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MOLECULAR MARKERS FOR PERSONALISED APPROACH TO PATIENTS WITH MELANOMA

Ivan Šamija

Department of Oncology and Nuclear Medicine,
University Clinical Hospital Center "Sestre milosrdnice", Zagreb, Croatia

Summary

The year 2011 was a breakthrough in melanoma treatment with two new targeted drugs, ipilimumab and vemurafenib, approved after showing overall survival benefit in patients with metastatic melanoma. Vemurafenib was approved only for treatment of patients with activating mutations in *BRAF* gene. Thus, the detection of activating *BRAF* mutations, which can be found in ≈50% melanomas, became a standard part of a routine protocol for the treatment of patients with metastatic melanoma. In this review, methods and protocols for the detection of *BRAF* mutations as an example of molecular marker for personalised approach to patients with melanoma are discussed, with an emphasis on the aspects that are still a matter of discussion and controversies. In addition to *BRAF* mutations, some other molecular markers for personalised approach to melanoma patients, such as predictive markers for ipilimumab therapy that are still not used routinely, are briefly discussed.

Keywords: melanoma; BRAF mutations; vemurafenib; ipilimumab; predictive markers; personalised medicine.

INTRODUCTION

Melanoma of the skin is the fifth most common cancer in the USA with the incidence rate of 21.3 per 100000 people per year [1]. The incidence of melanoma of the skin has been rising constantly with the average increase of 1.8% each year over 2002-2011 [1]. Unlike the incidence, the mortality was stable for the same period, the mortality rate being 2.7 per 100000 people per

year [1]. In the Republic of Croatia the incidence rate age-standardized to European Union population was 12.3 per 100000 in 2011 [2]. The discrepancy between incidence and mortality rate can be attributed to high survival rate for non-metastatic melanoma, the 5-year survival rate for localized melanoma being 98.1% [1]. However, the 5-year survival rate for melanoma patients with distant metastases is only 16.1% [1]. The poor outcome of patients with metastatic melanoma is mostly due to inefficient therapeutic options that were available until recently. The only two drugs approved and widely used for treating metastatic melanoma until 2011 were dacarbazine and interleukin-2, but neither of them showed improved overall survival benefit [3].

TARGETED THERAPY FOR MELANOMA

In 2011, two targeted therapy drugs, ipilimumab and vemurafenib, were approved for the treatment of metastatic melanoma. Unlike dacarbazine and interleukin-2, both ipilimumab and vemurafenib have shown overall survival benefit in patients with metastatic melanoma [4-6].

Ipilimumab, an immunotherapy drug, is a monoclonal antibody that targets immune checkpoint by binding specifically to cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 is an inhibitory receptor on T cells that binds CD80 and CD86 molecules on antigen-presenting cells during T-cell activation [7]. Blockade of CTLA-4 with specific antibodies like ipilimumab results with increased T cell activation and proliferation, and consequently with improved immune response against melanoma antigens [7]. Approval of ipilimumab for the treatment of unresectable or metastatic melanoma by the U.S. Food and Drug Administration (FDA) in 2011 was based on phase III clinical trial in which previously treated metastatic melanoma patients treated with ipilimumab and glycoprotein 100 (gp100) vaccine had improved survival compared to patients treated only with gp100 vaccine [4]. In another phase III randomized trial on previously untreated metastatic melanoma patients, median overall survival was significantly longer in patients treated with ipilimumab and dacarbazine compared to patients treated with only dacarbazine (11.2 months vs 9.1 months) [5]. In these clinical trials only 10-15% of melanoma patients responded to ipilimumab according to response evaluation criteria in solid tumors (RECIST) [4,5]. However, the response to ipilimumab treatment is different from typical response to cytotoxic cancer drugs. In some patients disease progression or development of new metastatic le-

sions was observed prior to ipilimumab induced disease control associated with improved survival [4,5,8]. Therefore different set of response criteria, immune-related response criteria (irRC), was developed as more appropriate to assess ipilimumab response compared to standard RECIST criteria [8]. The survival benefits in melanoma patients responding to ipilimumab seem to be long lasting. Pooled analysis of long-term survival data from different ipilimumab clinical trials has shown 22% three-year overall survival rate with survival curve reaching plateau around 3 years that extends through at least 10 years [9]. In clinical trials ipilimumab treatment was associated with more frequent and sometimes severe toxicities, notably specific immune-related adverse events [4,5]. Other targeted immunotherapy drugs have also been studied in melanoma patients. Most notable of them are drugs targeting programmed death 1 (PD-1) receptor, another inhibitory regulator of T cells, and it's ligand PD-L1 [10]. Examples of such drugs that have shown promising results in clinical trials on melanoma patients are nivolumab, lambrolizumab, and BMS-936559 [10-12].

Vemurafenib is a small-molecule inhibitor of V600 mutated BRAF kinase [13]. It was approved by FDA in 2011 for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation. In BRIM3 phase III trial on previously-untreated patients with BRAF (V600) mutation-positive metastatic melanoma both overall survival and progression free survival were significantly longer in patients treated with vemurafenib compared to patients treated with dacarbazine [6,14]. In that trial 57% of patients responded to vemurafenib treatment. In 2013 another small-molecule inhibitor of V600 mutated BRAF kinase, dabrafenib was approved by FDA for the treatment of treatment of BRAF V600E mutation-positive unresectable or metastatic melanoma. The approval was based on improved progression-free survival shown in a phase III trial comparing dabrafenib to dacarbazine [15]. In that trial 50% of patients responded to dabrafenib treatment [15]. In addition to other side effects, a significant proportion of patients treated with these BRAF inhibitors have developed cutaneous squamous-cell carcinomas (SCC) and keratoacanthomas [6,15]. That can be explained by the mechanism of paradoxical activation of RAS-RAF-MEK-ERK signaling pathway in cells with non-mutated BRAF by vemurafenib and dabrafenib [16]. A limitation of these BRAF targeted drugs is durability of response with median duration of response in different clinical trials being approximately 6 months and great majority of patients eventually progressing within 1 year [6,15]. Different

resistance mechanisms that can account for that have been described, some of them involving reactivation of mitogen activated protein kinase (MAPK) pathway [17,18]. Potential strategy of overcoming MAPK pathway reactivation resistance to BRAF inhibitors is MAPK pathway blockade downstream of BRAF. An example of such drug is a mitogen-activated protein kinase kinase (MEK) inhibitor, trametinib. Based on positive results of clinical trials trametinib was approved by FDA for the treatment of BRAF V600E or V600K mutation-positive unresectable or metastatic melanoma in 2013 as a single agent and in 2014 in combination with dabrafenib [19,20].

Drugs targeting other molecules and signaling pathways have also been studied in melanoma patients, like imatinib in melanoma patients with mutated *KIT*, drugs targeting vascular endothelial growth factor (VEGF), and drugs targeting PI3K-AKT-mTOR pathway [21-23].

BRAF FUNCTION AND *BRAF* MUTATIONS IN MELANOMA

Vemurafenib and dabrafenib are selectively targeting mutated and activated form of BRAF protein that is coded by *BRAF* gene [13,24]. BRAF is, together with ARAF and CRAF (RAF1), a member of RAF kinase family of serine/threonine protein kinases that are involved in RAS-RAF-MEK-ERK signaling pathway [25]. This signaling pathway is activated when an extracellular growth factor binds to a membrane-bound receptor with tyrosine kinase activity. Activation of different growth factor receptors leads to activation of RAS protein. RAS is a guanosine nucleotide-binding protein (G protein) that is critically involved in at least two different signaling pathways in addition to RAS-RAF-MEK-ERK pathway. In RAS-RAF-MEK-ERK pathway RAS activates RAF kinase, RAF phosphorylates and activates MEK and MEK phosphorylates and activates ERK mitogen activated protein kinases. ERK phosphorylates and activates different proteins: transcription factors that regulate gene transcription in the nucleus, and cytoplasmic proteins that regulate protein translation and other processes. In that way RAS-RAF-MEK-ERK pathway plays a central role in cellular proliferation, growth, differentiation and some other processes. This signaling pathway is often disrupted in cancer [25]. Due to activating mutations in genes coding for RAS and RAF and some other mechanisms, signaling trough this pathway is constitutive, unregulated and increased leading to uncontrolled cellular proliferation which is a hallmark of cancer [25,26].

Mutations in *BRAF* gene were found in different types of cancer, with high frequency in hairy cell leukemia (≈100%), melanomas (≈50%), papillary thyroid cancers (40-45%), colorectal cancers (8-15%), and ovarian cancers [26-30]. The most frequent *BRAF* mutation in different cancers is an amino acid substitution from a valine to a glutamic acid at position 600 in BRAF, known as V600E mutation. V600E mutation represents 80-90% *BRAF* mutations in melanomas [26,30,31]. Among remainder of BRAF mutations in melanomas the most frequent are other amino acid substitutions at the same position, V600K (found in up to 20% melanoma patients with *BRAF* mutations), V600D, and V600R [27,30,31]. BRAF mutations were more frequently found in younger patients and patients without chronic sun damage of the surrounding skin [27,32-35]. It was shown that presence of *BRAF* mutations is associated with worse prognosis in patients with metastatic melanoma not treated with BRAF-directed therapy [27].

TESTING FOR *BRAF* MUTATIONS

Testing for BRAF mutations is necessary before deciding about vemurafenib, dabrafenib, and trametinib therapy. All these drugs have shown activity and have been approved only for patients with melanoma that has V600 mutated BRAF. Furthermore, due to paradoxical activation of RAS-RAF-MEK-ERK signaling pathway in cells with non-mutated BRAF, vemurafenib and dabrafenib could promote cancer growth in patients with BRAF V600 non-mutated melanoma [16].

Different methods can be used to test for BRAF mutations: Sanger sequencing, pyrosequencing, mutation-specific real-time PCR, high resolution melting analysis, immunohistochemistry with VE1 antibody specific for V600E mutated *BRAF*, mismatch ligation assay, and others. These methods differ regarding sensitivity, specificity, cost, time and expertise required, and other parameters [36-38]. In the USA vemurafenib was approved by FDA for patients with BRAF mutations as detected by cobas® 4800 BRAF V600 Mutation Test (Roche Molecular Systems Inc.) as a companion diagnostic test, while dabrafenib and trametinib were approved for patients with BRAF mutations as detected by THxID™ BRAF Kit (bioMérieux, Inc.) as a companion diagnostic test. In the approval of these drugs in the European Union by the European Medicines Agency (EMEA) no companion diagnostic test was prescribed. The cobas test has shown very high specificity for *BRAF* V600

mutations (<1% false-positives), high sensitivity of >95% for *BRAF* V600E mutation, and capability to reliably detect *BRAF* V600E mutations present in as little as 5% of alleles present in a sample [39]. For comparison Sanger sequencing can reliably detect mutations if at least 20% of alleles present in a sample are mutant. The cobas method can detect also V600K and V600D mutations, but with lower sensitivity and limit for detection and it cannot distinguish between different mutations [39]. This is a potential disadvantage of that method because although vemurafenib was approved only for patients with V600E mutation, it was shown that melanoma patients with V600K mutation could also benefit from vemurafenib treatment (14,40).

BRAF mutations can be detected in fresh and frozen tissue samples but most often they are detected in paraffin-embedded tissue samples which are often only samples available. Parameters that could increase *BRAF* mutation detection failure rate are the age of samples, poor fixation of samples and high level of pigmentation. Another important parameter is percentage of cancer cells in the sample which should be high for *BRAF* mutation analysis to be reliable and representative. However, it was shown that cobas method has very low failure rate for *BRAF* mutation detection [39,41].

BRAF mutation testing is usually performed on patients with metastatic or unresectable melanoma who are candidates for therapy with vemurafenib, dabrafenib or trametinib. However, there are some valid arguments in favor of testing all patients with high-risk melanoma (American Joint Committee on Cancer stage IIb or higher) or even all patients upon diagnosis of melanoma [42]. When only patients who are candidates for therapy are tested, it is possible that time necessary to retrieve archived samples would delay initiation of therapy and thus decrease its potential benefit. It is also possible that only available archived samples from a patient would be old samples from initial biopsy what could increase *BRAF* mutation detection failure probability.

One finding that limits clinical value of *BRAF* mutation detection is intra-tumor and inter-tumor heterogeneity regarding the presence of *BRAF* mutations in a patient. It was shown that different regions within the same melanoma lesion can differ regarding the presence of *BRAF* mutations [43]. The results regarding the inter-tumor heterogeneity are conflicting. Several studies have shown relatively high discordance but other studies have shown high concordance between different melanoma lesions (primary and different metastases) in the same patients regarding the *BRAF* mutation sta-

tus [31,44-46]. Therefore, further studies are needed before making definitive conclusions regarding intra-patient *BRAF* mutation heterogeneity and its clinical consequences.

PREDICTIVE MARKERS FOR IPILIMUMAB THERAPY

Because of relatively low response-rate but durable and clinically significant response in patients responding to vemurafenib therapy, reliable predictive markers for vemurafenib would significantly improve management of patients with melanoma. However, in spite of different such potential markers being studied, none of them has shown the results that would justify its routine analysis. Markers that have shown promising results are increase in absolute lymphocyte count during treatment, presence of antibodies specific for NY-ESO1 antigen in serum, and baseline serum lactate dehydrogenase [47-49].

CONCLUSION

Analysis of *BRAF* mutations in melanoma patients prior to vemurafenib, dabrafenib, and trametinib therapy is one of a few examples of molecular predictive markers related to personalized cancer therapy that has become standard routine clinical practice. However, there are still controversies and opened questions regarding the optimal protocol for routine *BRAF* mutation testing in melanoma patients. Probably the most pertinent issue is intra-patient heterogeneity regarding the *BRAF* mutations. Also, there are different views on the best method for *BRAF* mutation detection and which patients to test. There would be a great clinical benefit from other types of molecular markers for personalized approach to patients with melanoma, like predictive markers for ipilimumab therapy or markers to predict resistance to vemurafenib therapy. No such marker has so far justified its routine analysis but several have shown promising results.

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Sažetak

Molekulski biljezi za personalizirani pristup bolesnicima s melanomom

2011. godina je bila prekretnica u liječenju bolesnika s melanomom budući da su te godine odobrena dva nova lijeka, vemurafenib i ipilimumab, koji su pokazali učinak na produženo preživljenje bolesnika s metastatskim melanomom. Vemurafenib je odobren za liječenje samo onih bolesnika koji imaju aktivirajuće mutacije u genu *BRAF*. Tako je detekcija aktivirajućih mutacija u genu *BRAF*, koje se mogu naći u ≈50% bolesnika s melanomom, postala standardni dio rutinskog protokola liječenja bolesnika s metastatskim melanomom. U ovom se radu raspravljaju metode i protokoli detekcije *BRAF* mutacija kao primjer molekulskog biljega za personalizirani pristup bolesnicima s melanomom, s naglaskom na one aspekte koji su još predmet rasprava i kontroverzi. Osim mutacija u genu *BRAF*, u radu se ukratko raspravljaju i neki drugi molekulski biljezi za personalizirani pristup liječenju bolesnika s melanomom, kao prediktivni biljezi za liječenje ipilimumabom, koji se još ne određuju rutinski.

Ključne riječi: melanom; BRAF mutacije; vemurafenib; ipilimumab; prediktivni biljezi; personalizirana medicina.

Corresponding author:
Ivan Šamija
E-mail: ivan.samija@kbcsm.hr