TRANSLATING
COLORECTAL
CANCER RESEARCH

WORKSHOP
FEBRUARY 9-10, 2017
PORTUGUESE ONCOLOGY INSTITUTE
PORTO, PORTUGAL

Organizers
Manuel Teixeira (Portugal)
Sergi Castellví-Bel (Spain)
Thomas Reid (USA)
Jordi Camps (Spain)

Session topics
Epidemiology
Tumor evolution and heterogeneity
Germline predisposition
Biomarkers
Cancer genome biology
Novel therapies
Precision cancer medicine
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Co-organized by COST ACTION BM1206

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program
Day 1 / February 9

8:00 REGISTRATION
9:00-9:15 WELCOME Manuel Teixeira (institutional host)

Inaugural talk
9:15-10:00 Ian Tomlinson, Welcome Trust Centre for Human Genetics, Oxford, UK
DNA polymerase proofreading mutations: from discovery to clinic in 3 years

Epidemiology
Chair: Victor Moreno
10:00-10:35 Kari Hemminki, German Cancer Research Center, Heidelberg, Germany
Epidemiology and genetics of familial colorectal cancer
10:35-11:10 Marc Gunter, International Agency for Research on Cancer, Lyon, France
Metabolic dysfunction and colorectal cancer: molecular epidemiologic approaches
11:10-11:30 Coffee break
11:30-11:45 Calogerina Catalano, German Cancer Research Center, Heidelberg, Germany
How can potentially functional variants in NOD like receptor genes affect colorectal cancer risk, survival and therapy response?
11:45-12:00 Victor Moreno, Catalan Institute of Oncology, Barcelona, Spain
Risk Model for Colorectal Cancer in Spanish Population Using Environmental and Genetic Factors. Results from the MCC-Spain study
12:00-14:00 Poster Presentation 1 (12:00-13:00, odd numbers) and Lunch (13:00-14:00)

Tumor evolution and heterogeneity
Chair: Marian Grade
14:00-14:35 Sabrina Arena, Bardelli’s group, Candiolo Cancer Institute, Torino, Italy
The impact of clonal evolution on colorectal cancer therapy
14:35-15:10 Thomas Ried, National Cancer Institute, NIH, Bethesda, USA
Intratumor heterogeneity and treatment response in colorectal carcinomas
15:10-15:25 Sanne ten Broeke, Leiden University Medical Centre, Netherlands
Comprehensive histological and molecular analysis of PMS2 associated malignancies; a separate entity among MMR deficient tumours?
15:25-15:40 Isabel Quintanilla, IDIBAPS, Barcelona, Spain
Whole genome duplication in colorectal cancer evolution and its physiological consequences
15:40-16:00 Coffee break

Germline predisposition
Chair: Sergi Castellvi-Bel
16:00-16:35 Evelien Dekker, Academic Medical Centre, Amsterdam, Netherlands
Serrated polyps and serrated polyposis syndrome: clinical and molecular risk
16:35-17:10 Roland Kuiper, Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands
DNA repair deficiency and polyposis
17:10-17:25 Carla Pinto, Portuguese Oncology Institute, Porto, Portugal
Molecular characterization of Portuguese Lynch syndrome families
17:25-17:40 Stephanie Schubert, Leiden University Medical Centre, Netherlands
Multiple evidence for involvement of chromosome 1q locus in predisposition to familial colorectal cancer and polyposis
17:50 GROUP PHOTO
Day 2 / February 10

Biomarkers
Chair: Jordi Camps

9:00-9:35  Linda Bosch, Meijer's group, Netherlands Cancer Institute, Amsterdam, Netherlands
Developing biomarkers for molecular early detection of colorectal cancer

9:35-10:10  Ulrike Peters, Fred Hutchinson Cancer Research Center, Seattle, USA
Comprehensive risk prediction for colorectal cancer risk-stratified screening

10:10-10:25  David Fiedler, University Medical Center Mannheim, Germany
Genomic instability in recurring and nonrecurring adenomas of the colon

10:25-10:40  Veronika Vymetalkova, Institute of Experimental Medicine, Prague, Czech Republic
Circulating miRNAs: a cancer screening biomarkers in rectal cancer

10:40-11:00  Coffee break

Cancer genome biology
Chair: Ian Tomlinson/Claire Palles

11:00-11:35  Daniele Tauriello, Batlle’s group, Institute for Research in Biomedicine, Barcelona, Spain
Genetic reconstitution of metastatic colorectal cancer in immunocompetent mice: a novel preclinical platform

11:35-11:50  Mariève Rocque, Norwegian University of Science and Technology, Norway
Generating model cells lines by CRISPR/Cas9 genome editing for functional studies of polymerase proofreading-associated polyposis

11:50-12:05  Sérgia Velho, Instituto de Investigação e Inovação em Saúde, Porto, Portugal
ITGA6 as a molecular mediator of KRAS-, BRAF- and PIK3CA-induced colorectal cancer stem cell phenotype

12:05-14:05  Poster Presentation 1 (12:05-13:05, even numbers) and Lunch (13:05-14:05)

Novel therapies
Chair: Tom van Wezel

14:05-14:40  Noel de Miranda, Leiden University Medical Center, Leiden, Netherlands
Next-generation immunotherapies for colorectal cancer

14:40-14:55  Flávia Castro, Instituto de Investigação e Inovação em Saúde, Porto, Portugal
Re-educating antigen-presenting cells: an interferon-gamma delivery system for cancer immunotherapy

14:55-15:10  Marta Pinto, Instituto de Investigação e Inovação em Saúde, Porto, Portugal
Human colorectal tumour decellularized matrices polarize macrophages towards a pro-invasive phenotype

15:10-15:30  Coffee break

Precision cancer medicine
Chair: Manuel Teixeira

15:30-16:05  Marian Grade, Georg-August-University Medicine, Göttingen, Germany
Targeting signaling pathways mediating treatment resistance in rectal cancer

16:05-16:40  Guro Elisabeth Lind, Oslo University Hospital, Norway
The prognostic value of DNA methylation biomarkers in colorectal cancer

16:40:16:55  Anna-Lena Jakubzik, University Medical Center Göttingen, Germany
HER-2 and HER-3 as promising therapeutic candidates in colorectal cancer

16:55:17:10  Sofia Fernandes, Institute of Molecular Pathology and Immunology, Univ. Porto, Portugal
Specific inhibition of p110α subunit of PI3K: putative therapeutic strategy for KRAS mutant colorectal cancers
Closing talk
17:10-17:55 Zoltan Szallasi, Dana-Farber/Harvard Cancer Center, Boston, USA
Exploiting next generation sequencing data to improve DNA repair pathway aberration
directed and cancer immunology therapy

18:00 CLOSING REMARKS Sergi Castellvi-Bel (COST Action BM1206 Chair)
talks
DNA polymerase proofreading mutations: from discovery to clinic in 3 years

Ian Tomlinson (Welcome Trust Centre for Human Genetics, Oxford, United Kingdom)

http://www.well.ox.ac.uk/tomlinson

Stratified medicine must ultimately identify small groups of patients with proven differential prognosis or response to treatment if it is to achieve "precision" or "personalised" status. This is often difficult, not least because lack of statistical power means that associations with clinico-pathological variables are hard to demonstrate formally. Here, I describe one example, POLE polymerase proofreading deficiency, where a rare group of patients has been identified, characterised and subjected to new management algorithms within a few years. POLE might provide an exemplar for biomarkers of rare patient groups in the future.
Epidemiology and genetics of familial colorectal cancer

Kari Hemminki and Asta Försti

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A family history of colorectal cancer (CRC) is well-established and several other, discordant cancers manifest in syndromes, such as hereditary nonpolyposis colorectal cancer (HNPCC). However, less is known about the possible familial associations of CRC with discordant cancers beyond HNPCC and other syndromes. Using the world’s largest database of familial cancer, the Swedish Family-Cancer Database covering 15 million individuals, we assessed the relative risks (RRs) for any cancer in families with increasing numbers of first-degree relatives diagnosed with CRC, and in reverse order RR for CRC in families of multiple discordant cancers. Several novel associations were found in CRC families, such as RR of 2.8 for melanoma and 1.9 for myeloma.

In the course of our whole genome sequencing efforts, we have developed a pipeline for analyzing germline genomes from Mendelian types of pedigrees. The variant calling step distinguishes three types of genomic variants: single nucleotide variants (SNVs), indels and copy number variants (CNVs). Segregation in the pedigree allows variants to be present in affected family members and not in old unaffected ones. The effectiveness of variant segregation depends on the number and relatedness of the family members; if over 5 third-degree (or more distant) relatives are available the experience has shown that the number of likely variants is reduced from many hundreds to a few tens. These are then subjected to bioinformatic analysis, starting with the combined annotation dependent depletion (CADD) tool, which predicts the likelihood of the variant being deleterious. The likelihood of success of the present genomic pipeline in finding novel high- or medium-penetrant genes depends on many steps but first and foremost, the pedigree needs to reasonably large and the assignments and diagnoses among the members need to be correct. We discuss our success with an experience from some 25 multiplex families.
Metabolic dysfunction and colorectal cancer: molecular epidemiologic approaches

Marc Gunter (EPIC consortium, International Agency for Research on Cancer, Lyon, France)


Obesity and Type 2 diabetes are established positive risk factors for colorectal cancer; however, the biological mechanisms that underlie these relationships are not fully understood. Obesity is associated with significant metabolic and endocrine abnormalities including alterations in sex hormone metabolism, insulin signalling, and adipokines/inflammatory pathways. All three mechanisms influence the balance between cell proliferation and apoptosis and have been linked to colorectal cancer development in both experimental and observational studies. However, it is likely that other, hitherto unrecognised, molecular pathways may mediate the adiposity-colorectal cancer association. In this presentation I will discuss new molecular epidemiologic approaches to understanding the link between obesity, metabolic dysfunction and colorectal cancer, highlighting our ongoing work that exploits metabolomics, genomic and epigenetic tools within the framework of prospective cohort studies, randomized controlled trials and clinical case series.
EPIDEMIOLOGY – selected talk from abstract

How can potentially functional variants in NOD like receptor genes affect colorectal cancer risk, survival and therapy response?

Calogerina Catalano (1), Filho Inacio da Silva M (1), Pavel Vodicka (2, 3), Ludmila Vodickova (2, 3), Alexander N.R. Weber (4), Kari Hemminki (1,5), Asta Försti (1,5)

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Background: Chronic inflammation is a preeminent driver of colorectal cancer (CRC). NOD-like receptors (NLRs) are key innate pattern recognition receptors (PRRs) and regulators of inflammation. If the intestinal epithelial barrier is disrupted, NLRs and other PRRs are activated by microbe- or endogenous damage-associated molecular patterns (MAMPs or DAMPs), leading to activation of several cell signaling pathways that participate in host defense and inflammation. A few members of the NLR family, especially NOD1, NOD2 and NLRP3, have already been associated with susceptibility, progression and treatment of human CRC and the development of colitis and colitis-associated colon cancer. Thus, the connection between NLRs and CRC seems to be of high interest. Aims: We investigate the influence of potentially functional variants in the NLR signaling pathway genes on the risk and survival of CRC. Furthermore, we study the interplay between the NLR variants and the previously analyzed Toll-like receptor and interferon variants in the development of CRC. Additionally, we evaluate whether and how these variants influence the response to a platinum-based therapy.

Materials and methods: We have conducted an association study on potential regulatory variants of the NLR signaling pathway genes in a Czech population (1540 cases and 1108 controls). The variants were selected using several in silico tools, such as UCSC browser, HaploReg, Regulome DB, PolyPhen, SIFT, Gtex Portal and microRNA binding site prediction tools. We used logistic regression models in the risk analysis and Kaplan-Meier method and Cox regression in the survival analysis. The identified CRC risk and survival variants will be functionally characterized for their influence on gene expression in primary human colon, rectum and immune cells as well as in primary human colon adenocarcinoma and CRC cell lines. Expected results: We expect to increase our knowledge about the potential influence of interactions of innate immunity genes on CRC development, response to platinum-based therapy and survival.
Risk Model for Colorectal Cancer in Spanish Population Using Environmental and Genetic Factors. Results from the MCC-Spain study

Gemma Ibáñez-Sanz, Anna Díez-Villanueva, Francisco Rodríguez-Moranta, Beatriz Pérez-Gómez, Vicente Martin, Javier Llorca, Pilar Amiano, Eva Ardanaz, Adonina Tardón, Jose J. Jiménez-Moleón, Rosana Peiro, Juan Alguacil, Carmen Navarro, Gemma Castaño-Vinyals, Nuria Aragonés, Manolis Kogevinas, Marina Pollan, Victor Moreno (presenting author)

Author affiliation(s): Institute, Country: CIBER Epidemiología y Salud Pública (CIBERESP), Spain

Colorectal cancer (CRC) screening of the average risk population is only indicated according to age. We aim to elaborate a model to stratify the risk of CRC by incorporating environmental data and single nucleotide polymorphisms (SNP). The MCC-Spain case-control study included 1336 CRC cases and 2744 controls. Subjects were interviewed on lifestyle factors, family and medical history. Twenty-one CRC susceptibility SNPs were genotyped. The environmental risk model, which included alcohol consumption, obesity, physical activity, red meat and vegetable consumption, and non-steroidal anti-inflammatory drug use, contributed to CRC with an average per factor OR of 1.36 (95% CI 1.27 to 1.45). Family history of CRC contributed an OR of 2.25 (95% CI 1.87 to 2.72), and each additional SNP contributed an OR of 1.07 (95% CI 1.04 to 1.10). The risk of subjects with more than 25 risk alleles (5th quintile) was 82% higher (OR 1.82, 95% CI 1.11 to 2.98) than subjects with less than 19 alleles (1st quintile). This risk model, with an AUROC curve of 0.63 (95% CI 0.60 to 0.66), could be useful to stratify individuals. Environmental factors had more weight than the genetic score, which should be considered to encourage patients to achieve a healthier lifestyle.
The impact of clonal evolution on colorectal cancer therapy

Sabrina Arena (Bardelli’s group, Candiolo Cancer Institute, Torino, Italy)

http://www.ircc.it/irccit/?q=Molecular-Genetics

Blockade of the Epidermal Growth Factor Receptor (EGFR) with monoclonal antibodies such as cetuximab or panitumumab is effective in a subset of colorectal cancers (CRC). Unfortunately, in the majority of the cases, this response is transient due to the progressive emergence of acquired resistance. How clonal dynamics affect the development of resistance during anti-EGFR therapy is poorly understood. Therefore digging into these still weakly characterized mechanisms is necessary in order to devise new strategies to overcome or even prevent resistance and to design new clinical trials. To address this issue and to investigate on the evolutive nature of colorectal cancer, we have expanded our analyses from preclinical models to patients who developed acquired resistance to anti-EGFR treatment. We find that clonal dynamics can be monitored in real time non only in cellular models, but also in the blood of patients, thanks to the liquid biopsy approach, that can be used to intercept the emergence of resistant clones before relapses are clinically manifest. We discovered that a multistep clonal evolution process driven by progressive increase in drug fitness underlies the development of resistance in cells and patients. Moreover, we observed that clonal evolution of drug-resistant cells is associated with the clinical outcome of CRC patients treated with anti-EGFR antibodies. These findings suggest that the use of targeted therapies, in order to reach long-term efficacy, needs to take into account the continuous evolution of cancer cells, ideally adapting to tumor evolution. In conclusion, rationally combined targeted therapies may restrain tumor evolution, and could limit the emergence of drug resistance thus leading to long-term effective responses.
TUMOR EVOLUTION AND HETEROGENEITY – invited talk

Intratumor heterogeneity and treatment response in colorectal carcinomas

Thomas Reid (National Cancer Institute, Bethesda, USA)

https://ccr.cancer.gov/Genetics-Branch/thomas-ried

Individual response to radiochemotherapy (RCT) in rectal cancer patients is highly variable and the underlying mechanisms of treatment resistance of cancer cells are poorly understood. Recent studies revealed a considerable degree of genomic tumor heterogeneity. We hypothesize that this heterogeneity has a direct impact on treatment response as subpopulations of cancer cells are resistant to currently used RCT and facilitate tumor growth under treatment. To address this highly relevant clinical issue, patient-derived rectal cell lines were established. The tissue was derived from a 59-year-old male presenting with an adenocarcinoma of lower rectum (T3,N1,M0), who was treated by neoadjuvant RCT (50.4 Gy plus 5-FU) and low anterior resection. The neoadjuvant treatment induced a shrinkage of the tumor (staging of the surgical specimen: ypT3,N0), suggesting a partial response to RCT. A biopsy of the treatment naïve tumor was implanted heterotopically into an immunodeficient nude mouse. After in vivo growth, the tumor was dissociated and introduced to an in vitro culture where murine stromal cells were depleted using antibody-based columns. The cell line proved to be of human origin by immunofluorescence for human Keratin 20 as well as genotyping using short tandem repeat profiling. The characterization of the genome by array CGH and spectral karyotyping (SKY) revealed a highly complex near-triploid karyotype with numerous chromosomal imbalances, which are specific for colorectal cancers, including gains in chromosomes 7, 13 and 20. We also observed other structural abnormalities including an isochromosome 1q. Multiplex-FISH by consecutive hybridization of probes for 15 gene loci, which are known to be relevant in colorectal cancer genesis, revealed a significant degree of clonal heterogeneity of the cell line. Single cell sorting was then performed to establish single cell derived cell lines from the genomically well characterized parental cell line. Exposure of the single cell derived cell lines to irradiation in combination with 3 µM 5-FU revealed substantial differences in treatment response. Analyses of the genome by array CGH and transcriptome by RNA-Seq of the respective single cell derived cell lines are currently underway. This will allow the comparison of the respective sensitivities to RCT to the identified aberration profiles. These data will facilitate the understanding of therapy resistance and potentially allow a reliable prediction of the patient’s response. A tailored therapy is an important step towards individualized treatment in colorectal cancer patients to avoid therapy resistance.
TUMOR EVOLUTION AND HETEROGENEITY – selected talk from abstract

Comprehensive histological and molecular analysis of PMS2 associated malignancies; a separate entity among MMR deficient tumours?

Sanne W. ten Broeke1,Tom van Bavel, Anne Jansen2 , E. Gomez Garcia3, L.P. van Hest4, T.G.W. Letteboer5, J.W. Olderode Berends6, Th. A. van Os7, L. Spruijt8, J.F.J. Tromp1, J.T. Wijnen1, H. Morreau2, T. van Wezel2, M. Nielsen1

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Background: Lynch syndrome (LS) related cancers have a different genetic background and histology compared to sporadic colorectal cancers (CRC) and show a different treatment response and survival. Up to now most studies on Lynch related tumours focused on MLH1, MSH2 and MSH6 deficient tumours, but data on PMS2 related tumours is currently lacking. We now aim to unravel the histological and molecular hallmarks of PMS2 associated CRC compared with other Lynch and sporadic tumours.

Methods: We obtained informed consent of PMS2 mutation carriers and were able to collect 21 CRCs for histological and molecular evaluation. Histological hallmarks were scored by an experienced pathologist. Moreover, to get an impression of the somatic tumour spectrum, we used the Ampliseq Cancer Hotspot panel (version 2) on isolated tumour DNA. This panel covers mutation hotspots in 50 genes (~2800 COSMIC mutations), including well known somatically mutated genes such as KRAS, APC and TP53.

Results: PMS2 associated CRCs showed a number of LS associated hallmarks: 81% were right-sided, 43% had Crohn’s like infiltrate (missing: 19%) and 81% (missing: 14%) showed microsatellite instability. However, a majority (63%, missing: 14%) hardly had any tumour infiltrating lymphocytes, a well-known hallmark of Lynch associated tumours. The molecular analysis showed a relatively low percentage of TP53 and APC mutations compared with controls and a high percentage of a specific FBXW7 mutation. Notably, 5/21 of CRCs had this c.1393C>T transition, where the controls had none. We also found a relatively rare KRAS hotspot mutation in exon 4 (c.436G>A, p.Ala146Thr) occurring three times in the PMS2 cohort but not in the control cohort.
Discussion: To our knowledge this is the first study to examine tumour characteristics of a cohort consisting solely of PMS2 mutation carriers. Several findings such as the specific KRAS and FBXW7 mutations might possibly help with the identification of PMS2 associated tumours. The finding of a less active immune response might negatively influence survival and treatment options, but this requires further investigation.
Whole genome duplication in colorectal cancer evolution and its physiological consequences

Isabel Quintanilla (1,8), Darawalee Wangsa (2), Elena Asensio (1), Keyvan Torabi (3), Maria Vila-Casadesús (1,4), Amaia Ercilla (5), Gregory Klus (2), Zeynep Yuce (2,6), Claudia Golofré (1), Miriam Cuatrecasas (7), Juan José Lozano (4), Neus Agell (5), Maria Pellisé (1), Daniela Cimini (8), Antoni Castells (1), Thomas Ried (2), and Jordi Camps (1,3)

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A considerable portion of tumors exhibits aneuploid karyotypes during their evolution, likely resulting from the progressive loss of chromosomes following whole genome duplication. In the case of colorectal cancer a multi-step progression is observed, with adenoma being the most well-known precursor lesion. In fact, malignant polyps are lesions that exemplify this process as they are adenomas containing a focus of adenocarcinoma. In this study, we sought to analyze the clonal evolution of colorectal cancer by performing sequential fluorescence in situ hybridization in the adenoma and carcinoma components of malignant polyps (n=23). Interestingly, in a significant proportion of cases we observed the gain of all the locus analyzed to be the decisive step in the transition from adenoma to carcinoma, suggesting a whole-genome duplication event. This finding supports previous evidence that suggested the importance of whole-genome duplication during carcinogenesis. Accordingly, we next sought to study tetraploidy in vitro in order to better understand the consequences of this phenomenon in cancer as well as its contribution to genome instability. To do so, we generated isogenic diploid and near-tetraploid clones derived from the colorectal cancer cell lines DLD1 and RKO and performed gene expression profiling, which revealed a significant enrichment of transcripts involved in replication stress. Then, we further characterized the replication stress phenotype and observed an association with the increased DNA damage and chromosome instability (CIN) in near-tetraploid clones. Our data also unveiled that near-tetraploid clones displayed increased migratory and invasive capacities, both in vitro and in primary colorectal tumors, thus providing physiological advantages to the cancer cells.
Serrated polyps and serrated polyposis syndrome: clinical and molecular risk

Evelien Dekker (Academic Medical Centre, Amsterdam, Netherlands)


Serrated polyps have been identified as precursors of colorectal cancer (CRC) and 15-30% of all CRCs may arise via the “serrated neoplasia pathway”. An even larger proportion of post-colonoscopy interval carcinomas, CRCs diagnosed within the time interval to the next surveillance colonoscopy, are suspected to arise from serrated polyps. This problem seems to be caused by an assemblage of several clinical as well as translational issues.

Firstly, serrated polyps are difficult to detect during colonoscopy, due to their discrete appearance. As a result, the detection rate of serrated polyps is widely variable among endoscopists. Secondly, not every serrated polyp is routinely removed during colonoscopy in daily practice, and especially small and diminutive hyperplastic polyps in the distal colon are often not resected by endoscopists. As the differentiation of premalignant sessile serrated polyps (SSP) and hyperplastic polyps tends to be difficult for endoscopists as well as for pathologists, also premalignant lesions might not be removed. Furthermore, as the genomic changes responsible for the serrated polyp to CRC progression have only been partially unravelled, identification of those serrated polyps truly at risk to develop into CRC remains a serious challenge. Thirdly, the indistinctive borders of SSPs cause a difficulty for complete resection, resulting in a higher risk for residual tissue. Lastly, in daily practice the diagnosis of serrated polyposis syndrome is often missed and therefore these patients are not surveilled adequately. As germline mutations for serrated polyposis syndrome are unknown, this diagnosis has been clinically defined by the World Health Organisation (WHO). Besides redefining the current WHO criteria for serrated polyposis syndrome, future studies should focus on the safety and feasibility of personalised treatment and surveillance for patients with serrated polyposis syndrome in order to decrease patient burden as well as the incidence of colonoscopy interval CRCs.
Polyposis is defined by the accelerated occurrence of polyps in the colon and rectum, and is strongly associated with genetic predisposition to colorectal cancer (CRC). Several of the underlying gene defects directly affect genome maintenance mechanisms. For example, polymerase proofreading associated polyposis (PPAP) is caused by mutations in the exonuclease domains of POLE and POLD1. MUTYH-associated polyposis (MAP) is a adenomatous polyposis syndrome with attenuated phenotype, and is caused by biallelic mutations in the base excision repair gene MUTYH (recessive inheritance). In addition, a second base excision repair gene, NTHL1, was found to act as a high-penetrant polyposis predisposing gene for which several families have now been described, and most recently, also biallelic mutations in the mismatch repair gene MSH3 were found in two families with CRC. These novel syndromes appear to share a much broader tumor spectrum and emphasize the role of DNA repair defects in adenomatous polyposis and CRC predisposition.
Molecular characterization of Portuguese Lynch syndrome families

Lynch Syndrome (LS) is an autosomal dominant syndrome of high penetrance mostly caused by germline mutations in the mismatch repair (MMR) genes, mainly in MLH1 and MSH2. Since the molecular characterization of LS was established, the identification of mutation carriers has become an important issue with major clinical implications. In the present study, 1147 individuals with clinical criteria for LS testing were included, 962 of whom had tumor available for prescreening methods by microsatellite instability or by immunohistochemistry. After this pre-selection, a total of 349 individuals were analyzed for MMR germline mutations. We characterized 126 families with LS, of which 38 (30.2%) presented a mutation in MLH1, 68 (54.0%) in MSH2, 18 (14.3%) in MSH6, and two (1.6%) in PMS2. Thirty-seven different mutations were found, 16 of which are not described in the Insight database. We also verified that six mutations (founders/recurrent) were responsible for 63.5% of all our confirmed LS families. These results suggest that the molecular analysis of LS in Portugal should be initiated by the screening of these six mutations. The implementation of this strategy will make the analysis of Portuguese families with clinical suspicion of LS faster and more cost-effective.
Multiple evidence for involvement of chromosome 1q locus in predisposition to familial colorectal cancer and polyposis

Stephanie A Schubert(1), Dina Ruano (1), Arnoud Boot (1), Fadwa A El Sayed (1), Stijn Crobach (1), Jan Oosting (1), Carli M Tops (1), Ronald van Eijk (1), Hans FA Vasen (1), Maartje Nielsen (1), Juul T Wijnen (1), Frederik J Hes (1), Hans Morreau (1), Noel F de Miranda (1), Rolf Sijmons (2) and Tom van Wezel (1)

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Despite great effort to elucidate the heritability of familial colorectal cancer (CRC) and polyposis, a substantial fraction of CRC heritability remains unexplained. Here we have set out to identify germline predisposing loci in these patient groups by employing various genomic methods. We performed homozygosity mapping in 302 CRC or (oligo)polyposis patients and 3,367 controls using 300K Illumina CytoSNP arrays. We identified a 1Mb region located on chromosome 1q32.2 in which runs of homozygosity were overrepresented in cases compared to controls (2.0% vs 0.1%), but which did not remain significant after adjusting for multiple testing. In parallel, linkage analysis in 15 individuals from a family with an extensive history of microsatellite stable (MSS) CRC and adenomas showed a linkage peak located on chromosome region 1q32.2-42.2 (maximum LOD score = 2.75). This 21.1Mb region overlaps with the region of homozygosity located on chromosome 1q identified with homozygosity mapping. Concomitantly, exome sequencing of five affected family members identified one shared, rare (population frequency <0.01) coding variant within the linkage peak, in the Melanoma Inhibitory Activity protein 3 gene (MIA3, NM_198551). MIA3 immunohistochemistry identified a possible tumorigenic role in adenoma development, independent of the germline variant. Subsequently, whole-genome sequencing identified over 500 shared, rare variants within the linkage peak in three family members. This strategy, however, did not identify any candidate variants. Taken together, this study shows, with two independent approaches, that a genomic region on chromosome 1q is associated with hereditary colorectal cancer and polyposis.
BIOMARKERS – invited talk

Developing biomarkers for molecular early detection of colorectal cancer

Linda Bosch (Meijer’s group, Netherlands Cancer Institute, Amsterdam, Netherlands)

https://www.nki.nl/people/bosch-linda/

http://www.nki.nl/divisions/diagnostic-oncology/meijer-g-group/

A number of unmet clinical needs exist for early detection of colorectal cancer, two of which will be addressed. The first is to improve the current stool test for colorectal cancer screening, i.e. the immunochemical fecal occult blood test (FIT). This test measures the presence of hemoglobin in stool samples. While this is a cost effective test, there is room for improvement as the current test still misses about 30% of the cancers, and about 70% of advanced adenomas. By using DNA and protein based biomarkers we aim to substantially improve this performance. The second unmet need is the identification of high-risk stage II colorectal cancer (CRC) patients who may benefit from future follow-up systemic therapy. Using molecular detection and monitoring of residual disease, through analysis of tumor DNA mutations and rearrangements as personalized biomarkers, we believe we can identify these patients. Based on established translational research from participating institutes from the USA and the Netherlands the biomarker solutions for addressing both these unmet clinical needs are currently under clinical validation in the context of a Stand Up To Cancer project with the aim to bring these developments to patients in the fastest possible way.
Despite declines in colorectal cancer (CRC) incidence, it remains one of the leading causes of cancer death. Paradoxically, it is among the most preventable and treatable of neoplastic diseases when detected early via screening. Multiple screening options are available, including colonoscopy, sigmoidoscopy or fecal occult blood test; however, overall utilization in the population remains suboptimal partly due to cost and invasiveness of the procedures. Current screening recommendations are based only on age and family history of CRC, whereas incidence of CRC varies substantially in the population and most cases occur in those without a positive family history (FH). To inform screening decisions, we have built a risk prediction model based on known genetic loci identified in genome-wide association studies (GWAS) as well as established or potential lifestyle and environmental risk factors, such as obesity, medication, smoking, alcohol, diabetes and diet. We utilized data from the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and the Colorectal Transdisciplinary study (CORECT), including 8421 CRC cases and 9767 controls. Genetic variants were combined by constructing a genetic risk score (G-score) as a sum of 64 GWAS loci for CRC weighted by their beta-coefficients from the multivariate logistic regression analysis. Similarly, we constructed an environmental risk score (E-score) based on 19 environmental and lifestyle risk factors for CRC. Compared with the risk model based on FH only the models based on FH+G-score+E-score significantly improved risk prediction: for men AUC 0.53 vs 0.64, p-value <10-12 and for women 0.53 vs 0.63, p-value <10-10, respectively. Calculating the 10-year risk estimates of developing CRC based on the FH+score+E-score model, the difference in age to start screening for the top 90% and the bottom 10% of risk score ranges from 12 to 14 years depending on sex and status of CRC family history. These results demonstrate that risk prediction models incorporating known genetic and environmental risk factors can more accurate estimate risk of CRC and potentially be used for risk stratified screening identifying individuals of higher risk of CRC for targeted screening and interventions, while reducing emphasis for those at low risk.
BIOMARKERS – selected talk from abstract

Genomic Instability in Recurring and Nonrecurring Adenomas of the Colon

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Objectives: Adenomatous polyps of the colon are widespread pre-cancerous lesions that might undergo malignant transformation developing into an invasive carcinoma. Despite endoscopical removal using polypectomy many polyps do recur. Aim of this study was to shed light on this issue and to understand which biomarkers are associated with the propensity of adenoma recurrence and might in future studies be of value for the individualization of the clinical management of patients with adenoma.

Methods: Forty-four colorectal adenomas with low-grade dysplasia and fourteen with high-grade dysplasia were collected from an FFPE archive. Tissue sections were taken for macro-dissection and DNA isolation. DNA copy numbers were analyzed using array CGH. Additionally, single-layer cytospins (Hedley-method) were prepared and analyzed for fourteen colorectal cancer-specific gene probes (COX2, PIK3CA, APC, CLIC1, EGFR, MYC, CCND1, CDX2, CDH1, TP53, ERBB2/HER2, SMAD7, SMAD4 and ZNF217) by multiplex fluorescence in situ hybridization (FISH) on single nuclei (>300 per case) adenomas.

Results: To date, we evaluated adenomas with recurrence (n=15) and nonrecurring adenomas (n=15). In particular, fourteen matched pair samples (consisting of the primary adenoma and its relapse), were analyzed. Array-based CGH revealed significant focal aberrations of 6p (52.5%) and 7q (42%). Gains (P<0.01) in 6p21.33, 7q22.1 and 17q12 existed predominantly in primary LG adenomas with recurrence but not in nonrecurring LG adenomas. We confirmed these findings using multicolor FISH and found high correspondence of both techniques (P<0.001, κ=0.847). CLIC1, EGFR and ZNF217 were the most common gains among all analyzed samples. Additionally, we identified CDX2 as a potential indicator for adenoma recurrence (P<0.05). Moreover, three genomically tetraploidized cases were identified. Chromosomal instability was increased in adenomas harboring one or more prominent aberrations (P<0.05). Matched pairs showed a high degree of clonality. Eleven of fourteen pairs comprised of at least one identical genomic imbalanced clone detectable in the primary adenoma and the relapse.

Conclusion: We conclude that there are characteristic amplifications in early stage colorectal adenomas that might drive adenoma recurrence.
Circulating miRNAs: A Cancer Screening Biomarkers in Rectal Cancer

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Early detection of colorectal cancer (CRC) is the main prerequisite for successful treatment and reduction of mortality. From a clinical point of view, malignancies in the colon (CC) and the rectum (RC) represent two distinct entities that require different treatment strategies with often different prognosis. Circulating microRNAs (miRNAs) in the bloodstream were previously identified as promising diagnostic, prognostic and predictive biomarkers for CRC in general, limited data are specifically available on miRNA deregulation in rectal cancer (RC) only. In the present study, we aimed to identify plasmatic miRNAs of RC and explored their expression profiles over time. First, miRNA array “unbiased” screening of almost all known miRNAs (2,555) in 69 paired rectal cancer samples (tumor and non-malignant mucosa) was used to select candidate markers. Several miRNAs expression levels were associated with recurrence free survival after adjuvant therapy. These candidate miRNAs associated with patient’s survival and treatment response were further followed in plasma samples from 40 subjects with RC collected at 3 time intervals. The first sampling was collected at the time of diagnosis, (i.e., active disease), the second after 6-9 months, depending on treatment (i.e., covering the tumor resection, administration of adjuvant chemotherapy) and the third sampling was conducted around 1 year after the diagnosis. The results from plasma samples of patients with RC at the time of diagnosis and after diagnosis will be compared to healthy controls. MiRNA expression profile was very similar in plasma and tumor tissue in RC patients. Up-regulated miRNAs in tumor tissue were also up-regulated in plasma samples from RC patients when compared to healthy volunteers, the same distribution was observed for down-regulated miRNAs. There was also a significant difference between samplings in RC patients for miRNA-19a and miRNA-19b. A year from diagnosis, miRNA expression profiles in all patients were no longer different from those measured in healthy volunteers. Our results provide evidence that circulating miRNAs might be a next-generation biomarker and contribute to cancer screening in non-invasive liquid biopsy.

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Genetic reconstitution of metastatic colorectal cancer in immunocompetent mice: a novel preclinical platform

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http://www.irbbarcelona.org/es/research/colorectal-cancer-laboratory

About 40%–50% of patients with CRC will develop metastasis either at the time of diagnosis or as recurrent disease after surgery. In the absence of genetic alterations to explain these processes, it remains a major challenge to predict which patients will develop metastatic disease or to design targeted therapies. Indeed, there are currently no effective therapeutic strategies to prevent or diminish metastatic burden. We have recently discovered that the formation of metastasis in CRC relies on TGF-beta activation of the tumour microenvironment. We identified several TGF-beta target genes in stromal cells that associate with short disease-free survival intervals after therapy and this improves on the current AJCC staging system. I will discuss our novel genetic mouse models of CRC and show that TGF-beta inhibition to block stromal signalling is a safe and promising therapeutic option to prevent metastasis formation.
Generating model cell lines by CRISPR/Cas9 genome editing for functional studies of polymerase proofreading-associated polyposis

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Background: Recently, a new colorectal cancer (CRC) syndrome has been described: the polymerase proofreading-associated polyposis (PPAP) syndrome. Mutations identified in the context of PPAP are typically in the exonuclease domain of the catalytic subunit of replicative DNA polymerase epsilon (POLE) or delta (POLD1). We previously reported the POLE c.1373A>T (p.Tyr458Phe) variant which was identified across multiple generations of a large Norwegian family with increased risk for CRC. Previous studies showed that mutating the corresponding tyrosine of orthologous polymerases resulted in decreased fidelity and thereby increased mutation rate. We aim to verify the increased mutation rate in human cells. For this purpose, model cell lines were created by CRISPR/Cas9 genome editing of HEK293T cells. At the same time, these model cell lines will serve as reference for future studies of novel POLE variants as well as to study in vitro cytotoxicity of drugs. Methods: Two different guide RNAs targeting the region near POLE c.1373 were selected according to their proximity to the desired mutation. A DNA repair template was designed so as to incorporate both the mutation of interest and silent mutations introducing a BfaI restriction site. Cleavage efficiency of Cas9 using the two guide RNAs was estimated by the T7 endonuclease 1 and precise gene modification was estimated by BfaI digestion. Limiting dilution was used to grow monoclonal cells, which were later screened by BfaI digestion and subsequent Sanger sequencing to confirm positive clones. Results: Despite similar cleavage efficiency between the two guide RNAs, precise editing of c.1373A>T was only detected using one of the guide RNAs. Clonal growth and subsequent screening lead to the identification of a number of monoclonal cells with the precise c.1373A>T mutation of one allele and/or a functional knock-out of the second allele. Conclusion: Cell lines obtained by CRISPR/Cas9 genome editing will allow future functional studies of the POLE c.1373A>T variant and POLE knock-out. Anticipated studies include the supF mutagenesis assay to study mutation frequency as well as cytotoxicity assays. Furthermore, cell lines obtained in the present study may also serve as a reference for novel POLE variants.
ITGA6 as a molecular mediator of KRAS-, BRAF- and PIK3CA-induced colorectal cancer stem cell phenotype

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Cancer stem cells have been recognized as the tumor cell of origin, the main responsible for metastasis, and the major drivers of tumor recurrence after treatment. Understanding the molecular and environmental factors regulating their origin and phenotype maintenance is therefore a major goal in cancer (CRC) research. In this work, we aimed at investigate the role that KRAS, BRAF, and PIK3CA oncogenic mutations play in the induction of a CRC cell stemness as well as to identify the downstream mediators of this effect. Oncogene mRNA expression was specifically silenced by RNAi in CRC cell lines harboring mutations in these genes. The stemness state of the silenced cells was subsequently analyzed in a sphere-forming assay. A decrease in the sphere-forming capacity was consistently observed upon KRAS, BRAF, and PIK3CA silencing, suggesting that the three oncogenes are comparatively able to support CRC cell stemness. In order to further characterize this effect, the expression of several cancer stem cell markers was evaluated by flow cytometry. Surprisingly, no consistent associations between changes in the expression of these markers and the general reduction in stemness were found. Thereafter, in order to find the molecular mediators of oncogene-induced cancer cell stemness, the expression of several intestinal stem markers, epithelial to mesenchymal-associated genes and other putative cancer stem cell markers, such as integrin alpha 6, was evaluated. Although changes in the expression of some intestinal stem cell markers and epithelial to mesenchymal markers were found, they were not common to all the cell lines and to all the inhibitions and therefore could not explain the decreased sphere-forming capacity. In contrast, integrin alpha 6 protein expression showed a consistent downregulation upon KRAS, BRAF, and PIK3CA inhibition. Although integrin alpha 6 role as a CRC stem cell marker remains to be confirmed, its function as an inductor of CRC cell migration and invasion has been reported. Together with our data, these evidences strengthen the assumption that integrin alpha 6 may act as modulator of colorectal cancer cell stemness, acting downstream of KRAS, BRAF, and PIK3CA. This hypothesis is currently being validated using in vitro and in vivo systems.
Next-generation immunotherapies for colorectal cancer

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Following the encouraging clinical responses observed in cancer patients treated with anti-CTLA-4, -PD-1, and -PD-L1 antibodies, immunotherapy shows great promise for the treatment of cancer. The blockade of co-inhibitory pathways in T-cells promotes their activation and triggers anti-tumour immunity. The latter was shown to be driven against tumour-mutated antigens (neo-antigens) and to be dependent on the existence of neo-antigen-specific, activated T-cells, prior to therapeutic intervention. This observation suggests the complementary enhancement of T-cell responses by means of neo-antigen vaccination and/or adoptive transfer of neo-antigen-specific T-cell clones.

The accumulated evidence on an association between the occurrence of natural anti-tumour immune responses in colorectal cancers (CRCs) and improved clinical prognosis, makes CRC patients excellent candidates to benefit from immunotherapy.

We are screening the coding genomes of CRCs by whole-exome and RNA next-generation sequencing (NGS). Somatic mutation profiles (mutanomes) are annotated and neo-antigens corresponding to the transcribed mutations are tested for their ability to induce activation of autologous T-cells derived from tumour infiltrating lymphocytes (TILs) and peripheral blood. The discovery of neo-antigen-specific T-cell clones in CRC patients would support the development of anti-cancer therapies consisting of neo-antigen-based vaccines and/or adoptive transfer of neo-antigen-specific T-cell clones.
Re-educating antigen-presenting cells: an interferon-gamma delivery system for cancer immunotherapy

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Anti-cancer immune responses depend on the efficiency of tumour antigens presentation and co-stimulatory signals provided by antigen-presenting cells (APCs). However, it is reported that dendritic cells (DCs) present at the tumour site have an immunosuppressive profile, which limit activity of effector T cells and support tumor progression. Additionally, macrophages were described to promote tumour progression and negatively impact on responses to therapy. Thus, APCs are promising targets to generate therapeutic immunity against cancer. We focused on the potential of Chitosan/Poly(γ-glutamic acid) nanoparticles incorporating interferon-gamma to modulate tumor cellular immunity and, consequently to affect cancer-cell related activities. Accordingly, we developed an interferon-gamma delivery system that modulates macrophage and DCs phenotype, promoting T cell activation and counteracting in vitro colorectal cancer cell invasion. We are currently testing this strategy in an in vivo model in order to re-educate resident, and recently recruited, immune cells towards a pro-inflammatory and anti-tumour phenotype.
NOVEL THERAPIES – selected talk from abstract

Human colorectal tumour decellularized matrices polarize macrophages towards a pro-invasive phenotype

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Tumors are complex microenvironments composed of cancer cells, stromal cells and a non-cellular component, the extracellular matrix (ECM). In this context, macrophages emerged as modulators of cancer progression, regulating breast cancer cell migration, invasion and metastasis. In a simplistic vision of macrophage function in tumors, these have been described as key elements for carcinogenesis, preventing the establishment and spreading of cancer cells – M1-macrophages – or supporting tumor growth and progression – M2-macrophages. Their remarkable plasticity makes them very sensitive to environmental factors, including the ECM. In the present work we evaluated the impact of human tumor colorectal ECM on macrophage polarization. Accordingly, we developed an innovative 3D-organotypic culture model, by decellularizing human colorectal cancer tissue fragments and by repopulating them with human monocytes, mimicking more closely the natural tumor microenvironment. DNA quantification and DAPI staining validated the efficiency of the decellularization protocol. SEM analysis revealed an ECM fiber meshwork with a similar architecture to the native tissues while immunohistochemistry confirmed that components, such as laminin and fibronectin, were retained. Native tissues’ mechanical properties, namely their rigidity, were also preserved. Notably, normal and tumor decellularized matrices distinctly promoted macrophage polarization, with macrophages in tumor matrices differentiating towards an anti-inflammatory M2-like phenotype. Matrigel invasion assays revealed that tumor ECM-educated macrophages efficiently stimulated cancer cell invasion through a mechanism involving CCL18. Interestingly, high CCL18 expression at the invasive front of human colorectal tumors correlated with advanced tumor staging. These results clearly evidenced that normal and tumor ECM harbor differences determinant for macrophage polarization and allowed the discovery of CCL18 as a potential player in CRC invasion and metastasis. Altogether, besides highlighting the relevance of using in vitro models that resemble the native tissue microenvironments, our work emphasises the critical role of the ECM on the stromal cells of a tumor with putative consequences for disease progression.
The standard treatment for locally advanced rectal cancers consists of preoperative 5-FU-based chemoradiotherapy followed by radical surgery. However, clinical response to chemoradiotherapy varies greatly, and a considerable percentage of rectal cancers are chemoradioresistant, even if intensified regimens are being pursued. This represents a substantial clinical and socioeconomic problem, as it exposes patients to the potential side effects of cytotoxic therapies and radiation without a clear benefit. Thus, it remains of utmost clinical importance to determine the molecular characteristics underlying this resistance, and to identify effective strategies to overcome it. We have therefore systematically explored genes and pathways that were suggested, based on high-throughput profiling analyses of primary rectal cancers and colorectal cancer cell lines, to mediate treatment resistance. Briefly, there is now convincing evidence that targeting Wnt/β-catenin and JAK/STAT signaling represents a promising strategy to increase therapeutic responsiveness to chemoradiotherapy.
The majority of colorectal cancers harbor a wide range of epigenetic aberrations, including frequent DNA methylation changes. Many of these changes have been shown to be clinically useful by representing biomarkers for early detection, risk assessment and prognostication.

Patients with early stage colorectal cancer generally have a good prognosis, but 15-20% experience relapse and eventually die of the disease. Occult metastases have been suggested as a marker for increased risk of recurrence in patients with node-negative disease. We have evaluated the prognostic value of a previously identified highly accurate epigenetic biomarker panel for early detection of colorectal tumors, in sentinel lymph nodes of early stage colon cancer patients. Although we were not able to identify the colon cancer patients that experienced relapse and that are in need of more aggressive treatment, the DNA methylation had a clear prognostic value. Patients with high levels of sentinel lymph node DNA methylation had an inferior prognosis compared to patients with a lower level of methylation.

The same association is seen for primary colorectal cancers, where patients with a CpG island methylator phenotype (CIMP), characterized by widespread tumor DNA promoter methylation, have an inferior prognosis. By analyzing more than 1100 tumor samples from two consecutive colorectal cancer series, we further demonstrate that the CIMP status can be used to identify high risk patients among the poor prognosis group of patients with MSS BRAF mutated tumors.
HER-2 and HER-3 as promising therapeutic candidates in colorectal cancer

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Purpose: Despite the implementation of multimodal treatment approaches and novel biological substances, a relevant proportion of patients with colorectal cancer (CRC) experience disease recurrence and require systemic treatment. Abrogation of growth factor-dependent signaling represents a promising strategy in this treatment. Here we aimed to evaluate the potential therapeutic effectiveness of targeting HER-2 and HER-3.

Methods: CRC cell lines were screened for protein expression of epidermal growth factor (EGF) receptors HER-2 and HER-3 using immunohistochemistry, and for gene amplification of HER-2 using silver in-situ-hybridization (SISH). Selected cell lines were subsequently subjected to treatment with various EGF inhibitors (trastuzumab, pertuzumab, TDM-1, lapatinib, and afatinib), either alone or in combination with chemotherapy. Western blot analysis was performed to measure the protein expression of Akt and pAkt, and cellular viability was assessed with a CellTiter-Blue assay.

Results: HER-3 expression (IHC ≥ 2+) was detected in five out of 12 cell lines (42%), while HER-2 was both expressed and amplified in three cell lines (25%) (IHC ≥ 2+ and SISH-positive). Treatment of LS513, LS1034 and SW837 with the HER-2-specific antibodies trastuzumab and/or pertuzumab resulted in a rather mild reduction of cellular viability. In clear contrast, the antibody-drug conjugate TDM-1 mediated a stronger and dose-dependent decrease of cellular viability, accompanied by reduced protein levels of pAkt. The most striking effects, however, were observed with the dual tyrosine kinase inhibitor lapatinib and the pan-HER inhibitor afatinib. Selectively, the effect of EGF receptor inhibition was augmented by a combination with 5-fluorouracil (5-FU) and oxaliplatin.

Conclusions: Inhibition of EGF receptors effectively blocks intracellular signaling and significantly impairs the cellular viability of CRC cells in vitro. However, the effectiveness of receptor inhibition highly depends on the inhibitors’ mode of action. Because both HER-2 and, especially, HER-3 are overexpressed in a relevant proportion of patients with CRC, HER-2-/HER-3-inhibition by targeting both receptors or their kinase activities may represent a potential therapeutic strategy.
Specific inhibition of p110α subunit of PI3K: putative therapeutic strategy for KRAS mutant colorectal cancers

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Colorectal cancer (CRC) is a leading cause of cancer mortality worldwide. It is often associated with activating mutations in KRAS leading to deregulation of major signaling pathways as the RAS-RAF-MAPK and PI3K-Akt. However, despite intensive research, information is scarce regarding the molecular mechanisms underlying the survival of mutant CRC cells that are mostly resistant to the available therapies. Moreover, CRC patients harboring somatic KRAS mutations are excluded from EGFR targeted therapies. It is therefore urgent to unravel novel therapeutic approaches for those patients. In this study, we have awarded PI3K p110α a key role in CRC cells harboring KRAS mutations or KRAS/PIK3CA mutations. Specific silencing of PI3K p110α by small interfering RNA (siRNA) reduced viability and induced apoptosis or cell cycle arrest. In agreement with these cellular effects, PI3K p110α silencing led to alterations in the expression levels of proteins implicated in apoptosis and cell cycle, namely XIAP and pBad in KRAS/PIK3CA mutant cells and cyclin D1 in KRAS mutant cells. To further validate our data, a specific PI3K p110α inhibitor, BYL719, was evaluated. BYL719 mimicked the in vitro siRNA effects on cellular viability and on the alterations of apoptotic- and cell cycle-related proteins in CRC mutant cells. Overall, this study demonstrates that specific inhibition of PI3K p110α could provide an alternative therapeutic approach for CRC patients, particularly those harboring KRAS mutations.
CLOSING TALK

Exploiting next generation sequencing data to improve DNA repair pathway aberration directed and cancer immunology therapy

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http://www.dfhcc.harvard.edu/insider/member-detail/member/zoltan-szallasi-md/

DNA repair pathway aberrations significantly contribute to the genomic instability underlying cancerous development. In fact, the actual DNA repair pathway aberration in a given cancer has a profound impact on the biology of the tumor and in cases it also determines the efficacy of a particular therapeutic approach. Certain DNA repair pathway aberrations, such as homologous recombination deficiency, make cancer vulnerable to certain forms of therapy, such as PARP inhibitors or platinum. Other forms of DNA repair pathway aberrations have a profound impact on the antigenic profile of the tumor. Therefore, developing reliable methods that determine the types of DNA repair pathway aberrations and mutagenic processes present in a given tumor will have a significant impact on selecting the most effective therapy in cancer. Next generation sequencing offers an efficient tool to achieve this goal by detecting distinct mutational signatures in the cancer genome. The identification of the relevant mutational signatures has been greatly facilitated by the creation of cellular model systems in which various DNA repair pathway related genes are disrupted, then the cells are grown in the presence and absence of mutagenic treatment in order to accumulate the mutational signatures. We will present our results of our model systems and show how those helped to evaluate next generation sequencing data of human tumor biopsies in order to prioritize patients for the most effective therapy.
POSTERS
Microsatellite instability-low and loss of heterozygosity in 2p may associate to increased susceptibility for familial rectal cancer

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Revised Bethesda guidelines (BG) identifies colorectal cancer (CRC) patients suspected of Lynch syndrome (LS), that do not fulfill Amsterdam criteria, to be tested for microsatellite instability (MSI) in the respective tumors, an hallmark of LS. Patients fulfilling BG whose tumors present MSI-high (MSI-H) should undergo germline mutation analysis for DNA mismatch-repair (MMR) genes. MMR gene mutations define LS and are found in approximately 40% of patients with MSI-H tumors. However, the molecular basis underlying increased CRC susceptibility in the remaining cases fulfilling BG is still unknown. We aimed to characterize patients with CRC fulfilling BG but without germline MMR gene mutations, for MSI status, namely regarding dinucleotide (DNR) or mononucleotide repeat (MNR) sequences, and for specific BG characterization, tumor stage and location in the colon and rectum. We selected 225 patients with CRC fulfilling the BG from our Familial Cancer Registry, having germline MMR gene mutations excluded: 121 microsatellite stable (MSS) and 104 MSI tumors-63 MSI-H and 41 MSI-low (MSI-L). MSI analysis have been performed by analyzing the Bethesda microsatellite markers (3 DNR–D2S123, D5S346, D17S250 and 2 MNR–BAT25, BAT26), using GeneScan. Loss of heterozygosity (LOH) at DNR was also evaluated. Correlation with clinical features was performed using Stata 12. Amongst MSI-L tumors, only two were MSI at MNR; in the MSI-H group, 3 presented MSI only at MNR, whereas S showed MSI only at DNR. Interestingly, MSI-L tumors were associated to BG#5 (family history of CRC in 3 family members irrespective of age at diagnosis) (44%) and MSS or MSI-H tumors were associated to BG#1-4 (65% and 87%, respectively), p=0.002. MSI-L was more frequent in DNR and in these cases it was associated to rectal tumors (71%). MSI at both MNR and DNR was associated to proximal colon (71%), p=0.003. MSI at DNR and LOH of D2S123 correlated with BC#5 (p=0.005 and p=0.045, respectively). D2S123 LOH also correlated with earlier stage tumors (p=0.035). Our results suggest that MSI-L at DNR and D2S123 LOH may be associated to increased susceptibility for familial rectal cancer, often early stage tumors.
Unexplained adenomatous polyposis: whole exome sequencing to identify new candidate genes


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In about 30% of patients diagnosed with colorectal adenomatous polyposis no germline pathogenic variants have been identified in the Mendelian susceptibility genes APC or MUTYH, although a hereditary predisposition is very likely. To identify new candidate genes associated to adenomatous polyposis and CRC, we performed whole-exome sequencing on germline DNA from 27 patients with unexplained polyposis (>10 adenomas, onset <60 years) from 19 families. All these patients tested negative for APC and MUTYH germline pathogenic variants and most of them were also diagnosed with CRC. To identify potential pathogenic variants we filtered for rare variants (MAF ExAC≤0.001 for heterozygous, or ≤0.01 for homozygous ones) with loss-of-function (LOF): frame shift, stop gain/loss, splicing ±1 or 2, and missense with high functional impact, predicted at least by three in silico analysis tools. To prioritize candidate genes we consider gene function, pathways related to cancer (KEGG, GO Annotations), expression in healthy colonic mucosa, overrepresentation of rare variants in candidate genes and/or common pathways, and co-segregation analyses. Although we did not find any LOF variants in the new genes lately associated to multiple colonic adenomas and CRC (POLE, POLD1, NTHL1 and MSH3) we found LOF in other genes involved in DNA repair such as NER, MMR or homologous recombination. Moreover, we found an enrichment of LOF variants in genes of the Fanconi anemia DNA repair pathway, recently related to familial CRC risk. These results might suggest the importance of DNA repair genes in polyposis etiology. Nevertheless, to confirm these findings we are sequencing our candidate genes in a validation cohort of about 300 unexplained polyposis patients.

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Delineating the phenotypic spectrum of the NTHL1-associated polyposis

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Germline biallelic mutations in NTHL1 have been recently identified in individuals with colorectal cancer (CRC) and polyposis. Four families with biallelic NTLH1 mutations have been reported so far, all of them with at least one allele carrying the c.268C>T (p.Gln90*) truncating mutation, three in homozygosis. The scarcity of cases reported so far prevents an accurate definition of the associated clinical phenotype. We aimed to assess the prevalence of the c.268C>T mutation in >500 non-polyposis CRC families and in 88 unrelated polyposis cases without mutations in known high-penetrance genes, and to help refine the clinical characteristics of biallelic NTHL1 mutation carriers. Two unrelated homozygous carriers of c.268C>T were identified (Spanish origin). While both carriers had multiple colonic adenomas and CRC, one of them showed a much more aggressive phenotype consisting of bilateral breast cancer, bladder cancer and 3 CRCs. Phenotypic data from all reported NTHL1 carriers (n=10) point to a phenotype characterized by attenuated adenomatous polyposis, CRC and high risk to multiple malignant primary tumors, all of them diagnosed in the adulthood.
Performance of Lynch syndrome predictive models in quantifying the likelihood of germline mutations in patients with abnormal MLH1 immunoexpression

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Lynch syndrome (LS) accounts for up to 4% of all colorectal cancers (CRC). Detection of a pathogenic germline mutation in one of the mismatch repair genes is the definitive criterion for LS diagnosis, but it is time-consuming and expensive. Immunohistochemistry (IHC) is the most sensitive prescreening test and its predictive value is very high for loss of expression of MSH2, MSH6, and (isolated) PMS2, but not for MLH1. We evaluated if LS predictive models have a role to improve the molecular testing algorithm in this specific setting by studying 38 individuals referred for molecular testing and who were subsequently shown to have loss of MLH1 immunoexpression in their tumors. For each proband we calculated a risk score, which represents the probability that the patient with CRC carries a pathogenic MLH1 germline mutation, using the PREMM1,2,6 and MMRpro predictive models. Of the 38 individuals, 18.4% had a pathogenic MLH1 germline mutation. MMRpro performed better for the purpose of this study, presenting an AUC of 0.83 (95% CI 0.67-0.9; P<0.001) compared with a AUC of 0.68 (95% CI 0.51-0.82, P=0.09) for PREMM1,2,6. Considering a threshold of 5%, MMRpro would eliminate unnecessary germline mutation analysis in a significant proportion of cases while keeping very high sensitivity. We conclude that MMRpro is useful to correctly predict who should be screened for a germline MLH1 gene mutation and propose an algorithm to improve the cost-effectiveness of LS diagnosis.
Role of miR-21 on apoptosis in SW480 colorectal cancer cells

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MicroRNA (miRNA) molecules represent endogenously expressed short noncoding RNAs that control gene expression at the posttranscriptional level by targeting mRNAs. The aberrant expression of certain miRNAs is implicated in tumor growth and carcinogenesis, as well as in response to chemotherapy, in different malignancies. miR-21 plays a significant role in tumorigenesis and it has been shown to be upregulated in various malignancies, including colorectal cancer (CRC). Moreover, miR-21 stimulates invasion and metastasis in CRC, but there is a limited amount of data how miR-21 affects the process of apoptosis in CRC. The aim of our study was to investigate the role of miR-21 in apoptosis of SW480 colorectal cancer cells as a model of invasive CRC. Cells were transiently transfected with lentivectors for miR-21 overexpression and inhibition. For apoptosis detection, transfected SW480 cells were stained with Annexin V-FITC and propidium iodide (PI) followed by flow cytometry analysis. At the same time, apoptosis was measured based on real-time PCR analysis of relative levels of Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) genes expression, with GAPDH used for normalization. Flow cytometry results showed that the difference between miR-21 overexpression and miR-21 inhibition treatments was 6% for early apoptosis and 2% for late apoptosis/necrosis. Results obtained by real-time PCR showed that Bax/Bcl-2 mRNA ratio was 15% lower for both miR-21 overexpression and inhibition in comparison with control cells. Surprisingly, the antiapoptotic signal was dominant among the cells for both miR-21 overexpression and inhibition treatments. Based on our preliminary results we can presume that miR-21 modulate apoptosis in SW480 colorectal cancer cells, through the regulation of other molecular pathways besides intrinsic apoptotic pathway. Further studies are needed to fully elucidate the role of miR-21 in apoptotic cell death. The future studies should also investigate if the effect of miR-21 can be modified by single or combination of different chemotherapeutic agents currently used for treatment of patients with CRC.
Integrated analysis of germline and tumor DNA identifies new candidate genes involved in familial colorectal cancer

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Colorectal cancer (CRC) is one of the most common and lethal neoplasms worldwide. Genetic factors account for 35% of its susceptibility. Hereditary forms are mainly due to highly penetrant variants in genes such as APC, MUTYH and the DNA mismatch repair family. Familial CRC shows also familial aggregation but no alterations in the hereditary CRC genes, remaining part of its heritability still unknown. Our study presents a new methodology to identify novel variants in genes linked to familial CRC germline predisposition. An integrated germline-tumor analysis based on Knudson’s two-hit hypothesis, which allows the assessment of the putative role as tumor suppressor genes (TSGs) of the candidates. Integration of germline and tumor whole exome sequencing data was performed in five unrelated families with strong CRC aggregation, compatible with an autosomal dominant pattern of inheritance and without alterations in the known hereditary CRC genes. Deleterious single nucleotide (SNVs) and copy number variants (CNVs) were considered as potential first germline or second somatic hits. A multi-step filtering pipeline programmed in R language was used in the case of SNVs and four different algorithms were implemented in order to infer CNVs. Sequencing quality, population frequency, family segregation, affected protein function and expression, pathogenicity and copy number status were taken into account as main variant features. Somatic mutational signatures characterization was also performed. Fourteen germline-somatic variant pairs were prioritized. Four corresponded to pairs of SNVs and ten were composed by a germline SNV and a somatic CNV. Among them, ADCY8 and DLEC1 were finally selected as the most promising candidates for TSGs related to CRC predisposition since they were previously involved in cancer. A hypermutator phenotype was suggested in one of the analyzed tumors, according to the large number of variants detected in the somatic mutational profile analysis. A putative TSG role in familial CRC of ADCY8 and DLEC1 was revealed by our analysis. Integrative Genomics Viewer and Sanger sequencing validations were successful for SNVs, whereas CNV verification and further functional studies will be required in order to confirm both candidate genes.
Association between cell-free DNA levels and histopathological characteristics of primary colorectal tumors

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Predictive and prognostic molecular markers are of great importance for patients with colorectal cancer (CRC) and can serve as precious tools in disease diagnosis and prognosis, as well as choice of treatment and follow up of response to the administered therapy. Cell-free DNA (cfDNA) in plasma or serum of CRC patients has been recently proposed as biomarker for disease monitoring, but its utility remains unclear. It has been extensively analyzed in metastatic disease, while the data for primary tumors are scarce. The aim of this study was to analyze the correlation between cfDNA levels in serum and histopathological characteristics of primary CRC. The study has included 53 individuals (mean age 60.6 years, 34% males) who underwent surgical removal of primary CRC. Standard histopathological analysis of tumor tissues was performed. Serum cfDNA levels were measured in triplicate on Real-Time PCR instrument using PicoGreen dsDNA quantitation reagent. The association of several histopathological characteristics with cfDNA levels was analyzed: tumor localization, primary tumor stage, regional lymph nodes, differentiation grade, production of mucus, prognostic category and Dukes classification. Differences between groups for categorical data were tested by χ2 analysis, while Independent Samples Mann Whitney U test and Kruskal Wallis test were used for continuous data. The values of cfDNA concentration measured in serum samples ranged from 10 to 60 ng/μL. Higher tumor differentiation grades correlated significantly with lower serum DNA levels (p=0.049). No association between other histopathological characteristics and the quantity of cfDNA in serum samples was observed. In this pilot study, lower levels of cfDNA were observed in patients with more differentiated primary tumors. We speculate that increase in cfDNA levels may indicate anaplastic changes in the tissue, and hence serve as a biomarker of carcinogenesis. However, the study was performed on a small group of subjects with cfDNA levels within a relatively narrow range. Use of cfDNA levels as a biomarker of anaplastic changes in patients with primary CRC should be investigated in a larger cohort of patients.
Validation of miR-1228 as an endogenous control for miRNA expression analysis in colorectal cancer plasma samples

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Introduction and objectives: Colorectal cancer (CRC) is the third most common cancer and the second leading cancer related death in developed countries. Therefore, there is an urgently need of new effective methods for the early detection of this disease. MicroRNAs (miRNAs or miRs) are small non coding RNAs that have been found deregulated in different pathologies such cancer. Recently, miRNAs have been found in different body fluids as blood making them potential noninvasive biomarkers for cancer detection. However, the main problem of miRNA quantification is the lack of standardized methodology. Part of this problem is due to the absence of a good endogenous miRNA that allow a reliable analysis method. An ideal normalization method should be stable between different groups and not affected by hemolysis. The aim of this study was to analyze different candidate miRNAs as endogenous controls in plasma samples from patients with colorectal neoplasia and healthy individuals.

Patients and methodology: Related with a project focused on the detection of new miRNAs biomarkers in CRC, we evaluated two candidate miRNAs as endogenous controls (miR-16 and miR-1228). Three hundred plasmas from patients of 8 different hospitals were included (100 Healthy, 100 Advanced Adenomas-AA and 100 CRC). MiRNA expression was quantified by RT-qPCR and data analysis was performed by logistic regression adjusted by age and gender. Results: The 2 candidate miRNAs analyzed as endogenous controls (miR-16 and miR-1228) showed good levels of Ct without significant differences between groups (CRC, AA and Healthy). However, miR-16 showed more variability and it was more affected by hemolysis than miR-1228. Conclusion: MiR-1228 is a good endogenous control for miRNAs study in CRC plasma samples.

Keywords: colorectal cancer, biomarker, plasma, microRNA, endogenous control
Investigating the mutation frequencies of POLE variants associated with PPAP

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Background: In light of the high incidence of colorectal cancer (CRC), identification of inherited causes is of great interest for targeted clinical management. In 2013, whole genome sequencing lead to the identification of exonuclease domain mutations in the catalytic subunit of DNA polymerase epsilon (POLE) and delta (POLD1) and thereby the description of a novel CRC syndrome: polymerase proofreading-associated polyposis (PPAP). Since then, the POLE c.1270C>G variant has been identified in 21 independent carriers. Recently, we reported another exonuclease domain mutation in POLE (c.1373A>T), which was identified across multiple generations of a large Norwegian family with high incidence of CRC. Mutations in the exonuclease domain of POLE are hypothesized to lead to a hyper-mutator phenotype and consequently cancer.

Methods: In order to investigate the hyper-mutator phenotype associated with POLE c.1270C>G and c.1373A>T, the supF mutagenesis assay was carried out in human cells. In this assay, the pSP189 reporter plasmid is replicated in HEK293T cells overexpressing the POLE gene (wild-type or mutant). Replicated copies of pSP189 are transformed in the MBM7070 strain of E. coli, which are then plated on indicator plates. Colonies will be either blue or white. A mutation event affecting the supF gene during replication in HEK293T results in a white colonies while functional supF genes will lead to blue colonies. Multiple biological replicates are used to estimate the frequency of white (mutant) colonies relative to the total number of transformants. Results: Preliminary results indicate a tendency towards increased mutation frequency of the supF gene in HEK293T cells overexpressing either of the POLE variants compared to POLE wild-type (regulated expression and overexpression). Additional results will be presented at the conference. Conclusion: The mutation frequency of POLE variants associated with PPAP was investigated in the present study. Future perspectives include direct sequencing of the reporter plasmid rather than transforming bacteria as well as utilizing cell lines endogenously expressing the mutations of interest (e.g. CRISPR/Cas9 genome editing or induced pluripotent stem cells).
Detection of variants in POLD1 in unexplained colorectal polyposis using targeted next generation sequencing

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Background and aims: Polyposis and colorectal cancer (CRC) are associated with heritable genetic variants, which represent 25-30% of colorectal cancer cases. While several high penetrance genes with germline mutations have been associated with high risk for polyposis and CRC, these explain only 5-10% of hereditary cases. The genetics of the remaining CRC heritability is still unexplained. Notably, the discovery of new genetic causes is important to improve CRC prevention and treatment. Here we aimed to identify new variants in unexplained colorectal polyposis patients. Methods: Leukocyte DNA of 370 unexplained polyposis index patients were used for this study. Next-generation sequencing was performed using a custom M13-tailed sequencing panel on the ion Torrent platform. The panel included: POLE, POLD1, APC, MUTYH, CHD4, BUB1 and BUB3. Variants were validated by Sanger sequencing. Results: We identified a heterozygous germline POLD1 c.961G>A, p.(Gly321Ser) variant in three patients from three families. The first patient was diagnosed with colorectal polyposis and CRC at age 35 and 37 respectively, the second patient developed CRC at age 41, unfortunately no DNA was available for co-segregation analysis of both patients. The third patient was diagnosed with CRC at age 61 and had two sisters with endometrial carcinoma. Furthermore, we identified POLD1 c.955T>G, p.(Cys319Gly) variant in a patient diagnosed with colorectal polyposis at age 51. Co-segregation was performed using available DNA from affected family members; an affected sister who developed CRC at age 38 did not carry the identified variant. Conclusions: Our finding suggests that the identified POLD1 variants may be involved in the susceptibility to colorectal polyposis. To evaluate the clinical relevance of the two variants functional studies are required. Identification of novel colorectal polyposis variants may play a role for increasing the understanding of mechanisms underlying colorectal polyposis initiation.
Exome sequencing data analysis to characterize rare germline copy number variants involved in colorectal cancer and serrated polyposis syndrome predisposition

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OBJECTIVES: Colorectal cancer (CRC) represents the third most common cancer worldwide. Next generation sequencing (NGS) has permitted to identify germline predisposition genes for this disease. Copy number variants (CNV) can be the mutational event involved in this predisposition, and they can be inferred from NGS data. METHODOLOGY: We analyzed germline DNA whole-exome sequencing (WES) data from 54 families with strong CRC or serrated polyposis syndrome aggregation without alterations in known hereditary genes. To infer rare candidate CNVs involved in this predisposition we used ExomeDepth and CoNIFER. Variants shared between family members were compared to Database of Genomic Variants catalog and our in-house database. Selected CNVs were validated and segregation analysis was performed using Comparative Genome Hybridization. In some cases, gene expression arrays, qRT-PCR and immunohistochemistry (IHC) were conducted to check for gene and protein expression alterations. RESULTS: Among them, 21 candidate CNVs corresponding to 16 duplications and 5 deletions were detected by calling tools. After multiple filtering steps, a duplication in chromosome 1 in one family stood out as interesting including TTF2, TRIM45, VTCN1 and miR942. Expression studies pointed out TTF2 and miR942 overexpression in carriers, and tumor IHC showed high levels of TTF2 protein and low levels of the TMEM158 protein, a predicted miR942 target. CONCLUSION: WES data can be used as a first approach to identify CNVs. Chromosome 1 duplication may correspond in the carrier family to the mutational event involved in their CRC predisposition.
Germline variants in homologous recombination (HR)-mediated DNA damage repair genes may contribute to increased CCR susceptibility in FCCTX families

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Familial colorectal cancer type X (FCCTX) families are clinically defined by the Amsterdam criteria, absence of germline mutations in mismatch repair (MMR) genes and the presence of microsatellite stable tumors. Previously, we have reported the presence of two distinct molecular entities amongst tumors from 15 FCCTX families: one (n=10) whose tumors presented frequent loss of heterozygosity in tumor suppressor genes (TSG+) and another (n=5) with tumors lacking this molecular feature (TSG-). Amongst TSG+, we found a subgroup (n=7) with a prevalence of APC/KRAS somatic mutations and MGMT/MMR methylation, and a second, where these features were almost absent. Here, we aimed to characterize these distinct subgroups at the germline level by the analysis of a panel of 94 genes associated to increased cancer risk. Next generation sequencing was performed using the TruSight Cancer panel (Illumina, Miseq platform), in the 15 index patients previously studied. Large deletions/duplications were evaluated for all genes associated with hereditary colorectal cancer syndromes by MLPA. In 7/15 families, all TSG+, we found one or more likely pathogenic germline variants in genes encoding proteins involved in double strand breaks (DSB) associated DNA repair pathways, secondary to DNA damage response to genotoxic stress, particularly in homologous recombination (HR)-mediated DNA damage repair. Five of the seven families belong to the subgroup whose tumors presented frequent KRAS somatic mutation and/or MGMT/MMR gene methylation. In two of these families we have also detected a likely pathogenic missense mutation in BMPR1A gene and a deletion of SMAD4 exons 5-8, respectively. The cytotoxic effects of alkylating agents, if not repaired by MGMT and MMR system, will eventually lead to DNA DSB. The latter, together with defects in HR-DNA repair pathways, will result in elevated chromosomal/DNA breakage and genome instability, which are consistent with the mutation signature previously reported by us in the FCCTX TSG+ subgroup. Therefore, germline defects in HR-DNA repair genes, identified in the present study, may contribute to increase colorectal adenoma/carcinoma risk in a subgroup of FCCTX families with TSG+ tumors, carrying frequent KRAS mutations and/or MGMT/MMR gene methylation.
The MSH2 exon 5 deletion (c.792+8_943-450del) is a founder mutation in Portuguese Lynch syndrome families with a Center-South ancestry

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Lynch syndrome (LS) is a hereditary colorectal cancer syndrome caused by germline mutations in the DNA mismatch repair (MMR) genes. Worldwide, large genomic deletions, particularly in MSH2 gene, account for ~17% of the mutational spectrum. A total of 14 unrelated families, with a recurrent exon 5 genomic deletion in MSH2 gene, were identified during genetic testing amongst those followed at the Portuguese Oncology Institute of Lisbon and Hospital de Sta. Maria, in Lisbon. This mutation was not identified in families followed at other Portuguese Oncology Institutes. After the confirmation, by Sanger sequencing, that all the families shared the same deletion breakpoints (c.792+8_943-450del) we aimed to evaluate a possible founder effect of this mutation. A haplotype analysis was performed using 9 microsatellite markers flanking MSH2 and 3 intragenic SNPs, in a total of 55 individuals (14 index patients and 41 relatives). The geographical origin of these families was also evaluated and the age of the mutation estimated. Five different haplotypes were phased for 6 of the 14 families, which share a common haplotype of 3.2Mb. Based on the mutation and recombination events, observed in the microsatellite haplotypes, and assuming 25 years per generation, it was possible to estimate that this mutation occurred 234±78 years ago. Our data suggests that the MSH2 c.792+8_943-450del is a founder mutation in Portugal, which is reinforced by the fact that the great majority of the families shared a common geographical origin in the center region of Portugal. Moreover, the prevalence of this mutation in our LS registry indicates that screening of this mutation, using Multiplex Ligation Probe dependent Amplification (MLPA), should be considered a first and cost-effective approach in the genetic diagnosis of suspected LS families with a Portuguese ancestry, especially in those with a Center-South origin.
Biallelic NTHL1 mutations predispose to a broad variety of tumors with a unique somatic mutational signature

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Biallelic germline mutations affecting the base excision repair gene NTHL1 predispose to the development of adenomatous polyposis and colorectal cancer. However, the clinical characteristics of previously identified NTHL1 mutation carriers (n=10) suggest that NTHL1 defects are also associated with extracolonic malignancies. Characterization of the somatic mutation spectrum in multiple colorectal carcinomas and a bladder cancer derived from individuals with biallelic germline NTHL1 mutations revealed a bias towards C:G>T:A (C>T) transitions. Yet, a unique mutational signature, i.e. the combined set of mutation types generated by a single biological process, associated with NTHL1 deficiency has not been described. Here, we explored the somatic mutation profile in five different malignancies of individuals with biallelic NTHL1 mutations and identified a distinct mutational signature. We identified three novel families in which biallelic NTHL1 mutation carriers were diagnosed with multiple tumor types. We selected six malignancies from four different tissues (colon (n=3), thyroid-gland, urothelium, and tonsil) in order to determine the somatic mutation profile. A bias towards C>T mutations was confirmed in all tissue types. Furthermore, in all four tissue types a bias was found towards C>T mutations at non-CpG sites, which is clearly distinct from sporadic cancers that show a bias towards C>T mutations at CpG sites caused by deamination of methylated cytosines (signature 1). Interestingly, a distinct somatic mutational signature was found in all six malignancies. These results demonstrate that NTHL1 deficiency is associated with a unique signature characterized by C>T mutations at non-CpG sites, and confirms the broad tumor spectrum found in biallelic NTHL1 mutation carriers. This finding provides an interesting strategy to establish whether a specific tumor is caused by a biallelic germline NTHL1 mutation.
POLE somatic mutations in advanced colorectal cancer

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Despite all the knowledge already gathered, the picture of somatic genetic changes in colorectal tumorigenesis is far from complete. Recently, germline and somatic mutations in the exonuclease domain of polymerase, epsilon, catalytic subunit (POLE) gene have been reported in a small subset of microsatellite stable and hypermutated colorectal carcinomas (CRC), affecting the proofreading activity of the enzyme and leading to misincorporation of bases during DNA replication. To evaluate the role of POLE mutations in colorectal carcinogenesis, namely in advanced CRC, we searched for somatic mutations by Sanger sequencing in tumor DNA samples from 307 cases. Microsatellite instability and mutation analyses of a panel of oncogenes were performed in the tumors harboring POLE mutations. Three heterozygous mutations were found in two tumors, the c.857C>G, p.Pro286Arg, the c.901G>A, p.Asp301Asn, and the c.1376C>T, p.Ser459Phe. Of the POLE mutated CRC, one tumor was microsatellite stable and the other had low microsatellite instability, whereas KRAS and PIK3CA mutations were found in one tumor each. We conclude that POLE somatic mutations exist but are rare in advanced CRC, with further larger studies being necessary to evaluate its biological and clinical implications.
Non-synonymous functional variants in DNA repair genes in sporadic colorectal cancer: Searching for predictive and prognostic markers

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Colorectal cancer (CRC) has one of the highest mortality due to the late diagnosis. More than 50% of patients are diagnosed in the higher stage of disease (III and IV) and the only improvement of the prognosis for these advanced stages can be achieved by surgery and/or an appropriate adjuvant therapy. Unfortunately, a significant percentage of patients (40-50%) do not benefit from the systemic therapy. Thus there is an urgent need for proper predictive and prognostic biomarkers that would be instrumental in the choice of optimal type, combination and dose of drugs for an individual patient. According to the current knowledge, DNA repair processes are involved in the treatment efficacy. In the present study, we analyzed the link between functional genetic polymorphisms (SNPs) in DNA repair genes covering the main DNA repair pathways relevant for therapy response in relation with clinical outcomes. Our set of candidate polymorphisms was selected according to several functional and genomic databases providing integrated information about the effects of SNPs which are predicted and indicated as functionally relevant. We have focused on those affecting protein coding and splicing regulation and we hypothesize that these modified proteins may modulate the function/efficiency of DNA repair, and thus may have an effect on CRC. Twenty-nine polymorphisms in 22 DNA repair genes were analyzed in DNA samples of 1172 cases and 1831 controls from the Czech Republic. Clinical data at diagnosis and complete information on follow up were provided for all patients. Genetic variations in several DNA repair genes were associated with clinical outcome. Interestingly the results also differ according to tumor localization. For example, in association with SNP rs3816032 rectal cancer patients displayed a worse survival and increased recurrence risk when receiving only 5-fluorouracil treatment while colon cancer patients showed a better survival with oxaliplatin treatment. Understanding the SNPs effect on the treatment response is regarded as important for the possibility to tailor patient specific treatment strategy. Individualized therapy will eventually help to improve therapeutic efficacy and to reduce toxicities.

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Impact of translation research in multidisciplinary approach on treatment strategies for colorectal liver metastasis: our experience.

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Colorectal Liver metastases (CLM) are a common event, 15% to 25% of patients have synchronous liver metastasis. A total of 30 to 50% of patients develop, either synchronous or metachronous, liver metastases. Liver metastasis are the main cause of death in CRC patients. Cure of such patients is possible when multimodal treatment strategies that evolved systemic therapy, ablative techniques and surgical treatment were performed. Improvements in patient’s selection, imaging staging, surgical techniques, and the integration of a more precision oncology, have improved the resectability rate. Intratumor heterogeneity (genetic, molecular and metabolic) determine treatment response. Thus, this information should be present in the dedicated multidisciplinary team (MDT) when deciding the best treatment. The aim of this study was to assess the impact of dedicated MDT on the overall survival of liver metastasis. A cohort of 216 consecutive patients from Portuguese Institute of Oncology in Porto, Portugal was divided in three different groups. First group treated by a general surgical team after digestive tract MDT decision strategy (between 1997 to 2002), second group treated by liver surgeons after digestive tract MDT decision strategy (2003 to 2009) and the last group treated by the same liver surgeons after a dedicated hepatobiliary and pancreatic MDT decision treatment and strategy (2010 to 2011). Comparison of survival between groups was performed.
GALNT12 inactivating D303N variant is not associated to polyposic colorectal cancer susceptibility

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Aberrant glycosylation is a pathological alteration widespread in cancer, which can affect cell growth, differentiation, transformation, adhesion, metastasis and tumor immune surveillance. A large family of polypeptide N-acetylgalactosaminyltransferases (GALNTs) catalyzes the first step in the mucin type O-glycosylation pathway by the transfer of monosaccharide N-acetylgalactosamine (GalNAc) to serine/threonine residues. GALNT12 is highly expressed in the digestive tract and a previous study has identified somatic and germline inactivating mutations in subjects with MMR proficient colorectal cancer (Guda, Proc Natl Acad Sci U S A. 2009). Trying to characterize a cohort of unexplained attenuated adenomatous polyposis we have detected the variant c.907G>A, p.Asp303Asn in GALNT12 (GALNT12_D303N) in the germline DNA of 4 out of 164 probands. This variant was previously detected; It is located in the catalytic domain of the protein encoded by this gene and it shows a partial activity of 37% when compared to the wild type protein (Guda, Proc Natl Acad Sci U S A. 2009). Moreover, it has been recently detected in three different Bethesda-positive families showing co-segregation with CRC and polyps (Clarke,Hum Mutat. 2012). In order to clarify the possible role of this variant in the susceptibility to polyposis colorectal cancer, we have analyzed the co-segregation of GALNT12_D303N with CRC/polyps in carrier families and we have conducted a case-control studies in a large cohort of attenuated polyposis and a set of cancer-free home controls. Segregation study’s results rule out the role of GALNT12_D303N as a high/moderate penetrance allele and case-control studies neither support the role of the variant as a risk allele. These results suggest that GALNT12 mutations, showing at least an activity of 37% of the wild type, display normal phenotypes and they are not associated with an increased risk of polyposic colorectal cancer when they are in heterozygosis.
Whole-exome sequencing approach for the identification of high-risk genetic variants in FCC-X families

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Almost half of the families with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) do not present the mismatch repair (MMR) defects that cause Lynch Syndrome, and have been therefore encompassed by the term Familiar Colorectal Cancer Type X (FCC-X). Identifying the genetic basis underlying FCC-X has been a challenge that many research groups have faced over the last years. Thanks to the arrival of Next Generation Sequencing (NGS), this goal has become more achievable. However, this type of studies have only succeeded in identifying a clear pathogenic mutation in a small fraction of these families, leaving the remaining – for now – with merely a list of candidate genes. Nevertheless, we believe it is still important to share this kind of information with the rest of the community, so as to keep expanding our knowledge of this heterogeneous group of families. In order to find new high-penetrance cancer-predisposing genes in this subgroup of hereditary CRC, whole-exome sequencing was performed in a total of 32 members from 13 FCC-X families. After thorough filtering, a number of candidate variants affecting interesting genes were selected for each family. Even though different tests to evaluate the effects of some of these mutations are still ongoing, here we present the most relevant results obtained so far in this tough search that aims to further understand the cancer hereditability of FCC-X.
Evaluation of a 25-gene panel in patients with suspected Lynch syndrome: FAMOSA study


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Introduction: The role of multigene panels for hereditary cancer risk assessment is yet to be established. We aimed at describing the prevalence of cancer predisposition gene mutations identified by a multigene panel in individuals with suspected Lynch syndrome (LS). Patients and methods: We performed germline analysis with a next-generation sequencing 25-gene-panel (Myriad myRisk™ Hereditary Cancer) using DNA from 95 patients with suspected LS (endometrial cancer <50 y-o and/or fulfillment of revised Bethesda criteria) from Nov-2014 through March-2015 within the FAMOSA study. We classified all identified germline variants for pathogenicity and calculated the prevalence of pathogenic mutations and variants of uncertain clinical significance (VUS). We analyzed data on patients’ personal and family history of cancer. Results: We included 95 patients [female: 46 (48.5%), mean age: 48.6 ± 12]; 8(8.5%) with endometrial cancer and 87(91.5%) with colorectal cancer. Multigene panel testing identified 20(21%) patients with LS syndrome mutations (8MLH1, 7MSH2, 4MSH6, 1PMS2) and 1(1%) with a mutation in BRCA2 in a 35 y-o woman without personal/familial history of breast/ovarian cancer. In patients diagnosed with mutations in the MMR genes and prior molecular screening (n=9), two displayed MMR proficiency and 5 patients had a negative prior genetic result by conventional techniques. Conclusions: In individuals with suspected Lynch syndrome, multigene panel testing identified unexpected high-penetration mutations in 1% of cases. Parallel sequencing also detected a meaningful number of cases with previous false negative results.
Elucidating the molecular basis of MSH2-deficient tumors by combined germline and somatic analysis


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In a proportion of patients presenting mismatch repair (MMR)-deficient tumors, no germline pathogenic mutations are identified in MMR genes, the so-called Lynch-like syndrome (LLS). Recently, germline mutations in POLE and MUTYH and double somatic events in MMR genes have been found in some of these patients. The aim of this study was to elucidate the molecular basis of MSH2-deficient LS-suspected cases using a comprehensive analysis of colorectal cancer (CRC)-associated genes at germline and somatic level. Fifty-eight probands harboring MSH2-deficient tumors were included. Germline mutational analysis of MSH2 (including EPCAM deletions) and MSH6 was performed. Pathogenicity of MSH2 variants was assessed by RNA analysis and multifactorial likelihood calculations. MSH2 gene cDNA and methylation of MSH2 and MSH6 promoters were studied. Matched blood and tumor DNA were analyzed using a customized next generation sequencing panel. Thirty-five individuals were carriers of pathogenic or probably pathogenic variants in MSH2 and EPCAM, and 5 were carriers of MSH2 variants of unknown significance (VUS). Two variants at the MSH6 promoter were identified. Pathogenicity assessment allowed the reclassification of 4 VUS and 6 probably pathogenic variants as pathogenic mutations. Pathogenic germline heterozygous mutations in BUB1, SETD2, FAN1 and MUTYH were identified in 5 cases. In addition, double somatic hits in MSH2 or MSH6 and somatic alterations in other MMR genes and/or proof-reading polymerases were detected. In conclusion, our comprehensive strategy combining germline and somatic mutational status of CRC-associated genes by means of a subexome panel allows the elucidation of up to 86% of MSH2-deficient suspected LS tumors.
The MSH2 nonsense mutation c.2152C>T shows a founder effect in Portuguese Lynch syndrome families

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The mutational spectrum of the MMR genes is highly heterogeneous, however specific mutations are observed at high frequencies in well-defined populations or ethnic groups, due to the occurrence of recurrent and/or founder effects. The identification of founder mutations can greatly facilitate the molecular diagnosis of Lynch syndrome by allowing targeted mutational gene analysis as the first step of the genetic testing strategy. Three founder mutations have already been described in Portuguese Lynch syndrome families, namely the MLH1 c.1896+280_OLRRFIP2:c.1750-678del, the MSH2 c.388_389del, and the MSH6 c.1030C>T mutations. The MSH2 c.2152C>T mutation has occasionally been described in Lynch families worldwide, including Portuguese Lynch syndrome families. During genetic testing for Lynch syndrome at the Portuguese Oncology Institute of Porto and Lisbon, this mutation was identified in 27 seemingly unrelated families. In order to evaluate if this alteration is a founder mutation, haplotype analysis using microsatellite markers flanking the MSH2 gene was performed in the 27 probands and 87 family members. Additionally, the geographic origin of these families was evaluated and the age of the mutation estimated. Twelve different haplotypes were phased for 13 out of the 27 families and shared a conserved region of ~3.6 Mb. Based on the mutation and recombination events observed in the microsatellite haplotypes and assuming a generation time of 25 years, the age estimate for the MSH2 mutation was 273±64 years. The geographic origins of these families were mostly from north and center of Portugal. Concluding, these results suggest that the MSH2 c.2152C>T alteration is a founder mutation in Portugal with a relatively recent origin. Furthermore, the high proportion of this mutation indicates that screening for this alteration, together with the previously reported Portuguese founder mutations as a first step, may be cost-effective in the genetic testing of Lynch syndrome suspects of Portuguese ancestry.
Contribution of MLH1 constitutional methylation for Lynch syndrome diagnosis in patients with tumor MLH1 downregulation

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Constitutional epimutation of the two major mismatch repair genes, MLH1 and MSH2, has been identified as an alternative mechanism that predisposes to the development of Lynch syndrome. In the present work, we aimed to investigate the prevalence of MLH1 constitutional methylation in colorectal cancer (CRC) patients with abnormal expression of the MLH1 protein in their tumors. In a series of 38 patients who met clinical criteria for Lynch syndrome genetic testing, with loss of MLH1 expression in the tumor and with no germline mutations in the MLH1 gene (35/38) or with tumors presenting the BRAF p.Val600Glu mutation (3/38), we screened for constitutional methylation of the MLH1 gene promoter using methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) in different biological samples. We found four (4/38; 10.5%) patients with constitutional methylation in the MLH1 gene promoter. RNA studies demonstrated decreased MLH1 expression in the cases with constitutional methylation when compared with controls. In two cases we could demonstrate the mosaic nature of MLH1 constitutional hypermethylation in tissues originated from different embryonic germ layers, and in one family we could show that it occurred de novo. We conclude that constitutional MLH1 methylation occurs in a significant proportion of patients who have loss of MLH1 protein expression in their tumors and no MLH1 pathogenic germline mutation. Furthermore, we provide evidence that MLH1 constitutional hypermethylation is the molecular mechanism behind about 3% of Lynch syndrome families diagnosed in our institution, especially in patients with early onset or multiple primary tumors without significant family history.
Novel BMPR1A mutation in Familial Colorectal Cancer Type X Family Identified by Diagnostic Next Generation Sequencing Strategy

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Next generation sequencing as a diagnostic tool can uncover novel predisposing genetic mutations associated with hereditary cancer syndromes for which the genetic etiology is unknown, such as familial colorectal cancer type X (FCCX). Recently, our group developed and validated a custom diagnostic NGS panel for hereditary cancers, comprised of 122 genes associated with cancer predisposition syndromes and RASopathies. Utilizing this NGS panel, we identified a previously unreported germline variant in BMPR1A (bone morphogenetic protein receptor type 1A) in a family fulfilling the clinical criteria of FCCX: BMPR1A c.233A>G (p.Thr75Ala). Although BMPR1A mutations are classically associated with juvenile polyposis syndrome and hereditary mixed polyposis syndrome, there are only two published reports of co-segregating BMPR1A variants in families with hereditary nonpolyposis CRC syndromes. The proband in the reported family was diagnosed with a microsatellite-stable, pTisN1 rectal adenocarcinoma at 46 y.o. Thirteen CRC-affected individuals were identified within the family, with age of diagnosis ranging from 22 y.o. to 79 y.o. (mean age = 48 y.o.). Review of family history and available pathology records showed no history of juvenile polyposis within the family. Co-segregation analysis revealed the presence of BMPR1A c.233A>G in the proband’s affected mother (diagnosed at 73 y.o.) and an affected second cousin (diagnosed at 44 y.o.), supporting the clinical significance of this variant. The reported variant changes an evolutionarily conserved amino acid within the extracellular Activin types I and II receptor domain. In silico pathogenicity analysis using SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, FATHMM all predicted deleterious effects on protein function. In silico structural analysis of the variant protein showed a destabilizing effect on the 3D structure of BMPR1A in five out of six predictors. Our group is currently evaluating the functional effects of the BMPR1A variants on bone morphogenetic protein signaling in vitro. Overall, this report illustrates how targeted NGS-based diagnostic strategies can be useful to refine clinical phenotypes associated with mutations in specific predisposing hereditary cancer genes.
Optimization of RAS mutational status screening in mCRC patients

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Colorectal cancer (CRC) is one of the most frequent and lethal cancer in developed countries being the third most commonly diagnosed cancer. The transmembrane protein epidermal growth factor receptor (EGFR) is over expressed in colon cancer, activating two pathways (RAF-MEK-ERK and PI3K/AKT) with important roles in cell division, apoptosis and growth. Given this, molecular targeting therapy to inhibit EGFR in metatastic colon rectal cancer (mCRC) is the most effective way to inhibit these pathways. Cetuximab and Panitumumab are antibodies that block EGFR activity by competing with its ligand. However, anti-EGFR therapy is not effective in about 50% of all mCRC due to the presence of KRAS and NRAS mutations, which are localized in hot-spots codons 12, 13, 59, 61, 117 and 146, more frequently in codons 12 and 13. Thus, according to mCRC treatment guidelines, RAS mutational status should be performed before any therapeudic decision. Before 2016, RAS mutational status was ascertained at IPO Porto by HRM (High Resolution Melting) and Sanger sequencing, but this year we implemented the IdyllaTM KRAS Mutation Test and IdyllaTM NRAS-BRAF-EGFR S492R Mutation Assay (RUO)(Biocartis). The aim of this work was to compare the results of the two methodologies. Between January and November 2016, 339 patients with mCRC were evaluated for RAS mutational status, being 132 studied by HRM and 207 by the IdyllaTM Assay (Biocartis). The use of recent technology for RAS screening allowed a significant decrease in median response time from 12 days to 5 days and an increase of the positive samples from 49,2% to 55,1%. We here demonstrate that IdyllaTM KRAS Mutation Test and IdyllaTM NRAS-BRAF-EGFR S492R Mutation Assay (RUO)(Biocartis) allow improvement of the response time and the sensitivity of RAS testing in mCRC.
Identification of a new POLE germline variant (c.1274A>G, p.K425R) associated with polyposis phenotype


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Germline mutations in the exonuclease domains of proofreading polymerases POLE and POLD1 have been recently associated with colonic polyposis and/or colorectal cancer predisposition, defining the autosomal dominant inheritance Polymerase Proofreading-Associated Polyposis (PPAP; OMIN # 615083). According to the literature the most frequent mutation is POLE p.L424V. Our study includes samples from a 600 polyposis patients cohort, previously tested negative for MUTYH and APC mutations. Phenotypic and familial aggregation data including polyp number, polyp histology, age of onset and colorectal cancer co-occurrence were collected. POLE and POLD1 proofreading domains mutations were screened using high resolution melting analysis and positive samples were confirmed using Sanger sequencing. POLE p.L424V mutation was not present on our cohort, neither other previously described mutations. However, the POLE c.1274A>G p.K425R variant was detected on two samples from non-family related patients. The carriers were diagnosed with attenuated adenomatous polyposis, belonging to families with aggregation of polyps. p.K425R shares POLE exonuclease domain localization and various bioinformatic predictor scores with p.L424V. Ongoing variant-phenotype cosegregation studies in these two families should help us to clarify the role of p.K425R in Polymerase Proofreading-Associated Polyposis.
Germline variants in DNA interstrand-cross link repair genes may contribute to increased susceptibility for serrated polyposis

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Serrated polyposis (SP) is characterized by development of multiple colorectal serrated polyps and increased predisposition to colorectal cancer (CRC). However, the molecular basis of SP, especially in cases with family history of polyps/CRC in first degree relatives (SP-FHP/CRC), is still unknown. We have reported that SP-FHP/CRC patients present clinical and histological differences when compared to apparently sporadic SP patients. We also identified two molecular entities amongst these families, proximal and distal SP-FHP/CRC, according to the preferential location of lesions and somatic events involved in tumor initiation: MGMT and mismatch repair (MMR) gene defects and Wnt gene mutations, in the former; RAS/RAF gene mutations in the latter. Our results suggested the involvement of distinct tumorigenic pathways in these two forms of SP-FHP/CRC and that early MGMT and MMR gene deficiency may be associated to inherited susceptibility to genotoxic stress in the proximal form. We aimed to characterize these distinct SP-FHP/CRC subgroups at the germline level by analyzing a panel of 94 genes associated to increased cancer risk (Trusight Cancer panel) by NGS, in 10 SP-FHP/CRC patients (6 with proximal and 4 with distal SP-FHP/CRC) and in 3 sporadic SP patients. Likely pathogenic germline variants in genes coding for proteins involved in the Fanconi Anemia (FA) pathway, that act downstream of FA complex to facilitate DNA Interstrand-Cross Link repair (ICLR), were detected in 4/10 SP-FHP/CRC patients. These variants were found only in the proximal group (4/6). We found mutations in genes coding for DNA nucleotide excision repair (NER) proteins in 2/3 apparently sporadic SP patients. DNA damage caused by alkylating agents, if not repaired by MGMT and MMR, may lead to DNA double-strand breaks. The latter, together with DNA-ICLR pathway defects, will result in elevated chromosomal/DNA breakage and genomic instability, consistent with the mutation signature previously reported by us in the proximal SP-FHP/CRC group. Therefore, germline defects in DNA-ICLR genes, identified in this study, may contribute to increase serrated colorectal polyps/carcinoma risk in a SP-FHP/CRC subgroup. Moreover, defects in NER genes may account for a subgroup of apparently sporadic SP patients.
Common genetic variation near CDKN1A is associated with colorectal cancer susceptibility in male PMS2 mutation carriers


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Background: Lynch syndrome (LS) patients are at high risk of developing colorectal (CRC) and show high phenotypic variability. This variability might in part be explained by common susceptibility loci identified in Genome Wide Association Studies (GWAS). Previous studies focused almost exclusively on MLH1, MSH2 and MSH6 carriers, but produced conflicting results. Here we investigate the role of GWAS SNPs in PMS2 carriers. Methods: A cohort of 521 PMS2 carriers, including 125 CRC cases, was assembled from Dutch family cancer clinics. The cohort was genotyped for 26 candidate GWAS SNPs; rs6687758, rs6691170, rs10936599, rs1321311, rs16892766, rs6983267, rs10795668, rs3802842, rs3824999, rs4444235, rs9929218, rs4939827, rs12953717, rs10411210, rs961253, rs4925386, rs1569686, rs2736100, rs1800734, rs1799945, rs1048943, rs4934683, rs1800562, rs7136702, rs4779584. Hazard ratios (HRs) were calculated using a weighted cox regression analysis to correct for ascertainment bias. Results: We found no evidence of an association between CRC risk and cumulative number of risk alleles (HR=1.05, 95%CI: 0.97-1.10). Male PMS2 carriers of the rs1321311 CA/CC genotype were at an increased risk of CRC (HR=2.81 (95%CI: 1.21-3.4, p=0.008). Moreover, the combination of rs1321311 with rs7136702 led to an increased HR for each additional risk allele of 1.58 (95% CI: 1.18-1.91, p=0.0010), a finding that also holds after correction for multiple testing (p<0.0015). Conclusions: Interestingly, two SNPs (rs3802842 and rs16892766) previously found to increase risk in MLH1 carriers do not appear to modify risk in this cohort. This, together with established lower penetrance and phenotypic variability, raises the question of whether PMS2-associated LS should be considered a separate disease entity.
Implication of germline mutations in POLE and POLD1 in several forms of hereditary cancer

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Germline mutations in the exonuclease domains of POLE and POLD1 predispose to adenomatous polyps, colorectal cancer and endometrial tumors. Recent findings suggest that the phenotypic spectrum associated to POLE and POLD1 mutations is not limited to the above-mentioned malignancies. To provide a definitive answer to this observation, we sequenced the exonuclease domains of both genes in 551 unrelated cancer-affected patients without mutations in known cancer-predisposing genes, studied according to the phenotypic characteristics of the family, including: 192 patients from high-risk breast/ovarian cancer families; 143 patients with personal and/or familial cancer history of breast/ovarian cancer and colorectal/endometrial/small intestine/gastric cancer; 123 patients fulfilling clinical criteria of Li-Fraumeni but without mutations in TP53; 34 patients with familial or personal history of multiple tumors; 34 patients with personal and/or familial cancer history of polyposis in combination with other tumor types (brain, breast, endometrium or skin); and 25 patients with personal and/or familial cancer history of brain cancer in combination with other tumors. Mutation screening was carried out by direct automated sequencing. Variants with a population MAF<1% and located within the exons or in the 10-nucleotide flanking regions were selected for further analyses. Synonymous and missense variants predicted to affect splicing were subjected to RNA study. POLE missense variants were functionally tested in Schizosaccharomyces pombe by analyzing ade6-485 allele reversion rates in mutant compared to wildtype strains. To assess the presence of the ultramutated phenotype associated to pathogenic POLE and POLD1 mutations, whole-exome sequencing in tumor DNA is being performed for all frameshift (n=2) and missense mutations (n=5), and for the synonymous variants with altered splicing or without available RNA (3). A germline POLD1 missense variant located outside the exonuclease domain and identified in an 8 year-old child with multiple brain tumors is also being functionally tested. This study will help refine the tumor spectrum associated to the syndrome, and will provide insight to the relevance of disrupting mutations and mutations located outside the exonuclease domain.
KIT and PDGFRA molecular characterization in Portuguese patients with GIST

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Gastrointestinal stromal tumors (GIST) represent the most common mesenchymal tumors of the gastrointestinal tract. The GIST diagnosis involves a multidisciplinary approach that combines clinical, pathological, and genetic features. Mutually exclusive activating mutations in KIT or PDGFRA genes occur in 85 to 90% of the cases and are considered primary events in GIST pathogenesis. Surgery remains the principal treatment for resectable primary cases. Recurrence following surgery occurs in 40 to 80% of cases and 5-year survival is 50% to 65%. Imatinib mesylate is a selective tyrosine kinase inhibitor of BCR-ABL, KIT and PDGFRA/B genes that competitively binds to the ATP binding pocket of the kinase domain, prevents substrate phosphorylation and inhibits downstream signaling, being an effective treatment for GIST. In this study, a consecutive series of 230 patients diagnosed with GIST at IPO-Porto was evaluated for KIT and PDGFRA mutations. Tumor DNA samples were screened, using Sanger sequencing, for mutations in exons 9, 11, 13, and 17 of KIT and, if negative, for mutations in exons 12, 14, and 18 of PDGFRA. The overall mutation frequency was 80.4%. KIT mutations were detected in 67% of cases, with 87.7% (n=135), 9.7% (n=15), 1.9% (n=3) and 0.6% (n=1) occurring in exons 11, 9, 13 and 17, respectively. No primary mutations were found in exons 13 or 17. Of all the KIT negative cases, PDGFRA mutations were present in 13.5% (n=31), occurring 64.5% (n=20), 29% (n=9), and 6.5% (n=2) in exons 18, 12 and 14, respectively. GIST molecular characterization is an important tool to assess a differential diagnosis, to predict the likelihood of imatinib mesylate target therapy response or resistance and dosage adjustment, and to the development of new therapeutic approaches for patients failing current therapy.
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venue
The meeting venue will be at the Portuguese Oncology Institute of Porto (IPO-Porto), located in Rua Dr. António Bernardino de Almeida, 4200-072 Porto (Google Maps: https://goo.gl/maps/86EnQ). IPO-Porto is the largest specialized Portuguese cancer institution with a triple role: patient care, research and teaching in Oncology (www.ipoporto.pt/en/). It is a member of the Organization of European Cancer Institutes (OECI) and it treats about 10.000 new patients per year. The meeting venue in IPO-Porto will take place at the facilities of EPOP (Escola Portuguesa de Oncologia do Porto), namely at the main auditorium and adjacent meeting rooms.

**Arriving at the meeting venue**

The hotels Eurostars Oporto and Ibis Porto S. João are within walking distance to the meeting venue (see map next page). Just find the IPO-Porto entrance just opposite the Metro IPO station and walk straight ahead inside IPO-Porto limits in direction of the building with a banner indicating EPOP - Escola Portuguesa de Oncologia do Porto. The other recommended hotels in the city center are nearby the Aliados Metro station (see map on page 7), which is 11 min. away from the meeting venue. Take the Metro line D (yellow) at Aliados station towards Hospital São João until the IPO station, which is just opposite the IPO-Porto entrance that allows direct access to the meeting venue (see above).
Entrance to the meeting venue (go straight ahead, stairs to the right, lift to the left, venue is one floor up in the 3rd floor

Local transport

The rechargeable ticket Andante can be bought at the automatic ticket machines at any Metro station and can be used both in the Metro and buses (STCP). To travel by Metro between the city center and the meeting venue (Metro every 6 min.) you need a Z2 ticket (1.20€ + 0.60€ the first time you buy the Andante rechargeable ticket). Validate your ticket before entering the Metro and inside the bus and every time you change lines. For further maps and schedules see the links below:


**Meeting rooms**

The major sessions will take place in the main auditorium and smaller meeting rooms are available if necessary.

**Lunch and coffee-breaks**

Lunch and coffee-breaks will be served on site in the facilities adjacent to the meeting venue.

**Internet access**

Wireless internet access is available at the meeting venue at no cost. If you have an eduroam connection at your home institution, you will be able to access the internet using the same login at the meeting venue by using the eduroam network access. If not, you will be able to access the internet via the WiFi Porto Digital network, which is also available free of charge in several hotspots in the city and in local buses.
notes
Sponsorship