Registry Protocol

Title: **Establishment of a tissue registry for hepatocellular carcinoma specimens for genetic and histologic research**

Study Key Name **TIR-HCC**

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SITE INVESTIGATORS SIGNATURE PAGE

Protocol Title	Establishment of a tissue registry for hepatocellular carcinoma biopsy specimens for genetic and histologic research
Short Title	TIR-HCC registry
Lead Investigator	Hasmik Ghazinyan, MD, PhD
Academic Affiliation	Yerevan State Medical University
Protocol Version 1.0	
Version Date	June 6, 2023

I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated.

Site Principal Investigator Name

Site Principal Investigator Signature

Date:

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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	*Adverse event*
AFP	*Alpha fetoprotein*
BIMS	*Biobank information management system*
EC	*Ethical committee*
GCP	*Good clinical practice*
HCC	*Hepatocellular carcinoma*
ICH	*International Council for Harmonization*
IRB	*Institutional review board*
POA	Powers of attorney
PHI	*Personal Health Identifiers*
SAE	*Serious adverse effect*
WHO	*World Health Organization*

SUMMARY

Aspect	Description
Registry title	Establishment of a TIssue Registry for HepatCcellular Carcinoma biopsy specimens for genetic and histologic research (TIR-HCC)
Background	Armenia has one of the highest incidence rates of primary liver cancer in the region according to WHO Globocan initiative.
Objective	To establish a cancer registry to facilitate research and assist in the identification of additional risk factors for hepatocellular carcinoma.
Study Design	Observational study with mixed retro- and prospective data elements.
Participants	Patients of Armenian descent with clinical, biochemical, and imaging evidence of hepatocellular carcinoma.
Data Collection	Tissue and blood samples will undergo histologic and genetic analyses, as well as relevant clinical history, laboratory and imaging results. Gut microbiota profiles will also be sampled. Samples will be collected from 8 institutions in Armenia.
Sample Size	The registry aims to collect data from 500 patients.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

According to the most recent epidemiological assessment conducted by the World Health Organization, hepatocellular carcinoma (HCC) emerged as a major threat for public health in Armenia. Indeed, Armenia has one of the highest incidence of primary liver between the Atlantic Ocean and the Chinese border[1]. The epidemiological bases of the problem are primarily linked to the spread of hepatitis C virus (HCV) epidemics in the country in the aftermath of the independence from the Soviet Union (September 1991)[2].

It is well known, however, that liver cancer is a multi-factorial disease that appears in patients submitted sequentially or concomitantly to several risk factors (e.g. hepatitis C virus infection and heavy alcohol intake, or hepatitis B virus infection and aflatoxin B1 intoxication). Moreover, recent publications from the International Cancer Genome Consortium (ICGC) have revealed that hepatitis viruses are not responsible of mutation fingerprints left on tumor genome as it is various chemical compounds rather than infectious agents that trigger mutations[3]. In Armenia, historical records suggest that chemicals (e.g. chloroprene) may play an active role in the liver tumorigenesis of some patients[4]. However, the spectrum of risk factors is poorly known in Armenia and hence public health measures aiming to prevent HCC development are difficult to implement.

Recent data taught us that the genome of HCC is characterized by a multiplicity of molecular signatures that provide solid clues concerning mutagenic factors involved in disease history from initiation to final progression[5].

There is scarce data on the pathologic and genetic subtypes as well as gut microbiota profiles of HCC in the Armenian patient population. Most pathologic exams are done for the confirmation of diagnosis, and there is no application of data in clinical or scientific research. Moreover, there is limited data on etiology and epidemiology of liver cancer in Armenia, even though the cancer profile is concerning compared to other similar countries[1]. Thus, the establishment of a cancer tissue registry ensures a long term availability of tissue samples for clinical as well as scientific research purposes. In particular, the analysis of molecular changes prevailing in tumors from Armenian patients will, help clinicians and public health stakeholders to identify additional effectors of liver tumorigenesis and to take appropriate measures to reduce the burden of severe liver diseases in the country. The translational data can be used by researchers from other areas to establish relevant clinical and scientific findings.

1.2 Compliance Statement

This study will be conducted in full accordance all applicable laws and regulations of the republic of Armenia and with the principles of ICH-GCP. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain the signed informed consent of the patients and will report unexpected problems in accordance with medical center, Ethical committee (EC) Policies and Procedures and all official requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to provide a mechanism to store the information about subjects with hepatocellular carcinoma.

2.2 Secondary Objectives

Secondary objectives are translational:

- identify the pathologic substance and the main gene targets of mutations (TP53, CTNNB1, TERT) in HCC from Armenian patients
- establish the nucleotide mutation spectrum of these alterations with the ultimate aim to identify what are the mutagenic agents responsible of these changes.
- to determine whether circulating genetic alterations in patients with HCC are suitable for diagnostic, prognostic or follow-up purposes.
- identify presence of specific gut microbiota profiles in patients with HCC

The samples may also be used for research purposes by professionals from multiple specialties, such as surgery, internal medicine/hepatology, genetics.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Registry/Repository Design

The repository will collect tumor tissue samples, buffy coat/plasma samples, and gut microbiota samples from patients with hepatocellular carcinoma. The patients will be introduced to the registry by assigned multidisciplinary teams from 8 institutions (*see **APPENDIX A***):

- Nikomed Medical Center,
- Violeta Medical Center,
- Wigmore Clinic - Medical Center,
- Mikaelyan Institute of Surgery of YSMU,
- Erebouni Medical Center,
- The National Center of Oncology after V.A.Fanarjyan,

- Nork Infection Clinic Hospital
- Keck Hospital of USC

The preliminary storage from the time of collection will be done at the pathologic laboratories HistoGen and Davidyans.

The final destination for tissue specimen storage is Ecosense laboratories in Armenia.

The specimens will be mailed to Institut Pasteur (through tissue mailing systems) for genetic typing, and then mailed back to Armenia for final storage in Ecosense laboratories.

Blood specimens will be stored at the Institute of Molecular Biology "Santé Arménie" Bio-bank in Yerevan, Armenia and undergo the same mailing and return mechanism as the tissue samples.

Gut microbiota samples will be stored in Biological Resource Centre of the Institute of Molecular Biology of Nation Academy of Sciences (NAS), in Yerevan, Armenia, and be profiled upon request from respective investigators and study groups.

3.1.1 Description of the Collecting Sites

Each participating institution based policies will apply for all procedures and workup.

3.1.2 Overview of the Data/Biospecimen Collection

The specimens will be collected from patients with biopsy-confirmed diagnosis of HCC. Registry organization systems will manage the tissue further. This is be addressed more detailed in section 5.

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Study

The repository will include both retrospective and prospective data. Retrospective data will include post year 2021 cases. The registry will also include prospective patients with new diagnoses of hepatocellular carcinoma, and their tissue samples will be histologically and genetically studied and recorded. Each participant is expected to be followed for 5 years. The duration will encompass the time from the inclusion of the first patient till the end of follow-up of the final patient and is estimated to be around 10 years (5 years of inclusion plus 5 years of follow-up of each patient).

3.2.2 Total Number of Study Sites/Total Number of Subjects Projected

We have identified 220 retrospective patients diagnosed with hepatocellular carcinoma in 7 investigative sites in Armenia (all patients are post-2021). The majority of the biopsy samples (160 out of 210 or 73%) have been genetically types and histologically studied as well. We have 50 samples that are pending genetic and histologic identification. In addition, we plan to include prospective cases and expand the data range up to 500 patients.

3.3 Study Population

3.3.1 Inclusion Criteria

i. Patients with biopsy-confirmed diagnosis of HCC
ii. Armenian descent
iii. Having given their signed Informed consent

3.3.2 Exclusion Criteria

i. Failure to obtain informed consent

4 STUDY PROCEDURES

4.1 Medical/Phenotype Data Collection Procedures

4.1.1 Data Collection

Initial part of the data will include epidemiologic and demographic information from patients. We will also include relevant clinical history as well as laboratory and imaging results (if applicable/necessary) to cases (*see **APPENDIX B***). Those include past medical and social histories, hepatitis and liver panels, US and CT scans, neo-adjuvant or any other form of therapy or surgical therapy, immune or hormone based therapy. Pathologic data about the tissue characteristics will also be included, and additional studies may be conducted to characterize the tissue further. DNA isolated (Insitut Pasteur) from the specimens will be studied for several mutations. All data will be recorded.

4.1.2 Data Elements

i. Tumor and liver tissue samples resulting from surgical resection
ii. 10 mL of whole blood collected on EDTA
iii. Stool samples for further gut microbiota profiling

4.1.3 PHI Elements Collected

The personal identifiers will include assigned gender, the date of birth, place of residence (to assess epidemiological exposure risk).

4.2 Biospecimens

4.2.1 Specimen and Collection Procedures for First Specimen

Pathologic identification of the tissue sampled will be done at Histogen and Davidyants laboratories in Yerevan, Armenia. Genetic studies will be carried out at the Pasteur Institute in Paris, France.

5 REGISTRY/PREPOSITORY ADMINISTRATION

5.1 Study Organization

The repository is planned to have 4 main parts: a director, a steering committee, a research committee and operational group(s).

The director will be the main representative and acting body of the repository, and the steering committee members would make all the relevant decision regarding different issues.

The research committee will monitor the data and identify any clinically or scientifically relevant information for further research purposes.

The operational group(s) is assumed to control the tissue collection and management activities, as well as ensure safety measures. The registry will work with multidisciplinary teams composed of radiologists, hepatologists, pathologists, surgeons, oncologists, and laboratory specialists to gain insights about possible donors, and will work on recruitment. A brief flowchart describing the process in presented in ***Figure 1***.

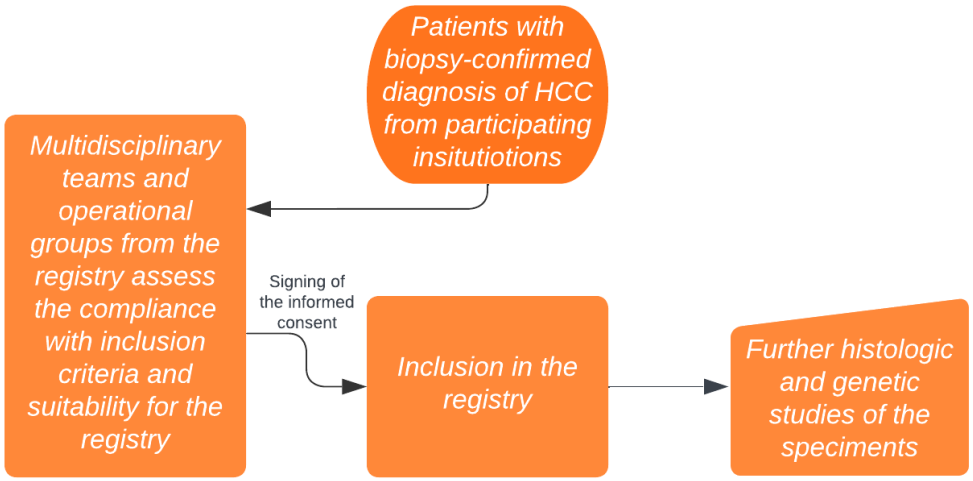

Figure 1: A flowchart overviewing the sample inclusion procedure in the registry

Any interested organization/researcher shall request a quote from the registry, and after careful review of the request, decision will be made as of whether the tissues should be granted for research or not. In additional, all applicants are required to submit an EC approval of their respective research projects.

5.2 Data Collection and Management

5.2.1 Computer Systems

All data will be stored on software platform (generated my Microsoft Excel©) for effective biobank information management systems (BIMS). The data will be accessible to the operational group(s) and the registry director.

5.2.2 Confidentiality of Subjects

The chief method for protection of confidentiality is the utilization of adequate BIMS technology. The labeling specimens with unique identification codes is one of the primary

protection mechanisms. The identification codes will be safely generated by a computer system and would not include any data about the donors whatsoever. Only limited number of personal will have access to the data management system, and the access to patient-identification code translation data would be granted by the director of the registry themselves (in case of data loss or mislabeling or any other pertinent issue such as usage of data in clinical management of particular patients).

5.3 Biospecimen Collection and Management

Tissue samples will be collected from the operation theater or from the pathology departments of all participating institutions. Identification codes (in a sequential order) will be assigned to the samples, and all further handling will only include the unique identification codes of the patient (decoding available only at the discretion of the biobank director).

The samples will be stored at -80°C or at -30°C if not -80°C is available. Genomic DNA will be subsequently extracted from tissues using the gold standard proteinase K digestion followed by phenol-chloroform extraction and cold ethanol precipitation. Samples will, then, be quantified, and quality controlled. Those suitable for further analysis will be sequenced or genotyped on selected hotspots known to be frequently altered in HCC (TP53, CTNNB1, TERT, AXIN1, ALB, H/K/N-RAS, NFE2L2/KEAP1, FGF19, CDKN2A). Mutations rate and spectra will be determined and correlation with clinical-pathological background will be actively searched through systematic statistical analysis and multidimensional investigation (hierarchical clustering, Bayesian analysis).

Biospecimen collection will take place from plasma and buffy-coats of the patients as well. Extraction of genomic DNA will be conducted similarly to that practiced on liver tissues except that precipitation will take place overnight in a -80°C freezer to ensure a thorough precipitation of small size DNA fragment shed in bloodstream as cell free DNA (cfDNA). DNA will be quantified using Qubit technique and analyzed by droplet digital PCR (DD-PCR) for the presence of TERT -124C>T or TP53 R249S mutations.

The fecal microbiota samples will be stored in cryovials under -80˚C conditions in the Biological Resource Centre of the Institute of Molecular Biology of NAS RA.

5.4 Providing Results to Subjects

The results of histologic identification may be used in diagnosis confirmation by oncologists and other relevant managing physicians. The genetic typing of tissues is done for research purposes and patients or physicians will not be informed about the results. However, if there are any incidental findings that have any clinical significance, patients or respective powers of attorney (POA) will be informed about it. The decision of whether any managing physician should be informed shall be decided by the patient/POA.

5.5 Regulatory and Ethical Considerations

5.5.1 Risk Assessment

The primary and most concerning risk is the breach of privacy and confidentiality of donors. Multiple systems of privacy management will be instituted to ensure that this is prevented, but there is still a small risk of mismanagement or staff mishandling that may result in a probable breach of confidentiality.

In addition, specimen management also includes risks of possible contamination of the products or mishandling by staff members. Likewise, appropriate training in laboratory skills and tissue management will ensure that these risks are minimized. There are minor risks with possible inoculation of staff members, in which up-to-date lab protocols are efficient prevention strategies as well.

5.5.2 Potential Benefits of Participation

The registry is primarily set up to foster future research in hepatology and hepatic oncology. Such research may prove to be beneficial for understandings in epidemiology, pathogenesis, diagnosis and treatment aspects, and prevention of hepatocellular carcinoma in the Armenian population.

There are also potential clinical benefits, in that the identification of several mutation or histologic subtype of the tissue might guide clinical management of the particular case of the cancer.

5.5.3 Risk-Benefit Assessment

Considering the benefits and the possible risks, we can conclude that proceeding with the creation of the registry includes more potential benefits than risks. With careful administration of management systems, the risks may be minimized while the benefits will be comparatively increased in magnitude.

5.5.4 Ethic considerations

This protocol will be evaluated by the ethical committee of the Yerevan State Medical University. The Ethics Committee will give its opinion as of whether or not the project correspond to ethical norms and are compliant with respective regulations.

The repository administrations would have the possibility to contact the Ethics Committee for any specific issue encountered during study activities.

5.6 Recruitment Strategy

The retrospective data that has been collected will be maintained. All patients have signed an informed consent form For prospective cases, the operational group(s) will work with multidisciplinary teams assigned to the registry. The teams (including radiologists, hepatologists, pathologists, surgeons, oncologists, laboratory specialists) will provide information about potential donors from their current patients, and the operation group members would identify compatibility with the registry's inclusion criteria and will start

working on recruitment. After gaining an informed consent from each prospective participating patient, sample procurement and storage would be initiated.

5.7 Informed Consent/Assent

The patients are given the form of informed consent. The form will inform them about the usage of their personal data in the registry, the pathologic and genetic workup of the specimens, the data protection and safety measures, and every other pertinent information. The form will be developed in accordance with European biobanking standards and institutional policies. If patients are unable to consent, respective powers of attorney will be asked for the consent.

5.8 Confidentiality

All information collected from participants in the registry will be kept strictly confidential and secure, and will only be used for research purposes as outlined in the informed consent documents. Access to the information will be restricted to authorized personnel only, such as operational team members, who are bound by strict confidentiality agreements. Any data or samples collected from donors will be de-identified and coded to protect their identity.

The information will not be shared with any third parties without patients' express permission or unless required by law. We will take all necessary precautions to safeguard the information against unauthorized access, use, or disclosure.

6 SAFETY MANAGEMENT

6.1 Clinical Adverse Events

Unanticipated problems involving risks to subjects and others will be monitored throughout the study.

6.2 Adverse Event Reporting

Since the study procedures are not greater than minimal risk and are limited to existing data and specimens, serious adverse effects are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study these will be reported to the EC.

7 PUBLICATION

Any finding that has possible clinical or scientific significance will be discussed in specially summoned meetings involving the director or the institution and the research committee members. Plan for further investigation and research will be discussed, and possible further research findings might be summarized in forms of articles. Ethical committee approvals should be sought for every such case.

8 REFERENCES

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5. Wang L, Qiu M, Wu L, Li Z, Meng X, He L, Yang B. Construction and validation of prognostic signature for hepatocellular carcinoma basing on hepatitis B virus related specific genes. Infect Agent Cancer. 2022 Dec 6;17(1):60. doi: 10.1186/s13027-022-00470-y. PMID: 36474267; PMCID: PMC9727957.

APPENDIX A

PARTICIPATING CENTERS :

Recruiting centers:

1. Nikomed Medical Center – contact person: Dr Hasmik Ghazinian, address: 93 Raffi St, Yerevan 0064, Armenia
2. Violeta Medical Center – contact person: Violeta Sargsyan, address: 2 Gusan Sherami St., Yerevan 0084, Armenia
3. Wigmore Clinic – contact person: Dr Haykuhi Geokchyan, address: 56 Pushkin St, Yerevan 0002, Armenia
4. Mikaelyan Institute of Surgery of YSMU – contact person: Dr Manik Gemilyan, address: 9 Ezras Hasratyan St, Yerevan 0052, Armenia
5. Erebouni Medical Center – contact person: Dr Aramayis Galumyan, address: 14 Titogradyan str., 0087, Yerevan, Armenia
6. The National Center of Oncology after V.A.Fanarjyan - 76 Fanarjyan str. 52 Yerevan Armenia – contact person: Dr Hasmik Ghazinian, address: 76 Fanarjyan str. 52 Yerevan Armenia
7. Nork Infection Clinic Hospital – contact person: Dr Hasmik Ghazinian, address: 153, Armenak Armenakyan St, Yerevan, Armenia
8. Keck Hospital of USC – contact person: Dr Saro Khemichian, address: 1520 San Pablo St #1000, Los Angeles, CA 90033, United States

Non-recruiting centers:

Tissue storage and genetic typing facility:

- INSERM U993, Institut Pasteur, Paris, France
  Contact person : Dr Pascal Pineau, Agnès Marchio, Unité « Organisation nucléaire et Oncogenèse »

Center for pathologic identification of biospecimens:

- HistoGen pathology center - address: 0014 Nikoghayos Adonts St, Yerevan, Armenia
- Davidyants Laboratories - address: 10, 3 Sasna Tzrer St, Yerevan 0054, Armenia
- Ecosense laboratories – address: 44 Komitas Ave, Yerevan 0051, Armenia

Advising and mentorship:

- Santé Arménie French-Armenian Academic Research center

Biobanking center:

- Biological Resource Centre of the Institute of Molecular Biology of the National Academy of Sciences of the Republic of Armenia, Santé Arménie biobanque; 7 Hasratyan St, 0014 Yerevan

APPENDIX B

A table with a list of historical, laboratory, and imaging data collected from the patients.

History/physical examination/imaging findings:
Assigned gender
Age
Place of residence
Place of living Armenia
Profession
Tobacco (type, daily consumption, years of smoking)
Coffee consumption
Alcohol consumption (volume, type)
Presence of type 2 diabetes mellitus
BMI
Status of treatment with antiviral medication
Grade of liver fibrosis estimated by ultrasonography/elastography
Presence of liver cirrhosis
Child-Pugh Score
MELD score
Estimated survival (months) Presence of liver steatosis
Presence of ascites
Presence of portal thrombosis
Presence of esophageal varices
History of bleeding
Presence of encephalopathy
Unintentional weight loss
Presence of right quadrant pain
Presence of cholestasis
Tumor histology if primary liver tumor (HCC, CCA, others)
Tumor Number
Tumor Diam (mm)
Lymph node involvement
Metastasis status
PIVKA II mAU/mL (≤ 50.9)
TNM stage
IVDU
Presence of tattoos, scars
History of blood transfusions
Presence of sexually transmitted infections
Known hepatitis
History of familial cancers
Previous personal tumors

Past medical / surgical history
Personal Status Score (ECOG, Karnofsky) ____

Lab data:

1. AFP 2. AgHBs 3. anti-HCV 4. AgHBe 5. anti-HBc 6. anti-HBe 7. anti-HBs 8. anti-Delta 9. HIV 10. HBV DNA load 11. HCV PCR 12. ALB (g /L) 13. BILI (microM) 14. INR 15. CREAT (microM) 16. AST (UI/mL) 17. ALT (UI/mL) 18. Platelets 19. GGT (UI/mL) 20. ALP (UI/mL) 21. Plasma Prot (g/%) 22. UREA (mMol/l) 23. Leukocytes 24. HGB (g/L) 25. CRP (mg/L)