

**Institutional Animal Care and Use Committee (IACUC)
Statement**

Name of journal: World Journal of Gastroenterology

Manuscript NO.: 76258, Basic Study

Manuscript title: Novel therapeutic Diiminoquinone exhibits anticancer effects on human colorectal cancer cells in 2D and 3D in vitro models

Alissar Monzer, Kevork Wakimian, Farah Ballout, Samar Al Bitar, Amani Yehya, Mariam Kanso, Nour Saheb, Ayman Tawil, Samer Doughan, Maher Hussein, Deborah Mukherji, Walid Faraj, Hala Gali-Muhtasib, Wassim Abou-Kheir

Prior to any mouse experiment, all mice protocols were reviewed and approved by the Institutional Animal Care and Use Committee (AUB, IACUC).

Signature,



Hala Gali-Muhtasib, PhD
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AMERICAN UNIVERSITY OF BEIRUT
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

REQUEST FORM FOR RESEARCH PROTOCOL APPROVAL

For IACUC Use Only:

Proposal No.:

Date of Approval:

The PI plans to perform / to use:

☐ Major Survival Surgery

☐ Pain Category E studies

☐ Hazardous Agents

TO BE COMPLETED BY PRINCIPAL INVESTIGATOR

A. RESEARCH PROTOCOL STATUS.

A1. Name of Principal Investigator (include middle initial) : **Hala Gali-Muhtasib**

A2. Faculty: **Arts and Sciences**

A3. a. Department: **Biology**

A3. b. Division: _____

A4. Telephone # in Office: **2513/4856** Mobile: **(03) 973820**

Beeper # _____

A5. Proposal Title: **The novel therapeutic Diiminoquinone exhibits anticancer effects on human colorectal cancer cells in 2D and 3D in vitro models**

A6. Animal Species (submit a separate protocol for each animal species): **Male NOD-SCID mice**

A7.a. Funding Source. Indicate the source(s) of funds that will be used to perform these animal procedures once approved by the AUB - IACUC:

- ☐ U. S. Public Health Service (e.g. NIH)
☐ URB
☐ MPP
☐ LNCSR
☐ Private Funding Agency. Identify:
☒ Other. Identify: CNRS

A7.b. Indicate Account Number(s) to be charged: **Proposal is under review**

A8. Is this a new Protocol?

- ☒ Yes.
☐ No. **This is a repeat of a previous experiment we did but this time with a larger number of animals per group**

If this is a one-year renewal, provide a progress report describing work accomplished during the last approval period. Include the number of animals used, the objectives that were met and how the work proposed in this renewal extends the previous studies.

A9. Do you plan on performing the animal procedures described in this form even if you do not receive intramural or extramural, or other funding?

- ☐ Yes.
☒ No.

A10. Indicate the reason for animal use:

- ☒ Research.
☐ Teaching or Training.
☐ Testing.
☐ Sentinel animal use.
☐ Breeding and colony management only; no experimental procedures.
☐ Breeding and colony management and experimental procedures
☐ Other. Please specify:

B. PERSONNEL

B1. Give the **names of all staff members** expected to work with animals in this study. For each person listed describe his/ her education, training, and experience with laboratory animals.

Alissar Monzer (PhD Candidate) have experience in handling animals and have successfully completed animal training.

Farah Ballout (PhD Candidate) have experience in handling animals and have successfully completed animal training.

C. PROPOSAL OVERVIEW**C1. Lay Description.**

Using non-technical (lay) language, briefly describe in not more than 50 words how this research project might improve the health of people and/or animals or contribute to science. A scientific abstract from a grant proposal is not acceptable.

In vitro analysis showed that DIQ is more potent than 5-fluorouracil against colon cancer cells. Considering that quinones-containing compounds are currently used as chemotherapeutics against different types of cancer, the successful completion of this project will offer cancer patients with a potent drug DIQ devoid of toxicity.

C2. Experimental Design.

C2.1. Using non-technical (lay) language, describe the experimental design (in not more 200 words max.):

Twenty mice will be randomly distributed into two groups (6-8 mice/group). 100 spheres of HCT116 colon cancer cells suspended in a total volume of 50 μ l growth media and Matrigel™ (1:1) will be injected subcutaneously into the flank of 4 to 6 week old mice. Once the tumor size, monitored daily, reaches 7 mm mice will be treated 3X per week for 21 days with intraperitoneal injections of vehicle (control group) or DIQ (20 mg/Kg).

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2.2. Describe, in scientific terms in not more than 200 words the experimental design for the planned animal experiments and the sequence of events to reveal what happens to the animals. Include all procedures and manipulations, and explain why they must be performed. Give your best estimate of how many animals will undergo the procedures or manipulations described.

Twenty mice will be randomly distributed into two groups (6-8 mice/group). Hundred HCT116-derived spheres in a total volume of 50 μ l growth media and Matrigel™ (1:1) will be injected subcutaneously into the flank of 4 to 6 week old mice. Once the tumor size, monitored daily, reaches 7 mm mice will be treated 3X per week for 21 days with intraperitoneal injections saline (control group) or different concentrations of DIQ (20 mg/Kg). 21 days post-treatment mice will be sacrificed. Tumor size will be measured every other day using Mitutoyo caliper throughout the study. Mice will be daily monitored for signs of morbidity. Body weight recordings will be carried out biweekly.

Treatment	Animals	Numbers	Method	Total
DIQ	Mice	20 mice	needle	20
Total animals				20

3. Characteristics and Number of Requested Animals

3.1. Complete the following table:

Strain, Stock, Mutant, or Breed	Gender	Age/weight	Source (Vendor) ACF or others	Health Status
NOD-SCID	Male	6 to 8 weeks, 25g	N/A	

3.2. Justify the number of animals requested and group sizes. Describe how the estimated number of animals needed for the experiments was determined. When appropriate, provide the number and type of experimental and control groups in each experiment, the number of experiments planned, and the number of animals in each group.

For this experiment we need two groups 6-8 mice/group, Therefore, a total of 20 animals will be used for this study. This is the minimum number of animals suitable to obtain statistical significance.

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3.3. Assign all requested animals to a category of pain / distress:

(Pain Categories A and B not applicable)

Pain Category C	Animals upon which teaching, research, experiments, or testing will be conducted involving no pain, distress, or pain-relieving drugs are used.
# of requested animals for Cat C	
Pain Category D	Animals upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used.
# of requested animals for Cat. D	
Pain Category E	Animals upon which teaching, experiments, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.
# of requested animals for Cat. E	

3.4. Are any Category D or E studies planned?

- ☐ No.
☐ Yes.

3.4.1. Categories D and / or E procedures - list and describe by filling out the table below

Procedure for Categories D and / or E studies	Analgesic, sedative, or anesthetic used, plus dose, route, and duration for Cat. D studies	Person(s) doing the monitoring for Cat. D and / or E studies	Frequency of monitoring after the procedure and how long animals will be monitored for Cat. D and / or E studies

3.4.2. Category E procedure(s) - describe each and justify fully why pain or distress relief cannot be provided.

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D. CONSIDERATION OF ALTERNATIVES AND PREVENTION OF UNNECESSARY DUPLICATION

Alternatives include methods that (1) *refine* existing tests by minimizing animal distress, (2) *reduce* the number of animals necessary for an experiment, or (3) *replace* whole – animal use with *in vitro* or other tests. See next page for references.

1. If any procedures fall into Pain Classification D or E, describe your consideration of alternatives and your determination that alternatives are not available.

2. Could any of the animal procedures described in this PROTOCOL be replaced by computer models or *in vitro* techniques? If not possible – justify:

No, because computer models would not allow us to investigate the outcome of DIQ with targeted therapies in xenograft models. Only animal experiments would address this question.

3. Could a different animal model or different animal procedure that involves 1) less distress, pain, or suffering, or 2) fewer animals substitute for any proposed animal model or animal procedure planned? Justify:

This model does not involve distress or pain to animals and has been widely used by others. Fewer animals would not allow us to attain statistical significance.

4. Does the proposed research duplicate previous work? If yes, justify.

This proposal does not duplicate previous work, there are no studies that address the antitumor effect of DIQ in *in vivo* model of colon cancer

FRAME Guide to Searching for Alternatives to the Use of Laboratory Animals

<http://www.frame.org.uk/Searching%20for%20Information//Search%20Guide%20Index.htm>

The statutory/policy bases for consideration of alternatives (U.S.)

<http://altweb.jhsph.edu/regulations/bases.htm>

Databases (AWIC list)

<http://www.nal.usda.gov/awic/alternatives/alternat.htm#databases>

Databases (UCCAA list)

http://www.vetmed.ucdavis.edu/Animal_Alternatives/database.htm

Thesaurus for Animal Use Alternatives (AGRICOLA)

<http://www.nal.usda.gov/awic/alternatives/altfact.htm>

Alternatives and the Animal Welfare Act (AWIC brochure)

<http://www.nal.usda.gov/awic/alternatives/Altbrochure.pdf>

Searching for Alternatives to Painful Procedures Used on Research Animals (NIH Library Handout)

<http://nihlibrary.ors.nih.gov/training/AlternativesSearchTips2-5-04.pdf>

AWIC workshops: Meeting the Information Requirements of the Animal Welfare Act

<http://www.nal.usda.gov/awic/awicworkshops/awicworkshops.htm>

If assistance is, at all, needed, please contact Animal Care Facility at:

Ext.: 4741, 4742, 4743

E. HUSBANDRY PROCEDURES

1. Fill out the "Request Form for Ordering Animals and Husbandry Services".
 - Name the person(s) from your staff who will be authorized to order and / or take animals from the Animal Care Facility

Name	Position	Signature
Alissar Monzer	PhD Candidate	

2. Will social animals be housed singly? **NO**
If YES - Please provide a justification for housing social animals singly.

3. Will rodents be housed on suspended wire mesh floors or other flooring in which the animals do not rest on bedding?
 - ☒ No.
 - ☐ Yes

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Is caging with wire mesh flooring necessary? If YES, why?

NO

3.2. Will genetically engineered or genetically modified animals be used that exhibit any characteristic clinical signs or abnormal behavior related to their genotype?

- ☐ No.
☐ Yes.

If YES - describe here:

4. Will major survival surgery be performed?

- ☒ No.
☐ Yes.

If YES- complete Attachment "Major Survival Surgery"

5. Will any cannulae, acrylic implants, venous catheters, or other similar medical devices be implanted into an animal such that the device extends chronically through the skin?

- ☒ No.
☐ Yes.

If YES - explain what implantation and wound management measures will be taken and what special husbandry are needed to minimize the chances of chronic infections around the device(s) where it/ they penetrate the skin.

6. Will any body fluids, tissues, or implanted devices or materials be collected from animals BEFORE euthanasia?

- ☒ No.
☐ Yes.

If YES- complete Attachment "Ante mortem Specimen Collection"

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F. SPECIAL HUSBANDRY AND PROCEDURES

Do you need any special husbandry procedure(s) as detailed below

- ☒ No

☐ Yes

If Yes – specify:

- temperature extremes
- food and / or water deprivation
- dietary manipulations
- calorie restrictions
- special housing / caging
- modified light cycle
- special animal monitoring
- special restraint practices,
- prolonged physical restraint practices
- unusual means of identification,
- use of noxious stimuli,
- forced exercise,
- behavioral manipulation.

Provide a complete description of any of the above and justify.

G. HOUSING SITES AND LOCATION OF PROCEDURES

1. Will all animals be housed only in the Animal Care Facility?

- ☒ Yes.
☐ No.

2. If NO - give the location(s) outside of the animal facility, where animals will be housed permanently or temporarily (more than 6 hours).

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3. Indicate **where** all non-surgical procedures and manipulations will be performed. Give the **location** of any laboratory or other areas outside the animal facility in which animals will be manipulated in any way.

- radiography,
- fluoroscopy,
- computed axial tomography (CT),
- magnetic resonance imaging (MRI),
- others-specify

Describe how animals will be transported to and from these sites.

(Such transport must be discreet such that hospital staff and patients are not aware of the transport, and are not exposed to allergens and/or body fluids from the transported animal(s).)

H. ANTIBODY PRODUCTION

Will animals be used to produce monoclonal or polyclonal antibodies, or will existing hybridoma cell lines be injected into animals to harvest antibody?

- ☒ No.
☐ Yes.

If YES – complete Attachment “Antibody Production”

I. TEST SUBSTANCES

Will Test Substances be administered to animals?

- ☐ No.
☒ Yes.

If YES - specify:

- ☐ radioisotopes,
- ☐ toxins,
- ☐ antigen,
- ☐ pharmacological agents,
- ☐ infectious agents,
- ☐ carcinogens or mutagens,
- ☐ biomaterials,
- ☐ prosthetic devices
- ☐ others **Diiminoquinone DIQ**

Complete Attachment “Test Substances”

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J. ENDPOINT CRITERIA

What specific endpoint criteria will be used for determining when sick animals, both on and off study, will be euthanatized or removed from a study?

Examples of appropriate criteria that should be considered include:

- Weight loss limit as a percentage of initial or expected body weight.
- Allowable duration(s) of anorexia.
- **Allowable tumor size or total tumor burden expressed**, for example as percent of body weight.
- The presence of health problems refractory to medical intervention.
- Severe psychological disturbances.

Other criteria appropriate for the species under consideration should also be considered.

1. **Weight loss: loss of 20-25% of body weight**
2. **Weakness and inability to obtain feed or water**
3. **Infection: infection involving any organ system in which the animal fails to respond to antibiotic therapy within an appropriate time.**
4. **Signs of severe organ system dysfunction non-responsive to treatment**

K. EUTHANASIA

Will animals be euthanatized as part of the planned study?

- ☐ No. (Describe the final disposition of the animals)
- ☒ Yes

1. Describe the exact method of euthanasia for each animal used. Include the agents used, their doses (as applicable), and route of administration:

Isoflurane (ISO) inhalation

2. List the personnel who will perform euthanasia. **Animal Facility Staff**

L. Attachments

- ☐ "Husbandry Service Request"
- ☐ "Surgery"
- ☐ "Ante mortem Specimen Collection"
- ☐ "Antibody Production"
- ☒ "Test Substances"

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M. CERTIFICATIONS

1. Certification by Principal Investigator(s).

I certify, to the best of my knowledge, that the information provided in this "Research Protocol Approval Form" (PROTOCOL) is complete and accurate.

I understand that IACUC approval:

- is valid for one year only,
- that approval must be renewed annually,
- that every third year the IACUC must perform a new review of my protocol,
- that I might be required to complete a newer version of the PROTOCOL and provide additional information at the time of the triennial review.

I also understand that IACUC approval must be obtained

- **before** I use additional animal species, increase the number of animals authorized, or increase the number of procedures performed on individual animals;
- **before** I change procedures in any way that might increase the pain/distress category in which the animals are placed, or might otherwise be considered a departure from this approved PROTOCOL;
- **before** I perform additional procedures not described in this PROTOCOL;
- **before** I allow other investigators to use these animals on other protocols, or use these animals on another of my IACUC-approved protocols.

I further certify that:

- No personnel will perform any animal procedures until they have been approved by the IACUC. When new or additional personnel become involved in this PROTOCOL, I will submit their qualifications, training, and experience to the IACUC and seek IACUC approval before they are involved in animal studies;
- I will ensure that all personnel are enrolled in an institutional Occupational Health and Safety Program prior to their contact with animals.
- I will provide my after-hours telephone numbers to the animal care staff in case of emergency.

Name of Principal Investigator(s)	Signature	Date
Hala Gali-Muhtasib	Hala Gali-Muhtasib	January 20, 2019

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2. Approval Signatures. To the best of their abilities, the undersigned verify that the IACUC has evaluated the care and use of the animals described in this PROTOCOL in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy and the Guide for the Care and Use of Laboratory Animals, and find the use of animals described in this PROTOCOL to be appropriate.

Name of Attending Veterinarian	Signature	Date

Name of IACUC Chair	Signature	Date



