The CpG Island Methylator Phenotype: What's in a Name?

Laura A.E. Hughes1,2, Veerle Melotte3, Joachim de Schrijver3, Michiel de Maat3, Vincent T.H.B.M. Smit4, Judith V.M.G. Bovée4, Pim J. French5, Piet A. van den Brandt1, Leo J. Schouten1, Tim de Meyer6, Wim van Criekinge6, Nita Ahuja7, James G. Herman7, Matty P. Weijenberg1, and Manon van Engeland2

Abstract

Although the CpG island methylator phenotype (CIMP) was first identified and has been most extensively studied in colorectal cancer, the term "CIMP" has been repeatedly used over the past decade to describe CpG island promoter methylation in other tumor types, including bladder, breast, endometrial, gastric, glioblastoma (gliomas), hepatocellular, lung, ovarian, pancreatic, renal, and prostate cancers, as well as for leukemia, melanoma, duodenal adenocarcinomas, adrenocortical carcinomas, and neuroblastomas. CIMP has been reported to be useful for predicting prognosis and response to treatment in a variety of tumor types, but it remains unclear whether or not CIMP is a universal phenomenon across human neoplasia or if there should be cancer-specific definitions of the phenotype. Recently, it was shown that somatic isocitrate dehydrogenase-1 (IDH1) mutations, frequently observed in gliomas, establish CIMP in primary human astrocytes by remodeling the methylome. Interestingly, somatic IDH1 and IDH2 mutations, and loss-of-function mutations in ten-eleven translocation (TET) methylcytosine dioxygenase-2 (TET2) associated with a hypermethylation phenotype, are also found in multiple enchondromas of patients with Ollier disease and Maffucci syndrome, and leukemia, respectively. These data provide the first clues for the elucidation of a molecular basis for CIMP. Although CIMP appears as a phenomenon that occurs in various cancer types, the definition is poorly defined and differs for each tumor. The current perspective discusses the use of the term CIMP in cancer, its significance in clinical practice, and future directions that may aid in identifying the true cause and definition of CIMP in different forms of human neoplasia. Cancer Res; 1–11. ©2013 AACR.

Introduction

Unraveling the complexities of the epigenetic code has been instrumental in advancing our understanding of cancer etiology. It is now clear that epigenetic modifications including aberrant DNA methylation, histone modifications, chromatin remodeling, and noncoding RNAs play a significant role in cancer development (1). Because such processes do not induce changes in the DNA sequence, but rather are self-propagating cationic variations, chromatin cations, including cations including DNA methylation of promoter-associated CpG islands, have been the most studied epigenetic alteration in human neoplasia (1). Wide-spread CpG island promoter methylation, also referred to as the CpG island methylator phenotype (CIMP), was first identified (6) and has been extensively studied in colorectal cancer. Recently, we systematically reviewed the body of colorectal cancer research and concluded that because there is no universal standard or consensus with respect to defining CIMP, establishing the true prevalence of CIMP in colorectal cancer will be challenging until its biologic cause is determined (7).

Despite these limitations identified in colorectal cancer research, the term "CIMP" has been repeatedly used over the past decade to describe the increased prevalence of CpG island promoter methylation in other tumor types, including bladder (8), breast (9–11), endometrial (12, 13), gastric (14–19), glioblastoma (gliomas; refs. 20–22), hepatocellular (23–26), lung (27, 28), ovarian (29), pancreatic (30), prostate (31), and renal cell (32) cancers, as well as in leukemia (33–36), melanoma (37), duodenal adenocarcinomas (38), adrenocortical carcinomas (39), and neuroblastomas (40, 41). The primary purpose of these studies was to determine if CIMP is also present in these cancers, and if it can be used to distinguish between known phenotypes of the respective cancer type. However, in many cases, the observation of CIMP for a tumor results from a self-fulfilling definition, where a subgroup of tumors with a greater
degree of DNA methylation than the remaining tumors constitutes CIMP.

Although CIMP has been associated with environmental and lifestyle factors (3, 42–48), the molecular basis for CIMP is only beginning to be explored. The first clues came from two studies showing that glioblastomas with a hypermethylator phenotype are associated with somatic mutations in isocitrate dehydrogenase-1 (IDH1; refs. 20, 21), and that somatic mutations in IDH1, IDH2, as well as loss-of-function mutations in ten-eleven translocation (TET)-methylcytosine dioxygenase-2 (TET2) establish a hypermethylator phenotype in leukemia (49). These are the first indications for a molecular basis of CIMP, and provide an explanation for a very distinct set of tumors with increased levels of hypermethylated DNA. Consequently, these studies have provided a framework for understanding the interplay between genetic and epigenetic changes, and also raise questions about the causes and importance of CIMP in other tumor types. Is “CIMP” a universal phenomenon across human neoplasia caused by similar defects and characterized by similar hypermethylomes, or are there tumor type–specific causes and tumor type–specific definitions of the phenotype?

Addressing these questions is essential for directing research at exploiting CIMP. Here, we discuss the evolution in our understanding of CIMP in various tumor types and how the recent characterization of the human cancer genome and epigenome may influence future research.

CIMP: Roots in Colorectal Cancer

Molecular characteristics of CIMP tumors

Before any discussion on CIMP, it is important to briefly describe CIMP in colorectal cancer, as much of the research surrounding CIMP in other cancer types is based on this body of evidence. It has been more than a decade since Toyota and colleagues first identified CIMP in colorectal cancer (6). Colorectal cancer tumors characterized by CIMP have distinctly different histology when compared with tumors derived from traditional adenoma-carcinoma pathway (50–53). An early event in CIMP tumors seems to be the V600E BRAF mutation (53). A tight association between the V600E BRAF mutation and CIMP, and mice data showing that the V600E BRAF mutation in the mouse gut induces increased DNMT3B expression, de novo methylation, and downregulation of specific CpG dinucleotides in p16INK4A exon 1, has been reported (54). However, there is no functional evidence supporting that the V600E BRAF mutation is a causal event for CIMP. Therefore, it remains possible that BRAF mutation is a surrogate marker for another causal gene. Furthermore, most CIMP colorectal cancers are characterized by promoter CpG island hypermethylation of the mismatch repair gene, MLH1, resulting in its transcriptional inactivation. Loss of MLH1 is thought to cause microsatellite instability (MSI), a form of genetic instability characterized by length alterations within simple repeated microsatellite sequences of DNA (51, 55). Once MLH1 is inactivated, the rate of progression to malignant transformation is rapid (53).

In 2006, a major advancement was made in CIMP research by using unsupervised hierarchical cluster analysis of methylation data; Weisenberger and colleagues identified a robust 5-gene panel that recognized a distinct, heavily methylated subset of colorectal tumors that were also characterized by the V600E BRAF mutation and MSI (56). This panel proved the validity of the phenotype in colorectal cancer, which has been further substantiated and validated in a large, population-based sample (57). Since then, the combinations of genes in addition to those proposed by the Weisenberger and colleagues have been suggested as the “best” panel (58–61), but the idea that CIMP is tightly linked with the V600E BRAF mutation remains consistent in all studies. However, a cause or molecular mechanism for CIMP in colorectal cancer has not yet been identified, and thus the sensitivity and specificity of this panel for defining CIMP remains to be established. Another aspect that needs to be resolved is the question of whether colorectal cancer CIMP cases should be further subgrouped in CIMP-high and CIMP-low colorectal cancers (58–60, 62–64). Although CIMP-low colorectal cancers have been associated with KRAS mutations, this group has many clinical and pathologic features in common with non-CIMP, and consensus on how to define CIMP-low is currently lacking.

CIMP translated to other cancer types

From the literature, it is evident that many studies have investigated CIMP on the premise that the phenotype and genes that quantify the phenotype are not cancer type specific, but rather universal. For example, studies involving breast and endometrial cancer have defined CIMP as “methylated multigenes in tumors” (11) and “when multiple genes are concurrently methylated” (13), respectively. The definition of “multiple” is defined by each investigator to provide separations into subgroups of patients. Furthermore, it is not uncommon for researchers investigating tumor types other than colorectal cancer to refer the study of Weisenberger and colleagues (56) as a rationale for studying CIMP as a marker of cancer, even though the results of that study were very specific for colorectal cancer, especially for tumors characterized by the V600E BRAF mutation.

In our recent review, we detailed the use of various techniques and multiple gene panels and cutoff thresholds used to classify a colorectal cancer tumor as CIMP-positive (7). Selection of gene panels and cutoff thresholds for defining CIMP and small sample sizes in other tumor types seems to be even more arbitrary than for colorectal cancer (Table 1). Studies in gastric cancer (14–19) have often been based on the “classic” gene panel first identified in colorectal cancer by Toyota and colleagues (6), before Weisenberger and colleagues (56). Studies in ovarian cancer (29), breast cancer (11), hepatocellular carcinoma (23, 26), and melanoma (37) have in part chosen gene panels based on observations from colorectal cancer or gastric cancer research. It is not our intention to imply that such studies are inherently flawed, but again, this type of selection assumes that CIMP is a universal process and not cancer specific.

Extensive studies of genetic and epigenetic changes in human cancers show that the transformation process differs greatly among tumors arising in different organs. Thus, if CIMP is ultimately organ or tissue specific, much of the true picture surrounding prevalence and prognostic value may not be
<table>
<thead>
<tr>
<th>Study characteristics</th>
<th>Assessment of CIMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Country</td>
</tr>
<tr>
<td>Adrenocortical carcinomas</td>
<td>Barreau and colleagues (39)</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>Maruyama and colleagues (8)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Bae and colleagues (9)</td>
</tr>
<tr>
<td></td>
<td>Jing and colleagues (11)</td>
</tr>
<tr>
<td></td>
<td>Fang and colleagues (10)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Whitcomb and colleagues (12)</td>
</tr>
<tr>
<td></td>
<td>Zhang and colleagues (13)</td>
</tr>
<tr>
<td>Duodenal adenocarcinoma</td>
<td>Fu and colleagues (38)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Toyota and colleagues (19)</td>
</tr>
<tr>
<td></td>
<td>Oue and colleagues (18)</td>
</tr>
<tr>
<td></td>
<td>Kim and colleagues (16)</td>
</tr>
<tr>
<td></td>
<td>Etoh and colleagues (15)</td>
</tr>
</tbody>
</table>

(Continued on the following page)
Table 1. Summary of studies of CIMP detection and status (Cont’d)

<table>
<thead>
<tr>
<th>Study characteristics</th>
<th>Assessment of CIMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Country</td>
</tr>
<tr>
<td>An and colleagues (14)</td>
<td>United States</td>
</tr>
<tr>
<td>Kusano and colleagues (17)</td>
<td>Japan</td>
</tr>
<tr>
<td>Giommas</td>
<td>Nourshmehr and colleagues (20)</td>
</tr>
<tr>
<td>van den Bent and colleagues (22)</td>
<td>Europe (EORTC study 26951, the Netherlands</td>
</tr>
<tr>
<td>Shen and colleagues (25)</td>
<td>China, England, United States</td>
</tr>
<tr>
<td>Zhang and colleagues (26)</td>
<td>China</td>
</tr>
<tr>
<td>Cheng and colleagues (23)</td>
<td>China</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Toyota and colleagues (36)</td>
</tr>
<tr>
<td>Garcia-Manero and colleagues (33)</td>
<td>United States</td>
</tr>
<tr>
<td>Roman-Gomez and colleagues (35)</td>
<td>Spain</td>
</tr>
</tbody>
</table>

(Continued on the following page)
<table>
<thead>
<tr>
<th>Study characteristics</th>
<th>Study</th>
<th>Country</th>
<th>N</th>
<th>Gene panela</th>
<th>Method</th>
<th>Marker threshold to assign CIMP-H</th>
<th>% CIMP-Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roman-Gomez and colleagues (34)</td>
<td>Spain</td>
<td>54</td>
<td>38 Genes involved in cell immortalization and transformation</td>
<td>MSP</td>
<td>≥3 Methylated genes</td>
<td>63%</td>
</tr>
<tr>
<td></td>
<td>Figueroa and colleagues (49)</td>
<td>United States</td>
<td>385</td>
<td>Genome-wide characterization of the methylome</td>
<td>Roche Nimblegen custom human promoter array covering 25,626 HpaII amplifiable fragments and MassArray Epityping</td>
<td>Clustering analyses</td>
<td>–</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Suzuki and colleagues (28)</td>
<td>Japan</td>
<td>150</td>
<td>TMEFF2 (HPP1), SPARC, RBP/RM (Reprimo), RBP1 (CBBP1), RARB (RARb)</td>
<td>MSP</td>
<td>–</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>Liu and colleagues (27)</td>
<td>China</td>
<td>60</td>
<td>OGG1 (hOGG1), VHL, RARB (RARb-B), MLH1 (MLH1), SMAD2, RASSF1A, ZMYND10 (BLU), FHIT</td>
<td>MSP</td>
<td>≥4.8 Genes methylated</td>
<td>57%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Tanemura and colleagues (37)</td>
<td>United States</td>
<td>122</td>
<td>WIF1, TFP2, RASSF1A, RARB (RARb-B), SOCS1, GATA4, MINT1, MINT2, MINT12, MINT17, MINT35, MINT31</td>
<td>MSP</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Abe and colleagues (40)</td>
<td>Japan</td>
<td>140</td>
<td>17 Members of PCDHB family, 13 members of PCDHA family, MST1 (HLP), DFK2b-4511127, CYP26C1</td>
<td>qMSP</td>
<td>Cutoff &gt;0% methylation of PCDHB family members</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Abe and colleagues (41)</td>
<td>Germany</td>
<td>152</td>
<td>17 Members of PCDHB family, MST1 (HLP), CYP26C1</td>
<td>qMSP</td>
<td>&gt;60% Methylation of PCDHB family 33% members and for samples with 40% to 60% PCDHB methylation, &gt;10% MST1 (HLP) methylation and/or &gt;70% CYP26C1 methylation</td>
<td>–</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Strathdee and colleagues (29)</td>
<td>Scotland</td>
<td>93</td>
<td>BRCA1, HIC1, MLH1, CDKN2A (p16), TERC (hTR), CASP8, MINT25, MINT31, CDKN2B (p15), TP73 (p73)</td>
<td>MSP</td>
<td>Unclear, although they do make a conclusion about CIMP</td>
<td>Unclear; 71% of tumors showed methylation</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Ueki and colleagues (30)</td>
<td>United States</td>
<td>45</td>
<td>RARB (RARb), THBS1, CACNA1G, MLH1, MINT1, MINT2, MINT13, MINT32</td>
<td>MSP</td>
<td>≥4.8 Genes methylated</td>
<td>14%</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Manyama and colleagues (31)</td>
<td>United States</td>
<td>101</td>
<td>RARB (RARb), RASSF1A, GSTP1, CDH13, APC, CDH1, FHIT, CDKN2A (p16RDRA), DAPK1 (DAPK), MGMT</td>
<td>MSP</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: EORTC, European Organization for Research and Treatment of Cancer; MSP, methylation specific PCR analysis; qMSP, quantitative methylation specific PCR analysis.
aGene names are reported as HUGO approved gene symbols, between brackets the gene symbols used in the original study.
bCIMP-H refers to either CIMP or in the instance that a study reported three CIMP categories, CIMP-high.
cData not reported.
recognized with the use of CIMP markers developed in another tumor type. For example, in a study of CIMP in endometrial cancer, genes were selected on the basis of their high degree of methylation in other malignancies, including colorectal cancer (13). However, a recent molecular characterization of endometrial tumors identified no V600E BRAF mutations in any of the 87 specimens considered (65). Therefore, selecting a CIMP panel tightly associated with BRAF mutation may not be entirely relevant to quantifying or identifying CIMP in endometrial tumors. Similarly, results from a recent study on duodenal adenocarcinomas suggest that BRAF mutations are not involved in duodenal tumorigenesis (38). If one hypothesizes that CIMP is a general phenomenon, then the cause of CIMP should also be general and similar across different cancer types.

To assess just how universal CIMP is across tumor types requires genome-wide characterization of the methylome. This is a relatively new direction in epigenetic research, and to our knowledge, has only been reported for gliomas (20), leukemia (49), breast cancer (10), benign nonhereditary skeletal tumors such as enchondroma (66), as well as, most recently, renal cell carcinoma (32), melanoma (67), gastric cancer (68), and oral squamous cell carcinoma (69).

CIMP: Genome-Wide Characterization of the Methylome

Glioma

Promoter-associated hypermethylation has been commonly reported in gliomas (70–76), but it was not until 2010, when Noushmehr and colleagues used Illumina array platform technology, that a CIMP specific for a group of gliomas with distinct molecular and clinical characteristics was established (20). They referred to this cluster of tumors as “G-CIMP” to imply its molecular and clinical characteristics was established (20). Furthermore, Noushmehr and colleagues showed that G-CIMP loci were then validated with MethyLight technology, and perfect concordance with G-CIMP calls on the array platforms versus with the MethyLight markers was observed. Consequently, similar prevalence of the phenotype was shown, providing validation of the technical performance of the platforms and of the diagnostic marker panel. Furthermore, Noushmehr and colleagues showed that G-CIMP was very tightly associated with the somatic IDH1 mutation, and validated this in an independent subset of tumors (20).

In 2012, additional evidence for a causal role of IDH1 in generating CIMP was presented. Using immortalized human astrocytes, Turcan and colleagues showed that the mechanistic process behind this involves the IDH1 mutation subtly remodeling the epigenome by modulating patterns of methylation on a genome-wide scale, thereby changing transcriptional programs and altering the differentiation state (21). The authors suggest that the activity of IDH1 may form the basis of an "epigenomic rheostat," which links alterations in cellular metabolism to the epigenetic state (21).

Mutations in IDH1 and IDH2 result in a reduced enzymatic activity toward the native substrate isocitrate. Mutant IDH1 catalyzes the reduction of α-ketoglutarate to 2-hydroxyglutarate (2-HG), a potential oncometabolite (77–80) affecting gene expression via various mechanisms. This is first accomplished via competitive inhibition of α-ketoglutarate–dependent dioxygenases including Jumonji-C domain-containing histone demethylases (JHDM), thereby altering histone methylation levels. In addition, 2-HG inhibits the TET family of 5-methylcytosine (5mC) hydroxylases that convert 5mC to 5-hydroxymethylcytosine (5hmC) via direct competition with α-ketoglutarate resulting in an accumulation of 5hmC and thereby potentially altering the expression levels of large numbers of genes (49, 80). Finally, a mechanism altering hypoxia-inducible factor (HIF) expression is involved (81).

In their recent study, Turcan and colleagues showed that the expression of wild-type IDH1 caused hypomethylation at specific loci, suggesting that both the production of 2-HG and the levels of α-ketoglutarate can affect the methylome (21). Furthermore, unsupervised hierarchical clustering of methylene data showed that the hypermethylated genes included both genes that underwent de novo methylation as well as genes that originally possessed low levels of methylation but subsequently acquired high levels of methylation. Control astrocytes did not undergo these methylene changes. Mutant IDH1-induced remodeling of the methylome was reproducible and resulted in significant changes in gene expression (21).

Leukemia

For leukemia, the same story can be told. CIMP, defined by methylation of candidate genes, was reported in 2001 and 2002 (33, 36). However, the mutational and epigenetic profiling data of Figueroa and colleagues in acute myelogenous leukemia (AML) for the first time identified a causal relationship between IDH1, IDH2, and TET2 mutations and (overlapping) hypermethylation profiles and global hypermethylation (49). Functional support for this relationship was provided in vitro in hematopoietic cells in which expression of mutant IDH1 and IDH2 leads to an increase in DNA methylation, indicating that IDH1/2 and TET2 mutations contribute to leukemogenesis through a shared mechanism that disrupts DNA methylation. In vivo evidence comes from a conditional IDH1(R132H) knockin mouse model, which develops increased numbers of early hematopoietic progenitors, splenomegaly, and anemia with extramedullary hematopoiesis. These alterations are accompanied by changes in DNA and histone methylation profiles (82).

Echondroma and spindle cell hemangiomma

Supporting the hypothesis that IDH1 mutation leads to DNA methylation, evidence shows that somatic mosaic mutations in IDH1, and to a lesser extent IDH2, cause enchondroma and spindle cell hemangiomma in patients with Ollier disease and Maffucci syndrome (66, 83). These are rare skeletal disorders in which there is also an increased incidence of glioma (66). Using Illumina HumanMethylation27 BeadChips, Sansuriya and colleagues examined possible differences in methylation between enchondromas with and without IDH1 mutations. Unsupervised clustering of the 2,000 most variable CpG methylation sites gave two subgroups, one of which showed an overall higher methylation at the examined CpG sites, and all but one enchondromas with an IDH1 mutation were positive for this "CIMP" (83).
IDH mutations in other cancer types

In addition to glioma (>70%), leukemia (AML: 15%–30%), echondroma (87%), and spindle cell hemangioma (70%), somatic IDH1 mutations are also found in sporadic chondrosarcoma (~50%; refs. 49, 84) and at lower frequencies in anaplastic thyroid carcinoma (11%; ref. 85), (intrahepatic) cholangiocarcinomas (10%–23%; refs. 86, 87), and melanoma (10%; ref. 88), whereas in other solid tumors IDH1 mutations are infrequent (<5%) or absent (89, 90). Interestingly, the IDH1/2 mutations in melanoma are also accompanied by a loss of 5mC in melanoma progression (67). Therefore, it is interesting to speculate whether or not future research to establish the cause of CIMP in other cancer types should focus on genes that are functionally similar to the IDH1 family, such as TET2, or on totally different genes. More specifically, it remains uncertain whether CIMP in other cancer types is also caused by inhibition of the conversion of 5mC to 5hmC and subsequent demethylation or that other factors are responsible for the accumulation of 5mC. In addition to colorectal cancer, another tumor type lacking IDH1/2 mutations, but with a putative CIMP phenotype, is breast cancer.

Breast cancer

To date, research that has investigated CIMP in breast cancer has not been conclusive (9, 91–94), with some studies going so far as saying that CIMP does not exist in breast cancer as a truly defined phenotype (9). Recently, Fang and colleagues used unsupervised hierarchical clustering from data collected with the Infinium Human Methylation27 platform in an attempt to clarify this dispute (10). Two DNA methylation clusters in a sample of breast cancer with diverse metastatic behavior were identified. One cluster encompassed a portion of hormone receptor (HR)þ tumors [defined as estrogen receptor (ER1)þ/progesterone receptor (PR)þ, cluster 2] and one encompassed tumors that were ER1/PR0 or ER1/PR– (cluster 1). Cluster 2 tumors had a highly characteristic DNA methylation profile with high coordinate cancer-specific hypermethylation at a subset of loci, similar to the CIMP phenotype seen in colorectal cancer. They referred to this as “B-CIMP,” and confirmed the composition of the phenotype through two independent clustering algorithms (10). Although this supports the idea that CIMP is a Prognostic Marker

Through their methodology, the studies of Fang and colleagues (10) and Noushmehr and colleagues (20) were able to clearly show distinct clinical characteristics of tumors characterized by B-CIMP and G-CIMP. For instance, B-CIMP tumors were associated with ESR1/PGR status, a lower risk of metastasis, and an improved clinical outcome (10). G-CIMP has been associated with improved survival, younger age at diagnosis, and histologic characteristics (20, 22). Furthermore, using the Infinium array, a recent methylome analysis in a study of patients with primary clear cell renal carcinoma showed that CIMP characterized a specific cluster of tumors associated with aggressiveness and patient outcome (32). Such findings reiterate a major motivation for establishing whether CIMP is universal or cancer specific is because of its potential use as a prognostic marker.

Table 2 shows that CIMP is associated with both favorable and unfavorable prognosis, as well as different clinical characteristics, depending on the type of tumors. There are several possible explanations for these discrepancies. First, although CIMP has been identified in different types of cancer, it may simply not be a universal marker of good or bad prognosis. Second, as previously noted, it could be possible that for some cancers, the gene panels and cutoff thresholds used to define CIMP are not accurate for defining the “true” phenotype. It is interesting to observe that CIMP is associated with a favorable prognosis for colorectal cancer and gliomas, two cancer types for which extensive research has been conducted with respect to identifying genes that are associated with clinical and molecular features of the tumors, and in studies that included a relatively large number of cases (20, 57).

Moreover, it has been noted that the association of methylation at CIMP genes with good clinical outcome is not universally applicable to methylation at all genes. Methylation of specific candidate genes or groups of genes has been associated with poorer prognosis, and these genes may have an effect on tumor aggressiveness independent of CIMP (10).

Conclusions and Future Perspectives

Much like what has been observed in the field of colorectal cancer research (7), the study of CIMP in other tumor types has been quite heterogeneous in terms of how the phenotype has been defined. Recent studies considering genome-wide characterization of the methylome in gliomas and leukemia have shown that CIMP is likely more than just a generic name to be used to describe aberrant methylation.

Although there is some overlap with respect to genes targeted by CIMP in colon cancer, breast cancer, and gliomas, and although IDH1 and genes that affect the same (metabolic) pathway, such as IDH2 and TET2, have been shown to be
causally involved in the generation of CIMP in gliomas and leukemia, cancer-specific differences still exist and the cause of CIMP in the majority of cancer types remains to be identified. The causal relationship between somatic mutations in genes such as \textit{IDH1}, \textit{IDH2}, and \textit{TET2} and altered genome-wide DNA methylation profiles generated by next-generation sequencing techniques is a promising clue on the cause of CIMP. The fact that these mutations impair histone demethylation and induce repressive histone methylation marks thereby blocking cell differentiation (95) provide clues on the complex relations between specific genetic alterations, CIMP, and clinical characteristics such as histologic features and prognosis.

In addition, analyzing the relationship between somatic mutations in chromatin remodeling genes and CIMP could yield interesting insights. For example, \textit{AT-rich interactive domain-containing protein 1a (ARID1a)}, a member of the switch/sucrose nonfermentable (SWI-SNF) complex, has been reported to be mutated and inactivated in a subset of gastrointestinal cancers, the majority of which also exhibit another characteristic of C-CIMP, namely MSI (96–98).

To unify the field and to establish a standard definition for CIMP, we present the following recommendations:

1. CIMP is not a single phenotype in all types of cancer. A simple variation from the standard nomenclature of "CIMP" to make this distinction, such as "C-CIMP" for colorectal cancer CIMP, "G-CIMP" for glioma CIMP, "L-CIMP" for leukemia CIMP, and "B-CIMP" for breast cancer CIMP should be adopted for clarity.

2. Multiple reports suggest a third category of CIMP in colorectal cancer by dividing CIMP into CIMP-high and CIMP-low. Although CIMP-low has repeatedly been associated with KRAS mutations, this group has many clinical and pathologic features in common with non-CIMP, and thus without evidence that this is a distinct phenotype and without consensus on how to define CIMP-low, the use of CIMP-low should be discouraged.

3. A consensus meeting should be organized to:
   a) Obtain recommended guidelines on the optimal CIMP marker panel for each tumor type. This includes the number of markers in the panel, the specific loci (genes) included, and the defined region examined for methylation in each gene.
   b) Obtain recommended guidelines on the method to measure CIMP. If quantitative methods are needed for CIMP classification, defined cutoffs must be established for each marker for subsequent validation.

### Table 2. CIMP and clinicopathologic features of different cancers

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Significant clinical associations</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenocortical carcinomas (39)</td>
<td>Subset of hormone positive tumors (ESR1⁺/PGR⁺)</td>
<td></td>
</tr>
<tr>
<td>Bladder cancer (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer (10)</td>
<td>Female, Older age, Proximal location, MSI, \textit{BRAF} mutation</td>
<td>+</td>
</tr>
<tr>
<td>Colorectal cancer (56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenal adenocarcinomas (38)</td>
<td>Early stage, COX-2 hypermethylation, MSI, Lymph node metastasis</td>
<td>±</td>
</tr>
<tr>
<td>Endometrial cancer (12, 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric cancer (14–19)</td>
<td>Younger age at diagnosis, \textit{IDH1} mutation</td>
<td>+</td>
</tr>
<tr>
<td>Gliomas (20)</td>
<td>Younger age at diagnosis, Serum α-fetoprotein (AFP), Metastasis, TNM staging, CIMP in serum</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma (23–26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia (adult acute lymphocytic; ref. 33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia (acute myeloid; ref. 36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia (T-cell acute lymphoblastic; ref. 35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia (childhood acute lymphoblastic; ref. 34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung cancer (27, 28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma (37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroblastoma (40, 41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate cancer (31)</td>
<td>High preoperative serum (PSA) levels, Advanced stage</td>
<td></td>
</tr>
<tr>
<td>Renal cell carcinoma (32)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hughes et al.
4. Once CIMP markers have been identified, they should be validated in large, independent, well-characterized patient series with clinical follow-up data (molecular pathologic epidemiology approach; refs. 99, 100).

5. A research effort for identifying the biologic cause of CIMP among tumor types should be implemented once standard criteria for CIMP are established and validated. Focus should be on establishing causal relationships to find the driver(s) of CIMP.

6. Dissemination of the recommended guidelines to the field, as was done for Bethesda MSI markers (101), is crucial in standardizing research in the field of CIMP.

Hopefully, these recommendations will help to establish the true causes, manifestation, and proper definitions of CIMP.

Disclosure of Potential Conflicts of Interest

W. van Criekinge is employed as CSO in MDX Health, J.G. Herman has a commercial research grant from MDX Health and is a consultant/advisory board member of the same. M. van Engeland has a commercial research grant from MDX Health. No potential conflicts of interest were disclosed by the other authors.

References


How to Define CIMP?

Authors’ Contributions

Conception and design: L.A.E. Hughes, V.T.H.B.M Smit, P.A. van den Brandt, N. Ahuja, M.P. Weijenberg, M. van Engeland

Development of methodology: L.A.E. Hughes, N. Ahuja, M.P. Weijenberg, M. van Engeland

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.P. Weijenberg

Analysis and interpretation of data (e.g., statistical analysis, bios-statistics, computational analysis): L.A.E. Hughes, J. de Schrijver, W. van Criekinge, N. Ahuja, J.G. Herman, M.P. Weijenberg

Writing, review, and/or revision of the manuscript: L.A.E. Hughes, V. Tu, S. Bonasio, D. Reinberg, D. Molecular signals of epigenetic states. Science 2010;330:612–6.

Acknowledgments

This study was financially supported by a Cancer Research Foundation Limburg grant (M. van Engeland, M.P. Weijenberg, and P.A. van den Brandt). J.V.M.G. Bovée is supported by the Netherlands Organization for Scientific Research (917-67-315). P.J. French is supported by ZonMW project numbers 90035560, 40-41200-98-9513, 95110051, and stophersentumoren.nl.

Received November 21, 2012; revised June 8, 2013; accepted June 8, 2013; published OnlineFirst June 25, 2013.


63. Dahlin AM, Palmqvist R, Henriksson ML, Jacobsson M, Eklof V, Rutegard J, et al. The role of the CpG island methylator phenotype...


The CpG Island Methylator Phenotype: What's in a Name?
Laura A.E. Hughes, Veerle Melotte, Joachim de Schrijver, et al.
Cancer Res Published OnlineFirst June 25, 2013.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-12-4306

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.