MELANOMA THERAPEUTICS: EXAMPLES OF CANCER THERAPY

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White Paper No 94
May, 2013

We examine several of the current therapeutics for melanoma. Our objective is to demonstrate how they function, not just to focus on this single cancer. Melanoma has been for a long while a difficult cancer to deal with. Recent progress demonstrates significant success in managing it for some.

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1 INTRODUCTION

There is an explosion of new cancer therapeutics. About ten years ago we saw imatinib for CML and now we have quite a few for metastatic melanoma, once a terminal disease for certain. In the Melanoma case we see some 20% may survive for extended periods of time. However the average life extension may be only 6 months at a cost that may exceed $100K. In addition there may need be several of the specific therapeutics used at one time.

A former Administration Health Care adviser has written on this of late[1]:

Many cancer patients, after getting a diagnosis of a terrifying disease, pursue any potentially promising therapy, regardless of the price. But the main cost driver is the fee-for-service payment system. The more doctors do for patients, the more reimbursement they receive. Surgeons earn more for every procedure. Oncologists typically make more money if they use newly approved drugs and the latest radiation treatments than if they use cheaper, older alternatives that work just as well. (This is because they get paid back the cost of the drug, in addition to an extra 6 percent of that cost — the more expensive the drug, the higher the compensation.)

His point is the 6% on the $100K charge. That is $6K per patient per six months. Take melanoma. The incidence is about 75K per year. Of that some 12K to 20K it the drug profile. At say $100K per person and assuming all persons, 20K, we would have in any one year $2B in costs and $120M paid to Oncologists. Is that too much? I guess it depends if you are in the 20% or the 80%.

He suggests changes:

First, over the next few years, the payment system needs to move away from fee-for-service toward a system of bundled payments, in which doctors are paid one fee for all the treatments involved in caring for a cancer patient.

This is a point well taken. But the problem is the way we compensate people based upon past assumptions.

Second, insurers have to give physicians information about where they are spending money.

I would suggest the patient also be informed. Patients all too often assume that the costs of the medication are low. They have no idea what the costs are. Moreover the basis for the costs should also be known. One must remember that the drug companies have gone through multi-Phase trials of hundreds of patients each at tens of thousands per patients just to the management of the Trial. Recall that the CROs, the Clinical Research Organizations, generate almost $30B in annual revenue just managing the Trials to comply with the FDA. That is not money in the pockets of the Pharmas.

[1] http://opinionator.blogs.nytimes.com/2013/03/23/a-plan-to-fix-cancer-care/

Third, any change in payment methods must be accompanied by rigorous quality monitoring to ensure that there is neither under- nor over-utilization of care.

Quality, now just what do we mean by that? This is what drove the character nuts in the Zen and the Art of Motorcycle Maintenance. Really, is it nothing more than what is in the eye of the beholder?

Fourth, we need more "high touch" oncology practices. In these practices, nurses manage common symptoms before they escalate to the point that they require visits to the emergency room...

Part of this is that the Oncologists are dealing with a mass amounts of new and different genetically targeted drugs which address pathways that they may have never been exposed to in Medical School. One melanoma drug leads to a new skin cancer, an unexpected effect.

Fifth, we need better incentives for research. Many expensive tests and treatments are introduced without evidence that they improve survival or reduce side effects, and with poor information about which patients should receive them.

Here I would disagree. The Trials are somewhat extensive but when you apply something used over 600 people to 20,000 you get a whole new set of issues. A drug may have to be withdrawn.

One key question is who should receive the new therapeutics? How do we manage them? Cancer is terrifying to the patient. But we now have an environment where people can find out about these new medications and demand them. Physicians are then pressed to use them, albeit with little significant survival benefit, on average. Yet that 20% who do survive contain valuable information for the next step.

Thus do we view use of the new therapeutics as the cost of continuing research or the cost of providing care?

1.1 WHAT IS CANCER

Let us begin by recalling the specific characteristics of what cancer is. As Hanahan and Weinberg state:

The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include

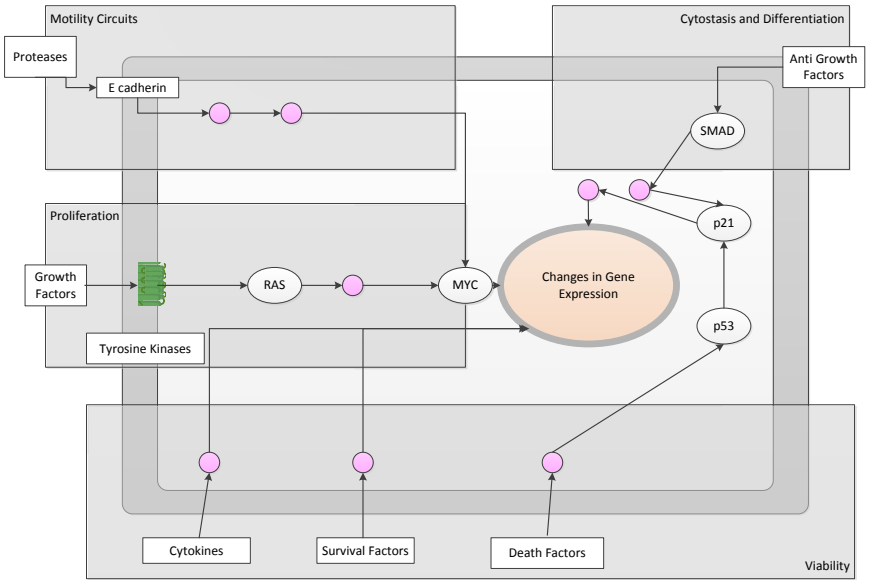
1. *sustaining proliferative signaling,*

2. *evading growth suppressors,*

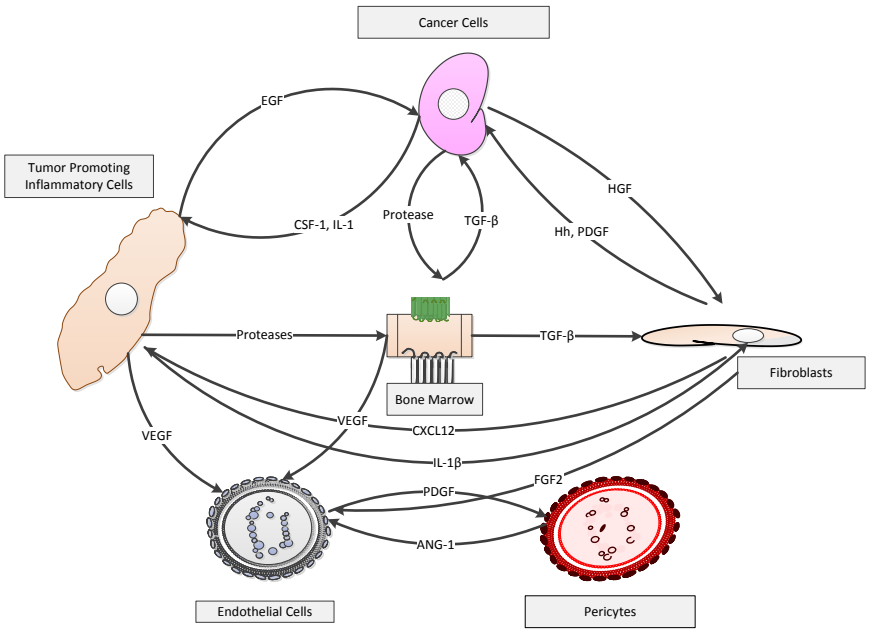
3. *resisting cell death,*
4. *enabling replicative immortality,*
5. *inducing angiogenesis, and*
6. *Activating invasion and metastasis.*

Underlying these hallmarks is genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions.

The Figure below is a depiction of these processes based upon the above mentioned paper. Note that this is a view of cancer from within the cell. The cell has the four characteristics shown: (i) motility, namely cancer cells move about such a melanocytes in melanoma in situ, where they move from the basal layer, (ii) proliferation, where they have activate mitotic behavior, as shown in the RAS-MEK pathway, (iii) differentiation, whereby the cell loses its functionality and becomes a nonfunctioning malignant cell, and (iv) loss of apoptosis, the cell essentially becomes immortal.



These characteristics map well onto the Hanahan-Weinberg terms. Cancer cells take on a life of their own. Therapeutics can then either attack them on the basis of the change in functionality or attack them outright. Classic chemotherapy used a meat cleaver approach, attacking any and all proliferating cells, including for example hair cells.



Thus the above depicts a somewhat global interrelationship between the cancer cell and the other cells within its environment. One of the key observations is that the cancer cells can also often take advantage of the surrounding cells and enlist them in the malignant cells own care and keep.

We are currently focusing on melanoma and from NCI we have the following estimated new cases and deaths from melanoma in the United States in 2013[2]:

- New cases: 76,690.
- Deaths: 9,480.

Furthermore the current therapeutics available, as described by NCI, are given as follows[3]:

Some melanomas that have spread to regional lymph nodes may be curable with wide local excision of the primary tumor and removal of the involved regional lymph nodes. A completed, multicenter, phase III randomized trial of patients with high-risk primary limb melanoma did not show a benefit from isolated limb perfusion with melphalan in regard to disease-free survival (DFS) or overall survival (OS) when compared to surgery alone.

Systemic treatment with high dose and pegylated interferon alpha-2b are approved for the adjuvant treatment of patients who have undergone a complete surgical resection but are considered to be at high risk for relapse. Prospective, randomized, controlled trials with both

[2] http://www.cancer.gov/cancertopics/pdq/treatment/melanoma/HealthProfessional

[3] http://www.cancer.gov/cancertopics/pdq/treatment/melanoma/HealthProfessional/page4

agents have shown an increase in relapse-free survival (RFS) but not OS when compared with observation.

Clinicians should be aware that high-dose and pegylated interferon regimens have substantial side effects, and patients should be monitored closely. Adjuvant therapy with lower doses of interferon have not been consistently shown to have an impact on either RFS or OS.

Although melanoma that has spread to distant sites is rarely curable, both ipilimumab and vemurafenib have demonstrated an improvement in progression-free survival (PFS) and OS in international, multicenter, randomized trials in patients with unresectable or advanced disease, resulting in U.S. Food and Drug Administration (FDA) approval in 2011.

Vemurafenib is a selective BRAF V600E kinase inhibitor, and its indication is limited to patients with a demonstrated BRAF V600E mutation by an FDA-approved test.

Interleukin-2 (IL-2) was approved by the FDA in 1998 on the basis of durable complete response (CR) rates in a minority of patients (0% –8%) with previously treated metastatic melanoma in eight phase I and II studies. No improvement in OS has been demonstrated in randomized trials.

Dacarbazine (DTIC) was approved in 1970 based on overall response rates. Phase III trials indicate an overall response rate of 10% to 20%, with rare CRs observed. An impact on OS has not been demonstrated in randomized trials.

Temozolomide, an oral alkylating agent, appeared to be similar to DTIC (intravenous administration) in a randomized phase III trial with a primary endpoint of OS; however, the trial was designed for superiority, and the sample size was inadequate to prove equivalency.

Thus there are now a significant number of options for treating melanoma, classic ones using alkylating agents and interferon or Interleukin-2, and more recent one based upon an understanding of pathways and the details of the immune system.

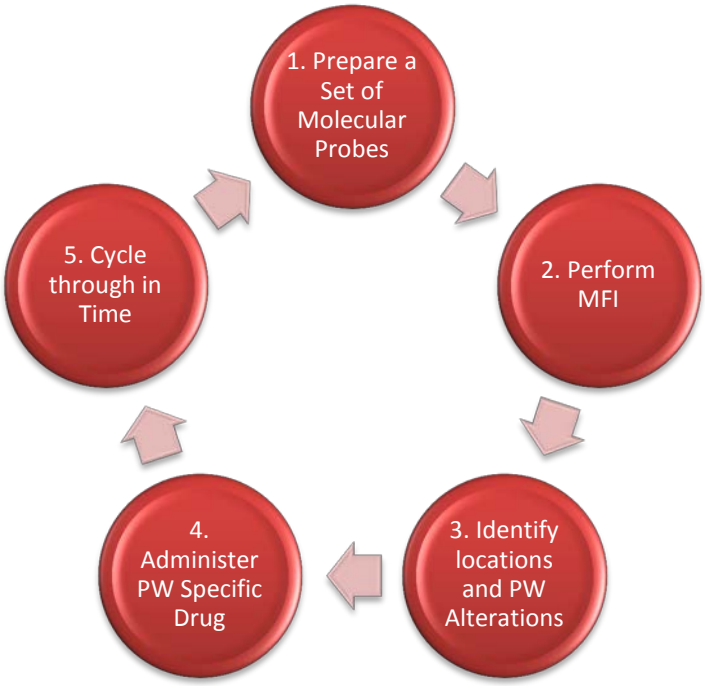
1.2 A NEW THERAPEUTIC PARADIGM

The classic therapeutic paradigm was to treat the disease systemically, namely suppress proliferation of cells, everywhere. Thus with something like methotrexate the cells stopped everywhere, including hair, and thus the patient went through an exhaustive and debilitating process. It also often times resulted in at best a suppression of the tumor for a short while and then a recurrence and death. With melanoma metastasis the process was often futile. Even with the early immune system approaches using interleukin and interferon, the side effects were present and the approach was for the most part systemic.

In the past decade with the understanding of pathways, in understanding the details of the immune system, and in being able to design targeted approaches to treatment we have now therapeutics that target the melanoma cells and not the entire system, almost.

The introduction of the use such as imatinib, a kinase inhibitor, for CML was a door opening step to dealing with cancers as genetically altered cells. Imatinib works to a degree, and when it fails another pathway element must be deployed.

We argue herein that there will be a new paradigm, which we depict below. It will be a paradigm based upon an understanding of cellular dynamics targeted at specific cells.



Namely:

1. Prepare a set of molecular probes which can tag the breakdown of specific pathway elements know to be specific to the metastasized cells. BRAF is but one example, and PTEN, cMyc, p53, are just a few others.

2. Perform a molecular functional imaging of the patient. This then allows for an identification of the location of the lesions, an assessment of the metabolic activity, and a clear indication of the gene expression aberrations in the tumor load.

3. Identify the specific localized PW aberrations and locations.

4. Prepare and administer therapeutics designed specifically to counter these aberrations.

5. Monitor patient and reiterate on periodic basis.

This is but one of the approaches, but it is an approach based on detailed understanding of the underlying malignancy at the gene level.

2 APPROACHES FOR THERAPEUTICS

The key question is; how does one develop a therapeutic for melanoma, as an example? The answer as with many such questions is; it all depends. There are several approaches as suggested by the Hanahan and Weinberg updates. Let us summarize a few:

1. Pathway Modulators: The assumption in this class of therapeutics is that we understand the cancer at a pathway level and that there is some specific point or collection of points in the pathways which are malfunctioning. We assume we can identify that malfunction and then we further assume we can develop a therapeutic to modify the malfunction to align with the proper homeostasis of the cell.

2. Immunological Control: This approach uses the immune system but does so with specific emphasis on the uniqueness of the tumor cells. If we can identify specific cell surface markers that more accurate targeting by T cells may be achieved.

3. Oncolytic Viruses: This is a novel approach that again uses knowledge of specific cell surface markers. One can engineer viruses that attach only to malignant cells and then enter, multiply, and destroy the cells.

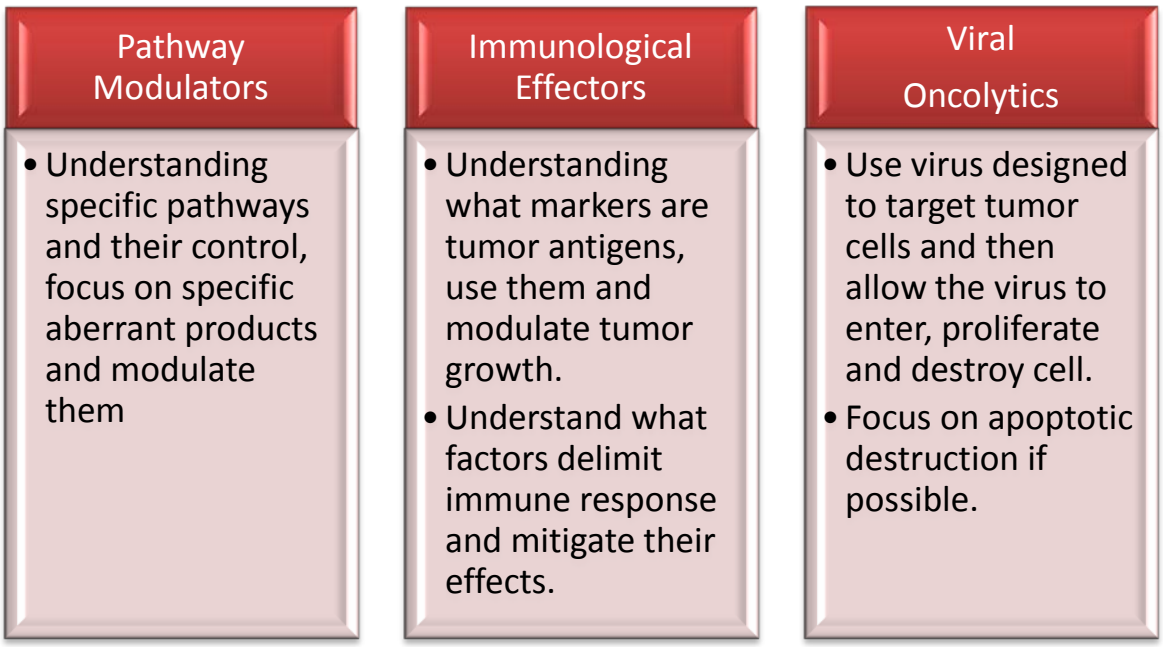
4. Extracellular Matrix Management: This is a more sophisticated approach using knowledge of the impact of the ECM on the cell.

5. Epigenetic Loss of Control: In this case we assume we are dealing with the genes in the pathways and that we have some epigenetic loss of control due to say miRNAs or methylation. Thus the therapeutic is one where we have a need to eliminate the methylation and thus reassert the gene expression or to likewise block the miRNA.

6. Gene Replacement: This approach assumes we have identified an aberrant gene, say resulting from some mutation or the like.

2.1 POSSIBLE MODALITIES

There are a set of putative therapeutic modalities for melanomas as well as cancers in general. We discuss them briefly here and detail them in the next section.

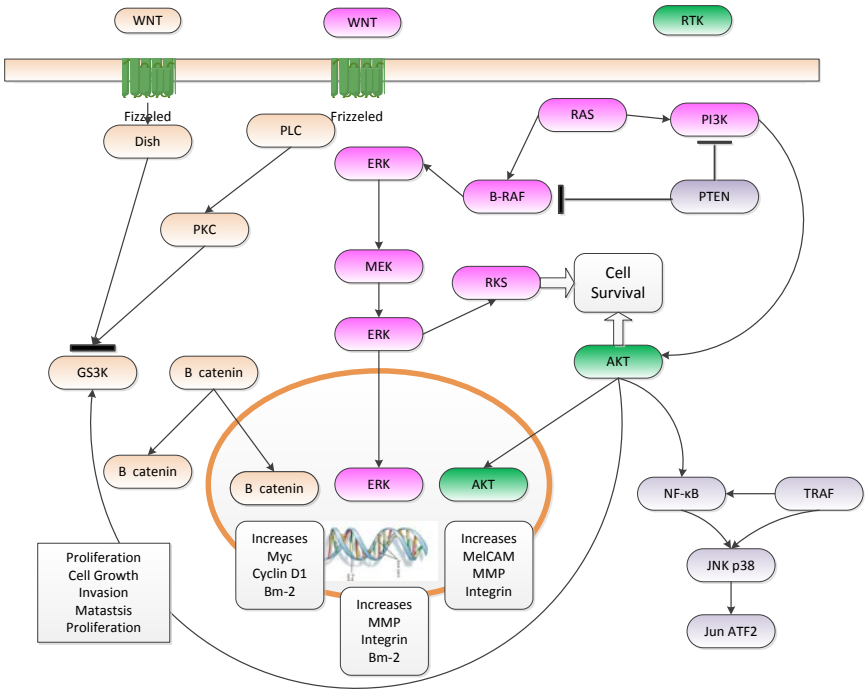


The above demonstrates the three directions we will focus on herein.

2.2 THE PATHWAY PARADIGM

The pathway paradigm is an articulation of our current understanding of pathways and how they can break down and result in excess proliferation, loss of functionality, movement, and all other characteristics of melanoma or cancer in general.

The following is an example of the classic pathway model as we now understand it.



Now pathways, as we have discussed in detail, control proliferation, movement, and functionality. The above is a graphic description of some of the genes related thereto.

3 IMMUNOLOGICAL

Using the body's own immunological system as a way to attack tumors has been an attractive option for decades. Rosenberg had been approaching this in a systematic way since the late 1960s, and as understanding of the immune system has developed there have been improved options to affect such an approach. Simply stated, tumor cells often express surface markers which are antigens which the T cells can recognize and become activated. This is why we often see clusters of lymphocytes around tumor clusters. However the tumor cells have developed means and methods to block the T cell from becoming activated and thus resulting in the digestion of the cell. The tumor cells become protected from the normal action of the immune system.

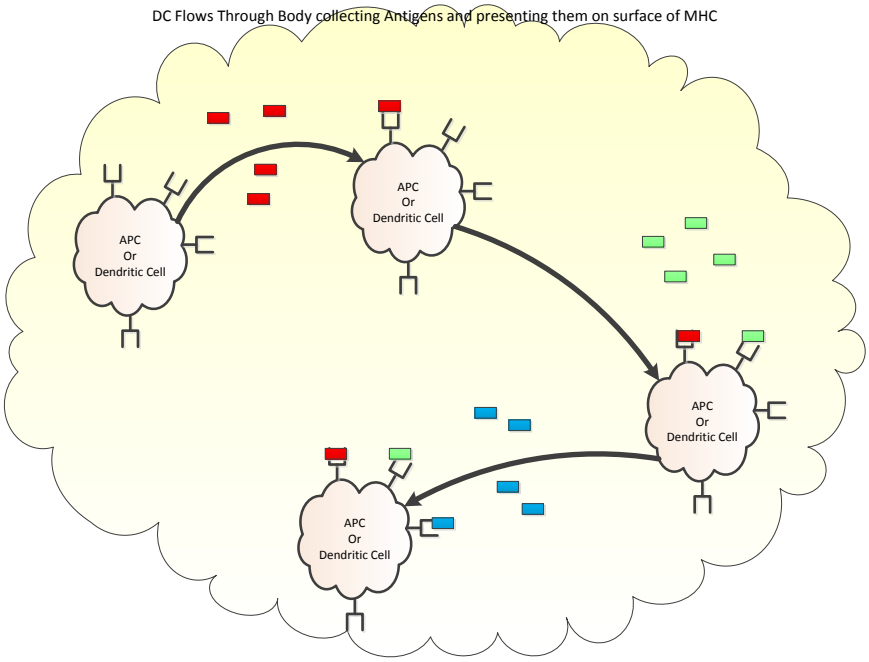
In melanoma the manner in which this happens is the use of a molecule the CTLA-4 which blocks a link normally attained with CD28 receptor. However by understanding this additional blockage one can then block the CTLA-4 from its blocking function and then allow normal operation of the immune T cell, namely destroying the melanoma cell.

3.1 IMMUNOLOGICAL SUMMARY

We begin with a brief summary of how the immune system works in the case of some invasion. We assume a viral invasion but a malignant melanoma cell works the same, almost. We look at three steps.

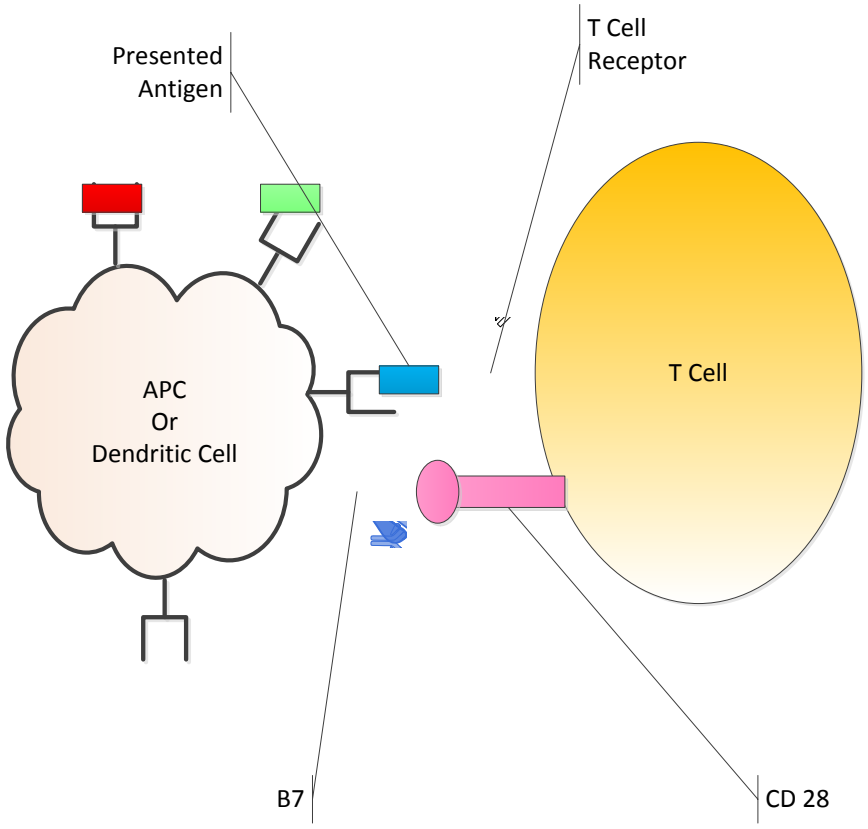
3.1.1 Step 1: Dendritic Cells and Antigen Presentation

First an antigen presenting cell collects antigens as it floats around the body. Seen below it collects several different types to be presented to other immune system cells. The APC or Dendritic Cells are the sensors of invaders into the body.



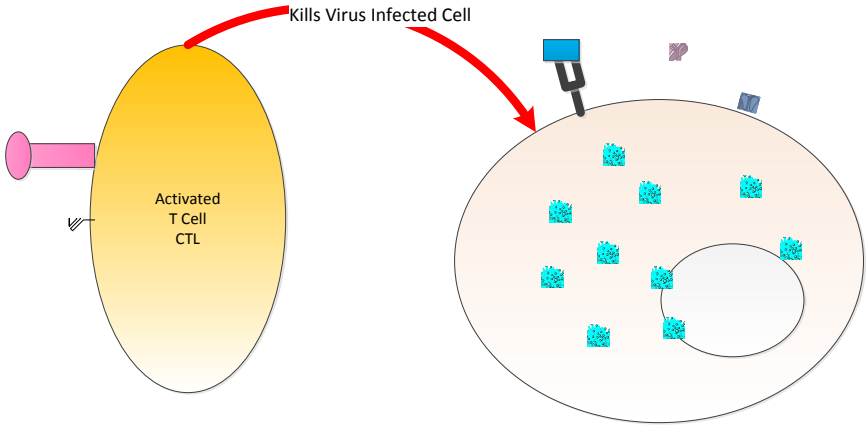
3.1.2 Step 2: Activation of T Cells

At a certain point the APC match up with a T cell and the antigen is then presented to the Tcell which in effect activates it to that specific “invader”



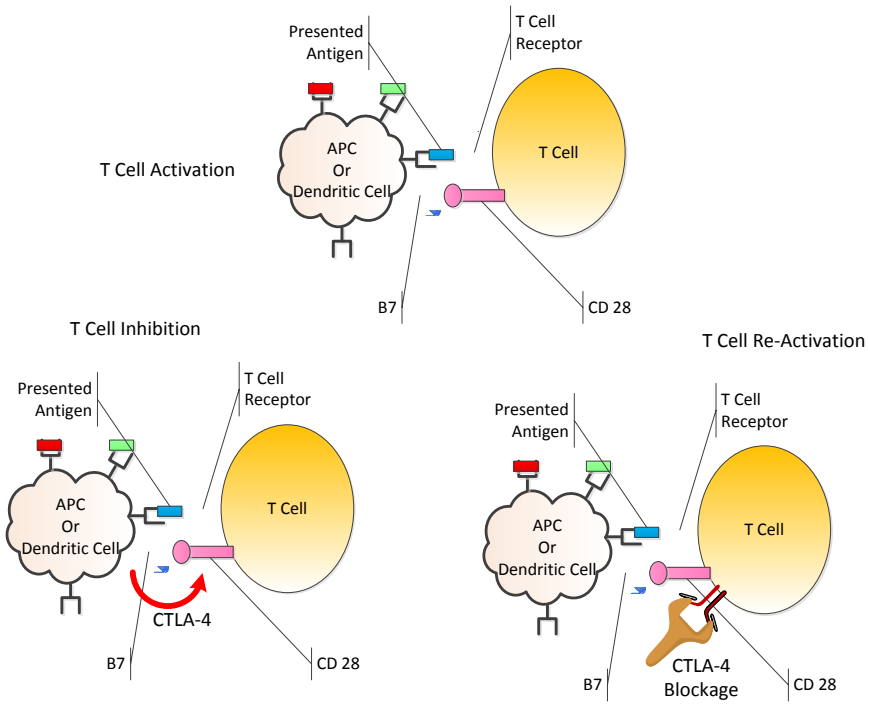
3.1.3 Step 3: T cell Destruction

The activated T cell now can roam about activated for attaching to the invader. When such an invader, in this case a virus infected cell is seen, it attacks and destroys the cell.



3.2 MELANOMA PARADIGM

Now we will apply this basic principle to the elimination of melanoma malignant cells. The Figure below depicts the three steps that are part of this process. First the APC sees the antigen. Now it is blocked by CTLA-4, which inhibits the destruction. Third, we find a molecule to block the attaching of the CTL-4 and thus reactivate the T cell. The 3 steps are shown below.



3.3 THERAPEUTIC ACTION

We now use the basic principles above to describe how ipilimumab functions blocking CTLA-4.

We start by quoting Robert et al who state:

In summary, this trial showed that there was a significant improvement in overall survival among patients with previously untreated metastatic melanoma who received ipilimumab plus dacarbazine as compared with dacarbazine plus placebo. Adverse events other than those typically seen with dacarbazine or ipilimumab therapy were not identified. An increase in liver-function values is an important side effect that was observed more frequently than expected with the combination therapy.

Other ipilimumab-associated adverse events (enterocolitis and endocrinopathy) were observed, albeit at a rate that was lower than expected. The key side effects of ipilimumab were managed

through adherence to treatment according to well established guidelines, including the administration of systemic glucocorticoids or other immunosuppressant agents.

Now we can examine the details of CTLA-4. As DeVita et al state:

CTLA-4 monoclonal antibody (ipilimumab) is a molecule expressed on lymphocytes that binds the B7-1 and B7-2 (CD80 and CD86) molecules on the surface of antigen-presenting cells. Engagement of the CTLA-4 molecule can suppress lymphocyte reactivity and interfere with IL-2 secretion and IL-2 receptor expression. The T-regulatory cells are the only lymphocytes in the resting circulation that constitutively expressed CTLA-4 on their surface; however, expression of CTLA-4 is transiently up-regulated after binding of the T-cell receptor. Multiple preclinical murine models have shown that CTLA-4 blockade can enhance immune-mediated tumor rejection when combined with vaccines.

Although the administration of anti–CTLA-4 monoclonal antibody to patients with metastatic melanoma has not been approved by the FDA as of the writing of this chapter, multiple clinical studies have shown that objective clinical responses can be achieved in patients treated with CTLA-4 blockade. In an updated study of 143 consecutive patients with metastatic melanoma treated with varying doses of anti–CTLA-4 either alone or in conjunction with peptide vaccination, an objective response rate of 17% was seen, including 10 patients (7%) with complete response. Substantial clinical experience with ipilimumab led to the observation that various unique patterns of clinical response could be observed in patients, including initial disease progression followed by tumor regression; mixed responses in which new lesions developed and subsequently stabilized or regressed; and late, slow continuous regression of metastatic disease.

The varied and delayed pattern of tumor response kinetics has been incorporated into strategies for clinical management of patients, for example, by observation of patients for 4-8 weeks beyond initial disease progression to detect late tumor responses. Preliminary data also indicate that a subset of patients achieving objective response or prolonged stable disease to an initial ipilimumab treatment course, who then subsequently demonstrate disease progression, can respond again to another treatment course of up to 4 doses.

*A multi-institutional prospective randomized trial was performed in 676 HLA-A*0201–positive patients with unresectable stage III or IV melanoma who received either (1) ipilimumab, (2) ipilimumab plus a gp100 peptide vaccine, or (3) the vaccine alone. Objective response rates were 11.0%, 5.7%, and 1.5%, respectively. Median overall survival was 10.1, 10.0, and 6.4 months, respectively (P = .003 for ipilimumab compared with vaccine). There were 14 (2.1%) study drug–related deaths.*

A second trial randomized 502 advanced melanoma patients without prior systemic treatment (except in the adjuvant setting) to dacarbazine (DTIC) every 3 weeks, up to 8 treatment cycles in combination with ipilimumab or placebo 10 mg/kg every 3 weeks up to 4 doses.

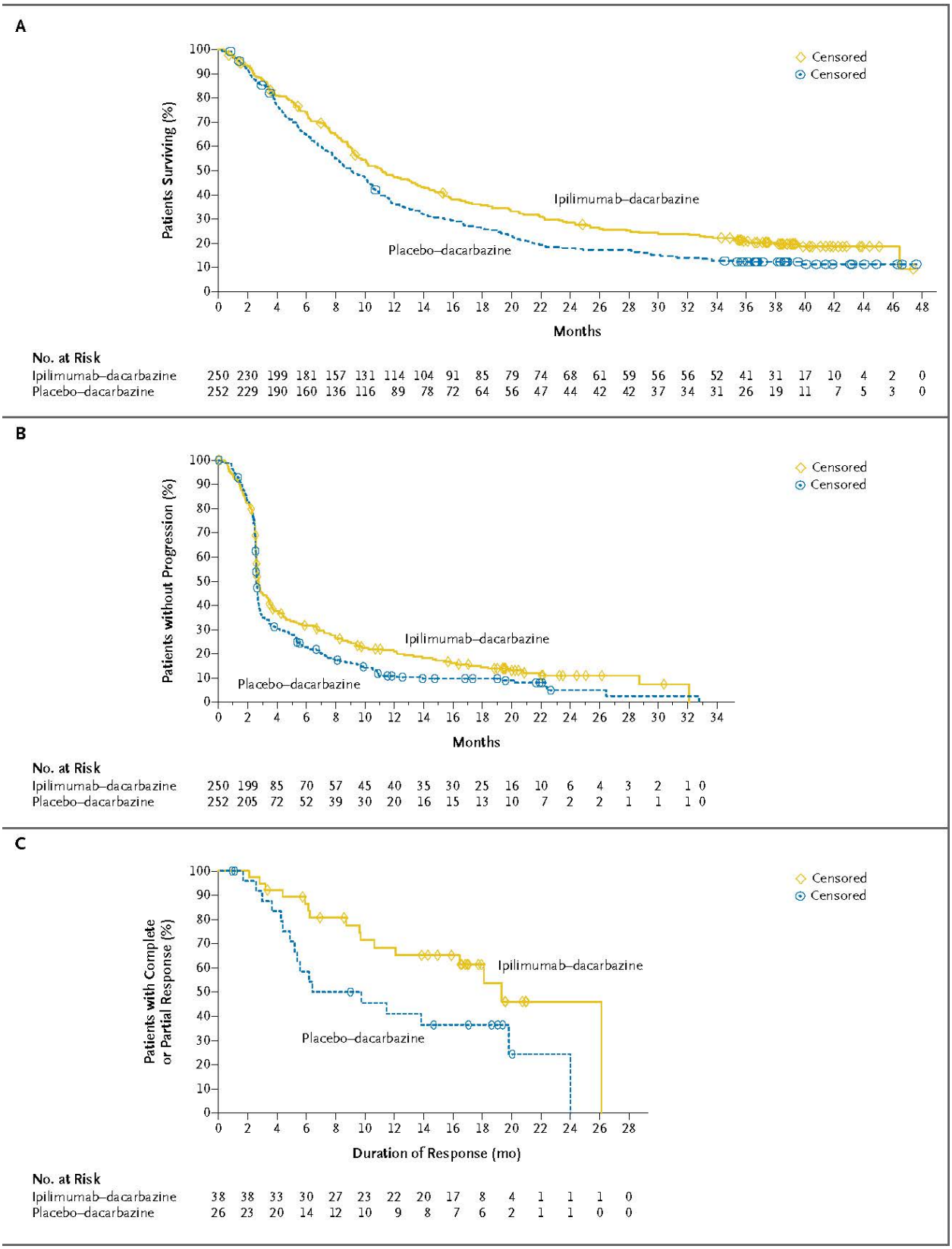
All patients without progressive disease or unacceptable toxicity were offered ipilimumab or placebo 10 mg/kg maintenance every 12 weeks. This trial also demonstrated improved median

survival of 11.2 versus 9.1 months for patients receiving ipilimumab (P < .0009). For both randomized studies, 2- and 3- year survival estimates were approximately 10% greater for the ipilimumab containing arms compared to control.

Ipilimumab administration is associated with induction of inflammatory/autoimmune adverse events, including dermatitis; diarrhea/colitis/enteritis; and less commonly hepatitis and endocrinopathies, including hypophysitis, adrenal insufficiency and thyroiditis. Other rare autoimmune/inflammatory toxicity has been observed including nephritis, pneumonitis, uveitis, motor neuropathies, and immune-mediated thrombocytopenia. The colitis can rarely be associated with life-threatening bowel perforation.

Most of these side effects could be abrogated by the administration of steroids, although some patients may require additional immunosuppression for variable periods with anti-TNF agents. At the 3 mg/kg and 10 mg/kg dose levels of ipilimumab as a single-agent, about 15-20% and 25% of patients respectively may develop grade 3-4 autoimmune adverse events. The toxicity profile of ipilimumab may be influenced by concurrently administered agents; for example, in combination with DTIC, the expected rates of colitis/diarrhea were lower but rates of transaminase elevations were higher than expected for single-agent ipilimumab. In some phase 2 trials, a strong association was found between the probability of achieving an objective antitumor response and the development of some form of autoimmune adverse event.

The Figure below presents the Kaplan Meir curves for ipilimumab. Note that it extends survival for the 50% group to about 6 months. However there is a 20% who have indefinite survival. The question is what makes the 20% so unique and can we reproduce this.


A
Patients Surviving (%)
Censored
Censored
Ipilimumab–dacarbazine
Placebo–dacarbazine
Months
No. at Risk
Ipilimumab–dacarbazine 250 230 199 181 157 131 114 104 91 85 79 74 68 61 59 56 56 52 41 31 17 10 4 2 0
Placebo–dacarbazine 252 229 190 160 136 116 89 78 72 64 56 47 44 42 42 37 34 31 26 19 11 7 5 3 0
B
Patients without Progression (%)
Censored
Censored
Ipilimumab–dacarbazine
Placebo–dacarbazine
Months
No. at Risk
Ipilimumab–dacarbazine 250 199 85 70 57 45 40 35 30 25 16 10 6 4 3 2 1 0
Placebo–dacarbazine 252 205 72 52 39 30 20 16 15 13 10 7 2 2 1 1 1 0
C
Patients with Complete or Partial Response (%)
Censored
Censored
Ipilimumab–dacarbazine
Placebo–dacarbazine
Duration of Response (mo)
No. at Risk
Ipilimumab–dacarbazine 38 38 33 30 27 23 22 20 17 8 4 1 1 1 0
Placebo–dacarbazine 26 23 20 14 12 10 9 8 7 6 2 1 1 0 0

4 PATHWAY MANAGED

We have examined many of the pathways which when broken can lead to tumor cells and their proliferation. We will examine several of the therapeutic possibilities here and consider future directions for development.

4.1 CURRENT DEVELOPMENTS

We begin by reviewing some of the most recent developments in pathway based therapeutics for melanoma.

As Chapman et al state:

Vemurafenib is a potent inhibitor of mutated BRAF. It has marked antitumor effects against melanoma cell lines with the BRAF V600E mutation but not against cells with wild-type BRAF. A phase 1 trial established the maximum tolerated dose to be 960 mg twice daily and showed frequent tumor responses. A phase 2 trial involving patients who had received previous treatment for melanoma with the BRAF V600E mutation showed a confirmed response rate of 53%, with a median duration of response of 6.7 months. We conducted a randomized phase 3 trials to determine whether vemurafenib would prolong the rate of overall or progression-free survival, as compared with dacarbazine.

The mechanism of the induction of cutaneous neoplasia is under investigation, but it is speculated to involve the activating effect of vemurafenib on preneoplastic cells in which wild-type BRAF is further primed by upstream pathway activation. Several investigators have shown that vemurafenib and other inhibitors of RAF kinases can potentiate the activity of the MAPK pathway in cells with wild-type BRAF.

This finding might explain the favorable therapeutic index of vemurafenib in patients who have melanoma with the BRAF V600E mutation but also suggests is further primed by upstream pathway activation. Several investigators have shown that vemurafenib and other inhibitors of RAF kinases can potentiate the activity of the MAPK pathway in cells with wild-type BRAF.

This finding might explain the favorable therapeutic index of vemurafenib in patients who have melanoma with the BRAF V600E mutation but also suggests that vemurafenib could accelerate the growth of some tumors with wild-type BRAF. An important, related ongoing effort by many research groups is to clarify how melanomas become resistant to vemurafenib. Initial studies from several groups have indicated that the MAPK pathway is reactivated in resistant tumors. Although the precise mechanisms of reactivation are still being investigated, gatekeeper mutations in BRAF, which would prevent vemurafenib from binding BRAF, have not been observed. Our results show that single-agent vemurafenib improved the rates of response and of both progression- free and overall survival, as compared with dacarbazine, in patients with metastatic melanoma with the BRAF V600E mutation. These findings provide a solid foundation for the development of future combination therapies.

As Sosman et al state:

In conclusion, this trial shows a high rate of response to vemurafenib in patients with metastatic melanoma and activating BRAF mutations. These results independently confirm the high response rate and response duration shown in a phase 1 trial. The long follow-up period in our study provides critical information on long-term overall survival, not yet shown in the phase 3 trial comparing vemurafenib with dacarbazine.19 Targeted therapy aimed at oncogenic BRAF V600 induces responses in half the patients and a median survival of 16 months.

As Flaherty et al state:

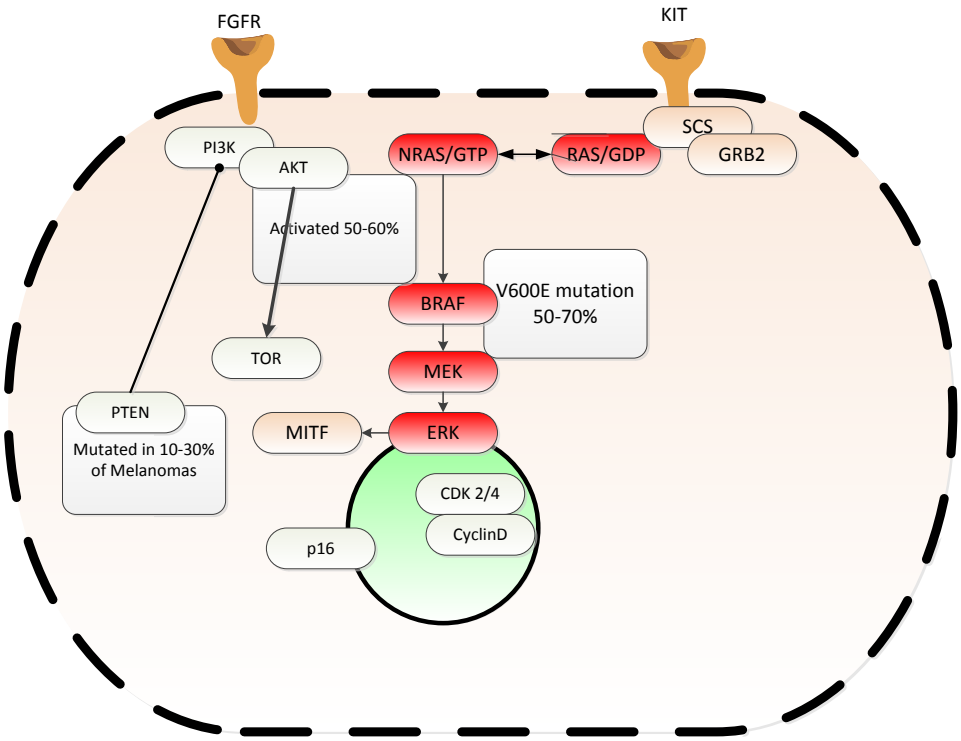
Pharmacologic inhibition of the mitogen-activated protein kinase (MAPK) pathway has proved to be a major advance in the treatment of metastatic melanoma. The use of vemurafenib and dabrafenib, agents that block MAPK signaling in patients with melanoma and the BRAF V600E mutation, has been associated with prolonged survival and progression-free survival, respectively, in randomized phase 3 trials involving patients with previously untreated melanoma. Trametinib mediates blockade of MAPK kinase (MEK), which is downstream of BRAF in the MAPK pathway and has been associated with improved progression-free and overall survival in BRAF V600 melanoma (comprising both V600E and V600K mutations).

In spite of these advances, 50% of patients who are treated with BRAF or MEK inhibitors have disease progression within 6 to 7 months after the initiation of treatment. Several mechanisms mediating resistance to BRAF inhibitors through MAPK reactivation have been described, including the up-regulation of bypass pathways mediated by cancer Osaka thyroid kinase (COT), development of de novo NRAS or MEK mutations, and dimerization or variant splicing of mutant BRAF V600. In addition, MAPK-independent signaling through receptor tyrosine kinases, such as platelet derived growth factor receptor β, insulin-like growth factor 1 receptor, and hepatocyte growth factor receptor, have been associated with resistance. New therapeutic strategies are needed to address these resistance mechanisms.

Despite successful development of oncogene targeted therapy for chronic myeloid leukemia, gastrointestinal stromal tumor, and subtypes of breast cancer and non–small-cell lung cancer, it has not yet been possible to develop combination targeted therapies that circumvent acquired resistance. The combination regimen of BRAF– MEK inhibitors described here represents a successful attempt to combine targeted therapies in an oncogene-defined patient population. Furthermore, as a consequence of unique biochemical effects observed with BRAF inhibitors, this combination appears to be associated with a reduced incidence and severity of some of the toxic effects of monotherapy with either a BRAF or MEK inhibitor. We believe that the combination of dabrafenib and trametinib warrants further evaluation as a potential treatment for metastatic melanoma with BRAF V600 mutations and other cancers with these mutations.

4.2 SOME PATHWAY ISSUES

Let us begin by considering some specific pathways, as relates to melanoma. We show below the B-RAF pathway with a V600 mutation.



All of the pathways shown above may be affected by mutations, suppression or over activation. We discuss here basically targets of opportunity.

4.3 WHY BRAF?

Is BRAF the most critical pathway to target or is it a target of opportunity. More than likely it is both easier to target and 40-50% of melanomas have seen this mutation. It should be noted, however, than for the Irish, it is only 10%. Now we begin by summarizing from the report by Haq et al:

Activating mutations in BRAF are the most common genetic alterations in melanoma. Inhibition of BRAF by small molecules leads to cell-cycle arrest and apoptosis. We show here that BRAF inhibition also induces an oxidative phosphorylation gene program, mitochondrial biogenesis, and the increased expression of the mitochondrial master regulator, PGC1α. We further show that a target of BRAF, the melanocyte lineage factor MITF, directly regulates the expression of PGC1α. Melanomas with activation of the BRAF/MAPK pathway have suppressed levels of MITF and PGC1α and decreased oxidative metabolism. Conversely, treatment of BRAF-mutated melanomas with BRAF inhibitors renders them addicted to oxidative phosphorylation. Our data thus identify an adaptive metabolic program that limits the efficacy of BRAF inhibitors.

As reported by Science Daily[4]:

A multi-institutional study has revealed that BRAF-positive metastatic malignant melanomas develop resistance to treatment with drugs targeting the BRAF/MEK growth pathway through a major change in metabolism. The findings, which will be published in Cancer Cell and have been released online, suggest a strategy to improve the effectiveness of currently available targeted therapies.

"We were surprised to find that melanoma cells treated with the BRAF inhibitor vemurafenib dramatically change the way they produce energy to stay alive," says David E. Fisher, MD, PhD, chief of Dermatology at Massachusetts General Hospital (MGH) and a co-corresponding author of the Cancer Cell paper. "While current BRAF inhibitor treatment is a major improvement -- shrinking tumors in most patients and extending survival for several months -- patients eventually relapse. So there is an ongoing need to improve both the magnitude and durability of these responses."

In about half the cases of malignant melanoma -- the most deadly form of skin cancer -- tumor growth is driven by mutations in the BRAF gene. Research by investigators at the MGH Cancer Center and elsewhere has shown that treatment with drugs that block BRAF activity temporarily halts tumor growth. Combining a BRAF inhibitor with a drug that targets MEK, another protein in the same growth pathway, strengthens and extends the antitumor response. The current study was designed to investigate how BRAF inhibition changes metabolic activity within melanoma cells and to find other possible treatment targets.

The most common way that cells convert glucose into energy is called oxidative phosphorylation and largely relies on the activity of the cellular structures called mitochondria. Many cancer cells use an alternative mechanism that produces the energy compound ATP without involving mitochondria. A series of experiments by the MGH team revealed that the elevated BRAF activity in BRAF-positive melanoma cells suppresses oxidative phosphorylation by reducing expression of a transcription factor called MITF.

Suppressing production of MITF reduced levels of a protein called PGC1α that regulates the generation and function of mitochondria. But melanoma cells treated with a BRAF inhibitor showed elevated MITF activity, along with increased expression of oxidative phosphorylation genes and greater numbers of mitochondria. By switching to oxidative phosphorylation to supply the energy they need, the tumor cells increased their ability to survive in spite of BRAF inhibitor treatment.

"These findings suggest that combination treatment with mitochondrial inhibitors could improve the efficacy of BRAF inhibitors in malignant melanoma," says Fisher, the Wigglesworth Professor of Dermatology at Harvard Medical School. "Several small molecules that target mitochondrial metabolism have been identified by investigators here at the MGH and elsewhere,

[4] http://www.sciencedaily.com/releases/2013/03/130308103416.htm

and laboratory investigations of specific combinations of BRAF inhibitors with mitochondrial antagonists are currently underway."

4.4 WHAT OTHER PATHWAYS

5 ONCOLYTIC VIRAL APPROACH

Viruses function in a manner whereby they use the host cell resources to proliferate and then spread. A virus can recognize an appropriate cell in which it can activate its reproduction via a cell surface marker and then manage its way into the cell and then capture the cell for its own purposes. A simple HPV type wart is an example. In that case we have the keratinocytes captured, and turned into a wart.

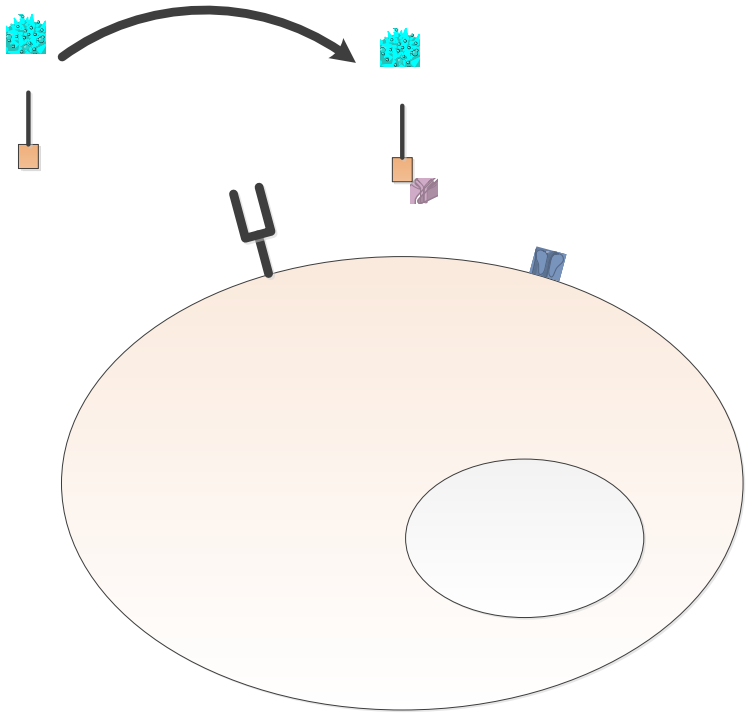
5.1 ONCOLYSIS EXAMPLE

We consider a simple three step process.

5.1.1 Step 1: Virus targets Specific Cell

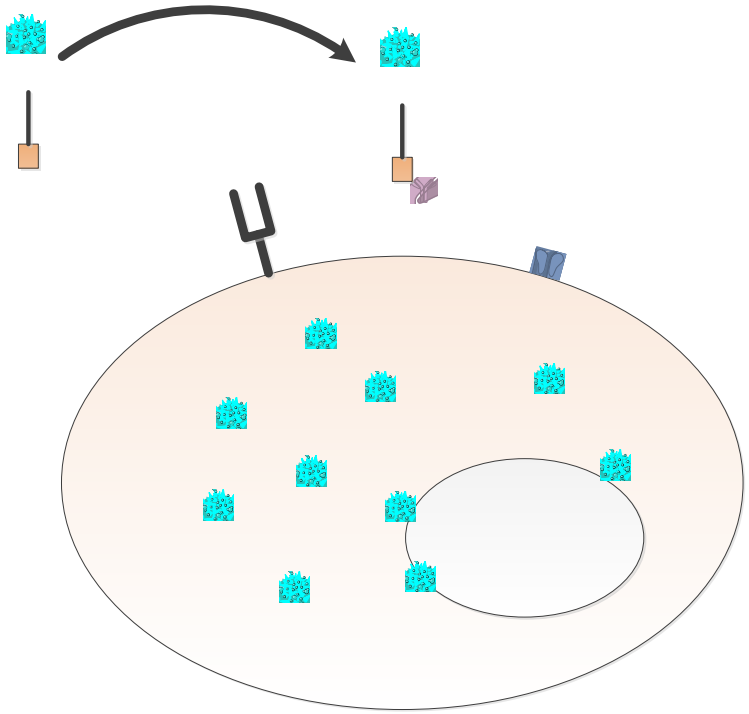
The figure below depicts an example of a virus which looks for a specific cell surface marker which it can then attach itself and enter the cell. For example HPV and HS-1 frequently attack specific epidermal cells and the generation of a wart is a classic example. The virus senses a specific cell type which it will use and then attaches and enters.

Now the problem with melanoma is that we have first to identify an appropriate cell surface marker unique to the melanoma cell and then engineer a virus to attack that specific cell. It becomes a targeted therapeutic.



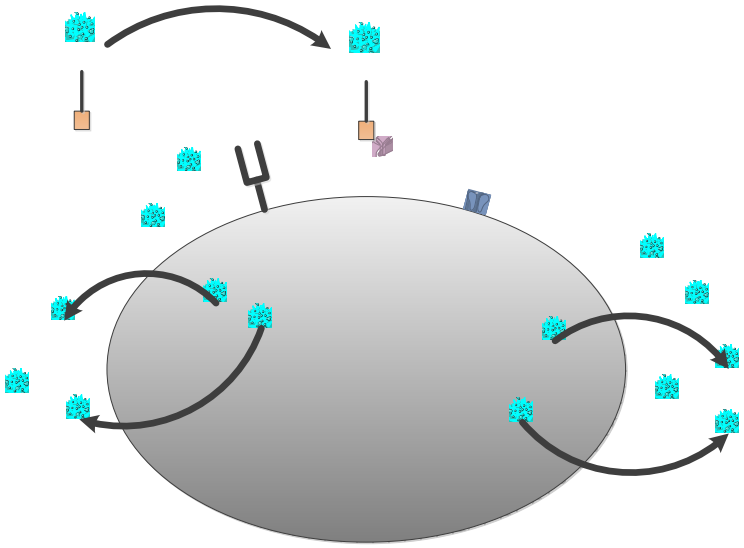
5.1.2 Step 2: Cell Enters and Proliferates

Viruses will then enter the cell and use the cells proteins to assist in its multiplication. In fact the cell becomes the host for this massive growth in the number of such cells.



5.1.3 Step 3: Virus Kills Cell

The final step is the killing of the cell by the explosive growth and expansion of the virus and then the virons go out and do the same with adjoining cells.



5.2 ANTIGENS AND CELL IDENTIFICATION

The key to identifying a tumor cell is the antigen it presents. From Abbas and Lichtman we have the following typical antigens[5]:

[5] See Abbas and Lichtman p 392.

Type of Antigen	*Examples of Human Tumor Antigens*
Products of mutated oncogenes, tumor suppressor genes	Oncogene products: Ras mutations (~10% of human carcinomas), p210 product of Bcr/Abl rearrangements (CML)
	Tumor suppressor gene products: mutated p53 (present in ~50% of human tumors)
Unmutated but overexpressed products of oncogenes	HER2/Neu (breast and other carcinomas)
Mutated forms of cellular genes not involved in tumorigenesis	Various mutated proteins in melanomas recognized by CTLs
Products of genes that are silent in most normal tissues	Cancer/testis antigens expressed in melanomas and many carcinomas; normally expressed mainly in the testis and placenta
Normal proteins overexpressed in tumor cells	Tyrosinase, gp100, MART in melanomas (normally expressed in melanocytes)
Products of oncogenic viruses	Papillomavirus E6 and E7 proteins (cervical carcinomas)
	EBNA-1 protein of EBV (EBV-associated lymphomas, nasopharyngeal carcinoma)
Oncofetal antigens	Carcinoembryonic antigen on many tumors, also expressed in liver and other tissues during inflammation
	α-Fetoprotein
Glycolipids and glycoproteins	GM_2, GD_2 on melanomas
Differentiation antigens normally present in tissue of origin	Prostate-specific antigen in prostate carcinomas CD20 on B cell lymphomas

Now the Amgen announcement states:[6]

Amgen today announced top-line results from the Phase 3 trial in melanoma, which evaluated the efficacy and safety of talimogene laherparepvec for the treatment of unresected stage IIIB, IIIC or IV melanoma compared to treatment with subcutaneous granulocyte-macrophage colony-stimulating factor (GM-CSF).

The study met its primary endpoint of durable response rate (DRR), defined as the rate of complete or partial response lasting continuously for at least six months. A statistically significant difference was observed in DRR: 16 percent in the talimogene laherparepvec arm versus two percent in the GM-CSF arm.

[6] http://www.amgen.com/media/media_pr_detail.jsp?releaseID=1798143

The analysis of overall survival (OS), a key secondary endpoint of the study, is event driven. A pre-planned interim analysis conducted with the analysis of DRR has shown an OS trend in favor of talimogene laherparepvec as compared to GM-CSF.

"These are the first Phase 3 results of this novel approach to cancer therapy," said Sean E. Harper, M.D., executive vice president of Research and Development at Amgen. "A high unmet need exists in melanoma and we believe the innovative mechanism of action of talimogene laherparepvec may offer a promising approach for these patients."

The most frequent adverse events observed in this trial were fatigue, chills and pyrexia. The most common serious adverse events include disease progression, cellulitis and pyrexia.
Among the various types of skin cancer, melanoma is the most aggressive and also the most serious. Although melanoma accounts for less than five percent of skin cancer cases, or 132,000 cases globally each year, melanoma accounts for 75 percent of all skin cancer deaths.
Talimogene laherparepvec is an investigational oncolytic immunotherapy designed to work in two important and complementary ways - to cause local lytic destruction of tumors while also stimulating a systemic anti-tumor immune response.

We summarize this in the Table below.

Cytokine	Tumor Rejection in Animals	Clinical Trials	Toxicity
Interleukin-2	Yes	Melanoma, renal cancer, colon cancer; limited success (<15% response rate)	Vascular leak, shock, pulmonary edema
Interferon-α	No	Approved for melanoma, carcinoid tumors	Fever, fatigue
TNF	Only with local administration	Sarcoma, melanoma (isolated limb perfusion)	Septic shock syndrome
GM-CSF	No	In routine use to promote bone marrow recovery	Bone pain

6 OTHERS

There has been and will continue to be a growing number of therapeutics. The older ones, such as decarbazine, have been somewhat useful. Interferon, also, has been around for quite a while. Other chemotherapeutics have been tried but to no avail. The difference with the newer ones we have discussed herein is that they are based upon specific characteristics of the melanoma cell.

The trend appears to be several folds:

(i) Targeting specific pathway modifications, as they appear,

(ii) An endogenous approach utilizing the person's own immune system as a targeting vehicle,

(iii) An exogenous approach using specific cell targeted viral probes.

We assume that many of the more broadly based approaches relating to cell proliferation modulation and angiogenesis modulation will continue to be explored but with greater knowledge of the cell dynamics the approaches discussed herein will be just as powerful if not more so.

6.1 CLASSIC THERAPEUTICS

Typical classic therapeutics for the treatment of melanoma have been based upon the principles of blocking general cell proliferation. Twenty five to thirty years ago (see Fitzpatrick et al p 963, 1987) the recommended treatments were spotty at best. They used:

1. DTIC, dimethyl-triazeno-imi-diazole-carboxamide.

2. Nitrosoureas

3. Cis-platin, vinblastine, and DTIC or bleomycin

4. BCG with immunotherapy

Needless to say these had little effect, even though some survival stories were reported.

6.1.1 Antimetabolites

Antimetabolites generally interfere with the availability of purine or pyrimidine nucleotide precursors. They may inhibit their synthesis or compete with them in DNA or RNA synthesis. At the present time they do not seem to be effective against melanoma. Methotrexate is a classic example of the antimetabolites.

6.1.2 Alkylating Agents

Alkylating agents attack cells by binding to nucleophillic groups on cell constituents. They alkylate DNA and it is that process that is lethal to the cell. Alkylating agents function on proliferating and non-proliferating cells.

Decarbazine is one of the few alkylating agents used in melanoma. Several therapeutic efforts described herein use decarbazine as an adjunct. Alkylating agents become cytotoxic via a covalent bonding to nucleophillic groups on cell constituents. Decarbazine works through a metabolite not via its own properties directly.

Temozolomide is also an alkylating agent which requires a biotransformation akin to decarbazine. It also functions in a broad systemic manner and thus frequently has significant secondary effects.

6.1.3 Immune System Modulators

There has been an ever increasing interest in using the immune system to attack malignant cells, as intruders in the body. T cell responses and the related cytokines such as Interleukin 2 and Interferon have been an area of intense interest for well over twenty years. The early approaches as best exemplified in Rosenberg's book from 1992 describing his earliest observations and use of IL 2 and interferon.

6.1.3.1 Interleukin-2

Interleukin 2, IL-2, is a cytokine which is a driver in the proliferation, growth and differentiation of T cells. IL-2 induces the proliferation of antigen primed T cells as well as enhancing the natural killer cells, NK, for the attacking of the tumor cells.

From DeVita, Chapter 45, we have:

IL-2 was the first agent available for the treatment of metastatic cancer that functions solely through the activation of the immune system. Originally described as a growth factor for activated T cells, IL-2 was later found to exert multiple effects on cellular immune function and to induce tumor regression in mice. Subsequent clinical trials involving patients with renal cell carcinoma and malignant melanoma have demonstrated sufficient efficacy to establish IL-2 as an FDA-approved treatment for both of these malignancies.

In 1976, Morgan et al. demonstrated the existence of a growth factor present in the conditioned medium of lectin-stimulated human peripheral blood mononuclear cells that could sustain indefinitely the ex vivo proliferation of human T cells. This initial report was followed in short order by the isolation, biochemical characterization, and ultimately, the cloning of what was then termed the T-cell growth factor. Subsequently designated IL-2, this factor was shown to be a 15-kD polypeptide made up of 153 amino acids, the first 20 of which form a signal sequence that is proteolytically cleaved during secretion. Natural IL-2 is glycosylated, although the attachment of sugar moieties is not essential for biologic activity.

They continue:

IL-2 was administered at 600,000 to 720,000 IU/kg IV every 8 hours on days 1 to 5 and 15 to 19 of a treatment course. A maximum of 28 to 30 doses per course was administered; however, doses were frequently withheld because of excessive toxicity. Treatment courses were repeated at 8- to 12-week intervals in responding patients. During initial studies, patients underwent daily leukapheresis on days 8 to 12 during which large numbers of lymphocytes were obtained to be cultured in IL-2 for 3 or 4 days to generate LAK cells; these LAK cells were then reinfused into the patient during the second 5-day period of IL-2 administration.

This high-dose IL-2 regimen with or without LAK cells produced overall tumor responses in 15% to 20% of patients with metastatic melanoma or renal cell cancer in clinical trials conducted at either the NCI Surgery Branch or within the Cytokine Working Group (formerly the Extramural IL-2 and LAK Working Group).61 Complete responses were noted in 4% to 6% of patients with each disease and were frequently durable. Rare responses, usually partial and of shorter duration, were also noted in patients with either Hodgkin's or non−Hodgkin's lymphoma, or non–small cell lung, colorectal, or ovarian carcinoma.

Randomized and sequential clinical trials comparing IL-2 plus LAK cells with high-dose IL-2 alone failed to show sufficient benefit for the addition of LAK cells to justify their continued use. Because of the quality and durability of tumor responses to this high-dose IL-2 regimen, IL-2 received FDA approval for the treatment of metastatic renal cell carcinoma in 1992 and for treatment of metastatic melanoma in 1998.

Long-term follow-up data for patients with melanoma and renal cell cancer treated in the initial high-dose bolus IL-2 trials presented to the FDA have confirmed the earlier findings of response durability, with median duration for complete responses yet to be reached and few, if any, relapses observed in patients free of disease for longer than 30 months. In fact, several patients have remained free of disease in excess of 20 years since initiating treatment. These data suggest that high-dose IL-2 treatment may actually have led to the cure of some patients with these advanced malignancies previously considered incurable.

The concept of a "cure" is thus achieved in a small group of patients using this modality. From DeVita, Chapter 19, we have:

The intravenous administration of high-dose IL-2 (aldesleukin) represents an effective treatment for patients with metastatic melanoma and the treatment most likely to provide long-term complete responses and cure in these patients.

IL-2 was first described as a T-cell growth factor in 1976. The DNA sequence of the gene coding for IL-2 was determined in 1983, and soon thereafter, the IL-2 gene was expressed in Escherichia coli, produced at high concentrations, and purified to homogeneity, and the biologic characteristics of this recombinant IL-2 were determined.261 Although early studies with IL-2 used material from mammalian sources, all clinical studies of IL-2 since 1985 have used the recombinant material.

The administration of IL-2 represented the first demonstration that purely immunotherapeutic maneuvers could mediate the regression of metastatic cancer. IL-2 has no direct effect on cancer cells, and all of its antitumor activity is a function of its ability to modulate immunologic responses in the host.

The FDA-approved regimen for the treatment of patients with metastatic melanoma using IL-2 involves the use of an intravenous bolus infusion of 600,000 to 720,000 IU/kg every 8 hours to tolerance using two cycles separated by approximately 10 days (maximum of 15 doses per cycle). Results of this treatment are evaluated at 2 months after the first dose, and if tumor is regressing or stable, a second course is then administered. This regimen was approved by the FDA for the treatment of patients with metastatic melanoma in January 1998 based on the ability of this IL-2 regimen to mediate durable responses.

The hallmark of IL-2 therapy is its ability to mediate durable complete responses in patients with widespread metastatic disease. In a report of the original 270 patients treated at 22 different institutions that was the basis of the approval of IL-2 by the FDA, a 16% objective response rate was obtained, with 17 complete responses (6%) and 26 partial responses (10%).264 At the last full analysis of these 270 patients, the median duration of response for complete responders had not been reached but exceeded 59 months, and disease progression was not observed in any patient who responded for more than 30 months.

However IL-2 has been used for several years now and does have a positive effect. Yet it is still not curative in most cases. Perhaps the characteristics of ipilimumab blocking are necessary to fine tune the approach for specific melanoma metastatic cells.

6.1.3.2 Interferon

Interferon is a cytokine and work by interacting with the surface receptors of cells. There are three types of Interferon; α, θ, γ. Interferon enhances the activity of macrophages and NK cells and increases the expression of MHC molecules and it further enhances the production of IgG2b.

As Lartigue states[7]:

Interferon clearly act upstream of many important signaling pathways, and researchers have elucidated a plethora of different cellular roles besides their namesake activity of viral interference. For example, they play vital roles in regulating both the innate and adaptive immune responses, and in the activation, migration, differentiation, and survival of various different types of immune cell. In the 1990s, the role of IFNs began to be further delineated, and there was much excitement as it became apparent that they had so-called non-antiviral effects, a variety of effects on cell growth, apoptosis, and angiogenesis (new blood vessel formation) were observed, and this is when clinicians began to realize the potential anticancer applications of IFNs.

[7] http://www.onclive.com/publications/oncology-live/2013/march-2013/interferon-therapy-a-growing-family-feeds-new-interest-in-an-older-treatment/1

Over the decades that followed the discovery of the cytotoxic effects of IFNs, they were touted as a potential "magic bullet" treatment for cancer. While they ultimately did not offer the cure-all that many had hoped, they did become the first treatment for numerous types of hematological cancers and solid tumors, and offered significant hope to patients. At one time or another, they were used clinically and were standard-of-care treatment for chronic myeloid leukemia (CML), hairy cell leukemia (HCL), T- and B-cell lymphomas, melanomas, renal cell carcinomas, and AIDS-associated Kaposi sarcoma.

Thus interferons are a broad based therapeutic for many cancers and operate by exciting the immune system broadly. Yet as with any broad based therapeutic, especially one having such a strong influence on the immune system, it does have side effects. The author states:

A significant issue with type I IFN therapy is the substantial side effects experienced by patients, which include myelosuppression and nervous system disorders, and likely occur as a result of the broad cellular activity of this group of IFNs. The recently identified third type of IFNs, the IFNλs, activate similar downstream signaling pathways to the type I IFNs and have been shown to share the same biological properties, including the antitumor activity. In fact, some studies suggest that IFNλ may have even more pronounced antiapoptotic and antiproliferative effects than IFNα. Since the lambda IFNs act through a unique receptor whose expression is limited to only certain cell types, it is possible that IFNλ could offer a less toxic therapeutic alternative for certain types of cancer. This is a hypothesis that is being heavily investigated.

As DeVita et al state:

Interferon alpha-2b was evaluated in three single-agent phase 2 trials in metastatic melanoma and was associated with a 22% objective response rate among 96 patients. No randomized trial comparing interferon-α with dacarbazine in metastatic disease has been conducted. On the basis of durable responses in some patients with metastatic disease, an adjuvant therapy trial was initiated in patients with high-risk stage 2 and stage 3 melanoma. Interferon-α was administered by intravenous infusion, 20 million U/m2, for 5 consecutive days every 7 days for 4 weeks during the "induction" phase. For a subsequent 48 weeks, 10 million U/m2 were administered by subcutaneous injection on alternate days for a total of three doses every 7 days in the "maintenance" phase.

The control arm was observation, the standard at the time that the trial was conducted. Two hundred eighty-seven patients were enrolled, 80% of whom had stage III melanoma; 20% had stage IIB melanoma. Pathologic staging was performed with regional lymph node dissection because sentinel lymph node biopsy had not yet been introduced. Overall survival was the primary end point, and the trial was designed to detect a 33% improvement.

Also from DeVita et al Chapter 19 we have:

Thus, interferon has been consistently shown to improve relapse-free survival compared to either observation or ganglioside GM2/keyhole-limpet hemocyanin vaccination. The longevity of this benefit has been established with 12.6 years of median follow-up … With twice the follow-up of the initial protocol-defined analysis, the improvement in relapse-free survival continued to be

statistically significant (28% reduction in risk by hazard ratio; P = .02). However, with longer follow-up or by pooled analysis of E1684 and E1690, a definitive benefit with high-dose interferon in overall survival is lacking. With long-term follow-up …, high-dose interferon was associated with a statistically insignificant 18% improvement (P = .18).

The consistency of relapse-free survival data across all trials, in the absence of a consistent or durable survival benefit, has raised speculation that interferon may contribute to causes of death that are unrelated to melanoma recurrence, such as cardiovascular disease. In addition to the negative result for low-dose interferon in E1690, another phase 3 trial evaluated intermediate-dose interferon compared to observation in the adjuvant setting.

A total of 1,388 patients were randomly assigned to one of three arms: interferon 10 million units daily for 5 days out of 7 repeated for 4 weeks followed by 10 million units 3 times weekly for 1 year; interferon 10 million units daily for 5 days out of 7 repeated for 4 weeks followed by 5 million units 3 times weekly for 2 years; or observation. Neither interferon arm was associated with a significant improvement in the distant metastasis-free interval (7% improvement for higher dose vs. observation; 3% improvement for lower dose vs. observation). Overall survival was slightly better for the higher-dose group (5% improvement compared to observation) but not different for the lower-dose group. As a consequence, such regimens remain investigational.

More importantly we have pegylated interferon, namely using polyethylene glycol, the "peg" term, coating to protect the therapeutic from degradation before being activated within the target cell, we have from DeVita et al:

Pegylation results in substantially slower clearance of interferon after administration. This allows for more stable drug exposure than can be achieved with the shorter-lived conventional interferon-α administered on alternating days by subcutaneous injection. To achieve a similar amount of drug exposure over the course of several days, pegylated interferon can be administered less frequently and at a lower dose per injection.

This results in a lower maximum concentration after each dose while increasing the percentage of the dosing interval for which interferon is at biologically active concentrations. Per month of therapy, this regimen is less toxic than the high-dose interferon regimen tested in E1684 and E1690. However, in EORTC 18991, 1,256 patients with resected stage III melanoma were randomized between observation and treatment with pegylated interferon 6 mcg/kg once weekly for 8 weeks by subcutaneous injection followed by maintenance at 3 mcg/kg weekly for 5 years. Given the long duration of therapy, it is not surprising that the cumulative toxicities reported were only marginally less than that observed with 1 year of high-dose therapy. Nonetheless, the dose intensity achieved during the induction phase was 88% of that intended, and for the maintenance phase it was 83%.

The primary end points of the trial were distant metastasis–free survival and relapse-free -survival. Patients treated with pegylated interferon had significantly reduced risk of relapse (18% improvement by hazard ratio; P = .01), but an insignificant improvement of distant metastasis–free survival (12% improvement; P = .11). Survival follow-up was immature at the

time of the analysis of the primary end points, but no significant difference in -survival was observed.

Given the substantially improved tolerability of pegylated interferon, the data supporting an improvement in relapse-free survival is being reviewed by the FDA and European regulatory authorities. Three years of pegylated (100 mcg subcutaneously once weekly) was compared to 18 months of low-dose interferon (3 million units subcutaneously 3 times weekly) in a recently reported randomized trial among 898 patients with primary melanomas greater than 1.5 mm in thickness with or without microscopic involvement of regional lymph nodes.

The peg approach is but one of several where a transport vehicle such as peg or a nano particle is used to movement of the therapeutic[8].

6.2 MULTIPLE PATHWAYS

The BRAF inhibitors can be combined with MEK inhibitors to block the progression of squamous cell cancers. However, this is a dual pathway approach but for two malignancies. The question that should be posed is; can we identify sequential pathway changes which we can then block as they occur? For example, do we expect to see changes in PTEN, p53, cMyc and other pathway elements as the tumor progresses? If so, we have certain therapeutics which may be applied to block the proliferation effects of the loss of such pathway changes.

6.3 STAGED AND COMBINED THERAPEUTICS

With many cancers there has been substantial success with combined or staged use of therapeutics. Recent efforts with melanoma have tried the BRAF and immunological approaches combined with more classic approaches such as interferon. However, recent efforts to use the newer BRAF and immunological approach have met with some problems.

As Ribas et al state:

There has been great interest in testing combination therapy with the BRAF inhibitor vemurafenib and the cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4)–blocking antibody ipilimumab, currently the only two agents approved for the treatment of advanced melanoma on the basis of improved overall survival.1 Vemurafenib and ipilimumab have different mechanisms of action, and preclinical studies have suggested that BRAF inhibitors may enhance immune-cell function and antigen presentation.2-5 The only clinically significant overlapping toxic effects for

[8] Again in the article by Lartigue it discusses such peg approaches as follows: Two pegylated IFNα agents, Pegasys and Pegintron, are approved for the treatment of chronic hepatitis B and C virus (HBV/HCV). The addition of several polyethylene glycol (PEG) molecules to IFNα helps to improve its pharmacokinetic and pharmacodynamic properties, shielding it from enzymatic degradation and increasing its half-life and stability. Since HBV and HCV infection is one of the leading causes of hepatocellular carcinoma (HCC), IFN treatment could help in the prevention of many cases of HCC, researchers hypothesize. Indeed, IFN treatment, either alone or in combination with the purine analogue ribavirin, has been shown to decrease the incidence of HCC in patients with chronic HCV and HBV.

these agents are in skin and liver, which rarely limit their use in patients. Therefore, ample rationale exists to investigate combined therapy with these two agents.

We conducted a phase 1 study of the concurrent administration of vemurafenib and ipilimumab. The primary goal was to evaluate safety and define an administration schedule for further clinical development. Patients were eligible to participate in the trial if they had metastatic melanoma with a BRAF V600 mutation and had not received previous therapy with a BRAF or MEK inhibitor or with CTLA-4 or programmed cell death protein 1 (PD-1)–blocking antibodies.

A second cohort of six patients was enrolled with the planned administration of a lower dose of vemurafenib (720 mg twice daily) together with the full dose of ipilimumab. Among the first four patients who were treated with this combination, elevations in aminotransferase levels (grade 3 in two patients and grade 2 in one patient) developed within 3 weeks after starting ipilimumab. After the toxic effects were reviewed, the remaining two patients in the second cohort received vemurafenib alone. In addition, two patients (one in each cohort) had elevations of grade 2 or 3 in the total bilirubin level with concomitant grade 3 elevations in aminotransferase levels…

Thus the simple and direct step of staging and integrating may have less than beneficial secondary effects. There may be a simple logic for each approach but the combination may introduce yet as identified responses that are not worth the use.

7 OBSERVATIONS

In the past few years there has been considerable success in designing and effecting therapeutics for metastatic melanoma.

We also have a set of choices. Consider the comments by Jang et al:

Patients with metastatic melanoma had few treatment options until 2011, when two drugs—ipilimumab and vemurafenib—were approved following advances in the understanding of melanoma biology and tumour immunology. Almost 50% of melanomas harbor mutations in BRAF, mainly at codon 600, which result in constitutive activation of the MAPK pathway.

The selective inhibitors of mutant BRAF Val600, vemurafenib and dabrafenib, showed major tumour responses, resulting in improved progression-free and overall survival in patients with metastatic disease, compared with chemotherapy. Antitumor activity was also recorded in brain metastases. The growth of cutaneous squamous-cell carcinomas is a unique side-effect of BRAF inhibitor therapy that is induced by the paradoxical activation of the MAPK pathway in cells with RAS mutations.

Trametinib, which targets MEK downstream of BRAF, also produced an overall survival benefit compared with chemotherapy, although tumour responses were less frequent than they were with BRAF inhibitors. Despite this robust antitumor activity, most responses to these drugs are partial and disease progression is typically seen at a median of 5—7 months. Multiple resistance mechanisms have been identified, including those that lead to reactivation of the MAPK pathway and other pathways, such as the PI3K-AKT-mTOR and VEGF pathways.

Some patients with BRAF Val600 mutant melanoma seem to also benefit from immunotherapies such as high-dose interleukin 2 and ipilimumab, which, by contrast with BRAF inhibitors, can produce durable complete responses. We review the available data to best guide initial treatment choice and the sequence of treatments for patients with BRAF Val600 mutant melanoma.

7.1 EXTENSIONS

This discovery leads to several observations of note:

1. One could have imagined something of this happening with Telomeres. It would almost be necessary to allow ongoing uncontrolled mitotic activity. Thus, despite the fact that there is no surprise here we do have a specific target, namely the activator of TERT.

2. Melanoma, as most other cancers, has a multiplicity of changes to genes. There are ligands, receptors, pathway elements, transcription factors, and the telomere issues as well. It is clear that no single factor is the dominant one as of yet. BRAF as a target works for a while and then there is a work around. Thus cancer is an evolving process, and one which may be highly adaptive.

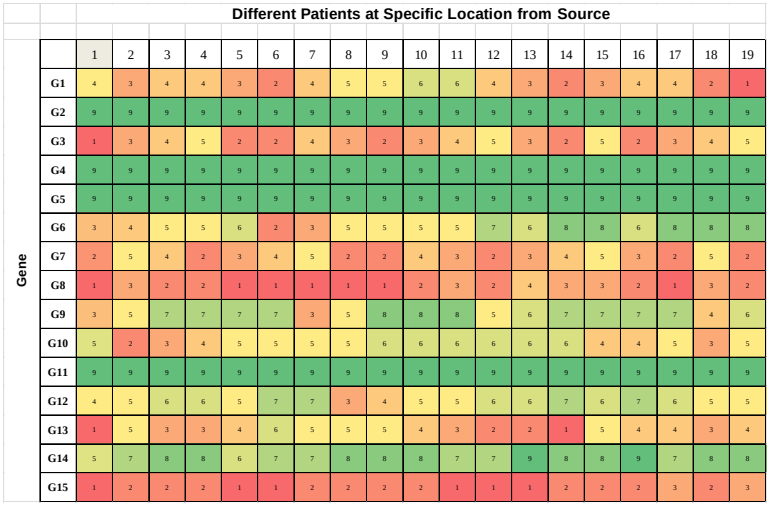
3. A Conjecture: As we have learned more and more as to aberrant genes and their products, as well as miRNAs, and their effects, one could envision several uses of malignancy profiling. We consider that in two steps.

Step 1: Profiling a Specific Patient at Various Locations. As shown below we consider a specific patient and then profile gene expression as a function of distance from the site of initiation, if such was possible. Then we can see how various aberrant genes are being expressed over the distances measure from the source. One would suspect that distance must be measured in some normalized manner but we leave that as an exercise for the student at this time. This gives us a profile for a specific patient, perhaps one for developing therapeutics.

Specific Patient at Different Locations From Source

Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
G1	7	6	6	5	5	5	5	4	4	4	3	3	3	2	2	2	1	1	1
G2	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G3	6	6	5	5	4	3	2	1	1	1	1	1	1	1	1	1	1	1	1
G4	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G5	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G6	6	6	6	5	5	4	4	3	3	2	2	1	1	1	1	1	1	1	1
G7	7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G8	7	4	4	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G9	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
G10	8	7	6	6	6	5	5	5	5	5	4	4	3	3	3	3	3	2	1
G11	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G12	5	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
G13	6	5	4	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G14	7	7	6	6	6	6	5	5	4	4	3	3	3	2	2	2	2	2	2
G15	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Step 2: The Same Location but across a Large Pool of Patients: Again we look now at the same distance from the source, perhaps at the same time, again an exercise for the student, and we get profiles of the expression of aberrant genes. This allows us to understand the between patient differences.



3. Is Seventy Enough? The study did an analysis on 70 lesions. Perhaps that is not enough. Furthermore based upon our previous comments perhaps a correlative study is demanded as well, by patient and by distance.

4. One of the problems I see is the continually hyping of the results as if this is finally the right answer. Anyone even slightly familiar with the field will understand that each input is vital but assembling them in a cohesive whole is essential. The systems approach is the sine qua non, but that cannot be done without the continual bench work required to understand the details.

For example in an article in the Boston Globe the reporter states[9]:

Now scientists working independently in Boston and Germany have made a surprising discovery: a set of genetic mutations found in most melanomas, the deadliest skin cancer. The presence of these mutations in the vast majority of tumors studied suggests that the researchers may have stumbled upon a fundamental mechanism involved in a hallmark trait of cancer cells—their ability to live forever—that could one day be targeted by drugs.

Outside researchers said the work, published online Thursday in the journal Science Express, is exciting because the conclusion is the opposite of what many exhaustive studies of cancers have shown.

In reality as we have discussed, it was imperative that the Telomeres be preserved in metastasis. Millions of rapid mitotic changes in a stem cell must survive and that means keeping Telomeres and that means lots of TERT. Somehow the conclusion was logical, consistent and not at all unexpected especially given what else has been found in the past decade.

[9] http://www.boston.com/news/science/blogs/science-in-mind/2013/01/24/boston-researchers-discover-mutations-that-underlie-melanoma-junk-dna/mNIYVavGfVsvstVj5eNfzO/blog.html

The article continues:

Both teams zeroed in on mutations in a part of the genome called a promoter, which acts like a volume knob on a stereo to control gene activity. The gene that the promoter controlled happened to be one that has long been of interest in cancer because it creates part of an enzyme called telomerase, which enables cancer cells to continue to divide indefinitely as one of its key jobs. Still, it wasn't easy for the researchers to convince themselves that what they found, underlying more than two-thirds of melanoma cases, was real.

One would expect this and if one looks at say the miRNA discoveries, they all add up to what controls the ultimate expression of mitotic survival.

5. Theraputics: Can we expect therapeutics from this understanding? Good question. Kinase inhibitors are now well understood, one could in theory build an inhibitor here as well. Is this the target, another target, necessary, helpful, we can only guess. Yet the above Conjecture may allow for the development of a therapeutic profiling plan for melanoma and other malignancies.

7.2 A SYSTEM VIEW OF THERAPEUTICS

We have developed models for cancer cell propagation and mutation. These models are based upon physical principles but clearly require experimental validation and verification. As one would expect, they are most likely first generation techniques, rough and requiring significant iterative modifications. However they are a paradigm for development.

They are also a paradigm for measuring progression and for determining what cellular modifications are where and when and thus being able to determine the best therapeutic practice.

In previous work we have developed a detailed temporal-spatial model for the propagation of cancer cells in a metastatic environment. We have also combined with that the effect of cellular mutations or equivalent expression changes, perhaps driven by epigenetic factors. We have further suggested that using molecular functional imaging that we could effectively profile the metastatic behavior consistent with the model. Having such a non-invasive data set, taken temporally over some period, could provide a powerful model for prognostic as well as putatively therapeutic usages.

Thus unlike the microarray approach, which is invasive, the molecular functional imaging, MFI, approach could provide a methodology that enable whole body assessment of the progression of the metastasis as well as the genetic alterations which are following the change.

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