Article

# The Potential of Epigenetic Therapy and the Need for Elucidation of Risks

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Epigenetic phenomena are known to be a root cause of many common diseases. To date, the FDA has approved four epigenetic therapies that show promising results for prolonging lives of terminal cancer patients. However, there is a relative lack of knowledge about long-term epigenetic effects, especially those that affect future generations. We propose a heightening of standards for epigenetic therapy: therapies should be targeted to specific genes in specific cells and cannot affect the germline and patients' epigenomes should be sequenced before and after treatment. Moreover, further research should be performed to answer questions about transgenerational epigenetic effects, to analyze the effects of altered epigenomes in the long term, and to develop superior assays for screening epigenomes. We highlight current research in the field, including the work of the Penn iGEM group.

## Epigenetics Background

**Introduction**. The code of life is more than a sequence of As, Cs, Ts, and Gs. Muscle cells in the human heart contain the same DNA as skin cells in the foot, yet these two cell types behave in radically different ways. Both contain the DNA for over 20,000 human genes but express only the ones needed for their own form and function. These differences in gene expression are modulated by epigenetic controls. Epigenetics refers to any heritable chemical modification of DNA that alters expression without changing genetic sequence. Neurodevelopmental disorders, immunodeficiency, cancer, and other illnesses can result when these mechanisms go awry.

**Methylation**. In humans, enzymes called methyltransferases add methyl groups to short DNA sequences abundant in the genome called CpG sites. Methyl groups block transcription factors (gene activators) from binding to DNA and performing their normal function. Although epigenetic factors do not change the sequence of DNA, they can affect the phenotype, the observable characteristics of the organism. Specific patterns of methylation are necessary for a cell to modulate the level of expression of each of its genes.

## Epigenetic Diseases

**Cancer**. DNA methylation has been referred to as the "hallmark of cancer" (Szyf 2004). Abnormal methylation patterns throughout the genome that cause blockage of tumor suppressor genes have been linked to many types of cancer. For instance, breast cancer generally exhibits inactivation of the gene BRCA1. In sporadic (i.e. non-familial) cases, this suppression is usually caused by hypermethylation rather than mutation of the gene (Rice 2000). In other cases, hypomethylation causes overexpression of the flap endonuclease 1 gene and lead to breast cancer in some patients (Singh 2008).

**Neurological**. Methylation abnormalities have been linked to a wide range of diseases. Fragile X syndrome, one of the leading genetic causes of intellectual disability, is characterized by hypermethylation, which disrupts the production of protein necessary for normal brain development. Patients suffering from this disorder are at risk for autism, ADHD, decreased IQ, infertility in females, and distorted facial features (Jacquemont 2011).

**Psychological**. Epigenetic mechanisms can also impact psychological states. In an animal study, rat pups that received better maternal care in the form of licking, grooming, and arched-back nursing had lower levels of methylation at the glucocorticoid receptor gene. These rats displayed less intense responses to stressful situations than those who received poor maternal care. The researchers were able to eliminate these differences via epigenetic interference (Weaver 2004).

## Epigenetic Therapy

**Fundamental Advantages.** The aforementioned epigenetic roots of disease are attractive targets for therapy. Aberrant DNA methylation patterns are more easily reversible than genetic mutations. In the case of cancer, epigenetic therapy coaxes tumor cells to return to a healthy state, rewiring their methylation patterns so the cells express genes that halt their cancerous uncontrolled growth. Traditional chemotherapy strategies, on the other hand, aim to kill cancer cells and are fundamentally more toxic to patients since healthy cells are also harmed.

**Recent Successes.** There have been some exciting clinical successes with the first generation of epigenetic

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therapies. To date, four epigenetics treatments have been approved by the FDA for use in cancer patients: Zolinza, Istodax, Vidaza, and Dacogen (Claus 2005).

The first two are histone deacetylase inhibitors and prevent histone modifications, one type of epigenetic change; the second two inhibit DNA methylation. These compounds are chemical analogues of the DNA base cytosine ("C"), and at low doses, they bind to the methyltransferases so that they don't come into contact with the patient's actual DNA, preventing the DNA's methylation. At high doses, they can incorporate into the DNA or RNA itself and reduce cell viability. These treatments are used, at low doses, to reverse hypermethylation in patients with myelodysplastics syndromes (MDS), a spectrum of blood cell disorders that can lead to anemia and heighted susceptibility to infections. The drugs effectively reverse cancerous hypermethylation and return standard function to cell-cycle genes, restoring normal growth rates. However, fewer than 10 percent of patients experience a "complete response," a total reversal from diseased to healthy bone marrow and blood (Silverman 2002).

**Problems**. The results of first generation epigenetic therapies are promising, but these drugs should not be seen as the ideal model for future developing therapies, especially if doctors want to treat younger patients with non-lethal epigenetic diseases. First generation drugs fail to satisfactorily address many issues. First, these compounds inhibit epigenetic methylation processes generally and may cause cancers which themselves develop in part by by low methylation levels and require additional therapies to counteract the effect (Feinberg 2004).

Second, the current drugs work by blindly affecting all genes in the genome of all the cells they encounter. One of the scientists behind the Dacogen studies noted there is a "potential for harm," but so far the adverse events, including red blood cell suppression, diarrhea, anorexia, and others, have been deemed acceptable by the FDA (Issa 2007). It is possible that the potential for greater harm will be realized if epigenetic therapies are used on patients with a much longer expected life span than the current patient population.

## Ethical Questions

**Long Term Risk.** Research has not yet fully elucidated the effects of DNA methylation (Rothstein 2009). Therapies may have off-target effects that are difficult to observe especially if they appear years after the initial therapy. To date, approved trials have been conducted with very sick, elderly cancer patients, and research has confirmed epigenetic modifications can affect us on a longer, even transgenerational, time-scale (Rothstein 2009). Clinical trials however do not normally track side-effects ten, twenty, or thirty years after treatment. If these treatments alleviate disease in the short term but eventually cause unforeseeable epigenetic abnormalities, are they acceptable for younger patients with non-terminal disease? Most would argue this depends on the gravity of the adverse effects—a risk/benefit analysis similar to that performed for any drug approval. Therefore, clinical trials must be designed to take these risks into account. Trials and follow-ups should last for a sufficient amount of time to asses these risks – on the decade time scale. If the drug does show an immediate benefit in the trial subjects, the fairness of keeping it off the shelf until the conclusion of the trial must be assessed. Certainly, trials of such durations with these looming questions would be unfavorable for the pharmaceutical investment community. Comprehensive diagnostic tests could help balance the need to push new drugs and ensure safe use. Methylation-sensitive genome sequencing in multiple cell types for each patient would reveal potential off-target effects. This kind of personalized medicine could obviate the need of a longitudinal clinical trial. This is difficult and expensive with existing technologies, and these procedural problems must be overcome so that adequate assessment of epigenetic therapies can be performed (Laird 2010). As more research is conducted, the balance between clinical trials and personal diagnostic tests and the responsibilities of those involved must be considered.

**Trans-generational Risk.** The issues discussed thus far are not unique to first generation epigenetic therapies. They act blindly not only on a genomic level, but also in terms of affected cell type. We cannot exclude the possibility that these treatments will affect the epigenomes of germline cells. In fact, recent studies demonstrate that Dacogen could interfere with embryo implantation and harm the fetus if given to a pregnant woman or to a man or woman planning on having children (Ding 2012). There is evidence that epigenetic modifications can be inherited by children and even grandchildren, so doctors need to keep the health of future generations in mind (Grossniklaus 2013). The effect of epigenetic therapies on germline cells should be measured not only while the drug is being administered, but also in follow-ups after the treatment is finished. It is critical that more basic research is performed to determine the extent of transgenerational epigenetic effects. If we acknowledge that some patients will have children regardless of any warnings, we must consider if it is acceptable to have synthetically altered epigenomes in the population.

## Second Generation Epigenetic Therapies

**Higher Standards.** Continued development of first generation epigenetic therapies that affect whole genomes and any cell type should be accompanied with open acknowledgement of potential undetectable harm. To date, epigenetic therapy has been held to the same safety standards of other cancer therapies. However, as these drugs affect gene expression, the basis of our existence, it is more appropriate to hold them to the standards of gene therapies. That entails an expectation to target only the genes, pathways, and cells relevant to the disease (U.S. Food and Drug Administration). Germline transmission of epigenetic modifications should be unacceptable. Animal model studies are always carried out before therapies are tested with humans—these must continue to be carried out rigorously before epigenetic therapies are more widely applied. In particular, researchers must consider non-mouse models as most mice can only live for around two years, which may not properly model long-term epigenetic effects. Patients' epigenomes should be screened, so doctors can be sure the proposed therapy is relevant to the individual, just as they would sequence a patient's genome to be sure of a genetic disease. Genome wide epigenetic sequencing has recently become feasible, although it is still difficult and somewhat error prone (Laird 2010). There is a need for further development of this technology to enable the promise of personalized epigenetic treatment.

**Research at Penn and Beyond.** These more stringent safety standards demand the development of second-generation epigenetic therapeutics that is properly targeted at the genomic and cellular level. In the past few months, we have seen promising initial published work on new tools for manipulating the genome in a careful, targeted manner. Histone methylases, histone demethylases, and DNA demethylases have all been engineered to act on specific DNA sequences (Konermann 2013, Mendenhall 2013, Maedner 2013). Our research team at the University of Pennsylvania, Penn iGEM, took initial steps to complete this suite of tools by designing a novel enzyme that selectively restores methylation at specific DNA sites. More importantly, we have developed an alternative DNA methylation assay, called MaGellin, which is significantly simpler, faster, and cheaper than methylation-sensitive sequencing for applications like ours to accelerate the optimization of these tools. These efforts would not have been possible without the expert advice of the University of Pennsylvania's epigenetics research labs. Dr. Marisa Bartolomei's lab is actively studying how methylation patterns are transmitted across generations. Additionally, Dr. Rebecca Simmons' lab in the Perelman School of Medicine has discovered a way to reverse epigenetic modifications including DNA methylation and prevent the onset of obesity in a rat model.

**Conclusion.** Epigenetic phenomena have a significant impact on the way we live and grow and can be responsible for the way we die. The need for epigenetic therapies is clear and the initial successes are promising for cancer patients, but the model for future developments is not yet set in stone. If doctors want to treat younger patients with non-lethal epigenetic diseases, the consideration of risks must include the long term, and the decisions in the clinic must be based on data from researchers asking fundamental questions. The proposed second generation epigenetic therapies could overcome the hurdles of restoring methylation, as opposed to only inhibiting methylation, and targeting specific genes as opposed to the entire genome. However, the issues of their delivery and cellular targeting still loom. While it will require significant effort from basic researchers to determine the relevant mechanisms researchers to optimize the clinical strategies, the path forward is promising.

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