Confidentiality Statement

- This information is meant to be presented to the participating members of the 5th Korea-US Nanoforum only.
- By having this information in possession, you are agreeing to keep this information non-disclosed.
- You may not share, use, claim or distribute the information contained in this file/slide/handout without a written consent from NanoMedicine Research Co. (consent via email accepted.)
- Violations of the terms above may result in legal actions.
- Should you come in possession of this information without the permission mentioned above, please contact j.f.chung@nmrpharma.com

NanoMedicine Research
Bringing Tomorrow

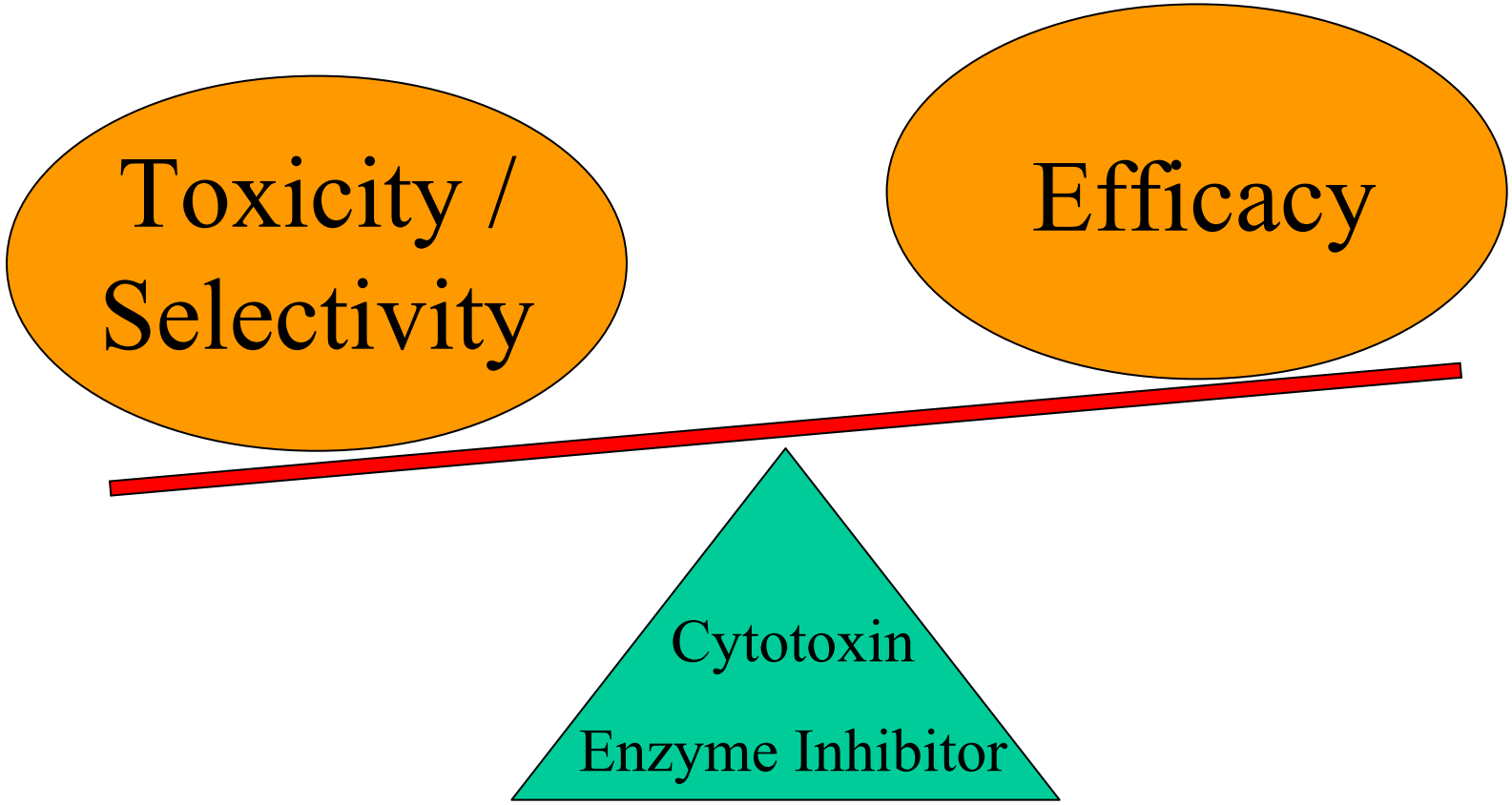
A Novel Anti-Cancer Strategy Through Biological Metal Overloading in Cancer Cells via pH-sensitive Metalo-organic Nanoparticles

Jinhyuk Fred Chung, Ph. D., CEO
NanoMedicine Research Co.
April 18th, 2008

The 5th Nanoforum
Jeju, Korea

NanoMedicine Research
Bringing Tomorrow

Challenges & Limitations in Anti-cancer Medicines: Seesaw Dillemma



Which side to leverage?

◈ Theory – A lesson from nature

Excitotoxic Calcium Overload in a Subpopulation of Mitochondria Triggers Delayed Death in Hippocampal Neurons

Natalia B. Pivovarova,[1] Huy V. Nguyen,[1] Christine A. Winters,[1] Christine A. Brantner,[1] Carolyn L. Smith,[2] and S. Brian Andrews[1]

[1]Laboratory of Neurobiology and [2]Light Imaging Facility, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892-4062

Journal of Neurochemistry, 2003, **85**, 443–453 doi:10.1046/j.1471-4159.2003.01691.x

Zinc toxicity on neonatal cortical neurons: involvement of glutathione chelation

Chun-Jung Chen and Su-Lan Liao

Department of Education and Research, Taichung Veterans General Hospital, Taiwan, Republic of China

1: Mol Med. 2008 Mar-Apr;14(3-4):98-108. Links

Iron-mediated inhibition of mitochondrial manganese uptake mediates mitochondrial dysfunction in a mouse model of hemochromatosis.

Jouihan HA, Cobine PA, Cooksey RC, Hoagland EA, Boudina S, Abel ED, Winge DR, McClain DA.

Departments of Medicine and Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah, USA and.

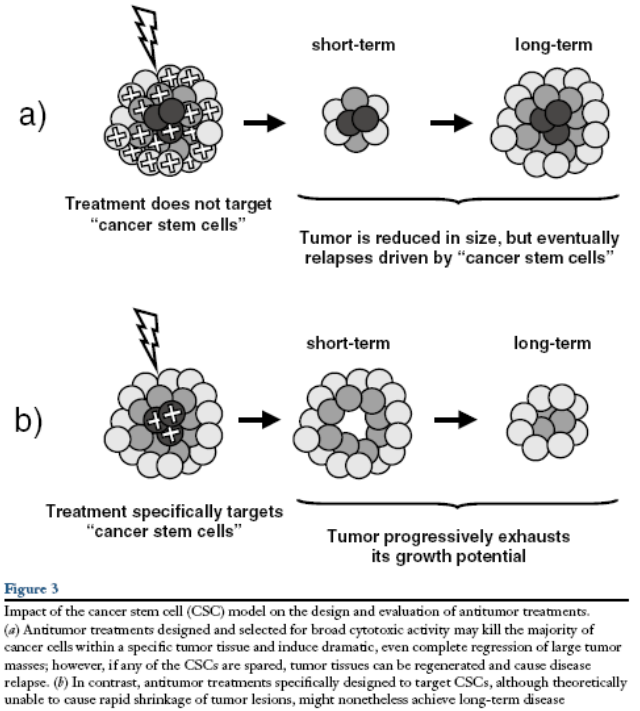


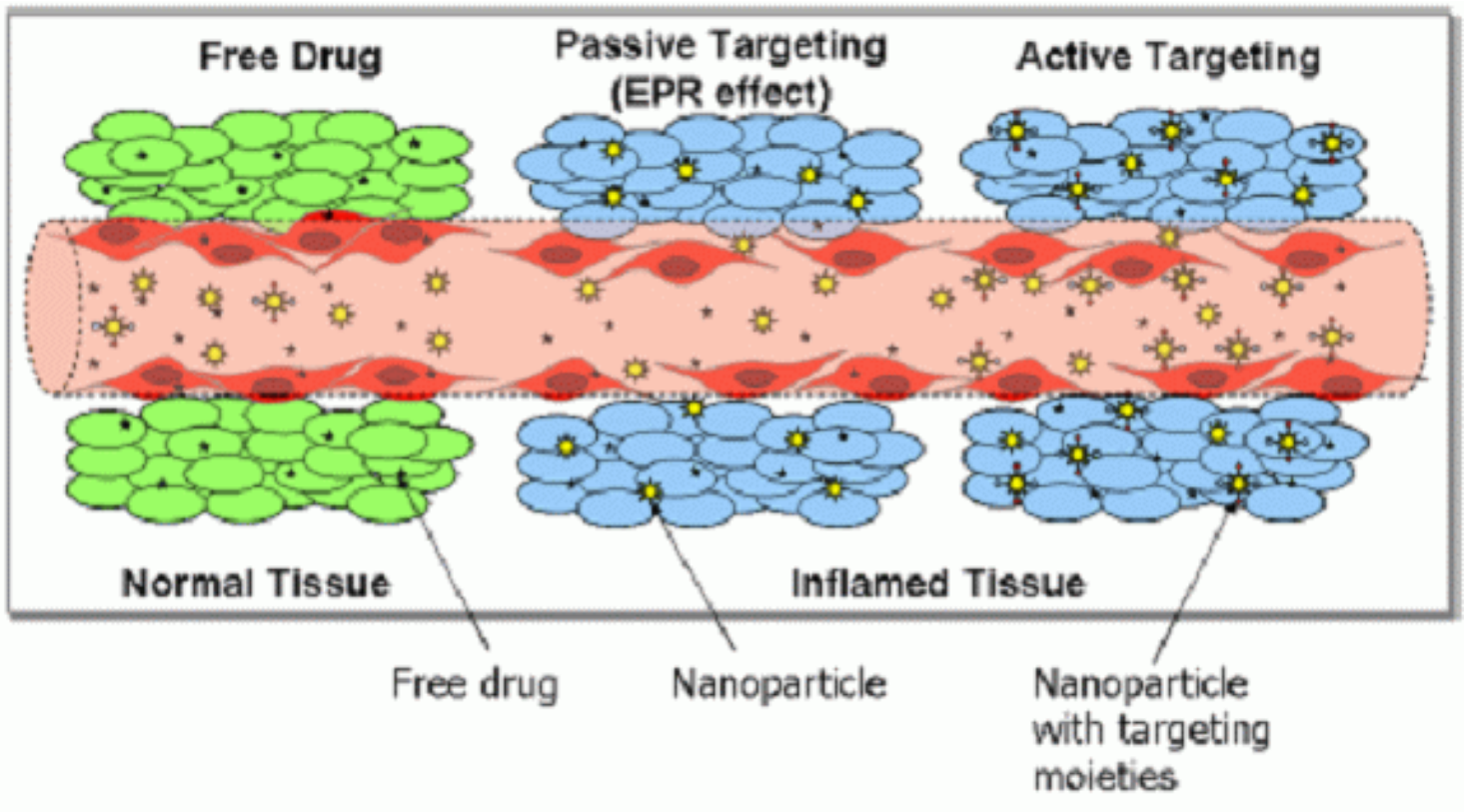
Figure 3

Impact of the cancer stem cell (CSC) model on the design and evaluation of antitumor treatments. (*a*) Antitumor treatments designed and selected for broad cytotoxic activity may kill the majority of cancer cells within a specific tumor tissue and induce dramatic, even complete regression of large tumor masses; however, if any of the CSCs are spared, tumor tissues can be regenerated and cause disease relapse. (*b*) In contrast, antitumor treatments specifically designed to target CSCs, although theoretically unable to cause rapid shrinkage of tumor lesions, might nonetheless achieve long-term disease eradication by exhausting self-renewal and growth potential of cancer tissues.

Benign biological metals can KILL UPON OVERLOADING!

NanoMedicine Research
Bringing Tomorrow

“Calcium & Iron Bomb” Strategy

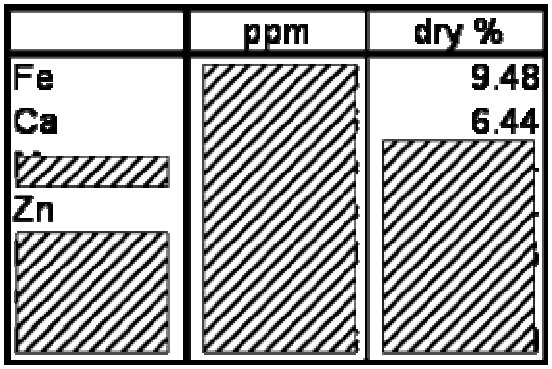


*Source of the depiction unknown

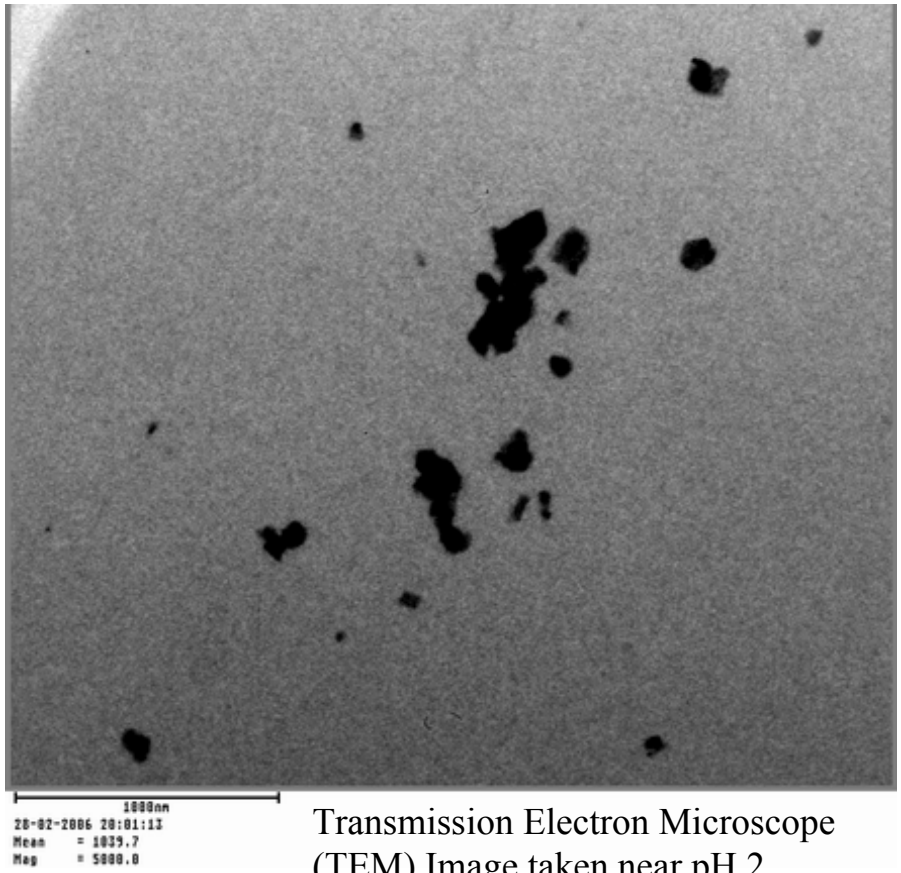
NanoMedicine Research
Bringing Tomorrow

◈ OFeCa-1: The Anti-Cancer Particle

- OFeCa-1
  - pH-sensitive organo-metallic nanoparticles with *iron & calcium* as the main therapeutic components.
  - Degrades near neutral pH, releasing metals.



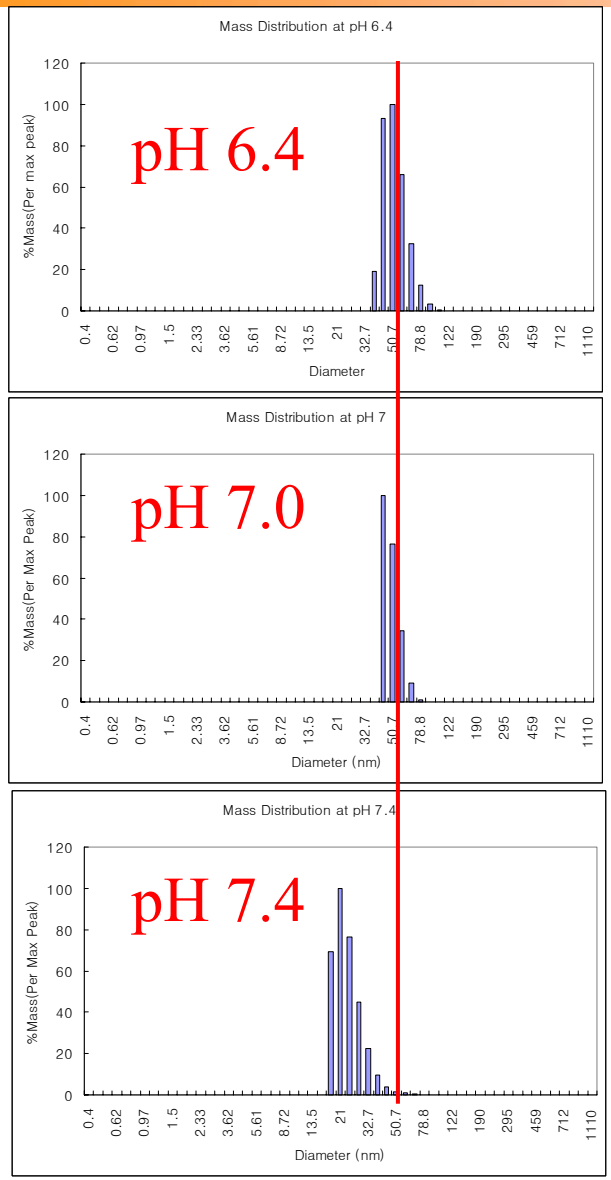
ICP-MS metal analysis of 11% w/w OFeCa-1 solution (same concentration solution as those used for cell & animal experiments)



Transmission Electron Microscope (TEM) Image taken near pH 2

NanoMedicine Research
Bringing Tomorrow

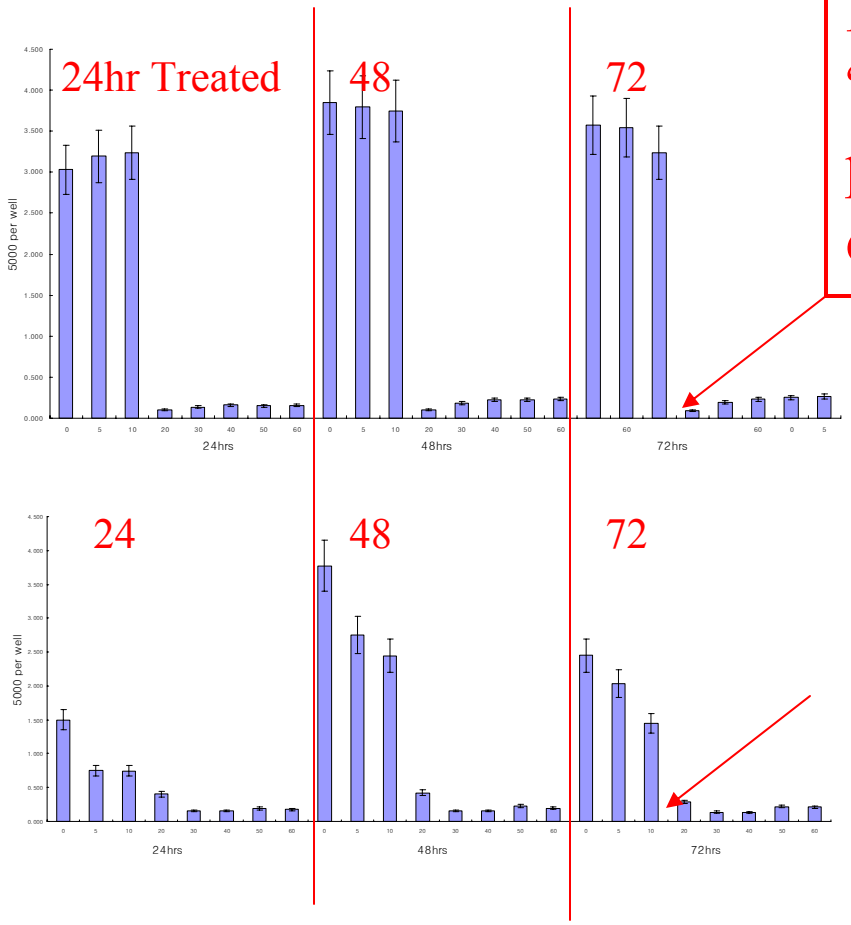
◈ OFeCa-1's Degradation via pH



- Dynamic light scattering results on OFeCa-1 at varying pH, showing pH-dependent degradation of OFeCa-1 near physiological ranges (~pH 7).
- Horizontal axis denote nanoparticle sizes in diameter, graphs denote mass distribution, normalized against the norm.
- This degradation property allows OFeCa-1 to "unload" bound metals that, in turn, kill target cancer mass

NanoMedicine Research
Bringing Tomorrow

◈ Human Cancer Cell Screening



Cancer Survival

OFeCa-1 Added (uL/mL Media)

Note this occurance of "critical dosage", at which point cancer suddenly disappear altogether

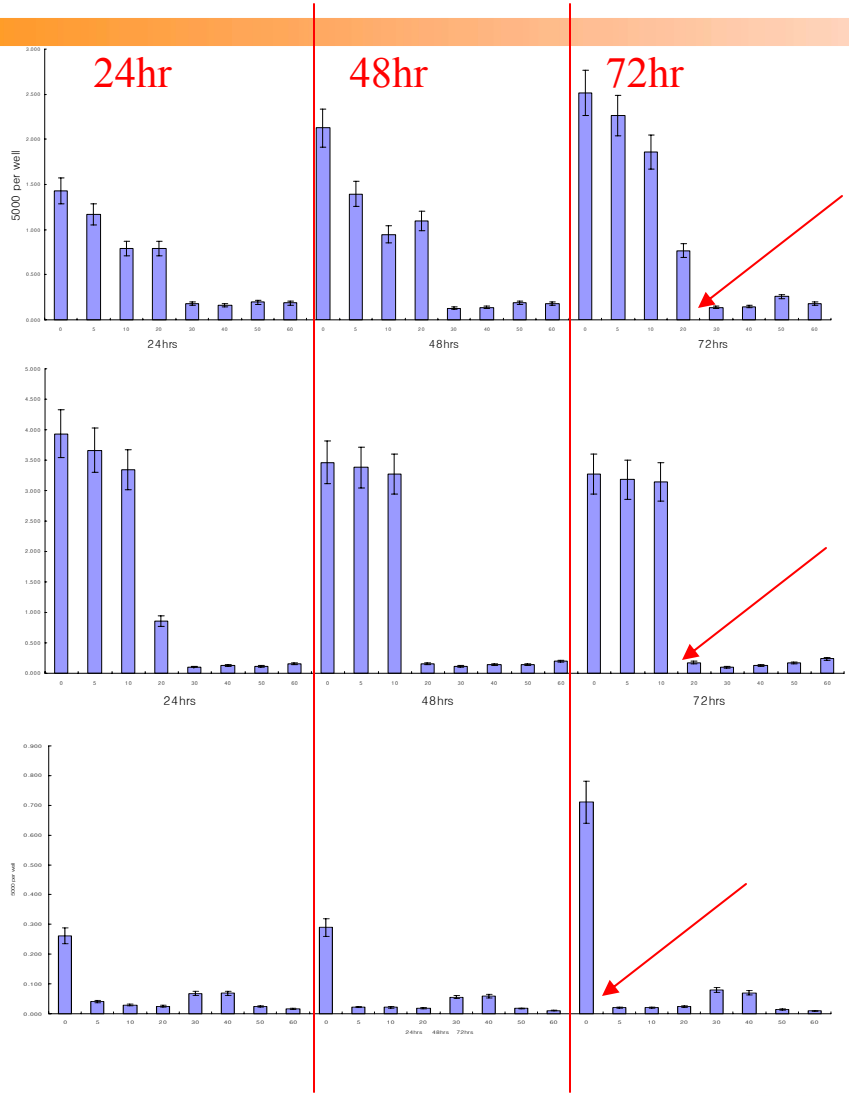
Hep2 (larynx cancer)

293T (kidney cancer)

*Cell counting kit-8 (Dojindo, Tokyo) was used to study cell viability.

NanoMedicine Research
Bringing Tomorrow

Continued



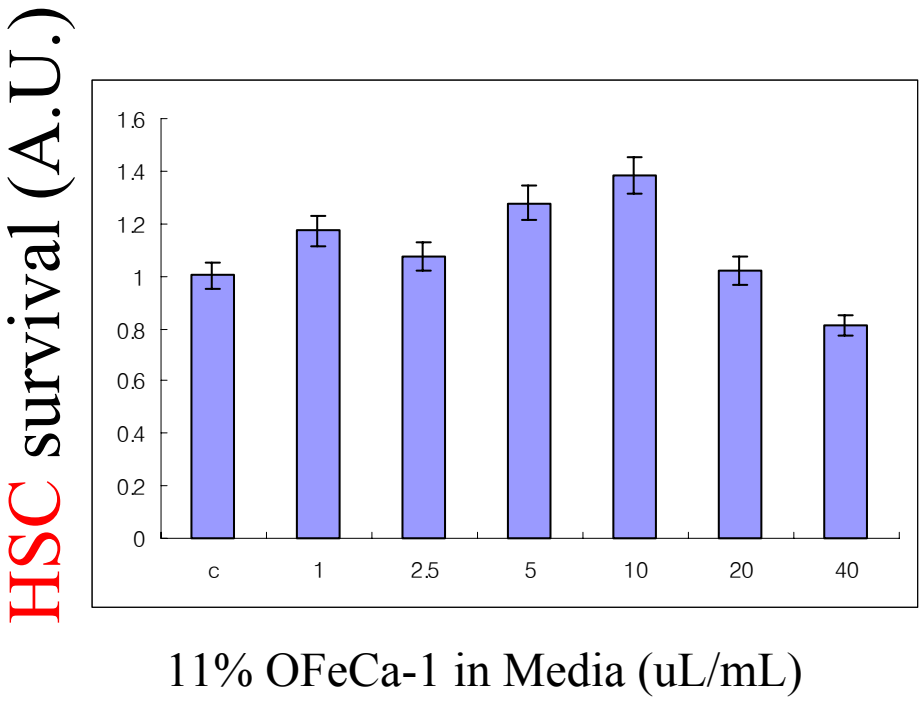
Ramos (Burkitt's lymphoma)

T98g (brain cancer)

H460 (lung cancer)

NanoMedicine Research
Bringing Tomorrow

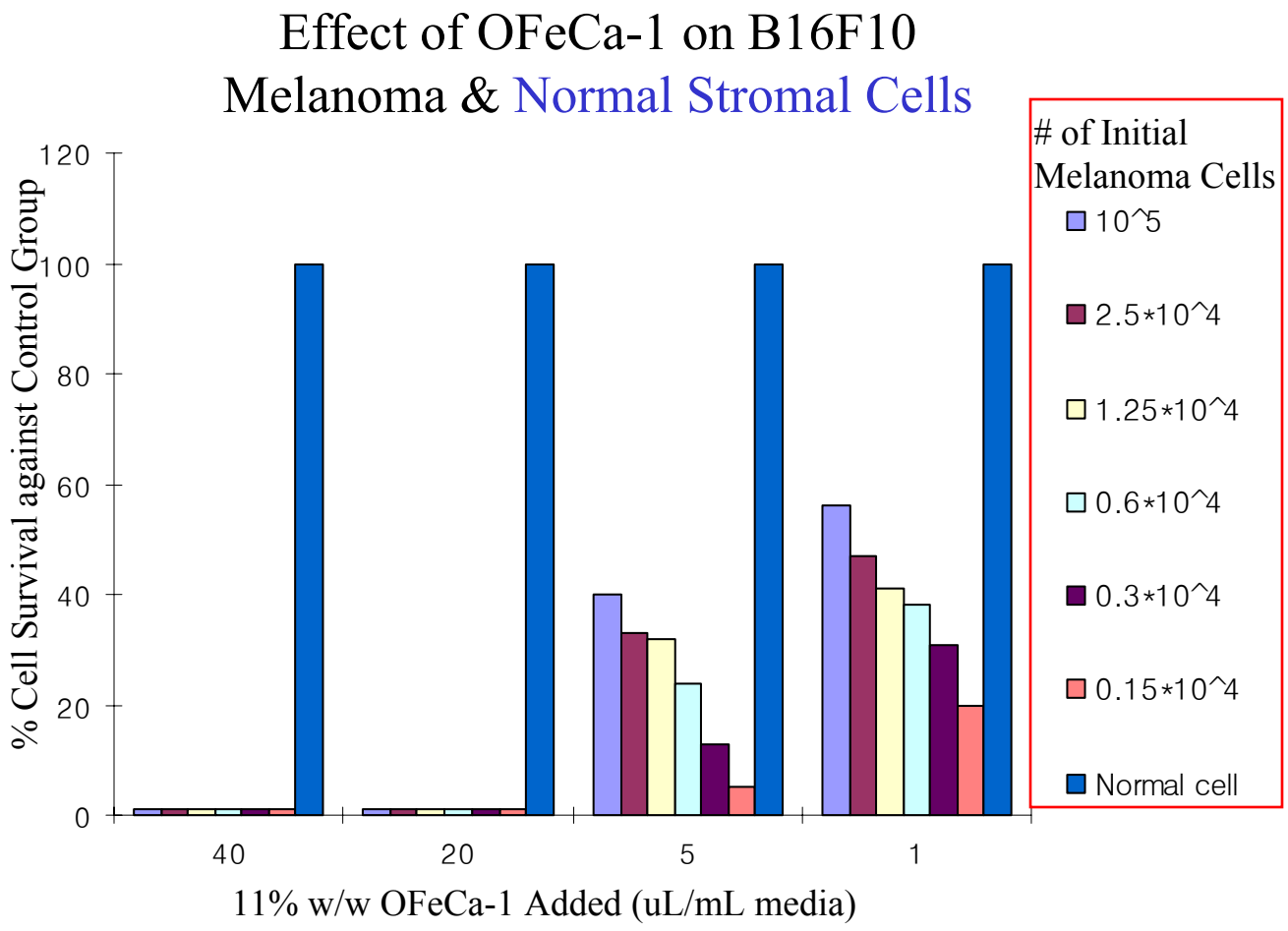
◈ Toxicity Testing against HSC



- Adult stem cells such as hair cells & hematopoetic stem cells (HSC: blood stem cell) are easily killed by conventional anti-cancer drugs, leading to hair loss & bone marrow suppression during cancer treatment.
- OFeCa-1, on the other hand, displays no signs of adverse effect on mouse HSC's survival.

NanoMedicine Research
Bringing Tomorrow

Efficacy against Mouse Cancer Cell

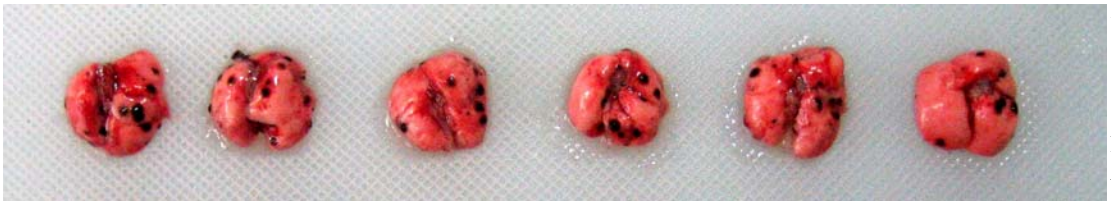


- OFeCa-1 shows no toxicity against non-cancerous normal mouse stromal cells.
- Within same range, OFeCa-1 shows powerful anti-cancer activity against mouse melanoma B16F10.

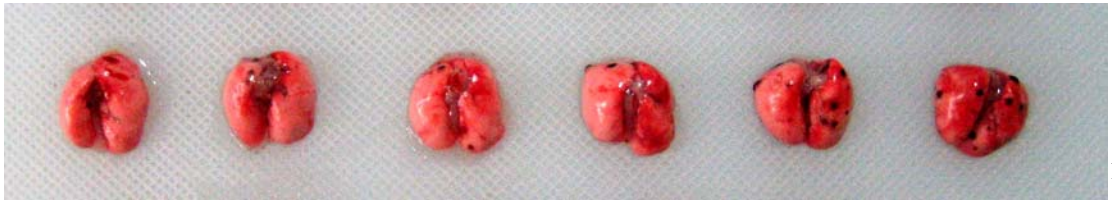
NanoMedicine Research
Bringing Tomorrow

Mouse Model Bearing Metastatic Lung Cancer

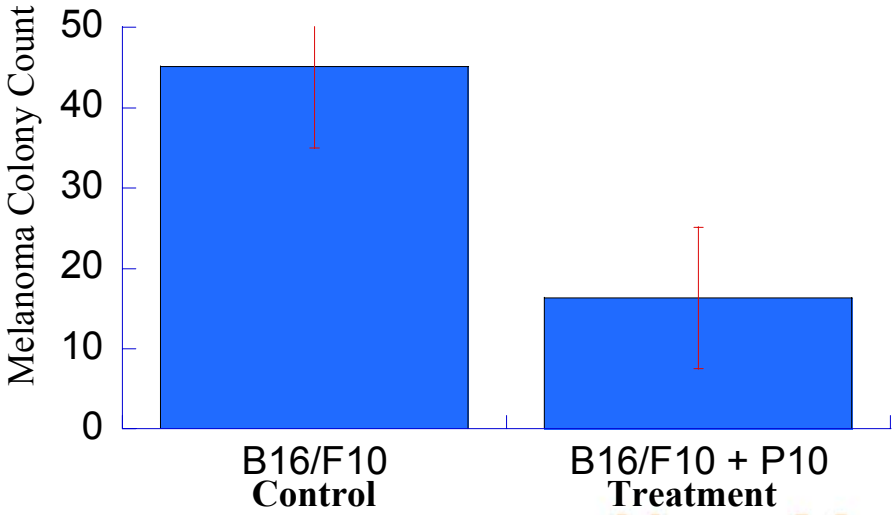
A



B



C



Effect of sustained administration of OFeCa-1 on intravanous metastatic mouse model using B16/F10. OFeCa-1 was passively administered as water-substitute by diluting 11 % OFeCa-1 ten-fold with dietary water. Quick survey showed that the mice consumed roughly 2~3 mL of the diluted OFeCa-1 daily. (A) B16/F10 control. (B) B16/F10 mice fed with 1/10 diluted OFeCa-1. (C) Quantified B16/F10 colonies found in the lungs of test subjects. P10 refers to passively fed 1/10 diluted 11% w/w OFeCa-1 against water.

Experiment terminated before the extinction of melanoma colonies in the treatment group for visual confirmation of successful metastasis

NanoMedicine Research
Bringing Tomorrow

◈ 2W Acute Toxicity Test using SD rats

1. SUMMARY

To evaluate the acute toxicity after a single oral administration of OFeCa-1, the test article was treated orally to 2 groups of male and female SD rats (5 animals/sex/group) at doses of 0 and 2000 mg/kg. Mortality, clinical signs, body weight changes and gross findings were continually screened for 15 days following the single dose. The results are summarized:

(1) There were no unscheduled deaths in any groups during the study period.
(2) In clinical signs, soft stool was observed in the 2000 mg/kg/day group on the dosing day.
(3) There were no treatment-related body weight changes in any groups during the study period.
(4) In the necropsy, no treatment-related gross findings were observed in any groups

On the basis of the above results, a single oral administration of the test article to SD rats at dose of 2000 mg/kg resulted in soft stool on the dosing day, and there were no treatment-related effects on mortality, body weight changes and gross findings. Therefore, it was estimated that the lethal dose of the test article might be over 2000 mg/kg for both sexes of rats.

STATEMENT

Study No.: G06005
TITLE: A Single Oral Dose Toxicity Study of OFeCa-1 in Rats

This study was carried out in compliance with GLP and Testing Guidelines as shown below:

(1) Good Laboratory Practice Regulation for Non-clinical Laboratory studies (Notification No. 2005-79) issued by Korea Food and Drug Administration on December 21, 2005 [1]

(2) OECD Principles of Good Laboratory Practice (1997) [2]

(3) Testing Guidelines for Safety Evaluation of Drugs (Notification No. 2005-60) issued by Korea Food and Drug Administration on October 21, 2005 [3]

The objectives laid down in the protocol were achieved, and as nothing occurred to adversely affect the confidence of the study, I consider the data generated to be valid.

3.3 Dosage and group assignment

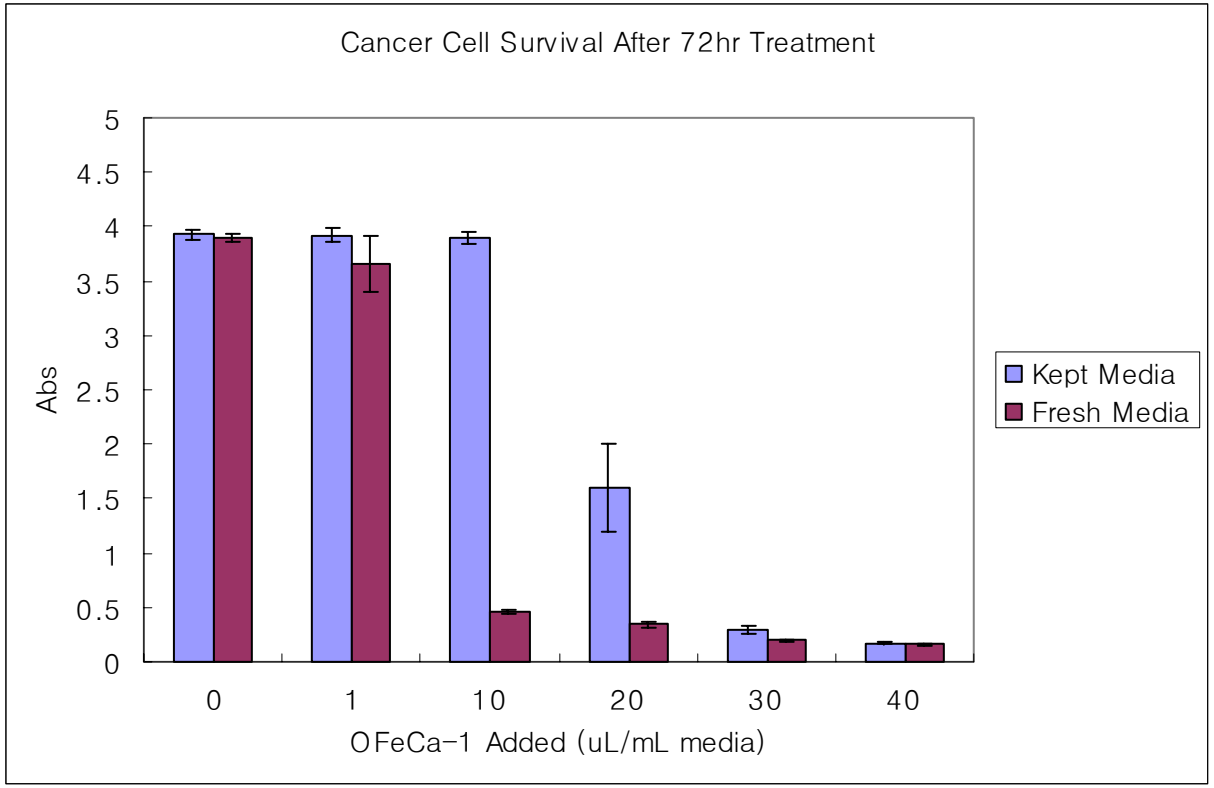
Dosage selection: According to request of the sponsor, 2000 mg/kg was selected as the limit dose, and the vehicle control group treated with distilled water for injection was added.

Group assignment, dosing volume, dosage:

Group	Sex	No. of animals	Animal No.	Volume (ml/kg)	Real Dose[a)] (mg/kg)	Dose (mg/kg)
Vehicle control	Male	5	1~5	20	0	0
	Female	5	11~15	20	0	0
T1	Male	5	6~10	20	15,385	2000
	Female	5	16~20	20	15,385	2000

[a)] Real dose was calculated in consideration for purity of the test article provided by the sponsor.

Replenishment Effect: More Effectiveness



- The graph left shows clear evidence of improvement in anti-cancer efficacy of OFeCa-1 upon daily replacement of the media containing OFeCa-1.
- This data indicates that frequent use of OFeCa-1 is likely to be recommended in the future.
- Considering the data thus far, greater dose and more frequent intake of OFeCa-1 is likely to be highly recommended, increasing the demand for OFeCa-1 for extra assurance in cancer treatment.

*B16F10 mouse melanoma as model. Media of appropriate condition were replaced daily for "fresh media" group.

NanoMedicine Research
Bringing Tomorrow

OFeCa-1: Ideal Anti-Cancer Agent

- Ultra-low toxicity*
- Composed only of biological metals & organic nanoparticle backbone that degrades into non-toxic biological metabolites
- Displays effectiveness against multiple aggressive cancer cell lines (both human & mouse origins)
- Designed to work in multiple modes, increasing effectiveness & reducing chances of resistance development
- Outlook of good efficacy against Lung cancer
- Significantly more effective upon frequent & prolonged use
- Expected to be compatible with conventional chemotherapeutic agents.
- And many more ideal behaviors & properties that qualify OFeCa-1 as an ideal candidate for future broad-spectrum cancer treatments.

*Based on toxicity tests against cell-based tests & acute toxicity test using SD rats