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unicancer RESEARCH

SOMMARY

Dr. Claire Labreveux
Director of the R&D Department of Unicancer

Identifying the onset of cancers or their relapse as early as possible is one of the challenges of oncology.

Unicancer, a pioneer in personalized medicine, has developed for several years, as part of its research strategy, a research program on circulating tumor DNA.

Several clinical studies or translational research have been and are being conducted at Unicancer, in different indications, in order to better characterize and validate the clinical utility of these biomarkers.

The impact is not only significant at the time of cancer diagnosis, but also when choosing treatment and throughout care.

We are pleased to present the results of these essential studies for effective personalized and egalitarian care for all patients.

One important Unicancer's line of research is to develop and promote the use of circulating tumor DNA (ctDNA) during cancer care. This DNA released from tumor cells into the bloodstream contains the mutations of the primary tumor. Detection of this particular DNA is a non-invasive technique for patients and may represent a new tool to help physicians in their endeavor to offer the best possible care for cancer patients.

To improve patient survival and quality of life, Unicancer promotes an increasing number of clinical studies to investigate the role of circulating tumor DNA detection in digestive, liver, colorectal, pancreatic, breast, and head and neck cancers.

Therefore, Unicancer investigates the value of circulating DNA detection at different cancer care stages:

- At diagnosis to facilitate and improve cancer detection. This can translate in early detection of the disease
- At treatment to help to decide the best treatment choice for each patient (personalized medicine) or to initiate a switch of treatment at the best possible timing, even before clinical detection of disease progression
- After treatment to determine the prognosis and thus to adapt supportive care for patients

édito

THE NIRVANA-LUNG STUDY

Unicancer, Radiation oncology Group, UNITRAD

Detect circulating tumor DNA to evaluate predictive biomarker of overall survival in patients with advanced non-small cell lung cancer included in the NIRVANA-Lung study: a collaboration with Natera™ company.

NIRVANALung study is an open-labelled, randomized, multicentric phase III study sponsored by Unicancer and endorsed by its Radiation oncology Group, UNITRAD, testing the addition of radiotherapy to pembrolizumab/chemotherapy in the first line setting in advanced non-small cell lung cancer (NSCLC). Accrual is ongoing, 110 patients have been recruited to date, and will include a total of 515 patients.

- The primary objective of this study is to evaluate the efficacy of a combination of multisite radiotherapy with pembrolizumab plus chemotherapy in terms of overall survival (OS).
- The secondary objectives are to compare toxicity, progression-free survival and quality of life between the two arms.
- In addition, the ancillary studies aim to evaluate predictive biomarkers of OS.

Indeed, the identification of markers that can predict outcome during immunotherapy and its association with radiation treatment are of high interest in order to identify the best responders to the treatment. Therefore, ancillary studies will comprise analysis of circulating tumor DNA (ctDNA) which is identified as good candidate for such surrogate markers.

While molecular diagnostics have traditionally been performed on biopsies of solid tumor tissue, blood-based tests or so-called "liquid" biopsies are gaining popularity as they provide the opportunity to genotype in a less invasive and less expensive manner, potentially in cases with insufficient tumor samples for tissue sequencing, and may offer a chance to monitor the molecular features of a cancer through the course of treatment, or predict relapse after adjuvant treatment. There are currently two US Food and Drug Administration (FDA)-approved ctDNA tests for lung cancer patients, both in the EGFR mutation-positive setting. It is likely that as more data emerge, the use of liquid biopsies to assess other molecular abnormalities will become more widespread.

The principle behind liquid biopsies is that cell-free ctDNA and/or circulating tumor cells (CTCs) are often present in the blood of patients with lung cancer. Platforms available for clinical use focus almost exclusively on isolating and detecting ctDNA, rather than CTCs. Polymerase chain reaction (PCR)-based platforms include allele-specific PCR, which preferentially amplifies a mutant DNA molecule over wildtype DNA, and emulsion PCR assays, which perform PCR reactions in thousands of droplets of a sample to quantify mutant and wildtype DNA. Next Generation Sequencing (NGS)-based plasma genotyping platforms are much broader in scope but currently take several weeks for results. In general, all of these methods are highly specific, although some platforms may detect allelic alterations that are present at such a low frequency that they may be clinically insignificant or represent low-level sequencing background noise.

In NSCLC patients receiving immune checkpoints inhibitors (ICI), ctDNA was up to now mainly used for tumour mutation burden (TMB) analyses. A high blood TMB has proven to be correlated with response to inhibitors of programmed cell death (PD)1 and its ligand (PD-L1), in particular with NSCLC and atezolizumab. However, the clinical adoption of TMB is challenging due to the high costs associated with whole-exome sequencing or broad panels.
Monitoring of response is another application of ctDNA during ICI treatment. Several reports have demonstrated a good correlation between ctDNA kinetics and clinical response. A complete or partial clearance at 4–8 weeks was strongly predictive of a durable response, whereas persistence of ctDNA had a detrimental impact. It was also showed that early changes in ctDNA burden may have the ability to discriminate between pseudo-progression and true progression.

These studies require confirmation of the prognostic impact of ctDNA in prospective cohorts with a significant number of patients, such as in our study.

Natera™ is a company based in USA leader in cell-free DNA (cfDNA) testing dedicated notably to oncology developing personalized genetic testing and diagnostics part of the standard of care. Signatera™ was developed by Natera™. It is a personalized, tumor-informed assay optimized to detect ctDNA for molecular residual disease (MRD) assessment and recurrence monitoring for patients previously diagnosed with cancer, with broad utility for cancer management.

By sharing the commun goal to detect responses to immunotherapy in assessing the predictive value of ctDNA, Unicancer and Natera™ join forces to obtain data allowing to develop testing for patients with advanced NSCLC and treated with anti-PD1 treatment or anti-PD1 treatment/radiotherapy.

To this end, plasma and PBMCs (only for the first timepoint) will be collected before the first injection of pembrolizumab, at week 3, at week 6 (first tumor evaluation) and at disease progression. A total of 100 patients (50 patients per arm) will be involved. The samples will be sent to Natera™ for ctDNA analysis and correlative analysis (tumoral response vs. ctDNA concentrations).

The early detection of patients who will not have clinical benefit with anti-PD1 treatment or anti-PD1 treatment and radiotherapy could offer the possibility of precociously alternative treatment strategies for these patients.

If promising results are obtained, possibilities of extending these tests to other stages of NSCLC, and even to other cancers in order to guide the prescription of anti-PD1 and / or the anti-PD1 / multifocal irradiation combination.

TARGET

Dr Esma SAADA, Medical Oncologist
Centre Antoine Lacassagne, Nice

Added value of liquid biopsy to tumour screening performed in the Molecular Tumour Board of PACA-East region.

Precision medicine aims to offer each patient the treatment adapted to his/her tumour characteristics. The development of high-throughput molecular tumour analysis has allowed the enrolment of patients in large molecular screening programs that suggested survival benefit conferred by targeted treatment to specifically selected patients (MOSCATO-01, PMID: 28365644).

The performance of such programs depends on the availability of tumour material, complete molecular analyses, and the access to clinical trials. For example, over the 1035 adult patients included in the MOSCATO-01 trial, tumour molecular analysis was not feasible for 1 out of 5 patients because of insufficient quantity or quality of tumour material.

The Molecular Tumour Board of PACA-East region (RTBmol) is a molecular screening program for advanced cancer patients treated into the REPOS Network (Réseau Essais Phases Précoces Oncologie Sud-Est). As of October 1, 2021, 1618 files have been discussed. The failure rates reported were 10% for unavailability of tumour material and 3% for non-effective molecular analysis.

These technical limitations could be overcome by analysis of circulating tumour DNA (liquid biopsy), which should better account for clonal heterogeneity and provide more accurate information on specificities of the dominant clone at the time of analysis. The performance of the most recent analysis techniques seems to meet the expectations of the molecular tumor board allowing high-throughput analysis of a panel of genes with targetable alterations.

Our objective is to compare the diagnostic value of liquid biopsy (NGS analysis) with analyses performed on solid biopsy in RTBmol, and to give patients from the PACA-Est area an equal chance to access these analyses opportunities.

The analysis of molecular anomalies on circulating tumour DNA will be performed on patients' blood samples. This new approach should allow to perform molecular analyses for patients with poor quality (bone metastases, old samples) or not available (exhausted material) samples and to avoid problems related to inter and intra-tumour heterogeneity. Molecular analyses performed on tumour material (less than 3 years old) will be used as comparators.

this technique, which is reproducible and less invasive, could eventually replace analyses on tumour samples in RTBmol.

REVEAL

Gustave Roussy

Reshape the EValuation Efficiency and Accuracy of non-small cell Lung cancer (REVEAL) - 5th Call for proposals RHU 2021.

Gustave Roussy was the first cancer center to formalize a consultation based on liquid biopsy for molecular research on circulating DNA (circDNA) in patients with lung cancer (ORACLE consultation). Patients followed by the Institute, or not, were offered a EFGR gene mutation testing in 5 days. It is thus possible to identify treatment's resistance and adapt the therapeutic choice. Since 2017, the research has been extended to other genes of interest in lung and breast cancers and since 2020 to all cancer type in a refractory situation. But circDNA can act way behind a surrogate tool to tissue biopsy. The goal of this new project, named REVEAL, is to substitute CT-scans by the analysis of circDNA, or liquid biopsy, in non-small lung cancer's monitoring.

Lung cancer is the leading cause of cancer mortality worldwide. Incidence in France is 46,300 cases diagnosed and 33,100 deaths each year. Non-small cell lung cancer (NSCLC) is the most prevalent subtype (85%). When diagnosis is made at an early stage (I,II, IIIa), while the disease is not obviously disseminated, treatment relies on surgical tumor resection and/or radiotherapy when feasible, which can be combined with systemic treatments. In Stage IV cases, first-line treatment was, 10 years ago, typically chemotherapy, with median overall survival approximately 8–10 months.

Despite poor historical outcomes, the management of NSCLC has been revolutionized by targeted therapy and immunotherapy. For both classes of drugs, analyses of tumor biomarkers are mandatory for drug selection. Development of circulating cell-free DNA (cirDNA) based assays, often referred to as liquid biopsies, have offered an alternative method to avoid repeated tissue biopsies. **REVEAL is an ambitious program that aims to take advantage of longitudinal cirDNA disease monitoring to move from treatment selection to strategic decisions.**

Imaging based approaches, in particular CT scans, remain the standard of care to detect lung cancer relapse after radical treatment or to measure response to systemic treatments. This method has become partially outdated in this immunotherapy setting due to its inability to characterize response in 15% of cases. CT scans have several additional limitations, including a poor definition (3 mm tissue threshold, corresponding to 7 million cancer cells), and safety issues (radiation-induced cancers and renal failure). No biological markers, such as PSA for prostate cancer, are currently available for biological follow-up of NSCLC. **As longitudinal cirDNA is directly associated with tumor burden, it offers a powerful potential alternative to imaging.**

In patients in whom the NSCLC was radically treated, the detection of minimal residual disease (MRD) by cirDNA analysis will be assessed by our project with two main objectives. First, the absence of MRD one month after the surgery could help avoid the need for adjuvant therapy, and thereby have an obvious impact on treatment de-escalation strategies. Commercially available assays have a low sensitivity. We will develop a unique combination device and plasmapheresis to tackle this issue. During follow-up, the current assays are designed to detect a molecular recurrence but not a second cancer, a frequent issue in patients who were or are smokers. To overcome this limitation, **we propose a second objective of the development of a cirDNA multiplex molecular biomarker based on mutation-agnostic cirDNA molecular features such as cirDNA quantification or size profile identification**. Mutation status will be simultaneously determined with concurrent techniques.

Since we anticipate that molecular recurrences will be detected by cirDNA before the event of metastasis on CT scans, there is a predicted need for circulating biomarkers able to predict response to immunotherapy without a need for tissue biopsies. We will investigate and validate candidate circulating biomarkers, primarily based on the patient's phenotype, including: immunosenescence markers, acceleration of telomere shortening, inflammation markers and neutrophil phenotyping.

For monitoring at the metastatic stage, our project will study a new cirDNA assay, specifically designed for NSCLC patients, with the aim to propose cirRECIST criteria. The assay will be developed with digital PCR (cdPCR) and will also combine mutation-agnostic cirDNA molecular markers. This easy-to-access and cost-effective test will allow us to evaluate treatment efficacy faster and more safely than CT scans. It should contribute to decreasing the inequality of cancer care in regions where access to CT scans is limited.

The consortium gathers recognized experts in thoracic oncology, immunology, cirDNA analysis and machine learning (Gustave Roussy Cancer Center, Université de Montpellier, Inserm, CentralSupélec, Université Paris-Saclay) and three biotech companies (Stilla® Technologies, IntegraGen, Cell Environment). The project will be carried out over 60 months.

A BLOOD TEST TO DIAGNOSE EYE CANCER IN CHILDREN

Dr. Gudrun Schleiermacher, pediatrician and researcher at Institut Curie

25/10/2021 - NEWS - MEETING OF THE INTERNATIONAL SOCIETY OF PEDIATRIC ONCOLOGY 2021

A blood test to diagnose retinoblastoma, the most common pediatric cancer: a new non-invasive method, based on analysis of circulating tumor DNA, is being studied at Institut Curie. The promising clinical results were presented at the meeting of SIOP 2021, an international event attended again this year by teams from Institut Curie dedicated to pediatric oncology.

Retinoblastoma is a cancerous tumor of the retina most frequently diagnosed before the age of 5. This serious disease can affect just one eye (in 60% of cases), or both eyes. With around 60 new cases every year in France, retinoblastoma is the most common pediatric cancer of the eye. Institut Curie is the leading treatment center for this retina cancer in France. Although the diagnosis is made following an examination of the optical fundus (along with an MRI or ocular ultrasound), access to the tumor tissue is very limited since biopsies are not possible, which means that the histopathological diagnosis of suspicious intraocular masses and onco-genetic trials for the family are difficult.

60% OF RETINOBLASTOMA CASES AFFECT ONE EYE ONLY

60 NEW CASES OF RETINOBLASTOMA EACH YEAR IN FRANCE

Within this context, the work of Dr. Irène Jimenez, pediatrician and researcher, conducted at Institut Curie in the Pediatric Oncology Translational Research team headed by Dr. Gudrun Schleiermacher, has enabled the development and discovery of a non-invasive method for analysis via a simple blood test. It is indeed possible to detect circulating tumor DNA in the plasma of patients suffering from non-hereditary intraocular retinoblastoma. This new technique will be invaluable for assisting in the diagnosis of suspicious cases, for family genetic counseling or for monitoring a residual intraocular disease.

> *Because accessing this tumor is a major difficulty for the treatment of retinoblastoma, diagnosis via detection of circulating tumor DNA in the plasma paves the way for some very promising concrete clinical options," declares Dr. Gudrun Schleiermacher, pediatrician and researcher at Institut Curie. "Today, liquid biopsies represent a significant breakthrough for children, first of all because they are non-invasive and can be used to characterize tumors.*

These results were presented orally at the SIOP 2021 meeting and were awarded the Young Investigator award from the International society of pediatric oncology.

TO GO FURTHER

The scientific committee of the SIOP selected the six highest-rated abstracts in the fields of basic and translational science, clinical trials and development of pediatric oncology programs that were presented. The highest-rated presentation in each category received a 1,000-euro prize.

Lisa Golmard, head of the Constitutional Genetics unit and specialist practician at Institut Curie's Hospital Group (Diagnostic and Theranostic Medicine division/ PMDT - Genetics) is the 2021 winner of the "Basic and translational sciences" award for her oral presentation on a highly sensitive method for detecting genetic predisposition and biomarkers for retinoblastoma. This high-throughput sequencing method with molecular barcodes has been applied to analysis of DNA extracted from leucocytes, tumoral fragments and free tumor DNA in the plasma and aqueous humor (liquid taken from the front chamber of the eye, allowing access to tumor DNA in children with retinoblastoma undergoing conservative treatment).

Reference: Molecular diagnosis of retinoblastoma by circulating tumor DNA Analysis. Irene Jimenez (…) Gudrun Schleiermacher. Eur. J. Cancer. 021 Sep;154:277-287. https://doi.org/10.1016/j.ejca.2021.05.039

PADA-1

French Breast Cancer Intergroup
Unicancer

The phase 3 PADA-1 trial demonstrates for the first time that changing hormone therapy following early detection of ESR1 mutations in plasma in some patients with hormone receptor-positive breast cancer treated with an aromatase inhibitor plus palbociclib (Ibrance) can delay the onset of cancer resistance to standard therapy.

The results were presented at San Antonio Breast Cancer Symposium 2021 (SABCS) by the study coordinator Prof François-Clément Bidard (Curie Institute). After a median follow-up of 26 months, the median progression-free survival significantly doubled (11.9 months vs 5.7 months) when treatment switched to fulvestrant (Faslodex) plus palbociclib before a detectable clinical disease progression.

Thanks to the commitment of 83 French centers, the PADA-1 trial recruited 1,017 patients. Patients with metastatic ERα-positive breast cancer that lacked expression of the growth factor receptor HER2, were treated in a first-line setting with an aromatase inhibitor plus palbociclib. The patients provided blood samples for ESR1 mutation screening every two months, by ddPCR. Two molecular platforms were involved to perform these tests and deliver the results in a short time : Curie Institute, Paris and IUCT-Oncople, Toulouse.

At rising ctDNA, patients who did not experienced a concurrent disease progression could be randomized to continuing palbociclib and their aromatase inhibitor or switch to palbociclib + fulvestrant. Of the recruited patients, 407 experienced disease progression in the absence of an ESR1 mutation, and a mutation was detected in 279 patients prior to (N= 219 pts) or concurrent (N= 60 pts) with disease progression. Patients who progressed after continuing aromatase inhibitor treatment were given the option to cross over to the fulvestrant arm of the study.

This French Breast Cancer Intergroup UNICANCER (UCBG) study, conducted in partnership with the GINECO intergroup, demonstrates the ability of these INCa labelized intergroups to conduct large-scale studies with complex logistics. PADA-1 received an institutional support from Pfizer.

Future directions include learning more about the clinical features of ESR1-mutated tumors and trying to predict which patients will develop mutations.

A SYNERGISTIC EFFORT BY THE ICM AND THE IRCM IN MONTPELLIER-1

ICM and IRCM, Montpellier

IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM U1194, Université de Montpellier ; and Institut régional du Cancer de Montpellier, Montpellier, F-34298, France.

Under the effort of Prof. M. Ychou and Dr. AR Thierry a multi-disciplinary team was created taking advantage of the viscinity of the ICM (Institute of Cancer of Montpellier) and IRCM (Institute of Research on Cancerology of Montpellier) within the same campus to conduct translational research and to carry out fundamental research mainly related to a emerging diagnostic tool: the circulating DNA (cirDNA).

I. Joint IRCM/ICM effort: Creation of the IRCM team "Biomarkers for Precision Oncology"

A new team associating clinicians and researchers was created in 2014 under the leadership of Pr M. Ychou, in order to conduct integrative GI (Gastro-Intestinal) cancer research towards personalized and optimized medicine. This new team gathered diverse range of skills and expertises from several institutions all dedicated to basic and translational research in GI oncology and individualized therapy. The team entitled "Integrated Research in Digestive Oncology for Precision Medicine".

The team first focused on discovering and developing early predictive and prognostic biomarkers for personalized management in GI oncology. Team's plan is to establish new ties between fundamental research and clinical needs in order to achieve significant and innovative practice-advances in digestive cancer research.

The team has high visibility in the research and application of circulating DNA (cirDNA) as a diagnostic tool in oncology. A.R. Thierry is a key leader and recognized as a pioneer in this field. Based on several discoveries about the structure and origins of cirDNA, the team developed specific and optimal methods to analyze cirDNA allowing the first demonstration of the clinical validation and utility of cirDNA in oncology. Since January 2014, the team has completed 3 clinical assays and is engaged in nine other clinical trials, three of which have resulted in two ERC H2020 grants.This program benefited greatly from the direction of Pr M. Ychou, Director of ICM and coordinator of the SIRIC grant, who is a recognized key leader in digestive oncology.

Dr A.R. Thierry co-leaded the team until 2020 and then leads the team entitled "Biomarkers for Precision Oncology". Considering the expertise of the new team's leader and the needs of the program development, the new team's scientific strategy will focuse more on biomarkers research but will no longer be limited to digestive oncology and will extend to other cancer diseases.

In view of the high visibility of the team production on circulating DNA research, the team will now mainly focus on that field which is considered as one the most promising in the last decade in oncology together with the immune-therapy. Consequently, the team specific objectives will be to, first, continue to study and evaluate cirDNA in basic, technological and transational research; and second, to implement projects on biomarker discovery to potentially nourish new cirDNA applications.

II. Circulating DNA

Circulating cell-free nucleic acids (cfNA) is one of the fastest growing and most exciting areas in oncology in recent years. Circulating cell-free DNA/RNA (cirDNA/RNA) is defined as extra-cellular DNA/RNA occurring in blood. Several observations, including ours, suggest that the study of these NAs, especially cirDNA, has a great potential, especially for cancer screening, prognosis and monitoring of the efficacy of anticancer therapies.

As liquid biopsy, cirDNA collected from blood, have many advantages over tissue biopsies:

- Plasma samples are compatible with genetic analysis. Genetic analysis from tissue biopsies face challenges in mostly using preserving reagents potentially damaging DNA. In contrast, the preservatives used in blood samples do not negatively affect current genetic analysis methods.
- Collection is less invasive: CirDNA is easily extracted from blood samples, and blood draw is minimally invasive as compared with surgical resection or needle biopsy, and as a result, lowering the risk to patients.
- Heterogeneity is detectable: liquid biopsies contain information about all parts of a tumour in patients and from multiple tumour sites in patients with multiple metastases. This wider genetic picture contributes to a more accurate characterization of the molecular profile, enabling therapy guidance.
- Samples include information about early metastatic lesions: tissue biopsies are performed only on tumours that have already been detected, by imaging or other analytical methods. Liquid biopsies include additionnal information about minimal residual disease and metastatic lesions that may not yet have been detected, enabling clinicians to identify patients in the need of treatment.

Because of the strong clinical need about theragnostics/drug predictive information, especially with the advent of the targeted therapy, detection of actionable mutations to guide clinician towards appropriate and personalized therapy, was historically the first cirDNA clinical objective in oncology. However, cirDNA potential should not be restrained as only a “liquid biopsy” but also as a powerful source of biomarkers and, as such, as a useful tool for patient follow up. Since blood samples can be collected at multiple time points during therapy and follow up, it shows higher advantages than invasive procedures. Thus, blood samples can be more easily and safely obtained than tissue biopsies during the course of therapy and follow up. Blood samples can be drawn regularly at routine clinical visits and do not require highly trained surgical personnel or specific equipment. This allows for dynamic monitoring of molecular changes in the tumour over the course of treatment rather than relying on a static time point at the initiation of treatment.

III. Contribution to the cirDNA research and applications

III.1 Team’s objectives

Following their first clinical evaluation in predictive medicine, the potential of cirDNA analysis now covers each phase of cancer patient management care by examining the predictive information, the detection of the minimal residual disease, the early detection of resistance, treatment monitoring, recurrence surveillance, and cancer early detection/screening.

However, the lack of standardization of the pre-analytical conditions hinders the use of cirDNA as a clinically robust biomarker in oncology. Lastly, features and potential biological functions of cfNA are poorly known. For instance, increased knowledge in specific cirDNA structure, as observed by size profile analysis, methylation or nucleosome positioning may benefit to higher capacity in diagnostics or cancer screening.

III.2 Pioneering findings and scientific achievements

We started to work in 2005 on circulating DNA despite no financial support up to 2010 since private and public agencies denied interest in this area; for instance, the refusal notice of two independent funding agencies we received at that time, indicated that cirDNA was “science fiction”.

Structure and origins of cirDNA

We initially provided pioneering observations on the structure and origins of tumor-derived ccirDNA.

1. We designed an experimental mouse model for studying ctDNA content variation with tumor progression. We found that plasma rather serum is the appropriate biological source. We determined the origin and performed quantification of circulating DNA in mice with human colorectal cancer xenografts (Nucleic Acid research, 2010).

2. We discovered that high fragmentation characterizes tumor-derived circulating DNA (PlosOne, 2011).

3. We pointed out that the proportion of circulating DNA originating from tumor, microenvironment and normal cells in colorectal cancer patients is of particular importance (Expert Opinion Mol Biol, 2012)

4. We reported a higher cirDNA analytical detection capacity when analyzing single strand DNA (NPJ Genomic Medicine, 2018).

5. We characterized the mitochondrial cirDNA release in healthy and cancer individuals (Sci.Rep. 2019).

6. We demonstrated the difference in cirDNA size fragment analysis (termed Fragmentomics) when comparing plasma of cancer and healthy individuals using qPCR, and Whole Genome Sequencing (WGS) from single or double strand DNA library preparations (NPJ Genomic Medicine, 2018).
7. We elucidated the structural forms of cirDNA nucleo-proteic complex in the blood stream (NPJ Genomic Medicine, 2018; Nucleic Acid Res, 2019).

8. We evidenced the influence of hypoxia on the cirDNA release from cancer cells in vitro and in vivo (British J Cancer, 2019).

9. We first revealed the full fragment size distribution of nuclear and of mitochondrial cirDNA (JCI Insight, 2021; J.Biol.Chem. 2022)

10. We demonstrated the presence of cell-free intact mitochondria circulating in blood (FASEB J., 2020).

Technological studies

1. We validated the multimarker approach of IntPlex. IntPlex is the first and so far unique technology enabling qualitative and quantitative multiplex analysis for ctDNA (Mol.Oncol., 2013)

2. We provided the first initial guidelines for the pre-analytical conditions for ctDNA analysis. (Clin.Chem.Acta, 2013)

3. We provided the first full guidelines on the pre-analytical conditions for cirDNA analysis (Clin.Chem. 2019).

4. We provided the first full guidelines on the pre-analytical conditions for cirDNA methylation analysis (Clin.Epigenetics, 2021).

5. We demonstrated a higher cirDNA analytical detection capacity when examining single strand cirDNA size profile with shallow Whole Genome Sequencing (sWGS) (Npj Genomic Medicine, 2018).

6. We demonstrated that fragmentomics as determined by sWGS and qPCR concurrent analysis showed strong potential in discriminating plasma from mCRC to healthy patients (JCI Insight, 2021.

7. By combining the MNR test, quantitative analysis and fragmentomics and assistance of machine learning (Artificial Intelligence), high level of performance in screening cancer individuals was obtained in a study involving more than 1200 individuals (Adv.Sci, 2020).

8. We have registered patents on the use of cirDNA for survival prognosis, biomarker and screening test on the use of circulating mitochondrial DNA, cancer screening test by examining cirDNA size profile with shallow Whole Genome Sequencing (sWGS) from single strand DNA library preparation and from double strand DNA library preparation; and on a q-PCR based test for detecting the EGFR mutations.

Translational studies

1. We highlighted that cell-free DNA from colorectal cancer patients may reveal high KRAS or BRAF mutation load. (Transl.Oncol. 2013)

2. We demonstrated that mutant cirDNA fragments are shorter than wild type cirDNA fragments in mCRC patient plasma (Transl.Oncol. 2013).

3. We were the first to clinically validate (in a prospective, blinded, large cohort and multicentric study) the analysis of ctDNA in oncology with a clinical prospective, blinded, multicentric study: Clinical validation of cirDNA analysis when detecting KRAS and BRAF mutations in metastatic colorectal cancer patients. (Nature Med. 2014).

4. We were the first to demonstrate (in a prospective, blinded, large cohort and multicentric study) the prognostic value of the cirDNA analysis in oncology: Circulating DNA as a Strong Multimarker Prognostic Tool for Metastatic Colorectal Cancer Patient Management Care (Clin.Cancer Res. 2015).

5. We detected the emergence of RAS and BRAF mutations following anti-EGFR therapy in mCRC patients and showed the convergent evolution and common resistance mechanisms during Treatment of Colorectal Cancer (Clin.Cancer Res. 2016).

6. We were the first to demonstrate (in a prospective, blinded, large cohort and multicentric study) the clinical utility of cirDNA analysis in oncology: Clinical utility of circulating DNA analysis for rapid detection of actionable mutations to select metastatic colorectal patients for anti-EGFR treatment (Ann.Oncol., 2017))

7. CirDNA as a follow up parameter to monitor Sorafenib activity in mCRC patients (Mol.Oncol. 2021).

8. Neutrophil extracellular traps (NETs) production is associated with metastatic colorectal cancer (iScience, 2022) and COVID-19 (J.Clin.Med., 2020, Clin.Cancer Res. 2021).

IV Projects and strategies

IV.1 Work plan

Basic research

Broadening our knowledge of the structural and biological features of cirDNA should provide new avenues of research in tumor biology and help in standardizing the pre-analytical conditions, which remain key steps to be taken towards the clinical implementation of cirDNA analysis. Objectives are to:

1. Decipher the structural features of circulating DNA (cirDNA) with using various and complementary methods of WGS and of Q-PCR.

2. Study the mitochondrial cirDNA release, structural forms and relation to disease stages and particular physiologic conditions (with inflammatory process, RO species, hypoxia or ischemia...).

3. Determine the respective proportions of the various cirDNA structural forms and nucleo-proteic complex.

4. Determine cirDNA peri-surgery kinetics in stage II-III cancer patients (collaboration with CHU Nimes).

5. Investigate/develop circulating messenger RNAs (cf-mRNAs) as cancer biomarkers,

Technological research

1. Implement novel analytical parameters for optimizing cfNA analysis.

2. Evaluate mitochondrial genes sequences as biomarkers for analyzing cirDNA.

3. Standardize cirDNA analysis

4. Set up a single strand DNA library preparation toward routine clinical use to analyse cirDNA (collaboration with Integragen, Paris, France)

5. Determine cirDNA fragmentation parametrics in healthy and cancer subjects (MiTest project, MSD Avenir)

Translational research

Assess further clinical unmet needs by examining in clinical studies: the detection of the minimal residual disease, the early detection of resistance, treatment monitoring, recurrence surveillance, and cancer early detection/screening. Objectives are:

1. Detection of the minimal residual disease and surveillance of the recurrence in stage II-III CRC postsurgery patients (DNAcirc, INCA PRTK). Ongoing and ending in 2021. N= 470 patients; Prospective and multicentric study. AR Thierry, co-coordinator.

2. First interventional study in using cirDNA as test for selecting patient for anti-EGFR therapy in mCRC patients (Panirinox, AMGEN). UniCancer, promotor. N= 480. Prospective, blinded and multicentric study T. Mazard, coordinator; AR Thierry, Scientific coordinator.

3. Comparison of cirDNA and radiological analysis in locally advanced breast and rectal cancer (LIMA, ERC H2020 EU). N= 220. Prospective, blinded and multicentric study (Utrecht University Hospital, ICM). AR Thierry, partner.
4. Evaluation and evolution of a personalized molecular tag in post-surgery stage III CRC patient (THRuST, ERC H2020 ERA-NET). N=320. Prospective, blinded and multicentric study (ICO, Barcelona; CIS, Torino; VHIO, Barcelona;) AR Thierry grant coordinator.

5. CirDNA as a follow up parameter to monitor Sorafenib activity in mCRC patients (Texcan, Gercor/Bayer).

6. CirDNA analysis in the course of pancreatic cancer treatment (Gabrinox assay, E. Assenat, CHU Montpellier)

7. Breast cancer prognosis by circulating tumor DNA analysis with using multiparametric structural parameters test (within MyProbe project, IGR, F. André). N=300. Retrospective, blinded and multicentric study. AR Thierry, partner.

8. Lung cancer prognosis by cirDNA analysis with using multiparametric agnostic parameters test (within the REVEAL project, IGR, B. Besse). N=1600. Prospective, blinded and multicentric study. AR Thierry, partner.

9. Evaluation of a multiparametric test for cancer screening in various cancers (MiTest, MSD MiTest and DepLR studies). N=1700. AR Thierry, coordinator.

10. Diagnosis and follow up of COVID-19 and cancer patients by NETs production analysis from various ancilliary studies (N~900).

IV. 2 Expected results and impacts in oncology

Deciphering the structural features of cirDNA upon disease stages and determining their respective proportion should directly improve the detection sensitivity of cirDNA and specifically improve their performance as diagnostic markers. In light of this, the study of the mitochondrial cirDNA release, structural forms and relation to disease stages and particular physiologic conditions would provide clues on links of cirDNA release and tumor progression with particular physiologic conditions. In addition, mitochondrial cirDNA may bear in some cases genetic alterations that would be of diagnostic interest. CirDNA half-life has been very poorly investigated although such knowledge is of obvious interest in regards to better collecting blood and about pre-analytical conditions, especially in respect to use of longitudinal analysis. Study the relationship between cirDNA release and NetoSis/DNA traps would provide clues in regards to metastasis formation and inflammatory process and immune defense.

We are convinced that the clinical trials will have a significant impact on the development of cirDNA in terms of clinical validation and utility as a novel diagnostic biological source towards more efficient cancer patient management care. For instance:

- The detection of the Minimal Residual Disease might help the oncologist to better select patients eligible for adjuvant therapy after surgery in stage I-III patients.
- The study of surveillance of the recurrence would be the first study to delineate the diagnostic and prognostic role of cirDNA in stage I to III CRC patients. Positive outcome of this study would subsequently enable to evaluate the clinical utility of the cirDNA as a new diagnostic approach to improve the patient surveillance tool arsenal. The surveillance of cirDNA for patients able to support a curative treatment should improve detection of relapses and decrease false positive and cost of the surveillance. This strategy could allow early systemic therapy and improve overall survival.
- We will propose the first interventional plasma DNA analysis for the detection of RAS/ BRAF hotspot mutations in a clinical trial on anti-EGFR therapies.
- The prospective study of the emergence of RAS/BRAF/EGFR/PIK3CA mutations in mCRC patients undergoing anti-EGFR therapy. The diagnostic technology developed by the project will also improve prognoses by providing clinicians with information about the biological and genetic characteristics of the disease in each individual patient over time, allowing them to prescribe the right therapy at the right time. Impacts would be: the validation of a strategy for earlier diagnosis, patient stratification and better prognosis, improved clinical decisions and better health outcomes more sustainable health care systems.
- Since prevention or early diagnosis are the most efficient means to fight cancer, it is worthwhile to evaluate a screening method based upon cirDNA for cancer patients. We are aware that we are in the prehistorical stage of developing of such a test but it is meaningful to build the fundations of such a challenge despite it requires important ressources.

V. Discovery to translational research

Biomarker research and development may be constituted by three themes respective to markers: Mechanistic, therapeutic, and clinical disease:

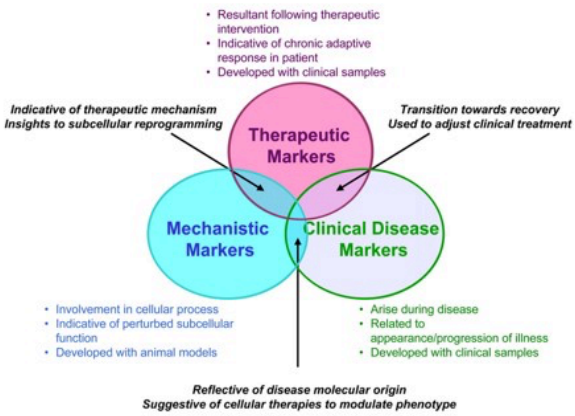


Figure 1

The originality of our research is located at the intersection of these three themes. It will foster research on circulating nucleic acid as a new diagnostic biological source and applying them with known or new genetic and epigenetic markers.

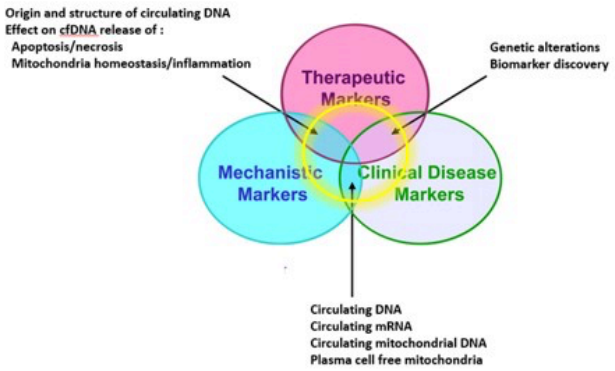
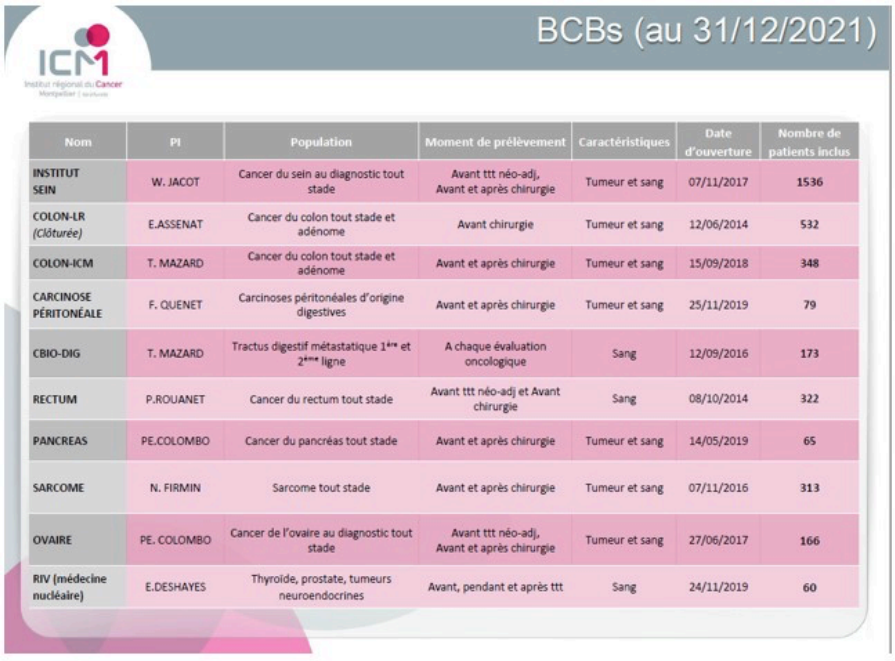


Figure 2

This strategy is only possible when uniting a multidisciplinary team with principal investigators with needed skills: molecular biology (P. Blache, AR Thierry), cellular biology and animal models (C. Prevostel), clinical biomarker (E. Crapez), biostatistics (C. Mollevi), and oncology (M. Ychou, A. Adenis, T. Mazard and B. Roch). The relationships with national and international networks in each domain especially in regards to the two European consortium and involvement in two RHU programs, enlarge the scope of knowledge/skills and vision of the biomarker field in concology. We are convinced that our strategy would contribute to successful research towards implementation of improved biomarkers in precision oncology.

VI. ICM efforts

Dr M. Ychou Director of the ICM and of the SIRIC network Grant is fully convinced on the potential of cirDNA in oncology. In addition to its input to the IRCM team, and in contributing and designing translational research programs, he favored building plasma banking of plasma to facilitate actual research programs and anticipate the future needs, especially in extending the scope of the research on various cancer types (Figure 2). Most of the clinical studies sponsored by the ICM proposed a plasma banking for anticipating the use of cirDNA analysis. The ICM has the objective to be a leading clinical center about the translational research on cirDNA.



Nom	PI	Population	Moment de prélèvement	Caractéristiques	Date d'ouverture	Nombre de patients inclus
INSTITUT SEIN	W. JACOT	Cancer du sein au diagnostic tout stade	Avant ttt néo-adj, Avant et après chirurgie	Tumeur et sang	07/11/2017	1536
COLON-LR *(Clôturée)*	E.ASSENAT	Cancer du colon tout stade et adénome	Avant chirurgie	Tumeur et sang	12/06/2014	532
COLON-ICM	T. MAZARD	Cancer du colon tout stade et adénome	Avant et après chirurgie	Tumeur et sang	15/09/2018	348
CARCINOSE PÉRITONÉALE	F. QUENET	Carcinoses péritonéales d'origine digestives	Avant et après chirurgie	Tumeur et sang	25/11/2019	79
CBIO-DIG	T. MAZARD	Tractus digestif métastatique 1ère et 2ème ligne	A chaque évaluation oncologique	Sang	12/09/2016	173
RECTUM	P.ROUANET	Cancer du rectum tout stade	Avant ttt néo-adj et Avant chirurgie	Sang	08/10/2014	322
PANCREAS	PE.COLOMBO	Cancer du pancréas tout stade	Avant et après chirurgie	Tumeur et sang	14/05/2019	65
SARCOME	N. FIRMIN	Sarcome tout stade	Avant et après chirurgie	Tumeur et sang	07/11/2016	313
OVAIRE	PE. COLOMBO	Cancer de l'ovaire au diagnostic tout stade	Avant ttt néo-adj, Avant et après chirurgie	Tumeur et sang	27/06/2017	166
RIV (médecine nucléaire)	E.DESHAYES	Thyroïde, prostate, tumeurs neuroendocrines	Avant, pendant et après ttt	Sang	24/11/2019	60

Figure 3

Conclusion

The cirDNA field is now popular in oncology and subject to exponential reporting in the literature. For instance, prescription in oncological theragnostic is allowed in several clinical conditions (lung cancer, melanoma and mCRC for guiding the clinicians about targeted therapy). Owing to our work and to the international efforts from leading teams (Among others: N. Rosenfeld, Cambridge, UK; L. Diaz, New York, USA; L. Bardelli, Torino, Italy; N. Turner, Manchester, UK; S. Kopetz, Houston, USA; S. Holdenrieder, Munchen, Germany; M. Diehn, San Francisco, USA; C. Andersen, Aarhus, Denmark; and P. Laurent-Puig P and V. Taly, Paris; …) other cirDNA potentials such as the detection of the Minimal Residual Disease or therapy efficacy monitoring, should extend their diagnostic applications in cancer management care. Furthermore, considering the intense cirDNA-related research now being carried out, especially with regard to cirDNA methylation or fragmentomics and assistance of Artificial Intelligence, we are convinced that the scientific community might be able to obtain in the next decade the oncology's 'grail': cancer screening.

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THE TRAK-ER STUDY: INTERVIEW OF PR. NICHOLAS TURNER

Nicholas Turner
The Institute of Cancer Research (UK)

What is TRAKER study, its purpose, technology used?

The TRAK-ER study will study the potential benefits of molecular relapse monitoring with circulating tumour DNA (ctDNA) assays in patients with ER positive HER2 negative breast cancer on adjuvant endocrine therapy. We have no way of adequality following these patients for future relapse. In the study we will offer patients, with higher risk disease, surveillance with personalised ctDNA assays. Those with a molecular relapse will be randomised between continuing standard adjuvant endocrine therapies and switching to palbociclib and fulvestrant. The aim of the study is to show that switching to palbcocilib and fulvestrant at the time of molecular relapse can delay metastatic relapse, and perhaps we can hope prevent a relapse in some patients.

How was the collaboration between the French and English centers born?

The TRAK-ER collaboration was born out of a shared interest between Unicancer and French PIs on one hand, and the Royal Marsden team and UK PIs on the other, to address the important question of whether molecular relapse monitoring can improve outcome for patients. The study will recruit up to 1100 patients into ctDNA surveillance, so combining our efforts is vital to answering this question for our patients.

Do you think that personalized follow-up could be considered in the future in standard practice?

The TRAK-ER study is going to be a leading international study to address whether molecular relapse monitoring has clinical utility. Although we know that ctDNA detection predicts for future relapse with high accuracy, we don’t know if treating people at the point of ctDNA detection – molecular relapse – can improve outcome. If TRAK-ER results are as we hope, such monitoring could become the standard of care in the future.

PANIRINOX

Unicancer - UCGI group

PANIRINOX is a first-line national randomized phase II study, sponsored by Unicancer and coordinated by Dr Thibault MAZARD (Montpellier Cancer Institute), and co-coordinated by Dr Alain THIERRY (Institute of Research on cancerology of Montpellier), evaluating FOLFIRINOX + Panitumumab vs mFOLFOX6 + Panitumumab in patients with unresectable metastatic colorectal cancer B-RAF and RAS wild type. The primary objective is to compare the complete response rate between treatment arms, defined as complete disappearance of metastatic lesions and normalisation of tumour marker level after 12 cycles of treatment. Secondary objectives include overall survival, progression free survival, secondary resection, and safety profile.

For the first time, circulating cell-free DNA analysis is used as a companion test for selecting patients towards anti-EGFR targeted therapy. B-RAF and RAS status is determined by Intplex technology in patient's plasma samples. Patients B-RAF and RAS wild type on liquid biopsy will be randomized in the PANIRINOX study.

Liquid biopsy through circulating cell-free DNA analysis could advantageously replace tumour-section analysis and expand the scope of personalized medicine for patients with cancer:

- Analysis could be performed on blood collected at time of treatment decision and not on tissue samples collected weeks to several years before;
- it prevents errors on mutation status due to intra-tumour heterogeneity that may arise in the selection of the region analysed on tumour tissue;
- it appears non-invasive, faster, and less expensive than conventional analysis on tumour tissue;
- blood tests can be repeated several times during the care of patients.

At the end of the study, sensitivity and specificity to discriminate RAS and BRAF mutated and wild type patients will be evaluated to compare the diagnostic performance of circulating DNA analysis with the gold-standard tumour-tissue analysis.

The Intplex technology used for patients screening is a quantitative PCR-based method allowing the simultaneous determination of five parameters (circulating DNA concentration, presence of mutation, mutant DNA concentration, proportion of mutant DNA, and DNA fragmentation index). This mutation-targeted test can be adapted to all mutations, genes, or cancers and enables rapid, highly sensitive, cost effective, and repetitive analysis.

It is planned to include 209 patients in the PANIRINOX study. The recruitment started in April 2017 and is currently ongoing in around 30 French hospitals and cancer centres, with 462 patients screened and 177 randomised.

An ancillary study is also planned to assess the onset of resistance under anti-EGFR treatment. For this purpose, detection of RAS mutations in circulating DNA will be performed on blood sampling at each radiologic assessment.