Published in final edited form as:

Curr Mol Med. 2011 October ; 11(7): 553–563.

Resistance to Chemotherapy and Molecularly Targeted Therapies: Rationale for Combination Therapy in Malignant Melanoma

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Abstract

Metastatic melanoma is one of the most intractable tumors, with all current regimens showing limited survival impact. Failure of most agents is attributed to development of therapy resistance. Accumulated evidence points to the apoptotic defect of melanoma cells and the surge of survival signals stimulated by cytotoxic drugs, as a way that tumors circumvent cytotoxic chemotherapy. An overview of inhibitors developed against these growth/survival factors, which are potential partners to be combined with systemic chemotherapy, will be discussed. The escape mechanism from molecular inhibitors also suggests a “vertical” or “horizontal” combination of molecularly targeted therapies. A better understanding of the interactions between simultaneously used regimens and of the rationale for combination therapy will provide new insights to improve survival and quality of life in patients with advanced melanoma.

Keywords

Melanoma; chemotherapy; drug resistance; targeted therapy

INTRODUCTION

Malignant melanoma is one of the fastest growing malignancies world-wide [1]. The life time risk of melanoma is currently estimated to be 1 in 75 and is rising each year [2] with over 60,000 Americans developing melanoma in 2010 [3,4]. The increasing incidence of malignant melanoma cannot be solely attributed to an increase in early diagnosis due to intensive screening, as most of these cases are thin melanomas [1,5]. Recent surveillance, epidemiology, and end-result (SEER) data reveal an increase in all major melanoma histological subtypes and thickness groups [6–8].

Cutaneous tumors (primary site) invade the local and regional lymphatics and eventually migrate to distant organs. Melanoma is highly curable when localized to the primary site, but after melanoma spreads to the regional lymph nodes the 5-year survival rate is only 29%

and once the disease has disseminated to major organs the rate falls to 7% [8]. Current adjuvant chemo- or immunotherapies are ineffective with response rates <20%. Surgery remains the cornerstone of curative treatment in early stages [9]. In-transit tumors are difficult to treat, with almost 50% recurring after surgical removal [10,11]. For melanomas of the head, neck, and trunk, wide local surgical excision of the primary lesion, including all involved sentinel and regional nodes, is the only treatment.

Systemic therapies for metastatic disease include chemotherapy, radiation, and immunotherapy options, none of which have demonstrated sustained long-term efficacy in more than 15–35% of patients, and all with moderate to significant toxicities and side effects. Metastatic melanoma is incurable in most affected patients because of its resistance to most systemic therapies (Table 1). Dacarbazine remains the first choice of systemic chemotherapy for advanced melanoma. The response rate for dacarbazine is 10% to 20%, and tumor relapse is reported to occur 5 to 6 months after treatment [12]. Combined chemotherapies, such as the “Dartmouth regimen” (dacarbazine, cisplatin, carmustine, and tamoxifen) demonstrated more toxicity rather than improved overall survival [13].

Traditional adjuvant therapies with biologics such as interferon-alpha have limited efficacy with less than 10% of patients showing benefit [14]. Recent clinical studies have looked at vaccine-based therapies as well as immunotherapies with monoclonal antibodies to Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) i.e. tremelimumab (CP-675,206; Pfizer) and ipilimumab (MDX-010; Medarex and Bristol-Myers Squibb). Early-phase (phase II) clinical trials have shown one-year survival rates of 47% to 51% for patients with stage III or IV melanoma treated with ipilimumab [15,16]. Long-term response rates with these novel agents are still unknown, and these drugs carry moderate to severe side effects indicating that improved therapeutics are a worthwhile pursuit.

Molecular inhibitors have been intensively studied in melanoma treatment, especially when recent positive results of a novel BRAF inhibitor PLX4032 (Plexxikon; RG7204, Roche Pharmaceuticals) shed light on targeted therapy strategies [17]. However, resistance is a common problem when tumor cells activate compensatory pathways to escape. Over the last three decades, despite all the advances we have made in cancer therapy in other disease sites such as breast and colon cancer, long-term survival data for melanoma has not significantly improved. There is a critical need, therefore, to identify or develop improved chemotherapeutic agents for melanoma that will improve survival and quality of life in patients with advanced disease.

The search for a novel agent with better efficacy than current therapies should remain a priority, as drugs such as ipilimumab are more effective than most other therapies in advanced disease, yet still have great room for improvement. As more drugs are developed and more positive findings are released, there are more options for combination therapies. Instead of blinded trials, there is an urgent need for understanding the interactions between simultaneously used regimens and the rationale for combination therapy.

RESISTANCE TO CHEMOTHERAPY, POSSIBLE COMBINATION OF CHEMOTHERAPY AND TARGETED THERAPY

Cytotoxic drugs exert their toxicity mainly by inducing DNA damage, followed by cell apoptosis. Melanoma cells are generally insensitive to chemotherapy-induced cell death; the mechanism of chemoresistance is still unknown. Possible explanations include disrupted accumulation of agents caused by drug pumps, upregulated DNA repair, defective apoptosis signaling, and survival factor activation (Table 1).

Drug Pumps

P-glycoprotein, a drug efflux pump, is the major mediator of multidrug resistance in tumor cells, but its role in melanoma drug resistance is limited. As reviewed in [18], P-glycoprotein is not detectable in most primary and metastatic melanomas, and it is not found to be induced by chemotherapies, including agents which are known to induce P-glycoprotein in other tumors. However, other drug transporters might be involved in melanoma resistance.

Recent studies found the novel ABCB5 member of the human P-glycoprotein family, which mediates melanoma resistance to doxorubicin as an efflux transporter [19]. Drug accumulation in ABCB5 positive cells is lower than their ABCB5 negative counterparts upon continuous drug exposure. In addition, ABCB5 blockade specifically enhanced doxorubicin accumulation in ABCB5-expressing tumor cells and reversed resistance. Based on these observations, the authors suggested that ABCB5 is a molecular marker for a distinct subset of chemoresistant cells among the bulk melanoma populations.

DNA Repair

The most widely used chemotherapy agents for melanoma are alkylating agents, and cytotoxicity of the most used alkylating agents dacarbazine/temozolomide is attributed to the induced *O*⁶-methylguanine lesion, a critical methyl-DNA-adduct [20]. Elevated DNA repair by *O*⁶-methylguanine-DNA-methyltransferase (MGMT) is yet another potential mechanism of drug resistance in melanoma. In contrast to this, deficiency of the mismatch repair system (MMR), which induces apoptosis in cells with the *O*⁶-methylguanine lesion, is also reported as a drug-resistant phenotype of melanoma cells [21].

Several studies in animal models have reported increased tumor sensitivity to the alkylating agent temozolomide by inactivating MGMT using gene therapy (reviewed in [22]), which validates MGMT as a target. However, low MGMT activity does not always predict better prognosis after chemotherapy in clinic [23], suggesting MGMT is only one of the multiple players in drug resistance.

Strategies based on deficiency in MMR are also evaluated. Decitabine is an epigenetic agent, which can induce re-expression of the epigenetically silenced MMR gene products. A phase I/II clinical trial is ongoing at the University of Pittsburgh, combining decitabine and extended-schedule temozolomide in patients with metastatic melanoma [20].

Anti-Apoptotic Pathway

Failure of melanoma cells to succumb to chemotherapy-induced cell death is largely due to defective cellular apoptotic signaling. Members of the B-cell lymphoma 2 (Bcl-2) family are critical in maintaining the survival of melanoma cells and even normal melanocytes [24]. Though some reports on the comparable levels of Bcl-2 in melanocytes, benign nevi, and melanoma cells of different stages argue against the role of Bcl-2 in melanoma formation and progression, Bcl-2 contributes to a survival advantage of melanocytes and melanoma cells upon adverse stimuli such as chemotherapeutic treatment (reviewed in [25]). Other inhibitors of apoptosis, such as survivin, melanoma inhibitor of apoptosis (ML-IAP), and FLICE-like inhibitory protein (FLIP) are also overexpressed in advanced melanoma [25].

Oblimersen sodium (Genasense; Genta International Inc.) is an antisense oligonucleotide, which specifically targets *Bcl-2* mRNA. In several clinical trials, oblimersen has been reported to enhance sensitivity to dacarbazine *via* increased apoptosis [26], and to improve overall survival in a phase III study of a subgroup of patients with advanced melanoma, compared with dacarbazine treatment alone [27]. 771 chemotherapy-naïve patients were enrolled onto this study. It was relatively well tolerated with neutropenia and thrombocytopenia increased in the oblimersen-dacarbazine group. The addition of oblimersen to dacarbazine yielded significant increases in progression-free survival (median, 2.6 v 1.6 months; $P < .001$), overall response (13.5% v 7.5%; $P = .007$), complete response (2.8% v 0.8%), and durable response (7.3% v 3.6%; $P = .03$). It was observed that patients whose baseline serum LDH was not elevated had significantly increased survival (median overall survival, 11.4 v 9.7 months; $P = .02$) from oblimersen. However, a discrepancy between Bcl-2 expression levels and oblimersen's inhibitory effect in several melanoma cell lines questioned its specificity [28].

ABT-737 is a novel synthesized inhibitor with higher specificity. This agent binds Bcl-2/Bcl-x(L) with higher affinity and downregulates its target more potently than previously designed inhibitors [29]. ABT-737 was widely tested in hematologic malignancies, demonstrating a synergistic effect with cytotoxic agents (dexamethasone, etoposide, fludarabine, and doxorubicin) in chronic lymphocytic leukemia patients [30]. Despite its promising effect in modulating apoptotic machinery, its failure to target myeloid cell factor-1 (Mcl-1) might account for the resistance to ABT-737 treatment in many cell lines [31]. As cytotoxic drugs including dacarbazine, fotemustine, and imiquimod are capable of downregulating Mcl-1 and upregulating pro-apoptotic Noxa [32], this compensatory mechanism strongly suggests a synergistic effect of cytotoxics and ABT-737 in melanoma therapy.

Experimental evidence of how cytotoxic agents alter the level of apoptosis-related proteins is necessary to develop a rationale for combining standard chemotherapy and apoptosis modulation. However, few studies address this question. One study revealed that chemotherapy-resistant cell lines derived from different cell origins showed variation in pro-apoptotic and anti-apoptotic gene expression [33], which reflects the complexity of the apoptotic system. Alteration of apoptotic factors might depend on the genetic background of specific cell systems.

Survival Pathway

Another way for cells to escape from chemotherapy is overproduction of survival signals. In melanoma, the Ras-Raf-MEK-ERK (MAPK) and the PI3K-AKT signalings, two major proliferation and survival pathways, are constitutively activated through activating mutations of *NRAS* or *BRAF*, loss of PTEN (a negative regulator for PI3K-induced signaling), exogenous stimulation of autocrine and paracrine growth factors, and adhesion receptor signaling, conversely regulating a series of proteins involved in cell proliferation, adhesion, survival and angiogenesis [34]. When tumors grow beyond a certain size, new vascular channels are recruited and formed to sustain tumor cell proliferation, local invasion, and hematogenous metastasis. Melanoma cells as well as host cells within the tumor microenvironment secrete angiogenic, growth, and survival signals which cooperatively regulate angiogenesis.

Anti-Angiogenic Therapies

Cytotoxic agents have been reported to select cells with more aggressive potential. Angiogenic factors, such as interleukin (IL)-8 (CXCL8) and vascular endothelial growth factor (VEGF) are correlated with response to chemotherapy in advanced melanoma patients [35]. Several studies confirm upregulation of CXCL8 and its receptors (CXCR1 and CXCR2) after chemotherapy in various tumors such as melanoma, breast cancer, and prostate cancer [36–38]. Given the fact that CXCL8 signaling is a key pathway that regulates multiple downstream signals involved in cell survival, and is linked to chemo-response [36], the CXCL8 pathway proves to be a good target for combined therapies. In support of this, preclinical data from our lab using small-molecule antagonists for CXCL8 receptors has shown promising effects in suppressing melanoma cell proliferation and angiogenesis [39]. Curiously, there are no reports combining chemotherapy with CXCL8 signaling neutralization. Possibly a different mechanism is involved when cells are under stress from cytotoxic treatment, and other compensatory growth factors can mediate the CXCL8 pathway downstream. Another consideration is the interaction between tumor cells and tumor microenvironment, as CXCL8 acts as both an autocrine and a paracrine factor, which is secreted and regulated by a diversity of cells.

Targeting VEGF is another choice for combination therapy. Bevacizumab (Avastin; Genentech Inc.), a monoclonal antibody against VEGF, showed significant efficacy in metastatic colorectal cancer when combined with fluorouracil-based chemotherapy [40]. Several phase II trials of bevacizumab plus dacarbazine, temozolomide, or fotemustine have been completed, showing clinical activity in advanced melanoma [41–43]. 62 previously untreated stage IV melanoma patients were given temozolomide and bevacizumab treatment in a multicenter phase II trial [42]. This regimen has a low toxicity profile, showing promising activity in this high-risk population. The overall response rate was 16.1% (1 complete response, 9 partial response), median progression-free survival and overall survival was 4.2 and 9.3 months respectively. Noticeably, response (chi-squared $P = .02$) and overall survival (14.8 v 9.2 months; $P = .02$) were higher in the BRAF V600E wild-type patients compared to mutated.

Targeting MAPK Pathway

Targeted therapy is most successful when delivered against molecules that tumors are dependent upon for growth and metastasis. BRAF mutations are the most common type of mutations in melanoma, present in 50% to 70% of melanomas [16,44]. The first generation BRAF kinase inhibitor sorafenib (Nexavar; Bayer HealthCare AG) was disappointing as a monotherapy in treating melanoma patients, and the preclinical success of its ability to enhance chemosensitivity [45] could not be translated in a clinical setting. In a double-blind randomized phase II study, dacarbazine and sorafenib were combined as a first-line therapy and compared to sorafenib and placebo in 101 chemotherapy-naïve patients with unresectable stage III or IV melanoma [46]. Improvement in progression-free survival (21.1 weeks vs 11.7 weeks, hazard ratio [HR], 0.665; $P = .068$) was observed, but there was no advantage in overall survival (median, 51.3 v 45.6 weeks in the placebo plus dacarbazine and sorafenib plus dacarbazine arms, respectively; HR, 1.022). A phase III trial of carboplatin and paclitaxel as chemotherapy partners for sorafenib, reported no improvement in progression-free survival (17.9 weeks for the placebo plus CP arm and 17.4 weeks for the sorafenib plus CP arm, HR, 0.91; $P = .49$), response rate (11% with placebo versus 12% with sorafenib), or overall survival as second-line treatment in 270 patients with advanced melanoma [47]. As this study was conducted in patients previously treated with cytotoxic therapies, the failure does not preclude recruitment of chemotherapy-naïve patients for a similar clinical trial.

Sorafenib's lack of efficacy might derive from its defect in specificity [48], as it has multiple targets, including CRAF, wild-type BRAF, VEGFR, c-KIT, and platelet-derived growth-factor receptor β (PDGFR- β). Another consideration is the insufficient downregulation of its target at the clinically tolerable dosage to inhibit tumor growth. Further, this agent might not target BRAF. This is strengthened by clinical studies of one group, who detected *BRAF* and *NRAS* mutational status in patients before treating them with temozolomide and sorafenib [49]. They found patients with a *BRAF* mutation did not benefit more from this regimen compared to those with wild-type *BRAF*, and they suggested that the observed response in cells was attributed to targeting VEGFR2 and inhibiting angiogenesis.

The more selective inhibitor against mutant BRAF, PLX4032, should be a better partner for cytotoxics. Indeed, we have witnessed the success of this agent, which generated a striking response (complete or partial tumor regression) rate of 81% among melanoma patients with the V600E BRAF mutation in a phase I dose-escalation trial [17]. The differentiation between normal and mutant targets by PLX4032 enables this drug to achieve high tolerable doses and kill tumor cells. Based on preliminary results, phase II and III trials are now underway. PLX4032 proved potent at the beginning of treatments, however, resistance to PLX4032 inevitably occurred, and duration of the response ranged from 2 to more than 18 months. Therefore, we still need to consider its combination with other drugs to overcome resistance, and ongoing trials will lay a basis for it.

Still the same pathway, targeting the downstream mitogen-activated protein kinase (MAPK/MEK) is unexpectedly disappointing. The MEK inhibitor AZD6244 (AstraZeneca) led to cell cycle arrest, but failed to generate sustained suppression of melanoma cell growth [50].

A similar cytostatic effect was seen in a mouse xenograft model. However, when co-treated with docetaxel, tumors shrank [50]. More data is needed and the development of novel MEK inhibitors, such as PD0325901 (Pfizer), might pave the way for a MEK inhibitor and a cytotoxic agent to enter clinic trials as partners.

Targeting PI3K/Akt Pathway

Phosphoinositide 3-kinase (PI3K)/Akt signaling is equally important in melanoma progression, but there is a lack of positive findings using targeted therapies. Most studies involve mammalian target of rapamycin (mTOR), which is a downstream substrate of Akt, and capable of regulating Akt activity. Inhibitors of mTOR include rapamycin and its analogues CCI-779 (Temsirolimus; Wyeth) and RAD001 (Everolimus; Novartis Pharmaceuticals). No antitumor effect was seen when they were used as monotherapy in metastatic melanoma [51]. Preclinically, CCI-779 stimulated a response to both dacarbazine and cisplatin [52,53]. Rapamycin shows similar activity in enhancing chemotherapy-induced apoptosis, especially when combined with temozolomide [54]. Unfortunately, clinical results did not prove an advantage of adding mTOR inhibitors to chemotherapy. In a phase II trial of 48 stage IV melanoma patients, only 4 of 48 (8%) patients showed a partial response when co-treated with temozolomide and RAD001 [55]. The median progression-free survival was 2.4 months, and the median overall survival was 8.6 months. The combination of temozolomide and RAD001 did not show a therapeutic advantage over temozolomide-alone historic controls. This study is still ongoing to determine other combination opportunities by analyzing changes in biomarkers before and after treatment. Since there are few clinical reports in this area, currently it is premature to conclude mTOR inhibitors are not a valid partner for cytotoxics.

Targeting Transcription Factor NF- κ B

The unique role of Nuclear Factor-kappa B (NF- κ B) makes it an attractive target for different malignancies including melanoma. This transcription factor regulates as many as 400 genes, including apoptosis and survival-related factors such as Bcl-2, survivin, IL-8, VEGF, Akt, and Cylin D1. It is part of the positive feedback loop in transcription and expression of these molecules (reviewed in [56]). Activation of NF- κ B is observed after treatment with many known cytotoxic agents [56], which might be the ultimate reason for chemoresistance induced by the effector molecules discussed above.

Physiologically, activation of NF- κ B involves release of inhibitory I κ B and its translocation from cytoplasm to nucleus. In this case, I κ B is phosphorylated by I κ B kinase (IKK) complex and then degraded by proteasome. Thus, inhibition of the NF- κ B pathway can be achieved by using a proteasome inhibitor (bortezomib (Velcade; Millennium Pharmaceuticals)) or kinase inhibitor of NF- κ B (a small molecule inhibitor of IKK β). Both inhibitors can reduce NF- κ B related gene products which are induced by cytostatic drugs, thus stimulating cell apoptosis, inhibiting tumor growth and metastasis in mouse models bearing melanoma [57–59]. Considering the physiological role of NF- κ B and hundreds of gene products tied up with it, targeting this transcription factor is associated with significant toxicity in clinic.

Single-agent bortezomib was not effective in the treatment of patients with metastatic melanoma in a phase II study [60]. No major clinical responses were observed in the 27 patients recruited, only 6 patients (22%) achieved stable disease, and 2 of them could not tolerate the toxicity after 4 cycles of treatment. The median time to disease progression was 1.5 months with a median overall survival of 14.5 months. Eleven patients (42%) had Grade 3 toxicities including sensory neuropathy, thrombocytopenia, constipation, fatigue, ileus, abdominal pain, and infection without neutropenia.

A 2-stage phase II trial of three-drug combination (paclitaxel, carboplatin, and bortezomib) was conducted in 17 patients, to evaluate its potential therapeutic advantages over inhibiting NF- κ B alone [61]. All patients in the study had metastatic melanoma and had received at most 1 prior chemotherapy for metastatic disease. Two partial responses were observed (11.8%). Four patients had stable disease at >12 weeks. The median progression-free survival was 3.2 months, and the median overall survival was 7.0 months. Grade $\geq$ 3 toxicities included neutropenia (71%), leukopenia (41%), thrombocytopenia (29%), and arthralgia (12%). The 2-stage design prevented recruiting large numbers of patients for a regimen with insufficient clinical efficacy yet significant toxicity.

RESISTANCE TO MOLECULARLY TARGETED THERAPIES WARRANTS DEVELOPMENT OF COMBINED TARGETED THERAPIES

Despite advances in the diagnosis and staging of advanced melanoma, survival has not significantly improved in the last 30 years [5], and despite using aggressive therapies the majority of these patients will die of recurrent or metastatic disease. Molecularly targeted therapies are being explored (as discussed above), but thus far have not shown the level of benefit in melanoma as they have in other cancers such as breast and colon cancer.

Based on our observations and published reports, we and others believe that combining targeted therapies through cooperative inhibition of multiple targets offers another approach (Table 2). Resistance to targeted therapies occurs in several ways (Table 1): secondary mutation in the same mutant target, such as *KIT* gene mutation after prolonged treatment of imatinib [62]; upregulation of downstream signals, which suggests targeting two molecules in the same pathway; and stimulation of parallel survival pathways, a rationale for attenuating signals in compensatory pathways (Fig. 1).

Inhibitors of mutant BRAF bring up two questions, one is activation of the pathway in cells with wild-type BRAF; the other is drug resistance. The CRAF isoform, is the culprit in both cases. Increased phosphorylation of MEK-ERK was observed in cells resistant to a RAF kinase inhibitor. However, BRAF was not the driving factor. Overexpression of CRAF was confirmed to rescue the pathway and activate downstream signaling when BRAF is targeted [63]. Thus, it makes sense to use a “vertical” combination of therapies against two points in MAPK pathway. This is supported by a preclinical study, which confirmed that co-treatment with BRAF and MEK inhibitors induced apoptosis and significantly suppressed resistant melanoma cells [64].

Another support of “vertical” combination within a signaling cascade comes from the study of the MEK inhibitor AZD6244 in a broad range of cancer cell lines with or without *BRAF* mutation [65]. In some cases, targeted therapy can lead to amplification of upstream signals, due to disrupted feedback loop within the pathway (Fig. 1). A physiological negative feedback exists between ERK and RAF, as increased levels of ERK lead to activation of Sprouty (Spry) protein, an inhibitor of upstream RAF, attenuating overactivation of the signaling. The feedback balance is disrupted by AZD6244, causing accumulated phospho-MEK instead. Interestingly, mutant BRAF also takes advantage of the impaired feedback in a different way. Spry cannot bind mutant BRAF, thus interrupting accumulation of phospho-MEK, in favor of restoring sensitivity to the MEK inhibitor. Therefore, cells harboring mutant BRAF are vulnerable to AZD6244 treatment [65]. Here, feedback regulation provides another mechanism of drug resistance and warrants multiple targeting within the same pathway to achieve sufficient inhibition.

Feedback regulation works in parallel pathways as well, which leads to aberrant activation of alternative signaling after suppressing one pathway (Fig. 1); an example is the interaction between MAP kinase and PI3K/Akt signaling [66]. According to this, a “horizontal” combination of agents against two points in compensatory pathways might synergize to inhibit tumor growth. Indeed, both sorafenib and MEK inhibitors (U0126 or PD98059) were able to cooperate with rapamycin or PI3K inhibitors in downregulating anti-apoptotic machinery and inducing cell death [67,68]. However, a similar effect was not achieved when a dual PI3K/mTOR inhibitor PI-103 was added to sorafenib in a mouse model [69].

Resistance to VEGF targeting has been studied for years. This therapy has even been reported to favor more invasive and aggressive tumor growth. Several other factors, such as PDGF, and basic fibroblast growth factor (bFGF), can rescue tumor angiogenesis when VEGF is blocked, which provides a mechanism for resistance to VEGF inhibitors [70]. In addition to combination with cytotoxic agents, dual inhibition of angiogenic factors or other survival factors is a possible therapeutic approach. Preliminary evidence comes from a synergistic anti-tumor effect of mTOR and VEGFR inhibitors in a murine melanoma model [71]. In a recent phase II trial, bevacizumab plus everolimus was well tolerated, and indicated moderate activity [72]. 57 patients with metastatic melanoma were recruited, they had no previous anti-angiogenesis or mTOR inhibitor treatment. Major responses occurred in 13% of the patients. Progression-free and overall survivals were 4 and 8.6 months, respectively. However, the majority of the responses in this trial were not sustained, and the estimated progression-free survival rate at 1 year was only 7%. Considering the modest activity of bevacizumab alone in advanced melanoma, the authors suggested that possibly no additive effect of everolimus to bevacizumab was achieved in this trial.

FUTURE DIRECTIONS

Despite the strong rationale for some combination therapies, preclinical and clinical outcomes are not satisfactory. Currently, the combination choices are simply based on examining the pattern of survival factors after using cytotoxic therapies. Less is known about how molecular inhibitors modulate the cytotoxic effect of chemotherapy. For example, some cell surface receptors might play a role in drug uptake; therefore, their

modulation can improve the effect of chemotherapy [73]. The mechanism of targeted therapy as a single agent versus as a combined agent can be different. Thus, understanding interactions of combined therapies is necessary for solving problems with drug resistance.

However effective a certain combined therapy can be clinically, patients should be carefully selected based on their molecular status before treatment, given the heterogeneity of the tumor itself. Tumor cells with different mutations respond distinctly to therapies, or even favor growth in response to certain drugs. For example, melanoma patients with mutant *RAS* cannot be treated with BRAF inhibitors [74]. At the bench, we also need to consider using cells with or without certain mutations, such as *RAF*, *RAS*, and *KIT* when we are studying new targets, considering the possibility that mutant gene products can modulate cellular responses to other molecular targets.

The problem of drug resistance results from the delicate signaling balance inside cells. When we look outside the cell, there is another view. The tumor microenvironment is a source of growth factors, and mediates many physiological and pathological activities of cells. In view of tumor therapies, the stromal environment has been reported to play a role in modulating drug responses [75]. Yet, stromal cells have relatively stable genomes, thus there is less potential to develop drug resistance compared to tumor cells. Targeting the tumor microenvironment in melanoma, such as matrix remodeling, or angiogenesis, is now underway, with no promising result yet [76]. But it's no doubt a promising field to investigate.

CONCLUSION

Whether melanoma cells are “born to survive” due to high endogenous Bcl-2 levels, or they are smart in escaping from therapies, melanoma seems to be a coreless disease with so many participants involved in cell survival. However, it is essential to investigate the tricks cancer cells are using to escape treatment, and find the core of the intricate pathways in tumors with specific genetic background. There has never been a better and more exciting time to work on developing combination therapies targeting multiple molecular targets and existing armamentarium of chemotherapeutics, which represent a significant progress made in understanding the biology of therapy resistance and in the management of malignant melanoma. The past decade has witnessed the discovery of new resistance mechanisms; at the same time the combination of selected chemotherapeutic drugs with molecularly targeted therapies has shown preliminary success in melanoma therapies. Ongoing studies from the bench to the bedside are bringing hope to patients with this intractable tumor. This suggests us to pursue mechanistic studies and biology-driven rational design of novel combination therapies. Our current studies should focus on the rapid translation into clinical applications of combined therapeutic strategies using chemotherapy and molecular inhibitors, and pursuing pathway-specific novel therapeutics for sensitization to existing therapies with lower toxicity, especially in a compassionate care setting where patients with malignant melanoma have such limited choices of treatment. Overcoming these challenges would offer patients a potentially effective therapeutic strategy to decrease the failure of currently available therapeutics, extend survival and improve quality of life, which is the ultimate goal.

Acknowledgments

This work was supported in part by Susan G. Komen for the Cure grant KG090860, and by grant CA72781 (R.K.S.), and Cancer Center Support Grant (P30CA036727) from National Cancer Institute, National Institutes of Health.

ABBREVIATIONS

CTLA-4	Cytotoxic T-Lymphocyte Antigen 4
MGMT	<i>O</i> ⁶ -methylguanine-DNA-methyltransferase
MMR	mismatch repair system
Bcl-2	B-cell lymphoma 2
ML-IAP	melanoma inhibitor of apoptosis
FLIP	FLICE-like inhibitory protein
Mcl-1	myeloid cell factor-1
CXCL8	CXC ligand 8, also known as Interleukin 8
VEGF	vascular endothelial growth factor
ERK	extracellular signal-regulated kinases
PDGFR	platelet derived growth factor receptor
MAPK/MEK	mitogen activated protein kinase
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa B
IKK	IκB kinase
Spry	sprouty
PI3K	phosphoinositide 3-kinase
FGF-2	fibroblast growth factor-2, also known as basic fibroblast growth factor
SEER	surveillance, epidemiology, and end-result

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Fig. 1. Potential mechanism for resistance to molecularly targeted therapies

A. Extracellular growth factors bind with cell receptors, activating a series of intricately regulated signaling. Tumor cells retain some levels of feedback inhibition, activation of one pathway might lead to inhibition of upstream substrates within the pathway, and/or the inhibition of compensatory pathways. Tumor cells depend on certain oncogenes for growth ("oncogene addiction"), these oncogene products provide good targets for molecularly targeted therapies. **B.** Molecular inhibitors suppress the overactivated growth/survival pathway, therefore tumors shrink. **C.** After prolonged treatment, induction of growth/survival signals of the same pathway and/or compensatory pathways leads to resistance against molecular inhibitors, which might be due to the disrupted feedback inhibitions. +++, overactivated signaling.

Table 1

Possible Mechanism of Resistance

Mechanism	Example	Comment	Relative therapies	Ref.
Resistance to chemotherapy:				
Drug efflux pumps	P-glycoprotein	Not detectable in most melanomas, not induced by chemotherapies		[18]
	ABCB5 (member of the P-glycoprotein family)	ABCB5 blockade enhanced doxorubicin accumulation	Doxorubicin + ABCB5 blockade	[19]
DNA repair	Elevated MGMT	Enhanced <i>O</i> ⁶ -methylguanine lesion repair after chemotherapy	Temozolomide + MGMT inactivation using gene therapy	[21,22]
	MMR deficiency	Lack of MMR activity causes deficient apoptosis in cells with the <i>O</i> ⁶ -methylguanine lesion	Extended-schedule temozolomide + decitabine	[20,21]
Defect in apoptosis	Endogenous high Bcl-2	Overexpression of anti-apoptotic proteins confers resistance to chemotherapy	Dacarbazine + oblimersen	[25,27]
Upregulation of survival signals	IL-8 & VEGF	Survival signals protect tumor cells from chemotherapy	Dacarbazine, temozolomide, or fotemustine + bevacizumab	[36,41-43]
Resistance to targeted therapy:				
Secondary mutation	Secondary <i>KIT</i> mutations after imatinib therapy	Re-activated signaling downstream of <i>KIT</i> confers resistance to imatinib		[62]
Activated downstream substrates	Recovery of ERK activity after BRAF inhibition	Re-activated MAPK pathway leads to resistance to BRAF inhibition	“Vertical” combination of inhibitors against the same pathway	[64]
Amplified upstream signals	Relief from feedback inhibition upstream of MEK induced by AZD6244	Re-activated MAPK pathway leads to resistance to AZD6244	“Vertical” combination of inhibitors against the same pathway	[65]
Rescue from compensatory pathways	Induction of PDGF, bFGF after VEGF inhibition	PDGF/bFGF-mediated signalings compensate for the inhibited VEGF signaling	“Horizontal” combination of inhibitors against parallel pathways	[70]

Table 2

Clinical Trials of Combined Therapies in Metastatic Melanoma

Regimen	Targets	Study design	Results	Comment	Ref.
Temozolomide + dacarbazine	DNA synthesis and MMR deficiency	Phase I/II		Ongoing study.	[20]
Dacarbazine +/- oblimersen	DNA synthesis and Bcl-2	Phase III (n=771)	OR 13.5 vs 7.5% PFS 2.6 vs 1.6 m	First-line treatment: Significantly increased survival from oblimersen in patients with normal serum LDH (OS 11.4 vs 9.7 m).	[27]
Temozolomide + bevacizumab	DNA synthesis and VEGF	Phase II (n=62)	OR 16.1% RD 5.0 m PFS 4.2 m OS 9.3 m	First-line treatment: Response and OS higher in the BRAF V600E wild-type patients compared to mutated.	[42]
Dacarbazine +/- sorafenib	DNA synthesis and BRAF	Phase II (n=101)	PFS 21.1 vs 11.7 w OS 45.6 vs 51.3 w	First-line treatment: Improved PFS, but no improvement in OS.	[46]
(Carboplatin + paclitaxel) +/- sorafenib	DNA synthesis and BRAF	Phase III (n=270)	OR 12 vs 11% PFS 17.4 vs 17.9 w	Progression after dacarbazine or temozolomide; No improvement in OR, PFS, or OS.	[47]
Temozolomide + RAD001	DNA synthesis and mTOR	Phase II (n=48)	OR 8% PFS 2.4 m OS 8.6 m	Pts had prior systemic therapy (excluding study agents); No therapeutic advantage over temozolomide-alone historic controls.	[55]
Paclitaxel + carboplatin + bortezomib	DNA synthesis and NF- κ B	2-stage Phase II (n=17)	PR 11.8% PFS 3.2 m OS 7.0 m	Pts received at most 1 prior chemotherapy; Insufficient clinical efficacy yet significant toxicity prevent second-stage accrual.	[61]
Bevacizumab + everolimus	VEGFR and mTOR	Phase II (n=57)	OR 13% PFS 4 m OS 8.6 m	Pts received up to 2 prior chemo and/or immunotherapy; Moderate activity, possibly no additive effect of everolimus to bevacizumab.	[72]

Abbreviations: OR, overall response; PR, partial response; PFS, progression-free survival; OS, overall survival; RD, response duration.