Brussels, 13 April 2018

COST 029/18

DECISION

Subject: **Memorandum of Understanding for the implementation of the COST Action "Identifying Biomarkers Through Translational Research for Prevention and Stratification of Colorectal Cancer" (TRANSCOLONCAN) CA17118**

The COST Member Countries and/or the COST Cooperating State will find attached the Memorandum of Understanding for the COST Action Identifying Biomarkers Through Translational Research for Prevention and Stratification of Colorectal Cancer approved by the Committee of Senior Officials through written procedure on 13 April 2018.

MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA17118
IDENTIFYING BIOMARKERS THROUGH TRANSLATIONAL RESEARCH FOR PREVENTION AND STRATIFICATION OF COLORECTAL CANCER (TRANSCOLONCAN)

The COST Member Countries and/or the COST Cooperating State, accepting the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action (the Action), referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any new document amending or replacing them:

a. "Rules for Participation in and Implementation of COST Activities" (COST 132/14 REV2);
b. "COST Action Proposal Submission, Evaluation, Selection and Approval" (COST 133/14 REV);
c. "COST Action Management, Monitoring and Final Assessment" (COST 134/14 REV2);
d. "COST International Cooperation and Specific Organisations Participation" (COST 135/14 REV).

The main aim and objective of the Action is to use innovative translational research to identify colorectal cancer biomarkers for personalized medicine that will improve screening, early detection and disease follow-up, and attain better tumor profiling, state-of-the-art functional characterization of genetic variants and new therapy approaches. This will be achieved through the specific objectives detailed in the Technical Annex.

The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 68 million in 2017.

The MoU will enter into force once at least seven (7) COST Member Countries and/or COST Cooperating State have accepted it, and the corresponding Management Committee Members have been appointed, as described in the CSO Decision COST 134/14 REV2.

The COST Action will start from the date of the first Management Committee meeting and shall be implemented for a period of four (4) years, unless an extension is approved by the CSO following the procedure described in the CSO Decision COST 134/14 REV2.

OVERVIEW

Summary

This Action aims at using **innovative translational research to identify colorectal cancer biomarkers for personalized medicine** that will improve screening, early detection and disease follow-up, and attain better tumor profiling, state-of-the-art functional characterization of genetic variants and new therapy approaches. It will be organized in the following working groups:

- *Disease risk profiling applied to the optimization of current screening programs*. Germline predisposition variants, environmental factors, epigenetics, microbiome and metabolomics biomarkers will be used to better select patients eligible to be screened.

- *Non-invasive biomarkers for early detection and disease follow-up*. Circulating tumor cells, circulating tumor nucleic acids, tumor-educated platelets and exosomes will be explored in order to identify new tools for early detection and monitoring of the disease.

- *Tumor profiling to identify biomarkers with prognostics and predictive value for patient stratification*. Intra-tumor heterogeneity will be considered and tumor mutational profiling, epigenetics, single-cell genomics sequencing used as instruments to better inform tumor and precursor lesion characterization.

- *Functional genomics and new therapies*. Candidate genetic variants will be validated and routes to novel therapies for this disease will be conceived. To do so, cutting-edge approaches such as CRISPR-Cas9 and immunotherapy will be applied.

The network will bring together participants from different COST countries and will facilitate the research interaction and collaboration between research groups and enterprises interested in the described objectives. Diverse expertise includes clinical practice, germline and somatic genetics, epigenetics, bioinformatics, cell and molecular biology, microbiology, immunology, biostatistics, epidemiology, health economy and the industrial sector.

Areas of Expertise Relevant for the Action	Keywords
● Biological sciences: Genomics, comparative genomics, functional genomics ● Basic medicine: Genetic epidemiology ● Clinical medicine: Gastroenterology and hepatology ● Basic medicine: Databases, data mining, data curation, computational modelling ● Medical biotechnology: Metabolomics for medical biotechnology	● Colorectal cancer ● Risk profiling ● Biomarker ● Tumor profiling ● Therapy

Specific Objectives

To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination

● To reduce the fragmented research and lack of communication between researchers working on CRC. Nowadays, CRC research needs to come together. This Action would allow the European scientists to embark in a thorough discussion of timely questions and methodological challenges, enable interdisciplinary networking and foster new collaborations.

● To permit the interconnection and interdisciplinary networking of researchers working in different CRC fields. By doing so, it will strengthen existing alliances and facilitate new collaborations. Most members will be active in more than one WG, thus allowing substantial crosstalk between groups and interactions between different disciplines.

● To optimize the technical and medical processes involved in the prevention, screening and CRC

management by using new approaches such as the emerging fields of microbiome characterization, metabolomics profiling, circulating tumor cells/nucleic acids, tumor mutational profiling, single-cell genomics, gene editing and immunotherapy. An important socio-economic impact is expected.
● To jointly develop scientific guidelines/recommendations for the different methodologies used in each WG through coordination among the involved participants. These documents will be later publicly available and back into the community, including patients, stakeholders and the general population.
● To aim towards FAIR data stewardship, i.e. making data Findable, Accessible, Interoperable and Reusable. Therefore, all data acquired within TRANSCOLONCAN will be uploaded in available research IT tools such as OpenClinica, TranSMART and cBioPortal, currently facilitated by Health-RI translational office suite (https://health-ri.org/).
● To use results derived from this Action as input for future market applications which could foster cooperation with private enterprises, being CRC screening devices a clear example. This Action is focused on timely and realistic research questions in the CRC field so intellectual property (IP) matters are expected.

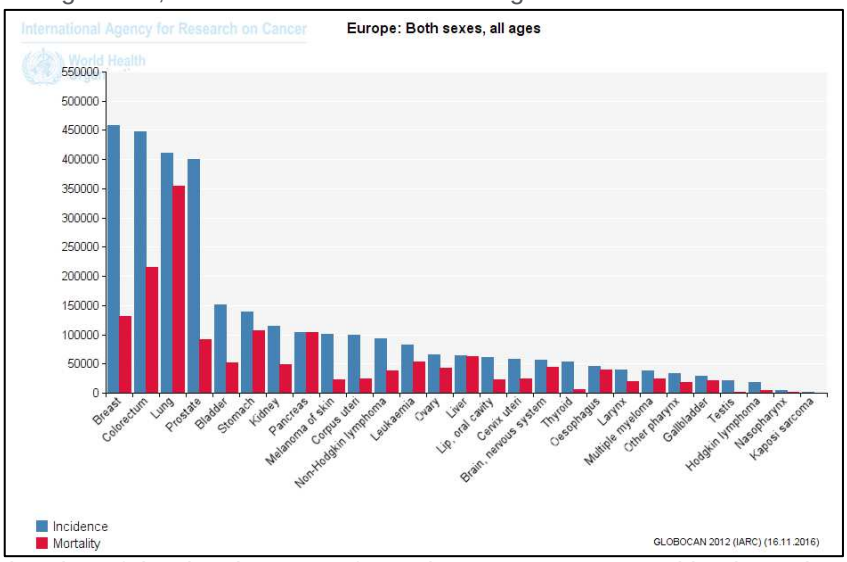
Capacity Building
● To foster knowledge, know-how, exchange and creation of synergies in a highly relevant topic in the scientific and socio-economic point of views to develop a joint research agenda.
● To bring into an interdisciplinary network separate scientific disciplines such as clinical CRC practice, germline and somatic genetics, epigenetics, bioinformatics, cell and molecular biology, microbiology, immunology, biostatistics, epidemiology and health economy and the industrial sector.
● To remain open for new interested groups and SMEs working on this field and facilitate their incorporation in a single effective and interactive European research network. This policy will include especially those from Inclusiveness Target Countries (ITC) or with less capacity in the field.
● To highlight the importance of training about the research topics developed, particularly for early career investigators (ECI) and ITC researchers. Training will take place during meetings, specialized workshops and conferences, STSM and training schools.
● To take into account constantly in the planned activities ECI and comply with the gender balance and ITC policies.
● To assure a future growth of scientific strength of the network, guaranteed by the educative and promoting role of the Action.
● To ensure the correct integration, dissemination and exploitation of all knowledge and data from TRANSCOLONCAN amongst the research groups of interest, industry sectors and users.

1. S&T EXCELLENCE

1.1. CHALLENGE

1.1.1. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

Aside from lung cancer, which has an avoidable environmental cause, colorectal cancer (CRC) is responsible for more deaths than any other malignancy in Europe. Indeed, this neoplasm is the second most common cancer in Europe when both genders are considered together (1). Also taking into account both genders, CRC ranks second after lung cancer as a cause of cancer related death (see Figure).



Worldwide, 1 million people each year develop CRC and the incidence of this tumor is increasing. Based on demographic trends with an ageing population due to rising life expectancy and declining fertility rates, the annual incidence for this disease is expected to increase by nearly 80% to 2.2 million cases over the next two decades (2).

It is generally acknowledged that a vast majority of CRC cases develops from non-malignant precursor adenomas (3). The average duration of the development of an adenoma to CRC transition is unobserved, but it is estimated to take at least 10 years (4). This long latent phase provides an excellent window of opportunity for early detection. Therefore, CRC is particularly suitable for screening. Keeping in mind the dimension of this disease, European national health systems have started population screening programs in order to increase early detection and improve prevention measures. Screening for CRC offers the possibility to identify the disease at an earlier stage or at a premalignant phase. For this reason, the evidence-based European Code Against Cancer recommended that men and women over 50 years of age should participate in CRC screening. This was given effect within the EU by the 2003 Council Recommendation on cancer screening (5). Indeed, CRC is highly preventable by detecting and removing adenomas through colonoscopy screening, but this procedure is very costly to be implemented as population screening and has an associated morbidity (6). Intermediate screens, such as fecal occult blood testing (FOBT), are therefore often used to select patients for colonoscopy with suboptimal sensitivity (7,8).

An adenoma grows in size and can develop high-grade neoplasia. The adenoma can invade the submucosa and become malignant. Initially, this malignant cancer is not diagnosed since it does not give symptoms (preclinical). However, it can progress from localized (stage I) to metastasized (stage IV) cancer, until it causes symptoms and is diagnosed. Only 14% of CRC cases are diagnosed at an early stage. The overall 5-year survival rate for patients with stage I CRC is high but declines as the lesion progresses. Only 12.5% of patients with distant metastasis live 5 years after diagnosis (2). A lack of early detection affects the survival of patients. Carcinoembryonic antigen (CEA) is the most widely used tumor marker in the clinical management of CRC but has limited specificity and sensitivity (9). Recently, “liquid biopsies” have become recognized as novel sources of biomarkers.

Approximately 70%-87% of CRCs are sporadic and occur in people without family history (10,11). In sporadic CRC, the progressive accumulation of genetic and epigenetic alterations leads to the transformation of normal colonic mucosa to adenoma and later to adenocarcinoma. The most frequently mutated genes in sporadic CRC are *APC*, *TP53*, *SMAD4*, *PIK3CA* and *KRAS* (12). The latest advances in the identification of CRC subgroups showed the presence of four molecular subtypes of tumors based on the mutational state, the genomic and epigenomic profiling, and gene expression signatures (13). Despite efforts in the stratification of patients according to the clinical characteristics, only a few biomarkers have been incorporated into the care routine of CRC patients. Genetic and phenotypic variation is observed between individuals with the same tumor type (intertumor heterogeneity), and subclonal diversity within a tumor may also be observed (intratumor heterogeneity). Inter- and intratumor heterogeneity has significant implications for the choice of biomarkers to guide clinical decision-making and prevent a more generalized clinical approach (14).

As for other complex diseases, CRC is caused by both genetic and environmental factors. Twin studies showed that around 13%-30% of the variation in CRC susceptibility involves inherited genetic differences (11,15). Some of the known CRC predisposition was already discovered in the past decade (16). However, most NGS studies identified candidate genetic variants predisposing to CRC but did not tackle its functional interpretation to unequivocally recognize a new hereditary CRC gene (17). Also, similarly to the germline counterpart, the same functional interpretation difficulty is encountered when somatic studies are pursued to identify clinically relevant variants.

Treatment modalities currently being used for metastatic CRC have been showing only modest efficacy and are associated with significant toxicities (18). Fluoropyrimidines, oxaliplatin and irinotecan represent the chemotherapy backbones for the treatment of metastatic CRC, and their sequential administration results in median overall survival of 18-20 months. Targeted agents such as vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) inhibitors rose median survival to 30 months (19). Various anti-angiogenic agents are approved for clinical use, but robust predictive biomarkers for treatment prioritization have not yet been identified. On the other hand, the selection of patients for anti-EGFR therapy is based on the absence of *KRAS* and *NRAS* mutations, which confer innate resistance (20). This unmet need for effective treatment of metastatic CRC has driven the search for novel strategies to improve survival while minimizing toxicities experienced by patients.

In summary, this Action will support trans-national cooperation to jointly develop translational research to identify CRC biomarkers that will improve screening, early detection and disease follow-up, and attain better tumor profiling, state-of-the-art functional characterization of genetic variants and new therapy approaches for this disease.

1.1.2. RELEVANCE AND TIMELINESS

CRC prevention is implemented in most European countries by establishing screening programs, mostly by using FOBT as an intermediate test for disease in medium-risk population. Colonoscopy is advised to those individuals with a positive FOBT although its sensitivity and specificity is suboptimal for disease detection. In few European countries, colonoscopy is directly offered as the main screening procedure or no program is established yet (21). Since colonoscopy is an expensive clinical procedure and FOBT is not highly sensitive, other biomarkers and procedures could be used to make CRC screening more reliable and less expensive facilitating its application in all European countries and beyond. Germline CRC genetic susceptibility variants, microbiome characterization, epigenetics, proteomics/metabolomics and environmental factors profiling are among the additional players that could be included nowadays in the improvement and personalization of CRC screening. By doing so, these biomarkers would determine CRC risk individually and screening could be personalized. Based on these risk estimates, it could be possible to tailor the screening protocol, in terms of starting age and/or screening interval, or to increase the positive predictive value of colonoscopy referral.

Regarding early detection, the ability to detect biomarkers in patient blood samples would provide the most practical screening tool for CRC. The detection of biomarkers such as circulating tumor cells, tumor nucleic acids, exosomes and tumor-educated platelets (TEP) can be considered among the most promising and offer many advantages, including minimal invasiveness and easy accessibility. Biomarkers with high specificity and sensitivity can enable the detection of CRC at an early stage, thereby improving prognosis, prediction of treatment response and recurrence risk (22).

On the other hand, high-throughput genomic tools can be applied nowadays to dissect somatic events in CRC tumors even at single-cell level. Approaches include the full characterization of genetic and copy number variants, coding and non-coding RNA, methylation and proteins. Also, due to inter-tumor heterogeneity within the disease, a careful stratification of CRC based on less frequently observed somatic mutations with driver properties for a very specific subtype of tumors should be pursued. By doing so, additional clinically relevant biomarkers with predictive potential for CRC prognosis and treatment response will be identified.

Finally, a functional interpretation of the identified variants involved in germline predisposition and somatic studies is essential to establish an unambiguous link to disease predisposition or progression. Approaches available today include bioinformatics, cell and molecular biology and animal models. Recent advances, such as the CRISPR-Cas9 system, have already showed high potential to be used in this direction (23). On the other hand, metastatic CRC is currently treated with modest efficacy and significant toxicities. Therefore, new therapy strategies are needed and immunotherapy represents a feasible alternative to be explored.

The TRANSCOLONCAN network is fundamental for Europe and the European Research Area. It will include diverse CRC expertise participants including clinical, germline and somatic genetics, epigenetics, bioinformatics, cell and molecular biology, microbiology, immunology, biostatistics, epidemiology, health economy and small and medium-sized enterprises (SMEs) with the final aim of identifying new CRC biomarkers for prevention, screening and disease management by using a multidisciplinary and multifaceted approach.

1.2. OBJECTIVES

The main aim of this Action is to develop an innovative translational research to identify CRC biomarkers, with the main objective of reducing CRC mortality by improving early detection as well as disease management.

1.2.1. RESEARCH COORDINATION OBJECTIVES

The research coordination objectives (RCO) are adjusted to the main aim of this Action and related also to the different Working Groups (WG) detailed in section 3.1.1.

- **RCO1**: To reduce the fragmented research and lack of communication between researchers and methodological areas working on CRC. As an example, some researchers excel in germline CRC genetics whereas others stand out in somatic studies for the same disease, without ever cooperating on a common project. However, it is known that both approaches can improve tremendously by interrelating to obtain mutual benefit. Nowadays, the fragmented research and methodological areas involved in the field of CRC need to come together. This Action would allow the European scientists to embark in a thorough discussion of timely questions and methodological challenges, enable interdisciplinary networking and foster new collaborations.
- **RCO2**: To permit the interconnection and interdisciplinary networking of researchers working in different CRC fields with final common objective of identifying new CRC biomarkers for prevention, screening and disease management as thoroughly described in the WG section. By doing so, it will strengthen existing alliances and facilitate new collaborations. Consequently, the COST framework represents an ideal instrument which allows bringing together many groups and experts in the diverse field of CRC research. Most TRANSCOLONCAN members will be active in more than one WG, thus allowing substantial crosstalk between groups and interactions between different disciplines.
- **RCO3**: To optimize the technical and medical processes involved in the prevention, screening and CRC management by using new approaches such as the emerging fields of microbiome characterization, metabolomics profiling, circulating tumor cells/nucleic acids, tumor mutational profiling, single-cell genomics, gene editing and immunotherapy. This RCO will have an important socio-economic impact. For example, CRC population screening could be optimized using new technological and scientific approaches, and, consequently, there could be substantial positive budgetary effects in Health departments by reducing the number of endoscopies.
- **RCO4**: To jointly develop scientific guidelines/recommendations for the different methodologies used in each WG through coordination among the involved participants. These documents will be later publicly available and back into the community, including patients, stakeholders and the general population.
- **RCO5**: To aim towards FAIR data stewardship, i.e. making data Findable, Accessible, Interoperable and Reusable. Therefore, all data acquired within TRANSCOLONCAN will be uploaded in available research IT tools such as OpenClinica, TranSMART and cBioPortal, currently facilitated by Health-RI translational office suite (https://health-ri.org/).
- **RCO6**: To use results derived from this Action as input for future market applications which could foster cooperation with private enterprises, being CRC screening devices a clear example. This Action is focused on timely and realistic research questions in the CRC field so intellectual property (IP) matters are expected to be dealt with and IP protection is conceivable.

1.2.2. CAPACITY-BUILDING OBJECTIVES

This Action will focus on the identification of new CRC biomarkers for prevention, screening and disease management. The following capacity-building objectives (CBO) will be pursued:

- **CBO1**: To foster knowledge, know-how, exchange and creation of synergies in a highly relevant topic in the scientific and socio-economic point of views to develop a joint research agenda.
- **CBO2**: To bring into an interdisciplinary network separate scientific disciplines such as clinical CRC practice, germline and somatic genetics, epigenetics, bioinformatics, cell and molecular biology, microbiology, immunology, biostatistics, epidemiology and health economy and the industrial sector.
- **CBO3**: To remain open for new interested groups and SMEs working on this field and facilitate their incorporation in a single effective and interactive European research network. This policy will include especially those from Inclusiveness Target Countries (ITC) or with less capacity in the field.
- **CBO4**: To highlight the importance of training about the research topics developed, particularly for early career investigators (ECI) and ITC researchers. Training will take place during meetings, specialized workshops and conferences, STSM and training schools.
- **CBO5**: To take into account constantly in the planned activities ECI and comply with the gender balance and ITC policies.
- **CBO6**: To assure a future growth of scientific strength of the network, guaranteed by the educative and promoting role of the Action.
- **CBO7**: To ensure the correct integration, dissemination and exploitation of all knowledge and data from TRANSCOLONCAN amongst the research groups of interest, industry sectors and users.

1.3. PROGRESS BEYOND THE STATE-OF-THE-ART AND INNOVATION POTENTIAL

1.3.1. DESCRIPTION OF THE STATE-OF-THE-ART

Disease risk profiling applied to the optimization of current screening programs

CRC is a high-prevalence disease which continues to have relatively high mortality and no simple avoidable cause (1). CRC is preventable by detecting and removing adenomas using sigmoidoscopy or colonoscopy screening. Due to the invasive nature and cost of endoscopy, intermediate screening methods, such as FOBT, are used to select patients for colonoscopy. The new immunochemical tests are showing a higher sensitivity for CRC and also for advanced adenomas, while maintaining a satisfactory specificity, but still the test can miss about 30% of CRC and 70% of advanced adenoma cases. Genetic and environmental factors are involved in the etiology of CRC. Of the proven risk factors for CRC, genetics is arguably one of the most powerful and easy to measure. In that sense, a minority of CRC cases (~5%) show strong familial aggregation and belong to the well-known hereditary CRC forms mainly caused by germline mutations in ~15 rare, high-penetrance hereditary genes such as *APC*, *MUTYH* and the DNA mismatch repair genes (10). Additionally, GWAS have identified more than 50 common, low-penetrance polymorphisms with moderate effects in predisposing to this disease (16). Recently, NGS approaches have permitted the identification of new high-penetrance CRC hereditary genes, being *MSH3* one of the most recent examples (24). On the other hand, non-genetic risk factors have also an indisputable contribution to CRC incidence and include medical/family history, gender, age, aspirin/NSAIDs, smoking, alcohol, diabetes and diet (25).

It is estimated that at least 15% of the cancer burden worldwide is attributable to known intestinal infectious agents that are often resident in the human gut together with other members of the intestinal microbiota (26). The diverse microorganisms and viruses inhabiting the gut collectively constitute the complex system known as the gut microbiome, which interacts in complex ways with various host processes including nutritional absorption, metabolism, immunity, carcinogenesis, and tissue development (27). Alterations in microbiome composition have been associated with a growing number of prevalent human diseases (28) including CRC, in which some typically rare gut microbes are thought to create a microenvironment more favorable to tumor development. Recent studies have elucidated specific alterations in the gut microbiome associated with CRC and explored its value for CRC screening (26,27,29).

Metabolomics is an innovative and powerful approach in which a large number of metabolites are systematically screened in blood, other biological fluids or tissues to characterize biological phenotypes with an unprecedented level of precision (30,31). As such, it is being increasingly applied as the method of choice for biomarker discovery, not only for the purposes of disease diagnosis and prognosis, but also to better understand underlying mechanisms of cancer development as well as etiological risk factors (32). Metabolomic-based approaches can be either targeted, i.e. measure a large number of a priori identified metabolites, or agnostic, i.e. measure a wide spectrum of metabolites in order to identify differences between study groups of interest. Both approaches have been successfully applied in cancer research projects, for example a targeted analysis exploring the etiology of primary liver (33) and breast

cancers (34) or exploring differences in the metabolomic profiles across the adenoma-carcinoma sequence of CRC development (35,36). Metabolomics techniques also empower the concept of the Exposome which refers to the totality of exposures from a variety of sources including, but not limited to, chemical agents, biological agents, radiation, and psychosocial components from conception onward, over a complete lifetime (37). The two main technologies currently being applied are high resolution nuclear magnetic resonance spectroscopy and chromatography with mass spectrometry. The field has also spurned the development of multivariable statistical methods, and novel analytical frameworks such as the meet-in-the-middle concept which aims to identify biomarkers associated with both lifestyle exposures and risk of disease development (38,39). Immediate challenges are annotation of the very large number of different metabolites identified, the incorporation of biostatistical models for analysis of huge metabolomic data and multi-omic datasets, better understanding of the underlying metabolic-dysfunction and pathways leading to CRC development, and the conceptualization of various models of life-course disease risk – all of which are being met with intense research (40). It is clear that the identification of susceptibility factors and metabolic pathways relevant to CRC development are key to identifying individuals at high risk and developing biomarkers for early detection, prognosis and follow-up of this important disease.

Non-invasive biomarkers for early detection and disease follow-up. Metastasis is the major cause of CRC-related death, even if the primary tumor was initially removed by surgery. To improve patient care and outcome significantly, early detection, monitoring, and therapeutic prediction need to become more effective. This might be achieved by implementing biomarkers, enabling longitudinal non-invasive monitoring of CRC in peripheral blood from early to late stages of the disease. The term “liquid biopsy” was originally used to describe the analysis of circulating tumor cells (CTC) in blood (41), but was later extended to other tumor derived blood-based biomarkers, such as cell-free tumor DNA (ctDNA), tumor-derived exosomes or TEP. These different types of circulating biomarkers might be complementary in different clinical situations. CTCs are epithelial cancer cells that entered the blood stream by active or passive mechanisms, detectable as ultra-rare cells in the peripheral blood by highly sensitive methods (approx. 1 CTC/10^9 blood cells) (42). Despite their rarity, CTCs are clinically relevant because they comprise the metastatic progenitor cells and their detection is of high prognostic value in most cancer types, including CRC (43). Furthermore, CTC enumeration might predict response to therapy in metastatic CRC (44,45). However, the greatest potential and highest clinical utility of CTCs lies in their molecular profiling to deliver clinically relevant information on protein, mRNA, and DNA level to guide therapy and inform on therapeutic resistance (42). Although this has not entered clinical routine yet, reliable CTC-profiling workflows have been reported (46). Since CTC numbers increase from benign to invasive CRC, their detection could also play a role in screening and early detection (47). However, this application is not well established yet. Compared to CTCs, the detection and analysis of ctDNA is less complex and probably closer to enter clinical routine. In addition, NGS and droplet digital PCR methods propelled the development of this blood-based biomarker during the last five years (48). The fragmented ctDNA is released from apoptotic/necrotic cancer cells and its amount increases with tumor burden (49). A major advantage of ctDNA is the specificity of cancer-related mutations that display high concordance with matched tumor tissue in metastatic CRC (22,50;51). Monitoring ctDNA allows assessing tumor dynamics in CRC patients undergoing surgery or chemotherapy (50,52) and can predict therapeutic resistance. Furthermore, ctDNA profiling seems more informative for the mutational profile than a conventional tumor biopsy (53). Despite the diagnostic potential of ctDNA for screening programs, its role for CRC diagnosis remains unclear. A third category of liquid biopsies are exosomes. These nano-sized extracellular vesicles are released by living cells, including cancer cells, and are involved in intercellular communication (54). Exosomes promote metastasis and are especially involved in preparing the pre-metastatic niche at distant sites (55). Due to their constant release, hundreds of billions cancer-derived exosomes can be found per milliliter of plasma (56), carrying tumor-derived proteins, coding and non-coding RNAs, as well as DNA fragments, which can be exploited for liquid biopsies. In comparison to CTCs and ctDNA, exosomes are the least developed biomarker, but promising data in CRC have been published for the prognostic relevance (57) and, importantly, for its potential use in early diagnosis (58). Finally, TEP have been recently demonstrated to sequester tumor RNA by a microvesicle dependent mechanism and can be used also as a tumor biomarker (59).

Tumor profiling to identify biomarkers with prognostics and predictive value for patient stratification. Recent advances in the characterization of CRC led to molecular-subtype-specific biomarkers to improve the prediction of CRC prognosis (60). Furthermore, the understanding of intratumor heterogeneity (ITH) is clinically relevant as it might also have prognostic value and can be the cause of therapeutic failure. Multiregional analysis with deep NGS approaches demonstrated extensive ITH already in the adenomas and showed that most known driver alterations were observed frequently in earlier-acquired alterations (61,62). Single-cell DNA sequencing approaches have recently revealed tumor lineages to metastasis, pointing to a late dissemination model (63). Altogether, in order to overcome the transient effect of targeted therapies, therapeutic approaches to monitor clonal

evolution and the evolving nature of CRC, may result in improved prognostic predictors and better survival outcomes (64,65).

Functional genomics and new therapies. NGS has revolutionized our ability to read information from the genome, including the DNA sequence itself, the state of the transcriptome and the epigenome, among others. Genetic alterations identified in recent germline and somatic studies need to be linked unequivocally to CRC predisposition or progression. Functional genomics approaches have an important role in identifying the causal links between genetic architecture and phenotypes, in order to decipher cellular function in health and disease. Therefore, a functional interpretation of identified genetic variants in NGS approaches is now essential. Approaches available today include bioinformatics, cell and molecular biology and animal models. Recent advances, such as the CRISPR-Cas9 system, have been already used as a powerful tool with this objective (23). Also, use of cell lines is of only limited value due to the CRC heterogeneity and its close interaction with microenvironment. Access to tridimensional (3D) cultures and xenograft models that mimic the *in vivo* tissue architecture could revolutionize functional analysis (66,67).

Finally, an effort needs to be made to improve current treatment strategies for metastatic CRC which show modest efficacy and significant toxicities. Modulation of the interaction between the immune system and the tumor microenvironment has long been a target of cancer research. Immunotherapy approaches for CRC explored until recently have shown only modest efficacy in reducing tumor burden. However, recent significant breakthroughs have been made. Immunogenic colorectal tumors show better disease-free survival (68) and immune checkpoint blockers such as anti-PD-1 antibodies have shown efficacy to treat advanced mismatch repair deficient tumors (69). Hence, immunotherapy now represents a possible avenue of curative treatment for those with chemo-otherwise refractory CRC tumors (70). While objective responses were observed in approximately half of mismatch repair deficient CRCs (69), it is expected that the combination of different checkpoint inhibitors (e.g. PD-1, CTLA-4, LAG-3) results in improved response rates. Nevertheless, a significant proportion of metastatic CRCs (~95%) are not yet amenable to such immunotherapeutic approaches and alternative approaches or novel immunomodulatory strategies have to be developed.

1.3.2. PROGRESS BEYOND THE STATE-OF-THE-ART

Disease risk profiling applied to the optimization of current screening programs. Screening to prevent CRC is cash-limited even in the wealthiest countries, with some evidence that too many individuals are screened. Whether or not this is true, there are excellent theoretical reasons to expect that the CRC screening programs could be improved by tailoring the prevention intensity and/or modality to groups that have different levels of risk.

Microbiome sequencing has proven very successful in uncovering novel candidate bacterial species that accurately discriminate neoplasia-free controls from CRC patients and to some extent also from advanced adenomas (27). It has further been suggested that microbiome-based tests could be effectively combined with FOBT to improve detection accuracy, increasing FOBT sensitivity at unchanged specificity. A combination of microbial CRC markers and FOBT has been also proposed recently (29). In concert with it, colonic microbiome characterization is increasingly seen as able to perform well in selecting general population groups with a higher risk of developing CRC.

The science of metabolomics, as the study of the comprehensive metabolite composition of biological samples, is maturing rapidly. The next challenges beyond the state-of-the-art are to harness this powerful technology for application to (a) improved understanding of CRC etiology – for individual/population risk stratification, and effective cancer prevention policies, (b) obtaining highly predictive metabolite profiles to identify early colorectal lesions – for distinguishing individuals at higher risk of CRC development or at early stages of tumorigenesis, and (c) enabling a better understanding of metabolic differences in molecular CRC subtypes – for better intervention, treatment modalities and prognostic follow-up. The analytical strategies, types of study designs needed and scientific expertise required to address each challenge are very different. Moreover, the application of various metabolomic methodologies may provide diverse findings. Thus, considerable advances can be envisioned if efforts are made to bring together scientists working in these diverse research areas in order to plan and implement complementary study designs with application of comparable metabolomic methodologies.

The development of risk models that incorporate the data of genetic, epigenetic and microbiome biomarkers together with information on metabolomics and environmental risk factors offers the possibility of improving the current CRC screening process, maximizing the detection of CRC risk lesions. A risk model that combines genomic and metabolomics information with environmental factors has the potential of a better selection of individuals who have to undergo a colonoscopy. In fact, some previous studies have already reported results that suggest that low-penetrance germline genetic variants could work in this direction, showing improvements in the discriminatory accuracy in relation to a model that did not include them (71,72).

Non-invasive biomarkers for early detection and disease follow-up. Despite the great progress over the last years, major challenges for liquid biopsy approaches are the (relative) low frequency of the tested analytes– except for mutated/methylated DNA or mRNA – and the lack of tumor specificity. Integrated approaches that combine CTCs, ctDNA, TEP and exosomes have not been established yet, but appear promising to increase sensitivity and specificity for non-invasive cancer detection. However, there is still a need for specific markers to detect tumor-derived exosomes, such as glypican-1 in pancreatic cancer (73). A recent study revealed CD147/CD9-positive exosomes as promising for non-invasive CRC detection (58). In the case of CTCs, enrichment and detection is facilitated by the biophysical and/or molecular differences between epithelial CTCs and normal blood cells (42). However, the greatest challenge remains the low detection rate, especially in early-stage cancer with ≤1000 CTCs circulating in their five-liter blood volume (74), decreasing the likelihood to detect them with regular CTC-assays. A potential solution for this problem is the clinically safe pre-enrichment resulting in a 250% increase in CTC detection frequency as well as an escalation of the median CTC numbers by 30-fold (74). However, in parallel with new technical developments, rigorous technical and clinical validation of the existing methods/technologies for liquid biopsies in CRC are still needed, which necessarily requires a multicentre and multidisciplinary effort.

Tumor profiling to identify biomarkers with prognostics and predictive value for patient stratification. The consequences of ITH are still under debate (75). Recent technological advances that allow profiling of intratumoral sub-populations and the measurement of clonal expansion will provide unprecedented relevant information to improve clinical outcome. Indeed, the genomic characterization of early CRC lesions using the actual–omics technologies will transform our approach to cancer prevention and detection, and will provide insights on the clonal evolution to CRC and their derived metastasis (76). There is a need to understand the extent to which genomic alterations simultaneously alter the expression of multiple genes and how this provides fuel to generate ITH. Furthermore, tackling CRC evolution demonstrated the route from the primary tumor to metastases and provided chances for therapeutic intervention (77).

Functional genomics and new therapies. Functional studies of genetic variants suspected of being involved in predisposition to a disease are still scarce in most research or hospital centers where NGS technologies are applied. However, the need for them is imperative for the correct interpretation of results in the fields of research and clinical diagnosis. With the aim of generalizing and facilitating this type of studies, tools have been recently developed that allow carrying them out massively and in parallel (78). On the other hand, the emergence of CRISPR/Cas9 technology is allowing a much more fluid modification of the genome at the time to introduce the genetic variants to be studied (23,79) and for its subsequent functional dissection in cellular models. Also, 3D models permit culture and outgrowth of intestinal crypt cells into organoids (66), recapitulating the physiology, shape, dynamics and cell make-up of the intestinal epithelium (80). The chorioallantoic membrane assay is a highly reproducible xenograft model (67). Such models play an important role in the screening and evaluation of new biomarkers and their functional characterization. Moreover, genes of interest can be knocked out and their functional effects on the gut can be studied (81).

The discovery of molecular mechanisms that dictate the successful employment of anti-PD-1 therapies in mismatch repair deficient CRC (only 50% respond) will be a major research focus in the near future. The profiling of such tumors is expected to deliver actionable biomarkers that sensitize an increased proportion of mismatch repair deficient CRC to immunotherapy. Among those, the expression of additional immunomodulatory, co-inhibitory ligands and receptors in the tumor microenvironment (e.g. TIM-3, LAG-3, CTLA-4), the secretion of immunosuppressive cytokines (e.g. TGF-β), and/or defects in antigen presentation in tumor cells might play a crucial role in response to anti-PD-1 therapies. All previous are amenable to therapeutic intervention and can contribute to improve response rates in mismatch repair deficient tumors.

A major challenge for immunotherapy in CRC resides in the apparent lack of immunogenicity of a large proportion of tumors (up to 80%). In these, complementary approaches explore somatic mutations in tumors that translate to mutated antigens (neo-antigens) for activation of anti-tumor immune responses. In this setting, cancer genomes are screened in a personalized setting and mutated proteins are tested for their ability to induce anti-tumor immune responses in autologous T-cells. Such approach has been successfully applied in a cholangiocarcinoma patient (82) but optimal procedures (NGS, bioinformatic analysis, formulation) are yet to be developed. Furthermore, while presenting a low mutation burden, mismatch repair proficient tumors often accumulate large chromosomal aberrations that result in aneuploidy. Such extensive DNA damage activates immune responses through stimulation of intrinsic cellular pathways such as the stimulator of *IFN* genes (STING), a transmembrane protein acting as a signaling adaptor (83). The identification of the molecular mechanisms that allow tumor cells with high chromosomal instability to escape the activation of such mechanisms could result in their sensitization to immunotherapeutic intervention (84). Finally, there is great promise in the combination of immunotherapeutic approaches with current (neo-) adjuvant therapies. Rectal cancer is a particularly

interesting group of tumors to explore such hypothesis as patients receive chemoradiotherapy in a neo-adjuvant setting, i.e., before surgical resection. Exposure of tumor tissues to such therapies has been shown to induce expression of inflammatory molecules, T-cell checkpoint ligands, and increase antigen presentation, representing a fantastic opportunity for combination of standard neo-adjuvant therapies with immunotherapy.

1.3.3. INNOVATION IN TACKLING THE CHALLENGE

The major innovation of the proposed COST Action lies in identifying biomarkers for CRC in order to improve screening, early detection and disease follow-up. Biomarker identification opens the door to innovation opportunities and it can have patent licensing prospects. The proposed Action will make available its data to markets, governments and society in order to improve CRC clinical and diagnostic management. Several European SMEs are supporting the present network.
Further innovative aspects are the anticipated concepts, products and technologies that are envisioned to come out from this consortium:

- CRC/advanced adenoma risk calculation: low-penetrance CRC genetic variants, microbiome characterization, epigenetics and metabolomics data and environmental factors will be used to calculate CRC/advanced adenoma risk in the analyzed individuals. The most likely application will be within CRC screening programs.
- Improved CRC screening: applying the previously mentioned risk calculation could permit a better selection of individuals undergoing CRC screening either by FOBT or colonoscopy. Nowadays, FOBT is not highly efficient to select individuals that should have a colonoscopy performed. When FOBT is positive, a majority of colonoscopies do not detect an advanced neoplasia. Therefore, directing the screening efforts to a better selection of the population undergoing colonoscopy is necessary to save resources and reduce the burden of the program.
- New CRC early detection devices: the ability to detect biomarkers in patient blood samples would provide the most practical early detection tool for CRC, and biomarkers such as CTC, ctDNA, methylation, RNA, proteins, exosomes and TEP can be considered among the most promising and offer many advantages, including minimal invasiveness and easy accessibility. Biomarkers with high specificity and sensitivity can enable the detection of CRC at an early stage, thereby improving prognosis, prediction of treatment response, and recurrence risk.
- Enhanced tumor characterization: approaches will be developed for a better CRC tumor characterization permitting a more accurate stratification of patients and personalized treatment. Inter- and intra-tumor heterogeneity will be taken into account. Methods will include tumor mutational profiling, epigenetics, single-cell genomics for the comprehensive characterization of genetic and copy number variants, coding and non-coding RNA, methylation and proteins.
- Pathogenicity assignment for genetic variants: NGS technology has permitted to detect germline and somatic variants with whole-genome coverage at an acceptable cost. Despite this progress, the unequivocal assignment of pathogenicity to the detected genetic variants is still a pending issue. The proposed network will develop approaches for a better functional characterization of variants by using bioinformatics, cell biology and gene editing. Solutions to perform variant evaluation in massive and parallel manner will be also pursued.
- Novel therapies for CRC: treatment for metastatic CRC has modest efficacy and it is associated with undesired toxicities. The network will work on the modulation of the interaction between the immune system and the tumor microenvironment and develop immunotherapy approaches more efficient and with less adverse effects.

1.4. ADDED VALUE OF NETWORKING

1.4.1. IN RELATION TO THE CHALLENGE

This Action will facilitate the research interaction and collaboration between research groups and SMEs interested in the described objectives and working groups (sections 1.2 and 3.1). Networking is the most suitable approach to reach more solid and reliable conclusions due to the following characteristics:

- Large cohorts of patients: Access to several cohorts of CRC screening programs and CRC patients lead by participants will allow increasing the number of studied individuals and samples in the different approaches developed. By doing so, results and conclusions reached will be more consistent. Validation of individual results will be also facilitated by being part of a consortium. Also, inclusion of large general population cohorts in the network will provide a unique tool to test preventive strategies at long term, together with the integration of life-course linked Electronic Health Registries.

• Interdisciplinary: Networking usually permits that different scientific disciplines work together with a common objective enriching the approach developed and facilitating more valuable results. Participants in the present Action will belong to different disciplines including clinical CRC practice, germline and somatic genetics, bioinformatics, cell and molecular biology, microbiology, immunology, biostatistics, epidemiology, health economy and SMEs.
• Transnational: the present pan-European network will bring together initially participants from 17 EU countries and 1 international partner country (IPC). Different approaches at the national level will be shared and harmonized. Conclusions reached will help establishing recommendations, standards and protocols available for the scientific community and society.
• Dissemination: Distribution of the research conclusions, protocols and recommendations back to the society is facilitated by the association of several research groups in the same network and the use of FAIR data stewardship.
• Mobility: several research groups working in the same consortium enables transnational mentoring and researcher mobility between the different participants. It ensures science excellence and a more general distribution of knowledge including to participants with less capacity in the field of the Action.

1.4.2. IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

There are some former existing efforts at the European and international level directed at specific parts of the proposed Action. Regarding germline CRC genetics, the more relevant are the European Hereditary Tumor Group (EHTG) and COGENT in Europe, Colon CFR in USA and InSiGHT and GECCO at the international level, as well as some national-level initiatives such as the Austrian CORSA. There are also national-level CRC screening initiatives such as the Dutch bowel cancer screening program. Likewise, the EU EPIC consortium works on cancer and other chronic diseases and their relationship with genetics, environment and metabolomics. On the other hand, the International Human Microbiome consortium works to understand the role of the human microbiome in the maintenance of health and causation of disease. MEDOCC (Molecular Early Detection of Colon Cancer) and CANCER-ID are consortia working on improving FOBT and CTCs/nucleic acid detection in blood. Finally, the International Cancer Genome Consortium and NIH TCGA/PCGA initiatives have been organized to elucidate the genomic changes present in many forms of cancers. COST Actions related are CA16113 (CliniMARK) and CA16120 (European Epitranscriptomics Network).
This Action will gather as many participants as possible from the previously mentioned consortia in order to work in the proposed objectives. Collaboration will be sought between the present Action and the existing consortia for mutual benefit. Integration of existing groups at the national-level in this international initiative will be also pursued, as well as the creation of new groups in countries where they do not exist such as screening programs. Interaction with running Actions will be also of interest if there are overlapping objectives.

2. IMPACT

2.1. EXPECTED IMPACT

2.1.1. SHORT-TERM AND LONG-TERM SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS

TRANSCOLONCAN is expected to have considerable impact from the scientific, technological and socioeconomic points of view.
Scientific impact (short-term): A risk model will be generated by using low-penetrance germline CRC predisposition variants, microbiome, epigenetics, metabolomics and environmental factors and these variables will be tested as predictors for CRC and advanced adenomas. Substantial improvement of the current methods used in CRC screening programs is expected. CTCs, circulating tumor nucleic acids, exosomes and TEP will be tested as non-invasive biomarkers for early detection of CRC. Novel approaches such as single-cell genomics will be used considering tumor heterogeneity and will permit a better tumor profiling, unraveling new tumor biomarkers with prognosis and predictive value. Novel methods will be developed for correct pathogenicity assessment of genetic variants and innovative CRC treatments such as immunotherapies will be verified. Participants will facilitate access to several CRC cohorts and screening programs in order to accomplish the aforementioned impacts.
Technological impact (long-term): Risk modeling methodology will be solidly confirmed for application in running CRC screening programs. The best CRC non-invasive test, tumor biomarkers, functional validation technology and novel therapies will be successfully validated and used in the clinical setting.

Socioeconomic impact (long-term): Societal benefits of this Action will focus on improving health for citizens of Europe and worldwide. Since CRC represents a highly frequent malignancy, seriously affecting quality of life of individuals, collaborative approaches may permit improvement of preventive measures, diminish CRC incidence and improve treatment efficacy. By doing so, economic benefits, such as reducing hospitalization and treatment expenses, could be expected and have an important impact in the healthcare systems. Also, since this Action will uncover new CRC biomarkers, there will be innovation opportunities and patent licensing prospects. Several European SMEs are supporting the present network and they could be involved in translating the achieved results to the market.

2.2. MEASURES TO MAXIMISE IMPACT

2.2.1. PLAN FOR INVOLVING THE MOST RELEVANT STAKEHOLDERS

This Action will facilitate research interaction and collaboration between research groups and SMEs interested in their objectives. The network will bring together participants from COST countries, including ITCs and International partners. The Action will remain open for new interested groups and SMEs from other countries especially ITC working on this timely topic.
Participants in the present Action will belong to different disciplines including clinical CRC practice, germline and somatic genetics, bioinformatics, cell and molecular biology, microbiology, immunology, biostatistics, epidemiology, health economy and SMEs. Additional participants from these fields will be actively sought. Initial participants will help recruiting new participants to widen the network, and meetings and scientific societies of the related fields can be used to contact them.
Moreover, the network will pursue the active participation of members from the most relevant existing consortia related to the field, as well as similar national-level initiatives. Importantly, the participation of national-level CRC screening programs will be essential and they will be invited to join the Action. Finally, representatives of the regulatory authorities in the EU and members of patient organizations will be asked to join meetings as independent ad hoc participants in order to be informed and bring their point of view.

2.2.2. DISSEMINATION AND/OR EXPLOITATION PLAN

The dissemination methods used for this Action will include:
- Announcements in specialized journals reaching to new participants when starting the Action.
- An Action-specific website: dedicated to communication and promotion of information on on-going research, meetings, publications and other activities. Part of the website will be accessible to the general public, whereas a section will be password-protected for the exchange of specific information and unpublished data between partners by using FAIR data stewardship.
- Scientific publications in specialized and peer-reviewed journals either in the form of original, review, or technical articles reporting recommendations, standards or protocols.
- Action activities (meetings, workshops, conferences, training schools, STSMs) will also help disseminating the obtained results.
- Press releases for events as well as advertisements in the local media whenever useful.
- Participation of members in international conferences of the related fields presenting research projects conducted by this Action.
- To promote the timely translation of the CRC research results of this Action into policies on national, European and international level, stakeholders will be invited to meetings including representatives from patient organizations.
- To increase the visibility of this Action, meetings will be preferably organized as satellites to major scientific conferences in the related fields.

The Action Management Committee (MC) will advise participants to protect IP rights, always respecting the COST Association principles.

2.3. POTENTIAL FOR INNOVATION VERSUS RISK LEVEL

2.3.1. POTENTIAL FOR SCIENTIFIC, TECHNOLOGICAL AND/OR SOCIOECONOMIC INNOVATION BREAKTHROUGHS

TRANSCOLOCAN will represent a European-based scientific and technological network committed to the identification of biomarkers for CRC. These disease indicators will be used to improve CRC screening, early detection and disease follow-up. A CRC risk profile will be developed by using low-

penetrance CRC genetic variants, epigenetics, microbiome, metabolomics and environmental factors to make CRC screening programs more selective. Non-invasive biomarkers will be established for early detection of CRC and early relapse. They will also enable earlier individualized measurements of therapy response leading to a better treatment optimization. Additional biomarkers will be identified through an enhanced profiling taking into account tumor heterogeneity. Also, state-of-the-art technology will allow correct pathogenicity assignment and the identification of new genes for germline CRC predisposition. Finally, novel immunotherapies for metastatic CRC will be developed, hopefully with increased efficiency and reduced toxicity. All the previously described objectives are very timely and represent breakthroughs in the CRC field. On the other hand, as stated in Section 1, they are based in previous seminal evidence, thus precluding a successful development of the Action.
Regarding the technological point of view, the identification of new biomarkers and the use of advanced methodologies will produce recommendations, standards and protocols to be established during the progress of the Action. Software for CRC risk profiling is also envisioned to facilitate its incorporation in running CRC screening programs.
As for the socioeconomic breakthroughs, this Action will work to reduce CRC incidence and mortality using prevention. Therefore, societal benefits will include improving health of citizens and patients, and economic benefits will comprise reducing hospitalization and treatment expenses, and consequently decreasing healthcare costs.
The Action is ambitious in the objectives but they have a clear potential for innovation. As detailed previously in section 1.3.3, the Action will lead to improvements in CRC screening, early detection and disease follow-up by biomarker identification for this disease. Opportunities for innovation include CRC/advanced adenoma risk calculation, improved CRC screening, new CRC early detection devices, enhanced tumor characterization, pathogenicity evaluation tools for genetic variants and novel effective therapies with less toxicity. Several European SMEs are supporting the present network and they could be involved in translating the accumulated know-how to the market.

3. IMPLEMENTATION

3.1. DESCRIPTION OF THE WORK PLAN

3.1.1. DESCRIPTION OF WORKING GROUPS

TRANSCOLONCAN will use innovative translational research to identify CRC biomarkers that will improve screening, early detection and disease follow-up, and attain better tumor profiling, state-of-the-art functional characterization of genetic variants and new therapy approaches. It will have four WG that are described below:
Working Group 1. Disease risk profiling. This WG will use low-penetrance germline genetic variants for CRC, microbiome characterization, epigenetics, metabolomics and environmental factors to model disease risk and apply it to better select individuals eligible to be screened for CRC or advanced adenomas.
Objectives: - To construct a CRC risk model using low-penetrance germline genetic variants, microbiome characterization, epigenetics, metabolomics and environmental factors. - To improve the current CRC screening strategies by using the new CRC risk model.
Tasks
- To genotype individuals for germline genetic variants for CRC and methylation markers.
- To gather environmental data for individuals to be analyzed such as age, gender, family history, alcohol, diet, weight and exercise habits.
- To characterize a gut microbiome profile that can be used to identify individuals at risk of CRC.
- To typify a metabolomics profile that can be used to identify individuals at risk of CRC.
- To produce the CRC risk model calculation for analyzed individuals and evaluate it.
- To pilot the risk model in country-specific CRC prevention programs, and to evaluate its performance and acceptability.
- To evaluate the incorporation of the risk model into large-scale CRC prevention programs, with a focus on health economics, and propose modifications to screening based on risk score.
- To develop a consensus model and adopt consistent measures of CRC screening performance across the consortium.
- To report to the bodies and individuals involved in planning, funding and running CRC prevention programs with the aim of introducing the risk score into clinical practice.
- To increase awareness of the importance and usefulness of CRC screening programs for CRC prevention by actively involving patient associations.

Activities Dedicated WG meetings will take place (1-2 per year). Workshops on the field will be included during meetings. A training school will be committed to the topic of risk profiling. Inter-laboratory exchanges in the form of STSMs are also envisioned especially for young researchers. A tight monitoring of the WG activities will be ensured by a strong WG committee.
Milestones
- Gut microbiome profile linked to CRC/advanced adenomas.
- Metabolomics profile linked to CRC/advanced adenomas.
- CRC risk modeling.
- Improvement of CRC screening programs.
Deliverables
- Research publications regarding microbiome, metabolomics profiling and CRC risk modeling.
- Software and protocols for CRC risk modeling.
- Guidelines for CRC screening.

Working Group 2. Non-invasive biomarkers. It will apply state-of-art liquid biopsies for the detection and characterization of CTCs, ctDNA, tumor-derived exosomes and TEP, and test diagnostic value for adenomas and early-stage CRCs.
Objectives:- To establish a set of validated standard operating protocols (SOPs) that can be used to assess circulating biomarkers from patients with CRC and adenomas. - To apply liquid biopsy SOPs to improve CRC screening, diagnosis, and monitoring.
Tasks
- To evaluate different systems and protocols for detection and characterization of CRC and adenomas CTCs, ctDNA, exosomes and TEP.
- To develop SOPs for detection of CRC and adenomas CTCs, ctDNA, exosomes and TEP.
- To create an infrastructure for multi-center trials and trainings to ensure compliance and performance of SOPs and to set criteria for assessing quality of clinical samples.
- To detect and characterize CTCs, ctDNA, exosomes and TEP in a multi-centre setup in defined sets of patients with advanced adenomas and CRCs, adenomas and healthy controls.
- To set quantitative criteria for classification and stratification of clinical samples.
- To compare data from CTCs, ctDNA, exosomes and TEP with clinical information derived from conventional diagnostic tools.
- To evaluate individual and multiple blood-based biomarkers for CRC diagnostic and monitoring.
- To evaluate the value of blood-based biomarkers as companion to the existing conventional diagnostic tools.
Activities: Dedicated WG meetings will take place (1-2 per year). Workshops on the field will be included during meetings. A training school will be committed to the topic of non-invasive biomarkers. Inter-laboratory exchanges in the form of STSMs are also envisioned especially for young researchers. A tight monitoring of the WG activities will be ensured by a strong WG committee.
Milestones
- Comparison of a pre-defined set of technologies and protocols for detection and characterization of CRC and adenomas CTCs, ctDNA, exosomes and TEP.
- Establishment of consensual SOPs for detection and characterization of CRC and adenomas CTCs, ctDNA, exosomes and TEP.
- Multi-centre comparison of SOPs.
- Establishment of criteria and cut-offs to classify samples according to their tumor content.
Deliverables
- Research publications regarding detection and characterization of CRC and adenomas CTCs, ctDNA, exosomes and TEP.
- SOPs for the detection of CRC and adenomas CTCs, ctDNA, exosomes and TEP.
- Guidelines for the inclusion of liquid biopsy for the diagnostic and follow-up of CRC.

Working Group 3. Tumor profiling. WG3 will focus on the genomic, epigenomic and transcriptional profiling of colorectal adenomas and carcinomas in a multiregional analysis fashion in order to identify novel biomarkers with prognosis and predictive value for CRC patient stratification.
Objectives: - To generate genomic, epigenomic and transcriptional profiling of adenomas and CRC.
- To integrate the data generated in this section with clinical features to identify new biomarkers for prognosis and prediction of treatment response.
Tasks
- To assess the mutational profiling and the genomic copy number alterations in early colorectal lesions and carcinomas using multiregional whole exome sequencing.
- To identify the methylation patterns by multiregional analysis which define the tumor sequence from adenoma to carcinoma.
- To perform single-cell RNA sequencing to decipher affected molecular and cellular pathways.
- To integrate the genomic and transcriptomic data with ITH and the patient clinical output.

- To generate a gene panel for cancer progression and perform ultra-deep sequencing in the bulk of the advanced adenomas.
- Tools to trace tumor evolution and assess the ITH based on the –omics data generated in this WG.
- Patient-derived xenografts and single-cell cloning to reproduce *in vitro* the primary tumor ITH.
- To correlate the tumor profiling with the germline variants and the microbiome present in each patient obtained in WG1.

Activities: Dedicated WG meetings will take place (1-2 per year). Workshops on the field will be included during meetings. A training school will be committed to the topic of tumor profiling. Inter-laboratory exchanges in the form of STSMs are also envisioned especially for young researchers. A tight monitoring of the WG activities will be ensured by a strong WG committee.

Milestones
- Identification of biomarkers involved in the adenoma-to-carcinoma transition.
- Deciphering the genomic make up of advanced adenomas.
- Utilization of ITH as a prognostic predictor.
- Patient triage based on cancer genomics.

Deliverables
- Publication of the adenoma and carcinoma genomic profiles and their associated transcriptomic signatures in high profile journals.
- Tools to delineate tumor evolution.
- Therapeutic impact of ITH.
- Bench-to-bed transferability of biomarkers for prognosis and treatment response prediction.

Working Group 4. Functional genomics and therapy. This WG will functionally validate candidate genetic variants from germline or tumor studies by using cutting-edge approaches such as CRISPR-Cas9 gene editing. On the other hand, it will conceive novel routes to CRC therapy including immunotherapy.

Objectives: - To link unequivocally genetic variants with an altered gene function or pathogenicity. - To develop treatments for metastatic CRC more efficient and with less adverse effects. - To develop immunomodulatory strategies sensitizing CRC not currently amenable to immunotherapy. - To optimize the combination of immunotherapy with current (neo-)adjuvant therapies.

Tasks
- To select the germline and somatic candidate genetic variants to be validated.
- To perform gene editing for the selected genetic variants by using CRISPR-Cas9 and reintroduce them in cell cultures.
- To study the alteration of cellular processes and the specific gene function in the transfected cells, comparing the studied genetic variant and their wild type counterpart.
- To assign pathogenicity to genetic variants if an alteration is detected in previous studies.
- To use 3D and xenograft models mimicking the *in vivo* CRC tissue for functional analysis.
- To define actionable biomarkers that explain resistance to checkpoint blockade in patients with mismatch repair deficient CRCs.
- To demonstrate the application of neo-antigen targeted therapies in low mutation load CRC.
- To identify mechanisms circumventing the activation of immune responses as a result of widespread chromosomal instability.
- To demonstrate the applicability of immunotherapy in the context of neo-adjuvant therapy in rectal cancer to improve response rates.

Activities: Dedicated WG meetings will take place (1-2 per year). Workshops on the field will be included during meetings. A training school will be committed to the topic of gene editing. Inter-laboratory exchanges in the form of STSMs are also envisioned especially for young researchers. A tight monitoring of the WG activities will be ensured by a strong WG committee.

Milestones
- New genetic variants involved in germline predisposition to CRC.
- New somatic genetic variants involved in CRC progression.
- Novel improved therapies for metastatic CRC.

Deliverables
- Research publications about new pathogenicity links for genetic variants and their involvement in germline or somatic CRC predisposition, and about novel CRC immunotherapies.
- Protocols for CRISPR-Cas9 gene editing.
- Guidelines for functional evaluation of candidate gene variants by gene editing.
- Optimized pipeline for neo-antigen screening in CRC patients.
- Clinical protocols for the introduction of immunotherapy in (neo-)adjuvant treatment setting.

3.1.2. GANTT DIAGRAM

	Year 1				Year 2				Year 3				Year 4			
Kick-off meeting																
MC meeting																
WG meeting																
Workshop																
Training schools																
STSM																
CRC Risk model																
Screening implementation																
Non-invasive biomarkers discovery																
Non-invasive biomarkers validation																
Tumor biomarkers discovery																
Tumor biomarkers validation																
New germline genes																
Improved CRC therapies																
Publications																
Protocols																
Risk profiling software																
Website/FAIR data stewardship																
Final conference																

MC, management committee; WG, working group; STSM, short-term scientific mission; CRC, colorectal cancer; FAIR, Findable, Accessible, Interoperable and Reusable.

3.1.4. RISK AND CONTINGENCY PLANS

It is worth highlighting that the proposed scientific objectives for TRANSCOLONCAN are mostly based on previous evidence, which diminishes the associated risk in developing them. However, there is always an associated risk in pursuing a research path. The MC and the involved WG will monitor the progress of each objective during the development of the Action and make the necessary modifications to circumvent risks.

One of the objectives of the Action is to create recommendations, standards and protocols around the CRC biomarkers identified. Agreements on final documents may be difficult to reach among the participants due to different scientific backgrounds, techniques or specific national policies. A consensus will be reached in the end taking into account simple majority after voting.

Networks bring together many participants from different backgrounds and diverse research funding opportunities. COST Actions only provide funding for networking activities. In other words, networking helps participants to progress on specific objectives but research funding needs to be provided by each participant individually from other sources in order to perform the described objectives. There is a risk that few participants have momentary difficulties in accessing to research funding compared to others. It represents a small risk to the development of the Action since the number of participants with the same expertise is expected to be high.

At the organizational level, this Action is proposing four WG that due to their complexity could suppose a risk for the correct development of the Action. For that reason, they will deserve each one of them a careful individual monitoring by a strong WG committee and dedicated meetings. In summary, risks can be mitigated and they can be traded off against a substantial and likely benefit for CRC patients, their families and healthcare systems.

3.2. MANAGEMENT STRUCTURES AND PROCEDURES

This Action will be steered by its MC, led by a Chair and vice-Chair according to COST rules. The MC will be responsible for the coordination, implementation and management of the Action activities and for supervising the appropriate allocation and use of funds in order to achieve their objectives. MC will consist of chairs and vice-chairs of WG, members of a Steering Committee for STSMs, and Action's website and publications boards for the full period of the Action. MC and WG will ensure fulfillment of the planned tasks needed to achieve the Action objectives by organizing meetings, workshops, training schools and final conference, conceiving publications, delivering reporting, and planning research activities at national level and collaborations between members inside and partners outside the Action.

This Action aims to organize two meetings per year with co-located activities including every time a MC meeting, dedicated WG meetings and a workshop. Whenever possible, and especially for the final conference, workshops will be international and open to the scientific community outside the Action and

will include outstanding experts invited to give plenary lectures. Four training schools are anticipated in this Action.
The Chair and MC will disseminate information electronically, and through an official Action's website with an open public access to ensure Action's visibility. The website will contain summaries of activities, minutes of meetings and future meeting plans, major achievements, list of published papers, and contact section for scientists interested to join the Action. A member-only restricted area for participating members will enable sharing knowledge and planning of future activities. Promising young scientists will be exchanged between participating laboratories to expedite technology transfer and accomplish specific and timely research tasks by means of STSM. The Action's organization schedule will greatly strengthen the existing collaboration of scientists from Europe and the rest of the world. Major Action's events will be organized with both academic and commercial participation to encourage transfer of scientific knowledge into practical applications.
The following activities are planned in order to achieve the scientific outputs:
1) Kick-off meeting: further definition of WG objectives, election of MC and leaders for WG and scientific committees and planning activities for the first year.
2) MC and WG meetings: One MC including also WG meetings is planned twice a year. Independent WG meetings may be also necessary throughout the year in order to facilitate progress for each objective.
3) Scientific workshops: Dedicated workshops including external invited speakers will facilitate the scientific progress and education of participants. They will be co-located with MC or WG meetings. Workshops on specific and relevant topics for existing WG are planned as well as one Final Conference to summarize and disseminate the conclusions of the Action.
4) Training schools: To widen the knowledge of the Action activities, providing intensive training on a new and emerging subject related to a WG. Training Schools in risk profiling, non-invasive biomarkers, tumor mutational profiling and CRISPR-Cas9 are planned to provide training or education to young researchers from across Europe.
5) Conference grants: To help PhD students and ECI from participating ITC countries attend international related conferences that are not specifically organized by the Action.
6) Short term scientific missions (STSM): They will be aimed at strengthening collaborative activities by allowing scientists to go to another laboratory to learn/perform a new technique. Again, they are particularly intended for young scientists.
7) FAIR (Findable, Accessible, Interoperable, Reusable) data stewardship: Use of available research IT tools with standardized annotations to facilitate data sharing.
8) Website and research publications: They will used to disseminate the outcome of the Action.
9) Special issue in a journal related to the discipline: It will be dedicated to the research outcomes of the Action as a final dissemination tool.

3.3. NETWORK AS A WHOLE

This Action is initially supported by more than 70 research groups and SMEs interested in the described objectives. The network will bring together members from COST countries, including ITCs and international partners. The participation of IPC participants from USA will be based on mutual benefit. They have relevant experience and expertise in this research topic and their participation will permit also to increase sample size, which is extremely important to the proposed objectives. Several SMEs will be also involved in this Action, anticipating a fruitful collaboration between researchers and business. CRC is a common cancer and its prevention and treatment is a general matter of interest. The Action will remain open for new interested groups and SMEs from other countries especially ITC working on this timely topic. Involvement of additional participants from other countries will be actively sought in order to increase the critical mass and make results more relevant. An ample geographical distribution in terms of countries reached by this Action will increase its impact. Also, participation of countries where CRC prevention and treatment is not so evolved (e.g. no running CRC screening programs) is required in order to contribute to their progress in this field.
TRANSCOLONCAN will include participants from different disciplines including clinical CRC practice, germline and somatic genetics, bioinformatics, cell and molecular biology, microbiology, immunology, biostatistics, epidemiology, health economy and SMEs. Additional participants from these fields will be actively sought. Initial participants will help recruiting new participants and meetings, workshops, conferences and scientific societies of the related fields can be used to contact them.

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