Translational Laboratory SS at UMGCC
7-010 BRB

- Make preclinical *in vitro* and *in vivo* model expertise available to UMGCC members and facilitate the translation of novel therapeutic concepts from bench to bedside

- Provide UMGCC clinicians with the means to perform early clinical trials of molecularly targeted drugs that require the assessment of pharmacodynamic endpoints

- Add value to advanced preclinical developments to create a pipeline of new drugs for UMGCC clinicians in Phase I and II studies

http://www.umgcc.org/research/translational_core_lab.htm
Outline of discussion

- Introduction to Drug Development
- Basic Research/Idea generation
- HTS/Lead optimization
- Pre-clinical Development
- Clinical Trials
- Repurposing/Translational Research
Drug Development Time Line

8-15 years or more

Discovery Phases
Pre-Clinical Phases
Clinical Testing
Commercial Phase

50 minutes
Where does it start?
Discovery: Idea Generation

- chemist, biologist, physician
- Usually based on
  - Target (protein/rna/dna)
  - Chemical (synthetic or natural)
  - Population (identify a problem to be fixed)
  - Observation
- Novel compounds/recycling of old drugs
- Natural Compounds

IN DRUG DEVELOPMENT, MOST ROADS LEAD TO BACK TO CHEMISTRY
Discovery: Typical Targets

Fractional content of marketed drugs according to their biochemical targets

Overington et al. Nature Reviews Drug Discovery 5, 993–996 (December 2006) | doi:10.1038/nrd2199
Drug Discovery

- Natural compounds;
- Synthetic chemical libraries;
- Combinatorial chemical libraries;
- Molecular modeling;
- Pharmaceutical biotechnology.

2-3 Years
“Hit” improvement
“Lead” improvement
Drug candidate

1-2 Years
Pre-clinical
- Toxicity;
- Efficacy;
- Dose response.

4-6 Years
Clinical
- Adverse response;
- Efficacy;
- Responder/non-responder
Discovery: High Throughput Screening

- Critical Step in process
- Results in **starting point** for drug design
- Protein, DNA, pharmacologic, siRNA based
- Screen libraries with millions of entities
- Microtiter plates
- Robotic/automated

- Start the chemistry process
- Results in a compound that chemists can modify
  - Rarely is this compound patentable
Discovery: Libraries of Compounds

➢ most chemical libraries focus on large groups of varied organic chemical series

➢ chemical structure, purity, quantity, and physiochemical characteristics of the compound.

➢ Virtual – “Computer Aided Drug Design”
Zimmer DB, Lapidus RG, Weber D. *In Vivo* Screening of S100B Inhibitors for Melanoma Therapy. In: Calcium-Binding Proteins: Methods and Protocols Ed. B.
Lead Optimization - 1

- MUST KNOW FINAL GOAL OF DRUG FOR THIS STEP TO DEFINE PARAMETERS
  - Target disease
  - Acute vs chronic administration
  - Best route of delivery
    - Intravenous
    - subcutaneous
    - Intramuscular (e.g., insulin)
    - Oral administration
Lead Optimization - II

- Unlikely that a perfect drug candidate will emerge from early screens.
- Several compounds are found to have some degree of activity.
- Similar structures - pharmacophores can then be developed.
- Medicinal chemists will attempt to use structure-activity relationships (SAR) to improve certain features of the lead compounds.
OTHER GOALS

- increase activity against the chosen target
- reduce activity against unrelated targets
- improve the “druggability” of the molecule
  - Increased solubility
  - Optimized pharmacokinetic parameters,
Tools for Lead Optimization

- **In Vitro Screens**
  - Target based assay - looking for increase affinity
  - Off target assays - looking for decreased affinity
  - Cell culture assays
  - Absorption/metabolisms

- **In Vivo Screens**
  - In Vivo Screen
  - Pharmacokinetics
  - Efficacy Model
Bench to Bedside: preclinical development (w/ continued lead optimization)
Preclinical Development

- Toxicology
  - Maximum Tolerated Dose
- ADME
  - absorption/distribution/metabolism/excretion
  - Route of administration (dependent on disease)
- Efficacy Models
  - Minimally Effective Dose
  - Therapeutic Window
- GLP Toxicology
- Formulation/CMC
  - (Chemistry, Manufacturing and Control)
Tolerability

- Usually start with rodents (mice/rats)
- Usually choose mice (less drug substance)
- Outbred inexpensive mice
  - Bridging tox study
  - Necropsy/IHC target organs
- Route of administration and dosing scheme for efficacy study
- Rule to thumb in cancer:
  - 20% body weight loss/LD10 is MTD
Predictive ADME

Absorption
Distribution
Metabolism
Elimination

Pharmacokinetic Bioavailability
pharmacokinetics and bioavailability

- Optimization problem
  - Poor systemic exposure
    - Distribution
      - Volume of distribution
    - Blood–brain barrier
    - Transporters
      - P-gp
      - MRP
      - OATP
      - OCTP
  - Clearance
    - Renal
    - Plasma
    - Hepatic
  - First-pass clearance
  - Absorption
    - Gut stability
      - Physicochemical properties
    - Membrane permeation
      - pKₐ
      - Solubility
      - LogP/D
      - Paracellular
      - Transcellular
    - Which conjugate?
      - Glucuronide
      - Sulphate
      - Amino acids
    - Others
      - Regiospecificity
      - Lability
      - Affinity
      - Induction
        - PXR
        - CAR
        - AHR
      - Type II binding
      - Inhibition
        - Mechanistic
For each actual marketed drug (*new chemical entity, NCE*) there have been more than 1000 substances that underwent screened *in vitro*. Without the use of available computer-based ADMET filters, this number would be even larger.
Efficacy Models

- Relevant Model
- Relevant Route of administration
- Minimally Effective Dose
- Therapeutic Window
  - depends on disease
GPI 21016 – lead candidate

- Oral (many species)
- Brain penetrable
- Accumulates in tumor and inhibits PARP for 24 hr
- Potent (Ki = 50 nM) and selective for PARP
- Efficacious in intracranial and xenograft models at doses of 10 – 40 mg/kg p.o.
- Well tolerated in rats and dogs; no exacerbation of TMZ toxicity
PK: GPI 21016 is brain penetrable
Efficacy: Oral GPI 21016 + TMZ Increases Survival Bearing SJGBM Glioma at CNS Site

![Graph showing percent survival over days for different treatments: Vehicle, TMZ 100 mg/kg IP qdx5, TMZ + 40 mg/kg GPI 21016 PO. The graph indicates a statistical significance of P<0.05.](image-url)
FDA PRECLINICAL PHARMACOLOGY & TOXICOLOGY REQUIREMENTS

• **DRUGS**
  – Two Species - Rodent & Non-rodent
  – Clinical Route & Schedule
    • Follow NCI Guidelines
  – Pharmacokinetics - Optional

• **BIOLOGICALS**
  – Most Relevant Species
  – Clinical Route & Schedule
Formulation/CMC

“Chemistry/Manufacturing and Control”
FILE AN IND

“investigational new drug application”
Terminology

• Translational
• Efficacy
• Pharmacodynamic (PD) – assay in development
• Biomarker – proven, used clinically (e.g., ER)
• Pharmacokinetics (PK)
• Therapeutic Window
• Maximum Tolerated Dose (MTD)
• CR = complete response
• PR = partial response
• OS = overall survival
• EFS = event-free survival
• PFS = progression free survival
Clinical Trials – Phase I

IND (investigational new drug application)

- first stage of testing in human subjects
- 1st use of a new agent or combo in humans
- small (20-100) group of healthy volunteers
- in cancer specifically – late stage disease
- Dose limiting toxicity (DLT)

- assess the safety (pharmacovigilance), tolerability, pharmacokinetics, and pharmacodynamics of a drug.
Clinical Trial: Phase I cont

- Classic dose escalation schedule
  - Treat three patients / dose level
  - Wait until all patients complete dose level
  - Grade toxicity: (NCI CTC, WHO, etc)
    - Grade 0: No toxicity
    - Grade 1: Adverse event not require Rx
    - Grade 2: Adverse event Rxable
    - Grade 3: Potentially Life threatening or cause hospitalization
    - Grade 4: Actually Life threatening
    - Grade 5: Cause death
Clinical Trials – Phase II

- Assess efficacy, feasibility, toxicity
- Larger groups (20-300)
- Assess how well the drug works
- Continue Phase I safety assessments in a larger group of volunteers and patients
- Many drugs fail in Phase II trials when the drug is discovered not to work as planned, or to have toxic effects
Clinical Trials – Phase III

- randomized controlled multicenter trials on large patient group
  - 300–3,000 or more depending upon the disease/medical condition studied
- definitive assessment of how effective the drug is in comparison with current 'gold standard' treatment.
Success Rates of Clinical Trials

1 in 6 drugs are Approved By FDA

New Drug Development Still Risky


* Data from CT run by top 50 Pharma Co – drug programs w/ > scrutiny
• 21 CFR 314.126: “Reports of *adequate and well controlled*\(^1\) investigations provide the primary basis for determining whether there is *substantial evidence*\(^2\) to support claims of effectiveness for new drugs and antibiotics.

\(^{1,2}\): Could be placebo controlled, no-active treatment controlled, comparison of doses, historical comparison.

• 2: Two adequate and well controlled trials although in some cases one trial with supporting studies may be sufficient.
FILE AN NDA
“New Drug Application”
Clinical Trials – Phase IV

- finding a new market for the drug
  - test for interactions with other drugs
  - test effect certain population groups such as pregnant women, who are unlikely to subject themselves to trials
  - Long term effects of treatment
  - Satisfy requirement for conversion from accelerated approval to full approval
A Success Story

The story of Gleevec
Chronic Myeloid Leukemia

• ~6600 newly diagnosed patients/year in US (2015)

• FEW EX of a MALIGNANCY where a SINGLE signaling pathway defect is cause of disease

• Reciprocal translocation between chromosomes 9 and 22 (Philadelphia chromosome)

• only occurs in the bone marrow, not in other organs
  • Not a germ line mutation – no risk of inheritance

• Results in formation bcr-abl oncogene

• bcr-abl protein, a tyrosine kinase, is produced in these cells
  • allow the white blood cells to grow uncontrollably (increased platelets, decreased RBCs)

• 95% of CML and 15 to 30% with ALL

• DISCOVERY OF Imatinib critical in treatment of disease

• Specifically targets bcr-abl
Imatinib = gleevec = ST1571
a great example

• A great example of rational design drug development and translational research
• Researchers discover the chromosomal translocation leading to bcr-abl protein (Philadelphia chromosome)
• Screening of chemicals to target this tyrosine kinase specifically
• ST1571 (gleevec, imatinib) identified
• Now, patients are screened for Philadelphia chromosome prior to treatment

http://www.gleevec.com/index.jsp
Drug Development of Gleevec
“a cinderella story”

STI571: An oral in vivo bcr-abl kinase inhibitor

N
N
N
NH NH O
Me
N
N
Me

Tyr phosphorylation in vivo

le Coutre et al, JNCI 91:163, 1999
Pathogenesis of Chronic Myeloid Leukemia before Imatinib

- Chronic phase: Median 4–6 years stabilization
- Advanced phases:
  - Accelerated phase: Median duration up to 1 year
  - Blastic phase (blast crisis): Median survival 3–6 months

Capdeville et al Nature Reviews 1: 493-502
PFS with Imatinib (Gleevec)

% without progression

Years since randomization

GLEEVEC arm: 81% estimated PFS rate (95% CI: 78, 85)
IFN-α arm: 61% estimated PFS rate (95% CI: 56, 65)

Gleevec.com
TWO LAST TOPICS

• Translational Research

• Repurposing
Translational Research
Definition of Translation Research in the cancer arena

• "Translational research transforms scientific discoveries arising from laboratory, clinical, or population studies into clinical applications to reduce cancer incidence, morbidity, and mortality."

NCI website

“translation” of scientific ideas into clinical practice

In clinical practice – use of scientific/basic Principles to push forward ideas

Definition of Translational Research

Can be used in the clinic and preclinically!

# Significance of Translational Research

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tumor</th>
<th>Survival Gain (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemcitabine (15)</td>
<td>Pancreas</td>
<td>1.5</td>
</tr>
<tr>
<td>Bevacizumab (16)</td>
<td>Colon</td>
<td>2.2</td>
</tr>
<tr>
<td>Erlotinib (19)</td>
<td>Pancreas</td>
<td>0.4</td>
</tr>
<tr>
<td>Bevacizumab (17)</td>
<td>NSCLC</td>
<td>2</td>
</tr>
<tr>
<td>Sorafenib (20)</td>
<td>renal</td>
<td>2</td>
</tr>
<tr>
<td>Temozolamide (18)</td>
<td>GBM</td>
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<td>Cervix</td>
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<tr>
<td>Bevacizumab (25)</td>
<td>Breast</td>
<td>1.5</td>
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Recent “Significant Advances” ($P < 0.05$) in Epithelial Malignancies in Patients Who **Had Not Been Selected** on the Basis of Molecular Characteristics

Erlotinib = Tarceva

• Another great example of rational design drug development and translational research
• Researchers determined that patients with mutant EGF receptors respond better to inhibitors than those with WT receptors
• Led to development of specific and ‘less specific’ TKI (Tyrosine kinases inhibitors)
• All lung cancer patients now screened for EGFR mutation status prior to treatment
Addition of erlotinib to frontline chemotherapy in advanced NSCLC had no apparent impact on PFS in the overall population, (13% w/ EGFR mutations and 21% w/ K-ras mutations).


Eberhard D A et al. JCO 2005;23:5900-5909

Lung Cancer 2012
New cases: 226,150.
5 yr survival – 15.7%
Cancer.gov
EGFR in Lung Cancer

• Mutations in the exons 18-21 around the ATP binding pocket of the TK domain leads to enhanced TK activity in response to EGF

• Mutations lead to increased sensitivity to EGFR inhibitors

Gefitinib (Iressa)

Erlotinib

Oral daily administration
Erlotinib in NSCLC
Effect of EGFR mutations

Eberhard D A et al. JCO 2005;23:5900-5909
Repurposing

• Definition

• Examples
  – Metformin
    • Epidemiologic studies in Type II diabetes patients with cancer
    • In vitro and in vivo data
    • Mechanistic hypotheses
    • Advantages of approved drug
    • Affordable (months supply $5)
SUMMARY

• Drug development is complex and involved:
  • New targets
  • New molecules
  • Better Understanding of Disease

• Life Scientists are the "gate keepers" for interesting opportunities for drug discovery and development

• A laboratory observation is conceivably a starting point for a team of investigations with scientific, clinical, ethical, and societal implications
Questions???
The drug discovery pipeline

Preclinical phase

Clinical trials

Market launch

Bench to Bedside

- Basic Research
  - mRNA Localization
- Translational Research
  - Drug Discovery
  - Therapeutics
- Applied Research
  - Disease Models
Steps in cancer drug discovery & development leading to clinical trials

- DEFINE DRUG TARGET OR DEFINE AN "ACTIVE" DRUG

- OPTIMIZE EVIDENCE OF ACTIVITY IN ANIMAL MODELS OF CANCER (DOSE / SCHEDULE)

- RELATE ACTIVITY (OR LACK THEREOF) IN ANIMAL MODELS TO CONCENTRATIONS AND DURATIONS OF DRUG EXPOSURE

- DEFINE IN ANIMALS A SAFE STARTING DOSE FOR HUMAN CLINICAL TRIALS

- THIS INFORMATION ASSEMBLED INTO AN "INVESTIGATIONAL NEW DRUG" ("IND") APPLICATION TO THE FDA
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In Vitro Assay for PARP inhibition

- Potency defined as ability to compete with 3H-NAD

IC50 40nM
PK: GPI 21016 is orally bioavailable

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<td>PO Tmax</td>
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nd: not determined
PK: GPI 21016 is brain penetrable
CANCER DRUG DEVELOPMENT:
PRACTICE & PITFALLS

• Cancer Drug Discovery
  • Historical trends: empirical / rational approaches
  • Target qualification

• Vetting a Molecule for transition to the clinic
  • Animal models
  • Pharmacology & Toxicology

• Clinical Drug Development Strategies
  • Phase I
  • Phase II
  • Phase III
  • Adjuvant
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<td><strong>PO Bioavailability</strong></td>
<td>4.9%</td>
<td>ND</td>
<td>25.6 - 60.0%</td>
<td>10 mg/kg: 13.0%</td>
<td>31.1%</td>
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<tr>
<td></td>
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<td>10 - 100 mg/kg: 40.1 - 45.7%</td>
<td>30 mg/kg: 21.1%</td>
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PK: GPI 21016 is brain penetratable

GPI 21016 Rat IV Single Dose PK DMPK04-106
30 mg/kg Males

[Graph showing time (hr) vs. [GPI 21016] (ng/mL or ng/g) for Plasma, Brain, and Heart. IC50 approx]
Cycle of optimization in the drug discovery pipeline

Fractional content of marketed drugs according to their biochemical targets

Clinical Trials – Phase II cont

- Single stage
  - Treat preset group to define level of activity for further testing
- Two Stage
  - Treat a small number of patients and if not observe activity above a certain level, close.
- Randomized Phase II:
  - powered to detect likely similarity between treatments – not powered for efficacy
  - Suggest basis for proceeding to formal Phase III
- If rare disease / response to standard therapy insufficiently documented / heterogeneity of prognostic factors / test multiple new therapies simultaneously
Discovery: Selection of compounds for High Throughput Screening (HTS)

- Project virtual library of 100,000 members

   - Clustering
   - Solubility model
   - Absorption model
   - Metabolism model
   - BBB model

   6000 Subset
   3000
   1450
   1150
   700
   500

   Selected for synthesis and screening

*Drug Discovery Today*