**Epigenetic Epidemiology of Cancer**

Timothy M. Barrow a,b,c, Karin B. Michels a,d,e\*,

aInstitute for Prevention and Tumor Epidemiology, Freiburg Medical Center, University of Freiburg, 79106, Germany.

bGerman Consortium for Translational Cancer Research (DKTK), Heidelberg, Germany

cGerman Cancer Research Center (DKFZ), Heidelberg, Germany

dObstetrics and Gynecology Epidemiology Center, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115, U.S.A.

eDepartment of Epidemiology, Harvard School of Public Health, Boston, MA 02115, U.S.A.

\*Corresponding author

Tel: +1 617 732 4895

Fax: +1 617 732 4899

E-mail: kmichels@research.bwh.harvard.edu

E-mail addresses of other authors:

timothy.barrow@uniklinik-freiburg.de

**Abstract**

Epigenetic epidemiology includes the study of variation in epigenetic traits and the risk of disease in populations. Its application to the field of cancer has provided insight into how lifestyle and environmental factors influence the epigenome and how epigenetic events may be involved in carcinogenesis. Furthermore, it has the potential to bring benefit to patients through the identification of diagnostic markers that enable the early detection of disease and prognostic markers that can inform upon appropriate treatment strategies. However, there are a number of challenges associated with the conduct of such studies, and with the identification of biomarkers that can be applied to the clinical setting. In this review, we delineate the challenges faced in the design of epigenetic epidemiology studies in cancer, including the suitability of blood as a surrogate tissue and the capture of genome-wide DNA methylation. We describe how epigenetic epidemiology has brought insight into risk factors associated with lung, breast, colorectal and bladder cancer and review relevant research. We discuss recent findings on the identification of epigenetic diagnostic and prognostic biomarkers for these cancers.

**Keywords**

Epigenetics; Epidemiology; Cancer; DNA methylation; Biomarkers.

**Abbreviations**

5-FU, 5-flurouracil; ACT, Adriamycin, Cytoxan and Taxol; ChIP-seq, chromatin immunoprecipitation sequencing; CIMP, CpG island methylator phenotype;

CRC, colorectal cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; EWAS, epigenome-wide association studies; HPLC, high performance liquid chromatography; HR, hazard ratio; LUMA, luminometric methylation assay; MBD-seq, methyl-CpG binding domain protein sequencing; MeDIP-seq, methylated DNA immunoprecipitation sequencing; NSCLC, non-small cell lung cancers; OR, odds ratio; RRBS, reduced representation bisulfite sequencing.

**Introduction**

Epigenetic epidemiology includes the study of variation in epigenetic traits and the risk of disease in populations. The merging of these two fields can facilitate insight into which epigenetic marks are associated with cancer, whether some of these marks explain the link between certain exposures and cancer, and how these epigenetic marks can be utilised as biomarkers. Epigenetic epidemiology can therefore serve to promote primary cancer prevention by identifying risk factors and their method of action, secondary prevention by establishing markers of early disease, and tertiary prevention by establishing markers of disease progression and drug resistance. Biomarkers can be identified by the adoption of appropriate study designs, in conjunction with a solid understanding of how any of the three cornerstones of epigenetics may be involved in carcinogenesis: DNA methylation; chromatin and histone modifications; and non-coding RNAs.

In addition to the identification of markers of drug resistance, insight into epigenetic dysregulation of the genome also provides new bases for therapies. For example, azacitidine is a demethylating agent that acts through inhibition of DNA methyltransferases and has been approved for use with myelodysplastic syndromes. It is currently in Phase I and Phase II clinical trials for use with diffuse large B-cell lymphomas, non-small cell lung cancers (NSCLC), breast cancer, pancreatic cancer, and oesophageal cancer. Histone deacetylase inhibitors, such as vorinostat and romidepsin, have been approved for use with the treatment of T-cell lymphomas. While no microRNA-based therapies have yet been approved for clinical usage, Phase I clinical trials are underway investigating the use of an miR-34 mimic, MRX34, with liver cancer and lymphoma patients. It is therefore evident that better understanding the epigenetic basis of cancer is enabling the development of a range of new therapeutic options.

 In this review, we will describe how epidemiology studies have related epigenetic variation with environmental factors and have identified diagnostic and prognostic biomarkers that can be applied in the clinical setting. We will describe the challenges in study design, and we will review progress that has been made in identifying biomarkers of disease risk, and especially the efforts in developing non-invasive means of screening patients. We will focus exclusively on work using primary human tissues, and we will pay particular attention to large-scale and prospective studies due to their relative strength in identifying biomarkers and the epigenetic dysregulation associated with cancer.

**The suitability of epigenetic biomarkers**

Epigenetic traits have the potential to serve as excellent diagnostic and prognostic markers of cancer. In addition to the stability of DNA methylation and the resistance of microRNAs to RNase-degradation, aberrant epigenetic events are frequently observed in early-stage cancers and in adenomas [1-7]. Significantly increased stochastic variation in DNA methylation has been observed in cervical cells of normal morphology in patients who went on to develop cervical cancer [8], and there is evidence that epigenetic-based tests may offer superior sensitivity to cytology-based ones in the early diagnosis of disease [6].

While gene-specific epigenetic modifications have been reported to occur with great frequency, such as hypermethylation of the *RASSF1A* promoter in >30% of lung [2], bladder [9] and breast [10-12] tumours, it is unlikely that a test based upon a single gene will suffice to identify a large proportion of early-stage cancers. Subsequently, many studies have aimed to establish panels of genes whose synergy offer the greatest sensitivity [12-14]. An alternative approach that has been employed is to utilise the global changes that are commonly observed in tumours. There is evidence that methylation boundaries are disrupted in cancer, such as those between CpG islands and shores, and that the cancer genome contains large regions of hypomethylated blocks [15].

Summation of total DNA genome methylation may therefore be able to serve as a comparatively simple diagnostic marker. While high performance liquid chromatography (HPLC) and mass spectrometry are considered the ‘gold standard’ for estimating global methylation levels, they are not readily applicable to the clinical setting. Repetitive elements, such as LINE-1 and *Alu*, have been proposed as surrogate markers of global DNA methylation [16], while the pyrosequencing-based luminometric methylation assay (LUMA) has been developed as a simpler direct approach to estimating global DNA methylation [17]. However, while pyrosequencing enables the potential interrogation of DNA methylation at any CpG site, LUMA specifically assesses methylation at CCGG motifs. LUMA is performed by restriction digests of genomic DNA using *Eco*RI in conjunction with one of *Hpa*II (methylation-insensitive) or *Msp*I (methylation-sensitive) that cleave DNA at CCGG motifs, and DNA methylation can then be quantified at these sites by the ratio of the peaks from the *Hpa*II and *Msp*I digests, as determined by pyrosequencing. However, results by these two methods often do not correlate well [18,19]. In a comparison of these approaches, Lisanti and colleagues [20] reported that LINE-1 methylation corresponded best to results by HPLC (r2 0.96), with *Alu* displaying a weaker correlation (r2 0.78), while LUMA performed very poorly (r2 0.04), although LINE-1 was found to overestimate hypomethylation and to underestimate hypermethylation. LINE-1 methylation is also advantageous over LUMA in displaying less variation between samples taken at different time points [21]. Other studies have reported weaker correlations (r2 0.51 – 0.70) between the methylation of LINE-1 and *Alu* elements and global DNA methylation measured by HPLC [22]. While the use of repetitive element methylation is preferable to LUMA, their use as surrogate markers for global DNA methylation remains controversial. Furthermore, a clear limitation of these surrogate markers of global methylation is their lack of specificity for different cancer types. However, LINE-1 methylation is in itself of interest, both in terms of its role in carcinogenesis and its potential use as a biomarker, as will be discussed later in this review.

**Epigenome-wide Association Studies**

It has become increasingly common for epigenetic epidemiology studies to use microarray or next-generation sequencing technology in order to assess epigenetic variation on a substantially larger scale, rather than using a surrogate marker of global methylation, such as LINE-1 (*Figure 1*). Referred to as epigenome-wide association studies (EWAS), these can offer a cost-effective means of interrogating large numbers of loci without requiring infeasible quantities of starting material, and can bring insight by identifying novel genes and pathways implicated in disease. The design and analysis of EWAS has been comprehensively reviewed by Michels *et al* [23].

 The most well-established of these approaches is the use of Illumina’s Infinium microarray technology, which can assess DNA methylation at single-nucleotide resolution. These microarrays offer a robust and cost-effective approach to epigenome-wide screening, and their use in epigenetic epidemiologic studies is increasingly common (*Figure 1*), but data analysis is complex and requires careful consideration. Whole-genome bisulfite sequencing is prohibitively expensive, and therefore sequencing-based approaches require stratification to enable their application to epidemiological studies. Methyl-CpG binding domain protein sequencing (MBD-seq) and immunoprecipitation-based approaches, such as methylated DNA immunoprecipitation sequencing (MeDIP-seq) and chromatin immunoprecipitation sequencing (ChIP-seq), require substantial quantities of starting material and therefore cannot be readily utilised. In contrast, reduced representation bisulfite sequencing (RRBS) can be utilised with as little as 10 ng of DNA and has been multiplexed to enable the co-preparation of 96 samples at once [24], thereby making it an attractive option to large-scale epidemiological studies.

**Study design**

The identification of epigenetic biomarkers requires an appropriate study design. Retrospective case-control studies cannot determine the temporality of epigenetic modifications with regard to disease occurrence. Moreover, in cancer development ‘drivers’ and ‘passengers’ cannot be distinguished, which prohibits identification of early markers of disease. Prospective studies permit temporal associations and are therefore highly preferable for the identification of biomarkers (a summary of prospective studies described in this review can be found in *Table 1*). However, these are substantially more expensive and time-consuming to perform, and the number of cases in case-control studies nested in prospective cohorts is commonly lower than that found with retrospective case-control studies. Subsequently, most studies in the field are of a retrospective nature, but any targets identified by these studies require validation in prospective studies.

 Further consideration must be paid to the tissues used for study. While the early involvement of epigenetic factors in carcinogenesis makes them highly suitable for roles as diagnostic markers, it also increases the likelihood of observing epigenetic field defects in adjacent normal tissue. For example, methylation of the *MGMT* promoter in colorectal tumours is frequently accompanied by the same defect in adjacent normal tissue [25] and that loss of *IGF2* imprinting is equally prevalent in distant normal tissue as in prostate tumours [26]. Microarray-analysis of adjacent bladder tissue revealed 142 loci that were aberrantly methylated in both the adjacent tissue and tumours in comparison with tissue from cancer-free individuals [27], while synchronous colorectal tumours display significantly correlated methylation at LINE-1 elements and CpG islands [28]. These field defects can inhibit the identification of biomarkers if adjacent tissue is used as a comparison. This is perhaps exemplified by a meta-analysis of studies investigating *MGMT* methylation in NSCLC tumours [29], in which all five studies using matched healthy individuals as controls did not report hypermethylation in any of the control samples, while 3 of the 10 studies using adjacent tissue from patients reported hypermethylation in >15% of controls. Furthermore, the use of normal tissue enables normal methylation ranges to be more accurately defined, and therefore for more accurate definitions of what constitutes hypo- and hypermethylation of the genes in cancers.

**The use of blood as a surrogate tissue**

Prospective epidemiology studies are commonly performed with several thousand participants. Since it is not feasible to collect tissue samples, such as biopsies, from target organs in healthy individuals, prediagnostic specimen collection in prospective studies is restricted to blood, urine and other easily accessible tissues. Blood-based biomarkers are also advantageous in the clinical setting as they facilitate easier and cost-effective methods of screening, and they substantially reduce the patient burden. However, the suitability of blood as a surrogate tissue is controversial as there is conflicting evidence as to whether alterations in DNA methylation observed in tumours can also be detected in the blood of the patients. For example, while there is evidence from case-control and prospective studies that the aberrant methylation of imprinted genes observed in breast and colon tumours is mostly not found in matched blood samples [30,31], a cross-sectional study reported loss of *IGF2* imprinting in both colon tumours and matched blood samples, and proposed its use as a blood-based diagnostic marker of disease [32]. While some epigenetic marks can be found across tissues, such consistency across tissues is rare and it remains unresolved whether these represent more predictive biomarkers.

Whether such marks can be observed in surrogate tissues may depend upon their origins. Alterations at the germline level may be seen across all tissues, while changes induced by *in vitro* exposures could be seen in tissues of the same developmental origins. However, acquired alterations may be limited to the tumour, depending upon the nature of associated risk factors. For example, the impact of airborne exposures will be most acute in the lungs due to the path they follow into the body. Whether changes in DNA methylation may still be observed in the blood will depend in part upon the size of the particulate matter and whether they are able to pass into the bloodstream.

Consideration must also be given to the cause and specificity of epigenetic changes observed in blood. DNA methylation profiles in peripheral blood are associated with changes in the distribution of leukocyte subpopulations [33], and accounting for differences in cell distribution statistically is therefore essential. It has been proposed that such changes can be utilised to identify patients with tumours, although it is not clear whether these differences in DNA methylation profiles between cancer and healthy individuals may simply be due to underlying inflammation in cancer patients, rather than the cancer itself. Koestler and colleagues [34] identified 50 differentially methylated regions associated with different leukocyte subtypes and reported that these could be used to discriminate between healthy controls and patients with head and neck squamous cell carcinomas and ovarian cancer. However, such changes were not unique to the cancer type, with both eliciting a similar change in leukocyte populations. Other blood-based diagnostic markers can similarly show a lack of specificity to tumour type. *SEPT9* has been proposed to be a blood-based marker of colorectal cancer (CRC) [35], but it has also been shown to be aberrantly methylated in lung and breast tumours [36,37], although the rate of positive detection in blood from patients with cancers other than CRC is only marginally higher than in controls [35].

Epigenetic changes observed in blood may also be induced by other risk factors as opposed to the tumours themselves, and therefore care must be taken to avoid confounding. LINE-1 methylation is associated with toenail arsenic levels and smoking blond tobacco in healthy individuals [38], which are also risk factors for bladder and lung cancers. While this may provide evidence of how such factors can influence the risk of disease, it may also underline the difficulty in identifying true blood-based diagnostic markers of disease, as opposed to markers of disease risk. Indeed, Widschwenter and colleagues [39] commented that prediction of risk by gene-specific methylation and other risk factors are not independent, with the former being both a marker of exposures and a measure of individual response.

While circulating cell-free tumour DNA is present in plasma, the relative abundance, especially with early-stage disease, it is sufficiently low to inhibit its application to diagnostics. However, the advent of next-generation sequencing has facilitated the development of approaches with significantly improved sensitivities that are able to utilise this material. Promising results have been reported for the detection of neoplasms using techniques such as methyl-BEAMing [40], shotgun massively parallel bisulfite sequencing [41], and chromatin immunoprecipitation sequencing [42]. Furthermore, as the levels of DNA in plasma correlate with tumour size, its quantification may provide insight into treatment efficacy.

**Non-invasive sample collection as an alternative to the use of biopsies**

Many tissues provide alternative non-invasive means to the use of peripheral blood. As will be described later, exfoliated cells can be obtained from sputum to study the lung, from urine to study the bladder, and from stool to study the colon. Further to these examples, a cytosponge method has been developed to retrieve cells from the oesophagus. A screening approach based upon this method has been estimated by microsimulation modeling to possibly reduce the number of cases of oesophageal cancer by 19% for a disease with a mortality rate of greater than 80% [43]. In addition to the potential to screen for adenocarcinomas, this approach offers sensitivity and specificity of >90% in the identification of patients with segments of >2cm of the premalignant condition Barrett’s oesophagus [44]. Radiofrequency ablation offers a means of eradicating Barrett’s with a high success rate and low rates of recurrence, but 9% of patients suffer complications [45]. However, work from a prospective cohort of 98 patients with Barrett’s oesophagus identified a four-gene panel (*GJA12*, *PIGR*, *RIN2*, and *SLC22A18*) that was able to stratify patients with Barrett’s into three risk groups, with 17 of the 20 patients with high grade dysplasia or adenocarcinoma displaying methylation of >2 genes (*Table 1*) [13]. Epigenetic markers used in conjunction with non-invasive approaches may therefore serve to identify at-risk individuals for treatment, thereby reducing the cost of screening and treatment programs, and reducing unnecessary and painful procedures for patients.

**Colorectal cancer**

Perhaps the most widely-studied cancer with regard to epigenetic changes is that of the colorectum. There is evidence that epigenetic silencing of genes may be an early event in the adenoma-carcinoma sequence, as suggested by the Vogelstein model [46]. The *MGMT* promoter has been reported to be methylated in 34% of adenomas [3], while Psofaki *et al* [47] reported aberrant methylation of the *CDKN2A/p16* and *MGMT* promoters in 67% and 76% tubulovillous and villous adenomas respectively. Together, this may suggest that epigenetic changes could serve as excellent markers of risk and for the diagnosis of early-stage tumours. Furthermore, distinct colorectal cancer subtypes can arise through epigenetic mechanisms, such as microsatellite instability through the silencing of mismatch repair genes such as *MLH1*, which occurs in 20% of tumours [48], and the classification of tumours by CpG island methylator phenotype (CIMP) status, first described in 1999 [49]. Interestingly, a prospective study identified extreme hypomethylation (<40%) of LINE-1 in colorectal tumour tissue that clustered as a distinct group, and that these patients were younger at diagnosis [50]. This interesting finding may suggest a defect in the maintenance of global DNA methylation that gives rise to a distinct subset of tumours, which could benefit from alternative therapeutic approaches.

Many risk factors for colorectal cancer have been demonstrated to be associated with epigenetic changes in tumours. Higher levels of processed meat intake and lower folate and fibre intake are associated with methylation of the *APC* 1A promoter in colorectal tumour tissue [51], smoking is associated with increased risk of CIMP-high tumours [52], while higher levels of alcohol intake are associated with increased global [53] and LINE-1 [54] hypomethylation in tumours. Perhaps one of the best studied risk factors is that of folate intake. Lower blood folate levels are associated with global DNA hypomethylation in both tumour tissue and blood [53,55], and lower folate intake with increased risk of LINE-1 hypomethylation [54]. However, there is conflicting evidence from studies nested within prospective cohorts as to whether high [56] or low [57] folate levels are associated with CIMP-positive tumours.

In addition to risk through exposures, individuals with a family history of CRC have a greater risk of developing tumours with lower LINE-1 methylation [58], which may explain how risk is conferred and how patients could be identified for surveillance. Indeed, tumours associated with predisposing conditions often show marked differences in comparison to sporadic tumours. Colorectal neoplasms associated with inflammatory bowel disease display distinct genetic and epigenetic profiles [59-61], while aberrant methylation of the first intron of the *PTPRG* gene is strongly associated with Lynch syndrome and is frequently present at the adenoma stage [62]. Even within sporadic tumours, consideration must be paid to genetic and epigenetic differences between proximal and distal tumours, such as the site-dependence and mutual-exclusivity of mutations in the *KRAS* and *BRAF* genes [63-65], and the associations between events, such as that between methylation of the *APC* and *CDKN2A/p16* genes with mutations in codons 12 and 13 of *KRAS* [66]. Understanding the variation in the genetic and epigenetic changes observed between different subtypes of the disease will greatly aid the development of a diagnostic test panel that will offer maximal sensitivity and which can guide treatment strategies.

Global DNA methylation, as measured by HPLC or liquid chromatography/tandem mass spectrometry, does not appear to offer a blood-based means of detecting CRC risk. Studies nested within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial and the Nurses’ Health Study, both using prospectively-taken blood samples from more than 350 cases, identified no association between global methylation levels and risk of cancer (*Table 1*) [55,67]. Smaller scale retrospective studies have observed associations between the risk of adenomas and increased leukocyte methylation levels [4] and, conversely, leukocyte hypomethylation [53], but the relative power of the prospective studies would suggest that global DNA methylation cannot serve as a marker of disease.

 Gene-specific approaches have offered more promise. *SEPT9* has been promoted as a diagnostic marker, with sensitivity and specificity of 70% and 90% reported from a case-control study [35]. However, when tested in a prospective study of asymptomatic individuals undergoing screening by colonoscopy, the sensitivity dropped to 48% (*Table 1*) [68]. This underlines the need for prospective studies to evaluate biomarkers of disease risk. This is perhaps further exemplified by the study of another potential marker, *IGF2*. A cross-sectional study of 172 patients identified loss of *IGF2* imprinting as potentially an excellent blood-based marker of risk (adjusted OR 21.7) with loss of imprinting observed in both blood and colon tissue [32]. However, a case-control study nested within the Northern Sweden Health and Disease Study prospective cohort reported no correlation between *IGF2* methylation in leukocytes and colon cancer risk [69], and nor was an association observed in prospectively-taken blood samples from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, despite hypermethylation of the locus being observed in 80% of tumours from the same patients [31] (*Table 1*). As the cross-sectional study by Cui *et al* [32] reported a stronger association for loss of *IGF2* imprinting with tumours than adenomas, and as no association was observed elsewhere in prospectively taken samples, it may therefore be that *IGF2* is not a driver of carcinogenesis and so is not useful as a biomarker of early disease. More promisingly, Lange *et al* [70] used publically available microarray data sets to identify *THBD* and *C9orf50* as potential CRC-specific tumour markers, ensuring that these genes are not aberrantly methylated in other tumour types, before investigating their performance in prospectively-taken serum and plasma samples. These two loci were shown to detect CRC tumours with sensitivity of 71% and specificity of 80% (*Table 1*).

The benefit of using stool samples to identify colonic neoplasms has recently been demonstrated. Following promising results in the identification of neoplasms in patients with inflammatory bowel disease [71], methylation of *BMP3* and *NDRG4* were taken forward for use in conjunction with an immunochemical assay for haemoglobin and the detection of *KRAS* mutations, using -actin as a reference gene to establish DNA levels, for the detection of colorectal tumours [72]. Using an algorithm to calculate a score based upon these measurements, this stool-based approach offered superior sensitivity to the faecal immunochemical test in the detection of tumours (92% *vs* 74%) and advanced precancerous lesions (42% *vs* 24%), at the expense of a reduction in specificity (87% *vs* 95%). Stool-based epigenetic markers therefore offer great promise as part of a cost-effective frontline test to identify patients for screening by colonoscopy, and this work demonstrates how epigenetic and genetic markers can complement each other to great effect.

There is substantial evidence that tumour subtypes associated with epigenetic alterations are associated with prognosis. Microsatellite-instable tumours are associated with favourable prognosis [73]. Work within the prospective Nurses’ Health and Health Professionals Follow-up studies has identified that CIMP-positive tumours are associated with poor differentiation [74] and significantly reduced cancer-related mortality [75]. Elsewhere, Han and colleagues [76] did not observe an association between CIMP status and disease-free survival, but did report that hypermethylation of two genes associated with CIMP status, *NEUROG1* and *CDKN2A/p16*, were associated with reduced survival. As this study focussed on a very distinct patient cohort, all with Stage II or Stage III CRC treated by FOLFOX, this finding may suggest that the relationship between CIMP status and progression may be complex.

Hypomethylation of LINE-1, but not *Alu*, is significantly associated with recurrence (HR 9.6) and impaired patient survival (HR 4.6) in early-stage rectal cancer [1], and with worse overall cancer-specific mortality for all colorectal tumours (HR 2.37 with a 30% decrease in LINE-1 methylation) [77]. It is not clear whether the discrepancy in the magnitude of these associations is the product of the difference in study design, with the retrospective study reporting greater risks than the prospective, or whether LINE-1 hypomethylation may be particularly implicated in rectal cancer.

*CDKN2A/p16* promoter hypermethylation has been reported in 25-30% of tumours [78,79], but there is conflicting evidence as to its utility as a prognostic marker. Retrospective studies of 212 colorectal patients [80], 285 Stage II and III colorectal cancer patients receiving fluoropyrimidine adjuvant therapy [66] and 381 rectal cancer patients [63] reported associations between the methylation of the gene promoter and recurrence, reduced survival and reduced recurrence-free survival. However, a prospective cohort study of 326 CRC patients [79] and a study nested within two prospective studies incorporating 902 colorectal tumours [78] both reported no association between hypermethylation of this gene and overall or recurrence-free survival. The strength of prospective studies and the relative sample sizes would suggest that *CDKN2A/p16* methylation is perhaps not of value as a prognostic marker. It is also unlikely that *MGMT* could serve as a prognostic marker, despite its usage with gliomas, as Shima *et al* [81] reported that it was not associated with cancer-specific mortality or survival [81], while Kim *et al* [66] similarly observed no association between *MGMT* promoter methylation and survival in patients with rectal cancer. More promisingly, low expression of *miR-128* has been reported to be associated with poor survival, which may be through the disrupted regulation of one of its targets, *NEK2*, which was identified by multivariate analysis to be associated with overall survival [82].

Histone modifications appear to be better indicators of prognosis than of risk. Approaches that require chromatin immunoprecipitation are not readily suitable for epidemiologic studies on account of requiring large quantities of DNA. Immunohistochemical detection of histone modifications is one approach that can facilitate their study due to the minimal sample requirements and cost. Furthermore, prognostic markers identified by such an approach could be readily utilised in the clinic by using commercially-available antibodies in conjunction with sections from tumour tissue taken for pathological analysis. Reduced levels of H3K27me2 and increased levels of H3Ac and H3K4me2 are associated with poor prognosis in CRC patients [83,84]. Benard *et al* [1] similarly reported that lower levels of H3K27me3 and higher levels of H3K9Ac are associated with worse survival in rectal cancer, but not distal recurrence. H3K27me3 was further associated with local recurrence, but, interestingly, H3K9Ac was only associated when LINE-1 methylation levels were high. In contrast to findings in NSCLC, overexpression of the histone modifier *EZH2* is associated with improved prognosis in CRC [85].

Epigenetic profiling of tumours may also provide insight into the efficacy of treatment strategies. A number of studies have concluded that tumours with microsatellite instability show reduced benefit from 5-flurouracil (5-FU) treatment [86-88], although improved benefit has been reported for 5-FU and irinotecan treatment [89]. However, a meta-analysis of 12,782 patients from 31 studies concluded that it was not possible to determine whether these tumours display reduced benefit or not [73]. In microsatellite-stable tumours, lower levels of LINE-1 methylation have been reported to be associated with improved survival of patients treated with 5-FU in comparison with those treated by surgery alone, while patients with high methylation levels received no significant benefit [90]. The authors demonstrated *in vitro* that 5-FU treatment induces DNA double-strand breaks via increased LINE-1 expression, thereby providing a mechanism for this action. Moutinho and colleagues [91] performed EWAS-based analysis of cell lines that are sensitive and resistant to oxaliplatin to identify the *SRBC* gene as conferring resistance to the drug. Methylation of this gene was then demonstrated to be frequent in primary tumours (39 of 131) and to be associated with impaired progression-free survival (HR 1.83).

Evidence from the study of colorectal cancer demonstrates how epigenetic epidemiology can provide tremendous clinical benefit. In addition to a greater understanding of risk factors for CRC, including familial history of the disease, there are promising results from the identification of blood- or, in particular, stool-based diagnostic markers that could be used in screening populations. Furthermore, insight into tumour subtypes, such as CIMP status, and the identification of markers of response to therapy should serve to reduce disease-related mortality.

**Lung cancer**

There is evidence that the leading risk factors for lung cancer, tobacco smoke and air pollution, can influence DNA methylation in the lungs and in blood. A study nested within the EPIC cohort reported hypomethylation of *F2RL3*, *AHRR* and two intergenic regions in the blood of smokers in comparison to non-smokers [92]. A follow-up study from the same group demonstrated that four loci, including *AHRR*, may serve as blood-based markers of tobacco smoke exposure [93]. While many exposure studies report small but statistically significant changes, the methylation of *AHRR* was 20% lower in current smokers than in individuals who had never smoked. Although the study size is small, the immediate biological relevance of the *AHRR* gene, which is involved in the metabolism of cigarette smoke, in conjunction with the substantial change in methylation levels in both lung tissue and blood would suggest that this could be a potential marker for disease risk. Furthermore, former smokers still displayed hypomethylation, if at a lesser degree, indicating that this gene could serve as long-term marker of exposure. Smoking during pregnancy has also been reported to be significantly associated with aberrant methylation of *AHRR* in cord blood, thereby linking *in utero* exposures to risk of disease [94].

In 2013, air pollution was officially classified as carcinogenic by the World Health Organisation, and there is increasing evidence to suggest that these pollutants induce epigenetic changes that may be implicated in carcinogenesis. Methylation of LINE-1 elements is decreased in leukocytes in response to recent exposures to high levels of black carbon and PM2.5 [95], while methylation of LINE-1 and *Alu* elements are significantly associated with long-term, but not short-term, exposure to PM10 pollutants in workers at a steel plant [96]. Interestingly, subfamilies of repetitive elements have different susceptibilities to changes following exposure to different air pollutants, with LINE-1 subfamilies with a greater evolutionary age displaying the greatest susceptibility following exposure to PM10 particles and benzene [97]. PM2.5 exposures have also been demonstrated to influence DNA methylation in a gene-specific manner, with *NBL2* methylation positively associated with silicon and calcium exposures in Chinese truck drivers [98].

As many lung cancers are detected at a late stage, it would be of enormous benefit to establish high-throughput and cost-effective means of screening for this disease. Subsequently there has been a substantial body of research into the use of blood, sputum and bronchial washings as a non-invasive means of screening. A nested case-control study reported no association between the methylation of five genes (*CDKN2A/p16*, *GSTP1*, *MGMT*, *MTFHR* and *RASSF1A*) in peripheral blood and the case/control status of the individuals (*Table 1*) [99]. However, a nested case-control study using sputum identified the methylation of three genes, *CDKN2A/p16*, *DAPK*, and *RASSF1A*, that conferred a significant increase in risk (OR > 1.5) in a high-risk population of smokers (*Table 1*) [14]. Furthermore, in sputum collected within 18 months of diagnosis, individuals with hypermethylation of at least three genes from a six-gene panel that also included *GATA5*, *MGMT* and *PAX5 * were observed to have a 6.5-fold increase in the risk of lung cancer, although 36% of controls similarly displayed hypermethylation of >3 genes. In samples taken 19-72 months prior to diagnosis, hypermethylation of *CDKN2A/p16* alone was observed to confer an 80% increase in risk of developing cancer. However, in contrast, another nested study observed that *CDKN2A/p16* and *RASSF1A* were each only hypermethylated in 1 of 18 sputum samples [100]. As these studies used the same technique to measure methylation (methylation-specific PCR) and similarly studied a cohort of smokers, it is not clear why this discrepancy exists. A test based upon the methylation of a single gene, *SHOX2*, was reported to offer sensitivity of 40% and specificity of 96% [101], which could therefore form the basis for the development of a multi-gene diagnostic test. However, it should be noted that 45% of samples produced an invalid result according to the manufacturer’s protocol, which would suggest that the assay is not yet ready for the clinical setting. MicroRNA expression levels have also shown promise, with a panel of five genes offering 83% sensitivity and 100% specificity in a small-scale prospective study (*Table 1*) [102]. Elsewhere, a two-gene panel consisting of *miR-31* and *miR-210* has alone been reported to provide sensitivity of 65% and specificity of 90%, and these genes are being carried forward into a prospective study investigating early detection of tumours in heavy smokers [103].

Promisingly, there is evidence that these non-invasive epigenetic tests can out-perform cytological ones. A case-control study reported that a test based upon the hypermethylation of four genes (*CDKN2A/p16*, *RASSF1A*, *TERT*, and *WT1*) in bronchial washings offered superior performance to a cytology-based test in the same samples [6]. This test had sensitivity and specificity of 82% and 91% respectively, with 64% sensitivity for tumours at stage T1 and positive test results in 74% of cytology-negative samples. A nested case-control study of 82 cytologically-negative sputum samples from cancer patients and 37 matched controls reported that 73-78% of the methylation changes observed in the *FHIT*, *CDKN2A/p16* and *RAR* genes in NSCLC tumours were also observed in sputum from the same patients (*Table 1*) [104]. In combination with five genetic markers, the authors described how methylation of *CDKN2A/p16* and *RAR* could be used as a diagnostic test with sensitivity of 82% and specificity of 75%, although it should be noted that the frequency of positive methylation test results in controls was high (14% and 22% for *CDKN2A/p16* and *RAR* respectively). Sputum has also been used to establish that *SULF2* and *PCDH20*, which are hypermethylated in lung cancer, are similarly so in chronic mucous hypersecretion, which is a risk factor for cancer development [105]. A prospective study would be required to establish whether these genes could identify patients for surveillance.

Repeated sampling has been demonstrated to improve the sensitivity of sputum-based approaches [106], while other refinements may also offer significant improvements in the accuracy of results. Nikolaidis and colleagues [6] accounted for the heterogeneity of cell types in sputum and bronchial washings by adjusting the methylation threshold for their four-gene test to account for the presence of lymphocytes. Differences between ethnicities may also need to be addressed. Leng *et al* [107] reported separate diagnostic panels for populations from Colorado and New Mexico, having previously observed that Native American ancestry in the Hispanic population in New Mexico reduced the risk of methylation of 12 genes associated with lung cancer [108]. This may suggest that differences in ethnicity and exposures may complicate the design of a single widely-used test for use in the clinic.

 In addition to non-invasive biomarkers for assessment of risk and early detection, promising work has targeted epigenetic dysregulation as prognostic markers. A meta-analysis of 18 studies identified hypermethylation of *CDKN2A/p16* as predictive of reduced disease-free survival [109], while Nadal *et al* [5] observed an association between methylation of *miRNA-34b/c* in 59 of 140 early stage lung cancers and significantly reduced disease-free survival. Following an EWAS approach, Sandoval *et al* [7] identified five genes that were significantly associated with recurrence of disease (HR 3.24) in both training and validation cohorts of Stage I tumours. Perhaps of particular interest is *HIST1H4F*, as NSCLCs are susceptible to the chemotherapeutic use of histone deacetylase inhibitors. The overexpression of another histone modifier, *EZH2*, which is a member of the Polycomb-group, is similarly associated with poor prognosis for NSCLC patients [85]. While most studies have focussed on epigenetics in isolation, Ko and colleagues [2] have demonstrated that the hypermethylation of *RASSF1A* can be used in conjunction with the expression of *TP63* to identify early non-small cell lung cancers without lymph node involvement that have poor rates of recurrence-free survival.

There is increasing evidence that the aberrant expression of microRNAs may serve as excellent biomarkers of disease progression. One of the most promising studies was that by Bediaga *et al* [110], in which a test based upon the expression of eight microRNA genes was found to provide sensitivity and specificity of 98% and 96% respectively. These genes were tested in both fresh-frozen and formalin-fixed paraffin-embedded tissue, and they are therefore excellent candidates for further investigation in a prospective study. Elsewhere, the expression of *miR-221* has been reported to be associated with recurrence [111], high levels of *miR-146b* expression are significantly associated with reduced survival (HR 2.7) [112], and the overexpression of *miR-21* and *miR-200c* in NSCLC tumours are similarly associated with impaired survival, while high levels of *miR-21* in serum is associated with lymph node metastases and advanced stage disease [113]. Further evidence for the utility of microRNA expression comes from a prospective study into response to cisplatin and vinorelbine, which identified that a two-gene panel, of *miR-149* and *miR-375*, that was significantly associated with response to therapy [114]. Furthermore, the authors reported a four-gene panel that was associated with overall survival.

Understanding how risk factors, such as tobacco smoke and air pollution, influence the epigenome has enabled potential diagnostic markers of early disease to be identified. Most promisingly, evidence from prospective studies suggests that aberrant DNA methylation and microRNA expression can be identified in the sputum and bronchial washings of high-risk individuals, and therefore could serve to identify lung tumours at an earlier stage.

**Breast cancer**

Breast cancer subtypes are epigenetically distinct, with tumours displaying differing LINE-1 methylation profiles according to the expression of the oestrogen and progesterone receptors [115-119] and *BRCA1* inactivation [119]. The identification of these differences in methylation profiles may suggest that different breast cancer subtypes may be candidates for azacitidine treatment, just as expression of the hormone receptors identify patients who will benefit from endocrine therapy. Furthermore, these observations may also bring insight into how the different subtypes arise. Silencing of the oestrogen receptor can occur through promoter methylation [120], and inactivation of *BRCA1* and *BRCA2* are also commonly through epigenetic mechanisms, although estimates of the frequency of this event vary substantially, from 17% of tumours to 60% [121,122]. Other epigenetic events are similarly frequent, with *RASSF1A* reported to be hypermethylated in 65-85% of breast tumours [10-12], *HIN1* hypermethylated in 63% [12], and RAR in 28% [12]. Interestingly, methylation of tumour-suppressor genes, such as *RAR*, *CDKN2A/p16* and *HIN1*, has been shown to be a common occurrence in women at high-risk of developing breast cancer due to their familial history of the disease, but who lack *BRCA1* and *BRCA2* mutations [123]. This may therefore provide evidence for alternative pathways involved in familial cases of disease, in addition to mutation or silencing of the BRCA genes.

There have been a large number of studies aiming to identify blood-based diagnostic markers for breast cancer. Brennan *et al* [124] observed an association between the hypermethylation of *ATM* in blood with increased risk of breast cancer in two of three prospective populations (*Table 1*). *SLC19A3* hypermethylation has also been demonstrated to be a promising marker in plasma, with a positive predictive value of 90% and negative predictive value of 85% [125]. One of the most promising, albeit small-scale, blood-based studies identified eight genes that are significantly differentially methylated between plasma samples from patients with cancer and healthy controls, seven of which also displayed a significantly higher level of methylation in tumour and serum samples compared to normal breast tissue in a second cohort [126]. *APC*, *CST6*, *BRCA1* and *CKDN1A/p21* displayed particular promise, with comparatively small ranges of values in controls and significantly higher methylation in samples from patients, and tests based upon the methylation of *BRCA1* and *CKDN1A/p21* displayed sensitivities of 75% and 88% respectively, based upon specificities of 90%. Furthermore, Chimonidou *et al* [127] reported that the *CST6* promoter was methylated in cell-free DNA from the plasma of 19% of patients within a pilot study and 40% in an independent cohort, while methylation was never observed in healthy controls. However, a nested case-control study using pre-diagnosis serum samples from the New York University Women’s Health Study cohort observed that *APC*, *GSTP1* (two of the eight genes from the Radpour *et al* study), *RAR* and *RASSF1A* were not differentially methylated between breast cancer patients, women with benign breast disease, and healthy controls (*Table 1*) [128], while a large case-control study of 902 blood samples from patients and 990 controls reported no significant association between hypermethylation of the *BRCA1* promoter and risk of breast cancer [129]. In addition to gene-specific changes, a population-based study of 1,055 cases and 1,101 controls reported that women in the highest quintile for global DNA methylation, as analysed by LUMA, had a 2.4-fold increase in the risk of developing breast cancer [19]. No such associations were observed with LINE-1 methylation.

Blood-based markers may also serve to identify patients with a familial risk of disease, as has been investigated within the prospective Sister Study, in which blood was taken from the sisters and half-sisters of patients with breast cancer. Epigenome-wide methylation scanning has revealed 250 differentially-methylated genes between individuals who went on to develop breast cancer and those who had not (*Table 1*) [130]. Although their suitability as biomarkers of risk may not be applicable to general population due to their association with familial disease, these could be useful in the surveillance of high-risk populations. Another study within the same cohort identified that lower quartiles of LINE-1 methylation are associated with increased risk of disease (*Table 1*) [131], although the range of methylation values within each quartile were extremely narrow. In contrast, results from within the Breast Cancer Family Registry suggest no correlation between breast cancer risk and the methylation of LINE-1 and *Alu* repetitive elements, but a significant correlation was observed with Sat2 methylation (*Table 1*) [132]. Considering the narrow range of LINE-1 values reported within the Sister Study, and the similar mean Sat2 methylation values for the cases and controls within the Breast Cancer Family Registry, it is perhaps unlikely that repetitive element methylation can act as a marker of breast cancer risk.

 As with other cancers, epigenetic events in breast tumours can provide insight into disease prognosis. Interestingly, the hypermethylation of *BRCA1* and *BRCA2* were significantly associated with high rates of overall and disease-free survival in a Tunisian population [121], but *BRCA1* hypermethylation is associated with worse prognosis in Taiwanese [133] and English [134] populations, despite the fact that the prevalence is similar across the populations (61%, 56% and 59% respectively). However, a meta-analysis of nine studies and 3,205 patients reported that hypermethylation of the *BRCA1* gene is significantly associated with impaired disease-free survival (HR 3.92) [122]. LINE1 hypomethylation is also significantly associated with reduced overall and disease-free survival, and with lymph node metastases [135]. A retrospective study of 670 tumours from an American cohort reported associations between the methylation of *GSTP1*, *RAR* and *TWIST1* and cancer-specific mortality, and the authors described how hypermethylation of 4-5 genes from a 10-gene panel was associated with a 1.66-fold increased risk of cancer-specific mortality, and 6 or more genes with a 2.45-fold increase in risk [12]. However, no association was observed between *RASSF1A* methylation and risk of mortality. This is in contrast to the finding of Buhmeida *et al* [11], who reported significant associations between the hypermethylation of this gene and impaired disease-free survival and risk of lymph node metastases in an Arabic population. Swift-Scanlan *et al* [136] similarly reported an association between hypermethylation of *RASSF1A* and lymph node positive disease, in addition to significant associations between hypomethylation of *ER* and disease recurrence, and hypomethylation of *CCND1* and *TWIST* with metastases.

 The associations with outcomes may in part be through mediation of drug response. Hypermethylation of *BRCA1* is associated with improved cisplatin response in triple-negative patients [137], *RASSF1A* methylation has been shown to be associated with resistance to docetaxel [138], while methylation of the *CDO1* promoter was associated with outcomes in a cohort of anthracycline-treated oestrogen receptor-positive tumours with lymph node involvement [139], although further work is required to establish whether methylation of this gene is associated with the tumour type, the therapy type, or both. Furthermore, *RASSF1A* methylation may also serve as a marker of response to ACT (Adriamycin, Cytoxan, and Taxol) therapy. In a pilot study of 21 patients, *RASSF1A* methylation was not detectable in serum from the 4 patients who achieved complete pathological response, but was still detectable in patients with a minimal or partial response [10]. A larger-scale follow-up is required, and attention must be paid as to how tumour stage and size influence the detection rates for this gene. While the retrospective nature of the study would not enable the deduction as to whether *RASSF1A* confers drug resistance or is merely serving as a marker of surviving tumour cells, the authors demonstrated that cell lines transfected with *RASSF1A* showed a synergistic effect with docetaxel in arresting the cell cycle.

There is a substantial body of evidence implicating the aberrant expression of microRNAs in the progression of breast cancer. In particular, they have been associated with tumour grade [140], subtype [141-143], survival [141,144] and response to radiotherapy [141]. MicroRNAs could therefore provide insight into disease progression and response to therapy. For example, the expression of *miR-27a*, *miR-30e*, *miR-155* and *miR-493* in tumours is associated with response to anthracycline treatment in triple-negative breast cancer [145]. Furthermore, there is evidence that these microRNAs can be detected in blood for use as non-invasive markers of disease. A meta-analysis has revealed that levels of miR-21 are associated with prognosis in a range of cancers, including those of the breast, colon, lung, liver and prostate [146]. The levels of miR-34a, miR-93 and miR-373 are higher in serum from cancer patients than healthy controls, and higher still in patients with metastases [142], while levels of miR-195 and let-7a have been observed to be 19- and 11-fold higher respectively in blood from breast cancer patients, with these levels decreasing to levels resembling those of controls following tumour resection [143]. Individually, miR-373 was able to detect disease with sensitivity of 77% and specificity of 100%, and miR-195 offered sensitivity of 86% and specificity of 100%. These two microRNAs are therefore excellent candidates to be taken forward for further investigation. However, while case-control studies have identified potential candidates for use as biomarkers, there have been few prospective studies investigating these. One of the few, a nested case-control study of 410 sisters of breast cancer patients, identified 21 miRNAs that were differentially expressed in serum from individuals who went on to develop cancer themselves and those who were cancer-free (*Table 1*) [147]. However, as the authors noted, the differences in expression were small (4 - 19%), which makes their use with individual patients.

The aberrant expression of microRNAs may result from epigenetic dysregulation of the genes. Aure *et al* [140] identified 70 miRNAs that were aberrantly expressed in breast tumours, of which 22 were primarily due to gene hypermethylation and a further 24 were the result of a combination of methylation aberrations and changes in copy number. Forty-one of these miRNAs were similarly aberrantly expressed in an independent validation set, and two were associated with tumour grade. Lehmann *et al* [148] observed that *miR-9-1*, *miR-124a3*, *miR-148*, *miR-152* and *miR-663* were each hypermethylated in >34% of breast tumours, with *miR-148* methylated in 86%, and that these events were significantly correlated with the methylation of *RASSF1A*, *CCND2*, *DAPK* and *SOCS1*.

In summary, subtypes of breast cancer have distinct epigenetic profiles, but there is limited evidence for blood-based diagnostic markers of disease that are applicable to the general population, although markers of risk have been identified in individuals with a familial history of the disease. Evidence from case-control studies suggests that microRNA expression may bring insight in prognosis and response to therapy, and these require further investigation in prospective studies.

**Bladder Cancer**

Exposure to arsenic and tobacco smoke have been established as leading risk factors for bladder cancer, and these have been demonstrated to be associated with epigenetic changes in healthy populations. A Spanish study of 892 healthy individuals observed that LINE-1 methylation levels in blood are associated with toenail arsenic levels and smoking blond tobacco [38], and the former is an observation that has also been made elsewhere in healthy individuals [149]. However, while statistically significant, some of these methylation changes were very small, with the mean methylation in non-smokers and individuals who smoke blond tobacco differing by only 0.3%. The biological significance of such small changes remains unclear, but studies of environmental exposures will seldom observe large changes in apparently healthy individuals. It is therefore important to view results in the light of current knowledge regarding risk factors and observations in tumour tissue, with exposure studies serving as a bridge to identify how these may be involved in the process of carcinogenesis and to help identify potential markers of early disease. Elsewhere, there is evidence that *in utero* arsenic exposures may influence DNA methylation in the unborn child. Using the Illumina 450K microarray platform, Koestler and colleagues [150] observed that 18% of interrogated CpG sites displayed significantly different methylation between quartiles of arsenic exposure, although these associations were no longer significant after correction for multiple hypothesis testing.

In tumours, epigenome-wide profiling of 14 arsenic-induced and 14 non-arsenic-induced urothelial carcinomas revealed 13 sites that significantly differed between the groups, with hypermethylation of *CTNNA2*, *KLK7*, *NPY2R*, *ZNF132* and *KCNK17* associated with cumulative arsenic intake, three of which had previously been implicated in bladder cancer and arsenic-induced carcinogenesis [151]. A case study of 351 tumours has shown that *CDKN2A/p16*, *RASSF1A* and *PRSS3* are hypermethylated in 30-34% of bladder cancers, and that current smokers have a significant 2.4-fold greater risk of *CDKN2A/p16* hypermethylation, while higher levels of arsenic measured in toenails significantly increase the risk of *RASSF1A* and *PRSS3* hypermethylation by 3.5- and 2.8-fold respectively [9]. Interestingly, the risk of *RASSF1A* hypermethylation was 50% lower among current smokers, but not significantly so.

 There has been a significant body of work investigating whether these epigenetic changes could prove to be useful as diagnostic and prognostic markers. A study of 285 cases and 465 controls identified that patients with LINE-1 methylation below 74%, as measured in peripheral blood, had an increased risk of developing bladder cancer (OR 1.80) [149]. However, this usefulness of this marker is questionable as these individuals represented the lowest decile of DNA methylation in the 750 subjects included in the study, and the odds ratio was lower than that for current smokers (OR 2.46). Perhaps more promisingly, there is evidence that the epigenetic profiling of tumour tissue can identify patients at risk of aggressive disease. In one microarray-based study of 310 bladder tumours, unsupervised clustering of the 267 genes displaying substantial changes ( > 0.2) between normal and tumour tissue revealed four distinct classes of tumour, two of which were significantly associated with increased risk of invasive disease (ORs of 3.93 and 4.89) [152]. Furthermore, water arsenic levels were associated with two of the four classes, which may suggest that arsenic-induced tumours develop through distinct pathways. This could serve to explain why hypermethylation of *RASSF1A* was observed to be associated with exposure to arsenic but not tobacco smoke by Marsit *et al* [9]. While interrogation of 267 loci may not be currently feasible for the clinical setting, a smaller panel of genes within this set could potentially be identified and used to identify aggressive tumours.

 Cells exfoliated from the bladder can be isolated from urine, and these offer an excellent means of developing non-invasive diagnostic tests for bladder cancer. Renard *et al* [153] adopted a candidate gene approach in urine samples taken from 157 patients with bladder cancer and 339 individuals with non-cancerous urological disorders, and they observed that the hypermethylation of *TWIST* and *NID2* together provided a potential diagnostic test for bladder cancer with sensitivity and specificity of 90% and 93% respectively. When taken forward to a prospective study, these decreased to 75% and 71% respectively (*Table 1*) [154]. However, as the authors described, the test may still have a clinical application by reducing the number of individuals referred for cystoscopy, as particular low-risk groups can be identified through low levels of methylation for these two genes. Furthermore, the prospective cohort used by Abern *et al* only contained 24 patients who went on to develop bladder cancer; something which is a problem for all but the largest of epidemiologic studies and which provides incentive to perform retrospective case-control studies, despite their own inherent drawbacks. In another prospective study of 248 patients with microscopic hematuria, 170 of whom were later diagnosed with bladder cancer, the methylation of 18 tumour-suppressor genes was investigated and *RUNX3* showed particular promise, being hypermethylated in 58 of 170 cases in comparison to only 8 of the 78 controls (*Table 1*) [155].

 As with lung cancer, epigenetic epidemiologic studies have provided insight into how risk factors, such as tobacco smoke and arsenic exposure, can affect the epigenome and subsequently how bladder tumours can arise. Gene-specific DNA methylation in exfoliated cells in urine holds particular promise for utilisation as diagnostic markers of disease, especially in high-risk populations.

**Insights into other cancers**

There have been promising developments into the identification of epigenetic biomarkers for a range of other cancers. In particular, the hypermethylation of *APC*, *GSTP1*, *RAR* and *RASSF1A* have shown promise in clinical trials aimed at identifying diagnostic markers for prostate cancer [156,157] and may be applicable to tests using urine from patients [158]. The hypermethylation of the *MGMT* promoter appears to be an excellent marker with gliomas, being associated with survival [159] and the response to therapy [160,161].

**Future prospects**

The prospects for the application of epigenetic biomarkers in the clinical setting are excellent. Increasing numbers of potential diagnostic and prognostic markers are being identified for a range of cancers, although many promising leads are still to be validated in prospective studies. The advent of increasingly sensitive technologies, and with these becoming ever more affordable, is facilitating the development of sensitive tests based upon a panel of genes. Furthermore, there have been promising results regarding the application of epigenetic testing to non-invasive sample collections. Questions remain over the potential for blood-based diagnostic tests, however.

 Non-invasive technologies may also improve the identification of biomarkers. Most large-scale prospective studies only collect blood samples due to the cost and effort of collecting other tissue samples. However, the development of methods that use non-invasive sample collections, such as sputum, urine and stool, may facilitate other approaches within prospective studies.

 Further aiding epigenetic epidemiologic research is the development of new, large-scale tissue banks that offer increasingly detailed profiling of tumours, thereby enabling greater stratification of studies. The need for cancer-free normal tissue, free from field defects, for comparison to tumour samples is being met by facilities such as the Susan G. Komen for the Cure Tissue Bank at Indiana University, which offers normal breast tissue and matched blood samples from healthy volunteers.

As new developments and questions arise in the field of epigenetics, so do new opportunities. New insight into the role of 5-hydroxymethylation and the TET family of enzymes will further our understanding of the maintenance of genomic DNA methylation, and how aberrant methylation may arise in (pre-)malignant cells. This could facilitate the identification of early markers of disease and disease risk.

**Conclusions**

Epigenetic epidemiology is providing important insight into how epigenetic alterations are associated with the development of cancer. This is being utilised to benefit patients through a better understanding of risk factors, and by the identification of novel biomarkers that enable early detection of disease and which can inform upon appropriate treatment strategies. The cost and duration of prospective studies can be prohibitive, but they remain the ‘gold standard’ for the identification and validation of biomarkers.

**References**

[1] A. Benard, C.J. van de Velde, L. Lessard, H. Putter, L. Takeshima, P.J. Kuppen, D.S. Hoon, Epigenetic status of LINE-1 predicts clinical outcome in early-stage rectal cancer, Br J Cancer 109 (2013) 3073-3083.

[2] E. Ko, B.B. Lee, Y. Kim, E.J. Lee, E.Y. Cho, J. Han, Y.M. Shim, J. Park, D.H. Kim, Association of RASSF1A and p63 with poor recurrence-free survival in node-negative stage I-II non-small cell lung cancer, Clin Cancer Res 19 (2013) 1204-1212.

[3] K.H. Lee, J.S. Lee, J.H. Nam, C. Choi, M.C. Lee, C.S. Park, S.W. Juhng, J.H. Lee, Promoter methylation status of hMLH1, hMSH2, and MGMT genes in colorectal cancer associated with adenoma-carcinoma sequence, Langenbecks Arch Surg 396 (2011) 1017-1026.

[4] U. Lim, A. Flood, S.W. Choi, D. Albanes, A.J. Cross, A. Schatzkin, R. Sinha, H.A. Katki, B. Cash, P. Schoenfeld, R. Stolzenberg-Solomon, Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women, Gastroenterology 134 (2008) 47-55.

[5] E. Nadal, G. Chen, M. Gallegos, L. Lin, D. Ferrer-Torres, A. Truini, Z. Wang, J. Lin, R.M. Reddy, R. Llatjos, I. Escobar, J. Moya, A.C. Chang, F. Cardenal, G. Capellà, D.G. Beer, Epigenetic inactivation of microRNA-34b/c predicts poor disease-free survival in early-stage lung adenocarcinoma, Clin Cancer Res 19 (2013) 6842-6852.

[6] G. Nikolaidis, O.Y. Raji, S. Markopoulou, J.R. Gosney, J. Bryan, C. Warburton, M. Walshaw, J. Sheard, J.K. Field, T. Liloglou, DNA methylation biomarkers offer improved diagnostic efficiency in lung cancer, Cancer Res 72 (2012) 5692-5701.

[7] J. Sandoval, J. Mendez-Gonzalez, E. Nadal, G. Chen, F.J. Carmona, S. Sayols, S. Moran, H. Heyn, M. Vizoso, A. Gomez, M. Sanchez-Cespedes, Y. Assenov, F. Müller, C. Bock, M. Taron, J. Mora, L.A. Muscarella, T. Liloglou, M. Davies, M. Pollan, M.J. Pajares, W. Torre, L.M. Montuenga, E. Brambilla, J.K. Field, L. Roz, M. Lo Iacono, G.V. Scagliotti, R. Rosell, D.G. Beer, M. Esteller, A prognostic DNA methylation signature for stage I non-small-cell lung cancer, J Clin Oncol 31 (2013) 4140-4147.

[8] A.E. Teschendorff, A. Jones, H. Fiegl, A. Sargent, J.J. Zhuang, H.C. Kitchener, M. Widschwendter, Epigenetic variability in cells of normal cytology is associated with the risk of future morphological transformation, Genome Med 4 (2012) 24.

[9] C.J. Marsit, M.R. Karagas, H. Danaee, M. Liu, A. Andrew, A. Schned, H.H. Nelson, K.T. Kelsey, Carcinogen exposure and gene promoter hypermethylation in bladder cancer, Carcinogenesis 27 (2006) 112-116.

[10] A. Avraham, R. Uhlmann, A. Shperber, M. Birnbaum, J. Sandbank, A. Sella, S. Sukumar, E. Evron, Serum DNA methylation for monitoring response to neoadjuvant chemotherapy in breast cancer patients, Int J Cancer 131 (2012) E1166-E1172.

[11] A. Buhmeida, A. Merdad, J. Al-Maghrabi, J. El-Maghrabi, F. Al-Thobaiti, M. Ata, A. Bugis, K. Syrjänen, A. Abuzenadah, A. Chaudhary, M. Gari, M. Al-Qahtani, A. Dallol, RASSF1A methylation is predictive of poor prognosis in female breast cancer in a background of overall low methylation frequency, Anticancer Res 31 (2011) 2975-2981.

[12] Y.H. Cho, J. Shen, M.D. Gammon, Y.J. Zhang, Q. Wang, K. Gonzalez, X. Xu, P.T. Bradshaw, S.L. Teitelbaum, G. Garbowski, H. Hibshoosh, A.I. Neugut, J. Chen, R.M. Santella, Prognostic significance of gene-specific promoter hypermethylation in breast cancer patients, Breast Cancer Res Treat 131 (2012) 197-205.

[13] M.A. Alvi, X. Liu, M. O'Donovan, R. Newton, L. Wernisch, N.B. Shannon, K. Shariff, M. di Pietro, J.J. Bergman, K. Ragunath, R.C. Fitzgerald, DNA methylation as an adjunct to histopathology to detect prevalent, inconspicuous dysplasia and early-stage neoplasia in Barrett's esophagus, Clin Cancer Res 19 (2013) 878-888.

[14] S.A. Belinsky, K.C. Liechty, F.D. Gentry, H.J. Wolf, J. Rogers, K. Vu, J. Haney, T.C. Kennedy, F.R. Hirsch, Y. Miller, W.A. Franklin, J.G. Herman, S.B. Baylin, P.A. Bunn, T. Byers, Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high-risk cohort, Cancer Res 66 (2006) 3338-3344.

[15] K.D. Hansen, W. Timp, H.C. Bravo, S. Sabunciyan, B. Langmead, O.G. McDonald, B. Wen, H. Wu, Y. Liu, D. Diep, E. Briem, K. Zhang, R.A. Irizarry, A.P. Feinberg, Increased methylation variation in epigenetic domains across cancer types, Nat Genet 43 (2011) 768-775.

[16] A.S. Yang, M.R. Estécio, K. Doshi, Y. Kondo, E.H. Tajara, J.P. Issa, A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements, Nucleic Acids Res 32 (2004) e38.

[17] M. Karimi, S. Johansson, D. Stach, M. Corcoran, D. Grandér, M. Schalling, G. Bakalkin, F. Lyko, C. Larsson, T.J. Ekström, LUMA (LUminometric Methylation Assay)--a high throughput method to the analysis of genomic DNA methylation, Exp Cell Res 312 (2006) 1989-1995.

[18] H.C. Wu, L. Delgado-Cruzata, J.D. Flom, M. Kappil, J.S. Ferris, Y. Liao, R.M. Santella, M.B. Terry, Global methylation profiles in DNA from different blood cell types, Epigenetics 6 (2011) 76-85.

[19] X. Xu, M.D. Gammon, H. Hernandez-Vargas, Z. Herceg, J.G. Wetmur, S.L. Teitelbaum, P.T. Bradshaw, A.I. Neugut, R.M. Santella, J. Chen, DNA methylation in peripheral blood measured by LUMA is associated with breast cancer in a population-based study, FASEB J 26 (2012) 2657-2666.

[20] S. Lisanti, W.A. Omar, B. Tomaszewski, S. De Prins, G. Jacobs, G. Koppen, J.C. Mathers, S.A. Langie, Comparison of methods for quantification of global DNA methylation in human cells and tissues, PLoS One 8 (2013) e79044.

[21] H.C. Wu, Q. Wang, L. Delgado-Cruzata, R.M. Santella, M.B. Terry, Genomic methylation changes over time in peripheral blood mononuclear cell DNA: differences by assay type and baseline values, Cancer Epidemiol Biomarkers Prev 21 (2012) 1314-1318.

[22] D.J. Weisenberger, M. Campan, T.I. Long, M. Kim, C. Woods, E. Fiala, M. Ehrlich, P.W. Laird, Analysis of repetitive element DNA methylation by MethyLight, Nucleic Acids Res 33 (2005) 6823-6836.

[23] K.B. Michels, A.M. Binder, S. Dedeurwaerder, C.B. Epstein, J.M. Greally, I. Gut, E.A. Houseman, B. Izzi, K.T. Kelsey, A. Meissner, A. Milosavljevic, K.D. Siegmund, C. Bock, R.A. Irizarry, Recommendations for the design and analysis of epigenome-wide association studies, Nat Methods 10 (2013) 949-955.

[24] P. Boyle, K. Clement, H. Gu, Z.D. Smith, M. Ziller, J.L. Fostel, L. Holmes, J. Meldrim, F. Kelley, A. Gnirke, A. Meissner, Gel-free multiplexed reduced representation bisulfite sequencing for large-scale DNA methylation profiling, Genome Biol 13 (2012) R92.

[25] M. Farzanehfar, H. Vossoughinia, R. Jabini, A. Tavassoli, H. Saadatnia, A.K. Khorashad, M. Ahadi, M. Afzalaghaee, E. Ghayoor Karimiani, F. Mirzaei, H. Ayatollahi, Evaluation of methylation of MGMT (O6-methylguanine-DNA methyltransferase) gene promoter in sporadic colorectal cancer, DNA Cell Biol 32 (2013) 371-377.

[26] S. Bhusari, B. Yang, J. Kueck, W. Huang, D.F. Jarrard, Insulin-like growth factor-2 (IGF2) loss of imprinting marks a field defect within human prostates containing cancer, Prostate 71 (2011) 1621-1630.

[27] E.M. Wolff, Y. Chihara, F. Pan, D.J. Weisenberger, K.D. Siegmund, K. Sugano, K. Kawashima, P.W. Laird, P.A. Jones, G. Liang, Unique DNA methylation patterns distinguish noninvasive and invasive urothelial cancers and establish an epigenetic field defect in premalignant tissue, Cancer Res 70 (2010) 8169-8178.

[28] K. Nosho, S. Kure, N. Irahara, K. Shima, Y. Baba, D. Spiegelman, J.A. Meyerhardt, E.L. Giovannucci, C.S. Fuchs, S. Ogino, A prospective cohort study shows unique epigenetic, genetic, and prognostic features of synchronous colorectal cancers, Gastroenterology 137 (2009) 1609-20.e1-3.

[29] C. Gu, J. Lu, T. Cui, C. Lu, H. Shi, W. Xu, X. Yuan, X. Yang, Y. Huang, M. Lu, Association between MGMT promoter methylation and non-small cell lung cancer: a meta-analysis, PLoS One 8 (2013) e72633.

[30] L. Barault, R.E. Ellsworth, H.R. Harris, A.L. Valente, C.D. Shriver, K.B. Michels, Leukocyte DNA as surrogate for the evaluation of imprinted Loci methylation in mammary tissue DNA, PLoS One 8 (2013) e55896.

[31] Y. Ito, T. Koessler, A.E. Ibrahim, S. Rai, S.L. Vowler, S. Abu-Amero, A.L. Silva, A.T. Maia, J.E. Huddleston, S. Uribe-Lewis, K. Woodfine, M. Jagodic, R. Nativio, A. Dunning, G. Moore, E. Klenova, S. Bingham, P.D. Pharoah, J.D. Brenton, S. Beck, M.S. Sandhu, A. Murrell, Somatically acquired hypomethylation of IGF2 in breast and colorectal cancer, Hum Mol Genet 17 (2008) 2633-2643.

[32] H. Cui, M. Cruz-Correa, F.M. Giardiello, D.F. Hutcheon, D.R. Kafonek, S. Brandenburg, Y. Wu, X. He, N.R. Powe, A.P. Feinberg, Loss of IGF2 imprinting: a potential marker of colorectal cancer risk, Science 299 (2003) 1753-1755.

[33] E.A. Houseman, W.P. Accomando, D.C. Koestler, B.C. Christensen, C.J. Marsit, H.H. Nelson, J.K. Wiencke, K.T. Kelsey, DNA methylation arrays as surrogate measures of cell mixture distribution, BMC Bioinformatics 13 (2012) 86.

[34] D.C. Koestler, C.J. Marsit, B.C. Christensen, W. Accomando, S.M. Langevin, E.A. Houseman, H.H. Nelson, M.R. Karagas, J.K. Wiencke, K.T. Kelsey, Peripheral blood immune cell methylation profiles are associated with nonhematopoietic cancers, Cancer Epidemiol Biomarkers Prev 21 (2012) 1293-1302.

[35] R. Grützmann, B. Molnar, C. Pilarsky, J.K. Habermann, P.M. Schlag, H.D. Saeger, S. Miehlke, T. Stolz, F. Model, U.J. Roblick, H.P. Bruch, R. Koch, V. Liebenberg, T. Devos, X. Song, R.H. Day, A.Z. Sledziewski, C. Lofton-Day, Sensitive detection of colorectal cancer in peripheral blood by septin 9 DNA methylation assay, PLoS One 3 (2008) e3759.

[36] R.H. Carvalho, V. Haberle, J. Hou, T. van Gent, S. Thongjuea, W. van Ijcken, C. Kockx, R. Brouwer, E. Rijkers, A. Sieuwerts, J. Foekens, M. van Vroonhoven, J. Aerts, F. Grosveld, B. Lenhard, S. Philipsen, Genome-wide DNA methylation profiling of non-small cell lung carcinomas, Epigenetics Chromatin 5 (2012) 9.

[37] D. Connolly, Z. Yang, M. Castaldi, N. Simmons, M.H. Oktay, S. Coniglio, M.J. Fazzari, P. Verdier-Pinard, C. Montagna, Septin 9 isoform expression, localization and epigenetic changes during human and mouse breast cancer progression, Breast Cancer Res 13 (2011) R76.

[38] S.M. Tajuddin, A.F. Amaral, A.F. Fernández, S. Rodríguez-Rodero, R.M. Rodríguez, L.E. Moore, A. Tardón, A. Carrato, M. García-Closas, D.T. Silverman, B.P. Jackson, R. García-Closas, A.L. Cook, K.P. Cantor, S. Chanock, M. Kogevinas, N. Rothman, F.X. Real, M.F. Fraga, N. Malats, Spanish Bladder Cancer/EPICURO Study Investigators, Genetic and non-genetic predictors of LINE-1 methylation in leukocyte DNA, Environ Health Perspect 121 (2013) 650-656.

[39] M. Widschwendter, S. Apostolidou, E. Raum, D. Rothenbacher, H. Fiegl, U. Menon, C. Stegmaier, I.J. Jacobs, H. Brenner, Epigenotyping in peripheral blood cell DNA and breast cancer risk: a proof of principle study, PLoS One 3 (2008) e2656.

[40] M. Li, W.D. Chen, N. Papadopoulos, S.N. Goodman, N.C. Bjerregaard, S. Laurberg, B. Levin, H. Juhl, N. Arber, H. Moinova, K. Durkee, K. Schmidt, Y. He, F. Diehl, V.E. Velculescu, S. Zhou, L.A. Diaz, K.W. Kinzler, S.D. Markowitz, B. Vogelstein, Sensitive digital quantification of DNA methylation in clinical samples, Nat Biotechnol 27 (2009) 858-863.

[41] K.C. Chan, P. Jiang, C.W. Chan, K. Sun, J. Wong, E.P. Hui, S.L. Chan, W.C. Chan, D.S. Hui, S.S. Ng, H.L. Chan, C.S. Wong, B.B. Ma, A.T. Chan, P.B. Lai, H. Sun, R.W. Chiu, Y.M. Lo, Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing, Proc Natl Acad Sci U S A 110 (2013) 18761-18768.

[42] U. Gezer, D. Ustek, E.E. Yörüker, A. Cakiris, N. Abaci, G. Leszinski, N. Dalay, S. Holdenrieder, Characterization of H3K9me3- and H4K20me3-associated circulating nucleosomal DNA by high-throughput sequencing in colorectal cancer, Tumour Biol 34 (2013) 329-336.

[43] T. Benaglia, L.D. Sharples, R.C. Fitzgerald, G. Lyratzopoulos, Health benefits and cost effectiveness of endoscopic and nonendoscopic cytosponge screening for Barrett's esophagus, Gastroenterology 144 (2013) 62-73.e6.

[44] S.R. Kadri, P. Lao-Sirieix, M. O'Donovan, I. Debiram, M. Das, J.M. Blazeby, J. Emery, A. Boussioutas, H. Morris, F.M. Walter, P. Pharoah, R.H. Hardwick, R.C. Fitzgerald, Acceptability and accuracy of a non-endoscopic screening test for Barrett's oesophagus in primary care: cohort study, BMJ 341 (2010) c4372.

[45] W.J. Bulsiewicz, H.P. Kim, E.S. Dellon, C.C. Cotton, S. Pasricha, R.D. Madanick, M.B. Spacek, S.E. Bream, X. Chen, R.C. Orlando, N.J. Shaheen, Safety and efficacy of endoscopic mucosal therapy with radiofrequency ablation for patients with neoplastic Barrett's esophagus, Clin Gastroenterol Hepatol 11 (2013) 636-642.

[46] E.R. Fearon, B. Vogelstein, A genetic model for colorectal tumorigenesis, Cell 61 (1990) 759-767.

[47] V. Psofaki, C. Kalogera, N. Tzambouras, D. Stephanou, E. Tsianos, K. Seferiadis, G. Kolios, Promoter methylation status of hMLH1, MGMT, and CDKN2A/p16 in colorectal adenomas, World J Gastroenterol 16 (2010) 3553-3560.

[48] X. Li, X. Yao, Y. Wang, F. Hu, F. Wang, L. Jiang, Y. Liu, D. Wang, G. Sun, Y. Zhao, MLH1 promoter methylation frequency in colorectal cancer patients and related clinicopathological and molecular features, PLoS One 8 (2013) e59064.

[49] M. Toyota, N. Ahuja, M. Ohe-Toyota, J.G. Herman, S.B. Baylin, J.P. Issa, CpG island methylator phenotype in colorectal cancer, Proc Natl Acad Sci U S A 96 (1999) 8681-8686.

[50] Y. Baba, C. Huttenhower, K. Nosho, N. Tanaka, K. Shima, A. Hazra, E.S. Schernhammer, D.J. Hunter, E.L. Giovannucci, C.S. Fuchs, S. Ogino, Epigenomic diversity of colorectal cancer indicated by LINE-1 methylation in a database of 869 tumors, Mol Cancer 9 (2010) 125.

[51] L.J. Gay, P.N. Mitrou, J. Keen, R. Bowman, A. Naguib, J. Cooke, G.G. Kuhnle, P.A. Burns, R. Luben, M. Lentjes, K.T. Khaw, R.Y. Ball, A.E. Ibrahim, M.J. Arends, Dietary, lifestyle and clinicopathological factors associated with APC mutations and promoter methylation in colorectal cancers from the EPIC-Norfolk study, J Pathol 228 (2012) 405-415.

[52] R. Nishihara, T. Morikawa, A. Kuchiba, P. Lochhead, M. Yamauchi, X. Liao, Y. Imamura, K. Nosho, K. Shima, I. Kawachi, Z.R. Qian, C.S. Fuchs, A.T. Chan, E. Giovannucci, S. Ogino, A prospective study of duration of smoking cessation and colorectal cancer risk by epigenetics-related tumor classification, Am J Epidemiol 178 (2013) 84-100.

[53] M. Pufulete, R. Al-Ghnaniem, A.J. Leather, P. Appleby, S. Gout, C. Terry, P.W. Emery, T.A. Sanders, Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study, Gastroenterology 124 (2003) 1240-1248.

[54] E.S. Schernhammer, E. Giovannucci, T. Kawasaki, B. Rosner, C.S. Fuchs, S. Ogino, Dietary folate, alcohol and B vitamins in relation to LINE-1 hypomethylation in colon cancer, Gut 59 (2010) 794-799.

[55] W.Y. Huang, L.J. Su, R.B. Hayes, L.E. Moore, H.A. Katki, S.I. Berndt, J.L. Weissfeld, S. Yegnasubramanian, M.P. Purdue, Prospective study of genomic hypomethylation of leukocyte DNA and colorectal cancer risk, Cancer Epidemiol Biomarkers Prev 21 (2012) 2014-2021.

[56] E.S. Schernhammer, E. Giovannucci, Y. Baba, C.S. Fuchs, S. Ogino, B vitamins, methionine and alcohol intake and risk of colon cancer in relation to BRAF mutation and CpG island methylator phenotype (CIMP), PLoS One 6 (2011) e21102.

[57] B. Van Guelpen, A.M. Dahlin, J. Hultdin, V. Eklöf, I. Johansson, M.L. Henriksson, I. Cullman, G. Hallmans, R. Palmqvist, One-carbon metabolism and CpG island methylator phenotype status in incident colorectal cancer: a nested case-referent study, Cancer Causes Control 21 (2010) 557-566.

[58] S. Ogino, R. Nishihara, P. Lochhead, Y. Imamura, A. Kuchiba, T. Morikawa, M. Yamauchi, X. Liao, Z.R. Qian, R. Sun, K. Sato, G.J. Kirkner, M. Wang, D. Spiegelman, J.A. Meyerhardt, E.S. Schernhammer, A.T. Chan, E. Giovannucci, C.S. Fuchs, Prospective study of family history and colorectal cancer risk by tumor LINE-1 methylation level, J Natl Cancer Inst 105 (2013) 130-140.

[59] A.S. Fleisher, M. Esteller, N. Harpaz, A. Leytin, A. Rashid, Y. Xu, J. Liang, O.C. Stine, J. Yin, T.T. Zou, J.M. Abraham, D. Kong, K.T. Wilson, S.P. James, J.G. Herman, S.J. Meltzer, Microsatellite instability in inflammatory bowel disease-associated neoplastic lesions is associated with hypermethylation and diminished expression of the DNA mismatch repair gene, hMLH1, Cancer Res 60 (2000) 4864-4868.

[60] M. Svrcek, J. El-Bchiri, A. Chalastanis, E. Capel, S. Dumont, O. Buhard, C. Oliveira, R. Seruca, C. Bossard, J.F. Mosnier, F. Berger, E. Leteurtre, A. Lavergne-Slove, M.P. Chenard, R. Hamelin, J. Cosnes, L. Beaugerie, E. Tiret, A. Duval, J.F. Fléjou, Specific clinical and biological features characterize inflammatory bowel disease associated colorectal cancers showing microsatellite instability, J Clin Oncol 25 (2007) 4231-4238.

[61] L. Tarmin, J. Yin, N. Harpaz, M. Kozam, J. Noordzij, L.B. Antonio, H.Y. Jiang, O. Chan, K. Cymes, S.J. Meltzer, Adenomatous polyposis coli gene mutations in ulcerative colitis-associated dysplasias and cancers versus sporadic colon neoplasms, Cancer Res 55 (1995) 2035-2038.

[62] E.H. van Roon, N.F. de Miranda, M.P. van Nieuwenhuizen, E.J. de Meijer, M. van Puijenbroek, P.S. Yan, T.H. Huang, T. van Wezel, H. Morreau, J.M. Boer, Tumour-specific methylation of PTPRG intron 1 locus in sporadic and Lynch syndrome colorectal cancer, Eur J Hum Genet 19 (2011) 307-312.

[63] M.R. Kohonen-Corish, J. Tseung, C. Chan, N. Currey, O.F. Dent, S. Clarke, L. Bokey, P.H. Chapuis, KRAS mutations and CDKN2A promoter methylation show an interactive adverse effect on survival and predict recurrence of rectal cancer, Int J Cancer 134 (2014) 2820-2828.

[64] A. Naguib, P.N. Mitrou, L.J. Gay, J.C. Cooke, R.N. Luben, R.Y. Ball, A. McTaggart, M.J. Arends, S.A. Rodwell, Dietary, lifestyle and clinicopathological factors associated with BRAF and K-ras mutations arising in distinct subsets of colorectal cancers in the EPIC Norfolk study, BMC Cancer 10 (2010) 99.

[65] H. Rajagopalan, A. Bardelli, C. Lengauer, K.W. Kinzler, B. Vogelstein, V.E. Velculescu, Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status, Nature 418 (2002) 934.

[66] J.C. Kim, J.S. Choi, S.A. Roh, D.H. Cho, T.W. Kim, Y.S. Kim, Promoter methylation of specific genes is associated with the phenotype and progression of colorectal adenocarcinomas, Ann Surg Oncol 17 (2010) 1767-1776.

[67] H. Nan, E.L. Giovannucci, K. Wu, J. Selhub, L. Paul, B. Rosner, C.S. Fuchs, E. Cho, Pre-diagnostic leukocyte genomic DNA methylation and the risk of colorectal cancer in women, PLoS One 8 (2013) e59455.

[68] T.R. Church, M. Wandell, C. Lofton-Day, S.J. Mongin, M. Burger, S.R. Payne, E. Castaños-Vélez, B.A. Blumenstein, T. Rösch, N. Osborn, D. Snover, R.W. Day, D.F. Ransohoff, PRESEPT Clinical Study Steering Committee, Investigators and Study Team, Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer, Gut 63 (2014) 317-325.

[69] R. Kaaks, P. Stattin, S. Villar, A.R. Poetsch, L. Dossus, A. Nieters, E. Riboli, R. Palmqvist, G. Hallmans, C. Plass, M.D. Friesen, Insulin-like growth factor-II methylation status in lymphocyte DNA and colon cancer risk in the Northern Sweden Health and Disease cohort, Cancer Res 69 (2009) 5400-5405.

[70] C.P. Lange, M. Campan, T. Hinoue, R.F. Schmitz, A.E. van der Meulen-de Jong, H. Slingerland, P.J. Kok, C.M. van Dijk, D.J. Weisenberger, H. Shen, R.A. Tollenaar, P.W. Laird, Genome-scale discovery of DNA-methylation biomarkers for blood-based detection of colorectal cancer, PLoS One 7 (2012) e50266.

[71] J.B. Kisiel, T.C. Yab, F.T. Nazer Hussain, W.R. Taylor, M.M. Garrity-Park, W.J. Sandborn, E.V. Loftus, B.G. Wolff, T.C. Smyrk, S.H. Itzkowitz, D.T. Rubin, H. Zou, D.W. Mahoney, D.A. Ahlquist, Stool DNA testing for the detection of colorectal neoplasia in patients with inflammatory bowel disease, Aliment Pharmacol Ther 37 (2013) 546-554.

[72] T.F. Imperiale, D.F. Ransohoff, S.H. Itzkowitz, T.R. Levin, P. Lavin, G.P. Lidgard, D.A. Ahlquist, B.M. Berger, Multitarget stool DNA testing for colorectal-cancer screening, N Engl J Med 370 (2014) 1287-1297.

[73] C. Guastadisegni, M. Colafranceschi, L. Ottini, E. Dogliotti, Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data, Eur J Cancer 46 (2010) 2788-2798.

[74] K. Nosho, N. Irahara, K. Shima, S. Kure, G.J. Kirkner, E.S. Schernhammer, A. Hazra, D.J. Hunter, J. Quackenbush, D. Spiegelman, E.L. Giovannucci, C.S. Fuchs, S. Ogino, Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample, PLoS One 3 (2008) e3698.

[75] S. Ogino, K. Nosho, G.J. Kirkner, T. Kawasaki, J.A. Meyerhardt, M. Loda, E.L. Giovannucci, C.S. Fuchs, CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer, Gut 58 (2009) 90-96.

[76] S.W. Han, H.J. Lee, J.M. Bae, N.Y. Cho, K.H. Lee, T.Y. Kim, D.Y. Oh, S.A. Im, Y.J. Bang, S.Y. Jeong, K.J. Park, J.G. Park, G.H. Kang, T.Y. Kim, Methylation and microsatellite status and recurrence following adjuvant FOLFOX in colorectal cancer, Int J Cancer 132 (2013) 2209-2216.

[77] S. Ogino, K. Nosho, G.J. Kirkner, T. Kawasaki, A.T. Chan, E.S. Schernhammer, E.L. Giovannucci, C.S. Fuchs, A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer, J Natl Cancer Inst 100 (2008) 1734-1738.

[78] K. Shima, K. Nosho, Y. Baba, M. Cantor, J.A. Meyerhardt, E.L. Giovannucci, C.S. Fuchs, S. Ogino, Prognostic significance of CDKN2A (p16) promoter methylation and loss of expression in 902 colorectal cancers: Cohort study and literature review, Int J Cancer 128 (2011) 1080-1094.

[79] S. Veganzones-de-Castro, S. Rafael-Fernández, M. Vidaurreta-Lázaro, V. de-la-Orden, B. Mediero-Valeros, C. Fernández, M.L. Maestro-de las Casas, p16 gene methylation in colorectal cancer patients with long-term follow-up, Rev Esp Enferm Dig 104 (2012) 111-117.

[80] H. Mitomi, N. Fukui, N. Tanaka, H. Kanazawa, T. Saito, T. Matsuoka, T. Yao, Aberrant p16((INK4a)) methylation is a frequent event in colorectal cancers: prognostic value and relation to mRNA expression and immunoreactivity, J Cancer Res Clin Oncol 136 (2010) 323-331.

[81] K. Shima, T. Morikawa, Y. Baba, K. Nosho, M. Suzuki, M. Yamauchi, M. Hayashi, E. Giovannucci, C.S. Fuchs, S. Ogino, MGMT promoter methylation, loss of expression and prognosis in 855 colorectal cancers, Cancer Causes Control 22 (2011) 301-309.

[82] Y. Takahashi, T. Iwaya, G. Sawada, J. Kurashige, T. Matsumura, R. Uchi, H. Ueo, Y. Takano, H. Eguchi, T. Sudo, K. Sugimachi, H. Yamamoto, Y. Doki, M. Mori, K. Mimori, Up-regulation of NEK2 by microRNA-128 methylation is associated with poor prognosis in colorectal cancer, Ann Surg Oncol 21 (2014) 205-212.

[83] T. Hashimoto, M. Yamakawa, S. Kimura, O. Usuba, M. Toyono, Expression of acetylated and dimethylated histone H3 in colorectal cancer, Dig Surg 30 (2013) 249-258.

[84] H. Tamagawa, T. Oshima, M. Numata, N. Yamamoto, M. Shiozawa, S. Morinaga, Y. Nakamura, M. Yoshihara, Y. Sakuma, Y. Kameda, M. Akaike, N. Yukawa, Y. Rino, M. Masuda, Y. Miyagi, Global histone modification of H3K27 correlates with the outcomes in patients with metachronous liver metastasis of colorectal cancer, Eur J Surg Oncol 39 (2013) 655-661.

[85] M. Takawa, K. Masuda, M. Kunizaki, Y. Daigo, K. Takagi, Y. Iwai, H.S. Cho, G. Toyokawa, Y. Yamane, K. Maejima, H.I. Field, T. Kobayashi, T. Akasu, M. Sugiyama, E. Tsuchiya, Y. Atomi, B.A. Ponder, Y. Nakamura, R. Hamamoto, Validation of the histone methyltransferase EZH2 as a therapeutic target for various types of human cancer and as a prognostic marker, Cancer Sci 102 (2011) 1298-1305.

[86] J.M. Carethers, E. Smith, C.A. Behling, L. Nguyen, A. Tajima, R.T. Doctolero, B.L. Cabrera, A. Goel, C.A. Arnold, K. Miyai, C. Boland, Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer, Gastroenterology 126 (2004) 394-401.

[87] C.M. Ribic, D.J. Sargent, M.J. Moore, S.N. Thibodeau, A.J. French, R.M. Goldberg, S.R. Hamilton, P. Laurent-Puig, R. Gryfe, L.E. Shepherd, D. Tu, M. Redston, S. Gallinger, Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer, N Engl J Med 349 (2003) 247-257.

[88] A. Zaanan, J.F. Fléjou, J.F. Emile, G.G. Des, P. Cuilliere-Dartigues, D. Malka, C. Lecaille, P. Validire, C. Louvet, P. Rougier, A. de Gramont, F. Bonnetain, F. Praz, J. Taïeb, Defective mismatch repair status as a prognostic biomarker of disease-free survival in stage III colon cancer patients treated with adjuvant FOLFOX chemotherapy, Clin Cancer Res 17 (2011) 7470-7478.

[89] M.M. Bertagnolli, D. Niedzwiecki, C.C. Compton, H.P. Hahn, M. Hall, B. Damas, S.D. Jewell, R.J. Mayer, R.M. Goldberg, L.B. Saltz, R.S. Warren, M. Redston, Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803, J Clin Oncol 27 (2009) 1814-1821.

[90] K. Kawakami, A. Matsunoki, M. Kaneko, K. Saito, G. Watanabe, T. Minamoto, Long interspersed nuclear element-1 hypomethylation is a potential biomarker for the prediction of response to oral fluoropyrimidines in microsatellite stable and CpG island methylator phenotype-negative colorectal cancer, Cancer Sci 102 (2011) 166-174.

[91] C. Moutinho, A. Martinez-Cardús, C. Santos, V. Navarro-Pérez, E. Martínez-Balibrea, E. Musulen, F.J. Carmona, A. Sartore-Bianchi, A. Cassingena, S. Siena, E. Elez, J. Tabernero, R. Salazar, A. Abad, M. Esteller, Epigenetic inactivation of the BRCA1 interactor SRBC and resistance to oxaliplatin in colorectal cancer, J Natl Cancer Inst 106 (2014) djt322.

[92] N.S. Shenker, S. Polidoro, K. van Veldhoven, C. Sacerdote, F. Ricceri, M.A. Birrell, M.G. Belvisi, R. Brown, P. Vineis, J.M. Flanagan, Epigenome-wide association study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) identifies novel genetic loci associated with smoking, Hum Mol Genet 22 (2013) 843-851.

[93] N.S. Shenker, P.M. Ueland, S. Polidoro, K. van Veldhoven, F. Ricceri, R. Brown, J.M. Flanagan, P. Vineis, DNA methylation as a long-term biomarker of exposure to tobacco smoke, Epidemiology 24 (2013) 712-716.

[94] B.R. Joubert, S.E. Håberg, R.M. Nilsen, X. Wang, S.E. Vollset, S.K. Murphy, Z. Huang, C. Hoyo, Ø. Midttun, L.A. Cupul-Uicab, P.M. Ueland, M.C. Wu, W. Nystad, D.A. Bell, S.D. Peddada, S.J. London, 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy, Environ Health Perspect 120 (2012) 1425-1431.

[95] A. Baccarelli, R.O. Wright, V. Bollati, L. Tarantini, A.A. Litonjua, H.H. Suh, A. Zanobetti, D. Sparrow, P.S. Vokonas, J. Schwartz, Rapid DNA methylation changes after exposure to traffic particles, Am J Respir Crit Care Med 179 (2009) 572-578.

[96] L. Tarantini, M. Bonzini, P. Apostoli, V. Pegoraro, V. Bollati, B. Marinelli, L. Cantone, G. Rizzo, L. Hou, J. Schwartz, P.A. Bertazzi, A. Baccarelli, Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation, Environ Health Perspect 117 (2009) 217-222.

[97] H.M. Byun, V. Motta, T. Panni, P.A. Bertazzi, P. Apostoli, L. Hou, A.A. Baccarelli, Evolutionary age of repetitive element subfamilies and sensitivity of DNA methylation to airborne pollutants, Part Fibre Toxicol 10 (2013) 28.

[98] L. Hou, X. Zhang, Y. Zheng, S. Wang, C. Dou, L. Guo, H.M. Byun, V. Motta, J. McCracken, A. Díaz, C.M. Kang, P. Koutrakis, P.A. Bertazzi, J. Li, J. Schwartz, A.A. Baccarelli, Altered methylation in tandem repeat element and elemental component levels in inhalable air particles, Environ Mol Mutagen 55 (2014) 256-265.

[99] P. Vineis, S.-C. Chuang, T. Vaissière, C. Cuenin, F. Ricceri, G. collaborators, M. Johansson, P. Ueland, P. Brennan, Z. Herceg, DNA methylation changes associated with cancer risk factors and blood levels of vitamin metabolites in a prospective study, Epigenetics 6 (2011) 195-201.

[100] R. Cirincione, C. Lintas, D. Conte, L. Mariani, L. Roz, A.M. Vignola, U. Pastorino, G. Sozzi, Methylation profile in tumor and sputum samples of lung cancer patients detected by spiral computed tomography: a nested case-control study, Int J Cancer 118 (2006) 1248-1253.

[101] P. Ilse, S. Biesterfeld, N. Pomjanski, C. Fink, M. Schramm, SHOX2 DNA methylation is a tumour marker in pleural effusions, Cancer Genomics Proteomics 10 (2013) 217-223.

[102] W.H. Roa, J.O. Kim, R. Razzak, H. Du, L. Guo, R. Singh, S. Gazala, S. Ghosh, E. Wong, A.A. Joy, J.Z. Xing, E.L. Bedard, Sputum microRNA profiling: a novel approach for the early detection of non-small cell lung cancer, Clin Invest Med 35 (2012) E271.

[103] J. Shen, J. Liao, M.A. Guarnera, H. Fang, L. Cai, S.A. Stass, F. Jiang, Analysis of MicroRNAs in sputum to improve computed tomography for lung cancer diagnosis, J Thorac Oncol 9 (2014) 33-40.

[104] H.S. Hsu, T.P. Chen, C.K. Wen, C.H. Hung, C.Y. Chen, J.T. Chen, Y.C. Wang, Multiple genetic and epigenetic biomarkers for lung cancer detection in cytologically negative sputum and a nested case-control study for risk assessment, J Pathol 213 (2007) 412-419.

[105] S. Bruse, H. Petersen, J. Weissfeld, M. Picchi, R. Willink, K. Do, J. Siegfried, S.A. Belinsky, Y. Tesfaigzi, Increased methylation of lung cancer-associated genes in sputum DNA of former smokers with chronic mucous hypersecretion, Respir Res 15 (2014) 2.

[106] A.J. Hubers, D.A. Heideman, G.J. Herder, S.A. Burgers, P.J. Sterk, P.W. Kunst, H.J. Smit, P.E. Postmus, B.I. Witte, S. Duin, P.J. Snijders, E.F. Smit, E. Thunnissen, Prolonged sampling of spontaneous sputum improves sensitivity of hypermethylation analysis for lung cancer, J Clin Pathol 65 (2012) 541-545.

[107] S. Leng, K. Do, C.M. Yingling, M.A. Picchi, H.J. Wolf, T.C. Kennedy, W.J. Feser, A.E. Baron, W.A. Franklin, M.V. Brock, J.G. Herman, S.B. Baylin, T. Byers, C.A. Stidley, S.A. Belinsky, Defining a gene promoter methylation signature in sputum for lung cancer risk assessment, Clin Cancer Res 18 (2012) 3387-3395.

[108] S. Leng, Y. Liu, C.L. Thomas, W.J. Gauderman, M.A. Picchi, S.E. Bruse, X. Zhang, K.G. Flores, D. Van Den Berg, C.A. Stidley, F.D. Gilliland, S.A. Belinsky, Native American ancestry affects the risk for gene methylation in the lungs of Hispanic smokers from New Mexico, Am J Respir Crit Care Med 188 (2013) 1110-1116.

[109] Z. Lou-Qian, Y. Rong, L. Ming, Y. Xin, J. Feng, X. Lin, The prognostic value of epigenetic silencing of p16 gene in NSCLC patients: a systematic review and meta-analysis, PLoS One 8 (2013) e54970.

[110] N.G. Bediaga, M.P. Davies, A. Acha-Sagredo, R. Hyde, O.Y. Raji, R. Page, M. Walshaw, J. Gosney, A. Alfirevic, J.K. Field, T. Liloglou, A microRNA-based prediction algorithm for diagnosis of non-small lung cell carcinoma in minimal biopsy material, Br J Cancer 109 (2013) 2404-2411.

[111] E. Duncavage, B. Goodgame, A. Sezhiyan, R. Govindan, J. Pfeifer, Use of microRNA expression levels to predict outcomes in resected stage I non-small cell lung cancer, J Thorac Oncol 5 (2010) 1755-1763.

[112] M. Raponi, L. Dossey, T. Jatkoe, X. Wu, G. Chen, H. Fan, D.G. Beer, MicroRNA classifiers for predicting prognosis of squamous cell lung cancer, Cancer Res 69 (2009) 5776-5783.

[113] X.G. Liu, W.Y. Zhu, Y.Y. Huang, L.N. Ma, S.Q. Zhou, Y.K. Wang, F. Zeng, J.H. Zhou, Y.K. Zhang, High expression of serum miR-21 and tumor miR-200c associated with poor prognosis in patients with lung cancer, Med Oncol 29 (2012) 618-626.

[114] T. Berghmans, L. Ameye, L. Willems, M. Paesmans, C. Mascaux, J.J. Lafitte, A.P. Meert, A. Scherpereel, A.B. Cortot, I. Cstoth, T. Dernies, L. Toussaint, N. Leclercq, J.P. Sculier, European Lung Cancer Working Party, Identification of microRNA-based signatures for response and survival for non-small cell lung cancer treated with cisplatin-vinorelbine A ELCWP prospective study, Lung Cancer 82 (2013) 340-345.

[115] N.G. Bediaga, A. Acha-Sagredo, I. Guerra, A. Viguri, C. Albaina, I. Ruiz Diaz, R. Rezola, M.J. Alberdi, J. Dopazo, D. Montaner, M. Renobales, A.F. Fernández, J.K. Field, M.F. Fraga, T. Liloglou, M.M. de Pancorbo, DNA methylation epigenotypes in breast cancer molecular subtypes, Breast Cancer Res 12 (2010) R77.

[116] M.J. Fackler, C.B. Umbricht, D. Williams, P. Argani, L.A. Cruz, V.F. Merino, W.W. Teo, Z. Zhang, P. Huang, K. Visvananthan, J. Marks, S. Ethier, J.W. Gray, A.C. Wolff, L.M. Cope, S. Sukumar, Genome-wide methylation analysis identifies genes specific to breast cancer hormone receptor status and risk of recurrence, Cancer Res 71 (2011) 6195-6207.

[117] W. Feng, L. Shen, S. Wen, D.G. Rosen, J. Jelinek, X. Hu, S. Huan, M. Huang, J. Liu, A.A. Sahin, K.K. Hunt, R.C. Bast, Y. Shen, J.P. Issa, Y. Yu, Correlation between CpG methylation profiles and hormone receptor status in breast cancers, Breast Cancer Res 9 (2007) R57.

[118] L. Li, K.M. Lee, W. Han, J.Y. Choi, J.Y. Lee, G.H. Kang, S.K. Park, D.Y. Noh, K.Y. Yoo, D. Kang, Estrogen and progesterone receptor status affect genome-wide DNA methylation profile in breast cancer, Hum Mol Genet 19 (2010) 4273-4277.

[119] K.P. Suijkerbuijk, M.J. Fackler, S. Sukumar, C.H. van Gils, T. van Laar, E. van der Wall, M. Vooijs, P.J. van Diest, Methylation is less abundant in BRCA1-associated compared with sporadic breast cancer, Ann Oncol 19 (2008) 1870-1874.

[120] J.S. Prabhu, K. Wahi, A. Korlimarla, M. Correa, S. Manjunath, N. Raman, B.S. Srinath, T.S. Sridhar, The epigenetic silencing of the estrogen receptor (ER) by hypermethylation of the ESR1 promoter is seen predominantly in triple-negative breast cancers in Indian women, Tumour Biol 33 (2012) 315-323.

[121] R. Ben Gacem, M. Hachana, S. Ziadi, K. Amara, F. Ksia, M. Mokni, M. Trimeche, Contribution of epigenetic alteration of BRCA1 and BRCA2 genes in breast carcinomas in Tunisian patients, Cancer Epidemiol 36 (2012) 190-197.

[122] L. Wu, F. Wang, R. Xu, S. Zhang, X. Peng, Y. Feng, J. Wang, C. Lu, Promoter methylation of BRCA1 in the prognosis of breast cancer: a meta-analysis, Breast Cancer Res Treat 142 (2013) 619-627.

[123] S.N. Vasilatos, G. Broadwater, W.T. Barry, J.C. Baker, S. Lem, E.C. Dietze, G.R. Bean, A.D. Bryson, P.G. Pilie, V. Goldenberg, D. Skaar, C. Paisie, A. Torres-Hernandez, T.L. Grant, L.G. Wilke, C. Ibarra-Drendall, J.H. Ostrander, N.C. D'Amato, C. Zalles, R. Jirtle, V.M. Weaver, V.L. Seewaldt, CpG island tumor suppressor promoter methylation in non-BRCA-associated early mammary carcinogenesis, Cancer Epidemiol Biomarkers Prev 18 (2009) 901-914.

[124] K. Brennan, M. Garcia-Closas, N. Orr, O. Fletcher, M. Jones, A. Ashworth, A. Swerdlow, H. Thorne, E. Riboli, P. Vineis, M. Dorronsoro, F. Clavel-Chapelon, S. Panico, N.C. Onland-Moret, D. Trichopoulos, R. Kaaks, K.T. Khaw, R. Brown, J.M. Flanagan, Intragenic ATM methylation in peripheral blood DNA as a biomarker of breast cancer risk, Cancer Res 72 (2012) 2304-2313.

[125] E.K. Ng, C.P. Leung, V.Y. Shin, C.L. Wong, E.S. Ma, H.C. Jin, K.M. Chu, A. Kwong, Quantitative analysis and diagnostic significance of methylated SLC19A3 DNA in the plasma of breast and gastric cancer patients, PLoS One 6 (2011) e22233.

[126] R. Radpour, Z. Barekati, C. Kohler, Q. Lv, N. Bürki, C. Diesch, J. Bitzer, H. Zheng, S. Schmid, X.Y. Zhong, Hypermethylation of tumor suppressor genes involved in critical regulatory pathways for developing a blood-based test in breast cancer, PLoS One 6 (2011) e16080.

[127] M. Chimonidou, A. Tzitzira, A. Strati, G. Sotiropoulou, C. Sfikas, N. Malamos, V. Georgoulias, E. Lianidou, CST6 promoter methylation in circulating cell-free DNA of breast cancer patients, Clin Biochem 46 (2013) 235-240.

[128] J.D. Brooks, P. Cairns, R.E. Shore, C.B. Klein, I. Wirgin, Y. Afanasyeva, A. Zeleniuch-Jacquotte, DNA methylation in pre-diagnostic serum samples of breast cancer cases: results of a nested case-control study, Cancer Epidemiol 34 (2010) 717-723.

[129] R. Bosviel, S. Garcia, G. Lavediaux, E. Michard, M. Dravers, F. Kwiatkowski, Y.J. Bignon, D.J. Bernard-Gallon, BRCA1 promoter methylation in peripheral blood DNA was identified in sporadic breast cancer and controls, Cancer Epidemiol 36 (2012) e177-e182.

[130] Z. Xu, S.C. Bolick, L.A. DeRoo, C.R. Weinberg, D.P. Sandler, J.A. Taylor, Epigenome-wide association study of breast cancer using prospectively collected sister study samples, J Natl Cancer Inst 105 (2013) 694-700.

[131] L.A. Deroo, S.C. Bolick, Z. Xu, D.M. Umbach, D. Shore, C.R. Weinberg, D.P. Sandler, J.A. Taylor, Global DNA methylation and one-carbon metabolism gene polymorphisms and the risk of breast cancer in the Sister Study, Carcinogenesis 35 (2014) 333-338.

[132] H.C. Wu, L. Delgado-Cruzata, J.D. Flom, M. Perrin, Y. Liao, J.S. Ferris, R.M. Santella, M.B. Terry, Repetitive element DNA methylation levels in white blood cell DNA from sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry, Carcinogenesis 33 (2012) 1946-1952.

[133] N.C. Hsu, Y.F. Huang, K.K. Yokoyama, P.Y. Chu, F.M. Chen, M.F. Hou, Methylation of BRCA1 promoter region is associated with unfavorable prognosis in women with early-stage breast cancer, PLoS One 8 (2013) e56256.

[134] X. Xu, M.D. Gammon, Y. Zhang, Y.H. Cho, J.G. Wetmur, P.T. Bradshaw, G. Garbowski, H. Hibshoosh, S.L. Teitelbaum, A.I. Neugut, R.M. Santella, J. Chen, Gene promoter methylation is associated with increased mortality among women with breast cancer, Breast Cancer Res Treat 121 (2010) 685-692.

[135] A.Q. van Hoesel, C.J. van de Velde, P.J. Kuppen, G.J. Liefers, H. Putter, Y. Sato, D.A. Elashoff, R.R. Turner, J.M. Shamonki, E.M. de Kruijf, J.G. van Nes, A.E. Giuliano, D.S. Hoon, Hypomethylation of LINE-1 in primary tumor has poor prognosis in young breast cancer patients: a retrospective cohort study, Breast Cancer Res Treat 134 (2012) 1103-1114.

[136] T. Swift-Scanlan, R. Vang, A. Blackford, M.J. Fackler, S. Sukumar, Methylated genes in breast cancer: Associations with clinical and histopathological features in a familial breast cancer cohort, Cancer Biology & Therapy 11 (2011) 853-865.

[137] D.P. Silver, A.L. Richardson, A.C. Eklund, Z.C. Wang, Z. Szallasi, Q. Li, N. Juul, C.O. Leong, D. Calogrias, A. Buraimoh, A. Fatima, R.S. Gelman, P.D. Ryan, N.M. Tung, A. De Nicolo, S. Ganesan, A. Miron, C. Colin, D.C. Sgroi, L.W. Ellisen, E.P. Winer, J.E. Garber, Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer, J Clin Oncol 28 (2010) 1145-1153.

[138] E.Y. Gil, U.H. Jo, H. Jeong, Y.M. Whang, O.H. Woo, K.R. Cho, J.H. Seo, A. Kim, E.S. Lee, I. Koh, Y.H. Kim, K.H. Park, Promoter methylation of RASSF1A modulates the effect of the microtubule-targeting agent docetaxel in breast cancer, Int J Oncol 41 (2012) 611-620.

[139] D. Dietrich, M. Krispin, J. Dietrich, A. Fassbender, J. Lewin, N. Harbeck, M. Schmitt, S. Eppenberger-Castori, V. Vuaroqueaux, F. Spyratos, J.A. Foekens, R. Lesche, J.W. Martens, CDO1 promoter methylation is a biomarker for outcome prediction of anthracycline treated, estrogen receptor-positive, lymph node-positive breast cancer patients, BMC Cancer 10 (2010) 247.

[140] M.R. Aure, S.K. Leivonen, T. Fleischer, Q. Zhu, J. Overgaard, J. Alsner, T. Tramm, R. Louhimo, G.I. Alnæs, M. Perälä, F. Busato, N. Touleimat, J. Tost, A.L. Børresen-Dale, S. Hautaniemi, O.G. Troyanskaya, O.C. Lingjærde, K.K. Sahlberg, V.N. Kristensen, Individual and combined effects of DNA methylation and copy number alterations on miRNA expression in breast tumors, Genome Biol 14 (2013) R126.

[141] L. Bojmar, E. Karlsson, S. Ellegård, H. Olsson, B. Björnsson, O. Hallböök, M. Larsson, O. Stål, P. Sandström, The role of microRNA-200 in progression of human colorectal and breast cancer, PLoS One 8 (2013) e84815.

[142] C. Eichelser, D. Flesch-Janys, J. Chang-Claude, K. Pantel, H. Schwarzenbach, Deregulated serum concentrations of circulating cell-free microRNAs miR-17, miR-34a, miR-155, and miR-373 in human breast cancer development and progression, Clin Chem 59 (2013) 1489-1496.

[143] H.M. Heneghan, N. Miller, A.J. Lowery, K.J. Sweeney, J. Newell, M.J. Kerin, Circulating microRNAs as novel minimally invasive biomarkers for breast cancer, Ann Surg 251 (2010) 499-505.

[144] A. Markou, G.M. Yousef, E. Stathopoulos, V. Georgoulias, E. Lianidou, Prognostic significance of metastasis-related microRNAs in early breast cancer patients with a long follow-up, Clin Chem 60 (2014) 197-205.

[145] P. Gasparini, L. Cascione, M. Fassan, F. Lovat, G. Guler, S. Balci, C. Irkkan, C. Morrison, C.M. Croce, C.L. Shapiro, K. Huebner, microRNA expression profiling identifies a four microRNA signature as a novel diagnostic and prognostic biomarker in triple negative breast cancers, Oncotarget 5 (2014) 1174-84.

[146] Y. Yang, J. Qian, Y. Chen, Y. Pan, Prognostic role of circulating microRNA-21 in cancers: evidence from a meta-analysis, Tumour Biol (2014) doi 10.1007/s13277-014-1846-8.

[147] A.C. Godfrey, Z. Xu, C.R. Weinberg, R.C. Getts, P.A. Wade, L.A. Deroo, D.P. Sandler, J.A. Taylor, Serum microRNA expression as an early marker for breast cancer risk in prospectively collected samples from the Sister Study cohort, Breast Cancer Res 15 (2013) R42.

[148] U. Lehmann, B. Hasemeier, M. Christgen, M. Müller, D. Römermann, F. Länger, H. Kreipe, Epigenetic inactivation of microRNA gene hsa-miR-9-1 in human breast cancer, J Pathol 214 (2008) 17-24.

[149] C.S. Wilhelm, K.T. Kelsey, R. Butler, S. Plaza, L. Gagne, M.S. Zens, A.S. Andrew, S. Morris, H.H. Nelson, A.R. Schned, M.R. Karagas, C.J. Marsit, Implications of LINE1 methylation for bladder cancer risk in women, Clin Cancer Res 16 (2010) 1682-1689.

[150] D.C. Koestler, M. Avissar-Whiting, E.A. Houseman, M.R. Karagas, C.J. Marsit, Differential DNA methylation in umbilical cord blood of infants exposed to low levels of arsenic in utero, Environ Health Perspect 121 (2013) 971-977.

[151] T.Y. Yang, L.I. Hsu, A.W. Chiu, Y.S. Pu, S.H. Wang, Y.T. Liao, M.M. Wu, Y.H. Wang, C.H. Chang, T.C. Lee, C.J. Chen, Comparison of genome-wide DNA methylation in urothelial carcinomas of patients with and without arsenic exposure, Environ Res 128 (2014) 57-63.

[152] C.S. Wilhelm-Benartzi, D.C. Koestler, E.A. Houseman, B.C. Christensen, J.K. Wiencke, A.R. Schned, M.R. Karagas, K.T. Kelsey, C.J. Marsit, DNA methylation profiles delineate etiologic heterogeneity and clinically important subgroups of bladder cancer, Carcinogenesis 31 (2010) 1972-1976.

[153] I. Renard, S. Joniau, B. van Cleynenbreugel, C. Collette, C. Naômé, I. Vlassenbroeck, H. Nicolas, J. de Leval, J. Straub, W. Van Criekinge, W. Hamida, M. Hellel, A. Thomas, L. de Leval, K. Bierau, D. Waltregny, Identification and validation of the methylated TWIST1 and NID2 genes through real-time methylation-specific polymerase chain reaction assays for the noninvasive detection of primary bladder cancer in urine samples, Eur Urol 58 (2010) 96-104.

[154] M.R. Abern, R. Owusu, B.A. Inman, Clinical performance and utility of a DNA methylation urine test for bladder cancer, Urol Oncol 32 (2014) 51.e21-51.e26.

[155] R. García-Baquero, P. Puerta, M. Beltran, M. Alvarez, R. Sacristan, J.L. Alvarez-Ossorio, M. Sánchez-Carbayo, Methylation of a novel panel of tumor suppressor genes in urine moves forward noninvasive diagnosis and prognosis of bladder cancer: a 2-center prospective study, J Urol 190 (2013) 723-730.

[156] J. Baden, S. Adams, T. Astacio, J. Jones, J. Markiewicz, J. Painter, C. Trust, Y. Wang, G. Green, Predicting prostate biopsy result in men with prostate specific antigen 2.0 to 10.0 ng/ml using an investigational prostate cancer methylation assay, J Urol 186 (2011) 2101-2106.

[157] G.D. Stewart, L. Van Neste, P. Delvenne, P. Delrée, A. Delga, S.A. McNeill, M. O'Donnell, J. Clark, W. Van Criekinge, J. Bigley, D.J. Harrison, Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study, J Urol 189 (2013) 1110-1116.

[158] T. Vener, C. Derecho, J. Baden, H. Wang, Y. Rajpurohit, J. Skelton, J. Mehrotra, S. Varde, D. Chowdary, W. Stallings, B. Leibovich, H. Robin, A. Pelzer, G. Schäfer, M. Auprich, S. Mannweiler, P. Amersdorfer, A. Mazumder, Development of a multiplexed urine assay for prostate cancer diagnosis, Clin Chem 54 (2008) 874-882.

[159] M. Esteller, J. Garcia-Foncillas, E. Andion, S.N. Goodman, O.F. Hidalgo, V. Vanaclocha, S.B. Baylin, J.G. Herman, Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents, N Engl J Med 343 (2000) 1350-1354.

[160] M.E. Hegi, A.C. Diserens, T. Gorlia, M.F. Hamou, N. de Tribolet, M. Weller, J.M. Kros, J.A. Hainfellner, W. Mason, L. Mariani, J.E. Bromberg, P. Hau, R.O. Mirimanoff, J.G. Cairncross, R.C. Janzer, R. Stupp, MGMT gene silencing and benefit from temozolomide in glioblastoma, N Engl J Med 352 (2005) 997-1003.

[161] G. Minniti, M. Salvati, A. Arcella, F. Buttarelli, A. D'Elia, G. Lanzetta, V. Esposito, S. Scarpino, R. Maurizi Enrici, F. Giangaspero, Correlation between O6-methylguanine-DNA methyltransferase and survival in elderly patients with glioblastoma treated with radiotherapy plus concomitant and adjuvant temozolomide, J Neurooncol 102 (2011) 311-316.

**Figure legends**

Table 1: The performance of potential epigenetic diagnostic markers as assessed within prospective studies. The sensitivities (Sens.), specificities (Spec.), odds ratios (OR) and hazard ratios (HR) are provided, with 95% confidence intervals (95% CI) where appropriate.

Figure 1: Utilisation of approaches for the study of global, repetitive element, genome-wide, and site-specific methylation by year. The number of publications by year are given for high performance liquid chromatography (HPLC), luminometric methylation assay (LUMA), LINE-1 and *Alu repetitive* elements, Illumina Infinium microarrays (Illumina) [Golden Gate, 27K, or 450K], reduced representation bisulfite sequencing (RRBS), and methylated DNA immunoprecipitation (MeDIP).

Table 1: The performance of potential epigenetic diagnostic markers as assessed within prospective studies.



Figure 1: Utilisation of approaches for the study of global, repetitive element, genome-wide, and site-specific methylation by year.

