Laboratory of Molecular Bioengineering & Protein Therapeutics

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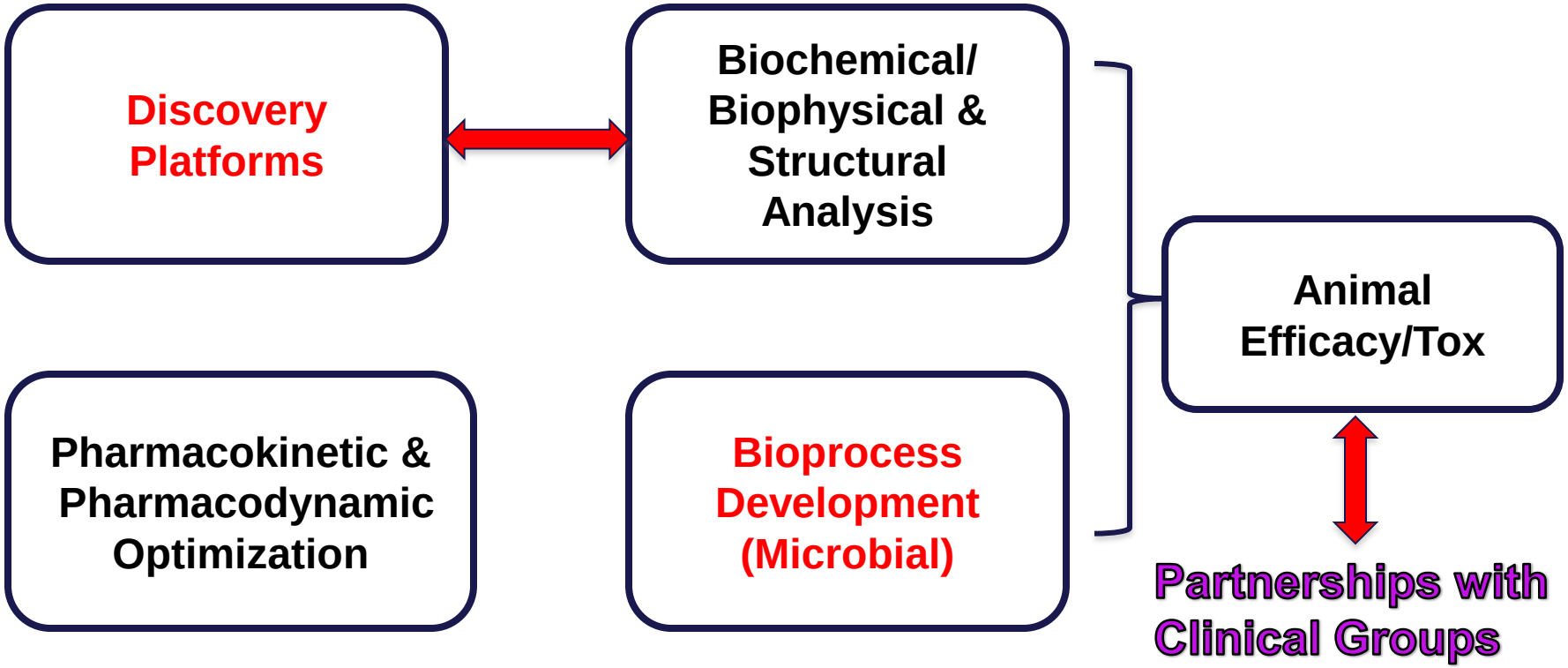
Support by NCI, NIGMS, Cancer Prevention & Research Initiative of Texas, DARPA, ONR, The Clayton Foundation

Discovery & Development of Protein Therapeutics; What do Engineers Do?

- **Understanding the biology & identification of therapeutic targets *(systems biology)***
- ***Early discovery*: HTS/platform technologies for therapeutic protein discovery**
- **Animal models of disease/toxicology**
- ***Lead optimization*: engineering proteins for enhanced therapeutic function, stability**
- ***Pharmacokinetics and Pharmacodynamic Optimization***
- ***Manufacturing/Formulation***
- **Clinical Evaluation**

Discovery Integration & Clinical Translation

GG Lab Therapeutics Program



Our Lab Pursues The Development of Protein Therapeutics from Discovery to Clinical Trials

(unique in engineering)

I. Enzyme Therapeutics for Systemic Metabolite Depletion in Cancer

I. Engineered Enzyme Therapeutic for Cancer

Rapidly proliferating cells have increased metabolic requirements

e.g. high glucose consumption (Warburg effect 1920; molecular mechanism discovered in 2008 by Cantley et al)

AMINO ACID AUXOTROPHIES IN CANCER CELLS

Many cancers are unable to synthesize certain amino acids instead relying on uptake from serum; systemic depletion of as induces selective apoptosis of tumor cells.

Therapeutic modalities for aa deprivation

- **Nutritional restriction**
  - **Difficult to achieve/compliance**
  - **Endogenous synthesis of metabolite can overcome nutritional limitation**

- **Pharmacological (drug-mediated) inhibition of biosynthetic pathways- affects normal and cancer cells, toxicity**

- **Eliminate essential metabolite by injecting an enzyme**

Intravenous Administration of Enzymes For the Systemic Removal of AA Essential for Cancer Survival

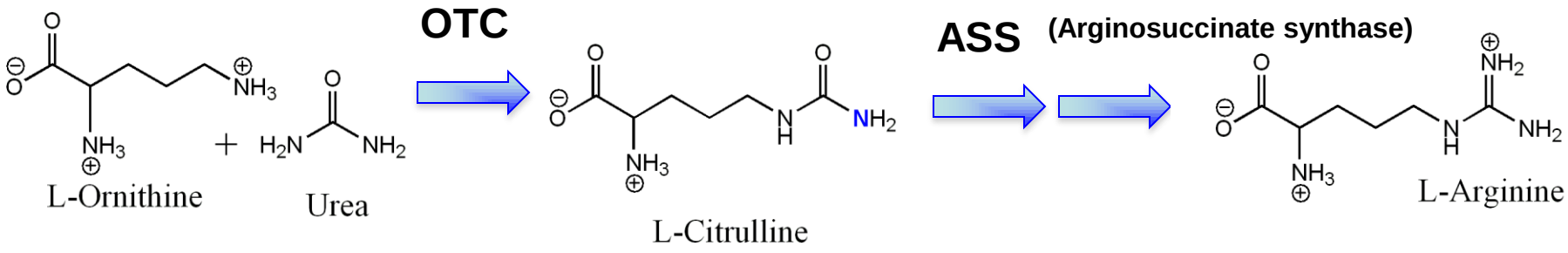
The human genome does not encode enzymes with <u>therapeutically relevant catalytic activity or pharmacological properties</u>

Non-human enzymes that exhibit the proper pharmacological action are immunogenic and elicit anti-enzyme antibodies

- Anaphylactic shock & death (bacterial L-methionine-γ-lyase)

- Inactivation and clearance of the therapeutic protein

Example: L- Argininine Auxotrophy in Cancer



Many high mortality tumors are deficient in ASS and/or OTC synthesis, cannot synthesize L-Arg and require on its uptake from serum

- **Hepatocellular carcinomas (60%)**
- **Metastatic melanoma (35%)**
- **Pancreatic carcinomas (25-30%)**
- **Small cell lung carcinomas (45%)**
- **Acute myeloid leukemias (60%)**
- **Prostate carcinomas**

GG Lab Protein Therapeutic Pipeline

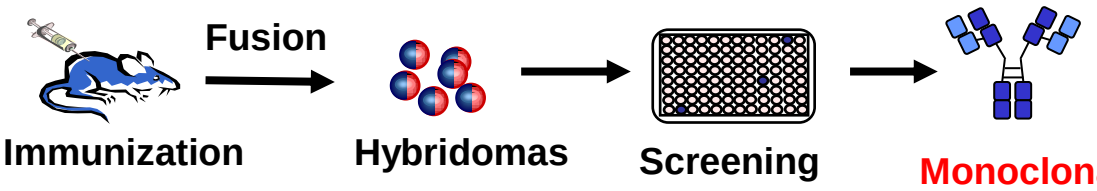
Early Stage Development				Late Stage		Clinical
Disease	**Lead Molecule**	**Mechanism of Action**	**Animal PK/PD & Efficacy**	**Bio-Processing**	**GMP/ Formal Tox**	**Phase I**
Metastatic melanoma **Hepatic carcinoma**	**Eng. hu Arginase I** ***[Mn-huArgI-PGE5K]***	**Systemic L-Arg depletion**	✓ ✓ ✓	**Yes**	***In progress*** **IND planned Sep '11**	**4th qt '11** **Melanoma AML, HCC**
CNS tumors (GB, NB)	**Eng hu Cystathione γ-Lyase**	**Systemic L-Met Depletion**	✓ ✓ ✓	***Planned***	***2nd Qt '12***	***3rd Qt 12***
Adult ALL, other lymphomas	**Eng hu Aspragi-nase**	**Systemic L-Asn Depletion**				
Inhalation Anthrax	**Anthim® (Eng Ab)**	**Anthrax Toxin neutralization**	***Elusys Inc***			***Completed***

II. Therapeutic Antibodies

1. Antibody Discovery Technologies

I. *Monoclonal Antibodies by B cell immortalization or cloning*

- Hybridoma technology



- Immortalization by EBV transfection
- Single B cell cloning using microfluidic platforms

II. Abs *by HTS of antibody ensembles (libraries) produced in microorganisms*

(multibillion dollar business)

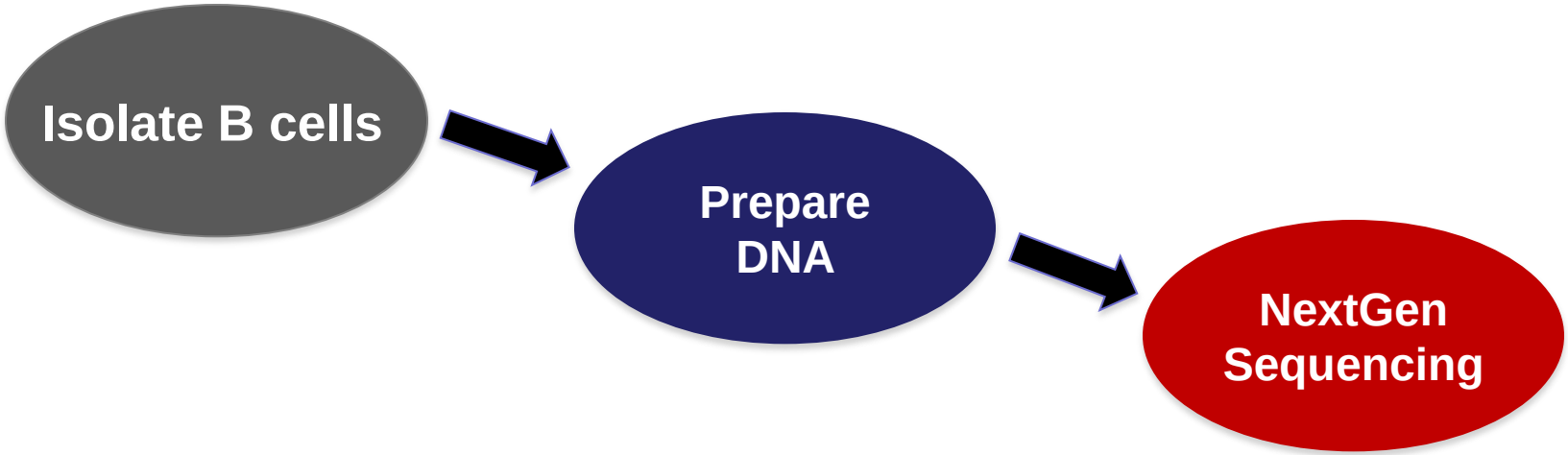
Ab libraries: >10^8 different proteins made by mol bio techniques from

- **B cells post immunization**
- **Unimmunized (naïve) individuals**
- ***Randomizing specific regions of Ab***

→ **High Throughput Screening**

III. "Third Wave": Ab discovery via NextGen DNA sequencing & bioinformatics

Reddy et al. *Nature Biotechnol* (Sept 2010)



- What antibodies are produced in higher amounts?
- How many different antibodies?
- Abs in secretory fluids (intestine, lung, mouth) vs blood?

What Molecules or Pathogens do they Recognize?

Converting Information to physical measurements

On Going Antibody Projects

Therapeutics and Diagnostics

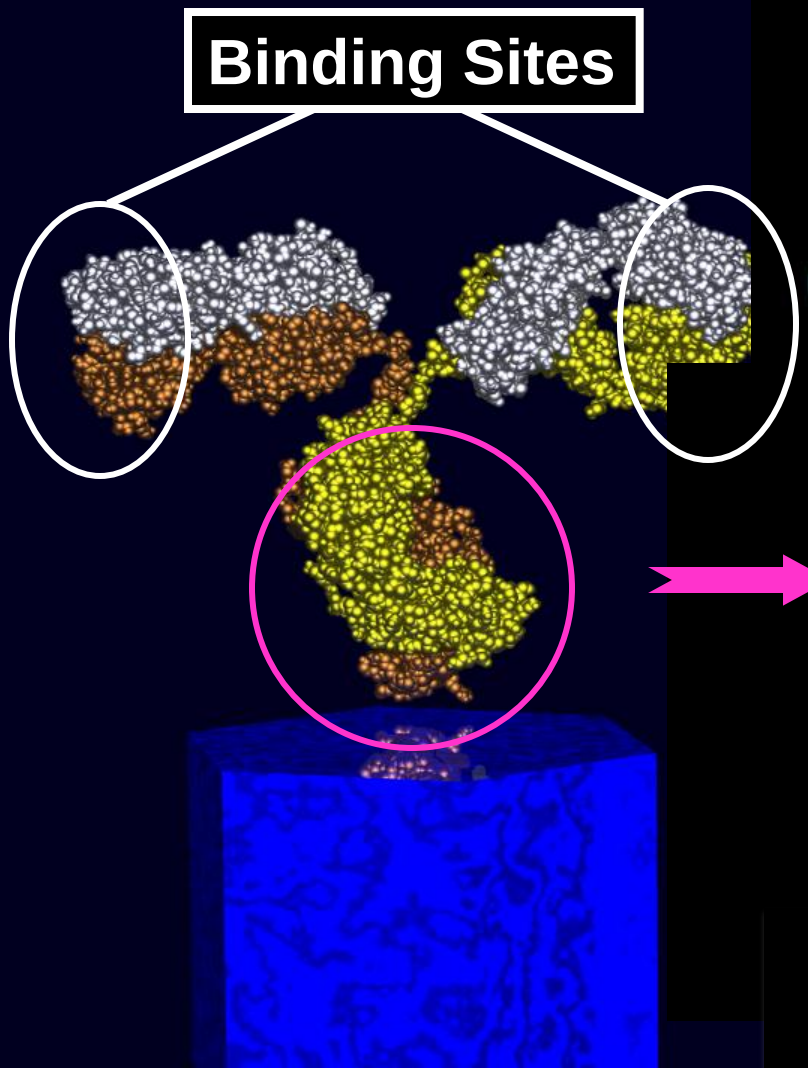
- ♠ Antibodies for neutralization of SARS-CorV
- ♠ Complement inhibition (ischemic reperfusion injury, etc)
- ♠ Breast cancer, Ovarian cancer, Lymphomas

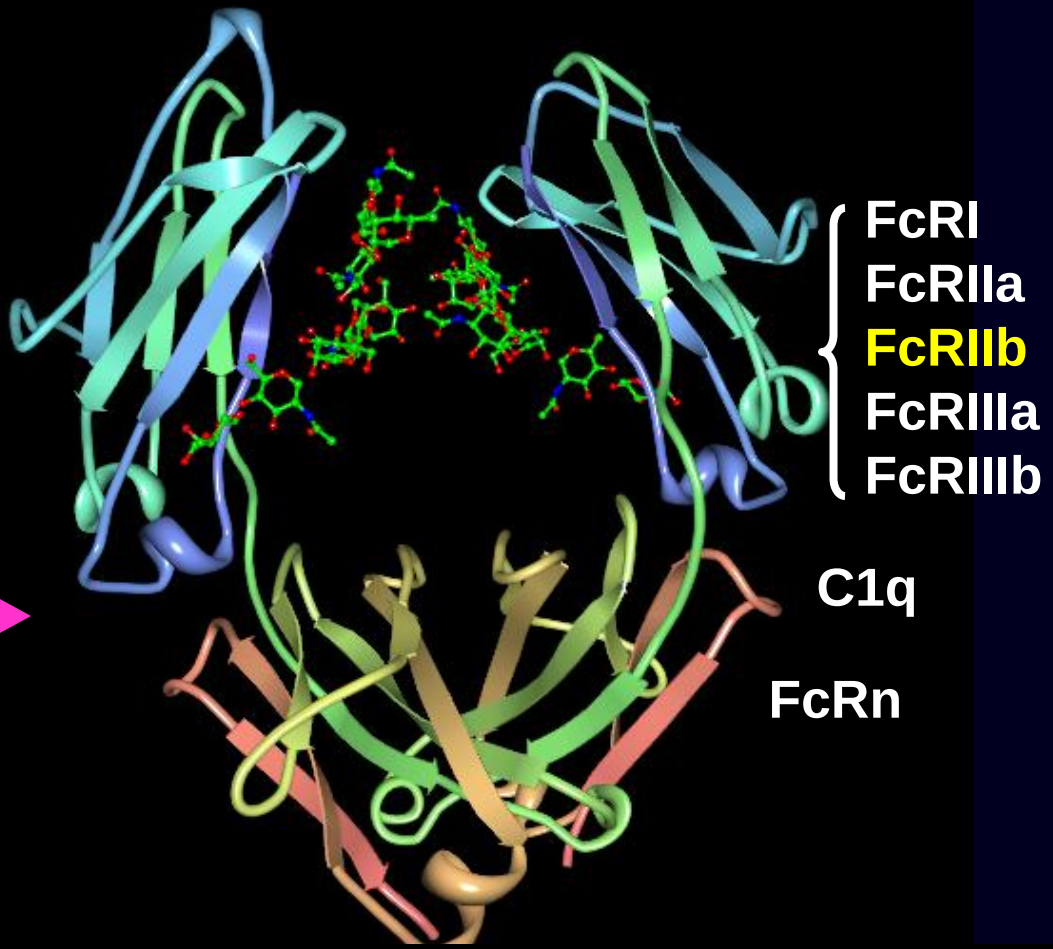
Understanding Mammalian Antibody Immunity

- ♠ How is the antibody repertoire formed

II. EngineeringAntibody Drugs Displaying Optimized Therapeutic Efficacy

II. Antibody Therapeutic Optimization: Engineering Antibodies for Target Cell Killing (ADCC) and the *Induction of Adaptive Immune Responses*



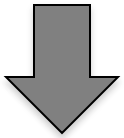


Fc Domain: Engaging the Immune System

II. Antibody Therapeutic Optimization

- **Antibody:antigen complex ligation of the activating FcγR receptors elicits potent target cell killing by macrophages, natural killer cells, dendritic cells and granulocytes (ADCC)**
- **Essential for the action of Rituxan, important for Herceptin, Erbitux**
- **Huge investment on engineered antibodies with improved cytotoxicity e.g. second generation Rituxan, *Roche GA101, P. Umana*)**

However, all antibodies engage the FcγRIIb receptor which mediates powerful anti-inflammatory responses, B cell apoptosis and inhibits immune complex mediated dendritic cell activation



Engineered first-in-class antibodies that bind exclusively to activating receptors and not to FcγRIIb (*Jung et al. PNAS 2010*)

- **Evidence for induction of adaptive immunity**
- **Engineered Herceptin in evaluation in humanized mice**

(*NOD scid IL*$\gamma 2^{-/-}$ engrafted with human HSC and bearing Her2 tumors)

Acknowledgements

Our incredible team of students, post-docs and research scientists

Our many, many collaborators

University of Texas, Austin

Andy Ellington –Gene Synthesis
Scott Hunicke-Smith; NextGen sequencing
Brent L. Iverson –Bioorganic chemistry
Ed Marcotte –Proteomics
Phil Tucker -Immunology
Carla VanDenBerg -Pharmacology
Jessie Zhang -Protein crystallography

Key Collaborators

Art Frankel, Scott & White
Jon Beckwith, Harvard

Also with various groups from

MD Anderson Cancer Center
Memorial Sloan Kettering
Harvard
Stanford Medical School
U. Chicago