Smoking-related Biomarkers of Effect/ Harm: Challenges and Opportunities

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G. L. Prasad
Research and Development
RJ Reynolds Tobacco Co.

Scope

Biomarkers of Potential Effect/ Harm

- Concepts
- Importance in harm reduction
- Development and complexities
- Current state and new paradigm
- Harmonization and collaboration

Background

- Context
- Objective

Harm Reduction

- Chronic smoking is a major cause of several diseases: COPD, cancer and CVD
  - Generally manifest after prolonged smoking
  - Difficult to predict morbidity – who and when
- Institute of Medicine (IOM) report "Clearing the Smoke" suggests epidemiology is best measure for evaluating harm
- Alternative harm reduction approaches
  - To the benefit of current smokers
  - Development of Potential Reduced Exposure Products (PREPs)
- Interim measures are important – Biomarkers

RAI's Guiding Principles and Beliefs

Selected from "Tobacco Use & Health" section:

1. **Quitting cigarette smoking significantly reduces the risk for serious diseases.**
2. **No tobacco product has been shown to be safe and without risks. The health risks associated with cigarettes are significantly greater than those associated with the use of smoke-free tobacco and nicotine products.**

Objective

- **To discuss the challenges and the opportunities in the development of smoking-related biomarkers of effect/ harm**

- **Biomarkers are critical elements of harm reduction strategies**
  - **predicting the health effects of smoking**
  - **PREP assessment**

- *Not* included in this perspective:
  - Biomarkers of exposure
  - Individual biomarkers of effect/ potential harm
  - Biomarkers (exposure and effect) of smokeless tobacco or nicotine replacement

Biomarkers

- Selected Definitions
- Fit-for-purpose

What are biomarkers?

- Widely utilized measures (of something) in health care and research
  - Prediction, diagnosis and prognosis
  - Drug discovery
  - Example, plasma/serum cholesterol
  - Used in decision making process
  - Regulatory implications
  - Should be robust
- Development and utilization of biomarkers is a rigorous process
- IOM committee on biomarkers and surrogate endpoints issued a report in 2010

Selected Definitions

Biomarker: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a(n) intervention." Example: cholesterol level.

Fit-for-purpose: being guided by the principle that an evaluation process is tailored to the degree of certainty required for the use proposed.

Selected Definitions

Analytical Validation: "assessing (an) assay and its measurement performance characteristics, determining the range of conditions under which the assay will give reproducible and accurate data."

Qualification: "evidentiary process of linking a biomarker with biological processes and clinical endpoints."

Guidance on smoking-related biomarkers

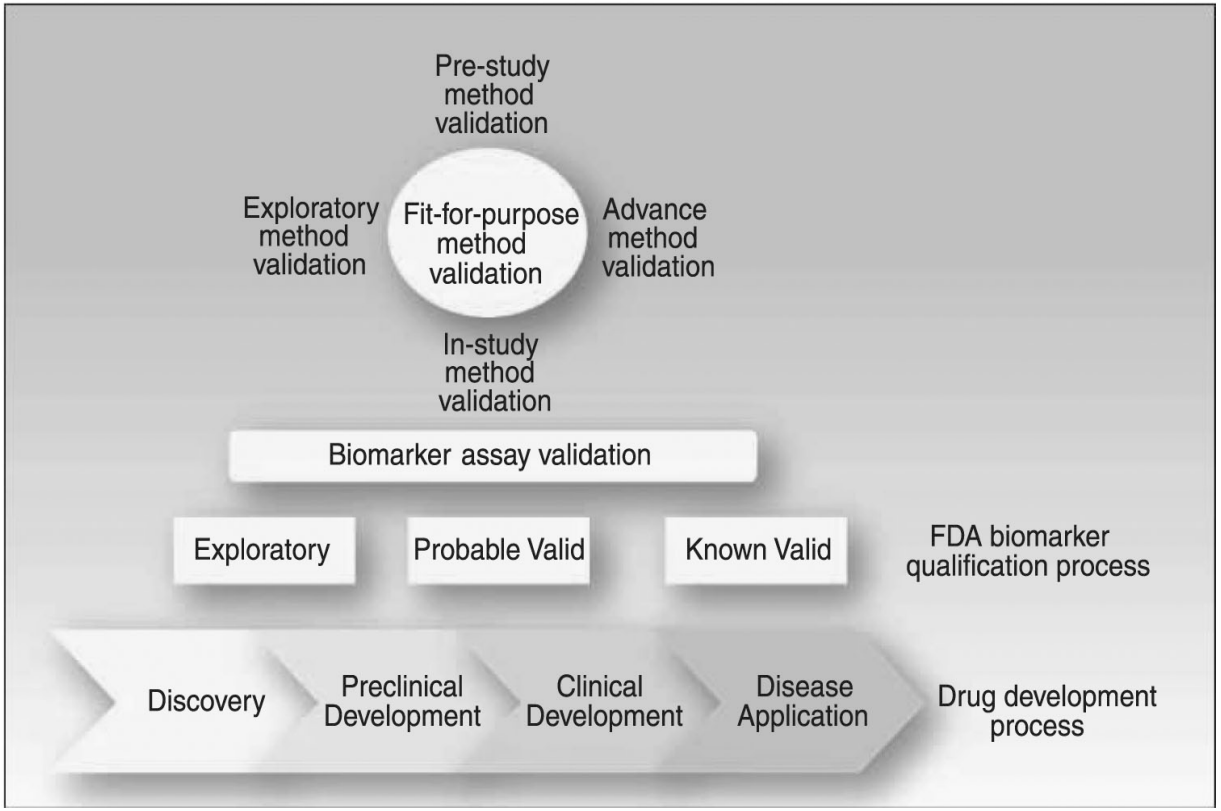
- Scientific basis for PREP assessment – five steps proposed by IOM ("Clearing the Smoke")
    1. Chemical analyses of cigarette smoke
    2. Biomarker development
    3. Preclinical research
    4. Clinical studies
    5. Long-term epidemiological studies and surveillance

No clear guidance exists on development of smoking-related biomarkers

A strategy for biomarker development

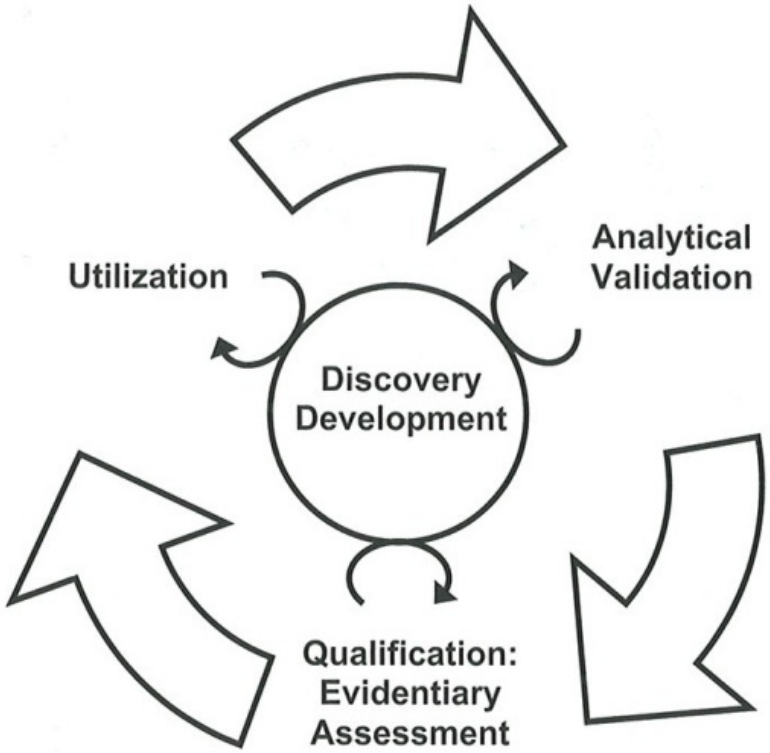
- Fit-for-purpose strategy – originally advocated to support drug development
  - Simple and continuously evolving
- Assay validation tailored to meet the intended purpose of the biomarker study
  - Conserves resources during the initial, exploratory stages
  - Increases rigor progressively as biomarker moves beyond initial discovery phase
- Context-based qualification

Fit-for-purpose strategy for biomarker development



Assay validation and biomarker qualification

Biomarker evaluation framework



Biomarker evaluation is an ongoing and context-based process

Smoking-related biomarkers of potential effect/ harm

- What, why and how

Potential applications of smoking-related biomarkers of effect

1. Predicting the onset of smoking-related illnesses in generally healthy smokers, and thus identifying at risk individuals
2. Distinguishing product categories, evaluation of PREPS and other modified risk products
3. Evaluating new products
4. Establishing a risk continuum across tobacco products
5. Understanding of pathophysiological effects of smoking
6. Likely to be important in tobacco regulation

Smoking-related biomarker definitions

Biomarker of exposure: a tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose.

Biomarkers of potential harm: a measurement of an effect due to exposure; these include early biological effects; alterations in morphology; structure; or function; and clinical symptoms consistent with harm; also includes "preclinical changes."

Biomarkers of effect and potential harm are synonymously used in this presentation

Smoking-related biomarker of harm

A working definition by Gregg *et al* (2006):

"A significant, objective, measurable, alteration in a biological sample, after smoking a tobacco product, that is known to be on a pathway predictive of pathologic change, or a surrogate for that pathway, which is altered in a proportion of smokers and is reversible on cessation of smoking."

What is desired of smoking-related biomarkers of effect?

Some features:

- Reflect the long-term effects of smoking and disease endpoints to be qualified as surrogate endpoints
- Distinguish smokers, former smokers, non-smokers, and PREP users
- Demonstrate adequate sensitivity, specificity and other analytical parameters
- Assayable in convenient assay matrix

Significant body of knowledge exists

- **Health effects of smoking are generally known**
  Examples: COPD, lung cancer and CVD
- **Mechanisms**
  Examples: Oxidative stress, inflammation, DNA damage
- **Many potential biomarkers exist and are under active investigation**

Yet, the need for new biomarkers and further research into existing biomarkers is widely recognized.

Challenges in the discovery of biomarkers of effect

Some potential challenges:

1. The complex chemical nature of cigarette smoke
2. A wide spectrum of smoking associated health effects and diseases, which compound elucidation of mechanisms
3. Local and systemic effects of smoking
4. Long latency observed in the manifestation of such effects
5. Limited availability/accessibility of tissues for research

Challenges in the discovery of biomarkers of effect

6. Life style
   Examples: diet, occupation, etc.
7. Gender and race of smokers
8. Inter-individual genetic variations
   Examples: genetic polymorphisms –
   may influence disease susceptibility of smokers
   a) xenobiotic metabolism
   b) toxin-induced repair of genetic lesions

Some strategies

For improving discovery, validation and qualification of biomarkers of tobacco effect/ harm include:

Experimental

- **Method development for assaying novel and putative biomarkers of effect in appropriate biological matrices**
- **Innovative design**
- **Standardization and harmonization of sample collection**
- **Generation of useful experimental models**

Some strategies

For improving discovery, validation and qualification of biomarkers of tobacco effect/ harm include:

Conceptual

- **Fit-for-purpose principles**
- **Better understanding of the smoke chemistry, and biological effects of smoking**
- **Integration of data across studies**

…collectively may expedite qualification of exploratory biomarkers and support the development of necessary tools for PREP assessment.

Important to note

Researchers are aware of the challenges.

Currently many of the strategies are being implemented towards biomarker discovery.

Active area of research.

Significant progress is being made…

Current approaches

- Cell culture studies
- Clinical studies

Cell culture studies

- Appropriate cell culture models
- Two dimensional vs. three dimensional “organoids”
- Cytotoxicity assays
  - Useful first set of studies – cumulative effect of high doses
  - May not provide mechanistic insights

Cell culture studies

- Long-term exposure at sub-cytotoxic doses desired
- Finer, defined molecular events may be more useful
  - Example: DNA damage, differentiation, motility, signaling, gene expression, inflammation and oxidative stress
  - Pathways to molecular events
- Quantify exposure – relate effects to dosage
  - Different products and fractions

Clinical studies

- Several cross sectional studies have been performed
- A number of biomarkers of potential effect have been evaluated in generally healthy study subjects
  - Biomarker examples: CRP, isoprostanes, IL8, 11-dehydro thromboxane B2 (TxB2)
- Generally, existing biomarkers of effect
  - *distinguish smokers vs. non-smokers*
  - partially distinguish effect of increased smoke exposure or reversibility (smoking cessation)

Clinical studies

CVD Cross-sectional Study – smokers, moist snuff consumers (MSC) and non-tobacco consumers (NTC); age stratified

- **Biomarkers of exposure: smokers, MSC and NTC could be distinguished**
- **Biomarkers of effect: elevated in smokers**
  - **8-epi-PGF2α and TxB2**
  - **Cytokines-IL12 (p70) and IL8**
  - **soluble intercellular adhesion molecule-1**
- **Physiological measures: less informative, although carotid intima-media thickness revealed an age effect**

Clinical studies

Total Exposure Study – effect of cigarette smoke exposure on US population

- 4 tar delivery categories
- 3585 adult smokers and 1077 non-smokers
- Gender, age and BMI
- Biomarkers of effect: CVD, oxidative stress, COPD
  - Many biomarkers of effect distinguished smokers and non-smokers
  - Top three: 8-epi-PGF2, TxB2 and white blood cell count
- For many others: BMI important factor

Clinical studies

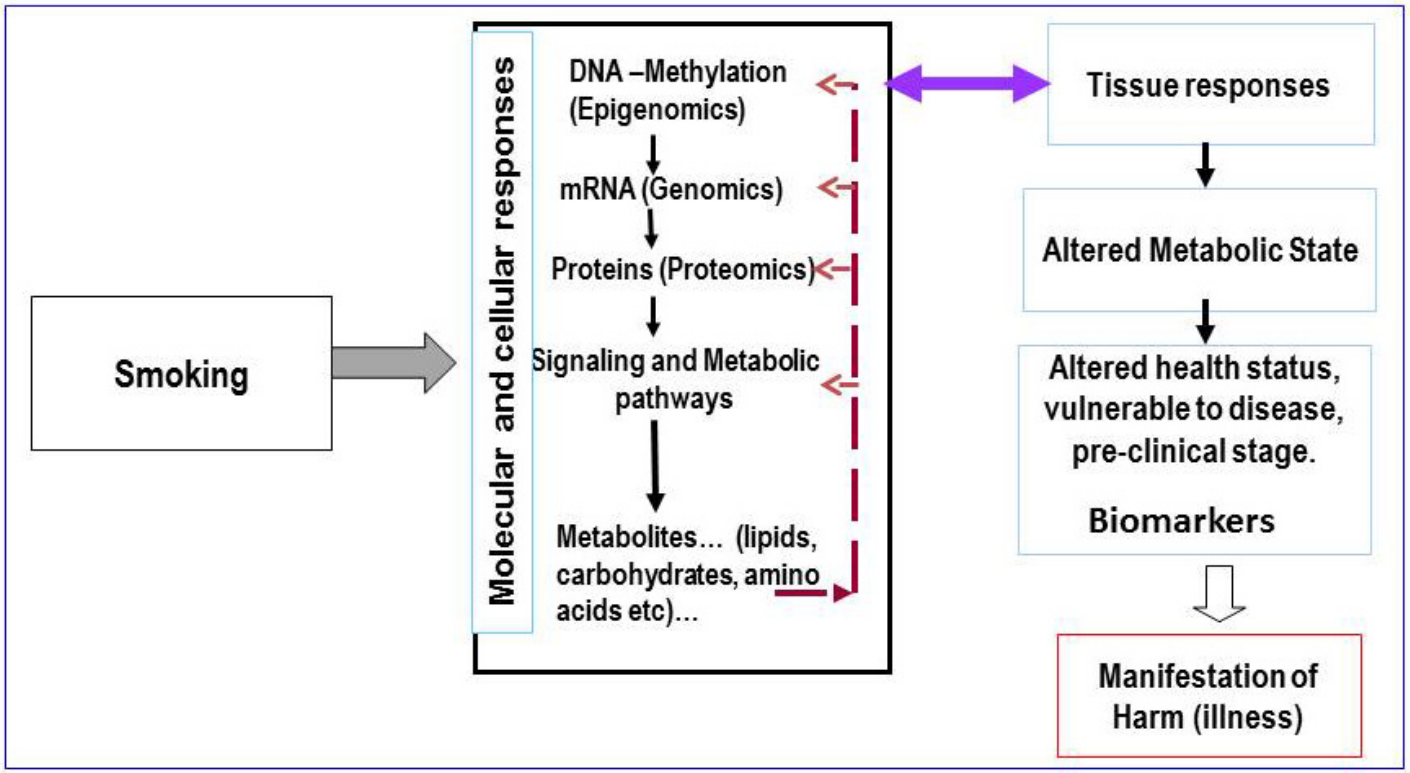
Some lessons

- Studies with healthy smokers valuable
- Yet no clear-cut disease link; age, duration, and cigarettes per day (CPD)
  - Oxidative stress and inflammation confirmed
- Limited number of biomarkers of effect exist
- New biomarkers
  - Lipid peroxidation products
  - In addition to 8-epi-PGF2α
- Other thoughts?

New technologies and opportunities

- The Omic approaches
- Omics and beyond
- Future opportunities

A different paradigm



New generation of biomarkers are likely to comprise a panel(s)/ signature(s).

How can we identify new biomarkers of effect?

- **Desired**

  Recruitment of subjects at preclinical disease state
- **Challenge**

  Not easy, lack of knowledge of this state
- **Solution**

  Enroll longer-term smokers, older and generally healthy. Disease state and treatment may *alter the biology and confound* the discovery of biomarkers of effect
- **Conduct**

  Carefully planned case-control studies;
  New global profiling technologies **AND** candidate pathways/markers

The Omics

- Mature
- Unbiased profiling
  - mRNA, proteins, post-translational modifications and metabolites
  - Epigenetics-methylation status of DNA
  - Post-transcriptional – micro RNA
- Hypothesis generating vs. hypothesis testing

Beyond Omics

- Need to confirm the large number of “hits”
  - Rerun profiling with a different sample set
  - Test the expression changes using targeted assays

- Biomarker levels → Biological significance
  ↑ ↓
  Disease mechanisms ← Pathophysiological changes

- Integrating data from different approaches to gain comprehensive understanding of the effects of smoking

Future opportunities

- Potential for discovery of many biomarkers of effect/ potential harm
- Method development and qualification can be demanding
- Collaboration is an attractive strategy for rapid progress towards harm reduction from smoking
- Industry-academic partnerships
  Example: Predictive Safety Testing Consortium with advisement of FDA and European Medical Agency

Future opportunities

- Additional biomarker category
- Patient Reported Outcomes (PROs) – questionnaires
- PRO consortium – FDA and Critical Path Institute
- Many PROs have been used
  - Product evaluations: liking/disliking, rewarding, sensory and physical effects
  - Quality of Life
- Need sensitive and validated PROs

Summary and conclusions

- Summary
- Conclusions
- Acknowledgements

Summary

- **The development and utilization of smoking-related biomarkers in harm reduction and PREP assessment can be markedly expedited by:**
  - **A better understanding of smoking-induced changes**
  - **The application of new technologies**
  - **Through the fit-for-purpose approach**

Conclusions

1. **Biomarkers** are key components of harm reduction strategies and PREP development.
2. **New biomarkers** to assess health effects related to tobacco use and evaluation of new products are necessary.
3. **Biomarker development** is an integrated, iterative and rigorous process.

Conclusions

4. New approaches (example, Omics) are powerful tools in the discovery of novel biomarkers of effect/ harm.
5. Emerging biomarkers are likely to consist of metabolites, nucleic acids (example, genomic, epigenomic and RNA) and proteins, and PROs.
6. Understanding the biology is critical.

….integrated and concerted efforts are key for the development and utilization of Smoking-Related Biomarkers of Effect/ Harm.

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THANK YOU