Chapter 19. BRAF-melanoma story 220724ea3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 19

The BRAF-melanoma story.

Introduction

Melanoma is a dangerous skin cancer often caused by excessive exposure to the sun. Unless surgically removed while the tumor is still small, the malignancy eventually spreads, leading to a fatal outcome. The lifetime risk of developing melanoma in the United States in 1999 was about one chance in 75 (Atkins et al., 1999). Early attempts to treat advanced melanoma with chemotherapy or immunotherapy only produced responses in about one-fifth of the patients and the responses rarely lasted more than a few months. Later years unveiled dramatic new ways to attack the disease. One of the most important was the discovery of the role of mutations of the BRAF oncogene. Equally important were discoveries about how the anti-cancer immune system is controlled. In 2012, both areas of investigation led to dramatic new paths of treatment for melanoma and other malignant tumors. The state of knowledge as of 2002, which is where this chapter begins, is summarized in Figures 19.1 and 19.2.

Before relating how all that developed over the years, however, here is how the story of BRAF mutation fits in the current sequence of chapters. Chapter 17 told the story of epidermal growth factor receptor (EGFR) mutations and signaling to RAS, and Chapter 18 told the story of RAS mutations and signaling to RAF. The current chapter (Chapter 19) focusses on RAF mutations.

Researchers found opportunities for therapy all along the signaling chains from EGFR (epidermal growth factor receptor) to the molecules that initiate cell proliferation (Figures 19.1 and 19.2). As information about this signaling accrued

over the years, it became more and more complex, as might be expected for a crucial biological system whose activity takes account of many conditions in the cell. A more recent view of the main part of the pathway and the mutations common in malignant melanoma is shown in Figure 19.3.

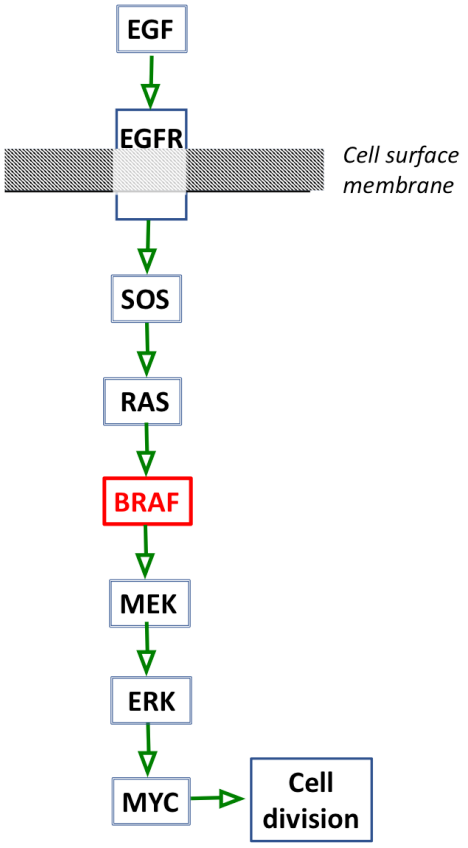

Figure 19.1. Pathway from receptor tyrosine kinase, EGFR, via the MAP kinase (mitogen-activated protein kinase) cascade to cell division-enhancing genes, as understood in 2002. A growth factor, such as epidermal growth factor, EGF, binds and stimulates EGFR, which in turn stimulates RAS via SOS (the human version of the fruit fly's "son of sevenless" – related in Chapter 18). RAS then stimulates BRAF (the most prominent member of the RAF family), which is mutated in about half of melanoma cases and is what drives the malignancy in those cases. Mutant BRAF is many times more active than the normal BRAF, and the mutant's activity is independent of RAS. BRAF then stimulates MEK and ERK, which are MAP kinases (mitogen-activated protein kinases). The signal is amplified at each step and then finally reaches growth factors, such as MYC, which stimulate the expression of cell division genes.

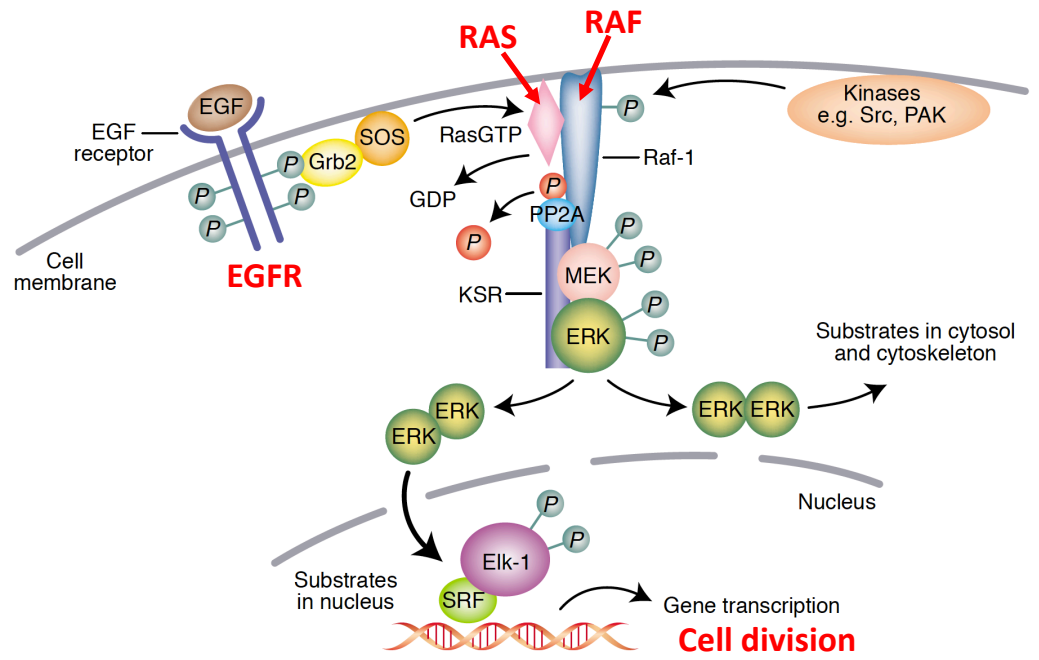

Figure 19.2. The state of knowledge in 2002 about how the EGFR-RAS-RAF-MEK-ERK pathway stimulates cell division. Signaling through the pathway was made efficient by all the components being held near each other. EGFR, SOS, RAS, and RAF are kept close to each other by binding to the cell surface membrane. RAF, MEK, and ERK are kept adjacent to each other through binding to a scaffold protein (KSR). (From (Kolch et al., 2002) with labels in red added.)

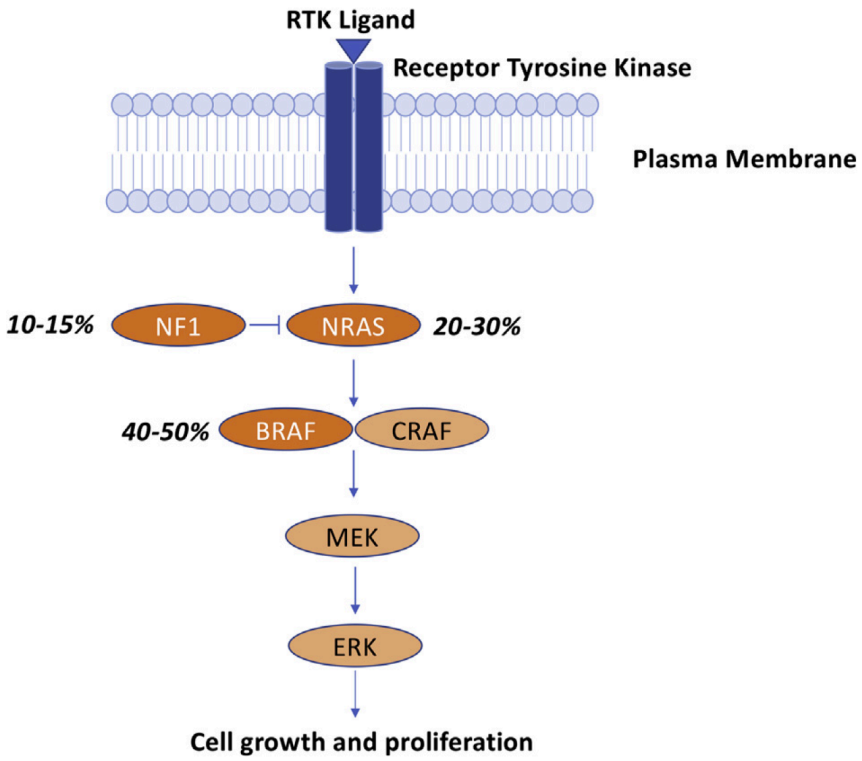

Figure 19.3. A recent overview of the pathway from receptor tyrosine kinases (such as EGFR) to cell proliferation, showing the frequencies of the mutations in melanomas (Jenkins and Fisher, 2020). The large majority of melanoma cases had one or another of these mutations. A recent finding was that the RAF proteins function as homo- or heterodimers. The COSMIC database of the Sanger Center listed the following frequencies of mutations in malignant melanoma (courtesy of Dr. Silvio Parodi): BRAF 45%, NF1 20%, and NRAS 17%. Also frequently mutated were LRP1B 36 %, FAT4 (29 %), PTPRT 25 %, GRIN2A 23 %, and others that may confer resistance to inhibitors of the NRAS-BRAF pathway.

Discovery of the BRAF *oncogene and its role in melanoma.*

In 2002, an international group led by researchers in the UK aimed to discover new cancer-causing mutations, *i.e.,* oncogenes (Davies et al., 2002). They did so by looking for DNA sequence differences between cancer and normal cells, focusing on the pathway from EGFR via RAS, RAF and MEK to ERK, a pathway that was known to push cell proliferation, a hallmark of cancer (Figure 19.1). Since *RAS*-mutant genes had been discovered to be oncogenes, they thought that other genes in the pathway might also sometimes be mutated to become oncogenes.

The UK investigators then went on to make a remarkable discovery: one of the three *RAF* genes, namely *BRAF*, was mutated in about half the cases of malignant melanomas (Davies et al., 2002)! Moreover, all of the mutations affected amino acids

in the protein kinase region of the BRAF protein, the region that activates MEK by adding phosphate groups to it. And 80% of the BRAF mutations were at a single site (V600E), where the mutation replaced an uncharged valine (V) with a negatively charged glutamate (E). They also found that the mutated BRAF protein was continually overactive and did not need activation signals from RAS or anything else.

The negatively charged glutamate was thought to mimic a negatively charged phosphate that normally activated BRAF (Vogelstein and Kinzler, 2004) (Sala et al., 2008). Since the glutamate was an integral part of the mutant BRAF protein, it was presumed to continually activate the protein's enzyme (kinase) activity. BRAF was known to be a protein kinase that phosphorylates and thereby activates MEK in the pathway to ERK and cell division, thereby over-stimulating the pathway to cell division and cancer (Figure 19.1).

Research then focused on mutations of *BRAF*, the first discovered melanoma-associated mutation. It was reasonable to suppose that BRAF-inhibitor drugs would have therapeutic potential, particularly against those melanomas that were dependent on or addicted to an overactive BRAF. But many questions had to be considered about whether or under what circumstances targeting BRAF mutation might become effective treatment.

BRAF mutation was found in about half of melanoma cases. But many questions remained. Was the mutation a cause of the malignancy, or an innocent bystander? Melanoma was known to be induced by sun exposure, but did sun exposure *cause* the mutation? Did the mutation occur also in benign melanocytic nevi (black birthmarks)? Did the mutation sometimes arise during the progress of the malignancy? In the tumors that had the BRAF mutation, did all of the cells have the mutation? These questions were debated for the first few years after the discovery of the BRAF mutation, until evidence and answers to most of them gradually emerged.

The V600E mutation of the *BRAF* gene was of the kind expected from exposure to sunlight because it entailed replacement of a T (thymine) by an A (adenine) in DNA, T being the nucleotide most susceptible to chemical change by ultraviolet light.

BRAF mutation was already present in many benign congenital melanocytic nevi and in benign nevi acquired due to sun exposure. The nevi usually remained benign, although they occasionally did go on to malignant melanoma (Roh et al., 2015). *BRAF* mutation therefore appeared to predispose a benign nevus to become malignant but did not by itself cause the malignancy.

Some melanoma tumors seemed to be composed of a mixture of cells that did and did not have the *BRAF* mutation (Helias-Rodzewicz et al., 2015). Researchers at the Cancer Institute in Santa Monica California found that the frequency of *BRAF* mutation was often higher in melanoma metastases than in the primary tumors

(Shinozaki et al., 2004). Investigating further, they were able to determine in 13 patients whether the mutation was present in the primary and metastatic tumors in the same patient. Of the 13 patients, 4 had the mutation in both the primary and metastatic tumors; however, 4 other patients had the mutation in a metastasis, but not in the primary tumor (Figure 19.4). It seemed that a malignant melanoma was sometimes initiated by sun exposure producing a BRAF mutation in a benign nevus.

Surprisingly, however, the mutation was occasionally found in the primary tumor but not in a metastasis (Sakaizawa et al., 2020) (Figure 19.5). ln such cases, the primary tumor (where the malignancy began) might have had a mixture of *BRAF* mutant cells and cells were driven to malignancy by a different mutation.

Melanomas sometimes occurred in members of families predisposed to the disease. In 2003, Peter Meyer and his colleagues in Tuebingen, Germany, reported that there was no *RAF* mutation in the melanomas of patients who had close relatives who had developed melanomas (Meyer et al., 2003). Instead, their inherited predisposition to melanoma may have come from a different mutation.

So, we see that the BRAF mutation story had a number of variations and complications. Nevertheless, the main picture that emerged was that the malignancy of melanomas was driven by mutated genes, about half of them mutated *BRAF*. That gave strong incentive to develop inhibitors of BRAF's protein kinase activity.

Patient	*BRAF* mutation of primary tumor	*BRAF* mutation in metastasis
1	Mutant	Mutant
2	Mutant	Mutant
3	Mutant	Mutant
4	Mutant	Mutant
5	Wild type	Mutant
6	Wild type	Mutant
7	Wild type	Mutant
8	Wild type	Mutant
9	Wild type	Mutant
10	Wild type	Wild type
11	Wild type	Wild type
12	Wild type	Wild type
13	Wild type	Wild type

Figure 19.4. Shinozaki and coworkers determined whether a *BRAF* mutation was present in the primary tumor and in a lymph node metastasis of the same melanoma patient (Shinozaki et al., 2004). Cases 5-9 suggested that the mutation arose in cells that produced or arose in the metastases. ("Wild type" is jargon from bacterial genetics. Here it means the normal case.)

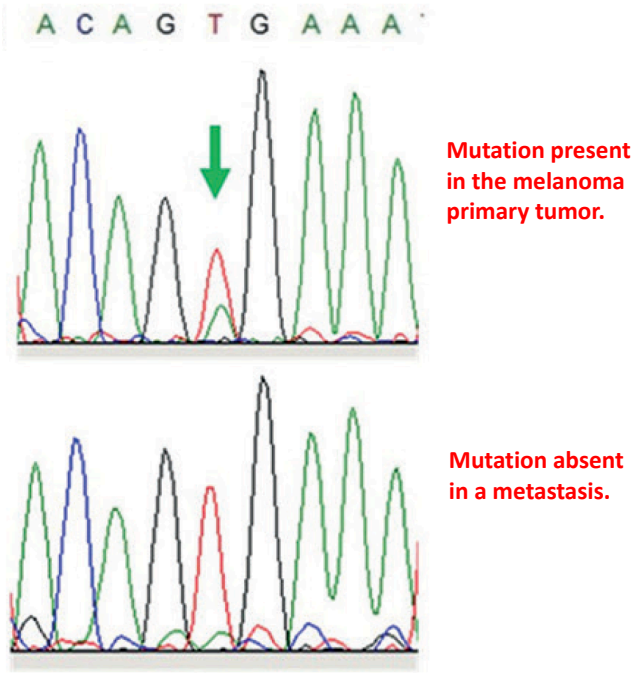

Figure 19.5. A case of malignant melanoma where the primary tumor had the *BRAF* V600E mutation (both the red and green peaks elevated), but a metastasis did not have the mutation (only the red peak elevated) (Sakaizawa et al., 2020). The arrow points to the mutated nucleotide at position 600: *red*, the normal nucleotide (T); *green*, the mutated nucleotide (A). The primary tumor had a both a mutated and a wild-type allele, whereas the metastasis had only wild-type alleles. This patient's metastases might have arisen from a different mutation.

Chemotherapy of metastatic melanoma.

Chemotherapy of metastatic melanoma began long before anything was known about the mutations. The earliest chemotherapy of melanoma began with a drug, initially called DTIC, later renamed dacarbazine (see Figure 2.5 in Chapter 2). It was synthesized in 1961 by Y. F. Shealy, John A. Montgomery and their coworkers at the Southern Research Institute and drew the attention of investigators when studies at the National Cancer Institute found the drug to be active against several mouse tumors. Initial clinical studies suggested that dacarbazine might be active against malignant melanoma (Carter and Friedman, 1972). Several further clinical trials, however, found that only about 20% of the patients responded to the drug, the responses lasted only a few months, and long survival was rare (Gerner et al., 1973). Dacarbazine, however, was the standard of care for metastatic melanoma, because there was nothing better. By the end of the 20th century, most patients with metastatic melanoma, despite treatment with dacarbazine, survived less than one year, and combinations of dacarbazine with other drugs were no better than dacarbazine alone (Sirott et al., 1993) (Chapman et al., 1999).

Before 2011, the only FDA-approved drugs for the treatment of metastatic melanoma were dacarbazine and immune system regulators of the interferon type. Few patients responded, toxicities were high, and there was little increase in survival time. However, in 2011 and the decade following, therapeutic options increased dramatically, and metastatic melanoma patients began to have a more optimistic outlook.

Two major advances were central to this advance. First, new chemotherapy drugs specifically targeted to the RAF and MEK components of the pathway from RAS to the cell proliferation stimulators in the cell nucleus. Second, new immunotherapy that increased the ability of cells of the immune system to act against the tumors.

Search for a specific inhibitor of the V600E mutant BRAF.

Researchers knew that cancers are often driven by overactivity of the pathway from receptor tyrosine kinases such as EGFR via RAS, RAF, MEK, and ERK to stimulation of cell division (Figure 19.1). An early attempt to inhibit this pathway in cancer patients used a drug called sorafenib that was originally aimed to inhibit RAF, but turned out to inhibit several different kinases as well. The drug did help against liver and kidney cancers, but, disappointingly, was useless for melanoma (Pratilas and Solit, 2010). What was needed for melanoma was a drug that inhibited specifically mutant BRAF and nothing else. That was asking for a lot! The drug would have to distinguish between similar sites in many protein kinases and also to distinguish between mutant and normal BRAF that differed by only a single amino acid change.

The challenge was undertaken by a consortium of investigators who reported their work in 2008 (Tsai et al., 2008). It was an extensive and intensive investigation with several stages of screening and molecular characterization that eventually yielded a highly specific inhibitor of the V600E BRAF mutant (which, as a reminder, had glutamate in position 600 in place of valine and was the most common mutation in melanoma). The success of this project showed how specific inhibitor drugs can be designed based on molecular structure analyses of the binding-interactions between drug and the amino acids at the active site of the enzyme and screening large numbers of candidates.

The researchers began by screening 20,000 small molecules for their ability to inhibit protein kinases among a large panel of both tyrosine- and serine/threonine-protein kinases. Of that large set of compounds, they found 238 that inhibited three of the kinases by about 30%. They then co-crystallized those compounds with the kinases to determine the molecular structure of how the compounds bound to the kinases (they used certain kinases that they found were relatively easy to crystallize). About half of the structures bound at the kinase site on the protein and revealed two hydrogen bonds between inhibitor and amino acids at the kinase site.

The structures of the active sites of the different kinases were similar enough to draw conclusions that would apply to many kinases. At this early stage, specificity for a particular kinase was not yet an aim. In order to obtain specificity, a compound that seemed suitable as an initial structure was modified by adding molecular groups to optimize how an inhibitor would fit at the active site of a particular kinase, aiming for best fit to the mutant BRAF. The binding of one of the intermediate compounds to the active site of a kinase is shown on the left panel of Figure 19.6. The Figure shows two of the compounds and how they bound to kinase domains along the way to the specific mutant-BRAF inhibitor vemurafenib. The paper reporting this long and tedious effort by many researchers had as many as 38 coauthors (Tsai et al., 2008).

Vemurafenib did well in clinical trials against advanced melanomas having the V600E-mutated BRAF and became standard treatment, although bedeviled by the development of resistance to the drug. The progression-free survival of the patients was usually about 6 months, which was better than the previously standard treatment with dacarbazine, for which progression-free survival was only 1 or 2 months (Figure 19.7) (Chapman et al., 2011). Clinical trials showed that vemurafenib held BRAF-mutated metastatic melanomas in check for a median of 6 months, and overall survival was a median of about 14 months (Dossett et al., 2015).

Vemurafenib was the first drug that targeted BRAF (V200E) mutant metastatic melanoma and was approved for treatment of those cancers by the U.S. Food and Drug Administration (FDA) in 2011. Although the median extension of progression-free survival was only 6 months, some patients survived up to 18 months. The cancer then became resistant to the drug and resumed its growth. Something obviously had to be done to counter the resistance to vemurafenib that limited the effectiveness of the drug. The first step, however, was to find out what caused the resistance.

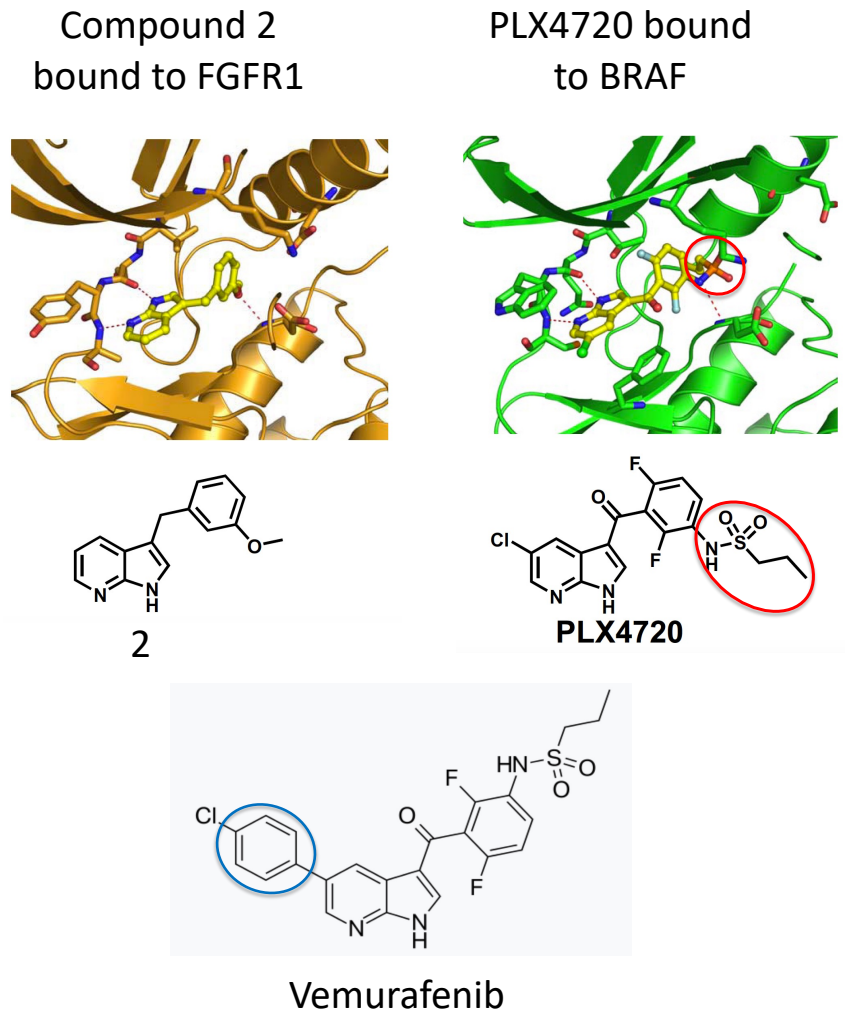

Figure 19.6. Two of the candidate structures on the way to vemurafenib, the specific inhibitor of the V600E mutant form of BRAF. Upper left shows the authors' compound 2 (yellow) and how it bound to the kinase domain of the fibroblast growth factor receptor, FGFR1. Upper right shows their compound PLX4720 and how it bound to the kinase domain of BRAF. (Notice the similarity of the protein structure around the kinase site of the two different kinases.) Below is the structure of vemurafenib. The red ovals show the SO_2-containing group that was added to compound 2 to make PLX4720. To make vemurafenib, an additional benzene ring (blue oval) was added to PLX4720: that is what made the drug specific for the V600E mutation. ("Vemurafenib" can be parsed as follows: VE[valine-glutamate]-mu[mutant]-raf[RAF]-enib[inhibitor].) (From (Tsai et al., 2008) with colored ovals added.)

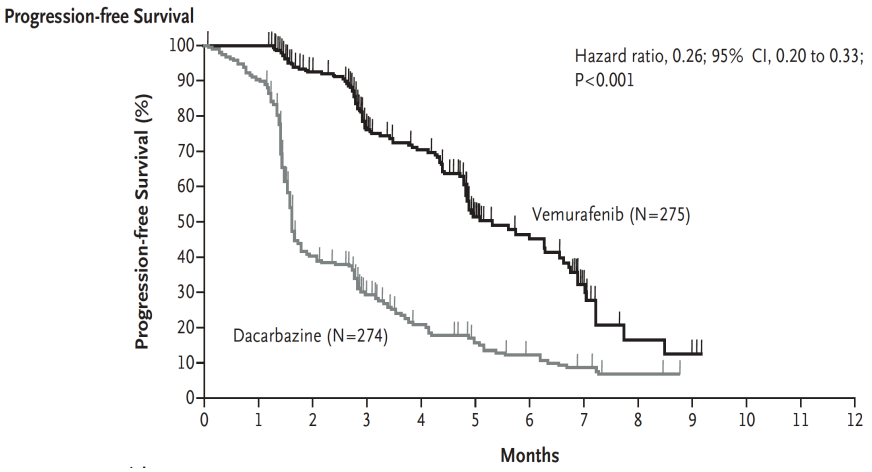

Figure 19.7. Vemurafenib was far better than standard dacarbazine therapy for treatment of metastatic melanomas that had the V600E BRAF mutation (phase III study), although the median time before the disease progressed was only 6 months. The graph shows the fraction of patients that remained free of progression of the cancer as a function time (Chapman et al., 2011).

What caused resistance to BRAF-targeted drugs?

BRAF-mutated metastatic melanomas responded to the BRAF inhibitor, vemurafenib, in over half the cases. After holding the cancer in check, typically for 5 to 7 months, however, the cancer became resistant to the drug, and the treatment was no longer effective. Very soon after those findings were reported, a flurry of papers appeared in high-profile journals in 2010 addressing this conundrum, and the findings were full of surprises (Flaherty et al., 2010) (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Livingstone et al., 2010; Nazarian et al., 2010; Poulikakos et al., 2010; Smalley, 2010; Sondergaard et al., 2010; Yang et al., 2010).

The essential findings were summarized by Solit and Sawyers (Solit and Sawyers, 2010). The first idea about the cause of the resistance was that -- as was the case in resistance to many other drugs -- the mutated target (*BRAF* in this case) would acquire an additional mutation that prevented the drug's effect. Shockingly however, vemurafenib *did* inhibit mutant BRAF in resistant melanoma cells even though the overactive cell division continued unabated. Moreover, DNA sequencing showed that there was no second mutation in the *BRAF* gene in drug-resistant melanomas (Nazarian et al., 2010). This finding was even more surprising, because engineered mutations at the binding pocket of mutant BRAF did in fact confer resistance to vemurafenib (Solit and Sawyers, 2010). Therefore, something else was going on to explain why vemurafenib remained fully effective in inhibiting the mutated BRAF, whereas the melanomas no longer responded.

But yet another surprise was in store: BRAF inhibitors actually *simulated* the MEK-ERK pathway in BRAF-mutated melanoma cells. This was found to be due to activation of another member of the RAF family, CRAF (also known as RAF1), which, like BRAF, signals cell division via the MEK-ERK pathway (Solit and Sawyers, 2010). Thus, resistance to inhibitors of mutant BRAF developed when CRAF became activated and bypassed the inhibition of mutant BRAF (Figure 19.3).

How all that happens was elucidated in 2013 by a large consortium of clinical and basic science investigators (Trunzer et al., 2013). They discovered that resistance to inhibition of mutant BRAF by vemurafenib was usually caused by one of two events: (1) activating mutation in *MEK*, or (2) activating mutation in a *RAS* gene, particularly *NRAS* (Figure 19.3).

How overactive MEK would bypass the inhibition of mutant BRAF was obvious, because MEK was downstream from BRAF in the signaling cascade (Figure 19.1). But how would an activating mutation of NRAS do that? The process was found to be quite simple: NRAS stimulates the activity of another member of the RAF family, the already mentioned CRAF, which bypasses mutant BRAF and stimulates MEK, which in turn directly stimulates proliferation of the melanoma cells (Trunzer et al., 2013). Activation of CRAF also accounted for most of the melanoma cases that had normal *BRAF* genes. (Some facts about protein kinases, such as BRAF, CRAF, MEK, and ERK, are summarized in Box 19.1.) That was the state of understanding of vemurafenib resistance as of 2013. During the next few years, several new inhibitors of BRAF and MEK were developed. Additional routes to resistance were uncovered and the full story became ever more complicated (Luebker and Koepsell, 2019).

Resistance thus developed in several different ways that became well understood, and countermeasures based on that knowledge were tested. The new treatments that prevented or delayed the onset of resistance involved combining a BRAF-inhibitor with another drug. How it all worked was based on a signaling cascade that went as follows:

The main parts of the signaling cascade are diagrammed, as they were understood at various levels of detail, in Figures 19.1, 19.2, and 19.3. It all begins with the activity of a receptor tyrosine kinase, such as epidermal growth factor receptor (EGFR, Chapter 17). EGFR is in the cell surface membrane and is activated when its extracellular part binds a growth factor that is floating around outside the cell. Several different receptor tyrosine kinases were known to funnel into the same signaling pathway via RAS, RAF, MEK, and ERK, and drive cell proliferation, although EGFR seemed to be the most important.

The signal from EGFR was known to go through a series of molecular steps that led to genes in the cell nucleus to become activated to promote cell division and to progress of cancer. First, the signal from EGFR activated, via SOS, members of the RAS family. (SOS is a human version of “son of sevenless” that was originally

discovered in a mutation of the fruit fly eye, as described in Chapter 18). From RAS, the signal activates a cascade of kinases, leading to transcription factors, such as MYC, that activate genes stimulating cell division and cancer progression.

So, how does resistance to BRAF inhibitors, such as vemurafenib, develop? First, a brief review: About half of melanoma cases have a BRAF mutation, over 80% of which are changes in a single amino acid in which valine is replaced by glutamic acid at amino acid position 600 (V600E; E is single-letter code for glutamic acid). The V600E mutation greatly increased the kinase activity of BRAF and promoted the growth of cancers that were dependent on signaling via MEK and ERK. Therefore, inhibiting the kinase activity of BRAF with vemurafenib was effective against cancers, particular V600E-mutated melanoma. Resistance developed when the cancer's dependence on the RAF-MEK-ERK pathway was overcome or circumvented (Trunzer et al., 2013). Overcoming the resistance was a difficult problem, however, because there were several different ways that resistance could develop. More than half a dozen molecular pathway changes were described, each of which could cause resistance to BRAF inhibitors (Johnson et al., 2015). Many studies were initiated to test various hypotheses about how to counter the different pathways to resistance.

Box 19.1. Some facts about protein kinases.

A protein kinase is an enzyme that adds phosphate groups to certain amino acids in proteins. There are two major classes of protein kinases: those that add a phosphate to tyrosine and those that add a phosphate to serine and/or threonine (serine and threonine are closely related amino acids, while tyrosine is special). One of the protein kinases in the cascade, MEK, adds phosphates to both a tyrosine and a serine in the next protein kinase in the cascade, but such dual tyrosine-serine kinases are uncommon. When a protein kinase adds one or more phosphates to the next protein kinase in the cascade, the latter becomes activated so as to enable it to phosphorylate the protein kinase that comes next in the chain. The major steps in the MAP kinase cascade are diagrammed in Figure 19.1. Each kinase in the sequence can activate several molecules of its target in the next step, thereby functioning as an amplifier of the signal, which is why the chain of protein kinases is aptly called a cascade.

How might the resistance of BRAF-mutated melanomas be overcome?

The most common cause of the resistance of BRAF-mutated melanomas to BRAF inhibitors was acquisition by the tumor of an activating mutation in the *MEK* gene. Since MEK is downstream of BRAF in the signaling sequence, overactive MEK would bypass BRAF in stimulating cell division (Figure 19.1 and Box 19.2). An obvious way

to counter or delay resistance due to mutation of *MEK*, therefore, was to add an inhibitor of MEK. When that was tested in a large randomized trial, the combination of vemurafenib and a MEK inhibitor added about 4 months (compared to vemurafenib alone) to the length of time that the malignancy was held in check (Figure 19.8) (Larkin et al., 2014). The combination therapy halted the progression of the disease by a median of 10 months, but the cancer would then resume its growth.

Therefore, additional paths to resistance were sought. One possibility that was investigated was activation of a pathway from EGFR to mTOR (mammalian target of rapamycin), which, like the pathway to ERK, stimulated genes that promoted cell proliferation (Figure 19.9). Drug combinations that would inhibit both the pathway to mTOR and the pathway to ERK were therefore proposed for treatment of mutant-BRAF cancers, (Taieb et al., 2019).

The pathway to mTOR branches at the EGFR level as an alternative to the branch leading to ERK (Figure 19.9). The first step in this alternative branch is binding and activation of PI3K (phosphatidylinositol-3'-kinase). PI3K binds to EGFR (at phosphotyrosines that are produced when EGFR is activated); from that perch, PI3K phosphorylates a membrane lipid (phosphatidylinositol) that binds and activates AKT (also known as protein kinase B). AKT then phosphorylates and activates mTOR, which proceeds to stimulate cell proliferation. This pathway was entirely independent of mutant-BRAF and therefore was considered as a possible way that resistance to inhibitors of BRAF and MEK could develop.

A further detail was that PI3K can be stimulated by EGFR via NRAS (this role of NRAS was omitted in Figure 19.9). An activating mutation of NRAS, which was sometimes found in malignant melanomas, therefore could stimulate PI3K and drive cell division via mTOR. In order to moderate the activity of PI3K, cells have evolved PTEN as a regulator (Figure 19.9). PTEN, however, was subject to inactivation by mutation, leading to enhanced cell division and enhanced malignancy (Nogueira et al., 2010). Various mutations in the positive and negative controllers of the pathway from EGFR to mTOR were being considered as possible causes of resistance that might be countered by adding inhibitors of NRAS and/or PI3K. That is where the investigations stood at the time of this writing.

BRAF mutation in other cancers.

The V600E-mutation of BRAF was occasionally found in cancers other than melanomas. (Why it was so common in melanomas – about 50% of cases – was unknown.) The mutation was found in 10% of metastatic colon cancers and portended a poor prognosis (Ducreux et al., 2019). Although that was a relatively low percentage, it amounted a large number of life-threatening cases, because colon cancer was the second most frequent cause of cancer deaths.

BRAF-mutated colon cancers, in contrast to melanomas, were not driven by mutated BRAF and did not respond to BRAF and MEK inhibitors. It seemed likely, therefore that colon cancers were often driven by a different pathway to uncontrolled cell proliferation. The pathway to mTOR was thought a good possibility (Taieb et al., 2019) (Ducreux et al., 2019) (Figure 19.9). Early clinical trials however were disappointing. Researchers therefore had to go back to the drawing board to find other routs to therapy.

An important consideration was that BRAF-mutated colon cancers were generally found to occur in the ascending part of the colon on the right side of the body, which is where about 15% of colon cancers happen. These cancers usually have defects in DNA mismatch repair, which causes instability of certain DNA sequences and leads to cancer. This defect was a possible lead to new therapy. The DNA mismatch repair story will be told in Chapter 25.

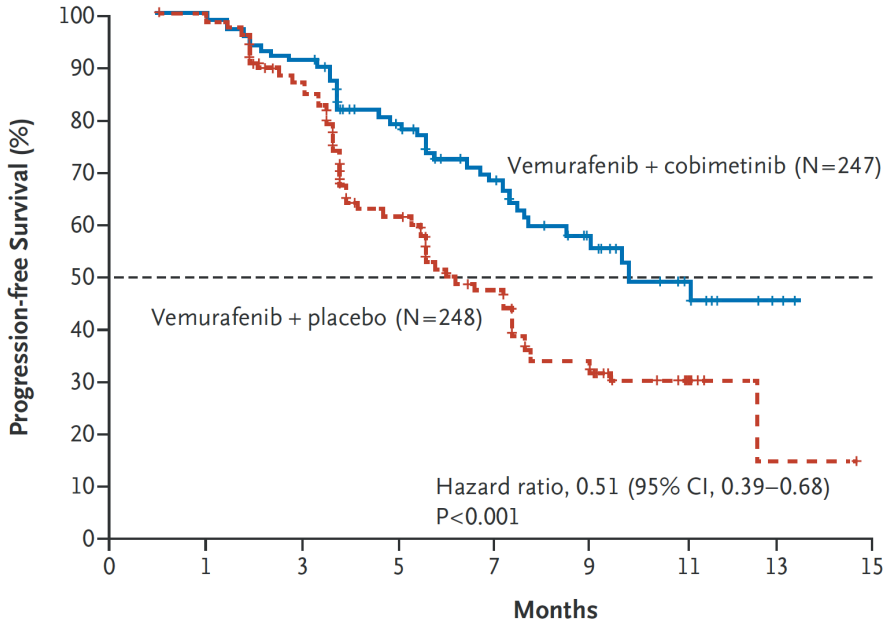

Figure 19.8. Metastatic melanoma patients whose tumor had a V600 BRAF mutation were randomized for treatment with vemurafenib with or without the addition of a MET inhibitor (cobimetinib) (Larkin et al., 2014). The combination of the two drugs, compared with vemurafenib alone, added about 4 months to the length of time before the malignancy progressed. The median progression-free survival was about 10 months for the combination, as opposed to only about 6 months for vemurafenib alone.

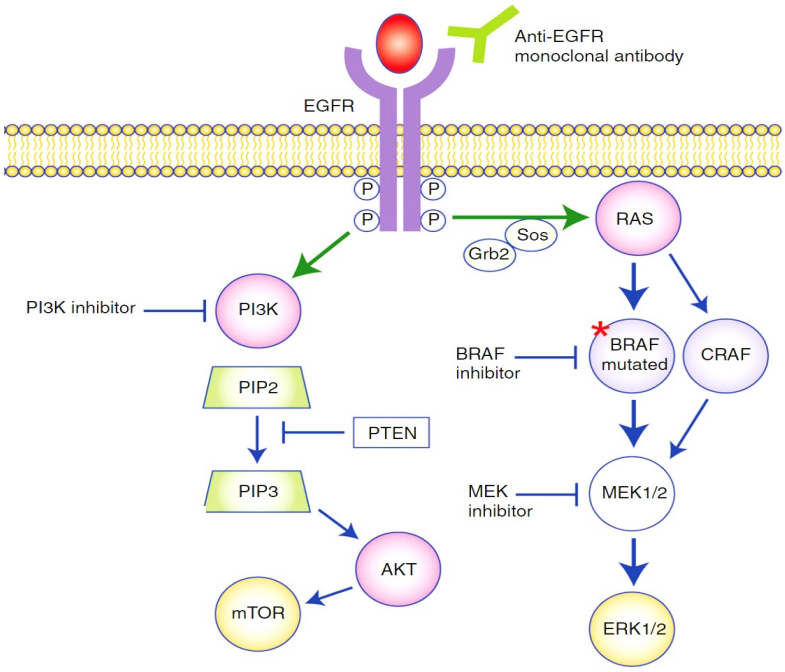

Figure 19.9. Two signaling pathways from EGFR that stimulated cell proliferation and caused resistance to BRAF inhibitors. In addition to the pathway leading to ERK, there was a separate branch from EGFR that led to mTOR. Cell proliferation was stimulated by mTOR as well as by ERK. This diagram is from a recent review by (Taieb et al., 2019), who proposed treatment of BRAF-mutant metastatic cancers (including some colon cancers) with drug combinations that would block both pathways and avoid or overcome drug-resistance: EGFR (by a monoclonal antibody), BRAF and MEK inhibitors, plus an inhibitor of PI3K.

What could be done for melanoma patients whose tumors had normal BRAF?

The question was what drives the malignancy of melanomas whose BRAF was normal, not mutated? Investigators found that the culprit was most often an activating mutation of one of the RAS genes, namely NRAS. Mutant NRAS was found to stimulate CRAF and the pathway to ERK and uncontrolled cell proliferation, as shown in Figures 19.3 and 19.9. In that case, drugs that inhibited BRAF were of no use – and, surprisingly, they might actually make things worse by *stimulating* the pathway through CRAF (Halaban et al., 2010).

Activation of the pathway through CRAF was also how mutant-BRAF melanomas could become resistant to drugs such as vemurafenib. These melanomas may initially respond to the drug, but might soon develop an activating mutation in NRAS, which drives uncontrolled cell proliferation via CRAF (Dossett et al., 2015). To make matters even more complicated, there were pathways from receptor

tyrosine kinases, other than EGFR, that could become mutated and stimulate cell proliferation by way of various other pathways The treatment of metastatic melanoma by inhibitors of specific molecular targeting therefore remained a work-in-progress in the quest for a cure. A different approach was also being investigated with similar or even greater intensity: immunotherapy, which is the subject of the next chapter.

Summary

About 50% of melanomas have a particular oncogenic mutation in BRAF, which is an unusually high incidence for a particular mutation in a particular disease. By far the most common mutation was a change in a single amino acid at position 600 in the amino acid chain of BRAF, a valine being replaced by a glutamic acid at that position (more rarely, the valine was replaced by aspartic acid, lysine or arginine). Thus, the mutation that made BRAF oncogenic was exquisitely specific. The mutation increased by a factor of 10 the ability of BRAF to signal faster cell proliferation (Dossett et al., 2015). The signal passed to MEK and from there down the chain to transcription factors that increase the production of proteins for cell proliferation. However, the multiple pathways, mutations, and by-pass possibilities that could occur presented a highly complicated picture that remained a great challenge.

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