AMITY UNIVERSITY MAHARASHTRA

Established vide Maharashtra Act No.13 of 2014, of Government of Maharashtra, and recognized under Section 2 (f) of UGC Act 1956.

Institutional Ethics Committee (IEC)

Amity University Maharashtra (AUM)

S.No.	Name & Affiliation	Role in IEC
1	**Dr. Dhananjaya Saranath,** Executive Director, Cancer Patients Aid Association (CPAA), Mumbai	Chairperson
2	**Dr.Deepak Modi**, Scientist-F, ICMR-National Institute for Research in Reproductive Health (NIRRH), Mumbai	Vice-Chairperson
3	**Dr. Aparna Khanna,** Dean (Research-S&T) Amity University Maharashtra &; Director, Amity Institute of Biotechnology (AIB), AUM, Mumbai	Member-Basic Medical Scientist
4	**Dr. Subhrajit Biswas,**Professor,Amity Institute of Molecular Medicine & Stem Cell Research (AIMMSCR), Amity University Uttar Pradesh, Noida.	Member-Basic Medical Scientist
4	**Dr. Rakesh Rai,**Senior Consultant Surgeon, Transplant program-Fortis Hospital, Mumbai	Member-Clinician
5	**Dr. Ekta Dubey,**Consultant Histopathologist, Fortis Healthcare, Mumbai	Member-Clinician
6	**Dr. Rajesh Korde,**Executive Director-Histopathology and Immunohistochemistry, iGenetic Diagnostics Private Limited, Mumbai	Member-Clinician
7	**Dr Parag Patil**, Assistant Professor, Department of Pathology & Laboratory Medicine, All India Institute of Medical Sciences (AIIMS), Bibinagar, Hyderabad	Member-Clinician
8	**Adv. Shruti Kanikdale,**Independent Practice, Mumbai	Member-Legal expert
9	**Dr. Mouleshri Vyas,**Professor, Centre for Community Organisation and Development Practice, School of Social Work, Tata Institute of Social Sciences, Mumbai.	Member-Social scientist
10	**Shri Chamu Rana,** Chairperson, Himalaya Parvatiya Sangh, (A registered NGO), Mumbai	Member-Layman
11	**Dr. Sujeet Kumar**, Principal Investigator & Head, Centre for Proteomics & Drug Discovery, AIB, AUM, Mumbai	Member Secretary

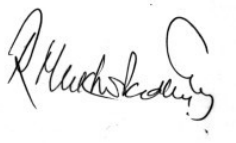
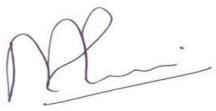
Date: April 1st , 2021

Registrar

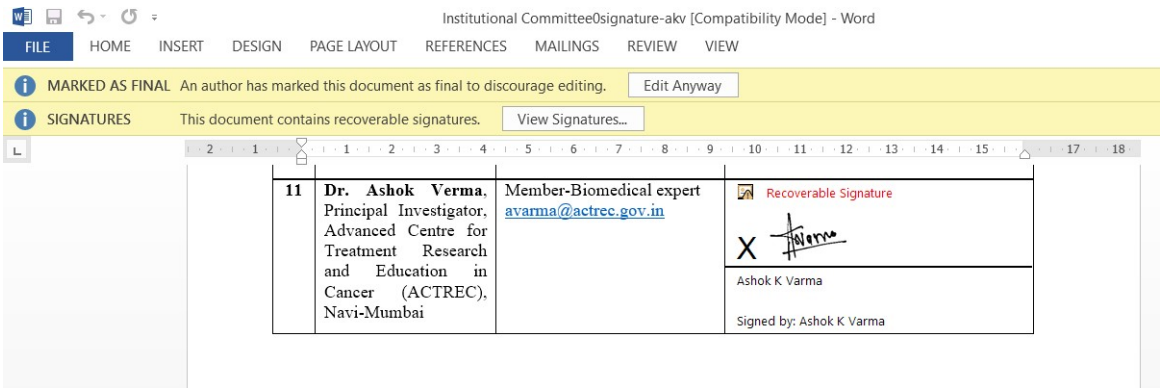
AMITY UNIVERSITY MAHARASHTRA

Established vide Maharashtra Act No.13 of 2014, of Government of Maharashtra, and recognized under Section 2 (f) of UGC Act 1956.

Institutional Committee on Stem Cell Research (IC-SCR)Amity University Maharashtra (AUM)

S.No.	Name & Affiliation	IC-SCR Designation	Signature
1	**Dr. Rabindranath Mukhopadhyaya,** Former Professor, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Navi Mumbai	Chairperson Rmukho.1951@gmail.com	
2	**Dr. Mohan Wani,** Scientist-G, National Centre for Cell Science (NCCS), Pune	Vice- Chairperson mohanwani@nccs.res.in	
3	**Adv. Shruti Kanikdale,** Associated with IPR law firm, Navi Mumbai	Member-Legal expert Shruti.kanikdale@gmail.com	MOM confirmed via email
4	**Dr. Navin Khattry,** Deputy Director, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Navi-Mumbai	Member- Ethics/Clinical Expert nkhattry@gmail.com	
5	**Dr. Bindhulakshmi P,** Associate Professor, Tata Institute of Social Sciences (TISS), Mumbai	Member-Social Scientist bindhulakshmi@tiss.edu	
6	**Dr. Rajarshi Pal**, Chief Scientist, Eyestem Research, Bangalore	Member-Stem Cell Expert palrajarshi12@gmail.com	MOM confirmed via email
7	**Dr. Prathibha Shetty,** Principal Scientist, Reliance Life Sciences, Mumbai	Member-Stem Cell Expert pratibha.shetty@relbio.com	MOM confirmed via email
8	**Dr. Jaya Lakkakula,** Assistant Professor, AIB-AUM, Mumbai	Member Secretary jrlakkakula@mum.amity.edu	
9	**Prof Ravindran K**, Assistant Professor, Tata Institute of Social Sciences (TISS), Mumbai	Member-Layman ravispif@gmail.com	MOM confirmed via email

10	**Dr. Geetanjali Sachdeva,** Scientist F, ICMR- National Institute for Research in Reproductive Health (NIRRH), Mumbai	Member-Biomedical expert sachdevag@nirrh.res.in	MOM confirmed via email
11	**Dr. Ashok Verma**, Principal Investigator, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Navi-Mumbai	Member-Biomedical expert avarma@actrec.gov.in	Unedited signature file Hence attached separately



AMITY UNIVERSITY MAHARASHTRA

Established vide Maharashtra Act No.13 of 2014, of Government of Maharashtra, and recognized under Section 2 (f) of UGC Act 1956.

Minutes of the 1st meeting of AUM Institutional Committee on Stem Cell Research (IC-SCR)

Date: April 23rd, 2021

Mode of meeting: Online platform (Zoom)

The meeting began with a brief introduction of all the members. The member-secretary welcomed the members and then requested the Chairman to conduct the meeting. The Chairman requested the members to self-introduce themselves. The Chairman then asked the Principal Investigator (PI) of the project to make the presentation. The Co-PI of the project was also present.

The proposal presented was, **"Use of human induced pluripotent stem cells derivedliver organoids in Bioartificial Liver device (BAL) as bridge device for acute liver failure".** IC-SCR Protocol Number: AUM-IITB/IC-SCR/2021-1. The project proposalaims to generate liver organoids from human induced pluripotent stem cells by developing hollow fibre membrane (HFM) based three dimensional Bioartificial liver Devices (BAL). The main aim of the study is to differentiate human induced pluripotent stem cell (hIPSC) lines into functional hepatocytes by organoid formation and incorporating them into extra corporeal device which will act as biological support, till the time an organ (liver) is available for transplantation. hIPSC lines will be differentiated into hepatocytes. A hollow- fibre membrane (HFM) based system will be developed. The expansion of the hepatocytes will be done to obtain sufficient numbers of functional hepatocytes. Finally, the *in vitro* functional assessment of the BAL will be performed.

Following recommendations were made by Chairperson and members:

1. It was suggested that more clarity on the differentiation protocol (Cytokines/growth factors used) for generation of hepatocytes should be included in the proposal.

The PI stated that the methodology for hepatic differentiation of hIPSC line will be the same as described elsewhere (Chitrangi et al., 2016; Kulkarni and Khanna, 2006) with minor modifications. Briefly cells will be cultured on Matrigel (BD Biosciences) coated plates (2D) for 28-30 days by using two step hepatic differentiation protocol. Matrigel is used as an ECM component to provide the microenvironment for hepatic differentiation. For differentiation the cells will be treated for 12 days using DMEM-LG media supplemented with 0.5% FBS (v/v), 20 ng/ml hepatocyte growth factor (HGF; R&D Systems), 10 ng/ml fibroblast growth factor 4 (FGF4; R&D Systems). Further, a maturation step will be induced by DMEM-LG supplemented with 0.5% FBS, 10^{-7}dexamethasone, (Sigma), 1× insulin–transferrin–selenium premix (ITS; BD Biosciences)and 10 ng/ml oncostatin M. For liver organoid formation, similar protocol will be followed with the suitable 3D scaffold/HFM device. The detailed methodology has now been incorporated in the revised submission (attached).

2. A clarification was sought with respect to whether hepatocytes alone are enough or other cell types will be required to maintain functionality of derived hepatocytes.

The PI mentioned that 70-80% of the cells in the liver are hepatocytes and are the functional cells. However, some other cell types (endothelial/macrophages) also constitute the remaining 20-30%, and may contribute to maintaining the functionality of the hepatocytes. Hence various strategies will be employed if required, like co- culture with the other relevant cell types to maintain functionality of generated hepatocytes. Hence care wouldbe taken to see that the liver organoids closely mimic liver tissue.

3. The details and the nature of procurement of the hIPSC lines to be used in the study was asked by the members and more clarity was sought.
The various sources of procurement of characterized hIPSC lines will be i) Instem, Bangalore; ii) WTSIi070-A: A hIPSC line from European Bank of Induced Pluripotent Stem Cells (EBiSC) procured from Sigma Aldrich and iii) A gift from Dr Rajarshi Pal.

4. The members mentioned that a positive control in the studies should be included.

The PI replied that both human hepatocellular carcinoma (hepG2) cell line and human primary hepatocytes purchased from commercial sources (Sigma Aldrich/ Cytesbiotechnologies) will be used, as a positive control in the study. She also stated thatmost of the available BAL devices use either porcine hepatocytes or hepG2 cell line as thesource of hepatocytes.

5. Another query was with respect to the percentage of differentiation and whether there will be any undifferentiated/mixed population of cells in the formed liver organoids. The PI mentioned that at different days of (Day 1 to Day 28) of differentiation the efficiency of differentiation will be checked using specific markers of hepatocyte differentiation to ensure maximum hepatic differentiation has occurred. Further to ensure a pure population of cells, sorting of the cells can be achieved by using a specific hepatocyte marker. However, at the stage of liver organoid formation that may be an issue since the cells will have to be dispersed. However, the percentage of various population of cells at the time of 2D differentiation will be optimised to determine a maximum number of differentiated cells at the end of differentiation (Day 28-30).

6. The members enquired with respect to the genetic alterations that may come up given that the cell line will be passaged for a number of times.
The PI answered that before initiating differentiation, the cell line will be characterized andas part of the characterization, karyotypic abnormality is checked to ensure no genetic abnormality has been introduced during passaging.

7. The members inquired to clarify at what stage of differentiation the liver organoids would be cultured on the BAL device.

The PI specified that at day 15-18 post-differentiation, the hiPSC-hepatocytes, will be harvested for the Hollow fibre membrane (HFM) bioreactor culture, for formation of liver organoids. The liver organoids will be kept in culture for 3 weeks. During this time expression of proteins such as CYP isoenzymes CYP1A2, CYP2B6, CYP2C9, and CYP3A4, ALBUMIN at transcript and protein level would be checked. Simultaneously, a 2D culture will also be differentiated to compare the hepatic maturation status between bioreactor cultured hepatocytes and 2D cultures. Cells will be seeded on the extra-capillary space of the HFMs and a bioreactor system is developed where media is passed continuously. The unique combination of the fibre matrix and the 3D engineered environment provided is expected to help the liver organoids to remain metabolically active.

8. The members asked to elaborate on how the functionality/viability of the derived hepatocytes would be shown.

As mentioned earlier, a gene expression as well as protein profiling will be done at various days of differentiation to understand whether hepatic differentiation occurs and also whether the mature hepatocytes are derived. For eg, AFP is a marker of immature hepatocytes (fetal endoderm marker) will determine that hepatocyte differentiation has taken place but at the later stages of differentiation there should be a down regulation of expression, indicating that now mature hepatocytes have been formed. Similarly, late-stage marker profiling will be done to ensure that maximum mature hepatocytes are formed by day 28-30. This will ensure detailed characterization of the hepatocytes before seeding on the HFM.

For functional assessment of liver organoids, urea synthesis, albumin secretion, measurement of metabolic activity of CYPs , staining for glycogen deposits etc will be performed. Hence a variety of functional assays will determine the viability of the derived hepatocytes.

9. The members asked to provide a more detailed assessment of the efficacy of the whole system on the scaffold with regard to the metabolites that will be measured to show that this system is effectively detoxifying harmful metabolites.

The development of an iHep-based bioartificial liver device is to be used as a substitute for the metabolic and detoxification functions of the liver. Hence, the liver CYP system is crucial for degradation and clearance of endogenous metabolites, hormones and xenobiotics will be tested by adding drugs and checking by metabolite profiling. The clearance performance and functionality of the HFMs seeded with cells will be evaluated using simulated fluids and it will be passed through the lumen of HFMs and the clearance performance will be evaluated of the stimulated fluids. The samples will be spiked with the pre-determined values of toxins to attain their levels in ALF patients. Along with the detoxification analysis (urea synthesis), protein synthesis and metabolic functions of liver cells will be evaluated by determining human plasm proteins, albumin secretion, apoB, and prothrombin. The liver

specific markers to present protein synthesis and metabolic functions of liver cells will be studied by determining albumin secretion, and urea synthesis in the spent cell culture media by assays/ detection kits.

Thus, the proposed HFM would be a three-dimensional matrix-based bioreactor and will offer both detoxification, secretory function by maintenance and viability of liver organoids. In the clinical scenario, impure blood will flow through the lumen of HFMs and cells will be cultivated on the extracapillary space of the HFMs. The purified pure blood will be again recirculated to the body.

10. It was commented that the role of the members of the constituted IC-SCR should be as per the NAC- SCRT guidelines.
The member- secretary mentioned that the composition, functions and responsibilities of the constituted AUM IC-SCR was based on the recommendations of the ICMR-DBT guidelines for Stem Cell Research.

Overall, the members commented that project was well-written with clear objectives and was presented well. The project is very relevant and has tremendous translational potential. Further, the inclusion of the clinical collaborator at the beginning of the project will definitely help in successful clinical translation. The members recommended that although the project has been submitted for funding, if seed money could be given from the University, preliminary work on the project could be initiated. One of the member couldn't attend the meeting due to miscommunication but has agreed to sign after going through to minutes of meeting and proposal

The meeting ended by thanking the members.

Member-Secretary

(Dr. Jaya Lakkakula)

Chairman

(Dr.Rabindranath Mukhopadhyaya)

AMITY UNIVERSITY MAHARASHTRA

Established vide Maharashtra Act No.13 of 2014, of Government of Maharashtra, and recognized under Section 2 (f) of UGC Act 1956.

Ref: AUM/AIB 001/IC-SCR//2021 Date: 10th May 2021

Amity University Mumbai- Institutional Committee on Stem Cell Research (AUM-IC-SCR)

To
Principal Investigator
AIB, AUM

Subject: Project Proposal No. AUM-IITB/IC-SCR/2021-1

The Committee has reviewed and approved the proposal entitled "Use of human induced pluripotent stem cells derived liver organoids in Bioartificial Liver device (BAL) as bridge device for acute liver failure" in its meeting held on 23rd April 2021 for a period of three years. The progress report and final report of the proposal should be submitted to the Committee.

Signature of Member Secretary,
AUM- IC-SCR
(Dr. Jaya Lakkakula)

Signature of Chairperson,
AUM- IC-SCR
(Dr. Rabindranath Mukhopadhyaya)