaling pathways.
 - Fewer genetic hits are required to immortalize mouse cells than human cells. Knowing these differences should allow scientists to create mouse models that more closely mimic the human cancer molecularly and therapeutically.
- Hann, B., & Balmain, A. (2001). Building ‘validated’ mouse models of human cancer. *Curr Opin Cell Biol*, 13(6), 778-784.
- Outlines the “ideal mouse model”, biologically, genetically, etiologically, and therapeutically.
 - Reviews current models and the importance of the tumor microenvironment.
 - Stresses the importance of using mice as preclinical models. Emphasizes the need to test the similarities between mice and humans therapeutically.
- <<http://www.cancer.gov>>. (2002). *Lung Cancer*. National Cancer Institute. Available: <http://www.cancer.gov>.
- <http://www.ensembl.org/Mus_musculus/>. (2002). Mouse Genome Center. Sanger Institute. Available: http://www.ensembl.org/Mus_musculus/.
- Hurley, L. H. (2002). DNA and its associated processes as targets for cancer therapy. *Nature Reviews/Cancer*, 2(March), 188-200.
- Review of how clinically important chemotherapeutic drugs target DNA in various ways.
 - Detailed description of how cisplatin forms intrastrand cross links and bends DNA thus trapping proteins, possibly explaining cisplatin’s cytotoxicity.
- Jacks, T. (1996). Tumor suppressor gene mutations in mice. *Annu Rev Genet*, 30, 603-636.
- Using gene targeting strategies one allele of Rb was disrupted in embryonic stem cells. These cells were used to make chimeric mice and germ line mice that were heterozygous for Rb. Mice were aged and characterized to study the role of Rb in tumorigenesis
 - Rb homozygous mice died in embryogenesis at E13.5 due to hematopoietic problems. Rb mutant mice have a different phenotype to humans and are not prone to retinoblastoma.
- Johnson, L., Mercer, K., Greenbaum, D., Bronson, R. T., Crowley, D., Tuveson, D. A., & Jacks, T. (2001). Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature*, 410(6832), 1111-1116.
- Authors constructed a K-ras mutant mouse model. Mice were genetically engineered that generate an oncogenic allele of K-ras sporadically by an *in vivo* recombination event. The authors mimic *in vivo* tumorigenesis with K-ras expression controlled by its endogenous promoter. This contrasts with other transgenic *ras* models in which *ras* expression is under the control of an exogenous promoter.
 - K-ras mutant mice are cancer prone. All mice get adenocarcinoma of the lung. In addition, 40% get thymic lymphoma and 20% get skin papillomas.
 - Demonstrates *in vivo* effects of K-ras mutations in tumorigenesis and represents an improved mouse model for human lung cancer.

- Kemp, C.J., Sun, S., & Gurley, K.E. (2001). P53 induction and apoptosis in response to radio- and chemotherapy in vivo is tumor-type-dependent. *Cancer Research*, 61(1),327-332.
- In vivo analysis of p53 induction by IHC following treatment with radiotherapy or chemotherapy. P53 induction occurred in a tissue specific manner. The lung was particularly resistant to showing any changes in p53 induction.
- Langendijk, H., Thunnissen, E., Arends, J. W., de Jong, J., ten Velde, G., Lamers, R., Guinee, D., Holden, J., & Wouters, M. (2000). Cell proliferation and apoptosis in stage III inoperable non-small cell lung carcinoma treated by radiotherapy. *Radiother Oncol*, 56(2), 197-207.
- Analysis of p53, Bcl-2, apoptosis, and topoisomerase in irradiated human NSCLC.
 - p53 expression and apoptosis were the only two prognostic indicators with radiotherapy allowing the prediction of local control.
- Loprevite, M., Favoni, R. E., de Cupis, A., Pirani, P., Pietra, G., Bruno, S., Grossi, F., Scolaro, T., & Ardizzoni, A. (2001). Interaction between novel anticancer agents and radiation in non-small cell lung cancer cell lines. *Lung Cancer*, 33(1), 27-39.
- Evaluated the response of lung cancer cell lines with numerous drugs and radiation.
 - All drugs disrupted the cell cycle. However, synergy with irradiation was found only in squamous cell lines and not adenocarcinomas.
- Lowe, S. W., Bodis, S., McClatchey, A., Remington, L., Ruley, H. E., Fisher, D. E., Housman, D. E., & Jacks, T. (1994). p53 status and the efficacy of cancer therapy in vivo. *Science*, 266(5186), 807-810.
- Demonstrated the importance of p53 and its induction of apoptosis in chemosensitivity to radiation and adriamycin therapy.
 - Xenograft tumors lacking p53 were less responsive to therapy. P53 mutations are very common in human cancers and possible explain the phenomena of chemoresistance.
- Macleod, K. F., & Jacks, T. (1999). Insights into cancer from transgenic mouse models. *J Pathol*, 187(1), 43-60.
- Reviews how important mice have been in the study of molecular alterations in tumorigenesis that reflect similar changes in human cancers.
 - Describes available transgenic models and tumor progression models.
 - Outlines how knockout mice have helped us understand *in vivo* gene function and cooperativity during tumorigenesis.
- Malkinson, A. M. (1998). Molecular comparison of human and mouse pulmonary adenocarcinomas. *Exp Lung Res*, 24(4), 541-555.
- Outline of the similarity between human and house lung tumors in histology and molecular alterations.
 - The mouse model of lung cancer is a valuable tool to discern the early events of lung tumorigenesis.
- Manegold, C. (2001). Chemotherapy for advanced non-small cell lung cancer: standards. *Lung Cancer*, 34 Suppl 2, S165-170.
- Describes the standard of care and regimens for lung cancer patients.
 - Outlines newly available drugs and discusses the difficulty in choosing appropriate regimens due to variability in how patients respond.
- Matsumoto, Y., Oka, M., Sakamoto, A., Narasaki, F., Fukuda, M., Takatani, H., Terashi, K., Ikeda, K., Tsurutani, J., Nagashima, S., Soda, H., & Kohno, S. (1997). Enhanced expression of metallothionein in human non-small-cell lung carcinomas following chemotherapy. *Anticancer Res*, 17(5B), 3777-3780.

- IHC of treated and untreated human NSCLC revealed higher levels of metallothionein in treated tumors.
- Results suggest that induced expression of metallothionein may grant chemoresistance in some tumors.

Monnet, I., de, C. H., Soulie, P., Saltiel-Voisin, S., Bekradda, M., Saltiel, J. C., Brain, E., Rixe, O., Yataghene, Y., Misset, J. L., & Cvitkovic, E. (2002). Oxaliplatin plus vinorelbine in advanced non-small-cell lung cancer: final results of a multicenter phase II study. *Ann Oncol*, *13*(1), 103-107.

- Clinical trial of a new platinum based therapy for advanced NSCLC.

Perdomo, J. A., Naomoto, Y., Haisa, M., Fujiwara, T., Hamada, M., Yasuoka, Y., & Tanaka, N. (1998). In vivo influence of p53 status on proliferation and chemoradiosensitivity in non-small-cell lung cancer. *J Cancer Res Clin Oncol*, *124*(1), 10-18.

- Paper highlights p53's importance in the sensitivity to therapy. Investigators injected two human NSCLC cell lines, one p53 WT, the other p53 deficient, into nude mice (xenograft) to study the *in vivo* effect of cisplatin treatment (two doses) and radiotherapy. Tumor proliferation, apoptosis, BrdU (labels cells in S-phase), and p53 staining were used as indicators of therapy efficacy and induction of the DNA damage response.
- p53 deficient xenografts are less sensitive to cisplatin and radiotherapy. Apoptosis induction by cisplatin correlated with p53 status of the tumors. Only p53 WT tumors showed significant apoptosis and decrease in S-phase. Neither tumor showed induction of apoptosis following radiation.

Ries, S., Biederer, C., Woods, D., Shifman, O., Shirasawa, S., Sasazuki, T., McMahon, M., Oren, M., & McCormick, F. (2000). Opposing effects of Ras on p53: transcriptional activation of mdm2 and induction of p19ARF. *Cell*, *103*(2), 321-330.

- Cells transformed by oncogenic Ras are more resistant to p53-dependent apoptosis upon DNA damage. Ras activates mdm2 that inhibits p53 and p19ARF that inhibit mdm2. Therefore, Ras activates two pathways with antagonistic effects on p53 levels.

Rodenhuis, S., Slebos, R. J., Boot, A. J., Evers, S. G., Mooi, W. J., Wagenaar, S. S., van Bodegom, P. C., & Bos, J. L. (1988). Incidence and possible clinical significance of K-ras oncogene activation in adenocarcinoma of the human lung. *Cancer Res*, *48*(20), 5738-5741.

- Clinical study revealing the incidence of K-ras mutation in NSCLC (25%).
- K-ras mutations are proposed to be an early event in lung tumorigenesis and perhaps induced by smoking and other environmental carcinogens.

Ross, J., & Rosen, G. (2002). The molecular biology of lung cancer. *Current Opinion in Pulmonary Medicine*, (8), 265-269.

- Review that summarizes the numerous and complicated molecular changes and environmental factors in lung tumorigenesis.
- Discusses the importance of K-ras, p53, and numerous genes controlling cell proliferation and apoptosis.

Rusch, V., Klimstra, D., Venkatraman, E., Oliver, J., Martini, N., Gralla, R., Kris, M., & Dmitrovsky, E. (1995). Aberrant p53 expression predicts clinical resistance to cisplatin-based chemotherapy in locally advanced non-small cell lung cancer. *Cancer Res*, *55*(21), 5038-5042.

- Implicates mutations of p53 rather than deficiency in chemoresistance.
- By staining human tumors pre and post cisplatin therapy for p53, authors expected to see a correlation of p53 expression with tumor response. P53 induction was expected

to occur in response to DNA damage in cisplatin sensitive tumors. Cisplatin therapy did not induce increased expression of p53 as expected in advanced NSCLC.

- However, p53 expression did correlate with resistance to cisplatin. The authors called this “aberrant” p53 expression, assuming that it was a mutated version of p53. Mutated p53 has a longer half-life than WT p53 and hence is positive for p53 IHC.

Schmitt, C. A., McCurrach, M. E., de Stanchina, E., Wallace-Brodeur, R. R., & Lowe, S. W. (1999). INK4a/ARF mutations accelerate lymphomagenesis and promote chemoresistance by disabling p53. *Genes Dev*, 13(20), 2670-2677.

- Using a mouse model of lymphoma, genetically defined tumors with mutations in p53 and Ink4a/ARF showed that mutations that failed to activate p53 resulted in apoptotic defects and chemoresistance *in vivo*.

Song, S., Wientjes, M. G., Gan, Y., & Au, J. L. (2000). Fibroblast growth factors: an epigenetic mechanism of broad spectrum resistance to anticancer drugs. *Proc Natl Acad Sci U S A*, 97(15), 8658-8663.

- Experiments illustrate the differences between *in vitro* and *in vivo* assays and implicate extra cellular factors in tumorigenesis.
- Authors cultured tumors in monolayer and histoculture. Both primary and metastatic tumors were treated with taxol, doxorubicin, and 5-fluorouracil. Differences in drug response implicated extra cellular factors in chemoresistance.
- By examining any differences in proteins secreted in culture media that had been in contact with the cells/histocultures for 24hrs, fibroblast growth factor (FGF) was identified as a chemoresistance inducer to taxol, doxorubicin, and 5-fluorouracil.

Takahara, P. M., Rosenzweig, A. C., Frederick, C. A., & Lippard, S. J. (1995). Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. *Nature*, 377(6550), 649-652.

- First demonstration of cisplatin/DNA adduct by x-ray crystallography.

Tsai, C. M., Chang, K. T., Li, L., Perng, R. P., & Yang, L. Y. (2000). Interrelationships between cellular nucleotide excision repair, cisplatin cytotoxicity, HER-2/neu gene expression, and epidermal growth factor receptor level in non-small cell lung cancer cells. *Jpn J Cancer Res*, 91(2), 213-222.

- High NER activity correlated with cisplatin resistance in NSCLC lines *in vitro*. This suggests an explanation for how tumor cells can increase repair to avoid inducing the DNA damage response in NSCLC by fixing cisplatin induced adducts.

Tuveson, D. A., & Jacks, T. (2002). Technologically advanced cancer modeling in mice. *Curr Opin Genet Dev*, 12(1), 105-110.

- Review on the current state of mouse modeling discussing various approaches used to mimic *in vivo* tumorigenesis.
- Outlines the important of the tumor microenvironment.

van de Vaart, P. J., Belderbos, J., de Jong, D., Sneeuw, K. C., Majoor, D., Bartelink, H., & Begg, A. C. (2000). DNA-adduct levels as a predictor of outcome for NSCLC patients receiving daily cisplatin and radiotherapy. *Int J Cancer*, 89(2), 160-166.

- Clinical data analyzed to provide prognostic information following combined radio and cisplatin therapy. Authors followed the outcome of 27 Dutch patients over 3 years following cisplatin and radiotherapy. The mean survival of patients was 9 months.
- Age, tumor stage, and DNA adducts were significantly associated with survival ($p < 0.05$, log rank test). In other words, younger patients with advanced stages of disease and low adducts had significantly shorter survival. Multivariate analysis

revealed that only adducts were an independent prognostic factor i.e. not related to tumor stage or patient age.

- Van Dyke, T., & Jacks, T. (2002). Cancer modeling in the modern era: progress and challenges. *Cell*, 108(2), 135-144.
- Excellent review of mouse models. Nice figure outlining the various strategies for creating mouse models of cancer.
 - Stresses the importance of cell-cell and cell-microenvironment interactions in tumorigenesis, an interaction that is inherent in mouse models.
 - Discusses the expectation of mouse models and how they should model the human counterpart molecularly and histologically. Outline the challenges of mouse models; species-specific differences, mouse tumors tend to represent early stages of human cancer.
 - Highlights the need for *in vivo* testing of drug efficacy.
- Waldman, T., Zhang, Y., Dillehay, L., Yu, J., Kinzler, K., Vogelstein, B., & Williams, J. (1997). Cell-cycle arrest versus cell death in cancer therapy. *Nat Med*, 3(9), 1034-1036.
- Evaluated the importance of p21 *in vitro* and *ex in vivo* in colon cancer cells following gamma irradiation.
 - In contrast to tumors with WT checkpoint status, some p21 deficient tumors were cured *ex in vivo*. This difference was not seen *in vitro* (clonogenic survival) illustrating the fact that checkpoint status is important to the *in vivo* response to therapy.
 - This paper also demonstrates the fact that drug sensitivity may differ between *in vivo* and *in vitro* models.
- Zamble, D. B., Jacks, T., & Lippard, S. J. (1998). p53-Dependent and -independent responses to cisplatin in mouse testicular teratocarcinoma cells. *Proc Natl Acad Sci U S A*, 95(11), 6163-6168.
- This paper sheds light on the mechanism of cisplatin cytotoxicity. The response of p53 wild type (WT) and deficient mouse teratocarcinoma cell lines to cisplatin therapy *in vitro* was studied.
 - Authors treated cell lines with low and high doses of cisplatin in order to study p53 induction (as well as some p53 target genes), apoptosis, and cell cycle response. IP western shows that p53 induction occurs in response to cisplatin.
 - p53 deficiency did not confer cisplatin resistance in testicular cell lines and cells responded in both a p53 independent and dependent manner. Therefore, WT p53 is not necessary for cisplatin cytotoxicity.
- Zamble, D. B., & Lippard, S. J. (1995). Cisplatin and DNA repair in cancer chemotherapy. *Trends Biochem Sci*, 20(10), 435-439.
- Tumor cells become resistant to cisplatin by various mechanisms, including enhanced DNA repair.
 - Interestingly, DNA repair has also been related to the cisplatin cytotoxicity.
- Zhou, B. B., & Elledge, S. J. (2000). The DNA damage response: putting checkpoints in perspective. *Nature*, 408(6811), 433-439.
- Excellent review exploring the role of the DNA damage response that controls cell arrest, apoptosis, and repair activation by unknown mechanisms.
 - Inability to repair damaged DNA leads to increased tumorigenesis.