Expression Profiling / Microarray / Data Analysis at Amgen

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Changes in Drug Development: Feeding the Beast

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Seminar Outline

- The current state of affairs, and where we want to go
- Instruments of change
- The Amgen experience

The High Stakes in Pharmaceuticals

- Global R&D increased 14% in 1999 to 24 billion.
- Top 11 geographical markets grew 9% to $202 billion in sales.
- U.S. market valued at $83 billion.

...And High Pressure

- **U.S. FDA approved 35 new molecular entities in 1999 (30 in 1998, 39 in 1997).**

- **36 branded pharmaceuticals ($1.9 billion in sales) came off patent protection in 1999.**

- **Between 2000 & 2005, 173 products representing $30 billion in sales to lose patent protection.**

C&E News, Jan. 17, 2000

The Gap Between R&D and New Drugs

Year	U.S. R&D (Billions)	New molecular entities FDA approved
1993	$10.5	25
1994	11.1	22
1995	11.9	28
1996	13.6	53
1997	15.5	39
1998	17.2	30
1999	20.1	35

Top Five Drug Targets

- 56 (11%) brands
- 29% of prescription sales
  - HMG CoA reductase *(hypercholesterolemia)*
  - Proton pump *(ulcers)*
  - Serotonin transporter *(depression)*
  - Calcium channel *(hypertension)*
  - Angiotensin converting enzyme *(hypertension)*

A Piece of the Pie

	Drug	Company
HMG-CoA Reductase Inhibitors	Zocor	Merck & Co.
	Lipitor	Warner-Lambert/Pfizer
	Pravachol	Bristol-Myers Squibb
	Mevalotin	Sankyo
	Mevacor	Merck & Co.
	Lescol	Novartis
	Baycol	Bayer/SKB
	Lodales	Sanofi

Why Pharmaceutical Executives Sleep like Babies at Night.

- 'Return' on R&D diminishing
- Potential for reduced government subsidization and/or HMO reimbursement
- Investor pressure
- Biologics

Biologics Come of Age

Drug	Company	Target	Indication
1. Abciximab	Centocor	Gp IIb/IIIa	Thrombosis Restenosis M.I./ Angina Stroke
2. Infliximab	Centocor	TNF	RA Crohn's Disease
3. Trastuzumab *(HERCEPTIN)*	Genentech	HER2 protein	Cancer
4. Rituximab	IDEC	CD20 antigen	Cancer Thrombocyt./Anemia Syst. Lupus Eryth.

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- Biologics
- The Red Queen

The Red Queen

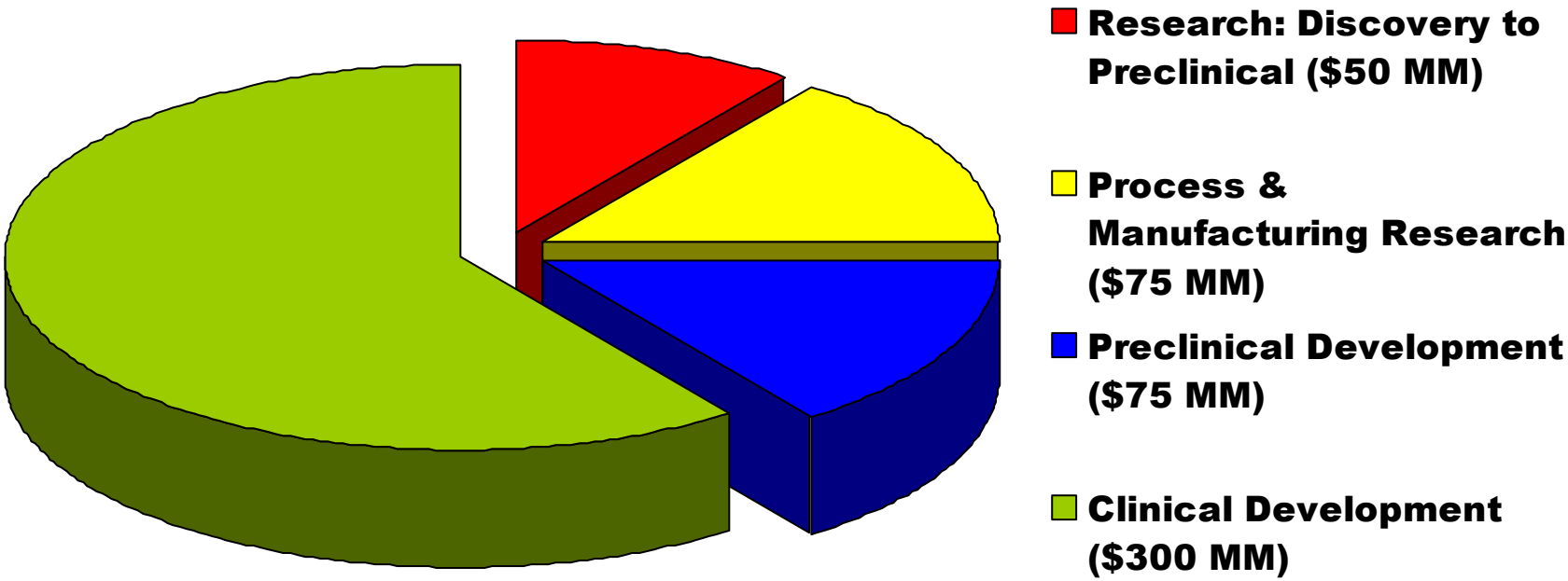
"A slow sort of country!" said the Queen. "Now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!"

Through the Looking Glass
Lewis Carroll

Four Obvious Words

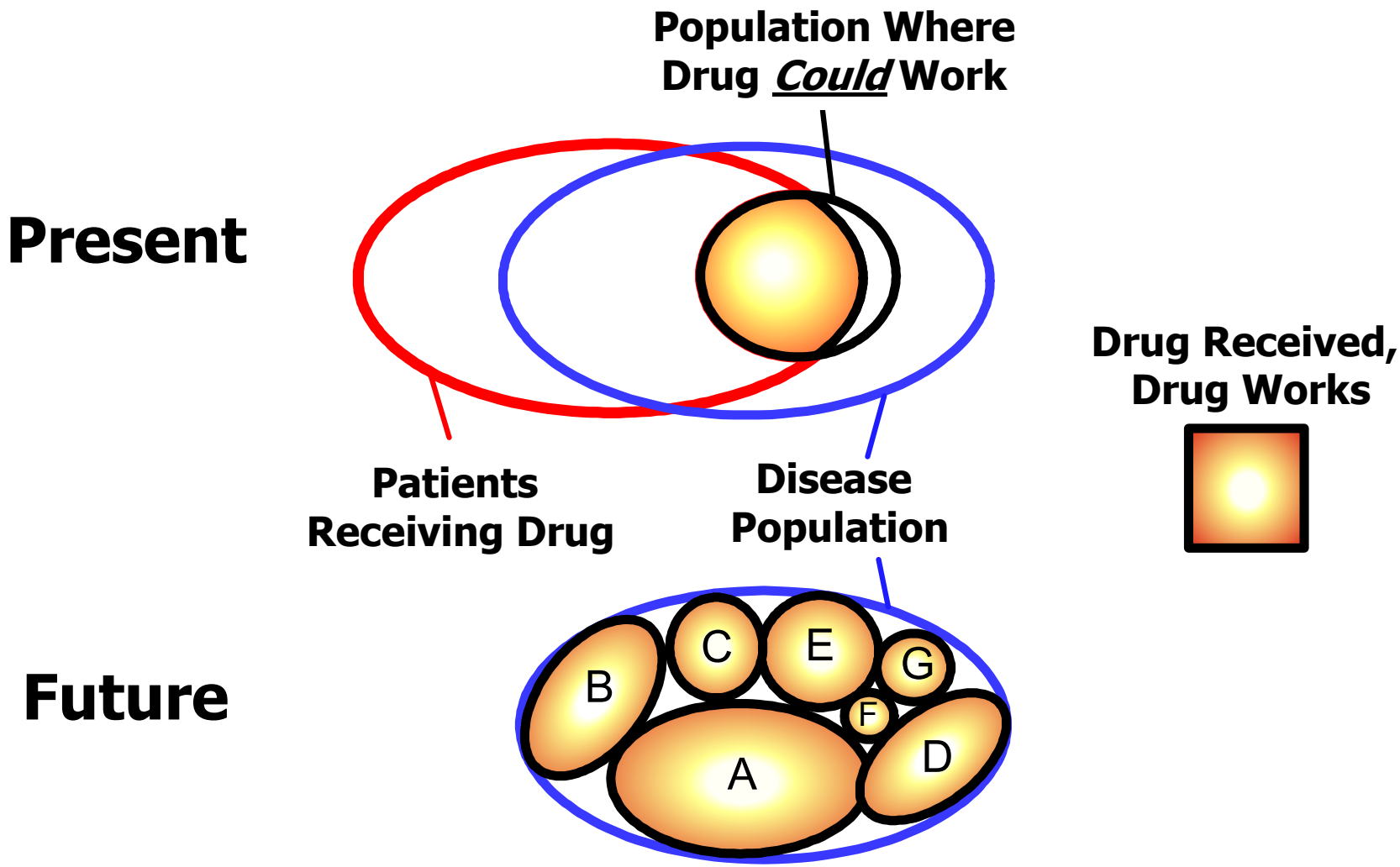
- Faster….
- More…
- Better…
- Cheaper

Cost of Drug Development



Total: $500 MM / 10 yrs

The Ultimate Goal



Changes in Drug Development

Parameter	Old	New
Drugs	Dominated by handful of "Blockbusters"	1000's of "Niche" drugs; few Blockbusters
Treat Underlying Cause	Generally NO	YES, with better understanding of disease
Target Identification & Validation	Academia; Classical Biochem, Mol Biol; One-off approach; Public Domain	Industry; Genomics; Systematic approach; Proprietary
Number of Targets	Less than a few hundred	1000's
Clinical Trials	High failure rate	Low failure by applying pharmacogenomics

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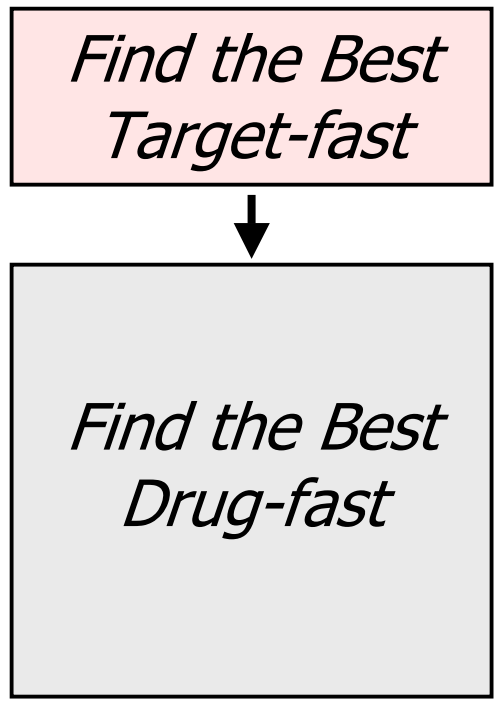
New Paradigm

- Better understanding of disease (genomics technologies)
- Selection of the best target from pool of all candidates
- Rapid identification & optimization of leads (drugs) for that target, in some cases will occur simultaneously with target selection/validation
- High rate of success in the clinic

Change Drivers

- Genomics Technologies (Pharmacogenomics)
- Combinatorial Chemistry
- Structural Chemistry
- Ultra-High Throughput Screening



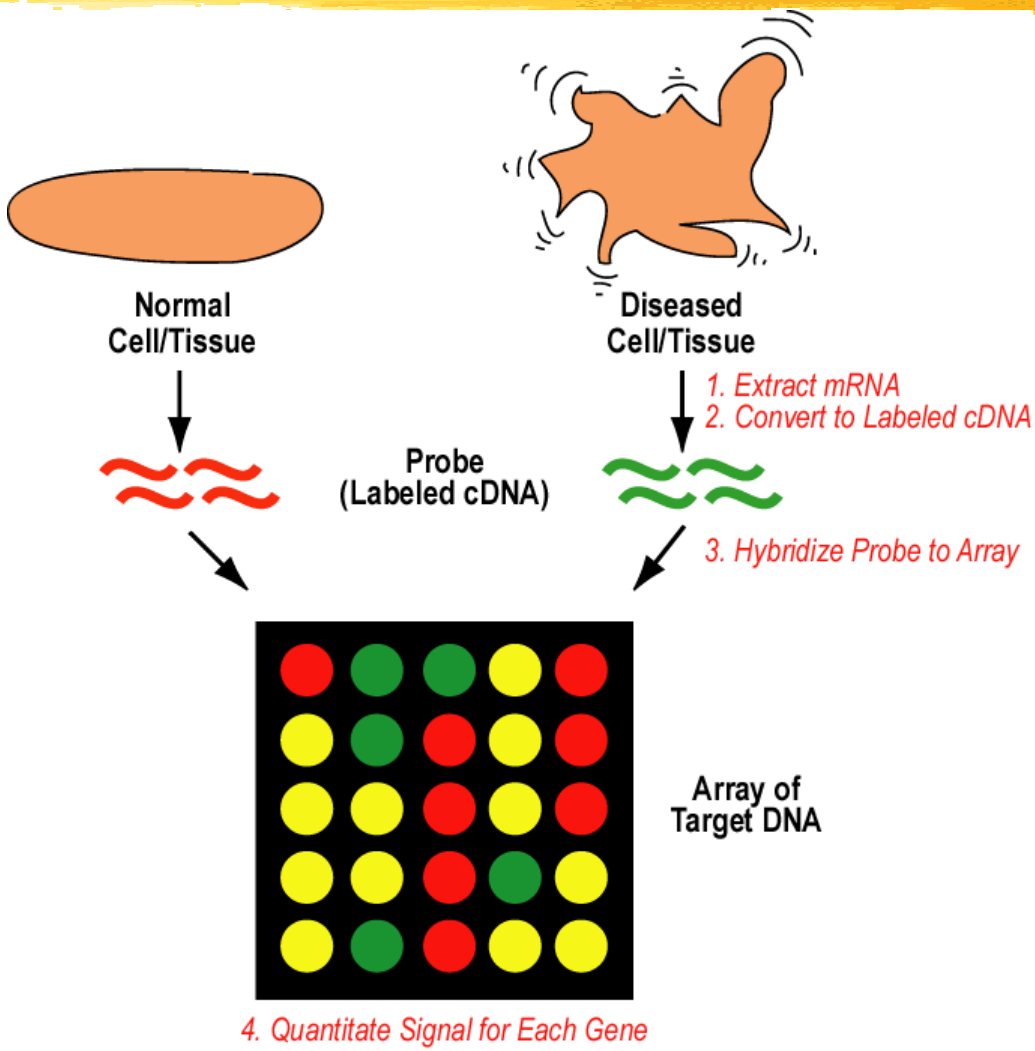
Pharmacogenomics

- The application of genomics technologies to the way that drugs are discovered, developed & used
  - Genome-wide sequencing
  - Expression profiling
  - SNP analysis (pharmacogenetics)
  - Proteomics

Expression Profiling

- High-throughput, parallel analysis of expression of 1000's of genes
- Variety of technologies
  - EST sequencing, SAGE, AFLP, **DNA Microarray**

Microarray Technology



Microarray: Modified Dot Blots....

Prepare multiple DNA ‘targets’.

↓

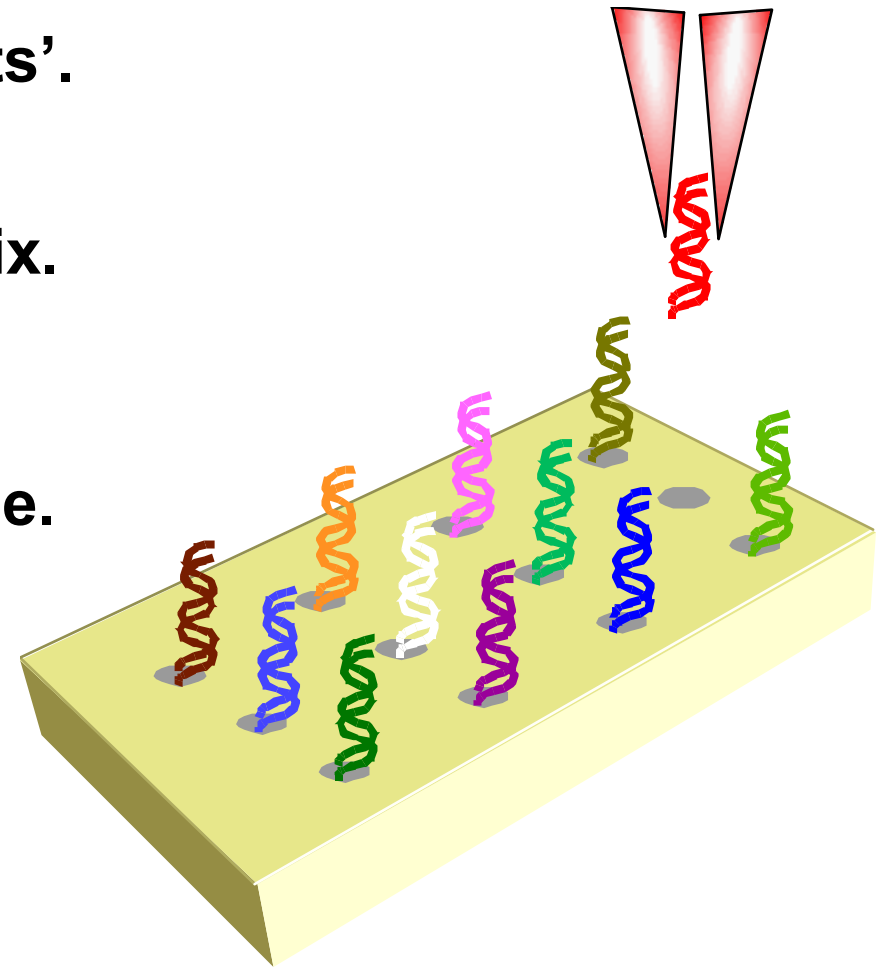
Spot DNA onto a surface. Fix.

↓

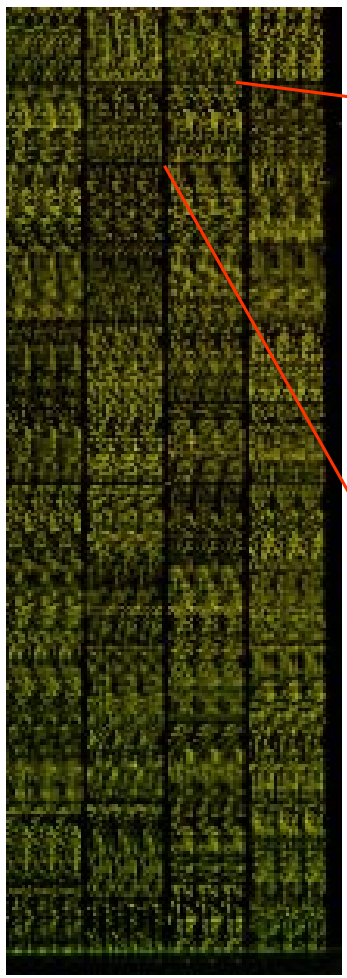
Synthesize labeled cDNA probe.

↓

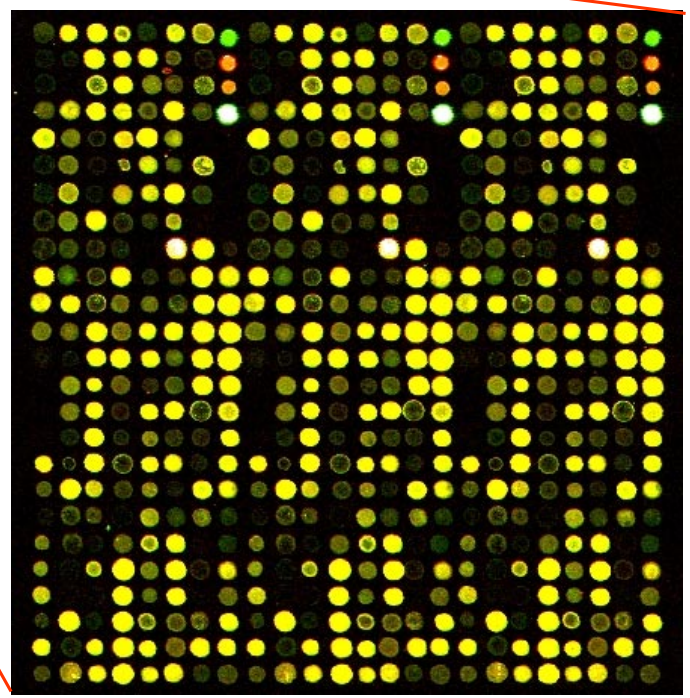
Hybridize probe.
Wash & detect bound fluor.



The Stanford/Incyte Approach to Microarray



Two Color Microarray Hybridization



Expression Profiling Component of Pharmacogenomics

- Target genes and pathways

- Toxicology / Off-target profiling
- Surrogate markers for clinical studies
- Stratify population for clinical trials
- Improve drugs/candidates/failures

Change Drivers

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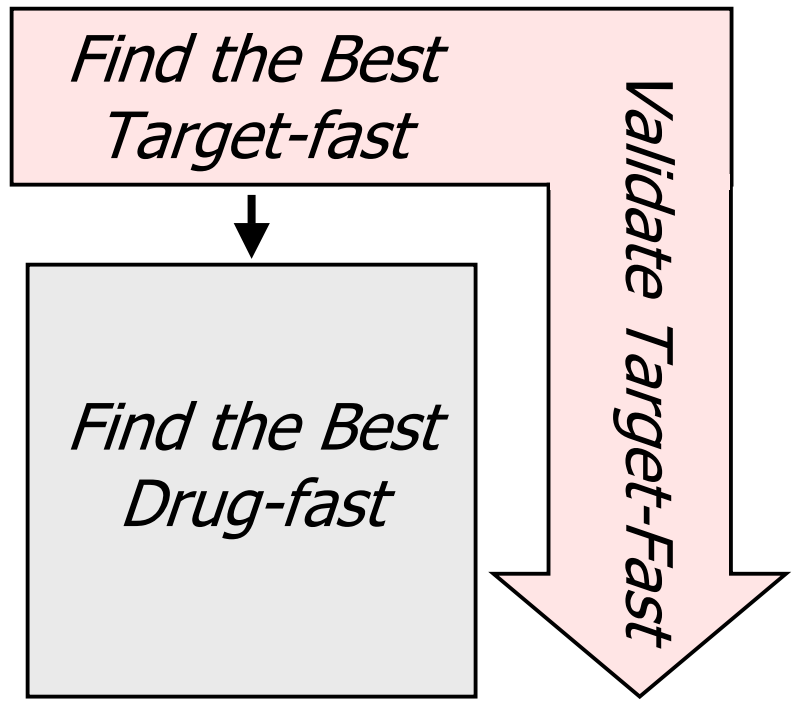

Proteomics

- High-throughput, parallel analysis of expression of 1000's of proteins
- Especially critical in cases where protein expression does not mirror mRNA levels or where information about post-translational structure is required
- Multiple roles in discovery/development: theoretically applicable to all phases that transcriptional expression profiling can address.

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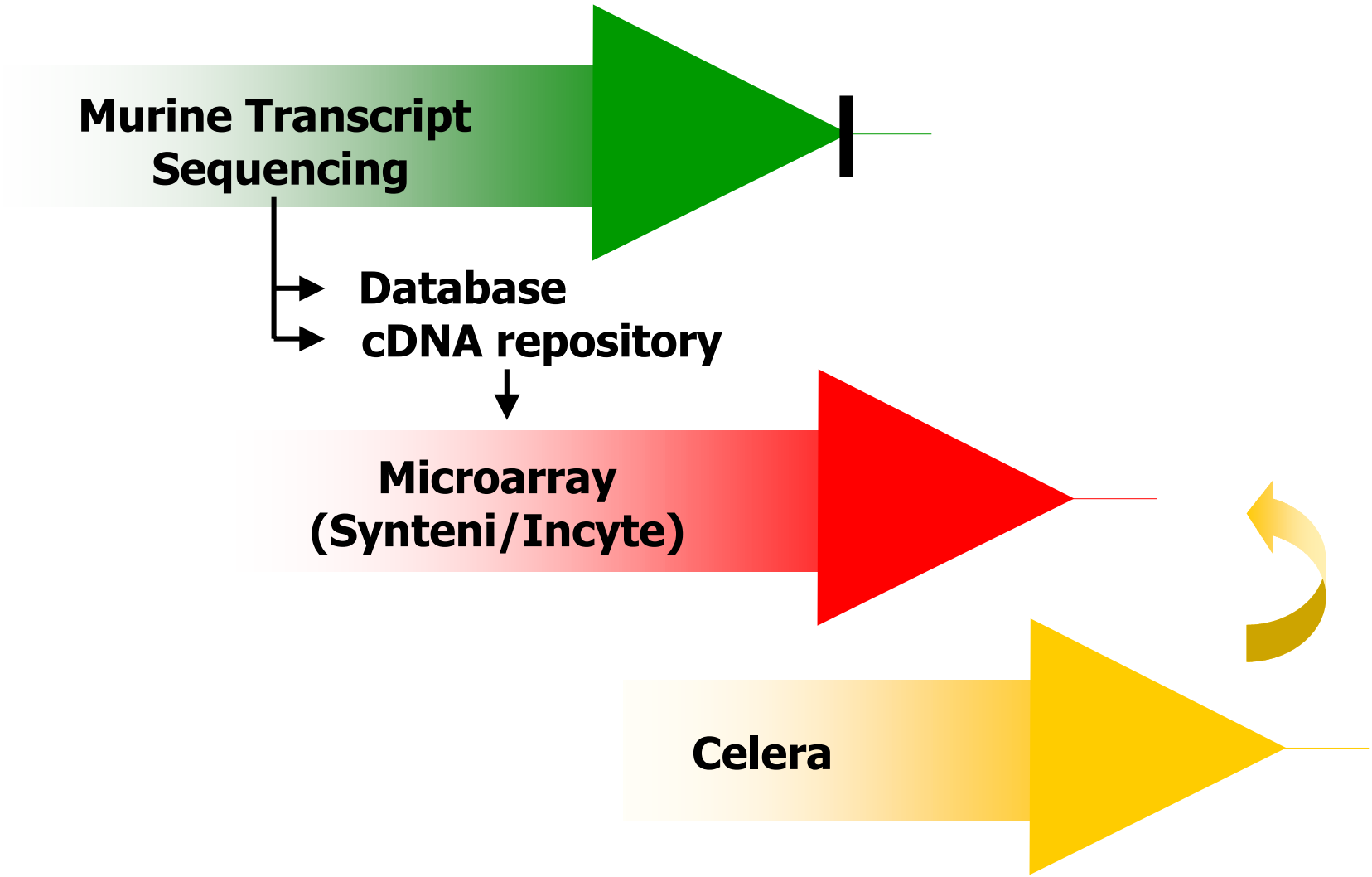
Expression Profiling History at Amgen



Examples of Expression Profiling Impact

Murine Genomic Project

- **OPG (osteoprotegerin)**
- **B7-RP1 (B7-related protein 1)**

Microarray

- **IL-13/Hodgkin's disease**
- **HER2 Study**

HER-2/*neu* Biology

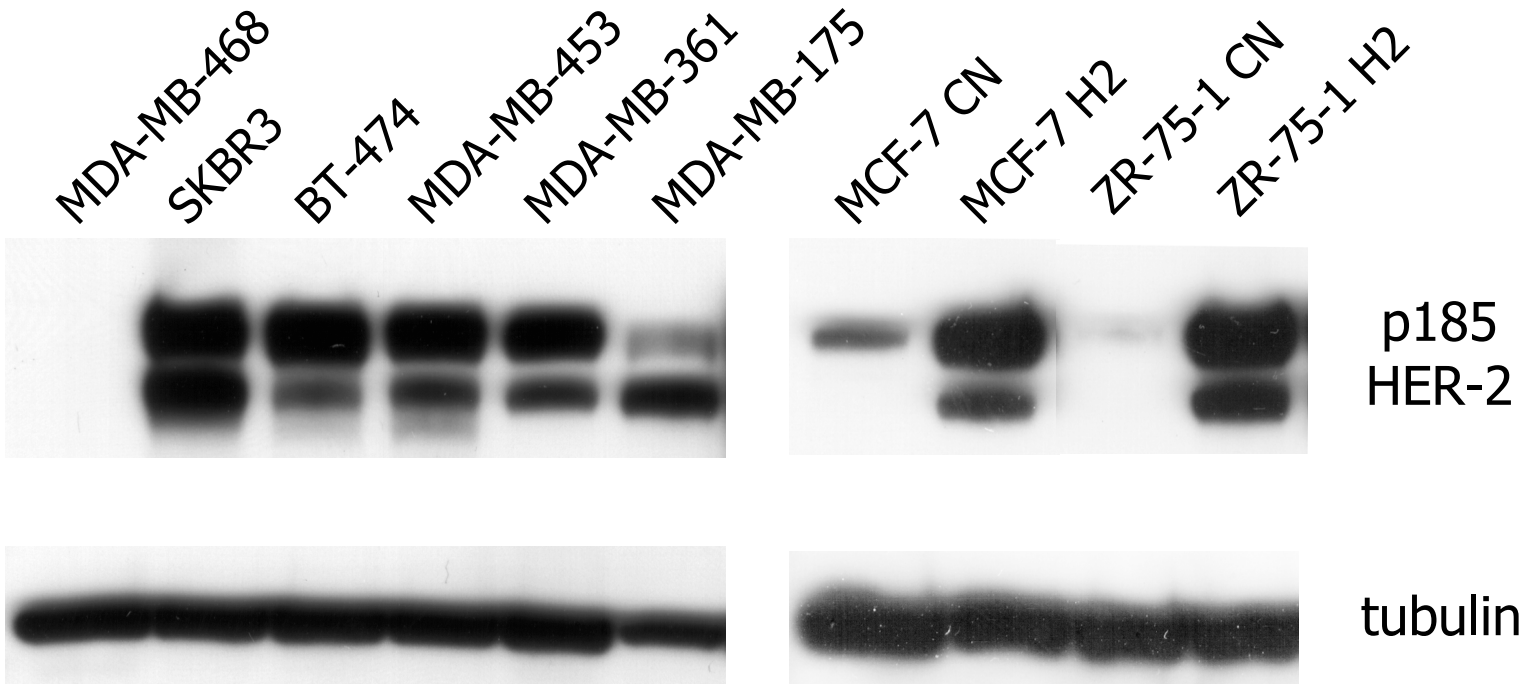
Clinical

- Amplified in ~25% of breast CA
- Poor prognosis: Shorter survival
- Correlation w/ ER negativity & failure of anti-estrogen therapy

Experimental

- Increased proliferation *in vitro* (plastic & soft agar)
- Increased growth in xenograft models
- Tamoxifen resistance
- Morphologic changes
- Others: motility, invasion, apoptosis, DNA repair

Cell Line Models

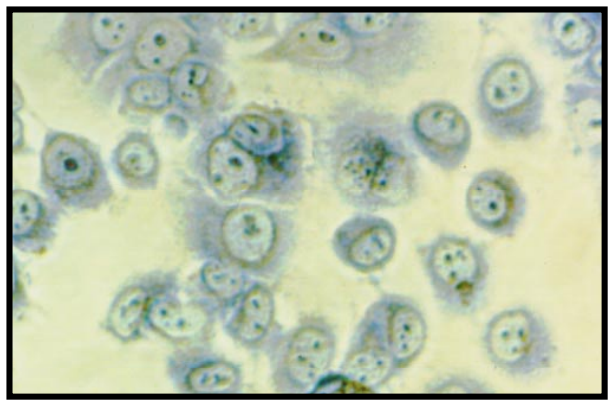


"natural"
over-expressing cells

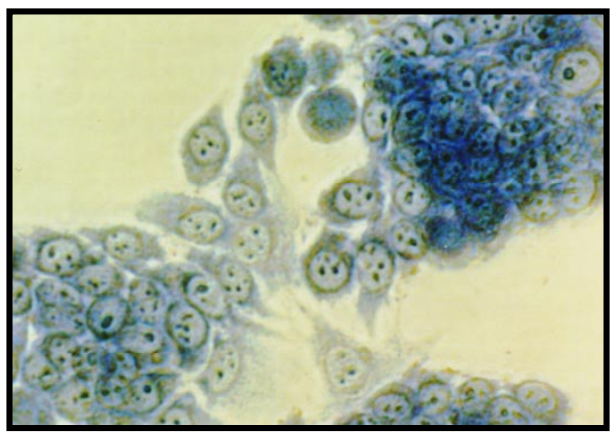
engineered
CN & H2 pairs

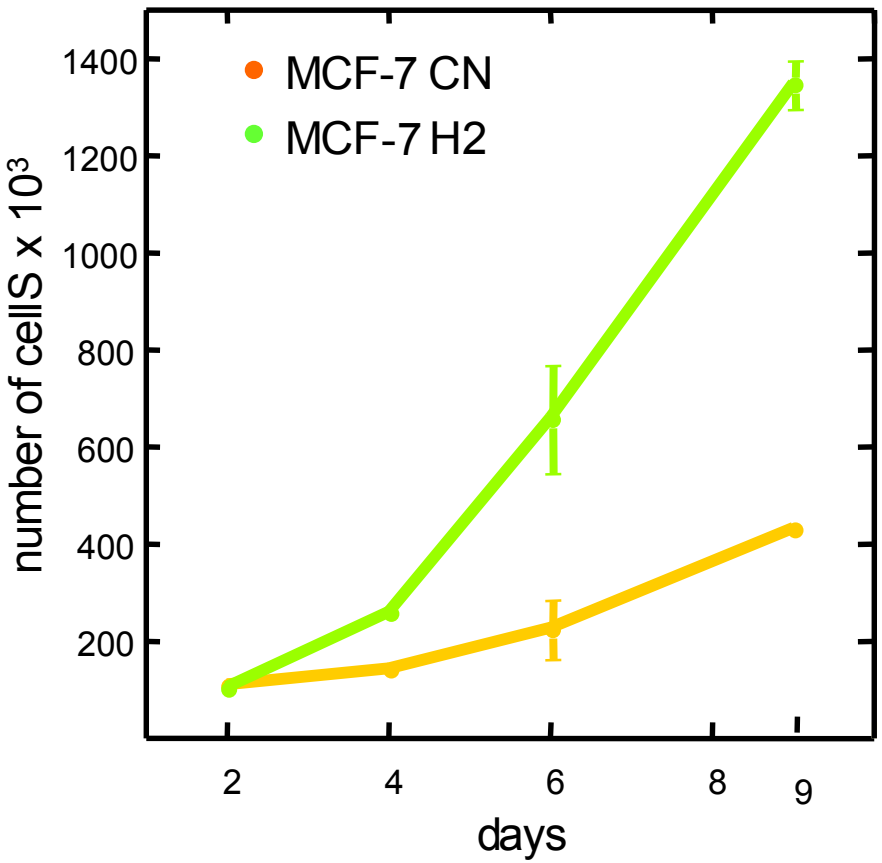
HER-2/*neu* Overexpression: Decreased Contact Inhibition and Increased Proliferation

MCF-7 CN

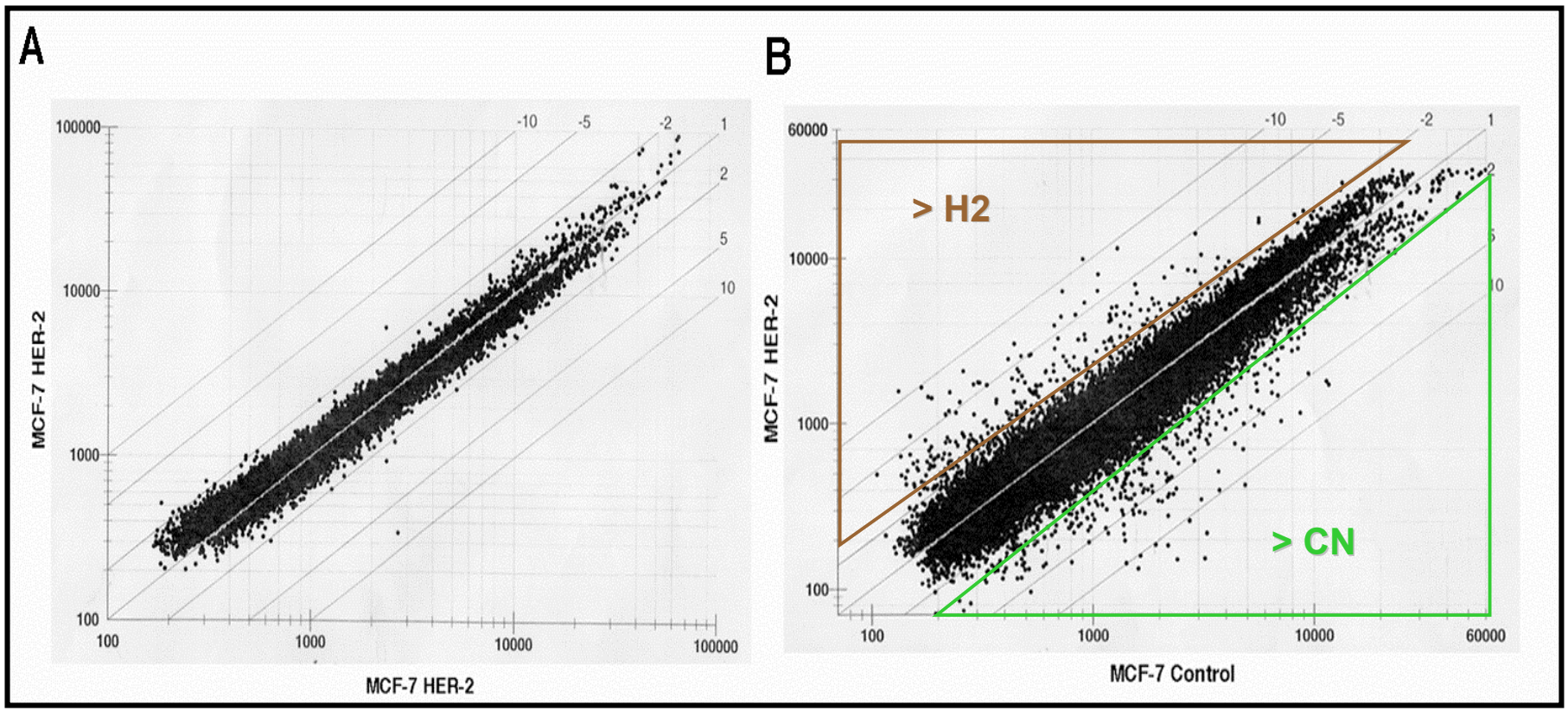


MCF-7 H2





cDNA Microarrays



Self RNA test

MCF-7/H2 vs CN

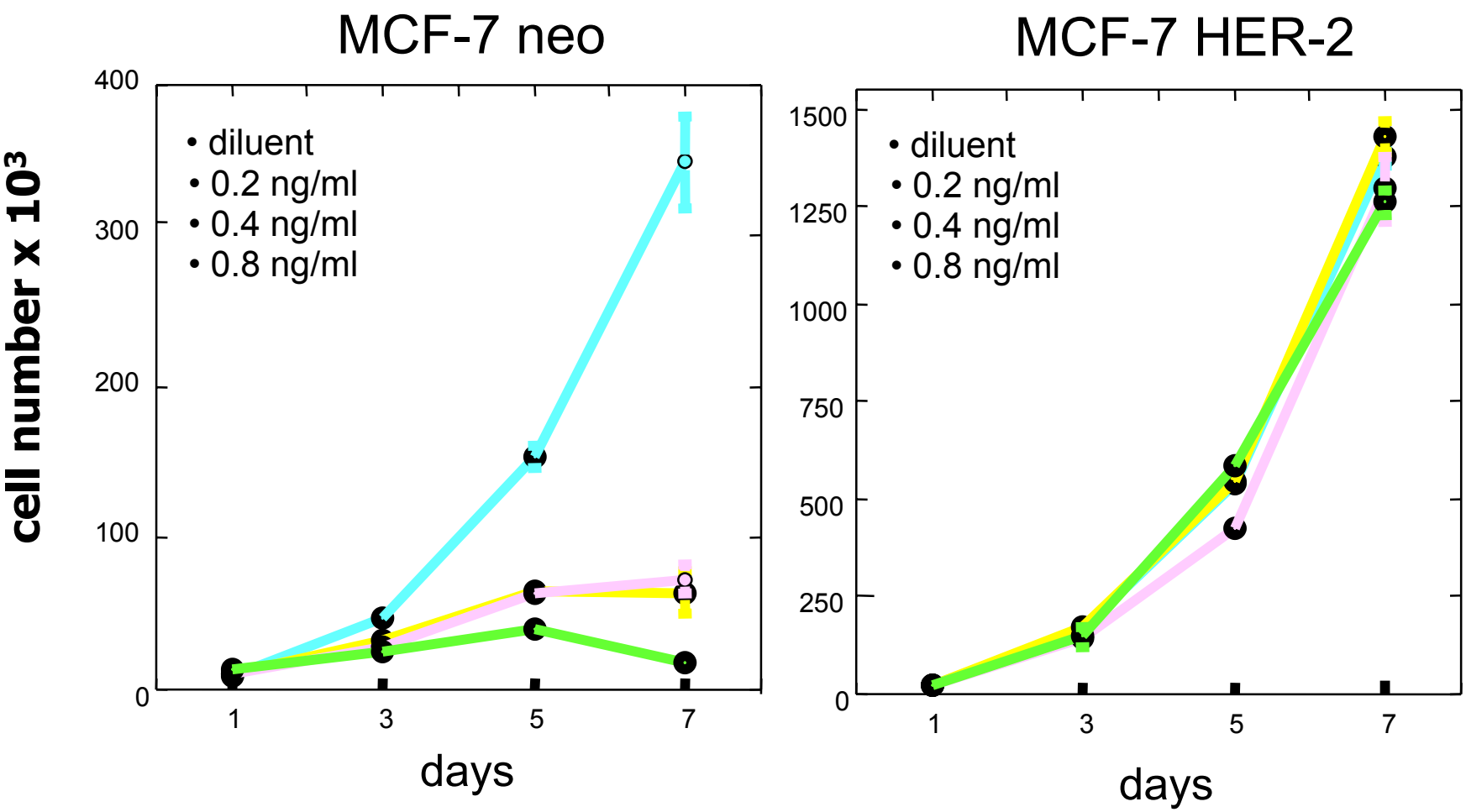
490 elements Δ > 2.5 fold

TGF-ß Pathway

Ligands	cDNA	filter
TGF-ß2 (M19154)	2.5	2.0
TGF-ß3 (J03241)	-	2.8
BMP-3 (M22491)	-	2.8
BMP-5 (M60314)	7.1	4.5
BMP-7 (M60316)	2.5	3.9
Receptors		
TBR II (D50683)	2.5	-
Endoglin (X72012)	2.9	-
Modulators		
Evi-1 (S82592)	3.4	-

Induced targets	cDNA	filter
CTGF (M92934)	5.2*3	4.4
Cyr61(AF031385)	3.8*2	-
B-Ig-H3 (M77349)	2.6	-
Endothelin1(J05008)	2.7	-
Timp-2 (J05593)	2.9	2.0
Col3A1(X14420)	2.9*2	4.4
Col5A1(M76729)	4.0*3	
Col18A1(AF018081)	3.7	4.7
Repressed targets		
hTRT-1(S82592)	8.9*4	-

MCF-7/HER-2 cells are Resistant to Growth Inhibition by TGF-ß1



HER2 Summary

- Lack of TGF-ß sensitivity may be an important component of the aggressive HER-2/*neu* phenotype.
- One aspect of Herceptin's efficacy may be its ability to increase sensitivity to growth inhibition by TGF-ß.
- The microarray profile may be a "signature" of TGF-ß resistance and thus a screening assay for the efficacy of anti-HER-2/*neu* therapeutics.

Expression Profiling at Amgen

Microarray Format:
Incyte-derived comprehensive chips
- Incyte catalog
- Amgen custom-made

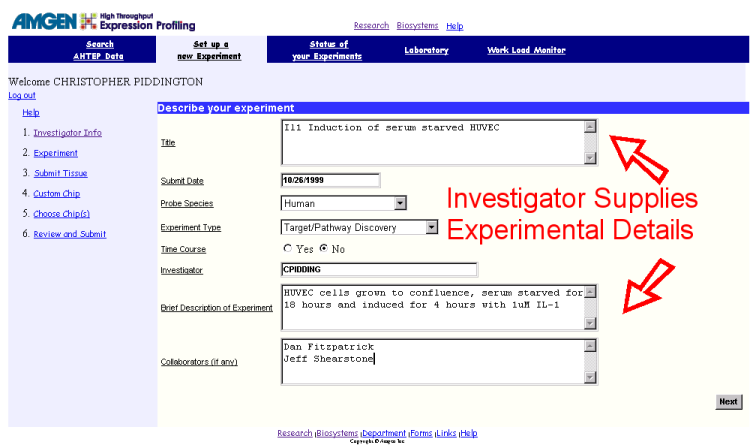
Model of expression profiling usage:
User-initiated

Analysis of Expression Data

- End-user sets up experiment through web-based front-end (EPIMS)
- Expression data managed using Resolver (Rosetta)
  - Basic sorting
  - Clustering:
    - by gene
      - within experiments
      - global
    - by chip
- Internal gene index
  - clones linked by live annotation
  - to link structure, chemoinformatics, external expression DB's

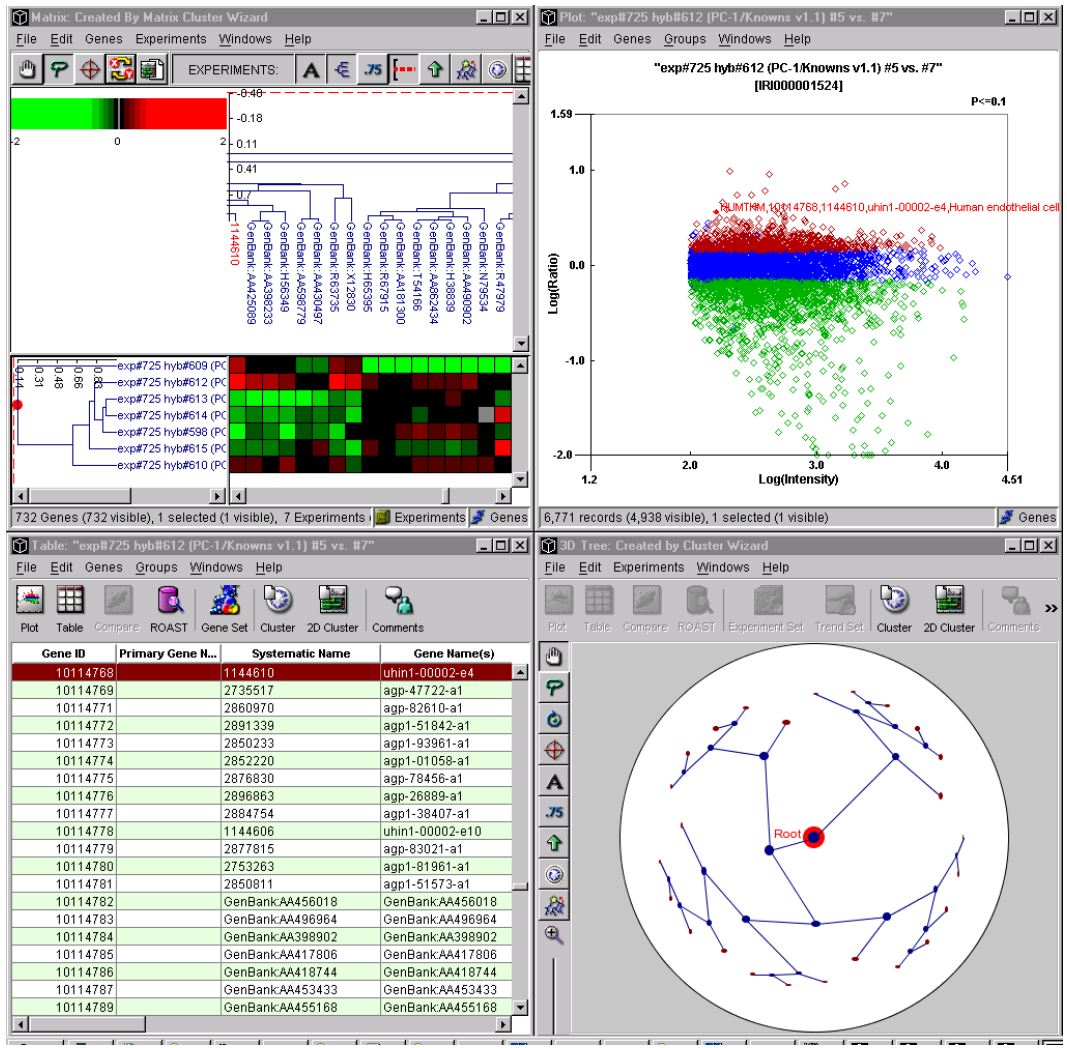
Amgen User Experience

Ordering experiment: web-based EPIMS (Expression Profiling Information Management System)

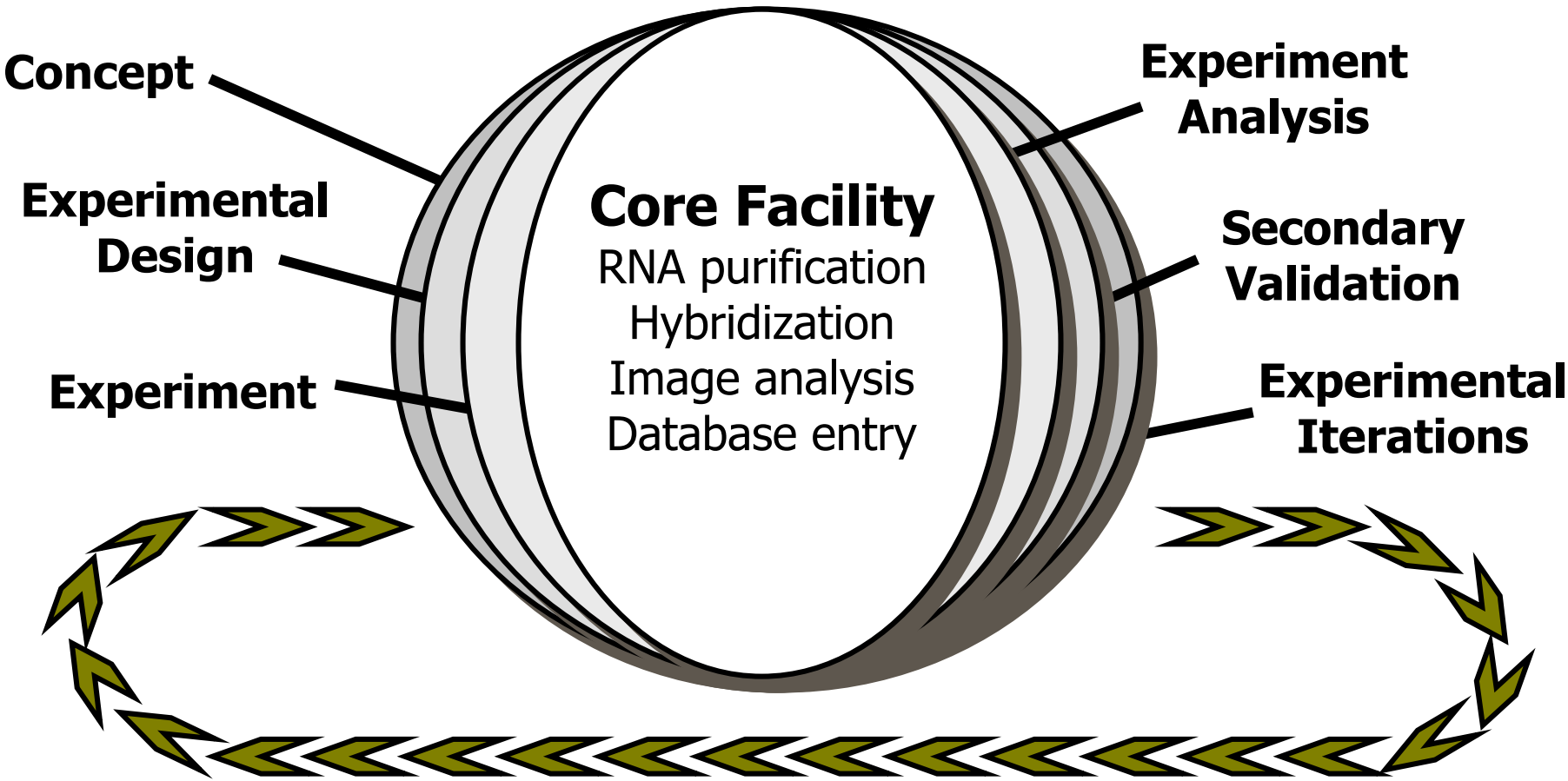


Experimental analysis: web-based Resolver suite.

Multiple Modules for Visualization & Analyses



Multi-tiered Contact Points for Customers



Expression Profiling Component of Pharmacogenomics

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- Toxicology profiling
- Surrogate markers for clinical studies
- Stratify population for clinical trials
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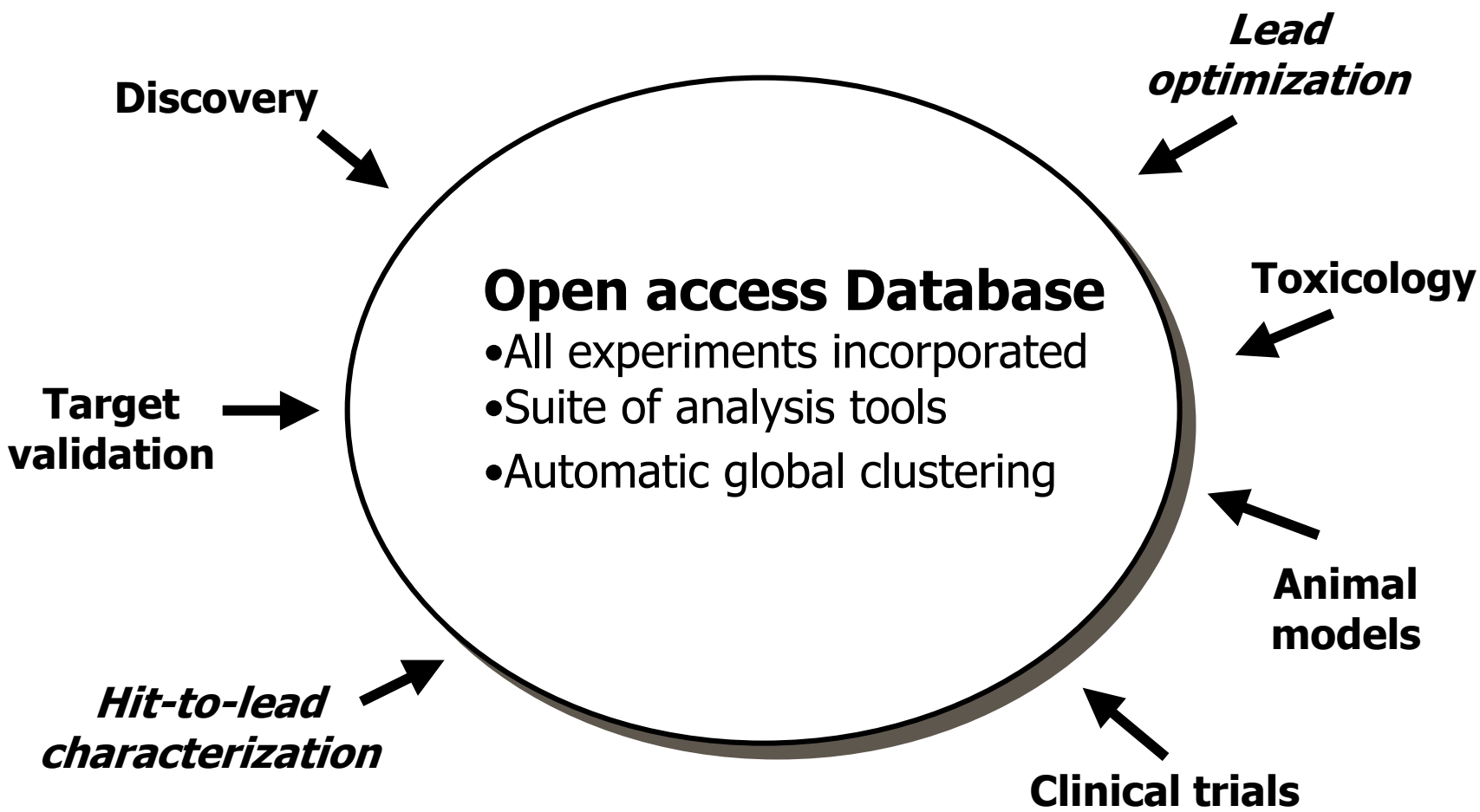
Expression Profiling in Drug Development

- Discovery

Progression

- Target validation
- Characterization of intervention leads
- Animal models
- Clinical trials

Open-access Centralization



Expression Profiling in Drug Development

- Discovery
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Progression

Expression Profiling

Until Now:

Enabling Technology for discovery

Vision for Future:

Interface Technology bridging multiple phases of drug development

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