Major advances in the treatment of advanced melanoma over the past 3 years are directly linked to advances in our understanding of how activating mutations in canonical signaling and tumor-mediated T-cell dysfunction moderate melanoma tumorigenesis and progression. Deep sequencing approaches established that activating mutations in MAPK pathway constituents (BRAF/NRAS) are common, mutually exclusive, and carry oncogenic potential in human cancers (1). RAS mutations (typically at G12, G13, or Q61 residues) impair GTP hydrolysis, resulting in constitutive activation of GTP-bound RAS. BRAF is a serine/threonine protein kinase, and mutations (most commonly a glutamic acid substitution for valine at codon 600, V600E) occur within the kinase domain’s activation segment. Oncogenic BRAF and NRAS mutations result in constitutive activation of downstream targets, including MAPK/ERK kinases 1 and 2 (MEK1/MEK2), and unrestrained proliferation. Seminal work by Curtin and colleagues identified distinct patterns of mutations in chronically sun-damaged skin (higher mutation load, lower BRAF/NRAS–mutant incidence) and intermittent sun-exposed skin (lower mutation load, BRAF/NRAS mutant)—underscoring the concept of melanoma as a heterogeneous disease with multiple genetically distinct subtypes (2). Concurrently, Allison and Honjo identified CTLA-4 and PD-1 to be key negative regulatory immune checkpoints in melanoma (3, 4). Several other agents in each class are under evaluation, including the BRAF inhibitor encorafenib (LGX818; Array BioPharma); MEK inhibitors cobimetinib (GDC-0973/SL18; Exelixis) and binimetinib (MEK162; Array BioPharma); PD-1 inhibitors AstraZeneca), atezolizumab (MPDL3280A; Roche), and avelumab (MSB0010718C; Merck).

The use of monoclonal antibodies targeting inhibitory immune checkpoints in melanoma is marked by relatively low response rates that, however, tend to be durable. Pooled analyses from multiple phase II/III trials reveal that ipilimumab has a lower response rate (11%–15%) compared with nivolumab (30%–35%) and pembrolizumab (45%–50%), with commensurately doubled 2-/3-year survival rates for PD-1 inhibitors compared with ipilimumab (5). Combined inhibition of both CTLA-4 and PD-1 increases response rates (to ~50%) and median progression-free survival (PFS), albeit with significant attendant toxicity (55% grade 3/4 adverse events; ref. 5). Conversely, BRAF inhibitor use is marked by higher initial response rates (45%–53%) with inevitable progression at a median of 6 to 9 months typically through the acquisition of resistance mutations that reactivate the MAPK pathway. The addition of MEK inhibitors improves response rate (64%–76%) and response duration (9–11 months), while reducing the incidence of cutaneous toxicities—although progression is similarly inevitable (5).

The initial development of MAPK pathway inhibitors proceeded concurrently with inhibitors of MEK (AZD8330/ARRY-704, PD0325901, XL518, RDEA119, AS703026, CH5126766, CL-1040, GSK1120212, and AZD6244) and RAF (GDC-0879, RAF265, ARQ680, XL281/BMS-908662, GSK118436, PLX4032, RAF053, and PLX4720).

In the January 15, 2012, issue of Clinical Cancer Research, Kirkwood and colleagues published a study comparing the MEK inhibitor selumetinib with temozolomide in unselected metastatic melanoma. Although selumetinib did not improve survival or response, most responders had BRAF-activating mutations, and selumetinib has since demonstrated efficacy in BRAF-mutant melanoma. This study laid the groundwork for the evaluation of BRAF/MEK inhibitors in BRAF-mutant melanoma. Clin Cancer Res; 21(24); 5412–4. ©2015 AACR.

See related article by Kirkwood et al., Clin Cancer Res 2011;17(2) January 15, 2012;555–67

Inhibition Redux


Diwakar Davar and John M. Kirkwood

Division of Hematology–Oncology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

Corresponding Author: John M. Kirkwood, Division of Hematology–Oncology, University of Pittsburgh Medical Center, 5171 Centre Avenue, Pittsburgh, PA 15232, Phone: 412-623-7707; Fax: 412-623-7704; E-mail: kirkwoodjm@upmc.edu
doi: 10.1158/1078-0432.CCR-14-3132
©2015 American Association for Cancer Research.
and sorafenib; ref. 6). Importantly, initial clinical development (C1-1040) occurred prior to the identification of BRAF mutations in human cancer, and initial trials were not selectively enriched for patients with NRAS/BRAF–mutated tumors or cancers in which these oncogenic mutations were most commonly detected, such as melanoma (6).

Haass and colleagues first reported the antitumor activity of AZD6244 (ARRY-142886) in melanoma in a cell culture model where BRAF V600E–mutant (WM35, WM793, 1205Lu, and 451Lu), NRAS Q61R–mutant (SbC12, WM2032, WM1361a, and WM852), and wild-type (C8161) lines revealed that AZD6244 inhibited melanoma growth additively with docetaxel in vitro and in vivo (7). Further studies characterized selumetinib (AZD6244/ ARRY-142886) as an inhibitor of both MEK1 (IC50 14 nmol/L) and ERK1/2 phosphorylation (IC50 10 nmol/L) and led to evaluation of selumetinib in phase I trials against multiple tumor types (6).

In the phase II study we reported, 200 patients with advanced stage III/IV melanoma were assigned at random to receive either temozolomide or selumetinib (8). Most patients had cutaneous primaries (73%), but uveal (10%), mucosal (3%), and unknown primaries (14%) were also included. BRAF/NRAS and GNAQ mutation status were retrospectively analyzed in the cutaneous and uveal primaries, although enrollment did not stratify patients by mutational status. Of the 158 samples that had adequate archival tissue for evaluation, 73 tumors (46% of tested samples or 50% of cutaneous primaries) were adequate archival tissue for evaluation, 73 tumors (46% of tested samples or 50% of cutaneous primaries) were selected (15). Melanoma patients were included in the latter three phases, although enrollment was stratified (15). Melanoma patients were included in the latter three phases, although enrollment was stratified (15). Melanoma patients were included in the latter three phases, although enrollment was stratified (15). Melanoma patients were included in the latter three phases, although enrollment was stratified (15).

The primary point of this trial was PFS. Sample size of 182 (91 per arm) was calculated to provide 80% power to detect improvement in PFS with a hazard ratio (HR) of 0.74 (overall) at a one-sided 20% significance level, which would represent a HR of 0.67 among BRAF-mutant tumors. PFS comparisons were based on the assumption that BRAF- and NRAS-mutant melanoma comprised 60% and 30% of study participants, respectively, and that selumetinib would have an equal effect in NRAS-mutant and BRAF wild-type patients, a reasonable assumption given prior reports of selumetinib efficacy in the BRAF/NRAS wild-type C8161 cell line.

We reported no significant difference in response or PFS between the treatment arms among unselected patients and in patients with tumors displaying BRAF mutation. Response rates in both arms were low (6% vs. 9%)—and surprisingly lower in the selumetinib arm. Furthermore, patients assigned to selumetinib therapy had lower overall survival even after adjusting for imbalances in adverse prognostic factors between the two arms. Notably, 5 of 6 selumetinib responders and 1 of the 2 responding patients who had been crossed over to selumetinib from temozolomide following progression were identified to be BRAF mutants.

Several aspects of clinical trial design may have contributed to these findings. First, the study was likely underpowered to detect a true difference between treatment arms, given the discrepancy between the assumed (prestudy) and observed (on-study) proportions of BRAF/NRAS mutations, despite the increase in enrollment (to 200) from an original accrual target (of 182). Second, subsequent reports of atypical BRAF/KRAS (G464E and G12D, respectively) mutations in the C8161 cell line raise doubt as to selumetinib’s efficacy in true BRAF/NRAS wild-type patients (9). Third, patients assigned to selumetinib had a higher incidence of known adverse prognostic features, including worse WHO performance status and higher lactate dehydrogenase levels.

Although these differences were adjusted for in the final analysis of overall survival, the nonsignificant P values preclude definitive conclusions regarding the efficacy or nonfutility of selumetinib, in either the overall or BRAF/NRAS–mutant populations. Finally, given that the study allowed crossover on investigator-assessed progression, selumetinib efficacy is difficult to ascertain as, although overall survival and PFS statistics were reported separately, unequal crossover suggests an element of investigator bias. Concurrently, two other phase II monotherapy studies in non–small cell lung cancer and colorectal cancer reported that selumetinib did not offer a response and/or survival advantage over standard chemotherapy (10, 11). Subsequent evaluation in metastatic uveal, BRAF wild-type (in combination with docetaxel), and BRAF mutant (in combination with dacarbazine) melanoma suggested that selumetinib improved PFS and response rates without significant improvements in overall survival (12–14).

Lessons learnt from these studies have fundamentally altered studies the next-generation MEK inhibitors. The MEK 1/2 inhibitor tretinom (GSK1120212) was evaluated in a tiered approach that is characteristic of the current paradigm of targeted therapy development: (i) highly specific and potent candidate (trametinib MEK 1/2 IC50 0.92 nmol/L/1.8 nmol/L vs. selumetinib MEK1 IC50 14 nmol/L) was defined through high-throughput screening; (ii) maximum tolerated dose (MTD) and recommended phase II dose (RP2D) was defined in a nonselected cohort; (iii) RP2D was evaluated in selected tumor types enriched for the target of interest; and (iv) biologically active dose ranges were further characterized (15). Melanoma patients were included in the latter select phases, although enrollment was stratified (15), reflecting our growing understanding of the importance of pre-treatment stratification by mutational status. Given reports of disease stabilization and tumor regression among BRAF-mutated (V600E/V600K) melanoma patients, subsequent phase II/III evaluation was restricted to BRAF V600E– or V600K-mutated melanoma (16).

Evaluation of BRAF inhibitors in melanoma has paralleled that of MEK inhibitors. Initial forays utilized sorafenib (BAY43-9006) in unselected patients—with only minimal (single-agent) or modest (in combination with chemotherapy) clinical activity that did not correlate with BRAF mutational status (17). Subsequent analysis suggested that while initially considered a marginally active agent) or modest (in combination with dacarbazine) melanoma suggested that selumetinib improved PFS and response rates without significant improvements in overall survival (12–14).
paraffin-embedded tissues and subsequently approved for this purpose concurrently with vemurafenib. Subsequently, vemurafenib was noted to have similar efficacy in patients with V600K BRAF mutations—missed in the prior report as the Cobas assay was poor at detecting these less common mutations. Dabrafenib’s approval in 2013 was accompanied by a companion diagnostic (THxID BRAF test) that detected both V600E and V600K mutations.

Since selumetinib was first evaluated for melanoma in 2006, the field has advanced rapidly, with the approval of multiple more highly selective BRAF and MEK inhibitors between 2011 and the present. Multiple modes of resistance to BRAF inhibition have been identified, including MAPK pathway–dependent (alternative splicing, increased gene amplification, and copy number gains, development of oncogenic mutations in NRAS or MEK), and MAPK pathway–independent [COT (MAP3K8) overexpression] mechanisms.

Combining MEK and BRAF inhibitors increases response rate and prolongs duration of response by subverting multiple escape mechanisms. BRAF/MEK inhibitors are being combined in BRAF-mutant malignancies (primarily melanoma) with a myriad of other agents, including small-molecule inhibitors of Bcl-2 (navitoclax, NCT01989585), Akt (GSK2414795, NCT01902173); autophagy inducers hydroxychloroquine (NCT02257424) and metformin (NCT02143050); VEGF antagonist bevacizumab (melanoma, NCT01459598); and EGFR antagonist panitumumab in colorectal cancer (NCT01750918). Furthermore, BRAF/MEK inhibitors are being combined with CTLA-4–blocking agents, including ipilimumab (NCT01940809) and tremelimumab (NCT02027961); PD-1/PD-L1–blocking agents, including nivolumab (NCT02357732), pembrolizumab (KEYNOTE-022, NCT02130466), and MPDL3280A (NCT01656642); and in a BRAF/MEK inhibitor versus CTLA-4/PD-1 inhibitor sequence study (NCT02224781). It is hoped that these combinatorial strategies will improve upon the outcomes achieved with MEK/BRAF inhibition alone.

### Disclosure of Potential Conflicts of Interest

J.M. Kirkwood is a consultant/advisory board member for Bristol-Myers Squibb, Merck, and Roche/Genentech. No potential conflicts of interest were disclosed by the other author.

### Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NCI or the NIH.

### Authors’ Contributions

Conception and design: D. Davar, J.M. Kirkwood
Writing, review, and/or revision of the manuscript: D. Davar, J.M. Kirkwood

### Grant Support

This work was supported by the NCI of the NIH under award number P50CA121973 (to J.M. Kirkwood).

Received August 24, 2015; accepted September 3, 2015; published online December 15, 2015.

---

**References**

Kinase Inhibition Redux
−−−−−−
Inhibition in Melanoma—Kinase Inhibition Redux

Diwakar Davar and John M. Kirkwood


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/21/24/5412

Cited articles
This article cites 19 articles, 7 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/24/5412.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.