Biomarkers in Drug Development

Thinking therapeutically, *not diagnostically*

Or

Thinking pharmacodynamically (finally!)

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Disclosure

Relevant Financial Relationships

None

Off label usage

None

Fellow Peanut Butter Lovers:

We know you probably have questions about the safety of peanut butter. And we take those questions very, very seriously.

Which is precisely why we can assure you Peter Pan Peanut Butter is *safe to enjoy with your family.*

Biomarker use in Drug Development Trials

- Characterize disease
- Select drug & dose
- Monitor drug activity
- Monitor disease changes

- *Little or no “diagnosis” is done in these trials*

Traditional characterization of assays developed for diagnostic use is the science of differences.

Longitudinal monitoring of endogenous substances is the science of change.

If we don't support diagnosis...

- Development differs:
  - Decision levels are not the same
  - Interpretive framework is not "normal"
  - Movie concept, not snapshot
- Validation differs:
  - Biological variability
  - Accuracy challenges (form not constant)
  - Outcomes are distant

Current Clinical Lab Assays

- Relatively stable analytes
- End products of biochemistry
- NOT physiologically active in circulation
- “LEAKAGE” proteins
- Defined structure
- Few isoforms

In Contrast

- “New” biomarkers are…
  - Physiologically active
  - Upstream from clinical expression
  - Highly subject to pathway acceleration, blocking or shunting
  - Short half-life
  - Multiple isoforms
  - Activation products, subject to depletion

Its all pharmacodynamics

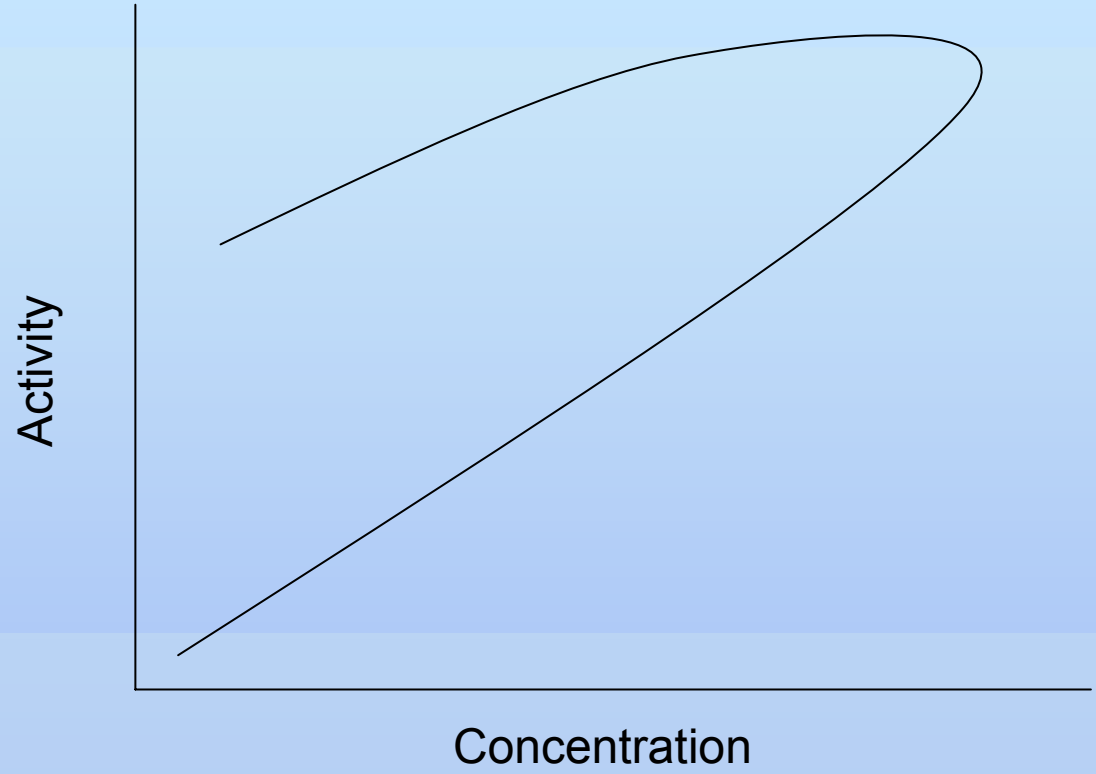
- The still embryonic science of linking drug levels to downstream changes will be the major effort of the next generation of laboratorians
- We will be applying PK concepts to endogenous analytes routinely

It really is a movie!

Terminology

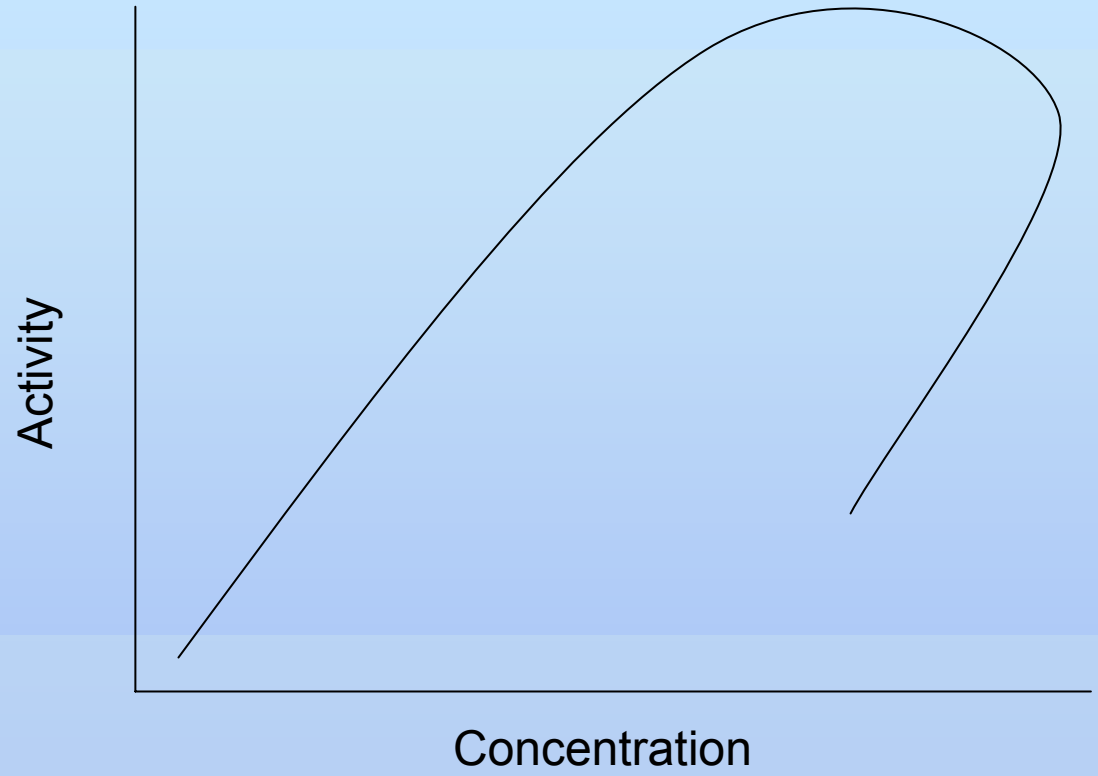
- Proof of Mechanism: dose-response relationship at the target
- Proof of Principle: evidence that the change in target response is related to disease parameters
- Proof of Concept: evidence that the change will improve clinically recognized symptoms, QOL, or endpoints.

Hysteresis

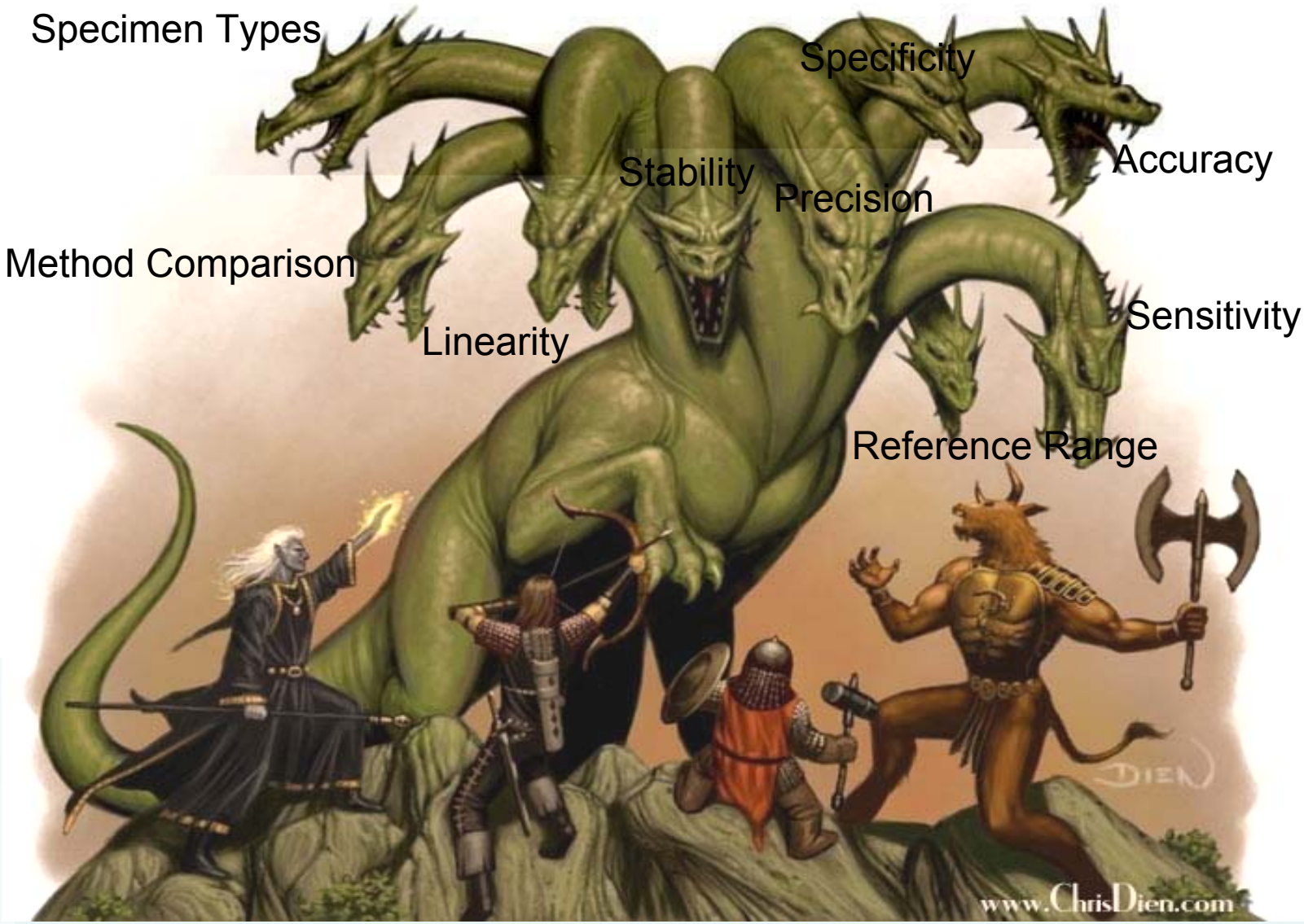


Found with: active metabolites, receptor up regulation, or increased receptor sensitization.

Proteresis



Found with: metabolism to antagonists, receptor down regulation or saturation, receptor tolerance.


Specimen Types
Specificity
Accuracy
Stability
Precision
Method Comparison
Sensitivity
Linearity
Reference Range
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Key validation issues

- Physiologic calibration
- Biologic variability
- Analyte alterations over time
- No longer organ-focused, but function-focused

Always Question the Assumptions:

- Therapeutic improvement is the biochemical reverse of disease
- Biochemical path to disease expression is constant
- In vitro reflects in vivo
- Dynamics cease after phlebotomy
- Analyte form is constant with progression

Definition of Biological Variability (BV)

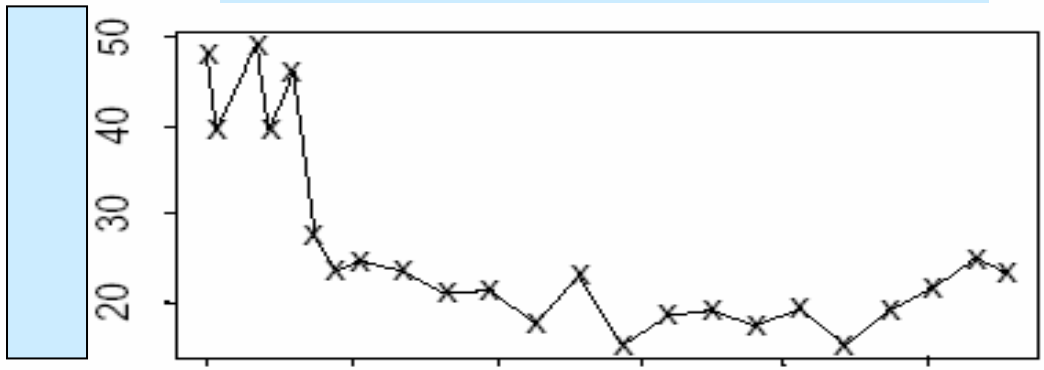
- The random fluctuations of the level of an analyte over time within an individual, in the absence of systematic changes such as due to disease progression.
- BV can be determined for healthy persons or for persons with disease.

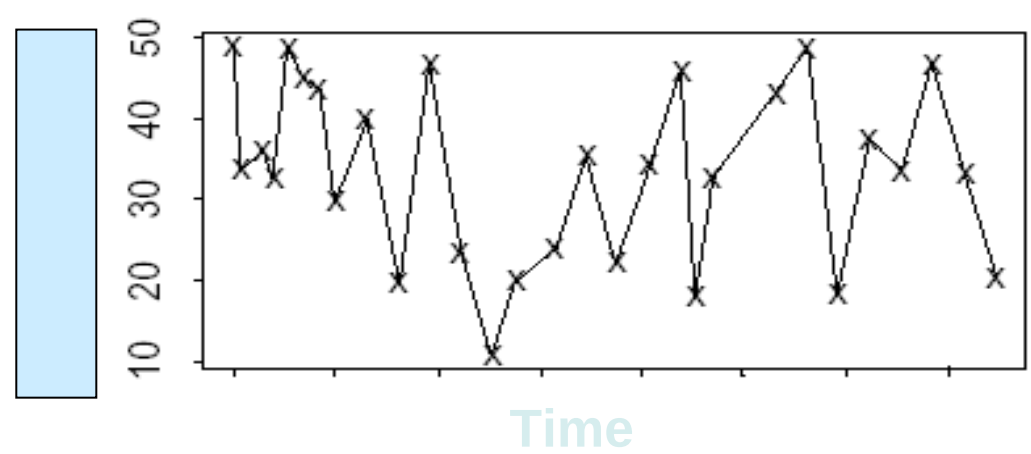
Biological Variability

- Intra-person variability in disease (defines drug activity detection)
- Inter-person variability in disease (defines selection criteria)
- Variability over time within and between days

Biologically significant change precedes clinically significant change

- A statistically significant change is
- $1.96\sqrt{2}\ SD_T$ (two sided)
- $1.65\sqrt{2}\ SD_T$ (one sided)
- Where $SD^2_T = SD^2_{BV} + SD^2_{AN} + SD^2_{pre}$





C.V. within

Person = < 10%

= 50%

Functions, not organs!

Inflammation
Lymphocyte activation
Immune recognition
Cell signaling

All are coherent biochemical processes that affect multiple organs

All are processes that can either be efficacy or toxicity responses

Physiological Forms of IL-6

- Pro-IL-6: 212 amino acids
- "Mature" IL-6: 184 (185?) amino acids substitution positions 73, 172, homodimer
- Complex with alpha-2 macroglobulin
- Complex with sIL-6R
- Heterogeneity at N-terminus
- Monocytes produce 5 forms
- Delta-4 splice variant

Short Half-life

Minutes to hours

- IL-6 = 4 hours
- IL-6/A2M increased
- IL-6/sIL-6R

Origin of analyte must be considered

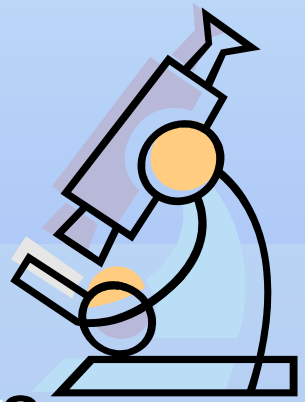
A substance acting like an endotoxin will, for example produce an IL-6 response…

a) Monocytes (m) IL-6
b) Endothelial cells (e) IL-6
c) Pituitary (p) IL-6

which are different immunologically

Studies In:

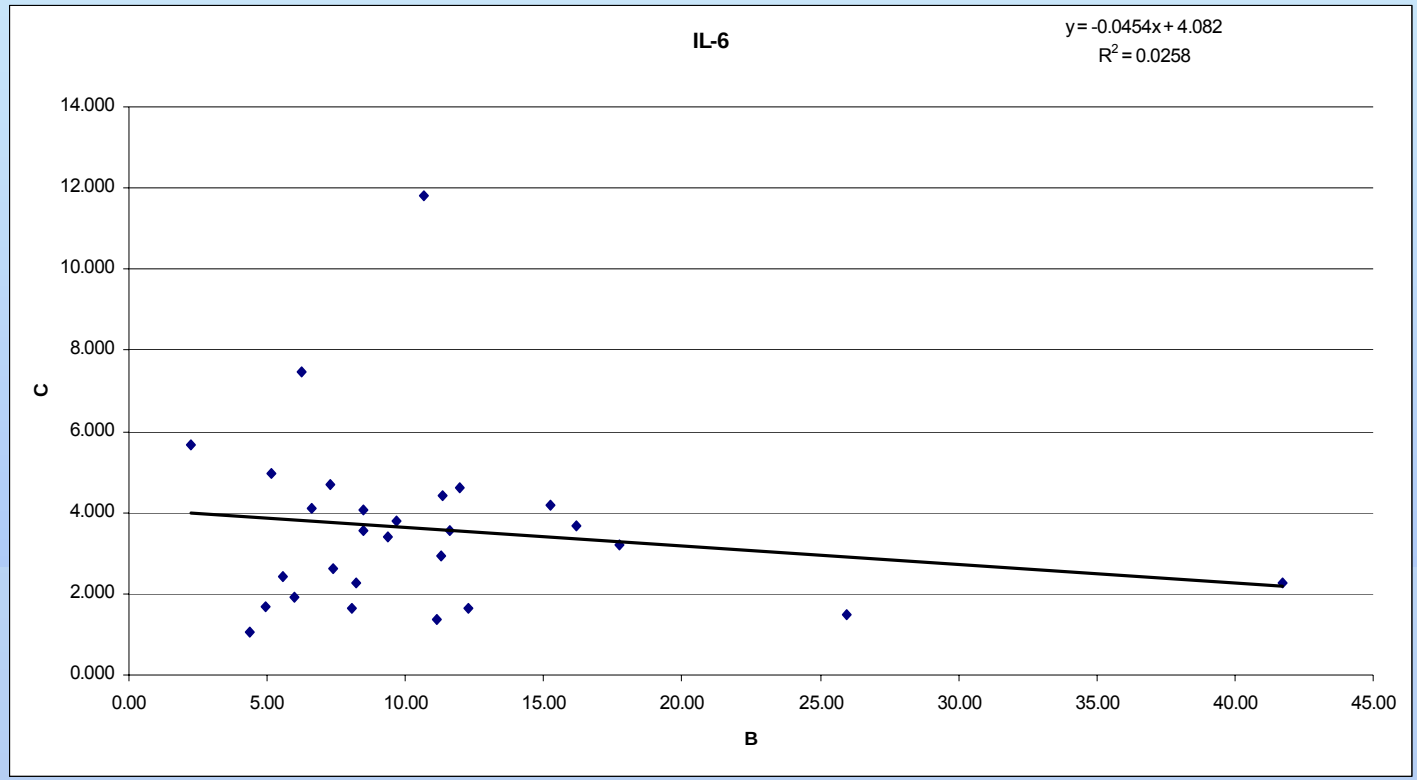
- Monocytes – instantly detectable
- Endothelial cells in the heart – minutes to an hour
- Osteoblasts – hours
- Pituitary – hours
- Gleoblastomas – hours to? days

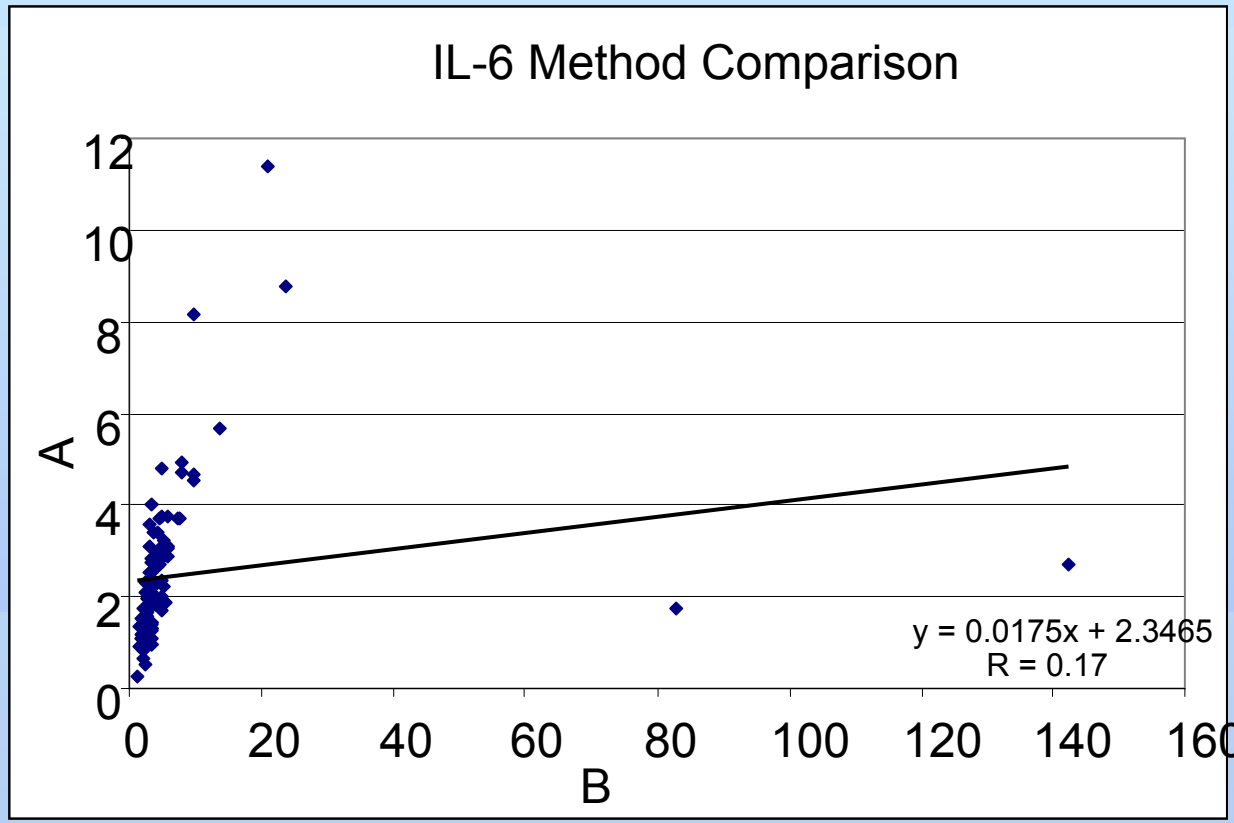


The Larger Picture:

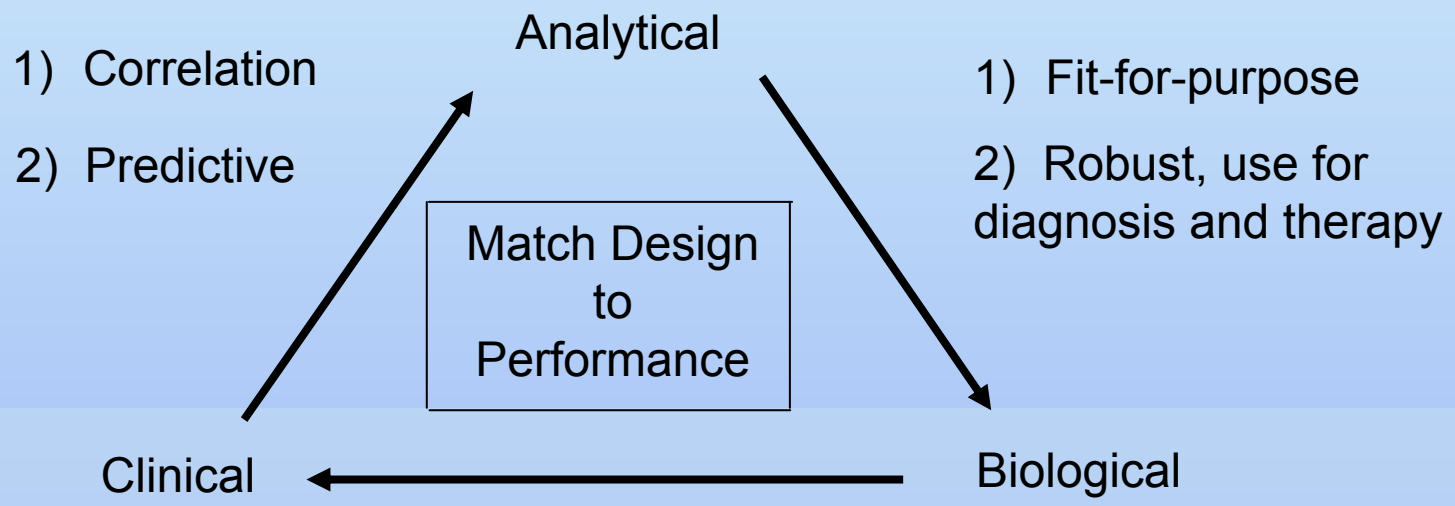
Sustained stimulation depletes the depot

- Pro-form release
- Loss of IL-6 reactivity
- Over-stimulation
- Change in substitution forms
- Pro-form release


IL-6
y = -0.0454x + 4.082
R2 = 0.0258
14.000
12.000
10.000
8.000
6.000
4.000
2.000
0.000
C
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
B


IL-6 Method Comparison
12
10
8
6
4
2
0
A
0
20
40
60
80
100
120
140
160
B
y = 0.0175x + 2.3465
R = 0.17

The New Validation Cycle



1) Characterize healthy state

2) Characterize disease state


corbis.
The End
is
Near.

Essence of Personalized Medicine

- Characterize disease
- Select drug & dose
- Monitor drug activity
- Monitor disease changes

All of this customized for the individual patient

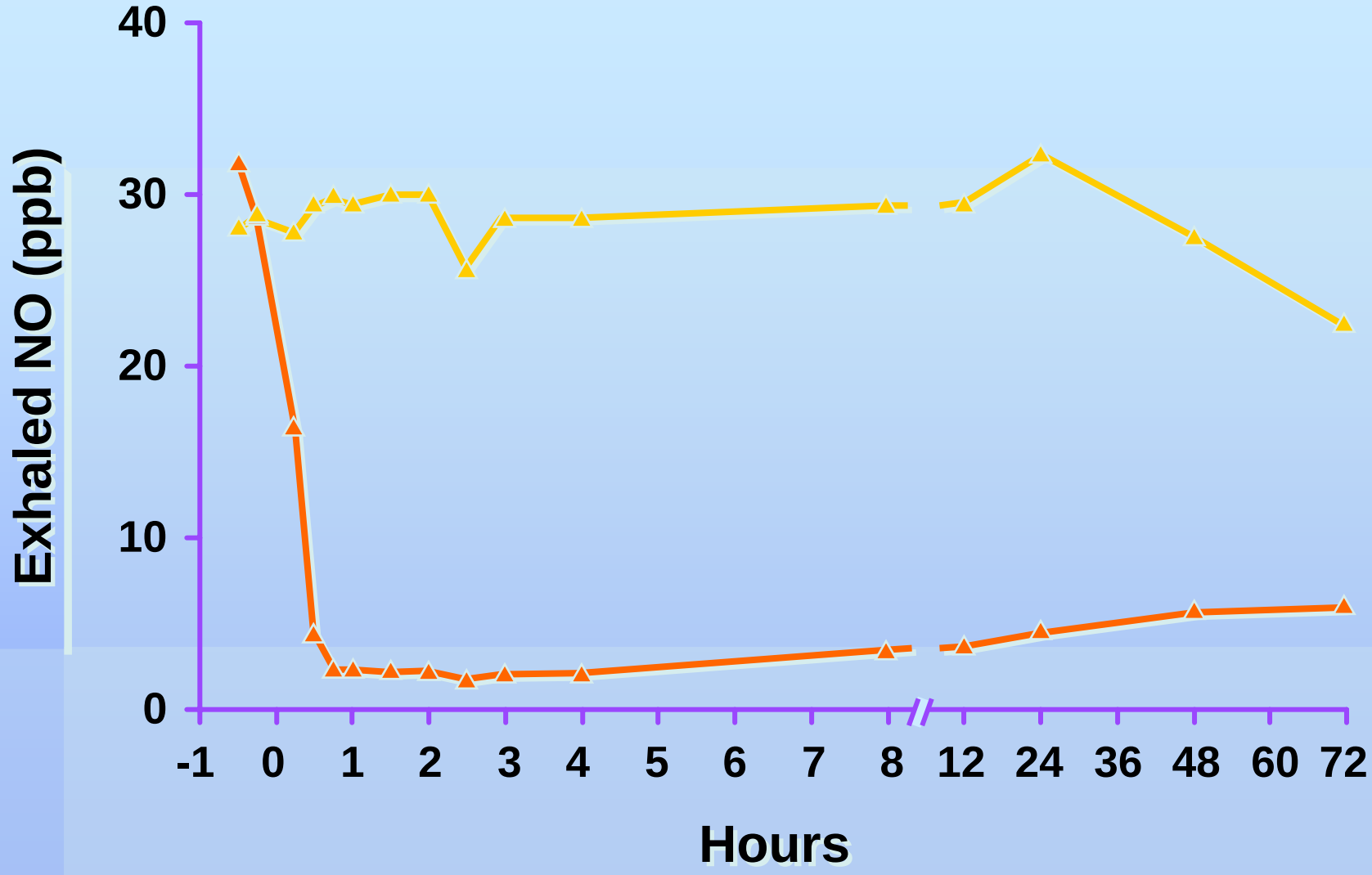
And is almost entirely post-diagnosis

Biologic Issues to solve for translation:

- Know what we are measuring, even if a mixture
- Document biologic variability, in stable disease
- Know causes for biologic variability
- Linking biochemistry with imaging

Example: iNOS inhibitors

- NO role in asthma
- L-N^6-(1-iminoethyl)lysine, selective for iNOS
- Animal models positive
- Tested in normals and asthmatics



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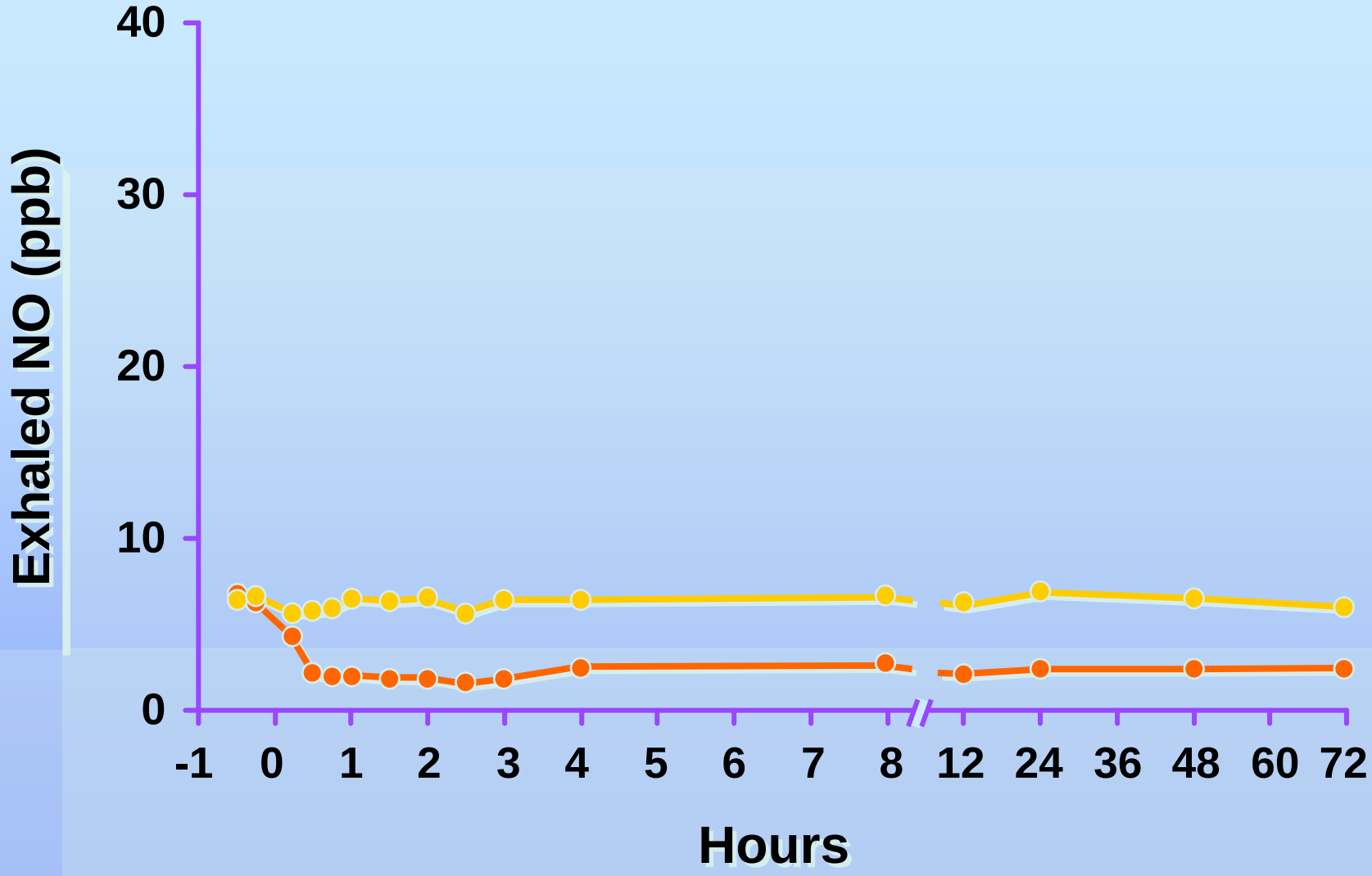

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Several years of work later…

- 2007 work with another iNOS inhibitor, GW274150
- Asthmatics, with and w/o allergen challenge
- “Positive” control = montelukast
- Endpoints: exhaled NO, FEV1

No change in FEV1, or no NO relationship

After $M’s, iNOS work is minimal

“There is nothing more difficult to carry out, nor more doubtful of success, nor more dangerous to handle, than to initiate a new order of things.”

Niccollò Machiavelli