BRAF inhibitors: two edged sword

Research Article

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Summary
Somatic mutations in BRAF occur in two thirds of cutaneous melanomas, leading to the activation of the RAF-MEK-ERK signalling pathway. Preclinical studies of BRAF mutations showed that inhibition of BRAF led to melanoma cell proliferation inhibition and apoptosis induction in vitro and blockade of melanoma xenograft growth in vivo. Clinical studies of BRAF inhibitors in BRAF-mutated metastatic melanoma revealed superior outcomes in patients treated with these agents when compared to cytotoxic chemotherapy. As a result, BRAF inhibitors are currently licenced by the regulatory authorities in the United States and Europe in BRAF-mutated metastatic melanoma. BRAF inhibitors paradoxically cause squamous cell carcinoma in melanoma patients treated with these agents. In this review we will discuss the mechanisms and the importance of this phenomenon. It is an example of the challenges that accompany the hope that targeted therapy brings to cancer patients as well as to their treating physicians.

I. Introduction:
In the past two decades there has been a revolution in the understanding of the pathogenesis of cancer leading to the discovery of more effective ways of cancer treatment. Although conventional chemotherapy remains the mainstay of the treatment of cancer in both adjuvant and metastatic settings, the so called new targeted agents are very promising¹. The concept of targeted therapy is based on the discovery of certain mutations or pathways that drive the uncontrolled growth of cancer cells and other hallmarks of cancer.

In 2000, Bernard Weinstein coined the term “oncogene addiction” meaning that despite the abundant genetic alterations in cancer cells, they can be dependent on a single mutation or protein that contributed to their malignant phenotype¹. The implication of this observation is that targeting this mutation can deprive cancer cells from their ability to survive and grow while sparing normal cells¹. Nowadays several targeted therapies are in clinical use in the treatment of human cancers including lung,
breast, chronic myeloid leukaemia (CML), gastrointestinal stromal tumour (GIST), ovarian, colorectal, melanoma and gastric cancers with wide range of success. Mutations and proteins with clinically available targeted agents include epidermal growth factor receptor (EGFR), human epidermal growth factor (HER2), vascular endothelial growth factor A (VEGF-A). These agents are generally well tolerated with less severe toxicity profile when compared to conventional chemotherapy, table 1 lists some of the adverse effects of some the commonly used molecularly targeted agents.

Most of the molecularly targeted agents are used in the metastatic setting; therefore, the quality of life issue is of extreme importance. It is important to note that many of targeted therapies gained an accelerated approval by FDA; hence the long term side effects of some them might not be well characterized. It is clear that building the knowledge and experience around their side effects will require time and constant review of data emerging from post licence follow up.

In this review we will discuss the side effects of BRAF inhibition, their incidence, pathogenesis, and management. BRAF inhibitors are currently licenced for BRAF-mutated metastatic melanoma, discuss BRAF mutations, the clinical trials led to their licence, mechanisms of adverse effects and their reported incidence.

Cutaneous findings in patients undergoing treatment with BRAF inhibitors are plentiful, and close dermatologic support with frequent clinical examination is recommended to ensure early identification of non-melanoma skin cancer. Photosensitivity, warts, and SCC were all reported. The focus point will be that BRAF inhibitors used to treat melanoma paradoxically cause squamous cell carcinoma, there is little role for therapeutic intervention. Mainstay of treatment is understating the pathophysiology of these side effects to detect them early, and treat them by simple resection. The development of these lesions in the presence of BRAF inhibition provides multiple avenues for further study regarding the molecular biology of these dermatological phenomena.

The development of SCC in patients with BRAF-mutated metastatic melanoma highlights the importance of understanding pathway signalling in clinical practice and of genotyping tumours prior to administering BRAF-selective drugs, to identify patients who are likely to respond and also to identify patients who may experience adverse effects.

Table 1:

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Cancer</th>
<th>Adverse effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumumab</td>
<td>HER2</td>
<td>Breast, gastric</td>
<td>Congestive heart failure</td>
<td>6</td>
</tr>
<tr>
<td>Bevacizumumab</td>
<td>VEGF-A</td>
<td>Colon, lung, renal, ovarian</td>
<td>Hypertension, proteinuria, GI perforation</td>
<td>7</td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR</td>
<td>Renal cell</td>
<td>Pneumonitis, Hypertension</td>
<td>8</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>EGFR</td>
<td>Colon, head &amp; neck</td>
<td>Acne skin rash</td>
<td>9</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR</td>
<td>Lung</td>
<td>Acne skin rash, pneumonitis</td>
<td>10</td>
</tr>
</tbody>
</table>
II. BRAF as a target

The RAF-MEK-ERK signal transduction pathway is a conserved RAS-activated protein kinase cascade that regulates cell growth, proliferation, and differentiation in response to growth factors, cytokines, and hormones\textsuperscript{11-13}. RAF activation is initiated by RAS-GTP association with the RAS binding domain (RBD) situated within the N-terminal regulatory region of the kinase\textsuperscript{14}. Concomitant conformational changes and recruitment to the cell membrane promote changes in RAF phosphorylation that combine to stimulate its serine/threonine kinase activity, triggering sequential phosphorylation and activation of MEK and ERK\textsuperscript{14,15}. The three functional RAF proteins in humans, ARAF, BRAF, and CRAF (also termed c-Raf-1), are dependent on activation segment phosphorylation for activity\textsuperscript{15}. However, the details of their regulatory mechanisms differ because CRAF and ARAF require additional serine and tyrosine phosphorylation within the N region of the kinase domain for full activity, and B-RAF has a much higher basal kinase activity than either A-RAF or C-RAF\textsuperscript{16}.

Sequence analysis of BRAF associated with human cancers has identified more than 30 single site missense mutations, mostly within the kinase domain\textsuperscript{17}. The V600E mutation accounts for 90\% of BRAF mutations in human cancers\textsuperscript{18}. The glutamic acid for valine substitution at residue 600 in the activation segment leads to constitutive activation of BRAF, leading to activation of downstream activity of ERK independent of RAS control\textsuperscript{17,18}. Other rare mutations identified have impaired kinase activity, yet able to activate ERK by triggering the activity of wild type CRAF, which in turn activates ERK\textsuperscript{17,18}.

Nearly two thirds of metastatic melanoma patients harbour an activating mutation at the kinase domain of BRAF, making BRAF an attractive target for treatment\textsuperscript{19-21}. These mutations are more common in melanomas occurring on skin that had been exposed to intermittent sun exposure. Most preclinical work studies examining the potential therapeutic value of targeting BRAF focused on BRAF-V600E mutation\textsuperscript{22}. In melanoma cell lines, it was found that inhibition of BRAF-V600E decreases ERK activity and induces apoptosis\textsuperscript{22}. In contrast to that, targeting wild type BRAF had minimal effects on melanoma cell lines. The encouraging results of preclinical work led to development of BRAF inhibitors which have been successfully used in phase III clinical trials with subsequent FDA approval\textsuperscript{20}.

III. Clinical Trials

Vemurafenib is a potent inhibitor of mutated BRAF, it has shown activity against BRAF mutant melanoma cell lines but not against wild type BRAF\textsuperscript{20,21}. Maximum tolerated dose has been established in a phase I trial and a phase II trial showed a response rate of more than 50\%\textsuperscript{15,20}. Based on those results, the phase III trial BRIM-3 was conducted to compare vemurafenib with dacarbazine, the only FDA licenced agent for metastatic melanoma when the trial was initiated\textsuperscript{20}. The trial included 675 patients with previously untreated metastatic melanoma with BRAF V600E mutation. Results showed that vemurafenib was associated with a relative reduction of 63\% in the risk of death and of 74\% in the risk of tumour progression in the experimental arm. Authors reported an impressive response rate of 48\% in the vemurafenib group compared to 5\% in the dacarbazine group. These results granted vemurafenib approval in BRAF-mutated metastatic melanoma.

The second selective BRAF inhibitor to enter clinical development was GSK2118436 (dabrafenib). Patients with metastatic melanoma harbouring BRAF mutations included in a phase I/II trial\textsuperscript{22}. At the two highest doses evaluated, 150 mg and 250 mg twice daily, objective responses were observed in 10 of 16 patients with BRAF V600E. Of note, five patients with the BRAF V600K mutations were enrolled at the highest doses evaluated, and all had evidence of tumour regression. Recruitment for a phase III trial has been completed\textsuperscript{22}.

IV. BRAF inhibitors toxicities

BRIM-3 study showed the following
adverse effects of vemurafenib: arthralgia, skin rash, fatigue, cutaneous squamous cell carcinoma, keratoacanthoma (a low grade variant of squamous cell carcinoma of the skin), nausea, alopecia, pruritus, diarrhoea, headache, vomiting and neutropenia\textsuperscript{20}. Adverse events led to dose modification or interruption in 38\% of patients receiving vemurafenib. The most common toxicities associated with GSK2118436 were mild-to-moderate fever, fatigue, headache, nausea, keratoacanthoma and vomiting\textsuperscript{22}.

The cutaneous side effects of BRAF inhibitors, namely squamous cell carcinoma (SCC), photosensitivity and keratoacanthoma drew a lot of interest. In the BRIM-3 trial 18\% of patients developed SCC, keratoacanthoma, or both\textsuperscript{15}. The obvious question what are the molecular mechanisms by which BRAF inhibitors can lead to those malignant side effects?

The incidence of cutaneous adverse effects of BRAF inhibitors was examined by Mattei and his colleagues\textsuperscript{23}. They retrospectively reviewed the medical notes of 53 patients that received vemurafenib or a combination of GSK2118436 and GSK1120212 (a MEK inhibitor). Authors found that 39\% of patients developed photosensitivity, 30.3\% developed actinic keratosis, 30.3\% developed warts and 18.2\% developed SCC. Other cutaneous side effects include erythema nodosum, genital herpes reactivation, hand-foot skin reaction, psoriasis flare and pruritus. The median time to first detection of cutaneous side effects was 1-6 months of therapy. An important observation was that SCC was seen at greater frequency and earlier in patients treated with vemurafenib. Another observational study of 15 patients who received vemurafenib for one month at least for BRAF-mutated metastatic melanoma found that 5 developed SCC\textsuperscript{24}. Other cutaneous side effects reported in small group of patients include: keratosis pilaris–like eruptions, seborrheic dermatitis–like rashes, and hand-foot skin reaction.

**IV. Mechanisms by which BRAF inhibition drives SCC development**

The paradoxical development of SCC in patients treated with BRAF inhibitors has drawn a lot of interest since the clear association between BRAF inhibition and SCC development was observed. The mechanisms by which BRAF inhibitors stimulate SCC growth are yet to be fully defined. The prevailing hypothesis is that in the context of a hyperactive mutant \textit{RAS} gene and a normal \textit{BRAF} gene, BRAF inhibitors including vemurafenib paradoxically activate ERK signalling by promoting the formation of CRAF-containing dimers, leading to hyperproliferation and tumour formation.

The hypothesis that those patients treated with vemurafenib who develop SCC have a \textit{RAS} mutation was examined in an interesting study by Su and his colleagues\textsuperscript{22}. Researchers performed a molecular analysis to identify oncogenic mutations (\textit{HRAS}, \textit{KRAS}, \textit{NRAS}, \textit{CDKN2A}, and \textit{TP53}) in the lesions from patients treated with vemurafenib. An analysis of an independent validation set and functional studies with BRAF inhibitors in the presence of the prevalent \textit{RAS} mutation was also performed. Samples from patients who participated in BRIM1,2 and 3 trials (the trails discussed earlier showing that vemurafenib is superior to dacarbazine in BRAF mutant metastatic melanoma) were used in the study. DNA extracted from the tumour specimens was sequenced for \textit{HRAS}, \textit{NRAS}, \textit{KRAS}, and \textit{CDKN2A} with the use of polymerase-chain-reaction (PCR) amplification. Results showed that \textit{RAS} mutations were present in approximately 60\% (total specimens=31) of cases in patients treated with vemurafenib. Authors suggested that pre-existing mutations may confer a predisposition to the development of SCC or keratoacanthomas. An important remark made by authors was that BRAF inhibitors should be used with caution in patients with cancers driven by mutations in...
**V. Therapeutic intervention in BRAF adverse effects**

Literature indicates that cutaneous toxicities of BRAF inhibitors can be easily treated. In the BRIM-3 trial all patients who had SCC, keratoacanthoma or both were treated successfully with simple excision. Other non-cutaneous toxicities required dose modification or interruption. In the case series of Massachusetts general hospital, none of the dermatologic side-effects required dose interruptions, modifications, or discontinuation of therapy. SCC was treated with surgical excision and warts with cryotherapy.

Photosensitivity is treated with sun avoidance and photoprotection. Regular skin examination and management by experienced dermatologists as well as continuous prophylactic photo protection including UV devices irradiation is mandatory. Therapeutic intervention has little role to play in cutaneous effects of BRAF inhibitors. The cornerstone is high clinical suspicion index and early diagnosis with treatment modification if necessary.

**VI. Conclusion**

BRAF inhibitors have shown excellent clinical efficacy in metastatic melanoma, the first BRAF inhibitor, vemurafenib was licenced following a phase III trial with initial reporting of six months follow up. Careful clinical monitoring for cutaneous adverse effects including squamous cell carcinoma is indicated. The paradoxical activation of MAPK by BRAF inhibitors in wild type BRAF, RAS mutant cells is a major concern. Several cancers are driven by RAS mutations. Therefore, BRAF inhibitors should be used with caution in this setting, preferably within a clinical trial.

Newer cancer treatment agents provide great hope to patients, their families and physicians, and a greater challenge to researchers and scientists to discover their molecular effects and possible toxicities.

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ESMO Glossary in molecular biology of cancer. The Translational research working group. P32.


