Modelling and predicting sensitivity to targeted therapies in colorectal cancers (COLTHERES)

Final Publishable Summary Report (1 January 2011 to 31 December 2014)

DATE	07/01/2015
PROJECT	Modelling and predicting sensitivity to targeted therapies in colorectal cancers (COLTHERES)
START DATE AND DURATION	1 January 2011 – 48 months
ABSTRACT	This final report comprises three separate parts: a final publishable summary report covering results, conclusions and socio-economic impact of the project, a plan for use and dissemination of foreground and a report covering the wider societal implications of the project. It also includes the final report on the distribution of the European Union financial distribution
AUTHOR, COMPANY	Alberto Bardelli (UNITO) and Herman Bertrand (ARTTIC)
FILING CODE	COLTHERES_Final_Publishable_Summary_Report
KEYWORDS	Final publishable summary report, final distribution, results, conclusions, socio-economic impact, use and dissemination, foreground, societal implications

DOCUMENT HISTORY

Version	Date	Reason of change	Status	Distribution
0.1	07/01/2015	First draft	DRAFT	ALL
1.0	11/05/2015	Final comments	FINAL	ALL

Table of Contents

PROJECT FINAL REPORT

Grant Agreement number: 259015

Project acronym: COLTHERES

Project title: MODELLING AND PREDICTING SENSITIVITY TO TARGETED THERAPIES IN COLORECTAL CANCERS

Funding Scheme: COLLABORATIVE PROJECT

Period covered: from 1 January 2011 to 31 December 2014

Name of the scientific representative of the project's co-ordinator, Title and Organisation:

Tel: +39 011 9933235

Fax: +39 011 9933225

E-mail: ALBERTO.BARDELLI@IRCC.IT

Project website address: WWW.COLTHERES.ORG

Abbreviations Used in this Document

Abbreviation / acronym	Description
BEAMing	Beads, emulsion, amplification and magnetics
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CRC	Colorectal Cancer
EGFR	Epidermal Growth Factor Receptor
FCGR	Fc gamma receptor
FCGR2	Fc gamma receptor type 2
FCGR3	Fc gamma receptor type 3
FCGR3A	Fc gamma receptor 3A
FFPE	Formalin-fixed paraffin embedded
HGF	Hepatocyte Growth Factor
ICO	Catalan Institute of Oncology
IPR	Intelectual Property Right
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
mCRC	Metastatic colorectal cancer
MEK	Mitogen-activated protein kinase kinase kinase 1 or MAP-ERK Kinase
NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
OS	Overall Survival
PIK3CA exon 20	Phosphatidylinositol 3-kinase exon 20
RFS	Relapse free survival
SAR	Survival After Relapse
shRNA	Short hairpin RNA
TGF	Transforming Growth Factor
VTB	Virtual Tumour Bank

1. Final Publishable Summary Report

Tumours are presently categorized and treated according to where they arose in the body. The revelation that cancer is a genetic disease and that accumulation of molecular alterations in the genome of somatic cells is the primary driver of tumour progression have revolutionized oncology. It is now manifest that cancers, which were thought to be indistinguishable based on light microscopy, are actually different diseases requiring distinctive ap**p**roaches to therapy. These findings lead to the concepts of precision oncology or precision cancer medicine. COLTHERES was conceived to apply these revolutionary concepts to colorectal tumours. A central goal of the consortium has been to define molecular signatures of sensitivity and resistance to agents targeting oncogenic nodes in the EGFR signaling pathway in Colorectal Cancer (CRC).

COLTHERES has achieved and in some instances exceeded its planned goals. Members of the consortium have shown that activating KRAS mutations are the cause of resistance to EGFR-inhibitors in 30-40% of patients but other molecular alterations can cause resistance as well and cause resistance in addition 30-40% of patients. These alterations can be mutations in downstream key molecules, over expression of activating or competing molecules or loss of inhibitors etc. The COLTHERES team has worked on identification of these alterations and on the development of comprehensive gene expression signatures that can be used to identify more accurately responders to cetuximab treatment than by assessing KRAS mutations alone (Tian et al Gut 2012; Popovic et al., JCO 2013).

Since patients with KRAS or BRAF mutations will not respond to EGFR inhibitors, alternative therapies for these patients are urgently required. Inhibition of the BRAF (V600E) oncoprotein by the small-molecule drug PLX4032 (vemurafenib) is highly effective in the treatment of melanoma, however, colon cancer patients harboring the same BRAF (V600E) oncogenic lesion have poor prognosis and show a limited response. Members of the COLTHERES consortium found that BRAF(V600E) inhibition causes a rapid feedback activation of EGFR, which supports continued proliferation in the presence of BRAF(V600E) inhibition (Prahallad et al., Nature 2011). Our data suggest that BRAF (V600E) mutant colon cancers for which there are currently no targeted treatment options available might benefit from combination therapy consisting of BRAF and EGFR inhibitors.

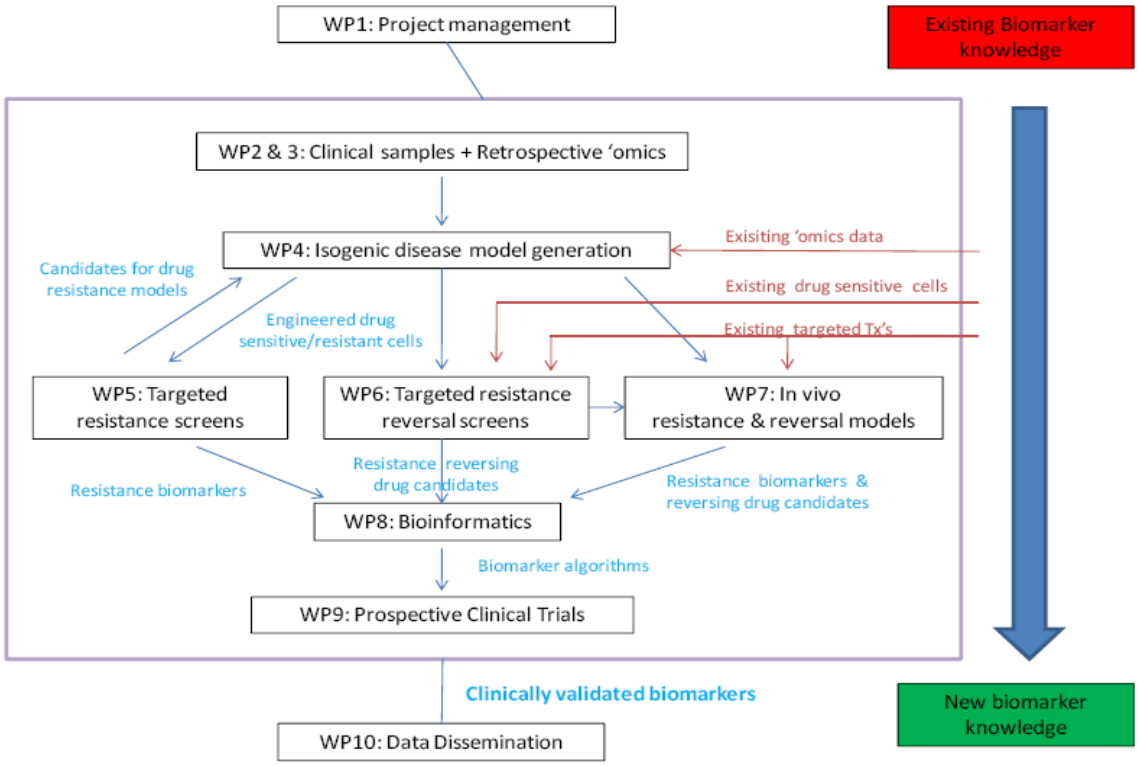
Research by the COLTHERES consortium has also shown that mutations in genes like KRAS, NRAS and BRAF are causally associated with acquired resistance to targeted therapies for colorectal cancer (Misale et al., Nature 2012, Misale et al., Science Translational Medicine). Our results demonstrate that resistance mutations in KRAS and other genes are highly likely to be present in a subpopulation of tumour cells before treatment. Resistance to therapy remains a fundamental obstacle to successful therapies and COLTHERES partners discovered recently reported how epigenetic modification modulates based-resistance to chemotherapy in CRCs (Moutinho et al., JNCI 2013). Notably, research by COLTHERES has found that resistance-associated mutations can be detected in the blood of patients (liquid biopsies) several months before radiographic evidence of disease progression is observable. This finding may offer an opportunity to anticipate and counter resistance by using combination therapies before patients relapse.

Perhaps the best testimony to the success of COLTHERES is the rapid translation of scientific findings generated by the consortium into clinical trials, which are already recruiting patients as described at: http://www.coltheres.org.

Executive summary

COLTHERES consortium meeting, fourth year, 10-11 March, 2014, Barcelona

COLTHERES is a consortium of EU-clinical centres and translational researchers who seek to define and perform biomarker driven clinical trials to improve cancer therapy outcomes. This 4-year programme has used comprehensively molecularly-annotated colon cancers as a 'test-bed' to define specific biomarkers of response or resistance to signalling pathway agents. This consortium is open to any pharmaceutical developer who wishes to determine which patients are most likely to respond to their novel cancer therapy and perform rapid proof-of-concept clinical trials.



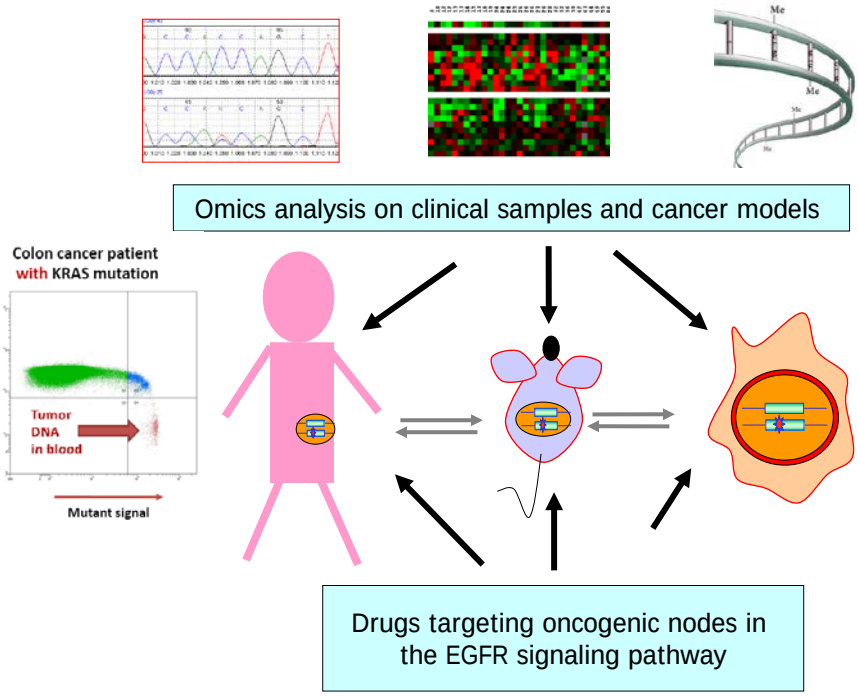
Summary description of project context and objectives

Precision cancer medicine based on the genetic milieu of individual colorectal tumours has long been postulated, but until recently this concept was not supported by clinical evidence. The observation that a subset of colorectal cancer (CRC) responds to anti-Epidermal Growth Factor Receptor (EGFR) antibody therapies, based on knowledge of which other mutant alleles are also present, has heralded the widespread realization that colorectal cancer medicine is now inevitably going to become more 'personalised'. This has therefore stimulated research and development of clinically validated diagnostic tools and biomarkers for the prospective identification of responder patients. Remarkably, these studies have also increased knowledge into the molecular basis of colorectal cancer.

At the root of understanding targeted drug sensitivity or resistance, is the concept that each tumour has an independent genetic identity. Intrinsic to this concept, therefore, is that molecular therapies targeting one specific oncoprotein are likely to be useful only in a fraction of patients, i.e. those who display the genetic lesion that is both drugable and is centrally important to the tumours' continued survival or growth. In addition to pre-existing, or de novo resistance mechanisms, it is now becoming apparent that secondary, or acquired mutations, during drug treatment, along with gene expression changes and/or epigenetic variations, also lead to resistance to targeted therapies in a remarkably short time. This makes it imperative to understand all the possible compensatory routes to acquired resistance, so that clinicians can be ready with rational combinations of targeted treatments to circumvent, reverse or even preclude resistance emergence. From the beginning COLTHERES had clear and focused objectives:

- To define routes of sensitivity and primary resistance to targeted agents mediated by oncogenic nodes in the EGFR signalling pathway in CRC;
- Identify biomarkers of secondary resistance that are likely to emerge for novel targeted therapies (such as RTK, *BRAF*, PI3K and MEK inhibitors)
- Identify genes mediating sensitivity and resistance to agents effectors of the EGFR signaling pathway.
- Define validated risk stratification criteria to be used in personalised patient-screening methodologies to predict individual therapy response and resistance profiles for colorectal cancer patients.

To achieve these objectives COLTHERES was organized in specific tasks. **Task 1** was set to define a comprehensive algorithm of positive and negative predictors by integrated mutational, gene copy number, epigenetic, transcriptional analyses and pathway-specific (phospho)protein measurements of responsive and non-responsive tumours. This will be achieved applying state of the art '–omics' technologies (described in the next sections) to fully characterize colorectal tumour samples and therapeutic response data that are already available through the clinical units of the consortium. Specifically COLTHERES consortium members have large retrospective series available. To overcome these limitations COLTHERES applied a new approach described in the figure below in which molecular lesions (biomarkers) and compounds targeting the corresponding oncogenic proteins are functionally assessed using novel in vitro and in vivo models



The COLTHERES approach: parallel clinical, omics and functional analyses of molecular markers of resistance and sensitivity in CRCs

Task 2 was focused on developing cellular models that closely recapitulate the genetic milieu of individual colorectal tumours. The approach allows for the routine and precise mutation or correction of any endogenous gene within a human cell-line grown in culture, enabling the creation of gold-standard 'isogenic' in vitro disease models that: a) accurately recapitulate the genetics present in real cancer patients; and b) provide a matched normal genetic background for referencing the effects of targeted drugs and/or rationally determining mutational-based resistance mechanisms Using this approach the mutations found in the genes representing key nodes in the EGFR signalling pathway will have been inserted (knock-in). Among these are the hotspot mutations found in KRAS, PIK3CA, BRAF, PTEN beta catenin etc. This strategy ultimately lead to isogenic mutant vs wild type cellular models in the same context that they occur in real patients. A variety of models will be created covering the variety of known and newly discovered mutant oncogenes; in both isolation and in specific resistance imparting combinations. For example, there are 7 major variants of mutant *KRAS*, which at this time are assumed (probably erroneously) to be equal in imparting resistance to Cetuximab. COLTHERES will insert all these mutations and many others emanating from the research into novel isogenic cell-lines for further study.

These cellular tools have been explored by COLTHERES partners to identify the oncogenic events and the signalling pathways that contribute to primary resistance to targeted therapies in CRCs for subsequent assessment in clinical trials. Key proof-of-concept data that isogenic models could have predicted the now known clinical data that specific mutations can cause resistance to EGFR-therapy is provided in figure 7; and Di Nicoloantonio 2009). HD is now providing most of the world's major pharma and biotech companies with these tools to rationalise targeted drug discovery; many of which will be tested in trials conducted by this consortium.

Prospective models of secondary or 'acquired' drug resistance are also sorely needed, as it is clear that secondary resistance is responsible for rapid treatment failure and its molecular bases are presently largely unknown. COLTHERES has explored the mechanisms of secondary resistance on two levels, each of them novel 1) Running two dedicated trials in patients; sampling the patients at repeated time points to study changes in treatment efficacy and acquired resistance (genotyping these samples is part of task 1); and 2) Using cell-based models of acquired resistance in vitro (**task 3** cellular models) and *in vivo* (**task 4**

'xenopatients'). Functional genetic screens have been performed to validate putative primary markers of resistance from task 2; and prospectively predict candidate biomarkers of secondary resistance to targeted therapies within task 3. To achieve this, the consortium includes the laboratory that initially developed the siRNA bar code screening technology to rapidly identify genes whose suppression cause resistance to cancer drugs. This approach was successfully used by COLTHERES members to identify a major pathway of resistance to HER2-targeted therapy in breast cancer. This strategy and complimentary gene-overexpression strategies will be used in CRC cell lines that are highly sensitive to EGFR and other targeted agents to find genes causing resistance to molecularly targeted therapies.

To further identify and validate candidate secondary resistance mechanisms, Task 4 exploited sub-cutaneous implantation of fresh tumour samples (liver metastases from CRCs) in immunocompromised mice ('xenopatients') followed by the establishment of continued 'cancer lines' in experimental animals. From individual patient-derived material a large cohort of tumour-bearing animals have already been generated by members of the consortium; these will represent 'xeno-patients' undergoing control treatment or systemic treatment with Cetuximab, or other targeted agents. The 'xeno-patients' approach was used to understand the mechanisms of secondary resistance (by chronic treatment of 'responsive xeno-patients' with targeted agents) and for the preclinical validation of combinatorial treatments aimed at targeting multiple signalling networks.

Next targeted functional-genomic and drug combination approaches were employed based on pathway/systems analysis of COLTHERES data to find rational strategies to reverse drug resistance. This approach is based on the notion that acquisition of resistance by mutation, up-regulation or down-regulation of parallel pathway targets, could be reversed directly (or indirectly downstream in specific pathway) by rational combinations of targeted agents already in existence. To exploit this feature, **Task 5** will search for genes that when inhibited with targeted siRNAs or inhibitor compounds either reverse resistance to EGFR signalling inhibition in cellular models. These will then offer new targets for rational therapeutic strategies to be used for combinatorial pharmacological 'attacks' on core oncogenic signalling pathways in CRC. The results of the 'omics analysis on clinical samples from CRC patients as well as the results of the functional analysis (in vitro and in vivo) generated were integrated at multiple levels through biostatistical analysis in **task 5** which includes experts in statistical data analysis. These analyses will generate risk stratification algorithms for personalised screening methodologies and prediction of individual therapy response and resistance for CRC patients receiving drugs targeting the oncogenic nodes of the EGFR signalling pathway. The results were disseminated through peer reviewed publication by COLTHERES members. More importantly, the results have been directly translated into practical approaches for the benefit of patients though the design of novel biomarker **hypothesis** driven trials. These are presently ongoing and are based on the knowledge gathered on the mechanisms of sensitivity and resistance to agents targeting the EGFR oncogenic signaling on defined patient populations.

Description of the main S&T results / foregrounds

The COLTHERES tumour database

At the roots of COLTHERES was the idea of building a tumour sample database of FFPE CRCs samples and their related clinical data, in order to use these samples & data retrospectively, if needed, to define and validate signatures of sensitivity and resistance to agents targeting oncogenic nodes in the EGFR signalling pathway in CRC To successfully achieve this milestone and the assurance of the best clinical advisory and assessment in the case that clinical trials are developed, the COLTHERES consortium established three main objectives for the WP: i) to manage the ethical approval/regulatory issues; ii) to provide logistic and dedicated sample tracking support and iii) to build and to maintain databases including sample information, images and related clinical data. To make sure that the objectives were realistic and their degree of accomplishment could be measured, four tasks and three deliverables were defined. It is important to remark that all tasks have been completed and no major deviations need to be reported.

The first objective was to develop the inventory of available samples and data, with prior engagement of the clinical partners, who confirmed their ability to provide a unique set of CRC samples. Available samples were re-assessed by the institutions' pathologists (a first assessment was carried out while drafting the proposal). The final database included tumour specifications (primary, non-relapsed from a single anatomical site), histological classification and clinical data (demographic, diagnostic, follow-up therapeutic and outcome data). To date, the database is being maintained a COLTHERES partner, the Data Coordination Centre at the Swiss Institute of Bioinformatics (SIB). The final number of samples and clinical data collected was lower than previously expected after careful revision of all the samples, as some were discarded for quality or quantity reasons. However, the database is subject to periodic updates from the clinical sites, so the number is expected to grow. The second task is closely related to the setting of the samples & clinical data. Under the title "Legal/ethical issues management", the task's objective was to produce and approve all necessary documents related to ethical issues by the participant institutions (i.e. patient informed consent or case report form), and to ensure that the samples and clinical data comply with all applicable local and international regulation. An ethical committee was set-up to deal with the ethical, legal and societal issues which might arise during the project.

The construction of a Virtual Tumour Bank by the SIB was crucial to centralise all the information on the samples in a web-based format containing the following information: basic sample data (i.e. local inventory code, type, etc.), tumour specifications data (primary, non-relapsed from a single anatomical site), histological classification and sample quality information. To successfully do so, data was confidentially collected from different clinical centres. Once collected, data was cleaned and consolidated in a homogeneous format. Finally, all the information was uploaded in a website only accessible to partners.

The final VTB is available for end users. The page includes the heading, menu bar and footer as specified in the template file, as well as the custom page contents in the central part of the page, as specified in the homepage source file.

Also, the option of a Java-based implementation of the VTB was explored. It was however discarded after development, security and maintenance costs considerations. The database includes all the clinical variables of interest and a specific protocol which was decided under consensus by all the clinical groups and institutions providing clinical data within COLTHERES. All samples not complying with the requirements after second or further checks and/or with incomplete clinical data were rejected. The clinical database was created as stated in the proposal. All patient clinical data are coded keeping patient's identifiers to a minimum (the consortium members use sequential identification numbers to identify the samples).

During the final part of the project, when the scientific impact became remarkable because of high impact publications and conferences, various COLTHERES partners (AG, NKI, VHIO, SIB, KUL) were invited to join the *colorectal cancer subtype consensus consortium (CRCSC)* hosted by SAGE: (doi:10.7303/syn2623706).

SAGE is a non-profit research organization based in Seattle, US and collaborates with a worldwide network. SAGE is using the Synapse platform to store and manage large datasets (**http://sagebase.org/synapse/**).

In addition, SAGE provides bioinformatics analysis tools and support.

The *CRCSC* members have agreed to study the use of the Coltheres database in larger academic-based datasets like SAGE's in the benefice of CRC research.

The goals of the CRCSC are perfectly aligned with those of COLTHERES:

(i) to compare and validate the major published colorectal subtypes;
(ii) to conduct an integrative analysis across the pooled data sets to establish a robust consensus of molecular subtypes;
(iii) to define the clinical and molecular hallmarks of these subtypes;
(iv) to investigate the clinical value of these subtypes in patient studies.

This alignment, together with the fact that and the SAGE consensus effort can achieve much larger numbers of samples than the COLTHERES consortium in their own, has led to the agreement of the COLTHERES partners to study the inclusion of the COLTHERES database within the SAGE database in the near future.

Development of positive predictors to therapies targeting the EGFR pathway

The presence or the absence of activating mutations in KRAS, BRAS, PIK3CA exon 20 and NRAS can help to predict the efficacy of cetuximab before treatment in mCRC, thus improving the cetuximab therapeutic index. Analysis of these mutations in randomized trials indeed confirms these initial findings, presentation of extended KRAS, BRAF and NRAS mutation analysis on the OPUS randomized trial of Folfox +/- cetuximab at ASCO GI 2014 by Coltheres member (Tejpar S et al, Effect of KRAS and NRAS mutations on treatment outcomes in patients with metastatic colorectal cancer (mCRC) treated first-line with cetuximab plus FOLFOX4: New results from the OPUS study., ASCO GI Jan 2014) as well as publications on non Coltheres series (Douillard et al NEJM 2013). A novel mechanism of primary resistance was identified by the Coltheres consortium. In a study led by UNITO, KRAS gene amplifications were found to predict primary resistance to anti –EGFR therapy. We performed a screening of 1,039 CRC samples to assess the prevalence of KRAS amplification in this tumour type and further evaluated the role of this genetic alteration on the sensitivity to anti EGFR therapies. We detected KRAS amplification in 7/1,039 (0.67%) and 1/102 evaluable CRC specimens and cell lines, respectively. KRAS amplification was mutually exclusive with KRAS mutations. Tumours or cell lines harbouring this genetic lesion are not responsive to anti-EGFR inhibitors. Although KRAS amplification is an infrequent event in CRC, it might be responsible for precluding response to anti-EGFR treatment in a small proportion of patients.

Construction of cellular models recapitulating the genetic milieu of CRCs

Coltheres generated isogenic cancer models carrying specific genetic alterations and expression changes present in target patient populations. These lines have been used as tools for performing functional genomics (gene over-expression and siRNA approaches) approaches to dissect and validate candidate resistance biomarkers and tools prospectively screen for other markers of primary or secondary drug resistance e.g., signalling pathway proteins, drug transporters & metabolisers. The aim was also to generate second-generation 'isogenic' cancer models carrying secondary resistance genes/alterations to screen for resistance reversing drug combinations in vitro and in vivo and to perform 'omics on these disease models for subsequent correlation and validation with patients sample data. Over 50 isogenic cell lines were generated and made available to Coltheres partners.

Functional-genomic analysis of cellular models identify new markers of primary/secondary resistance

Within Coltheres a tumour-stromal cell co-culture assay system was developed. HD successfully established an imaged-based approach to analysing tumour-stromal cell co-cultures, where the tumour cells were labelled with GFP to enable their growth to be differentiated from that of the stromal cell component of the co-culture. As proof-of-concept HD co-cultured GFP-expressing LIM1215 CRC cells with MRC5 stromal fibroblasts and where able to demonstrate that the stromal cell population drove resistance to cetuximab in the tumour cell population. HD demonstrated that MRC5 fibroblasts secrete high levels of HGF and that addition of exogenous HGF to LIM1215 monocultures also rendered them less sensitive to cetuximab, which are consistent with the possibility that HGF secretion by the stromal cell population could be rescuing the LIM1215 cells from the effects of cetuximab inhibition in the co-culture. We extended the analysis of this co-culture system to other MAPK pathway-targeted agents and similarly found that the presence of the stromal cell population also reduced the sensitivity of the tumour cells to these agents.

As a proof-of-concept for the co-culture system HD set out to test the effect whether the presence of stromal cells have an effect on the sensitivity of LIM1215 CRC cells to EGFR inhibition with the monoclonal antibody drug cetuximab.

Reversing primary and secondary resistance with targeted siRNAs and compounds

HD and NKI analysed a large panel of colon cancer lines for Mek-inhibitor sensitivity, with two lines (SW480 and SW620) successfully selected as being resistant were used in the resistance reversing screens in the next period. To avoid duplication of effort with NKI, who made significant progress with these types of screens in 2D, and in recognition of the emerging importance of the tumour microenvironment in the response to KRAS pathway-targeted agents, HD decided to focus effort on the development of more complex 3D assays formats. These changes were officially ratified with the EU. A pair of DLD1 (CRC) cell lines isogenic for the presence (parental) or absence (KRAS G13D KO) of a KRAS G13D mutation was used to set up 3D soft agar assays and to study KRAS dependency in 2D versus 3D assay formats. These results were proof-of-concept to the importance of performing screens for MAPK pathway-target compounds under 3D in addition to 2D assay conditions. To extend the observations made with the DLD1 KRAS isogenic pair and to build a panel of cell lines that could be used as screening tools > 40 (mainly non-isogenic) cell lines were screened for ability to grow under 3D conditions; suitable conditions were identified for >20 cell lines. A subset of these cell lines was evaluated for MEK inhibitor sensitivity in 2D versus 3D (soft agar) to determine their dependency on the MEK/ERK pathway. This revealed that similar to the DLD1s all cell lines tested that carry a KRAS mutation were more sensitive to MEK inhibition in 3D further supporting the rationale for perform a screen in 3D.

Predicting sensitivity and resistance to targeted agents using phase 0 clinical trials of CRCs

Coltheres we exploited xenogafted tumors derived from bioptic samples of CRC patients who initially responded to anti EGFR therapies and then relapsed with the aim to unveil the molecular bases of acquired resistance and to test new therapeutic combinations according to the genomic profile of the resistant tumors characterized. Established xenopatients retained the histopathologic and genetic characteristics of the original samples. Preclinical study and validation of potential new therapeutic targets or pharmacological combinations can take a strong advantage from well-designed xenopatients platforms as demonstrated in previous works (Bertotti et al., Cancer Discovery 2011).

Within Coltheres we proceeded with the accrual of bioptic material of colorectal cancer (CRC) patients who relapsed upon cetuximab (or panitumumab) treatment and their implantation into severely immunocompromised animals (NOD/SCID mice). Samples were transplanted in mice within 3-5 hours after biopsy. Upon transplantation, tumour pieces were allowed to engraft within 35~40 days without anti-EGFR treatment of xenopatients. After the first engraftment, tumours were expanded in cohort of 6 mice each and after 3 weeks, treatment with cetuximab started. Notably, we found that xenopatients derived from relapsed metastatic CRC (mCRC) patients showed full resistance to cetuximab, therefore confirming the optimization and validation of the procedure. Using the approach described above, we generated a first example of mouse xeno-transplant (patient -derived xenograft, or PDX) from a lung metastasis of a CRC patient who responded and subsequently relapsed upon anti-EGFR therapy (cetuximab). This tumor carried a KRAS exon4 mutation A146T. After implantation and engraftment the xenografted tumor was serially transplanted

until production of four cohorts. Mice were then randomized to vehicle alone, cetuximab monotherapy, pimasertib monotherapy and their combination. Notably, cetuximab alone or the MEK inhibitor pimasertib alone had limited effectiveness, while combinatorial (cetuximab-pimasertib) treatment prominently impaired tumor growth and induced moderate shrinkage.

We also established a second type of xeno-transplanted mouse from a mCRC patient horbouring a different genetic lesion as mechanism of acquired resistance to anti-EGFR treatment. Notably we demonstrated that MET amplification was driving acquired resistance in this particular case (Bardelli et al., Cancer Discovery 2013). Therefore, as for the choice of therapeutic regimens, we focused on small-molecule inhibitors of MET that were administered individually or in combination with cetuximab. We selected JNJ-38877605, the MET-specific tool compound (not in clinical use), and crizotinib, a dual MET/ALK inhibitor that has shown promising antitumor activity in MET-amplified esophagogastric adenocarcinomas (Lennerz et al., J Clin Oncol 2011). The xenopatients showed cetuximab resistance also after different transplantations and expansions in mice, therefore confirming the value of this preclinical system. All treatments potently delayed tumor growth compared to vehicle or cetuximab alone. In detail, the antitumor activity of crizotinib was not enhanced by the addition of cetuximab, and the most effective modality in producing durable disease stabilization proved to be the JNJ-38877605–cetuximab combination.

Integrative bioinformatics data analysis

Coltheres investigated how knowledge of molecular characteristics of clinical CRC tumor samples, in particular their gene expression profiles, could contribute to the identification of determinants of resistance to pathway-targeting chemotherapies. A specific objective was to establish a detailed description of heterogeneity of primary CRC, test for the existence of “intrinsic” disease subgroups and propose a system of molecular subgroups that could be used directly as biomarkers or could be used as stratification factors when studying potential biomarkers. A second specific objective was to find new candidate resistance biomarkers by correlating molecular features with relapse and response data. A third objective was to deduce markers of resistance by investigating molecular variation in model systems or in patients under drug pressure. Finally, there was the intention to see if signatures derived from models were helpful in interpreting signatures found in human tumor data. The investigations performed in COLTHERES include:

- Subtype discovery in gene expression from primary CRC tumors.
- Consolidation of different subtype systems into a consensus system.
- Integrative study of multiple data sources (mutation, copy number, methylation, microRNA, proteomics) to characterize the proposed subtypes.
- Analysis of association between gene expression and response to anti-EGFR therapy in gene expression profiles of clinical cohorts.
- Analysis of correlation btw molecular characteristics and resistance in mouse xenografts.
- Analysis of tumor evolution and resistance emergence in patients using liquid biopsies.
- Functional genetic screens revealing causative mechanisms of resistance.

Given the successes of the functional screening studies and of the analysis of xenopatients cohorts (WP7), whose relevance for the clinical applications was compelling, biochemical pathway analysis related to resistance has been driven by those empirical-experimental analyses.

The analysis of diversity among gene expression patterns of primary CRC suggests a continuum of variance with a few (3-7 depending on the desired level of resolution) very distinct characteristic types. The situation in CRC is similar to that seen in many other solid tumors, for example ovarian cancer or glioblastoma. A subdivision of CRC in 3 subtypes differing in levels of stroma-EMT and levels of immunological infiltrations is quite clear, while a finer subdivision is based on more subtle multigenic differences that correspond less well to well-defined biomolecular patterns.

After the publication of subtype systems by six groups, a common consensus was found that encompasses the description of four subtypes, which we consider sufficiently well and clearly defined, that their roles can now be investigated in any research direction concerning properties of primary colon tumors. The validity and extension of these systems to metastatic CRC is an open question. The consensus system with four

consensus molecular subtypes (CMS) with distinguishing features:

1) CMS1 (“MSI Immune”), strong immune components, high prevalence of MSI and CIMP, few somatic copy number alterations (SCNA) but many point mutations (hypermutation);
2) CMS2 (“Canonical”), epithelial, most highly chromosomally unstable (frequent SCNA), marked WNT and MYC signaling activation;
3) CMS3 (“Metabolic”), epithelial, with evident metabolic dysregulation, some overlap with CMS1 in relation to MSI status and hypermutation;
4) CMS4 (“Mesenchymal”), prominent transforming growth factor β activation, stromal invasion, and angiogenesis.

Survival analysis shows that patients with CMS4 tumors have significantly worse relapse-free survival and overall survival, CMS2 patients longer survival after relapse and a larger proportion of long-term survivors. The CMS1 group has the worst survival after relapse.

In the analysis of association between gene expression data and response to anti-EGFR therapy it appeared that there was little new information that could be exploited for an efficient discrimination of the tumors that are sensitive from those that are resistant to the treatment, in addition to what was already known or to the information incorporated in the subtype system. This might be due to the fact that one key determinant of resistance for metastatic tumors are minor tumor subclones harboring resistance-conferring genetic aberrations and for the primary tumor patients the existence of such aberrations in tumor cells that are seeding metastatic sites or are quiescent somewhere in the body. These cells do not impact the gene expression pattern of the bulk tumor tissue used for profiling. Deep sequencing technologies might bring some improvement in the future for the detection of minor tumor subclones. These subclones are small but can grow out quickly under treatment when most of the cells in the tumor mass stop growing or undergo apoptosis.

COLTHERES has identified key emerging factors of resistance in the amplification of growth pathway-controlling genes such as HER2, KRAS and MET and in the accumulation of activating KRAS mutations. These observations also suggest therapeutic options that might help to overcome resistance and to prolong patient life. In Genotype-Response correlation studies in mouse xenografts HER2 amplifications correlated with resistance and with constitutive activation of the receptor. A multi-arm trial in xenopatients showed that combined inhibition of HER2 and EGFR induced a more long-lasting regression. In longitudinal analysis of DNA from serum, detection of activating KRAS mutations could be causally associated with acquired resistance directly in metastatic CRC patients. Analysis of metastases from patients who developed resistance to cetuximab or panitumumab showed the frequent acquisition of secondary KRAS mutations or, more rarely, KRAS amplifications. Functional studies showed that cells with expression of mutant KRAS remained usually sensitive to combinatorial inhibition of EGFR and of the mitogen-activated protein-kinase kinase (MEK). Another less frequent event associated with acquired resistance was amplification of the MET gene and this was also detectable in circulating tumour DNA before relapse was clinically evident. Also in patient-derived colorectal cancer xenografts, MET amplification correlated with resistance to EGFR blockade, which could be overcome by MET kinase inhibitors. These results highlight the role of MET in mediating primary and secondary resistance to anti-EGFR therapies in colorectal cancer and encourage the use of MET inhibitors in patients displaying resistance as a result of MET amplification. Similarly, functional genetic screens suggested that BRAF mutant colon cancers for which there are currently no effective targeted treatment options available, might benefit from combination therapy consisting of BRAF and EGFR inhibitors.

These findings may offer an opportunity to anticipate and counter resistance by using combination therapies before patients relapse. KRAS mutant alleles could be detected in the blood of patients several months before radiographic detection of disease progression, thus revealing also the potentially huge value of regular blood monitoring for the clinical management of colorectal cancer patients at high risk of metastasis. These findings also offer immediate opportunities to design various clinical studies aimed at determining the optimal personalized treatment.

Design of innovative hypothesis-driven clinical Studies

Approximately 40% of mCRC show a silencing of the MGMT gene. In particular, in a retrospective analysis on 244 CRC samples. COLTHERES Partners (Idibell) found that 71% of tumors with G>A mutation of KRAS showed asimultaneous epigenetic inactivation of MGMT, thus demonstrating a strong association between the MGMT promoter hypermethylation and the presence of KRAS mutations. Furthermore, the same Authors described that MGMT hypermethylation is present also in 35% of CRC with KRAS wild type.

It has also been showned that MGMT hypermethylation is associated with mutations G:C > A:T in KRAS but not in adenomatous polyposis gene (APC), suggesting that MGMT hypermethylation may succeeds APC mutations but precedes KRAS mutations in colorectal carcinogenesis. In conclusion, the loss of MGMT expression compromises DNA repair in tumor cells and play a significant role in the progression of solid tumors, particularly for CRC, and in sensitivity to anticancer therapy based on DNA damage. The mechanism of action of temozolomide is DNA methylation at O6-guanine site, leading to a base pair mismatch.

For this reason, COLTHERES partners (Idibell and Niguarda Hospital) hypothesized that MGMT inactivation through hypermethylation may confer sensitivity to alkylating agents such as temozolomide or to its analog dacarbazine in mCRC. From the above observations, temozolomide could represent a useful therapeutic alternative in patients relapsed/refractory to conventional second-line therapies.

Based on this observation COLTHERES partners launched a clinical trial entitled ‘Phase II study of temozolomide in metastatic colorectal cancer patients resistant to standard therapies and with O6-methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation.

The pre-selection of patients based on MGMT hypermethylation could be applied to improve the efficacy of this drug in mCRC. The purpose of this study is to elucidate the value of patients selection for MGMT status and to consolidate the role of temozolomide as a single agent treatment in this mCRC setting.

Data coordination center and dissemination

The final goal of COLTHERES was to develop clinically relevant personalized algorithms to be used for the prediction of individual therapy response to molecularly targeted therapies in CRCs. Throughout the project, the consortium was committed to rapidly releasing data both to the scientific community and to the general public. Primary data has been made available to the scientific community by publication in field-specific scientific / peer-reviewed journals and a dedicated dynamic web site – including both public and private sections – has been set up early in the project to publicise the results generated by the consortium. Articles, conferences, press releases and other publications were posted on the home page. Users were able to circulate posts (using Twitter, posting on their Facebook page or emailing the post to others), follow COLTHERES on Twitter (http://twitter.com/#!/coltheres) and subscribe to the COLTHERES newsletter. Posts were categorised (articles, conferences, “Nature Paper”, press releases and publications) and archived for easy access.

The generation and publishing of a rational strategy or ‘cascade’ of in vitro and in vivo tests using defined disease models and markers to direct future drug research and development activities has been achieved. Scientific results have also been presented during international scientific conferences and special care has of course been paid to the use of unpublished data by partners. Results have attracted the interest of both specialized and lay media as well as that of pharmaceutical industries.

Data produced by the consortium has been stored in databases (data coordination center) and consistent datasets downloadable by the scientific community has been balanced with the protection of patient interests. The confidentiality of the data produced has been rigorous.

Dissemination was done via press releases, organisation of seminars, poster discussion / presentation and talks /lectures at conferences. The dissemination activities are listed in section 2 of this report.

Potential impact

The results of COLTHERES show convincingly that there are strategies to improve patient survival based on the molecular characterization of the disease and the selection of an appropriate combination of existing drugs. This could have a potentially quick positive clinical and therapeutic impact and strongly advocates for the rapid implementation of molecular tumour characterization in clinical practice. While the reported studies already suggest some therapeutic options (clinical trials ongoing), they call for further studies to use the new technologies to determine which is the optimal treatment depending on the molecular signature of the tumor and which is optimal use of the new profiling and monitoring strategies in patient management.

The functional and model system studies suggested immediate opportunities for testing the utility of rational combination therapies, so for example the combination of EGFR and BRAF inhibitors in BRAF mutated CRCs or of HER2 inhibitors and MEK inhibitors in KRAS mutated CRCs. Beyond these first propositions, even more importantly, they show a way how good combination therapies could be determined.

The CRC subtype system that was developed could help in this problem of mapping model systems back to the relevant patient group. A first application of the system is in prognostic prediction, as survival patterns differ significantly even if not hugely across subtypes. A second application is in the retrospective analysis of clinical trials to test, if they can yield relevant information on treatment benefit. The CMS1 group appears to be associated to a constitutive over-activation of the MAPK pathway and to be more resistant to current anti-growth factor receptor treatments like cetuximab. On the other hand the hypermutability goes along with the generation of a rich repertoire of cancer-specific antigens and immune reactivation strategies might be most promising in this CRC subtype. Tumors of the CMS4 group might rely on active angiogenesis to support their growth and anti-angiogenic therapies might be more successful in this subtype, maybe in combination with TGF-β pathway inhibition.

Globally, the most important impacts are the contribution to innovative paradigms for the characterization and treatment of cancer. Liquid biopsies allow patient monitoring to optimize interventions and can revolutionize clinical oncology. Model system and functional screening studies can suggest how to match best treatment options to molecular profiles and make these more widely actionable than they are today. In this regard, discoveries made within COLTHERES are presently being tested in ad hoc clinical trials (designed by COLTHERES partners).

Address of the project public website

The COLTHERES public website is www.coltheres.org

Below is the list of the Beneficiaries:

Partner number	Partner name	Partner short name	Contact person	Email
P1	UNIVERSITA DEGLI STUDI DI TORINO	UNITO	Alberto Bardelli	alberto.bardelli@ircc.it
P2	FUNDACIO INSTITUT D'INVESTIGACIO BIOMEDICA DE BELLVITGE	IDIBELL	Manel Esteller Anna Martinez-Cardus	mesteller@idibell.cat amartinezc@idibell.cat
P3	HORIZON DISCOVERY	HD	Charlotte Batley	c.batley@horizondiscovery.com
P4	AGENDIA BV	AG	Inès Goossens-Beumer	ines.goossens@agendia.com

P5	STICHTING HET NEDERLANDS KANKER INSTITUUT	NKI	Rene Bernards	r.bernards@nki.nl
P6	FUNDACIO PRIVADA INSTITUT D'INVESTIGACIO ONCOLOGICA DE VALL-HEBRON	VHIO	Josep Tabernero	jtabernero@vhio.net
P7	AZIENDA OSPEDALIERA OSPEDALE NIGUARDA CA' GRANDA	ONCG	Salavatore Siena	Salvatore.Siena@OspedaleNiguarda.it
P8	KATHOLIEKE UNIVERSITEIT LEUVEN	KUL	Sabine Tejpar	sabine.tejpar@uz.kuleuven.ac.be
P9	THE UNIVERSITY OF LIVERPOOL	UNILIV	Michael Clague	clague@liv.ac.uk
P10	SWISS INSTITUTE OF BIOINFORMATICS	SIB	Mauro Delorenzi	Mauro.Delorenzi@isb-sib.ch
P11	ARTTIC IN BRUSSELS SPRL	ARTTIC	Paul Crompton	pdc@arttic.be

2. Use and dissemination of foreground

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided ?	Internet link to the publication
1	Genomic Classifier ColoPrint Predicts Recurrence in Stage II Colorectal Cancer Patients More Accurately Than Clinical Factors.	Kopetz *et al.*	Oncologist	February	Alphamed Press	US	2015	127-133	10.1634/theoncologist.2014-0325	YES	http://www.ncbi.nlm.nih.gov/pubmed/25561511
2	Independent Validation of a Prognostic Genomic Signature (ColoPrint) for Patients With Stage II Colon Cancer	Maak *et al.*	Annals of Surgery	January 9	Lippincott Williams & Wilkins	US/UK	2013	1053-8	10.1097/SLA.0b013e31827c1180	NO	http://www.ncbi.nlm.nih.gov/pubmed/23295318
3	Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition.	Roepman *et al.*	Int J Cancer	February	John Wiley & Sons	US	2014	552-62	10.1002/ijc.28387	YES	http://www.ncbi.nlm.nih.gov/pubmed/23852808

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided ?	Internet link to the publication
4	A robust genomic signature for the detection of colorectal cancer patients with microsatellite instability phenotype and high mutation frequency.	Tian *et al.*	J Pathol	December	John Wiley & Sons	US	2012	586-95	10.1002/path.4092	YES	http://www.ncbi.nlm.nih.gov/pubmed/22926706
5	A combined oncogenic pathway signature of BRAF, KRAS and PI3KCA mutation improves colorectal cancer classification and cetuximab treatment prediction.	Tian *et al.*	Gut	April	BMJ Group	UK	2013	540-9	10.1136/gutjnl-2012-302423	YES	http://www.ncbi.nlm.nih.gov/pubmed/22798500
6	Gene Expression Signature to Improve Prognosis Prediction of Stage II and III Colorectal Cancer	Salazar *et al.*	Journal of Clinical Oncology	November 23	American Society of Clinical Oncology	US	2010	17-24	0.1200/JCO.2010.30.1077	YES	http://www.ncbi.nlm.nih.gov/pubmed/21098318
7	DUSP 4 expression identifies a subset of colorectal cancer tumors that differ in MAPK activation, regardless of the genotype.	De Vriendt *et al.*	Biomarkers	September	Informa Pharmaceutical Science	UK	2013	516-24	10.3109/1354750X.2013.819038	YES	http://www.ncbi.nlm.nih.gov/pubmed/23875912

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided?	Internet link to the publication
8	A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer.	Bertotti A, et al.	Cancer Discov.	1(6)	America Association for Cancer Research	US	2011	508-523	doi: 10.1158/2159-8290	yes	http://cancerdiscovery.aacrjournals.org/content/1/6/508.long
9	Targeted therapies: how personal should we go?	Martini M, et al.	Nat Rev Clin Oncol.	Feb;9(2)	Nature Publishing Group	US	2012	87-97	10.1038/nrclinonc.2011	No	http://www.nature.com/nrclinonc/journal/v9/n2/full/nrclinonc.2011.164.html
10	Toll-like receptor 9 agonist IMO cooperates with cetuximab in K-ras mutant colorectal and pancreatic cancers	Rosa R, et al.	Clin Cancer Res.	Oct 15;17(20)	America Association for Cancer Research	US	2011	6531-41	10.1158/1078-0432.CCR-10-3376	yes	http://clincancerres.aacrjournals.org/content/17/20/6531.long
11	Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer.	Misale S, et al.	Nature	486(7404)	Nature Publishing Group	US	2012	532-536	doi: 10.1038/nature11156	yes	http://www.ncbi.nlm.nih.gov/pmc/articles/pmid/22722830/
12	Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR.	Prahallad A, Sun C, et al.	Nature	483(7387)	Nature Publishing Group	US	2012	100-103	doi: 10.1038/nature10868	No	http://www.nature.com/nature/journal/v483/n7387/full/nature10868.html

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided?	Internet link to the publication
13	Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas.	Migliardi G, et al.	Clin Cancer Res	May 1;18(9)	America Association for Cancer Research	US	2012	2515-25	10.1158/1078-0432.CCR-11-2683	Yes	http://clincancerres.aacrjournals.org/content/18/9/2515.long
14	Targeting oncogenic serine/threonine-protein kinase BRAF in cancer cells inhibits angiogenesis and abrogates hypoxia	Bottos A, et al.	Proc Natl Acad Sci U S A	Feb 7;109(6)	National Academy of Sciences	US	2012	E353-9	10.1073/pnas.1105026109	Yes	http://www.pnas.org/content/109/6/E353.long
15	Amplification of the MET Receptor Drives Resistance to Anti-EGFR Therapies in Colorectal Cancer	Bardelli A, et al.	Cancer Discov.	3(6)	America Association for Cancer Research	US	2013	658-73	doi: 10.1158/2159-8290	Yes	http://cancerdiscovery.aacrjournals.org/cgi/pmidlookup?view=long&pmid=23729478
16	Oncogenes and angiogenesis: a way to personalize anti-angiogenic therapy?	Bottos A, Bardelli A.	Cell Mol Life Sci	Nov;70(21)	Springer	Germany	2013	4131-40	Doi: 10.1007/s00018-013-1331-3	No	http://link.springer.com/article/10.1007%2Fs00018-013-1331-3

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided?	Internet link to the publication
17	Targeted Knock-in of the Polymorphism rs61764370 does not Affect KRAS Expression but Reduces let-7 Levels	Crowley EH, et al.	Human Mutation	Feb;35(2)	Wiley Periodicals Inc.	UK	2013	208-14	Doi: 10.1002/humu.22487	Yes	http://onlinelibrary.wiley.com/doi/10.1002/humu.22487/abstract;jsessionid=480BC8B84571E1EB7DA58708E147C5DD.f03t04
18	Modeling Tumor Progression by the Sequential Introduction of Genetic Alterations into the Genome of Human Normal Cells	Zecchin D, et al.	Human Mutation	Feb;34(2)	John Wiley & Sons, Inc.	US	2013	330-7	10.1002/humu.22234	Yes	http://onlinelibrary.wiley.com/doi/10.1002/humu.22234/abstract
19	KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy	Valtorta E, et al.	International Journal of Cancer	Sep 1;133(5)	John Wiley & Sons, Inc.	US	2013	1259-65	10.1002/ijc.28106	Yes	http://onlinelibrary.wiley.com/doi/10.1002/ijc.28106/abstract
20	BRAF V600E is a determinant of sensitivity to proteasome inhibitors	Zecchin D, et al.	Mol Cancer Ther	Dec;12(12)	AACR	US	2013	2950-61	10.1158/1535-7163	Yes	http://mct.aacrjournals.org/cgi/pmidlookup?view=long&pmid=24107445
21	Liquid biopsy: monitoring cancer-genetics in the blood	Crowley E, et al.	Nat Rev Clin Oncol	Aug;10(8)	Nature Publishing Group	US	2013	472-84	10.1038/nrclinonc.2013.110	No	http://www.nature.com/nrclinonc/journal/v10/n8/full/nrclinonc.2013.110.html

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided?	Internet link to the publication
22	Climbing RAS, the Everest of Oncogenes	Russo M, et al.	Cancer Discovery	Jan;4(1)	AACR	US	2014	19-21	10.1158/2159-8290	yes	http://cancerdiscovery.aacrjournals.org/cgi/pmidlookup?view=long&pmid=24402942
23	Liquid Biopsies: Genotyping Circulating Tumor DNA.	Diaz LA Jr, Bardelli A.	J Clin Oncol	Feb 20;32(6)	ASCO	US	2014	10.1200/JCO.2012.45.2011	10.1200/JCO.2012.45.2011	No	http://jco.ascopubs.org/content/32/6/579.long
24	Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies	Bettegowda et al.	Sci Transl Med	Feb 19;6(224)	AAAS	US	2014	224ra24	10.1126/scitranslmed.3007094	Yes	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4017867/
25	Blockade of EGFR and MEK Intercepts Heterogeneous Mechanisms of Acquired Resistance to Anti-EGFR Therapies in Colorectal Cancer	Misale S, et al.	Sci Transl Med	eb 19;6(224)	AAAS	US	2014	224ra26	10.1126/scitranslmed.3007947	No	http://stm.sciencemag.org/content/6/224/224ra26.short
26	Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma.	Sun C, et al.	Nature	Apr 3;508(7494)	Nature Publishing Group	US	2014	118-22	10.1038/nature13121	No	http://www.nature.com/nature/journal/v508/n7494/full/nature13121.html

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided?	Internet link to the publication
27	Circulating pEGFR is a Candidate Response Biomarker of Cetuximab Therapy in Colorectal Cancer	Katsila T, et al.	Clin Cancer Res	Dec 15;20(24)	AACR	US	2014	6346-56	10.1158/1078-0432.CCR-14-0361	No	http://clincancerres.aacrjournals.org/content/20/24/6346.long
28	Resistance to Anti-EGFR Therapy in Colorectal Cancer: From Heterogeneity to Convergent Evolution	Misale S, et al.	Cancer Discov	Nov;4(11)	America Association for Cancer Research	US	2014	1269-1280	10.1158/2159-8290.CD-14-0462	No	http://cancerdiscovery.aacrjournals.org/content/4/11/1269.long
29	Mutational profiling of kinases in glioblastoma	Bleeker FE, et al.	BMC Cancer	Sep 26;14	BioMed Central	UK	2014	718	10.1186/1471-2407-14-718	Yes	http://www.biomedcentral.com/1471-2407/14/718
30	Genotyping cell-free tumor DNA in the blood to detect residual disease and drug resistance.	Siravegna G, Bardelli A	Genome Biology	Aug 30;15(8)	BioMed Central	UK	2014	449	10.1186/s13059-014-0449-4.	Yes	http://genomebiology.com/content/15/8/449
31	RAF suppression synergizes with MEK inhibition in KRAS mutant cancer cells	Lamba S, et al.	Cell Rep	Sep 11;8(5)	Cell Press	US	2014	1475-83	10.1016/j.celrep.2014.07.033	Yes	http://www.sciencedirect.com/science/article/pii/S2211124714006172

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided?	Internet link to the publication
32	TGF-α and amphiregulin paracrine network promotes resistance to EGFR blockade in colorectal cancer cells	Hobor S, et al.	Clin Cancer Res	Dec 15;20(24)	America Association for Cancer Research	US	2014	6429-38	10.1158/1078-0432.CCR-14-0774	No	http://clincancerres.aacrjournals.org/content/20/24/6429.long
33	Acquired resistance to EGFR-targeted therapies in colorectal cancer	Van Emburgh BO, et al.	Mol Oncol	Sep 12;8(6)	Elsevier	UK	2014	1084-1094	10.1016/j.molonc.2014.05.003	Yes	http://www.sciencedirect.com/science/article/pii/S1574789114000969
34	Intrinsic resistance to MEK inhibition in KRAS mutant lung and colon cancer through transcriptional induction of ERBB3	Sun C, Hobor S, et al.	Cell Reports	Apr 10;7(1)	Cell Press	US	2014	86-93	10.1016/j.celrep.2014.02.045	Yes	http://www.sciencedirect.com/science/article/pii/S2211124714001612
35	Minimal residual disease in breast cancer: in blood veritas	Siravegna G, Bardelli A	Clin Cancer Res	May 15;20(10)	America Association for Cancer Research	US	2014	2505-7	10.1158/1078-0432.CCR-14-0370	No	http://clincancerres.aacrjournals.org/content/20/10/2505.long
36	Epigenetic Inactivation of the BRCA1 Interactor SRBC and Resistance to Oxaliplatin in Colorectal Cancer.	Moutinho C. et al	Journal of the National Cancer Institute	106 (1)	Oxford Journals	US	2014			yes	http://jnci.oxfordjournals.org/content/106/1/djt322.long

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided ?	Internet link to the publication
37	A DERL3 associated defect in the degradation of SLC2A1 mediates the Warburg effect	Lopez-Serra P.	Nature Communications	5:3608	America Association for Cancer Research	US	2014		10.1038/ncomms4608.	Yes	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3988805/
38	Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer	Budinska E, et al.	J Pathol	Sep;231(1)	John Wiley & Sons, Inc.	US	2013	63-76	10.1002/path.4212		http://www.ncbi.nlm.nih.gov/pubmed/23836465
39	Identification of a poor prognosis BRAF-mutant-like population of colon cancer patients	Popovici V, et al.	J Clin Oncol	30	ASCO	US	2012	1288-95	10.1200/JCO.2011.39.5814		http://www.ncbi.nlm.nih.gov/pubmed/22393095
40	A robust genomic signature for detection of colorectal cancer patients with microsatellite instability phenotype and high mutation frequency	Tian S,	Journal of Pathology	228	John Wiley & Sons, Inc.	US	2012	586-595	10.1002/path.4092		http://www.ncbi.nlm.nih.gov/pubmed/22926706
41	Context-dependent interpretation of the prognostic value of BRAF and KRAS mutations in colorectal cancer	Popovici V, et al.	BMC Cancer	231(1)	BioMed Central	UK	2013	63-76	10.1186/1471-2407-13-439		http://www.biomedcentral.com/1471-2407/13/439

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available) - DOI	Open access provided?	Internet link to the publication
42	Distal and proximal colon cancers differ in terms of molecular, pathological and clinical features	Missiaglia E, et al.	Ann Oncol	Oct;25(10)	Oxford University Press	UK	2014	1995-2001	10.1093/annonc/mdu275		http://www.ncbi.nlm.nih.gov/pubmed/25057166
43	A comprehensive characterization of genome-wide copy number aberrations in colorectal cancer reveals novel oncogenes and patterns of alterations	Xie T, et al.	PLoS One	7	Public Library of Science	UK	2012	e42001	10.1371/journal.pone.0042001		http://www.ncbi.nlm.nih.gov/pubmed/22860045
44	Test of four Colon Cancer Risk-Scores in FFPE Microarray Gene Expression data	Di Narzo AF, et al.	J Natl Cancer Inst	Sep 22: 106(10)	Oxford University Press	UK	2014	dju247	10.1093/jnci/dju247		http://www.ncbi.nlm.nih.gov/pubmed/25246611

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
1	Abstract & Oral presentation	KUL/Agendia	Colorectal cancer subtyping consortium (CRCSC) identifies consensus of molecular subtypes	2014	ASCO annual 2014				

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
2	Abstract & Poster presentation	Agendia	Molecular subtyping of colorectal cancer identifies patients with a mesenchymal tumor type who might benefit from TGF-beta inhibition	2014	ASCO annual 2014				
3	Abstract & poster presentation	Agendia	Comparison of ColoPrint risk classification with clinical risk in the prospective PARSC trial	2014	ASCO annual 2014				
4	Abstract & poster	NKI	RANBP2 knock-down is synthetic lethal with BRAF V600E in colon cancer	2014	ENA 2014				
5	Abstract	Agendia	Role of Intraepithelial Lymphocyte Density in the Transcriptome of Microsatellite Stable Primary Colon Cancer.	2014	USCAP 2014				
6	Abstract &	Agendia	Molecular subtyping of colorectal cancer identifies a mesenchymal tumour type that might benefit from TGF-beta pathway inhibition	2014	ASCO GI 2014				
7	Abstract &	Agendia	Excellent concordance of ColoPrint and MSI-Print classification in paired endoscopic-surgical specimens of Stage I-III colorectal cancer (CRC)	2014	ASCO GI 2014				
8	Abstract	Agendia	Comparison of ColoPrint risk classification with clinical risk in the prospective PARSC trial	2014	ASCO GI 2014				

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
9	Poster presentation	HD	3D: an informative approach for KRAS drug discovery	2014	EACR 2014				
10	Poster presentation	HD	Isogenic cell lines and complex culture systems enable more accurate modelling of patient responses to targeted therapies	2014	BACR 2014				
11	Press release	Agendia	Multiple Studies on Agendia's MammaPrint and BluePrint Tests to Be Featured at ASCO Annual Meeting	May 22, 2014					
12	Press release	Agendia	Molecular Subtyping Can Help Determine Prognosis and Chemotherapy Benefit in Colorectal Cancer Patients	June 5, 2013					
13	Abstract & poster	Agendia	Genomic classifier (ColoPrint) predicts outcome and chemotherapy benefit in stage II and III colon cancer patients	2013	ESMO 2013				
14	Abstract	Agendia	Use of genomic classifiers (ColoPrint) for personalized treatment decisions in stage II and stage III colon cancer patients	2013	ASCRS 2013				

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
15	Abstract & Poster discussion	Agendia	Colorectal cancer intrinsic subtypes are associated with prognosis, chemotherapy response, deficient mismatch repair and epithelial to mesenchymal transition (EMT)	2013	ASCO Annual 2013				
16	Abstract & poster presentation	Agendia	Genomic classifiers (ColoPrint/ MSI-Print) predict outcome and chemotherapy benefit in stage II and III colon cancer patients		ASCO Annual 2013				
17	Press release	Agendia	Ground-breaking Research Identifies Molecular Subtyping as a Key to Determining Prognosis and Benefit of Chemotherapy for Breast and Colorectal Cancers	May 31, 2013					
18	Abstract & presentation	Agendia/VHIO	Colorectal cancer intrinsic subtypes are associated with prognosis, chemotherapy response, deficient mismatch repair and epithelial to mesenchymal transition (EMT)	2013	ASCO GI 2013				
19	Abstract & poster	NKI/KUL	Identification of synthetic lethal interactions with the BRAF oncogene in colorectal cancer	2013	ASCO GI 2013				

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
20	Abstract & poster	Agendia	Genomic classifiers (ColoPrint/ MSI-Print) predict outcome and chemotherapy benefit in stage II and III colon cancer patients	2013	ASCO GI 2013				
21	Abstract & poster	KUL/Agendia	DUSP4 expression as a marker of heterogeneous signaling in colorectal cancer patients	2012	FASEB 2012, Colorado				
22	Abstract & presentation	Agendia	Personalized treatment planning of stage II and IIIA colon cancer patients using genomic classifiers (ColoPrint/ MSI-Print)	2012	ESMO world GI 2012				
23	Abstract & poster discussion	Agendia	Development and Validation of a robust molecular diagnostic test (ColoPrint) for predicting outcome in stage II colon cancer patients	2012	ESMO annual 2012				
24	Abstract & Poster presentation	Agendia	The PARSC trial, a prospective study for the assessment of recurrence risk in stage II colon cancer patients using ColoPrint.	2012	ASCO 2012				
25	Abstract & Poster presentation & discussion	Agendia	Validation of a genomic classifier (ColoPrint) for predicting outcome in the T3-MSS subgroup of stage II colon cancer patients.	2012	ASCO Annual 2012				
26	Press release	Agendia	Agendia Announces Launch of ColoPrint for Colon Cancer Prognosis and Prediction	June 1, 2012					

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
27	Publication	VHIO/Agendia	ColoPrint gene assay can guide treatment decisions in stage II colon cancer	May 1, 2012	The ASCO Post				
28	Abstract	Agendia	Development and validation of a genomic signature to identify colorectal cancer patients with Microsatellite Instability	2012	ASCO GI 2012				
29	Abstract	Agendia	The PARSC trial, a prospective study for the assessment of recurrence risk in stage II colon cancer (CC) patients using ColoPrint	2012	ASCO GI 2012				
30	Abstract & presentation	VHIO/Agendia	Clinical and technical validation of a genomic classifier (ColoPrint) for predicting outcome of patients with stage II colon cancer	2012	ASCO GI 2012, JCO abstract				
31	Press release	Agendia	Agendia Announces Nine Studies in Breast and Colon Cancer for Presentation at 2012 Annual Meeting of the American Society of Clinical Oncology (ASCO)	May 29, 2012					

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
32	Press release	Agendia	Agendia receives 1.27 M Euros of funding from The Seventh Framework Programme to develop companion diagnostics within consortiums of leading European cancer centers and translational research groups	July 21, 2011					
33	Abstract & presentation	Agendia	Signal transduction of activating mutations of *KRAS*, *BRAF* and *PIK3CA* pathway converges at transcriptional level and predicts treatment response to cetuximab in colorectal cancer	2011	ASCO annual 2011				
34	Abstract	Agendia	Independent validation of a prognostic genomic profile (ColoPrint) for stage II colon cancer (CC) patients	2011	ASCO GI 2011				
35	Abstract	Agendia	Independent validation of a prognostic genomic signature (ColoPrint) for stage II colon cancer patients	2011	ESA annual 2011				
36	Co-Chair and SpeakerLecture in a conference	UNITO	26th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics	18-nov-14	Barcelona (Spain)	Scientist		5000	International
37	Speaker in a conference	UNITO	23rd Biennial Congress of EACR	5-jul-14	Munich (Germany)	Scientist		5000	International

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
38	Speaker in a conference	UNITO	4th EACR-OECI Joint Training Course “Molecular Pathology approach to cancer”	5-may-14	Amsterdam (The Netherlands)	Scientist		5000	International
39	Speaker in a conference	UNITO	AACR Annual Meeting 2014	5-apr-14	San Diego, CA (USA)	Scientist	General Public	15000	International
40	Speaker in a conference	UNITO	Oncology Update 2012	23-feb-14	Jeddah (Saudi Arabia)	Scientist		1000	International
41	Speaker in a conference	UNITO	CGC.nl meeting - Translational cancer genomics	21-nov-13	Amsterdam (The Netherlands)	Scientist		1000	International
42	Speaker in a conference	UNITO	Epidermal Growth Factor Receptor - Future Directions - Joint International Research Conference of The Institute for Advanced Studies and the Israel Science	17-nov-13	Jerusalem (Israel)	Scientist		1000	International
43	Speaker in a conference	UNITO	William Guy Forbeck Research Foundation XXVIII Annual Forum	7-nov-13	Hilton Head, SC (USA)	Scientist		1000	International
44	Seminar	UNITO	Istituto Pascale	29-oct-13	Napoli (Italy)	Scientist		200	Italy
45	Speaker in a conference	UNITO	17th ECCO-38th ESMO-32nd ESTRO European Cancer Congress - Reinforcing multidisciplinarity	27-oct-13	Amsterdam (The Netherlands)	Scientist		5000	International
46	Seminar	UNITO	University of Pittsburgh Cancer Institute - Hillman Cancer Center	23-oct-13	Pittsburgh, PA (USA)	Scientist		200	US

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
47	Seminar	UNITO	Program for Evolutionary Dynamics - Harvard University	21-oct-13	Cambridge, MA (USA)	Scientist		500	US
48	Speaker in a conference	UNITO	AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics Conference	19-oct-13	Boston, MA (USA)	Scientist		5000	International
49	Speaker in a conference	UNITO	The 3rd Global Cancer Genomics Consortium Symposium - From Oncogenomics to Cancer Care	19-sept-13	Lisbona (Portugal)	Scientist		5000	International
50	Speaker in a conference	UNITO	Cancer Pharmacogenomic and Targeted Therapies	15-sept-13	Cambridge (UK)	Scientist		1000	International
51	Keynote Lecture in a conference	UNITO	ESMO 15th World Congress on Gastrointestinal Cancer	5-july-13	Barcelona (Spain)	Scientist		5000	International
52	Speaker in a conference	UNITO	2nd Michelangelo International Conference - Promises and challenges of developing nwe drugs in oncology	4-july-13	Milano (Italy)	Scientist		1000	International
53	Speaker in a conference	UNITO	Systems Medicin in Cancer	14-june-13	Berlin (Germany)	Scientist		1000	International
54	Seminar	UNITO	14th International Medical Education Workshop on Molecular Targeted Therapy of Cancer(MTTC)	10-may-13	Sorrento (Italy)	Scientist		100	Italy
55	Speaker in a conference	UNITO	AACR Annual Meeting 2013	6-apr-13	Washington, DC (USA)	Scientist	General Public	15000	International

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
56	Poster presentation	HD	X-MAN™ reporter cell lines: Enabling the study of endogenous promoter activity and protein dynamics + 3-Dimensional growth reveals KRAS dependency.	April-2013	AACR Annual Meeting 2013, Washington, DC (USA)	Scientist	General Public	15000	International
57	Seminar	UNITO	MITHO-Master in Thoracic Oncology	22-mar-13	Torino (Italy)	Scientist		100	Italy
58	Speaker in a conference	UNITO	SEMM - European School of Molecular Medicine	12-mar-13	Milano (Italy)	Scientist		1000	International
59	Speaker in a conference	UNITO	The 1st International Congress on Controversies in Personalized Oncology (CONPO) - Breast, Colon, Lung, Melanoma	8-mar-13	Barcelona (Spain)	Scientist		1000	International
60	Speaker in a conference	UNITO	ESMO Symposium on Signaling Pathways in Cancer - Targeting the HER/EGFR family in breast, lung and colorectal cancers	1-mar-13	Barcelona (Spain)	Scientist		1000	International
61	Seminar	UNITO	IEO - Istituto Europeo di Oncologia	23-jan-13	Milano (Italy)	Scientist		200	Italy
62	Speaker in a conference	UNITO	Adoptive Clinical Trial Design	17-jan-13	Boston, MA (USA)	Scientist		1000	International
63	Poster presentation	HD	X-MAN™ NanoLuc™ Reporter Cell Lines:Tools for High Throughput Screening	12-17-jan-13	SLAS	Scientist		1000	International

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
64	Speaker in a conference	UNITO	IDIBELL Cancer Conference on Personalized Medicine	4-dec-12	Barcelona (Spain)	Scientist		1000	International
65	Speaker in a conference	UNITO	5th Annual Cancer Symposium - Cancer Center/Beth Israel Deaconess Medical Center/Harvard Medical School	30-nov-12	Boston, MA (USA)	Scientist		5000	International
66	Keynote Lecture in a conference	UNITO	1st Joint BioCARE, StratCan and UCan Cancer Research Meeting	9-nov-12	Malmö (Sweden)	Scientist		1000	International
67	Speaker in a conference	UNITO	24th EORTC-NCI-AACR Symposium on 'Molecular Targets and Cancer Therapeutics'	7-nov-12	Dublin (Ireland)	Scientist		5000	International
68	Poster presentation	HD	X-MAN™ NanoLuc™ Reporter Cell Lines: Applications to drug-discovery	5-9th Nov 2012	EORTC	Scientist			
69	Speaker in a conference	UNITO	54th SIC Annual Meeting	4-oct-12	Bologna (Italy)	Scientist		5000	International
70	Chairperson in a conference	UNITO	ESMO Congress 2012	29-sept-12	Vienna (Austria)	Scientist		5000	International
71	Speaker in a conference	UNITO	24th Pezcoller Symposium	15-june-12	Trento (Italy)	Scientist		5000	International
72	Speaker in a conference	UNITO	2012 ASCO Annual Meeting	2-june-12	Chicago, IL (USA)	Scientist		5000	International

No	Type of activities	Main leader	Title	Date	Place	Type of audience	Additional type of audience	Size of audience	Countries addressed
73	Speaker in a conference	UNITO	Liverpool Cancer Research UK Centre 2012 Annual Meeting	14-may-12	Liverpool (UK)	Scientist		1000	International
74	Speaker in a conference	UNITO	AACR Special Conference on Molecularly Targeted Therapies	10-may-12	San Diego, CA (USA)	Scientist		5000	International
75	Speaker in a conference	UNITO	IFOM-IEO Visit	26-apr-12	Bangalore (India)	Scientist		1000	International
76	Speaker in a conference	UNITO	2012 AACR Annual Meeting	3-apr-12	Chicago, IL (USA)	Scientist	General Public	15000	International
77	Speaker in a conference	UNITO	13th World Congress on Gastrointestinal Cancer	24-june-11	Milano (Italy)	Scientist		5000	International
78	Co-Chair and Speaker Lecture in a conference	UNITO	26th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics	18-nov-14	Barcelona (Spain)	Scientist		5000	International
79	Abstract & poster	IDIBELL	2014 AACR Annual Meeting	April 2014	San Diego, CA (USA)	Scientist			International

Section B (Confidential or public: confidential information to be marked clearly)

Part B1

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.

Type of IP Rights[1]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	NO		WO2010074573 AU2009330807 CA 2748224 CN 102325902 EP2379740 JP 2012-513752 MX/a/2011/006926 NZ 593847 US20110319285	Methods and means for typing a sample comprising colorectal cancer cells. (ColoPrint)	AGENDIA B.V.
Patent	NO		WO2012044167 AU 2011308134 EP 2622099 US-20130302321*	Methods and means for typing a sample comprising cancer cells based on oncogenic signal transduction pathways. (EGFR pathway)	AGENDIA N.V.
Patent	NO		WO2012/087144 EP2655661 US-20130296191*	Methods and means for molecular classification of colorectal cancers. (Molecular subtyping & MSI)	AGENDIA N.V.
Patent	NO		GB1418460.0	Methods and methods for treating KRAS mutated cancer	HDL and UNILIV
Trademark	NO		EU: CTM4075321 Ger: 30413491 31 USA: 3212036	ColoPrint	AGENDIA N.V.
Trademark	NO		EU CTM5954854 International registration designating: AU, CN, JP, RU, US	ColoPrint	AGENDIA N.V.

Part B2

[1] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Type of Exploitable Foreground[2]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[3]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
None								

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[3] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

3. Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	259015
Title of Project:	Modelling and predicting sensitivity to targeted therapies in colorectal
Name and Title of Coordinator:	Alberto Bardelli

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	NO
2. Please indicate whether your project involved any of the following issues (tick box) :	YES
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	YES
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	YES
• Did the project involve Human data collection?	YES
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	

• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	YES
• Were those animals transgenic small laboratory animals?	YES
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	NO
• Research having the potential for terrorist abuse	NO

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	1
Work package leaders	2	8
Experienced researchers (i.e. PhD holders)	8	4
PhD Students	0	1
Other	4	4

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	
Of which, indicate the number of men: **5/1**	

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?	○ ○X	YES NO

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
X	Set targets to achieve a gender balance in the workforce	○	X	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

X Yes- please specify: We hosted high school students in the participant laboratory

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

X No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

X Main discipline[4]: Medical Sciences

○ Associated discipline[4]: Natural Sciences ○ Associated discipline[4]: 3.1

[4] Insert number from list below (Frascati Manual).

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*	○ X	YES NO

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

- ○ No
- ○ Yes- in determining what research should be performed
- ○ Yes - in implementing the research
- ○ Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	YES NO

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- X Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- X Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- X European level
- X International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	44
To how many of these is open access[5] provided?	25
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	
X publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[6]: …….	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		3
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	2
	Registered design	0
	Other	0
17. How many spin-off companies were created / are planned as a direct result of the project?		None
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies

[5] Open Access is defined as free of charge access for anyone via Internet.

[6] For instance: classification for security project.

❑	Decrease in employment,	X	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
	Full time
Difficult to estimate / not possible to quantify	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes X No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes X No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

X	Press Release	X	Coverage in specialist press
X	Media briefing	X	Coverage in general (non-specialist) press
❑	TV coverage / report	X	Coverage in national press
❑	Radio coverage / report	X	Coverage in international press
❑	Brochures /posters / flyers	X	Website for the general public / internet
❑	DVD /Film /Multimedia	X	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	X	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

4. FINAL REPORT ON THE DISTRIBUTION OF THE European Union FINANCIAL CONTRIBUTION

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1.	
2.	
n	
Total	