Epigenetics in Pharmaceutical Development & Discovery

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Robert Georgantas is an employee and shareholder of AbbVie.
### Biomarkers Span a Broad Spectrum of Roles in Drug Development and Personalized Health Care

<table>
<thead>
<tr>
<th>Biomarker Type</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Diagnostic</td>
<td>Indicates presence or absence of a specific physiological or pathophysiological state or disease</td>
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<tr>
<td>Prognostic</td>
<td>Baseline characteristics that categorizes patients by degree of risk for disease occurrence or progression of a specific aspect of a disease</td>
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<tr>
<td>Predictive</td>
<td>Baseline characteristics that categorizes patients by their likelihood of response to a particular treatment relative to no treatment. May predict favorable or unfavorable response (i.e. AEs)</td>
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<tr>
<td>Pharmacodynamic or activity</td>
<td>Change in biomarker shows that a biological response has occurred in a patient who has received a therapeutic intervention and for which the magnitude of the change is considered pertinent to the response</td>
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<tr>
<td>Surrogate</td>
<td>Predict expected clinical benefit</td>
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* Categories are not mutually exclusive

Biomarkers Affect All Stages of the Pharmaceutical Pipeline

PHC/CDx study initiation point decision is influenced by:
1. Discovery Research Goals
2. Development Research Goals
3. Business Development input
4. Previous Clinical Data & Experience
Clinical Assay Availability and Invasiveness Yields a Rank Order of Biomarker Clinical Utility

Protein ELISA
↓
Blood Based Genetics & Genomics
↓
IHC, MRI, X-Ray, ETC
↓
Invasive Observations
↓
Simple Biopsies
↓
Complex Biopsies

Normal
Diseased

Synovial thickening, Effusion
Bone marrow lesion

Molecular signatures

Normal
Diseased

Skin sample is removed
Skin is removed

Lesion

Skin
Fat

Joint space
Trochar
DNA Methylation is a prime target for peripheral blood biomarkers

- DNA Methylation is highly stable
- Assay methods are robust and standardized
- High complexity of assayable elements
  - Current chip technologies assay 800,000 methylation sites
  - Roughly 28 million CpG in human genome
  - More probable to find cell and pathway specific methylation patterns
- Easily assayable from whole blood or purified cell populations
- Sample collection is clinical standard
  - Just a whole blood tube at the most basic
- Sample prep is automated
- Methylation changes have been robustly correlated with disease states
Many caveats may affect the study of methylation patterns

- Methylation Changes with age
- Overall cell composition greatly affects observed methylation patterns
- Large methylation changes in a small population of cells may be masked by other cells
- Peripheral blood samples may or may not reflect pathology at the site of disease action
Many caveats may affect the study of methylation patterns

- **Methylation Changes with age**
- Overall cell composition or the effect of disease on methylation patterns
- Large methylation changes in a small number of cells may be masked by other cells
- Peripheral blood samples may or may not reflect pathology at the site of disease
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*Jaffe and Irizarry Genome Biology 2014, 15:R31*  
http://genomebiology.com/2014/15/2/R31

**Accounting for cellular heterogeneity is critical in epigenome-wide association studies**

Andrew E Jaffe¹* and Rafael A Irizarry²*
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Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDL2 and other loci


Affiliations | Contributions | Corresponding authors

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A Handful of Clinical Trials are Examining Methylation for Biomarkers and Target ID

**Methylation as a Diagnostic Biomarker**
- Early Diagnosis of Oral Cancer by Detecting p16 Methylation
- Validation of DNA Methylation Biomarkers for Oral Cancer Detection
- DNA Methylation Biomarkers for Cervical Cancer Screening
- Peripheral Blood DNA Methylation Markers for the Early Detection of Colorectal Carcinoma

**Methylation as a Pharmacodynamic Biomarker**
- Methylation Bio-signature in Childhood Chronic Kidney Disease
- DNA Methylation Biomarkers and Metastasis of Gastric Carcinoma

**Methylation for Drug Target Discovery**
- Identification and Characterization of the Methylation Abnormalities on Whole Genome Among Infertile Men
- Studying DNA in Patients With Stage I, Stage II, Stage III, or Stage IV Ovarian Epithelial Cancer
The Abbvie Experience with Drug Responder Methylation Biomarkers

Methylation predicts patient response peg-IFN for HCV treatment
The genetic polymorphism rs12979860 within the IFNλ3 gene has been shown to have a large effect on response to treatment with pegylated interferon/ribaviron (pegIFN/RBV) in HCV-infected subjects. The functional role of the rs12979860 single nucleotide polymorphism (SNP) has not been fully elucidated. Epigenetic analysis of the IFNλ3 gene may provide functional information for this SNP, as well as identify additional factors involved in treatment response to pegIFN/RBV, and ultimately may be relevant for newer therapies directly targeting the HCV virus.
Analysis of a CpG Island in the IFNλ3 Promoter

• The IFNλ3 CpG island investigated is in the 5’ promoter region, approximately 1000 base pairs proximal to the rs12979860 polymorphism

• Working Hypotheses
  • RS12979860 SNPs may affect promoter methylation
  • Differential promoter methylation may correlate patient with response to pegIFN/RBV
Analysis of IFNλ3 CpG Island

• DNA samples from whole blood (N=629) were assayed for methylation levels in the IFNλ3 promoter by pyrosequencing
  • 127 healthy subjects
  • 465 subjects infected with HCV genotype 1
    • 359 subjects were treatment naïve
    • 106 subjects were prior treatment failures
  • 16 subjects infected with HCV genotype 2
  • 21 subjects infected with HCV genotype 3

• DNA samples from HCV-infected subjects came from clinical trials AVIATOR, Navigator, M11-602, M12-114

• rs12979860 allele status also determined

• Methylation levels varied considerably from subject to subject, ranging from 14% to 80%
Methylation Levels are Associated with rs12979860 Allele Status - HCV Genotype 1

- C/C: N=107
- C/T: N=255
- T/T: N=103

Response to pegIFN

p<0.001
Reduction in HCV Levels with pegIFN/RBV Treatment is Associated with IFNλ3 Genotype Status

![Graph showing log change in HCV RNA levels over time. Two lines represent different genotypes (C/C and non C/C) and show the decline in HCV RNA levels from baseline to day 14.]
Reduction in HCV Levels with pegIFN/RBV Treatment is Associated with IFNλ3 Genotype and Methylation Status

Baseline
Day 1
Day 7 or 9
Day 14

Log Change HCV RNA

Methylation above 39.5% (5 C/T, 2 T/T)
Methylation below 39.5% (3 C/T)

[-0.5]

[-0.7]

[-1.2]
Higher % Failures in Subjects with High Methylation Levels after 8-Week Treatment in AVIATOR

Methylation at IFNλ3 may distinguish difficult to treat subjects

Failure to achieve SVR due to premature discontinuation or lost to follow-up was not included in the analysis.
Hypothesis – are other DNA methylation regions potential biomarkers for defining response to therapy in treatment naïve vs. experienced subjects

450k methylation chips were run on treatment-naïve and treatment-experienced subjects.

DNA samples from HCV-infected subjects came from clinical trials AVIATOR, NAVIGATOR, M11-602, M12-114.

DNA Methylation Changes in HCV Treatment Naïve vs. Treatment Experienced Subjects

Whole Methylome analysis (450k chips)

Validated in an Independent Cohort of Subjects (PSQ)
The Abbvie Experience with Drug Responder Methylation Biomarkers

DNA Methylation as a Pharmacodynamic (PD) Biomarker of Drug Action
Strong significantly differential methylation was detected in Healthy Subjects treated with drug

- This Drug should affect T Cell activity
- Experiment compared whole blood DNA samples from baseline and D55 post-treatment
- A number methylation sites were found to be significantly differentially methylated
  - Top 10 hits range from P-values of $10^{-11}$ to $10^{-26}$

- Analyzed:
  - Correlation of individual SNPs to D1 or D55 samples
  - Bioinformatic analysis of nearest genes to the methylation sites
  - Pathways analysis of the nearest neighbor genes

- Ongoing analysis
  - Clustering of differentially methylated sites into promotor islands
  - Comparison to ENCODE methylation maps for known functional sites
The top Methylation site show strong differentiation of baseline and Day 55 time point

Top 10 Methylation sites showed nearly bimodal methylation

cg06834912 (FOXF1) Scatter Plot

cg22588144 (ISM1) Scatter Column

cg06292898 (TCP11L1) Scatter
Top nearest neighbor hits are among known T cell function genes – TRIM27 as an example

- TRIM27 was the top hit gene
  - 3 most significant single methylation site
  - 9 different local methylation sites were affected
The nearest neighbor genes to the methylation sites were analyzed with Ingenuity Pathway Analyst to predict those pathways affected by drug.

The results were then filtered on pathways with known immunologic function:

- CXCR4 Signaling
- PTEN Signaling
- NF-kB Signaling
- STAT3 pathway
- NFAT signaling
- fMLP signaling in Neutrophils
- IL-8 signaling
- Leukocyte Extravasation Signalling
- PIK3 signalling in B cells
- CCR3 signaling in eosinophils
- IL-1 signaling
Four immune cellular functions were predicted to be controlled by the affected pathways.

Nearest neighbors genes to the differentially methylated sites indicate four immune pathways affected by drug:

- T cell homeostasis ($p=1.86^{-7}$)
- T cell development ($p=1.89^{-7}$)
- Differentiation of T lymphocytes ($p=8.08^{-7}$)
- Migration of memory T lymphocytes ($p=5.05^{-5}$)
Using Methylation in Pharmacogenetics

Factors to control for & tools for methylation
Tools for Methylation Assays and Data Analysis

Assay Methods

- **Illumina Infinium HumanMethylation450 BeadChip**
- **Whole Genome Bisulfide sequencing**

```
ACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCG
sequencing
ACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCG
bisulfide conversion
ACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCG
reconstruct sequence
ACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCGACGACTACGCG
```

- **DNA Methylation PCR**

*Proc. Natl. Acad. Sci. USA*  
Vol. 93, pp. 9821-9826, September 1996  
Medical Sciences

**Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands**  
(DNA methylation/tumor suppressor genes/p16/p15)  

*James G. Herman*,†, *Jeremy R. Graff*,* Sanna Myöhänen*,* Barry D. Nelkin*, and *Stephen B. Baylin*†
Tools for Methylation Assays and Data Analysis

Data Analysis

- ENCODE project (Encyclopedia of DNA Elements)

- methylANALYSIS: an R Package for DNA Methylation Data

Modified from PLoSBiol 9:e1001046
We’d like to thank

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