EUROPEAN CHOLANGIOCARCINOMA NETWORK (EURO-CHOLANGIO-NET)
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HIGHLIGHTS FROM THE FIRST WORKING GROUP MEETING Action Chair: Vincenzo Cardinale (Italy), Vice-Chair: Jesus Banales (Spain)

The first Working Group Meeting was held at the historical Old University Campus of the University of Malta in Valletta from 12-13 September. Nearly 80 participants from 22 Countries attended this meeting.
Specific objectives of the meeting were to foster and dynamically implement a pan-European multidisciplinary collaboration to overcome the limitation of the small number of cases, improve translation by generating consensus on appropriate experimental models of CCA (WG#1), set up interconnected digitalized European registries for correlation studies on: i) epidemiology, risk factors, and clinical presentation, ii) histomorphology, and iii) radiologic imaging of tumor and preneoplastic tissue (WG#2,#4,#5), define driver mutations, epigenetic alterations, and the transcriptome of each CCA histomorphological subtype to rationalize target therapies (WG#4), gain insights into the determination of new sensitive and specific non-invasive biomarkers for the early diagnosis of each CCA subtype (WG#5), and develop or define novel drugs and strategies (WG#6).
WG parallel sessions and a plenary session were conducted as discussion tables where participants faced the questions: "What is the question behind the question? What is missing? What is next?". In order to facilitate dynamic implementation and the achievement of deliverables and network growth, invited presentations included: Network Medicine and AI in Cancers (Manuela Petti, IT), Tips from a successful COST Action Chair (Bruno Botta, IT), and COST Connect Report on the Future of European Cancer Research (Pedro Rodrigues, SP).
Moreover, a poster session was held to showcase proposals from early career investigators to increase the visibility of participating centers, expand the network, discuss new discoveries and/or new scientific tools applicable to CCA, organize mentoring programs, and promote multidisciplinary and innovative collaboration. Eighteen applications from early career investigators were received from Poland (3), UK (2), Netherlands (3), Spain (3), Sweden, Italy (4), Romania, and Portugal.
This article discusses what has been generated in terms of projects and deliverables by each specific Working Groups (WGs).
Highlights from WG#1 PRECLINICAL, Leader: Diego Calvisi (Germany), Vice-Leader: Laura Fouassier (France)
WG1 aims to review current knowledge and expertise on preclinical models, including in vitro and in vivo models, and technologies that assess experimental systems of CCA, such as 3-D culture and organoids. This WG is essential to support translational research in CCA and understand molecular mechanisms of CCA in terms of i) development, ii) progression, and iii) drug resistance, and to design and test new drugs. In particular, WG1 addresses issues regarding the histomorphology, pathological background, cells of origin, transcriptome, proteome, and molecular profiling of the distinct models of CCA with respect to human subtype counterparts.
Scientists developing models of CCA, particularly animal models, primary and immortalized human cell cultures, organoids, and related patient-derived xenograft (PDX) may contribute to WG#1 by sharing data using customized sheet forms. The creation of these dedicated ad hoc sheet forms was one of the issues discussed during the meeting. Since fundamental research is performed primarily with human CCA cell lines in 2-D culture (one of the most common preclinical models), we plan to establish a registry of commonly used available cell lines and primary cells with standardized criteria including information on phenotype epithelial/mesenchymal and tumorigenic potential, and immune-related receptors. In the cell registry, cellular models to study the microenvironment, including cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and vessel cells, will be included. In order to make preclinical in vitro models more similar to the in vivo situation in patients, organoids and 3-D culture have emerged as alternative models to study CCA biology, drug efficacy, and resistance. Methodologies to revise current literature and reach a consensus have been initiated in collaboration with WG#6.
Translational research is mandatory and has to follow in vivo step wise; development of animal models is therefore crucial because studies in these systems provide a validation step before possible translation to patients. Like in vitro models, a data registry based on the standardized sheet form will be adapted to provide information with respect to human CCA, and will be validated by pathologists and molecular biologists with a transcriptional analysis to gain insight into the models and for comparison with human data. To mimic the human situation, the background of the liver on which the tumor develops has to be taken into consideration. Considering the use of immune therapies in cancer, a better characterization of the immune component is needed for in vivo models. For all models, a major issue is control, which is still a matter of debate. A training

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school planned for 2020 will focus on preclinical and animal models and organoids and will be attended by early career investigators who will develop non-invasive tools for monitoring tumor growth.

Highlights from WG#2 IN-DEPTH HISTOMORPHOLOGICAL PHENOTYPING Leader: Guido Carpino (Italy), Vice-Leader: Benjamin Goeppert (Germany)

CCAs are characterized by a high intertumoral and intratumoral heterogeneity at anatomical and histopathological levels. The histomorphological heterogeneity of these tumors could reflect differences in cells of origin and in the pathological background from which the tumors arose. WG#2 will deal with the histological characterization of CCA in regard to pathological background, molecular alterations, involvement of cancer stem cells, relevant signaling pathways, and potential therapeutic targets.

Apart from the current WHO classification of CCA, many questions remain regarding histological classification and subclassification of CCAs. This is a pressing need for intrahepatic CCA, in which two main histological subtypes have been recognized, i.e. large duct type and small duct type. These two subtypes show different molecular profiles, have different prognoses, and arise from different pathogenetic backgrounds and from different cells of origin. However, no uniform criteria are used to define them and there are tumors that do not fit in the current classification. Moreover, an in-depth correlation of histomorphology with clinical traits and molecular profiles is still missing. Finally, it has recently been demonstrated that machine/deep learning approaches could predict genomic alterations from tumor histomorphology, which has not yet been implemented in CCA research.

The primary action of WG#2 will be to collect histological samples from European centers and set up a digitalized and online European histological registry. Collected samples should be obtained from patients included in the clinical registry developed within the European Network for the Study of Cholangiocarcinoma (ENS-CCA). Samples included in the ENS-CCA histological registry will be used to build up a parallel molecular registry (please refer to WG#3). Histological samples will be collected in a single highly experienced center (Sapienza University of Rome). Samples will be digitalized and analyzed by international expert participants among the network centers. Data obtained will be correlated to clinical, molecular, and radiologic data provided by other WGs. This WG will interact with the rest of the network to promote multidisciplinary translational actions.

The presence of the ENS-CCA histological registry represents a tool to harmonize nomenclature, definitions, classification, and outcomes. To date, this Action has already included nearly 100 patients in the registry. Upcoming efforts will be: i) to expand the number of cases from non-represented European countries, ii) to generate data and perform an integrative correlation with the available clinical/molecular registries, iii) to apply artificial intelligence and machine/deep learning approaches to the morphological phenotypes of included samples.

Highlights from WG#3 MOLECULAR PROFILING Leader: Jesper Andersen (Denmark), Vice-Leader: Trine Folseraas (Norway)

The main goal of this working group is to promote initiatives and actions to gain insights into the molecular profiles of the distinct histological and clinical subtypes of CCA. In order to achieve this objective, the main effort of the WG participants will be to create a genomic data registry. As such, participants include experts in genetics, cell biology, molecular pathogenesis, translational biomedicine, and gastroenterology. To ensure a far-reaching scientific and clinical impact of the data registry, we will cooperate closely with the European Network for the Study of Cholangiocarcinoma (ENS-CCA) and utilize patient data included in the ENS-CCA epidemiological and histomorphological registries. Patients will be recruited through the ENS-CCA epidemiological registry, and referred from European centers participating in the EURO-CHOLANGIO-NET Action. Long-term, these efforts aim to define specific CCA subtypes, prognostic classes with a distinct molecular pathogenesis, and molecular targets, thus introducing the possibility to search for specific etiopathogenetic mechanisms with druggability.

During the WG meeting deliberations were taken to define the initial study phases, including considerations on patient selection, data focus, and acquisition, as well as funding schemes that the group assumes to successfully achieve our deliverables. Discussions were initiated with AMMF - The Cholangiocarcinoma Charity in the UK as a future partner in this innovative initiative.

A set of patient sample criteria were discussed with the histomorphology WG#2 and a decision was reached to focus on intrahepatic CCA patients with the aim of understanding the differences in carcinogenesis in small and large intrahepatic ducts. The decision to initially focus on intrahepatic CCA was reached based on the unclear genetic mechanisms, heterogenous nature, and lack of efficient therapies in this subtype, leading to high morbidity and mortality. Moreover, an initial emphasis will be given to cryptogenic cases though the inclusion of patients with sporadic etiology. We will use the patient epidemiology registry to select patients without a documented history of alcohol abuse, viral hepatitis B or C infection, or inflammatory primary sclerosing cholangitis (PSC). It was decided that patients may include recorded obesity, diabetes, and non-alcoholic

steatohepatitis (NASH). A second sample set will include patients with PSC-associated intrahepatic CCA to advance the understanding of the PSC-derived etiology of CCA and potentially define common molecular subsets and targets in PSC and sporadic intrahepatic CCA cases.
A second key task is a genome-wide association study (GWAS) to identify inherited genetic variants associated with biliary tract cancer risk (CCA and gallbladder carcinoma). This initiative is coordinated by the International Cholangiocarcinoma Research Network (ICRN) under the Cholangiocarcinoma Foundation in a joint effort with many COST Action members. A current update showed a total global enrollment in the discovery phase in excess of 3200 bile duct patients.

Highlights from WG#4 EPIDEMIOLOGY, CLINICAL CHARACTERIZATION AND TRIALS Leader: Juan Valle (UK), Vice-Leader: Bas Groot Koerkamp (The Netherlands)

The primary aim of WG#4 is the creation of an online data registry of patients with cholangiocarcinoma (CCA). All centers are encouraged to participate by sharing their de-identified clinical data. The second aim is to use data from this registry to improve clinical guidelines for the diagnosis and treatment of patients with CCA. The third aim is to set standards for clinical trial design and establish precise endpoints to assess the efficacy of treatments and biomarkers in CCA. Subtypes of CCA (intrahepatic, perihilar, and distal) have often been misclassified because of ambiguous definitions and inconsistency across coding systems. The new ICD-11 classification should go a long way to resolve these issues. The registry is particularly valuable in combining clinical data with biological samples and the imaging repository being developed by other WGs. An important milestone is to set standard operative procedures (SOPs) for the collection and storage of biological samples in collaboration with other WGs.
Initial analyses of the clinical data registry have already been performed and will be further improved with more centers participating. The clinical registry will expose considerable heterogeneity in the management of patients with CCA. Analyses of heterogeneity in patient management may identify treatments with superior outcome and change guidelines accordingly. Moreover, many prognostic models have been published for patients with CCA. The registry will be the largest source to validate and compare existing models, as well as develop better models to guide patient management. We will further expand the registry with detailed surgical characteristics and outcomes.
ENS-CCA is a great collaborative platform for clinical trials, including those in partnership with Industry Partners. Clinical trials are required to compare existing treatments and evaluate future (biomarker-based) treatments. Setting standards for clinical trials is the first step to ensure the quality of evidence. Several clinical trials have been proposed (e.g., neoadjuvant chemotherapy for resectable pCCA) and will be evaluated and prioritized.

Highlights from WG#5 EARLY DIAGNOSTIC BIOMARKERS Leader: Rocio Macias (Spain), Vice-Leader: Marcin Krawczyk (Poland)

The aim of WG5 is to identify and validate histological, imaging, and serum biomarkers for the diagnosis, prognosis and staging of CCA. To achieve this aim, this WG can benefit from the three collaborative registries created in the framework of the ENS-CCA/COST Action; i) the clinical registry with the demographic and clinical information of patients and available samples (tissue, serum/plasma, bile, etc.) with their characteristics, ii) the histology registry, with information obtained after stains performed on CCA specimens and surrounding tissue, and iii) the radiology registry, with magnetic resonance imaging (MRI) analysis.
Since CCA is a rare tumor, it is difficult to obtain enough samples from a single hospital, especially when specific characteristics of the patients or tumors are required. The European CCA registry, supported by 2016 and 2019 EASL Registry Awards, which includes approximately 2000 patients with CCA from 20 institutions in 11 countries on the secure online platform REDCap, facilitates well-powered studies based on thoroughly characterized samples. Participants in WG#5 were encouraged to include information in the registry, especially those from Eastern European countries.
Several collaborative project proposals were presented. The aim of one of them was to perform international validation of a panel of protein biomarkers present in serum extracellular vesicles for the diagnosis and follow up of patients with PSC, CCA, and hepatocellular carcinoma. A second proposal was an international validation of a panel of selected prognostic biomarkers. To participate in this work, mRNA from resected tumors (iCCA, pCCA, or dCCA) is required, together with 5-year follow-up information (overall survival and disease-free survival). A third proposal, funded by "PSC Support" and "PSC Partners Seeking a Cure", focused on CCA patients with a PSC background. Serum samples from patients with PSC who did or did not develop CCA within two years and from patients with PSC who underwent liver transplantation with or without disease recurrence in two years will be used to identify metabolites that might be useful in the prediction of prognosis and in the early diagnosis of CCA. Inclusion and exclusion criteria for each proposal were explained.

Part of the WG#5 session was dedicated to the presentation of high-quality selected abstracts of early career investigators. The involvement of junior investigators through career development programs (high-level training on research methodology, one-to-one mentorship from senior members of the network, mentorship lectures, and courses on grantsmanship and scientific writing), the offer of a mouse model of female gallbladder carcinoma (GBC), the possibility to include human GBC samples in the European CCA registry, the elaboration of guidelines for screening patients with PSC and identifying new early biochemical tumor markers, the establishment of the best technique to detect early changes in the bile duct wall, and a proposal of collaboration to validate a panel of fibrosis and cancer-associated biomarkers as a risk stratification tool for patients with PSC were discussed. Because these proposals could be of interest for researchers attending other parallel WG sessions or who could not attend the Malta meeting, it was decided to summarize each of the proposals and make them accessible to all who may be interested in participating.

Highlights from WG#6 DEVELOPMENT AND NOVEL THERAPEUTIC TARGETS AND TOOLS Leader: Chiara Braconi (UK), Vice-Leader: Joachim Martens (Switzerland)

CCA incidence is increasing worldwide while survival remains remarkably poor. Despite recent advancements in systemic therapies, overall survival of inoperable patients remains lower than 18 months, underlining the need for better therapeutics. The aim of WG#6 is to develop and define novel drugs and therapeutic strategies at the preclinical level and facilitate the translation from bench to bedside through the integration of cutting-edge basic technologies with the use of patient samples and patient-derived models. Chaired by Dr. Chiara Braconi, the discussion of WG#6 focused on the need for 1) integrating molecular knowledge of CCA with chemical design in order to select the most promising pathways to target an effective response, 2) testing novel and repurposed compounds in CCA preclinical models that mimic human CCA, 3) testing multiple pre-clinical models to overcome the limitations associated with single models, and 4) understanding the mechanisms of chemoresistance for the identification of therapeutics that could reverse drug resistance and provide potential predictive biomarkers. It was concurred that the effort in drug discovery in CCA has so far been limited, with little data on the possibility to repurpose compounds useful in other diseases. This may be linked with the lack of preclinical models that can reproduce limiting issues in human CCA, such as recapitulation of the 3D architecture and genomics of CCA subtypes, intratumor deliverability of compounds, and interaction with the microenvironment. It was acknowledged that there has been a progressive advancement in the generation of novel preclinical models over recent years with the introduction of novel animal models, 3D spheroids, and patient-derived primary cultures in forms of cell lines, organoids, and co-cultures. WG#6 has accepted the challenge to promote a large drug discovery project in CCA where, based on the molecular knowledge derived from other WGs, the effort is focused on the generation of a large compound library that builds on the preliminary work of WG members in the generation of novel compounds targeting a plethora of signaling pathways that were proven to be drivers in different subtypes of CCA. The expertise of this and other WGs will generate complex patient-mimicking CCA models that can be used as a testing platform. It was agreed that drug discovery projects should use a comprehensive platform comprising a pool of models that combine different advantages to select the most promising candidate to be used in clinical experimentation.

As a next action, based on the proposal of junior researchers affiliated with the group, it was decided that a virtual registry of all models would be created so that the screening of novel compounds could be used in multiple and appropriate conditions, which would be aligned with a registry of clinical samples where the clinical relevance of potential novel therapeutic targets could be validated for comprehensive hypothesis-driven projects before beginning human investigation. In addition, a partnership with industry will be sought to overcome the issues of translating preclinical compounds into clinical investigation.

EURO-CHOLANGIO-NET dissemination and exploitation plan: highlights from the set-up meeting in Malta, Science Communications Manager Rui Casto (Portugal)

The European Cholangiocarcinoma Network (EURO-CHOLANGIO-NET) Cost Action (CA18122) aims to establish a pan-European-wide interdisciplinary co-operative network of stakeholders, ultimately focusing on the resolution of cholangiocarcinoma (CCA). Acknowledging the importance of communication, both within the network and externally (overall society, patients, governing bodies, potential novel stakeholders), EURO-CHOLANGIO-NET has been developing a comprehensive website platform, as well as reinforcing social media presence in line with its dissemination and exploitation plan. The website will contain multiple unified and dedicated tools to facilitate interaction and the exchange of information among participants, while also providing assessible and relevant content to support patients and raise awareness in the general population. In this regard, EURO-CHOLANGIO-NET has partnered with Scientific Education Support, a multidisciplinary team of communication and education experts with experience in medical education, scientific publishing, journalism, project management, event management, and digital communication, to help ensure a high-level and widespread EURO-CHOLANGIO-NET presence. The Global Cholangiocarcinoma Alliance (GCA), the Cholangiocarcinoma

Charity (AMMF), and the Cholangiocarcinoma Foundation (CCF) are also supporting EURO-CHOLANGIO-NET in this goal. The progress of the website, which is set to go live in November, was presented at the Malta meeting. The proposal from one early career investigator (Ana Landa Magdalena (Biodonostia Research Institute, SP) to create an online, interactive map of every relevant EURO-CHOLANGIO-NET stakeholder was selected as the winner of the poster competition and its implementation is now being planned.
Several manuscripts by EURO-CHOLANGIO-NET members, particularly from ENS-CCA participants, have already been published or submitted for publication, which prompted the network to discuss continued efforts to keep the momentum going, including a special issue or collection of articles to be published in the next couple years. At the same time, the Action committed to developing educational material for patients, in close collaboration with AMMF, to be prepared in different languages in order to extend their reach.
Last but not least, it was also decided to reach out to both the European Association for the Study of the Liver (EASL) and United European Gastroenterology (UEG) to strengthen EURO-CHOLANGIO-NET visibility and further help its dissemination efforts.

References

1. Memorandum of Understanding EUROPEAN CHOLANGIOCARCINOMA NETWORK (EURO-CHOLANGIO-NET) www.cost.eu/actions/CA18122
2. Banales JM, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). Nat Rev Gastroenterol Hepatol. 2016 May;13(5):261-80. doi: 10.1038/nrgastro.2016.51.
3. Banales JM, Cardinale V, Macias RIR, Andersen JB, Braconi C, Carpino G, Alvaro D, Calvisi DF. Cholangiocarcinoma: State-of-the-art knowledge and challenges. Liver Int. 2019 May;39 Suppl 1:5-6. doi: 10.1111/liv.14101.
4. Banales JM, Marin J, Lamarca A, Rodrigues P, Khan S, Roberts L, Cardinale V, Carpino G, Andersen J, Braconi C, Calvisi D, Perugorria M, Fabris L, Boulter L, Macias R, Gaudio E, Alvaro D, Gradilone S, Strazzabosco M, Marzioni M, Coulouarn C, Fouassier L, Raggi C, Invernizzi P, Mertens JC, Moncsek A, Rizvi S, Heimbach J, Koerkamp B, Bruix J, Forner A, Bridgewater J, Valle J, and Gores G. Cholangiocarcinoma 2020: next Horizon. Nat Rev Gastroenterol Hepatol. 2019, Accepted Manuscript.

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