PROJECT FINAL REPORT

MErCuRIC

Grant Agreement number: 602901
Project acronym: MErCuRIC
Project title: A phase Ib/II study of MEK 1/2 inhibitor PD-0325901 or MEK-162 with c-MET inhibitor PF-02341066 in *RAS*MT and *RAS*WT (with aberrant c-MET) Colorectal Cancer Patients
Funding Scheme: HEALTH 2013.2.4.1-1
Project Start Date: 1/12/2013
Project End Date: 31/12/2019

Name, title and organisation of the scientific representative of the project's coordinator:

Dr. Sandra van Schaeybroeck

Clinical Reader, School of Medicine, Dentistry and Biomedical Sciences

Centre for Cancer Research and Cell Biology

The Queen's University of Belfast

Tel: +44 (0)28 9097 2954

Fax: +44 (0)29 9097 2776

E-mail: s.vanschaeybroeck@qub.ac.uk

Project website address: http://www.mercuric.eu/

1. FINAL PUBLISHABLE SUMMARY REPORT 3

1.1 EXECUTIVE SUMMARY 3

1.2 A SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES SUMMARY 4

1.3 A DESCRIPTION OF THE MAIN S&T RESULTS/FOREGROUNDS 5

1.3.1 WP1 PHASE I TRIAL OF THE MEK1/2 INHIBITOR PD-0325901 OR MEK-162 WITH THE CMET/ALK INHIBITOR CRIZOTINIB IN MCRC 5

1.3.2 WP3 SEQUENCING AND MRNA PROFILING OF TUMOURS TO FIND MOLECULAR SIGNATURES OF RESPONSE TO MEKI/METI TREATMENT 7

1.3.3 WP4 GENOMIC ANALYSIS OF CELL FREE CIRCULATING TUMOUR DNA (CTDNA) TO MONITOR RESPONSE/RESISTANCE TO MEKI/METI TREATMENT 8

1.3.4 WP5 USE OF PATIENT-DERIVED XENOGRAFTS (XENOPATIENTS; PDX) TO ASSESS NOVEL THERAPEUTIC STRATEGIES FOR PATIENTS RELAPSING FOLLOWING INITIAL RESPONSE 8

1.3.5 WP6 BIOINFORMATICS ANALYSIS AND DATA MANAGEMENT 9

1.3.6 WP7 BIOREPOSITORY AND SAMPLE DISTRIBUTION MANAGEMENT 11

1.3.7 CONCLUSION 11

1.4 IMPACT, DISSEMINATION AND EXPLOITATION 12

1.5 WEBSITE AND CONTACT DETAILS 14

1. Final publishable summary report

1.1 Executive summary

Colorectal Cancer (CRC) is any cancer of the large bowel, including colon and rectal cancer. It is a common, and often deadly, form of cancer. In Europe, around 450,000 people are diagnosed with CRC every year. With approximately 200,000 deaths per year, it remains the second most common cause of cancer death. More than half of all CRC patients develop cancer in other parts of their bodies. Only just over half of CRC patients live longer than five years after their diagnosis.

Surgery, chemotherapy and radiotherapy are used to treat CRC but the long-term outcome for many patients is very poor. Cancer is a complex disease – cancer cells can be different between patients or tumours in the same patient. So, many patients do not respond to standard treatments, based on "one-size-fits-all" approaches. Cancer cells also change with time, and can become resistant to treatments that were once effective. While some patients may be cured by current treatments, many patients do not benefit at all, yet may still suffer the unpleasant side effects associated with a therapy. Giving patients a therapy that is not effective for them also means that they lose precious time before a more effective second treatment is given.

Researchers are learning more about CRC and developing tests to profile cancer in individual patients. To provide CRC patients with better options, new "personalised medicine" treatments must be developed using this knowledge and tested in selected groups of patients most likely to benefit. The goal of "personalised medicine" is to improve survival outcomes and quality of life. In particular, novel treatments are needed for subgroups of CRC patients with poor outcomes, such as those with tumours where the RAS gene is mutated.

Previous work by the MErCuRIC team showed that in cancers with a mutated RAS gene, a second gene called MET could cause resistance to therapies that target a third gene called MEK. They found that adding a second therapy that targeted MET to the MEK therapy caused the cancer cells to die. This combination of anti-MEK and anti-MET therapies was the focus of the MErCuRIC clinical trial, conducted in five EU countries. The trial looked at two combinations of drugs: (1) Crizotinib with PD-0325901 and (2) Crizotinib with Binimetinib. A Phase Ia clinical trial was carried out during the project to determine the safe, recommended doses for the two combinations. A Phase Ib clinical trial was then conducted, focused on Crizotinib with Binimetinib, assessing the response of CRC patients with RAS mutated tumours or normal RAS but abnormal MET. The trials found that the drugs can be given together at doses where they should have an effect, but that the treatment can be hard for patients to tolerate and the best response was stable disease. So, overall the trial did not show any evidence of patient benefit from this approach.

In addition to the clinical trial, MErCuRIC included important laboratory work to increase our understanding of CRC and a biobank of clinical samples was created that can be used in future research. Using genetic material from tumour samples obtained in the MErCuRIC trial, researchers looked at whether they could identify "molecular signatures" that could identify groups of patients that might respond to anti-MEK and anti-MET therapies. Unfortunately, no predictive signatures were found, but the results were consistent with other studies and increased our understanding of the differences in cancer cells found within the same patient.

The project also developed a test for tumour DNA found circulating in blood ("liquid biopsy") that could be used to monitor whether a treatment is working or not, and is less invasive than tumour biopsies. Patients that are unlikely to respond or develop resistance to a therapy could be identified, saving them from unnecessary and ineffective treatments, and enabling them to start a more effective treatment without delay. Using this new test, researchers were able to measure level of disease and track mutations in individual genes.

Patients are at the centre of the MErCuRIC project. A patient representative joined the consortium to provide input into the clinical trial and help communicate the project's work to CRC patients across Europe. The team

also engaged with key patient organizations across Europe, including EuropaColon and the European Cancer Patient Coalition.

1.2 A summary description of project context and objectives summary

CRC is a significant health issue for men and women worldwide, with > 1 million cases and over 680,700 deaths each year.[1] CRC remains the second most common cause of cancer death within the Western world.[2] The incidence increases significantly with age, with >80% of cases occurring in the 60 years or older population. Given the ageing population in Europe, CRC's already considerable health and societal burden is expected to increase significantly.

As patients often do not often have symptoms of the disease, CRC is frequently diagnosed only after the cancer has spread. Patients with metastatic colorectal cancer (mCRC), where the cancer has spread throughout the body, have very poor survival rates. Standard chemotherapies for advanced CRC include combination of 5-Fluorouracil (5-FU) with Irinotecan (FOLFIRI) or Oxaliplatin (FOLFOX). Resistance to chemotherapy, either intrinsic or acquired, ultimately results in treatment failure for the majority of CRC patients. New and more effective therapies are urgently needed to help patients with this type of cancer. Understanding how mCRC works at the molecular level gives researchers and drug companies a better chance to develop such therapies and to identify sub-groups of patients that will benefit most from a given therapy using "personalised medicine approaches". Efficient development of novel targeted therapies for specific sub-groups of patients requires biomarkers to stratify patients, clearly distinguishing between "responders" and "non-responders", based on their molecular characteristics.

MErCuRIC sought to identify novel genetic signatures to select patient subgroups which can maximally benefit from specific therapeutic combinations. The project was a pan-European collaboration which assembled European and global leaders with expertise in the biology, pathology and clinical treatment of CRC. In a multi-country clinical trial in eight clinics across five European countries, the project tested two combinations of drugs that target two key proteins, MEK and MET**,** with the aim of improving patient survival rates. These proteins are known to be abnormal in mCRC and pre-clinical studies in cells and animal models carried out before the project started had shown that cancer cell death is higher when drugs targeting MEK and MET are given together than when given alone (Figure 1**Error! Reference source not found.**).

In addition to the clinical trial, the project also sought to further increase our understanding of the biology of mCRC and develop new diagnostic and predictive tools to improve how patients are treated in clinics. MErCuRIC researchers aimed to advance the state-of-the-art by: (i) exploring a novel treatment strategy targeting the biology of mCRC; (ii) using next generation sequencing (NGS), RNAseq and immunohistochemical strategies to identify CRC patient subgroups who will maximally benefit from this novel treatment strategy, and (iii) deploying (a) non-

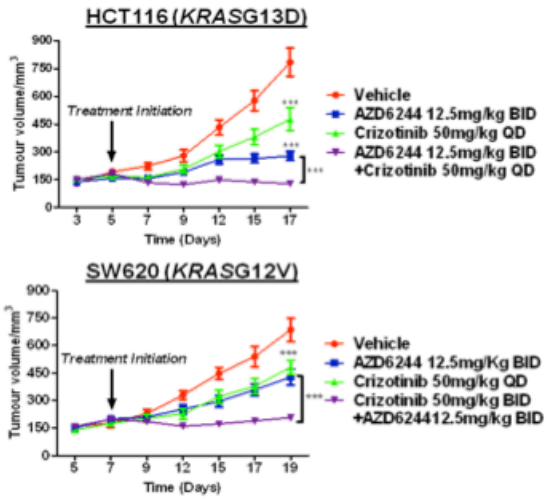

Figure 1 Pre-clinical in vitro and in vivo data of combined treatment with MEK inhibitor AZD6244 and c-MET inhibitor crizotinib in KRASMT CRC models

[1] Malvezzi M, Bertuccio P, Levi F, La Vecchia C, Negri E. (2012) European cancer mortality predictions for the year 2012. Ann Oncol. 23: 1044-52.

[2] Siegel, R., Naishadham, D., Jemal, A. (2013). Cancer statistics, 2013. CA: Cancer J Clin *63*, 11-30.

invasive detection tools (b) relevant preclinical models to underpin novel stratified solutions for patients with progressive disease.

1.3 A description of the main S&T results/foregrounds

1.3.1 WP1 Phase I trial of the MEK1/2 inhibitor PD-0325901 or MEK-162 with the cMET/ALK inhibitor Crizotinib in mCRC

MErCuRIC conducted a number of Phase I clinical studies, looking at combination MEKi/METi therapies. The first Phase Ia (dose escalation) studies looked at METi Crizotinib in combination with MEKi PD-0325901. During the study, development of the drug PD-0325901 was discontinued by the manufacturer, so an alternative MEK inhibitor was needed to continue the project. The team identified Binimetinib as a suitable replacement drug, as there was already substantial data available on its safety and Phase III studies of Binimetinib with other drugs were already on-going. A second Phase Ia (dose escalation) study was conducted to determine what doses of Crizotinib with Binimetinib. Both Phase Ia studies determined what doses could be safely given together and how often. Approvals were granted to run the study in the United Kingdom, Belgium and Spain. The studies recruited patients with any type of advanced solid tumour, for whom inhibition of MEK and MET were a treatment option based on genetic screening and treatments they had previously received. Patients were assigned to cohorts and treated with different dosing regimens (Figure 2). Blood samples, tumour biopsies and other clinical data were collected and analysed before and during treatment. Pharmacokinetics (PK), the way the body absorbs, distributes, and clears a drug, were studied using blood samples.

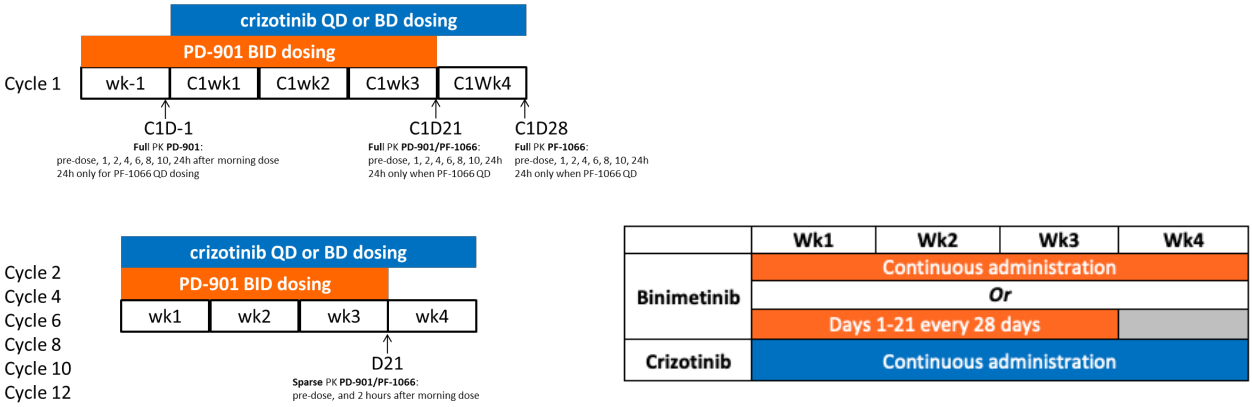

Figure 2 Schedule for PD-0325901/Crizotinib (left) and Binimetinib/Crizotinib (right) in MErCuRIC phase I studies

25 patients were registered in the Phase Ia study of Crizotinib with PD-0325901. In total, four doses were studied and it was found that the two drugs could be given together at doses expected to have an effect. The maximum tolerated dose (MTD) was determined as PD-0325901 8 mg twice daily (BD) over days 1 - 21 and Crizotinib 200mg BD continuously in a 28 day cycle.

20 patients were registered the Phase Ia study of Crizotinib with Binimetinib. In total, three doses were studied and, as for Crizotinib with PD-0325901, it was found that the two drugs could be given together at doses expected to have an effect. The MTD was determined as Binimetinib 30 mg BD over days 1 - 21 and Crizotinib 250 mg once daily (OD) continuously in a 28 day cycle.

Having identified the MTD for Crizotinib with Binimetinib, a Phase Ib (dose expansion) study was conducted to look at this combination of drugs in CRC patients with defined molecular sub-groups. Based on the latest knowledge from published studies and results from MErCuRIC partners, researchers identified characteristics of patients most likely to respond to METi/MEKi and selected three sub-groups to target in the study: (1) normal RAS gene (*RAS*WT) with MET super expression ('cappuccino' group), (2) normal RAS gene (*RAS*WT) with MET amplification or mutation ('espresso' group) and (3) mutated RAS gene (*RAS*MT) (Figure 3). One sub-group (*RAS*MT) was known to be more common than the other sub-groups, and if a response to treatment was seen in this group in Phase Ib, a Phase II study would be conducted.

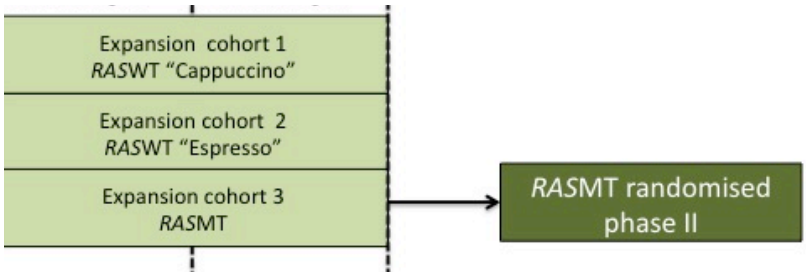

Figure 3 Molecular sub-groups, phase I/II trials

The Phase Ib study aimed to look at the response to treatment and to further investigate safety, toxicity and tolerability. Response to treatment was assessed in terms of clinical and radiological response (tumour size) looking to see if the disease was stable, partially-responsive or completely-responsive. Progression-free survival (PFS, length of time where disease does not worsen) and overall survival (OS, length of time from diagnosis or start of treatment) were also examined. As in the Phase Ia study, PK studies were conducted to look at drug absorption, distribution and clearance. Additional pharmacodynamic (PD) studies were performed using skin and tumour biopsies, looking at the biochemical and physiological effects of the drugs. Approvals were granted to run the study in the United Kingdom, Belgium, Spain, Ireland and France.

37 eligible patients were identified and enrolled in the Phase Ib (dose expansion study): 36 patients in the *RAS*MT sub-group and one in the *RAS*WT 'cappuccino' sub-group. These were patients that received several, and often all available, lines of chemotherapies before entry in the MErCuRIC phase Ib study. In the *RAS*MT sub-group, once 30 patients had completed a response assessment after cycle 1 of treatment or had disease progression, an interim analysis was conducted to determine if there was enough evidence of clinical and radiological response to treatment to continue with the study. The PK analyses found that giving Binimetinib with Crizotinib did not affect the PK of Binimetinib compared to giving Binimetinib alone, and PD results showed evidence of inhibition of the targeted molecular pathways. However, the radiological response criteria were not met and the treatment combination was difficult for patients to tolerate, so recruitment to this sub-group was closed. As a result, the planned Phase II trial was not conducted. Recruitment to the *RAS*WT sub-groups continued after the *RAS*MT sub-group closure, but not enough patients were identified to continue the study.

While the Phase Ib study was closed early, valuable clinical samples and data were collected during both the Phase Ia and Phase Ib studies that were used to further our understanding of mCRC, as described in the following sections. The lack of tumour response meant that translational work on study samples focused on the biological effects of treatment and the natural history of the disease, since relationships with efficacy could not be explored.

1.3.2 WP3 Sequencing and mRNA profiling of tumours to find molecular signatures of response to MEKi/METi treatment

To adopt "personalised medicine" approaches for the treatment of CRC in clinics, we need ways to identify which patients are most likely to respond to specific treatments. One way to do this is to classify or stratify patients based on the molecular signatures of their tumours. Two classification systems have been previously developed and published for CRC: (1) consensus molecular subtypes (CMS), and (2) colorectal cancer intrinsic subtypes (CRIS).[3] Both systems use gene signatures specific to CRC tumours and may be able to predict outcomes, such as OS, PFS or treatment effect in mCRC.[4] Most of the work related to these classification systems has been performed on primary tumour samples, before exposure to chemotherapy. Little is known about if these systems are also predictive when using tumour samples from patients who have undergone chemotherapy, in particular for patients with advanced disease who have had multiple types of treatment, like those enrolled in the MErCuRIC clinical trials.

DNA and RNA were extracted from biopsies of MErCuRIC *RAS*MT patients in the Phase Ib study were analysed using next generation sequencing (NGS) that targets 92 amplicons and over 500 'hot spot' mutations in genes linked to CRC. 67 samples from 26 patients were analysed, with samples collected before treatment at baseline screening and after 15 or 21 days of treatment. Using validated software, the NGS data was used to classify samples into CMS and CRIS sub-types. For 19 patients enrolled in Phase Ib study where a pre-treatment biopsy sample was available and whose samples showed more than 30% of tumor cells, both CMS and CRIS subtypes were determined. Using the data, we tested whether the molecular subtypes are associated with disease stabilization using RECIST 1.1 criteria for the target lesions. After removing the patients with missing endpoints or with non-assessable target lesions, 14 cases could be used to test the association between molecular classification and clinical outcomes. No correlation between CMS or CRIS groups and (benefit) effect of treatment with Binimetinib/Crizotinib was identified.

HGF/MET targeted therapies have been assessed as new paradigm in the treatment of number of cancer types, including CRC. A number of efforts have been made over the last few years to identify the patient population(s) most likely to derive benefit from HGF/c-MET targeted therapies. A number of biomarker platforms have been assessed in clinical trials, in particular for c-MET immunohistochemistry (IHC). Although MET IHC scoring optimization, has been able to identify the patient population benefitting from MET targeting therapies in phase II clinical trial, the same manually scored immunohistochemistry (IHC) for cMET failed as predictive marker in phase III clinical studies. These studies indicated that cMET IHC alone is insufficient to identify the MET dependent cancer.

Using CRC liver biopsies, available in the NI Biobank, we developed and optimized MET protein (IHC) and gene (RNA and DDISH) scoring algorithm. Subsequently,this scoring algorithm was applied to the pre-treatment biopsies obtained during the phase Ib clinical trial with Binimetinib and Crizotinib in RASMT advanced CRC patients. These data showed that 10/33 patient's tumour were grouped as superexpressors (IHC/RNA scope 3+), one patient's tumour was MET amplified. There was no correlation between MET expression levels and clinical outcome.

We subsequently stained and applied MET scoring algorithm to 3 large stage II/III patient cohorts. Initial data would indicate that MET RNA scope, and not IHC, correlates with poor outcome.

[3] Guinney et al. Nature Med 2015, Isella et al. Nature Comm 2017

[4] Guinney et al. Nature Med 2015, Dunne et al. Nature Comm 2017, Stintzing S et al. J Clin Oncol. 2017, Okita et al. Oncotarget 2018

1.3.3 WP4 Genomic analysis of cell free circulating tumour DNA (ctDNA) to monitor response/resistance to MEKi/METi treatment

In addition to studying the molecular nature of tumours in CRC, MErCuRIC looked at cell-free circulating tumour DNA (ctDNA) found in patient blood samples. ctDNA shed by tumours into the blood stream of patients can be analysed by NGS and used to monitor tumour growth and identify molecular biomarkers associated with response and acquired resistance ("liquid biopsies", Figure 4). As the procedure is less invasive than tumour biopsies, liquid biopsies can be used more frequently to monitor treatment. In CRC, liquid biopsies can be used to study how tumours develop resistance to treatments and help clinicians select therapies that may improve clinical outcomes earlier than with tumour biopsies.

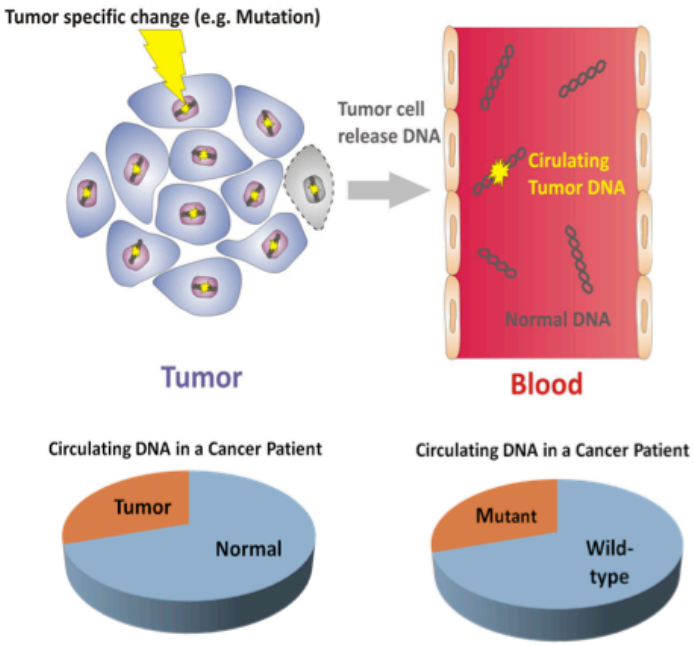

Figure 4 Detection of ctDNA in plasma of cancer patients

ctDNA analysis was performed on plasma samples collected from 36 patients from the Phase Ib study using NGS and Droplet Digital PCR. The analyses of ctDNA were performed longitudinally to follow the patients during therapy, with 92 samples being tested. In all patients, we identified mutations related to the tumour and confirmed the RAS mutation.

In the vast majority of the patients, no drop in the level of RAS mutations was observed when compared to baseline levels and the samples taken prior to cycle 2 of treatment. This is consistent with the clinical data, where none of the patients in the Phase Ib study showed a partial radiological response. Blood marker parameters parallel the lack of clinical benefit seen in these patients, and the dynamics of mutations in liquid biopsy mirrored the PD biomarkers.

In addition to the Droplet Digital PCR assay, a high sensitivity liquid biopsy target panel (LB-panel) was developed and validated.[5] The LB-panel looks at 'hot spot' regions in 44 genes relevant for CRC. Samples from three Phase Ib patients that had received at least four cycles of treatment and had sufficient DNA available were analysed using the LB-panel. In all patents, the presence of RAS mutations was confirmed. Two patients showed an increased tumour load, while one showed a stable tumour load at the start and end of treatment.

1.3.4 WP5 Use of patient-derived xenografts (xenopatients; PDX) to assess novel therapeutic strategies for patients relapsing following initial response

Representative models are needed to study the biology of CRC in laboratory settings to support the development and testing of new drugs before clinical trials. To be effective, the models must reflect the clinical features of patients and the molecular pathways involved in the disease. One type of model commonly used in biomarker discovery and drug development is cancer cell lines. The Cancer Cell Line Encyclopedia, the Genomics of Drug Sensitivity in Cancer (GDSC) Sanger Institute project, the National Cancer Institute-60 (NCI-60) cancer cell line screen and the Cancer Therapeutic Response Portal (CTRP) are cell lines and initiatives that

[5] R. Kennedy et al., Nat Protoc 9, 2586-2606 2014; M. W. Schmitt et al., Nat Methods 12, 423-425 2015

have been used to systematically screen compounds and provide a platform for pharmacogenomic analysis. While useful, there are some drawbacks with using cancer cell lines. For example, they do not represent the genetic differences found between and within tumours, and they do not replicate tumour microenvironments.

An alternative model is patient patient-derived tumour xenografts (PDXs, xenopatients), where tumour material from patients are engrafted in animal models. The resulting PDXs maintain the genetic heterogeneity of the patient's tumour and the molecular pathway activity of the patient is replicated. MErCuRIC planned to engraft tumour material from patients who responded to MEKi/METi therapy and then developed resistance to treatment, in order to study novel therapies in resistant patients. It was not possible to conduct this study as no patients in the Phase Ib responded and then acquired resistance. Instead, existing CRC PDXs were used to model the MErCuRIC clinical setting. A PDX model of a patient with metastatic CRC with MET gene amplification and overexpression of MET protein was selected for similarity with the 'espresso' sub-group (*RAS*WT with MET amplification or mutation) of the Phase Ib study, and a patient-derived cell line (PDC) was derived. This cell line model, called WiDr res, was treated with: (1) Crizotinib alone, and (2) Crizotinib with Binimetinib to see if the response differed. The data showed that adding Binimetinib markedly increased the response to Crizotinib. The results obtained helped to refine the hypothesis that led to the clinical trial, in particular the combination of Binimetinib and Crizotinib in MET amplified *RAS*WT CRC.

1.3.5 WP6 Bioinformatics analysis and data management

Bioinformatics involves collecting, classifying, storing and analysing data, using specific high-performance hardware and advanced software tools, in order to better understand biology and disease. In MErCuRIC, bioinformatics work focused on analyses of NGS and RNA profiling data to help understand the mechanisms that lead to the development of resistance to combined MET/MEKi treatment and integrative analysis of the various data sources (clinical data, NGS, miRNA, ctDNA and xenografts) in order to discover biomarkers predicting response, as well as developing and maintaining a database and web portal for data hosting and sharing between partners across Europe.

Bioinformatics relies on data, and in the case of MErCuRIC, the planned bioinformatic focus was on response and resistance emergence. As the Phase Ib study did not yield any responders in terms of clinical response, the bioinformatics focus was shifted to molecular changes and to the co-development of a better approach to look at cMET with immunohistochemical (IHC) analysis of pathology slides.

The data for the bioinformatics analyses in MErCuRIC was collected at multiple sites, so a platform to bring the data together was necessary. A data warehouse was developed and maintained to support data sharing among partners. After a few iterations, the final solution adopted was based on an open source project supported by Kitware Inc (Resonant - https://resonant.kitware.com/). The software has been deployed on Masaryk University's secure servers and provided access to all data produced within the project. A notable feature of this solution, leading to its selection, was the ability to display virtual pathology slides and to allow their annotations remotely. Thus, the IHC slides stained for cMET could be assessed by experts from various participating institutions.

The main data sources available for the bioinformatics analyses were:

- clinical data, including demographics, diagnosis and endpoint information;
- ctDNA data used for monitoring response to treatment and exploration of potential predictors; and
- IHC virtual pathology slides.

After initial cleaning and re-formatting, the clinical data was immediately usable for statistical analyses, the other data types more explorations/adjustments.

The liquid biopsy platform allows quasi-real-time measurement of various blood markers for achieving precision medicine in the clinical management of cancer. One of the means for performing a liquid biopsy is the profiling of ctDNA which could provide information about the genetic make-up of the tumour(s) currently present in the patient. ctDNA is released in the blood stream by the tumour cells via various mechanisms and is present in the blood at low to very low concentrations, requiring highly sensitive detection techniques. In MErCuRIC, ctDNA profiling was performed on blood samples obtained from the patients enrolled in the Phase Ib clinical trial, as described above (WP4).

The blood samples were drawn at the beginning of each of the treatment cycle (6 cycles) and at the end of the treatment (EOT). Due to various reasons (including patient withdrawing from study, adverse reactions, etc.), for most of the patients, there were only a few of time points available, leading to a very sparse data structure. At each time point, the presence and abundancy of a number of target mutations (of the cancer-related genes KRAS and NRAS) were assessed. Further, in many cases the targeted mutations could not be detected and the time lapse between the treatment cycles was not constant. Thus, we attempted to use some derivate features as potential predictors for the outcome. We explored three main such features: for each patient and target mutation, computing: (i) the average abundance; (ii) direction of change (i.e. slope of the linear regressor fitted on the abundances across time points); and (iii) presence indicator (i.e. simple binary variable indicating whether the mutation was detected or not).

With these ctDNA-based variables we attempted to fit predictors for the "disease stabilisation" endpoint (with and without major clinical parameters). Models have been fitted in both scenarios, without any significant difference. Due to the small sample size and the sparsity of the data, no statistically significant model could be identified.

In an attempt to understand the mechanisms leading to lack of response to MET/MEKi combined treatment in patients, we have analysed cMET staining in FFPE tissue sections. Both RNAscope (in situ hybridisation) and classical IHC staining were performed and scored on a semi-quantitative scale. In Figure 5, a cMET stained tissue section and the distribution of cMET scores are shown. The correlation between RNAscope and IHC scores is relatively low (0.6) and there is no association between these scores and the treatment outcome.

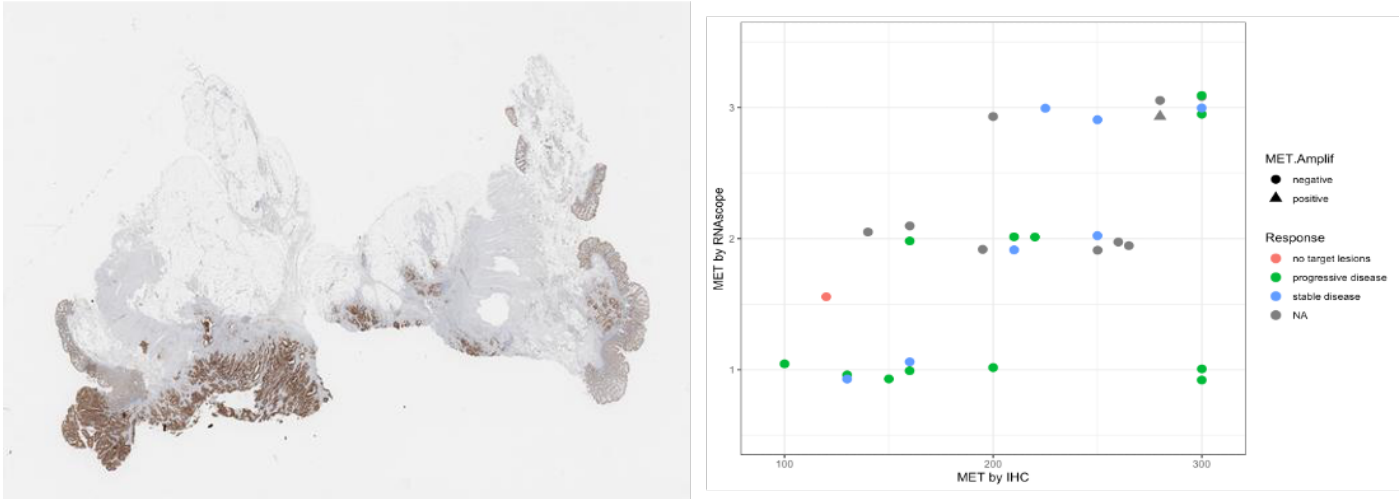

Figure 5 (Left) Tissue section stained for cMET (IHC) with high staining grade (300). (Right) Distribution of IHC and RNAscope scores in the context of clinical response.

Integrative analyses: We have also attempted to combine in the same model both ctDNA-derived variables and cMET scores, but the cMET scores have not been selected (in the automatic variable selection procedure) indicating lack of predictive power in the given context (variables and sample size).

The bioinformatics analyses were limited by the amount of available data. The small sample size and lack of clinical response did not allow the development of any definite predictive model. However, the several tentative models indicate that there is still a need for more detailed analyses for understanding the lack of response in patients with RAS mutations (KRAS (NRAS) mutations) and analyses of immune response may shed light onto this issue.

1.3.6 WP7 Biorepository and sample distribution management

MErCuRIC set out to develop of a collaborative infrastructure that allowed for the successful transfer and processing of clinical samples for quantitative experimental analysis, and the secure exchange and compilation of detailed clinical data documentation. The collection and exchange of patient tissue and corresponding clinical data was crucial to the successful implementation of MErCuRIC. Therefore, the consortium partners implemented a mutually agreed workflow for tissue collection and exchange, in addition to data transfer. This workflow has been applied to the clinical tissue and blood samples collected during the phase Ib study in BHSCT, OXFORD, VHIO, UZA, VCC, RCSI (Beaumont Hospital) and APHP (Hospital St Antoine and Hospital European George Pompidou). Subsequent work using tissue samples and blood samples in WPs 3, 4 and 5, as well as associated data integration work based on these analyses in WP6, were dependent on the accomplishment of the objectives of WP7.

The successful exchange of clinical data and patient samples between the partners was crucial for the progression of the project. MErCuRIC has successfully implemented the necessary workflows for the collection, processing, labelling, transfer and movement of samples of both phase Ia and Ib clinical trials (Binimetinib/Crizotinib) between partners, and the creation of an appropriate and effective biorepository. These achievements are fundamental to MErCuRIC, and provided the essential groundwork for the work of the other WPs in the project. Details of sample collection, processing, storage, shipment addresses and timing and couriers were updated in sample handling manuals for dose escalation and expansion. The PK samples of the dose expansion have been successfully collected and analysed. All PD samples (screening and post-treatment FF skin biopsies, FFPE tumour biopsies) from the 37 RASMT/WT patients have been analysed. All ctDNA samples, serum samples and FF tumour samples have been analysed.

1.3.7 Conclusion

MErCuRIC set out to investigate a novel MEKi/METi combination therapy for mCRC patients, who currently have poor clinical outcomes and few treatment options. The combination therapy was proposed based on our best understanding of mCRC disease mechanisms and focused on specific patient groups, with the aim of enabling “personalised medicine”. A Phase Ia (dose escalation) study was completed for two MEKi/METi combinations (PD-0325901/Crizotinib and Binimetinib/Crizotinib) in patients with advanced solid tumours, with the finding that both combinations could be given safely at doses expected to have a clinical effect. The PK analyses found that giving Binimetinib with Crizotinib did not affect the PK of Binimetinib compared to giving Binimetinib alone. A Phase Ib (dose expansion) study was undertaken with Binimetinib and Crizotinib with mCRC patients, with the aim of looking at three sub-groups of mCRC patients expected to respond to the therapy. Interim analyses found no clinical response in one sub-group, though PD results showed evidence of inhibition of the targeted molecular pathways. Recruitment to the remaining two sub-groups proved infeasible due to the rarity of patients.

Valuable clinical samples and data were collected during both the Phase Ia and Phase Ib studies that were used to further our understanding of mCRC, focusing on the biological effects of treatment and the natural history of the disease:

- Molecular signatures were used to classify MErCuRIC tumour samples using established CMS and CRIS systems to examine if the classifications could be used predictively in patients with advanced disease who have received multiple treatment types. No correlation between CMS or CRIS groups and (benefit) effect of treatment with Binimetinib/Crizotinib was identified. Nonetheless, an interesting result was obtained – metastatic tumours, in spite of the patients being *RAS*MT, classified differently compared to what is observed for *RAS*MT primary tumours. This is notable as most treatment decisions for patients presenting with metastatic disease are made based on molecular characteristics of their primary resected tumours.
- Liquid biopsies (ctDNA analyses) were performed to follow patients during therapy. In the vast majority of the patients, no drop in the level of RAS mutations was observed when compared to baseline levels and the samples taken prior to cycle 2 of treatment. This was consistent with the clinical data, where none of the patients in the Phase Ib study showed a partial radiological response. Blood marker parameters parallel the lack of clinical benefit seen in these patients, and the dynamics of mutations in liquid biopsy mirrored the PD biomarkers.
- Based on the results of the NGS analysis of the ctDNA samples, novel potential treatment combination strategies for RASMT patients were identified.
- A cell line model similar to the 'espresso' sub-group (*RAS*WT with MET amplification or mutation) of the Phase Ib study showed that adding Binimetinib markedly increased the response to Crizotinib, helping to refine the hypothesis that led to the clinical trial, in particular the combination of Binimetinib and Crizotinib in MET amplified *RAS*WT CRC.
- Bioinformatics analyses were conducted but were limited due to the small sample size and sparsity of data available due to early closure of the Phase Ib study. Liquid biopsy and histochemical data were analysed but no statistically significant model could be developed. However, the several tentative models indicate that there is still a need for more detailed analyses for understanding the lack of response in patients with RAS mutations (KRAS (NRAS) mutations) and analyses of immune response may shed light onto this issue.
- A novel MET scoring algorithm was developed. Further data showed that MET scope and not protein levels correlate with patient outcome in early stage CRC.

1.4 Impact, dissemination and exploitation

While surgery can be curative in the early stages of CRC, treatment of advanced and metastatic disease is more difficult as there are no effective therapies. Therapies targeting pathway biology have brought some improvements in PFS and OS, but intrinsic or acquired drug resistance is common and severely limits the success of these approaches. Thus, new drugs to overcome treatment resistance are needed. The lack of treatment options is a major problem for health systems as mCRC accounts for >50% of all CRC cases. CRC is an increasing health problem with the ageing populations of Europe and the Western World, with over one million new cases and over 680,700 deaths each year.[3]

MErCuRIC investigated a novel "personalised medicine" approach in mCRC patients, aiming to improve both PFS and OS in patient groups with very poor clinical outcomes. While the MEKi/METi combination therapy could be safely given at doses expected to have an effect, no clinical response was observed and the therapy was difficult for patients to tolerate. However, MErCuRIC included translational and pre-clinical research in addition to the clinical trial, which have furthered our understanding of the underlying biology in mCRC. Based on the results of the NGS analysis of the ctDNA samples, novel potential treatment combination strategies for

poor prognostic *RAS*MT CRC patients were identified. These novel treatment combinations will be further explored in pre-clinical *RAS*MT *in vivo* models. In addition, a novel MET scoring algorithm was developed. Further data showed that MET scope and not protein levels correlate with patient outcome in early stage CRC. The data will be validated using a tissue microarray including 1800 stage II/III CRC patients.

Through MErCuRIC, new strategic research collaborations were founded and existing ones strengthened, an additional impact of the EU funding received. In the closing months of the project, the partners established a Virtual Research Community and developed a Joint Action Plan for Research based on potential avenues for future collaborative research.

In addition to clinical and research activities, MErCuRIC included a public patient involvement (PPI) initiative, to maximise social benefit, patient benefit and research value. Patient representatives were active in review of materials for the MErCuRIC clinical trials, as well as communications activities. We established and fostered links with key organisations, such as Digestive Cancer Europe, that could act as communications amplifiers. Through a number of public events, partners promoted the value of publicly-funded clinical and basic research, as well as the importance of clinical trials to advancing personalised medicine (Figure 6). An 'explainer' video was also developed to make clinical and translational work more accessible to general audiences (https://youtu.be/BF66gx__plo,

Figure 7) and links to information on CRC for patients and the general public were included on the project website.

Figure 6 Bowel Cancer Public Information Evening 2016 (top left), International Clinical Trials Day 2019 (top middle) 1st European Alliance for Personalised Medicine Congress - Personalising Your Health: A Global Imperative!, 2017, (Top right), Bowel Cancer Public Information Evening 2015 (bottom)

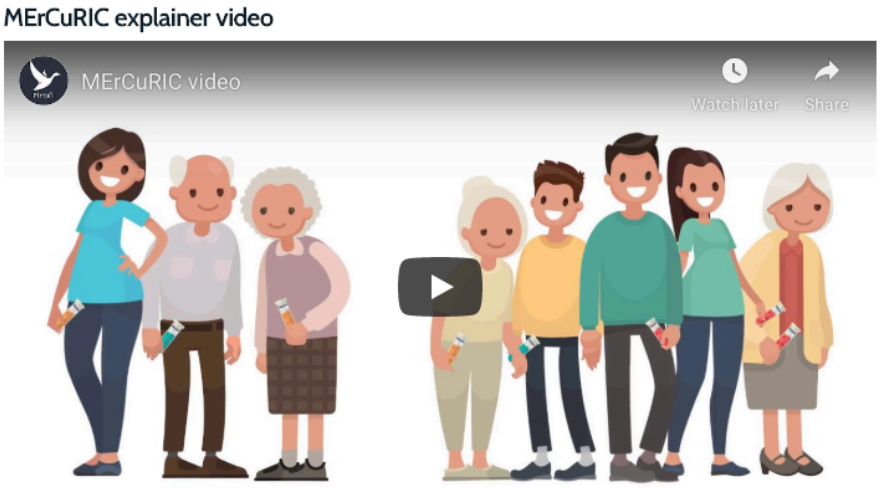

Figure 7 MErCuRIC explainer video

MErCuRIC researchers actively disseminated the results of the project through peer-reviewed publications. 34 publications were published in total, in journals such as Nature Communications, Nature Medicine, Molecular Oncology, Clinical Cancer Research, Genome Medicine and Cancer Discovery. The website includes a full list of all MErCuRIC publications (http://mercuric.eu/project/publications/). The team has also made presentations at major international and national scientific conferences, such as the European Society for Medical Oncology (ESMO) Congress, American Association for Cancer Research (AACR) Annual Meeting, American Society of Clinical Oncology (ASCO) Meeting and the UK National Cancer Research Institute Conference.

1.5 Website and contact details

Dr. Sandra van Schaeybroeck

Clinical Reader, School of Medicine, Dentistry and Biomedical Sciences

Centre for Cancer Research and Cell Biology

The Queen's University of Belfast

s.vanschaeybroeck@qub.ac.uk

http://mercuric.eu/