Application Note

NCI-H69V Small Cell Lung Cancer (SCLC) Mini-tumor Model in the ClinoStar System

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Introduction

- This application note details the process of developing an SCLC tumor model using the NCI-H69V cell line in the ClinoStar.
- The established model is reactive to treatment with the standard chemotherapy drug irinotecan, leading to a reduction in cell growth, viability, and glucose consumption.
- Viability of the model is demonstrated by measuring the following parameters: soluble protein content, planar surface area, intracellular adenosine triphosphate, extracellular adenylate kinase levels, and glucose consumption.

Small cell lung cancer (SCLC) is an aggressive disease. Despite extensive research and multiple clinical trials, it's standard of care has remained unchanged for the past three decades. To address the lack of a functional *in vitro* model to study the disease, a mini tumor model for SCLC was developed using NCI-H69V cell line in the ClinoStar. The ClinoStar is a clinostat-based rotating bioreactor system, designed to produce uniform 3D constructs reproducibly and in a physiologically relevant manner.

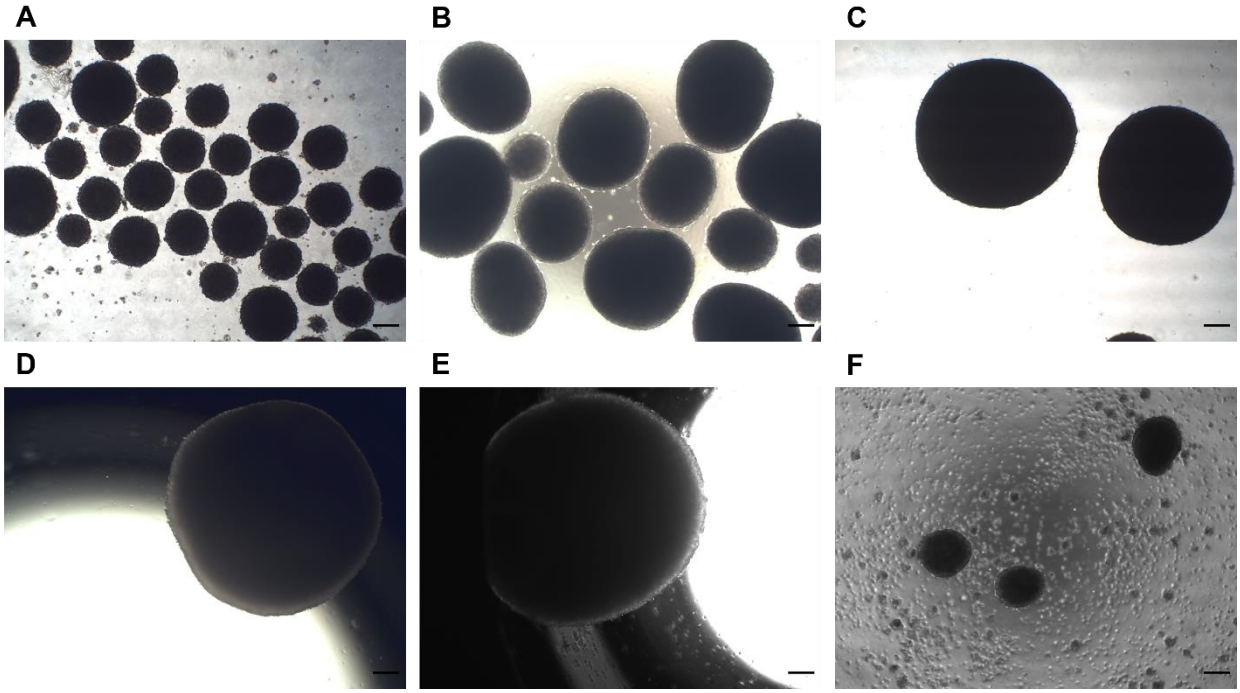


FIGURE 1 Photomicrographs of NCI-H69V spheroids during characterization, taken on (A) day 0, (B) day 8, (C) day 18, (D) day 22, and (E) day 30. (F) Shows the formation of "daughter" spheroids from day 22 until day 30 of culture in the rotating bioreactors (scale bar = 200 µm). Day 0 was defined as the day the formed spheroids were transferred to the newly prepared ClinoReactors after 72 h.

Culturing spheroids in the ClinoStar

1) The culture medium containing a base of RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids (NEAA), 1% penicillin/streptomycin, 2 mM L-glutamine and 25 µg/ml ascorbic acid was prepared.
2) A single cell suspension of NCI-H69V was prepared by trypsinization using 0.25% trypsin-versene upon reaching 80-90% confluency.
3) After obtaining cell count, a 1 x 10^5 cells/ml suspension was prepared and added to each prepared ClinoReactor with a total volume of 10 ml. (1x10^6 cells/ClinoReactor)
4) ClinoReactors were rotated at 2.5 rpm. The spheroids were monitored daily, and the rotational speed of the ClinoReactors was adjusted to counteract their increasing size.
5) Following an incubation period of 72 h, the formed spheroids were transferred to a 6-well plate and visually inspected for uniformity and perceived density.
6) Spheroids of similar sizes were then selected and counted, before being placed in a newly prepared ClinoReactors with pre-warmed culture medium supplemented with 5 µg/ml ascorbic acid for maintenance.
7) The ClinoReactors were returned to the ClinoStar and rotated at 8.0–8.5 rpm.
8) pH equilibrated culture medium, containing 5 µg/ml ascorbic acid, was exchanged every second day and the rotation speed adjusted daily to keep the spheroids suspended in the culture medium.

Response to Chemotherapeutic drug – Irinotecan

To validate the model for cancer treatment applications, the standard chemotherapy drug irinotecan was used. The response of the NCI-H69V spheroid model to treatment with two separate concentrations (IC_{25} and IC_{50}) of the drug for a period of 72 hours was measured. The dosages of IC_{25} and IC_{50} were determined by the MTT assay in flat cultures. For detailed protocols and quantification methods, please refer to the publication.

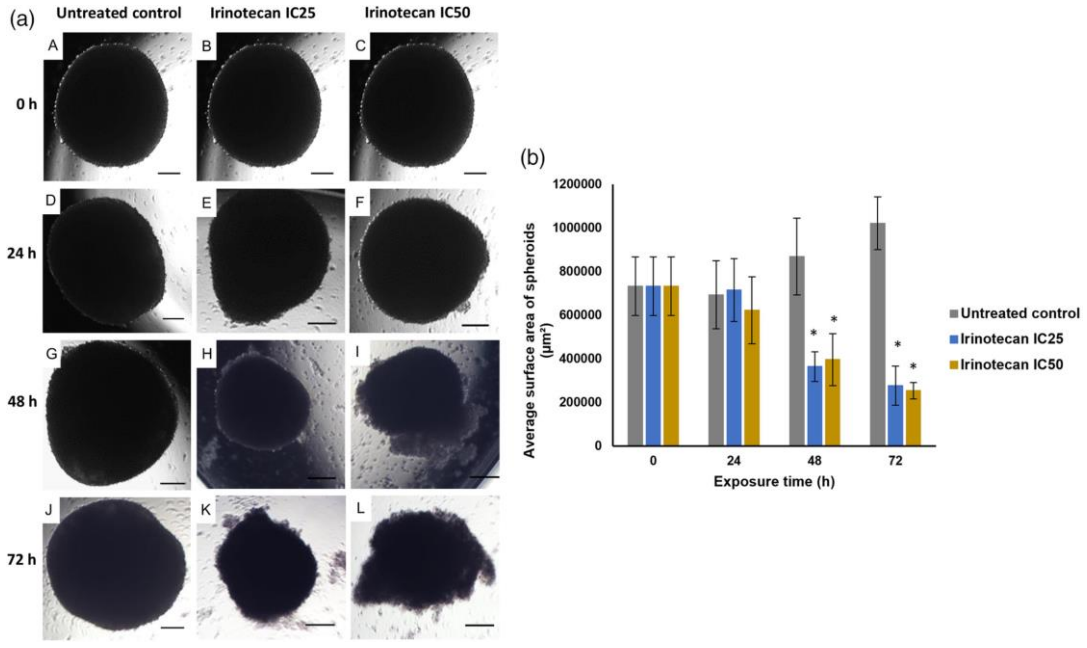


FIGURE 2 (i) Photomicrographs of spheroids during 72 h treatment (time 0 – a, b, c; 24 h – d, e, f; 48 h – g, h, i; 72 h – j, k, l) with irinotecan hydrochloride for the following treatment groups: untreated control (a, d, g, j) irinotecan IC25 (b, e, h, k) and irinotecan IC50 (c, f, i, l) (scale bar = 200 μ m). (a), (ii) The average surface area per spheroid from each treatment group at each time point is shown (error bars = SD; n = 6; * = statistically significant, $p < 0.05$ [one-way ANOVA followed by the Tukey post hoc test for comparison with the untreated control at each time point])

Validation of SCLC model

Efforts to validate this model included:

1. Quantification of soluble protein content in spheroids via a Bradford protein assay
2. Histological analysis to identify expression of relevant protein markers
3. ATP quantification assay to determine cell viability
4. Glucose consumption quantification to determine metabolic activity
5. Quantification of cell death to determine health of the spheroid population

Detailed procedures are described in:

"A novel NCI-H69V small cell lung cancer functional mini-tumor model for future treatment screening applications" 2022 Liezaan van der Merwe, Hanna Svitina, Clarissa Willers, Krzysztof Wrzesinski, Chrisna Gouws. PMID: 35362670 DOI: 10.1002/btpr.3253

Notice

All data presented in this Application Note was produced in the 1st generation ClinoStar (BAM – Bio Array Matrix). The same Clinostat principle is used in the 2nd generation ClinoStar.

For additional product or technical information, visit www.celvivo.com or consult CelVivo Aps at info@celvivo.com or +45 70 228 228