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Epigenetics and Precision Oncology

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Abstract

Epigenetic alterations such as DNA methylation defects and aberrant covalent histone modifications occur within all cancers and are selected for throughout the natural history of tumor formation with changes being detectable in early onset, progression and, ultimately, recurrence and metastasis. The ascertainment and use of these marks to identify at-risk patient populations, refine diagnostic criteria, and provide prognostic and predictive factors to guide treatment decisions is of growing clinical relevance. Further, the targetable nature of epigenetic modifications provides a unique opportunity to alter treatment paradigms and provide new therapeutic options for patients whose malignancies possess these aberrant epigenetic modifications, paving the way for new and personalized medicine. DNA methylation has proven to be of significant clinical utility for its stability and relative ease of testing. The intent of this review is to elaborate upon well-supported examples of epigenetic precision medicine and how the field is moving forward, primarily in the context of aberrant DNA methylation.

Introduction

Epigenetics can be thought of as the factors that affect gene expression and cellular phenotypes other than DNA sequence. The best understood epigenetic determinants of phenotype include methylation of DNA on cytosine residues, addition of acetyl and methyl groups onto histone tails, non-coding RNA expression, and chromatin structural remodelers. Collectively, the epigenetic status within cells is tightly controlled to maintain a proper differentiation state. In cancer, this finely-tuned genomic programming is disrupted, leading to uncontrolled cell division, differentiation defects, and resistance to apoptosis (1). Since the discovery of aberrant DNA methylation in malignancy, epigenetic errors and their causes have become a major focus in cancer research over the past four decades. Alterations of the epigenome have been shown to impact virtually every step in tumor formation, progression, and treatment. Accordingly, such changes are prime targets for cancer detection, diagnosis, stratification, and therapeutic intervention. With the advent of high-throughput sequencing technologies as well as techniques requiring very small amounts of biological samples, testing of epigenetic status of individual patients is now common in some contexts. In addition, new methodologies, such as ATAC-seq and ChIPmentation are unraveling unique and distinct chromatin interactions and gene accessibility (2,3). With this enhanced understanding we can further identify specific and personalized targets within an individual

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malignancy. In this review we aim to highlight specific examples of risk stratification, diagnostic, prognostic, predictive, as well as interventional precision epigenetic therapies in clinical oncology, and comment on promising areas for future investigation. Key examples are summarized in Table 1.

Methods for measuring epigenetic status in cancer

The use of epigenetic status in precision oncology is dependent on measurement of epigenetic marks present on tumor chromatin in an accurate and reproducible manner. Although gene expression is the functional reflection of epigenetic states, measuring epigenetic information may provide additional information such as stability over time (4). There are several technologies primed for clinical applications, most of which query DNA methylation status. As a very stable epigenetic modification, DNA methylation is well suited for transition into clinical applications. DNA methylation can be assessed using methods employing i) bisulfite conversion of cytosine residues to uracil, ii) restriction enzymes with specificity for methylated or unmethylated cytosines, iii) antibodies with specificity to methylated cytosines, or iv) nanopore-based single DNA molecule sequencing. A recent study compared various DNA methylation assays for their potential adoptability in clinical practice, and concluded that while several methods were accurate and reproducible, bisulfite pyrosequencing and amplicon bisulfite sequencing were best in terms of also balancing cost and scale (5). Another recent feasibility study reported that nanopore sequencing could be very rapidly applied at low cost in a clinical setting (6). In a research context, histone modifications can be measured using chromatin immunoprecipitation followed by high-throughput sequencing or PCR; however, because histone marks are relatively dynamic and immunoprecipitation is only partially quantitative, they may be less reliable to interrogate in the clinic. Finally, long-distance chromatin interactions can be measured using chromosome conformation capture, in which DNA is covalently cross-linked in its native form before digestion into small fragments and random ligation (such that covalently linked fragments will be ligated together). High-throughput sequencing can then be used to identify interacting segments of DNA. In any clinical application of these assays, the cost, accuracy, and reproducibility must be thoroughly evaluated – at present DNA methylation studies are the most thoroughly validated, thus we focus on clinically relevant examples in subsequent sections.

Cancer risk assessment

Constitutional epimutations

Although most epigenetic changes in cancer occur as somatic events, there are examples of inherited or sporadic constitutional epigenetic defects (“epimutations”). Constitutional epimutations are alterations in the epigenome throughout all tissues in the body that result in abnormal gene expression (7). An example of such epimutations can be seen in Lynch syndrome (LS), which is strongly associated with colorectal cancer, and other malignancies. The MLH1 and MSH2 proteins are core components of DNA mismatch repair (MMR). Deficiencies in MMR are detected through changes in short microsatellite repeats, with MMR deficient tumors showing microsatellite instability (MSI) (8,9). Cancer-specific

methylation-induced silencing of the *MLH1* gene has been identified in close to 75% of sporadic MSI+ colorectal cancers, and is also present in a subset (~20%) of LS patients (9). A few families with Lynch syndrome were described where the defect appeared to be inherited methylation of *MLH1* (10). *MSH2* has also been shown to harbor epimutations secondary to deletions in the nearby gene, *EPCAM*. Such an epimutation is associated with early-onset colorectal cancer and increased risk of several other cancers (7,9,11,12). Thus, testing peripheral blood DNA for *MLH1* or *MSH2* promoter hypermethylation may be useful in cases of familial colon cancer. Furthermore, such analyses can aid in distinguishing sporadic somatic inactivation of MMR genes from germline mutations when the MLH1 protein is absent. Indeed. it has been shown that testing for *MLH1* promoter methylation is highly cost-effective in selecting patients with strong family histories of colorectal cancer to be referred for germline analysis (13). Other examples of constitutional epimutations in cancer risk can be seen in familial breast and ovarian cancer syndrome with *BRCA1* and *RAD51C* showing promoter hypermethylation, and subsequent down-regulation at the protein level in a small number of cases (14). Collectively, such epimutations may be useful to evaluate risk for patients with a strong family history of cancer, or patients who have early onset disease.

Age-related DNA methylation changes

In addition to gene specific epimutations, it is now widely accepted that the epigenome changes with age. Specifically, numerous studies have been published documenting age-related DNA hypermethylation and acceleration of this process could be a biomarker of cancer risk (15-18). Data from an *in vivo* model demonstrated that age-related methylation changes in blood accelerate during the development of myelodysplastic syndromes and acute myeloid leukemia (AML) (15). In a case-control study of breast cancer, peripheral blood DNA methylation was evaluated with respect to an age estimator; residuals in the model defined a measure of intrinsic epigenetic age acceleration (IEAA). A one unit increase in IEAA as determined by regression of epigenetic on chronological age, was reported to increase the risk of developing breast cancer by 4% (OR, 1.04; 95% CI, 1.007-1.0076) (17,18). Another recent study reported that the extent of age-related DNA hypermethylation varies by tissue and is correlated with site-specific lifetime cancer risk (15). Collectively, these data suggest that interrogating DNA methylation at age-related genomic loci may aid in determining risk of development of cancer.

Diagnostic applications of DNA methylation assays

In recent years several diagnostic applications for DNA methylation analysis have been reported. Below we highlight some of the most extensively studied examples, including tumors arising from colorectal, prostate, pancreatic and unknown primary tissues-of-origin.

DNA methylation in diagnosis of colorectal cancer

Though *MLH1* promoter methylation is frequently cited in the colon cancer literature and is characteristic of a subset of Lynch syndrome patients, it is important to note that it is not a singular event in colorectal cancer. Many studies have identified hundreds of genes that are aberrantly methylated in the archetypal colorectal cancer genome. The list includes genes

central to the Wnt signaling pathway (*APC, AXIN2, DKK1, SFRP2, SFRP2, WNT5A*), components of DNA repair processes (*MGMT, MSH2*), cell cycle-related genes (*CDKN2A and CDKN2B)*, and components of the RAS signaling cascade (*RASSF1A* and *RASSF1B*) (19).

The significance of this cancer-specific DNA hypermethylation in the onset and progression of CRC has led to development of non-invasive CRC detection biomarkers. The first FDA-approved blood assay for CRC screening measures the presence of aberrantly methylated septin 9, a gene involved in control of cellular proliferation (Table 1). Hypermethylation of CpG island 3 in the promoter of the *SEPT9* gene can be detected through its release into the peripheral blood from necrotic and apoptotic cancer cells. Thus, detection of *SEPT9* methylation reflects the likely presence of CRC (20,21). Initial studies evaluating the detection of *SEPT9* promoter methylation in the plasma of CRC patients reported a sensitivity of 72% and specificity of 90% (22,23). A prospective trial evaluating screening in asymptomatic individuals showed a CRC detection rate up to 48.2% and specificity of 91.5% (24). A large meta-analysis has also confirmed the utility of *SEPT9* promoter methylation measurement in the detection of cancer amongst symptomatic patients, with the test showing superiority to that of protein and fecal immunohistochemical testing (20). In asymptomatic patients, the sensitivity and specificity of the test was lower than FIT, underscoring the need for further research and refinement of methods as well as the careful selection of the algorithm in which to define the threshold for *SEPT9* methylation detection (20). Despite data supporting its use as a diagnostic tool, from a large-scale screening perspective *SEPT9* methylation status is still considered inferior to other modalities, and is only recommended for patients who refuse other options (25).

In addition to blood-based DNA methylation diagnostics, another promising application is in the context of fecal DNA analysis. It was recently reported in a large clinical trial that a targeted fecal DNA test which included interrogation of *NDRG4* and *BMP3* methylation status, along with other colon cancer gene mutations could identify significantly more colorectal cancers compared to the standard fecal immunochemical test (26). In this study, the fecal DNA test had sensitivities of 92.3% for detecting overt cancer, 42.4% for advanced precancerous lesions, and 42.4% for sessile polyps >1 cm. A key result was the number of patients screened with the fecal DNA test to detect one cancer was nearly as few as for colonoscopy screening, suggesting a high degree of sensitivity. The major limitation thus far is specificity as the false-positive rate was significantly higher than that observed in colonoscopy. These data led to the FDA approval of this screening assay, and it is an accepted screening modality by the National Comprehensive Cancer Network, however, the issue of a high false-positive rate remains to be resolved (25,26).

Prostate cancer diagnosis

Screening for prostate cancer based on prostate specific antigen (PSA) remains controversial, as the natural history of the disease suggests many patients with overt malignancy will not die because of it. Thus, any reduction in cancer-specific mortality must be considered in the context of the increased risk of complications caused by unnecessary biopsies and interventions (27,28). Accordingly, there remains an unmet need for diagnosis

of clinically-relevant prostate cancer, and several studies have proposed using epigenetic status as a marker. Somatic hypermethylation of CpG island sequences at glutathione S-transferase gene (*GSTP1*) is found in up to 90% of prostate cancer cases and is far more specific than traditional PSA screening when detected in plasma, serum or urine, potentially offering an adjunct to PSA levels. The level of promoter DNA methylation of *GSTP1* has been shown to differentiate between benign prostatic hyperplasia and different grades of adenocarcinoma, as the gain of methylation and progressive loss of protein expression correlates with prostate carcinogenesis (29-31). A multicenter study evaluated methylation of two genes in addition to *GSTP1*; namely *RARβ2* and *APC*. The interrogation of these three markers in urine samples increased diagnostic accuracy to greater than that of total serum PSA from 51.7% to 61.7% (32). As such, addition of methylation-specific analysis for these three genes could improve diagnostic accuracy. Future studies, however, are still needed to address whether the detected prostate cancers are clinically relevant in terms of cancer-specific mortality.

Pancreatic cancer diagnosis

Early diagnosis of pancreatic adenocarcinoma remains a major clinical problem as most patients present with advanced disease (33). There is potential for DNA methylation analysis to aid in early diagnosis and decrease cancer-specific mortality in this disease. A clinical cohort study published in 2016 demonstrated that interrogation of circulating blood DNA for methylation can identify patients with pancreatic adenocarcinoma (34). By examining methylation status at *BMP3*, *RASSF1A*, *BNC1*, *MESTv2*, *TFPI2*, *APC*, *SFRP1*, and SFRP2, overt cancer was distinguished from healthy controls, as well as patients with chronic or acute pancreatitis with a sensitivity of 76% and specificity of 83% (34). Diagnostic predictive accuracy was independent of tumor stage, suggesting that circulating DNA methylation may aid in early diagnosis of pancreatic cancer. Future studies will be needed to validate this model, and also demonstrate that the genes interrogated are specific for pancreatic cancer versus other malignancies.

Tissue-of-origin diagnosis of occult metastatic tumors

Metastatic tumors of unknown origin present a unique clinical challenge because of the inherent absence of site-specific therapy options. These tumors have consistently been shown to confer a dismal prognosis in patients treated with empiric chemotherapy. Gene expression profiling has attempted to address this unmet need by aiding in occult tumor classification. Some promising retrospective analyses have been published and the concept is being tested in an ongoing phase III clinical trial (NCT01540058). Extending from this idea, Moran, et al. developed and validated a DNA methylation-based classifier intended to elucidate primary tissues of origin in occult tumors (35). In their study, correct tissue-of-origin predictions were made in the vast majority of cases, with 99.6% specificity, 97.7% sensitivity, 88.6% positive predictive value, and 99.9% negative predictive value. Importantly, patients who were treated with chemotherapy indicated for the predicted tissue-of-origin had significantly improved overall survival compared to patients whose chemotherapy was not tailored toward tissue-of-origin predictions. This concept of occult tumor classification was also recently explored by Guo, et al. where a novel metric of regional DNA methylation - methylation haplotype blocks - was shown to be tissue specific,

and was able to accurately classify the primary tissue of origin using circulating tumor DNA from patients with lung or colorectal cancer (36). Collectively, these data suggest that DNA methylation status is a useful tool for chemotherapy regimen selection in the context of occult malignancy, although further prospective studies are needed to verify the superiority of this approach over gene expression-based methods. It is plausible that because DNA methylation is a relatively stable epigenetic mark, it may be superior to mRNA profiling for identification of tumor tissue-of-origin in this context.

Prognostic applications of DNA methylation analysis

Hypermethylation of CpG islands in the promoter of key genes has been shown to differentiate between cancer patients at low or high risk of adverse clinical outcomes. Key examples are seen in leukemia and breast cancer; however, DNA methylation defects with prognostic and/or therapeutic importance are present in many other cancers. In the following sections, we highlight advancements seen in the risk stratification of acute myeloid leukemia (AML) and breast cancer, by way of epigenetic research.

CpG island methylator phenotype is favorable in acute myeloid leukemia

Recent data in AML have supported the potential use of DNA methylation status and mutations in epigenetic regulators for risk stratification and treatment selection. Aberrant DNA hypermethylation at many CpG island promoters (A-CIMP) was shown to confer an overall survival advantage to patients treated with chemotherapy (37). In this study, a distinct methylation signature also associated with mRNA down-regulation of many pluripotency genes was correlated with aberrant hypermethylation at their promoters, suggesting a less dedifferentiated phenotype in A-CIMP+ patients. Separately, a prognostic seven gene expression-based signature was identified using DNA methylation studies of hypermethylated genes in cancer (38). Although these data have not yet been integrated into the standard-of-care for AML, an important opportunity for clinical contribution of epigenetic status is in determining which patients should receive hematopoietic stem cell transplants (39). Because the risk of treatment-related mortality is high, a longstanding question is whether DNA methylation could help identify patients curable with chemotherapy alone. Recent data from our group and others suggest this is a likely possibility.

Mismatch repair deficiency is prognostic in colorectal cancer

Clinical presentation of MSI+colorectal cancer patients shows a striking demarcation with LS patients presenting earlier in life and are predominantly male whereas non-LS *MLH1* hypermethylated patients are older and female. This age disparity in presentation may arise in part due to the changes seen in methylation and inactivation of genes associated with the processes of normal aging. Despite differences in initial presentation and in the origin of MLH1 suppression, there is little difference in prognosis by LS status with both showing a similar cancer-specific survival when analysis is adjusted for age and stage at diagnosis (40). Compared to MMR proficient patients, however, individuals whose malignancy is deficient in MMR, dMMR, show reduced rates of tumor recurrence, delayed time to treatment failure, and improved survival rates (8,41).

Interestingly, studies of *MLH1* methylation in tumors of other origin have revealed a different prognostic impact than that seen in colorectal cancer. For example, in esophageal squamous cell carcinoma, *MLH1* promoter methylation was significantly associated with poor overall survival in male patients. So although the methylation-induced silencing of *MLH1* and concomitant deficiency of MMR is present, these epigenetic changes confer a varying prognosis dependent on the context of the tissue of origin, as well as the patient population (42).

Breast cancer gene expression classifiers and DNA methylation

A number of gene expression classifiers have been marketed since the derivation and subsequent FDA approval of MammaPrint dx, a 70-gene signature that classifies patients as either high or low risk for metastatic relapse within 5 years of diagnosis (43). The clinical utility of this 70-gene signature when added to standard clinical and pathological criteria in selecting patients for adjuvant chemotherapy was recently evaluated in a phase 3 study (EORTC 10041/BIG 3-04 MINDACT). This study determined that among women with early-stage breast cancer with high clinical risk and low genomic risk for recurrence, the absence of chemotherapy as determined by the 70-gene signature led to a 5-year rate of survival without metastasis 1.5 percentage points lower than those who had received chemotherapy. As such, nearly 46% of women with breast cancer who are at high clinical risk may receive little to no benefit from chemotherapy. Refinement and addition of new signatures to the current paradigm of treatment will continue to inform whether adjuvant treatment is needed and, if so, which treatment will be most efficacious in the treatment of early-stage breast cancer (44). It remains unknown what role the epigenome participates in these signatures and whether evaluation of the epigenetic status of these patients may augment the clinical utility of expression patterns. At least one study suggests that this could be the case with DNA methylation alterations associated with CIMP in breast cancer, denoted B-CIMP, triggering the gene expression differences captured within the MammaPrint signature (45). The B-CIMP signature derived by the authors included key genes which are known to contribute to metastasis through their involvement in EMT and Wnt signaling pathways. As such B-CIMP+ status, as defined within a subset of hormone receptor-positive breast cancer tumors, was found to correlate with a decreased propensity for metastasis and a better clinical outcome than B-CIMP- tumors. Another application of DNA methylation analysis to monitoring breast cancer status was recently reported by way of circulating DNA methylation levels. Patients with breast cancer who responded to neoadjuvant chemotherapy and had a methylated *RASSF1A* promoter in circulating DNA were found to have significantly decreased methylation after treatment, while patients who were chemoresistant did not (46). Thus it is possible that *RASSF1A* promoter methylation in circulating DNA could be used as a measure of neoadjuvant efficacy via a non-invasive test.

Epigenetic predictive biomarkers

DNA methylation has been proposed to serve as a predictive biomarker in some instances, where epigenetic status is significantly correlated with likelihood of response to various therapeutic interventions. We highlight below several examples of predictive DNA

methylation-based biomarkers which have or should be evaluated in clinical trials and possibly adopted clinically.

BRCA1 methylation and PARP inhibitor sensitivity

BRCA1 is a protein involved in double strand DNA break repair and is often mutated in hereditary breast and ovarian cancer syndrome (47). When BRCA1 is deficient at the protein level, cells are unable to repair double strand breaks in DNA via the homologous recombination pathway. This creates synthetic lethality with inhibition of selected DNA repair pathways and this is exploited therapeutically by targeting the single-strand repair protein PARP1 using small molecule inhibitors such as olaparib (48). In addition to inactivating mutations, CpG island hypermethylation of the *BRCA1* promoter can cause protein down-regulation, and subsequent sensitivity to PARP inhibition. In an *in vitro* setting it was found that *BRCA1* promoter methylation in the wild-type UACC3199 breast cancer cell line was equally sensitive to PARP inhibition as the *BRCA1* mutant MDA-MB-436 line (49). In addition, a patient-derived xenograft model confirmed the presence of BRCA1 methylation in a subset of tumors, and that loss of methylation was associated with PARP inhibitor resistance in this context (50). These data hint at a possible epigenetic role in determining likely sensitivity to PARP inhibitors that could be used in treating patients with breast or ovarian cancer.

MGMT promoter methylation in glioma

Despite aggressive treatment regimens, the median survival for glioblastoma patients remains stagnant at no more than 12 months (51). Much evidence has supported the role of methylation of the promoter for the gene encoding O^6-methylguanine-DNA methyltransferase (MGMT) as a biomarker of response to the alkylating agent, temozolomide (52,53). MGMT is a ubiquitously expressed DNA repair enzyme that removes alkyl adducts from the O^6-position of guanine (54). The presence of O^6-alkylated guanine in DNA leads to double-strand breaks resulting in apoptosis and cell death. Hence, MGMT serves to protect normal cells from carcinogens. In cancer, MGMT counteracts TMZ-induced damage, and serves as a mechanism of chemoresistance. As such, methylation of the *MGMT* promoter leads to decreased protein expression and higher levels of O^6-methylated guanine, inducing cell death particularly in the context of alkylating agents such as TMZ. The correlation of *MGMT* promoter methylation with gene silencing and sensitivity to alkylating agents has been extensively validated by a number of groups in both pediatric and adult glioblastoma (55-57). For the 40% of GBM patients that present with a methylated *MGMT* promoter, the prognosis, independent of therapeutic regimens, provides for both a better progression free survival (58) as well as overall survival (OS). When treated with alkylating agents, *MGMT* promoter methylation is associated with longer OS (58). As for the other 60% of GBM patients who present with an unmethylated *MGMT* promoter, treatment paradigms are focusing on MGMT inhibition as well as TMZ analogues that show *MGMT* promoter status-independence (52). Based on these data the most recent update to the NCCN guidelines for glioblastoma treatment include interrogation of the *MGMT* promoter (59).

TET2 target methylation and chemotherapy response in AML

TET2 is a protein involved in DNA demethylation and is often mutated in hematologic malignancies (60). It has been shown that such mutations result in aberrant hypermethylation of specific CpG sites in blood cancers, including chronic myelomonocytic leukemia, and AML (61). Recent data in a large retrospective cohort of clinical AML patient samples identified a distinct subset of cases defined by their hypomethylation of four TET2 targets: *SP140*, *MCCC1*, *EHMT1*, and *MTSS1* (62). The AML patients within this hypomethylated group had significantly improved overall survival after cytarabine-based chemotherapy treatment compared to patients who did not have hypomethylation at these loci (62). This methylation based signature outperformed mutation based assays for predicting outcomes in this cohort. Because of the limited number of sites interrogated, and the ease of the bisulfite pyrosequencing assay, this epigenetic signature may be a strong candidate to aid in plan-of-care decisions in AML, and should be prospectively validated.

MLH1 promoter methylation in cancer treatment

In addition to serving as a biomarker for diagnosis and risk assessment of colorectal cancer, a number of therapeutic predictive associations have been made with *MLH1* promoter methylation (and subsequent MMR deficiency). MMR deficiency has been reported as a predictive marker for lack of efficacy of 5-fluorouracil-based adjuvant chemotherapy in colorectal cancer (8,63). Colorectal tumors with MMR deficiency are also significantly enriched for CIMP-positivity, which may have different therapeutic implications (64). A recent study evaluated the potential of the demethylating agent, azacitidine, to sensitize refractory CIMP-high patients to capecitabine and oxaliplatin-based chemotherapy (65). Despite promising pre-clinical results, patients enrolled on this phase I/II trial showed no objective responses by RECIST criteria, and study endpoints were not correlated with CIMP positivity, however, the investigators did identify a potential predictive biomarker in serum vimentin. Vimentin methylation was significantly correlated with both baseline tumor volume and obtainment of stable disease, suggesting it may be useful to evaluate independently of *MLH1* and other CIMP markers (65,66). Beyond colon cancer, *MLH1* methylation may also be relevant for therapeutic efficacy prediction because of its association with MMR status. In particular, MMR deficiency has been shown across several clinical trials to confer sensitivity to pembrolizumab irrespective of cancer type. These data led to the recent FDA approval of pembrolizumab for use in MMR deficient tumors of any site - the first such tissue-agnostic approval for adult and pediatric patients (67).

Epigenetic features and therapeutic combinations

One of the most exciting areas of translational epigenetic research attempts to use epigenetic drug combinations in genetically or epigenetically-defined groups of patients. Conceptually the hypothesis of priming cancer cells to either chemotherapy sensitivity or immunogenicity has been proposed, with preliminary data suggesting feasibility in multiple *in vivo* models (68-70). The combination of the hypomethylating agent azacitidine with the immune checkpoint inhibitor ipilimumab has been shown to improve tumor control in mouse models via induction of an immunologic gene expression signature (68,69). In addition, combination regimens of cytotoxic chemotherapy and DNA methylation inhibitors have

been shown to cause cell death in multiple *in vitro* settings, via a distinct calcium-dependent mechanism (70). Finally, recent *in vitro* data has highlighted the epigenetic specificity of different combinations of therapies in different cancer types (71). For example, in acute promyelocytic leukemia, the primary defect causing cancer is known to be a block in hematopoietic differentiation, which in the HL-60 cell line appeared to be most strongly affected by the combination of decitabine and the KDM1A inhibitor, S2101. In contrast, the greatest effect on re-expression of silenced tumor suppressor genes was seen in a colon cancer cell line, YB5, with the combination of decitabine and the EZH2 inhibitor GSK343 (71). These data suggest that specific combinations of targeted agents could be used to address the primary defects present in specific cancers. In addition to these examples, there are many other drugs and drug combinations targeting the epigenome that are currently under clinical development. In these cases, future clinical trials should study whether patients with an identifiable tumor defect (e.g. gene expression implying a block in differentiation) may benefit from one combination over another.

Clinically available epigenetic assays and future directions

Epigenetic defects are now understood to exist in virtually all forms of cancer, and have been shown to aid in risk assessment, diagnosis, prognostication, and treatment selection in some contexts. Although the existing data point to the utility of DNA methylation as a clinical tool, its widespread adoption has been limited to relatively few indications. Those which have reached standard-of-care status include *MLH1*, or *MSH2* methylation in colorectal cancer genetic screening or MMR status evaluation; BMP3 and NDRG4 methylation as part of the Cologuard fecal DNA colon cancer screening test; and MGMT promoter methylation in glioblastoma treatment selection (25,59,72). In these settings, the NCCN guidelines suggest a DNA methylation-specific PCR assay, bisulfite pyrosequencing, or in the case of colon cancer, the Cologuard fecal DNA test. Despite the limited current clinical adoption of epigenetic tests, there are exciting new applications in different malignancies that merit clinical consideration, especially in the context of combinations of epigenetic and cytotoxic or immunologic therapies. To this point, the FDA recently approved the immunologic checkpoint inhibitor, pembrolizumab for tumors that are mismatch repair deficient of any primary tissue of origin (67). It remains to be seen whether *MLH1* or *MSH2* promoter methylation can be used as a predictive biomarker in this context, but the literature in colorectal cancer suggest it may be a valuable tool, as they are associated with MMR deficiency. Going forward new clinical trials will be needed to evaluate the role of epigenetic biomarkers in diagnosis of cancer, and in defining likely therapeutic responders. An additional consideration with respect to diagnostic markers is whether improvements in detecting clinically relevant disease can be made (i.e. whether cancer-specific mortality can be decreased using epigenetic status). Such a consideration is especially relevant in prostate cancer, which is now understood to have limited disease-specific mortality in many men. One possibility is that an approach incorporating both PSA levels and circulating DNA methylation status could stratify tumor natural history in addition to diagnosis. There remains a need for much additional work, but the recent progress in incorporating epigenetic information in clinical management of cancer should not be understated as it is already being incorporated into the standard-of-care.

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Table 1
Applications of epigenetics to precision oncology

Type of clinical tool	Tumor Type	Management implications	Status/Study Type	References
Risk assessment				
Constitutional epimutations in *MLH1, MSH2, BRCA1, RAD51C*	Colorectal, Breast, Ovarian	Predisposition to cancer development	NCCN recommendations (*MLH1, MSH2*)Prospective clinical cohort study (*BRCA1, RAD51C*)	7-14
Age-related DNA methylation changes	MDS, AML, Breast	Accelerated aging phenotype and increased overall risk	*In vivo* pre-clinical study (MDS, AML)Prospective clinical case-control study (Breast)	15-18
Diagnostic				
SEPT9, NDRG4 and *BMP3* methylation	Colorectal	Detection using stool DNA; test is sensitive but lacks specificity as a screening modality	Phase III clinical trial (*NDRG4, BMP3*)FDA approved	19-26
GSTP1, RARβ2 and *APC* methylation	Prostate	Aberrant methylation in three markers increased diagnostic accuracy above PSA alone	Prospective clinical case-control study	27-32
BMP3, RASSF1A, BNC1, MESTv2, TFP12, APC, SFRP1 and *SFRP2* methylation	Pancreatic	Distinguish cancer from healthy controls, as well as those with chronic or acute pancreatitis	Prospective clinical case-control study	34
Occult tumor classifier	Many	Methylation classifiers used to identify tumor-specific treatment	Retrospective clinical cohort study	35,36
Prognostic				
CpG island methylator phenotype	AML, Colorectal, Breast	Improved overall survival and decreased risk of metastasis (breast)	Retrospective clinical cohort studies	37,38,40,45
RASSF1A methylation	Breast	Circulating DNA methylation associated with neoadjuvant response	Prospective clinical cohort study	46
Deficiency of MMR	Colorectal	Reduced rates of tumor recurrence, delayed time to treatment failure, and improved survival rates	Clinical trials	8,41
Predictive				
BRCA1 promoter methylation	Breast	Sensitivity to PARP inhibition	*In vitro* and *in vivo* pre-clinical studies	49,50
MGMT promoter methylation	Glioblastoma	Sensitivity to temozolomide	Randomized clinical trials	55-59
TET2 target hypomethylation signature	AML	Improved survival with cytarabine-based chemotherapy	Retrospective clinical cohort study	62