doi.org/10.36721/PJPS.2025.38.5.REG.13432.1

Cytotoxicity of bovine serum albumin nanoparticles loaded with vincristine sulfate for intravenous drug delivery: *In vitro* and *in vivo* studies

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Abstract: One common approach for treating cancer is chemotherapy. The extremely low distribution efficiency and lack of specificity of anticancer medications might occasionally limit systemic chemotherapy due to the lack of targeting and the existence of severe toxic side effects. Bioengineered from bovine serum albumin, the protein's viability was assessed by loading it into nanoparticles intended for intravenous delivery of vincristine sulfate for cancer treatment. Utilizing the desolvation process, cross-linked nanoparticles containing vincristine sulfate and made of albumin were created. This work examined the produced nanoparticles' *in vitro* and *in vivo* properties. After effective preparation, the bovine serum albumin nanoparticles loaded with vincristine sulfate had a spherical shape and a small particle size of 162.70 nm, as confirmed by a transmission electron microscope. In comparison to the standard vincristine solution, the nanoparticles showed sustained cytotoxicity on the MCF-7 breast cancer cell line and primary dermal fibroblast normal cell line (HDFn) cells. The nanoparticle-treated group did not exhibit appreciable histological alterations compared to the control group. In conclusion, albumin nanoparticles can enhance the distribution of vincristine sulfate to cancer cells, thereby increasing the drug's lethal effect. One promising therapeutic strategy for cancer treatment involves the development of injectable nano-based medications.

Keywords: Vincristine sulfate, bovine serum albumin, desolvation method, histopathological study

Submitted on 18-09-2024 – Revised on 20-11-2024– Accepted on 21-11-2024

INTRODUCTION

Cancer represents a group of disorders characterized by the uncontrollable growth and spread of abnormal cells, which, if unchecked, can be fatal (Phuah & Nagoor, 2014). It stands as one of the most common causes of death globally, with 19.3 million new cancer cases diagnosed annually, leading to nearly 10 million reported fatalities (Vazquez et al., 2023). Current cancer care primarily relies on cytotoxic chemotherapy, immunotherapy, targeted therapy, endocrine therapy, radiation therapy, and surgery. Despite significant progress in cancer treatment over recent decades, challenges such as relapses, resistance to traditional chemotherapies and novel targeted drugs persist as major obstacles (Wang *et al*., 2019).

Systemic toxicity from anticancer medications gives rise to side effects like nausea, vomiting, fatigue and hair loss, impacting both normal tissues and tumor sites (Hashim & Rajab, 2021). Systemic drugs, when administered, not only target malignant cells but also inadvertently kill adjacent healthy cells (Bhattacharya, 2022). Vincristine sulfate (VCS), a potent anticancer agent belonging to the vinca alkaloids class, operates by inhibiting cancer cell growth, thereby impeding cancer progression and metastasis. Marketed under the brand name Oncovin, VCS is commonly used in conjunction with other drugs to treat various cancers, including brain, lung and Hodgkin's disease (Hasan & Ghareeb, 2024; Al-Musawi *et al.*, 2021).

Extended exposure to VCS enhances its in vitro cytotoxicity in cells due to the cytotoxic effects being exerted after many cells have undergone mitosis. Chemotherapy resistance in VCS treatment arises through multiple mechanisms, including drug inactivation, multi-drug resistance, apoptosis suppression, drug metabolism alterations, epigenetic changes, enhanced DNA repair, and gene amplification. Overexpression of P-glycoprotein (P-gp) remains a significant hurdle in effective VCS cancer therapy (Silverman *et al.*, 2013; Mansoori *et al.*, 2017). Due to its robust P-gp-mediated efflux, resulting in rapid clearance and limited absorption, oral administration of VCS is uncommon (Ling *et al.*, 2010).

Various types of nanoparticles serve as carriers for cancer therapies, including lipid-based nanoparticles such as liposomes, solid lipids, and nanostructured lipid carriers, as well as lipid-polymer hybrids and polymeric micelles like cationic, unimolecular, dual-responsive, and triple-responsive micelles. Additionally, metal nanoparticles, dendrimers, polymer-based nanoparticles (e.g., poly lactide co glycolide, polycaprolactone, polylactide,

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chitosan), protein-based nanoparticles (e.g., albumin, gelatin nanoparticles) and polymer-based nanoparticles are utilized (Kudarha & Sawant, 2017).

Protein nanoparticles are a highly advantageous drug delivery technology due to their biodegradability, stability, surface modification capabilities, ease of size control, and lack of toxicity-related issues like immunogenicity (Hong *et al.*, 2020). Over the past two decades, albumin nanoparticles have primarily been employed for targeted therapy and drug delivery. Circulating albumin nanoparticles can accumulate subtly in tumor tissue through enhanced penetration and retention (EPR). Active targeting of nanoparticles enhances the therapeutic impact of drugs, with albumin being favored as a drug delivery vehicle due to its availability, biodegradability, non-toxic nature, immunogenicity and improved accumulation in both tumors and inflammatory tissues (Qu *et al.*, 2024).

Bovine serum albumin (BSA) and human serum albumin (HSA), due to their similar structures, are frequently used as model proteins (Jahanban-Esfahlan *et al.*, 2019). BSA is chosen as the protein model for its medical significance, abundance, affordability, ease of purification, and stability. BSA and HSA are homologous proteins (Hu *et al.*, 2006).

The current study aims to develop and characterize site-specific nanoparticles based on albumin for delivering vincristine sulfate in cancer treatment, while also evaluating the cytotoxicity of these nanoparticles in vitro and in vivo.

MATERIALS AND METHODS

Materials

Vincristine sulfate, bovine serum albumin, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) (EDC) were purchased from Hangzhou Hyper Chemicals Limited/ China. Acetone for HPLC-Isocratic grade was obtained from Carlo Erba. Deionized water was provided by Rafidain Environment Company, Iraq. Modified Eagle Medium (DMEM) was supplied by Giboco/ USA and MTT (3-(4, 5-Dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide) from Promega/ USA.

Animals

The inclusion criteria for the animal study involved the selection of nine Swiss albino (BALB/c) male rats sourced from the Animal House in the Cancer Research Center at Al Mustansiriah University, Baghdad, Iraq. These rats, aged 8 weeks and weighing an average of 300 grams, were specifically chosen for histopathological studies. In selecting the rats for the study, exclusion criteria were applied to ensure the integrity of the research. Rats with pre-existing health conditions, outside the specified age range or significantly deviating from the average weight of 300 grams were excluded. They were housed in polycarbonate cages under controlled environmental conditions, maintaining a temperature of 24±1 °C, humidity levels between 35-50% and a 12-hour light/dark cycle. The rats had ad libitum access to water and food, with a standard protocol of food removal from cages 2 hours prior to each treatment. Approval for all animal experiments was obtained from the ethics committee of the University of Baghdad (permission number: RECAUBCP282023M) and the study was conducted in full compliance with the ethical guidelines set forth by the World Medical Association, as outlined in the Declaration of Helsinki.

Preparation of VCS loaded BSA nanoparticles (BSA NPs)

Acetone was used as a desolvating agent, and EDC was used as a across-linker in the desolvation procedure to create BSA NPs. Using a hotplate magnetic stirrer (Joanlab/ China), 5 ml of 20 mg/ml of BSA in an aqueous solution and 5 ml of 1 mg/ml of VCS were continuously stirred at 1250 rpm for one hr. at room temperature to create nanoparticles. With a steady flow rate of 1 ml/min, approximately 5.5 ml of acetone were added to this solution drop-wise using a syringe pump (Beijing KellyMed Co. Ltd/China). 240µl of 0.1% EDC in deionized water was added to the solution for cross-linking once it turned turbid. For three hours, the mixture was stirred at 1250 rpm. This volume of cross linker was calculated depending on equimolar mass ratio of both albumin and EDC, the molecular weight of BSA was 66430 g/mole, while the molecular weight of EDC was 15524 g/mole.

Next, a cooling centrifuge (Harmle Labortechnik/ Germany) and 3 cycles of centrifugations at 15,000 rpm for 15 min were used to remove the unbound VCS, free albumin, excess cross-linker, and acetone. Next, using an ultrasonic cleaner (Fuyang Technology China), the pellets were re-disseminated in 10 ml of water to their original volume and sonicated for 10 min (Prajapati *et al.*, 2021). Using a Zetasizer (Malvern, UK) (Naji & Al Gawhari, 2024), the prepared NPs were examined for particle size, particle size distribution, and zeta potential. Additionally, the entrapment efficiency (%EE) of VCS (Mohammad *et al.*, 2022), and morphological analysis were conducted using a transmission electron microscope (TEM) (Zeiss Libra 120 PLUS, Carl Zeiss GmbH, Germany) (Albassam & Kassab, 2023).

In vitro cytotoxicity assay

In vitro cytotoxicity trials were measured by means of MTT reduction. MCF-7 and HDFn cells were seeded in 96- well plates for up to 24 h at 37°C using incubator, memmert/ Germany. The cells were sub-cultured in media (DMEM) comprising VCS, BSA NPs and VCS-loaded

BSA NPs at various concentrations of VCS (25, 50, 100, 200 and 400µg/ml) under sterile conditions using laminar flow hood (Labconco/ USA). After 48 h, the DMEM medium was changed with a fresh medium loaded with 10% MTT (5 mg/ml) then, the plates were incubated for additional 4 h at 37°C for the cytotoxicity study. Then, the medium was removed with the addition of 100 µl DMSO to every well. Absorbance at 560 nm was measured using multimode detector (Glomax/ USA) and cell viability was computed using the following equation (Shihab & Mohammed, 2019; Kadhim & Rajab, 2024):

$$\text{The cell viability}\,(\%) = \frac{\text{ABS(Treated cells)}}{\text{ABS(Control cells)}} \times 100 \quad \ldots\ldots\text{Q (2)}$$

The IC_{50} was computed using GraphPad Prism 10 Software from La Jolla, CA, USA.

In vivo biosafety study

Determination and preparation of VCS doses

Adults are typically administered 1.4 mg/m2 of VCS weekly by intravenous injection (Silverman & Deitcher, 2013). For 1.73m^2 which is normal body surface area for 70kg adult, the dose equals to 2.42mg. The VCS dose in rats was calculated based on its recommended dose in humans (2.42 mg per week; or 34µg/kg). It was determined using the following equation:

HED (mg/kg) × Km ratio (H/A) = AED (mg/kg) … Q (1)

Where:

Km is a correction factor calculated by dividing the mean body weight (kg) of human by its body surface area (m^2). The values of human Km and animal Km are equal to 37 and 6, respectively. The Km ratio is expressed as the ratio between human and rat Km factor (37/6 = 6.2) (Fareed & Kassab, 2023).

$AED_{\mu g/kg}$ = 34µg/kg × 6.2 = 210 µg/kg

Histopathology

To study the in vivo biosafety of VCS-loaded BSA NPs, the histomorphology of tissues from different organs (brain, lung, liver and kidney) of animal models (rats) was examined (Balli *et al.*, 2019).

Nine Swiss albino male BALB/c rats were used for histopathology studies. Animals were randomly assigned into three groups to receive the treatment as follows: three rats were given phosphate buffer as a control, second group received blank NPs (without VCS) and the last group received VCS-loaded BSA NPs. The weights of the animals were recorded throughout the entire experiment to monitor any obvious signs of suffering. The animals were exposed to the treatments through direct intravenous route (fig. 1).

The animals were anaesthetized intraperitoneally with a mixture of xylazine (10 mg/kg) (Laboratories Caller, Barcelona, Spain) and ketamine (80 mg/kg) (Hameln Pharmaceutics, Germany) before the medications were instilled in all groups (Fareed & Kassab, 2023). The animals in the control group were given intravenous access to 250µL of phosphate buffer. Animals in the other groups received 250µL of the diluted formulations containing the prescribed dosages of the medication using the same procedure.

One animal per group was put to death by cervical dislocation after 14 days of instillation. After that, the organs were removed and immediately preserved in 10% neutral buffered formalin. The organ tissue was then placed in paraffin for histological analysis. Five-micrometre tissue slices were attached to glass slides and stained with hematoxylin and eosin (H and E) per standard procedures to observe their morphology. With the Nikon Eclipse 90i microscope, images of every section were taken. A certified veterinary pathologist examined the slides (Elbardisy *et al.*, 2022).

Fig. 1: Intravenous administration of phosphate buffer, BSA NPs and VCS loaded BSA NPs to rats

In the histopathology study assessing the in vivo biosafety of VCS-loaded BSA NPs in rats, a scoring system was employed to evaluate tissue samples from various organs post-treatment. The scoring criteria encompassed assessments of tissue morphology, inflammation, necrosis, and fibrosis. Each criterion was assigned a score ranging from 0 to 3, with higher scores indicating more severe histopathological changes. The total score for each animal was calculated by summing the scores across the criteria, allowing for a comprehensive evaluation of the tissue responses to the treatments. Validation measures included ensuring inter-observer agreement and conducting comparative analyses among the control, blank NPs, and VCS-loaded BSA NPs groups to discern the effects of the treatments on the tissues. This systematic scoring system provided a standardized

approach to quantifying and grading histopathological alterations, aiding in the assessment of the nanoparticle formulations' biosafety profile in the experimental setting.

STATISTICAL ANALYSIS

The SPSS® IBM® 29.0 versions were used for the statistical analysis. The data was expressed as mean ± (SD). ANOVA, or one-way analysis of variance, was used to examine the variations between the repeated measures. Differences that were deemed statistically significant were defined as ($p<0.05$) (Malik & Al-Khedairy, 2023).

RESULTS

Table 1 and (fig. 2) illustrates the characteristics of the prepared VCS-loaded BSA NPs. The particle size was 162.70 nm with nearly homogeneous and uniform particle distribution in an aqueous medium without any aggregation (Poly dispersity index equal to 0.15 and zeta potential equal to -21.97). The EE% of VCS in NPs was nearly 58% (therefore; each one ml of formulation contains approximately 0.289 mg of VCS.

The MTT assay was utilized to examine and compare the in vitro cytotoxicity of VCS-loaded BSA NPs with that of unloaded blank NPs and free VCS using the primary dermal fibroblast normal cell line (HDFn) and breast cancer cell line MCF-7. As demonstrated in fig. 3, at all concentrations used (25-400 µg/ml), VCS-loaded NPs significantly ($p<0.05$) outperform pure VCS and blank NPs in terms of cytotoxicity against HDFn and MCF-7 cells 48 hours after exposure. The IC_{50} (inhibitory concentration of 50%) of VCS-loaded BSA NPs was (215.5 and 16.77 µg/ml) which is significantly lower than that of the free VCS (313.5 and 148 µg/ml) against HDFn and MCF-7 cells respectively. These results are similar to that in the literature. As demonstrated in fig. 4, non-significant ($p>0.05$) cytotoxic activity was seen for the drug-free NPs at different concentrations compared to others, suggesting that the synthetic blank NPs are harmless in cell culture, this means that the BSA NPs are safe and suitable as a drug carrier for therapeutic uses (Zhao *et al.*, 2014).

To examine the histological alterations in the different organs, sections obtained from rat's organs following various intravenous administrations were subjected to H and E staining. The histopathological changes of organs after intravenous administrations of the control, blank NPs, and VCS loaded BSA NPs treatments were showed in the next figs. in details. The histopathological figs. of liver, kidney, brain and lung of control group were illustrated in figs. 5, 6, 7, and 8 respectively. The results showed no animal died in 14 days of treatment for all groups. H and E staining indicated that VCS loaded BSA NPs treatment did not induce any significant histological change in the rat organs after 14 days post administration as compared with blank NPs and phosphate buffer (control) treatments.

DISCUSSION

The assessment of the physicochemical properties of (NPs) exhibited spherical morphology when observed under TEM with constant shape, this observation was consistent with previous work mentioned in literature (Shukla *et al.,* 2024). Besides a small range of particle sizes (162.70 nm), a high degree of homogeneity (PDI = 0.15), and an EE% of almost 58%. The zeta potential was nearly -22 mV which approximately same as stated in literature (Danafar Hossein *et al.,* 2023). The MTT experiment in (HDFn) and MCF-7 cell cultures demonstrated the long-lasting lethal effects of BSA NPs loaded with VCS. This implies that the nanocarrier can transport VCS in a controlled way, resulting in maintained drug concentrations. This observation suggests that the cells have the potential to break down the NPs, so enabling the liberated VCS to diminish in cell viability.

VCS loaded albumin NPs can enter cancer cells by endocytosis and avoid the efflux pumps that cause VCS therapeutic resistance, which is one reason for their superiority over free VCS which enters by passive diffusion (Elbatanony *et al.*, 2021).

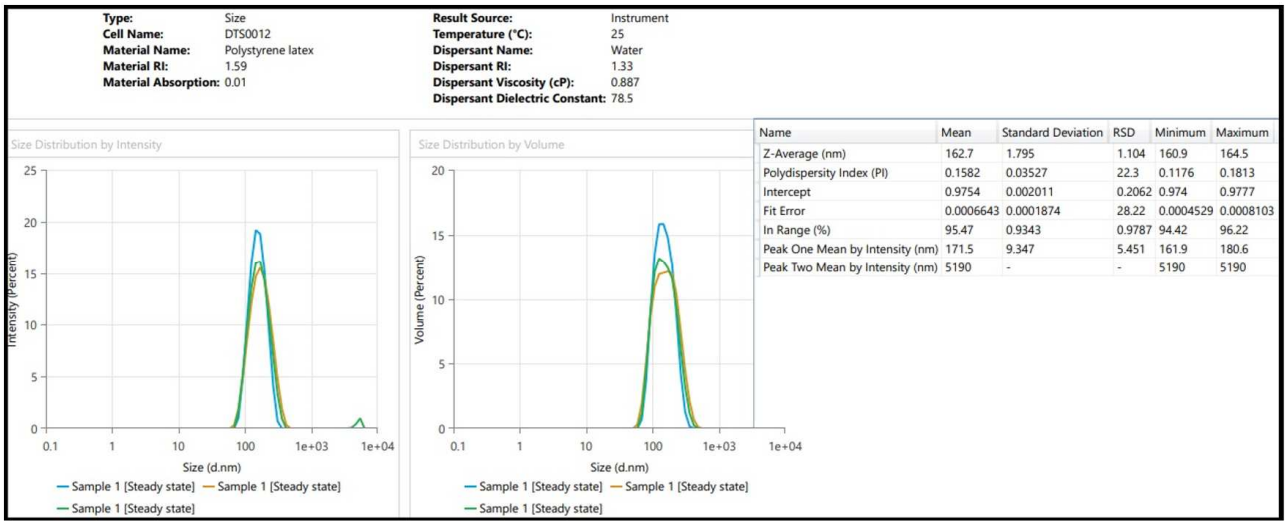
The non-significant ($p>0.05$) cytotoxic activity in primary dermal fibroblast normal cell lines and breast cancer cell lines was seen for the VCS-free NPs at different concentrations compared to others, suggesting that the synthetic blank NPs are harmless in cell culture and proved nanoparticle formulations' safety.

To combat multidrug resistance in cancer, Wang *et al*. (2014) developed multifunctional NPs and assessed their lethal effects on MCF-7 and MCF-7/Adr cells. These nanocarriers were then used to transport vincristine sulfate. At various drug doses ascertained by the MTT technique, the study demonstrated a significant increase in cytotoxicity compared to the free VCS (Wang *et al.*, 2014). Drug-loaded albumin NPs were found to have a potent anticancer effect due to increased intratumoral accumulation caused by enhanced permeability and retention (EPR)-mediated uptake of albumin-based NPs in solid tumors. The literature stated that NPs with a size of 100-200 nm are thought to be optimal for achieving a maximized EPR effect (Leporatti, 2022). The size of NPs in this study was 167 nm.

The selective accumulation of a certain size of molecules was due to the highly permeable vasculature present in solid tumors.

Table 1: Parameters of the prepared VCS-loaded BSA NPs (mean± SD, n=3)

Particle size (nm)	PDI	Zeta potential (mV)	EE%
162.70 ± 1.795	0.1582 ± 0.03527	-21.97 ± 2.9560	57.88 ± 0.031



Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	162.7	1.795	1.104	160.9	164.5
Polydispersity Index (PI)	0.1582	0.03527	22.3	0.1176	0.1813
Intercept	0.9754	0.002011	0.2062	0.974	0.9777
Fit Error	0.0006643	0.0001874	28.22	0.0004529	0.0008103
In Range (%)	95.47	0.9343	0.9787	94.42	96.22
Peak One Mean by Intensity (nm)	171.5	9.347	5.451	161.9	180.6
Peak Two Mean by Intensity (nm)	5190	-	-	5190	5190

Fig. 2: The particle size distribution of selected VCS-loaded BSA NPs

The prepared NPs were spherical in shape as inferred from TEM image of the VCS loaded BSA NPs shown in fig. 3.

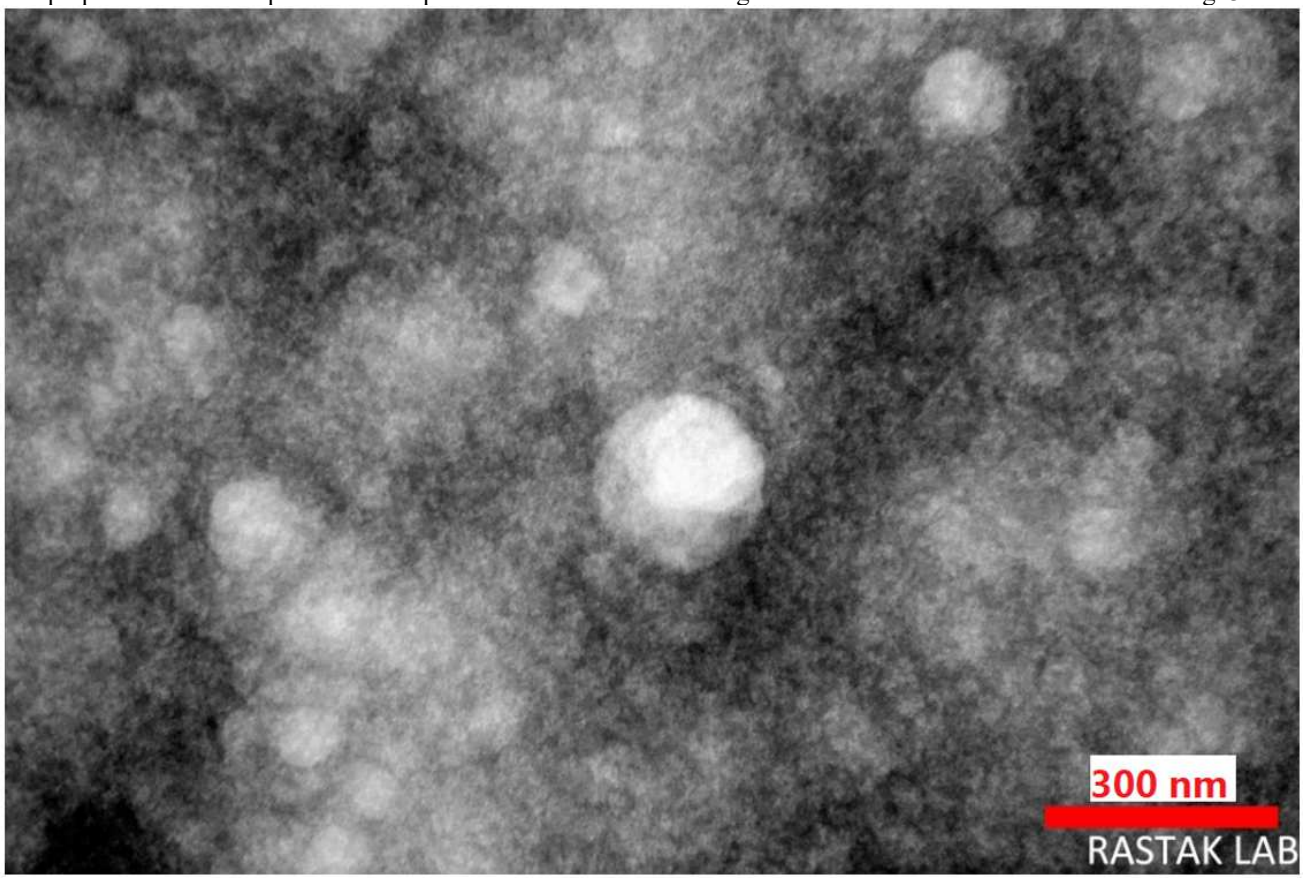


Fig. 3: TEM image of VCS loaded BSA NPs, the scale bar was 100 nm, stained with phospho-tungstic acid, dropped on a copper grid, dried at 60º C, the used voltage was 100 kv

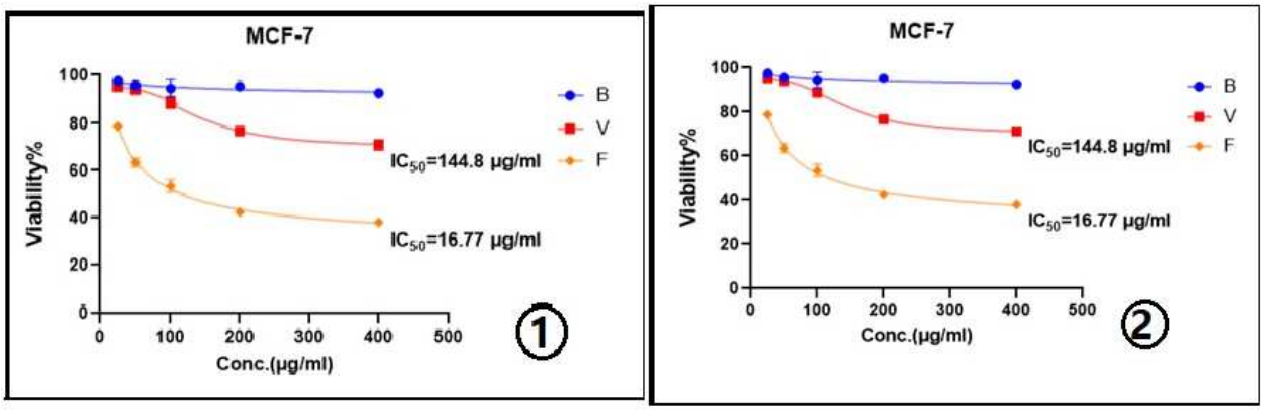


Fig. 4: Dose-response curve and half maximal inhibitory concentration (IC_{50}) values of B: BSA-NPs, V: pure VCS and F: VCS loaded BSA NPs on (1) primary dermal fibroblast normal cells (HDFn) and (2) human breast cancer cells (MCF-7)

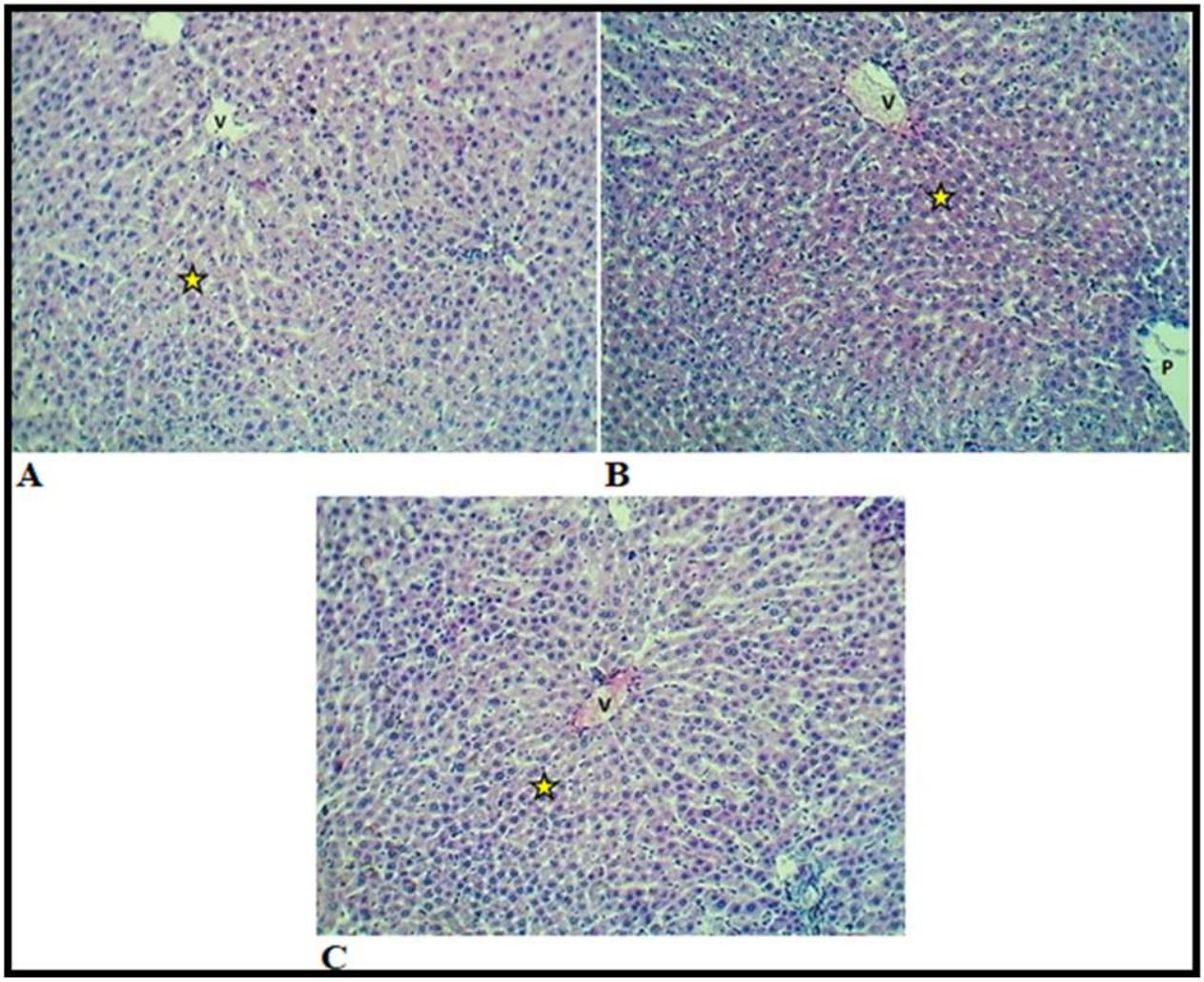


Fig. 5: Histopathological investigations of the liver (A): segment of the hepatic lobule (Control) displays a normally arranged hepatic cord (Asterisk), normal portal vein (P) and normal central vein (V). H&E is shown at a 10x magnification. (B): A portion of the hepatic lobule (Blank NPs group) displays a normal hepatic cord arrangement (Asterisk) and a normal central vein (V).H&E.100x. Section of the hepatic lobule (VCS loaded NPs) (C): H&E stain at 100x; the normal arrangement of hepatic cords (Asterisk) and central vein (V) is shown.

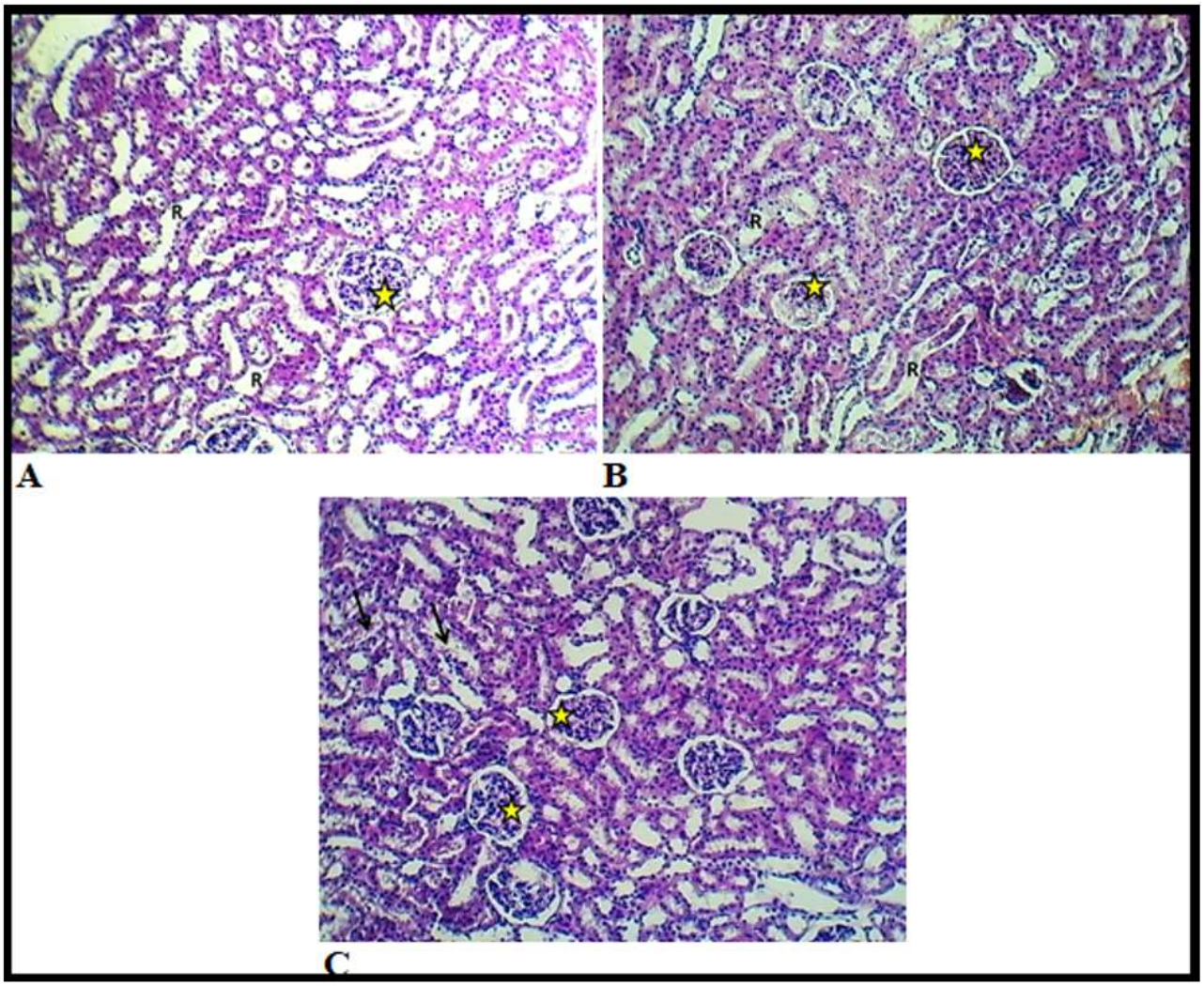


Fig. 6: Kidney Histopathological Alterations (A) An H&E-stained section of the renal cortex (Control) displays the usual appearance of the renal tubules (R) and glomeruli (Asterisks).100 times. (B) The H&E stain section of the renal cortex (Blank NPs group) displays the normal appearance of the renal tubules (R) and glomeruli (Asterisks). 100 times. (C) An H&E-stained section of the renal cortex (VCS loaded NPs) displays modest vascular degeneration of the lining cells of some renal tubules (Arrows) and a normal appearance of glomeruli (Asterisks). 100 times.

In addition, these molecules retained in the tumor due to the poor lymphatic drainage. The physiological basis of the EPR effect is caused by abnormal tumor vasculature. The vasculature in solid tumors is characterized as an unevenly distributed and highly disorganized vascular network, which is dilated, tortuous and saccular channels with haphazard patterns of interconnection and branching (Islam *et al.,* 2022).

The active targeting approach entails modifying the surface with a selected ligand that specifically attaches to cancer cells, therefore facilitating the efficient transportation of the nanocarriers. Cancerous cells exhibit over expression of several targets, while neighboring healthy cells display decreased expression of these targets. Ligands enhance the transportation of NPs by facilitating a specific contact with the surface of cells (Chen *et al.,* 2023).

The important consideration in the architecture of these NPs was the presence of protein albumin. The presence of albumin served as homing device to target specific cancer tissues that highly expresses SPARC and gp60 receptors, these receptors are abundantly expressed in cancer cells and exert substantial cellular uptake of the formulation containing protein via the caveolin-mediated transcytosis pathway (Meng *et al.,* 2024). Enhancing cellular accumulation of VCS was anticipated by exploiting of albumin's intrinsic active targeting modality through the interaction between gp-60 and SPARC.

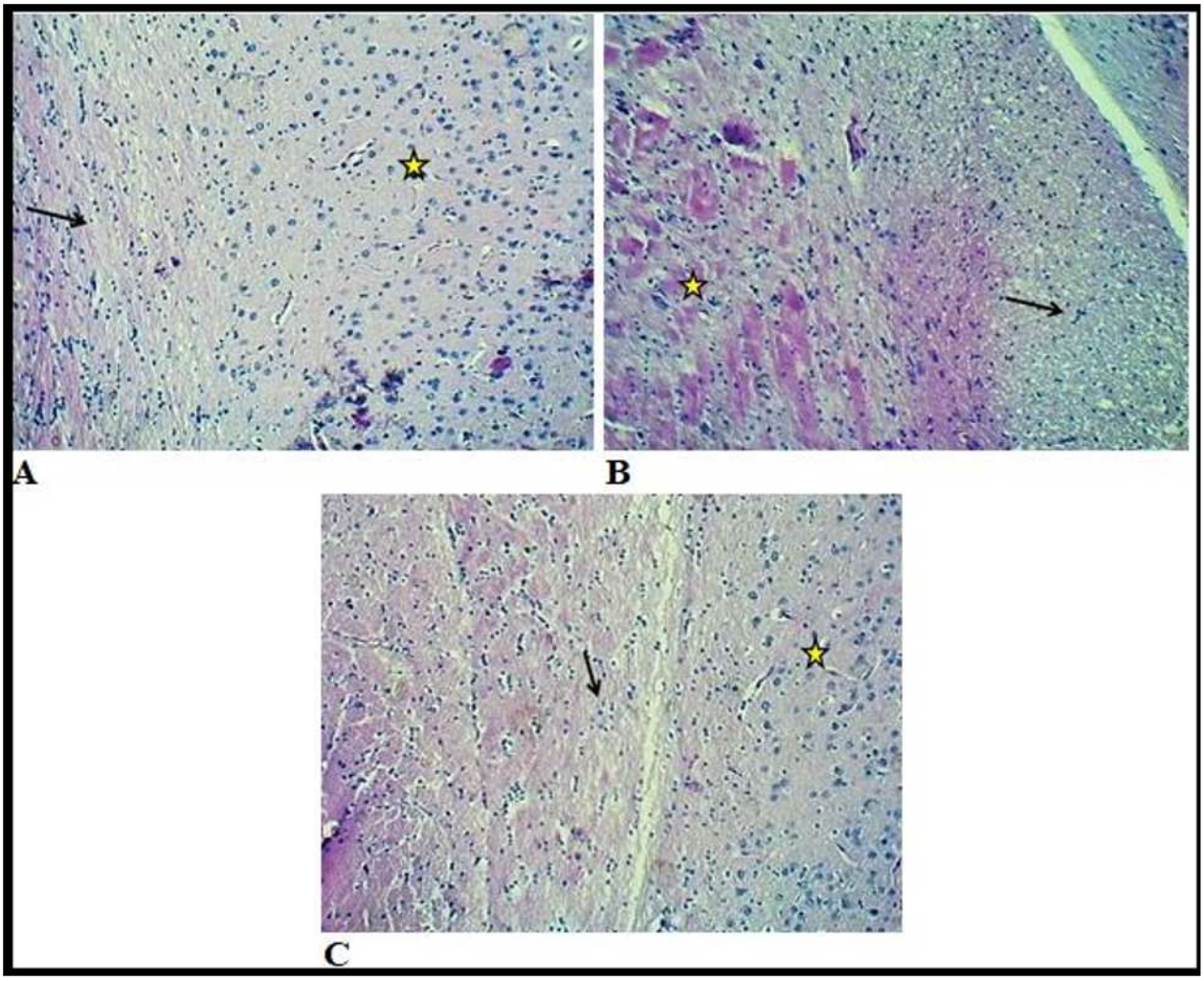


Fig. 7: Brain Histopathological Alterations (A) H&E stain at 100x magnified view of the cerebral cortex (Control) demonstrates the usual appearance of the multiform layer cells (arrow) and the midbrain tract (asterisk). (B) A section of the cerebral cortex (blank NPs group) displays the multiform layer (asterisk) and the midbrain tracts (Arrow), both of which appear normal at 100x magnification. (C) H&E stain at 100x reveals a section of the cerebral cortex (VCS loaded NPs group) with the multiform layer (asterisk) and midbrain tracts (Arrow) appearing normally.

The findings of this study could ultimately result in the creation of a new therapeutic option for different cancers which is both more efficient and less harmful compared to existing therapy choices. When albumin NPs loaded with VCS exhibit ideal characteristics, drug-loaded NPs will release their payload directly to cancer cells through both active and passive targeting as discussed previously. This allows the cancer treatment to be focused on specific regions, improve anticancer activity, lessen systemic side effects, conquer multi-drug resistance (MDR). Thus, adopting this approach might enhance the well-being of individuals with cancer, augment their chances of surviving, and extend their lifespan.

These results also show that the produced NPs were safe, essentially non-toxic, and highly compatible with living things because it was made from biodegradable and biocompatible natural macromolecular carrier. Furthermore, is consider as non-immunogenic and non-toxic carrier (Sristi *et al.,* 2023). Patients with cancer have lower serum albumin levels because their growing tumor mass needs to digest albumin to meet its amino acid needs (Elzoghby et al., 2015; Kratz, 2014).

While our study demonstrates the potential of BSA NPs loaded with Vincristine sulfate for drug delivery, several limitations should be considered like the method used to produce BSA NPs in this study may not be easily scalable for large-scale production. The techniques employed, such as solvent evaporation or high-pressure homogenization, might require specialized equipment and could be cost-prohibitive for widespread clinical application.Variability in Clinical Settings: The *in vitro* results observed in controlled laboratory conditions may not fully translate to clinical settings.

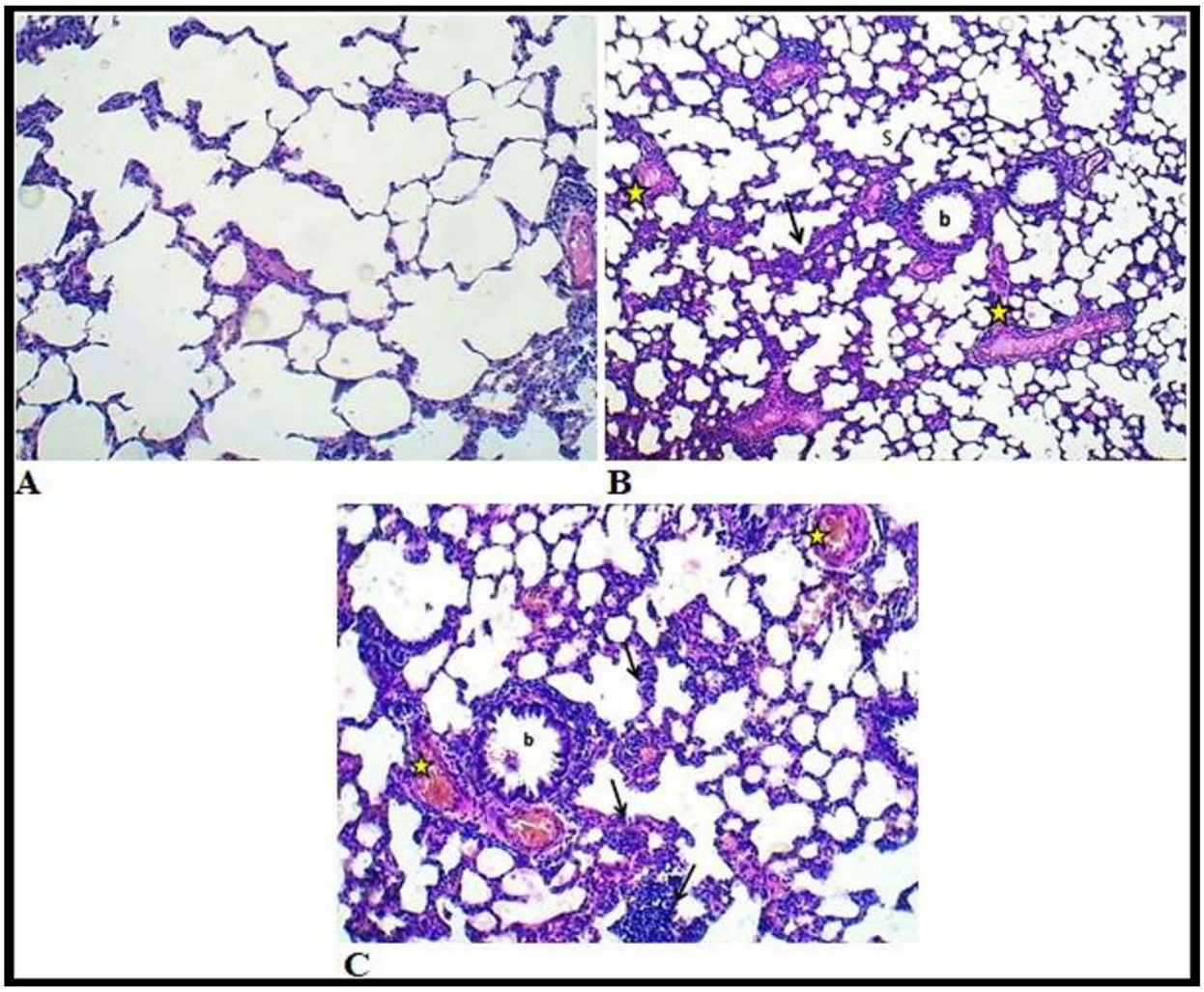


Fig. 8: Histopathological changes in Lungs (A) section of lung (Control) shows normal appearance of the alveoli and alveolar wall, H&E stain.100x. (B) Section of lung (Blank NPs group) shows: normal appearance of bronchial tree (b), alveolar sacs (S), and pulmonary vessels (asterisk) with mild thickening of alveolar wall (arrow), H&E stain. 100x. (C) Section of lung (VCS loaded NPs) shows: mild congestive interstitial pneumonia characterized by congestion of pulmonary arteries (asterisks), thickening of pulmonary interstitum associated with congestion and infiltration of MNCs (Arrows), H&E stain. 100x.

Factors such as patient-specific variables (e.g., differences in metabolism, immune response, and tumor microenvironment) can lead to variability in the effectiveness of the treatment when administered to patients. Additionally, the pharmacokinetics and biodistribution of the NPs may differ *in vivo* compared to our experimental models. Beside the i*n vivo* studies were conducted with a relatively small number of animal subjects.

This limited sample size may affect the statistical power of our findings and could lead to variability in the observed responses. Finally, the duration of our *in vivo* study may not be sufficient to fully assess the long-term effects and safety of the BSA NPs loaded with Vincristine sulfate. Future studies should include longer follow-up periods to evaluate potential delayed adverse effects.

CONCLUSION

The findings of our study on BSA NPs loaded with Vincristine sulfate reveal promising advancements in targeted drug delivery systems for cancer treatment. The demonstrated cytotoxicity of these NPs suggests their potential to enhance the therapeutic efficacy of Vincristine while minimizing systemic side effects commonly associated with conventional chemotherapy. This research lays the groundwork for further exploration into the use of BSA NPs as a versatile platform for delivering a variety of chemotherapeutic agents, potentially leading to improved patient outcomes in oncology.

Moreover, our *in vivo* result indicate that these NPs could be developed for clinical applications, paving the way for

future studies focused on optimizing formulation parameters and assessing long-term safety and efficacy in human trials. By addressing the challenges of drug solubility and targeted delivery, this work not only contributes to the field of nanomedicine but also opens new avenues for personalized cancer therapies tailored to individual patient profiles.

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