

***‘From Pathway Biology to Pathway Medicine:
Challenges Associated with Infectious Disease’***

Existing Clinical practice:

Stepwise tests derived from reductionist research
Diagnoses - Experience of the practitioner

Isolation of a single factor

Homeostasis

Single Risk Factor-Disease Analysis

Treatment Partitioning

Highly Successful:

***BUT can disregard Component Interactions
and their dynamics***

Clinical practice, the Systems Alternative:

*Strategies beyond linear relationships and single
parameters*

Evidence-based clinical practice

Simultaneous evaluation of multiple factors

Recognition of Robustness

Multidimensional & Synergistic Treatments

Individualised diagnosis & prognosis

***More effective in chronic and
complex diseases?***

How do we merge Research and Clinical Practice?

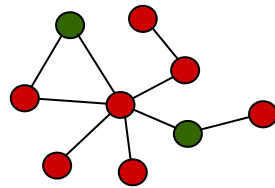
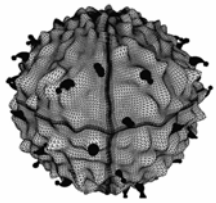
Hepatitis C: A Unique opportunity to exploit host-pathogen interplay:

Infectious agent

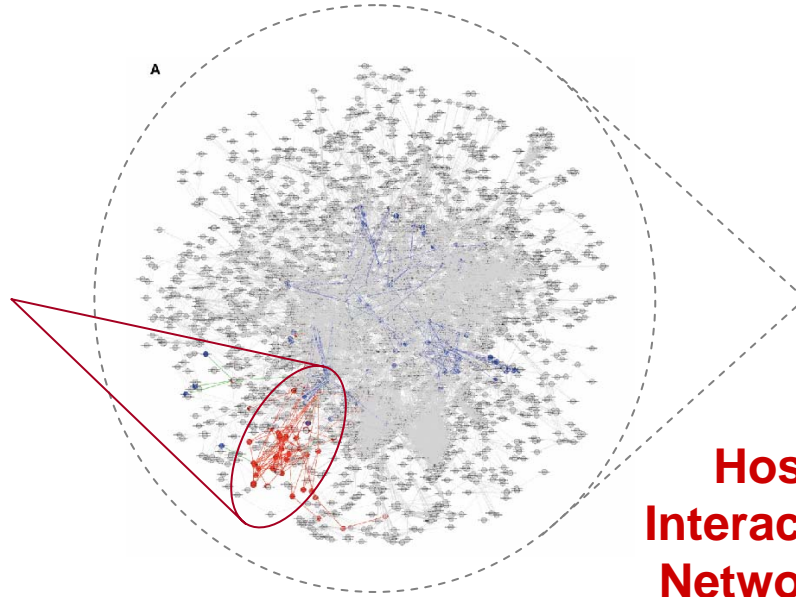
Antiviral

Systemic Therapeutic

Host



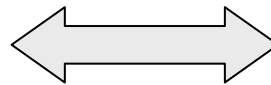
**Viral
Interaction
Networks**



**Host
Interaction
Networks**

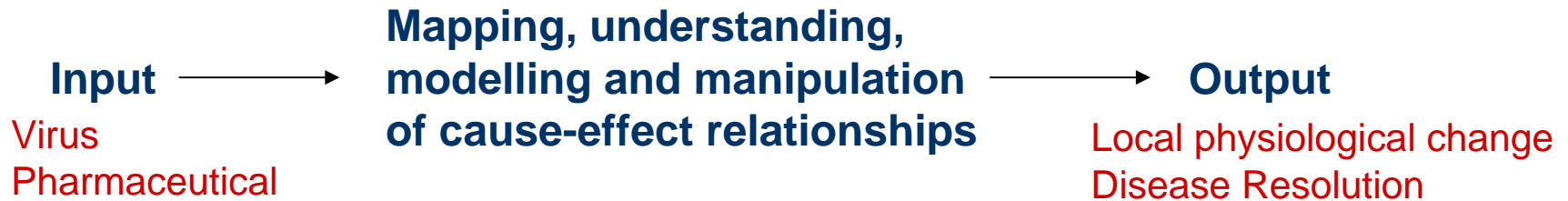


Research:
Knowledge acquisition
Understanding
Open ended and flexible

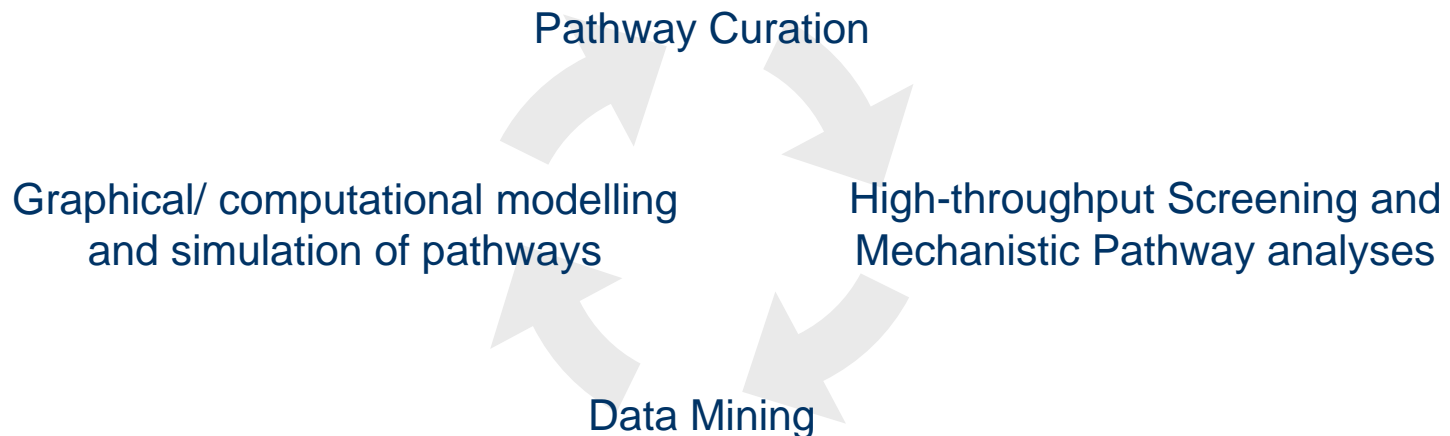


Medicine:
Applied
Standard practice
Ethical, safety, budget

Our Pathway Approach:



Key Aspects:

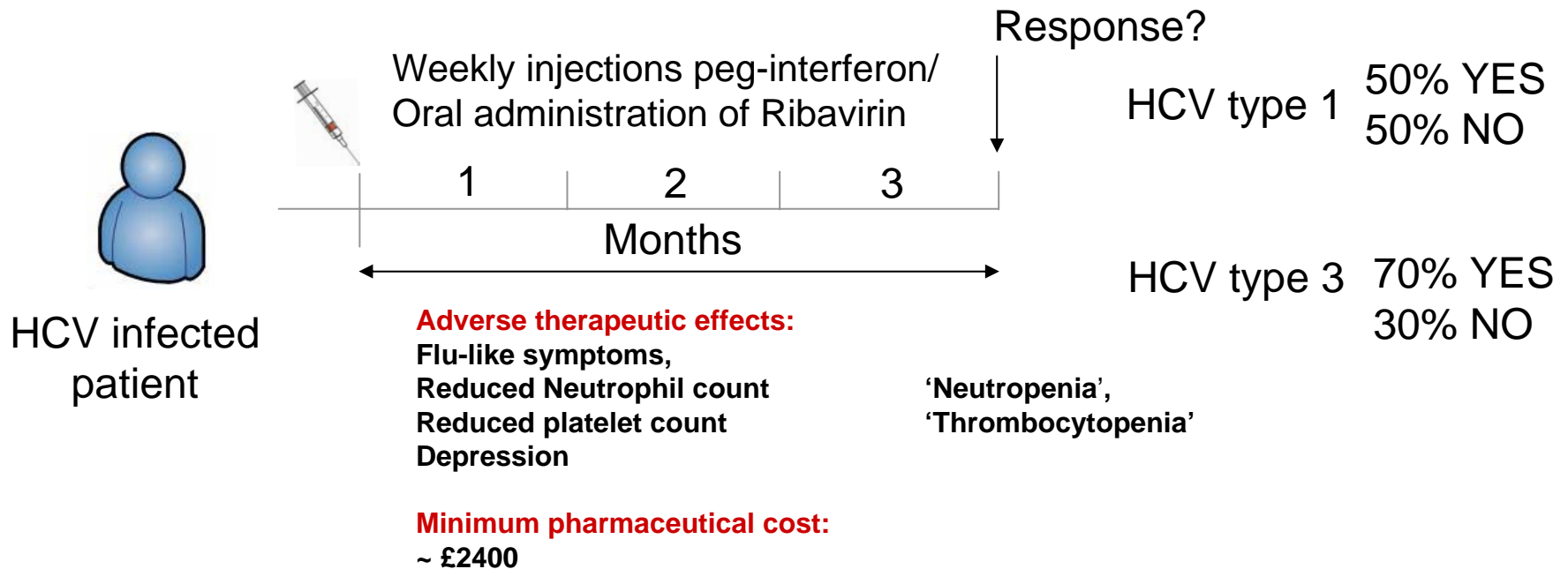


Benefits

- Provides a practical tangible focus
- Provides a common language between clinical and technological disciplines

Hepatitis C: A Systemic Problem & Enormous Challenge

- 120-170 million people infected worldwide (20% cirrhosis and hepatocellular carcinoma)
- **NO Effective Vaccine**
- Very High Mutation Rate [1 nucleotide change/ replication] – Drug escape mutants
- 6 Major Genotypes: 31% to 33% difference at nucleotide level
- **Varying sensitivity to ANTIVIRAL AND HOST therapy**



By Integrating Pathways and Medicine

Systemic Molecular profiling:
Host-Pathogen Interplay

Pathway Biology

Rapid prediction of
response to interferon
therapy

Viral Genotyping

Clinical Parameters


Immediate Objective:

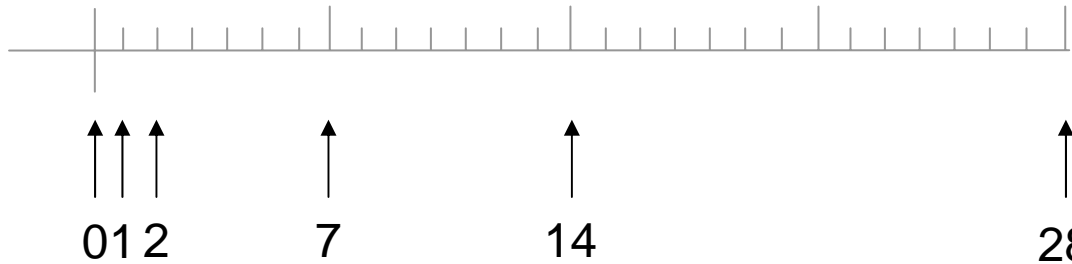
Define

Molecular Basis of Positive Treatment Outcome

Collaboration with Taylor *et al.*




HCV infected
patients



Positive treatment
> 3.5 log₁₀ IU/ml drop or
to undetectable on day 28

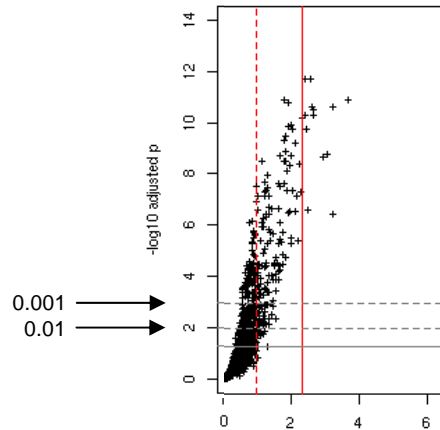
Non-Response
< 1.4 log¹⁰
IU/ml decline on day 28
relative to baseline.

Method:

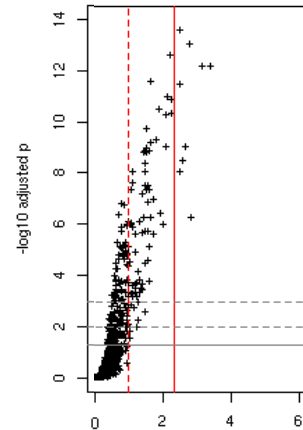
Full genomic expression profiling of HOST PBMC pre- & post- treatment

Hepatitis C: Responder and Non-Responder Analysis

Positive treatment outcome



Non-Responders



Day 0 to Day 1

Twice as many genes significantly changed in expression ($p < 0.01$, $FC > 2$) in positive treatment patients (147 vs 76)

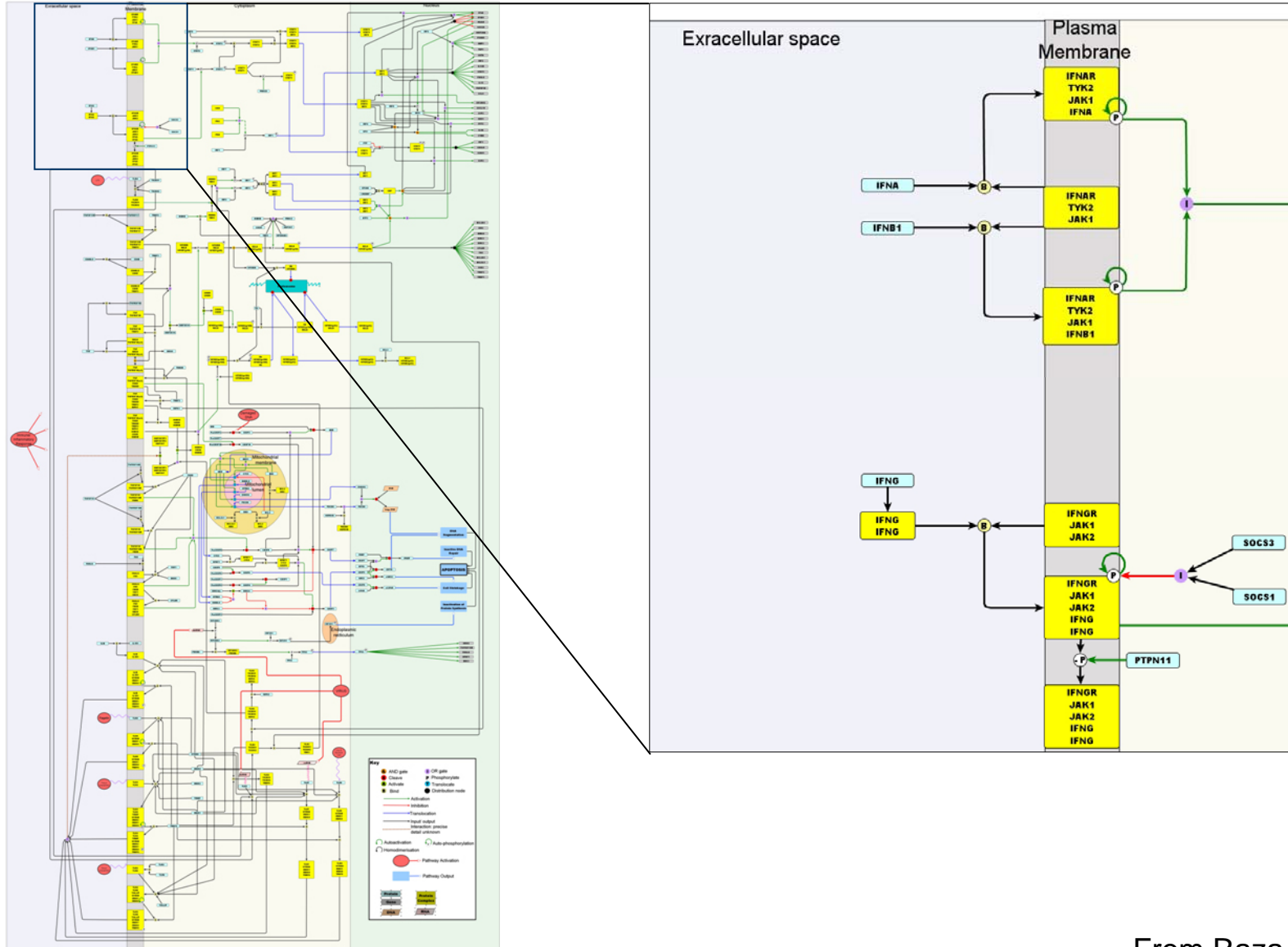
Common response:

74 genes significantly changed ($p < 0.01$, $FC > 2$)
Positive treatment outcome & Non-responders

Positive Treatment Outcome ONLY:

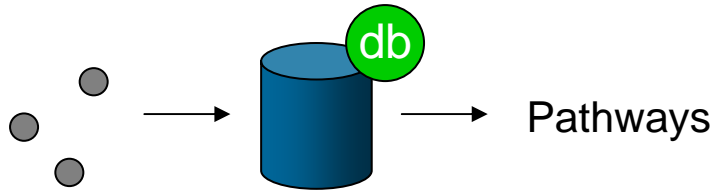
26 genes

Hepatitis C: Responder and Non-Responder Re-analysis: *Pathway Context*

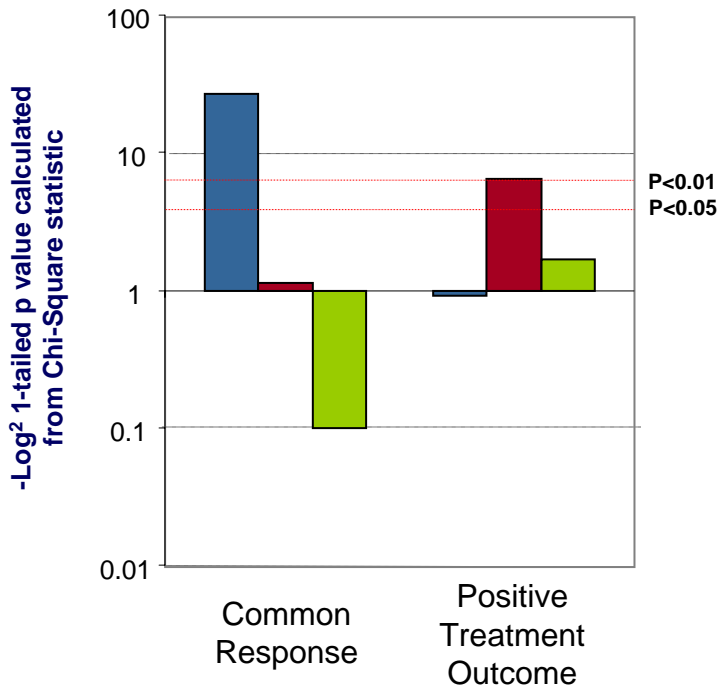


From Raza *et al.* (2007)

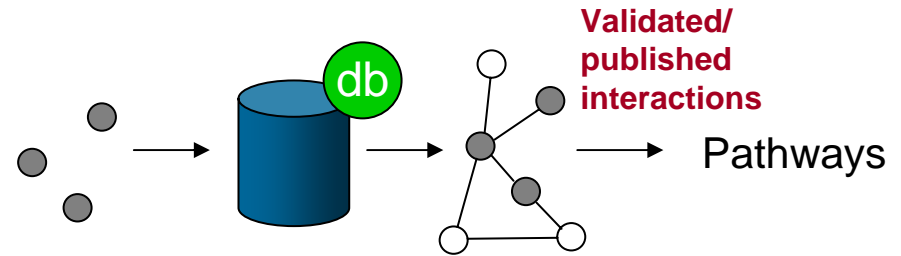
Hepatitis C: Differential Pathway responses may allow us to discriminate between Responders and Non-Responders



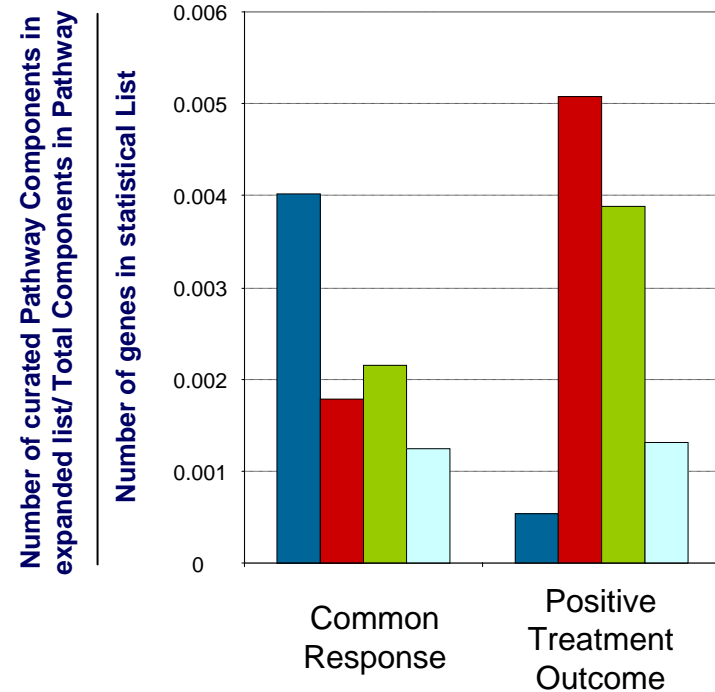
Chi-Squared Analysis of differential Pathway responses in **Statistically Significant** genes Day 0 and Day 1



■ Interferon ■ TLR ■ Apoptosis ■ HCV Targeted Proteins



Qualitative analysis of differential Pathway responses in **expanded** protein network



Hepatitis C: *Differential Pathway responses may allow us to discriminate between Responders and Non-Responders*

Toll-like Receptors:

Family of Molecular Sensors

Recognise molecular patterns: TLR3 (dsRNA), TLR7-8 (ssRNA), TLR9 (CpG)

Expressed by immune cells

Induce Inflammatory Responses

'New' Therapies for HCV

Small Molecule

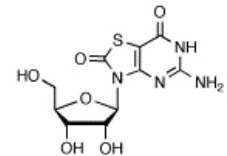
- NS3-4A protease inhibitors – BILN2061*, VX-950*
- NS5B RdR polymerase inhibitors – Nucleoside (NM283*) & Non-nucleoside Inhibitors (JTK-109, JTK-003)

Nucleic Acid Antivirals

- Anti-sense oligos, ribozymes and siRNA [*in vivo* delivery & resistance problems]

Novel Immunomodulatory Agents

- **Synthetic Agonists of TLR 9 (CPG-10101*) and TLR7 (ANA245*, Isatoribine*)**



'Combinations of multiple agents (targetting host and virus) will be required to treat chronic HCV'

Francesco and Migliaccio (2005) Nature **436**, 953-960

* Clinical effect, issues with toxicity

Computational Modelling of Infectious Disease Pathways

Network of Molecular Dependencies

Pros

Computationally efficient – Allows large Network analysis
Progress in domain where Rate constants etc are unavailable
Allows all or subnetwork analysis

Cons

Suboptimal approximation

Binary read-out on proteins present or absent (defined by X and Y)

Run simulation

Binary read-out on predicted proteins present at end of simulation

Discretised REAL data Comparison

Iterating through upper and lower input and output thresholds relating to gene expression values defined by microarray experiment.

Pathway Curation

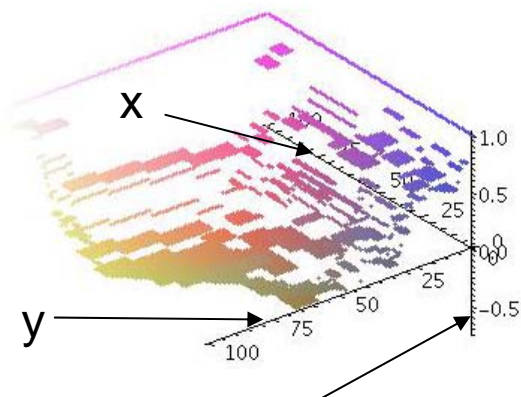
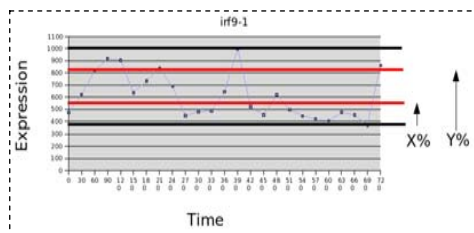
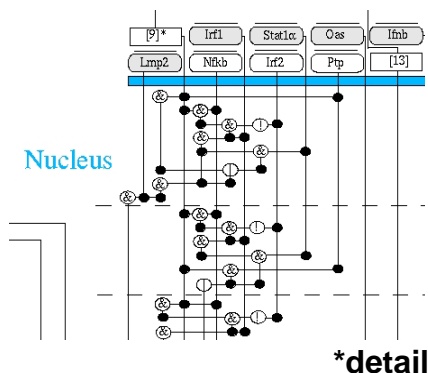
Map to Boolean Representation

Boolean Framework representing Interferon signalling

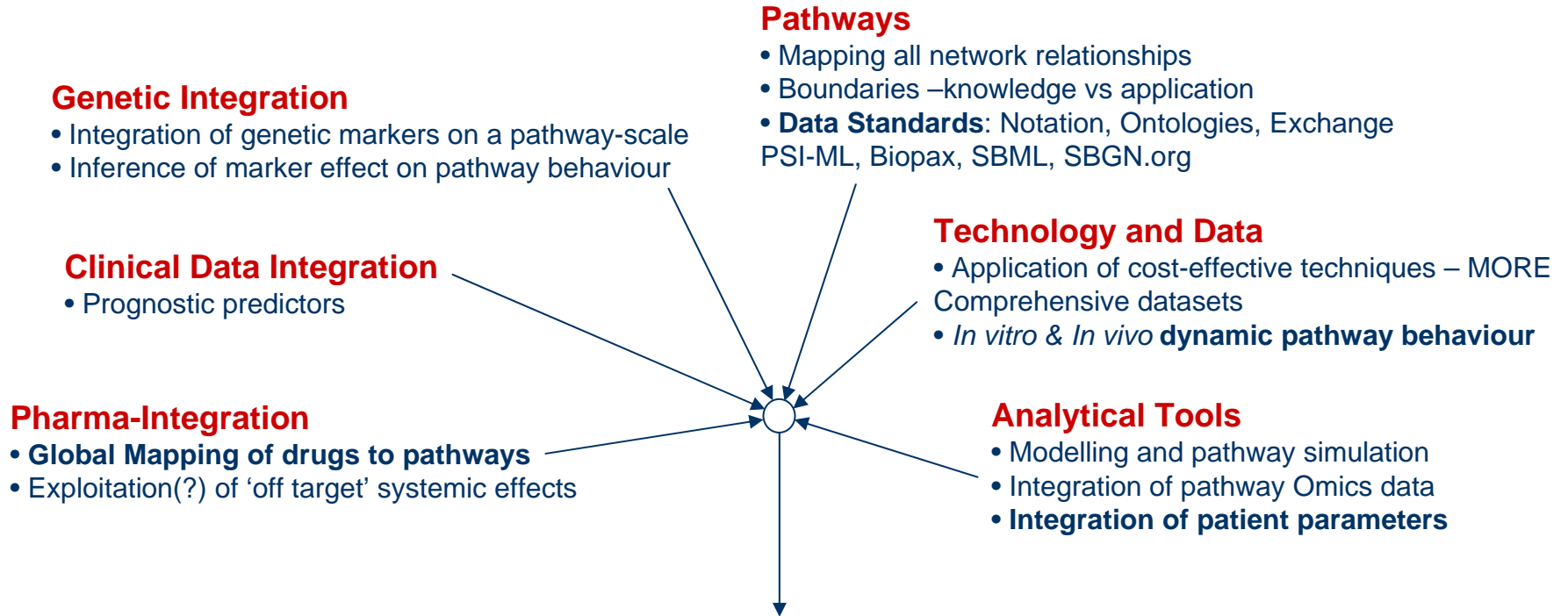
Discretisation of *in vitro* data on IFN treatment of Macrophages

Experimental testing and optimisation of model

Iterative testing of viral/ pharmaceutical pathway perturbation



Correlation: simulation and real data



‘Use of **clinical data** and **current best evidence** coupled with **pathway knowledge** to make **decisions** about the care of **individual patients**’



Prof. Peter Ghazal
Director of Division

Dr. Tom Freeman, Sobia Raza
Biolayout, Pathway Mapping & High Throughput *in vitro* analyses

Thorsten Forster
Statistician

Dr Steven Watterson
Computational Pathway Modelling & Simulation



Professor Milton Taylor
Microarray Analysis of Responses to Interferon Therapy