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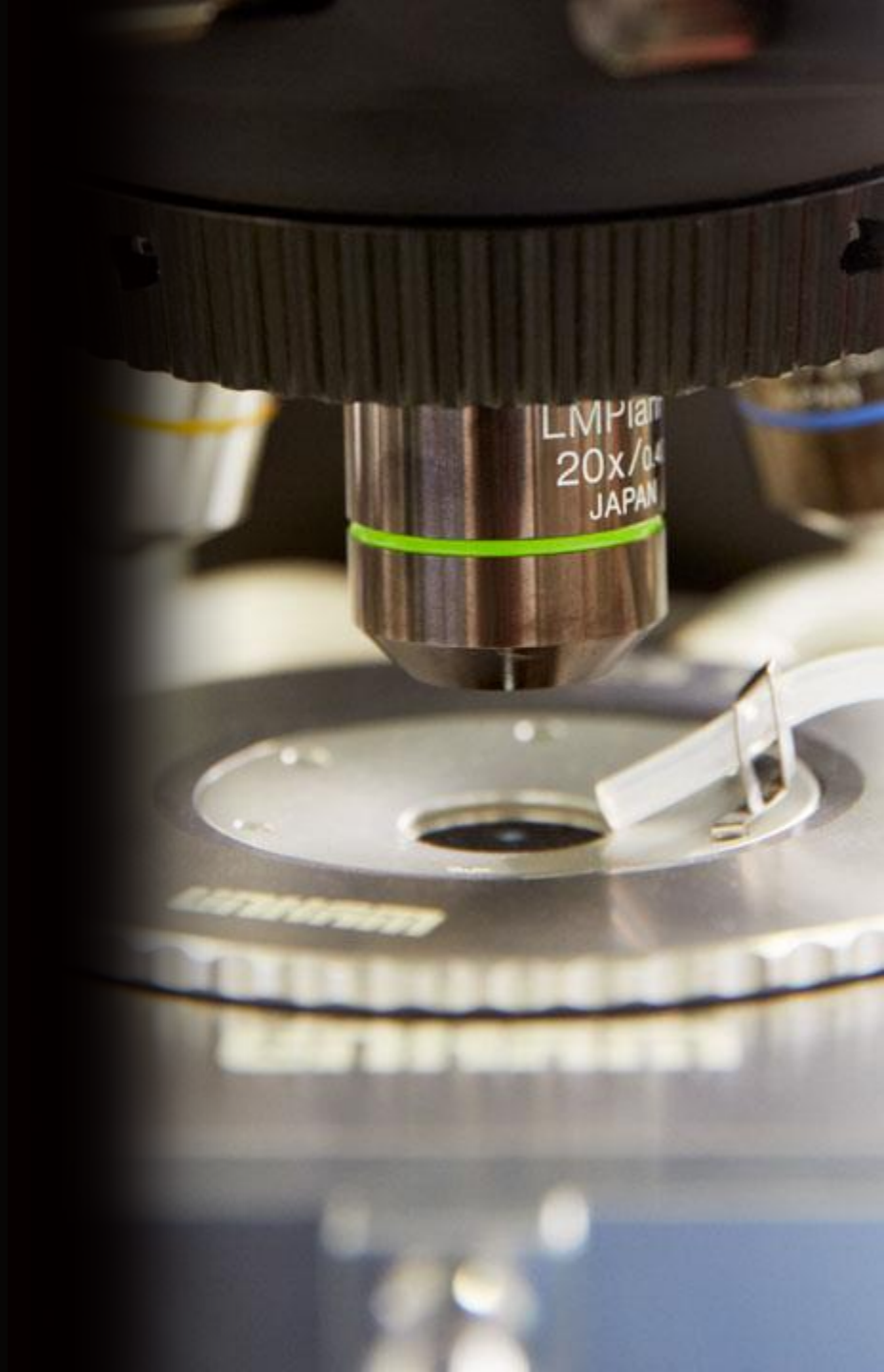
Epigenetics in Pharmaceutical Development & Discovery

Robert W. Georgantas III, PhD

Associate Director
Immunology Programs
Pharmacogenetics & Pharmacogenomics
Abbvie, inc.

Thurs, March 10th, 2016



Disclosures

These studies were funded by AbbVie.

AbbVie contributed to the study design, research, and interpretation of data, writing, reviewing, and approving the publication.

Robert Georgantas is an employee and shareholder of AbbVie.

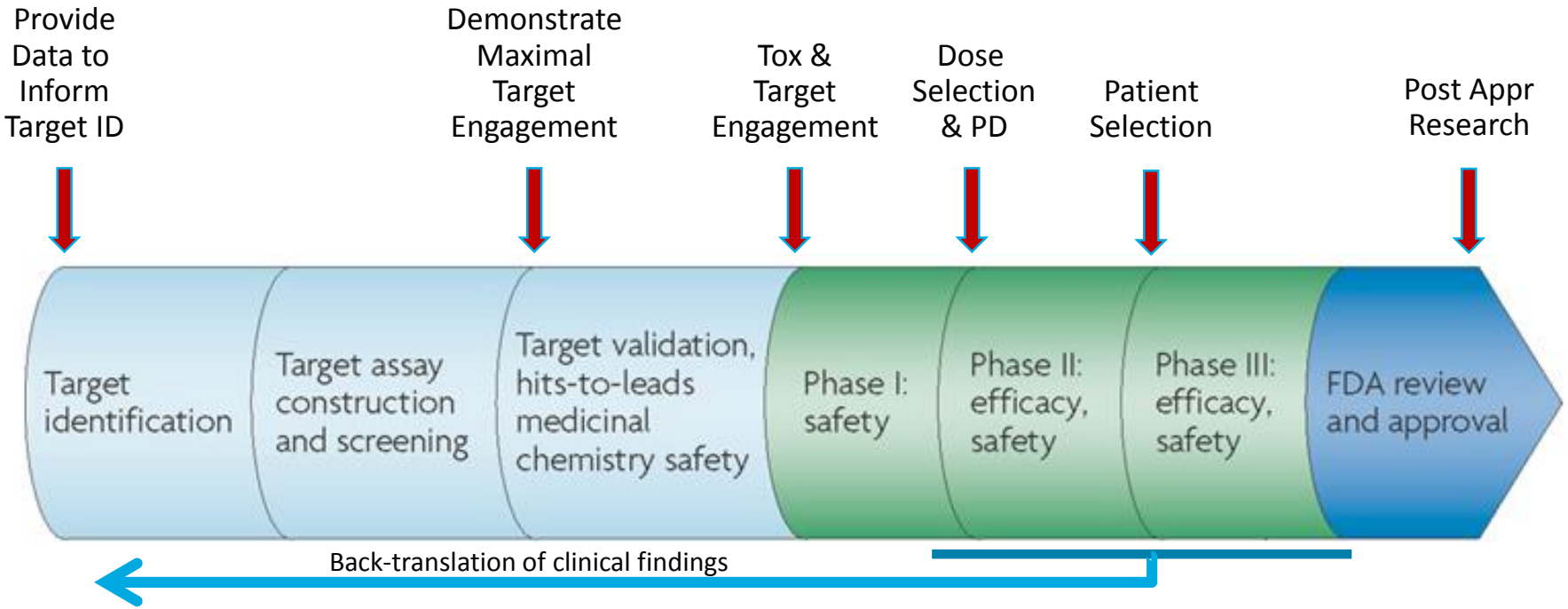
Biomarkers Span a Broad Spectrum of Roles in Drug Development and Personalized Health Care

Diagnostic	• Indicates presence or absence of a specific physiological or pathophysiological state or disease
Prognostic	• Baseline characteristics that categorizes patients by degree of risk for disease occurrence or progression of a specific aspect of a disease
Predictive	• 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)
Pharmacodynamic or activity	• 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
Surrogate	• Predict expected clinical benefit

* Categories are not mutually exclusive

Guidance for Industry and FDA Staff. Qualification Process for Drug Development Tools Jan 2014

Biomarkers Affect All Stages of the Pharmaceutical Pipeline

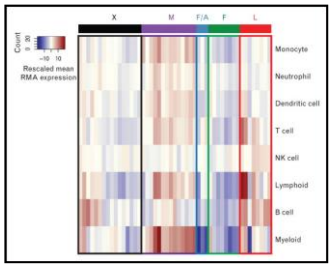


PHC/CDx study initiation point decision is influenced by:

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

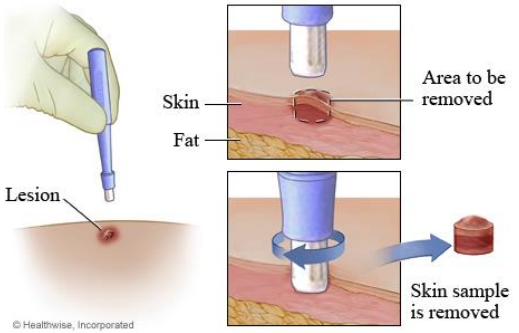


Molecular signatures

Blood Based Genetics & Genomics

IHC, MRI, X-Ray, ETC

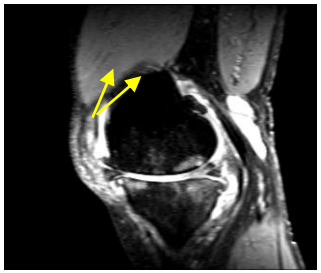
Invasive Observations



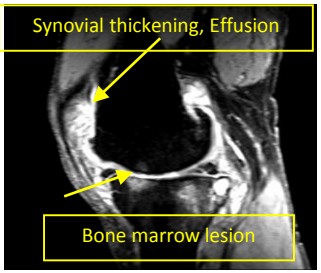
Simple Biopsies

Complex Biopsies

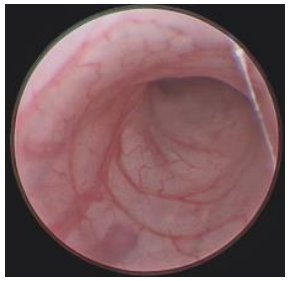
Normal



Diseased



Normal



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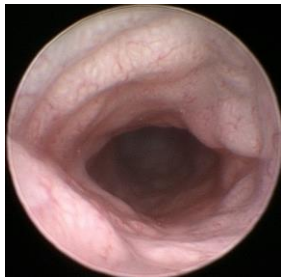
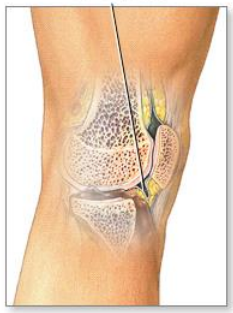


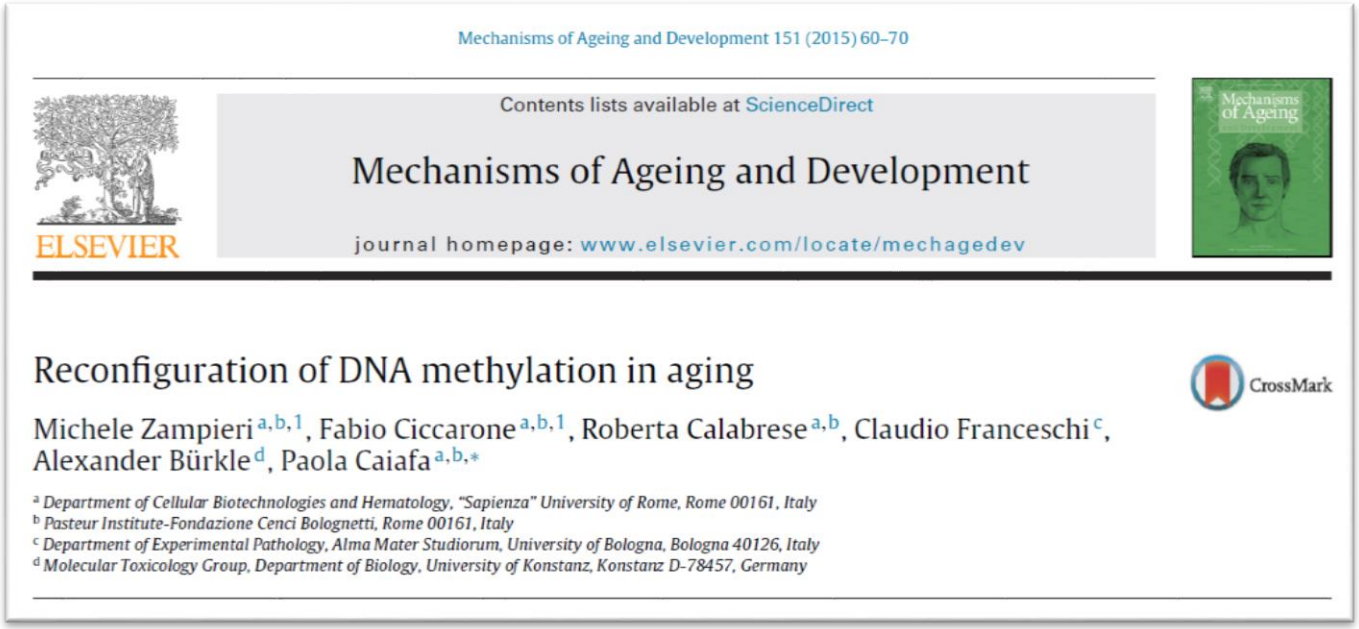


DNA Methylation is a prime target for peripheral blood biomarkers

- 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



Mechanisms of Ageing and Development 151 (2015) 60–70

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Reconfiguration of DNA methylation in aging

Michele Zampieri[a,b,1], Fabio Ciccarone[a,b,1], Roberta Calabrese[a,b], Claudio Franceschi[c], Alexander Bürkle[d], Paola Caiafa[a,b,*]

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[b] Pasteur Institute-Fondazione Cenci Bolognetti, Rome 00161, Italy
[c] Department of Experimental Pathology, Alma Mater Studiorum, University of Bologna, Bologna 40126, Italy
[d] Molecular Toxicology Group, Department of Biology, University of Konstanz, Konstanz D-78457, Germany

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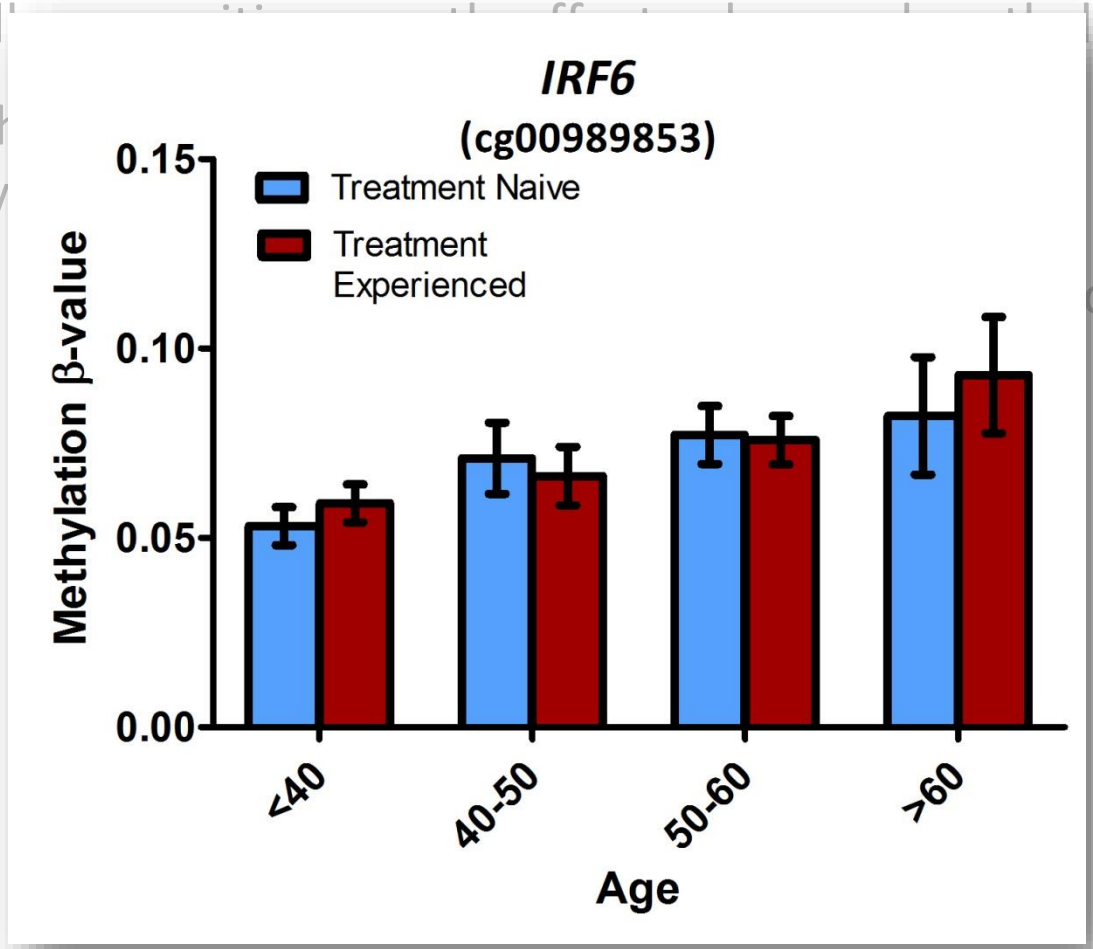

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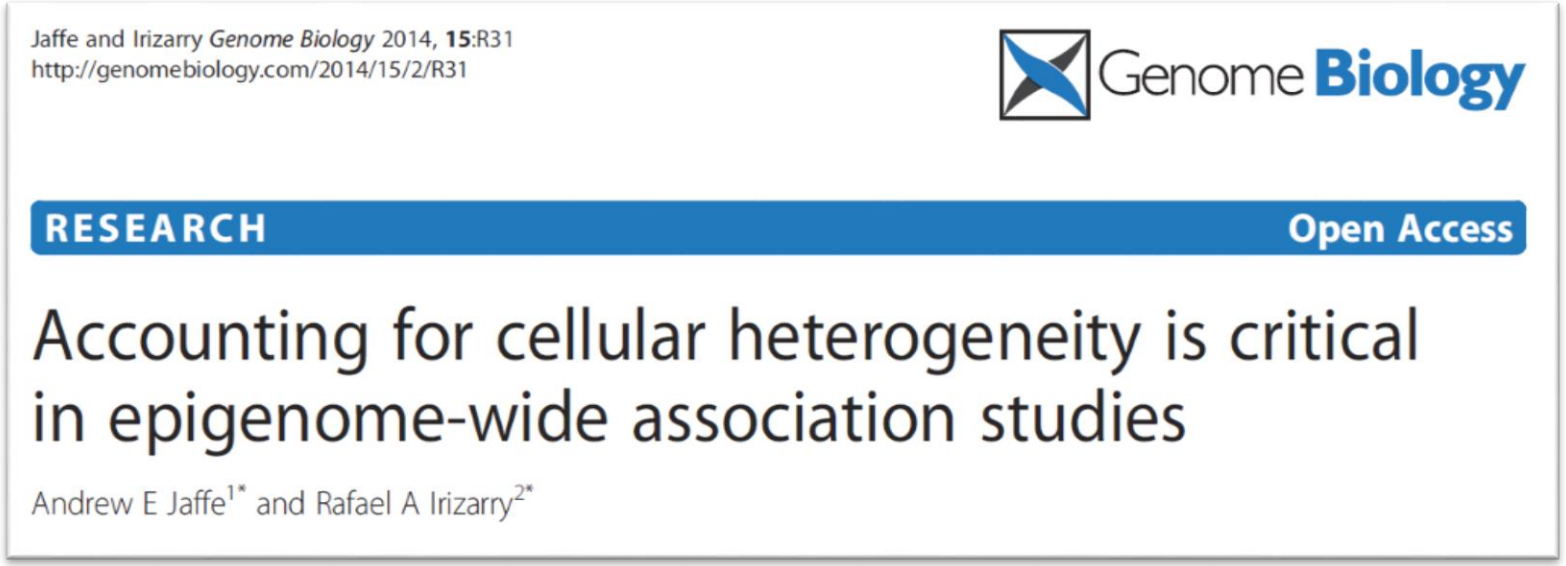

Jaffe and Irizarry *Genome Biology* 2014, **15**:R31
http://genomebiology.com/2014/15/2/R31

Genome Biology

RESEARCH Open Access

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

Andrew E Jaffe[1*] and Rafael A Irizarry[2*]

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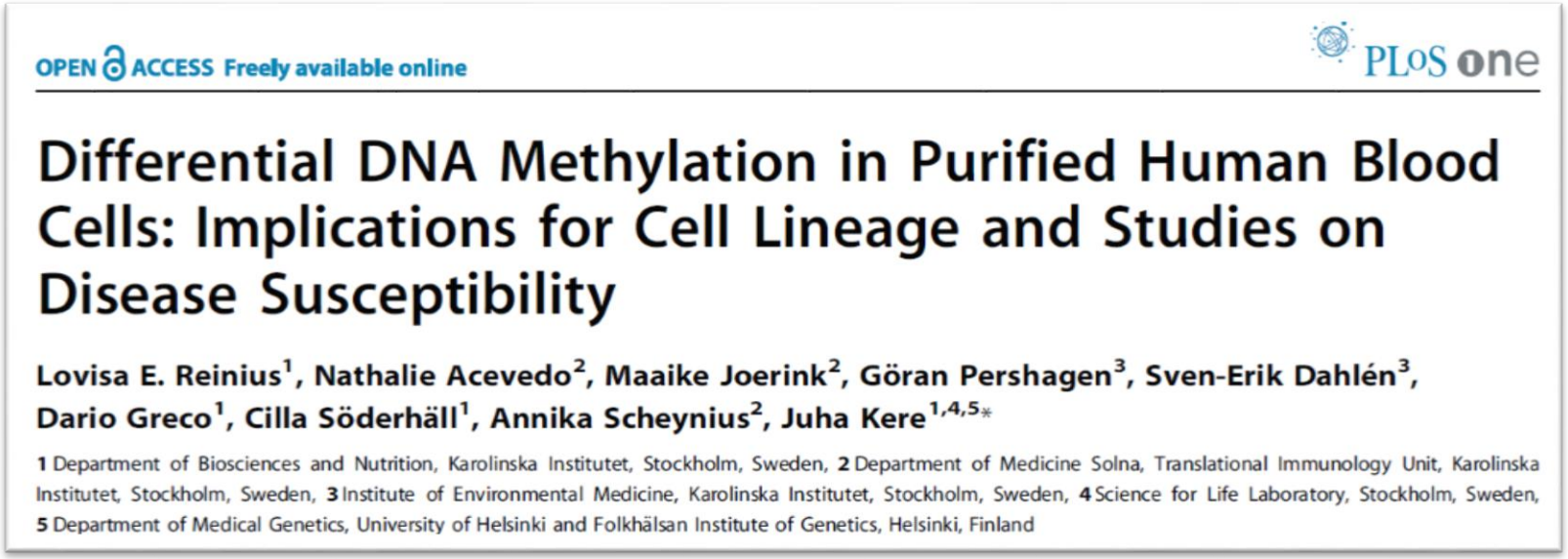

OPEN ACCESS Freely available online

PLOS one

Differential DNA Methylation in Purified Human Blood Cells: Implications for Cell Lineage and Studies on Disease Susceptibility

Lovisa E. Reinius[1], Nathalie Acevedo[2], Maaike Joerink[2], Göran Pershagen[3], Sven-Erik Dahlén[3], Dario Greco[1], Cilla Söderhäll[1], Annika Scheynius[2], Juha Kere[1,4,5]*

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Many caveats may affect the study of methylation patterns

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- Large methylation changes in a small population of cells may be masked by other cells
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NATURE NEUROSCIENCE | ARTICLE

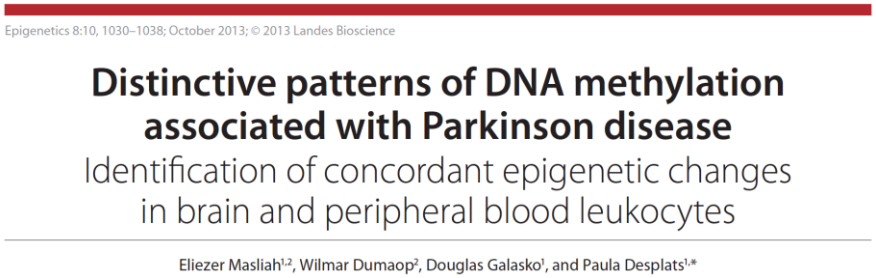
Alzheimer's disease: early alterations in brain DNA methylation at *ANK1*, *BIN1*, *RHBDF2* and other loci

Philip L De Jager, Gyan Srivastava, Katie Lunnon, Jeremy Burgess, Leonard C Schalkwyk, Lei Yu, Matthew L Eaton, Brendan T Keenan, Jason Ernst, Cristin McCabe, Anna Tang, Towfique Raj, Joseph Replogle, Wendy Brodeur, Stacey Gabriel, High S Chai, Curtis Younkin, Steven G Younkin, Fanggeng Zou, Moshe Szyf, Charles B Epstein, Julie A Schneider, Bradley E Bernstein, Alex Meissner, Nilufer Ertekin-Taner + *et al.*

Affiliations | Contributions | Corresponding authors

Nature Neuroscience **17**, 1156–1163 (2014) | doi:10.1038/nn.3786

Received 05 May 2014 | Accepted 16 July 2014 | Published online 17 August 2014



Epigenetics 8:10, 1030–1038; October 2013; © 2013 Landes Bioscience

Distinctive patterns of DNA methylation associated with Parkinson disease

Identification of concordant epigenetic changes in brain and peripheral blood leukocytes

Eliezer Masliah[1,2], Wilmar Dumaop[2], Douglas Galasko[1], and Paula Desplats[1,*]

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

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The Abbvie Experience with Drug Responder Methylation Biomarkers

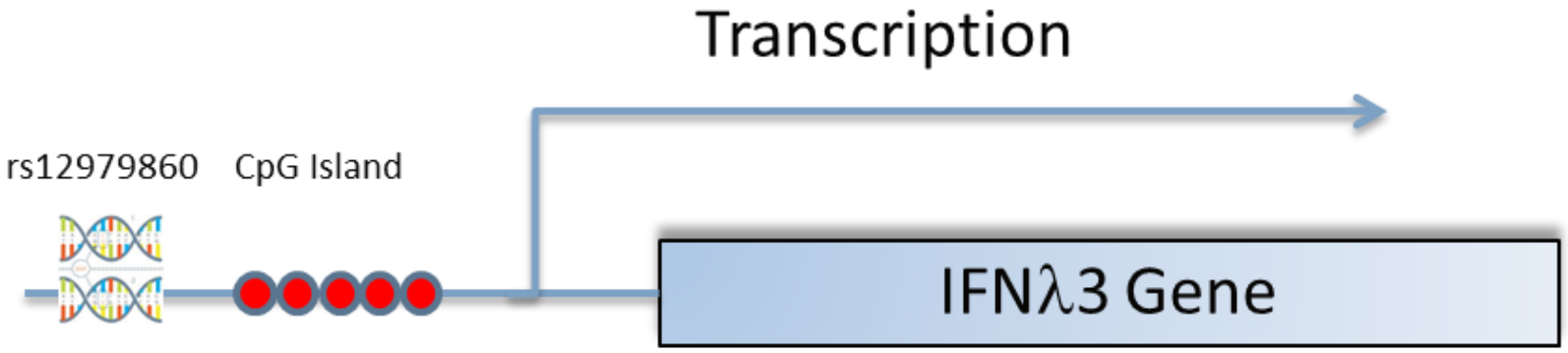
Methylation predicts patient response peg-IFN for HCV treatment

HCV
Peg-IFN

Rs12979860 Polymorphisms Affect pegIFN/RBV Response

- 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

HCV
Peg-IFN

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

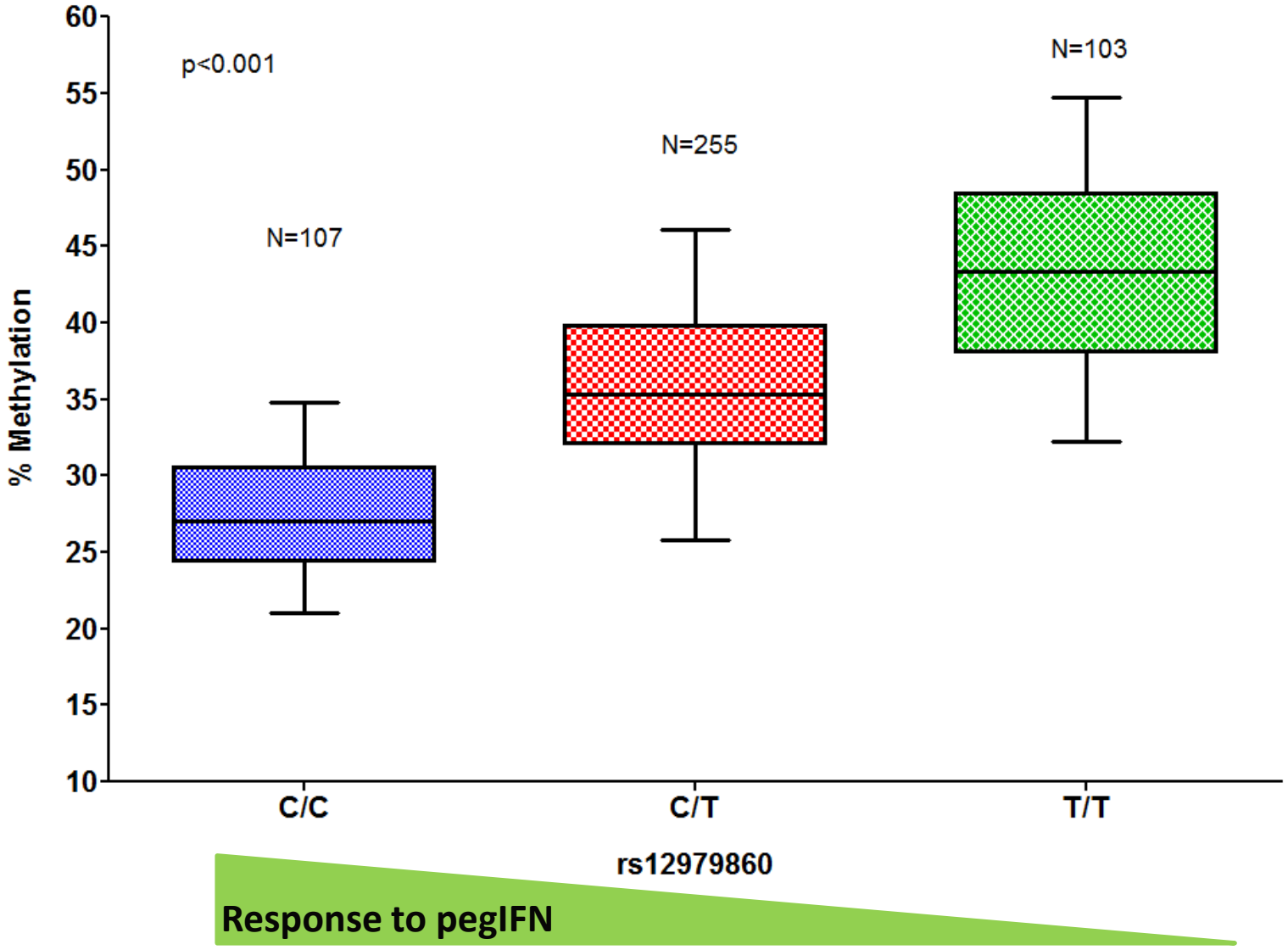
- DNA samples from HCV-infected subjects came from clinical trials AVIATOR, Navigator, M11-602, M12-114

- rs12979860 allele status also determined

- Methylation levels varied considerably from subject to subject, ranging from 14% to 80%

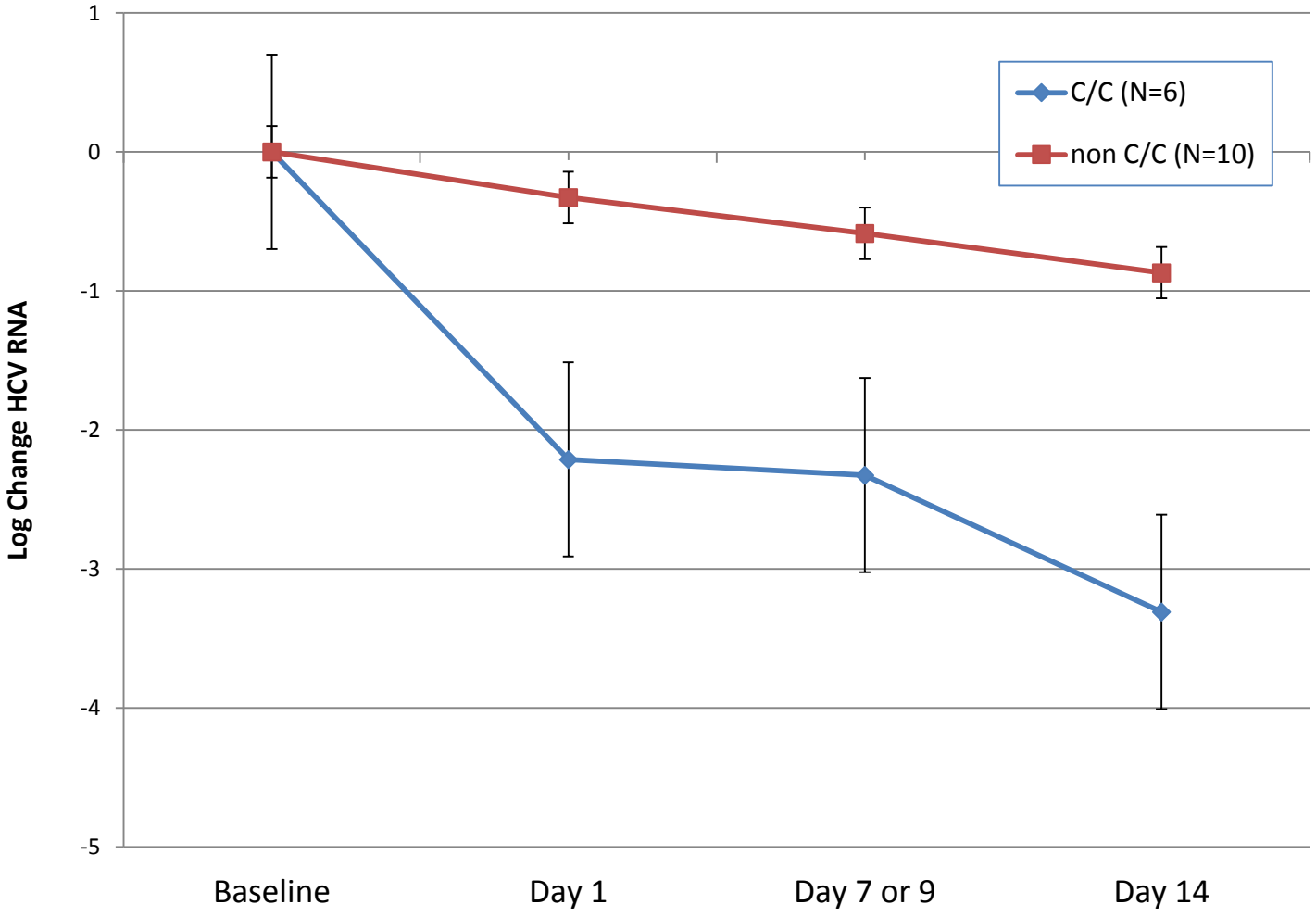
HCV
Peg-IFN

Methylation Levels are Associated with rs12979860 Allele Status - HCV Genotype 1



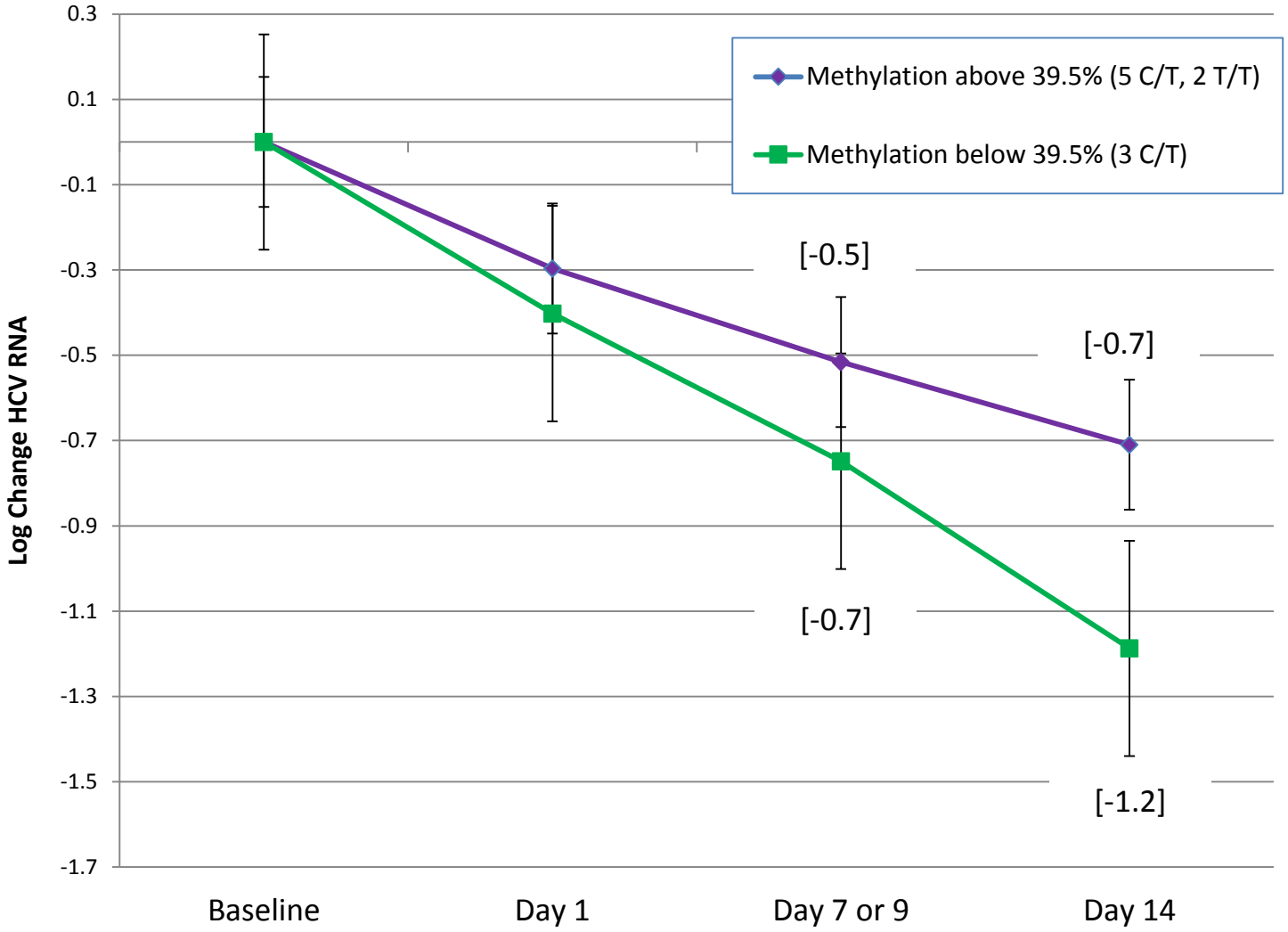
HCV
Peg-IFN

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



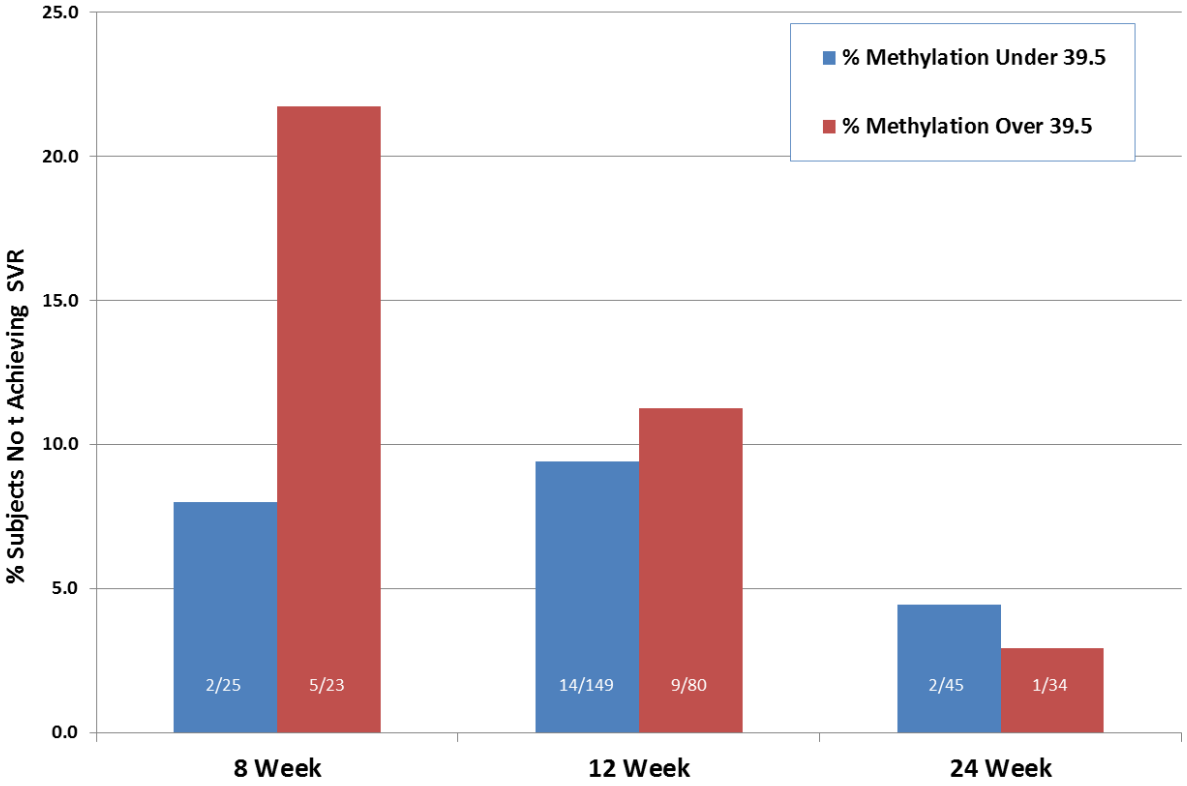
HCV
Peg-IFN

Reduction in HCV Levels with pegIFN/RBV Treatment is Associated with IFNλ3 Genotype and Methylation Status



HCV
Peg-IFN

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



Failure to achieve SVR due to premature discontinuation or lost to follow-up was not included in the analysis

Methylation at IFNλ3 may distinguish difficult to treat subjects

HCV
Peg-IFN

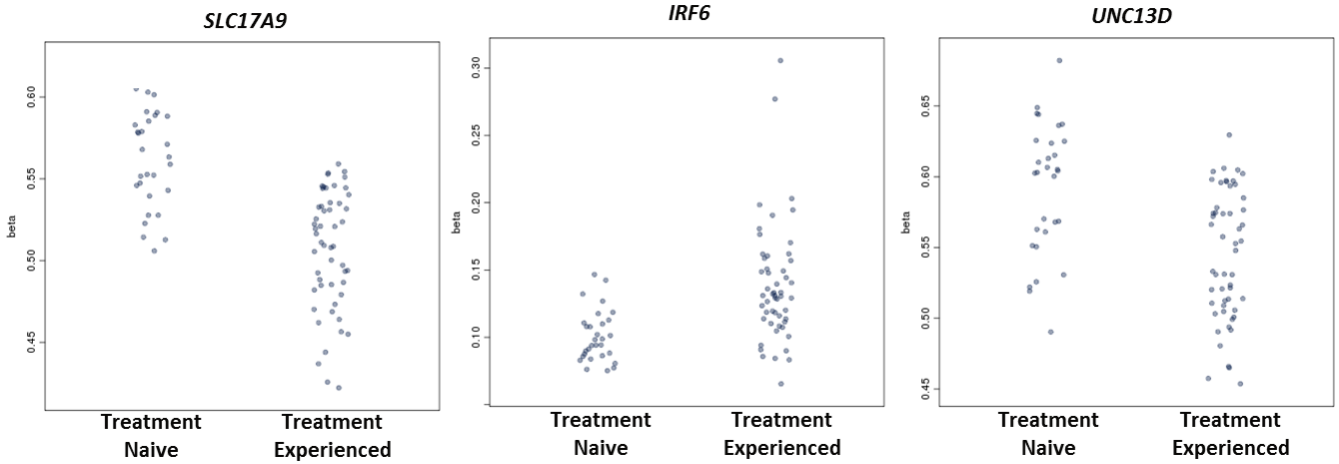
Identification of Additional Gene Methylations Correlating with Response to HCV Therapy

- 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.
  - *N Engl J Med 16:222; N Engl J Med 16:222; N Engl J Med 370:1604; N Engl J Med 370:1594; N Engl J Med 370:1973; N Engl J Med 370:1983*

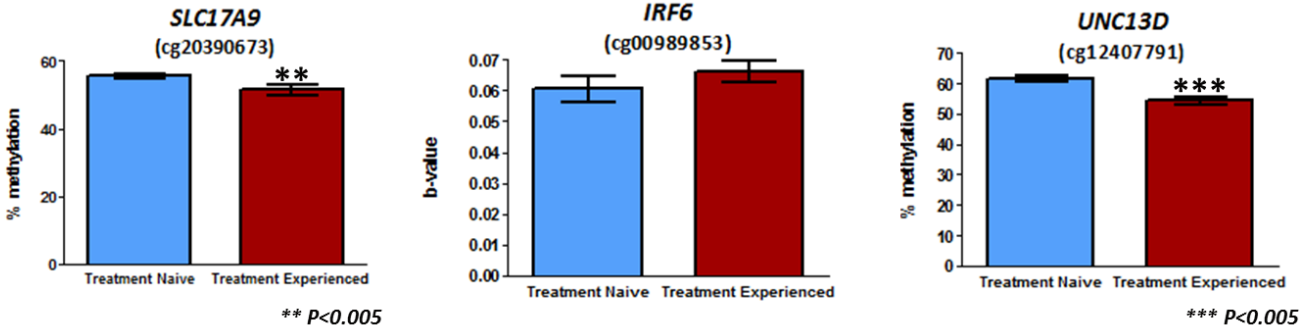
HCV
Peg-IFN

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

Whole Methylome analysis (450k chips)



Validated in an Independent Cohort of Subjects (PSQ)



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The Abbvie Experience with Drug Responder Methylation Biomarkers

DNA Methylation as a Pharmacodynamic (PD) Biomarker of Drug Action

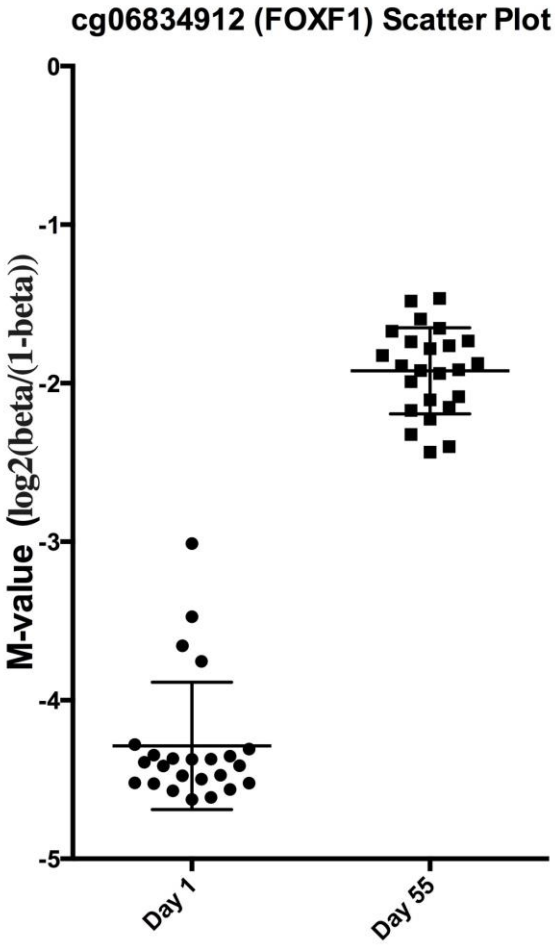
Methylation PD Marker

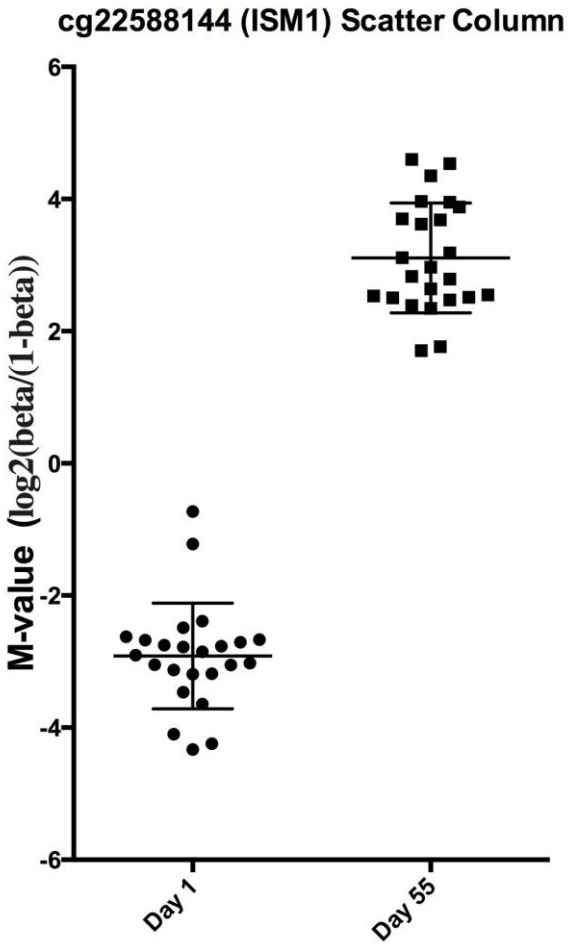
Strong significantly differential methylation was detected in Healthy Subjects treated with drug

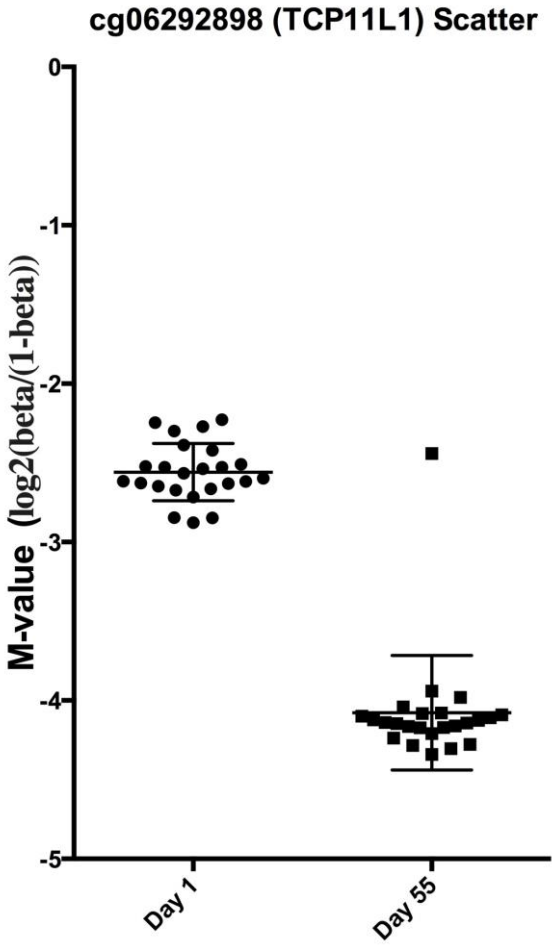
- This Drug should affect T Cell activity
- Experiment compared whole blood DNA samples from baseline and D55 post-treatment
- A number methylation sites were found to be significantly differentially methylated
  - Top 10 hits range from P-values of 10^{-11} to 10^{-26}
- Analyzed:
  - Correlation of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







Top nearest neighbor hits are among known T cell function genes – TRIM27 as an example

Tripartite motif containing protein 27 negatively regulates CD4 T cells by ubiquitinating and inhibiting the class II PI3K-C2β

Xinjiang Cai[a,b], Shekhar Srivastava[a,b], Yi Sun[a,b], Zhai Li[a,b], Haiyan Wu[c], Ljiljana Zuvela-Jelaska[d], Jun Li[d], Rachel S. Salamon[c], Jonathan M. Backer[c], and Edward Y. Skolnik[a,b,e,1]

[a]The Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute for Biomolecular Medicine, [e]Division of Nephrology, Department of Medicine, [b]Department of Pharmacology, New York University Langone Medical Center, New York, NY 10016; [c]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461; and [d]Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT 06877

- TRIM27 was the top hit gene
  - 3 most significant single methylation site
  - 9 different local methylation sites were affected

Many Canonical Pathways of T cell function are predicted by the Methylation Patterns to be 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 ($p=5.05^{-5}$)

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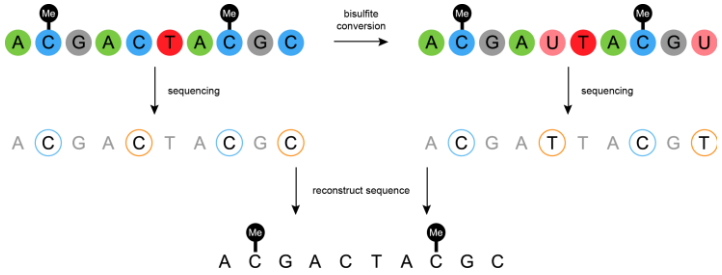
Using Methylation in Pharmacogenetics

Factors to control for & tools for methylation

Tools for Methylation Assays and Data Analysis

Assay Methods

- Illumina Infinium HumanMethylation450 BeadChip
- Whole Genome Bisulfide sequencing



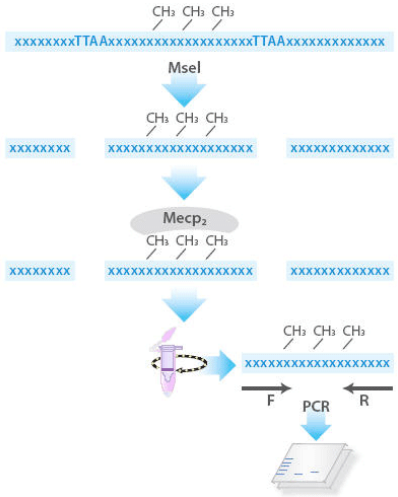
- DNA Methylation PCR

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*)

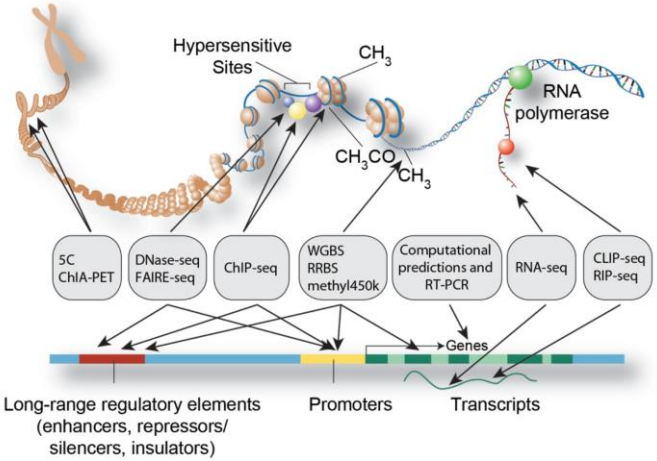
JAMES G. HERMAN*†, JEREMY R. GRAFF*, SANNA MYÖHÄNEN*, BARRY D. NELKIN*, AND STEPHEN B. BAYLIN*‡



Tools for Methylation Assays and Data Analysis

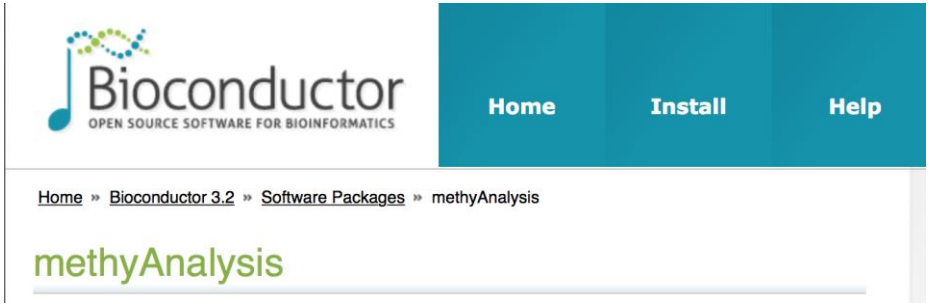
Data Analysis

- ENCODE project (Encyclopedia of DNA Elements)



Modified from PLoSBiol 9:e1001046

- methylANALYSIS: an R Package for DNA Methylation Data



We’d like to thank

- The many patients who participated in these clinical studies
- The Abbvie Clinical Pharmacology Research Unit
- Abbvie Clinical Development
- Abbvie Infectious Disease Development
- Abbvie Discovery Immunology
- The Abbvie Department of Pharmacogenetics & Pharmacogenomics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