A recent review of our last 35 years of phase 2 clinical trials for metastatic melanoma demonstrates that there has been no statistically significant improvement in 1-year overall survival or 6-month progression-free survival with any intervention tested in multicenter trials to date (1). An understanding of the basis of failure for these tested agents has generally been lacking, and few of these trials involved laboratory corollary studies of mechanism. Many cell survival and death pathways that are dysregulated in melanoma [reviewed in (2)] and other new pathways that might explain the refractoriness of melanoma to current treatments are under exploration (3). Melanoma repopulating (stem) cells that are resistant to chemotherapy may also be culpable (4). Targeting molecules critical for melanoma cell survival in rational combinations may improve the efficacy of chemotherapy, and this is currently being tested in relation to sorafenib.

Nuclear factor kappa B (NF-κB) is the general designation for a family of dimeric complexes of members of the Rel protein family, which are important in melanoma [reviewed in (5,6)]. This transcription factor is activated in response to diverse stimuli, including proinflammatory cytokines, “nonsell” antigens, lymphocyte mitogens, and cellular stress (ultraviolet and ionizing radiation, as well as chemotherapeutic agents). In resting cells, NF-κB remains cytoplasmic through its association with one of three inhibitory IκB proteins (IκBα, IκBβ, or IκBε). It can be activated through at least three different pathways. The canonical pathway exhibits rapid kinetics and is dependent on the stimulation of the inhibitor of IκB kinase (IKK), a complex composed of the enzy-
matically active subunits α and β and the regulatory subunit γ/NEMO. This pathway controls the activation of p50 heterodimers and is essential for cell survival and innate immunity. The noncanonical pathway exhibits slower kinetics and uniquely uses the IKKα protein to control the proteolytic processing of p100 into the active p52 isoform. This pathway is important for development of lymphoid organs and adaptive immune response. Finally, the incompletely understood double-strand DNA break pathway results in a number of different posttranslational modifications of IKKγ/NEMO, leading to its export from the nucleus back to the cytosol, where it activates IKK and causes NF-κB activation. Activation of the canonical and the double-strand DNA break pathways results in IkB phosphorylation followed by polyubiquitination of IkB at Lys-48 and subsequent proteasomal degradation of IkB. In the noncanonical pathway, the p100 itself acts like an IkB protein and retains its partner in the cytosol. Proteasomal degradation of IkB proteins frees NF-κB dimers, which enter the nucleus where they coordinate the transcriptional activation of a large set of genes that encode proteins involved in regulation of cell death (eg, inhibitors of apoptosis proteins and bcl-2-like proteins), proliferation (cycin D1- and cyclin-dependent kinase 2), adhesion (cell adhesion molecules and selectins), cytokine (interleukin-1 [IL-1], IL-2, IL-6, and tumor necrosis factor-α [TNF-α]) and chemokine (MIP-1α, MCP1, RANTES, and eotaxin) production, invasion, and angiogenesis.

In this issue of the Journal, Schön et al. (7) present a thorough series of in vitro and in vivo investigations of a small-molecule inhibitor of IKK catalytic subunit beta (IKKβ), termed kinase inhibitor of nuclear factor-kappa B-1 (KINK-1), which suppresses NF-κB activity in melanoma cells. KINK-1 is a highly selective for the different subunits of IKK (concentration that causes 50% inhibition of growth [IC_{50}] = 8.5 nM [IKKβ], IC_{50} = 250 nM [IKKα]) (8) and does not inhibit other enzymes tested. The inhibitor suppressed constitutive and induced phosphorylation of IkB, as well as the expression of known downstream NF-κB targets represented in the 54K gene Affymetrix platform. Short (up to 24 hours) exposure of melanoma cell lines to KINK-1 alone did not affect cell proliferation or apoptosis except at higher concentrations (≥10 μM). However, KINK-1 acted synergistically with multiple cytotoxic and cytostatic agents in vitro and decreased the number of lung metastases in a preclinical melanoma xenograft model without inducing secondary drug resistance.

Targeting the NF-κB signaling pathway is not a novel concept. Many well-established drugs interfere with NF-κB signaling with variable success in the clinic. These drugs may inhibit membrane signaling from major cytokines that activate NF-κB, such as TNF-α; inhibit activation or the assembly of individual IKK subunits to prevent phosphorylation of IkBα; prevent proteasomal degradation of IkBα; or interfere with NF-κB binding to DNA [reviewed in (9)]. In metastatic melanoma, three drugs that target NF-κB—bortezomib, arsenic trioxide, and thalidomide—have previously been tested and failed to demonstrate clinical antitumor efficacy. It is unclear whether their low affinity for the target or distal impact on NF-κB signaling accounts for their lack of efficacy. Targeting IKKβ is a selective and effective approach to inhibition of NF-κB signaling for at least two reasons. First, there is little evidence that IKKβ phosphorylates proteins that are not involved in NF-κB signaling. Second, IKKβ is preferentially involved in the canonical pathway, avoiding unwanted side effects related to inhibition of the noncanonical pathway, such as adaptive immunity. In fact, the recent report of the use of a less selective IKKβ inhibitor than KINK-1 (BMS-345541, IC_{50} = 300 nM) in melanoma provided proof of the concept that targeting IKKβ in vitro and in vivo is a promising strategy in melanoma (10). Guided by the evidence that NF-κB is a common denominator of response to chemotherapeutic agents, Schön et al. (7) have shown that inhibiting IKKβ potentiates the cytotoxic effect of a range of chemotherapeutic agents for melanoma in vitro and in vivo.

The safety and efficacy results of the first clinical trials of the multiple IKKβ inhibitors currently in development are awaited with interest. However, the mechanism of action of these agents raises a number of issues. The first relates to the obvious association between the canonical pathway of NF-κB activation and chronic inflammation as well as innate immunity. These two issues cannot be studied in preclinical in vivo melanoma xenograft model systems. Although suppressing chronic inflammation may be desirable, the potential interference with activation and differentiation of lymphocytes, macrophages, dendritic cells, and granulocytes for antitumor immune responses may have adverse consequences. Second, if IKKβ inhibitors prove not to be successful against metastatic melanoma due to immunosuppression that may be associated with these agents, this does not preclude potential benefits earlier in disease progression, either in the adjuvant setting or as chemoprevention. A recent case–control study in patients with primary cutaneous melanoma who were compared with age-, sex-, and neighborhood-matched control subjects showed decreased melanoma incidence with extended use of nonsteroidal anti-inflammatory agents, which are weak inhibitors of IKKβ (11). Third, it is interesting that the less selective IKKβ inhibitor BMS-345541 was more cytotoxic to melanoma cells in vitro and in vivo, compared with KINK-1 when administered alone. Does that imply that highly selective IKKβ inhibitors may be less toxic and less effective? Fourth, although Schön et al. (7) noted no secondary drug resistance following reexposure of melanoma cells from tumors upon reexposure to KINK-1, the counter-regulatory response of melanoma cells following IKKβ inhibition will be of interest. Extensive preclinical research upon the impact of the proteasome inhibitor and inhibitor of NF-κB signaling bortezomib in melanoma suggests that unless all proteins that protect melanoma cells against death are targeted in combination, the treatment will be clinically ineffective—as in the case of bortezomib (12). Finally, not all chemotherapeutic agents tested by Schön et al. (7) showed synergy with IKKβ inhibition.

Cytotoxic drug sensitivity of melanoma in vitro may not be dependent upon NF-κB, and we have recently shown that suppression of Ubc9, the single SUMO E2–conjugating enzyme in melanoma cell lines, enhances the cytotoxicity of temozolomide, cisplatin, and paclitaxel (3). We have found that Ubc9 suppression does not alter p65 levels, although it increased rather than decreased NF-κB activity, based on a NF-κB response element luciferase assay (S.J. Moschos, 2008, unpublished data). This suggests that further research should be conducted to define the spectrum of chemotherapeutic–or molecularly targeted (to proteins other than IKKβ) agents with which IKKβ inhibitors should be combined.
In summary, the study by Schön et al. (7) is the first to describe the preclinical efficacy of a highly selective IKK\(\beta\) inhibitor in melanoma. Because no clinical studies of IKK\(\beta\) inhibitors as treatments for cancer have been conducted to our knowledge, it is hard to predict the ultimate role of IKK\(\beta\) inhibitors. These new agents may be most beneficially combined with chemotherapeutic agents to which melanoma has been refractory in the past. Obvious concerns regarding the “collateral damage” that may result from their inhibition of the immune system, and potential enhancement of tumor escape mechanisms, may lead to their deployment at earlier stages of melanoma progression, and the clinical exploration of these agents will ideally be addressed through carefully designed clinical trials that evaluate both the antitumor and immunologic consequences of this new family of agents.

References