considered as accurate in that fraction of patients who present at diagnosis with lymph node involvement and lack of any available primary melanoma tissue (due to a missing surgically excised sample, primary tumor regression, or DNA degradation).

CONFLICT OF INTEREST

PAA has had a consultant and advisory role for Bristol Myers Squibb, Merck Sharp and Dohme, Roche-Genenetech, Novartis, Ventana Medical Systems, and Amgen. He received research fund from Bristol Myers Squibb, Roche-Genetech, and Ventana. The rest of the authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.05.103.

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MEK Is a Therapeutic and Chemopreventative Target in Squamous Cell Carcinoma

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TO THE EDITOR

Cutaneous squamous cell carcinoma (cuSCC) is diagnosed more than 700,000 times annually, claiming up to 8,800 lives annually, in the United States alone (Karia et al., 2013). No standard targeted therapy exists for cuSCC. Exome sequencing of cuSCC suggests that loss-of-function mutations in major tumor suppressor genes such as *NOTCH1/2, TP53*, and *CDKN2A* drive tumor development (Li et al., 2015; Pickering et al., 2014; South et al., 2014; Wang et al., 2011). No activated oncogene is consistently present in cuSCC. EGFR/HER2 inhibitors, the most tested targeted therapy to date, have had limited success, and whether responses correlate with mutation, amplification, or overexpression of *ErbB* family genes is unresolved (Stratigos et al., 2015).

BRAF inhibitors induce cuSCC formation (Oberholzer et al., 2012; Su et al., 2012) by increasing mitogen-activated protein kinase/ERK kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling in *BRAF* wild-type contexts (Menzies et al., 2013). Although other mechanisms contribute (Vin et al., 2013), coadministration of MEK inhibitors (MEKi) with BRAF inhibitors dramatically abrogates cuSCC

Abbreviations: cuSCC, cutaneous squamous cell carcinoma; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/ERK kinase; MEKi, MEK inhibitors

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induction (Flaherty et al., 2012). Elevated phospho-MEK/ERK is also seen in sporadic human cuSCC (Dajee et al., 2003; Einspahr et al., 2012). With these rationales in mind, we tested if MEK signaling is necessary for cuSCC induction and maintenance, and whether MEK inhibition is an actionable approach for treatment and chemoprevention of sporadic cuSCC.

To test the effects of MEK inhibition across cuSCC cases with different etiologies and mutational profiles, we tested responses to two MEKi, trametinib and cobimetinib, in 10 lines from both immunocompromised and immunocompetent patients (Vin et al., 2013; Watt et al., 2011). Of the 10 lines, 9 responded to both trametinib and cobimetinib at the highest concentrations tested (1 μM and 10 μM, respectively), but sensitivity between lines at lower doses was heterogeneous (Figure 1a, Supplementary Figure S1 online). No clear segregation of sensitive and insensitive lines was revealed, and mutational status of *RAS* or *EGFR* did not correlate with sensitivity (Supplementary Table S1 online).

To confirm the on-target activity of trametinib and cobimetinib, signal transduction pathway changes in MEK/ERK were probed. Downstream phospho-ERK was strongly suppressed at 72 hours by MEKi (Figure 1b), although phosphorylated MEK increased with MEKi treatment. Similar results were obtained with cobimetinib after 72 hours, although the levels of phospho-ERK in SRB1 and SRB12, the least sensitive lines tested, were more modestly suppressed with 25 nM treatment (Figure 1c), suggesting that incomplete signaling inhibition could explain differences in sensitivity between lines.

We next sought to characterize the cellular response that accompanied the effectiveness of MEK inhibition. In four cuSCC cell lines spanning a range of sensitivities to MEKi, cell cycle progression as measured by 5-ethynyl-2′-deoxyuridine (EdU) nucleotide incorporation was strongly (from 2.5- to 37.9-fold) downregulated by treatment with both MEKi (Figure 1d, Supplementary Figure S2 online), with no apoptosis detected by western. Consistent with this, we observed a dose-dependent decrease in cyclin D1 levels after both trametinib and cobimetinib treatment (Figure 1f). No change in cyclin D1 was detected in SRB12 with either treatment, consistent with this line being the least sensitive in our viability screen (Figure 1a, Supplementary Figure S1).

MEK inhibitor-treated cuSCC cells became enlarged and flattened (Supplementary Figure S3 online), a morphological hallmark of senescence (Munoz-Espin and Serrano, 2014). Staining for senescence-associated β-galactosidase activity revealed induction in 9.2 ± 2.0 to $18.6 \pm 1.8\%$ of cells in treated populations ($P < 0.05$, Figure 1e, Supplementary Figure S3). In addition, p21 (*CDKN1A*), a cell-cycle inhibitor and marker of senescence (Munoz-Espin and Serrano, 2014), was induced in all tested lines after trametinib and cobimetinib treatment, except in cobimetinib-treated SRB12 cells, which were relatively resistant (Figure 1g). We also observed that phospho-AKT levels were unchanged only in relatively resistant lines, and that cotargeting AKT resulted in enhanced responses (Supplementary Figure S4 online).

To test if MEK inhibition could reduce tumor growth in vivo, we established SRB1 tumor xenografts in NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice and treated them with oral trametinib (2 mg/kg/day). At killing, the average vehicle tumor volume was 3.1-fold larger than trametinib-treated tumors ($P < 0.0001$, Figure 1h and i). Western-blot analysis of tumor lysates confirmed that trametinib significantly reduced phospho-ERK/total ERK levels in vivo on an average of 9.8-fold ($P = 0.03$, Figure 1j and k), demonstrating successful target engagement in tumors. Together, these data suggest that MEK tumor signaling drives proliferation and prevents tumor suppressive senescence induction in cuSCC cells and tumors (Figure 1l), an effect that can be exploited by targeting MEK in vivo.

To better study the effects of MEK inhibition on both cuSCC induction and growth, oral trametinib (2 mg/kg/day) and cobimetinib (10 mg/kg/day) were tested in a UV-driven hairless mouse model of cuSCC using chronic, low-dose, solar simulated UV light (12.5 kJ/m^2 UVB weekly administered across three doses, Figure 2a), which more faithfully recapitulates human cuSCC molecularly than chemical carcinogenesis models (Vin et al., 2013). Over the course of treatment, control mice formed substantially more tumors than those treated with trametinib or cobimetinib (Figure 2b). Spaghetti plots of individual lesions and comparisons of lesion sizes at killing confirmed that trametinib completely suppressed detectable net tumor induction, whereas cobimetinib reduced tumor number versus baseline (Figure 2c and d).

Tracking of individual tumors revealed that trametinib-treated tumors had a 2.4-fold reduced tumor volume increase versus control, whereas cobimetinib-treated tumors showed 5.0-fold growth suppression (Figure 2e). Ki67 staining was reduced by 24% for trametinib and 18% for cobimetinib ($P = 0.002$, $P = 0.02$, Figure 2f and g), and target pathway engagement was confirmed by suppression of ERK activation by up to 39% (Supplementary Figure S5 online). Overall, 62−69% of papillomas responded and 50−75% of cuSCCs responded to MEKi (Supplementary Figure S6 online).

Our results suggest that MEK is an effective target for preventing and treating cuSCC. Inhibition of MEK causes senescence, but not apoptosis, of cuSCC cells, with observed synergism with AKT inhibition. The near-complete abrogation of cuSCC induction in our UV-driven model with MEKi indicates that MEK activation is rate limiting for sporadic cuSCC induction, as it appears to be for BRAF inhibitor-induced lesions (Flaherty et al., 2012). Although responses of existing tumors were heterogeneous, significant suppression of proliferation and phospho-ERK was observed in tumors of treated mice. We conclude that MEK inhibition may be a basis for molecularly targeted chemoprevention and therapy of cuSCC.

CONFLICT OF INTEREST

The authors state no conflicts of interest.

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