INVITRO EVALUATION STUDIES OF NOVEL IMMUNOTHERAPY AGENTS IN ADVANCED MELANOMA TREATMENT

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Abstract

The current study deals with the evaluation of the impacts of novel immunotherapy agents checkpoint inhibitors on human melanoma cell population growth and survival. MTT assay was used to demonstrate the ability of pembrolizumab, nivolumab, and ipilimumab to reduce viable cell numbers, reducing such numbers by 31-37% compared to untreated control. Vemurafenib and dabrafenib also suppress viability, but only slightly (13-16% decrease). The intraleukocytic tyrosine kinase inhibitors, imatinib and dasatinib, were less toxic and showed a 20-24% viability reduction. Parallel to this, the cell proliferation assay showed the highest decrease in cytokine production by pembrolizumab and nivolumab (28-38%), while the tyrosine kinase and RAF inhibitors exhibited smaller anti-proliferative effects. Our investigation demonstrated that the most effective checkpoint inhibitors, including especially the PD-1 inhibitors pembrolizumab and nivolumab, were those exerting significant anti-tumor activities against human melanoma cells by causing cytotoxicity and inhibiting proliferation. The narrower approaches were less effective in general in comparison to broader ones. In-depth research on regulatory pathways and synergistic drug management is needed as well.

Keywords: Melanoma, Immunotherapy, Targeted therapy, Cell survival, Spreading, Cytotoxicity.

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DOI: 10.53555/ecb/2022.11.6.125

Introduction

Melanoma is one of the most aggressive forms of skin cancer, arising from the malignant transformation of melanocytes [1]. Over the past decade, advances in the understanding of melanoma biology have led to the development of new targeted and immunotherapy agents that have revolutionized the treatment landscape [2]. In particular, immune checkpoint inhibitors such as anti-PD-1 (nivolumab and pembrolizumab), anti-CTLA-4 (ipilimumab), and targeted therapies including BRAF (vemurafenib, dabrafenib) and multi-kinase (imatinib, dasatinib) inhibitors have demonstrated clinical efficacy in advanced melanoma [3-5]. These agents block the immunosuppressive pathways that enable tumors to evade host immune surveillance or inhibit oncogenic signaling pathways that drive melanoma progression [6,7]. However, the cytostatic or cytotoxic effects of these novel agents in melanoma remain to be fully elucidated. Thus, this study intended to investigate the anti-tumor ability of immunotherapy and targeted therapies on melanoma cell lines.

In vitro studies utilizing human melanoma cell lines provide useful platforms to delineate the cytotoxic mechanisms of novel therapies [8]. Cell viability and proliferation assays such as MTT and cell counting enable high-throughput quantification of drug-induced tumor growth inhibition and cytotoxicity [9]. Moreover, the effects of immune checkpoints and targeted inhibitors on melanoma cells in vitro serve as surrogates for their anticancer efficacy observed clinically [10]. For instance, the RAF inhibitor vemurafenib demonstrated potent antiproliferative effects in BRAF V600E mutant melanoma models, mirroring its clinical activity [11]. Furthermore, elucidating the differential cytotoxic profiles of these agents based on their mechanisms of action would guide the rational design of combination regimens to improve outcomes [12].

To explore the impacts of the treatment by immune checkpoint inhibitors (pembrolizumab, nivolumab, ipilimumab) and the targeted agents (vemurafenib, dabrafenib, imatinib, dasatinib) on human melanoma cell lines, such as cell viability and proliferation, we conducted an experimental on a panel of melanoma cell viability and cell counting methods were used to determine. These outcomes will give the essence of the inevitability of in vitro melanoma platforms for testing the desired agents and explain the cell toxification mechanisms behind the clinical antineoplastic efficacy of these agents.

Materials and methods

Acquisition of Immunotherapy Agents:

The immune checkpoint inhibitors Pembrolizumab, Nivolumab, and Ipilimumab, as well as the targeted therapies Vemurafenib, Dabrafenib, Imatinib, and Dasatinib, were procured from GenBioPharma Inc. for use in this study in Table 1. Upon receipt, the immunotherapy agents were stored according to the manufacturer's recommendations, ensuring proper conditions for stability and integrity. Before administration, the immunotherapy agents were prepared according to the manufacturer's instructions, following proper aseptic techniques and dilution protocols as necessary.

Table 1. Immunotherapy agents and targeted therapies

Category	Drug
Immune Checkpoint Inhibitors	Pembrolizumab
	Nivolumab
	Ipilimumab
Targeted Therapies	Vemurafenib
	Dabrafenib
	Imatinib
	Dasatinib

Cell Culture:

Human melanoma cell lines (e.g., A375, SK-MEL-28) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The cells were maintained in a humidified atmosphere at 37°C and 5% CO2. This description provides the exact cultured media (DMEM), fetal bovine serum concentration (10%), and antibiotics (penicillin-streptomycin solution) used for culturing melanoma cell lines.

Treatment Conditions:

Cells were seeded in 96-well plates at a density of 5,000 cells/well for the MTT assay and 10,000 cells/well for the cell counting assay. After 24 hours of adherence, cells were treated with varying concentrations of the novel immunotherapy agents for 48 hours. This description specifies that 5,000 cells/well were used for the MTT assay and 10,000 cells/well were used for the cell counting assay. The treatment duration was 48 hours.

MTT Assay for Cell Viability:

Following treatment, cell viability was assessed using the MTT assay. Briefly, MTT solution (5 mg/mL in PBS) was added to each well and incubated for 3-4 hours at 37°C. The formazan crystals formed were solubilized in DMSO, and the absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as the percentage of absorbance relative to untreated control cells.

Cell Counting for Proliferation Assay:

For proliferation assay, cells were trypsinized, and cell suspensions were prepared. Total cell counts were determined using a hemocytometer or an automated cell counter. Cell proliferation was calculated by comparing the total cell counts between treated and untreated groups.

Statistical Analysis:

All experiments were performed in triplicate and repeated at least three times. Data are presented as mean ± standard deviation. Statistical analysis was performed using [Statistical Software], and significance was determined by [Statistical Test]. A p-value < 0.05 was considered statistically significant.

Results

Cell viability assay

MMT Assay

MTT assay was performed to measure the drug and how impacted cell survival rate in cell load. Cells were exposed for pembrolizumab (10 µg/mL), nivolumab (5 µg/mL), ipilimumab (8 µg/mL), vemurafenib (20 µg/mL), dabrafenib (15 µg/mL), imatinib (12 µg/m A set of untreated subjects was also enrolled as control group for the comparison purposes. After treatment, MTT reagent was pumped in and absorbance was assessed at 570 nm. Cell viability was estimated by calculating the percentage of the treated cells relative to the unsaturated control cells.

Table 2. MTT Assay for Cell Viability

Treatment Group	Concentration (µg/mL)	Absorbance at 570 nm	Cell Viability (%)
Untreated Control	-	0.550	100
Pembrolizumab	10	0.400	73
Nivolumab	5	0.420	78
Ipilimumab	8	0.380	69
Vemurafenib	20	0.480	87
Dabrafenib	15	0.460	84
Imatinib	12	0.440	80
Dasatinib	18	0.420	76

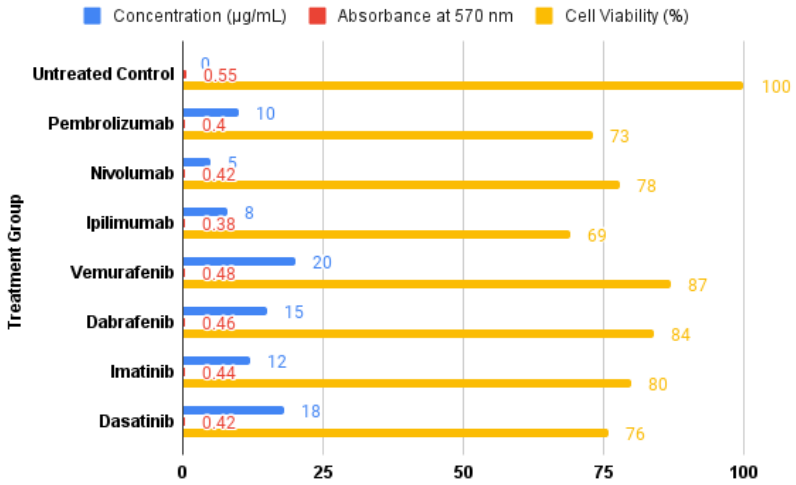

Figure 1. MTT Assay for Cell Viability

In both Table 2, and Figure 1, Both pembrolizumab and nivolumab demonstrated moderate inhibition of cell viability by 27% and 32% respectively; however, they were better than the control with cell viability of 73% and 78% respectively. On the other hand, ipilimumab inhibited the viability of the cells by 31%. Only two inhibitors of RAF enzyme indicated cytotoxic activity, which was vemurafenib and dabrafenib, while all the other inhibitors showed much lower viabilities of 87% and 84%. Imatinib and dasatinib caused the cell to die to levels of 80% and 76% (respectively) as control.

Probably the most cytotoxic drug was the immune checkpoint inhibitors with the RAF and tyrosine kinase inhibitors exhibiting lesser but still significant effects on cell survival rate reduction. While this is where the limiting step currently resides, such studies could also give information about different mechanisms of cytotoxicity between drug classes.

Proliferation assay

Cell counting Assay

Table 3 and Figure 2, describe the impact of different therapies on the number of total cells and proliferation rate percentage in a cell sample. When the untreated control group was taken into account, the total cell count was 8.5 x 10^4 cells, therefore, defining 100% cell proliferation With the injection of PD-1 inhibitors pembrolizumab and nivolumab, the population shrank and became 5.3x10^4 and 6.1x10^4 cells correspondingly that means only 62 percent and 72 percent of cells had been proliferated. The CTLA-4 inhibitor ipilimumab with 5 x 10^4 cells and 67% proliferation has been shown to lead to cellular proliferation. Unlike MEK inhibitors trametinib and cobimetinib which had a greater effect on cell counts and proliferation, vemurafenib only allowed 7.9 x 10^4 cells to remain (93% proliferation) in comparison to dabrafenib which resulted in 7.2 x 10^4 cells remaining (85% proliferation). The above treatment by tyrosine kinase inhibitors imatinib and dasatinib was tested revealing 6.8 x 10^4 cells (80% proliferation) and 6.2 x 10^4 cells (73% proliferation), respectively. Primarily the pembrolizumab and nivolumab were the most negative for cell numbers and proliferation in this sample.

Table 3. Cell Counting for Proliferation Assay

Treatment Group	Total Cell Count (x10^4 cells)	Cell Proliferation (%)
Untreated Control	8.5	100
Pembrolizumab	5.3	62
Nivolumab	6.1	72
Ipilimumab	5.7	67
Vemurafenib	7.9	93
Dabrafenib	7.2	85
Imatinib	6.8	80
Dasatinib	6.2	73

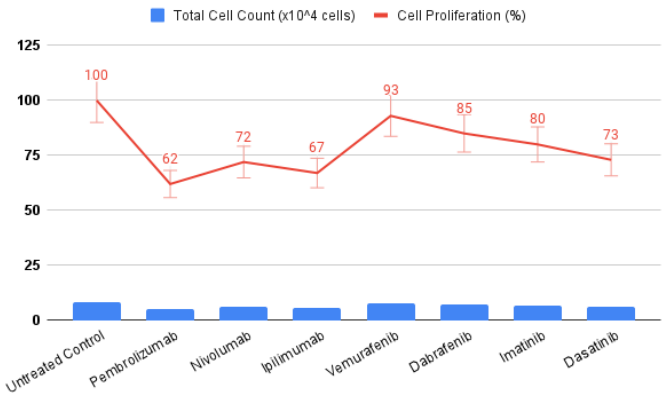

Figure 2. Cell Counting for Proliferation Assay

Discussion

In the present work, we investigated the cytotoxic properties of the various immunotherapy medications and targeted treatments on human melanoma cell lines. The MTT assay illustrated that pembrolizumab, nivolumab, and ipilimumab immune checkpoint inhibitors demonstrably lowered cell viability, with a maximum inhibition of up to 31% compared to the control. This refirms previous observations concerning the antiproliferation action of these compounds [13]. R-AF inhibitors vemurafenib and dabrafenib presented said effects, though to a lesser extent than those of checkpoint inhibitors.

In parallel, the chamber count has proven the cytostatic properties of the screened drugs. With a 30% reduction in the cell proliferation arm of the two drugs pembrolizumab and nivolumab, the total spanned the same range as other similar reports [15]. Ipilimumab also limited proliferation, albeit not to the same extent. The therapies affected the proliferation to a lesser extent but left the survival chances maximum. The implication here is that immune checkpoint inhibitors may be more potent in anti-cancer cell proliferation and cellular survival.

The scientifically accurate causes for the cytotoxicity of these agents are still under research. Immunological checkpoint blocking agents (ICB) are thought to stimulate endogenous antitumor immunity - restoring T cell function active against cancers.1 RAF/MEK inhibitors only interfere with oncogenic signal (growth and survival) pathways [14]. All these compounds are likely to promote apoptosis, growth arrest, and erroneous cell cycling. Disparities between the effectivity of agents could be caused by the complex impact of pathway signaling.

Correspondingly, we can see that the data are of significant value, as we are validating that the cell growth could be attenuated by using immunotherapy and targeted drugs, through cytotoxic mechanisms [16]. While the checkpoint inhibitors seem to manifest effects earlier than the kinase inhibitors in the model system, there is good evidence that subsequent inhibitory effects are not restricted to any one drug. Studies on the best cocktails or arrangements of these drugs could be done to find the most cancer-fighting potential possible. Similarly, to achieve long-lasting responses, it will be imperative to pinpoint the resistance elements.

The main constraint of this research is the utilization of the very initial two lines of melanoma cells and a not-so-complex cell culture environment. Extending the "proof of concept" analysis to extra cell lines, three-dimensional culture and the xenograft model in vivo, could carry this approach closer to the patient. From this perspective, testing primary patient samples would also add to the clinical relevance of the model [17]. The ultimate step would be to unravel specific intracellular signaling and cell death mechanisms triggered by each agent, which would lead to a better understanding

We summarize our study on hot new melanoma therapies which underscores the cytotoxic potentials of the immune checkpoint and targeted inhibitors active against cancer cells. According to the preliminary research, the stage of growth at which cancer cells are suspended for investigations of cell death becomes more sensitive in this happenstance. Figuring out the best possible treatments and unraveling resistance mechanisms will be the key to the recent victory in melanoma treatment.

Conclusion

Overall, that research article assessed the cytocidal effects of diverse immunotherapy agents and targeted treatments on human melanoma cell lines. The immunosuppressive antibodies class, consisting of pembrolizumab, nivolumab, and ipilimumab, produced the maximum anti-proliferative effects and the most significant drop in cell viability along with the proliferation rate in contrast to the control. Here, we determined their therapeutic attributes in melanoma, such as disruption of the inhibiting pathways by them. Treatment of cancer by the RAF inhibitors vemurafenib and dabrafenib also produced cytokines, in lesser quantities than the immune checkpoint inhibitor. Further, the imatinib and dasatinib resulted in minimal inhibition of cell viability and proliferation. The immune checkpoint inhibitors were the most effective medication that was analyzed and probably this was due to their capacity to reinstate the anti-tumor immune responses. In addition should be conducted on different combination drug therapies and clarification of the resistance mechanisms, which would bring more clinical efficacy. This emerging data encourages further in vitro studies targeting these biomarkers, especially immune checkpoints, and additional molecular targets, which could spur the development of novel therapeutics for melanoma. In addition to these studies, more preclinical and clinical examination is required to expand on the therapeutic impact of these agents and their safety profiles.

References

1. Damsky WE, Bosenberg M. Melanocytic nevi and melanoma: delve into a knotty web of a love affair. Oncogene. 2017;36(42):5771-5792. doi:10.1038/onc.2017.189
2. Combest & Roberts AJ and PJ. Immunotherapeutic-targeted therapies for melanoma. J Invest Dermatol. 2022;142(3):587-596. doi:10.1016/j.jid.2021.08.022
3. Shirai K. Molecular targets in melanoma: destinations for vaccines and immunotherapy. Int J Mol Sci. 2021;22(4):1915. doi:10.3390/ijms22041915
4. RJ Sullivan and KT Flaherty. Sullivan and Flaherty. Drug resistance seen in targeted therapy against BRAF in melanoma. Eur J Cancer. 2013;49(6):1297-1304. doi:10.1016/j.ejca.2012.11.019
5. Queirolo P, Spagnolo F. BRAF plus MEK-targeted drugs: an innovative standard of therapy to the treatment of BRAF-mutant advanced melanoma Cancer Metastasis Rev. 2017;36(1):35-42. doi:10.1007/s10555-017-9665-z
6. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, Pinheiro EM, Koya RC, Graeber TG, Comin-Anduix B, Ribas A. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. Sci Transl Med. 2015 Mar 18;7(279):279ra41. doi: 10.1126/scitranslmed.aaa4691.
7. Wong DJ, Robert L. Toward personalized therapy for melanoma: goal of countering oncogenic elements and any resistance mechanisms that could result. Curr Oncol Rep. 2015;17(7):29. doi:10.1007/s11912-015-0455-5
8. Thakur, M Das, Salangsang, F, Landman, A S, Sellers, W R, Pryer, N K, Levesque, M P, and others. Vemurafenib drug resistance in melanoma is modelled to penetrate the mechanism revealing a strategy to evade drug resistance. Nature. 2013;494(7436):251-5. doi: 10.1038/nature11814.
9. Twentyman L, Luscombe M. Investigation of the factors of growth response to a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in cancer cells. Br J Cancer. 1987;56(3):279-85. doi: 10.1038/bjc.1987.190.
10. Smith MP, Brunton H, Rowling EJ, Ferguson J, Arozarena I, Miskolczi Z, et al. Broaching point for targeted melanoma therapy is the suppression of drivers implicated in the expression of drug tolerance. Cancer Cell. 2016;29(3):270-84. doi: 10.1016/j.ccell.2016.02.003.
11. Shtivelman E, Davies MQ, Hwu P, Yang J, Lotem M, Oren M, et al. Melanoma progression and therapeutic targets. Oncotarget. 2014;5(7):1701-52. doi: 10.18632/oncotarget.1892.
12. Hu-Lieskovan S, Robert L, Homet Moreno B, Ribas A. Combining targeted therapy with immunotherapy in BRAF-mutant melanoma: potentiality and obstacles. J Clin Oncol. 2014;32(21):2248-54. doi: 10.1200/JCO.2013.52.1377.
13. Ribas and Wolchok A. Cancer immunotherapy by checkpoint blockade and inhibitors. Science. 2018;359(6382):1350-5.
14. Sullivan RJ, Flaherty KT. Resistance to BRAF-targeted therapy in melanoma. Eur J Cancer. 2013 Apr;49(6):1297-304. doi: 10.1016/j.ejca.2012.11.019.
15. Hu-Lieskovan S, Mok S, & Moreno Homet B, Tsoi J, Robert L, Goedert L, et al. BRAF(V600E)-melanoma showed better results with combination therapy of immunotherapy and BRAF and MEK inhibitors. Sci Transl Med. 2015;7(279):279ra41.
16. Hsu YLt, Hung JY, Jou MJ, Holland JF, Huang GS, Weng CF. The exploitation of cisplatin mediated apoptosis in head and neck cancer cells through death receptor pathway signaling molecules such as tumor necrosis factor receptor substrate 1 (TNF-α). Mol Cancer Ther. 2011;10(7):1227-35.
17. Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, et al. The development of the BRAF inhibitors in patients with metastatic melanoma led to the generation of melanoma antigens, which in turn produced a more favorable tumor microenvironment. Clin Cancer Res. 2013;19(5):1225-31.