A COMPUTATIONAL METHOD TO ASSESS THE EFFECTS OF COMBINATION DRUGS ON LEUKEMIA CELL LINES

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Abstract

One of the most common, prominent and lethal disease known to humans nowadays is cancer. It is the 2nd cause of death of the entire human population, whilst also causing suffering, pain and an overall decrease of life quality and expectancy. To combat this affliction, one of the best methods up to date has been chemotherapy. But even though there has been some success, the limitations are quite clear. Low drug efficacy, ever increasing dosage or concentration to achieve some result and an inherent resistance from drugs by the cancer cells have raised the issue to find something better with minimal risk and damage. Predicting methods for drug combination has achieved great results. Whilst being mainly on paper, with the laboratory work still ongoing, the predicting possibilities have been outstanding, with zero cost, resource or deleterious effect. In our study we took 8 approved (cytarabine, dasatinib, dexamethasone, ibrutinib, imatinib, midostaurin, prednisone, venetoclax), and 1 un-approved (quizartinib) FDA drug combined them with each other and predicted the possible outcomes. This, in combination with the results for toxicity of each individual drug, resulted in creating viable multi-drug combination with the ability to destroy cancer cells. These drug combos (drug A 20μM/ drug B 1μM) were tested in silico on three different leukemia cell lines, MV4-11, LAMA84, U-937. Cytarabine showed the highest inhibition of the proliferative leukemic cells when combined with both prednisone, and dexamethasone followed closely by venetoclax, while imatinib had the lowest.

Key words**:** *Drug, Combination, Synergy, Leukemia, Cell line.*

Përmbledhje

Një nga sëmundjet më të zakonshme, të njohura dhe vdekjeprurëse e njohur sot nga njeriu është kanceri. Është shkaktari i 2-të i vdekjes në popullatën njerëzore, duke shkaktuar gjithashtu vuajtje, dhimbje dhe ulje të cilësisë dhe gjatësisë së jetës. Për të luftuar këtë sëmundje një nga metodat më të mira deri më sot ka qenë kimioterapia. Edhe pse ka patur disa suksese, kufizimet janë mjaft të qarta. Efikasiteti i ulët i barit, dozat ose përqëndrimet përherë në rritje për të marrë rezultat, si dhe rezistenca që shfaqin qelizat kancerogjene ndaj barit kanë ngritur çështjen për gjetjen e diçka më të mirë me rrezik dhe dëmtim minimal. Metodat parashikuese për kombinimin e bareve ka sjellë rezultate shumë të larta. Edhe pse kryesisht në letër, me punën laboratorike ende ne vijim, mundësitë parashikuese kanë qenë të shkëlqyera, me kosto dhe burime zero, pa efekte dëmtuese. Në studimin tonë u morën 8 bare (cytarabine, dasatinib, dexamethasone, ibrutinib, imatinib, midostaurin, prednisone, venetoclax) të aprovuar nga FDA dhe 1 të pa aprovuar (quizartinib), i kombinuam me njëri tjetrin dhe u parashikuan përfundimet e mundshme. Kjo, e kombinuar me rezultatet e toksicitet për secilin bar individual, solli krijimin e kombinimeve multi-bar të vlefshme me aftësinë për të shkatërruar qeliza kancerogjene. Këto kombinime bari (bari A 20μM/ bari B 1μM) u testuan në mënyrë in silico në tre linja qelizore të ndryshme leuçemie, MV4-11, LAMA84, U-937. Cytarabine shfaqi inhibimin më të lartë të qelizave proliferative kur kombinohej me prednsisone dhe dexamethasone, ndërsa imatinib kishte me inhibimin më të ulët.

Fjalë kyçe: *Bar, Kombinim, Sinergji, Leuçemi, Linjë qelizore*

Introduction

Cancer is the 2nd leading cause of deaths in the world. The number of new cases is ever on the rise. The number of de novo cancer cases in 2020 was estimated around 19 million, with a prediction of 29 million in the year 2040 (Bray et al., 2021). The treatments for these patients present an issue every single day. Even though the number of anticancer drugs has seen a rapid increase during the last years, still their advantages remain limited. The most prominent challenges that the medical world faces during patient caregiving are low drug efficacy, especially after the initial treatment regimen; the high dosages/concentrations leading to hepato- or cytotoxicity of the patient and lastly, mechanisms of resistance which are either acquired or intrinsic after several administrations of monotherapy drug treatment (Wang et al., 2019). Two other key factors affecting drug resistance have been attributed to the

inter- and intra-tumoral heterogeneity of the patient (Chatterjee and Bivona., 2019; Vasan et al., 2019).

To circumvent these issues, the usage of combination therapy has resulted very successful, by decreasing dosages, increasing drug efficacy and overcoming resistance mechanisms (Mokhtari et al., 2017). Several studies have shown that the usage of combination therapy vastly improves the survival chances of patients compared to the ones receiving monotherapy. (Das et al., 2019; Ma et al., 2021). Although the number of drug combinations is high, the cost of testing is extremely high as well. Another limitation is the low number of patients willing to participate in these clinical trials, rendering most results unavailable. To deal with these hurdles, a series of computational methods have been developed making it possible to analyse and evaluate the possible combinations of effective drugs (Fan et al., 2021; Duarte and Vale., 2022; Torkamannia et al., 2022). These machine learning vectors for drug combination have resulted quite effective on paper, the actual laboratory work is either lacking or still in progress. Apart from the aforementioned problems, there are still two main points to look at. The first is the fact that most of these combination methods only use one synergy metric to evaluate drug efficacy, while offering no other alternative. And the second point to address is the fact that these methods require huge datasets and databases to give reliable conclusions. This is a double-edged sword, because on the one hand the required number is high, but on the other the final results offer high reliability and confidence.

In our study we focus on combining seven different anticancer drugs with two support (anti-inflammatory) drugs to analyse and predict the best possible interaction to inhibit cancer cell proliferation. The cancer we assessed was leukemia, being the 13th cancer affecting people globally and 10th in line for mortality. In silico analysis on three different leukemia cell lines, MV4-11, LAMA84 and U-937 were used as models to predict the most favourable outcome and the best possible treatment for the afflicted patients. The usage of these predicting algorithms allows a huge amount of combinations without ever putting the patient at risk or causing him/her further damage or lowering of life quality.

Materials and methods

In order to assess the toxicity of antineoplastic drugs, we used ProTox-II software to generate the possible combinations. ProTox-II (https://tox-new.charite.de/protox_II/) serves as a machine-learning predictor of

compound toxicity. This algorithm is based on a variety of endpoints such as toxicity targets and pathways, hepatotoxicity, carcinogenicity, cytotoxicity, immunotoxicity and mutagenicity (Banarjee, et al., 2018).

DruComb (https://ai.drugcomb.org/) is a software used to collect, assess and generate data by combining different types of drugs in cell lines. The algorithm consists in analysing combined drugs and evaluate drug synergism (Zagidullin et al., 2019).

Cytarabine is a cytidine antimetabolite analogue containing an arabinose sugar instead of ribose. Arabinose is converted as a triphosphate in the cell, increasing its affinity for DNA. This allows it to sterically inhibit the helical turn of the DNA, thus stopping replication in the S phase. This antineoplastic agent inhibits DNA polymerase by lowering its replication and repair.

Dasatinib- a selective inhibitor of the tyrosine receptor kinase used to treat Philadelphia chromosome positive chronic myeloid leukemia patients. This drug targets and inhibits the high proliferative activity of TKs. Because of its not so strong affinity for the BCR-Abl kinase, dasatinib is able to circumvent mechanisms of resistance that mutated CML cells have for imatinib.

Dexamethasone- is synthetic adrenal corticosteroid with powerful anti-inflammatory characteristics. It can also target specific steroidal nuclear receptors, blocks NFKB signalling and modifies apoptosis pathways.

Ibrutinib- is a small antineoplastic inhibiting molecule of the Bruton tyrosine kinase (BTK). Administered orally, this drugs irreversibly targets and inhibits BTK activity by preventing activation of B cells and their signalling pathway. This leads to fewer malignant B cells overexpressing BTks to mature. Expression and overexpression of BTKs in tumor cells leads to an increased proliferation and survival.

Imatinib- is a pharmaceutical antineoplastic compound of the BCR-Abl proteins, responsible for CML. It also inhibits PDGF and SCF receptors, leading to inhibition of proliferation and induction of apoptosis of cells overexpressing these proteins.

Midostaurin- is a synthetic multikinase inhibitor derived from indolocarbazole, with antiangiogenic and antineoplastic characteristics. It inhibits protein kinase C-alpha (PKC-α), VEGFR2, c-Kit PDGFR and FLT3. This leads to a disrupted cell cycle, inhibited proliferation, apoptosis and inhibition of angiogenesis in tumors.

Prednisone- is a synthetic glucocorticoid anti-inflammatory and immunomodulating drug. After it attaches to the surface receptor and enters the cell, prednisone penetrates the nucleus and targets the specific receptors by modulating gene expression and inhibiting production of proinflammatory cytokines. Prednisone lowers the overall number of free lymphocytes, induces cell differentiation and stimulates apoptosis in sensitive tumor cells.

Quizartinib- an orally administered small molecule with potentially antineoplastic activity. It selectively inhibits third class TKs, including FLT3, CSF1R, SCFR and PDGFRs by inhibiting proliferation and inducing apoptosis of free ligand leukemia cells.

Venetoclax- a small molecule which is administered orally, with high inhibiting attributes for the anti-apoptosis Bcl-2 protein. It is also considered an antineoplastic drug for its role in chronic lymphocytic leukemia (CLL) treatment. Venetoclax is an apoptosis inducer exhibiting immunosuppressing characteristics for Bcl-2, but not Bcl-XL.

All drugs' dosages in this study use the standard model for drug administration, male model weighing 70 kilograms. This was evaluated as optimal to assess the pharmaco-kinetic and -dynamic characteristics of the drugs in analysis.

Results and discussion

To assess drug efficacy, a number of broad-spectrum drugs used to treat cancer cells were used. These drugs are known for their inhibiting activity of mutated kinases, as well as lowering inflammation in the human body (prednisone and dexamethasone). Their antineoplastic and antiproliferative effects make them ideal compounds for the treatment of leukemia patients. Dexamethasone, imatinib and dasatinib are currently being used to treat 3 of the four major leukemia, whereas prednisone is used in all leukemia cases.

Cytarabine is mostly used in treating acute leukemia. Dexamethasone and venetoclax have the lowest toxicity (5), whereas imatinib is considered the most toxic drug (3). This evaluation is because of their lethal doses (LD50) for each specific drug. LD50 is the median lethal dose, where 50% of the experimented population dies upon exposure to the drug after a certain amount of time (Morris-Schafer & McCoy., 2021). Imatinib has a very low LD50 (100mg/kg), rendering it toxic if administered in these doses.

This drug is a class 3 toxicity compound, with high toxicity and fatal in low doses (<300mg/kg). On the other hand, dexamethasone and venetoclax have a high therapeutic index, since their LD50 falls into the 3000-3600mg/kg range. This classifies them as relatively safe drugs if administered in these doses, minimizing the damage of wrong administering. The remaining drugs are categorised as class 4 toxicity, since their LD50 falls between the safety ranges of 300mg/kg – 2000mg/kg. The normal expected effect should be the one that is observed the more frequently, but there still is room for a level of tolerance for each specific drug in regards to their prediction accuracy.

Table 1: Data for drugs used in leukemia 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; Pred- prednisone; Ima- imatinib; Ibr- ibrutinib; Das- dasatinib; Cyt- cytarabine; Dex- dexamethasone; Ven; venetoclax; Mid- midostaurin; Sor- sorafenib

	Pred	Ima	Ibr	Das	Cyt	Dex	Ven	Mid	Sor
LD50 mg/kg	1680	100	1000	1000	826	3000	3600	1000	800
TC	4	3	4	4	4	5	5	4	4
A %	71	54.26	54.26	23	100	100	23	54.26	67.38
M.W	358.4	493.6	440.5	488	243.22	392.46	868.44	570.64	464.8
HT	0.99I	0.71A	0.69I	0.63I	0.78A	0.99I	0.6A	0.72I	0.82A
CG	0.94I	0.67I	0.6A	0.5A	0.57A	0.72I	0.55I	0.57I	0.5I
IT	0.99A	0.66A	0.66I	0.66A	0.99I	0.99A	0.99A	0.99A	0.92A
MG	0.53I	0.73I	0.54I	0.67I	0.96I	0.69I	0.54A	0.57I	0.79I
CT	0.76I	0.53I	0.66I	0.63I	0.94I	0.72I	0.55I	0.62A	0.77A

Table 1 shows a high active hepatotoxicity from drugs imatinib, cytarabine, venetoclax, and sorafenib; 0.71, 0.78, 0.6 and 0.82 respectively. Imatinib exhibits three types of liver damage with different consequences: increased hepatic enzymes in the serum which is usually asymptomatic, acute hepatitis or reactivation of a latent B type hepatitis (Bethesda., 2018). In a study by Han et al., 2020, of 177 patients, 33.9% of them resulted with hepatotoxicity 90 days after imatinib administration. According to the same study, patients carrying hepatitis B or suffering another form of liver damage, were 8 times more at risk for increased hepatotoxicity (Han et al., 2020). A study by Johnson

et al., in 2003 have stipulated that 0.5% of CML patients must immediately and indefinitely suspend the usage of imatinib because of its high toxicity.

Sorafenib, a drug commonly used for the treatment of a variety of cancer cells, has also shown a considerable number of side effects (Parikh et al., 2017). In its highest concentration, sorafenib has been shown to cause high hepatotoxicity. This is believed to be caused by mitochondrial toxicity (Zhang et al., 2020). Venetoclax induces a low-level increase of hepatic enzymes in the serum during treatment, but does not cause acute damage or jaundice (Bethesda., 2017). Pharmacokinetic analysis of venetoclax administered in patients with normal or abnormal hepatic function (mild, average or severe) showed no side effects of hepatotoxicity, regardless of their condition (Salem et al., 2019).

The last drug, cytarabine, is a commonly used drug for the treatment of AML patients. Even though this drug is considered safe and not toxic for the liver, treatment of relapsed AML patients has shown considerably high levels of hepatotoxicity (Fu et al., 2019). This study supports the findings of Grigorian and O'Brien in 2014, which demonstrated that a number of patients had high hepatotoxicity levels from cytarabine, but there was no need for modifications in their treatment.

Another key aspect that was taken into consideration in this study is the resistance tumor cells develop against drugs, apart from the existing issue of low efficacy. Combination of two or more drugs has shown to improve overall efficacy of treatment for cancer. This combo creates a synergistic effect, since several studies have shown that synergy can be affected by factors such as drug-drug ratio, type or order of administration (Shuhendler et al., 2010; Piccolo et al., 2015; Li et al., 2016).

To evaluate the effect of drugs in leukemia cell lines, we analysed combination of 2 drugs, drug A which is variable, and drug B which is unchanged. All compounds used as drug A are all approved from the U.S. Food and Drug Administration (FDA), apart from Quizartinib. The inclusion of this drug in our study was because of the good results and high efficacy in the second phase of clinical trials of AML patients in Japan (Takahashi et al., 2019). Drug B was dexamethasone in one case and prednisone in the other. The usage of these two compounds as support drugs was done in accordance to the regimens set by the European Hematology Association (EHA) and European Society for Medical Oncology (ESMO).

Testing was done in three different cell lines: MV4-11, U-937 and LAMA84, to screen for: 1- the level of inhibition of the proliferation of leukemia cells; 2- destruction of leukemia cells; and 3- synergistic values of drugs. MV4-11 is a cell line taken from a 10-year-old patient suffering from biphenotypic B-myelomonocytic leukemia, with continuous proliferative capabilities (ATCC., 2020). U-937 is a cell line derived from a patient suffering from histiocytic lymphoma. These cells are used to study the behaviour and differentiation of monocytes (ATCC., 2020). LAMA84 is a CML cell line able to differentiate into different B cell subtypes. This cell line is used as model to assess sensitivity and resistance of tyrosine kinase inhibitors (ATCC., 2020).

The concentration of drug A was set at 20μM and drug B was set at 1μM. this ratio was considered optimal because further analysis with different concentrations showed a lower inhibiting effect of the combined drugs (data not shown).

Graph 1 shows that the highest inhibition effect in the MV4-11 cell line is from the cytarabine+prednisone/dexamethasone combination, with 52% in both cases. In the case of sorafenib it is shown that the combination with dexamethasone has a higher inhibition compared to its combination with prednisone. Imatinib has the lowest inhibition rate when compared to the other drugs (38% with dexamethasone and 35% with prednisone). These values correlate with previous studies that sorafenib and cytarabine have a higher inhibiting effect than imatinib for the MV4-11 cell line (Hu et al., 2008; Nik et al., 2015). It should be noted that different studies have shown that leukemia cells develop a resistance mechanism from treatment with imatinib, especially after relapse (Lierman et al., 2007; Stolzel et al., 2010).

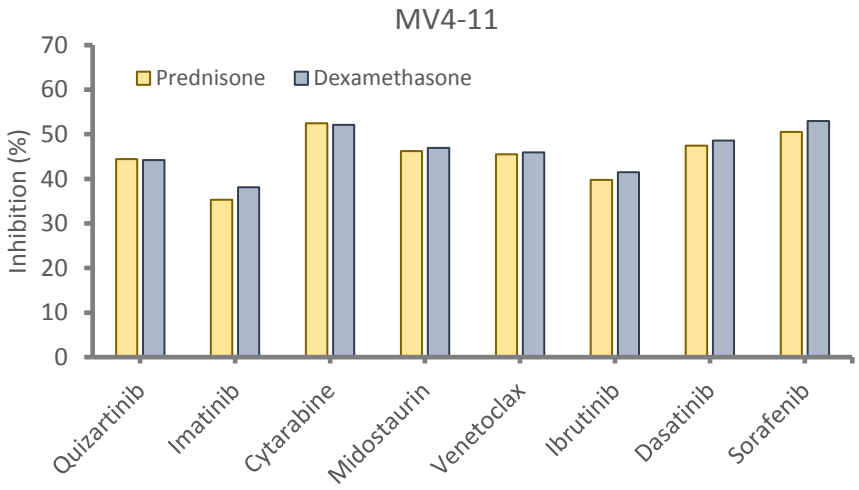

Figure 1: Level of inhibition of MV4-11 cell line from prednisone and dexamethasone

Lower concentrations showed lower inhibiting values and higher concentrations lead to high drug toxicity. Low concentrations of drug A led to lower proliferation inhibition, but this is attributed to the insufficient drug dosage. The same protocol was used for the other two cell lines, U-937 and LAMA84. When comparing the different cell lines with each other and their level of inhibition, it is shown that cytarabine exhibits the highest inhibiting effect compared to the other drugs. Previous studies support these results, stating that usage of high doses of cytarabine displays good inhibiting effect of leukemia cells (Lowenberg et al., 2011; Ma et al., 2017).

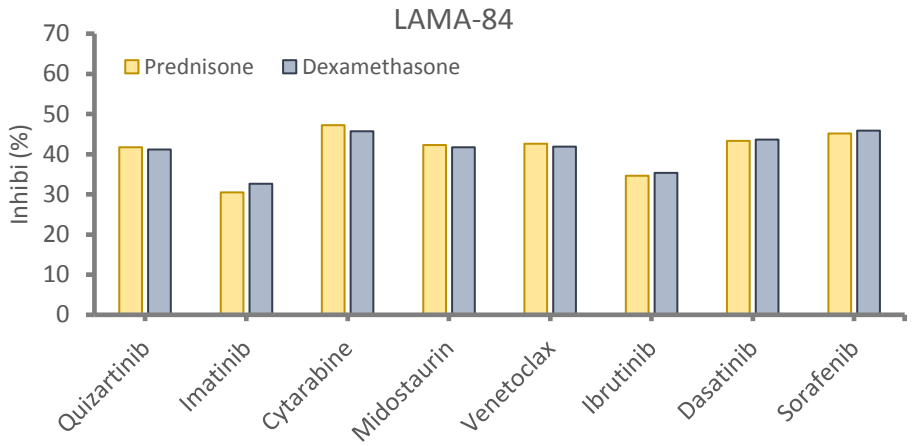

Figure 2: Level of inhibition in LAMA84 cell line

Another major aspect to take into consideration is the effect of imatinib in the inhibition of the cell line U-937. In the other two cell lines, the inhibiting effect of imatinib is relatively low, whereas in line U-937 a 10% increase is seen. This fact can be attributed to the high affinity imatinib has for CML cells, consequently the inhibiting effect of imatinib is always increased in these cells (Hakansson et al., 2004; Hochhaus et al., 2017). One key feature that must be mentioned is the simplicity of the cell lines. This important feature means that these cell lines only carry leukemia cells and are unencumbered by outside or internal elements. Apart from these, these cell lines are also not impeded by other signalling pathways which could affect the overall efficiency and

efficacy of the drug.

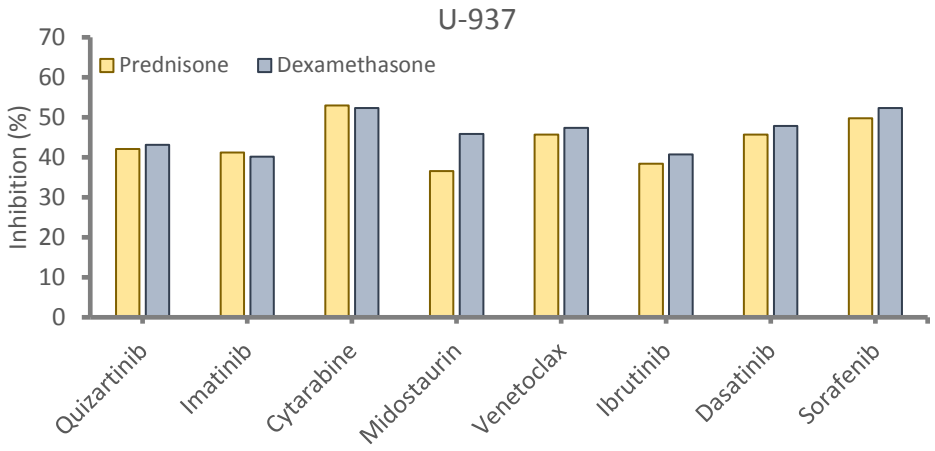

Figure 3: Level of inhibition in U-937 cell line.

Upon verification of the drug-drug combination and their inhibiting effect, the next step was the verification of their synergism. This effect is measured by comparing the values of the drug combination with synergy evaluation methods. The standard accepted methods for assessing synergy effects are: ZIP, HSA, BLISS and LOEWE (Ianevski et al., 2107).

In all cell lines, the combination of drug A with dexamethasone generates a positive evaluation for ZIP, HSA and BLISS, whereas all combinations give a negative assessment for LOEWE. This assessment is based on a point system determined by SynergyFinder:

- When synergy values are lower than -10: the drugs interaction can result in an antagonistic effect.
- When synergy values range between -10 and 10: the drugs interaction can result in an additive effect.
- When synergy values are higher than 10: the drugs interaction can result in a synergistic effect.

Data shows that most of the drug's combinations for the treatment of cancer cell lines have an additive effect. The exemptions are quizartinib and cytarabine for all three lines, and venetoclax for U-937. The highest additive evaluation if from HSA, because the drugs are taken into account as independent from each other and the highest effect is defined by probability.

This model analyses all combination options and the highest evaluation is for the value that manifests the most frequently.

The LOEWE model manifests all results with a negative value, rendering all the drugs as antagonists of each other. Even though this model has been taken into account, it does not constitute as an important part of the study. According to this model all drugs are the same, because of their mechanism of action, which would be acceptable in sham experiments. But in *in silico* models and especially in in vivo or in vitro experiments, it rarely happens to combine identical or the same drugs. It's for this reason that this model is faulty and generates fake results (Yadav et al., 2015). Even though none of the results showed a synergistic effect, an additive effect is seen leading to a higher efficacy of the combined drugs with fewer side effects for the treatment of leukemia (Zhou et al., 2019; Tusa et al., 2020).

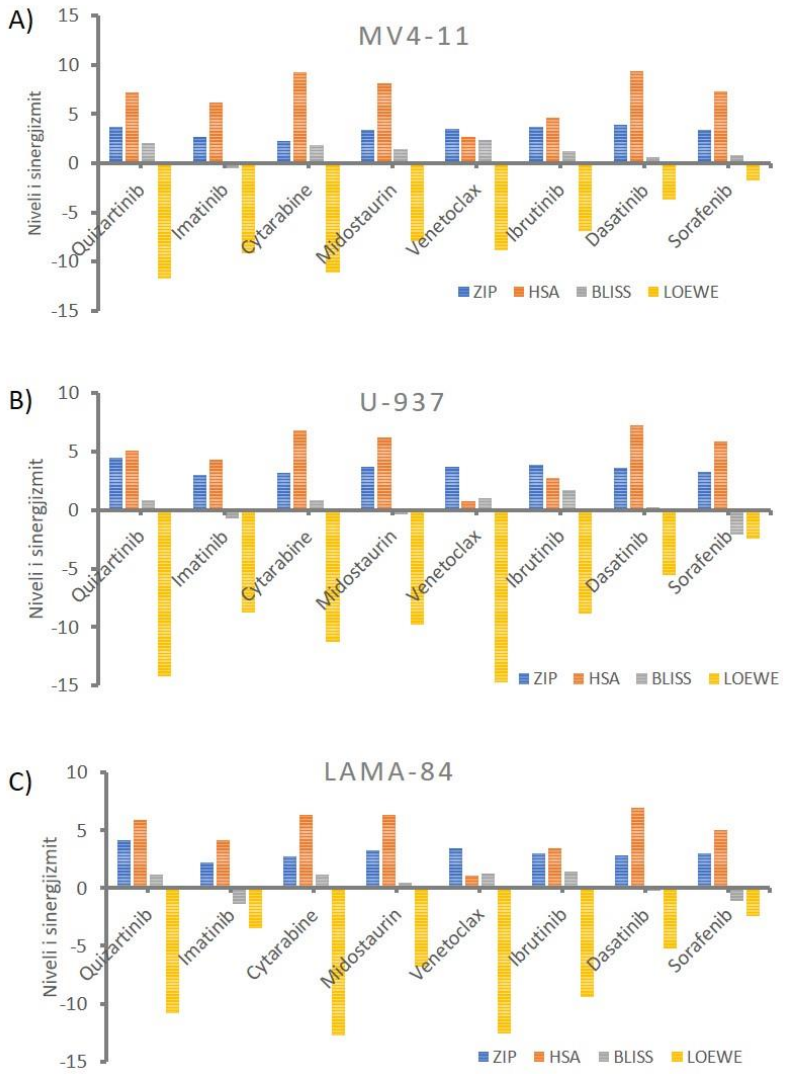

Figure 4: Evaluation of the synergistic effects of drugs with ZIP, HSA, BLISS & LOEWE; A- MV4-11; B- U-937; C- LAMA84

Conclusions

The results of our study focus on the inhibition of leukemia cells via an in-silico approach. By using the data and algorithms to predict the toxicity effects and level of inhibition of cancer cells it is possible to find appropriate drug

combinations with the highest efficacy, lowest concentrations, lower doses and fewer side effects for the treatment of the patient. Even though the results were acceptable, it must be noted that none of the inhibitions surpassed the 55% mark. These results constitute one of the limitations of the in silico approach. The numerous databases and datasets used, take into account the level of resistance of the drug in the cell lines, as well as their cytotoxicity. For this particular reason, an experimental approach is essential to further analyze and predict both the therapeutic effect and damage these drug combinations can do. Integrating these several methods should be paramount for finding and improving novel and existing treatments for leukemia cell patients.

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