Liposome Drug Delivery in Cancer Chemotherapy: Review and Multifactorial Analysis

Tatiara Régis[1]*; Luciana Knop[2]; Bruna Machado[2]; Valter de Senna[3]
[1]SENAI CIMATEC, Doctorate Computational Modelling and Industrial Technology; [2]SENAI CIMATEC, Technology Institute of Health; [3]SENAI CIMATEC, Computational Modelling and Industrial Technology; Salvador, Bahia, Brazil

We reviewed the use of liposomes for cancer therapy using computational biostatistics. We used virtual libraries, such as PubMed, LILACS, National Health Institute (NHI) and the Food Drug Administration (FDA) to conduct the review. Cluster analysis and correlation were developed using the Tanimoto coefficient (TC) of 0.7 using the modeling tool ChemMine Tools from databases (PubChem, DailyMed, DrugBank, Drug @ FDA). The results pointed fifteen molecules in the pharmaceutical form of liposome for the oncological clinic. Of these, 13 are classified by size into small-molecules and were analyzed by computational statistical modeling. Of these, only 4 were approved for use by the FDA, and 9 are in phases of research by the pharmaceutical industry as liposomal formulations. Essential differences in physical-chemical properties and molecules structure were observed, indicating original proposal in the development of liposomal as an anticancer drugs.
Keywords: Bioinformatics. Cancer. Drug delivery. Liposomal.

The cancers have in common the disordered and malignant growth of several cell lines with the capacity to invade other tissues far from the place of origin, forming secondary tumors or metastases [1]. The disease is currently one of the world's public severe health problems. According to Globocan 2018, a project by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), there were 14.7 million new cases of cancer and a total of 9.8 million cancer deaths worldwide in 2018, and a projection of almost 21.4 million new cases until 2030 with 13.2 million cancer deaths [2].

So, new antitumoral drugs are in tests as well as new methods to deliver these drugs. Due to the limitations and toxic events of conventional chemotherapies, several nanocarrier delivery systems have been developed and extensively used for drug delivery to cancer cells [3-5]. A large number of nanocarriers such as liposomes, polymeric nanoparticles, micelles, nanotubes, are already in the market, or under research and evaluation for cancer treatment [6]. Reports reveal an estimated global growth of liposome drug delivery (LDD) around $ 3.6 billion by 2020 in the global economic market and pharma industry [7].

The structural diversity and encapsulated drugs, as well as the mechanics of action liposomes, created a new technological perspective. However, there are challenges in methods for the preparation of liposomes, methods for efficient drug delivery, and procedures for the entrapment the drugs in the liposome. So, the innovations in liposome technology target researching that make it possible to predict new formulations and drugs for antitumoral treatments.

This review summarizes the types of methods used for the preparation of liposomes, mechanism of drug loading and potential therapeutic applications in cancer therapy, and provide current information on the liposomal products as a potent anticancer delivery drugs, either in clinical use or in clinical trials.

Liposomes

Liposomes have been widely used for several therapeutic applications, primarily as delivery drugs to reduce the toxic effects in the standard cell. They have also been used to optimize the bioavailability

of drugs in areas such as pharmacology, oncology, cell biology, immunology, genetic engineering, and therapeutic and preventive medicine.

Liposomes have a larger surface area as compared to bigger particles, which can be easily modified to encapsulate a high amount of drug, increasing the blood circulation time, and enhancing the accumulation of drugs in solid tumors by permeability and retention (EPR) effect as well as selective targeting of tumor cells [8-10]. They also improve the solubility, bioavailability and pharmacokinetic properties of chemotherapeutics [9,11,12].

Liposomes have an unusual sphere structure, globular lipid bilayers of 50-1,000 nm. They are versatile, notably in size, surface charge and have phospholipid bilayer membranes similar to plasmatic membranes, which favored the permeability of the molecules to get into the cell [13]. Therefore, these structures can encapsulate and deliver both hydrophilic and hydrophobic substances.

Drug Delivery Systems (DDS)

DDS are methods of administering drugs to reduce side effects and increase therapeutic efficacy, such as reduction of toxicity for anticancer agents [7,13]. DDS represents an advance in pharmaceutical technology compound to achieve a therapeutic effect in drug delivery for several treatments [13,14].

Liposomes are a potential DDS [15] (Figure 1). For example, niosome are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or their lipids [16] by sub-micron (colloidal) of types MLVs (size ≥ 0.05 µm), LUVs (size ≥ 0.10 µm), SUVs (size $= 0.025$-0.05 µm) [17], and consist of advanced DDS liposome (Figure 2) [18].

Gregoriadis and colleagues [19] consider two aspects that justify the promising future of liposomes drug delivery (LDD): reducing toxic side effects, and incoming of the drug in tumor cells. The studies by liposomes have been showing that the absorption of the drug by tumor cells is superior to drugs used in chemotherapies [20].

Designing Liposomes Drug Delivery (LDD)

The physicochemical properties of the liposomes are generally useful for characterizing a drug formulation, which must be more stable to avoid changes in product quality, including leakage of the drug. So, chemical, manufacturing and control (CMC); pharmacokinetics and bioavailability; and labeling documentation for liposome products submitted to new drug applications (NDAs) and abbreviated to new drugs (ANDAs) have been reviewed by the Center for Drug Evaluation and Research (CDER) [21] and recommended by the FDA for Industry.

The production of liposomes involves the use of an active agent and a phase lipid/lipid drug. So, the variety of natural or synthetic lipids, the different sizes, and molar rays, lead to a diversity of liposomes. In this aspect, the inherent properties of the carriers' structures and the nature of the drug to be encapsulated could affect biological half-life and modify drug absorption due to the relation between such structures and physicochemical characteristics of the drug substance (Table 1) [22].

At present, the designing stage of the liposome is still in the beginning. So, the choice of the

Figure 1. Illustration of liposome for drug delivery.
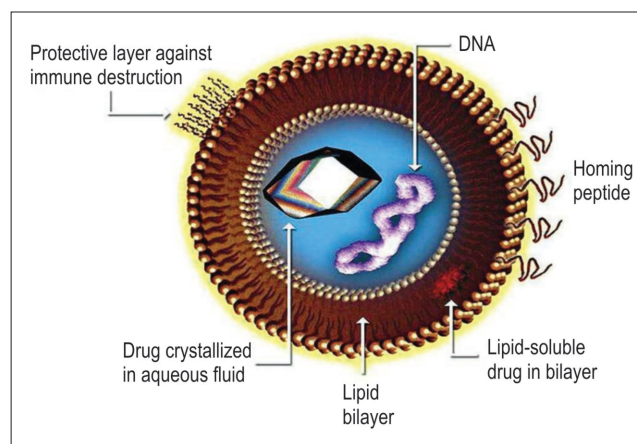
Source: Çağdaş M. et al. (2010) [15].

Figure 2. A schematic structures of non-ionic surfactant vesicle.
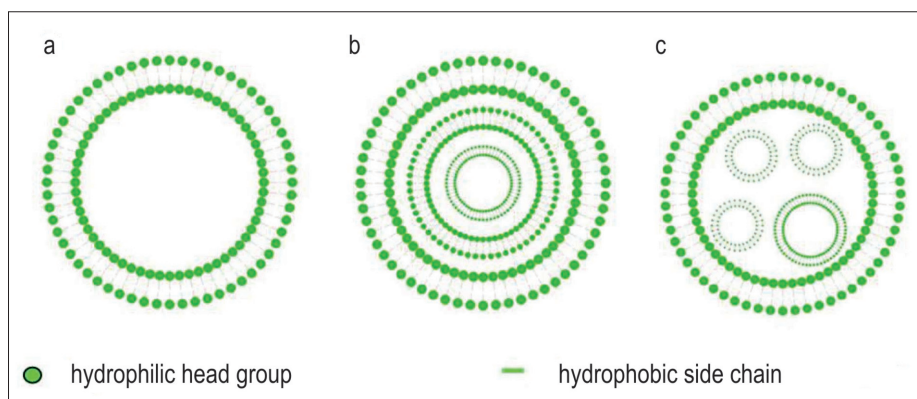
(a) unilamellar vesicle, (b,c). multi-lamellar vesicle [18].

best liposome design and reagent quality may be decisive for the testing phases for drug approval.

Liposomes Drug Delivery in Cancer

FDA approvals 16 news therapies for cancer with new pharmaceuticals, including new molecular entities (NMEs) and new Biologic License Applications (BLAs) between 2013-2016. The pipeline of 836 drugs for cancer using liposomes in 2016, 123 were for lung cancer, 106 for leukemia, 92 for lymphoma (including non-Hodgkin lymphoma), 82 for breast cancer, 58 for brain tumors, 53 for skin cancer (including melanoma) [23].

According to Institute for Healthcare Informatics (IMS) on Global Use of Drugs [24], many potential applications of liposomes in immunotherapies and targeted therapies will be a promising field in oncology and drug delivery in 2020 [25].

Regarding clinically used liposome-based products in cancer chemotherapy active agent indicated for the treatment of different types of cancer are Doxorubicin (DOX) (Doxil®/Myocet®), Daunorubicin (DAU) (DaunoXome®), Cytarabine/Ara-C (Depocyt®), Mifamurtide (Mepact®), Vincristine (Marqibo®), Irinotecan (Onivyde™). Also, the pharmaceutical industry is testing for some new active substance (NAS) Tecemotide, T4 endonuclease V, Cisplatin, Lurtotecan, Paclitaxel, all-trans retinoic acid for treating different types of cancer [26]. For example, Idarubicin (IDA), a drug exclusively used for the treatment of leukemia through thermosensitive liposomes (TSL) in combination with hyperthermia (HT) demonstrated to be more prominent tumor growth inhibition in leukemia than in the conventional form [27].

Some of these drugs have already in Phase II in Japan, as Stimuvax® (Tecemotide/L-BPL

Table 1. Effect of the nature of the drug on the formation of niosomes.

Nature of drug	Leakage from the vesicle	Stability	Oher properties
Hydrophobic drug	Decreased	Increased	Improved transdermal delivery
Hydrophilic drug	Increased	Decreased	-
Amphiphilic drug	Decreased	-	Increased encapsulation, altered electrophoretic mobility
Macromolecule	Decreased	Increased	-

Modified from KM Kazi et al. (2010) [22].

25) [28]: a liposome vaccine against the cancer cells that overexpress Mucin 1 (MUC-1) (Figure 3), a glycoprotein antigen expressed in cancers such as multiple myeloma and colorectal, breast cancer, prostate and ovarian cancers. However, the results of Stimuvax® were disappointing to the investigational therapeutic vaccine to enter Phase III (PhIII) studies in Non-Small Cell Lung Cancer (NSCLC), mainly due to flops in Phase III lung cancer trial and breast tumors [28-30]. Even so, the pharmaceutical company, Merck, will be responsible for the clinical development, commercial producing and marketing, estimated the sales to reach about €350m in 2019 [28].

Pfizer developed and produced the drug Xalkori® (Crizotinib) [31] for the treatment for Non-Small Cell Lung Cancer (NSCLC) and metastatic anaplastic lymphoma kinase (ALK) approved by FDA in August 2011 using liposomes [32]. Crizotinib is a receptor tyrosine kinase inhibitor that inhibits ALK and c-MET, and it is also been using in a liposome as a target-antitumoral drug [33,34].

Darolutamide by Bayer and Orion [33] have reported positive results in Phase III clinical trial (ARAMIS) in patients with non-metastatic castration-resistant prostate cancer using liposomes [35].

Avapritinib, an inhibitor of KIT and PDGFRα D842V mutant kinases, is under clinical development in China at present (2019) for GI Stromal tumors [33,36].

Analysis of Liposome Drug Delivery in Anticancer Therapy

We performed a database of chemical molecules, substances, compounds, bioassay, drugs and drugs for therapeutic or clinical medicine in liposomes using (PubChem) (National Institutes of Health - NIH), DailyMed [37], DrugBank [38,39], Drug@FDA [40], Pubmed (Medline), Cochrane Foundation, Isi Web of Science. Our focus was to find molecular similarities and correspondence for both liposome drug and drug delivery molecule for anticancer therapies, both approved and already in use, as well as those that are released by the FDA for in phases 3,2,1 or investigative research.

Fifteen molecules were found in the pharmaceutical form of liposome for oncological clinic. Of these, 13 are classified by size into small-molecules and were analyzed by computational statistical modeling. Of these only four were approved for use by the FDA, and nine are in phases of research by the pharmaceutical industry as liposomal formulations (Table 2). Tecemotide (Stimuvax®), Vincristine (Marquibo®), and

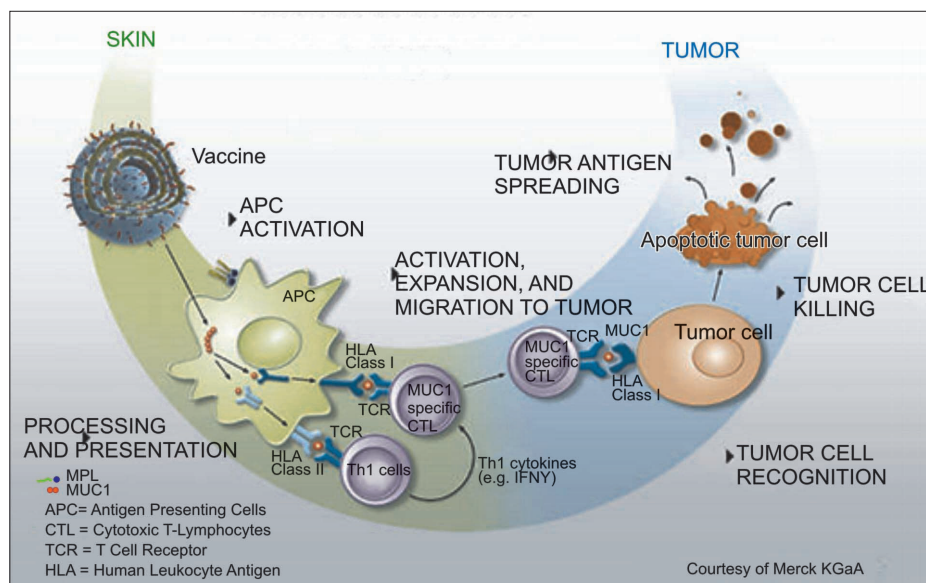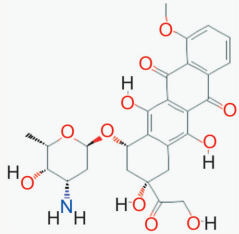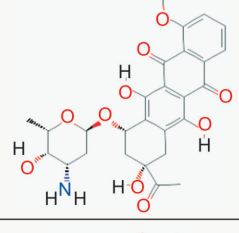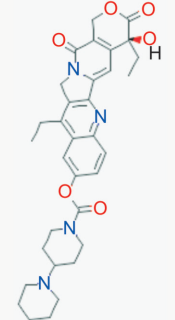**Figure 3.** Proposed mode of action of L-BPL 25.
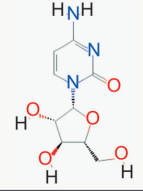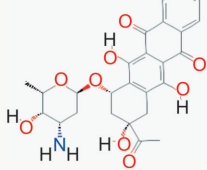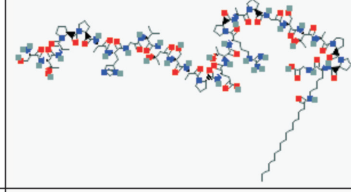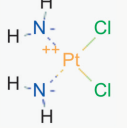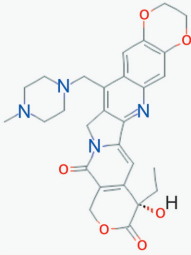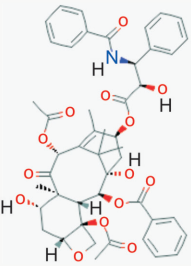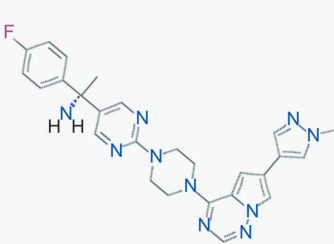
Table 2. Therapy and anticancer LDD approved by FDA and in investigative research.

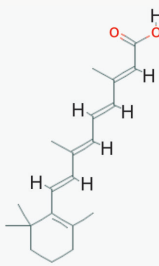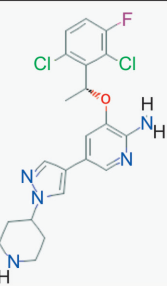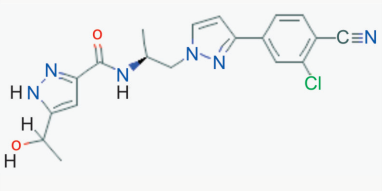| Active Agent	Formula	Molecular Weight g/mol	Structure Molecular	Treatment	FDA
Doxorubicin 31703	$C_{27}H_{29}NO_{11}$	543.520		Several solid tumors	Prescription
Daunorubicin 30323	$C_{27}H_{29}NO_{10}$	527.520		Leukemia	Discontinued
Irinotecan 60838	$C_{33}H_{38}N_4O_6$	586.678		Colorectal cancer	Prescription
Cytarabine 6253	$C_9H_{13}N_3O_5$	243.2166		Acute non-lymphocytic leukemia, acute lymphocytic leukemia and blast phase of chronic myelocytic leukemia	Discontinued
Idarubicin 42890	$C_{26}H_{27}NO_9$	497.5		Acute myeloid leukemia (AML) in adults	Investigation
Tecemotide 16208013	$C_{124}H_{203}N_{33}O_{38}$	2764.181		Prostate câncer, rectal carcinoma	Investigation
Cisplatin 57021978	$Cl_2H_4N_2Pt$	298.035		Treat various types of cancers, including sarcomas, e.g. small cell lung cancer, and ovarian cancer, lymphomas and germ cell tumors Phase 01	Investigation

The pink's lines is active agent approved FDA and used liposome-based products for treatment various types of cancer.

(continued Table 2)

Active Agent	Formula	Molecular Weight g/mol	Structure Molecular	Treatment	FDA
Lurtotecan 60956	$C_{28}H_{30}N_{406}$	518.57		Antineoplastic activity	Investigation / BioAssay
Paclitaxel 36314	$C_{47}H_{51}NO_{14}$	853.918		Carcinoma of the ovary, and other various cancers including breast cancer	Investigation for liposome form
All-trans Acid Retinoic 444795	$C_{20}H_{28}O_2$	300.442		Acute promyelocytic leukemia (APL)	Investigation
Crizotinib 11626560	$C_{21}H_{22}Cl_2FN_{50}$	450.339		Non-small cell lung cancer	Prescribed
Darolutamide 67171867	$C_{19}H_{19}ClN_6O_2$	398.851		Prostate câncer	Descontinued
Avapritinib 118023034	$C_{26}H_{27}FN_{10}$	498.57			Investigation

Mifamurtide (Mepact®) were not evaluated in this analysis.

We use ChemMine® for small molecules data analysis relevant in chemical biology, chemical genomics, and drug discovery [41]. ChemMine® proved to be an excellent software to study similarity, clustering, prediction of a small molecule, integrating the physicochemical and bioactivity properties through PubMed [42].

Few similarities are found between the liposome-based products in Cancer Chemotherapy approved (Graphic 1). So, the diversity physicochemical characteristics of a drug molecule in terms of therapeutic class and chemical structures, involving different functional groups are factors considered essential to meet FDA recommendations about liposomes as a tool with anticancer drugs.

The graphics multidimensional scaling (MDS) is an array of item distances. The coordinates are assigned to each item in a low-dimensional space to represent the distances graphically in a scatter plot.

The distance matrices required for the clustering of MDS were calculated by comparisons of all against all compounds using measures of similarity of atom pairs, the similarity of substructures and transforming similarity scores generated in distance values. According to the model proposed by ChemMine®, basic descriptor type that is defined by the shortest paths among the non-hydrogen atoms in a molecule are atom pair. The TC calculated the similarity and the distance values were calculated by subtraction (1 - Tc). Then, the coordinates are assigned to each item in a low-dimensional space to plot the distances in a scatter plot. We determined Tc = 0.7. The coefficient varies between 0-1, the higher, the greater, and the greater the similarity. The axes V1 and V2 represent the chemical space in two directions. A higher correlation between the structures contained in the pink band on Graphic 2.

HC results were developed based on the molecular weight and physicochemical properties heat map Joe Lib descriptors. The JOELib tool computes 38 descriptors for each compound. The result showed the most similar of all molecular, almost identical is cluster hierarchical formed by DOX/ DAU and IDA. IDA is closer in physicochemical properties of DAU than dox. DAU has been discontinued for economic issues unrelated to the product's quality, safety or efficacy.

Boron (B), Bromine (B), Iodine (I), Phosphorus (P), Sulfur (S), SO2, -SO2, -OSO, -NO2, and hydrophilic groups are not present in all active agents.

The molecular weight, an import characteristic for liposome development varies in green range by almost all molecules, except Cytarabine, All-trans- Acid retinoic, Darulutamide and Paclitaxel.

Several halogen atoms are down (blue for green), but more in the cluster formed by Crizotinib, Daurulutamide, and Avapritinib.

The number of primary groups and acid groups between the active agents, Lurtotecan showed the most significant amount of basics groups. However, with the predominance of acid groups, eight of the fifteen compounds analyzed contained more acid groups, highlighting all trans-acid-retinoic.

Lurtotecan and Irinotecan were outside the correlation range due to out of the correlation range due to the presence of a primary group in the first and greatest geometrical diameter in second. On the other hand, Cytarabinestands out from all other compounds by having lower: molecular weight, P-hydroxybenzaldehyde, heavy bonds, number of atoms, number of bonds, geometrical diameter, and other descriptors.
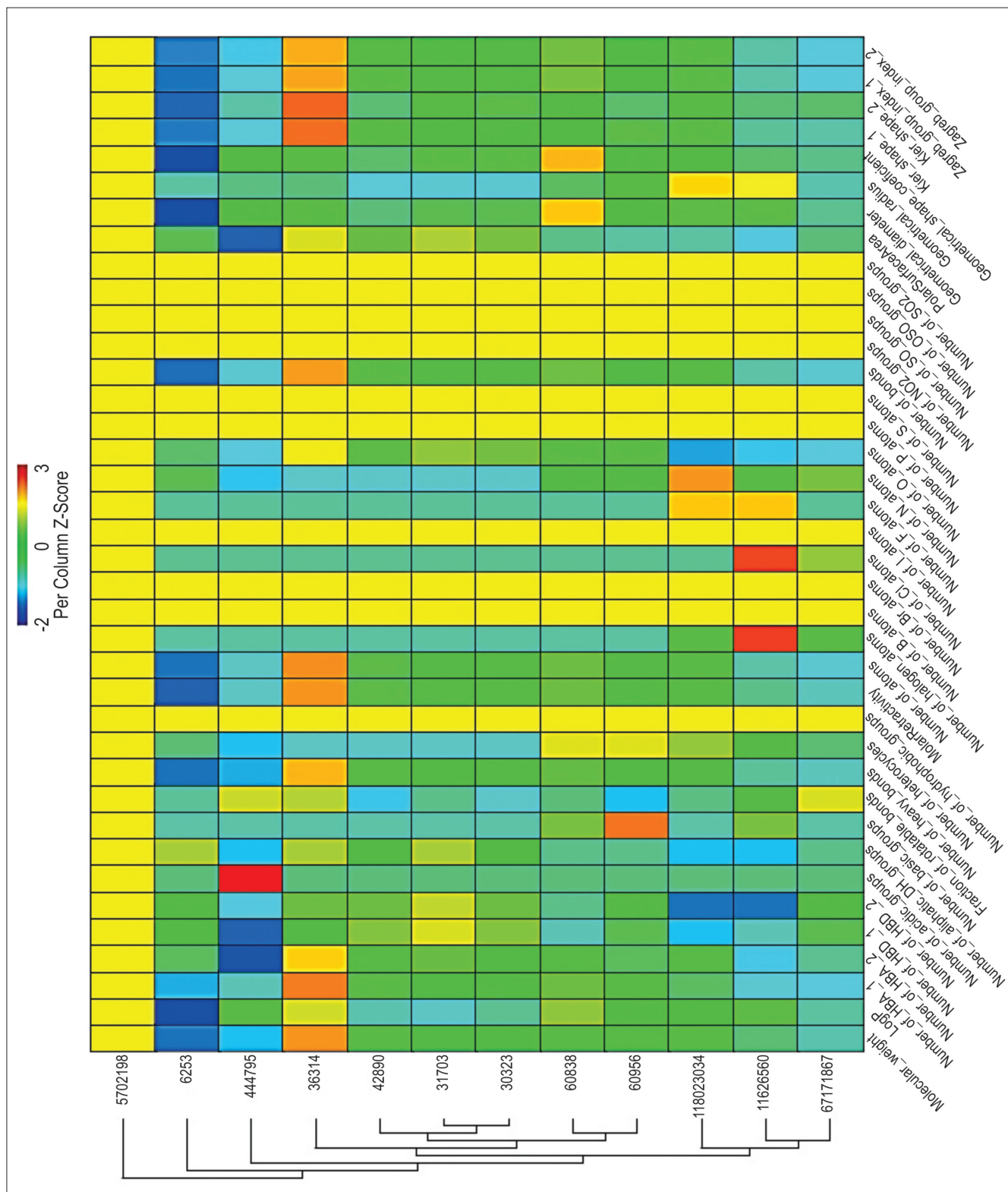
Conclusion

The use of liposome as anticancer therapy is restricted. However, liposomes are an essential research niche for NAS prospecting

Graphic 1. Multidimensional Scaling Clustering (MDS) of the NAS liposome in clinical oncology.
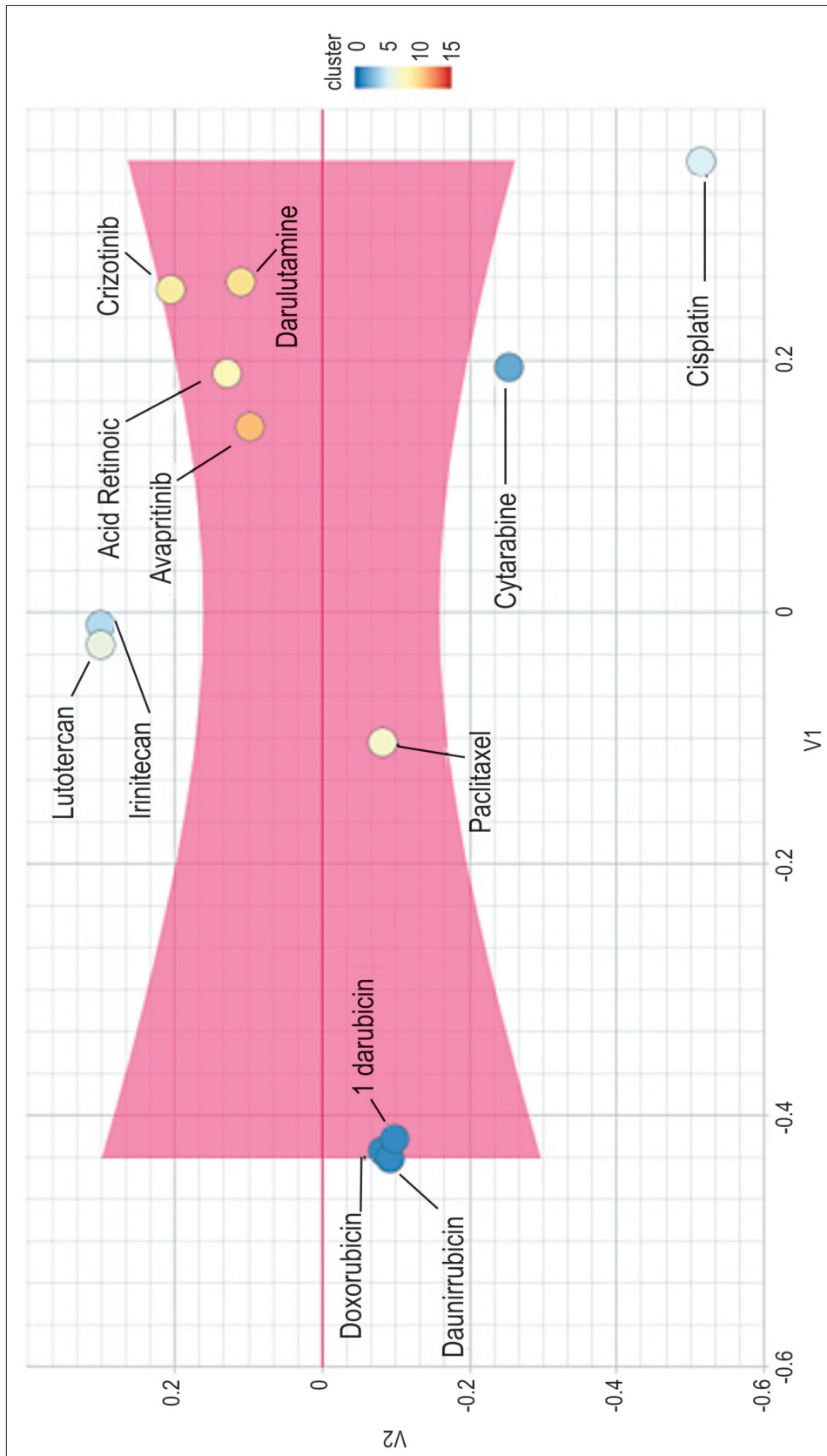
Graphic 2. Hierarchical Clustering Results of the NAS liposome in clinical oncology.

in which the evaluation of the physical and chemical characteristics of the pharmaceutical by computational methods can provide valuable information for the development of LDD in antineoplastic therapy. Nevertheless, it is essential to make a point of time-based understanding of physicochemical and bioactivity properties may be useful in finding methods of predicting more assertive experimental bed paths.

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