dinitrochlorobenzene have been used for melanoma therapy in both preclinical and clinical studies (Terheyden *et al.*, 2007; Wack *et al.*, 2002). However, as elegantly demonstrated by van den Boorn *et al.* (2011, this issue), monobenzone is more specific than dinitrochlorobenzene because it is active only in pigmented cells. Moreover, it is already available as a registered drug with a well-established toxicity profile. It is hoped the present report by van den Boorn *et al.* will prompt clinical trials testing this concept for immunotherapy of melanoma.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related article on pg 1291

Fisetin: A Natural Fist against Melanoma?

Jack L. Arbiser[1] and David E. Fisher[2]

Melanoma has now become the subject of targeted therapies, based upon the high prevalence of B-raf mutations in melanoma. However, while initial responses to B-raf inhibitors are impressive, resistance is extremely common, suggesting that melanoma is not addicted to B-raf. In their report, Syed *et al.* demonstrate that fisetin, a natural product without well established mechanisms, has activity against melanoma. Their report suggests that "nontargeted therapies" need to become part of our armamentarium against melanoma, given that targeted therapies do not target all of the pathways required for melanoma growth.

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Several common genetic events in melanoma have been elucidated in recent years. These include oncogenic events, such as the B-raf V600E mutation, amplification of the microphthalmia transcription factor (MITF), and Nras mutations. Common tumor suppressor events include inactivation of the p16ink4a tumor suppressor gene, often by deletion or hypermethylation, and loss and/or mutation of PTEN, a common event in melanomas with B-raf mutations. Melanomas have been shown to exhibit differing genetic pathways to reach these mutations, depending on their anatomic location. The pathways involve a complex interplay among pigmentation genes such as melanocortin 1 receptor, c-kit, and DNA repair genes.

As our understanding of melanoma genetics has increased, so has our understanding of the signaling pathways relevant to melanoma progression. The current concept of atypical nevi giving rise to a noninvasive melanoma (radial growth phase) and then to an invasive and potentially metastatic phenotype has been confirmed with distinct signaling events, although melanomas may also arise in the absence of preexisting nevi. Atypical nevi are clonal neoplasms with a high frequency of B-raf mutations, but despite the activation of B-raf, they do not exhibit stable activation of MAP kinase (p42/44 ERK). Radial growth melanoma demonstrates high levels of expression of activated MAP kinase, as well as of Id-1 and telomerase. Invasive melanoma

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Clinical Implications

- Forty years ago, novel and effective therapeutic agents were derived most often from biologically active natural products, usually without knowledge of mechanisms.
- More recently, new information about regulatory aspects of the human genome have led to the development of "signaling-specific" agents for cancer, but only some of them have proven useful.
- Syed *et al.* now demonstrate antimelanoma activity of fisetin, a tetrahydroxyflavone found in the Rhus family (including mangoes), suggesting that the older methods may retain significant merit.

demonstrates high levels of Akt activation, through either loss of PTEN (thus causing Akt1 activation) or amplification of Akt3. Akt activation has been shown to be transforming for human melanoma, resulting in the activation of reactive oxygen. Indeed, the phenotype of loss of p16ink4a, Akt activation, and reactive oxygen generation has been termed the "reactive oxygen–driven tumor," and reactive oxygen is probably a major source of NF-κB activation through oxidative inactivation of IκB.

Although the genetics and signaling of melanoma are increasingly well understood, directing treatment against these events has lagged. The discovery of B-raf mutations led to clinical testing of a first-generation B-raf inhibitor, sorafenib. Although relatively potent, sorafenib lacks selectivity, and its clinical activity against melanoma in humans was disappointing. Nevertheless, this lack of specificity turned out to be beneficial because sorafenib is a potent inhibitor of VEGFR2 and is therefore used to treat the highly angiogenic neoplasm renal cell carcinoma. More selective mutant-specific B-raf inhibitors have been developed, including PLX4720, and these have entered clinical trials. PLX4032 has resulted in remission of advanced melanomas in ~80% of patients, but not in cures (Flaherty *et al.*, 2010).

Single-agent targeted therapies have only rarely (if ever) cured patients with cancer. The clinical efficacy of B-raf(V600E) selective antagonists has met with great enthusiasm in recent months. Yet the precise mechanism of killing remains uncertain, and the magnitude of clinical responses seems to be less than might have been predicted, despite strong suppression of B-raf(V600E). Moreover, the failure of B-raf inhibition alone to produce more substantial responses or cure suggests that other lesions/pathways within melanomas play roles in controlling tumorigenic behavior. Indeed, PLX4032-resistant melanomas arise all too readily, displaying a variety of mechanisms of resistance, including Nras mutations, Craf overexpression, COT amplification, and activation of other tyrosine kinase receptors. This suggests that B-raf-independent pathways are also likely to play important roles in melanoma—roles that may require broader therapeutic approaches.

In this issue, Syed *et al.* demonstrate antimelanoma activity of fisetin, a tetrahydroxyflavone that is found in the Rhus family (including mangoes and other plants). Flavones are a family of compounds composed of polyphenols, which include other biologically active compounds, such as curcuminoids, epicatechins, resveratrols, and honokiols. Syed *et al.* have demonstrated that fisetin exhibits several activities that are potentially beneficial in treating melanoma. These include downregulation of nuclear β-catenin with concomitant downregulation of Wnt signaling pathways, resulting in suppression of MITF, an amplified oncogene in ~20% of metastatic melanomas. Fisetin-induced MITF suppression was found to be dependent on glycogen synthase kinase activity. Importantly, fisetin was active in xenograft models of melanoma at doses of 45 mg/kg.

Despite the extensive studies of polyphenols, this vast group of compounds is underrepresented in clinical medicine. The only polyphenol-based drug currently used is Veregen (15% polyphenon E), for the treatment of external genital warts. Lack of enthusiasm for the development of polyphenol-based drugs appears to be widespread among drug companies and academic institutes as well as in the National Institutes of Health–based Developmental Therapeutic Program.

The most active decade in neoplastic drug development was 1960–1970. During this time, most of the currently available antineoplastic agents were synthesized, especially antimetabolites and alkylating agents. Investigators knew little of the mechanisms of actions of drugs, they had not yet sequenced any genomes, and they performed less sophisticated assays. A few cell lines were used, and if a compound showed activity *in vivo*, animal testing was performed, often on the rapidly growing L1210 leukemia cell line. They did not know whether the observed activities were due to apoptosis, autophagy, mitotic catastrophe, or cell cycle arrest. Promising animal data were followed by clinical trials. Drugs that are still useful today—e.g., methotrexate, 5-fluorouracil, cisplatin, and vinca alkaloids—were all developed in this way. Efficacy was the main concern, and mechanisms were studied later.

By contrast, the decade 2000–2010 witnessed an explosion of new information. Investigators sequenced numerous genomes, including the human genome. One can take a portion of a human melanoma specimen from a patient and find not only B-raf or Nras mutations but also amplifications and mutations in multiple tyrosine kinases in a single specimen. Potent inhibitors of each of the tyrosine kinases, for example, as has been done with B-raf and EGFR, may be taken to the clinic. This more sophisticated approach has brought significant clinical benefit to small groups of cancer patients, although cure remains rare. Many investigators have been profoundly energized by this progress because it is mechanism based and therefore highly predictive. It also permits a significantly more focused approach to understanding mechanisms of resistance to such targeted small molecules. Yet, despite the elegance of this approach, the ultimate significance of these inhibitors has lagged behind some of the older standbys: methotrexate, Cytoxan, and cisplatin. An important question is whether investigators are accomplishing less but with more resources.

One component to this question involves the decreased use of natural products as drugs or drug leads. The movement away from natural products probably reflects the complex synthetic chemistry and medicinal chemistry requirements to optimize delivery and oral bioavailability and to minimize toxicity. Yet natural products tend to produce significant biological activity, presumably because nature has evolved three-dimensional configurations that fit the "grooves and turns" of biochemically important molecules. In addition, many natural products are likely to target multiple species, thereby making the elucidation of their mechanism of action more challenging to dissect.

Although it is difficult to fully define mechanism, we know it when we see it. Mechanism is often presented as a crystal structure of a compound nestled in the crevices of a mutant protein, with hydrogen bonds of the target compound closely approximating critical catalytic residues in the target protein. Polyphenols do not fit this conception of mechanism, because in most cases, including that of fisetin, we don't even know the target protein. In fact, we don't know whether it is a single target protein or multiple target proteins or whether the compounds inhibit protein–protein interactions. We do know that the compound has downstream signaling activities that are important, such as upregulation of E-cadherin and downregulation of c-myc and N-cadherin. Thus, efficacy in the absence of knowledge of preconceived mechanism makes investigators uncomfortable, because it diminishes predictability. Apparently the same may be true of regulatory agencies, such as the Food and Drug Administration in the United States.

Better treatments for melanoma are possible, and they may be right around the corner. Perhaps a rejuvenated interest in natural products would provide a boost—especially if combined with modern methods of target identification, pathway analysis, biomarker discovery, and combinatorial treatments. The power of nature's tools is remarkable. Investigators understood this 40 years ago; revisiting the concept more vigorously might benefit patients.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Fumarate Esters as Angiogenesis Inhibitors: Key to Action in Psoriasis?

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Fumarate esters—an oral therapy for psoriasis—are used primarily in Europe, but not at all in the United States. Given that biological therapies are exceedingly expensive and pose an increased risk for infections and malignancy, the need for safer and less expensive therapies for psoriasis is compelling. Nonbiological therapies for psoriasis, including methotrexate and systemic retinoids, carry potentially severe side effects and relatively high cost. Fumarate, a natural product that is generated internally in humans during the Krebs cycle, is an attractive alternative to these therapies. However, the mechanism for fumarate's activity in psoriasis remains unknown.

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Two reports in this issue of the *Journal of Investigative Dermatology* shed light on possible mechanisms of action of fumarate esters. Meissner *et al.* (2011) demonstrate that dimethylfumarate causes a decrease in tube formation in human endothelial cells *in vitro*. Analysis of angiogenic factors in endothelial cells treated with dimethylfumarate revealed a decrease in vascular endothelial growth factor receptor 2 (VEGFR2) protein but not in VEGFR1 or neuropilin-1. Because VEGFR2 transcription is dependent on the Sp1 transcription factor, the researchers analyzed the effect of dimethylfumarate and demonstrated decreased binding of the Sp1 transcription factor to the VEGFR2 promoter. García-Caballero *et al.* (2011) also demonstrated inhibition of tube formation on Matrigel by dimethylfumarate, but not by monomethylfumarate or free fumaric acid itself. They found that dimethylfumarate does not inhibit the kinase activity of VEGFR2, and they demonstrated antiangiogenic activity in two *in vivo* models: the quail chorioallantoic membrane and a transgenic zebrafish in which the endothelial cells are labeled with green fluorescent protein. Thus, it is safe to say that angiogenesis inhibition probably plays a role in the activity of dimethylfumarate.

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