

1.1. What Epigenetics is All About

Scientists are increasingly recognizing that to understand human health and disease—not just what causes disease but how to prevent and reverse it—they need to know more than what kind of genes someone has, or what the DNA sequence of their genome is. Equally, if not more important, they need to know how cells use that genomic information.

That is what epigenetics is all about: how genomic information encoded in the DNA sequence is regulated by other, nonDNA-sequence factors, each of which plays a key role in gene expression by allowing or preventing transcription to proceed—that is, by turning genes on and off. These other factors include:

- Methylation of cytosine bases
- Various types of covalent modifications to the histone proteins around which the DNA double helix is wound
- The activity of various nonhistone proteins that bind to the DNA
- Small, noncoding RNA molecules

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More formally, epigenetics is the study of heritable alterations in gene expression not coded in the actual DNA sequence of any gene(s). Epigenetic alterations, or information, encompass all of the various chemical and physical mechanisms that affect development and inheritance *in addition to* the myriad of cellular information encoded by the DNA sequence of the genome. In fact, the word *epigenetics* is derived from the Greek prefix *epi-*, which means *above* or *in addition to*.

To say that something is heritable means that it can be passed down generation to generation, either through mitosis (in which case it is “mitotically heritable” or “somatically heritable”) or through meiosis (in which case it is “meiotically heritable”). Usually in biology, the word “heritable” refers to the latter. Throughout this report, however, it refers to the former: the transmission of nonDNA-sequence-related information through mitosis, from 1 cellular generation to the next.

Heritability is an essential component of anything and everything genetic. As Peter Jones, PhD, said in his keynote address at the Cambridge Healthtech Institute Epigenomics conference in March 2007 in San Diego, CA, “It’s not epigenetic unless it’s heritable.”

Table 1.3. Total Sales for FDA-Approved Epigenetic Therapeutics Already on the Market

Drug	Brand	Type of epigenetic drug	Company	3Q '07 sales	3Q '06 sales
vorinostat	Zolinza	HDAC inhibitor	Merck	\$3 million	N/A
decitabine	Dacogen	Methylation inhibitor	MGI Pharma	\$34.6 million	\$11.9 million
azacitidine	Vidaza	Methylation inhibitor	Pharmion*	\$42.3 million	\$36.6 million

* In November, 2007, Celgene agreed to acquire Pharmion for a total of \$2.9 billion in cash and stock. The deal is expected to close by 2Q '08.

Source: Insight Pharma Reports

1.3. The Etiology of Cancer: From a Mutational to Epigenetic Understanding

Most of the interest and investment in epigenetic-based diagnostics and therapeutics has been in oncology. This is true even though cancer has long been considered a “mutational disease.”

Cancer as a Mutational Disease

DNA mutations are a normal part of life, and their occurrence does not necessarily signify cancer. When cells replicate, their nuclear DNA nearly always naturally undergoes random chemical changes. But our cells are able to quickly repair most errors. Changes that are not repaired become mutations: permanent alterations in the DNA sequence of a gene. Most mutations are harmless, and even among those that can cause harm, rarely does a single “bad” mutation lead to cancer. Rather, malignant transformation—the process by which a precancerous cell becomes an actual tumor cell—is believed to result from the accumulation of multiple DNA sequence changes. Some scientists have argued that most common adult cancers are probably caused by the accumulation of 5 to 7 random mutational events; others suspect the number is greater.

Histone Modifications as Biomarkers: A Field in its Infancy

While DNA methylation dominates the epigenetic R&D landscape and is the focus of this chapter for that reason, it is worthwhile to note that there has also been a recent surge of interest and investment in histone modifications as biomarkers. As was (and remains) the case with DNA methyl biomarkers, the reasons are twofold:

- **Advances in science:** Scientists are gaining tremendous ground in their understanding of histone modifications as key modifier roles in health and disease. Indeed, this understanding is a key component, or premise, of the so-called “histone code hypothesis”: that posttranslational histone modifications mediate distinct nuclear processes in a selective, code-like manner.
- **Advances in technology:** The new high-throughput, genomewide epigenetic detection approaches made possible by the next-generation deep-sequencing technologies made possible by the new “deep sequencing” platforms on the market promise to be just as, if not more, useful decoding the histone code as they will be for analyzing genomewide methylation patterns. Regarding the latter, in October 2007, the National Human Genome Research Institute (NHGRI), part of the National Institutes of Health (NIH), announced that it would be rewarding more than \$80 million in grant money over the next 4 years to expand its ENCyclopedia Of DNA Elements (ENCODE) project to include epigenetic-related technology development tasks. One of the grants was awarded to John Greally, PhD, of the Albert Einstein College of Medicine, for a project involving mapping genomewide histone modifications through the use of CHromatin ImmunoPrecipitation, followed by high-throughput DNA SEquencing, or ChIP-Seq. Greally and colleagues will be using the Solexa high-throughput sequencing platform. Other institutions receiving technology development grants from the same \$80 million package include Stanford University (Palo Alto, CA), University of Washington (Seattle), Indiana University (multiple campuses), University of Chicago, and the Genome Institute of Singapore.

Alarming, in December 2006, Roche decided to terminate its 4-year collaboration with Epigenomics after deciding that the latter's Septin 9 biomarker assay for the detection of colorectal cancer didn't meet Roche criteria for development as an in vitro diagnostic test.

While some experts anticipated that the end of the Roche collaboration might be too great a setback for Epigenomics, the latter responded by shifting its focus primarily to late-stage product development, a move that led to the layoff of approximately one-third of its Berlin staff. The company also began seeking other diagnostic partners.

As part of their intensified focus on late-stage product development, by the end of 2007, scientists at Epigenomics had successfully modified the Septin 9 assay, resulting in a significantly improved success rate: 70% sensitivity, compared to 50%-60% sensitivity previously. In other words, the assay was shown to correctly detect 70% of all colorectal cancers (91 out of 130). More importantly, the 70% detection rate was achieved in patients with early stage disease (stage I-III). The new data were announced at the 2007 annual meeting of the American Association for Cancer Research (AACR), held in Los Angeles, CA, in April 2007.

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Current company efforts are being directed at streamlining the sample processing workflow, as a way to reduce costs and facilitate transfer of the assay to clinical use. In 3Q '07, Epigenomics partnered with Abbott Molecular (Des Plaines, IL) in a nonexclusive collaboration and licensing agreement for the improved product. Both partners anticipate launching a CE-marked test on Abbott's automated m2000 instrument platform in Europe in 2009, followed by regulatory filing for US approval in 2010. Epigenomics plans to partner with a US reference laboratory for early market introduction of its Septin 9 biomarker for in-house testing.

So despite Roche's withdrawal, by shifting its focus to late-stage product development and clinical validation, Epigenomics was able to strengthen its entry position into the molecular diagnostics market with what could be one of the first commercially available epigenetic-based diagnostic tools. Abbott's backing is testament to the validity of the approach.

The Septin 9 colorectal detection test remains one of Epigenomics' lead products. Another lead product, arguably further along, according to Dr. Lofton-Day, is the company's prostate cancer molecular classification test (which is also DNA methylation-based). See the interview with Lofton-

Interview with Cathy-Lofton Day, PhD

*Vice President of Molecular Biology, Diagnostics
Epigenomics*

Headquartered in Berlin, Germany, with a wholly owned subsidiary in Seattle, WA, Epigenomics is a molecular diagnostics company focused on developing and commercializing DNA methylation tests for cancer screening and diagnostics.

CHI: *When and how was Epigenomics founded?*

Cathy Lofton-Day: The company is a merger of 2 different companies. The first was a technology-based company, Epigenomics AG, which was started in Berlin in 1999 by a group of scientists led by Dr. Alex Olek [PhD] who were interested in methylation. Epigenomics AG was founded on the premise of developing tools for looking at differences in methylation among tissue samples.

About the same time, another company, Orca Biosciences, led by Dr. Robert Day [MD, MPH, PhD], was starting up in Seattle. Dr. Day had been President of the Fred Hutchinson Cancer Center for about 25 years and after retirement remained very involved in the biotech community, starting up several companies and sitting on the board of many others. His vision was to use some sort of technology for early detection of cancer, when it is curable. So he collaborated with Dr. Peter Jones [PhD], Director of the Norris Comprehensive Cancer Center at USC, who had done a lot of research on methylation and epigenetic drugs, and founded Orca Biosciences.

After people from both companies met at a conference and realized that together they had both a technology [Epigenomics' proprietary technology] and a really good idea [Orca Biosciences' premise], they merged in early 2001. At the time, Epigenomics AG was funded predominantly by the German government and actually had more money than Orca Biosciences, so the merger meant an influx of money into the Seattle-based side so the company was able to maintain a presence in the United States.

Other companies, including publicly traded Montreal-based MethylGene (Montreal, Quebec), privately funded Gloucester Pharmaceuticals (Cambridge, MA), and publicly traded CuraGen (Branford, CT), are hot on Merck's trail with lead HDAC inhibitor compounds in pivotal clinical trials. Additionally, at least 4 companies have HDAC inhibitors in Phase I or II clinical trials: Johnson & Johnson, Novartis, Pharmacyclics, and Syndax.

Some experts predict that Merck will need to work hard to ensure Zolinza retains its first-in-class market advantage when similar drugs, such as MethylGene's MGCD0103, earn approval. Others point to the reality that there is plenty of room for multiple novel therapies, particularly for difficult-to-treat cancers, arguing the possibility that approval of Zolinza has opened a floodgate.

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Biologically, as with demethylation agents, a major potential shortcoming of any HDAC inhibitor is that it could elicit nonspecific, genomewide epigenetic alterations resulting in the reactivation of silenced oncogenes. Some scientists also point to HDAC inhibitors' pharmacokinetic profiles and the fact that inhibition of HDAC activity typically requires continuous drug exposure and systemic circulation, which is often only achieved through daily intravenous administration, the inconvenience of the which is partially why clinical development of Zolinza shifted from a Phase I evaluation of daily intravenous infusions to an oral formulation.⁴⁸ It is also why some scientists are investigating the potential of novel drug delivery systems, such as the use of liposomes for controlled release.

3.4. Combination Therapy: Epigenetic Drugs Make Good Partners

Pharmion, MGI Pharma, and Merck are not the only companies actively pursuing combination strategies for their respective epigenetic therapeutic products or lead candidates. MethylGene is doing the same with its lead HDAC inhibitor compound, MCGD0103, and, in fact, just announced in January 2008 enrollment of the first patient in a Phase 2 clinical trial evaluating the combined use of MCGD0103 and Vidaza in patients with relapsed or refractory Hodgkin's lymphoma or non-Hodgkin's lymphoma. Altogether, MethylGene is running 4 single-agent clinical trials with MGCD0103 (among patients with elderly AML, B-cell lymphoma, CLL, and Hodgkin's) and 5 combination clinical trials, including 3 with Vidaza (among patients with AML/MDS, lymphomas and AML), 1 with Eli Lilly's Gemzar (gemcitabine; patients with pancreatic cancer), and 1 with Sanofi-Aventis' Taxotere

It is not just that knowledge is increasing. It is also expanding. Decades ago, when the science was truly in its infancy, most researchers involved studied DNA methylation; in fact, some scientists still associated the word *epigenetics* with *DNA methylation*. Over the past several years, however, the science has expanded to include not just histone modifications but also nonhistone proteins with epigenetic control functions; small, noncoding RNAs (although most biologists and life sciences industry companies treat the world of small RNA as a separate discipline); and, most recently, stem cells. Regarding the last, 11.9% of our survey respondents identified tissue repair/regeneration as an application likely to benefit the most from continuing advances in epigenetic science and technology.

New Technology

Technological advances in biomarker discovery, detection, validation, and analysis are moving forward at an exhilarating pace, creating novel opportunities for new types of research and clinical tools. In particular, the development of “deep sequencing” technologies, including ChIP-Seq, promises to dramatically accelerate the genomewide study of epigenetic phenomena. As discussed in Chapter 2, it is widely believed that further advances in epigenetic diagnostics and therapeutics will depend on the development of genomewide approaches for studying epigenetic control mechanisms—both by enabling the rapid identification of biomarker or therapeutic target candidates *and* advancing the development of safe, effective clinical tools.

Several respondents to our survey listed next-generation sequencing or ChIP-Seq as “the technology of choice for research purposes in the next 5 years.” When asked which of several next-generation technological categories have the greatest potential to dramatically increase the commercial availability of epigenetic products over the next 5 to 10 years, the 2 most popular answers, both with 39.1% response rates, were “technologies enabling simultaneous monitoring of epigenetic modifications” and “technologies enabling whole profiling of single cells.” Both responses reflect a growing interest, investment, and belief that genomewide epigenetic technologies will dramatically change the commercial landscape.

In separate questions, while a handful of respondents predict that these same epigenomic research tools will also emerge as technologies of choice for clinical purposes within the next 1 to 5 years, clearly the primary applications will be research-based, at least initially (see Figures 14 to 17 in Appendix).

...further advances in epigenetic diagnostics and therapeutics will depend on the development of genomewide approaches for studying epigenetic control mechanisms...

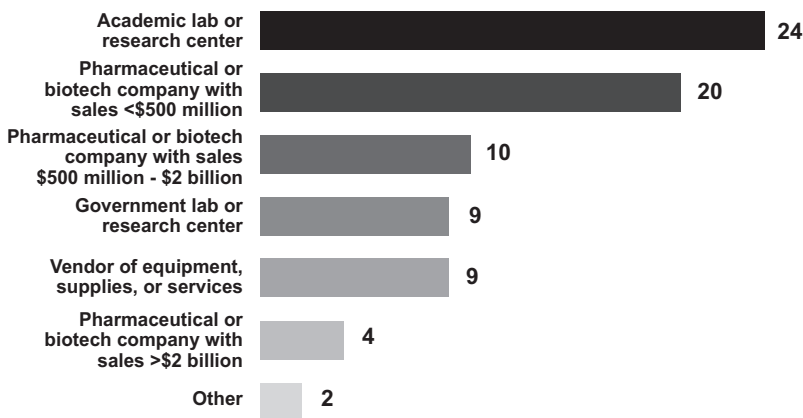
Appendix

INSIGHT PHARMA REPORTS EPIGENETICS SURVEY– JANUARY 2008

In January 2008, CHI Insight Pharma Reports conducted an online survey containing 22 questions related to research and development in epigenetic diagnostics and therapeutics. Invitations to participate were sent to people in the pharmaceutical/biopharmaceutical industry, academic research centers, and governmental research centers. The results are tabulated below.

Figure 1A. Types of Organizations

Please classify your organization



N= 78

Source: *Insight Pharma Reports Epigenetics Survey–January 2008*
