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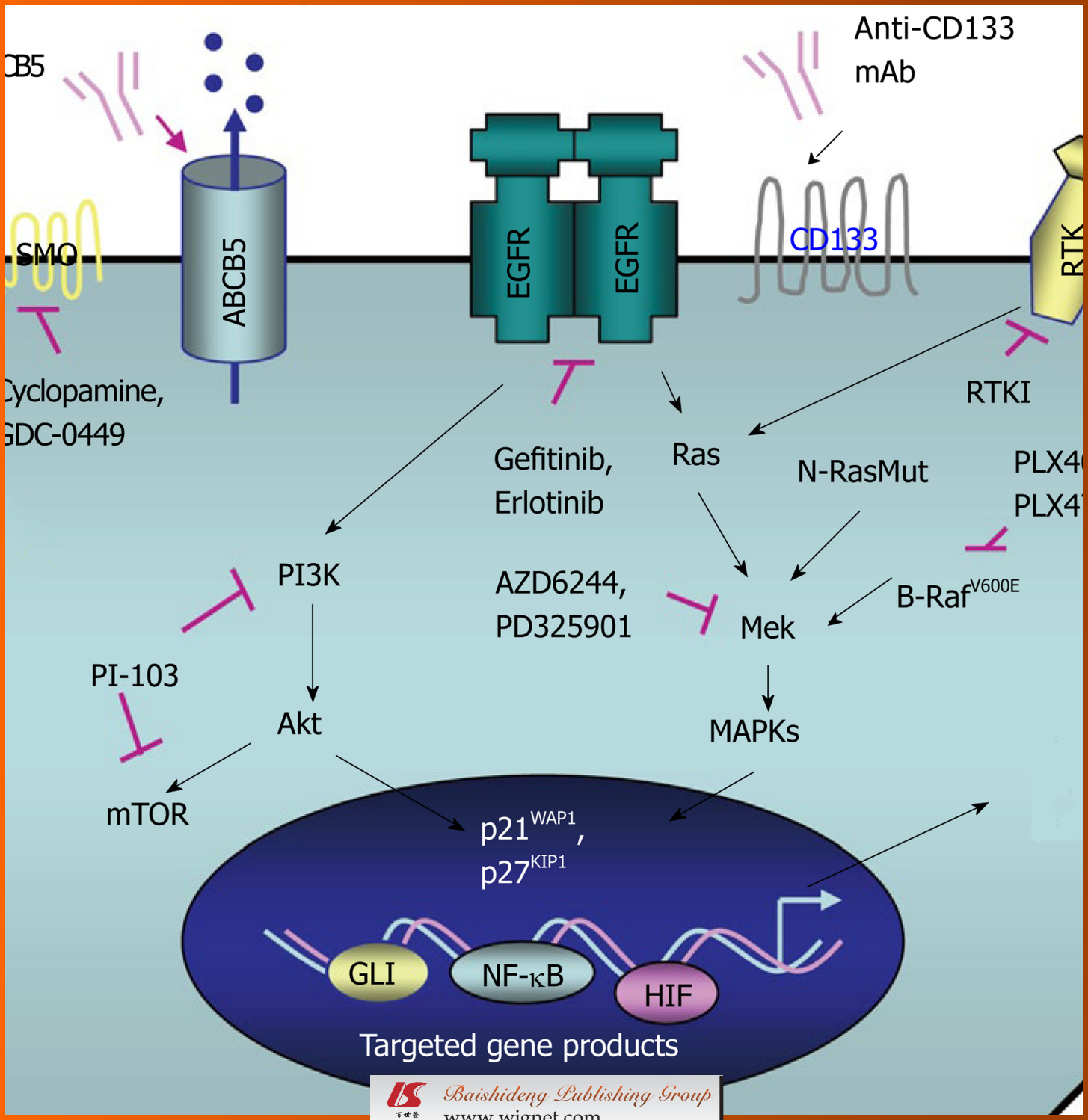

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Room 903, Building D, Ocean International Center,
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Telephone: +86-10-85381892
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World Journal of Clinical Oncology
Room 903, Building D, Ocean International Center,
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Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893
E-mail: wjco@wjgnet.com
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REVIEW

Novel biomarkers and therapeutic targets for optimizing the therapeutic management of melanomas

Murielle Mimeault, Surinder K Batra

Murielle Mimeault, Surinder K Batra, Department of Biochemistry and Molecular Biology, College of Medicine, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198-5870, United States

Author contributions: Mimeault M searched and reviewed the literature and wrote the manuscript; Batra SK reviewed the literature and revised the manuscript; both authors read and approved the final version of manuscript.

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Correspondence to: Murielle Mimeault, PhD, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198-5870, United States. mmimeault@unmc.edu
Telephone: +1-402-5595455 Fax: +1-402-5596650

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Abstract

Cutaneous malignant melanoma is the most aggressive form of skin cancer with an extremely poor survival rate for the patients diagnosed with locally invasive and metastatic disease states. Intensive research has led in last few years to an improvement of the early detection and curative treatment of primary cutaneous melanomas that are confined to the skin by tumor surgical resection. However, locally advanced and disseminated melanomas are generally resistant to conventional treatments, including ionizing radiation, systemic chemotherapy, immunotherapy and/or adjuvant stem cell-based therapies, and result in the death of patients. The rapid progression of primary melanomas to locally invasive and/or metastatic disease states remains a major obstacle for an early effective diagnosis and a curative therapeutic intervention for melanoma patients. Importantly, recent advances in the melanoma research have led to the identification of different gene products that are often implicated in the malignant transformation of melanocytic cells into melanoma cells, including melanoma stem/progenitor cells, during melanoma initiation and progression to locally advanced and metastatic disease states. The frequent deregulated genes products encompass the oncogenic B-RafV600E and N-RasQ61R mutants, different receptor tyrosine kinases and developmental pathways such as epidermal growth factor receptor (EGFR), stem cell-like factor (SCF) receptor KIT, hedgehog, Wnt/β-catenin, Notch, stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor-4 (CXCR4) and vascular endothelial growth factor (VEGF)/VEGFR receptor. These growth factors can cooperate to activate distinct tumorigenic downstream signaling elements and epithelial-mesenchymal transition (EMT)-associated molecules, including phosphatidylinositol 3'-kinase (PI3K)/Akt/ molecular target of rapamycin (mTOR), nuclear factor-kappaB (NF-κB), macrophage inhibitory cytokine-1 (MIC-1), vimentin, snail and twist. Of therapeutic relevance, these deregulated signal transduction components constitute new potential biomarkers and therapeutic targets of great clinical interest for improving the efficacy of current diagnostic and prognostic methods and management of patients diagnosed with locally advanced, metastatic and/or relapsed melanomas.

Key words: Cutaneous melanomas; Melanoma stem/progenitor cells; Biomarkers; Screening tests; Diagnosis; Prognosis; Molecular targets; Targeted therapy

Peer reviewer: Simone Mocellin, MD, PhD, Department of Oncological and Surgical Sciences, University of Padova, via Giustiniani 2, 35128 Padova, Italy

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INTRODUCTION

Cutaneous malignant melanoma represents the major cause of mortality among skin cancers and its incidence rate is increasing during last years[1-5]. Although the localized cutaneous melanomas diagnosed in the early stages are usually curable by surgical resection of malignant tumors, the rapid progression to invasive and metastatic disease states is generally associated with a poor median survival of 6 mo to 12 mo and a five year survival rate of less than 10%[1,2,6-8]. The therapeutic options for the patients with unresectable melanomas and metastases at distant organs such as lungs, liver and brain consisting to the radiation therapy and/or chemotherapy are only palliative, aiming to improve the quality of life of patients[8-10]. Especially, the standard treatment with alkylating agent, dacarbazine or its orally active analog temozolomide, alone or in combination with other cytotoxic agents, is ineffective in the most cases and culminate to the development of drug resistance, disease relapse and the death of melanoma patients[11-13].

Importantly, recent advances in melanoma research have led to the establishment of the molecular oncogenic events that may contribute to melanoma initiation and progression and treatment resistance of melanoma cells. It has been observed that the persistent activation of different oncogenic signaling cascades initiated in an autocrine or a paracrine manner by distinct growth factors and cytokines through their cognate receptors is typically involved in the sustained proliferation, survival, invasion and metastases at near lymph nodes and distant sites of melanoma cells and angiogenic process[2,14-23]. These deregulated gene products include B-Raf^{V600E}, N-Ras^{Q61R}, epidermal growth factor receptor (EGFR), hepatocyte growth factor (HGF) receptor MET, platelet-derived growth factor receptors (PDGFRs), sonic hedgehog, Wnt/β-catenin, Notch, Nodal/Cripto, hyaluronan (HA)/CD44, stem cell-factor (SCF) receptor KIT, stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor-4 (CXCR4), and vascular endothelial growth factor (VEGF)/VEGFR receptor (Figure 1)[13,14,17,19,22,24-47]. These tumorigenic pathways can cooperate for the sustained activation of downstream signaling effectors such as mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3'-kinase (PI3K)/Akt, nuclear factor-kappaB (NF-κB) and hypoxia-inducible factors (HIFs) for the acquisition of a more malignant behavior by melanoma cells during disease progression to locally advanced and metastatic states.

In addition, recent advances in skin stem/progenitor cell research have led to the identification of melanoma cells endowed with stem cell-like properties and which can provide critical functions for tumor growth, metastases at distant sites, treatment resistance and disease relapse[17,18,20,21,23,48,49]. More specifically, highly tumorigenic melanoma stem/progenitor cells have been identified *in situ* and isolated from primary and secondary melanoma tumors, circulating melanoma cells and established melanoma cell lines[20,21,50-64]. Melanoma stem/progenitor cells may express different stem cell-like markers such as CD133, nestin, aldehyde dehydrogenase (ALDH-^{high}), CD166, neural crest nerve growth factor receptor (CD271) and/or ATP-binding cassette (ABC) multidrug resistance transporters such as multidrug resistance-1 encoding P-glycoprotein (P-gp), ABCG2 and ABCB5. It has been shown that highly tumorigenic melanoma stem/progenitor cells can give arise to the total tumor cell mass *in vivo* with the phenotypic features resembling to original patient's melanomas and metastasize at distant sites[50-58,60,61,63-65]. In this matter, we review the most recent advancements on the gene products that are often altered during melanoma initiation and progression to locally invasive and metastatic disease states and which may be exploited to develop novel multiplex biomarker detection methods for optimizing diagnosis and prognosis and multitargeted therapies for a more effective management of melanoma patients.

NEW BIOMARKERS FOR OPTIMIZING DIAGNOSIS, PROGNOSIS AND INDIVIDUALIZED TREATMENT OF MELANOMA PATIENTS

The clinical diagnosis of cutaneous malignant melanomas at early stages retains a big challenge for the experimented pathologists and is generally made only after they become visible on skin[66]. Moreover, a skin biopsy and different tumor imaging tests such as X-rays, computed tomography (CT) scan, magnetic resonance imaging (MRI) and positron emission tomography (PET) tests are often performed to establish the grades and stages of melanomas and screen for metastatic melanomas[66,67].

In addition, the immunohistochemical staining of tissue specimens with different antibodies directed against different melanocytic markers such as S-100 and melanoma-associated antigen recognized by T-cells (MART-1) also designated as melanocyte antigen (Melan-A), which is expressed by melanoma cells, is useful for improving the accuracy of the pathological diagnosis and prognosis of melanoma patients[68-70]. Moreover, monoclonal antibody gp100 corresponding to clone HMB-45, which is highly specific and sensitive for melanocytic tumors but does not react with other non-melanoma malignancies such as carcinomas, lymphomas and sarcomas and normal melanocytes, may be used for the pathological diagnosis to distinguish poorly differentiated melanoma subtypes of other tumor types[69,71]. The immunohistochemical analysis of the vimentin expression in primary melanoma tissues, which is frequently overexpressed in primary melanoma patients with hematogenous metastasis, also may help to establish the melanoma patients with a high risk to develop hematogenous metastasis[72]. Although this importance advance, few biomarkers in melanoma stem/progenitor cells and their progenies have been validated in the clinics to use in combination in screening methods for an early

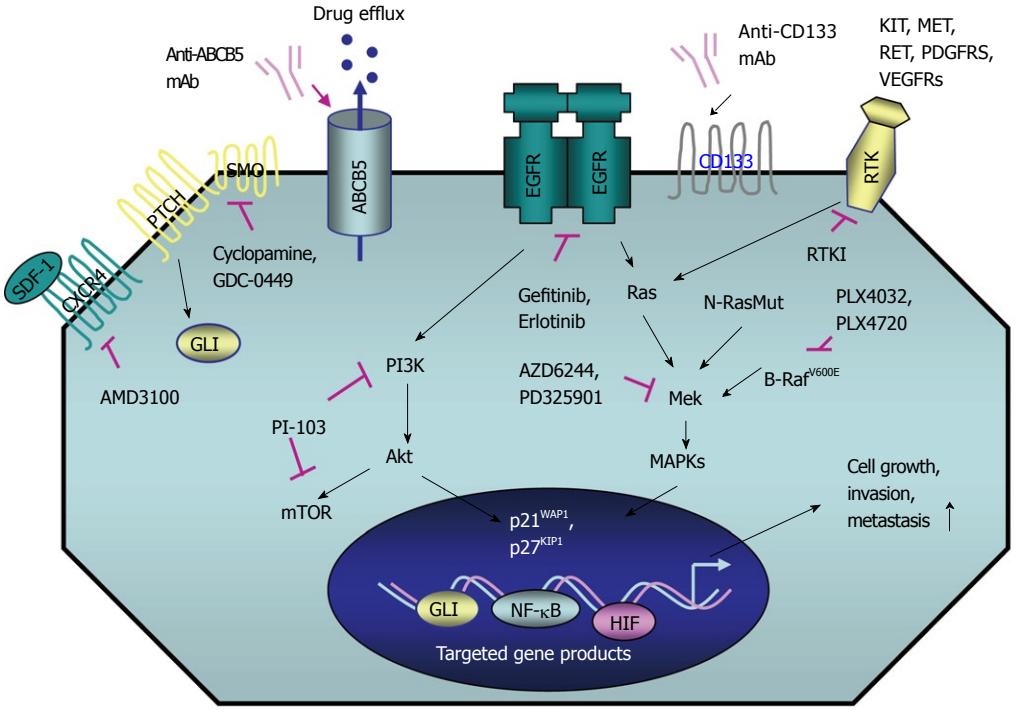

Figure 1 Novel multitargeted strategies against locally advanced, aggressive and metastatic melanomas. The scheme shows the intracellular signaling cascades induced through the activation of distinct growth factor pathways which may provide critical roles for the sustained growth, survival, migration, invasion, metastases and/or drug resistance of melanoma cells, including melanoma-initiating cells, through the up-regulation of the expression levels of different oncogenic gene products. The oncogenic gene products include c-Myc, Bcl-2, N-cadherin, snail, twist, matrix metalloproteinases (MMPs), urokinase-type plasminogen activator (uPA), cyclooxygenase-2 (COX-2) and vascular endothelial growth factor (VEGF). The potential therapeutic agents that may be used to block these tumorigenic signaling pathways, including a selective inhibitor of receptor tyrosine kinases (RTKI), EGFR (gefitinib or erlotinib), smoothened (SMO) hedgehog signaling element (cyclopamine or GDC0049) PI3K/mTOR (PI-103), oncogenic B-Raf^{E600V} mutant (PLX4032 or PLX4720) and MEK (AZD6244) as well as a monoclonal antibody (mAb) directed against stem cell-like markers, CD133 and ABCB5 multidrug transporter are also indicated. NF-κB: Nuclear factor-kappaB; HIF: Hypoxia-inducible factors; EGFR: Epidermal growth factor receptor.

and non-invasive detection of cutaneous melanomas and the establishment of the risk of the disease progression, metastases at near lymph nodes and distant sites and relapse. Consequently, the identification and validation of novel molecular biomarkers associated with the melanoma initiation and progression to locally invasive and metastatic disease states and response of melanoma patients to the clinical treatment is of great interest for improving the efficacy of current diagnostic and prognostic methods and therapeutic management of melanoma patients.

Numerous cytogenetic analyses in malignant melanoma tissues and serum samples *vs* benign melanocytic naevi and normal tissues and serum samples using microarray, immunohistochemical and polymerase chain reaction (PCR)-based techniques have led to the discovery of novel deregulated genes in melanoma cells[18,23,42,73-83]. The gene products often altered during melanoma progression constitute potential biomarkers for a more early diagnosis and accurate prognosis of melanoma patients and effective personalized medicine. The potential biomarkers that may be detected in malignant tissues and/or serum samples, either alone or in combination, to establish the risk of disease progression and as prognostic indicator of melanoma patients include different oncogenic products. Among the more promising molecular biomarkers, there are EGFR, activated pAkt phosphorylated form, microphthalmia-associated transcription factor (MITF), serum amyloid, MIC-1 also designated as growth and differentiation factor-15 (GDF-15), VEGF, interleukin-8 (IL-8) and/or twist[18,23,42,73-79,84-88].

More specifically, it has been observed that the EGFR expression was enhanced in primary and metastatic melanoma tissues from patients relative to non-malignant tissues suggesting that the detection of EGFR could be used as a prognostic indicator to predict the risk of disease progression to metastatic disease states and poor outcome of melanoma patients[86;87]. Moreover, the overexpression of MITF protein has also been detected in 62 of 104 tumor tissues obtained from metastatic melanoma patients and correlated with the chemotherapeutic response and reduced disease-specific survival of melanoma patients[73]. Importantly, the secreted MIC-1 cytokine has also been observed to be overexpressed in 66% of 53 melanoma cell lines analyzed as compared to normal melanocytes[76]. Moreover, the immunohistochemical analyses have indicated that the MIC-1 protein was expressed at low levels in primary melanoma biopsies (15 of 22) while all metastatic melanoma biopsies examined (16 of 16) exhibited strong expression of MIC-1[76]. The results from another study have also indicated that

MIC-1 was overexpressed in approximately 67% cases of advanced melanomas and secreted MIC-1 protein levels detected in serum samples of melanoma patients were 5-6 fold higher as compared with serum samples from normal individuals[42]. In this matter, it has been reported that the enhanced MIC-1 expression in melanoma cells may be induced at least in part through the constitutively active mutant B-Raf^{V600E} and activation of MAPKs, and to a lesser extent *via* the activated PI3K/Akt pathway[42,76]. The stimulation of SCF receptor KIT, which may contribute to the activation of MAPK pathway and the phosphorylation of MITF, also may result in an up-regulation of the MIC-1 expression[76]. Hence, together these results combined with the fact that the secreted MIC-1 cytokine has been observed to promote the tumorigenicity of melanoma cells *in vivo*[42;76], support the clinical interest to detect MIC-1 in melanoma tissue biopsies or serum samples for improving the diagnosis and prognosis of melanoma patients.

On the other hand, the occurrence of polymorphisms in the melanocortin-1-receptor (*MC1R*), which may lead to the *MC1R* variants encoding a non-functional MC1R protein and the acquisition of a red hair color (RHC) phenotype, fair skin, freckles and poor tanning ability of individuals, has also been associated with a high risk of developing melanoma[89]. Interestingly, the combined immunohistochemical analyses of expression levels of different cell cycle modulators (p21, p27, p53 and retinoblastoma proteins) and pro-apoptotic factors (Bax and Bak) in primary cutaneous melanoma tissues at stage Ⅱa from 31 patients performed during a 10-year follow-up period have also indicated that the down-regulation of these markers may be more appropriate than the detection of a single molecular marker for assessing the risk of melanoma progression and metastases[83].

Of particular therapeutic interest, a multicenter phase Ⅱ trial has also been undertaken in order to investigate the efficacy of a sensitivity-directed, first-line chemotherapy in patients with metastasized melanomas by performing an *in vitro* assay using an ATP-based luminescence viability test for evaluating the chemosensibility of viable melanoma cells obtained from metastatic lesions to seven single drugs and five drug combinations[90]. The results have revealed that among the 53 patients evaluable for all study end points, 22 (42%) were chemosensitive and 31 (58%) chemoresistant patients and the chemosensitive patients showed an increased overall survival of 14.6 months compared with 7.4 mo in chemoresistant patients[90]. In the same way, the results from a recent study have also indicated the possibility to establish the B-Raf^{E600V} mutation status in the tissue biopsies and circulating free DNA samples from melanoma patients to assess the patients that could be susceptible to respond to the pharmacological agents targeting oncogenic B-Raf^{E600V} mutant[91]. In addition, it has also been noted that the serum concentrations of diverse angiogenic factors such as VEGF, basic fibroblast factor (bFGF) and IL-8 were increased in melanoma patients relative to healthy individuals and associated with advanced stages and poor overall and progression-free survival of melanoma patients[18,23]. More particularly, a study carried out with 35 patients with stage Ⅳ melanoma has indicated that 15 patients who responded to chemotherapy showed a significant decrease in the serum IL-8 level while non-responders with progressive disease did not[92]. These data suggest that the detection of serum IL-8 level could serve as an indicator of the potential response of melanoma patients to the chemotherapeutic treatment.

Potential biomarkers in melanoma stem/progenitor cells

Of great clinical interest, the results from recent studies have also indicated the possibility to detect the stem cell-like markers such as ABCB5, nestin, CD133 and CD166 in primary and metastatic melanoma tissue specimens and/or circulating melanoma stem/progenitor cells in combination with current clinical biomarkers to predict the risk of the metastasis formation and overall survival of melanoma patients[51,55,59,63,93-95]. For instance, it has been observed that highly tumorigenic circulating melanoma cells isolated from the peripheral circulation of melanoma patients expressing the stem cell-like marker, ABCB5 multidrug transporter were tumorigenic and able to form the metastases in animal model *in vivo*[63]. Furthermore, it has been observed that the expression of ABCB5 protein was enhanced in primary and metastatic melanoma specimens as compared to normal skin and benign nevi[51,55,94]. Then, these data support the interest to detect the ABCB5 multidrug transporter in primary melanoma tissue specimens and circulating melanoma cells to predict the risk of progression to metastatic disease states.

The immunohistochemical analysis of nestin, which is a neuroepithelial intermediate filament expressed in proliferative neuroectodermal progenitor cells during embryonic development and adult bulge areas-resident stem cells in hair follicle, has also indicated that its expression was significantly enhanced in primary and metastatic melanoma tissue specimens as compared to benign and normal melanocytes[96-101]. Nestin was also co-expressed with SOX9 and SOX10, which may contribute to its transcriptional up-regulation, in primary and secondary melanoma specimens and associated with a poor survival of melanoma patients[98-101]. The analyses by flow cytometry and quantitative reverse transcription-PCR (qRT-PCR) of the expression level of nestin performed on 23 tissue specimens from patients with stage Ⅲ-Ⅳ melanoma has also indicated that this stem cell-like marker was expressed at a higher level in stage Ⅳ patients compared to stage Ⅲ/Ⅳ with no evidence of disease[93]. It has also been noted that the expression of nestin positively correlated with the tumor burden and tyrosinase and melan-A co-expression in malignant tissues[93]. Nestin has also been detected with tyrosinase in a proportion of circulating melanoma cells enriched from peripheral blood samples while no cells expressing nestin were detected in peripheral blood of healthy volunteers[93].

Additionally, it has also been reported that the percentage of circulating melanoma cells expressing stem cell-like markers, nestin and CD133, detected in 32 melanoma patients correlated with tumor burden and number of metastatic sites, and was associated with a shorter overall survival of patients[59]. The immunohistochemical analyses of co-expression of different stem cell-like markers, including nestin, CD133, ABCB5 and CD166 have also indicated that these biomarkers were significantly enhanced in primary and metastatic melanoma specimens as compared to melanocytic nevi[102,103]. On the other hand, a higher proportion of melanoma cells coexpressing stem cell-like markers, CD271 and SOX10, has also been detected within melanoma biopsies of primary tumors, melanoma metastases and melanoma cell lines and associated with higher metastatic potential and poor tumor-specific survival of melanoma patients[64].

Collectively, the recent advancements on the identification of distinct potential biomarkers in melanoma stem/progenitor cells and their differentiated progenies offer now the possibility to assess their expression levels in primary and metastatic melanoma tissue specimens, serum samples and/or circulating melanoma cells detected in peripheral circulation from patients in the clinics. The simultaneous analyses of the expression of these novel molecular biomarkers could be exploited to develop more effective and non-invasive screening tests for improving the current diagnostic and prognostic methods. Moreover, these novel molecular biomarkers could be used to predict the potential response of melanoma patients to the inhibitory agents targeting these deregulated signaling elements, and thereby lead to an optimization of the choice of cytotoxic drugs for their therapeutic treatment in the clinics. In this matter, we review data from recent *in vitro* and *in vivo* studies and clinical trials carried out to validate new potential therapeutic targets in melanoma stem/progenitor cells and their progenies for improving current treatments of patients diagnosed with aggressive melanomas.

NEW THERAPEUTIC STRATEGIES AGAINST AGGRESSIVE AND METASTATIC MELANOMAS

Molecular targeting strategies

Recent investigations in melanoma research have led to the identification of several molecular pathways and specific gene products that are often deregulated during melanoma initiation and progression to locally advanced and metastatic disease states. The oncogenic products constitute new potential therapeutic targets to eradicate the total melanoma cell mass, including melanoma stem/progenitor cells, and prevent disease progression and relapse. These deregulated gene products include B-Raf^{V600E}, N-Ras^{G61K}, different receptor tyrosine kinases (RTKs) such as EGFR, KIT, MET, PDGFRs and VEGFRs as well as sonic hedgehog, Wnt/β-catenin, Notch, Nodal/Cripto, HA/CD44 and SDF-1/CXCR4 and their downstream signaling effectors such as PI3K/Akt, NF-κB and MIC-1 as well as ABC multidrug resistance transporters (Figure 1; Table 1)[13,17,22,24-46]. The blockade of these tumorigenic pathways and targeting of drug resistance-associated molecules by using specific inhibitory agents has been shown to suppress the growth, invasion and/or metastases of melanoma cells and angiogenesis process *in vitro* and *in vivo*[17,24-46,104]. For instance, a combination of EGFR tyrosine kinase inhibitor, erlotinib plus adenoviral vector-mediated IL-24 expression was more effective as individual agents at inhibiting growth and inducing apoptosis of different melanoma cell lines *in vitro*[105]. In the same way, the combined treatment with erlotinib and a monoclonal antibody (mAb) termed bevacizumab that binds to and inhibits VEGF, also induced supra-additive inhibitory effect on the tumor growth of melanoma cell-derived xenografts and reduced the metastatic spread of melanoma cells to lymph nodes and lungs in mice as compared to single agents[106]. The antitumoral effects of the combined drugs was mediated in part through the inhibition of proliferation and increase of apoptosis of melanoma cells as well as a reduction in tumor angiogenesis[106]. Moreover, it has been reported that the activation of Ras/MAPK and PI3K/Akt pathways may contribute to the up-regulation of GLI transcriptional effector of hedgehog cascade in melanoma cells and the inhibition of smoothened (SMO) co-receptor for sonic hedgehog ligand using cyclopamine reduced the growth of melanoma cell-derived xenografts and metastases in mice and prevented disease recurrence (Figure 1)[104].

Importantly, the targeting of the stem cell-like marker CD133 using mAbs has also been reported to induce the cytotoxic effects in FEMX-I melanoma cells *in vitro* and reduce their metastatic spread in mice *in vivo*[57]. Moreover, the inhibition of ABCB5 multidrug transporter using a mAb also inhibited the tumor growth of CD133$^+$/ABCB5$^+$ melanoma stem cell-derived xenografts *in vivo*[55]. A combination of a CXCR4 inhibitor AMD3100 plus current chemotherapeutic drug, darcarbazine was also more effective at reducing the tumor growth and metastases of chemoresistant CD133$^+$/CXCR4$^+$ melanoma cells *in vivo* as compared to single drugs[65]. Additional studies, however, are necessary to further establish the molecular mechanisms at the basis of the cytotoxic effects of these therapeutic agents, alone or in combination therapies with current chemotherapeutic drug, dacarbazine on different melanoma cell models.

In addition, several clinical trials have also been carried out or are undergoing to investigate the anticarcinogenic efficacy of new chemopreventive and anticarcinogenic agents and diverse immunosuppressive therapeutic strategies such as the use of dentritic cells, high-doses of interferon-α (IFN-α) and/or IL-2 and anti- cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) antibody, alone or in combination with current therapies for treating locally advanced, metastatic and recurrent melano-

Table 1 Potential therapeutic targets in melanoma stem/progenitor cells and their progenies

Targeted deregulated element	Name of inhibitory agent
mAb against stem cell-like surface marker	
CD133	Anti-CD133 mAb
ABCB5	Anti-ABCB5 mAb
Growth factor signaling inhibitor	
EGFR (erbB1) antibody	mAb-C225, cetuximab (IMC-C225), IMC-1121B
EGFR-TKI	Gefitinib, erlotinib, AG1478, PD153035
Anti-EGF antibody	ABX-EGF
Pan-erbB1/erbB2/erbB3/erbB4-TKI	Cl1033
MET	SUI1274
Hedgehog	Anti-SHH antibody, SMO inhibitor (cyclopamine, GDC-0449, BMS-833923, NVP-LDE225, IPI-926 IPI-269609)
Wnt/β-catenin	Anti-Wnt antibody, WIF-1
Notch	γ-secretase inhibitor (DAPT, MK-0752,GSI-18)
Nodal/Cripto	LEFTY, Anti-Cripto mAb
KIT	Imatinib mesylate, dasatinib
HA/CD44	Anti-CD44 mAb, soluble CD44 protein
VEGF	Anti-VEGF antibody (bevacizumab)
VEGFR2	Anti-VEGFR-2 mAb (DC101)
VEGFR2/EGFR/RET	Vandetanib (ZD6474)
VEGFRs, PDGFRs, KIT	Sunitinib
B-Raf, C-Raf, KIT, PDGFRs, VEGFR2 and 3	Sorafenib
ECM component/integrin	Anti-integrin antibody
CXCR4	AMD3100
Intracellular signaling inhibitor	
B-RafE600V	PLX 4032, PLX4720
MEK1/2	AZD6244 (ARRY-142886), PD0325901
PI3K	LY294002
mTOR	Rapamycin, CCl-779,
PI3K/mTOR	PI-103
NF-κB	IkBα inhibitor, sulfasalazine, bortezomib (PS-341) salinosporamides A (NPI-0052), parthenolide
COX-2	NS-396, etodolax, celecoxib, rofecoxib
Immunomodulatory agent	
Immune and/or vascular systems	Imiquimod, INF-α, IL-2, IL-21, anti-CTLA-4, mAb (ipilimumab "MDX 010"and tremelimumab "CP-675, 206")

CTLA-4: Cytotoxic T lymphocyte-associated antigen 4; DAPT: N-(N-3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; ECM: Extracellular matrix; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; IκBα: Inhibitor of nuclear factor-κBα; IL: Interleukin; INF: Interferon; mAb: Monoclonal antibody; NF-κB: Nuclear factor-kappaB; PDGFRs: Platelet-derived growth factor receptors; MET: Hepatocyte growth factor receptor; PI3K: Phosphatidylinositol 3'-kinase; SMO: Smoothened; TKI: Tyrosine kinase inhibitor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; WIF-1: Wingless inhibitory factor-1; Wnt: Wingless ligand.

mas[33,66,107-119]. The cytotoxic drugs include the specific inhibitors of B-Raf^{E600V}, N-Ras^{G61K}, KIT, EGFR and hedgehog signaling elements. Importantly, the results from phase I clinical studies with a orally active inhibitor of oncogenic B-Raf^{V600E} product, PLX4032 carried out with 32 patients with metastatic melanomas harboring the B-Raf^{V600E} mutation have revealed that this treatment led to a substantial tumor regression including 24 patients that showed a partial response and 2 had a complete response[120]. The trials are now undergoing to determine the long-term effect of PLX4032, alone or in combination with other agents such as MEK inhibitor, on the survival of melanoma patients[121,122]. In regard with this, the clinical responses have also been observed in a phase II study with orally active and highly selective inhibitor of MEK1/2, AZD6244 (ARRY-142886) or temozolomide performed with 200 patients with advanced melanoma harboring B-Raf^{E600V} mutation[123,124]. On the other hand, it has also been reported that the melanoma patients harboring the activating mutations in the KIT receptor exhibited a partial or complete response to imatinib mesylate[33]. It has however been noted that dasatinib was more effective than imatinib at reducing the viability of melanoma cells in two melanoma patients harboring the KIT^{L576P} mutation which is the most frequent KIT mutation occurring in approximately 30%-40% cases of melanoma[44]. Furthermore, the data from a multi-institutional phase II trial with an oral multikinase inhibitor termed sorafenib, which targets different tyrosine protein kinases, including wild-type and mutant B-Raf and C-Raf kinases, PDGFRs, KIT, VEGFR2 and VEGFR3, performed with 36 patients with advanced melanomas have indicated that 1 patient showed a partial response for 175 d and 3 patients had stable disease with a mean duration of 37 wk[125].

Immunotherapy-based strategies

Among other promising experimental strategies, the results from the clinical trials with a experimental treatment consisting to a topical application of a cream containing 5% an immunomodulatory agent, imiquimod (Aldara) after surgical excision of tumors have revealed that this treatment reduced some melanocytic nevi and melanoma-in-situ (lentigo maligna)[9,10,107-109,126,127]. Moreover, the data from a Phase Ⅰ/Ⅱ study of a combination of topical imiquimod and intralesional IL-2 have also revealed that its treatment induced a significant clinical response in patients with multiple accessible melanoma metastases by increasing the activated lymphocytes and the production of IFN-γ by peripheral blood mononuclear cells as well as by restoring the Th1/Th2 balance[110,111]. In addition, a therapeutic treatment consisting of an adjuvant immunotherapy with high doses of immunosuppressive agents, IL-2 and/or IFN-α, alone or in combination with chemotherapy or adoptive cell therapy, has also been observed to result in a complete and long-lasting remission in a small subset of melanoma patients[1,9,10,66,113,116,117,128-130]. In particular, it has been reported that the melanoma cell density in metastases and angiogenesis was significant reduced after a treatment with IFN-α[131]. Moreover, the results of phase Ⅱ trials with 28 patients with stage Ⅳ

melanoma without brain metastases have revealed that a combination of dacarbazine plus pegylated IFN-α2a was well tolerated and associated with a response rate of 24% in 25 patients evaluable for response, including 2 long-lasting complete responses[132]. Interestingly, the results of a phase II trial with an oncolytic herpes simplex virus type 1 encoding granulocyte macrophage-colony stimulating factor (GM-CSF), designated as Oncovex (GM-CSF), have also indicated a 28% objective response rate occurred in patients with melanomas which was accompanied by a tumor regression of both injected and non-injected lesions[126,127]. These data suggest that the treatment with Oncovex (GM-CSF) can induce a direct oncolytic effect in injected tumors as well as a secondary immune-mediated anti-tumor effect on non-injected tumors[126,127].

CONCLUSION

Significant advancements made in last few years have provided important information on the molecular signaling pathways and gene products that are frequently deregulated in melanoma stem/progenitor cells and their progenies during melanoma formation and progression to locally advanced and metastatic disease states. Consequently, the combination of different molecular biomarkers or cytotoxic agents targeting distinct gene products altered during melanoma development may constitute more promising therapeutic strategies as the use a single biomarker or monotherapy for improving the accurate of current diagnostic and prognostic methods and efficacy of the treatment of melanoma patients.

REFERENCES

1 **Tsao H**, Atkins MB, Sober AJ. Management of cutaneous melanoma. *N Engl J Med* 2004; **351**: 998-1012
2 **Gray-Schopfer V**, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. *Nature* 2007; **445**: 851-857
3 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**: 277-300
4 **Garbe C**, Leiter U. Melanoma epidemiology and trends. *Clin Dermatol* 2009; **27**: 3-9
5 **Houghton AN**, Polsky D. Focus on melanoma. *Cancer Cell* 2002; **2**: 275-278
6 **Balch CM**, Buzaid AC, Soong SJ, Atkins MB, Cascinelli N, Coit DG, Fleming ID, Gershenwald JE, Houghton A, Kirkwood JM, McMasters KM, Mihm MF, Morton DL, Reintgen DS, Ross MI, Sober A, Thompson JA, Thompson JF. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol* 2001; **19**: 3635-3648
7 **Francken AB**, Bastiaannet E, Hoekstra HJ. Follow-up in patients with localised primary cutaneous melanoma. *Lancet Oncol* 2005; **6**: 608-621
8 **Bastiaannet E**, Beukema JC, Hoekstra HJ. Radiation therapy following lymph node dissection in melanoma patients: treatment, outcome and complications. *Cancer Treat Rev* 2005; **31**: 18-26
9 **Buzaid AC**. Management of metastatic cutaneous melanoma. *Oncology (Williston Park)* 2004; **18**: 1443-150; discussion 1443-150
10 **Danson S**, Lorigan P. Improving outcomes in advanced malignant melanoma: update on systemic therapy. *Drugs* 2005; **65**: 733-743
11 **Comis RL**. DTIC (NSC-45388) in malignant melanoma: a perspective. *Cancer Treat Rep* 1976; **60**: 165-176
12 **Hill GJ**, Metter GE, Krementz ET, Fletcher WS, Golomb FM, Ramirez G, Grage TB, Moss SE. DTIC and combination therapy for melanoma. II. Escalating schedules of DTIC with BCNU, CCNU, and vincristine. *Cancer Treat Rep* 1979; **63**: 1989-1992
13 **Tawbi HA**, Buch SC. Chemotherapy resistance abrogation in metastatic melanoma. *Clin Adv Hematol Oncol* 2010; **8**: 259-266
14 **Liu ZJ**, Xiao M, Balint K, Smalley KS, Brafford P, Qiu R, Pinnix CC, Li X, Herlyn M. Notch1 signaling promotes primary melanoma progression by activating mitogen-activated protein kinase/phosphatidylinositol 3-kinase-Akt pathways and up-regulating N-cadherin expression. *Cancer Res* 2006; **66**: 4182-4190
15 **Mehnert JM**, McCarthy MM, Jilaveanu L, Flaherty KT, Aziz S, Camp RL, Rimm DL, Kluger HM. Quantitative expression of VEGF, VEGF-R1, VEGF-R2, and VEGF-R3 in melanoma tissue microarrays. *Hum Pathol* 2010; **41**: 375-384
16 **Gangjee A**, Kurup S, Ihnat MA, Thorpe JE, Shenoy SS. Synthesis and biological activity of N(4)-phenylsubstituted-6-(2,4-dichloro phenylmethyl)-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamines as vascular endothelial growth factor receptor-2 inhibitors and antiangiogenic and antitumor agents. *Bioorg Med Chem* 2010; **18**: 3575-3587
17 **Mimeault M**, Bonenfant D, Batra SK. New advances on the functions of epidermal growth factor receptor and ceramides in skin cell differentiation, disorders and cancers. *Skin Pharmacol Physiol* 2004; **17**: 153-166
18 **Rofstad EK**, Halsør EF. Vascular endothelial growth factor, interleukin 8, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor promote angiogenesis and metastasis in human melanoma xenografts. *Cancer Res* 2000; **60**: 4932-4938
19 **Pinnix CC**, Herlyn M. The many faces of Notch signaling in skin-derived cells. *Pigment Cell Res* 2007; **20**: 458-465
20 **Fernandez F**, Caygill CP, Kirkham JS, Northfield TC, Savalgi R, Hill MJ. Faecal bile acids and bowel cancer risk in gastric-surgery patients. *Eur J Cancer Prev* 1991; **1** Suppl 2: 79-82
21 **Mimeault M**, Batra SK. Recent advances on skin-resident stem/progenitor cell functions in skin regeneration, aging and cancers and novel anti-aging and cancer therapies. *J Cell Mol Med* 2010; **14**: 116-134
22 **Ohshima Y**, Yajima I, Takeda K, Iida M, Kumasaka M, Matsumoto Y, Kato M. c-RET molecule in malignant melanoma from oncogenic RET-carrying transgenic mice and human cell lines. *PLoS One* 2010; **5**: e10279
23 **Ugurel S**, Rappl G, Tilgen W, Reinhold U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. *J Clin Oncol* 2001; **19**: 577-583
24 **Iivanainen E**, Lauttia S, Zhang N, Tvorogov D, Kulmala J, Grenman R, Salven P, Elenius K. The EGFR inhibitor gefitinib suppresses recruitment of pericytes and bone marrow-derived perivascular cells into tumor vessels. *Microvasc Res* 2009; **78**: 278-285
25 **Djerf EA**, Trinks C, Abdiu A, Thunell LK, Hallbeck AL, Walz TM. ErbB receptor tyrosine kinases contribute to proliferation of malignant melanoma cells: inhibition by gefitinib (ZD1839). *Melanoma Res* 2009; **19**: 156-166
26 **Amin DN**, Bielenberg DR, Lifshits E, Heymach JV, Klagsbrun M. Targeting EGFR activity in blood vessels is sufficient to inhibit tumor growth and is accompanied by an increase in VEGFR-2 dependence in tumor endothelial cells. *Microvasc Res* 2008; **76**: 15-22
27 **Chin L**, Garraway LA, Fisher DE. Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev* 2006; **20**: 2149-2182

28 **Curtin JA**, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* 2006; **24**: 4340-4346
29 **Guo Y**, Ma J, Wang J, Che X, Narula J, Bigby M, Wu M, Sy MS. Inhibition of human melanoma growth and metastasis in vivo by anti-CD44 monoclonal antibody. *Cancer Res* 1994; **54**: 1561-1565
30 **Ahrens T**, Sleeman JP, Schempp CM, Howells N, Hofmann M, Ponta H, Herrlich P, Simon JC. Soluble CD44 inhibits melanoma tumor growth by blocking cell surface CD44 binding to hyaluronic acid. *Oncogene* 2001; **20**: 3399-3408
31 **Weeraratna AT**, Jiang Y, Hostetter G, Rosenblatt K, Duray P, Bittner M, Trent JM. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* 2002; **1**: 279-288
32 **Smalley KS**, Contractor R, Nguyen TK, Xiao M, Edwards R, Muthusamy V, King AJ, Flaherty KT, Bosenberg M, Herlyn M, Nathanson KL. Identification of a novel subgroup of melanomas with KIT/cyclin-dependent kinase-4 overexpression. *Cancer Res* 2008; **68**: 5743-5752
33 **Hodi FS**, Friedlander P, Corless CL, Heinrich MC, Mac Rae S, Kruse A, Jagannathan J, Van den Abbeele AD, Velazquez EF, Demetri GD, Fisher DE. Major response to imatinib mesylate in KIT-mutated melanoma. *J Clin Oncol* 2008; **26**: 2046-2051
34 **Bartolazzi A**, Peach R, Aruffo A, Stamenkovic I. Interaction between CD44 and hyaluronate is directly implicated in the regulation of tumor development. *J Exp Med* 1994; **180**: 53-66
35 **Knight LA**, Di Nicolantonio F, Whitehouse P, Mercer S, Sharma S, Glaysher S, Johnson P, Cree IA. The in vitro effect of gefitinib ('Iressa') alone and in combination with cytotoxic chemotherapy on human solid tumours. *BMC Cancer* 2004; **4**: 83
36 **Kim Y**, Lee YS, Choe J, Lee H, Kim YM, Jeoung D. CD44-epidermal growth factor receptor interaction mediates hyaluronic acid-promoted cell motility by activating protein kinase C signaling involving Akt, Rac1, Phox, reactive oxygen species, focal adhesion kinase, and MMP-2. *J Biol Chem* 2008; **283**: 22513-22528
37 **Qiu L**, Wang Q, Di W, Jiang Q, Schefeller E, Derby S, Wanebo H, Yan B, Wan Y. Transient activation of EGFR/AKT cell survival pathway and expression of survivin contribute to reduced sensitivity of human melanoma cells to betulinic acid. *Int J Oncol* 2005; **27**: 823-830
38 **Kumano K**, Masuda S, Sata M, Saito T, Lee SY, Sakata-Yanagimoto M, Tomita T, Iwatsubo T, Natsugari H, Kurokawa M, Ogawa S, Chiba S. Both Notch1 and Notch2 contribute to the regulation of melanocyte homeostasis. *Pigment Cell Melanoma Res* 2008; **21**: 70-78
39 **Postovit LM**, Seftor EA, Seftor RE, Hendrix MJ. Targeting Nodal in malignant melanoma cells. *Expert Opin Ther Targets* 2007; **11**: 497-505
40 **Postovit LM**, Margaryan NV, Seftor EA, Hendrix MJ. Role of nodal signaling and the microenvironment underlying melanoma plasticity. *Pigment Cell Melanoma Res* 2008; **21**: 348-357
41 **Nickoloff BJ**, Hendrix MJ, Pollock PM, Trent JM, Miele L, Qin JZ. Notch and NOXA-related pathways in melanoma cells. *J Investig Dermatol Symp Proc* 2005; **10**: 95-104
42 **Huh SJ**, Chung CY, Sharma A, Robertson GP. Macrophage inhibitory cytokine-1 regulates melanoma vascular development. *Am J Pathol* 2010; **176**: 2948-2957
43 **Puri N**, Ahmed S, Janamanchi V, Tretiakova M, Zumba O, Krausz T, Jagadeeswaran R, Salgia R. c-Met is a potentially new therapeutic target for treatment of human melanoma. *Clin Cancer Res* 2007; **13**: 2246-2253
44 **Woodman SE**, Trent JC, Stemke-Hale K, Lazar AJ, Pricl S, Pavan GM, Fermeglia M, Gopal YN, Yang D, Podoloff DA, Ivan D, Kim KB, Papadopoulos N, Hwu P, Mills GB, Davies MA. Activity of dasatinib against L576P KIT mutant melanoma: molecular, cellular, and clinical correlates. *Mol Cancer Ther* 2009; **8**: 2079-2085
45 **Tsai J**, Lee JT, Wang W, Zhang J, Cho H, Mamo S, Bremer R, Gillette S, Kong J, Haass NK, Sproesser K, Li L, Smalley KS, Fong D, Zhu YL, Marimuthu A, Nguyen H, Lam B, Liu J, Cheung I, Rice J, Suzuki Y, Luu C, Settachatgul C, Shellooe R, Cantwell J, Kim SH, Schlessinger J, Zhang KY, West BL, Powell B, Habets G, Zhang C, Ibrahim PN, Hirth P, Artis DR, Herlyn M, Bollag G. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc Natl Acad Sci U S A* 2008; **105**: 3041-3046
46 **Halaban R**, Zhang W, Bacchiocchi A, Cheng E, Parisi F, Ariyan S, Krauthammer M, McCusker JP, Kluger Y, Sznol M. PLX4032, a selective BRAF(V600E) kinase inhibitor, activates the ERK pathway and enhances cell migration and proliferation of BRAF melanoma cells. *Pigment Cell Melanoma Res* 2010; **23**: 190-200
47 **Frank NY**, Schatton T, Kim S, Zhan Q, Wilson BJ, Ma J, Saab KR, Osherov V, Widlund HR, Gasser M, Waaga-Gasser AM, Kupper TS, Murphy GF, Frank MH. VEGFR-1 expressed by malignant melanoma-initiating cells is required for tumor growth. *Cancer Res* 2011; **71**: 1474-1485
48 **Ruiter D**, Bogenrieder T, Elder D, Herlyn M. Melanoma-stroma interactions: structural and functional aspects. *Lancet Oncol* 2002; **3**: 35-43
49 **Merighi S**, Simioni C, Gessi S, Varani K, Mirandola P, Tabrizi MA, Baraldi PG, Borea PA. A(2B) and A(3) adenosine receptors modulate vascular endothelial growth factor and interleukin-8 expression in human melanoma cells treated with etoposide and doxorubicin. *Neoplasia* 2009; **11**: 1064-1073
50 **Fang D**, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005; **65**: 9328-9337
51 **Frank NY**, Margaryan A, Huang Y, Schatton T, Waaga-Gasser AM, Gasser M, Sayegh MH, Sadee W, Frank MH. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. *Cancer Res* 2005; **65**: 4320-4333
52 **Dou J**, Pan M, Wen P, Li Y, Tang Q, Chu L, Zhao F, Jiang C, Hu W, Hu K, Gu N. Isolation and identification of cancer stem-like cells from murine melanoma cell lines. *Cell Mol Immunol* 2007; **4**: 467-472
53 **Monzani E**, Facchetti F, Galmozzi E, Corsini E, Benetti A, Cavazzin C, Gritti A, Piccinini A, Porro D, Santinami M, Invernici G, Parati E, Alessandri G, La Porta CA. Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. *Eur J Cancer* 2007; **43**: 935-946
54 **Keshet GI**, Goldstein I, Itzhaki O, Cesarkas K, Shenhav L, Yakirevitch A, Treves AJ, Schachter J, Amariglio N, Rechavi G. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008; **368**: 930-936
55 **Schatton T**, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, Fuhlbrigge RC, Kupper TS, Sayegh MH, Frank MH. Identification of cells initiating human melanomas. *Nature* 2008; **451**: 345-349
56 **Grichnik JM**, Burch JA, Schulteis RD, Shan S, Liu J, Darrow TL, Vervaert CE, Seigler HF. Melanoma, a tumor based on a mutant stem cell? *J Invest Dermatol* 2006; **126**: 142-153
57 **Rappa G**, Fodstad O, Lorico A. The stem cell-associated antigen CD133 (Prominin-1) is a molecular therapeutic target for metastatic melanoma. *Stem Cells* 2008; **26**: 3008-3017
58 **Kupas V**, Weishaupt C, Siepmann D, Kaserer ML, Eickelmann M, Metze D, Luger TA, Beissert S, Loser K. RANK is expressed in metastatic melanoma and highly upregulated on melanoma-initiating cells. *J Invest Dermatol* 2011; **131**: 944-955
59 **Fusi A**, Reichelt U, Busse A, Ochsenreither S, Rietz A,

Maisel M, Keilholz U. Expression of the stem cell markers nestin and CD133 on circulating melanoma cells. *J Invest Dermatol* 2011; **131**: 487-494
60 **Boonyaratanakornkit JB**, Yue L, Strachan LR, Scalapino KJ, LeBoit PE, Lu Y, Leong SP, Smith JE, Ghadially R. Selection of tumorigenic melanoma cells using ALDH. *J Invest Dermatol* 2010; **130**: 2799-2808
61 **Boiko AD**, Razorenova OV, van de Rijn M, Swetter SM, Johnson DL, Ly DP, Butler PD, Yang GP, Joshua B, Kaplan MJ, Longaker MT, Weissman IL. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. *Nature* 2010; **466**: 133-137
62 **Zabierowski SE**, Herlyn M. Learning the ABCs of melanoma-initiating cells. *Cancer Cell* 2008; **13**: 185-187
63 **Ma J**, Lin JY, Alloo A, Wilson BJ, Schatton T, Zhan Q, Murphy GF, Waaga-Gasser AM, Gasser M, Stephen Hodi F, Frank NY, Frank MH. Isolation of tumorigenic circulating melanoma cells. *Biochem Biophys Res Commun* 2010; **402**: 711-717
64 **Civenni G**, Walter A, Kobert N, Mihic-Probst D, Zipser M, Belloni B, Seifert B, Moch H, Dummer R, van den Broek M, Sommer L. Human CD271-positive melanoma stem cells associated with metastasis establish tumor heterogeneity and long-term growth. *Cancer Res* 2011; **71**: 3098-3109
65 **Kim M**, Koh YJ, Kim KE, Koh BI, Nam DH, Alitalo K, Kim I, Koh GY. CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. *Cancer Res* 2010; **70**: 10411-10421
66 **Algazi AP**, Soon CW, Daud AI. Treatment of cutaneous melanoma: current approaches and future prospects. *Cancer Manag Res* 2010; **2**: 197-211
67 **Patnana M**, Bronstein Y, Szklaruk J, Bedi DG, Hwu WJ, Gershenwald JE, Prieto VG, Ng CS. Multimethod imaging, staging, and spectrum of manifestations of metastatic melanoma. *Clin Radiol* 2011; **66**: 224-236
68 **Nonaka D**, Chiriboga L, Rubin BP. Differential expression of S100 protein subtypes in malignant melanoma, and benign and malignant peripheral nerve sheath tumors. *J Cutan Pathol* 2008; **35**: 1014-1019
69 **Mahmood MN**, Lee MW, Linden MD, Nathanson SD, Hornyak TJ, Zarbo RJ. Diagnostic value of HMB-45 and anti-Melan A staining of sentinel lymph nodes with isolated positive cells. *Mod Pathol* 2002; **15**: 1288-1293
70 **Ben-Izhak O**, Stark P, Levy R, Bergman R, Lichtig C. Epithelial markers in malignant melanoma. A study of primary lesions and their metastases. *Am J Dermatopathol* 1994; **16**: 241-246
71 **Gown AM**, Vogel AM, Hoak D, Gough F, McNutt MA. Monoclonal antibodies specific for melanocytic tumors distinguish subpopulations of melanocytes. *Am J Pathol* 1986; **123**: 195-203
72 **Li M**, Zhang B, Sun B, Wang X, Ban X, Sun T, Liu Z, Zhao X. A novel function for vimentin: the potential biomarker for predicting melanoma hematogenous metastasis. *J Exp Clin Cancer Res* 2010; **29**: 109
73 **Ugurel S**, Houben R, Schrama D, Voigt H, Zapatka M, Schadendorf D, Bröcker EB, Becker JC. Microphthalmia-associated transcription factor gene amplification in metastatic melanoma is a prognostic marker for patient survival, but not a predictive marker for chemosensitivity and chemotherapy response. *Clin Cancer Res* 2007; **13**: 6344-6350
74 **Matharoo-Ball B**, Ratcliffe L, Lancashire L, Ugurel S, Miles AK, Weston DJ, Rees R, Schadendorf D, Ball G, Creaser CS. Diagnostic biomarkers differentiating metastatic melanoma patients from healthy controls identified by an integrated MALDI-TOF mass spectrometry/bioinformatic approach. *Proteomics Clin Appl* 2007; **1**: 605-620
75 **Utikal J**, Schadendorf D, Ugurel S. Serologic and immunohistochemical prognostic biomarkers of cutaneous malignancies. *Arch Dermatol Res* 2007; **298**: 469-477
76 **Boyle GM**, Pedley J, Martyn AC, Banducci KJ, Strutton GM, Brown DA, Breit SN, Parsons PG. Macrophage inhibitory cytokine-1 is overexpressed in malignant melanoma and is associated with tumorigenicity. *J Invest Dermatol* 2009; **129**: 383-391
77 **Ugurel S**, Thirumaran RK, Bloethner S, Gast A, Sucker A, Mueller-Berghaus J, Rittgen W, Hemminki K, Becker JC, Kumar R, Schadendorf D. B-RAF and N-RAS mutations are preserved during short time in vitro propagation and differentially impact prognosis. *PLoS One* 2007; **2**: e236
78 **Findeisen P**, Zapatka M, Peccerella T, Matzk H, Neumaier M, Schadendorf D, Ugurel S. Serum amyloid A as a prognostic marker in melanoma identified by proteomic profiling. *J Clin Oncol* 2009; **27**: 2199-2208
79 **Busch C**, Geisler J, Knappskog S, Lillehaug JR, Lønning PE. Alterations in the p53 pathway and p16INK4a expression predict overall survival in metastatic melanoma patients treated with dacarbazine. *J Invest Dermatol* 2010; **130**: 2514-2516
80 **Jönsson G**, Busch C, Knappskog S, Geisler J, Miletic H, Ringnér M, Lillehaug JR, Borg A, Lønning PE. Gene expression profiling-based identification of molecular subtypes in stage IV melanomas with different clinical outcome. *Clin Cancer Res* 2010; **16**: 3356-3367
81 **Grafström E**, Egyházi S, Ringborg U, Hansson J, Platz A. Biallelic deletions in INK4 in cutaneous melanoma are common and associated with decreased survival. *Clin Cancer Res* 2005; **11**: 2991-2997
82 **Blokx WA**, van Dijk MC, Ruiter DJ. Molecular cytogenetics of cutaneous melanocytic lesions - diagnostic, prognostic and therapeutic aspects. *Histopathology* 2010; **56**: 121-132
83 **Tchernev G**, Orfanos CE. Downregulation of cell cycle modulators p21, p27, p53, Rb and proapoptotic Bcl-2-related proteins Bax and Bak in cutaneous melanoma is associated with worse patient prognosis: preliminary findings. *J Cutan Pathol* 2007; **34**: 247-256
84 **de Wit NJ**, Rijntjes J, Diepstra JH, van Kuppevelt TH, Weidle UH, Ruiter DJ, van Muijen GN. Analysis of differential gene expression in human melanocytic tumour lesions by custom made oligonucleotide arrays. *Br J Cancer* 2005; **92**: 2249-2261
85 **Dai DL**, Martinka M, Li G. Prognostic significance of activated Akt expression in melanoma: a clinicopathologic study of 292 cases. *J Clin Oncol* 2005; **23**: 1473-1482
86 **Udart M**, Utikal J, Krähn GM, Peter RU. Chromosome 7 aneusomy. A marker for metastatic melanoma? Expression of the epidermal growth factor receptor gene and chromosome 7 aneusomy in nevi, primary malignant melanomas and metastases. *Neoplasia* 2001; **3**: 245-254
87 **Rákosy Z**, Vízkeleti L, Ecsedi S, Vokó Z, Bégány A, Barok M, Krekk Z, Gallai M, Szentirmay Z, Adány R, Balázs M. EGFR gene copy number alterations in primary cutaneous malignant melanomas are associated with poor prognosis. *Int J Cancer* 2007; **121**: 1729-1737
88 **Hoek K**, Rimm DL, Williams KR, Zhao H, Ariyan S, Lin A, Kluger HM, Berger AJ, Cheng E, Trombetta ES, Wu T, Ninobe M, Yoshikawa K, Hannigan GE, Halaban R. Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Res* 2004; **64**: 5270-5282
89 **Williams PF**, Olsen CM, Hayward NK, Whiteman DC. Melanocortin 1 receptor and risk of cutaneous melanoma: a meta-analysis and estimates of population burden. *Int J Cancer* 2011; **129**: 1730-1740
90 **Ugurel S**, Schadendorf D, Pföhler C, Neuber K, Thoelke A, Ulrich J, Hauschild A, Spieth K, Kaatz M, Rittgen W, Delorme S, Tilgen W, Reinhold U. In vitro drug sensitivity predicts response and survival after individualized sensitivity-directed chemotherapy in metastatic melanoma: a multicenter phase II trial of the Dermatologic Cooperative Oncology Group. *Clin Cancer Res* 2006; **12**: 5454-5463

91 **Board RE**, Ellison G, Orr MC, Kemsley KR, McWalter G, Blockley LY, Dearden SP, Morris C, Ranson M, Cantarini MV, Dive C, Hughes A. Detection of BRAF mutations in the tumour and serum of patients enrolled in the AZD6244 (ARRY-142886) advanced melanoma phase II study. *Br J Cancer* 2009; **101**: 1724-1730

92 **Brennecke S**, Deichmann M, Naeher H, Kurzen H. Decline in angiogenic factors, such as interleukin-8, indicates response to chemotherapy of metastatic melanoma. *Melanoma Res* 2005; **15**: 515-522

93 **Fusi A**, Ochsenreither S, Busse A, Rietz A, Keilholz U. Expression of the stem cell marker nestin in peripheral blood of patients with melanoma. *Br J Dermatol* 2010; **163**: 107-114

94 **Vásquez-Moctezuma I**, Meraz-Ríos MA, Villanueva-López CG, Magaña M, Martínez-Macias R, Sánchez-González DJ, García-Sierra F, Herrera-González NE. ATP-binding cassette transporter ABCB5 gene is expressed with variability in malignant melanoma. *Actas Dermosifiliogr* 2010; **101**: 341-348

95 **Laga AC**, Zhan Q, Weishaupt C, Ma J, Frank MH, Murphy GF. SOX2 and nestin expression in human melanoma: an immunohistochemical and experimental study. *Exp Dermatol* 2011; **20**: 339-345

96 **Flørenes VA**, Holm R, Myklebost O, Lendahl U, Fodstad O. Expression of the neuroectodermal intermediate filament nestin in human melanomas. *Cancer Res* 1994; **54**: 354-356

97 **Brychtová S**, Fiurásková M, Brychta T, Hirnák J. [The role of intermedial filament nestin in malignant melanoma progression]. *Cesk Patol* 2005; **41**: 143-145

98 **Piras F**, Perra MT, Murtas D, Minerba L, Floris C, Maxia C, Demurtas P, Ugalde J, Ribatti D, Sirigu P. The stem cell marker nestin predicts poor prognosis in human melanoma. *Oncol Rep* 2010; **23**: 17-24

99 **Kanoh M**, Amoh Y, Tanabe K, Maejima H, Takasu H, Katsuoka K. Nestin is expressed in HMB-45 negative melanoma cells in dermal parts of nodular melanoma. *J Dermatol* 2010; **37**: 505-511

100 **Tanabe K**, Amoh Y, Kanoh M, Takasu H, Sakai N, Sato Y, Katsuoka K. Prognostic significance of the hair follicle stem cell marker nestin in patients with malignant melanoma. *Eur J Dermatol* 2010; **20**: 283-288

101 **Bakos RM**, Maier T, Besch R, Mestel DS, Ruzicka T, Sturm RA, Berking C. Nestin and SOX9 and SOX10 transcription factors are coexpressed in melanoma. *Exp Dermatol* 2010; **19**: e89-e94

102 **Klein WM**, Wu BP, Zhao S, Wu H, Klein-Szanto AJ, Tahan SR. Increased expression of stem cell markers in malignant melanoma. *Mod Pathol* 2007; **20**: 102-107

103 **Sharma BK**, Manglik V, Elias EG. Immuno-expression of human melanoma stem cell markers in tissues at different stages of the disease. *J Surg Res* 2010; **163**: e11-e15

104 **Stecca B**, Mas C, Clement V, Zbinden M, Correa R, Piguet V, Beermann F, Ruiz I Altaba A. Melanomas require HEDGEHOG-GLI signaling regulated by interactions between GLI1 and the RAS-MEK/AKT pathways. *Proc Natl Acad Sci U S A* 2007; **104**: 5895-5900

105 **Deng WG**, Kwon J, Ekmekcioglu S, Poindexter NJ, Grimm EA. IL-24 gene transfer sensitizes melanoma cells to erlotinib through modulation of the Apaf-1 and Akt signaling pathways. *Melanoma Res* 2010

106 **Schicher N**, Paulitschke V, Swoboda A, Kunstfeld R, Loewe R, Pilarski P, Pehamberger H, Hoeller C. Erlotinib and bevacizumab have synergistic activity against melanoma. *Clin Cancer Res* 2009; **15**: 3495-3502

107 **Turza K**, Dengel LT, Harris RC, Patterson JW, White K, Grosh WW, Slingluff CL. Effectiveness of imiquimod limited to dermal melanoma metastases, with simultaneous resistance of subcutaneous metastasis. *J Cutan Pathol* 2010; **37**: 94-98

108 **Buettiker UV**, Yawalkar NY, Braathen LR, Hunger RE. Imiquimod treatment of lentigo maligna: an open-label study of 34 primary lesions in 32 patients. *Arch Dermatol* 2008; **144**: 943-945

109 **Wolf IH**, Cerroni L, Kodama K, Kerl H. Treatment of lentigo maligna (melanoma in situ) with the immune response modifier imiquimod. *Arch Dermatol* 2005; **141**: 510-514

110 **Green DS**, Dalgleish AG, Belonwu N, Fischer MD, Bodman-Smith MD. Topical imiquimod and intralesional interleukin-2 increase activated lymphocytes and restore the Th1/Th2 balance in patients with metastatic melanoma. *Br J Dermatol* 2008; **159**: 606-614

111 **Green DS**, Bodman-Smith MD, Dalgleish AG, Fischer MD. Phase I/II study of topical imiquimod and intralesional interleukin-2 in the treatment of accessible metastases in malignant melanoma. *Br J Dermatol* 2007; **156**: 337-345

112 **Krauze MT**, Tarhini A, Gogas H, Kirkwood JM. Prognostic significance of autoimmunity during treatment of melanoma with interferon. *Semin Immunopathol* 2011; **33**: 385-391

113 **Weide B**, Eigentler TK, Pflugfelder A, Leiter U, Meier F, Bauer J, Schmidt D, Radny P, Pföhler C, Garbe C. Survival after intratumoral interleukin-2 treatment of 72 melanoma patients and response upon the first chemotherapy during follow-up. *Cancer Immunol Immunother* 2011; **60**: 487-493

114 **Bedikian AY**, Johnson MM, Warneke CL, Papadopoulos NE, Kim KB, Hwu WJ, McIntyre S, Rohlfs M, Homsi J, Hwu P. Does complete response to systemic therapy in patients with stage IV melanoma translate into long-term survival? *Melanoma Res* 2010

115 **Ridolfi L**, Petrini M, Fiammenghi L, Granato AM, Ancarani V, Pancisi E, Scarpi E, Guidoboni M, Migliori G, Sanna S, Tauceri F, Verdecchia GM, Riccobon A, Valmorri L, Ridolfi R. Unexpected high response rate to traditional therapy after dendritic cell-based vaccine in advanced melanoma: update of clinical outcome and subgroup analysis. *Clin Dev Immunol* 2010; **2010**: 504979

116 **Finkelstein SE**, Carey T, Fricke I, Yu D, Goetz D, Gratz M, Dunn M, Urbas P, Daud A, DeConti R, Antonia S, Gabrilovich D, Fishman M. Changes in dendritic cell phenotype after a new high-dose weekly schedule of interleukin-2 therapy for kidney cancer and melanoma. *J Immunother* 2010; **33**: 817-827

117 **Schadendorf D**, Algarra SM, Bastholt L, Cinat G, Dreno B, Eggermont AM, Espinosa E, Guo J, Hauschild A, Petrella T, Schachter J, Hersey P. Immunotherapy of distant metastatic disease. *Ann Oncol* 2009; **20 Suppl 6**: vi41-vi50

118 **Becker JC**, Bröcker EB, Schadendorf D, Ugurel S. Imatinib in melanoma: a selective treatment option based on KIT mutation status? *J Clin Oncol* 2007; **25**: e9

119 **Agarwala SS**. Novel immunotherapies as potential therapeutic partners for traditional or targeted agents: cytotoxic T-lymphocyte antigen-4 blockade in advanced melanoma. *Melanoma Res* 2010; **20**: 1-10

120 **Flaherty KT**, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 2010; **363**: 809-819

121 **Bollag G**, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, Burton EA, Wong B, Tsang G, West BL, Powell B, Shellooe R, Marimuthu A, Nguyen H, Zhang KY, Artis DR, Schlessinger J, Su F, Higgins B, Iyer R, D'Andrea K, Koehler A, Stumm M, Lin PS, Lee RJ, Grippo J, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, Chapman PB, Flaherty KT, Xu X, Nathanson KL, Nolop K. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* 2010; **467**: 596-599

122 **Ledford H**. Rare victory in fight against melanoma. *Nature* 2010; **467**: 140-141

123 **Dummer R**, Robert C, Chapman PB, Sosman JA, Middleton M, Bastholt L, Kemsley K, Cantarini MV, Morris C, Kirkwood JM. AZD6244 (ARRY-142886) vs temozolomide (TMZ) in pa-

tients (pts) with advanced melanoma: an open-label, randomized, multicenter, phase II study. J Clin Oncol 2008; 26: abstr 9033

124 **Banerji U**, Camidge DR, Verheul HM, Agarwal R, Sarker D, Kaye SB, Desar IM, Timmer-Bonte JN, Eckhardt SG, Lewis KD, Brown KH, Cantarini MV, Morris C, George SM, Smith PD, van Herpen CM. The first-in-human study of the hydrogen sulfate (Hyd-sulfate) capsule of the MEK1/2 inhibitor AZD6244 (ARRY-142886): a phase I open-label multicenter trial in patients with advanced cancer. *Clin Cancer Res* 2010; **16**: 1613-1623

125 **Ott PA**, Hamilton A, Min C, Safarzadeh-Amiri S, Goldberg L, Yoon J, Yee H, Buckley M, Christos PJ, Wright JJ, Polsky D, Osman I, Liebes L, Pavlick AC. A phase II trial of sorafenib in metastatic melanoma with tissue correlates. *PLoS One* 2010; **5**: e15588

126 **Kaufman HL**, Bines SD. OPTIM trial: a Phase III trial of an oncolytic herpes virus encoding GM-CSF for unresectable stage III or IV melanoma. *Future Oncol* 2010; **6**: 941-949

127 **Kaufman HL**, Kim DW, DeRaffele G, Mitcham J, Coffin RS, Kim-Schulze S. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. *Ann Surg Oncol* 2010; **17**: 718-730

128 **Dréau D**, Foster M, Hogg M, Swiggett J, Holder WD, White RL. Angiogenic and immune parameters during recombinant interferon-alpha2b adjuvant treatment in patients with melanoma. *Oncol Res* 2000; **12**: 241-251

129 **Fateh S**, Schell TD, Gingrich R, Neves RI, Drabick JJ. Unsuccessful high dose IL-2 therapy followed immediately by near continuous low dose temozolomide can result in rapid durable complete and near-complete remissions in metastatic melanoma. *Cancer Biol Ther* 2010; **10**: 1091-1097

130 **Hong JJ**, Rosenberg SA, Dudley ME, Yang JC, White DE, Butman JA, Sherry RM. Successful treatment of melanoma brain metastases with adoptive cell therapy. *Clin Cancer Res* 2010; **16**: 4892-4898

131 **Håkansson A**, Gustafsson B, Krysander L, Håkansson L. Effect of IFN-alpha on tumor-infiltrating mononuclear cells and regressive changes in metastatic malignant melanoma. *J Interferon Cytokine Res* 1998; **18**: 33-39

132 **Hauschild A**, Dummer R, Ugurel S, Kaehler KC, Egberts F, Fink W, Both-Skalsky J, Laetsch B, Schadendorf D. Combined treatment with pegylated interferon-alpha-2a and dacarbazine in patients with advanced metastatic melanoma: a phase 2 study. *Cancer* 2008; **113**: 1404-1411

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BRIEF ARTICLE

Correlation between the proportion of breast volume involved by locally advanced tumors and invasion of the skin and posterior structures

Parastoo B Dahi, Komal P Dhiran, Constantine A Axiotis, Jeremy Weedon, Mahmoud El-Tamer, Gurinder Sidhu, Albert S Braverman

Parastoo B Dahi, Gurinder Sidhu, Albert S Braverman, Division of Hematology/Oncology, Department of Medicine, Downstate Medical College of the State university of NY, Brooklyn, NY 11203, United States
Komal P Dhiran, Constantine A Axiotis, Division of Surgical Pathology, Kings County Hospital Center, Department of Pathology, Downstate Medical College of the State University of NY, Brooklyn, NY 11203, United States
Jeremy Weedon, Scientific Computing Center, Downstate Medical College of the State university of NY, Brooklyn, NY 11203, United States
Mahmoud El-Tamer, Breast Surgery Service, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, United States
Author contributions: Dahi PB, El-Tamer M and Braverman AS designed the project and wrote the manuscript; Dahi PB, El-Tamer M, Sidhu G and Braverman AS conducted the research; Dhiran KP and Axiotis CA provided pathological expertise; Dahi PB, Dhiran KP, Axiotis CA, Weedon J, El-Tamer M, Sidhu G and Braverman AS approved the manuscript.
Correspondence to: Albert S Braverman, MD, Division of Hematology/Oncology, Department of Medicine, Downstate Medical College of the State University of NY, Box 55, DMC SUNY, 450 Clarkson Ave, Brooklyn, NY 11203, United States. abraverman@downstate.edu
Telephone: +1-718-2701500 Fax: +1-718-2701544
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Abstract

AIM: To evaluate any differences between the percentages of involved breast volume, pathologic attributes, and tumor marker expression of T3 and T4a-c tumors in locally advanced breast cancers (BC).

METHODS: All patients with T3N > 0 and T4a-c BC without evidence of distant metastasis (M0), presenting to the Breast Clinic from 1980 to 2010, were examined to determine whether their BC's involved ≥ 50% of their breast volumes, defined by gross replacement of at least one hemisphere. Core needle biopsy or post-mastectomy specimens from tumors involving a known percent of breast volume were evaluated for: (1) pathological grades and lympho-vascular invasion (LVI); (2) hormone receptor (ER/PR) expression > 0; and (3) epidermoid growth factor 2 (her2) over-expression (3+) by immune-histochemical staining or fluorescent *in situ* hybridization.

RESULTS: The data base included 98 patients with T3N> 0 M0 and 120 with T4a-c, any N disease, M0 disease. T3 tumor masses involved 50% or more of the breast in 23/98 (24%), and T4a-c tumors 65/120 (54%) ($P < 0.001$). Only 1% of T3 tumors and 23% of T4a-c tumors presented with total breast replacement. There were no significant differences between the pathological attributes and marker expression of the T3 and T4a-c tumors.

CONCLUSION: These data suggest that erosion of the overlying skin or underlying chest wall by some BC may be due to neglect and delay, rather than inherent biological aggressiveness.

Key words: Breast cancer; Locally advanced breast cancer; Breast cancer size

Peer reviewer: Lu-Zhe Sun, PhD, Professor, Dielmann Endowed Chair in Oncology, Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., MC7762, San Antonio, TX 78229, United States

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INTRODUCTION

Locally advanced (stage Ⅲ) breast cancer (BC) refers to attributes of primary tumors and/or draining nodes which imply the probability of early local and distant relapse. The relevant attributes of the primary tumors are: (1) At least one diameter > 5 cm with associated local nodal involvement (Stage ⅢA; T3N1); (2) Invasion of the chest wall (T4a), skin (T4b) or both (T4c) (stage ⅢB); and (3) Diffuse breast inflammation with rapid growth and early nodal involvement (T4d)[1]. T4d tumors are a distinct clinical and pathological entity[2]. As such tumors are often diffuse, determination of their size, and amount of breast volume they encompass, is not possible.

T3N1 tumors have a significantly better prognosis than T4a-c tumors[3]. Some of the latter are node negative. The size of many of these tumors suggests very prolonged growth prior to presentation, but histories obtained from patients are not a reliable method of determining their duration. Local invasion may not always be due to intrinsic aggressiveness. Prolonged growth may ultimately lead to replacement of most of the breast by tumor, leading to proximity to adjacent structures, which may facilitate local invasion.

The purpose of this study is to compare local growth, pathological attributes and marker expression in stage Ⅲ patients with T3 or T4a-c tumors. The data obtained may help elucidate the mechanism of local invasion by breast tumors.

MATERIALS AND METHODS

Patient information was obtained from the Kings County Hospital Locally Advanced Breast Cancer Database, including all patients who presented with T3N > 0M0 or T4a-c, any N, M0 tumors. All patients were initially staged by radionuclide bone scanning and either chest films or computerized axial tomography; those with detectable distant metastases were excluded. Patients with diffuse inflammation (T4d) were excluded, but those with localized inflammation due to ulceration were considered T4b or c.

The two longest diameters of each tumor were measured and their product determined. The dimensions from mammography were also noted; these usually corresponded with the measurements from physical examination, but where these parameters were discordant those from mammography were employed. The breast was similarly measured. Data from mastectomy specimens was not usable because almost all of the patients had received neoadjuvant systemic therapy. From these data the volumes of the tumor and the breast were estimated, and confirmed by a second physician. The cases in which the tumor volume was equal to or greater than 50% of the breast volume could be reliably distinguished from those in whom a smaller proportion of the breast volume was involved; in such cases at least one breast hemisphere was fully replaced. In many cases almost the whole breast was involved by tumor.

The tumors, either as sampled by multiple core needle or incisional biopsies at presentation, or from mastectomy specimens before systemic therapy, were also evaluated for pathological grade, lymphovascular invasion (LVI), hormone receptor and epidermoid receptor-2 (her-2) over-expression. The latter determination was based on American Society of Clinical Oncology (ASCO) criteria[4]. Estrogen (ER) and progesterone receptor (PR) expression was determined by immunohistochemical (IHC) staining of nuclei and cytoplasm. The tumor specimens were considered positive (1+) if >10% of the tumor cells displayed nuclear ER or PR. Triple negative tumors were those whose ER/PR scores were 0, and which were < 3+ for her2.

The presence or absence of LVI was not noted on many pathology reports, and in such cases was determined from review of slides, or preparation of new slides from archival material. Core needle biopsy material was not deemed sufficient for evaluation of LVI, so that only mastectomy specimens were used for this purpose. Neoadjuvant therapy, mostly with anthracycline based chemotherapy, was administered to 98% of the T3N > 0, and 91% of the T4a-c patients, which may have altered tumor morphology in mastectomy specimens. Evaluation was limited to tissue peripheral to carcinoma, and LVI defined as tumor cells within vessels. Minimal residual disease or ductal carcinoma *in situ* (DCIS) only, were present in some specimens. Equivocal morphologic results were obtained in some cases. The latter were IHC stained with monoclonal antibodies directed against podoplanin, expressed by lymphatic endothelium, and platelet endothelial cell adhesion molecule 1, expressed by blood vessel endothelium[5].

Determinations of relapse incidence and relapse free survival (RFS) were based on those ⅢA and ⅢB patients who had neoadjuvant or adjuvant therapy, and definitive surgery. All patients were asked to report to clinic for follow-up every 6 mo for one year following primary treatment, and yearly thereafter, or when they noted signs and symptoms of local and distant relapse.

RESULTS

The criteria for inclusion in the study were patients with T3N > 0M0 or T4a-c, any N, M0. Only patients for whom percentage of breast volume replaced by tumor had been recorded at presentation were included, 98 of whom had T3 and 120 T4a-c tumors. For this reason 10 of 108 T3 and 7 of 127 T4 tumors were excluded. We included patients whose tumor grade was not known, and those for whom LVI could not be determined. We also included those without adequate data concerning ER/PR

and her2 expression.

In some cases these data had not been entered in the database. This was the case with many patients who presented before 1993 for ER/PR, and before 1996 for her2. Electronic charts were introduced in 1997, and hard copy charts for patients presenting earlier were unavailable. Some of these data were obtained from archival material, when available.

The youngest and oldest T3N > 0M0 patients were 26 and 77 years of age, and 26 and 92 for the T4a-c, any N, M0 group. The mean ages of the T3N > 0M0 and the T4a-c, any N, M0 groups are presented in table 1. The oldest T3 patient was 77, while 11% of the T4 patients were > 77 (78-92). The T4a-c patients were significantly older than the T3N > 0 patients. *P* value (< 0.001) was calculated by the Wilcoxian test.

Most T3 tumors involved less than 50% of the breast volume, and most T4a-c tumors involved 50% or more ($P < 0.001$); that is, at least one breast hemisphere was replaced by tumor. The whole breast was involved in a fifth of the T4a-c cases, but in only 1% of the T3 tumors ($P < 0.001$) (Table 1).

The two groups were almost identical in pathological grade and hormone receptor expression (Table 1). There were no significant differences between her2 expression, triple negativity and LVI. Of the T3 patients 13 (22%) and of the T4a-c 17 (28%) were triple negative. Of 96 available Hematoxylin-Eosin stained slides, 18 contained only minimal residual disease or DCIS. Of the 78 specimens containing sufficient tumor for evaluation, 31 cases were positive and 21 cases negative for LVI. The morphology of 26 cases was equivocal, and these were IHC stained to determine whether or not LVI was present; 22 were informative. The results are presented in Table 1. P values were calculated by Fisher's exact test.

More than 90% of all patients received neoadjuvant systemic therapy, and the rest post-operative treatment. Relapse was defined as either distant metastases, confirmed by imaging, or by regional recurrence, such as supraclavicular nodes or the brachial plexus syndrome; chest wall recurrences were not included. The analysis of the two patient groups for relapse incidence and RFS was limited to those who had definitive surgery: 72/98 (74%) of the stage ⅢA and 81/120 (68%) of the ⅢB patients. Of the ⅢA patients 38%, and of the ⅢB patients 51% ultimately relapsed at distant or regional sites. The Kaplan-Meier curves of RFS are presented in Figure 1. Although more of the ⅢB patients relapsed the difference was not significant ($P = 0.097$).

Table 1 Comparison between the attributes of 98 patients with T3N > 0M0 breast tumors and 120 patients with T4a-c, any N, M0 tumors

BC	T3N>0	%	T4a-c	%	*P* value
Age Mean	51	-	58	-	< 0.001
⩾ 50%BV	23/98	24	65/120	54	< 0.001
WB	1/98	1	21/99	21	< 0.001
Grade Ⅲ	51/82	62	51/84	61	0.874
ER> 0	36/63	57	38/67	57	1
PR> 0	29/63	46	31/67	46	1
ER/PR> 0	27/63	43	33/67	49	0.486
Her2+	25/58	42	21/60	35	0.455
Triple neg	13/59	22	17/60	28	0.528
LVI	6/33	18	11/40	28	0.403

BC: Breast cancers; BV: Breast volume; WB: Whole breast; ER: Estrogen receptor; PR: Progesterone receptor; Her2+: Epidermoid growth factor receptor 2; LVI: Lymphovascular invasion.

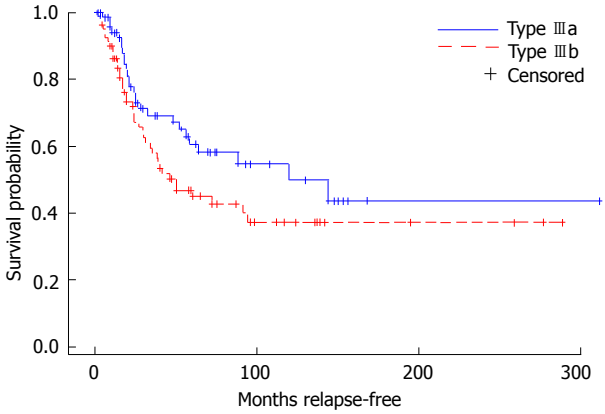

Figure 1 A Kaplan-Meier plot was constructed to compare relapse rate of type Ⅲa (*n* = 72) *vs* type Ⅲb (*n* = 81). The log-rank test of comparison of groups yielded $P = 0.097$.

DISCUSSION

Prolonged growth resulting from neglect has been assumed to be an explanation for local invasion by BC's, but the unreliability of patient histories has made it difficult to confirm this hypothesis[6-7]. It is supported by the much higher incidence of locally advanced BC's amongst women in non-industrialized nations, or in those with limited use of, or access to screening or regular medical care[8].

The percentage of breast volume involved by a tumor is a function of the ratio of tumor to breast size. We compared the amount of breast volume replaced by tumor in patients with large breast cancers (T3) and in those whose cancers, regardless of size, had grossly invaded the skin or chest wall (T4a-c). We found that cancers which invaded local structures more often replaced 50% or more of the breast volume than large cancers (T3) which remained within the breast. Replacement of most of the breast may bring the tumor cells close enough to adjacent structures to facilitate their invasion. We and others[9-11] have found physical examination a reliable method of determining breast tumor size and percentage of the breast involved by tumor; the results correlate well with those obtained by mammography[12].

Pathological attributes and marker expression were similar in the two groups. Thus, an important role for neglect and prolonged tumor growth in the pathogenesis of local invasion is suggested by these data. But other biological differences may exist between them; we have not excluded differences between gene expressions in the

two groups.

There may still be a role for constitutively rapid growth in the tendency of some T4a-c tumors to occupy large breast volumes, and for intrinsic differences in the ability of tumor cells to invade adjacent structures.

The mean age of our patients with locally invasive tumors was 58, while that of the patients with T3 tumors was 51. Yet the tumors of younger BC patients are generally more aggressive[13]. For example, the mean age of 127 patients with constitutively aggressive inflammatory carcinomas (T4dM0) was 50 (data not presented). Our T4a-c patients may have been older than T3 or T4d patients because more years had elapsed between tumor onset and presentation for medical treatment.

The incidence of distant metastases at presentation or subsequent distal relapse of T4a-c tumors is similar to that of inflammatory carcinomas[3]. Prolonged growth and local invasion may themselves facilitate the development of such metastases. Alternatively, tumors which invade local structures may be constitutively prone to distant spread. Of interest is the fact that T4 tumors are the only subsets of BC whose distant relapse rate may be independent of nodal involvement[3].

As previously reported[3], there is a significant difference between the incidences of distant metastases at presentation in patients presenting with T3N1 and T4a-c tumors; 8.8% and 42% in this study. Thus, the stage ⅢB (T3N1M0) patients in this study may represent a subset of T4a-c patients with less aggressive disease. This may, in part, explain the fact that the differences between relapse rates and survival curves of the ⅢA and B patients were not significant. Earlier studies[3] showed a poorer prognosis for ⅢB (T4a-c) patients, but the adjuvant therapy for many of the patients reported here included innovations, such as taxanes, anti-her2 agents, and aromatase inhibitors. The longest recorded follow-up periods were for patients who had relapsed. Many of our patients reside in the Caribbean; although some continue to report for yearly follow-up at our clinic, those who relapse are more likely to do so. Most of these data were obtained from patients of African-Caribbean origin, and may not be the same in other groups.

We conclude that the behavior of locally advanced primary BC's is likely to be related, in part, to their duration and growth. Intracellular attributes facilitating invasion of adjacent structures may exist, but their existence cannot be assumed until demonstrated.

COMMENTS

Background

Any or all of the following attributes define locally advanced (stage Ⅲ) breast cancer: (1) large (> 5) cm size with involved lymph nodes in the armpit (stage ⅢA); (2) tumors which have grossly invaded adjacent skin or chest wall (stages ⅢBa-c), tumors which are inflammatory (stage ⅢBd); (3) locally advanced breast tumors are also defined by the absence of distant metastases at presentation (stage Ⅳ), as detected by imaging of bones, lungs or liver. Stage Ⅲ tumors are more likely to relapse at distant sites after surgery and radiation than stage Ⅰ or Ⅱ tumors; (4) the cells of inflammatory (stage ⅢBd) tumors are known to be rapidly growing, abnormally invasive and aggressive. This is not necessarily true of stage ⅢA and ⅢBa-c tumors. In such patients large size and local invasion may be due to prolonged neglect of the tumor by the patient or her physicians, or the inaccessibility of adequate screening procedures. But because of the unreliability of patients' histories, it is difficult to reliably document neglect in most cases.

Research frontiers

An approved molecular technique called gene expression analysis can give us detailed information about which genes are active in specific breast tumors. Especially in the case of tumors which have not involved lymph nodes in the armpit, expression of some genes is correlated with subsequent distant relapse. But there is little data correlating gene expression with the prognosis of locally advanced breast tumors. Thus, whether any tumor became large or locally invasive because of neglect, or due to intrinsic aggressiveness, cannot be determined. Some of the factors which seem to correlate with aggressiveness are: Failure to express hormone receptors. Over-expression of the epidermoid growth factor receptor 2 (her2). Failure to express both her2 and hormone receptors: triple negativity

Innovations and breakthroughs

T3N1 tumors have a significantly better prognosis than T4a-c tumors. Local invasion may not always be due to intrinsic aggressiveness. Prolonged growth may ultimately lead to replacement of most of the breast by tumor, leading to proximity to adjacent structures, which may facilitate local invasion.

Applications

In this study authors compared several hundred patients with either large (ⅢA) and/or locally invasive (ⅢBa-c) tumors by the following criteria: (1) did the tumor occupy > 50% of the total breast volume? (2) the pathological grade of the tumor; high grade tumors may be more aggressive; (3) invasion of small blood or lymphatic vessels by tumor; (4) hormone receptor expression by tumor cells; (5) her2 expression by tumor cells; (6) triple negativity of the tumor for estrogen and progesterone receptors and her2. None of these criteria distinguished stage ⅢA and ⅢBa-c tumors except the first. That is, stage ⅢA tumors, however large, usually involved < 50% of breast volume, whereas most stage IIIBa-c tumors did involve > 50% of the breast volume, and many involved the whole breast.

Peer review

This paper is well written and interesting to the readers.

REFERENCES

1 **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474

2 **Lucas FV**, Perez-Mesa C. Inflammatory carcinoma of the breast. *Cancer* 1978; **41**: 1595-1605

3 **El-Tamer M**, Hussain S, Weedon J, Chalchal H, Chakrabarti A, Sohn C, Braverman AS. Prognoses of T4 breast cancer subsets. *Ann Surg Oncol* 2002; **9**: 340-345

4 **Wolff AC**, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; **131**: 18-43

5 **Mohammed RA**, Ellis IO, Lee AH, Martin SG. Vascular invasion in breast cancer; an overview of recent prognostic developments and molecular pathophysiological mechanisms. *Histopathology* 2009; **55**: 1-9

6 **Buttlar CA**, Templeton AC. The size of breast masses at presentation. The impact of prior medical training. *Cancer* 1983; **51**: 1750-1753

7 **Bloom Hj**. The influence of delay on the natural history and prognosis of breast cancer: a study of cases followed for five to twenty years. *Br J Cancer* 1965; **19**: 228-262

8 **Ward E**, Jemal A, Cokkinides V, Singh GK, Cardinez C, Ghafoor A, Thun M. Cancer disparities by race/ethnicity and

socioeconomic status. *CA Cancer J Clin* 2004; **54**: 78-93
9 **Müller C**, Caputo A, Schumacher M, Raab G, Schütte M, Hilfrich J, Kaufmann M, von Minckwitz G. Clinical response by palpation during primary systemic therapy with four dose-dense cycles doxorubicin and docetaxel in patients with operable breast cancer: further results from a randomised controlled trial. *Eur J Cancer* 2007; **43**: 1654-1661
10 **Kaufmann M**, von Minckwitz G, Smith R, Valero V, Gianni L, Eiermann W, Howell A, Costa SD, Beuzeboc P, Untch M, Blohmer JU, Sinn HP, Sittek R, Souchon R, Tulusan AH, Volm T, Senn HJ. International expert panel on the use of primary (preoperative) systemic treatment of operable breast cancer: review and recommendations. *J Clin Oncol* 2003; **21**: 2600-2608
11 **Gralow JR**, Burstein HJ, Wood W, Hortobagyi GN, Gianni L, von Minckwitz G, Buzdar AU, Smith IE, Symmans WF, Singh B, Winer EP. Preoperative therapy in invasive breast cancer: pathologic assessment and systemic therapy issues in operable disease. *J Clin Oncol* 2008; **26**: 814-819
12 **Fiorentino C**, Berruti A, Bottini A, Bodini M, Brizzi MP, Brunelli A, Marini U, Allevi G, Aguggini S, Tira A, Alquati P, Olivetti L, Dogliotti L. Accuracy of mammography and echography versus clinical palpation in the assessment of response to primary chemotherapy in breast cancer patients with operable disease. *Breast Cancer Res Treat* 2001; **69**: 143-151
13 Benchmark Reports, V. 9.0. National Cancer Data Base, Commission on Cancer, American College of Surgeons, 2009. Available from: URL: http: //www.facs.org/cancer/public-ncdb.html

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Simone Mocellin, MD, PhD, Department of Oncological and Surgical Sciences, University of Padova, via Giustiniani 2, 35128 Padova, Italy

Lu-Zhe Sun, PhD, Professor, Dielmann Endowed Chair in Oncology, Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., MC7762, San Antonio, TX 78229, United States

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Events Calendar 2012

January 16-17, 2012
Biomarkers Summit Egypt
London, United Kingdom

January 25-26, 2012
Multi-Disciplinary Appoaches to Cancer Therapy
Dubai, United Arab Emirates

January 26-27, 2012
3rd National Conference: Renal and Bladder Cancer 2012
London, United Kingdom

January 30-31, 2012
2nd Annual Clinical Trials in Oncology
Rome, Italy

February 2-3, 2012
Stem Cells 2012 Conference and Exhibition
San Diego, CA, United States

February 6-8, 2012
Mahidol International Conference on Infections and Cancers 2012
Bangkok, Thailand

February 12-17, 2012
Keystone Symposia: Cancer and Metabolism
Alberta, Canada

February 22-25, 2012
Excellence in Oncology
Istanbul, Turkey

March 8-10, 2012
10th International Congress on Targeted Anticancer Therapies
Amsterdam, Netherlands

March 9-10, 2012
13th European Congress: Perspectives in Lung Cancer
Amsterdam, Netherlands

March 14-16, 2012
BTOC-11 Biological Therapy of Cancer
Munich, Germany

March 15-17, 2012
3rd Conference on Therapeutic Resistance in Cancer
Quebec, Canada

March 29-30, 2012
Modern methods of diagnosis and treatment of malignant tumors
Kiev, Ukraine

April 13-15, 2012
Asian Oncology Summit 2012
Singapore, Singapore

April 20-21, 2012
Diagnosis and treatment of advanced forms of prostate cancer, bladder cancer and kidney cancer
Kiev, Ukraine

April 20-22, 2012
The 9th Meeting of Asian Society for Neuro-Oncology
Taipei, Taiwan

April 26-28, 2012
3rd International Video Workshop on Radical Surgery in Gynaecological Oncology
Prague, Czech Republic

April 28, 2012
Issues in Pediatric Oncology
Kiev, Ukraine

May 5-6, 2012
Radiation Research Methods as A Diagnostic and Therapeutic Support in Oncology
Kiev, Ukraine

May 17-18, 2012
Eurasian forum on the management of patients with tumors of the gastrointestinal tract
Uman, Ukraine

June 16-17, 2012
Issues of Neurosurgery, vascular neurosurgery, neurooncology, spinal surgery and spinal cord
Kiev, Ukraine

July 7-10, 2012
22nd Biennial Congress of the European Association for Cancer Research
Barcelona, Spain

July 21-28, 2012
Cancer In Women
Hawaii, HI, United States

July 25-27, 2012
5th Latin American Conference on Lung Cancer
Rio de Janeiro, Brazil

August 27-30, 2012
UICC World Cancer Congress 2012
Québec, Canada

September 6-8, 2012
The 8th International Jordanian Oncology Society Conference
Amman, Jordan

September 27-28, 2012
Current issues of diagnosis and treatment of oncogynecology diseases
Ivano Frankivsk, Ukraine

September 27-29, 2012
European Conference of Oncology Pharmacy
Budapest, Hungary

October 5-8, 2012
44th Congress of the International Society of Paediatric Oncology
London, United Kingdom

October 13-16, 2012
14th Biennial Meeting of the International Gynecologic Cancer Society
Vancouver, Canada

October 19, 2012
Modern aspects of diagnosis and treatment of breast cancer
Kiev, Ukraine

October 23-26, 2012
Sydney International Breast Cancer Congress 2012
Sydney, Australia

October 27-28, 2012
Optimization methods for radiation diagnosis in oncology
Odessa, Ukraine

November 6-9, 2012
24th EORTC-NCI-AACR Symposium on "Molecular Targets and Cancer Therapeutics"
Dublin, Ireland

November 16-17, 2012
17th Annual Perspectives in Thoracic Oncology
New York, NY, United States

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GENERAL INFORMATION

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Editor-in-chief

Stuart K Calderwood, PhD, Associate Professor, Director
Molecular and Cellular Radiation Oncology, Department of Radiation Oncology, Beth Israel Deaconess Medical Center,

Harvard Medical School, 99 Brookline Avenue, Boston, MA 02215, United States

Editorial Office
World Journal of Clinical Oncology
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
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There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

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Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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