UTILIZATION OF EXTREME DRUG RESISTANCE TESTING IN MALIGNANT MELANOMA: NEW IS NOT ALWAYS BETTER

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This thesis represents my own work and contains no material which has been previously submitted for a degree or diploma at this University or any other institution, except where acknowledgement is made.

Signature ___________________________ Date ___________________________
To Alice and all my heroes

In appreciation to my parents, grandparents, siblings and nieces, my friends and to Joe

Gracious a mi amor verdad
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Abstract

This research considers the treatment of malignant melanoma. Data were collected from patient records for 78 individuals treated within the Yale Cancer Center Melanoma Unit. The patients were diagnosed with malignant melanoma prior to 1994 and progressed to stage III or IV disease before their deaths.

Due to the rapid progression of malignant melanoma, treatments are initiated at the time of diagnosis. Results of experimental Extreme Drug Resistance (EDR) tests subsequently become available. Physicians are warned the test results are not intended to guide therapy; however, assay directed therapies arguably result in better outcomes with other cancers. Thus, the question arises of whether the use of these tests might benefit patients in this context.

This study evaluates the treatment decisions made using a multi-disciplinary approach within the Yale Cancer Center Melanoma Unit regarding patients with malignant melanoma relative to information contained in EDR tests conducted by Oncotech Inc. Within this comparison, three specific outcomes consistent with hypotheses of the study were assessed: the utilization of test results, drug toxicity and cost effectiveness and survival.

Results were found to suggest that the initial treatment decisions of the Yale Cancer Center Melanoma Unit were in accord with tests results that were received henceforth for 74 of 78 patients. Two of those patients were in terminal stages of the disease thus treatments were unchanged; however two patients received a change in therapy.

It is suggested that physicians made use of the tests as they became available. However, only two patients with therapies altered by the test results were shown to face reduced costs, drug toxicity, or have the benefit of improved survival. From the patient data collected, four patients receiving drugs to which their tumors exhibited EDR were found to exhibit shorter survival times. Literature review studies conducted to evaluate physician treatment approach and patient preference rate favorably the consideration of quality of life issues. The principle finding of this observational study which focuses upon the development of the Yale Cancer Center Melanoma Unit, suggest that a multi-disciplinary approach to the treatment of malignant melanoma may offer quality of life benefits to the patient.
INTRODUCTION

Melanoma is the most common malignancy in humans (Gallagher, 2003). Melanoma is the leading cause of death from skin tumors worldwide with an annual increase in incidence over the past decade (Stahl et al., 2004). The incidence of malignant melanoma in the United States has doubled each decade for the past fifty years (Berger et al., 2004; Tarhini and Agarwala, 2005) and the number of people that have developed melanoma is increasing at a faster rate than any other form of cancer (Kuhn and Harke, 2002). In 2005, melanoma is estimated to affect 55,000 American lives. Of these, 7,700 are estimated to die from the disease (Tarhini and Agarwala, 2005). In the United States, the incidence of melanoma in women is increasing at a rate second only to lung cancer. Early stage melanoma is curable however advanced metastatic melanoma is almost uniformly fatal (Agarwala, 2003). Approximately 20% of people who develop the cutaneous form of melanoma will progress to malignant metastatic disease (Buzaid and Adkins, 2001). Patients with stage IV melanoma have an overall five year survival of less than 10% and a median survival of 8.5 months (Prignano et al., 2002).

Immunological approaches have yielded the only newly approved agents for malignant melanoma in 30 years which includes high-dose bolus Interluekin-2 with limited durable clinical response rates (Tarhini and Agarwala, 2005). To date, no standard adjuvant therapy has demonstrated increased overall survival (Ascierto et al., 2005). The agents clinically available for malignant melanoma are associated with high cost, drug toxicity and drug resistance. Despite a number of novel therapeutics undergoing active clinical investigation (Agarwal, 2003), malignant melanoma is at
present intractable. The single greatest deterrent to effective treatment is the drug
resistance which malignant melanoma exerts.

The research contained in this document focuses on the utilization of diagnostic
testing for drug resistance in malignant melanoma. The primary focus of the research is
the utilization of in vitro extreme drug resistance (EDR) micro-arrays by the physicians
who treat malignant melanoma. The micro-array tests are performed in clinical
laboratories to determine drug resistance on individual tumor tissue samples. In vitro
EDR testing is commercially available to physicians who treat malignancies to guide
treatment choices for the individual patient. Parameters including accuracy, feasibility
and effectiveness of the micro-array EDR testing method are the subject of the research.
While the research in the document is focused principally on utilization of the EDR in
vitro micro-array testing, it also contains research for in vitro testing of biomarkers in
malignant melanoma.

The research contained in the document was conducted at the Yale Melanoma
Cancer Center. The center is comprised of physicians who treat stage III and IV
malignant melanoma. The physicians in the center consider the utilization of in vitro
EDR testing in their treatment determination for malignant melanoma. The practice style
employed by the physicians encompasses a multi-disciplinary approach which is
inclusive to this research. The aim of the research was to evaluate the consideration of
the utilization of the in vitro EDR tests and the practice style employed by the physicians
at the Yale Cancer Center for a disease which demands a departure from conventional
medical practice.
The research deliberates on the decision making process of therapeutic choice for malignant melanoma and the challenges inherent to the variety of novel diagnostic tests available to augment this process. The research includes discussion of the relative predictive accuracy or value on in vitro micro-assays and biomarker testing for malignant melanoma. The document is foreshadowed by the overarching global problem of drug resistance. Disease processes and diagnostic tests that address issues of drug resistance are provided in the document to highlight the emerging global challenges facing clinicians who treat chronic illness and the aspects which parallel malignant melanoma.

The document discusses the problematic issues of medical surveillance in drug resistance research. A conventional problem with medical surveillance programs that apply diagnostic testing and biomarkers is determining the optimal frequency of such testing to minimize adverse health effects and cost (Judd et al., 2003). Considerable economic and health problems emanate from drug resistance as seen in bacterial infection, viral illness and in oncologic disease. Research to accurately quantify problems of drug resistance and proposals to evaluate practicable solutions are needed (Okeke, 2005). This research attempts to identify economic issues regarding in vitro testing and the difficulties and realities of clinician choice in the therapeutics of malignant melanoma. The research conducted as contained in this document suggests the need for further study to identify and quantify the diagnostic testing and therapeutic intervention for malignant melanoma.

Vaccines are being tested in patients with metastatic melanoma to determine their immune effects and to define their activity in combination with other immunotherapeutic agents. The two most widely investigated immunotherapy drugs for melanoma are
interferon-alpha (INF-alpha) and interleukin-2 (IL-2). Their therapeutic success in malignant melanoma is limited. Recombinant IL-2 demonstrates an overall response rate of 15-20% with resultant complete and durable remissions in only six per cent of patients (Agarwala, 2003). Additionally the economic burden and drug toxicity of recombinant IL-2 is excessive and is a concern shared by both the patient and treating physician (Sun and Schucter; 2001; Garle and Ergentler; 2004). This study will highlight physician choice in therapeutic treatment for malignant melanoma.

Melanoma cells exhibit a high level of intrinsic or acquired resistance to the cytotoxic agents often associated with the over-expression of drug transporters (Molinari et al., 2005). Malignant melanoma represents a very difficult challenge for the medical oncologist despite varied chemotherapeutic approaches. Treatment of melanoma in the stage of distant metastasis aims on palliation and achievement of durable tumor remission by prolongation of survival (Crosby et al., 2000; Garle and Ergentler, 2004).

If metastasis is confined to one organ system and is removable, surgery remains the treatment of choice (Garle and Ergentler, 2004). Unfortunately, a majority of post-surgical metastasis relapses and succumbs to distant disease (Spanknebel and Kaufmann, 2004). In limited metastasis, radiation therapy for bone and brain metastasis offers palliation (Garle and Ergentler, 2004). Whole-brain radiotherapy has had limited success to alleviate palliative symptoms (Eedy, 2003). According to the 3rd revision of the Guidelines for Melanoma (van Everdingen et al., 2005) treatment for metastatic melanoma should include inclusion into clinical trials.

Malignant melanoma is aggressive and refractory with a futile prognosis. In patients who progress to advanced melanoma, only low response rates (between 10-15%)
have been achieved by the single-agent cytostatics, leading to a mean five-year survival in less than five percent of patients (Buzaid and Atkins, 2001; Garle and Ergentler, 2004) with durable remissions occurring in less than two percent of the patients (Buzaid and Atkins, 2001). More aggressive treatment regimens using multi-drug chemotherapy yielded response rates of up to 40%, but failed to show a significant benefit in overall survival compared to single agent therapy (Buzaid and Atkins, 2001; Garle and Ergentler, 2004). Monotherapy with dacarbazine, temozolomide, fotemustine or vindestine or its combination with Interferon-alpha are the current preferred agents; however all have failed to prolong survival (Garbe and Eigentler, 2004). This document further discusses issues of drug treatment in advanced melanoma.

The untold velocity which malignant melanoma progresses in the human body undermines clinical attempts in chemotherapeutic intervention. Given the rapid transit of the tumor's activity, timing and accuracy of treatment are ideal but generally unrealistic parameters in treatment consideration. The empiric approach to chemotherapy has limitations. The malignancy of melanoma is felonious and its tenancy is proven by its extreme drug resistance. Individually the tumor burden is so high as to resist drug effectiveness. It is very difficult to determine on case-by-case basis which drug or drug agents are effective. It is difficult to determine drug effectiveness when factoring in confounding variables of co-morbidity, drug toxicity and rapid progression from diagnosis to death. In effect the human body (in vivo) of a patient with advanced melanoma becomes a less than ideal medium from which to observe drug behavior (sensitivity and resistance) and drug success.
A minimum of two months of in vivo chemotherapeutic administration is needed in each individual patient to determine drug response and high tumor resistance to cytotoxic agents for malignant melanoma and most other malignancies (Fruehauf and Bosanquet, 1993). In malignant melanoma the interval between diagnosis and death does not lend itself to accurate or feasible in vivo testing for individual tumor drug resistance. Several pernicious scenarios can arise alone or in concert during this interval. During this interval many issues are salient in the treatment determination.

Quality of life is one primary concern to the treating physician and to the patient with malignant melanoma. Quality of life encompasses many facets and components and is a consideration that is difficult to measure clinically as it varies considerably from individual to individual yet its importance is pervasive in devising a treatment plan. Quality of life issues constitute personal issues ranging from economic burden, family, employment, religion, and coping skills. The prognosis of the disease and the side effect profile of the treatment drugs confer psychological challenge, morbidity, and often worsening of co-morbidity. Their effects can be toxic and without benefit.

The range of potential side effects from cytotoxic agents and particularly immunotherapy include blood dyscrasias which may lead to mortality. It may become difficult to decipher clinically whether systemic affects are due drug toxicity or disease progression. Disease progression may occur during the evaluation period to first-line therapy. It may be the case that ineffective therapy may induce cross-resistance to subsequent agents that initially may have been effective, thus decreasing the probability of a clinical response. These results can be reversible or non-reversible stemming solely drug administration. The clinical course of illness in the progression of malignant
melanoma induces challenge in clinical treatment determination. This challenge is enhanced by the economic burden which accompanies the consideration of drug choice. The therapeutic choices made by the treating physician are not made in exclusion to the cost to the patient and should not be made in exclusion to the cost of experimental trial to the society.

The economic burden for treatment of malignant melanoma for the patient and society is high. The cost for an individual patient may exceed financial insurance coverage allotment and individual finances may be absent from time of diagnosis. The cost includes hospitalization necessary to administer some types of therapy, the cost of the drugs itself, the cost of toxic effects and cost to quality of life.

Cost as a variable is inclusive to scientific drug discovery with many permutations. It is not a novel concept that the treatment of cancer can be costly for the individual and for society. Yet as the arena of healthcare continues to evolve as a prime concern on global scale, the ante is raised as is people’s awareness of limited financial resources. Insurance coverage is not consistent as people would like especially when confronted with a devastating illness such as malignant melanoma. As such, the cost of cancer treatment and the accompanying insurance coverage renders itself less bedfellows. The drug resistance seen in advanced melanoma and the actual cost to attempt to treat it are in direct inverse proportion to the insurance coverage realized for its course.

Precision and economics of drug testing become very important tools as the climate of healthcare continues and as people continue to live longer and demand healthier lifestyles. There is limited research on malignant melanoma and cost of treatment. Laha and Grob (2003) conducted a literature review of clinical trials and
economic studies published on the use of IFN-α as an adjuvant therapy in stage II-III (AJCC 1992) malignant melanoma. The authors selected large clinical trials with sufficient follow-up to assess efficacy of trial organization. Medico-economic studies, based on the results of several of these trials, were analyzed to estimate the cost-effectiveness ratios of IFN in this disease. Interferon-α as adjuvant therapy used to treat malignant melanoma, demonstrated efficacy with high-dose regimens in patients with overt regional nodal disease (high-risk) and with low-dose regimens in stage IIA and–B patients without clinically detectable nodes (intermediate-risk). Studies such as these can offer economic analysis performed in varied settings and using several methods to extrapolate clinical results which can lend data and in this case are producing similar results of the extra costs for IFN-associated treatment. The incremental cost-effectiveness ratios provided in the study were U.S. $50,000/per life/per year gained in widely used medical strategies for different disease settings. This study suggests the recommendation of IFN-α therapy in malignant melanoma, specifically high doses in high-risk patients and low doses in intermediate-risk patients. In order that the treatment decision in whether or not to treat is made, however, the patient will need to be informed that IFN (and IL-2) may only delay progression of disease with the possibility of any curative effect being uncertain. This limited effect must be balanced with the potential impact on quality of life from the high side-effect profile of IFN and especially IL-2 and with the findings by Lafma and Grob that many patients in whom low doses are indicated would not recur in the absence of treatment.

The cost of drug resistance can be reduced by effective diagnostic tools that improve drug treatment success. Metastatic melanoma exhibits a highly metastatic
character and resistance to radio and chemotherapy (Dai et al., 2004). Diagnostic tools that improve drug response and address drug resistance are needed to augment the diagnosis and treatment of malignant melanoma. The physicians who treat advanced melanoma are charged with the responsibility to consider diagnostic tools that are economic, feasible, assessable, accurate and effective. The treating physician must choose wisely from diagnostic tools that address a time-sensitive treatment plan yet one that focuses on the individual patient. Diagnostic tools that satisfy these variables for clinical utilization in malignant melanoma are limited or nearly non-existent.

This document and the research contained therein examine the use of a diagnostic tool under consideration and availability to the physicians to enhance empiric treatment of the disease. The remainder of the document will describe in vitro EDR testing in malignant melanoma. In short, the assays are used to identify the most effective treatment for an individual when many options exist, much like the use of estrogen-receptor expression and tamoxifen (Wieland, 2005). Trial designs more typical of diagnostic assays can provide important clinical information regarding their predictive accuracy. Patients and treating oncologists can benefit from chemotherapy sensitivity and resistance assay results obtained from non-interventional studies even with limited findings as supplements to other clinical data when deciding on a treatment.

The poor drug response and survival outcomes for malignant melanoma are very likely related to the heterogeneity of chemosensitivity as well as frequent constitutive resistance to individual cytotoxic drugs. Several mechanisms may account for the phenomenon of resistance that includes failure of the drug to reach and/or affect its intracellular target (Dhar et al., 2003; Dunn et al., 2004; Tas et al., 2005). Tumor cells
display a variety of mechanisms by which they evade immune detection and destruction and render the immune response ineffective.

The development of chemoresistance is a persistent problem during the treatment of local and disseminated disease. Therapeutic agents selectively, but not exclusively, target actively proliferating cells and include at present, DNA alkylating agents, antimetabolites, intercalating agents and cytokines. Resistance constitutes a lack of response to drug-induced tumor growth inhibition; it may be inherent in a subpopulation of heterogeneous cancer cells or be acquired as a cellular response to drug exposure. Although regulatory approval may require efficacy in as few as 20% of the trial cohorts, a drug may subsequently be used in unselected patients displaying resistance to the treatment (Luqmai, 2005). Principle mechanism may include altered membrane transport via the P-glycoprotein product of the multi-drug resistance (MDR) gene as well as other associated proteins, altered target enzyme (i.e. muted topoisomerase II), decreased drug activation, increased drug degradation due to altered expression of drug-metabolizing enzymes, drug inactivation due to conjugation with increased glutathione, sub cellular redistribution, drug interaction, enhanced DNA repair or failure to apoptosis as a result of muted cell cycle proteins such as p53 (Senchenkov, Litvak and Cabot, 2001; Hussein et al., 2003; Tas et al., 2005). Attempts to overcome resistance mainly involve the use of combination drug therapy using different classes of drugs with minimally overlapping toxicities to allow maximal dosages and with narrow cycle intervals necessary for bone marrow recovery.

The combination of genetic instability together with molecular heterogeneity displayed by malignant cells render the construction of effective treatment programs
difficult. A non-trivial problem is the drug development system itself which for reasons of scientific necessity does not assume answers in the short time frame seen in the course of metastatic melanoma. Unraveling the complexities of cellular behavior to intercede in tumor cell proliferation and yield effect tumor response to cytotoxic agents is a therapeutic goal. The complexities of cellular behavior and drug resistance include apoptosis.

The ability to escape suicide (apoptosis) is a hallmark of most cancer cells and often correlates with tumor aggressiveness and resistance to traditional anticancer drug treatments. According to Catherine Denicourt (Science 2004) (Catherine Denicourt of Howard Hughes Medical Institute and Steven F. Dowdy of the Department of Cellular and Molecular Medicine, University of California San Diego School of Medicine, “academic and industrial laboratories are engaged in a Herculean effort to develop new molecules that reactivate the apoptotic program in tumor cells by specifically targeting protein-protein interactions. Modulating or mimicking protein-protein interactions with biologically active peptides or chemical compounds offers an attractive strategy for therapeutic intervention in specific disease pathways.” Targeting growth factor receptors within the endothelial cells, for example, with anti-angiogenesis therapies have been shown to affect the apoptotic pathway response. (Dauffenbach L, Torres C, and Fruehauf J American Association for Cancer Research (AACR), 2003 Endothelial Cells Co-Cultured with Breast or Ovarian Cancer Demonstrated Differential Gene Expression and Apoptotic Responses, Abstract #3325). Many types of cancer take advantage of immune modulating activities of cytokines because of their capacity to act on gene expression and
to down-regulate certain immune responses that might destroy cancer cells (Roth, et al., 1983; Ouaissi and Ouaissi, 2005).

A likely reason for ineffective therapy for metastatic melanoma is the lack of specificity for melanoma cells. Biomarker expression as determined by immunohistochemistry is an efficient and effective tool for screening and discovering new targets in melanoma based on outcomes (Fruehauf, Fruehauf J 2004; Krishnansu S, et al, 2005). An example of this are C-Kit expression markers in solid tumors of differing histologies, including carcinomas of the breast, colon, lung (small cell), endometrium, ovary, prostate, and melanoma may be linked to drug resistance and other prognostic factors. In one study (Fruehauf et al., Fruehauf J 2004) DNA alkylators used to treat solid tumors were found to be significantly less active against tumors that expressed CD117. The investigators also found that increased C-Kit expression was significantly associated with increased MDR-1, HER2 and mutant p53 suggesting that C-Kit expression may be linked to drug resistance and other adverse prognostic factors.

A likely reason for ineffective therapy for metastatic melanoma is the lack of specificity for melanoma cells. The utilization of drugs exploiting targets preferentially expressed in melanoma may be found to be beneficial. Kluger et al. (2004) studied a large cohort tissue micro-array and found that drugs that specifically target HER-2/neu were not likely to be useful for the treatment of metastatic melanoma or as adjuvant therapy for melanoma patients at high risk for recurrence. However, Chung et al. (2004) found evidence to support a potential synergistic effect of abnormal HER-2/neu and EGFR and p53 status in the pathogenesis and natural history of lymph node-negative breast carcinoma. Furthermore, these authors found that a combined analysis of multiple
markers may enhance the prognostic capabilities compared with individual markers. This finding has implications for melanoma as its potential targets through combined analysis of multiple markers in malignant melanoma.

Despite the aggressive nature of advanced melanoma there are no standard biological assays in clinical usage that can predict metastasis and few molecular biomarkers have yet to achieve acceptance in the clinical setting. Tissue-based markers evaluated by immunohistochemistry suffer from a high degree of inter- and intra-observer variability due, in part perhaps, to the inadequacy of reproducible assessments of protein expression using traditional immunohistochemistry (Rubin et al., 2004). One recent advance in this field that promises to automate this process is the development of AQUA, a molecular-based method of quantitative assessment of protein expression. This system integrates a set of algorithms that allows for the rapid, automated, continuous, and quantitative analysis of tissue samples, including the separation of tumor from stromal elements and the sub-cellular localization of signals. The methodology and development of AQUA will be discussed in further detail later in this document.

Rimm and colleagues (Hoek et al., 2004) used AQUA to assess global differential gene expression comparing normal human melanocytes with six independent melanoma cell strains from advanced lesions. The data, validated at the protein level for selected genes, confirmed the over-expression in normal cells relative to normal melanocytes of several genes in the growth factor/receptor family that confer growth advantage and metastasis. Some differentially expressed genes reside on chromosomal regions displaying common loss or gain in melanoma or are known to be regulated by CpG promoter methylation. These results provide a comprehensive view of changes in
advanced melanoma relative to normal melanocytes and reveal new targets that can be
used in assessing prognosis, staging and therapy of melanoma patients. Berger,
Harigopal, Martens, et al. (2003 Appendix A) at Yale Medical School sought to
determine if Ki-67 expression correlated with extreme drug resistance in a cohort of 100
melanoma specimens, all tested with the EDR assay at Oncotech Laboratory.

Immunohistochemistry was performed on the tissue micro-array slide with a
monoclonal antibody to Ki-67. While there was no relationship between either Ki-67 or
EDR and the time to first recurrence, either test may be valuable in terms of predicting
response to therapy. Future studies will include this analysis upon collection of treatment
and outcome data. Novel targets in melanoma will be discussed in the paper.

The focus of this paper discusses the method of in vitro micro array EDR testing
conducted by Oncotech Laboratory Incorporated. This laboratory is located in Orange
County California and offers a full line of laboratory tests designed to provide
information to oncologists that can be used to tailor treatment to individual patient’s
cancer, based on the unique biological and genetic properties of the malignant cells
obtained from the patient. The tissue arrays performed at this research lab yield results
that enable the oncologists and surgeons ordering the tests to stratify their individual
patients into good or poor prognostic groups and thus avoid use of specific chemotherapy
agents to which the patient’s tumor is resistant. The many tests offered at Oncotech will
be discussed with emphases given to the Extreme Drug Resistance (EDR) Assay and
immunohistochemistry prognostic and predictive marker testing and its relevance to
malignant melanoma.
Predictive chemosensitive assays that individualize chemotherapy by drug sensitivity and resistance testing were discovered over two decades ago when a group of scientists raised the possibility that tumor tissue could be cultured with in the laboratory (in vitro) setting in order to yield data about drug response the results of which could be transferable the individual patient (in vivo). Such chemosensitivity assays were challenged and impeded by technical difficulties over the next 25 years. As a result, the in vitro assays maintained attention with limited success in the initial years, lost hold in the mid-1980’s, but gained a resurgence of attention and success in the last 15 years. Efficacy and turn-around time for in vitro testing prompted this success.

Presently there is a variety of drug sensitivity and resistance methods and laboratories that conduct them through which surgeons and oncologist can from provide tissue samples for testing. Results from in vitro testing can be provided to the oncologist for utilization in the determination of therapeutic intervention for the individual patient with malignant melanoma. The benefit in asserting drug response to individual tumor in a laboratory setting prior to administration of chemotherapy for a patient potentially can forestall negative consequences associated with drug administration. Side effects can be deleterious or fatal. The contribution to cost savings can be readily argued. At present use and overuse of medication is of realistic concern as exemplified when considering the state of our society in relation to antibiotic use. A profound example of the implications and conservation of diagnostic testing and use of therapeutic agents is found with antimicrobial prescription determination. The effect of the treatment decision is in large part made for the individual patient, yet the overall effect on the population should not be excluded. The treating clinician is charged with keeping abreast the economic concerns
and consequences of her or his therapeutic decisions. It is for this reason that the treating clinician needs to maintain the ability to decipher the benefits and disadvantages of diagnostic tools that constantly enter into therapeutic choice. The astute clinician continues to utilize accurate tools based on critical evaluation of such tools and the moral obligation to use them when indicated. The same is true when prescribing medication. Practicing medicine does not occur in a vacuum. Moral obligation dictates consideration of therapeutic choice as it affects the individuals within the community of practice as well as the greater community on global scale. Therapeutic choice must balance the individual needs of the patient within the context of the population being treated. There is a compelling need to standardize testing procedures and establish conditions for laboratory detection and accurate identification of disease processes.
Antimicrobial Use on Global Scale and In Vitro Diagnostic Testing Utilization

Efforts that monitor over-treatment with antibiotics are an imperative that face the medical community at large. Effective and accurate in vitro diagnostic testing methods found to identify bacterial organisms are proving advantageous on global scale in these efforts. The discovery and identification of in vitro diagnostic tools which may augment treatment decisions for difficult diseases or diseases with high incidence offer tremendous contribution to the overarching goal of disease management. Evaluations of in vitro diagnostic tool utilization by clinicians which prove accurate diagnostic and rational use of effective therapeutic approaches for disease processes with high rates of morbidity and mortality and cost containment for the populations under treatment will contribute to this goal. Such evaluations are made on a scientific and clinical continuum in order to best identify and clarify their advantage and disadvantage.

The clinical utilization of rapid diagnostic tests for the detection of specific bacterial antigens in serum to supplement therapeutic decision making for acute respiratory infectious (ARI) is proving useful in some developing countries. ARIs are one of the most important causes of morbidity and mortality in children throughout most of the world (MartinsTeixeira, 1999). More than four million children under five years of age are estimated to die from ARI every year. This represents about 30% of the 14.25 million deaths of children under five years of age that occur in the developing world each year (Gwatkin, 1980; World Health Organization, 1983; MartinsTeixeira, 1999). Most of the rapid diagnostic tests have been based on particle agglutination or enzyme-linked immunosorbent assay (ELISA) tests and the utilization of these in vitro diagnostic testing method have been well documented for example in the treatment of acute respiratory
infections (ARI). The success of the ELISA serological tests is limited as they present certain technical difficulties. According to Lu et al. (2005) protein micro array assays present a higher positive rate and sensitivity (86.1% and 1: 2000) compared with traditional ELISA screening methods for SARS and could provide a rapid, parallel and high-throughput antigen screening platform. The discovery of novel diagnostic testing methods and the enhancement of existing methods provide ongoing evidence for disease management. Studies which evaluate test utility identify strengths and weaknesses of the effectiveness, accuracy and economic benefit in the overall improvement of therapeutic intervention. This information can offer direct benefit to the clinicians who treat the diseases and the patients and the population for whom such utilization may benefit.

Choices made about diagnostic test utilization depend on the prevalence of disease and the value placed on increased diagnostic accuracy and efficacy. Risk adjustment assessments which evaluate test utilization consist of a series of techniques that account for the health status of patients when predicting or explaining costs of health care and effectiveness of treatment for defined populations or for evaluating retrospectively the performance of providers who care for them. Inclusion of assessments incorporates patient preference, their perception of treatment and quality of life needs. The clinician treating disease is charged with consideration of the effects of treatment decision on an individual basis as well as its effect on the population. This balance is maintained and enhanced by renewed consideration of diagnostic techniques that improve upon the collective goal to contain disease on global scale and to make it affordable and accessible where possible.
According to a paper published by Bryce et al. (2003), "In 42 countries with 90% of child deaths worldwide in 2000, 63% if these deaths could have been prevented through full implementation of a few known and effective interventions." Health systems are constantly inequitable, providing more and higher quality services to the well-off, which need them less, than to the poor, who are unable to attain them (Gwatkin et al, 2004). According to Gwatkin (2004) the inequities are likely to continue in the absence of concerted efforts to ensure that health systems reach disadvantaged groups more effectively. Gwatkin contends that this situation need not be accepted as inevitable, for there are many promising measures that might be pursued, one of which is use of one or more of the several techniques that seem to be effective in at least some of the settings where they have been tried. Gwatkin adds that the empowerment of people to have a more central role in health system design will also improve the health care system.

There are examples in the literature, as cited later in this document, to indicate that in general patients do prefer to be included in therapeutic decision making. One paper by Tang et al. (2005) examining patient satisfaction with "information exchange" domain from their doctors during the SARS outbreak in Taiwan in 2003, showed patients were most satisfied with understanding their treatment plan about their illness (100%) and doctor being honest about their illness (97%) and being understood regarding their illness (96%). Knowledge is power. The more accurate clinical data available to the treating clinicians the more confidence existing with which the clinician can inform their patient.
Diagnostic Testing and Treatment of *Helicobacter pylori*

This document includes discussion of the value of diagnostic testing methods in the detection of gastric cancer (Sierra et al., 2003). The discussion includes citation from current research as it pertains to therapeutic choice in the setting of limited resources and with regard to economic burden in the treatment of *Helicobacter pylori* (*H. pylori*) and its association with gastric cancer. Studies are cited in this document demonstrating evidence that genetic mutation may prove etiologic in the transformation of cells found in *H. pylori* bacterial disease which result in gastric malignancy. Diagnostic testing to identify the presence of *H. pylori* in humans to augment therapeutic decision attempts to uncover effective methods which can demonstrate accuracy and economic benefit for the patients being treated. Scrutiny as to the utilization of diagnostic testing methods involves modification of treatment regimen based on the population being treated and the needs of the individuals within that population. *H. pylori* and malignant melanoma are no doubt two very different disease processes yet a percentage of the selected challenges inherent to *H. pylori* regarding drug resistance and therefore treatment, may offer insight when discussing the spectrum of therapeutic challenge for malignant melanoma. Lessons learned in view of the challenges in one disease that may be of value in another are discussed. An example of the discussions contained within the document comparing the two diseases and the utilization of diagnostic testing to augment treatment determination follows.

The colonization of the human gastric mucosa with *H. pylori* bacterium invariably results in the development of chronic gastritis and subsets of patients have a progression of the chronic gastritis to either ulcer or cancer (Malferthere et al., 2005). Epidemiological
evidence indicates that the proportion of all gastric cancers attributable to \textit{H. pylori} infection, and hence potentially preventable upon elimination of this risk factor, is somewhere in the range of 60-90\% (Malfertheir et al., 2005). This portends significant benefit in terms of morbidity and mortality, not least in populations with high prevalence of \textit{H. pylori} infection coupled with high incidence of gastric cancer. The utilization of the C-urea breath test after unsuccessful treatment of \textit{H. pylori} is increasing in clinical practice (Gisbert, 2005). The observation of a pattern of histological (active) gastritis without the concomitant finding of \textit{H. pylori} raises the suspicion of diagnostic error (Gisbert, 2005). Antimicrobial resistance incompletely explains eradication failure in treatment of \textit{H. pylori} (Borody et al., 2002) rather an impaired immune response may contribute to failed eradication after standard therapy. \textit{H. pylori} can induce apoptosis of gastric cancer cells (Chen et al., 2005). The mechanism of process still needs further elucidating but its similarities to the challenges inherent to the behavior of malignant melanoma will be brought forth in this document. The burgeoning problem of drug resistance is salient and is translated to drug choice and treatment determination. These decisions may be made less problematic with the utilization of diagnostic tests if found to be accurate, effective and economic. A discussion of these variables and consideration of the advantages and disadvantages of available diagnostic tests is the specific aim of the research contained within this document as may be beneficial in clinical practice.

In vitro testing may demonstrate economic incentive to medical insurers. If it can be shown that in vitro testing of tumor drug response in the lab yields effective and accurate advantage, then its utilization may in fact translate from the US outward globally. In this way, discovering a test that is may demonstrate economic benefit is a
huge success in the fight against the devastation of a disease like malignant melanoma. Economic concerns are a reality in the U.S. and especially abroad where resources are limited. There are still challenges involved with in vitro testing however its acceptance is increasing. As of September 2000, Medicare began to cover certain of the in vitro testing of tumor tissue in the U.S. (Medicare Newsletter, 2000). Until this coverage, the cost of the testing was absorbed either by the patient or the surgeon and/or oncologist directing therapy. Coverage for single agent testing was approved nationally if that testing occurred in the state of California. Medicare reimbursement for multi-agent testing is under consideration and likely to be approved at this writing. Insurance reimbursement is a factor where economic benefit may be realized as the widespread utilization of in vitro testing assays in the U.S slowly gains momentum.
Evaluation Cohort Study of Drug Resistance in Malignant Melanoma Using EDR In Vitro Testing

The evaluative research for which this document is written was conducted to learn further about utilization of in vitro testing for malignant melanoma and establish cause for ongoing consideration of its utilization in clinical practice for those who treat melanoma. The research examined clinical decision making and in vitro drug testing utilization within a medical community who comprise the Yale Cancer Center Melanoma Unit. The research focused on therapeutic choices and decision making processes made in concert with a multidisciplinary approach of surgical and medical specialists. The research is retrospective and is evaluative in method and involves a small cohort of 78 patients. All of the patients in the study were diagnosed or progressed to stage III or IV melanoma during the years from 1994 through 2000. The patients primarily received medical treatment through Yale University Medical School and Yale New Haven Hospital. Patient histories, progression of disease, physician treatment, tumor tissue drug resistance testing results, and patient decedent dates were collected via medical clinic and hospital medical records, serology and pathology results, physician interview and participation in Yale Cancer Center Melanoma Weekly Conference. The data collection effort provided medical data prior to surgical evaluation and including course of disease through death. By the time of the data collection effort, all of the patients in the study were deceased.

Each patient in the cohort had tumor tissue surgically removed by a surgeon, Dr. Stephan Ariyan. The tumor tissue from each patient was sent by overnight express to Oncotech Laboratory Incorporated for EDR in vitro assay testing. Results from
Oncotech Inc. EDR testing were provided to Dr. Ariyan and to Yale oncologist Dr. Leonard Farber. Dr. Farber was the primary oncologist who oversaw treatment for each patient in the study through to their demise. The specific aim of the research was to obtain understanding and data regarding utilization of the EDR test results provided by Oncotech Lab for the surgeons and oncologists who are members of the Yale Cancer Center Melanoma Unit for their patients who received treatment who diagnosed with stage III and IV malignant melanoma.

The surgeon and oncologists who treated each patient in the study comprise the multi-specialty team at the Yale Melanoma Unit at the Yale Cancer Center. The director of the Yale Cancer Center Melanoma Unit is Dr. Stephan Ariyan who established the Unit in 1976 and continues to hold the same title. Dr. Ariyan is surgeon specializing in the treatment of head and neck cancer, breast cancer, and malignant melanoma. The Yale Cancer Center Melanoma Group meets weekly for clinical conferencing for the purpose of presenting individual patient cases of advanced stage III and IV melanoma, in particular for those patients in whom treatment is difficult or rendering limited progress.

Inclusive to the research presented in this study are observations made during two years of observation of the weekly conference and interviews conducted with its members. Collectively this group of medical experts participates in an exchange of medical expertise with the goal to improve patient outcome through a multi-disciplinary approach. In addition to the presentation of individual patient cases and resultant treatment plans, the members discussed the utilization of the in vitro Oncotech EDR results inclusive to treatment determination. Each member participates in clinical trial to varying degree and discussion of such participation contributes to the conference format.
Each member is involved in a clinical research in addition to the treatment of melanoma. The observations and interviews were conducted to obtain information about the multidisciplinary approach employed for those patients in the practice with stage III and IV melanoma. The observations and interviews also sought to ascertain data about the utilization of in vitro EDR testing.

The research in this document sought to determine whether the in vitro testing was considered and/or utilized by the surgeons and oncologists in the group and how variables such as drug toxicity, quality of life issues and economic burden of treatment affect patient preference. It sought to determine methodology as to how these variables are executed in decision making for the members of the Yale Cancer Center Melanoma Unit within the context of a multi-disciplinary approach for patients with advanced melanoma who may not respond to conventional therapies. The multi-disciplinary approach can serve as a model which proves very effective for individualized treatment of advancing melanoma where drug resistance is extreme and quality of life issues and economics of treatment are of significant relevance.
Global Practice Measures in Drug Treatment

The research presented in this document contains research conducted in Costa Rica by Drs. Rafaela Sierra on *H. pylori* and Rodrigo Zeledón on Chagas Disease infections, respectively. These diseases are presented in this document as examples of the interface between immunological function and carcinogenesis when faced with drug resistance and clinical treatment determination. A portion of the discussion in this document focuses on the potential for the treatment of these diseases in relation to drug resistance and global clinical practice measures. Treatment approaches to these diseases confers immunologic challenge that can be seen in both diseases in consideration of the utilization of diagnostic tools by treating clinicians. There are similarities in the current challenges that are comparable to that of malignant melanoma especially in regard to diagnostic tool consideration. However, it should be emphasized that the scope of these comparisons is restricted to clinical practice measures and disease management and not specific mechanisms of melanogenesis.

The data collected from Costa Rica, Kenya and Burma is incorporated into this document to discuss drug resistance and immune system evasion strategies in cancer disease and in consideration of containment-strategies in drug prescribing practice and drug testing development and utilization. This research in Kenya was conducted in the summer of 1987, in Burma in the summer of 1998 and in Costa Rica during the years of 2001 through 2004. This data collection offers enhancement to the discussion of the disease management challenges that confront clinical medicine on global scale.

The purpose of the research contained in this thesis is to demonstrate the value of and consideration for utilization of drug resistance testing in malignant melanoma.
The thesis hypotheses are as follows:

(1) Clinicians do not use the results of in vitro testing provided by research laboratories.

(2) If in vitro EDR results are used by the clinician, their use will reduce cost and toxicity to the patient.

(3) If in vitro tissue results are used by the clinician, the patient will benefit through increased progression to survival.

The data collected for this evaluative study which considers the utilization of EDR testing results by physicians in the Yale Cancer Center Melanoma Unit was used concurrently in a study which examined biomarker identification in metastatic melanoma conducted at Yale Medical School by David Rimm and colleagues (Berger, Harigopal and Martens et al., 2003, Appendix A, abstract). The tumor tissue samples surgically obtained from the cohort of patients for both studies was catalogued and preserved on slides at Yale Medical School in the pathology laboratory of Dr. Rimm.

Dr. David Rimm is a Yale Medical School pathologist who developed an automated method technique for analysis of tissue arrays called AQUA: Automated Quantitative Analysis (Dolled-Filhart, Rimm, 2002). The AQUA method allows for systematic pathologic identification and rapid assessment of tissue biopsies. The technique consists of a set of algorithms that provides a reproducible, automated, quantitative analysis of expression for a given biomarker from tissue samples. The AQUA method was used to produce tissue microarray cores using tumor tissue collected from the same patient cohort to assess nuclear antigen Ki-67 correlation with EDR (Berger et al 2003, abstract). Although Ki-67 and extreme drug resistance are both predictors of aggressive tumors, no correlation was found between Ki-67 expression and drug resistance in eight of nine drug assays tested (Berger, Harigopal and Martens et al.,
2003, Appendix A, abstract). The results of the study and relevance for EDR testing will be further discussed in this paper.

What follows is a brief description of the principal conclusions of the study and the remainder of its organization. The evidence from the study did not support the hypothesis that clinicians do not use the results of in vitro testing. In this small cohort sample, it was not proven that the surgeon and oncologist did not utilize the Oncotech Inc. testing results. Based on interviews with the treating surgeon and oncologist and through individual chart review, it was determined that the drug(s) chosen for treatment for the individual patient was shown to be resistant in the EDR assay panel testing results provided by Oncotech. This view would disprove the hypothesis indicating the conclusion that the clinicians did utilize the extreme drug resistance test results to augment treatment determination. Further evaluative studies may substantiate these findings.

In vitro drug resistant testing asserts to avoid direct costs of ineffective therapies and costs of managing treatment related morbidity. Based on the findings of the study, it is reasonable to surmise that cost savings and reduction in morbidity may be incurred through utilization of EDR in vitro testing; however the hypothesis that in vitro EDR results if used by the clinicians will reduce cost and toxicity to the patient was not definitively proven. The summarization that EDR test utilization may provide evidence for economic benefit as well as reduced toxicity to the patient may stimulate further study.

No standard adjuvant therapy has shown increased overall survival in malignant melanoma (Asceirto et al., 2005). The evaluative study conducted did not prove nor
disprove with certainty the hypothesis that utilization of in vitro EDR test results will increase survival for patients with advanced melanoma. However, the findings in the evaluative study does submit that the EDR test results in specific cases may be useful in physician decision making in the treatment of patients with stage III and IV melanoma. Further research is needed to ascertain and secure parameters of accuracy and feasibility of EDR testing for confident utilization in clinical practice for this malignancy.

Beyond this introduction, this paper will further be divided into five chapters. Chapter 1 provides a discussion of the diagnosis, treatment, and epidemiology of malignant melanoma. The chapter discusses drug resistance for malignant melanoma and the challenge in drug treatment determination for the treating clinician. Drug resistance, drug prescribing practices and social responsibility are discussed in the chapter to provoke questions as drug resistance soars at alarming rate on global scale. Chapter 2 delineates the benefits and limitations of extreme drug resistance testing assays. Chapter 3 depicts the research evaluation cohort design, data and results undertaken for the research contained in this thesis. Chapter 4 discusses the multi-disciplinary approach of a heuristic clinical medical model and eventiary medicine for the treatment of malignant melanoma. Observations collected from Yale Cancer Center Melanoma Unit and their relevance is developed herein. Chapter 5 includes a discussion of the current utilization of lymphodepletion and autologous T-cell transfer regimens in evaluating new therapies for malignant melanoma. Chapter 5 further discusses challenges in drug resistance and treatment on global scale. The chapter concludes with a discussion for a proposed model for a melanoma unit using the elements and data defined from both the cohort study and
the observations of the Yale Cancer Center Melanoma Unit as it may stimulate further and provocative research.

The topical elements discussed in this thesis include: drug resistance testing and screening for novel therapeutic agents; the identification of patterns of resistance for melanoma malignancy; patterns of cross-resistance and sensitivity in treatment naïve and relapsing tumors; identification of genomic and proteomic profiles associated with resistance; correlations of in vitro drug response; preclinical in vivo effect; and clinical outcome associated with a therapeutic tailoring of individual chemotherapy treatment regiments whose end-goal is reduced morbidity and increased survival in malignant melanoma.

Various assays are under scientific scrutiny for clinical utility and accuracy to achieve these endpoints, including several in vitro clonogenic and proliferation assays, cell metabolic activity assays, molecular assays that monitor expression of markers for responsiveness, in vivo tumor growth and survival assays in metastatic and orthotopic models, and in vivo imaging assays. Descriptors and the advantages and disadvantages of these assays will be included in this document which will commence following the discussion of melanoma and the drug resistance that beleaguer this malignancy.