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Radiosensitization by BRAF inhibitor therapy - mechanism and frequency of toxicity in melanoma patients

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Running Title: Radiosensitization by BRAF inhibitor therapy
Abstract

**Background:** Recent evidence suggests that ionizing radiation may be associated with unexpected side effects in melanoma patients treated with concomitant BRAF inhibitors. A large multi-center analysis was performed to generate reliable safety data and elucidate the mechanism.

**Methods:** A total of 161 melanoma patients from eleven European skin cancer centers were evaluated for acute and late toxicity, of whom 70 consecutive patients received 86 series of radiotherapy with concomitant BRAF inhibitor therapy. To further characterize and quantify a possible radiosensitization by BRAF inhibitors, blood samples of 35 melanoma patients were used for individual radiosensitivity testing by fluorescence-in-situ-hybridization of chromosomal breaks after *ex vivo* irradiation.

**Results:** With radiotherapy and concomitant BRAF inhibitor therapy the rate of acute radiodermatitis ≥2° was 36% and follicular cystic proliferation was seen in 13% of all radiotherapies. Non-skin toxicities included hearing disorders (4%) and dysphagia (2%). Following whole brain radiotherapy, rates of radiodermatitis ≥2° were 44% and 8% (*p*<0.001) for patients with and without BRAF inhibitor therapy, respectively. Concomitant treatment with vemurafenib induced acute radiodermatitis ≥2° more frequently than treatment with dabrafenib (40% versus 26%, *p*=0.07). In line with these findings, analysis of chromosomal breaks *ex vivo* indicated significantly increased radiosensitivity for patients under vemurafenib (*p*=0.004) and for patients switched from vemurafenib to dabrafenib (*p*=0.002), but not for patients on dabrafenib only. No toxicities were reported after stereotactic treatment.

**Conclusion:** Radiotherapy with concomitant BRAF inhibitor therapy is feasible with an acceptable increase in toxicity. Vemurafenib is a more potent radiosensitizer than dabrafenib.

Key words: Radiosensitization, radiotherapy, radiation, BRAF, vemurafenib, dabrafenib
Introduction

BRAF-inhibitors are a standard treatment for patients with metastatic BRAF V600-mutated melanoma [1-3]. Frequently, radiotherapy is also required in these patients [4]. Recently, radiosensitizing effects of both BRAF inhibitors vemurafenib and dabrafenib have been described [5-10]. In addition, after sequential radiotherapy and BRAF inhibitor treatment, radiation recall phenomena have been reported [11-13]. However, some cancer centers reported good tolerability [14, 15].

Currently, there is no standard approach with regard to interruption of the systemic therapy with BRAF inhibitors, while patients undergo radiotherapy. Since the interruption in treatment could potentially lead to progression, an analysis of toxicity was called for. The aim of this study was to provide reliable data on the frequency and severity of radiosensitizing effects of vemurafenib and dabrafenib in a sufficient number of patients as basis for rational decisions on treatment algorithms.
Methods

Patients

In total 161 metastatic melanoma patients from nine German, one Austrian and one Swiss skin cancer centers were analyzed retrospectively. Toxicity of 177 radiotherapies in those 161 patients was fully documented. Among these patients 86 radiotherapies were applied in 70 patients with concomitant BRAF inhibitor therapy. Patients’ characteristics are shown in Table 1. Regarding the sites of the radiotherapies the largest subgroup received WBRT with or without stereotactic boost (n=32). These patients were compared to a control group of melanoma patients treated with WBRT without BRAF inhibitors between 1998 and 2014 at the University Hospital Erlangen (n=91) (Table 1).

Individual radiosensitivity was studied in 35 blood samples of melanoma patients with or without BRAF inhibitor therapy. Approval by the Ethics Committee at the University of Erlangen was obtained and all patients gave written informed consent. Blood samples were taken during necessary blood draw at regular follow-up visits.

Materials

Acute radiodermatitis of the 177 radiotherapies was scored according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [16]. Other toxicities were documented descriptively.

Individual radiosensitivity was determined in freshly drawn heparinized peripheral blood from 35 melanoma patients. After dividing the blood sample in two aliquots, one was not irradiated and the other irradiated with a dose of 2Gy. Ionizing radiation was generated by a 6MV linear accelerator (Mevatron; Siemens, Germany) with a dose rate of 2.2Gy per minute. After irradiation, lymphocytes were stimulated with phytohemagglutinin and cultured for 48h. The preparation for three-color fluorescence in situ hybridization (FISH) followed a previously described standard
technique [17]. Chromosomal aberrations were scored as breaks per metaphase (B/M). At least 200 metaphase spreads were scored for the unirradiated control and 100 metaphases after 2Gy. The 0Gy value was subtracted to correct the influence of spontaneous aberration frequencies. The assessment was performed in a blinded manner.

**Statistical analysis**

Data analysis was performed using SPSS 19.0 (IBM Corporation, Armonk, New York) and the Mann-Whitney U-test. Two sided p-values were evaluated and a p-value of <0.05 was considered statistically significant.
Results

Toxicity analysis of all radiotherapies

Any acute or late toxicity appeared in 57% of radiotherapies with concomitant BRAF inhibitor therapy. Skin toxicity appeared frequently whereas other toxicities were rare (Table 2). There were no differences in skin toxicity based on the sites of radiotherapy. The most frequent toxicities were acute radiodermatitis with radiodermatitis ≥2° in 36% (Fig 1A, B) and follicular cystic proliferation (FCP) in 12.8% (Fig 1C). One case of hand-foot syndrome occurred after irradiation of the foot (Fig 1D) and one patient developed a maximal form of FCP, which has been described before as cutis verticis gyrate-like toxicity [10, 13]. But despite this high rate of acute skin toxicities, no severe sequelae were reported after a mean follow up time of 6.6 months (95%CI: 4.8 - 8.3 months). Non-skin toxicities were rare and included hearing disorders (4%) and dysphagia (2%). BRAF inhibitor therapy was interrupted due to toxicity in 9% and irradiation was interrupted in 4% of all cases.

The frequency of radiodermatitis was further analyzed depending on the type of BRAF inhibitor. In patients treated with vemurafenib (n=63) acute radiodermatitis ≥2 occurred in 40%, whereas in the dabrafenib group (n=23) in only 26% (p=0.07) (Fig 2A). Follicular cystic proliferations (FCP) only appeared in patients taking vemurafenib. In several patients the BRAF inhibitor dose was reduced precautionary due to the upcoming radiotherapy (n=5) or after prior adverse events induced by the BRAF inhibitor (n=10). These dose reductions did not reduce radiation-induced skin toxicity during concomitant treatment compared to full dosage (p=0.4) (Fig 2B).

The largest subgroup of patients treated with radiotherapy and concomitant BRAF inhibitors received WBRT. These 32 patients were compared to 91 patients treated with WBRT only. In patients receiving WBRT with concomitant BRAF inhibitor therapy acute radiodermatitis ≥2 according to RTOG criteria occurred in 44% of cases compared to 8% of patients with WBRT only (p<0.001) (Fig 2C).
Rates of acute radiodermatitis of conventionally fractioned radiotherapies \((n=67)\) and stereotactic treatments \((n=19)\) were also compared. No increased skin toxicity and no other severe adverse events were reported after stereotactic radiotherapy with concomitant BRAF inhibitor therapy (Fig 2D). In contrast, acute radiodermatitis \(\geq 2\) was reported in almost every other patient \((46\%)\) who received a conventionally fractioned radiotherapy with concomitant BRAF inhibitor therapy \((p<0.001)\).

**Individual radiosensitivity *ex vivo***

Individual radiosensitivity was assessed in peripheral blood lymphocytes of melanoma patients after *ex vivo* irradiation. Three-color FISH was used to analyze the cells’ ability to respond to ionizing radiation-induced DNA damage. Misrepair, impaired signaling and dysfunctional cell cycle control results in chromosomal aberrations. Color changes along chromosomes indicate these aberrations (Fig 3A, B). The chromosomal aberrations were expressed as mean breaks per metaphase \((B/M\)-value\) and were scored in the blood of melanoma patients without BRAF inhibitor therapy \((n=15)\), patients taking vemurafenib \((n=8)\) or dabrafenib \((n=9)\) and patients who were switched from vemurafenib to dabrafenib \((n=3)\). B/M-values of less than 0.5 indicate an average radiosensitivity and B/M-values between 0.5 and 0.6 increased radiosensitivity. Patients with B/M-values higher than 0.6 have a clearly increased radiosensitivity with an increased risk for severe toxicities during radiotherapy \([17-19]\). In the control group the B/M-values of none of the patients was higher than 0.6 B/M (Fig 3C). By contrast 50\% \((4/8)\) of patients under vemurafenib had strongly increased B/M-values. Interestingly, the B/M-value was increased only in 11\% \((1/9)\) of patients under dabrafenib. The patient of the dabrafenib group with the dramatically increased B/M-value of 1.0 developed 17 HPV acanthomas and one squamous cell carcinoma three months after start of therapy with dabrafenib. Patients who were currently taking dabrafenib and had previously been treated with vemurafenib, had very high B/M-values, even though vemurafenib treatment was stopped on average 5.2 months before. Patients under vemurafenib \((p=0.004)\) and patients who were switched from vemurafenib to dabrafenib \((p=0.002)\) had significantly increased B/M-values compared to patients without BRAF inhibitor therapy. Patients taking vemurafenib had significantly higher B/M values than
patients under therapy with dabrafenib ($p=0.04$). There was no correlation of B/M-values with BRAF inhibitor dose, dose per body weight or dose per body mass index. Eight of the patients in which a radiosensitivity testing was performed were also treated with radiotherapy. Patients with average B/M values had no skin toxicities, whereas patients with increased B/M values suffered much more frequently from acute and late skin toxicities $\geq 2^\circ$ (Fig 3D).
Discussion

This analysis of a large patient cohort showed an increased rate of acute radiodermatitis ≥°2 of 36% in patients treated with radiotherapy and concomitant BRAF inhibitor therapy. Despite the high rate of acute radiodermatitis, no severe skin related late toxicities were reported during an average follow up time of 6.6 months. Follicular cystic proliferations (FCP), a characteristic late reaction of concomitant BRAF inhibitor therapy and WBRT [9, 13], was reported in 13% of our patient cohort. Other reactions like hand-foot syndrome are reported here for the first time after radiotherapy. In our patients these skin reactions were strictly limited to the irradiated areas. But it has to be considered, that BRAF inhibitors frequently induce follicular dermatitis and hyperkeratosis without ionizing radiation [1-3, 20]. It can be speculated, that some of these adverse events might have also happened without ionizing radiation. Reports on radiation-induced visceral reactions such as pneumonitis or anorectitis [7, 11] and potentially liver toxicity exist [5]. However, in this patient population non-skin toxicity was rare. Another finding of the study was that radiation-induced toxicity only appeared in patients, who received conventionally fractioned radiotherapy with concomitant BRAF inhibitor therapy. No skin or other toxicity appeared after stereotactic treatment. This is in line with previous case reports [15] and an earlier retrospective analysis of twelve patients (n=3 WBRT; n=3 WBRT+stereotactic boost; n=6 stereotactic radiotherapy) with no reported toxicities except for brain necrosis in one patient [14].

So far, it was unclear whether additional toxicity was induced by BRAF inhibitors and if so, whether this increased toxicity was mediated by an immunologic boost [21] or whether the effect was direct. To establish the pathogenic mechanism the radiosensitivity in patients taking BRAF inhibitors was investigated ex vivo and clearly showed a radiosensitizing effect of vemurafenib but not of dabrafenib. These ex vivo findings are in line with the patient data that also showed a higher rate of acute radiodermatitis ≥°2 in vemurafenib-treated patients (40%) compared to dabrafenib-treated patients (26%). Interestingly, photosensitization is almost exclusively reported in vemurafenib-treated patients [22]. One might speculate that this is a consequence of the very selective binding affinity of dabrafenib to mutant BRAF, whereas
vemurafenib also has a low affinity to CRAF, wild-type BRAF and possibly other enzymes [23].

The radiosensitizing effect of BRAF inhibitors probably also sensitizes melanoma cells, maybe even to a greater extent than keratinocytes. In vitro the radiosensitizing effect of BRAF inhibitors in BRAF-mutated melanoma cells has already been proven [24, 25]. This might enhance the antitumor effect of both radiotherapy and BRAF inhibitors, which is especially valuable for patients with multiple brain metastases, when no stereotactic radiotherapy is possible. Both, whole brain radiotherapy and BRAF inhibitor therapy improve cerebral tumor control [26-28]. Nevertheless, the prognosis of melanoma patients with multiple brain metastases is still poor. Synergistic effects of ionizing radiation and BRAF inhibition within a concomitant treatment regime could improve the prognosis of these patients.

Whether the BRAF inhibitor therapy should be interrupted during radiotherapy, has to be discussed in light of these data. Radiation recall phenomena have been reported up to one month after radiotherapy [11-13]. Consequently, if maximal safety is favored, therapy interruption of systemic treatment would last several weeks and might lead to progression of non-irradiated metastases. Whereas when radiotherapy is performed with concomitant BRAF inhibitor therapy, systemic tumor control is maintained. Furthermore, a radiosensitizing effect might improve (local) tumor control. Our data demonstrate that stereotactic radiotherapy with concomitant BRAF inhibitor therapy does not increase the risk of toxicity. Patients receiving conventionally fractioned radiotherapy with concomitant dabrafenib have a moderately increased risk of acute radiodermatitis compared to a larger increase in patients taking vemurafenib. Thus, in patients with planned radiotherapy the choice of BRAF inhibitor with respect to toxicity favors dabrafenib. Switching patients from vemurafenib to dabrafenib before starting radiotherapy cannot be recommended, as these patients showed the highest individual radiosensitivity ex vivo. Particularly patients under treatment with vemurafenib should be monitored closely for skin and non-cutaneous radiation toxicities and receive early supportive care, if necessary. Nevertheless, the results of this analysis show the feasibility of radiotherapy with concomitant BRAF inhibitor therapy.
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Disclosure

Seventeen authors report collaborations with different pharmaceutical companies, partially with the manufacturers of BRAF inhibitors, outside the project of this manuscript.
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Table 1:
Patient characteristics
Characteristics of 86 radiotherapies in 70 patients with any radiotherapy and concomitant BRAF inhibitor therapy and 91 patients with whole brain radiotherapy (WBRT) without BRAF inhibitor therapy. BRAFi = BRAF inhibitor; STX = stereotactic radiotherapy; WBRT = whole brain radiotherapy.
<table>
<thead>
<tr>
<th></th>
<th>Radiotherapy with concomitant BRAFi</th>
<th>WBRT without BRAFi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>70 pts.</td>
<td>91 pts.</td>
</tr>
<tr>
<td>Radiotherapies per patient:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- One</td>
<td>56 pts.</td>
<td>91 pts.</td>
</tr>
<tr>
<td>- Two</td>
<td>12 pts.</td>
<td></td>
</tr>
<tr>
<td>- Three</td>
<td>2 pts.</td>
<td></td>
</tr>
<tr>
<td><strong>Number of radiotherapies</strong></td>
<td><strong>86</strong></td>
<td><strong>91</strong></td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>53y (19-85)</td>
<td>60y (25-87)</td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>61</td>
</tr>
<tr>
<td>Irradiated sites:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- WBRT</td>
<td>32</td>
<td>91</td>
</tr>
<tr>
<td>- Bone metastases</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>- STX brain</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>- Lymph node metastases</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>- Soft tissue metastases limbs</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>- Mediastinal metastases</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>- Others</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Stereotactic radiotherapy (STX)</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>WBRT dosage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Mean dose</td>
<td>33.6Gy</td>
<td>33.0Gy</td>
</tr>
<tr>
<td>- With boost</td>
<td>8</td>
<td>25% of WBRT</td>
</tr>
<tr>
<td>Prior radiotherapy of the same site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- All patients</td>
<td>12</td>
<td>16% of WBRT</td>
</tr>
<tr>
<td>- Subgroup WBRT</td>
<td>5</td>
<td>14% of WBRT</td>
</tr>
<tr>
<td>Concomitant therapy:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Vemurafenib 960mg bid</td>
<td>51</td>
<td>59%</td>
</tr>
<tr>
<td>- Vemurafenib reduced dose</td>
<td>12</td>
<td>14%</td>
</tr>
<tr>
<td>- Dabrafenib 150mg bid</td>
<td>20</td>
<td>23%</td>
</tr>
<tr>
<td>- Dabrafenib reduced dose</td>
<td>3</td>
<td>4%</td>
</tr>
<tr>
<td>- Fotemustine</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>- Temozolomide</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>- Others</td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

**Table 1**
Table 2:

Adverse events

Adverse events in 86 radiotherapies of 70 patients treated with radiotherapy and concomitant BRAF inhibitor therapy. *Includes one case of cutis verticis gyrate-like toxicity as the maximal form of follicular cystic proliferation (FCP).

<table>
<thead>
<tr>
<th>Adverse events in 86 radiotherapies (100%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin toxicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute radiodermatitis ≥ RTOG °2</td>
<td>31</td>
<td>36%</td>
</tr>
<tr>
<td>Follicular cystic proliferation*</td>
<td>11</td>
<td>13%</td>
</tr>
<tr>
<td>Hand-foot syndrome (irradiated area)</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Impaired wound healing</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td><strong>Other toxicities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing disorder</td>
<td>3</td>
<td>4%</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Hemorrhagic intracranial metastasis</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Polyneuropathy</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Taste disorder</td>
<td>1</td>
<td>1%</td>
</tr>
</tbody>
</table>
Figure 1:
Skin toxicities of patients treated with radiotherapy with concomitant BRAF inhibitor therapy.
(A) Acute radiodermatitis °3 of a patient treated for axillary metastases. (B) Acute radiodermatitis °3 of a patient treated for a soft tissue metastasis of the ankle. (C) Follicular cystic proliferation (FCP) of a patient after WBRT. (D) Hand-foot syndrome of a patient treated for a soft tissue metastasis of the foot.
Figure 2

Acute radiodermatitis of patients treated with radiotherapy with concomitant BRAF inhibitor therapy.

Acute radiodermatitis of 86 radiotherapies (RT) with concomitant BRAF inhibitor therapy divided in subgroups of BRAF inhibitor (BRAFi) type (A) and BRAFi dose (B). Acute radiodermatitis after WBRT of 32 patients with and 91 patients without concomitant BRAF inhibitor therapy. Acute radiodermatitis of 86 conventionally fractioned or stereotactic radiotherapies with concomitant BRAFi inhibitor therapy (D). Grading of skin toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (1° Faint erythema or dry desquamation; 2° Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate edema; 3° Moist desquamation in areas other than skin folds and creases; bleeding induced by minor trauma or abrasion; 4° Life-threatening consequences; skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site; skin graft indicated).
Figure 3
Figure 3:  
**Individual radiosensitivity testing of melanoma patients with or without BRAF inhibitors *ex vivo***.

Three-color FISH painting of chromosomes 1 (red), 2 (green) and 4 (yellow). (A) A metaphase without aberrations and (B) a metaphase with one dicentric chromosome and two acentric fragments are displayed. The aberrations were scored as 2 breaks per metaphase (B/M). (C) Lymphocytes were irradiated *ex vivo* with 2Gy. B/M found in non-irradiated metaphases were subtracted from those scored in the irradiated samples. B/M-values of patients treated with vemurafenib, dabrafenib, and dabrafenib after vemurafenib were compared to melanoma patients without BRAF inhibitor therapy. *The patient with a dramatically increased B/M-value of 1.0 developed 17 HPV acanthomas and one squamous cell carcinoma three months after start of therapy with dabrafenib. (D) Correlation of acute and late skin toxicity of irradiated patients with their B/M-values.