Searching for the Best Drug Combination to Treat Melanoma Patients: From Lab to Bedside

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ABSTRACT

Despite the initial encouraging clinical results that emerged from the vast arsenal of different novel targeted therapies for melanoma, long-term outcomes seem less auspicious. Recently, new drug combinations seem to better benefit melanoma patients. The present article explores the combination effects of the second-generation Polo-Kinase 1 (PLK1) inhibitors volasertib (BI6727) and GSK461364 on human melanoma cell lines and on primary melanoma cell cultures. The effects on cell viability with these new PLK1 agents were studied alone or in combination with some classical chemotherapy drugs (cisplatin, temozolomide, and doxorubicin) frequently employed in melanoma treatment. Additionally, the radiosensitizing effects of both PLK1 inhibitors were assessed.

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The search for the optimal drug combinations for treating melanoma is a relevant debate from the clinical perspective. Since the promising results with targeted therapy for treating unresectable or metastatic melanoma patients with Ipilimumab, a monoclonal antibody against CTLA-4 in 2011,[1] several studies have focused on drug combination to enhance sustained clinical response. Achieving long-term and continued tumor response for advanced melanoma patients receiving new targeted therapies remains challenging. As recently revised by Niezgoda,[2] an ample list of different drugs, either alone or combined, are in clinical testing for this purpose. Some FDA (US Food and Drug Administration) and EMA (European Medicines Agency) novel agents for treating advanced melanoma include BRAF (Vemurafenib, Dabrafenib, and LGX818), MEK (Trametinib, Selumetinib, Cobimetinib, and MEK162), C-KIT (imatinib, sunitinib, nilotinib, and masatinib) inhibitors, and immunomodulating drugs (CTLA-4 monoclonal antibodies, PD-1 inhibitors, and PD-L1 inhibitors). Far less explored is the association of some of these new inhibitors with classic chemotherapeutic compounds, particularly their radiosensitizing effects for melanoma cells.

In a previous volume of JDD,[3] we addressed the prospective use of the PLK1 inhibitor BI 2536 as an attractive strategy to impair melanoma progression and dissemination. However, regardless of promises, the clinical use of BI 3526 has been restricted by low intratumor levels,[4] acquired resistance,[5] and mild antitumor activity with drug-related adverse events in clinical trials.[6]

We would like to share our approach in treating human melanoma cell lines and two primary melanoma cell cultures with a combination of the second-generation PLK1 inhibitors volasertib (BI6727) and GSK461364 with classical chemotherapy drugs for treating melanoma. Additionally, the radiosensitizing effects of both PLK1 inhibitors were evaluated.

The human melanoma HT144 (HTB-63™) cell line was purchased from the American Type Culture Collection, (ATCC, Rockville, MD). The LB373-MEL cell line was kindly provided by Dr. Dimas Tadeu Covas (Regional Blood Center of Ribeirão Preto). HT-144 is a malignant human melanoma cell line derived from a subcutaneous metastatic site of a 29-year-old Caucasian male.[7] The LB373-MEL cell line was obtained from an in-transit metastasis (N2cM0III) at the lower limb of a 32-year-old female.[8] Additionally, two different primary melanoma cell cultures were studied: TU2000 cells were cultured from a metastatic melanoma of a 40-year-old male, and TU2284 cells derived from a locally invasive melanoma diagnosed in a 64-year-old male. Cells were cultured in HAM-F10 (Life Technologies® #11550043, Carlsbad, CA) supplemented with 10% fetal bovine serum (Life Technologies® #A12618DG), penicillin (100 U/mL; Sigma-Aldrich, P3032), and streptomycin (100 ug/mL; Sigma-Aldrich, S9137) at 37˚C in a humidified 5% CO2 incubator. For viability experiments, cells were treated with concentrations ranging from 10 to 150 nM of BI 6727 or GSK461364 (Axon Medchem® #1473 and #1688) or combinations with Cisplatin (CDDP), Temozolomide (TMZ), and Doxorubicin (DXR) (Sigma-Aldrich, P4394, T2577, and D1515), and analyzed after 24, 48, and 72 hours thorough the XTT® assay as described before.[9] To test the effect of PLK1 inhibition on radioresistance, clonogenic assays were performed as previously reported.[10] Our results showed significantly reduced proliferation in both melanoma cell lines ($P<0.05$), however, compared to BI 3526, the antiproliferative effects of BI 6727 and GSK461364 were more moderate, with the maximum

FIGURE 1. Antiproliferative effects of PLK1 inhibition in melanoma cell lines and primary cultures. The LB373-mel (A) and HT144 (B) cell lines were treated with concentrations ranging from 10 to 150 nM of BI 6727 or GSK461364, and analyzed after 24, 48, and 72 hours thorough the XTT® assay. Primary cultures (C) were exposed to the same concentrations and analyzed after 72 h. Results are expressed as mean +/- SD. *P<0.05.

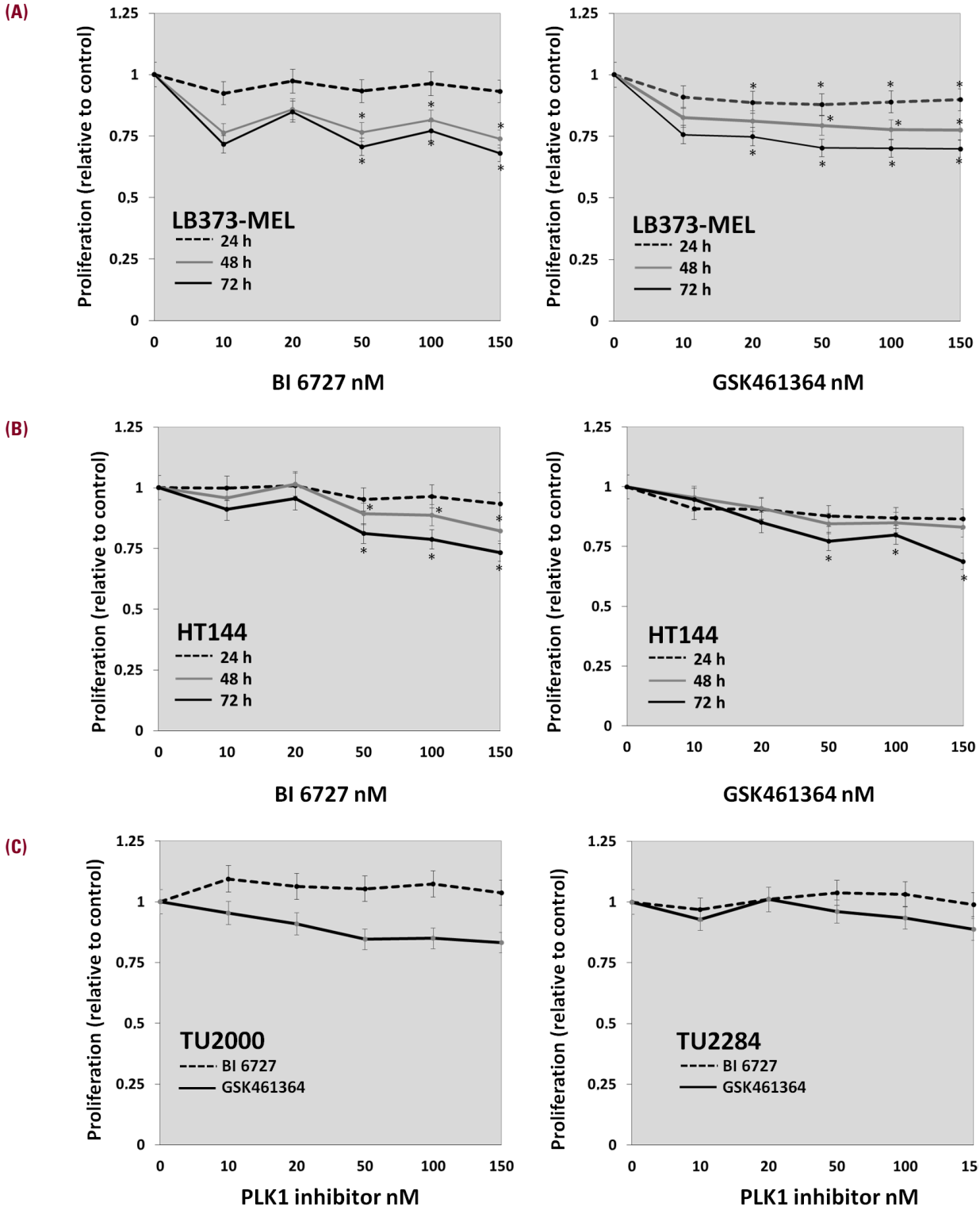

TABLE 1.

Median Dose Effect Analysis Used to Characterize Interactions Between the PLK1 Inhibitors BI 6727 and GSK461364 With Conventionally Used Drugs (CDDP, TMZ, and DXR)

CI values >1 indicate antagonistic effects. Dose-enhancement ratios (DER) for melanoma cell lines pretreated with 20 nM of BI 2767 or GSK461364 were analyzed by a clonogenic assay. DER >1 indicates suppra-additive effects.

LB373-MEL cell line												
BI 6727 nM	TMZ uM	FA BI 6727 + TMZ	CI	DXR uM	FA BI 6727 + DXR	CI	CDDP ug/ml	FA BI 6727 + CDDP	CI	BI 6727 nM	Radiation	DER
50	200	0.135	4.17	4	0.308	5.1	8	0.109	10.25	20 +	2 Gy	1.687
50	400	0.15	3.35	8	0.32	7.11	16	0.141	7.82	20 +	4 Gy	1.243
50	800	0.259	0.69	16	0.355	5.39	32	0.262	4.4	20 +	6 Gy	2.534
GSK461364	TMZ uM	FA GSK461364 +TMZ	CI	DXR uM	FA GSK461364 + DXR	CI	CIS ug/ml	FA GSK461364 + CDDP	CI	GSK461364 nM	Radiation	DER
50	200	0.086	1846.45	4	0.3219	3.29	8	0.1085	278.62	20 +	2 Gy	4.078
50	400	0.131	57.64	8	0.3126	8.56	16	0.1406	35.53	20 +	4 Gy	1.647
50	800	0.143	28.3	16	0.332	9.89	32	0.2623	4.08	20 +	6 Gy	14.076
HT144 cell line												
BI 6727 nM	TMZ uM	FA BI 6727 + TMZ	CI	DXR uM	FA BI 6727 + DXR	CI	CIS ug/ml	FA BI 6727 + CDDP	CI	BI 6727 nM	Radiation	DER
50	200	0.117	1.72	4	0.22	2.07E+14	8	0.084	4.96	20 +	2 Gy	1.807
50	400	0.136	2.24	8	0.222	2.88E+14	16	0.127	5.21	20 +	4 Gy	2.068
50	800	0.226	1.88	16	0.246	8.95E+12	32	0.257	4.03	20 +	6 Gy	3.519
GSK461364	TMZ uM	FA GSK461364 +TMZ	CI	DXR uM	FA GSK461364 + DXR	CI	CIS ug/ml	FA GSK461364 + CDDP	CI	GSK461364 nM	Radiation	DER
50	200	0.111	2.84	4	0.115	6.02E+24	8	0.075	7.04	20 +	2 Gy	3.618
50	400	0.126	3.38	8	0.105	2.89E+26	16	0.124	6.25	20 +	4 Gy	4.438
50	800	0.142	4.55	16	0.147	3.71E+21	32	0.173	7.39	20 +	6 Gy	3.233

FA=Fraction affected; CI=combination index; DER= Dose enhancement ratio

effect represented by a viability decrease of only ~30% after 72 hours of treatment with the highest concentration tested (Figure 1A and 1B). Moreover, the lack of response was more evident when treating primary cultures, where even after 72 hours, GSK461364 was able to reduce viability in merely ~10%. Of note, BI 6727 was highly inefficient, increasing viability in 5% (Figure 1C). In addition, combinations with CDDP, TMZ, or DXR were highly antagonistic (Table 1). Conversely, PLK1 inhibition by low concentration (20nM) of either PLK1 inhibitor led to radiosensitization for both HT144 and LB373-MEL, showing supra-additive effects at all doses tested (Table 1). It should be considered, however, that melanoma cells are highly resistant to standard anticancer drugs and in our model, drugs were administered simultaneously. Perchance, other alternative combination schemes at varying sequences and different time intervals might lead to dissimilar results.

Only recently, the radiosentizing effect of PLK1 inhibitors has gained attention. A growing number of evidences place PLK1 inhibitors as a radiosensitizing agent for squamous cell carcinomas,[11-12] medulloblastomas,[13] glioblastomas,[14] nosopharyngeal carcinoma xenografts,[15] and osteosarcoma.[10] Moreover, PLK1 seemed to be a predictive marker for radiation response when assessed in pretreatment biopsies of patients with advanced rectal cancer.[16] Although the radiosensitizing effect of PLK1 inhibition seem well documented, Lund-Andersen et al.,[17] evaluating osteosarcoma and colorectal cancer cells, demonstrated that either radiosensitization or radioresistance may be achieved, dependending on the delivered treatment schedule. This observation turns clinical treatment with PLK1 inhibitors far more complex. Of note, no previous studies to date have focused on the radiosensitization effect of PLK1 inhibition for human melanoma. As recently stated by Ascierto et al.,[18] combination seems to represent the most up-to-date treatment approach for treating melanoma. Besides new compounds, our results point to the potential radiosentizing effects of these drugs for melanoma cells in vitro. This property, if correctly explored, may be an important strategy to be studied among

these new drugs repertoire and systematically evaluated by clinical models.

Disclosure

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