Melanoma: Narrowing the sights on an evasive enemy

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Abstract

Much of the resistance of melanoma to immunotherapy, radiotherapy and cytotoxic treatment is due to an impressive array of molecular defences that derive ultimately from the essential molecular structure of the melanocyte and its biological requirement for defence against apoptosis. The exploration of melanoma susceptibility genes like CDKN2A, CDK4 and MC1R has highlighted a number of key pathways in melanomagenesis. Others have been revealed by a systematic exploration of somatic chromosomal and genetic abnormalities in naevi and melanomas.

Constitutive activating mutations in Nras and BRAF are the most common somatic oncogene mutations in melanoma, indicating the importance of the Ras-RAF pathway in the deregulation of melanocyte growth. Downstream targets of this signalling pathway include the cell cycle regulator cyclin D1 and the melanocyte-specific transcription factor, Mitf. Newly tested inhibitors of the RAF pathways, like sorafinib, may sensitize melanoma cells to cytotoxic attack.

Inhibitors of apoptosis, like Bcl-2 and Mcl-1 are frequently over-expressed in established melanomas. Antagonists of the Bcl-2 family of proteins offer exciting potential for synergy with cytotoxic drugs. Other pathways highly relevant to melanoma tumour progression and its targeted therapy include the PI3K-PTEN-Akt-mTOR pathway and pathways of angiogenesis, which may be inhibited by molecules like bevacizumab and bosentan. Considerable hope is also provided by recent Phase II trials with monoclonal antibodies such as ticilimumab and ipilimumab, which inhibit immnosuppressive cell signalling.

Metastatic melanoma

Melanoma is remarkable for variability in its pattern of spread. In selected patients the disease remains confined to loco-regional lymphatics for extended periods and some such patients have achieved long-term remissions even after hind-quarter amputation. In others, haematogenous dissemination occurs early and widely. Certain patients may have many years between the primary presentation and the development of metastases. Others may have serial presentations, each with relatively isolated metastases, remaining in clinical remission for many years between serial metastasectomy. Some patients present with fulminant disease in many organs simultaneously with a very rapid demise. The disease may have particular affinity for a specific organ or organs. Thus, certain individuals may develop extensive pulmonary involvement without ever developing liver metastases. Others will succumb to cerebral metastases without any extra-cranial disease. This wide spectrum of variability confounds the ability to make accurate prognosis. However, some broad guidelines may be drawn from statistical analyses of large numbers of patients who have died from metastatic melanoma.

The most common initial sites of metastasis are skin, subcutis, distant lymph nodes, lung, liver, bone, small intestine and brain. Approximately 4% of patients present with widespread metastases as the initial manifestation of metastatic disease. About 15% of patients presenting with metastatic melanoma in Australia have no identifiable primary site (occult primary melanoma). These patients show no discernible differences in pattern or prognosis from those with known primary sites. Psycho-social factors that show independent correlation with longer survival from metastatic melanoma include a positive perceived outcome from treatment, minimisation of perceived threat, anger and presence of a stable partner.

In a recent revision of the American Joint Committee on Cancer (AJCC) Staging System for Melanoma, Stage IV melanoma has been subdivided into three prognostic groups. The M1 category includes those patients with lymph node and/or subcutaneous metastases and has a median survival of >12 months and a two-year survival of 15-20%. The M2 category has pulmonary metastases +/- subcutaneous or lymph node involvement, and has a median survival of 9-12 months and a two-year survival of 10%. The M3 category has other visceral involvement, or any site with an elevated serum lactate dehydrogenase (LDH). Although non-specific, the LDH is an independent prognostic factor for patients with metastatic disease and is frequently used in stratifying patients in clinical trials. M3 patients have a median survival of four to six months and a two-year survival of 5%.

Current status of drug treatment for metastatic melanoma

Metastatic melanoma is relatively resistant to treatment with cytotoxic drugs. No form of systemic therapy prolongs overall survival. Single agent treatment with dacarbazine (dimethyl triazeno imidazole carboxamide or DTIC), discovered in 1961, has been standard best systemic therapy for metastatic melanoma since the early 1970s and its use in Australia was pioneered by Gerald Milton and William McCarthy at Sydney Melanoma Unit. Partial responses to dacarbazine and two other commonly used single-agent cytotoxic drugs, temozolomide and fotemustine, occur in less than 25% of treated patients and complete responses in less...
than 5%. However, in recent Phase III prospective randomised trials, in which dacarbazine has been standard therapy, response rates were 6.8-13%.14,15 The use of combinations of cytotoxic drugs, such as the widely used ‘Dartmouth’ regimen - consisting of cisplatin, dacarbazine, carmustine and tamoxifen, show no advantage over dacarbazine alone.15 The addition of potent cytokines like interleukin-2 and interferon-alpha to cytotoxic drugs (“biochemotherapy”) produces slightly higher transient response rates, but at considerable cost in toxicity and with no overall survival benefit.15

Predictors of response to dacarbazine include good performance status and disease confined to the skin, subcutis, lymph nodes and lungs.19,20 The median duration of response is five to six months.12 Only 1-2% of patients treated with dacarbazine sustain long-term complete responses, but those in complete remission more than two years after treatment tend not to relapse.12,21

A major advantage of dacarbazine is that it is simple, ambulatory treatment, being administered intravenously on a three week schedule. It is associated with minimal toxicity when given with serotonin receptor antagonist anti-emetics. Alopecia does not occur with dacarbazine therapy and the drug is minimally myelosuppressive. Acute photosensitivity reactions may occur.

Both temozolomide and dacarbazine are prodrugs of the active alkylating agent 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MTIC). Unlike dacarbazine, which requires metabolic activation, temozolomide spontaneously converts to MTIC under physiological conditions. It has the advantage over dacarbazine of being orally administered. However, it is expensive and there is little difference from dacarbazine in toxicity and no difference in activity against metastatic melanoma.14 Temozolomide is not available under the Australian Pharmaceutical Benefits Scheme (PBS) for metastatic melanoma. The fact that temozolomide penetrates the central nervous system is widely used to justify its preferential use over dacarbazine in patients with brain metastases. However, the blood-brain barrier is nearly always disrupted in cerebral metastases from melanoma, demonstrated by the fact that they are nearly always strongly contrast-enhancing.

Fotemustine was superior to dacarbazine in inducing tumour responses in a Phase III trial, but its use is limited by severe and occasionally unpredictably protracted myelosuppression. Fotemustine, which is lipid soluble, also reaches high concentrations in the cerebrospinal fluid. It is PBS listed for metastatic melanoma.

**Refining existing chemotherapy**

The cytotoxic activity of the active metabolite of dacarbazine and temozolomide is probably mainly mediated through methylation of DNA at the O6 position of guanine bases. The DNA repair enzyme O6-alkylguanine-DNA alkyltransferase (AGT) is thought to be the main determinate of resistance to dacarbazine and temozolomide. AGT detects and specifically removes alkylated base damage, effectively reversing cytotoxicity. Phase II trials are currently underway with lomeguatrib, an agent that inhibits AGT and therefore may sensitise melanoma cells to these cytotoxic drugs. There is no rationale however, for this approach to be tumour specific and improved therapeutic ratios may therefore not be achieved.

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**Figure 1. Molecular targets in melanoma: the RAS/RAF pathway**

Targets undergoing experimental inhibition in melanoma therapy (diamonds) are:

1) ligands for receptor tyrosine kinases (bevacizumab);
2) receptor tyrosine kinase inhibitors (imatinib);
3) farnesyl transferase inhibitors (tipifarnib);
4) RAF inhibitors (sorafenib);
5) MEK inhibitors;
6) mTOR inhibitors (temsirolimus). Red asterisks denote genes commonly altered in established melanomas.

Legend: RAS: retrovirus associated sequence oncogene; BRAF: v-raf murine sarcoma viral oncogene homolog B1; MEK, mitogen-activated protein kinase kinase (MAP2K); ERK, extracellular signal-regulated kinase, also known as mitogen-activated protein kinase (MAPK); Mitf: microphthalmia transcription factor; PTEN: phosphatase and tensin homolog; PI3K: phosphatidylinositol-3 kinase; Akt: murine v-akt oncogene homologue, also known as protein kinase B; mTOR: mammalian target of rapamycin.
Targeted drug treatment

The molecular pathways so far identified as being central to the regulation of melanoma cellular proliferation and apoptosis are the subjects of intense investigation for their potential as therapeutic targets.

The Ras/RAF pathway

Growth factors, such as stem cell factor (SCF), fibroblast growth factor (FGF) and transforming growth factor-alpha (TGF-alpha) are produced by the action of solar radiation on melanocytes and surrounding keratinocytes and fibroblasts (Figure 1). Resulting signals are transduced and amplified via the kinase signalling pathways NRas, then the RAF kinases BRAF and c-RAF and subsequently MEK-ERK-Mitf, or PI3K-Akt-mTOR. Mitf triggers the transcription of a suite of genes involved in regulation of cellular proliferation, apoptosis and migration. mTOR promotes the translational efficiency of growth regulatory gene products. PI3K is inhibited by PTEN.

Constitutive activating mutations in NRas, BRAF and PTEN are among the most common somatic oncogenic mutations in established melanomas, indicating the importance of these pathways in the deregulation of melanocyte growth:24-26 The pan-RAF inhibitor sorafenib (BAY 43-9006) has minimal activity in metastatic melanoma as a single agent,27,28 but in a Phase II trial in combination with the cytotoxic drugs carboplatin and paclitaxel in patients with metastatic melanoma, 60% of whom had received prior therapy, 14 of 35 patients achieved partial responses.29 Response did not depend upon the presence of an activating RAF mutation,30 as sorafenib is “promiscuous” in its effects against RAF family members. The combination of carboplatin/paclitaxel +/- sorafenib is now in Phase III clinical trial in many centres in Australia. Cutaneous reactions constitute the major toxicity of sorafenib.

Apoptosis regulators

The genetic locus CDKN2A is a melanoma susceptibility gene31 and it is also altered in a large number of established melanomas. It produces two protein products, p16INK4A (p16) and p14ARF (ARF) (Figure 2). When defective, p16 is unable to inactivate CDK4 and 6, which phosphorylate Rb, releasing the transcription factor E2F leading to cell cycle progression.32

The molecule usually central to the DNA damage response, p53, is rarely altered in melanoma. However, alterations and gene deletions affecting ARF permit degradation of p53 by releasing its binding partner hdm2.33 This probably contributes to the natural resistance of melanoma cells to apoptosis (programmed cell death) in response to cytotoxic, radiation and immunological attack. As a further defence, melanoma cells frequently express high levels of the anti-apoptotic Bcl-2 family of proteins which include Bcl-XL and Mcl-1.34 These are important molecular vulnerabilities in melanoma. Oblimersen is an antisense oligonucleotide to Bcl-2, which is over-expressed in many melanomas. It was the first of this class of drugs to enter clinical trial.

Figure 2: Molecular targets in melanoma: apoptosis and cyclin kinase inhibitors

Targets undergoing experimental inhibition in melanoma therapy (diamonds) are:

1) antisense oligonucleotide to bcl-2 (oblimersen, “Genasense”);
2) CDK inhibitors (flavopiridol). The asterisk denotes a gene commonly altered in established melanomas.

Legend: CDKN2A: cyclin dependent kinase inhibitor-2A gene; CDK: cyclin dependent kinase; Rb: retinoblastoma protein; p16: p16INK4A, 16,000 MW protein; ARF (p14ARF): 14,000 MW alternate reading frame protein; bcl-2: B-cell lymphoma derived sequence 2; Mcl-1: myeloid cell leukaemia sequence 1; hdm2: human double minute chromosome-associated protein 2; E2F: E2F cell cycle regulated transcription factor; p21: 21,000 MW protein; RB: retinoblastoma protein; Bax: Bcl-2 associated X protein.
in melanoma. In the largest Phase III trial ever conducted in metastatic melanoma (771 patients), incremental benefits in progression-free survival and response rate were demonstrated for the combination of dacarbazine plus oblimersen versus dacarbazine alone. Overall survival benefit was similar for the two arms, but a pre-stratified subgroup of 500 patients with normal LDH showed a statistically significant survival benefit in the combination arm and seven of 11 patients with complete remission on the combination arm remained disease free at >24 months. However, this study was marred by failure to select patients with Bcl-2 over-expressing tumours. Furthermore, much better inhibitors of the Bcl-2 family of proteins are now in advanced development. Many of these specifically target the BH3 domain of the Bcl-2 family of proteins, releasing bound pro-apoptotic proteins, like Bax, and thereby sensitising cells to cytotoxic attack. Native inhibitors of Bcl-2, like Bim and Noxa, may also be inducible with proteosome inhibitors like bortezomib. It is likely that a multi-pronged attack on the redundant anti-apoptotic pathways in melanoma cells will be necessary to achieve significant tumour remissions.

**Anti-angiogenic agents**

Thalidomide has a variety of anti-tumour effects, which include immuno-modulation and anti-angiogenesis. It has been tested in small cohorts of pre-treated patients with metastatic melanoma, but failed to show convincing evidence of activity. A large Phase III trial of a potent thalidomide analogue, lenalidomide, showed no benefit over placebo. Thalidomide has been tested in combination with a number of agents, including interferon-alpha and dacarbazine. Only a small trial in combination with temozolomide showed some trend towards improved response rates and survival in a preliminary report. Bevacizumab is a monoclonal antibody against Vascular Endothelial Growth Factor (VEGF), a mediator of tumour angiogenesis. It has shown significant benefit when combined with chemotherapy in colorectal cancer. Phase II trials in metastatic melanoma showed good tolerability and some responses.

The monoclonal antibody MEDI-522 targets integrin alphaVbeta3, which plays a critical role in angiogenesis, tumour growth and metastasis and is highly expressed in melanoma. Preliminary results of a randomised Phase II trial of MEDI-522 with or without dacarbazine in previously untreated patients suggest potential clinical activity of MEDI-522. Bosentan, an endothelin receptor antagonist used in the treatment of primary pulmonary hypertension, may modulate anti-proliferative and anti-angiogenic activities in melanoma. A Phase II Trial of bosentan in patients with metastatic melanoma suggested some clinical activity and Phase III Trials are now underway testing the combination of dacarbazine with or without bosentan.

**Immunomodulators**

Immunotherapy continues to be investigated intensively in metastatic melanoma and attempts are being made to target the major defences that melanoma mounts against an effective immune response. These defences include development of host tolerance to melanoma antigens, production of immunosuppressive factors by melanoma cells and clonal selection of melanoma cells that are resistant to apoptosis. Despite the presence of detectable immune responses in 30–60% of patients, tumours regress in only a few vaccine-treated patients with metastatic disease. The cytokine interleukin 2 has FDA approval for high-dose intravenous use in treating metastatic melanoma, on the basis of durable responses in some patients. However, the overall response rate is low (16%) and systemic toxicity is high.
and includes hypotension, capillary leak syndrome, sepsis and renal failure. Innovative immunotherapy approaches include the use of monoclonal antibodies such as ticilimunab (CP-675206) and ipilimumab (MDX-010) to inhibit immunosuppressive cell signalling (Figure 3). Both these monoclonal antibodies have been associated with durable remissions in patients with metastatic melanoma, and are in Phase II and III Trials in many Australian centres. The major toxicity involves autoimmune-type reactions in skin, colon and endocrine organs.

Conclusion

The field of experimental therapies for melanoma has never been richer. Melanoma medical oncologists face increasingly difficult decisions about the choice of agents for clinical trials. The traditional endpoints of Phase II and Phase III trials (tumour response and survival) are stringent in the context of highly advanced tumours with an extensive repertoire of defences against cytotoxic attack. This is particularly so for biological agents, like anti-angiogenic drugs, that are likely to induce stable disease rather than obvious tumour regressions. New trial platforms are urgently required. One such design is ‘Treat, Resect, Analyse for Melanoma’ (TRAM), which proposes the use of relatively short-term biological response indicators in patients treated for short periods (several weeks) prior to surgical resection of in-transit or lymph node metastases. This type of clinical design would also permit the testing of multiple novel agents simultaneously, allowing selection of only the most promising for formal Phase II testing.

Advanced metastatic melanoma has attained its notoriously treatment-resistant phenotype through acquisition of a bewildering array of molecular advantages. An understanding of the details of these specific molecular abnormalities and the means for targeting them is finally enabling the sights to be narrowed on an elusive enemy.

References


