Nonclinical Drug Development

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Translational Medicine Early Development
Oncology Therapeutic Area
Janssen R&D/Johnson & Johnson
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Disclosure Information
Chris H. Takimoto, MD, PhD

- **Employment**: Janssen R&D/Johnson & Johnson
- **Stockholder**: Johnson & Johnson
- **Off Label Use**: I will not discuss off label use of any products, but I will discuss an experimental study with Carlimab, an anti-CCL2 antibody

Lecture Outline

- Nonclinical Drug Development Definitions & Scope
- Components of Nonclinical Drug Development
  - Pharmacology Studies
  - Safety Pharmacology
  - PK/ADME Studies
  - Toxicology
  - Starting Dose Selection and Study Design Issues for FIH
- Nonclinical Translational Research Strategies
  - Targeted therapies/Biomarkers
  - Pharmacological Audit Trail/Model-based drug development
  - Translational clinical development plans
  - PK-PD Modeling in clinical trial interpretation
Nonclinical Drug Development

- **Broad Definition**: All the activities required before a new molecular entity can be administered to humans
  - Spans gap between discovery/screening to FIH clinical trials
- **Current Discussion**
  - Focus on pharmacology, safety, toxicology, and translational research strategies in nonclinical development
  - Will not discuss API, CMC, and formulation issues

*Bias Warning!: Large pharmaceutical, small molecule anticancer drug development perspective*

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Nonclinical Drug Development

An Industrial Perspective

![Diagram](image)

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Components of Nonclinical Drug Development

- Pharmacology studies/Model selection
- Safety pharmacology
- PK/ADME studies
- Toxicology
- Starting dose selection and study design issues for FIH
S9 Oncology Specific Guidance

- Applies to targeted small molecules and biopharmaceuticals used for treating “patients with advanced disease and limited therapeutic options”
  - Advanced cancer is a progressive, fatal disease
  - Existing therapies have limited effectiveness
  - Treatment at or close to adverse effect dose levels
- Type, timing, and flexibility of oncology studies may differ from other therapeutic areas
- Does NOT apply to cancer prevention, supportive care, healthy volunteers, radiopharmaceuticals, vaccines, cellular or gene therapies

-- S9 Guidance for Industry, 2010

S9 Oncology Specific Guidance

Goals of Nonclinical Testing

1. Identify the pharmacologic properties of a pharmaceutical
2. Understand the toxicological profile of a pharmaceutical
3. Establish a safe initial dose level of the first human exposure

-- S9 Guidance for Industry, 2010
Nonclinical Drug Development
In Vitro Pharmacology Models

- In vitro studies performed in cell lines or cell-free systems
  - Often form the basis for screening and optimization during discovery
- Oncology uses human tumor cell lines for evaluation of:
  - Mechanism of action
  - Evaluation of potency and selectivity
  - Early indication selection
  - Predictive biomarker discovery

In Vitro Cell Line Analyses

Limitations of 2D Tumor Models
Tumor Microenvironment

**Humanized 3D Models**
(for Advanced Biomarker and Drug Discovery Applications)

<table>
<thead>
<tr>
<th>Standard 2D Culture</th>
<th>3D-TGA</th>
<th>&quot;Humanized&quot; 3D-TGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cells alone or co-culture</td>
<td>Tumor cells alone in 3D BiPME</td>
<td>Tumor cells + (10:1) hMSC/hCAF in 3D BiPME</td>
</tr>
</tbody>
</table>

"TME-Aligned" 3D-TGA

<table>
<thead>
<tr>
<th>&quot;Humanized&quot; xenografts</th>
</tr>
</thead>
</table>

Tumor cells + (10:1) hMSC/hCAF in 3D BiPME

**Abbreviations:** TGA, tumor growth assay; IrBME, Irradiated basement membrane extract; hMSC, human mesenchymal stem cells; hCAF, human cancer associated fibroblasts; TME, tumor microenvironment

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**In Vivo Animal Models**

- The ideal animal model should be:
  - Valid
  - Selective
  - Predictable
  - Reproducible

- There is no perfect tumor model
  - All models are wrong, some are useful

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**Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth**

**In Vivo Efficacy Models in Cancer**

- Spontaneous tumors
  - Idiopathic
  - Carcinogen-induced
  - Transgenic/gene knockout animals: p53, RB, etc

- Transplanted tumors
  - Syngeneic animal tumors: Lewis lung, S180 sarcoma, B16 melanoma murine tumors
  - Human tumors growing in vivo in implantable hollow fibers

**Human Tumor Xenografts Models**

- Most common in vivo preclinical efficacy models in oncology
  - Current NCI standard in vivo efficacy testing system
- Consist of human tumor cells implanted in immunocompromised animals
  - Nude mice
  - SCID mice
  - Nude rats
- Diverse human tumor cell lines propagated in vitro can grow as xenograft models

**Nude Mouse Hosts for Xenograft Studies**

- Athymic “nude” mice developed in 1960’s
- Mutation in nu gene on chromosome 11
- Phenotype: retarded growth, low fertility, no fur, immunocompromised
  - Lack of a thymus gland causing impaired T-cell immunity
- First human tumor xenograft of colon adenocarcinoma by Rygaard & Poulson, 1969
Differential Tumor Growth of Prostate Cancer Xenografts

(Mahajan, Cancer Res 2005;65:10514)

Xenograft Advantages

- Diverse selection of different human tumor types
  - Molecular characterization, GEP, available in public databases
- Ease and speed of start up and conduct of studies
- Simultaneous evaluation of safety and efficacy (therapeutic index)
- Some correlation with clinical activity lung, colon, breast, and melanoma cancers
- Although subcutaneous implantation is most common, orthotopic injections are possible
  - Mammary fat pad, CNS, intraperitoneal, etc
- Wide accessibility
- Many decades of experience

Xenograft Disadvantages

- Atypical biological behavior
  - Metastases are rare
  - Survival not an ideal endpoint, with historical deaths from tumor bulk, not invasion
  - Short doubling times
  - Less necrosis, better blood supply
- Positive predictive value is poor
- Poorly mimics the tumor microenvironment
  - Human tumor cells with murine stroma
  - Host directed therapies (immunomodulation, stromal tissue targets) may not be applicable
  - Species specific differences between humans and mice
  - Examples: Antibody biopharmaceutics that only recognize human targets
Low Passage Patient Derived Xenografts

Primary human tumors

Patient Derived Xenograft Clinical Correlations

Colorectal Tumograft

Myoepithelial Salivary Gland Tumograft

Transgenic Animal Models of Cancer

- p53 or other tumor suppressor gene knockout animals have high incidence of endogenous tumor development
  - Advantages
    - Theoretically more analogous to humans
    - May preserve the immune system
    - Murine tumor and stroma
    - Better for cancer prevention
    - May be engineered for specific purposes
  - Disadvantages
    - Long experimental start up times
    - Variable penetrance
    - Monitoring tumor growth in individual animals is challenging

- Knock-in animals engineered to express human targets
  - Example: hHGF engineered animals (ligand for cMET receptor)
Components of Nonclinical Drug Development

- Pharmacology Studies/Model Selection
- Safety Pharmacology
- PK/ADME Studies
- Toxicology
- Starting Dose Selection and study design issues for FIH

Safety Pharmacology Studies

- For non-oncology agents, a core battery of safety pharmacology tests is required (ICH S7A, Section 2.7)
  - Central Nervous System
  - Cardiovascular System
  - Respiratory System
- Additional supplemental studies must be individualized for each drug
  - May incorporate into general toxicology studies
- Oncology recommendations (S9 Guidance)
  - Vital organ assessment still required, but may not need stand alone safety studies in the absence of specific risk
  - Incorporate core vital organ evaluation into cGLP toxicology studies
- References
  - S9 Guidance 2010
  - S7A Safety Pharmacology Studies for Human Pharmaceuticals, 2000

Safety Pharmacology Studies

- QTc Prolongation Risk Assessment
  - Prolonged QTc caused by delayed ventricular repolarization
    - Increased risk of ventricular arrhythmias, especially Torsade de Pointes
    - Increased risk with hypokalemia, structural heart disease, or bradycardia
  - Late repolarization of cardiac action potential
    - Mediated by efflux of K+ (I_Na and I_L) through delayed rectifier K+ channels
  - Human ether-a-go-go-related gene (hERG)
    - Encodes the alpha subunit of the human K+ channel proteins responsible for IKr
    - Basis for propranolol in vitro testing for QTc prolongation risk
  - Pharmaceuticals that prolong QTc can have proarrhythmic effects
- References
  - S7B, Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization, 2000
Nonclinical QTc Testing Strategy (ICH S7B, 2005)

- Routine Nonclinical Tests
  - In Vitro \( I_{\text{sc}} \) (hERG) assay, and
  - In vivo QT assay in nonrodent species
    - May incorporate CV core battery study
    - Assess chemical/pharmacological class for choice of reference compounds

- Integrated Risk Assessment
  - Consider all relevant nonclinical information
  - Consider follow up studies
    - Action potential, Rabbit wedge, etc

- Determine Evidence of Risk

Components of Nonclinical Drug Development

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Nonclinical PK/ADME Studies for Oncology Studies

- Limited pharmacokinetic parameter estimation in nonclinical animal species
  - \( C_{\text{max}}, AUC, \) and half-life

- Use to facilitate dose selection, schedule, and escalation in Phase 1

- Additional nonclinical ADME studies should be generated in parallel with clinical development (!)

- Reference
  - S9 FDA Guidance 2010
Nonclinical PK/ADME Studies

- Cellular uptake and membrane transport
  - MDR (P-glycoprotein), MRP, etc.
  - Predictions of bioavailability and distribution

- In vitro drug metabolism
  - P450 isoenzyme metabolism, inhibition or induction

- Plasma protein binding studies

Components of Nonclinical Drug Development

- Pharmacology Studies/Model Selection
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Nonclinical Toxicology Studies in Oncology

- IND-enabling general toxicology studies
  - Use the same route and formulation as clinical trial
  - Approximate the clinical schedule

- Small molecule toxicology testing usually includes rodents and non-rodents (i.e., dogs)
  - Non-human primates for biologicals

- Assess the potential to recover from toxicity
  - Terminal non-dosing period recommended
  - Complete recovery demonstration is not essential

- Toxicokinetics evaluations as appropriate

— S9 Guidance for Industry, 2010
Good Laboratory Practice (GLP)

- Most safety pharmacology and toxicology studies should be conducted with GLP
  - Full GLP may not be feasible in some safety pharmacology studies
- All core battery safety pharmacology studies should be GLP
- Primary pharmacodynamic (general pharmacology) studies do not need to be conducted in compliance with GLP

--- S7A Guidance Section 2.11

Reproductive Toxicology for Oncologic Agents (S9 Guidance)

- Embryonic and fetal toxicology studies required at the time of marketing application
  - In rare cases may not need at all for genotoxic agents that target rapidly dividing cells or known developmental toxins
- Typically conducted in two different species
  - Biologicals may use one relevant species
- Fertility and early embryonic development studies are not required for use in advanced cancer patients
- Pre- and post-natal toxicology studies not warranted for oncology

--- S9 Guidance for Industry, 2010

Other Toxicology Studies for Oncology Agents (S9 Guidance)

- Genotoxicity
  - Not essential for oncology clinical trials
  - Should be performed to support marketing application
- Carcinogenicity
  - Not warranted for marketing in oncology patients
- Immunotoxicity
  - May evaluate in general toxicology studies for oncology
  - May require more extensive study for known immunomodulators
- Photosafety testing
  - Initial phototoxic potential assessment prior to Phase 1 based upon known photochemical properties

--- S9 Guidance for Industry, 2010
## Components of Nonclinical Drug Development

- Pharmacology Studies/Model Selection
- Safety Pharmacology
- PK/ADME Studies
- Toxicology
- Starting Dose Selection and Study Design

### Issues for FIH

- **Starting Dose & Schedule for First in Human Oncology Studies**
  - **Goal:**
    - Select a start dose & schedule that is expected to generate pharmacological effects yet is reasonably safe to use
  - Based on all available nonclinical data
  - Scale up from animal studies
  - For small molecules, normalize to body surface area

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### Treatment Schedules to Support Initial Oncology Trials

*(S9 Guidance for Industry, March 2010)*

<table>
<thead>
<tr>
<th>Clinical Schedule</th>
<th>Nonclinical Treatment Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once every 3-4 wks</td>
<td>Single dose</td>
</tr>
<tr>
<td>Daily for 5 days every 3 wks</td>
<td>Daily for 5 day</td>
</tr>
<tr>
<td>Daily for 5-7 days, alternating wks</td>
<td>Daily for 5-7 days, alternating wks (2-dose cycles)</td>
</tr>
<tr>
<td>Once a week for 3 wks, 1 wk off</td>
<td>Once a week for 3 weeks</td>
</tr>
<tr>
<td>Two or three times a week</td>
<td>Two or three times a week for 4 wks</td>
</tr>
<tr>
<td>Daily</td>
<td>Daily for 4 wks</td>
</tr>
<tr>
<td>Weekly</td>
<td>Once a week for 4-5 doses</td>
</tr>
</tbody>
</table>
Duration of Nonclinical Toxicology Studies

- Treatment duration in Phase 1 oncology may be extended according to patients' response
  - New toxicology studies not required
- Phase 2 studies may be supported by existing nonclinical and clinical Phase 1 data
  - Additional toxicology not required
- Phase 3 studies may require repeat dose studies of 3 months duration
  - Sufficient to support marketing
- New drug combination regimens do not require specialized toxicology studies
  - In vivo pharmacology studies of the combination may suffice

-- S9 Guidance for Industry, 2010

Oncology Small Molecule Dose Selection

- In oncology, the start dose at 1/10 the severely toxic dose in 10% of animals (STD10) in rodents
- If non-rodent is most appropriate species, then 1/6 the highest non-severely toxic dose (HNSTD)
  - HNSTD is the highest dose level that does not produce evidence of life-threatening toxicities or irreversible findings

-- S9 Guidance for Industry, 2010

Biologicals: MABEL Instead of NOAEL, MAYBE?

- MABEL: minimal anticipated biological effect level
  - Consider differences in sensitivity for the mode of action across species
- European recommendations based upon Tegenero FIH disaster
  - EMEA Guidelines, 2007
- Consider selection of starting doses based upon reduction from the MABEL
Calculation of MABEL  
(EMEA Guidelines, 2007)

- MABEL calculations should utilize nonclinical data available, including…
  - Target binding and receptor occupancy data in target cells in vitro in human and animals
  - Concentration-response curves in vitro
  - Dose/exposure-response in vivo in relevant animals

- Wherever possible an integrated PK/PD modeling approach should be used

- Apply a safety factor to the MABEL for the recommended starting dose (i.e., 1/10 MABEL)

Nonclinical Translational Research Strategies in Drug Development

The Drug Discovery & Development Pipeline

Discovery & Development Pipeline

Total time = 13.5 years  
Total cost = $1.778 billion*

* Modified from Paul et al. Nature Rev Drug Discov 2010
A Blueprint for a Restructured Drug Development Organization

Our Translational Strategy

Characteristics of Molecularly Targeted Therapies (adapted from Paoletti 2005)
**Translational Research Timelines**

- **Pharmacodynamic/Mechanism of Action Biomarkers**
  - Inform about a drug's pharmacodynamic actions
  - What is the drug doing in the patient and/or tumor?
- **Predictive Biomarkers**
  - Optimize patient selection by selecting subpopulations for treatment
  - Who should or should not get this drug?
  - Basis for stratified/personalized medicine strategies

**The Pharmacological Audit Trail**

Commentary

Auditing the Pharmacological Accounts for Hsp90 Molecular Chaperone Inhibitors: Updating the Relationship between Pharmacokinetics and Pharmacodynamics

Paul Workman


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**The Pharmacological Audit Trail**

(from Workman et al, Mol Cancer Therap 2003)

- In the target expressed or activated?
- Adequate drug dose & schedule?
- Active concentrations in plasma?
- Active concentrations in tumor?
- Active against the molecular target?
- Modulation of downstream pathways?
- Biological effect achieved?
- Clinical response of benefit?
- Predictive biomarkers of activity?
- Proof of Concept achieved?

Reduce Uncertainty

Unknown

Weak

Established

Strong
Model Based Drug Development Example

**cMET Inhibition**

- Sacrifice a subset at 1, 4, 8, and 24 h (n = 3 per time point)
- Dose at 3.1, 6.3, 12.5, 25, and 50 mg/kg
- Plasma PK Analysis
- Tumor Growth Inhibition
- Assay: Tumor PD Biomarker

Adapted from Yamazaki et al Drug Met Dispos 2008

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Model Based Drug Development

**Pharmacokinetics**
- PK in Plasma
- PK Simulation in Tumor

**Pharmacodynamics**
- cMET Phosphorylation
- Tumor Growth Inhibition

Plasma PK → Tumor PK → Biomarker Change → Antitumor Activity

(Yamazaki et al Drug Met Dispos 2008)

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Translational Phase I Study with Biomarker-Defined Endpoints

"Biological Activity" → "MTD" → "DLT"

- Starting Dose Levels
- Target PD biomarker effect in surrogate tissues or if any clinical activity
- Potential Phase 2 Dose Range
- Expansion Cohort 1
- Expansion Cohort 2
- Expansion Cohort 3
- Tumor biopsies and/or Predictive biomarker selected pts
- Maximum Tolerated Dose
Use of PK/PD Modeling Analyses to Interpret Clinical Data: Carlumab Phase 1 Trial

**Carlumab (CNTO 888)**
- Anti-human CCL2 IgG1κ mAb previously in development as an antitumor agent
- Target: CCL2 (aka monocyte chemoattractant protein-1), an 8.5 kDa β chemokine
  - Promotes tumor proliferation, migration, and metastases and angiogenesis
- Carlumab demonstrates potent inhibition of CCL2 in cell-based bioassays
- Biological activity in nonclinical tumor models
- Phase 1 program in cancer patients completed in 2009

**Carlumab Phase 1 Results**
- Multi-dose FIH Phase 1 study in advanced cancer patients
  - Standard 3+3 dose escalation design
  - Five dose levels: 0.3, 1, 3, 10 and 15 mg/kg IV every 2 weeks
- Well tolerated with no dose limiting toxicities
- No biomarker or clinical safety/efficacy drug related effects observed
  - Unclear as to why
- Evaluation of PK/PD in 21 patients
  - Total CCL2, free CCL2, and carlumab PK measured in serum
Mechanistic PK/PD Modeling

-- Fetterly et al 2013, in press.

Observed and Predicted PK/PD Profiles

-- Fetterly et al 2013, in press.

Carlumab PK/PD Summary

• Only transient suppression of free CCL2 in serum after each antibody dose
• Sustained suppression estimated to require dosing at 25 to 50 mg/kg per week
  -- Exceeds maximal economic toxicity!
  -- Confirmed in later primate studies
• Provides potential explanation for clinical and biomarker findings
• Carlumab experience supports future preclinical PK/PD testing in primates prior to initiating clinical trials
**Clinical Development in 2013 and Beyond**

<table>
<thead>
<tr>
<th>Nonclinical Trials</th>
<th>PBM Selected Trials</th>
<th>Small Enriched Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 Trials</td>
<td>Phase 2 Trials</td>
<td>Phase 3 Trials</td>
</tr>
<tr>
<td>Multiple Tumors</td>
<td>PBM Disease</td>
<td>No efficacy</td>
</tr>
<tr>
<td>Assess PK, MTD, biological activity &amp; pt selection</td>
<td>Phase 2 Poc Disease</td>
<td></td>
</tr>
<tr>
<td>Predictive Biomarker Identification</td>
<td>Phase 2 Poc Disease</td>
<td></td>
</tr>
<tr>
<td>Qualification</td>
<td>Phase 2 Poc Disease</td>
<td></td>
</tr>
<tr>
<td>Patient selection</td>
<td>Phase 2 Poc Disease</td>
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**Summary**

- Nonclinical drug development involves the collection of key pharmacology, safety, toxicology, and PK/ADME data prior to the initiation of FIH studies
- Strict regulatory requirements regarding data needed for IND submission
- Key period for formulating Translational Research plans for clinical development
  - Generate scientific data to support clinical development strategies

**And Finally…**

<table>
<thead>
<tr>
<th>Translational Medicine</th>
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<tbody>
<tr>
<td>Nonclinical Pharmacology</td>
</tr>
<tr>
<td>Efficacy/Safety</td>
</tr>
<tr>
<td>Traditional animal studies</td>
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<tr>
<td>PK/PD Toxicology</td>
</tr>
<tr>
<td>Biomarkers &amp; Molecular targets</td>
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<tr>
<td>Clinical Pharmacologist</td>
</tr>
<tr>
<td>“Model-based drug development”</td>
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<tr>
<td>Early Clinical Trials</td>
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<tr>
<td>Traditional dose and toxicity endpoints</td>
</tr>
<tr>
<td>Traditional PK/PD Biomarkers &amp; Molecular endpoints</td>
</tr>
<tr>
<td>Patient selection</td>
</tr>
</tbody>
</table>

*It is a great time to be in drug development!*