AN EVALUATION OF THE EFFICACY OF ADENOVIRUS-MEDIATED GENE THERAPY WITH P53 FOR THE TREATMENT OF CANCER

by

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ABSTRACT

Cancer is the second leading cause of mortality in the United States today, and equally prevalent throughout the world. Traditional treatments such as chemotherapy and radiotherapy have thus far proven unable to treat the disease with high efficacy, with different cancer types often requiring different treatments providing a spectrum of results. Cancer types in the late stages often have no adequate treatment at all. Over the past two decades, research in the field of gene therapy has created new hope in finding a remedy for cancer that displays a high efficacy in treating many different types and stages. The p53 tumor suppressor gene has garnered a great deal of interest, as p53 mutation or inactivation is present in approximately 50% of all cancers. The loss of p53 activity can be attributed to several different causes, including mutation of the p53 gene or overexpression of p53 inhibitors. Research has illustrated that the p53 protein plays an important role in tumor suppression by inducing senescence, cell cycle arrest, or cell apoptosis. Studies have shown that
reactivation of p53 in tumor cells leads to tumor cell apoptosis and overall tumor regression. The focus of p53 research has now shifted to strategies of reintroducing or reactivating the gene in tumor cells so that it may carry out its anti-tumor functions. Of the strategies proposed, the use of adenovirus to introduce p53 shows the most promise. Adenoviruses bind to and enter the cell, and, after escaping proteasomal degradation, travel to the nucleus where they inject their genetic material. By delivering wild-type p53 gene into tumor cells using adenovirus, large amounts of p53 protein are transcribed in the cell and initiate its antitumor properties. Many clinical trials using adenovirus-mediated p53 gene transfer (Ad-p53) have been performed with generally positive results across a variety of cancer types. Ad-p53 in combination with more traditional treatments like chemotherapy and radiotherapy has been especially promising. The engineering of both adenoviral vectors and the p53 gene to be delivered presents new options for further increasing the efficacy of this therapeutic approach. Both Onyx-015, a selectively replicating adenovirus, and Ad-p53vp, a p53 gene that avoids inhibition, have been used in clinical trials with success. As a whole the field of adenovirus-mediate p53 gene transfer is promising and holds many advantages to classical treatments, but is still in the early stages of research. Further research must be completed so this therapy may be widely approved and used. The specific combination of Ad-p53 and traditional therapies has proven highly effective and should be used in clinical settings immediately.
# TABLE OF CONTENTS

Title Page i  
Copyright ii  
Readers Approval Page iii  
Abstract iv  
Table of Contents vi  
List of Tables viii  
List of Figures ix  
List of Abbreviations x  
Introduction 1  
  Cancer History 1  
  p53 2  
    Structure of p53 4  
    Regulation of p53 6  
    Activation of p53 7  
  Roles of p53 in the cell 10  
    p53 regulation of the cell cycle 12  
    p53 regulation of cell senescence 14  
    p53 regulation of apoptosis 15  
    p53 regulation of cell metabolism 17  
    p53 role in antioxidant defense 19
Cancer treatment strategies utilizing p53 21

Small molecules 21

MDM2/MDM2DX inhibitors 22

Reactivation of wild-type function 25

Inhibiting the proteasome 26

Gene therapy 27

Retrovirus 28

Adenovirus 29

Adenovirus biology 30

Adenovirus Structure 30

Mechanism of entry 34

Evaluation of Ad-p53 in cancer treatment 36

Non-small cell lung cancer 36

Breast cancer 39

Gastric cancer 41

Osteosarcoma 44

Hepatocellular carcinoma 45

Head and neck cancer 47

Oncolytic virotherapy 48

Conclusion, future aims 51

References 55

Vita 65
<table>
<thead>
<tr>
<th>Number</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Evaluation of the efficacy of Ad-p53 treatment of NSCLC</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>Evaluation of the efficacy of rAd-p53 in combination with bronchial arterial infusion in treating NSCLC</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>Evaluation of the efficacy of Ad-p53 treatment for locally advance breast cancer</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Evaluation of the efficacy of Onyx-015 in the treatment of head and neck squamous cell carcinoma</td>
<td>50</td>
</tr>
<tr>
<td>Number</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Map of the common region of deletions of the short chromosome 17</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Basic structure of p53 protein</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Structure of MDM2</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>An overview of p53 function and regulation</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>The anti-tumorigenic functions of p53</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>The role of p53 in regulating cell cycle arrest</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>The various roles of p53-induced p21 in the cell</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>Downstream target genes of p53 that induce apoptosis</td>
<td>16</td>
</tr>
<tr>
<td>9</td>
<td>The many roles of p53 in regulating cellular energy metabolism</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>p53 regulation of ROS and oxidative stress in the cell</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>Strategies for inducing apoptosis with Nutlins</td>
<td>23</td>
</tr>
<tr>
<td>12</td>
<td>Strategies for inducing apoptosis using PRIMA-1 or MIRA-1</td>
<td>25</td>
</tr>
<tr>
<td>13</td>
<td>Basic diagram of gene therapy</td>
<td>27</td>
</tr>
<tr>
<td>14</td>
<td>The capsid structure of human adenovirus</td>
<td>32</td>
</tr>
<tr>
<td>15</td>
<td>Geometric representation of the human adenovirus</td>
<td>33</td>
</tr>
<tr>
<td>16</td>
<td>Mechanism of entry of an adenovirus</td>
<td>35</td>
</tr>
<tr>
<td>17</td>
<td>Efficacy of Ad-p53 in combination oxaliplatin in treating gastric cancer</td>
<td>42</td>
</tr>
<tr>
<td>18</td>
<td>Map of adenoviral vectors used in p53 gene therapy</td>
<td>46</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full name</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------------------------------------------------</td>
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<td>Ad</td>
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<td>Ad-p53</td>
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<td>Ad-p53vp</td>
<td>Recombinant adenovirus with transactivation domain deleted</td>
<td></td>
</tr>
<tr>
<td>Ad-RLP23/p53</td>
<td>Adenovirus carrying both p53 and RLP23</td>
<td></td>
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<td>AMPK</td>
<td>AMP-activated protein kinase</td>
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<td>Arf</td>
<td>Alternative reading frame</td>
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<td>CAR</td>
<td>Coxsackie and AD receptor</td>
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<td>LABC</td>
<td>Locally advanced breast cancer</td>
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<td>MDM2</td>
<td>Murine double minute 2</td>
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<td>MDMX</td>
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<td>NPC</td>
<td>Nuclear pore complex</td>
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<td>NSCLC</td>
<td>Non-small cell lung cancer</td>
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<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
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<td>pRB</td>
<td>Retinoblastoma protein</td>
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<td>rAd-p53</td>
<td>Replication-deficient Ad-p53 (Gendicine)</td>
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</tr>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<td>SIPS</td>
<td>Stress-induced premature senescence</td>
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</tr>
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<td>wt-p53q</td>
<td>Wild-type p53</td>
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</tbody>
</table>
III. INTRODUCTION

A. Cancer History

Throughout history societies have attempted to understand and find a cure for what we now describe as cancer. Hippocrates initially hypothesized cancer was caused by an excess of black bile, one of the four humors believed to be present in the human body. Later theories would blame an excess of lymph fluid, chronic irritation, and trauma. In Holland in the seventeenth century, doctors Zacutus Lusitani and Nicholas Tulp posited that cancer was contagious based on the observation that breast cancer was often present in members of the same household (“The history of cancer,” 2012). The eighteenth century saw a movement away from this view to one that was caused by environmental and physiological factors; it was not until Virchow’s invention of the microscope in nineteenth century that physicians and scientists were able to truly examine and understand the damage caused by cancer in the human body (“The history of cancer,” 2012). Today cancer is one of the leading causes of mortality in the United States, second only to cardiac disease and responsible for the deaths of nearly 600,000 people annually (CDC.gov, 2010). Given its widespread prevalence and high rate of mortality, a tremendous effort has been made to find a cure for this disease. In 2012, over 1.3 billion dollars was spent on lung, prostate, breast, and colorectal cancer alone (cancer.gov, 2012). As our understanding of cancer at a cellular level has increased, new targets for
possible cancer treatment have arisen. One of these is the tumor-suppressor gene p53.

B. p53

The p53 tumor-suppressor protein is the subject of an incredible amount of research because of the role it plays in many different forms of malignant neoplasms. So frequently are mutations in the p53 protein seen that Bert Vogelstein, M.D., observes “it seems nearly impossible for a normal cell to become a cancer cell unless is inactivates the p53 network” (Vogelstein, 2010). Though originally believed to be an oncogene given its elevated levels in tumor cells (Vuong et al., 2012), landmark research by Baker and colleagues in the late 1980s showed p53 served a much different function (Baker et al., 1989). Prior research showed that over 75% of colorectal carcinomas demonstrated allelic deletions on the short arm of chromosome 17, seen in Figure 1 below; furthermore, these deletions were frequently late events corresponding to the transition of a benign adenoma to a malignant carcinoma (Vogelstein et al., 1988); Baker and colleagues set out to identify the common region of deletion in these carcinomas, eventually finding that the region coded for p53. Further study showed that malignant tumors containing an allelic deletion of p53 allele also contained a mutant form of the p53 gene on the remaining allele (Baker et al., 1989). Corresponding data suggested the mutations led to a loss of tumor suppressor function of the p53 protein (Baker et al., 1989). For the first time, the
p53 gene was shown to be a tumor suppressor gene, not an oncogene, and in the case of colorectal cancer its loss of function appeared to play a decisive role in the progression and growth of the tumor.

**Figure 1.** Map of the common region of deletions of the short arm of chromosome 17. These deletions are present in a majority of colorectal cancers, with 20 markers indicated. Eight separate tumors are shown, with hybridization results given at the far right. Numbers at the bottom identify patients. A loss of one parental allele is denoted with a completely filled circle, while retention of both parental alleles is denoted by a crosshatched circle. An empty circle denotes the marker was not informative. As shown in the hybridization result, in all tumors the region between markers YNZ22.1 and EW505 was deleted, denoting mutations of the p53 encoding region (Figure taken from Baker et al., 1989).
The discovery made by Baker and colleagues caused a fundamental shift in the view of p53 and lead to a great deal of research examining its anti-tumorigenic properties.

1. Structure of p53

p53 plays several highly important roles in normal cells, ranging from its regulation of cellular metabolism to its best known function as a tumor suppressor protein; p53 also participates in the transcriptional control of angiogenesis, cell migration, autophagy, stem cell renewal, and innate immunity (Wachter et al., 2013). The structure of normal wild-type p53 (wt-p53) contains three distinct regions as seen in Figure 2 below: the N-terminus transactivation domain, central binding domain, and C-terminus tetramerization domain (Mirzayans et al., 2012). The central binding domain of p53 is highly-specific to particular sequences of DNA. Upon binding to the gene in question, the transactivation domain of p53 transcribes downstream proteins which lead the cell senescence, cell cycle arrest, or apoptosis depending on the nature and extent of DNA damage or cellular stress (Wachter et al., 2013). It is the specificity of p53 transactivation activity that allows it to efficiently maintain genomic integrity in the cell (Ozaki et al., 2013). The tetramerization domain allows p53 to form dimers, optimizing the activity of p53 (Devine and Dai, 2013). Activation of p53 is achieved primarily through post-translational modifications in response to cell stress or DNA damage (Gostissa et al., 2012).
Figure 2. Basic structure of p53 protein. Shown are the three basic regions of the p53 protein: the N-terminus transactivation domain, central binding domain, and C-terminus Tetramerization domain. Also shown are common sites of post-translational modification: circles represent sites of Ser/Thr phosphorylation, rectangles represent acetylation sites, and the hexagon represents a SUMOylation site. The box below shows several proteins that phosphorylate p53 and the amino acids they target, while the box above the central binding domain shows the distribution of point mutations of p53 in human cancers (Figure taken from Mirzayans et al. 2012).
2. Regulation of p53

Research has shown that “levels of p53 protein are extremely low in normal conditions, but p53 becomes stabilized and activated by a variety of posttranslational modifications in cells subjected to different types of DNA damage as well as upon overexpression of oncogenes” (Gostissa et al., 2012). Given the numerous and complex actions of p53, it is clear that careful regulation inside the cell is necessary to avoid unwanted activation. p53 protein is constitutively present and held at low levels in the cell and transported between the cytoplasm and nucleus in a manner dependent on the stage of the cell cycle, localizing in the cytoplasm during G1, nucleus during the G1 to S transition, and back to the cytoplasm after the beginning of S phase (Vuong et al., 2012). The major regulator protein responsible for holding p53 at low levels is Murine Double Minute 2 (MDM2, HDM2 in humans), via a negative feedback loop: as levels of p53 rise it increases the transcription of MDM2. MDM2, the structure of which can be seen in Figure 3 below, inhibits p53 in several ways including binding to its transactivation domain, targeting p53 for ubiquination or inhibiting acetylation of p53 by shuttling it to the cytoplasm (Balint and Vousden, 2001).
Prior research has shown that in the absence of MDM2, p53 levels increase in an unrestrained manner and leads to inhibition of the normal growth and development of the cell (Balint and Vousden, 2001), illustrating its role in p53 regulation. Acting as an ubiquitin E3 ligase, MDM2 marks the protein for proteasomal degradation via the 26S proteasome, avoiding an accumulating of excess levels of p53 in the cytosol by facilitating its turnover (Koom et al., 2012). Similar to MDM2, a second protein, MDMX, functions in much the same way and works to regulate p53 (Jhang et al., 2013). MDMX is an MDM2 homolog and often partners in binding with MDM2, increasing its efficacy in regulating p53 (Divine and Dai 2013).

3. Activation of p53

In response to cell stress or DNA damage, p53 is activated and its half-life in the cell increases, stabilizing levels in the cell (Liang et al., 2013). Specifically in regards to cancer, if there is an overexpression of oncogenes such as c-Myc
or Ras, the Arf (alternative reading frame) of the INK4A locus will bind to the central binding domain of p53, blocking ubiquination by MDM2 and avoiding the proteasomal degradation of p53 (Devine and Dai, 2013). Arf can also act to sequester MDM2 in the nucleus of the cell, inhibiting it from binding to and regulating p53. (Devine and Dai, 2013). Furthermore, phosphorylation of both p53 and MDM2 by kinases blocks the protein-protein interaction between the two and allows for the stabilization of p53 (Balint and Vousden, 2001). In addition to these forms of regulation of p53 there are several co-activators that activate or modify p53 in the cell (Balint and Vousden, 2001). For example, in tumor cells Myc, an oncogenic transcription factor, induces the phosphorylation of p53, preventing it from binding to its negative regulator MDM2, and allowing it to mediate the mitochondrial-apoptotic pathway, thus destroying the tumor cell (Nieminen et al., 2012). A summary of the regulation and functions of p53 in the cell can be seen in Figure 4 below.
Figure 4. An overview of p53 function and regulation. p53 is normally regulated by several proteins including MDM2, Yyl and others that lead to its ubiquination and subsequent degradation. However, in response to stress, p53 levels stabilize and it goes on to affect the transcription of numerous downstream products involved in regulating the cell cycle and apoptosis (Figure taken from Vuong et al., 2012).
4. Functions of p53 in the cell

The roles of p53 are incredibly diverse and important to normal cellular function. p53 is involved in DNA repair, angiogenesis in the vascular system, cell migration, cell metabolism, stem cell renewal, and embryogenesis; it is best known, however, for the multiple functions it performs which aid in the blocking of both the initiation and progression of tumor growth within the cell (Wachter et al., 2013). For the purposes of this review only four areas of p53 activity will be discussed as they are primary targets of p53-related research in regards to cancer therapy. Topics to be discussed will include the major anti-tumorigenic functions of p53 (seen in Figure 5 below) including its regulation of the cell cycle, cell senescence and apoptosis, as well as its regulation of cell metabolism and energy use.
Figure 5. The anti-tumorigenic functions of p53. This figure shows the major functions of p53 in response to DNA damage or oncogenic growth. Based on the extent of the damage, p53 will induce cell cycle arrest and direct the cell to one of three options: senescence, DNA repair, or apoptosis. Above is shown the basic regulation of p53, a feedback loop with MDM2 and the Arf (Figure taken from Essmann and Schulze-Ostoff, 2010).
a. p53 regulation of the cell cycle

One of the primary functions of p53 activity is to induce cell cycle arrest in a cell that has experienced stress or has accumulated mutations in its genome that need repair. To avoid replication of these mutations, stabilized levels of p53 induce the transcription of a cyclin-dependent kinase inhibitor, known as factor p21, which is able to induce both G1 and G2 cell-cycle arrests as seen in Figure 6 below (Balint and Vousden, 2001). p21 acts by blocking the CD4/cyclin D complex; the CD4/cyclin D complex normally phosphorylates pRb (retinoblastoma protein), inducing the transcription factor E2F1 to trigger the progression from G1 to S (Vuong et al., 2012). In regards to cancer therapy, it has been shown that the ability of p53 to arrest the cell cycle in the G1 phase promotes radiosensitivity to fractionated radiation in certain lung cancers, thereby causing a much better response to radiation treatment (Perez et al., 2013). p21 can also arrest the cell cycle in the S phase by inhibiting PCNA (proliferating cell nuclear antigen) (Vuong et al., 2012).
Figure 6. The role of p53 in regulating cell cycle arrest. Through the transactivation of downstream targets such as p21 and Gadd45, p53 can induce cell cycle arrest at both the G1/S and G2/M checkpoints (Figure taken from Denaro et al., 2011).
b. p53 regulation of cell senescence

p53 induces cell senescence, in response to an accumulation of genomic mutations, DNA damage, or over-expression of oncogenes (Vuong et al., 2012). Its mode of action is, like its regulation of the cell cycle, to transactivate downstream targets such as p21. It is now widely accepted that p21 plays an important regulatory role in several types of cell senescence, such as stress-induced premature senescence (SIPS) and replicative senescence (Mirzayans et al., 2012). Its various roles in senescence can be seen in Figure 7 below.

Figure 7. The various roles of p53-induced p21 in the cell. p21 positively regulate cell senescence genes, p53 and ATM. It inhibits cell-cycle progression through its interaction with cyclins/CDK complexes and PCNA. It has shown anti-apoptotic effects through its interaction with ASK-1 and Capsase-3 (Figure taken from Mirzayans et al., 2012).
c. p53 regulation of apoptosis

In cases where the DNA damage is beyond repair or severe stress has completely compromised the cell, p53 will induce apoptosis, programmed cell death, in order to protect genomic integrity in the organism and avoid neoplastic growth (Balint and Vousden, 2001). The mode of action of p53, as in its other regulatory roles, is to transactivate downstream targets which will exert the desired effect, which can be seen in Figure 8 below. In activating target genes such as Bax, Noxa and PUMA, and by inhibiting anti-apoptotic genes such as bcl-2 (which suppresses the glutamine starvation mediated apoptotic pathway and inhibits the pro-apoptotic Bax), p53 induces cell apoptosis (Nieminen et al., 2012, Wachter et al., 2013). These downstream targets of p53 typically operate by destabilizing the mitochondrial membrane potential and the release of cytochrome C, thus activating the Apaf-1/caspase-9 apoptotic cascade leading to the directed destruction of the cell (Balint and Vousden, 2001). It is via apoptosis that p53 primarily exerts its anti-tumorigenic effect, halting progression and even causing regression of the cancerous tumor (Gostissa et al., 2012). However, research has shown that p53 also displays anti-apoptotic properties, illustrating its complex roles in programmed cell death (Wachter et al., 2013).
Figure 8. Downstream target genes of p53 that induce apoptosis. Events in apoptosis are shown in the center pathway and include disruption of the mitochondrial membrane potential (mitochondria ΔΨ) followed by cytochrome C release and activation of caspases. The left cascade shows the anti-apoptotic actions of p53 by transactivating IGF-BP3 which inhibits IGF, in turn inhibiting apoptosis (Figure taken from Balint and Vousden, 2001).
d. p53 regulation of cell metabolism

Recent research has shown p53 an important regulator of a cell’s energy metabolism, playing a role in oxidative phosphorylation, glycolysis, glutamine metabolism, lipid metabolism, and the antioxidant defense of the cell as shown in Figure 9 below (Liang et al., 2013). In 1926, Otto Warburg first characterized what became known as the Warburg effect, describing how a cancer cell primarily meets its energy needs from glycolysis as opposed to oxidative phosphorylation (Liang et al., 2013). Given the relative inefficiency of glycolysis when compared to oxidative phosphorylation, the tumor cell must commensurately increase its uptake of glucose to compensate, providing the basis for many diagnostic tests looking for cancerous tumors (Liang et al., 2013). In the normal cell, p53 acts to downregulate the glycolytic and pentose phosphate pathways while simultaneously upregulating oxidative phosphorylation in the mitochondria, directing the cell to primarily use oxidative phosphorylation for its energy needs (Feng and Levine, 2010). In tumor cells, AMP-activated protein kinase (AMPK) monitors the energy status of the cell by monitoring the ratio of ATP to AMP/ADP. When this ratio falls due to a lack of glucose, AMPK phosphorylates and activates p53, which in turn induces apoptosis in the tumor cell (Nieminen et al., 2012). This regulatory function is often lost with the deletion or inactivation of p53 in many tumor cells, allowing the tumor cell to meet its high energy needs and grow uncontrollably. p53 function in energy metabolism has thus been established as a potential target for cancer therapy.
Figure 9. The many roles of p53 in regulating cellular energy metabolism. p53 functions in the regulation of processes such as glycolysis, oxidative phosphorylation, fatty acid oxidation and glutaminolysis. p53 induces the transcription SCO2, AIF, p53R2 and mtDNA to stabilize the mitochondrial membrane and promote the use of oxidative phosphorylation in the cell. At the same time, p53 represses the transcription of GLUT 1 and 4 and inhibits GLUT 3 to reduce the uptake of glucose into the cell and decrease glycolysis (Figure taken from Liang et al., 2013).
e. p53 role in antioxidant defense

p53 also plays an important role in the regulation of antioxidant defense in the cell. Oxidative stress and the accumulation of reactive oxygen species (ROS) is a source of DNA damage and often plays a role in the genesis of tumors in humans (Liang et al., 2013). ROS are generated primarily by mitochondrial oxidative phosphorylation, a process p53 normally promotes in the cell while inhibiting glycolysis. In order to avoid the buildup of ROS, p53 transactivates several antioxidant genes including TIGAR, ALDH4 and sestrins1/2 which act to lower ROS levels in a variety of ways (Liang et al., 2013). p53 antioxidant effects can be seen in Figure 10 below, in response to low oxidative stress. In response to high levels of oxidative stress in the cell which produces DNA damage or mutations, p53 induces a pro-oxidant response that induces the increase of ROS levels in the cell (Liang et al., 2013). These increased levels of ROS in turn further activate p53, activating its anti-tumorigenic function in the cell and leading to cell senescence or apoptosis, as can be seen in Figure 10 below (Liang et al., 2013).
Figure 10. p53 regulation of ROS and oxidative stress in the cell. In response to low levels of oxidative stress p53 transactivates antioxidant genes to lower ROS levels and avoid DNA damage or mutation. In response to high levels of oxidative stress p53 transactivates pro-oxidant genes which increase ROS species, leading to increased p53 activation. Increased p53 activity will eventually lead to apoptosis or cell senescence, thus avoiding tumorigenesis in the organism (Figure taken from Liang et al., 2013).
C. Cancer treatment strategies utilizing p53

Animal models have consistently shown that p53 activation in tumors halts tumor progression and can be curative, even in later stages of cancer (Martins et al., 2006). This discovery has led to a considerable amount of research in search of a viable method for utilizing p53 in the treatment of cancer, and to date several strategies have been developed with varying success. The following sections will give an overview of different therapeutic approaches to treat cancer by reactivating p53 function.

1. Small molecules

A variety of small peptides have been developed with the intent of reactivating p53 function when it has been lost in tumor cells. There are several different causes of p53 inactivation in cancer; commonly, allelic mutations or deletions of the p53 gene have removed its regulatory functions in the cell. Even in the event that wild-type p53 can be properly transcribed from one normal allele, it will incorporate mutant-type p53, transcribed from the mutated allele, during oligomerization and thus dampen any response to DNA damage or mutations that occur (Liu et al., 2000). In this condition, the concentration of wild-type p53 needs to be higher than endogenous mutant p53 to overcoming its dampening effect (Chan et al., 2004). In many tumors, however, the inactivating of p53 is not caused by a p53 mutation or deletion, but by an overexpression of MDM2, which acts to inactivate p53 and induce its proteasomal degradation, thus
removing p53 function in the cell (Balint, 2001). Small molecules have been developed targeting these causes of p53 inactivation.

a. MDM2/MDMX inhibitors

A common dysfunction in the p53 signaling system found in various cancer is the inactivation of p53 due to an upregulation of MDM2 or MDMX, the primary negative regulator of p53 (Devine and Dai, 2013). When p53 binds to a p53 responsive element in its gene, MDM2 transcription is activated and MDM2 binds to the N-terminal domain of p53, inactivating it (Saha et al., 2013). This is purposed to avoid an unregulated p53 response in the cell. However, in cancer MDM2 can be constitutively activated, thus entirely removing the ability of p53 levels to stabilize in response to DNA damage or oncogenic activation; the loss of p53 response allows for accumulation of genetic mutations and progression of the tumor (Essmann and Schulze-Ostoff, 2010). Compounds such as Nutlins, benzodiazepinediones and sulfonamides directly inhibit MDM2 from binding to p53, avoiding proteasomal degradation of p53 and allowing its levels to stabilize in the cell (Ozaki et al., 2013).
**Figure 11. Strategies for inducing apoptosis with Nutlins.** Cells with wt-p53 can be induced to undergo apoptosis via p53 transcription-dependent or independent pathways. Nutlin can induce apoptosis in mutant-p53 expressing cells via p53 or p73, but only to a partial extent (Figure taken from Saha et al., 2013).

Research has shown that treatment of various hematological malignancies, in which overexpression of MDM2 is common, with Nutlins alone reproduces the apoptotic and cytotoxic response in cells that is expected with wt-p53 as seen in Figure 11 above (Saha et al., 2013). Another peptide, RITA, directly interacts
with p53, causing a conformational shift that blocks the MDM2-p53 interaction, producing a similar effect to Nutlins (Essmann and Schulze-Osthoff, 2010). While promising, the one major shortcoming of small molecules such as Nutlins or RITA is their inability to reactivate mutant-type p53 in tumor cells where p53 has been mutated or deleted (Saha et al., 2013).

b. Reactivation of wild-type function

Several small molecules have been designed to reactivate some wild-type function of p53, including PRIMA-1 and MIRA-1. Both PRIMA-1 and MIRA-1 reconstitute wild-type function of mutant p53 in cancer cells by refolding the unfolded protein (Ozaki et al., 2013). This process is selective, leaving normal cells with wt-p53 unaffected (Ozaki et al., 2013). Their effect can be seen in Figure 12 below.
Figure 12. Strategies for inducing apoptosis using PRIMA-1 or MIRA-1. PRIMA-1 can induce apoptosis in p53 mutant cells. In certain cancer types, PRIMA-1 may induce apoptosis regardless of p53 status (shown in center cascade). In certain cancers, MIRA-1 has been shown to induce apoptosis regardless of cell type, though it primarily acts to reactivate mutant-type p53 (Figure taken from Saha et al., 2013).

As opposed to MDM2 inhibitors, however, there is little evidence to show PRIMA-1 and MIRA-1 have any effect on cancer cells where p53 has been inactivated for reasons other than a mutation (in certain hematological cancers this has been observed regardless of p53 status) (Essmann and Schulze-Osthoff, 2010).

c. Inhibiting the proteasome

An additional target of small molecules is the proteasome. The proteasome functions to degrade many cyclical molecules in the cell to allow cell cycle progression; in an continuously proliferating cell like a tumor cell, the
proteasome must work overtime in order to degrade tumor suppressor proteins like p53 (Shen et al., 2013). Small molecules have been developed which inhibit both the 20S and 19S proteasomes. Compounds such as Bortezomib and peptide boronates have been developed to inhibit the 20S proteasome, while b-AP15 inhibits the 19S proteasome (Shen et al., 2013). All three inhibitors cause stabilization of p53 levels in cells expressing the wild-type form, restoring p53 function and leading to apoptosis of tumor cells (Shen et al., 2013). The shortcoming of proteasome inhibitors, like MDM2 inhibitors, is their therapeutic potential is limited to cells that express wild-type p53 and does not extend to cells with p53 mutations or deletions.

In short, there are several promising small molecule treatments for cancer, including some like Nutlins and PRIMA-1 that have reached clinical trials, however they are very specific to certain p53 deficiencies and therefore are limited in potential.

2. Gene therapy

Research in animal models has clearly shown that transfection of p53 into tumor cells with p53 mutations or deletions restores p53 function and leads to a halt of tumor progression and often tumor cell apoptosis (Lane et al., 2010). Other research has also shown that the presence of wild-type p53 increases the sensitivity of tumor cells to fractionated radiation, promoting the anti-tumorigenic effects of radiation therapy (Perez et al., 2013). Given the frequency of mutations
or deletions of p53 in human cancer (approximately 50% of human cancers as stated earlier), the goal of reactivating p53 in tumor cells using gene therapy is an emerging field full of potential in cancer therapy. Gene therapy is the transfer or insertion of a desired foreign gene into the genome of a cancer cell, with the hope that activation and replication of the gene will destruction of the tumor cells, as seen in Figure 13 below (Cross and Burmester, 2006).

Figure 13. Basic diagram of gene therapy (Figure taken from Cross and Burmester, 2006)

Many different gene types, including suicide genes and antiangiogenesis genes, have been proposed as viable options; given the well-established anti-tumorigenic functions of p53, current research is exploring the possibility of inserting this gene into cancer cell as a therapeutic treatment. At question is the most efficient vector of introducing p53, several of which will be discussed below.

a. Retroviral vector

One vector that has been used in gene therapy is that of a retrovirus. Retroviruses carry a diploid RNA genome, within a viral capsid, that is integrated
into a host cells chromosome upon entry into the cell (Lim, 2012). Unlike many other vectors used in gene therapy, retroviruses have the capacity to be expressed in the host cell genome for long periods of time given the stability of their integration (Lim, 2012). Retroviruses were first used to introduce p53 into tumor cells of patients with non-small cell lung cancer by Dr. Jack Roth in 1996; of nine patients for whom traditional treatment failed, three saw tumor regression and another three tumor stabilization (Roth et al., 1996). Importantly, apoptosis was the primary response seen when wild-type p53 was integrated into the tumor cell genome (Roth et al., 1996). Research has shown that the integration of a retroviruses genetic material is not random but targets parts of the genome near transcriptional start sites as well as CpG islands, which are rich in regulatory elements (Lim et al., 2010). Despite the early success shown by Jack Roth in using retrovirus vectors, substantial research has shown that retroviruses often display oncogenic properties in humans when integrated into a host genome, itself causing cells to transform and become malignant (Essmann and Shulze-Osthoff, 2011). The promoter and enhancer components of the retrovirus long-terminal repeat (LTR), used by the virus to insert its genetic material, have the ability to activate host cell oncogenes, thus initiating tumorigenesis (Hacein-Bey-Abina et al., 2003). The retrovirus’ propensity for inserting its genetic material near transcriptional start sites and CpG islands further exacerbates this oncogenicity (Lim, 2012). Given the serious threat of oncogenesis when using
After the early success of retroviral gene therapy, other vectors have been sought out and largely replaced retroviruses for gene cancer therapy.

**b. Adenovirus vector**

The most common vector used to introduce wild-type p53 into tumor cells is human adenovirus (Cross and Burmester, 2006). Adenoviruses are composed of a capsid shell typically containing double stranded DNA. Adenoviruses bind to and are taken up by a host cell where, once inside the cell, they inject their nucleic acid content into the host cell’s nucleus for reproduction (Scherer et al., 2011). An early trial using adenoviral vectors for gene therapy led to devastating consequences, when in 1999 Jesse Geisinger was treated for ornithine transcarbamylase (OTC) deficiency (Stolberg, 1999). The treatment caused a massive immune response, leading to “multiple-organ system failure” and the patient’s death (Stolberg, 1999). To avoid these terrible side effects, today’s adenoviruses are engineered to lack certainly proteins necessary for replication (Lane et al., 2010). This replication deficiency allows for large scale GMP production of the desired gene product, such as wild-type p53, for a transient period of time without the mass immune response or oncogenic risk seen in retroviruses (Essmann and Shulze-Osthoft, 2011). Following is an overview of human adenovirus biology and function which will serve as an introduction to a discussion on the use of adenovirus vectors to introduce or reactivate p53 in clinical settings.
i. Adenovirus biology

Adenoviruses must be engineered to deliver genes such as p53 to cancer cells. The most common form of adenovirus used is human adenovirus serotype 5; human adenovirus serotype 5 is one of 51 adenoviruses in the Adenoviridae family that has been characterized by its ability to be neutralized by animal antisera (Beatty and Curiel, 2012). The serotypes in this family are further subcategorized into six different species based on factors such as oncogenicity and genomic structure (Davison et al., 2003).

ii. Adenovirus structure

The human adenovirus is a 36 kilodalton genome of double-stranded DNA (Nemerow et al., 2012), encapsulated in a non-enveloping icosahedral capsid (Beatty and Curiel, 2012). For many years the structure of the capsid itself proved difficult to elucidate, but with the help of X-ray crystallography and electron microscopy this difficulty has been overcome and we know have a much greater understanding of its structure and composition (Beatty and Curiel, 2012). The capsid is composed primarily of three different proteins, hexon, penton base and fiber, and each of these proteins has a specific role in the adenovirus and its function (Nemerow et al., 2012). Based on information gleaned from X-ray crystallography, it is now believed hexon provides the sites of contact between neighboring particles in the crystal lattice of the capsid, as well as with neighboring loops or cement proteins (Reddy et al., 2010). Data has shown that
the penton base protein serves to attach surface integrins, which act as receptors that mediate the internalization of the virus, to the capsids outer surface (Wickham et al., 1994). Finally, the primary role of fiber protein is as the site of cellular attachment (Zubieta et al., 2005). The structure of human adenovirus and the roles of its integral capsid proteins can be seen in Figures 14 and 15 below.
Figure 14. The capsid structure of human adenovirus. The capsid is composed primarily of the proteins hexon, penton base, and fiber which are labeled. Also labeled is a minor protein in the capsid structure, polypeptide IX, which has been hypothesized as a possible locus for targeted incorporation in addition to the three primary components. (Figure taken from Beatty and Curiel, 2012)
Figure 15. Geometric representation of the human adenovirus. This representation shows the arrangement of hexon, penton base, and fiber proteins, including the icosahedral lattice of hexon trimmers in red, yellow and green, and the penton base subunits at the vertices of the capsid, shown in magenta. (Figure taken from Nemerow et al., 2012).
iii. Mechanism of entry

In order for the adenovirus to deliver its contents into the target cell’s genome it must first enter the cell and reach the nucleus. The first step in the entry of an adenovirus is the attachment to its primary receptor on the cell surface (Beatty and Curiel, 2012). The binding of an adenovirus to its receptor is carried out by fiber proteins on the capsid surface; depending on the subcategory of adenovirus being used, the receptor may vary but is most commonly the coxsackie and AD receptor (CAR) (Beatty and Curiel, 2012), a 46 kilodalton immunoglobulin that is normally found in the formation of tight junctions between cells (Coyne and Bergelson, 2006). Secondly, the adenovirus must then be internalized into the cell. Upon binding to the cell, surface integrins interact with the penton base of the adenovirus, inducing the cytoskeleton of the cell to alter itself and internalize the virus in a clathrin coated vesicle (Li et al., 1998). The virus is carried by this clathrin-coated vesicle to the endosome within the cell, from which it eventually escapes and is released into the cytoplasm (Meier et al., 2002). Once in the cytoplasm, the virus must reach the nucleus. Recent research has shown that once in the cytoplasm, adenovirus recruits the motor protein dynein, which it uses to travel along microtubules towards the centrosome in the vicinity of the nucleus (Scherer et al., 2011). Once it has reached the nucleus, the virus binds to a nuclear pore complex (NPC) on the surface of the nuclear membrane and injects its contents into the nucleus (Scherer et al., 2011). The
mechanism of adenovirus binding to CAR, endocytosis, and movement to along microtubules to the nucleus can be seen in Figure 16 below.

**Figure 16. Mechanism of entry of an adenovirus.** Adenovirus binds to CAR on the plasma membrane and is endocytosed by the host cell. After it is released form the endosome it then moves along microtubules, using dynein, until it reaches the nuclear membrane, at which point it injects its contents through a nuclear pore (Figure taken from Scherer et al., 2011).
II. Evaluation of Ad-p53 in cancer treatment

A. Non-small cell lung cancer

After research by Dr. Jack Roth showed that retroviral introduction of wild-type p53 caused tumor apoptosis and regression in non-small cell lung cancer (NSCLC) (Roth et al., 1996), trials using adenovirus-mediated transfer of wild-type p53 were begun in order to avoid the negative oncogenic effects of retroviruses. Research using animal models showed that adenovirus carrying p53 (Ad-p53) maintained the anti-tumor properties seen with retroviral vectors (Swisher et al., 1999). In 1999 a phase 1 clinical trial was conducted on 28 patients whose NSCLC had progressed in spite of conventional therapeutic approaches by injecting Ad-p53 monthly for a total of up to 6 treatments (Swisher et al., 1999). A range of doses was used to evaluate if any p53 response was dose-dependent. Results showed adenovirus vector DNA in 86% of patients with evaluable biopsy specimens, supporting the efficacy of adenovirus as a vector for gene therapy in cancer treatment, though p53 expression was seen more frequently in patients receiving higher doses, indicating a dose-dependency (Swisher et al., 1999). Most importantly, of the 25 evaluable patients, tumor stabilization was seen in 16 patients and tumor progression seen in only 7 patients (the final 2 patients showed only partial responses), all with minimal to no adverse effects accompanying treatment (Swisher et al., 1999). These results, which can be seen in Table 1 below, indicate that Ad-p53 is a viable treatment
option for NSCLC, even in advanced stages, with minimal negative effects seen in therapeutic approaches.

Table 1. Evaluation of the efficacy of Ad-p53 treatment of NSCLC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Viral dose, plaque-forming units</th>
<th>No. of courses</th>
<th>DNA-PCR†</th>
<th>RT-PCR†</th>
<th>TUNEL % positive cells†</th>
<th>Response</th>
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<tr>
<td>A</td>
<td>10⁶</td>
<td>4</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>B</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>Stable</td>
</tr>
<tr>
<td>C</td>
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<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NT†</td>
</tr>
<tr>
<td>D</td>
<td>10⁶</td>
<td>3</td>
<td>(2)</td>
<td>NT†</td>
<td>NT†</td>
<td>Progression</td>
</tr>
<tr>
<td>F</td>
<td>10⁶</td>
<td>2</td>
<td>(2)</td>
<td>(2)</td>
<td>2</td>
<td>Progression</td>
</tr>
<tr>
<td>G</td>
<td>10⁴</td>
<td>2</td>
<td>(1)</td>
<td>(1)</td>
<td>78</td>
<td>Not evaluable†</td>
</tr>
<tr>
<td>H</td>
<td>10⁵</td>
<td>5</td>
<td>(2)</td>
<td>(2)</td>
<td>81</td>
<td>Stable</td>
</tr>
<tr>
<td>I</td>
<td>10⁵</td>
<td>4</td>
<td>(1, 2)</td>
<td>-</td>
<td>50†</td>
<td>Stable</td>
</tr>
<tr>
<td>J</td>
<td>10⁴</td>
<td>2</td>
<td>(1, 2)</td>
<td>-</td>
<td>10†</td>
<td>Stable</td>
</tr>
<tr>
<td>K</td>
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<td>(1, 2)</td>
<td>-</td>
<td>NT†</td>
<td>Progression</td>
</tr>
<tr>
<td>L</td>
<td>10⁵</td>
<td>4</td>
<td>(1, 2)</td>
<td>-</td>
<td>4</td>
<td>Partial response</td>
</tr>
<tr>
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<td>(3, 4)</td>
<td>(2)</td>
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<td>(1, 2)</td>
<td>(1)</td>
<td>4</td>
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</tr>
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<td>O</td>
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<td>Partial response</td>
</tr>
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<td>-</td>
<td>3</td>
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</tr>
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<td>-</td>
<td>50†</td>
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</tr>
<tr>
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<td>(1)</td>
<td>45†</td>
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</tr>
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<td>S</td>
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<td>5</td>
<td>NT†</td>
<td>(1, 2, 3, 4)</td>
<td>71†</td>
<td>Stable</td>
</tr>
<tr>
<td>T</td>
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<td>2</td>
<td>NT†</td>
<td>(1)</td>
<td>4</td>
<td>Progression</td>
</tr>
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<td>3 x 10⁴</td>
<td>6</td>
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<td>(1, 2, 3)</td>
<td>0†</td>
<td>Not evaluable†</td>
</tr>
<tr>
<td>W</td>
<td>10⁵</td>
<td>3</td>
<td>NT†</td>
<td>(1, 2, 3, 4, 5, 6)</td>
<td>71†</td>
<td>Stable</td>
</tr>
<tr>
<td>X</td>
<td>10⁵</td>
<td>5</td>
<td>NT†</td>
<td>(1)</td>
<td>2</td>
<td>Stable</td>
</tr>
<tr>
<td>Y</td>
<td>10⁶</td>
<td>6</td>
<td>NT†</td>
<td>(2, 3)</td>
<td>3</td>
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<td>3</td>
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<td>(2, 3)</td>
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</tr>
<tr>
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<td>1</td>
<td>NT†</td>
<td>(1)</td>
<td>1</td>
<td>Progression</td>
</tr>
<tr>
<td>BB</td>
<td>10⁵</td>
<td>2</td>
<td>NT†</td>
<td>(1)</td>
<td>1</td>
<td>Progression</td>
</tr>
</tbody>
</table>

*Sequential letters were assigned during manuscript editing to ensure confidentiality of patients; the letter do not represent identifiers used during the trial.†Course number during which DNA-polymerase chain reaction (PCR) or reverse transcriptase (RT)-PCR was positive is given in parentheses.

Maximum percentage of cells staining positive by terminal deoxynucleotidyl transferase-mediated biotinyl uridine triphosphate nick-end labeling (TUNEL) posttreatment. Mean pretreatment apoptotic index was 3.6% with 95% confidence interval = 1.5%–5.8%.

†Partial response = decrease ≥50% in size of tumor for minimum of 4 weeks; complete response = complete disappearance of tumor as judged by computed tomography scan and physical examination for a minimum of 4 weeks; progression = increase ≥25% in size of tumor; stable = any variation in size not meeting criteria of complete response, partial response, or progression.

‡Pretreatment apoptotic index above 95% confidence interval of pretreatment apoptotic index.

NT† = not tested because of insufficient quantity or quality of biopsy specimen.

†Not evaluable because patients died of non-treatment-related causes prior to 30-day follow-up computed tomography scan.

(Table taken from Swisher et al., 1999)

Another trial performed at the West China Hospital of Sichuan University combined a replication deficient adenovirus expressing wild-type p53 (rAd-p53), known as Gendicine, with bronchial arterial infusion. Gendicine in combination with chemotherapy and radiotherapy has previously been shown to significantly improve therapeutic effects in head and neck cancer (Guan et al., 2009). Two groups, one receiving the combination of treatments and the other receiving only
the bronchial arterial infusion, were examined; results showed a 47.3% response rate with the combination group, and a 38.4% response rate with the control group (Guan et al., 2009). The rAd-p53 lead to a slowed progression of the disease, however it did not produce better survival results (Guan et al., 2009). The results of this study can be seen in Table 2 below.

**Table 2. Evaluation of the efficacy of rAd-p53 in combination with bronchial arterial infusion in treating NSCLC.**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Gender</th>
<th>Age (year)</th>
<th>Histology</th>
<th>Stage</th>
<th>Gene transfer access</th>
<th>rAd-p53 Dose (x10¹⁵ VP)</th>
<th>Time (days)</th>
<th>BAI times</th>
<th>Local response</th>
<th>TTP (months)</th>
<th>Survival duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>40</td>
<td>Adeno</td>
<td>IIIb</td>
<td>TA</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>SD</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
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<td>M</td>
<td>56</td>
<td>Squam</td>
<td>IIIb</td>
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<td>Large cell</td>
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<td>PI</td>
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<td>2</td>
<td>2</td>
<td>PR</td>
<td>10</td>
<td>12</td>
</tr>
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<td>M</td>
<td>53</td>
<td>Squam</td>
<td>IIIb</td>
<td>PI/TA</td>
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<td>CR</td>
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<td>71</td>
<td>Adeno</td>
<td>IV</td>
<td>PI &amp; TC</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>SD</td>
<td>5</td>
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<td>PI</td>
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<td>2</td>
<td>SD</td>
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<td>IIIb</td>
<td>PI &amp; LN</td>
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<td>3</td>
<td>3</td>
<td>PR</td>
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<td>CR</td>
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<td>59</td>
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<td>2</td>
<td>2</td>
<td>PR</td>
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<td>3</td>
<td>PR</td>
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<td>TA</td>
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<td>2</td>
<td>1</td>
<td>PR</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

M: male; F: female; Adeno: adenocarcinoma; Squam: squamous; TA: transarterial infusion; TC: thoracic cavity; PI: percutaneous injection; LN: lymph nodes; BAI: bronchial arterial infusion; SD: stable disease; PD: progressive disease; PR: partial response; CR: complete response; TTP: time to progression

(Taken from Guan et al., 2009)

Similar results were seen in a phase II clinical trial combining Ad-p53 with traditional chemotherapy for the treatment of NSCLC, in which a response rate of 52% was seen in patients treated with the combination (Shuler et al., 2001).
However there was no statistical distinction between this group and the control group receiving only chemotherapy, in which a 48% response was seen, indicating there was no advantage to receiving Ad-p53 in treating NSCLC (Shuler et al., 2001).

After several studies, there is no consensus as to the efficacy of Ad-p53 gene therapy for the treatment of NSCLC. Though adenovirus DNA, and in turn p53 messenger RNA, was consistently seen, this therapeutic approach did not consistently provide better outcomes when compared to traditional treatment.

B. Breast Cancer

Breast cancer is the most common cancer seen in women. Currently the primary mode of treatment is the use of cytotoxic chemotherapy, and response to this treatment is the most important prognostic indicator for long term outcomes (Cristofanilli et al., 2006). Several clinical studies have been or are currently being performed to evaluate the use of gene therapy, targeting several different genes commonly seen mutated in breast cancer (Takahashi et al., 2006). Given the high incidence of mutation of the p53 gene in breast cancer, a phase II clinical trial using adenovirus mediated p53 gene transfer was performed. The drug Advexin is a non-replicating adenovirus carrying the wild-type p53 gene and was given in combination with traditional chemotherapy to treat locally advanced breast cancer (LABC) (Cristofallini et al., 2006). In the 13 patients treated with the combination treatment, none presented with a complete clinical response,
defined as the complete disappearance of the localized tumor; however, of the 12 evaluable patients, all showed a partial clinical response representing a 50% reduction in tumor size, as can be seen in Table 3 below (Cristofallini et al., 2006).

**Table 3. Evaluation of the efficacy of Ad-p53 treatment for locally advance breast cancer. (Taken from Cristofallini et al., 2006)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of evaluable patients</td>
<td>12</td>
</tr>
<tr>
<td>Median no. of cycles treated (range)</td>
<td>6 (4–6)</td>
</tr>
<tr>
<td>Breast clinical response (%)</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PR</td>
<td>12 (100)</td>
</tr>
<tr>
<td>SD</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PD</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pathologic residual breast disease (size range)</td>
<td></td>
</tr>
<tr>
<td>No. with residual breast disease ≤1 cm</td>
<td>8 (0.1–1.0 cm)</td>
</tr>
<tr>
<td>No. with residual breast disease &gt;1 cm</td>
<td>4 (2.0–6.0 cm)</td>
</tr>
<tr>
<td>Pathologic lymph node status (%)</td>
<td></td>
</tr>
<tr>
<td>No. with 0 lymph nodes</td>
<td>2 (17)</td>
</tr>
<tr>
<td>No. with 1–3 lymph nodes</td>
<td>4 (33)</td>
</tr>
<tr>
<td>No. with &gt;4 lymph nodes</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Median follow-up (range), mo</td>
<td>37 (13–41)</td>
</tr>
<tr>
<td>Estimated 3-year overall survival (95% CI), %</td>
<td>77 (57–100)</td>
</tr>
<tr>
<td>Estimated 3-year breast cancer-specific survival (95% CI), %</td>
<td>84 (67.5–100)</td>
</tr>
</tbody>
</table>

CR indicates complete clinical response; PR, partial clinical response; SD, stable disease; PD, progressive disease; 95% CI, 95% confidence interval.

Further research has shown that Ad-p53 treatment sensitizes breast cancer cells to adriamycin, a traditional chemotherapy treatment to which breast cancer tumor cells show a resistance (Qi et al., 2011). The effect of this loss of resistance led
to apoptosis of the tumor cells (Qi et al., 2011). Clinical trials using this treatment approach have yet to be performed.

Though the use of adenovirus-mediated p53 gene transfer to treat breast cancer is still in the early stages, initial results have been very positive. Specifically the combination of Ad-p53 and traditional chemotherapy seems to be very effective. Further research and clinical trials will be needed to fully evaluate this therapeutic approach, but the potential for breast cancer treatment appears high.

C. Gastric Cancer

Gastric cancer, the most common form of cancer in the digestive system, has been the subject of numerous studies involving adenovirus-mediated p53 gene therapy. Early studies on four different gastric cancer cell lines showed that introduction of wild-type p53 to lines with mutated p53 led to tumor regression via apoptosis of tumor cells (Ohashi et al., 1999), while lines already expressing wild-type p53 did not show tumor regression (Ohashi et al., 1999). These early results indicated a great amount of promise for Ad-p53 treatment of gastric cancer. More recent research comparing the anti-tumor effects of Ad-p53 in combination with oxaliplatin, a common chemotherapeutic drug used in digestive cancer, showed significant synergistic effects between the two (Chen et al., 2011). The study, the results of which can be seen below in Figure 17 below,
also confirmed that Ad-p53 treatment alone leads to gastric tumor regression, as shown prior (Chen et al., 2011).

**Figure 17. Efficacy of Ad-p53 in combination oxaliplatin in treating gastric cancer.** Groups a, b, c, and d were treated with increasing doses of oxaliplatin, illustrating a dose dependency of oxaliplatin. Groups A, B, C, and D were treated with increasing doses of Ad-p53, also displaying a dose dependency. Importantly, the synergistic effects of combining Ad-p53 and oxaliplatin can be seen when comparing groups A, B, C, and D to groups E and F, which did not receive Ad-p53. (Figure taken from Chen et al., 2011)
The recombinant adenovirus Gendicine has also been used, in combination with the cytotoxic chemotherapy agent epirubicin hydrochloride, to treat gastric cancer in animal models both in vitro and in vivo (Xie et al., 2010). Much like the results seen when used in combination with oxaliplatin, Gendicine not only led to tumor regression when used alone but displayed synergistic effects when combined with epirubicin hydrochloride (Xie et al., 2010).

Though no human trials have yet to be completed using Ad-p53 to treat gastric cancer, in vitro and in vivo studies have been extremely promising. Not only has traditional Ad-p53 therapy been effective, but recent strides have seen the development of an adenovirus which co-transduces p53 with RPL23 (Ad-RLP23/p53), a gene that has been shown to protect wild-type p53 from MDM2 inactivation (Zhang et al., 2013). The benefits of this co-transduction are tremendous. Unlike prior studies which saw a resistance to Ad-p53 treatment by cancer cell lines expressing wild-type p53 (Ohashi et al., 1999), Ad-RLP23/p53 induced antitumor responses in both cell lines expressing mutant p53 and wild-type p53, likely by rescuing wild-type p53 from the inactivating effects of MDM2 overexpression (Zhang et al., 2013). This is an exciting breakthrough in gene therapy because it allows for a single drug to treat multiple genetic mutations that inactivate p53, increasing its efficacy in treating a broad range of patients. Multi-gene targeting such as this may lead to new and more efficient therapeutic approaches in cancer treatment.
D. Osteosarcoma

One of the most common bone cancers, osteosarcoma is another target of gene therapy using adenovirus-mediated p53 gene transfer. Current therapeutic approaches including surgery, radiation, and chemotherapy have proven insufficient in the treatment of osteosarcoma and the prognosis of this disease remains poor, making the need for a novel approach such as gene therapy evident (Ternovoi et al., 2006). The use of Ad-p53 has been shown to exhibit anti-tumor effects in canine osteosarcoma grafts on nude mice via apoptosis of the tumor cells (Kanaya et al., 2011). Research has also shown that while Ad-p53 induces apoptosis in human osteosarcoma cell lines alone, it also enhances the sensitivity of osteosarcoma cells to cisplatin and doxorubicin, traditional chemotherapeutic drugs (Oshima et al., 2007, Ternovoi et al., 2006). However, it has also been noted that certain osteosarcoma cell lines are resistant to wild-type p53 introduction, likely due to the inactivating effect of overexpressed MDM2 (Hellwinkel et al., 2005). Despite this issue, Ad-p53 has proven itself a viable potential treatment for osteosarcoma, especially when given in combination with traditional chemotherapy, but further research must be undertaken to better understand the effect of Ad-p53 in osteosarcoma cells before it can be taken to the clinical trial stage.
E. Hepatocellular carcinoma

Hepatocellular carcinoma is one of the most common cancers in the world, especially in regions of the world where infection with hepatitis is at epidemic levels (Habib et al., 2002). Early research examining the efficacy of adenovirus-mediated p53 gene therapy, while well-tolerated and with minimal side effects, showed a very minimal anti-tumor effect in most subjects (Habib et al., 2002), leading researchers to speculate that perhaps a more effective vector for was needed. Subsequent clinical trials, however, have shown that Ad-p53 in combination with traditional chemotherapeutic agents has been highly effective and led to cancer cell apoptosis and tumor regression (Koom et al., 2012, Guan et al., 2011). One clinical trial combining Ad-p53 with transcatheter hepatic arterial chemoembolization saw a success double that of the control group treated with hepatic arterial chemoembolization alone (Guan et al., 2011). Importantly, the survival rates of patients treated with the combination were significantly higher than those of the control group (Guan et al., 2011). A separate study illustrated a similar synergistic effect between Ad-p53 and radiotherapy in vivo using animal models, especially in tumor cells with low levels of MDM2 expression (Koom et al., 2012). Perhaps more importantly, this study also examined the use of an adenovirus carrying a modified p53 gene designed with the transactivation domain replaced with the herpes simplex virus vp16; this modified p53 gene, seen in Figure 18, no longer possesses the MDM2 binding site and avoids the negative feedback loop of MDM2, leading to a high efficacy in
treating hepatocellular carcinoma in tumor cells with high levels of MDM2 being expressed (Koom et al., 2012).

Figure 18. Map of adenoviral vectors used in p53 gene therapy. Both Ad-p53 and the recombinant adenovirus Ad-p53vp are illustrated. The E1 region in both is deleted, making the virus replication-deficient. In the Ad-p53vp, the transactivation domain has been replaced with the herpes simplex virus vp16, freeing p53 from MDM2 inactivation. (Figure taken from Koom et al., 2012)

Recombinant adenoviruses, similar to Gendicine (originally authorized in China for the treatment of head and neck cancer) have been evaluated as a treatment for hepatocellular carcinoma as well. Two trials used recombinant adenoviruses in combination with two different traditional treatments. When combined with fractionated stereotactic radiotherapy, the treatment led to a higher response rate than with radiotherapy alone, as well as a better survival rate (Yang et al., 2010).
Treatment in combination with the chemotherapy agent 5-fluorouracil and following transcatheater arterial chemoembolization led to higher responses to treatment as well as better survival rates (Tian et al., 2009).

In treating hepatocellular carcinoma, Ad-p53 in combination with traditional treatments such as chemotherapy or radiation therapy has shown high efficacy and minimal adverse effects, following the pattern seen when treating other forms of cancer. The use of a modified adenovirus like Ad-p53vp to avoid MDM2 inhibition is another positive step towards the development of a virus that may target a wide range of p53 mutations or causes of inactivation, thus potentially treating a wide array of cancers.

**F. Head and neck cancer**

Some of the earliest attempts to use adenovirus mediated p53 gene therapy was for head and neck cancers. After seeing tumor regression in animal xenografts models using Ad-p53, a clinical trial was performed to evaluate the efficacy of Ad-p53 in human subjects with head and neck squamous cell carcinoma, in which traditional treatments caused high rates of morbidity (Clayman et al., 1998). Results were positive, with no adverse side effects and tumor regression or stabilization in half of evaluable patients (Clayman et al., 1998). A separate clinical trial evaluated the efficacy of SBN-1, a recombinant adenovirus carrying p53, in combination with traditional radiotherapy to treat head and neck squamous cell carcinoma. When compared to a control group
receiving radiotherapy alone, patients receiving the combination of treatments showed significantly higher rates of tumor regression with no toxic side effects (Zhang et al., 2003). These findings are consistent with results from the use of Ad-p53 in combination with traditional chemotherapy or radiotherapy to treat many other cancer types, as discussed prior.

1. Oncolytic virotherapy

Another direction taken by researchers to treat head and neck cancer with adenovirus-mediated p53 is in the field of oncolytic virotherapy. Onyx-015 is an oncolytic virus that has been heavily researched as a potential treatment for head and neck cancer, as well as a variety of different cancers. Onyx-015 was developed with the E1B 55K gene deleted; this gene codes for a 55K protein that binds directly to and degrades p53, thus allowing the virus to replicate in the cell while avoiding the p53 induction and inhibition that would normally occur upon infection (McCormick, 2003). The effectiveness of Onyx-015 comes from the fact that it selectively replicates in cells where p53 is mutated or inactivated, such as tumor cells, while being unable to inhibit p53 and avoid degradation in normal cells (McCormick, 2003). This allows for specific targeting of tumor cells while avoiding unwanted p53 activation in normal cells. A phase II clinical trial evaluated the combined treatment of Onyx-015 with the traditional chemotherapeutic agents cisplatin and 5-fluorouracil. The responses, described as “substantial” by the research team, saw 0% tumor progression in patients
responding to the combined treatment, while the control group had a tumor progression rate of 100% after 6 months (Khuri et al., 2000). A separate phase II clinical trial evaluating the efficacy of Onyx-015 alone to treat head and neck squamous cell carcinoma showed tumor regression or stabilization in 55% of patients receiving the standard treatment of intratumoral injection for 5 consecutive days (Nemunaitis et al., 2001). This study also evaluated the administration of Onyx-015 in a fractionated manner (twice daily for two consecutive weeks) and saw a remarkable 72% tumor regression or stabilization, indicating that Onyx-015 infection is transient and its effects pronounced when given more frequently (Nemunaitis et al., 2001). Previous studies had shown that Onyx-015 induced apoptosis was highly selective to cancer cells (Nemunaitis et al., 2000), an observation seen in this trial. One possible shortcoming of Onyx-015 that has been observed is its apparent inability to induce tumor necrosis of cancer cells that possess wild-type p53, as seen in Table 4 below (Nemunaitis et al., 2000), slightly limiting its efficacy in treating tumors with inactivated p53.
Table 4. Evaluation of the efficacy of Onyx-015 in the treatment of head and neck squamous cell carcinoma. (Taken from Nemunaitis et al., 2000)

Response was assessed by radiologic scanning in 22 patients and by physical exam in 2 patients.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Total no.</th>
<th>CR</th>
<th>PR</th>
<th>NR</th>
<th>SD</th>
<th>PD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 gene sequence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>0.017</td>
</tr>
<tr>
<td>Wild type</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Not evaluable</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
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<tr>
<td>Neutralizing antibodies (baseline)</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>0.76</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Tumor diameter (centimeters)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 cm</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>0.34</td>
</tr>
<tr>
<td>&gt;2 cm</td>
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<td>1</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>CD4 cell count (cells/μl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
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<td>5</td>
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<td>1.00</td>
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<tr>
<td>≥500</td>
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<td>1</td>
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<td>2</td>
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</tr>
<tr>
<td>Not evaluable</td>
<td>3</td>
<td>0</td>
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<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Treatment regimen</td>
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<tr>
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<td>2</td>
<td>2</td>
<td>6</td>
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<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*CR, complete response; PR, partial response; NR, minor response; SD, stable disease; PD, progressive disease.

P based on comparison of tumors demonstrating antitumor activity (CR, PR, NR) versus no activity (SD, PD) for each evaluable subgroup (Fisher’s exact test).

At the same time, the effectiveness of Onyx-015 in combination with traditional chemotherapy is extremely promising for the field of oncolytic virotherapy.

Recent research has also illustrated that the effects of Onyx-015 are not solely due to its ability to selectively replicate in mutant p53 expressing cells but an overall stress response of the infected cell (Lane et al., 2013). Regardless, this approach holds great potential for cancer treatment.

The use of adenovirus-mediated p53 gene transfer has shown promising results for head and neck cancer. As in the treatment of other forms of cancer using this method, development is still progressing, with phase II and III clinical trials representing the furthest advancement. The use of oncolytic virotherapy with drugs such as Onyx-015 remains promising. H101, a virus similar to Onyx-
015, in combination with chemotherapy has been approved for treatment of certain cancers in China. This field continues to be developed (Lane et al., 2013).

III. Conclusion, future aims

The search for a cure for cancer remains a top priority in the medical community. The pervasiveness of this disease throughout society and its devastating consequences continue to plague families across the world. Unfortunately, as our understanding of the disease has progressed, it has become apparent that there is not likely a “one-size-fits-all” cure. The complex molecular genetics behind cancer illustrate the wide range of mutations and molecular abnormalities that can lead to tumorigenesis. In combination with environmental and predisposing factors, the potential causes of cancer seem limitless and likely to continue increasing in tandem with our knowledge of the disease. Traditional treatments have given many patients a fighting chance but have still come woefully short of stemming the tide of cancer mortalities occurring each day. Furthermore, the toxic effects of traditional treatments like chemotherapy and radiotherapy often hinder more than help an already weakened patient; simultaneously killing cancer and patient.

The field of gene therapy has made great strides over the course of the past decade. As our understanding of the field of genetics has increased, researchers have been able to develop treatments that target cancer more directly while avoiding the systemically toxic effects often seen with more
traditional treatments like chemotherapy and radiotherapy. This factor alone gives much promise to gene therapy. Gene therapy targeting p53 reactivation, specifically, allows for the potential treatment of half of all cancer cases seen, a vast swathe of the disease.

Of the current possibilities for p53 reactivation being researched, the option displaying the greatest potential is using adenoviruses as a vector for p53 introduction. While small molecule treatment options do show promise, their focus is too narrow. Nutlins and RITA, the two MDM2/MDMX inhibitors discussed, can be effective in wild-type 53 expressing where MDM2 overexpression is inhibiting the normal p53 protein, however they exert no therapeutic effects in cells expressing a mutant p53 variant, a very common mutation in cancer. PRIMA-1 and MIRA-1, on the other hand, reactivate mutant p53 but have no effect in cells expression wild-type p53 and high levels of MDM2/MDMX. Small molecules inhibiting proteasomal degradation of p53, like Nutlins and RITA, are unable to exert therapeutic effects in cells expressing a mutated variant of p53. In order to have the greatest possible impact on cancers involving p53 inactivation, all three types of small molecules would need to be researched and developed further, an already expensive process compounded by the need for each separate drug. The flexibility of gene therapy seems to be a much better option given its ability to target all forms of p53 inactivation with one type of treatment. Of the two primary options for transmission of the p53 gene, adenoviruses and retroviruses, the oncogenicity of retroviruses is too great a risk
factor, especially when compared to the safety and overall lack of toxic effects seen from adenoviral vectors.

With the use of adenovirus as the best course for p53 introduction established, results from clinical trials suggest that the strategy of using adenovirus-mediated p53 gene therapy in combination with either chemotherapy or radiotherapy is currently the best course of treatment for cancers showing p53 mutation or inactivation. In every study evaluated, the use of a combination treatment showed statistically greater efficacy, as measured by tumor regression or stabilization, than the traditional treatments alone. Furthermore, a crosscomparison of studies evaluating combination treatments with studies evaluating the efficacy of Ad-p53 alone showed the combination largely led to a greater percentage of positive responses to treatment, suggesting a synergistic effect between the gene therapy and traditional cytotoxic therapy. The combination of treatments also permits for the reduction of the amount of traditional chemotherapy or radiotherapy given, allowing physicians to tailor treatment protocols based on a patient’s current health and ability to withstand the potential side effects of cytotoxic treatment. These combination treatments must progress beyond the clinical trial phase and be approved for clinical use so they may begin to improve outcomes for those suffering from cancer, even as further refining of gene therapy continues.
The true future of cancer treatment lies in the development of recombinant adenoviruses carrying engineered p53. A slight shortcoming of normal Ad-p53 has been its decreased efficacy when treating tumor cells already expressing wild-type p53 but overexpressing MDM2/MDMX. Because adenovirus-mediated p53 is translated to high titer, the resulting concentration is able to escape complete MDM2/MDMX inhibition, but not without a dampening effect on its ability to induce tumor regression or stabilization. The engineered Ad-p53vp, however, escaped this inhibition and displayed an equivalent efficacy in treating both mutant p53 expressing and wild-type p53/overexpressed MDM2 expressing cancer cells. Ad-RLP23/p53 was also able to escape MDM2/MDMX inhibition by co-transducing a second gene, RLP23, along with the p53 gene that protected p53 and allowing it to exert its full anti-tumor effects. Both engineered products enabled adenovirus-mediated p53 to maximize its anti-tumor potential regardless of the cause of p53 inactivation. And oncolytic viruses like Onyx-015 represent another, more refined step towards selectively targeting and destroying cancer cells, taking advantage of molecular interactions in p53 regulation to allow for viral reproduction only in tumor cells, avoiding any negative side effects in normal, wild-type p53 expressing cells. Engineered viruses such as these represent the future of cancer treatment, but currently a great deal more research is necessary to continue refining these treatments and maximizing their ability to treat a wide range of cancers.
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Career Focus
- I am committed to my goal of going to medical school and becoming a practicing physician.

Education and Coursework
2011 University of California, Davis
Davis, CA 95616
Neurobiology, Physiology, and Behavior
Bachelor of Science

Experience
- Clinical
  - Pediatric Emergency Room Volunteer: Volunteered at the UC Davis Medical Center over the summer of 2010. Duties included cleaning gurneys, transporting samples to the laboratory, and interacting and entertaining patients.
  - Emergency Department Scribe: Transcription in a Sacramento, CA emergency department. I work closely with doctors, filling out all patient charts and helping keep track of patient care

- Research
  - Over three years of undergraduate research on the effects of hypoxia on hamster and rat hippocampi.
  - PUF Grant recipient for my original research hypothesis on the effects of calcium ion levels on hamster hippocampi.
  - UC Davis Undergraduate Research Conference presenter in 2011.

- Leadership
  - Head researcher on an original hypothesis. Duties included organizing the research schedule and setting the vision for the team.
  - President, Sigma Nu Fraternity (2010-2011): Duties included running all chapter meetings, setting the goals and vision of the fraternity, and acting as liaison between the fraternity and the school.
  - Risk Reduction Chair, Sigma Nu Fraternity (2009-2010): Duties included creating risk reduction plans and managing all public events.

Work Experience

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Emergency Medicine Scribe Systems
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Emergency Department Scribe

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Davis, CA
Building Manager

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Member Services Attendant