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Prospects of 'Omics Based Molecular Approaches in Colorectal Cancer Diagnosis and Treatment in the Developing World: A Case Study in Cape Town, South Africa

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1. Introduction

The emergence of the field of genomics, proteomics and more recently lipidomics in science has advanced diagnostic and therapeutic medicine in no small measure. These fields typically deal with the documentation of the identity, abundance and localization of DNA, RNA, protein and lipid biomolecules in a given cell, tissue or organism. An in-depth knowledge of the biologic and physiologic localization, chemistry, and methodology for isolation of these essential biomolecules is key to a successful analysis and interpretation of information retrieved in the 'omics field.

The recent rapid development of these fields can be accounted for by the concurrent development of new state of the art, high throughput technologies such as real time qualitative polymerase chain reaction (RTqPCR), microarrays, flow cytometry, mass spectrometry and sequencing. These high throughput technologies have found extensive utility in diverse areas of human biology, particularly following the completion of the human genome project (HGP) in 2003. This project, which successfully documented the full complement of genes present physiologically within the human cell, gave a scientific platform to newer experimental initiatives thereafter.

Clinical application of 'Omics based approaches have gained popularity and are believed to be the future of medicine because of its inherent ability to determine disease-associated changes in the human genome, transcriptome, proteome, lipidome and metabolome. Docu-

mentation of these changes in principle enables the identification of disease-associated biomarkers for use in diagnostic tests, as well as with identifying molecular mechanisms of disease.

Establishment of the HGP was then followed by the initiation of the Cancer Genome Atlas project to create a repertoire of genomic profiles for 20 different types of cancers. This project is designed to evaluate, across-the-board, the molecular and genomic depiction of different cancer types and to delineate the networks and maps for disease pathogenesis; the first results to be published from this project were for human glioblastomas [1], followed by ovarian carcinomas. [2]

Most recently, human colon and rectal cancers have been added to the Cancer Genome Atlas Project [3], the aim being to evaluate somatic modifications in such carcinomas using genome level methodologies to assess variation in DNA copy number, methylation patterns, micro-RNA expression, exon utilization; and acquired mutations: To date, genetic mutations have been found in 29 genes and amplifications of ERBB2 and IGF2 have also been observed. This large scale identification of novel genetic changes in human colorectal cancer (CRC) may in due course enable the identification of underlying molecular mechanisms of cancer development and new therapeutic targets, as well as the development of diagnostic and/or predictive tests for CRC.

Current knowledge of cancer cell biology shows that the complex carcinogenesis process is made up of several intricate molecular pathways and that different cancer cells express a heterogeneous array of signals. Hence individualized administration of beneficial treatment regimens to patients, rather than a 'one-size-fits-all' approach, is becoming more acceptable as a generic principle to strive towards, not least since it would be similar to the use of specific antibiotics based on microscopic culture and drug sensitivity testing instead of using a broad spectrum/empirical antibiotic approach.

According to the GLOBOCAN cancer fact sheet of the International Agency for Research on Cancer (IARC), over 12 new cancer cases and 7 million cancer deaths are reported worldwide annually. Colorectal cancer is the third most common cancer in males (663,000 new cases representing 10.0% of the total cancer cases) and the second most common cancer in females (571,000 new cases representing 9.4% of the total cancer cases). [4] Roughly 600,000 cases of colorectal cancer deaths are expected annually, accounting for almost a tenth of all cancer deaths, making it the fourth leading cause of cancer death worldwide. In trend, mortality rates are higher in males than in females except in the Caribbean regions.

Over half of the global CRC burden is occurs in developed countries and its incidence is known to be lowest in third world countries. The lowest incidence is reported in Africa; however in South Africa the incidence of CRC more closely resembles that of developed countries. Even though the mortality and incidence figures are poorly reported and cannot be harmonized reliably, reports from the currently defunct National Cancer Registry of South Africa revealed that in 1999 CRC was the sixth commonest cancer in the general population, accounting for about 2,367 cases of the total 26,606 new cancer cases reported. [5]

Whilst research and health institutions in the developed world are replete with ongoing studies and discoveries of candidate disease biomarkers, significant attendant challenges are associated with 'omics based studies in a developing world setting, *inter alia*: infrastructure, human capacity and funding. Here we provide a synoptic overview of the prospects, challenges and benefits of 'omics based approaches in the diagnosis and treatment of CRC in a developing world, using our Cape Town experience as a case example.

2. Diagnostic inaccuracies in colorectal cancer

The intricacies of CRC are due in part to its multiple implicated aetiologies and the heterogeneity of the tumour. Important aetiologic considerations include; location (right sided or left sided, surface or cryptic, colonic or rectal), whether sporadic or hereditary, de novo or sequel to adenomatous polyp; intra-tumour heterogeneity (ITH) also plays an important role in the accuracy of histopathologic and molecular diagnosis. The classical theory of 'field cancerisation' [6] presupposes that cancer develops from multifocal areas of precancerous changes; this concept is supported by latter studies which demonstrated that true cancer boundaries often exceed the physically discernible margin taken out at surgery, with histological analyses showing that these multiple abnormal tissues constituting premalignant cancer cell field changes persist and surround the tumor and may form multiple independent lesions that sometimes coalesce. The implications of such findings are profound when differentiating the cancer field recurrence from second primary tumours [6-8]. Subsequently, others have considered the concept of 'sequential carcinogenesis' which suggests that cancers develop as a progression from patchy premalignant lesions which later show evidence of mild, then moderate and then severe epithelial dysplasia. [7] These dysplastic lesions eventually progress to carcinoma-in-situ (high grade intraepithelial neoplasm), then to microinvasive carcinoma and advanced invasive carcinoma.

The literature is replete with the extensive application of the field concept of Slaughter in surgical management of head and neck cancers and is gradually becoming a major consideration in the management of cancer of other organs such as the stomach, skin, cervix, lungs, vulva, bladder, colon, breast, and ovaries. [9, 10] In addition, molecular investigations have been used to assess correlation between phenotypic stages of cancer progression and expression of cancer specific genes and mutations; these studies have demonstrated significant correlations between both [7, [11], implying that molecular markers have the potential to significantly enhance the precise diagnosis, staging and treatment monitoring of cancers.

The possibility of a physically normal tissue adjacent to the tumour area and expressing cancer specific genetic profiles has spurred interest in the origin of molecular level heterogeneity and histologic variability in the immediate field surrounding the cancer area. Some researchers have suggested that the heterogeneity in cancer growth follows a Darwinian evolutionary theory of natural selection [12], whilst others have concluded that there are two plausible origins of diversity in tumour cells expansion, described by the 'clonal evolution' theory and the 'cancer stem cell' theory. [13-15] The clonal evolution theory (cancer monoclonality)

presupposes that tumour cells result from a solitary clone of mitotically unstable cell that differentiate into different offspring lineage clones that have developed additional unique genetic damage down the lineage. [13] By contrast, the cancer stem cell theory (cancer polyclonality) is premised on the possibility of cancer developing from multiple cancer stem cells that proliferate concurrently and drive the expansion of the tumour. Both theories have therapeutic implications in that only a fraction of the tumour bulk drives its expansion, hence targeted molecular therapies at these 'driver cells' could in principle be established for cancer treatment.

Phylogenetic evidence suggests that well-characterized subpopulations of tumour cells, including annotations of genetic mutations, have been derived from sequential genetic events [16] and mathematical models have been described to account for this, but to date have mostly provided a one-dimensional insight into the complexities of ITH. [17, 18] The mechanistic development of cancer is a multi- dimensional event and multiple factors have been established to govern its progression such as: the shape of the organ in which it occurs; blood supply; surgical interference; the consistency of the surface on which it occurs; tumour microenvironment; and the genetic nature of the cell. Clearly ITH is a reality that affects tumour diagnosis, classification, prognosis, and treatment; and requires further understanding.

2.1. Reliability of histopathology reports

Introduction: Histopathology is the science of utilising classical histological techniques to assess micro- and macroscopic evidence of potential disease. Classical histology routinely utilises microscopic observation of micrometer cross-sections of tissue, differential staining techniques, as well as immunohistochemical assays, to assess the tissue specimen in question. The visual evidence provided by each technique, or combination thereof, allows a pathologist to identify potential evidence of pathology, thereby providing a pathological diagnosis for a given specimen.

Classical histological techniques are inherently limited in their scope for the detection of pathology as they rely on a microscopically visible presentation of clear or strong evidence of pathology, e.g. in the case of advanced disease. Furthermore, any aberrant change at the sub-microscopic level, i.e. the molecular level, needs to have translated into morphological change at the subcellular and/or overall cellular morphological level, or to have produced a variation in the abundance of a particular protein, or set of proteins, that is detectable through immunohistochemistry.

Evidence of molecular changes that are not yet detectable at the histological level: In molecular studies of tumour biopsies and adjacent "paired" normal samples, researchers often seek to identify molecular signatures that can distinguish tumour samples compared to histologically normal samples obtained from anatomical sites distal to the primary lesion. However, recent studies have investigated the possibility of significant molecular differences between histologically normal colorectal tissue from patients with polyps, or colorectal carcinoma, versus the same tissue from healthy individuals [19], suggesting that significant aberrant molecular changes occur in apparently normal colorectal tissue, despite being apparently distinctly located from the original lesion. In turn, this suggests that classic histopathological techniques are limited

in their scope to identify such clinically relevant aberrant changes in tissue that appears morphologically normal; interestingly, these molecular observations are entirely consistent with Slaughter's 'field cancerisation' theory. [6] However, whether or not such a field has a gradually tapering aberrant effect as function of distance from the tumour remains incompletely answered. For instance, it has been found that there was no significant correlation between the degree of aberrant gene expression perturbation and distance from a polyp or tumour. [20] Irrespective, of the specific characteristics of any field effect, it is interesting to note that supporting evidence of aberrant perturbations in histologically normal colorectal mucosa appeared five decades after Slaughter's original hypothesis as a direct result of modern genomic, epigenomic, and proteomic research.

In a study of normal colonic mucosa from individuals with a family history of sporadic cancer conducted by Hao et al [21], it was found that there was a significant difference in the expression of several genes in these individuals' normal mucosa relative to the same tissue from healthy controls. In particular, the gene expression levels of PPAR-gamma, SAA1, and IL-8 were found to be significantly different in the morphologically normal rectosigmoid tissue samples in the individuals with a family history of CRC. Furthermore, a follow-up study in individuals with adenomatous polyps with or without familial history of colorectal carcinoma again found that there was a difference between gene expression in normal rectosigmoid mucosa from these individuals and healthy controls, regardless of the presence of a familial history of cancer. [22]

Polley et al [19] observed significantly different proteomic signatures in morphologically normal mucosa from patients with colorectal neoplasia compared to the same tissue from healthy subjects. It therefore appears that a larger than anticipated field of tissue in the colorectum may be affected by the presence of a neoplastic lesion, implying in turn that the method used to determine clear margins estimated during surgical resection may need the support of molecular assays in the future. However, whether or not the molecularly perturbed normal mucosa will progress to disease remains to be determined.

In addition to genomic and proteomic perturbations, epigenetic changes have also been reported in CRC tissues, one example being the hypomethylation of L1 promoter sequences in colorectal tumours and in adjacent normal tissue of 6 out of 19 cancer patients, but not in colonic mucosa of 14 healthy individuals. Furthermore, genomic CpG methylation appeared to be lower in normal colorectal tissue from diseased patients, compared to healthy subjects, and significantly lower in patients with hypomethylation of the L1 promoter sequences. [23]

The above examples at the genomic, proteomic and epigenetic level provide substantial evidence of molecular aberrations in morphologically normal tissue sample adjacent to a tumour. These changes might be subtle and may not effect a microscopically visible phenotype, but could well represent significant perturbations that impart normal tissue samples with precancerous characteristics. It is therefore important that such findings are considered when assessing individual biopsies by histopathology since these samples may in fact have underlying molecular signals of disease that, if interpreted correctly, could provide insight into the disease.

Molecular Intratumour heterogeneity due to branched evolutionary clonal expansion: Intratumour heterogeneity in terms of cellular morphology and types of cells, is a well-established observation in anatomical pathology. However, it is only recently that a further layer of complexity has been introduced through the discovery of an added layer of heterogeneity at the genomic and epigenomic level. This molecular heterogeneity further complicates biopsy selection and subsequent histopathological assessment because a specific lineage of clones, with a distinct genomic profile, may occur in physical clusters on a given tumour and thus be physically separated from other clonal populations. Therefore, each biopsy taken may present a uniform or slightly variable morphological appearance but might be distinct at the molecular level with unique functional potential and characteristics. This has fundamental ramifications when it comes to assessing the severity of disease and the prognosis, as well as deciding the appropriate course of treatment. Furthermore, intra-tumour heterogeneity governs how each unique tumour lineage evolves and adapts to therapeutic interventions and should therefore ideally be understood when developing new chemo- and biological treatments.

In a landmark study by Gerlinger et al [14], multiple spatially separated biopsy samples obtained from primary renal carcinomas and associated metastatic sites showed evidence of branched evolutionally growth by unique clonal population lineages. Furthermore, the majority of all somatic mutations (63 – 69%) were not found to be present across all tumour biopsies. One clinically relevant consequence of these findings was the identification of both good and poor prognostic signatures in different biopsies of the same tumour. This study highlights the inherent complexity of utilising a single biopsy for biomarker development, prognostic or predictive measures based on molecular markers to guide treatment regimen. It is clear that future biopsy collection - for the purpose of treatment planning or for biomarker research - should ensure collection and molecular assessment of multiple biopsies from physically separate sites on the same tumour.

A recent study by Kreso et al [24] has provided supporting evidence, in the context of colorectal carcinoma, that the distinct genetic profile of each intratumoural clonal subpopulation has implications in growth potential and chemotherapy tolerance and therefore has an impact on treatment outcomes. This appears to be due to Darwinian style natural selection, with certain subpopulations becoming more dominant by leveraging their intrinsic tolerance to selective pressure such as chemotherapeutic intervention. These findings highlight the fact that different tumour subpopulations have distinct proliferative potentials and chemotherapy tolerance mechanisms; as such, treatment regimens in the future my need to target each type of cellular population individually in order to prevent disease recurrence.

Future directions for modern pathological assessments of cancer tissue samples: Given the recent developments in the field of intratumour heterogeneity, and the evidence of significant molecular aberrations in histologically normal mucosa, it is clear that classical histopathological techniques are limited in their ability to assess the underlying clinically relevant heterogeneity in tumour and normal tissue samples, suggesting that modern, validated, cost-effective molecular assays should be integrated into histopathological assessments. Amongst others, this would ensure that clinically relevant phenotypic or functional characteristics - whether dormant or active - which may directly govern a tumours response to therapeutic intervention

can be identified. This enhanced approach should facilitate the development of novel treatment regimens that efficaciously target all heterogeneous subpopulations for a given tumour and, in doing so, potentially also thwart aberrant histologically normal tissue from progressing to a malignant lesion.

The possibility that distinct subpopulation of cells and/ or genetic signals exist in different areas of a particular biopsy specimen poses a major challenge in implementing personalized therapy. [25] 'Omics based research can in principle generate a more robust predictor of therapeutic benefits, but this often involve an extensive sample collection for discovery and validation, adequate funding and availability of appropriate manpower: an example of one such initiative is the 'Personalized RNA Interference to Enhance the Delivery of Individualized Cytotoxic and Targeted Therapeutics' (PREDICT) consortium [26] that aims to identify reliable biomarkers of different cancer types.

2.2. Tumour classification and staging

The earliest classification system for CRC by Dukes [27], modified by Astler [28], considered the staging of CRC severity to be based on its depth of invasion. More recently, a standardized widely accepted staging system of the American Joint Committee on Cancer (AJCC) also known as the TNM system which incorporated the tumour size, nodal involvement and presence of distant metastasis was introduced [29]: These three classifications are largely based on morphologic evidence and can be subject to inaccuracies. To minimize errors in diagnosis, these phenotypically driven systems of staging colorectal tumour could now in principle be usefully complemented with a validated molecular diagnostic method.

Newer and often more reliable molecular based assessment of tumour staging and prognosis are beginning to emerge for CRC based on new molecular cancer knowledge and 'omics based techniques and complement existing orthodox morphology based methods. One of such molecular classification is based on CpG island methylator phenotype (CIMP), as well as microsatellite instability (MSI), and is scored as type 1-5 based on different combinations of these molecular features. [11] Chromosomal instability (CIN) has also been identified as an important global molecular classifiers of CRC. [30] In addition, KRAS mutations have emerged as the most common prognostic biomarker of CRC for anti-EGFR therapy patients and many more candidate markers for prediction of therapeutic outcomes in colorectal cancer are being discovered now using 'omics based techniques, including loss of PTEN signals, PI3KCA mutation and BRAF gene mutation. [31]

Identification of cancer specific genes, proteins, lipids and metabolites is increasingly regarded as a promising route to early diagnosis, treatment and monitoring of disease progress. For instance, the following genes have been recognized as associated with the risk of developing colorectal cancer; TP53, MLH1, MSH2, MSH6, MYH, EPCAM, KIT, BLM, SMAD4, PGDFRA, BMPR1A, APC, AXIN2, and STK11. *Vogelstein et al* [32] In a recent meta-analysis of 25 different genetic expression studies of colorectal cancer, a statistically significant down-regulation of carbonic anhydrase II (CAII) and CEACAM1 was observed, while there was up-regulation of TGFβ1, IFITM1, SPARC, GDF15 and MYC genes. [33] Based on these molecular patterns, a novel staging and classification system that is devoid of errors can now potentially be evolved.

2.3. Role of surgical pathology

The rationale behind the histopathologic use of slide sections from biopsy samples has been to evaluate for diagnostic purposes a representative microcosm of disease from a routinely processed, waxed, and miniaturized specimen blocks. However this has sometimes led to mis- or under- diagnosis of tumour depending on the exact site from which the biopsy was taken from. In principle, the goal of surgical resection is to take adequate 'tumour-free' margin; however achievement of this goal in practice is at best an estimated blind procedure, since cancer specific molecular alterations in the 'apparently' tumour free regions are largely unknown. This makes it difficult to readily determine the adequacy of a surgically resected margin. Not least, pathologists have had to take multiple biopsies from different sites in a resected tumour mass, trying to maximize the chances of locating the accurate tumour areas. This blind sampling procedure is a major potential source of diagnostic inaccuracies in practice and it is therefore important to detect colorectal cancer early to improve the chances of getting an adequate 'tumour free' surgical margin. Prompt surgical intervention, coupled with accurate determination of the most beneficial adjuvant therapy for the specific patient, appears to hold the key. Surgical pathology - which is the interface between surgery and pathologic specimen processing - is thus a vital control point for the eradication of diagnostic inaccuracies. Given the current molecular evidences on intratumour and interbiopsy heterogeneity, an exclusive morphology based sampling technique is a potential minefield for diagnostic errors, even with the best mastery.

A delicate balance needs to be achieved during sampling of surgical specimens for the assessment of tumour heterogeneity. The most prominent, average pattern of expression is most likely to be identified during sampling of a large tumour biopsy sample, but this approach risks masking the less prominent but potentially equally important information about sub-populations of tumour cells in the biopsy sample. By contrast, signal-to-noise ratios have to be carefully balanced when analysing smaller biopsy samples since this approach presents a smaller sampling frame within which it may be impractical to identify all possible biomarker signals a tumour could express. [34] Surgeons and pathologists also need to bear in mind the downstream requirements and applications of 'omics based research when surgical biopsy samples are taken, especially since mining the molecular archives of formalin fixed paraffin embedded (FFPE) specimens through genomics, proteomics and lipidomics research is beginning to gain traction now. However, factors such as age of tissue, condition of storage, tissue sample size, fixation time, pH influences and buffers are known to influence the outcome of 'omics based analyses on surgical specimens. [35] In particular, RNA degrades rapidly at room temperature, whilst formalin damages nucleic acids within the specimen by forming sclerotic crosslinks of DNA and RNA *via* methylol adducts and methylene bridges. [35] Thus, immediate snap freezing of fresh sections in liquid nitrogen or dry ice is good practice, whilst use of newer alcohol-based kits [36], or of phosphate buffered formalin [37, 38], provide useful alternatives to standard formalin for rapid fixation of surgical specimens prior transport to the lab.

2.4. Patient evaluation and therapeutic loopholes

Biomarker discovery research will in principle enable accurate stratification of patients into appropriate risk categories. The orthodox clinical approach of prescribing a common thera-peutic cancer regimen to all colorectal cancer patients is fast becoming a subject of evidence-based debates. Certain differences exist between patients who come from demographically, geographically and genetically divergent backgrounds. Such inter-patient variability may also present significant differences in tumour phenotype, behavior and natural histories across a population, an important observation that is referred to as single nucleotide polymorphism (SNP) noise. [39] Patient stratification based on these natural clusters can enable meaningful biomarker discovery using 'omics based techniques. For instance, in a culturally heterogene-ous South African population composed of Caucasians, Indigenous African, Indian, and Mixed Ancestries, the *a priori* expectation is that different racial groups may express different biomarkers of disease. In the same light, candidate biomarkers discovered from studies on developed world patient cohorts may not necessarily be effective for the management of colorectal cancer in a developing world situation due to differing ethnicities, which has clear implications for the planning and execution of 'omics based discovery and validation of biomarkers.

In principle, it is possible to predict response to specific targeted therapies (e.g. Herceptin) using 'omics based approaches, with patients who would not respond to specific targeted therapies being identified at the outset and given alternative therapies. Patient dependent source of variation for biomarker discovery includes individual genetic make-up, metabolism, stage of disease, health, immunocompetence, nutritional factors, and environmental factors. Careful patient selection to eliminate confounders must be carried out prior to experiments and biomarkers must be validated in a standardized acceptable manner.

Genetic profiling of patients for KRAS mutation, BRAF genes, Microsatellite instability (MSI) and CpG island methylator phenotypes (CIMP) is now being increasingly carried out for colorectal cancer patients in the developed world and this has contributed significantly to treatment planning. Patients that exhibit MSI have been found to possess better overall survival than those with chromosomal instability and are less affected by p53 mutations [40], whilst CRC with p53+, MSI+ profiles are usually more aggressive than those with p53-, MSI+ profiles. [41] Biomarkers such as telomerase and survivin have been used to assess the long term risk of CRC development [42] whilst morphologic biomarkers in patients include neoplastic colorectal polyposis and the presence of aberrant cryptic foci (AFC); the presence of adenomas with Intraepithelial Neoplasm in the colorectal region can also be used in surveillance as surrogate endpoint biomarkers. [43] Paradoxically, most anti-cancer agents do not have well-established single predictors of individualized response, however with the advances in 'omics based approaches it should be possible to provide such molecular predictors. For example, MSI has been documented to be an effective predictor of response to fluoropyrimidine therapy, whilst ERCC1 was found to be beneficial in patients using platinum containing anticancer regimen. [31]

'Omics based techniques relevant to colorectal cancer management

The management of CRC has to date been based on fairly invasive techniques for diagnosis and treatment. In contrast, 'omics based techniques in most instances are non- or minimally invasive, thereby improving patient compliance, eliminating surgical morbidities and ultimately reducing the burden of disease through early diagnosis and effective treatment monitoring. The potential utility of increasingly common-place 'omics based techniques in the diagnosis, surveillance, treatment, and prevention of colorectal cancer is thus discussed below.

2.5. Genomics and epigenomics

Introduction: In genomic investigations, high-throughput technologies such as microarray platforms or DNA/ RNA sequencing are now commonplace. These technologies are now routinely applied to large sample collections, with complete clinical annotations, and aim to produce profound insights into disease at the resolution of single nucleotide polymorphisms, gene expression, and the status of epigenetic regulatory mechanisms such as hypo- and hypermethylation.

Extensive bioinformatic analysis of these high-throughput, multi-level, datasets has provided in-depth insights into the mechanisms of disease and treatment resistance. These findings have been translated into biomedical research aimed at addressing the significant challenge in treating cancer of the colorectum. While it is well established that cure rate for treating TNM stage I colorectal cancer is over 90%, the clinical management of stage II CRC is more complicated. [44, 45] Furthermore, improved chemo- or biological treatment regimens are needed to address the high rates of recurrent disease observed with stage II and III CRC disease, as well as for TNM stage IV disease which is generally considered incurable at present. [46, 47]

The overall disease-free survival statistics for TNM stage II disease, treated with surgery alone, are as high as 75%. [44] However, certain sub-populations of patients experience a worse prognosis and have clinical outcomes more similar to TNM stage III disease. Therefore, much focus has been on finding genetic markers to guide treatment regimen selection in stage II and III disease, the goal being to improve overall efficacy, decrease treatment failures, reduce the incidence of recurrent disease, whilst at the same time lowering the cost of treatment by limiting costly chemotherapeutics to those patients with predicted benefit.

High-throughput genomic studies today provide a comprehensive means to analyse amongst others the expression level of every individual gene, as well as to assess chromosomal segment copy number- and DNA methylation pattern variations and to determine single nucleotide polymorphism and mutation frequencies, all in a genome-wide manner. Biomarker research can thereafter be carried out to identify and validate distinct signatures derived from integrated value measurements associated with each gene, as a prelude to translating such findings into a clinical setting, as either biomarkers or novel therapeutic targets.

3. Transcriptomics

The field of transcriptomic research, within the context of prognosis and treatment outcome prediction, has seen much attention in recent years. High-volume real-time gene expression

assays, gene expression microarrays and, more recently, 'next generation sequencing' based methods have provided the necessary platforms to investigate putative prognostic and predictive genetic markers. These platforms, combined with known clinical outcomes, enable panels of genes to be significantly correlated with prognosis, or the outcome of treatment with various chemotherapeutic agents. However, genetic association studies require large datasets in order to identify putative prognostic and predictive markers with significance worthy of clinical utility. As such, there are to date a limited number of landmark publications that present such multi-gene panel lists associated with prognosis and treatment outcome prediction in TNM stage II and III colon and rectal cancers.

A study by O'Connell et al [48] involved the combined analyses of four independent studies of colorectal cancer patients. Samples were obtained from 1851 CRC patients in the United States, with stage II or III disease, who participated in the National Surgical Adjuvant Breast and Bowel Project (C-01/C-02 and Cleveland Clinic (CC); C04; C-06) and 1136 candidate genes (761 genes assessed in C-01/C-02; 375 genes in CC/C-06) were evaluated. The aim of the study was to establish a panel of markers that could be associated with the risk of disease recurrence and that could determine the likelihood of patients benefitting from adjuvant 5-fluorouracil/leucovorin adjuvant therapy. The analyses resulted in the identification of 48 genes significantly associated with the risk of disease recurrence and 66 genes significantly associated with 5-FU/LV benefit (with four genes in common between the two sets). For practical reasons a gene panel of 7 genes predictive of disease recurrence, 6 predicted of 5-FU/LV benefit, and 5 reference genes were selected. The clinical utility of this predictive panel was then independently evaluated in the Quick and Simple and Reliable (QUASAR) study. [49] The aforementioned study validated the use of this gene panel, and it's consequent recurrence score, as an independent predictor of the risk of recurrent disease in stage II colon cancer patients who had undergone surgery. This gene panel was then commercialized by Genomic Health by the production of a multi-gene panel called the Oncotype DX® Colon Cancer Assay.

In separate work, Salazar et al [50] described a gene expression signature - named ColoPrint - in early 2011 that allowed for improved prediction of prognosis in TNM stage II and III colorectal cancer. The gene signature consists of 18 genes that were identified from analysis of 188 frozen tumour samples (TNM stage I-IV; 78.7% not treated with adjuvant chemotherapy), and a cross-validated on 206 independent tumour samples (TNM stage I-III; 60.7% not treated with adjuvant chemotherapy) originating from three sites in the Netherlands. This panel showed better predictive accuracy, in comparison to the American Society of Clinical Oncology criteria for assessing the risk of cancer recurrence without prescreening for microsatellite instability (MSI), with a hazard ratio of 2.69 (95% CI, 1.41 to 5.13; P = 0.003) for patients with stage II disease. Results such as these are encouraging, and provide strong supporting evidence for the utility of molecular approaches over purely clinical markers.

In a related study, a panel of 13 genes - referred to as ColoGuideEx - was reported by Ågesen *et al* [51] to be significantly associated with predicting prognosis for stage II colorectal cancer patients. This study was conducted on an initial dataset obtained from 207 colorectal samples originating from three independent Norwegian patient series, and validated on a 108-sample gene expression dataset originating from the USA and Australia. The independent prognostic

value of the panel of genes was confirmed by multivariate Cox regression analyses (p≤0.004), which included various clinicopathological variables and all three-sample series.

O'Connell et al (2010)	O'Connell et al (2010) Oncotype DX® Colon Assay	Salazar et al (2011) ColoPrint®	Ågesen et al (2012) ColoGuideEx
5FU benefit	Disease recurrence	Disease recurrence	Disease recurrence
ATP5E	ATP5E	CA4388O2	AZGP1
AXIN2	BGN	CTSC	BNIP3
BIK	C-MYC	CYFIP2	CXCL10
EFNB2	FAP	EDEM1	CXCL13
GPX1	GADD45B	HSD3B1	DSC3
HSPE1	GPX1	IL2RA	ENPP3
MAD2L1	INHBA	IL2RB	EPHA7
PGK1	Ki-67	LAMA3	KLK6
RUNX1	MYBL2	LIF	MMP3
UBB	PGK1	MCTP1	PIGR
VDAC2	UBB	PIM3	SEMA3A
	VDAC2	PLIN3	SESN1
		PPARA	TUBA1B
		PYROX D1	
		SLC6A11	
		THNSL2	
		ZBED4	
		ZNF697	

Table 1. A list of multi-gene panels that are significantly associated with disease recurrence, or benefit from adjuvant 5-fluorouracil (5-FU) and leucovorin (LV) chemotherapy, as published in three independent studies.

Table 1 illustrates the various gene panels from the four studies described above and it is noteworthy that there are no genes in common between these gene panels. Furthermore, the biological relevance the genes utilised in each panel has yet to be fully explained and may represent an opportunity to identify pathologically associated genes and pathways when molecular enrichment analyses are applied across multiple gene panel studies in the context of prognostic and predictive biomarkers used in recurrent colorectal cancer. For example, there are genes across the lists in Table 1 that share a relationship by virtue of their KEGG pathway associations: (1) *Cytokine-cytokine receptor interaction* (n=6 genes; CXCL10 and CXCL13 [Chemokines; CXC subfamily] from Ågesen et al (2012), LIF [gpl30 shared] from Salazar et al (2011), IL2RA and IL2RB [IL2RG shared] from the Hematopoietins from Salazar et al (2011), and

INHBA [TGF-ß family] O'Connell et al (2010); (2) *Axon guidance* (n=3 genes; EPHA7 from Ågesen et al (2012), EFNB2 from O'Connell et al (2010), and SEMA3A from Ågesen et al (2012); (3) *Pathways in Cancer* (n=3 genes; LAMA3 from Salazar et al (2011), AXIN2 from O'Connell et al (2010), RUNX1 from O'Connell et al (2010)); (4) *p53 signaling pathway – target genes* (n=2 genes; SESN1 from Ågesen et al (2012), and GADD45B from O'Connell et al (2010)). These KEGG pathway association mappings were adapted from outputs generated by GeneCodis. [52-54]

In a novel study that utilised eight published prognostic and predictive gene expression signatures, Shi et al [55] combined the datasets and integrated them with publically available protein-protein interaction network data in order to identify candidate molecular markers associated directly with the recurrent colorectal cancer phenotype. As a result, they were able to not only infer pathophysiological mechanisms underpinning the recurrent disease phenotype, but also used the augmented and cross-study integrated signature to identify both a prognostic signature and a multi-gene signature to predict treatment outcome. The resultant gene signature consists of 487 genes and is referred to as the NEM signature (as it integrates information from Network, Expression, and Mutation datasets).

It is clear therefore that gene expression panels are developing to the point where they are close to translation into a clinical setting and adoption into routine clinical practice. It remains to be seen though how many of the published gene panels will make it into the clinic after appropriate validation studies have been carried out to assess performance across larger and more ethnically diverse patient populations. In this context, it is important to note that such signatures might yet be inherently compromised by the likely existence of multiple, possibly opposing, signatures in the same tissue sample (*vide supra*). Therefore, testing of multiple biopsies from the same tumour may be necessary in order to generate a holistic prognosis and to provide guidance on treatment strategy. Furthermore, in developing nations where the incidence of colorectal cancer is not yet decreasing and cases consistently present at more advanced stages of disease, it remains to be seen whether the cost of such diagnostic or prognostic panels will be affordable in the public sector where there is the most need and where the greatest diversity exists.

Single nucleotide polymorphisms: Colorectal cancer has classically been treated by 5-fluorouracil (Capecitabine; Xeloda®; Hoffmann-La Roche Inc.), combined with either Oxaliplatin (Eloxatin®; Sanofi-Synthélabo Inc.) or Irinotecan (Camptosar® or CPT-11; Pfizer Pharmaceuticals Inc.). Over the past decade these regimens have been augmented by the addition of biological therapeutic agents, such as the monoclonal antibodies Cetuximab (Erbitux®; ImClone Systems Inc.), Panitumumab (Vectibix®; Amgen Inc.), and Bevacizumab (Avastin®; Genentech Inc).

Each drug has a different mechanism of action and a unique set of molecular targets, as well as distinct sets of enzymes responsible for its metabolism; furthermore, particularly in the case of biological agents, specific enzymes that are functionally important to the pathways targeted by the biologic are major determinants of response. It is well-established that polymorphic variations in enzymes involved in drug metabolism can alter drug availability, thereby causing a variation in clinical response, by altering the rate and specificity of drug metabolism. Such variant drug metabolizing enzymes are generally encoded by single nucleotide polymorphisms (SNPs) that result in changes in the amino acid composition of the relevant gene

product (i.e. protein), resulting in altered enzymatic activity. As such, the biomedical community has utilised an arsenal of molecular techniques, including high-throughput genomic platforms, to identify such SNPs and to quantify their frequency in populations in order to correlate with treatment outcomes and thereby to identify biomarkers that are predictive of therapeutic response.

This area of research has been comprehensively reviewed recently by Asghar et al [56], Bandrés et al [57], Benheim et al [58], Coate et al [59], De Roock et al [60], and Ross et al [61] and forms the basis of the rich pharmacogenetic resource, PharmGKB® (http://www.pharmgkb.org) which documents each therapeutic agent together with an aggregated list of SNPs reported in the literature to be associated with treatment outcome.

4. Pharmacogenomics

4.1. Markers of treatment outcomes when treated with classical chemotherapeutics

5-Fluorouracil: 5-Fluorouracil (5-FU) was introduced more than 50 years ago by Heidelberger et al [62] and is the foundational cytotoxic agent used in the treatment of colorectal neoplasia. 5-FU can be administered in three different physical forms: either through an intravenous solution, or as an oral compound (Capecitabine, and Tegafur). The metabolism of either of these compounds results in the formation of fluoronucleotides. A subset of these molecules, fluorouridine triphosphate (FUTP) and fluorodeoxyuridine monophosphate (FdUMP) are misincorporation into DNA and RNA during their *in vivo* biosynthesis. In addition, FdUMP inhibits thymidylate, synthase thereby resulting in a nucleotide imbalance due to a depleted intracellular reserve of thymidine for DNA synthesis. The enzymes responsible for producing the active metabolites have been studied for polymorphic variation and analysed for their correlation to treatment response (as seen in Table 2).

Gene	SNP	Molecular effect	Associated treatment outcome	Reference
TP		Increased expression	Increased response	Bandrés et al (2007)
TS	TSER2R	Decreased expression	Increased response	Bandrés et al (2007)
DPD	DYPD*2A	Decreased activity	Increased response Increased toxicity	Bandrés et al (2007)
MTHFR	677T	Increased production of CH_2FH_4	Increased response	Bandrés et al (2007)

Table 2. A list of single nucleotide polymorphism (SNP) markers associated with treatment outcome, as reported by Bandrés et al (2007), when using a 5-fluorouracil-based regimen.

Oxaliplatin: Oxaliplatin is a platinum analog that results in inter- and intra-molecular DNA cross-links, resulting in the inhibition of DNA synthesis, transcription and repair processes.

This compound has been extensively utilised in combination with 5-FU and Leucovorin, a regimen referred to as FOLFOX and commonly prescribed for treatment of advanced colorectal cancer.

There are two groups of genes that are primarily responsible for altered response to Oxaliplatin, namely genes involved in DNA repair and in glutathione conjugation reactions. In the former group, a polymorphism in the X-ray repair cross-complementing group 1 enzyme (XRCC1) - which is part of the base excision repair system - has been associated with variable initiation of DNA repair. [57] In addition, a polymorphism in a component of the ubiquitous nucleotide excision repair pathway - the excision repair cross complementing group 2 (ERCC2) gene - has been significantly associated with a clinical response to platinum-based chemotherapy. [57] However, there are no known polymorphisms in the DNA mismatch repair pathway associated with variation in treatment response with Oxaliplatin. In the second group, it has been reported that platinum compounds are inactivated by glutathione conjugation and therefore the enzymes responsible for catalyzing this reaction have been investigated. In particular, SNPs in several glutathione-S-transferase (GST) genes have been implicated in conferring resistance to Oxaliplatin. [57]

Gene	SNP	Molecular effect	Associated treatment outcome	Reference
GSTP1	613G	Decreased activity	Increased response	Bandrés et al (2007)
GSTT1	Deletion	Decreased activity	Increased response	Bandrés et al (2007)
GSTM1	Deletion	Decreased activity	Increased response	Bandrés et al (2007)
XRCC1	388Gln	Decreased activity	Increased response	Bandrés et al (2007)
ERCC2	751Gln	Decreased activity	Increased response	Bandrés et al (2007)

Table 3. A list of single nucleotide polymorphism (SNP) markers associated with treatment outcome, as reported by Bandrés et al (2007), when using a chemotherapeutic regimen that includes Oxaliplatin.

It is noteworthy that in a recent study by Fernandez-Rozadilla et al [63], seven SNPs (rs16857540, rs2465403, rs10876844, rs10784749, rs17626122, rs7325568, rs4243761) were found to be significantly associated with adverse drug reactions in the context of singular 5-FU or FOLFOX treatment. Given the relatively large sample size of 221 CRC patients and a validation set of 791 patients, these results hold strong statistical significance and provide potential predictive capacity for toxicity response on an individual patient basis if validated through a larger cohort.

Irinotecan (CPT-11): Irinotecan is a camptothecin analogue with well-established anti-neoplasia activity exerted though stabilization of the ordinarily transient DNA topoisomerase I-DNA complex, thus preventing the repair of temporary single stranded breaks during DNA replication and leading to cell death. [64] There are two primary pathways that have been implicated in variable response to Irinotecan treatment: drug transport into the extracellular

environment, and metabolism of Irinotecan into its active (SN-38 and SN-38G) and inactive metabolites.

Hydrolysis of Irinotecan by carboxylesterases: CES1 and CES2 results in the production of its active metabolite, SN-38. This metabolite then undergoes detoxification via the process of glucuronidation, catalyzed by uridine diphosphate-glucurono-syltransferase 1A (UGT1A), to produce a metabolite called SN-38G which then interacts directly with the DNA topoisomerase I enzyme. However, Irinotecan can also be oxidized by members of the Cytochrome P450 3A subfamily (CYP3A) to produce inactivate metabolites. Each of these metabolites is transported out of the cell by the adenosine-triphosphate (ATP) binding cassette (ABC) transporter transmembrane proteins. As such, polymorphic variation in these enzymes has been associated with altered metabolism, transport and therapeutic efficacy of this drug and its metabolites. A selection of these SNPs is detailed in the Table 4.

Gene	SNP	Molecular effect	Associated treatment outcome	Reference
CES2	IVS10-88	Decreased expression	Decreased response	Bandrés et al (2007)
CYP3A	?	Decreased activity	Decreased response	Bandrés et al (2007)
UGT1A1	UGT1A1*28	Decreased activity	Increased response Increased toxicity	Bandrés et al (2007)
ABCB1	1236C"/>T	Decreased activity	Increased response Increased toxicity	Bandrés et al (2007)
ABCB1	3435C"/>T	Decreased activity	Increased response Increased toxicity	Bandrés et al (2007)
ABCC2	3792T		Increased response	Bandrés et al (2007)
ABCG2	421A	Decreased activity	Increased response	Bandrés et al (2007)

Table 4. A list of the single nucleotide polymorphism (SNP) markers associated with treatment outcome, as reported by Bandrés et al (2007), when using a chemotherapeutic regimen that includes Irinotecan (CPT-11).

Markers of treatment outcomes when treated with biological agents: In recent years there has been an emergence of a new class of antibody-based therapeutics that directly and specifically target molecules belonging to processes fundamental to cancer pathophysiology. Comprehensive reviews of the hallmarks of the cancer phenotype have been updated recently by Hanahan and Weinberg [65, 66]; in addition, comprehensive reviews of biomarkers associated with monoclonal antibody therapies that are currently in being used in the treatment of this disease have also been published recently. [56, 60]

Vascular endothelial growth factor as a target: It is well understood that tumours have angiogenic potential, i.e. they possess the ability to induce the production of new blood vessels, and thereby increase their supply of oxygen and nutrients; this characteristic provides an opportunity for therapeutic intervention, targeting the vascular endothelial growth factor (VEGF) and associated VEGF receptors (VEGFR-1, -2, and -3). In particular, overexpression of VEGF has been associated with vascularity, endothelial cell migration and invasion, poor prognosis

and aggressiveness in most malignancies [67]; mAbs have thus been designed to target VEGF, thereby reducing the amount of free VEGF, reducing VEGF receptor activation, and ultimately reducing angiogenesis. [68] A recombinant humanized IgG1 monoclonal antibody, Bevacizumab, that specifically targets VEGF is currently indicated as part of combination therapy in metastatic colorectal cancer; however, a biomarker that is predictive of response to anti-VEGF therapy is yet to be discovered.

Epidermal growth factor receptor as a target: Another fundamental hallmark of the cancer phenotype is the ability for tumours to alter their response to growth factors through differential gene expression of growth factor receptors. This too presents the opportunity interrupt aberrant growth mechanisms. In colorectal cancer, it has been shown that there is an abnormal activation of epidermal growth factor receptor (EGFR). [69] As such, a humanized monoclonal antibody, Cetuximab, targeted at the extracellular domain of EGFR has been evaluated in clinical trials. This mAb blocks specific EGF-mediated signal transduction events [70], thereby inhibiting cellular proliferation and inducing apoptosis. [71] Treatment with Cetuximab also leads to increased responses to classic chemotherapeutic agents and radiotherapy, as well as inhibiting cellular proliferation, angiogenesis and metastasis. [72] Another recombinant human IgG2 monoclonal antibody, Panitumumab, which also targets EGFR is currently indicated in metastatic colorectal carcinoma where there has been resistance to fluoropyrimidine, Oxaliplatin, and Irinotecan containing regimens.

To date there have been a small but useful number of genes harboring mutations that provide insight into the outcome of anti-EGFR therapy. For example, the mutational status of the Kirsten-ras (KRAS) gene is currently being used in predicting treatment benefit in the context of anti-EGFR therapy for patients with metastatic disease. Other common genes with mutations relevant to anti-EGFR mAb therapy include BRAF and PIK3CA, as well as loss of expression of PTEN.

Conclusion: It is clear that SNP biomarkers associated with treatment outcome are generally found in genes with specific characteristics of cancer pathophysiology, or with drug metabolism and transport, and numerous low-throughput, focused, yet fruitful studies have resulted in clinically translatable SNP biomarkers being identified. This contrasts with the high-throughput transcriptomic approach to identification of biomarker gene panels that is typically initially blind to the functional significance of each individual gene. As such, these simple SNP markers present a cost-effective option to predicting the efficacy of a specific therapeutic agent, or combination thereof, prior to prescription, enabling design of individualized therapy that will result in increased efficacy and improved treatment outcomes.

5. Epigenomics and epigenetics

Molecular studies of cancer have revealed the presence of not only of genetic mutations, copy number alterations, altered gene expression, but also of aberrant epigenetic changes. Intense investigation in recent years has shown that epigenetic regulation of gene expression plays a crucial role in embryonic development, imprinting, and tissue differentiation. [73] Therefore,

deregulation of such a fundamental mechanism might offer insight into the driving molecular mechanisms associated with the development, and regulation, of a carcinoma phenotype, particularly in cases where other genomic perspectives have not yet provided an answer.

In cancer studies, the field of epigenomics has encompassed investigations into aberrant DNA methylation, post-translational modification of histones that affects chromatin structure, altered expression of microRNAs and non-coding RNAs, and nucleosome positioning. [73-75] For complete reviews of this specific topic see Ballestar et al [76], Hatziapostolou et al [77], Khare et al [74], Lao et al [75], Liu et al [78], Sawan et al [79], Sharma et al [73], Ting et al [80], and van Engeland et al [81].

DNA methylation studies have focused particularly on the methylation patterns of cytosine and guanine (CpG)-rich DNA sequences. In particular, regions of the genome that have a higher than expected number of CpG nucleotides compared to the rest of the genome – so-called "CpG islands" – are of particular interest because they have been shown to overlap the promoter regions of 60-70% of genes. [75] An extension of this concept has been the discovery of CpG sites outside of promoter regions, referred to as "CpG Island shores", that are within two kilobases of the 5′ end of a CpG island. [75] Methylation of CpG islands is largely associated with transcriptional repression [75, 82] and, in general, CpG islands are protected from aberrant methylation but in cancer this does not appear to be the case. Since methylation changes can significantly alter gene expression profiles and thereby deregulate important biological pathways, a sound understanding of which genes and which associated pathways are affected in which individual patients might in the future be applied in the clinic to guide the selection of appropriate treatment regimens.

The first report of epigenetic alterations in tumours of the colon revealed an extensive loss of 5′-methylcytosine when compared to normal colon tissue. [83] However, it is only recently that this area of research has gained increased attention. Today, high-throughput methylation-specific microarray and sequencing technologies, together with a well-established array of commercially available methylation assay kits, have facilitated large-scale epigenomic investigations and contributed to an increased understanding of the methylome on a multi-gene scale.

Arguably the most important epigenetic finding to date, in the context of colorectal cancer, has been the identification of a unique molecular subtype characterized by a high frequency of gene methylation. Colorectal tumours of this variety are now referred to as having the CpG island Methylator Phenotype (CIMP). The exact panel for diagnosing CIMP varies from study to study, but the panel of genes proposed by Weisenberger et al [84] has been commonly used, i.e. *NEUROG1, SOCS1, RUNX3, IGF2,* and *CACNA1G.* In general, if a panel has more than 60% of its genes methylated, then is considered to be CIMP positive. Despite, the lack of a stand-ardized CIMP diagnostic panel, this type of tumour is reported to be predominantly associated with right-sided colon cancer, and tends to be more common in woman. [84, 85]

Diagnosing CIMP tumours is clinically relevant because approximately 20% of colorectal tumours have this phenotype and generally share a high frequency of the BRAF c.1799T>A (p. V600E) mutation [75] that has been reported to negatively impact treatment outcomes in anti-

EGFR mAb-mediated therapy in patients with KRAS wild-type tumours and metastatic disease. [60, 86, 87] As such, classifying a patient's tumour as having the CIMP phenotype could be used in the future to guide the selection of biological therapeutics to ensure that the most efficacious treatment regimen is utilised.

The current lack of a standardized panel of genes from which to assess the status of genomic methylation highlights the fact that this is an emerging field of study. As such, the translation of these panels into the clinic as diagnostic or prognostic biomarkers is still premature. However, as the field progresses and more literature-based evidence is published in support and validation of a particular panel, the full potential of such epigenomic biomarkers may ultimately be translated into patient benefit.

While the field is currently in its infancy, there are a number of encouraging studies that represent good examples of novel discoveries of methylation markers that have highly significant associations. For example, Wang et al [88] examined the methylation status of five tumour suppressor genes in eighty-five paired colorectal tumours and normal mucosa samples from Chinese patients and showed that the methylation status of two genes, CDH13 and FLBN3, were significantly associated with stage of disease and prognosis. In particular, CDH13 was significantly associated with poor differentiation (p=0.019) and had a relatively strong association with advanced stage of disease (p=0.084). In a similar fashion, FLBN3 was significantly associated with advanced disease (p=0.027) and with the presence of lymph node metastasis (p=0.029). Furthermore, CDH13 and/or FLBN3 methylation status was found to be predictive of a poor overall survival (p=0.001) and conversely the presence of methylated hMHL1 indicated a better chance of survival (p=0.046).

In a clinical setting, samples that are obtained in a non- or minimally invasive manner are preferred and there have been a number of studies based on stool and blood plasma samples to assess aberrant methylation patterns. One example is the clinically validated methylation status of the Vimentin gene (*VIM*) - which has been found in the majority of colorectal tumours (53 – 84%; Lao et al 2011) – with assays conducted on stool samples, thus providing a non-invasive mechanism for early detection of colorectal cancer. This assay is currently commercially available in the United States as the ColoGuard assay (LabCorp), and reports a sensitivity of 83% and a specificity of 82%. [89, 90] Outside of the United States, in Europe and the Middle East there is now an additional non-invasive test for early detection of colorectal cancer by assessing the methylation status of *SEPT9*. This assay is currently commercially available as Epi *pro*Colon (Epigenomics AG).

The field of epigenomics, and epigenetics, applied to colorectal cancer is providing valuable insights into underlying mechanisms of this disease and seems to be a promising avenue of research. It is clear that assessing the status of DNA methylation alone holds prognostic and predictive value, and as such we would expect this field to gain much momentum towards translating these findings into a routine clinical setting. As this field of study can be conducted on a broad spectrum of sample types, along with commercially available kits to assess methylation, this field is likely to see an increased number of significant findings in the near future. However, as in the case of gene expression-based biomarkers, each epigenetic finding will need to be assessed in patients of diverse ethnicities. A recent study by Nieminen et al [91]

highlighted this in observing that colorectal carcinomas in an Egyptian cohort had a significantly higher state of methylation, in microsatellite stable tumours, compared with sporadic colorectal cancers in a Finnish cohort.

5.1. Proteomics

The field of proteomics deals with identification of the total complement of peptides and proteins expressed in a cell, tissue or an organism and is in principle more directly related to phenotypic changes associated with disease pathogenesis. Proteomic studies are able to define: the functional state of protein activities; protein-ligand interactions; protein-protein interactions; and a host of dynamic post-translational modifications such as glycosylation, phosphorylation, ubiquitinylation, SUMOylation, proteolytic cleavage, lipoylation and acetylation of proteins. The proteome of a cell may vary from one time point to another and in different states of health and disease so proteomics techniques have been employed to identify cancer specific proteomes as a means to identify candidate biomarkers of early disease. A huge amount of data is generated from single proteomic experiments and these are typically analyzed to understand the mechanistic pathways of pathologic events in protein networks using various databases, workflows and algorithms. For proteomics analysis, complex mixtures of proteins derived from a given biological sample are typically rendered into a set of peptides via proteolysis, most commonly using the enzyme trypsin; direct liquid chromatography-tandem mass spectrometry (LC-MS/MS) based methods are then typically employed to separate, quantify and identify thousands of individual tryptic peptides in a sample [92-94], from which the identity and quantity of the parent proteins in the original biological sample can be inferred. For example, one notable recent study identified and quantified >7,000 unique proteins from FFPE tissue blocks from individual colorectal cancer patients [95], amply demonstrating the potential of the technology.

Common sources of current proteomics biomarkers for CRC include stool, blood, biopsy and urine samples. A common fecal proteomics biomarker in use today is hemoglobin [96], while carcinoembryonic antigen (CEA) is a common blood-based biomarker currently in use. [97] Tissue inhibitor of metalloproteinase 1 (TIMP-1) has also been used and has been found useful in the early detection of cancer although, paradoxically, other studies using multivariate analysis have described TIMP-1 as independent of the stage of cancer. [98] Proteasome activator complex subunit-3 (PSME-3), nicotinamide N-methyltransferase (NNMT), collapsin response mediator protein-2 (CRMP-2), MIF, M2-PK, M-CSF, HNP 1-3, CCSA-2, CCSA-3, CCSA-4, laminin, MMP-9, MMP-7, and a host of other serum proteomics biomarkers are being developed and optimized for clinical use. For example, surface enhanced laser desorption-ionisation (SELDI) mass spectrometry was used to analyze the serum of 62 CRC patients compared to 31 controls, and four proteomic markers were found in detectable levels in the serum of CRC patients: apolipoprotein C1, alpha-1-antitrypsin, C3a-desArg and transferrin. [99] A separate study carried out at the Mayo Clinic revealed elevated levels of 5 serum biomarkers in CRC: DcR3, TRAIL-2, spondin-1, MIC 1 and Reg IV [100]; usefully, this '5-biomarker panel' has been reported to exceed the performance of CEA both in specificity and sensitivity. Immunoproteomics - involving techniques such as immunoblotting, ELISA and

immunocapture-mass spectrometry - has also yielded candidate biomarkers in CRC, including antibodies against inosine monophosphate dehydrogenase II [101], MUC-5A, MUC-1, MAPKAPK3, AVCR2B, HlpA, RpL7/L12, and nucleobindin- 1. [102-106]

5.2. Lipidomics

Lipidomics is a novel 'omics field which deals with complex large scale analysis of the full complement of various classes of lipids and lipid networks expressed by a cell, tissue or organism (the 'lipidome') and involves the high throughput systems-level identification and quantification of lipid metabolic pathways that may be involved in disease using chromatographic methods coupled to mass spectrometry. Generally, lipids are hydrophobic molecules that are involved in energy storage, structural components of a cell, cell signaling, endocrine actions, signal transduction, membrane trafficking, and morphogenesis. Structurally, lipids can be classified as: fatty acids; glycerolipids; glycerophospholipids; sphingolipids; sterol lipids; prenol lipids; saccharolipids; and polyketides. These 8 classes of lipids can also be further subdivided into several subclasses.

Whilst there is currently a paucity of clinically validated lipidomics biomarkers for colorectal cancer, the prospects of this field to complement proteomics and genomics in a combined 'systems biology' approach for disease detection, monitoring and treatment is worth consideration since over the past two decades, numerous publications have described the perturbation of lipid metabolism and signaling in colorectal carcinogenesis. [107-110] Lipidomics analysis of primary and metastatic colorectal cancer cell lines (SW480 and SW620) identified 600 and 694 lipids respectively in 'shotgun' study [111]; increased level of triglyceride lipid and plasmacholine were observed, while a decrease in the level of C-16 containing sphingomyelin, ceramide lipid and plasmenylethanolamine were observed in the metastatic CRC cell line compared to the primary isogenic CRC cell line, implying that lipidomic biomarkers of metastatic CRC disease might be plausible.

Empirically, polyunsaturated fatty acids have been known to be more beneficial in the prevention of colorectal diseases compared to saturated long-chain types, but the pathways related to this were largely unclear. The emergence of lipidomics techniques however has revealed that metabolic control of long chain fatty acids is an important factor in development of CRC, with short chain fatty acids from the gut microflora/ microbiome being described as onco-preventive. [112]

Elevated level of lysophosphatidic acid - a phosphoglyceride - has been described as a prospective cancer biomarker of ovarian tumours [113-115] but, paradoxically, a marked decrease in the serum level of lysophosphatidylcholine has been reported in CRC. [116] Similarly, elevated profiles of phosphatidylcholine and choline kinase activity have been demonstrated it colon cancers [117] and a high ratio of phosphatidylcholine to phosphatidylethanolamine has been used to differential metastatic colon cancers from localized ones. [118] Elevated levels of sphingomyelin have also been reported to characterize human colon cancer, based on nuclear magnetic resonance (NMR) studies [119], whilst cancer cell motility was shown to be down-regulated by the interaction between CD9 and sialoglycosphingolipid GM3 using CRC cell lines [120] and ceramides have been found to induce apoptosis in CRC cell lines

(HT-29, LOVO, and HCT-116). [121-123] Urinary phospholipids analysis using nanoflow LC-ESI MS/MS has been previously used for the analysis of breast [124, 125]) and prostate cancer [124], but there is a dearth of literature on the application of this method to colorectal cancer. Interestingly though, urinary levels of metabolites of prostaglandin E_2 have been used as a biomarker for colorectal cancer risk evaluation. [126, 127] Overall, these observations of disease-associated variation in colorectal cancer lipid profiles provide a sound precedent for the future development of reliable lipidomics biomarkers.

5.3. Metabolomics

The relatively new field of 'omics techniques that investigates the presence and abundance of low molecular weight metabolites in cells and body fluids is known as 'metabolomics'. This new branch in the 'omics world has emerged to address molecular biologic problems that have hitherto not been amenable to genomics or proteomics approaches. Common specimens compatible with metabolomics experiments include urine, serum and tissue. As is the case for genomics, proteomics and lipidomics, the 'metabolome' changes depending on physiologic and pathologic states of an individual and identification of unique metabolites provides potentially useful insight into pathogenetic mechanism of disease.

A number of analytical techniques have been used for metabolomics research, including: gas chromatography mass spectrometry (GC-MS); liquid chromatography mass spectrometry (LC-MS); capillary electrophoresis mass spectrometry (CE-MS); matrix assisted laser desorption-ionization mass spectrometry (MALDI-MS); and nuclear magnetic resonance (NMR). [128] By way of example, metabolomics studies on CRC patient serum samples, using a combination of proton-NMR and GC-MS techniques, was used to differentiate locoregional CRC from metastatic types as well as to identify CRC that metastasized to the liver. [129] Separately, in a review of eight metabolomics studies on CRC for diagnostic accuracy and distinguishing metabolites, twelve metabolites were found to be elevated. [130] In a further study using GC-MS, 34 endogenous metabolites were found significantly elevated in CRC compared with health individual, whilst the serum 3-hydroxybutyric acid level was noted to be reduced. [131] A predictive model developed in yet another study comprised of 2-hydroxybutyrate, kynurenine, cystamine and aspartic acid and was found to have specificity, sensitivity and accuracy as high as 85%, 85%, and 85% respectively. [128] Finally, Cheng et al [132] found evidence of dysregulation of several metabolic pathways through urine analysis of colorectal cancer patients, whilst Qui et al [133] also observed evidence of similar perturbations in tricarboxylic acid (TCA) and tryptophan metabolic pathways. With a solid foundation, a panel of metabolite markers may ultimately be developed for metabolomic profiling of colorectal patients as a means to improve diagnosis.

5.4. Molecular Imaging

Visualisation of precursor lesions and of malignant tissue is a major aspect of diagnosis and monitoring of therapeutic interventions in oncology. Classically this has been carried out using anatomical and functional technologies, such as Ultrasound (US), Computerised Tomography (CT), and Magnetic Resonance Imaging (MRI). While these approaches have been the mainstay

of medical imaging, they are limited to information at the gross anatomical and physiological levels respectively. Recent years have however seen the emergence of molecular imaging and its addition to the repertoire of techniques to visualise disease.

In previous sections we have discussed how a range of different 'omics technologies have facilitated an in-depth view of the pathophysiology and molecular pathology of cancer. These insights provide candidate biomarkers based on the identification of differentially expressed genes and proteins between tumours and normal tissue. These biomarkers have either been associated with prognosis, stage of disease, molecular subtypes, predictors of treatment outcome, and markers of discriminating carcinoma from benign lesions at the molecular level.

In this light, molecular imaging is then the science of utilising the ever-increasing knowledge of cancer molecular pathology to: identify the appropriate molecular targets; design molecular constructs to selectively and specifically interact with them; and produce a visual signal that is measurable through an imaging technology. In order for this methodology to succeed at the molecular level, the target should be available for interaction and should be unique enough to ensure selective and specific interactions. Furthermore, once a signal has been generated from the appropriate interaction, it is important that a suitably sensitive imagining technology exists in order to visualise, and quantify, signal emission.

In the context of colorectal cancer, plasma-membrane associated proteins are generally considered good candidate targets since they are reasonably accessible and a subset of them play vital roles in signal transduction. In a similar fashion, aberrantly expressed or deregulated intracellular and secreted enzymes represent potentially useful targets for molecular imaging, based on their ability to catalyse formation of spectroscopically-measurable products. Both of these types of targets are accessible through the extensive vascularisation of the colorectum, but more importantly these targets can be accessed via the luminal surface through topical application of an appropriately designed molecular construct.

Molecular imaging provides tremendous opportunities to enhance the early detection of CRC, as well as potentially aiding the demarcation of clear margins during surgical resection, as reviewed recently by Abdullah [134], Seaman et al [135] and Akin et al [136].

By way of illustration of the potential of molecular imaging methods, colorectal cancers have been found to naturally emit a red fluorescent signal due to the accumulation of protopor-phyrin IX (PpIX) in primary colorectal tumours and associated metastases located in lymph nodes. [137] These authors postulated that endogenous PpIX accumulates as a result of aberrant metabolic changes in the CRC cells; Kemmner et al [138] subsequently provided supporting evidence for this hypothesis by showing that there is a significant down-regulation of ferrochelatase (FECH) mRNA expression in gastric, colon, and rectal carcinomas, leading to accumulation of PpIX. In an effort to utilise this information Moesta et al [137] found that metastatically involved lymph nodes could be identified compared to all other palpable nodes; in the context of previously untreated patients (n=24), this observation had a sensitivity of 62% and a specificity of 78% (p < 0.0001). However, in a neoadjuvant setting there was a reduction in PpIX fluorescence in primary tumours, and a drastic reduction of fluorescent signal in metastases that resulted in not being able to discriminate between lymph nodes containing

metastatic cells. In a follow-up study by Wan et al [139], conducted in xenografted nude mice, a novel siRNA-mediated knockdown of FECH was used to enhance the accumulation of PpIX, thereby increasing the endogenous fluorescence in tumour cells. While these results still need to be developed further, and tested in a clinical trial, they do hold promise for increasing the accuracy of early detection of primary and metastatic lesions and monitoring therapeutic response based on the size of the visualised tumour.

In different work, differential gene expression profiling, confirmed by immunohistochemistry, demonstrated that matrix-metalloproteases (MMPs) are differentially expressed in the context of colorectal adenocarcinoma by macrophage subpopulations and, at times, by the tumours themselves. MMPs are a family of zinc-dependent endopeptidases with multiple human peptidase members. For example, MMP-9 is able to degrade specific components of the extracellular matrix, including type IV collagen, after activation of the secreted zymogen [140] and as a direct consequence, malignant cells are thereby able to become mobile and achieve metastatic potential.

Of particular interest to surgical resection was the finding that colorectal adenocarcinomas express MMP-9 *via* a distinct macrophage subpopulation found at the edge of primary tumours and local lymph node metastases. [140] Fudala et al [141] have subsequently designed a dual fluorophore beacon molecule that is specifically cleaved by MMP-9, resulting in the emission of a specific fluorescent signal, suggesting that if a suitably non-invasive and anti-immuno‐genic method is developed to administer the beacon molecule to an anatomical structure under investigation, then accurate measurement of the tumour edge may be possible during surgical resection procedures.

As can be seen from the above examples, molecular imaging holds considerable promise for application in CRC. As this field becomes more developed and is validated through clinical trials, it should provide improved visualisation ability coupled with the ability to quantify specific molecules, enabling novel insights into diagnosis, prognosis, treatment response monitoring, and underlying tumour physiology and molecular pathology in CRC.

5.5. Cancer nanotechnology

Nanotechnology as a division of engineering concerned with the manipulation of atomic and subatomic molecules has recently found its place in the detection, staging, imaging, and management of human cancers. Various physical, chemical and biologic principles have been applied to improve the diagnosis and treatment using elements and molecules in the Nano-range ($\sim 10^{-9}$). [142]

For diagnosis of CRC, nanoparticles have been used to enhance the precision and reliability of colonoscopy and other conventional diagnostic methods, largely resulting in earlier detection and obviating variables such as operator skills and speed of examination. Quantum dots (QD) and surface-enhanced Raman scattering (SERS) are two important nano- method‐ologies used to improve tissue based diagnosis of cancer, avoiding the cumbersome protocols and lower reliability of multiplexed tissue staining. Both methods have the ability to detect multiple biomolecular signals in a single cancer cell. Gold and silver particles have been

derivatized and stabilized with Raman active particles and silica respectively for used in generating composite organic and inorganic nanoparticles (COINs) for potential improvement of biopsy diagnosis. [143] Simultaneous Multiple Aptamers and RGD Targeting (SMART) cancer probes have also been used to detect multiple cancer biomarker signals using currently available imaging techniques. [144] Superconducting quantum interference device (SQUID) sensors and magnetic relaxometry - which are both nanotechnology based techniques - have been reported to be more accurate in the diagnosis of breast cancer than mammography and MRI respectively. KRAS mutant alleles have been detected in gastrointestinal malignancies, including colorectal carcinoma, using nanofluidic digital PCR which showed a better performance in detection of mutation KRAS in colorectal adenomas compared to conventional PCR. [145] Nanoparticles have been coupled with short cancer specific oligonucleotides (aptamers) for targeted binding to prostate specific membrane antigen positive cells in prostate cancer cell lines. [146] Finally, serum detection of colorectal cancer has been achieved with gold nanoparticles using SERS spectroscopy in a study which exemplified the use of this approach as a viable, minimally invasive screening method for colorectal cancer. [147]

Nanotechnology also has significant potential in the development of targeted therapeutics for cancer, with many studies currently at experimental- and a few at clinical validation stages. Different types of nanostructures, including mesoporous silica nanoshells, dendrimers, supramagnetic iron cores, nanosuspensions, gold nanoparticles, nanolipogels, nanoemulsions, carbon nanotubes, titanium oxide nanoparticles, liposomes, polymeric miscelles, and other lipid based nanoparticles have been used as drug delivery vehicles and facilitators of targeted cancer therapies. [148-155] Although most of the cancer nanodiagnostic and nanotherapy studies are still in their infancy, it seems clear that nanotechnology will play an important role in colorectal cancer diagnostics and therapeutics in the future.

6. Prospects of 'Omics based molecular approaches in colorectal cancer diagnosis and treatment in a developing country: A case study in Cape Town

Groote Schuur Hospital, situated in Cape Town, South Africa, is a quaternary hospital where the many colorectal cancer patients from the Western Province region are treated by combinations of radiotherapy, colonic resection and standard chemotherapeutic regimens. However, there are two complicating factors involved in treating these patients with the greatest efficacy: (1) the relatively high cost of utilising platinum- or modern monoclonal antibody-based regimens; and (2) the fact that the majority of patients present with advanced stages of disease, typically between TNM stage II and III. As mentioned earlier, these stages of disease have relatively high recurrence rates and as such timely diagnosis and efficacious treatment schedules are needed to reduce disease recurrence and improve patient prognoses.

To illustrate this point, consider the cost of the standard chemotherapeutic regimen of 5-fluorouracil (5-FU) alone versus Oxaliplatin: One cycle of Oxaliplatin costs approximately ZAR 5,000.00 (~USD 560), while one cycle of 5-FU is drastically less at a cost of ZAR 200 (~USD 22).

The response rate obtained with 5-FU alone can be improved by utilising Leucovorin (LV) in a combination therapy approach. However, it has been observed that the further addition of Oxaliplatin or Irinotecan can improve the stage II/III CRC response rates drastically to around 40 – 50%. [156, 157] The relatively recent appearance of monoclonal antibody based therapies has also offered significant gains in treatment response rates.

South Africa is a developing nation with a limited budget for treating non-communicable diseases such as cancer, not least because a large proportion of the country's healthcare budget is understandably spent on addressing the concurrent HIV/AIDS and TB epidemics (see for example the Lancet series on "Health in South Africa" published in 2009, with particular emphasis on the following publications: Abdool et al [158]; Chopra et al [159]; Coovadia et al [160]; and Mayosi et al [161]). Considering these financial constraints, two possible situations can be envisaged in which a patient treated in the public sector (i.e. subject to government healthcare budgets) could access platinum-based regimens and/or modern biological thera‐ peutic agents:

The first opportunity for a patient to access medication with greater efficacy would be through participation in clinical trials conducted by pharmaceutical companies either wanting to assess their treatment in ethnically diverse cohorts (which could be required by local regulatory authorities) or to explore a new disease indication for an existing therapeutic agent. The second opportunity might be one afforded by the use of 'omics approaches, leveraging biomarker panels to provide predictive indications of whether or not a patient might benefit from a particular chemo- or biological therapeutic agent. Furthermore, given the heterogeneity of tumours and the unique molecular subtypes of colorectal cancer, it would be instructive to assess the relative likelihood of recurrent disease. Such a measurement could be used to motivate the aggressiveness of the treatment regimen prescribed.

In terms of possible cost effective benefits from the addition of platinum-based drugs to the standard regimen of 5-FU and LV, a trial conducted in the United Kingdom found that the relative levels of topoisomerase-1 (Topo1), assessed by routine immunohistochemistry, could be used to identify patient subpopulations who could potentially benefit from the addition of Oxaliplatin to their 5-FU/LV regimen [162]. Furthermore, Paré and colleagues [163] reported that a particular polymorphism in the excision repair cross-complementing 1 *ERCC1* gene (codon 118) can predict response and overall survival in patients treated with an Oxaliplatin/ 5-FU/LV regimen. Provided that these markers are further validated in a clinical setting, it then stands to reason that a simple immunohistochemical or real-time polymerase chain reaction assay could therefore be routinely requested to determine likely response to Oxaliplatin and therefore to motivate additional expenditure on an Oxaliplatin-supplemented regimen. This type of personalised approach has the obvious advantage of improving treatment efficacy, and reducing the risk of disease recurrence with a concomitant cost saving for the hospital authority from not having to conduct lengthy additional treatments, after possible first round treatment failure.

Similarly, assessment of the common SNPs that are predictive of benefit from the limited array of biological agents (e.g. the mutational status of the KRAS and BRAF genes) could provide

an indication of whether or not the comparatively expensive anti-epidermal growth factor receptor mAb therapy should be prescribed.

As discussed above, a number of gene expression panels could also be used to predict patient prognosis and the associated likelihood of disease recurrence, with results being used to design a personalised treatment regimen in which the aggressiveness of the treatment schedule correlates with the severity of disease, the most likely prognosis and the likelihood of recurrence. In this context, Genomic Health's Onco*type* DX® Colon Cancer Assay costs ~USD 3,200 [45], which translates to approximately ZAR 28,560 in South Africa; this assay could provide a very useful clinical metric of the likelihood of recurrence, particularly in complicated TNM stage II cases and stage III cases where recurrence rates are still unfavorably high, but in reality such a test is beyond the financial means available for treatment of CRC disease at a public facility in South Africa today, not least since its cost dwarfs even that of Oxaliplatin-based treatments. The prospects for wide uptake of the Onco*type* DX® Colon Cancer Assay in South Africa therefore seem remote.

As the complexities of biopsy heterogeneity are addressed by the biomedical community, it is thus clear that local validation studies of simple and cost-effective, assays will be necessary to ensure that the prognostic and treatment outcome biomarkers reported in the literature apply to the diverse ethnicities in South Africa. Given the financial constraints imposed by the government funded healthcare system, it appears that relatively inexpensive techniques such as PCR based SNP assays would be well-suited to the public sector since, despite being a simple, low cost assay, the results could have a profound impact on treatment outcomes for the patients afflicted with this disease. Importantly, if utilised correctly such a molecular diagnostics strategy could actually result in significant cost savings mid-term for the hospitals administering care by avoiding the complexities of treatment failures.

7. Conclusion

'Omics based techniques represent novel, scientifically sound approaches to the diagnostic and therapeutic aspects of managing colorectal cancer patients, but there remain very significant challenges regarding their uptake and wide utilisation in developing world healthcare settings, primarily due to financial considerations. None-the-less, surgeons, clinicians, basic medical researchers and all other healthcare workers at the cutting edge of colorectal cancer management need to remain abreast of the prospects and potential effectiveness of integrating molecular approaches in to colorectal cancer management. The old paradigm where patients had no active choice or participation in their disease management, with treatment choices being exclusively the decision of the clinician, is under threat today since many patients now have access to information about emerging therapeutic options *via* the internet. It is therefore important that surgeons and clinicians, in spite of their invariably tight schedules, consider some form of participation in basic medical research in order to contribute clinical perspectives as well as to improve their understanding of molecular approaches to diagnosis and treatment.

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References

[1] Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008; 455: 1061-68.

[2] Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011; 474: 609-15.

[3] Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487: 330-37.

[4] GLOBOCAN cancer fact sheets 2008 (IARC). Available at: http://globocan.iarc.fr/fact-sheets/cancer/colorectal.asp. Accessed June 26, 2012.

[5] Mqoqi N, Kellet P, Sitas F et al. Cancer in South Africa, 1998-1999. National Cancer Registry of South Africa, National health Laboratory Service, Johannesburg, 2004.

[6] Slaughter DP, Southwick HW, Smejkal W. "Field Cancerization" in oral stratified squamous epithelium. Cancer 1953; 6 (5): 963-968.

[7] Tabor MP, Braakhuis BJ, Van der Waal JE et al. Comparative molecular and histological grading of epithelial dysplasia of the oral cavity and the oropharynx. J Pathol 2003; 199: 354-360.

[8] Braakhuis BJ, Brakenhoff RH, Leeman CR. Second field tumors: a new opportunity for cancer prevention. The Oncologist 2005; 10: 493-500.

[9] Bernstein C, Bernstein H, Payne CM et al. Field defects in progression to gastrointestinal tract cancers. Cancer Letters 2008; 260: 1-10.

[10] McDonald SA, Greaves LC, Gutierrez- Gonzalez L et al. Mechanism of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells. Gastroenterology 2008; 134: 500-510.

[11] Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology 2007; 50: 113-130.

[12] Yap TA, Gerlinger M, Futreal PA et al. Intratumor heterogeneity: Seeing the wood for the trees. Sci Transl Med 2012; 4 (127): 127ps10.

[13] Michor F, Polyak K. The origin and implication of intratumor heterogeneity. Cancer Prev Res (Phila) 2010; 3(11): 1361-1364.

[14] Gerlinger M, Rowan AJ, Horswell S et al. Intratumour heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012; 366 (10): 883-93.

[15] Rocco A, Compare D, Nardone G. Cancer stem cell hypothesis and gastric carcinogenesis: experimental evidence and unsolved questions. World J Gastrointest Oncol 2012; 4 (3): 54-59.

[16] Luo W, Wu F, Atlas SR et al. Defining genetic intra-tumour heterogeneity: a chronological annotation of mutational pathways. Proceedings in Obstetrics and Gynecology 2010; 1 (1): 9.

[17] Durrett R, Foo J, Leder K et al. Intratumor heterogeneity in evolutionary models of tumor progression. Genetics 2011; 188 (2): 461-77.

[18] Iwasa Y, Michor F. Evolutionary dynamics of intratumor heterogeneity. PLoS One 2011; 6 (3): e17866.

[19] Polley AC, Mulholland F, Pin C et al. Proteomic analysis reveals field-wide changes in protein expression in the morphologically normal mucosa of patients with colorectal neoplasia. Cancer Res. 2006 Jul 1;66(13):6553-62.

[20] Chen LC, Hao CY, Chiu YS et al. Alteration of gene expression in normal-appearing colon mucosa of APC(min) mice and human cancer patients. Cancer Res. 2004 May 15;64(10):3694-700.

[21] Hao CY, Moore DH, Wong P et al. Alteration of gene expression in macroscopically normal colonic mucosa from individuals with a family history of sporadic colon cancer. Clin Cancer Res. 2005 Feb 15;11(4):1400-7.

[22] Hao CY, Moore DH, Chiu YS et al. Altered gene expression in normal colonic mucosa of individuals with polyps of the colon. Dis Colon Rectum. 2005 Dec;48(12): 2329-35.

[23] Suter CM, Martin DI, Ward RL. Hypomethylation of L1 retrotransposons in colorectal cancer and adjacent normal tissue. Int J Colorectal Dis. 2004 Mar;19(2):95-101. Epub 2003 Oct 8.

[24] Kreso A, O'Brien CA, van Galen P et al. Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. Science 2013 Feb 1;339(6119):543-8. doi: 10.1126/science.1227670. Epub 2012 Dec 13.

[25] Fisher R, Larkin J, Swanton C. Inter and intratumour heterogeneity: a barrier to individualized medical therapy in renal cell carcinoma. Frontiers in oncology 2012; 2 (49): 1-5.

[26] Swanton C, Larkin JM, Gerlinger M et al. Predictive biomarker discovery the parallel integration of clinical trial and functional genomics datasets. Genome Med 2010; 2: 53-63.

[27] Duke CE. The classification of Cancer of the rectum. Journal of Pathologic Bacteriology 1932; 35: 323-32.

[28] Astler VB, Coller FA, The prognostic significance of direct extension of carcinoma of the colon and rectum. Am Surg 1954; 139 (6): 846- 852.

[29] Edge SB, Byrd DR, Compton CC et al. Cancer staging manual. AJCC (American joint committee on cancer). 7th ed. New York, Springer 2010.

[30] Ogino S, Goel A. Molecular Classification and Correlates in Colorectal Cancer. Journal of Molecular Diagnostics 2008; 10 (1): 13-27.

[31] Bohanes P, LaBonte MJ, Winder T et al. Predictive Molecular Classifiers in Colorectal Cancer. Seminars in Oncology 2011; 38 (4): 576-87.

[32] Vogelstein B, Kinzler KW. Cancer genes and the pathway they control. Nat Med 2004; 10 (8): 789-99.

[33] Chan SK, Griffith OL, Tai IT et al. Meta-analysis of Colorectal Cancer Gene Expression profiling studies identifies Consistently Reported Candidate Biomarkers. Cancer Epidemiol Biomarkers Prev 2008; 17: 543-52

[34] Diaz- Cano SJ. Tumour Heterogeneity: Mechanism and bases for a reliable application of molecular marker design. Int. J. Mol. Sci. 2012; 13: 1951-2011.

[35] Nirmalan NJ, Harnden P, Selby PJ et al. Mining the archival formalin-fixed paraffin-embedded tissue proteome: opportunities and challenges. Mol BioSyst 2008; 4: 712-20.

[36] Dotti I, Bonin S, Basili G et al. Effect of formalin, Methacarn, and FineFIX fixatives on RNA preservation. Diagn Mol Pathol 2010; 19 (2): 112-22.

[37] Chung JY, Braunschweig T, Williams R et al. Factors in tissue handling and process‐ ing that impact RNA obtained from formalin-fixed, paraffin-embedded tissue. J His‐ tochem Cytochem 2008;56:1033–42.

[38] Gnanapragasam VJ. Unlocking the molecular archive: the emerging use of formalin-fixed paraffin-embedded tissue for biomarker research in urological cancer. BJU In‐ ternational 2009; 105: 274-78.

[39] Bakay M, Chen Y, Borups R et al. Sources of variablility and effect of experimental approaches on expression profiling data interpretation. BMC Bioinformatics 2002; 3:4.

[40] Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005; 23: 609-18.

[41] Mori S, Ogata Y, Shirouzu K et al. Biological features of sporadic colorectal carcino‐ ma with high-frequency microsatellite instability: special reference to tumor prolifer‐ ation and apoptosis. Int J Clin Oncol 2004; 9: 322-29.

[42] Soreide K, Gudlaugsson E, Skaland I et al. Metachronous cancer development in pa‐ tients with sporadic colorectal adenomas- multivariate risk model with independent and combined value of hTERT and surviving. Int J Colorectal Dis 2008; 23: 389-400.

[43] Soreide K, Nedrebo BS, Knapp JC et al. Evolving molecular classification by genomic and proteomic biomarkers in colorectal cancer: potential implication for the surgical oncologist. Surgical Oncology 2009; 18: 31-50.

[44] Markowitz SD, Dawson DM, Willis J et al. (2002). Focus on colon cancer. Cancer cell; 1: 233-6.

[45] Carlson B. Putting a lid on chemotherapy costs. Biotechnol Healthc. 2011; 8(2):15-8.

[46] Libutti SK, Saltz LB, Tepper JE. Colon cancer. In: DeVita, V. T. Jr, Lawrence, T. S., Rosenberg, S. A. eds. DeVita, Hellman, and Rosenberg's cancer: principles and prac‐ tice of oncology. Vol. 1. Philadelphia: Lippincott Williams & Wilkins 2008, 1232-1284.

[47] Compton C, Hawk ET, Grochow L et al. Colon cancer. In: Abeloff, M.D., Armitage J., Niederhuber J. E et al, eds. Abeloff's clinical oncology. Philadelphia: Churchill Liv‐ ingstone 2008, 1477-1534.

[48] O'Connell MJ, Lavery I, Yothers G et al. Relationship between tumor gene expression and recurrence in four independent studies of patients with stage II/III colon cancer treated with surgery alone or surgery plus adjuvant fluorouracil plus leucovorin. J Clin Oncol 2010 Sep 1;28(25):3937-44. doi: 10.1200/JCO.2010.28.9538.

[49] Kerr D, Gray R, Quirke P, Watson D, Yothers G, Lavery IC, Lee M, O'Connell MJ, Shak S, Wolmark N, and Genomic Health and QUASAR Colon Teams.A quantitative multigene RT-PCR assay for prediction of recurrence in stage II colon cancer: Selec‐ tion of the genes in four large studies and results of the independent, prospectively designed QUASAR validation study Journal of Clinical Oncology, 2009 ASCO Annu-

al Meeting Proceedings (Post-Meeting Edition). Vol 27, No 15S (May 20 Supplement), 2009: 4000

[50] Salazar R, Roepman P, Capella G et al. Gene expression signature to improve prognosis prediction of stage II and III colorectal cancer. J Clin Oncol. 2011 Jan 1;29(1): 17-24. doi: 10.1200/JCO.2010.30.1077.

[51] Agesen TH, Sveen A, Merok MA et al. ColoGuideEx: a robust gene classifier specific for stage II colorectal cancer prognosis. Gut. 2012; 61(11): 1560-67.

[52] Tabas-Madrid D, Nogales-Cadenas R, Pascual-Montano A: GeneCodis3: a non-redundant and modular enrichment analysis tool for functional genomics. Nucleic Acids Research 2012; doi: 10.1093/nar/gks402.

[53] Nogales-Cadenas R, Carmona-Saez P, Vazquez M et al: GeneCodis: interpreting gene lists through enrichment analysis and integration of diverse biological information. Nucleic Acids Research 2009; doi: 10.1093/nar/gkp416.

[54] Carmona-Saez P, Chagoyen M, Tirado F et al: GENECODIS: A web-based tool for finding significant concurrent annotations in gene lists. Genome Biology 2007 8(1):R3.

[55] Shi M, Beauchamp RD, Zhang B. A network-based gene expression signature informs prognosis and treatment for colorectal cancer patients. PLoS One 2012; 7(7): e41292. doi: 10.1371/journal.pone.0041292.

[56] Asghar U, Hawkes E, Cunningham D. Predictive and prognostic biomarkers for targeted therapy in metastatic colorectal cancer. Clin Colorectal Cancer. 2010 Dec;9(5): 274-81. doi: 10.3816/CCC.2010.n.040.

[57] Bandrés E, Zárate R, Ramirez N et al. Pharmacogenomics in colorectal cancer: the first step for individualized-therapy. World J Gastroenterol. 2007 Nov 28;13(44): 5888-901.

[58] Benhaim L, Labonte MJ, Lenz HJ. Pharmacogenomics and metastatic colorectal cancer: current knowledge and perspectives. Scand J Gastroenterol. 2012 Mar;47(3): 325-39. doi: 10.3109/00365521.2012.640832.

[59] Coate L, Cuffe S, Horgan A et al. Germline genetic variation, cancer outcome, and pharmacogenetics. J Clin Oncol. 2010 Sep 10;28(26):4029-37. doi: 10.1200/JCO. 2009.27.2336.

[60] De Roock W, De Vriendt V, Normanno N et al. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol. 2011 Jun;12(6):594-603. doi: 10.1016/S1470-2045(10)70209-6.

[61] Ross JS, Torres-Mora J, Wagle N et al. Biomarker-based prediction of response to therapy for colorectal cancer: current perspective. Am J Clin Pathol. 2010 Sep;134(3): 478-90. doi: 10.1309/AJCP2Y8KTDPOAORH.

[62] Heidelberger C., Chaudhuri NK, Danneberg P et al. Fluorinated Pyrimidines, A New Class of Tumor Inhibitory Compounds. Nature 179: 663-666.

[63] Fernandez-Rozadilla C, Cazier JB, Moreno V et al.Pharmacogenomics in colorectal cancer: a genome-wide association study to predict toxicity after 5-fluorouracil or FOLFOX administration. Pharmacogenomics J. 2012. doi: 10.1038/tpj.2012.2.

[64] Hsiang YH, Liu LF, Wall ME et al. DNA topoisomerase I-mediated DNA cleavage and cytotoxicity of camptothecin analogues. Cancer Res. 1989; 49(16): 4385-9.

[65] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100(1):57-70.

[66] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5): 646-74. doi: 10.1016/j.cell.2011.02.013.

[67] Takahashi Y, Kitadai Y, Bucana CD et al. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995; 55: 3964-3968.

[68] Presta LG, Chen H, O'Connor SJ et al. Humanization of an anti- vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 1997; 57: 4593-4599.

[69] Mayer A, Takimoto M, Fritz E et al. The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and mdr gene expression in colorectal cancer. Cancer 1993; 71: 2454-2460.

[70] Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res 2001; 7: 2958-70.

[71] Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008; 358: 1160–74.

[72] Baselga J. The EGFR as a target for anticancer therapy--focus on cetuximab. Eur J Cancer 2001; 37 Suppl 4: S16-S22

[73] Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2010; 31:27–36.

[74] Khare S, Verma M. Epigenetics of colon cancer. Methods Mol Biol 2012;863:177-85. doi: 10.1007/978-1-61779-612-8_10.

[75] Lao VV, Grady WM. Epigenetics and colorectal cancer. Nat Rev Gastroenterol Hepatol. 2011 Oct 18;8(12):686-700. doi: 10.1038/nrgastro.2011.173.

[76] Ballestar E, Esteller M. Epigenetic gene regulation in cancer. Adv Genet. 2008; 61:247–267.

[77] Hatziapostolou M, Iliopoulos D. Epigenetic aberrations during oncogenesis. Cell Mol Life Sci. 2011;68(10):1681-702. doi: 10.1007/s00018-010-0624-z.

[78] Liu M, Chen H. The role of microRNAs in colorectal cancer. J Genet Genomics. 2010 Jun;37(6):347-58. doi: 10.1016/S1673-8527(09)60053-9.

[79] Sawan C, Herceg Z. Histone modifications and cancer. Adv Genet. 2010; 70:57–85.

[80] Ting AH, McGarvey KM, Baylin SB. The cancer epigenome--components and functional correlates. Genes Dev. 2006; 20:3215–3231.

[81] Van Engeland M, Derks S, Smits KM et al. Colorectal cancer epigenetics: complex simplicity. J Clin Oncol. 2011; 29:1382–1391.

[82] Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002; 16:6–21.

[83] Feinberg A, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. 1983; 301:89, 92.

[84] Weisenberger DJ, et al. DNA methylation analysis by digital bisulfite genomic sequencing and digital MethyLight. Nucleic Acids Res. 2008; 36:4689–4698.

[85] Curtin K, Slattery ML, Samowitz WS. CpG island methylation in colorectal cancer: past, present and future. Patholog Res Int. 2011; 2011:902674.

[86] Mao C, Liao RY, Qiu LX et al. BRAF V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis. Mol Biol Rep 2011 Apr;38(4):2219-23. doi: 10.1007/s11033-010-0351-4.

[87] Tol J, Dijkstra JR, Klomp M et al. Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. Eur J Cancer 2010 Jul;46(11):1997-2009. doi: 10.1016/j.ejca.2010.03.036.

[88] Wang Z, Yuan X, Jiao N et al. CDH13 and FLBN3 gene methylation are associated with poor prognosis in colorectal cancer. Pathol Oncol Res. 2012 Apr;18(2):263-70. doi: 10.1007/s12253-011-9437-0.

[89] Itzkowitz SH, Jandorf L, Brand R et al. Improved fecal DNA test for colorectal cancer screening. Clin Gastroenterol Hepatol. 2007; 5:111–117.

[90] Itzkowitz S,Brand R, Jandorf L et al. A simplified, noninvasive stool DNA test for colorectal cancer detection. Am J Gastroenterol. 2008; 103:2862–2870.

[91] Nieminen TT, Shoman S, Eissa S et al. Distinct genetic and epigenetic signatures of colorectal cancers according to ethnic origin. Cancer Epidemiol Biomarkers Prev 2012 Jan;21(1):202-11. doi: 10.1158/1055-9965.EPI-11-0662.

[92] Mallick P, Kuster M. Proteomics: a pragmatic perspective. Nature Biotech 2010; 27: 695-709.

[93] Domon B, Aebersold R. Options and considerations when selecting a quantitative proteomics strategy. Nature Biotech 2010; 27: 710-721.

[94] Duncan MW, Aebersold R, Caprioli RM. The pros and cons of peptide-centric proteomics. Nature Biotech 2010; 27: 659-664.

[95] Wisniewski JR, Ostasiewicz P, Dus K et al. Extensive quantitative remodeling of the proteome between normal colon tissue and adenocarcinoma. Molecular Systems Biology 2012; 8: 611.

[96] Huang CS, Lal SK, Farraye FA. Colorectal cancer screening in average risk individuals. Cancer Causes Control 2005; 16: 171-88.

[97] Bates J. Cancer Biomarkers: adoption is driving growth. Insight Pharma Reports 2008, Needham, MA, USA.

[98] Holten-Andersen M, Christensen IJ, Nilbert M et al. Association between preoperative plasma levels of tissue inhibitor of metalloproteinases 1 and rectal cancer patient survival: a validation study. Eur J. cancer 2004; 40: 64-72.

[99] Ward DG, Suggett N, Cheng Y et al. Identification of serum biomarkers for colon cancer by proteomic analysis. Br J cancer 2006; 94: 1898-1905.

[100] diaDexus Inc (2007). Clinical application of diaDexus diagnostics in colorectal cancer. http://www.diadexus.com/products/research/oncology_diagnostics.php

[101] He YJ, Mou ZR, Li WL et al. Identification of IMPDH2 as a tumor-associated antigen in colorectal cancer using immunoproteomics analysis. Int J. Colorectal Dis. 2009; 24: 1271-79.

[102] Tjalsma H. Identification of biomarkers for colorectal cancer through proteomics-based approaches. Expert Rev. Proteomics 2010; 7 (6): 879-95.

[103] Kocer B, McKolanis J, Soran A. Humoral immune response to MUC5AC in patients with colorectal polyps and colorectal carcinoma. BMC Gastroenterol 2006; 6: 4.

[104] Chen Y, Lin P, Qui SM et al. Autoantibodies to Ca+ binding protein Calnuc is a potential marker in colon cancer detection. Int J. Oncol 2007; 30: 1137-44.

[105] Babel I, Barderas R, Diaz-Uriarte R et al. Identification of tumor-associated autoantigens for the diagnosis of colorectal cancer in serum using high density protein microarrays. Mol. Cell Proteomics 2009; 8: 2382-95.

[106] Silk AW, Schoen RE, Potter DM et al. Humoral immune response to abnormal MUC1 in subjects with colorectal adenoma and cancer. Mol Immunol 2009; 47: 52-56.

[107] Merchant TE, Kasimos JN, de Graaf PW et al. Phospholipid profile of human colon cancer using 31P magnetic resonance spectroscopy. Int J Colorectal Dis 1991; 6 (2): 121-6.

[108] Dueck DA, Chan M,Tran K et al. The modulation of choline phosphoglyceride metabolism in human colon cancers. Mol Cell Biochem 1996; 162 (2): 97-103.

[109] Schonberg SA, Lundemo AG, Fladvad T et al. Closely related colon cancer cell lines display different sensitivity to polyunsaturated fatty acids, accumulate different lipid

classes and downregulates sterol regulatory element-binding protein 1. FEBS J 2006; 273 (12): 2749-65.

[110] White- Gilbertson S, Muller T, Senkal C et al. Ceramide synthase 6 modulates TRAIL sensitivity and nuclear translocation of active caspase-3 in colon cancer cells. Oncogene 2009; 28 (8): 1132-41.

[111] Fhaner CJ, Liu S, Ji H et al. Comprehensive Lipidome Profiling of Isogenic Primary and Metastatic Colon Adenocarcinoma Cell Lines. Anal Chem. 2012; 84: 8917-26.

[112] Gassler N, Klaus C, Kaemmerer E et al. Modifier-concept of colorectal carcinogenesis: Lipidomics aa a technical too in pathway analysis, World J Gastroenterol 2010; 16 (15): 1820-27.

[113] Sutphen R, Xu Y, Wilbanks GD et al. Lysophospholipids are potential biomarkers for ovarian cancer. Cancer Epidemiol Biomarkers Prev 2004; 13: 1185-91.

[114] Pozlep B, Melch M, Kobal B et al. Use of lysophosphatidic acid in the management of benign and malignant ovarian tumors. Eur J Gynaecol Oncol 2007; 28: 394-99.

[115] Melch M, Pozlep B, Mlakar A et al. Determination of serum lysophosphatidic acid as a potential biomarker for ovarian cancer. J Chromatogr B Analyt Technol Biomed Life Sci 2007; 858: 287-91.

[116] Zhao Z, Xiao Y, Elson P et al. Plasma lysophosphatidylcholine levels: potential biomarkers for colorectal cancer. J Clin Oncol 2007; 25: 2696-701.

[117] Nakagami K, Uchida T, Ohwada S et al. Increased choline kinase activity and elevated phosphocholine levels in human colon cancer. Jpn J Cancer Res 1999; 90: 419-24.

[118] Dobrzynska I, Szachowicz-Petelska B, Sulkowski S et al. Changes in electrical charge and phospholipids composition in human colorectal cancer cells. Mol Cell Biochem 2005; 276: 113-19.

[119] Merchant TE, Diamantis PM, Lauwers G et al. Characterization of malignant colon tumors with 31P nuclear magnetic resonance phospholipid and phosphate metabolite profiles. Cancer 1995; 76 (10): 1715-23.

[120] Ono M, Handa K, Sonnino S et al. GM3 ganglioside inhibits CD9- facilitated haptotactic cell motility: coexpression of GM3 and CD9 is essential in the downregulation of tumor cell motility and malignancy. Biochemistry 2001; 40: 6414-21.

[121] Zhang XF, Li BX, Doug CY et al. Apoptosis of human colon carcinoma HT-29 cells induced by ceramide. W J Gastroenterol 2006; 12: 3581-84.

[122] Tan X, Zhang Y, Jiang B et al. Changes in ceramide level upon catechins-induced apoptosis in LoVo cells. Life Sci 2002; 70: 2023-29.

[123] Ahn EH, Schroeder JJ. Sphingoid bases and ceramide induce apoptosis in HT-29 and HCT-116 human colon cancer cells. Exp Biol Med (Maywood) 2002; 227: 345-53.

[124] Min HK, Lim S, Chung BC et al. Shotgun lipidomics for candidate biomarkers of urinary phospholipids in prostate cancer. Anal Bioanal Chem 2011; 399 (2): 823-30.

[125] Kim H, Min HK, Kong G et al. Quantitative analysis of phosphatidylcholines and phosphatidylethanolamine in urine of patients with breast cancer by nanoflow liquid chromatography/ Tandem mass spectrometry. Anal Bioanal Chem 2009; 393 (6-7): 1649-56.

[126] Johnson JC, Schmidt CR, Shrubsole MJ et al. Urine PGE-M: A metabolite of Prostaglandin E2 as a potent biomarker of Advanced Colorectal Neoplasia. Clinical Gastroenterology and Hepatology 2006; 4: 1358-1365.

[127] Cai Q, Gao Y, Chow W et al. Prospective Study of Urinary Prostaglandin E2 Metabolite and Colorectal cancer Risk. Journal of Clinical Oncology 2006; 24 (31): 5010-16.

[128] Nishiumi S, Kobayashi T, Ikeda A et al. A Novel Serum Metabolomics-Based diagnostic Approach for Colorectal Cancer. PloS ONE 2012; 7 (7): e40459.

[129] Farshidfar F, Weljie AM, Kopciuk K et al. Serum metabolomic profile as a means to distinguish stages of colorectal cancer. Genome Medicine 2012; 4: 42.

[130] Wang H, Tso VK, Slupsky CM et al. Metabolomics and detection of colorectal cancer in humans: a systematic review. Future Oncology 2010; 6 (9): 1395-1406.

[131] Ma YL, Liu WJ, Peng JY et al. Study on specific metabonomic profiling of serum from colorectal cancer patients by gas chromatography-mass spectrometry. Zhonghua Wei Chang Wai Ke Za Zhi 2009; 12 (4): 386-90.

[132] Cheng Y, Xie G, Chen T et al. Distinct Urinary Metabolic Profile of Human Colorectal Cancer. J Proteome Res 2012; 11: 1354-62.

[133] Qui Y, Cai G, Su M et al. Urinary metabonomic study on colorectal cancer. Journal of Proteome Research 2010; 9: 1627-1634.

[134] Abdullah B. Molecular imaging: spawning a new melting-pot for biomedical imaging. Biomed Imaging Interv J 2006; 2(4): e28. doi: 10.2349/biij.2.4.e28.

[135] Seaman ME, Contino G, Bardeesy N et al. Molecular imaging agents: impact on diagnosis and therapeutics in oncology. Expert Rev Mol Med. 2010 Jul 15;12:e20. doi: 10.1017/S1462399410001511.

[136] Akin O, Brennan SB, Dershaw DD et al. Advances in oncologic imaging: update on 5 common cancers. CA Cancer J Clin. 2012 Nov-Dec; 62(6): 364-93. doi: 10.3322/caac.21156.

[137] Moesta KT, Ebert B, Handke T et al. Protoporphyrin IX occurs naturally in colorectal cancers and their metastases. Cancer Res. 2001; 61(3):991-9.

[138] Kemmner W, Wan K, Rüttinger S et al. Silencing of human ferrochelatase causes abundant protoporphyrin-IX accumulation in colon cancer. FASEB J. 2008; 22(2): 500-9.

[139] Wan K, Ebert B, Voigt J, Wang Q, Dai Y, Haag R, Kemmner W. In vivo tumor imaging using a novel RNAi-based detection mechanism. Nanomedicine. 2012; 8(4): 393-8. doi: 10.1016/j.nano.2012.02.004.

[140] Illemann M, Bird N, Majeed A et al. MMP-9 is differentially expressed in primary human colorectal adenocarcinomas and their metastases. Mol Cancer Res. 2006 May; 4(5):293-302.

[141] Fudala R, Ranjan AP, Mukerjee A et al. Fluorescence detection of MMP-9. I. MMP-9 selectively cleaves Lys-Gly-Pro-Arg-Ser-Leu-Ser-Gly-Lys peptide. Curr Pharm Biotechnol. 2011; 12(5):834-8.

[142] Fortina P, Kricka LJ, Graves DJ et al. Application of nanoparticles to diagnostics and therapeutics in colorectal cancer. Trends in Biotechnology 2007; 25 (4): 145-52.

[143] Sun L, Sung K, Dentinger C et al. Composite organic-inorganic nanoparticles as Raman labels for tissue analysis. Nano Letters 2007; 7 (2): 351-56.

[144] Ko HY, Choi K, Lee CH et al. A multimodal nanoparticle-based cancer imaging probe simultaneously targeting nucleolin, intergrin $\alpha v\beta 3$ and tenascin-C proteins. Biomaterials 2011; 32: 1130-38.

[145] Azuara D, Ginesta MM, Gausach M et al. Nanofluidic digital PCR for KRAS mutation detection and quantification in gastrointestinal cancer. Clin Chem 2012; 58 (9): 1332-41.

[146] Farokhzad OC, Jon S, Khademhosseini A et al. Nanoparticle-Aptamer Biconjugates: a New Approach for Targeting Prostate cancer Cells. Cancer Res 2004; 64: 7668-72.

[147] Lin D, Feng S, Pan J et al. Colorectal cancer detection by gold nanoparticle based surface-enhanced Raman spectroscopy of blood serum and statistical analysis. Opt Express 2011; 19 (14): 13565-77.

[148] Jordan M, Navel A, Brownlow B et al. Development and evaluation of tocopherol-rich argan oil-based nanoemulsions as vehicles possessing anticancer activity. J Biomed Nanotechnol 2012; 8 (6): 944-56.

[149] Goncalves AS, Macedo AS, Souto EB. Therapeutic nanosystems for oncology nanomedicine. Clin Transl Oncol 2012. 14: 883-90.

[150] Ruggeiro A, Villa CH, Holland JP et al. Imaging and treating tumor vasculature with targeted radiolabeled carbon nanotubes. Int J Nanomedicine 2010; 5: 783-802

[151] Gianella A, Jarzyna PA, Mani V et al. Multifunctional nanoemulsion platform for imaging guided therapy evaluated in experimental cancer. ACS Nano 2011; 5 (6): 4422-33.

[152] Yao L, Zhao X, Li Q et al. In vitro and in vivo evaluation of camptothecin nanosuspension: a novel formulation with high antitumor efficacy and low toxicity. Int J Pharm 2012; 423 (2): 586-88.

[153] Komizu Y, Ueoka H, Goto K et al. Remarkable inhibitory effects of hybrid liposomes on growth of human colon cancer cells through induction of cell cycle arrest along with apoptosis. Int J Nanomedicine 2011; 6: 1913-20.

[154] Cho H, Kwon GS. Polymeric miscelle for neoadjuvant cancer therapy and tumor-primed optical imaging. ACS Nano 2011; 5 (11): 8721-29.

[155] Zhang AP, Sun YP. Photocatalytic killing effect of TiO2 nanoparticles on Ls-174-t human colon carcinoma cells. World J Gastroenterol 2004; 10 (21): 3191-93.

[156] Giacchetti S, Itzhaki M, Gruia G et al. Long-term survival of patients with unresectable colorectal cancer liver metastases following infusional chemotherapy with 5-fluorouracil, leucovorin, oxaliplatin and surgery. Ann Oncol. 1999; 10(6): 663-9.

[157] Douillard JY, Cunningham D, Roth AD et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000;355(9209):1041-47.

[158] Abdool Karim SS, Churchyard GJ, Karim QA, Lawn SD. HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response. Lancet. 2009; 374(9693): 921-33. doi: 10.1016/S0140-6736(09)60916-8.

[159] Chopra M, Daviaud E, Pattinson R et al. Saving the lives of South Africa's mothers, babies, and children: can the health system deliver? Lancet 2009; 374(9692): 835-46. doi: 10.1016/S0140-6736(09)61123-5.

[160] Coovadia H, Jewkes R, Barron P et al. The health and health system of South Africa: historical roots of current public health challenges. Lancet. 2009; 374(9692):817-34. doi: 10.1016/S0140-6736(09)60951-X.

[161] Mayosi BM, Flisher AJ, Lalloo UG et al. The burden of non-communicable diseases in South Africa. Lancet 2009; 374(9693): 934-47. doi: 10.1016/S0140-6736(09)61087-4

[162] Braun MS, Richman SD, Quirke P, Daly C, Adlard JW, Elliott F, Barrett JH, Selby P, Meade AM, Stephens RJ, Parmar MKB, Seymour MT. Predictive Biomarkers of Chemotherapy Efficacy in Colorectal Cancer: Results From the UK MRC FOCUS Trial. JCO JUNE 1, 2008 vol. 26 no. 16 2690-2698

[163] Paré L, Marcuello E, Altés A, del Río E, Sedano L, Salazar J, Cortés A, Barnadas A, Baiget M. Pharmacogenetic prediction of clinical outcome in advanced colorectal cancer patients receiving oxaliplatin/5-fluorouracil as first-line chemotherapy. Br J Cancer. 2008 Oct 7;99(7):1050-5. doi: 10.1038/sj.bjc.6604671. Epub 2008 Sep 16.