

In Vivo Antitumor Activity of Neratinib and Paclitaxel Loaded Cubosomes in an MCF-7 Breast Cancer Xenograft Model

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ABSTRACT

Background: Cubosomes are bicontinuous lipid nanocarriers capable of incorporating poorly water-soluble drugs and modifying their release. This study investigated the antitumor performance of neratinib-loaded cubosomes administered alone or together with separately prepared paclitaxel-loaded cubosomes in an MCF-7 breast cancer xenograft model. **Methods:** Fifteen female BALB/c nude mice bearing MCF-7 xenografts were allocated to five groups (n=3): saline control, neratinib suspension (10 mg/kg), paclitaxel suspension (5 mg/kg), neratinib loaded cubosomes (10 mg/kg), and co-administration of separately formulated neratinib and paclitaxel-loaded cubosomes (10 and 5 mg/kg). Five intraperitoneal administrations were given at three-day intervals during a 14-day observation period. Tumor volume and body weight were assessed serially. **Results:** Day-14 mean tumor volumes were 1021.79 ± 49.54 , 559.07 ± 32.24 , 462.57 ± 27.68 , 275.59 ± 20.48 and 127.20 ± 29.02 mm³, respectively. Relative to the control, end-point mean tumor volumes were lower by 45.29%, 54.73%, 73.03% and 87.55% in the treated groups. Body-weight changes were -2.58%, -3.26%, -8.18%, +0.83% and -0.40%, respectively. **Conclusion:** The co-administered cubosomal formulations were associated with the lowest mean end-point tumor volume. These findings support further investigation of combined cubosomal delivery; the design does not establish pharmacological synergy or systemic safety.

Keywords: cubosomes; neratinib; paclitaxel; breast cancer; MCF-7 xenograft; lipid nanoparticles; antitumor activity

1. INTRODUCTION

Breast cancer remains a major therapeutic challenge because tumor heterogeneity, resistance mechanisms and limitations of drug exposure can constrain treatment response. In addition to drug discovery, pharmaceuticals offers opportunities to improve the delivery of established anticancer agents through rational formulation design. Cubosomes are dispersed lipid nanoparticles derived from bicontinuous cubic liquid-crystalline phases. Their interconnected aqueous channels and lipid domains can accommodate drugs of differing physicochemical properties, while formulation composition influences entrapment, dispersion stability and release [1,2].

Neratinib is an irreversible inhibitor of HER-family receptor tyrosine kinases, particularly EGFR/HER1,

HER2 and HER4, suppressing downstream proliferative signaling. Paclitaxel acts through a distinct mechanism by stabilizing microtubules and disrupting mitosis. These complementary pharmacological targets provide a rationale for investigating their combined administration, but therapeutic interaction depends on tumor receptor biology, exposure and dosing [5–7].

The formulation-development component of this research previously addressed Quality-by-Design optimization of separately prepared neratinib- and paclitaxel-loaded cubosomes [4]. The present investigation focuses on in vivo antitumor outcomes following neratinib cubosomes alone and co-administration of the two distinct drug-loaded cubosomal dispersions. The formulations were not co-encapsulated in a single cubosome. Tumor growth,

body-weight changes and tumor-tissue histology were evaluated in an MCF-7 xenograft model.

2. MATERIALS AND METHODS

2.1 Cubosomal formulations

Neratinib-loaded and paclitaxel-loaded cubosomal dispersions were developed as separate formulations using a glyceryl monooleate–Poloxamer 407 lipid-based platform. Their prior formulation development and physicochemical evaluation are described in the related Quality-by-Design study [4]. For the present experiment, neratinib-loaded cubosomes were evaluated as a single-agent formulation and were also administered alongside separately prepared paclitaxel-loaded cubosomes. The combination group therefore received two distinct formulations rather than a co-loaded nanoparticle.

2.2 Animals and husbandry

Fifteen female BALB/c nude mice, aged approximately 6–8 weeks and weighing approximately 20–25 g, were used. Animals were acclimatized for at least seven days and maintained at 22 ± 2 °C and 55 ± 5% relative humidity under a 12-hour light/dark cycle, with

appropriate feed, water and sanitation. Handling was performed with attention to minimizing animal stress and discomfort. The study was conducted at Systemic Life Sciences & Research Pvt. Ltd. under IAEC protocol 01/IAEC-I/SLSRPL/2026; the facility CCSEA registration number was 2232/PO/RcBt/S/2023/CCSEA. Reporting was guided by ARRIVE 2.0 principles [3].

2.3 Establishment of MCF-7 xenografts and dosing

MCF-7 human breast cancer cells were maintained in Dulbecco’s modified Eagle medium supplemented with 10% fetal bovine serum and antibiotics. A suspension containing 1 × 10⁶ cells in 0.1 mL phosphate-buffered saline was inoculated subcutaneously in the flank. Following establishment of tumors of approximately 80–100 mm³, mice were allocated to five treatment groups (n=3 per group). Five intraperitoneal doses were administered at three-day intervals during the 14-day study period. The hormone-support conditions used to establish this estrogen-responsive xenograft model should be specified from the experimental record in the final methods.

Table 1. Experimental treatment groups and doses.

Group	Treatment	Dose	Animals
G1	Saline tumor control	—	3
G2	Neratinib suspension	10 mg/kg	3
G3	Paclitaxel suspension	5 mg/kg	3
G4	Neratinib-loaded cubosomes	10 mg/kg	3
G5	Separate neratinib- and paclitaxel-loaded cubosomes administered together	10 + 5 mg/kg	3

2.4 Tumor-volume and body-weight assessment

Tumor length and width were measured by electronic calipers on Days 0, 3, 6, 9, 12 and 14. Tumor volume (mm³) was calculated as length × width²/2. Body weight was recorded at corresponding intervals. End-point relative tumor-volume reduction was calculated as 100 × [1 – (treated-group mean Day-14 tumor volume/control-group mean Day-14 tumor volume)]. This calculation describes a between-group end-point difference and is not equivalent to baseline-adjusted tumor growth inhibition.

2.5 Tumor-tissue histology

Excised tumor tissues were processed for hematoxylin-and-eosin (H&E) examination following formalin fixation and paraffin embedding. Representative micrographs were organized by experimental group for descriptive comparison.

3. RESULTS

3.1 Antitumor activity

At baseline, group mean tumor volumes were similar, ranging from 87.86 to 92.29 mm³. In the saline-treated tumor-control group, mean tumor volume increased from 87.96 ± 4.76 mm³ on Day 0 to 1021.79 ± 49.54 mm³ on Day 14. All active-treatment groups exhibited lower mean end-point volumes than the control. Neratinib suspension and paclitaxel suspension resulted in Day-14 mean tumor volumes of 559.07 ± 32.24 and 462.57 ± 27.68 mm³, respectively. Neratinib-loaded cubosomes yielded a lower mean volume of 275.59 ± 20.48 mm³. The group receiving separately prepared neratinib- and paclitaxel-loaded cubosomes together had the lowest mean end-point volume, 127.20 ± 29.02 mm³ (Table 2; Figure 1).

Table 2. Mean tumor volumes ($\pm$ SD; n=3) and descriptive end-point comparisons.

Treatment	Day 0 (mm ³)	Day 14 (mm ³)	Reduction vs control
Saline control	87.96 $\pm$ 4.76	1021.79 $\pm$ 49.54	—
Neratinib suspension	90.98 $\pm$ 5.55	559.07 $\pm$ 32.24	45.29%
Paclitaxel suspension	92.26 $\pm$ 4.87	462.57 $\pm$ 27.68	54.73%
Neratinib cubosomes	87.86 $\pm$ 2.92	275.59 $\pm$ 20.48	73.03%
Separate cubosomes administered together	92.29 $\pm$ 6.51	127.20 $\pm$ 29.02	87.55%

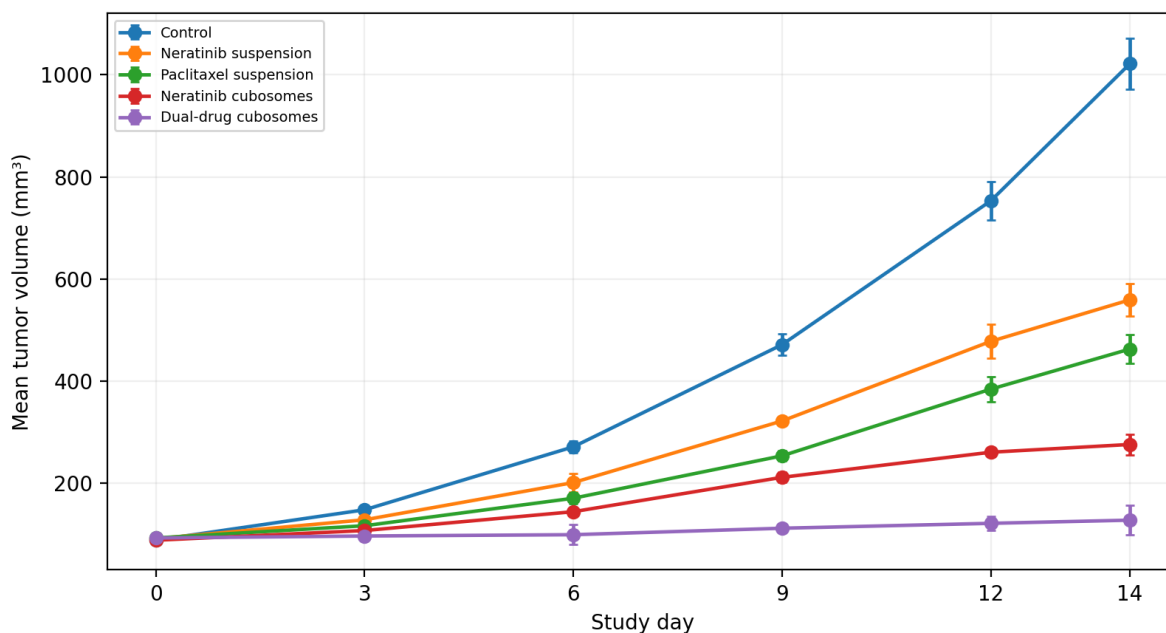


Figure 1. Longitudinal mean tumor volumes during the 14-day study (mean $\pm$ SD, n=3 per group).

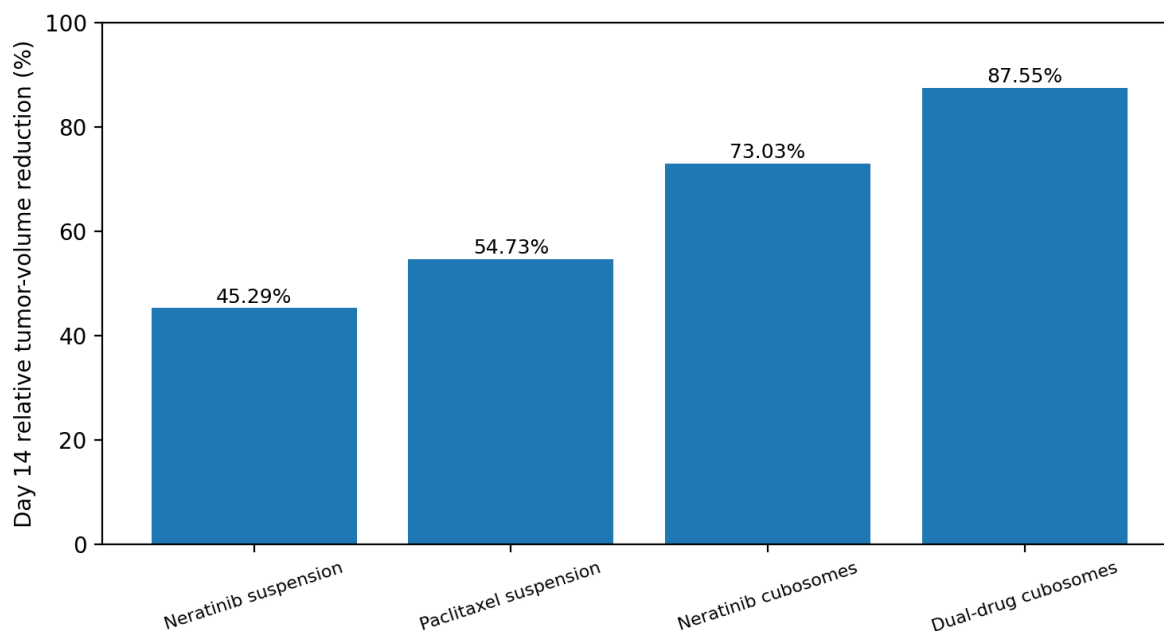


Figure 2. Percentage reduction in Day-14 mean tumor volume relative to the saline-treated control. Percentages describe end-point comparisons.

3.2 Body-weight changes

Day-14 changes in body weight relative to baseline were -2.58% for the saline control, -3.26% for neratinib suspension, -8.18% for paclitaxel suspension, $+0.83\%$ for neratinib-loaded cubosomes and -0.40% for the combined cubosomal treatment. Body weight remained comparatively stable in both cubosome-treated groups, whereas the largest mean reduction was observed in the paclitaxel-suspension group (Figure 3). These observations reflect gross weight trajectories and do not independently establish the absence of systemic toxicity.

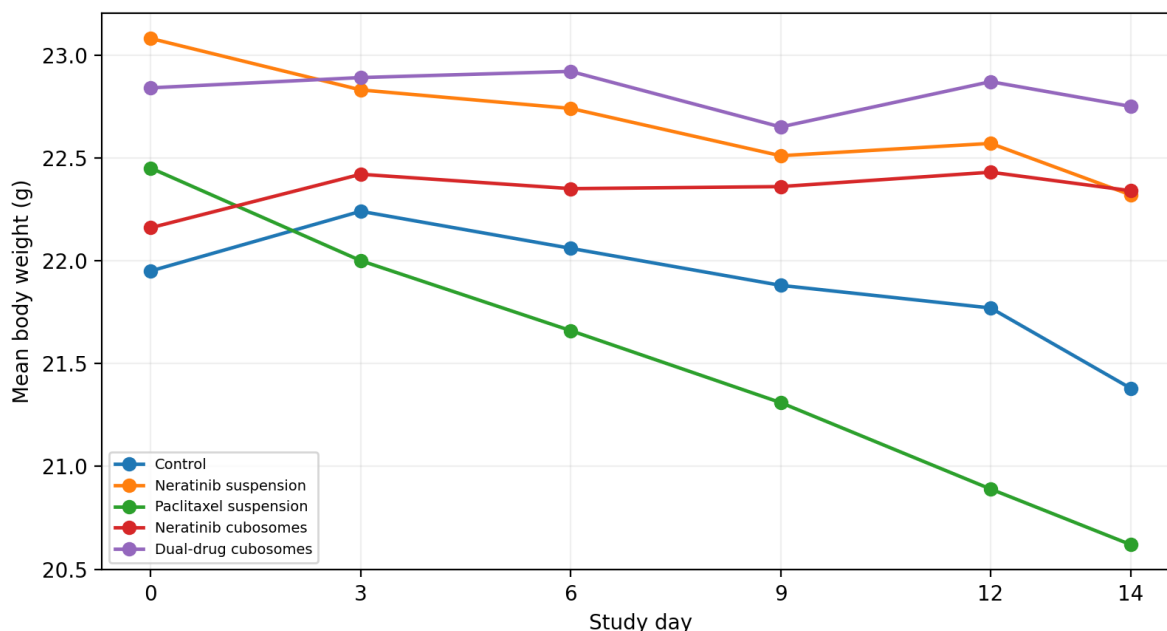


Figure 3. Mean body-weight trajectories during the treatment period ($n=3$ per group).

3.3 Experimental observations and tumor histology

Representative study photographs document animal handling, the presence of a flank tumor, tumor excision and specimen weighing (Figure 4). The photographs illustrate the experimental workflow; they are not used to assign tumor weights to individual treatment groups. H&E-stained tumor sections from all five groups are presented in Figure 5. The sections demonstrate tumor-tissue morphology across treatment conditions, but qualitative appearances alone do not establish a specific mechanism of tumor-cell death or provide a validated necrosis score.



Figure 4. Representative animal-study documentation: (A) handling, (B–C) flank tumor, (D) excised tissue and (E) specimen weighing. Panels illustrate procedures and are not assigned to specific animals or treatment days.

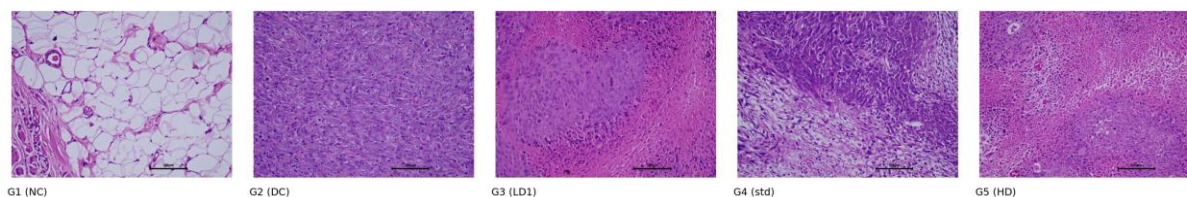


Figure 5. Representative H&E-stained tumor sections: (A) G1, saline control; (B) G2, neratinib suspension; (C) G3, paclitaxel suspension; (D) G4, neratinib cubosomes; (E) G5, separately formulated neratinib and paclitaxel cubosomes administered together. Magnification and scale were not documented.

4. DISCUSSION

This study provides a comparative in vivo assessment of free-drug suspensions and lipid-based cubosomal formulations in an MCF-7 xenograft model. The most prominent finding was the lower mean tumor volume in the combined cubosomal treatment group across the observation period, culminating in an 87.55% reduction in the Day-14 group mean relative to the saline control. Neratinib-loaded cubosomes alone also showed a lower mean end-point volume than the corresponding neratinib suspension (275.59 versus 559.07 mm³), supporting the relevance of formulation strategy to observed antitumor performance.

From a pharmaceuticals perspective, cubosomes provide a bicontinuous lipid architecture with substantial internal interfacial area, which can favor incorporation of lipophilic molecules and influence their apparent dispersion and release [1]. Such attributes may affect drug availability at the biological interface, but the present animal outcomes do not directly establish increased tumor accumulation, altered pharmacokinetics or a particular cellular uptake pathway. Accordingly, a formulation-mediated benefit is a plausible interpretation rather than a measured mechanism.

The combined treatment has a pharmacological rationale because neratinib inhibits HER-family kinase signaling, whereas paclitaxel perturbs microtubule dynamics and mitosis [5–7]. The difference in their mechanisms supports exploration of co-administration. Nevertheless, the combination group received two separately formulated cubosomal products and should not be characterized as a single co-loaded formulation. Furthermore, the absence of a free neratinib-plus-paclitaxel combination comparator and a paclitaxel-only cubosome group means that improved efficacy cannot be partitioned between the effects of formulation, the additional drug and a pharmacological interaction. Synergy therefore cannot be concluded from these data. The body-weight profile provides useful complementary context. The cubosome-treated groups showed relatively small changes in mean body weight, while paclitaxel suspension was associated with the largest decline. However, systemic tolerability requires assessment beyond body weight, including clinical observations, hematological or biochemical parameters and non-tumor tissue pathology. Histological micrographs of excised tumors provide morphological documentation but cannot substitute for validated pathological scoring or mechanistic markers of apoptosis and necrosis.

The modest sample size of three mice per group and the 14-day duration limit the precision and generalizability of the comparisons. MCF-7 tumors are also influenced by hormonal conditions; documentation of estrogen supplementation and receptor phenotype is important

when interpreting the activity of a HER-family inhibitor [8]. Future work should include appropriate combination controls, a larger prospectively justified sample size, a fully specified longitudinal statistical model, pharmacokinetic/biodistribution analysis and blinded histopathological scoring. Despite these limitations, the results establish a useful experimental basis for further development of separately formulated cubosomal anticancer therapies.

Strengths of the Study

1. The study provides an in vivo comparison of free-drug suspensions and cubosomal formulations in an MCF-7 breast cancer xenograft model.
2. It evaluates neratinib-loaded cubosomes as a single-agent formulation and in combination with separately formulated paclitaxel-loaded cubosomes.
3. Serial tumor-volume measurements over 14 days enabled the assessment of tumor growth patterns across the five experimental groups.
4. Body-weight monitoring provided an additional preliminary indicator of treatment tolerability during the study period.
5. The inclusion of representative H&E-stained tumor sections provided supportive morphological observations across the treatment groups.
6. The study highlights the potential of cubosomal drug delivery systems for further investigation in breast cancer therapy while appropriately recognizing the need for additional efficacy, toxicity, and pharmacological interaction studies.

5. CONCLUSION

In the MCF-7 xenograft model, separately formulated neratinib- and paclitaxel-loaded cubosomes administered together produced the lowest Day-14 mean tumor volume among the five groups investigated. Neratinib-loaded cubosomes alone also demonstrated a lower mean end-point tumor volume than neratinib suspension. The comparatively stable body-weight trajectories of the cubosomal groups further support their evaluation in larger studies. These results indicate the promise of lipid cubic-phase delivery systems for anticancer formulation research, while formal statistical significance, pharmacological synergy and systemic safety require dedicated confirmation.

Declarations

Funding: The authors received no external funding. The study was self-funded.

Data availability: Data supporting the findings are presented in the tables and figures. Additional underlying animal observations, tumor measurements, body-weight records and analysis files are available from

the corresponding author upon reasonable request, subject to institutional and ethical requirements.

Author contributions: Ayesha Farhath Fatima contributed to conceptualization, methodology, formulation development, investigation, data collection, analysis, interpretation and original manuscript preparation. Kumaraswamy Gandla contributed to conceptualization, methodology, supervision, validation, interpretation and critical manuscript review and editing. Both authors contributed to the study, reviewed and approved the final manuscript, and accept accountability for the work.

Ethics and animal welfare: Animal procedures were conducted at Systemic Life Sciences & Research Pvt. Ltd. under IAEC protocol 01/IAEC-I/SLSRPL/2026 (CCSEA registration 2232/PO/RcBt/S/2023/CCSEA). Animals were acclimatized and housed under controlled temperature, humidity and light-cycle conditions with routine husbandry. Animal handling and serial monitoring were undertaken with attention to minimizing distress and supporting animal welfare.

Conflicts of interest: The authors declare no known financial or non-financial conflicts of interest.

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