9. APPENDICES
APPENDIX-1

Appendix

Department of Pharmacology
Kasturba Medical College
A Constituent College of Manipal Academy of Higher Education (A Deemed University)

Proceedings of the Institutional Animal Ethics Committee (IAEC) meeting, KMC, Manipal


To,

Raghavendra Shetty
Scientist - III
Manipal Acunova
KH Clinical Research Centre
Shirdi Sai Baba Cancer Hospital
Manipal.

Through,

Dr. K. L. Bairy
Professor
Dept. of Pharmacology
KMC, Manipal.

Sir,

Subject: - Approval of your project entitled "Liposome Formulation of Anticancer drug and its Pharmacokinetic and Toxicokinetic evaluation" by IAEC, MAHE.

The Institutional Animal Ethics Committee has scrutinised the above mentioned project following clarification regarding the details of study design to justify the number of animals required. You are permitted to proceed with your project.

Dr. Vasanthi Kumar
Chairman
IAEC, MAHE
Manipal.
Form B [per rule 8(a)]

APPLICATION FOR PERMISSION TO CONDUCT ANIMAL EXPERIMENTS

To
Dr. Vasanth Kumar,
Chairman,
Institutional Animal Ethics Committee,
K.M.C., Manipal

Part A
1. Name and address of establishment: Manipal AcuNova Ltd, Manipal.
2. Registration number: PhD/Ph/NU/VK/23/06-549
3. Name, address and registration number of breeder from whom animals required (or to be acquired) for experiments mentioned in Parts B and C: Central Animal House, Manipal University, Manipal- Reg. No. 94/1999/CPCSEA
4. Place where the animals are presently kept (or proposed to be kept):
   Central animal house, Manipal University, Manipal.
5. Place where the experiment is to be performed:
   Central animal house, Manipal University, Manipal.
6. Date on which the experiment is to commence and Duration of experiment:
   Start: May 2008
   Duration: 3 months

Date: 23/04/08
Place: Manipal

Dr. K.L Bairy
Professor
Department of Pharmacology
KMC Manipal
Part B
1. Project Title: “Liposome Formulation of Anticancer drug(Irinotecan) and its Pharmacokinetic and Toxicokinetic evaluation”

2. Chief Investigator:
   a) Name: Dr. K L Bairy
   b) Designation: Professor
   c) Dept/Div/Lab: Department of Pharmacology, KMC Manipal
   d) Telephone number: 0820-2922928

3. List of names of all individuals authorized to conduct procedures under this Proposal:
   Mr. Raghavendra Shetty

4. Funding source: Manipal AcuNova KH Clinical Research Centre.

5. Duration of the project:
   a) Number of months: 12
   b) Date of Initiation: June 2008.
   c) Date of Completion: June 2009.

6. If date by which approval is needed is less than six weeks from date of submission, justification for the same: NA

7. Study Objectives:
   a) Aim of study
   To study the Pharmacokinetic and toxicokinetic evaluation of the developed formulations.

   b) Justification

   In the present scenario there is an increasing demand for newer formulations of the existing drugs. Carboplatin is an anticancer drug used for the treatment of ovarian cancer. Despite considerable progress in the treatment search for newer applications of the existing drugs continues because of time consuming and expensive process involved in the development of newer molecules and developing new technology for the existed drugs offer an alternative safer mode of treatment.
8. **Animal Required:**
   
a) Species: Albino Rat  
b) Age/Weight/Size: 180-250g  
c) Gender: Male  
d) Number to be used: 100  
e) Number of day’s animal will be housed: 2-3 Months

9. **Rationale for animal use:**
   
a) **Why is animal usage necessary for these studies?**
   It is mandatory to use animals to study pharmacokinetic and Toxicokinetic parameters and to assess the efficacy of the drugs /formulations. The design of the experiment involves administration of active pharmaceutical ingredient and developed formulation in aqueous suspension forms. Subsequent periodic sampling of blood is required for measurement of required parameters. Therefore, experiments essentially require animals, albino rats are well accepted and routinely used by researches for aforesaid activity.

b) **Why are the particular species selected required?**
Rats are suitable for these experiments because of their small size, genetic similarities to man, amenability to experimentation, and availability of abundant published data acquired from previous experiments.

c) **Why are the estimated numbers of animals essential?**
The estimated numbers of animals are essential to (I) To minimize the biological variations (II) To apply appropriate statistical tests to analyze the data obtained (III) To account for the number of animals to be used to assess the pharmacokinetic and histopathological parameters.

d) **Similar experiments conducted in past. If so, the number of animals used and results obtained in brief:** NO

e) **If yes, why new experiment is required?** NA

f) **Have similar experiment/s been made by any other organization/agency? If yes, their results in your knowledge?** NO
10. Description of procedures to be used

**Pharmacokinetic study**

The study involves nasal/intramuscular/subcutaneous administration of the developed formulation to overnight fasted animals and pharmacokinetic parameters are determined by withdrawing blood sample from each animal at predetermined time intervals.

11. Does the protocol prohibit the use of anesthetic and analgesic for the conduct of painful procedures (any which cause more pain than that associated with routine injection or blood withdrawal? If yes, explanation and justification)- NO

12. Will survival surgery be done?

NO

13. Methods of disposal, post-experimentation:

To monitor the parameter in above mention models the animals need to be sacrificed by excess of pentobarbitone. Thus the procedure involves no rehabilitation. Carcass disposal is by Incineration.

14. Animal transportation methods- Institutional transport is envisaged: NA

15. Use of hazardous agents: NA
**Investigator’s declaration:**

1. I certify that I have determined that the research proposal herein is not unnecessarily duplicative of previously reported research.

2. I certify that all individuals working on this proposal, and experimenting on the animals, have been trained in animal handling procedures.

3. For procedures listed under item 11, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.

4. I will obtain approval from the IAEC/CPCSEA before initiating any significant changes in this study.

5. Certified that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body

6. Institutional Biosafety Committee’s (IBC) certification of review and concurrence will be taken. I shall maintain all the records as per format.

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Signature

Raghavendra Shetty  
Scientist-III  
Manipal AcuNova KH Clinical Research Centre  
Sirdi Sai Baba Cancer Hospital  
Manipal

Name of Chief Investigator  
(Dr. K.L Bairy)

Date: 23/04/2008  
Place: Manipal
(For IAEC/CPCSEA)

Proposal Number

Date first received

Date received after modification (if any)

Date received after second modification (if any)

Approval date

Expiry date

Name of IAEC/CPCSEA Chairperson

Date: Signature

Place:
Figure i. Representative Chromatogram of $C_{\text{max}}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan conventional i.v. formulation to Rat_1
Figure ii. Representative Chromatogram of $C_{\text{max}}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan conventional i.v. formulation to Rat_2
Figure iii. Representative Chromatogram of $C_{\text{max}}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan conventional i.v. formulation to Rat_3
Figure iv. Representative Chromatogram of $C_{max}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan conventional i.v. formulation to Rat_4

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Figure v. Representative Chromatogram of $C_{max}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan conventional i.v. formulation to Rat_5

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Figure vi. Representative Chromatogram of C<sub>max</sub> concentration obtained from intravenous administration of 20 mg/mL Irinotecan conventional i.v. formulation to Rat_6
Figure vii. Representative Chromatogram of $C_{\text{max}}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan liposome formulation to Rat_1

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Figure viii. Representative Chromatogram of $C_{\text{max}}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan liposome formulation to Rat_2
Figure ix. Representative Chromatogram of $C_{max}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan liposome formulation to Rat_3

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Figure x. Representative Chromatogram of $C_{\text{max}}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan liposome formulation to Rat_4
Figure xi. Representative Chromatogram of $C_{\text{max}}$ concentration obtained from intravenous administration of 20 mg/mL Irinotecan liposome formulation to Rat_5.
Figure xi. Representative Chromatogram of Cmax concentration obtained from intravenous administration of 20 mg/mL Irinotecan liposome formulation to Rat_6

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APPENDIX-3

FORMULAE USED

Mean: \[ \text{Sum of all values} / \text{Number of values} \]

Standard Deviation (S.D (±)):
\[ \sqrt{\frac{\sum (x - \overline{x})^2}{(n-1)}} \]

Precision: Coefficient of variation (CV %): \[ \frac{\text{Standard deviation}}{\text{Mean}} \times 100 \]

Accuracy: % Nominal concentration: \[ \frac{\text{Concentration found}}{\text{Nominal concentration}} \times 100 \]

Percent of recovery: \[ \frac{\text{Extracted peak area}}{\text{Unextracted peak area}} \times 100 \]

Mean Percent of Change:
(\text{Mean or corrected mean concentration of stability samples} - \text{Mean or corrected mean concentration of comparison samples})
\[ \frac{\text{Mean or corrected mean concentration of comparison samples}}{X 100} \]

Percent of change of stock solutions:
(\text{Mean peak response (stability samples)} - \text{Mean peak response (comparison sample)})
\[ \frac{\text{X 100}}{\text{Mean peak response (Comparison samples)}} \]

Percentage deviation:
\[ \frac{\text{Calculated Conc.-Actual Conc.}}{\text{Actual conc.}} \times 100 \]