A BIOINFORMATICS FRAMEWORK FOR EVALUATING DACARBAZINE COMBINATION THERAPY

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Abstract

Melanoma is the most aggressive form of skin cancer, characterized by rapid metastasis, early dissemination, and high resistance to systemic therapies. Although it represents less than 5% of all skin cancer cases, it accounts for the majority of skin cancer–related deaths. Its development is driven by complex interactions between environmental factors, particularly ultraviolet radiation, and genetic alterations affecting key oncogenes and tumor suppressor genes such as BRAF, NRAS, KIT, PTEN, and *CDKN2A. Among these,* the *BRAF V600E mutation is the most prevalent, leading to constitutive activation of the MAPK/ERK signaling pathway and promoting melanoma progression. While targeted therapies such as BRAF inhibitors have improved patient outcomes, acquired resistance frequently emerges, highlighting the need for effective combination treatment strategies.*

This study evaluates the therapeutic potential of drug combinations in three melanoma cell lines—LOX-IMVI, SK-MEL-28, and M14—using predictive modeling and synergy quantification methods (LOEWE, BLISS, ZIP, and HSA) implemented through SynergyFinder. The combination of vemurafenib and dacarbazine demonstrated a synergistic effect in LOX-IMVI cells according to the ZIP model, while consistently showing additive effects across other models. SK-MEL-28 cells exhibited predominantly additive responses, reflecting their distinct molecular profile with BRAF V600E mutation and wild-type NRAS. The M14 cell line displayed response patterns similar to LOX-IMVI, likely due to shared BRAF V600E mutations. Although strong synergy was limited, the reproducible additive effects observed across cell lines suggest clinically relevant benefits, supporting combination therapy as

a promising approach to enhance efficacy and reduce toxicity in melanoma treatment.

Key words**:** *Melanoma, Drug, Toxicity, Cell line, Synergy.*

Përmbledhje

Melanoma është forma më agresive e kancerit të lëkurës, e karakterizuar nga metastazim i shpejtë, përhapje e hershme dhe rezistencë e lartë ndaj terapive sistemike. Megjithëse përfaqëson më pak se 5% të të gjitha rasteve të kancerit të lëkurës, ajo shkakton shumicën e vdekjeve të lidhura me kancerin e lëkurës. Zhvillimi i saj përcaktohet nga ndërveprime komplekse midis faktorëve mjedisorë, veçanërisht rrezatimit ultravjollcë, dhe ndryshimeve gjenetike që prekin onkogjene kyçe dhe gjene supresore të tumorit si BRAF, NRAS, KIT, PTEN dhe *CDKN2A. Ndër këto, mutacioni BRAF V600E është më i përhapuri, duke çuar në aktivizim të vazhdueshëm të rrugës sinjalizuese MAPK/ERK dhe duke nxitur zhvillimin e melanomës. Megjithëse terapitë e targetuara, si inhibitorët e BRAF, kanë përmirësuar rezultatet klinike, rezistenca e fituar shfaqet shpesh, duke theksuar nevojën për strategji efektive të terapisë së kombinuar.*

Ky studim vlerëson potencialin terapeutik të kombinimeve të barnave në tre linja qelizore të melanomës—LOX-IMVI, SK-MEL-28 dhe M14—duke përdorur modele parashikuese dhe metoda të kuantifikimit të sinergjisë (LOEWE, BLISS, ZIP dhe HSA) të vlerësuara përmes SynergyFinder. Kombinimi i vemurafenibit dhe dakarbazinës tregoi një efekt sinergjik në qelizat LOX-IMVI sipas modelit ZIP, ndërsa në mënyrë të qëndrueshme shfaqi efekte shtuese në modelet e tjera. Qelizat SK-MEL-28 shfaqën kryesisht përgjigje shtuese, duke reflektuar profilin e tyre molekular të veçantë me mutacion BRAF V600E dhe NRAS të tipit të egër. Linja qelizore M14 tregoi modele përgjigjeje të ngjashme me LOX-IMVI, me shumë gjasa për shkak të mutacioneve të përbashkëta BRAF V600E. Megjithëse sinergjia e fortë ishte e kufizuar, efektet shtuese të vëzhguara në mënyrë të përsëritur në të gjitha linjat qelizore sugjerojnë përfitime klinikisht të rëndësishme, duke mbështetur terapinë e kombinuar si një qasje premtuese për rritjen e efikasitetit dhe uljen e toksicitetit në trajtimin e melanomës.

Fjalë kyçe: *Melanoma, Bar, Toksicitet, Linja Qelizore, Sinergji.*

Introduction

Melanoma is a malignant tumor originating from melanocytes, specialized skin cells responsible for the production of melanin. The primary function of melanocytes is to protect the skin from harmful UV radiation, but when these cells sustain irreversible genetic damage, they may begin to proliferate uncontrollably leading to melanoma (Dummer et al., 2020). This neoplasm is the most aggressive skin cancer due of its ability to metastasize quickly and at early stages to other tissues as well as developing a high resistance to systemic treatment (Garbe et al., 2020). Although melanoma accounts for less than 5% of all skin cancer cases, it is responsible for the highest mortality rate among skin malignancies.

The development of melanoma is a result of a number of complex factors ranging from environmental to genetical. Exposure to UV radiation is the most commonly known environmental factor contributing to melanocyte damage. These harmful effects, combined with the lack of appropriate efficient repair mechanisms, can lead to mutations in specific genes responsible for regulating cell cycle, cell proliferation and survival (Elder et al., 2020; Guo et al., 2021).

Melanoma can develop on existing or de novo skin lesions. Malignant transformation of melanocytes is frequently associated, but not limited, to mutations of specific oncogenes and tumor suppressor genes like BRAF, NRAS, KIT, PTEN and CDKN2. Their mutated activation dysregulates the downstream pathways of MAP Kinases and PI3K/AKT, which are critical for melanoma growth and progression (Smalley et al., 2014).

Melanoma harboring the BRAF mutation account for 40-50% of all cases. The most common single nucleotide polymorphism mutation of BRAF V600E, constitutively activates the MAPK/ERK pathway, promoting cell survival and proliferation, making it the most prevalent melanoma subtype (Cancer Genome Atlas Network., 2015). In contrast, melanoma positive for RAS mutations- particularly NRAS- represent 20% of cases worldwide. These mutations lead to the downstream signaling of both the MAPK and PI3K/AKT pathway. These mutations are associated with a more aggressive behavior and a reduced response to targeted therapy (Zhou et al., 2021; Jaeger et al., 2023).

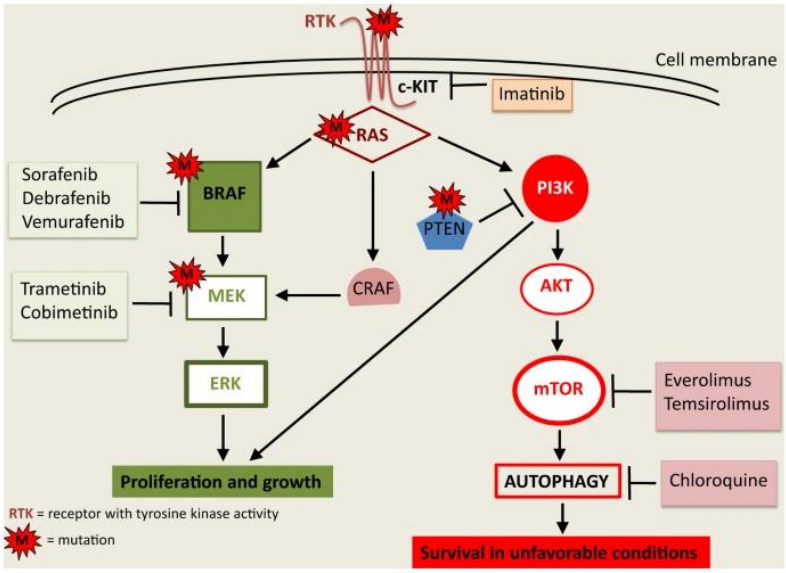

Figure 1. Mechanism of action for targeted therapy in melanoma (Matia G et al., 2018)

Targeted therapy involves the use of pharmaceutical drugs to specifically target compounds actively participating in tumorigenesis as shown in Figure 1. The standard care treatment involves the administration of BRAF inhibitor drugs for their melanoma BRAF positive cells killing capabilities (Chapman et al., 2011). However, numerous studies have shown that patients acquired specific drug resistance within months of treatment initiation (Luebker and Koepsell., 2019; Zhong J et al., 2022). One of the primary mechanisms underlying resistance is the reactivation of the MAPK pathway through secondary mutations of the NRAS gene, which promote signaling of RAF, therefore bypassing BRAF inhibition.

Additionally, amplification of the mutant allele of BRAF V600E leads to overexpression of the protein and significantly reduces sensitivity to inhibitors (Corcoran et al., 2010; Zecchin et al., 2013; Darabi et al., 2025). Given the high drug resistance, rapid growth and development of the tumor and the poor prognosis associated with melanoma, an ever-increasing demand for suitable, efficient and working drug combination therapies is required. The use of predictive modeling and toxicity assays offers a promising possibility to identify and evaluate potential drug combinations that could give a synergistic

effect, reducing drug toxicity while enhancing treatment efficacy against melanoma cells.

Methodology

Prediction of the toxicity of anti-cancer drugs was done by using the ProTox 3.0 in silico modeling program to calculate and assess the combinations of anti-neoplastic drugs. ProTox 3.0 (https://tox.charite.de/protox3/#) predicts toxicity endpoints such as acute and organ toxicity as well as adverse outcome pathways and toxicity targets based on CLUSTER cross-validation (Banerjee P et al., 2024)

SynergxDB (https://www.synergxdb.ca/) is a database used to explore and evaluate the synergistic drug combinations for the discovery of cancer biomarkers. The application allows the identification of new synergistic drugs by predicting potential biomarkers (BHK Lab., 2020).

Azacitidine- an anti-neoplastic drug able of embodying into DNA and RNA, thus disrupting the metabolic pathways of RNA and inhibiting DNA synthesis, and also disrupting DNA methylation (Garcia-Manero G et al., 2008). Its primary role is in the anti-cancer activity against acute myeloid leukemia and myelodysplastic syndromes, but it has been repurposed for usage in patients with advanced melanoma coupled with resistance to immune checkpoint blockade (van der Westhuizen A et al., 2022)

Celecoxib- is a nonsteroidal anti-inflammatory drug that works as a cyclooxygenase inhibitor and is known to have a reduced toxic effect as causative for gastro-intestinal bleeding. It has also been shown to reduce cell viability and migration, as well as induce apoptosis in some melanoma cell lines (Pagliarulo V et al., 2013; Venuta A et al., 2023).

Cabazitaxel- a FDA approved anticancer drug used for treating primarily prostate cancer. Its mechanism of action consists in inhibiting cell mitosis via regulation and stabilization of cell microtubules, leading to apoptosis (Food and Drug Administration., 2023). Even though it is not standard treatment for melanoma patients, cabazitaxel has shown effective inhibiting capabilities of melanoma cells in mouse models B16/TXT (Vrignaud P et al., 2013).

Dacarbazine- a synthetic intravenous drug used for the treatment of malignant melanoma and Hodgkin's disease. It's mechanism of action in unknown, but it alkylating capabilities show a cytotoxic effect (Reid JM Et al., 2022).

Dasatanib- this oral drug is a tyrosine kinase inhibitor for the treatment of leukemia patients positive for the Philadelphia chromosome (Dohse M et al.,

2010). Even though as a single agent it has shown severe limitations in treating melanoma, in cells exhibiting a c-Kit mutation has shown to have a metabolic activity (Malak Sabbah et al., 2021).

Decitabine- a drug that works by inhibiting the methyltransferase of DNA. It incorporates in the DNA and leads to the demethylation of DNA, thereby allowing inhibited suppressor genes to be reactivated (Chenlin Te et al., 2024).

Doxorubicin- is a drug used for a multitude of cancers, including but not limited to melanoma. Its mechanism of action is dependent on the intercalation of the drug to the DNA of the cancer cells leading to the inhibition of topoisomerases II, creating ROS and finally activating apoptosis (Rostami Z et al., 2025)

Fluorouracil- a topically administered antimetabolite drug which a wide range of uses for different types of skin cancer, by interfering with DNA/RNA synthesis and destroying mitotic cancer cell. Its systemic use is not recommended because of its severe side effects (Pourmanoucheri Z et al., 2022).

Vemurafenib- a competitive drug of the tyrosine kinase inhibitor family that has extensive use in patients who suffer from a positive BRAF V600 mutation melanoma. The drug blocks the MAPK kinase, responsible for cell proliferation, stopping the rate of cancer cell division (Funck-Brentano E et al., 2015).

Datasets were obtained from the NCI-ALMANAC database and different combinations were tested to understand their synergy scores and the possibility of overcoming single drug usage mechanism of resistance from the melanoma cells.

Analysis and discussion

Drug combination is a very important therapeutic strategy for the treatment of melanoma patients, especially in the advanced stages of the disease or in case of drug resistance of single agents. Combining different drugs allows an increase in efficacity for the therapy by using drug synergy, all the while minimizing side effects of individual drugs or development of resistance by melanoma cells. The drugs taken into consideration are known for their anti-neoplastic activity either by stopping cell division entirely, or by interfering with the methylation and synthesis of DNA and proteins. Dacarbazine is the model drug used for the treatment of melanoma and will also be the primary drug to be used in the potential synergistic combination with the other anti-

neoplastic chemotherapy/immunotherapy compounds. Dacarbazine by itself is the leading chemotherapy used for treating malignant melanoma, but its chemical properties have shown limitations in this aspect because of drug induced resistance (Xiong Wei, et al., 2022).

One of the most prominent side effects of dacarbazine is hemopoietic depression because of its toxicity and there have also been reports of hepatic necrosis (Hospira Inc., 2019). In order to assess the toxicity issue of the drugs to be paired, an evaluation of the toxicity class and LD50 of each drug was taken. Dacarbazine is considered the least toxic drug (5), whereas all the other drugs all fall in the same category of toxicity (class 4). The difference in the toxicity levels for the class 4 drugs is in reference to their lethal dose (LD50). This parameter shows the percentage of the tested population expected to die after a period of time following drug administration (Morris-Schafer & McCoy., 2021). Cabazitaxel has the lowest LD50 (560mg/kg), closely accompanied by azacitidine (572mg/kg).

Cabazitaxels' low LD50 values show a high toxicity potential, which also coincides with its cytotoxic taxanic nature with limited use because of occurrence of neutropenia (Eisenberger MA et al., 2012). Azacitidine on the other hand is considered a generally safe to use with a relative safe toxicity profile with rare occurrences of hepatotoxicity (LiverTox., 2023). Fluorouracil and dacarbazine and show the highest values for LD50 (1923mg/kg and 2032mg/kg respectively), showing a good systemic tolerance and making them good options for long-term combinations.

Analysis of the other parameters reveal a number of other differences. Regardless of its high LD50, fluorouracil exhibits high cytotoxicity values (0.931), mutagenicity (0.881) and immunotoxicity (0.991), indicating that its prolonged usage can be associated with DNA damage and compromising of the immune system in specific dosages (Longley DB et al., 2003). Vemurafenib, a BRAF inhibitor, shows lower values for systemic and hepatic toxicity, rendering it a drug with a more balanced efficacy/safety profile. This profile favours its usage in targeted therapy, especially in patients positive for BRAF V600E mutations (Ascierto et al., 2013). Cabazitaxel and celecoxib exhibit an increased risk for hepatotoxic and mutagenic effects, with values of HT >0.6 and MG >0.75. These sets of values suggest that their usage should be closely monitored for clinical implications, particularly in patients with hepatic complications or previous oncologic history as shown in table 1.

Table 1. Data for drugs used in melanoma treatment; LD50- lethal dose; TC- toxicity class; A- accuracy; M.W- molecular weight; HT- hepatotoxicity; CG- carcinogenicity; IT- immunotoxicity; MG- mutagenicity; CT- citotoxicity; A -active; I- inactive; Vem- Vemurafenib; Cel- Celocoxib; Cab- Cabazitaxel; Flu- Fluorouracil; Dac- Dacarbazine; Aza- Azacitidine; Dec- Decitibine

	Vem	Cel	Cab	Flu	Dac	Aza	Dec
LD50 mg/kg	910	1400	560	1923	2032	572	826
TC	4	4	4	4	5	4	4
A %	54.26	54.26	67.38	67.38	100	100	68.07
M.W	489.92	519.56	835.93	130.08	182.18	244.21	228.21
HT	0.59A	0.6I	0.66I	0.78I	0.63A	0.72A	0.5A
CG	0.6I	0.56A	0.62I	0.85A	0.66A	0.73A	0.56A
IT	0.8A	0.99I	0.99A	0.99I	0.99I	0.96I	0.98I
MG	0.63I	0.75I	0.77I	0.88I	0.78A	0.63A	0.74I
CT	0.65I	0.91I	0.56A	0.93I	0.84I	0.94I	0.91I

To assess the synergy score of these drugs combination of two drugs was performed for each of them. Studies have shown that combining two or more pharmaceutical anti-cancer compounds that have distinct mechanisms of action can potentially lead to better effects, efficacy and reduced toxicity for the treatment of cancer (Foucquier and Guedj., 2015; Sun et al., 2016; Cheng et al., 2019; Paltun et al., 2021). The evaluation was made for a combination of two drugs, Drug 1 which is changeable and Drug 2 which remains non variable. All the drugs taken into account are FDA approved and already in use in clinical trials or in afflicted patients as single therapeutic agents.

Predictive testing was undertaken in three different cell lines: LOX-IMVI, SK-MEL-28 and M14. LOX-IMVI is a melanoma cell line extracted from a 58-year-old white human male that has excellent characteristics for

chemotherapeutic experimentation (Sigma Aldrich., 2025). SK-MEL-28 is a cell line extracted from the skin of 51-year-old patients afflicted with melanoma. These cells are very sensitive to toxicologic research (ATCC., 2025). The M14 cell line is derived from an adult patient suffering from metastatic melanoma with skin lesions. This cell line has a variety of uses, ranging from tumor progression to therapeutic assessment (Cytion., 2025).

To assess the effect of combination drugs, their synergy scores were measured and verified. To evaluate the results, synergism in drug combinations quantification comparative methods were used (Duarte and Vale., 2022). The most common methods used are LOEWE, BLISS, ZIP and HSA and the final assessment is on a point-based system conditioned by SynergyFinder:

Synergy values more than 10, the combination results in a synergistic effect.

Synergy values between -10 and 10, the combination results in an additive effect.

Synergy values lower than -10, the combination results in an antagonistic effect.

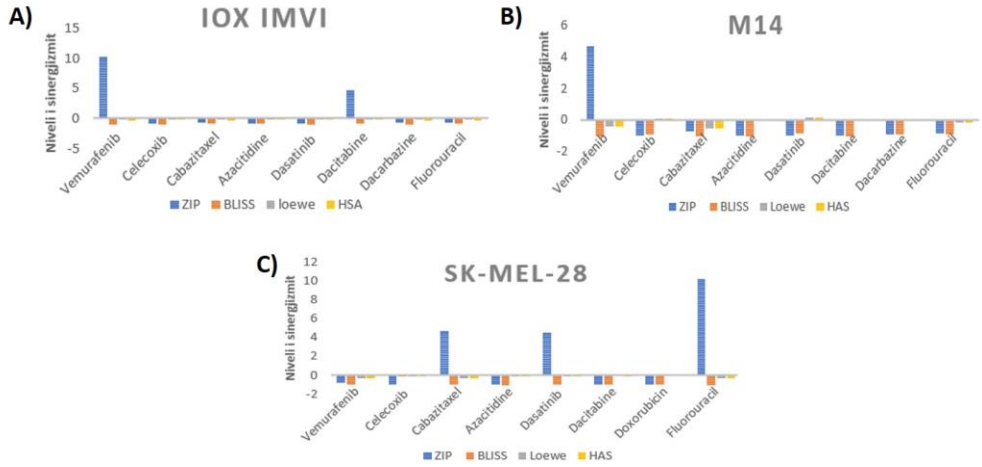

Figure 2. Synergistic effect assessment of anti-neoplastic drugs for treatment of melanoma cell lines

The score from the LOX-IMVI testing (Figure 1) indicates that only the ZIP method shows a synergistic effect between the combination of vemurafenib and dacarbazine with a score of 10.31. ZIP also reports a positive value for the dacarbazine/decitabine combination (score= 4.7). The other methods do not support the synergy score, but nonetheless HSA, LOEWE and BLISS still

show an additive effect for all the drug combinations involved, vemurafenib included.

SK-MEL-28 portrays a different response profile to LOX-IMVI. The vemurafenib/dacarbazine combination demonstrates only an additive effect in the negative values, yet still achieves improved efficacy relative to single-agent treatments. Conversely, in the case of cabazitaxel, dasatinib and fluorouracil a positive additive effect is shown from the ZIP model with values ranging from +4 to +10. But these positive additive effects are not corroborated from the other synergistic methods, but still the score is between the values determining additive drug effect. This cell line is of particular interest because of the dual molecular profile- harboring mutant BRAF V600E and wild type NRAS, making it a perfect cell line to understand the signaling pathway driven by BRAF for melanoma proliferation.

The M14 cell line exhibits a response pattern similar to the LOX-IMVI cell line for the dacarbazine-vemurafenib combination. This could be associated with the fact that both cell lines possess a mutated BRAF V600E point mutation, but LOX-IMVI also lacks the p53 mutation. It must be mentioned that even though the synergy scores don't show a synergistic effect, the consistently observed additive effects remain clinically relevant, proving that the combination of these drugs could lead to a better regimen for the therapeutic approach, as well as reduced toxicity from single drug usage.

Conclusions

Overall, the LOX-IMVI, SK-MEL-28, and M14 cell lines demonstrate that while strong synergistic interactions between the tested drug combinations are largely limited and model-dependent, additive effects are consistently observed across multiple synergy assessment methods. The ZIP model alone identifies a synergistic interaction for the vemurafenib–dacarbazine combination in LOX-IMVI cells and modest positive interactions in select combinations in SK-MEL-28 cells; however, these findings are not uniformly supported by HSA, Loewe, or Bliss analyses. Importantly, the presence of additive effects across all models suggests that combination therapies can still offer therapeutic benefit over single-agent treatments.

The differential responses observed among cell lines highlight the influence of distinct genetic backgrounds—particularly BRAF V600E status, NRAS expression, and p53 alterations—on drug interaction outcomes. Taken together, these results support the potential clinical relevance of additive drug

combinations in melanoma, emphasizing the need for careful model selection in synergy analysis and further validation in biologically and clinically relevant systems

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