Nonclinical Drug Development:
With Examples from Oncology Therapeutics

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Disclosure Information
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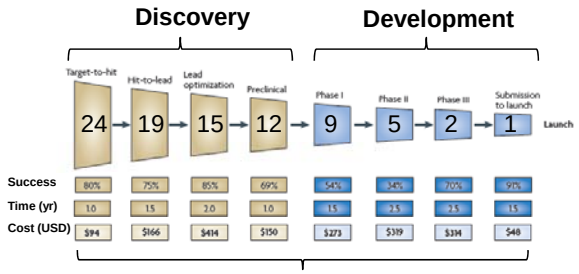
- **Employment**: Ortho Biotech Oncology R&D/Centocor R&D, Inc., a member of the Johnson & Johnson family of companies
- **Stock**: Johnson & Johnson
- **Off Label Use**: I will not discuss off label use of any product but I will refer to previously presented Phase I investigational study data

The Drug Discovery & Development Funnel



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

-- Paul et al, Nature Rev Drug Discov 2010

*** Capitalized costs**

Drug Development

- Drug discovery & screening
- Nonclinical development
- Animal scale up
- Phase I studies
- Phase II studies
- Phase III studies

Specific examples from anticancer drug development

Guidance for Industry

S9 Nonclinical Evaluation for Anticancer Pharmaceuticals

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

March 2010
ICH

Goals of Nonclinical Testing of Small Molecule Drugs and Biologicals

- Identify the pharmacologic properties of a pharmaceutical
- Establish a safe initial dose level of the first human exposure
- Understand the toxicological profile of a pharmaceutical
  - e.g., identification of target organs, exposure-response relationships, and reversibility

-- S9 Guidance for Industry, 2010

Anticancer Therapeutics

- Desirable to provide new, effective anticancer drugs more expeditiously
- Used to treat cancer in patients with serious and life threatening malignancies
- Treatment at or close to adverse effect dose levels is common
  - Design and scope of nonclinical studies to support anticancer pharmaceuticals may differ from other theapeutic areas
- Flexible nonclinical data to support Phase 1 studies (in patients)
  - Clinical Phase 1 data sufficient for moving to Phase 2 in 1st or 2nd line therapy in advanced cancer patients

-- S9 Guidance for Industry, 2010

Nonclinical Pharmacology Evaluation

- Select appropriate models based on target and MofA
- These studies can:
  - Provide nonclinical proof of principle regarding mechanism of action and efficacy
  - Guide schedule and dose escalation schemes
  - Provide information for selection of test species
  - Aid in start dose selection
  - Selection of investigations biomarkers
  - Justify pharmaceutical combinations
  - Understand pharmacodynamic properties

-- S9 Guidance for Industry, 2010

Nonclinical Pharmacology Evaluation: In Vitro Studies

- In vitro studies performed in cell lines, cell-free systems
  - Often form the basis for screening and optimization during discovery
- Cellular uptake and membrane transport
  - MDR, MRP, etc
  - Predictions of bioavailability and distribution
- In vitro drug metabolism:
  - P450 isoenzyme inhibition or induction
- Effects on hERG channels (prolonged QT interval risk)
- Preliminary protein binding studies

-- S9 Guidance for Industry, 2010

Nonclinical Pharmacology Evaluation: In Vivo Studies in Oncology

- Animal screening is too expensive for routine use
- Efficacy demonstrated in disease specific animal models: Proof of therapeutic principle
  - Groundwork for clinical development planning
- Evaluation of therapeutic index
  - Toxicity versus efficacy
- Animal pharmacokinetics can guide dose and schedule selction
  - ADME data can be generated in parallel with clinical development
- Preliminary evaluation of candidate biomarkers

-- S9 Guidance for Industry, 2010

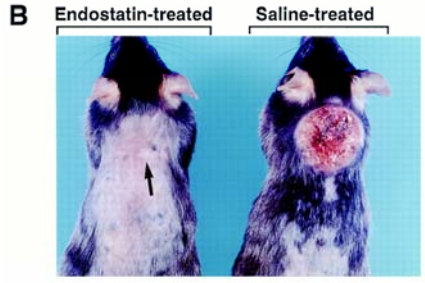
Ideal Animal Model

- Validity
- Selectivity
- Predictability
- Reproducibility

"There is no perfect tumor model"

Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth

O'Reilly et al, Cell 88:277-285 (1997)



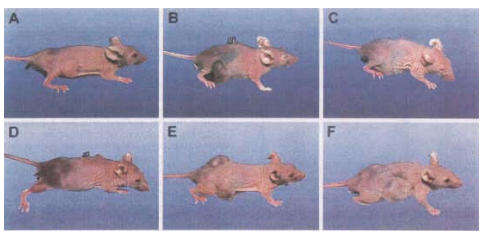
In Vivo Efficacy Models in Cancer

- Spontaneous tumors
  - Idiopathic
  - Carcinogen-induced
  - Transgenic/gene knockout animals: p53, RB, etc
- Transplanted tumors
  - Animal tumors: Lewis lung, S180 sarcoma, etc
  - Human tumor xenografts: human tumor lines implanted in immunodeficient mice (current NCI standard in vivo efficacy testing system)
  - Human tumors growing in vivo in implantable hollow fibers

Human Tumor Xenografts

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

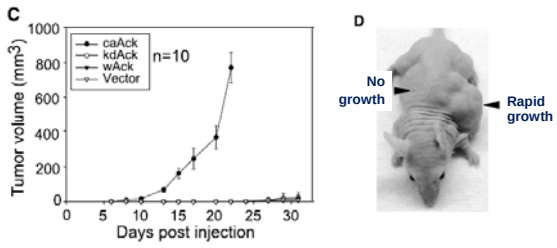
Athymic Nude Mice



Murine Xenograft Sites

- Subcutaneous tumor (NCI method of choice) with IP drug administration
- Intraperitoneal
- Intracranial
- Intrasplenic
- Renal subcapsule
- Site-specific (orthotopic) organ inoculation

Inhibition of Tumor Growth in Human Prostate Cancer Xenografts



(Mahajan, Cancer Res 2005;65:10514)

Xenograft Advantages

- Many different human tumor cell lines transplantable
- Wide representation of most human solid tumors
- Allows for evaluation of therapeutic index
- Good correlation with drug regimens active in human lung, colon, breast, and melanoma cancers
- Several decades of experience

Xenograft Disadvantages

- Brain tumors difficult to model
- Different biological behavior, metastases rare
  - Survival not an ideal endpoint: death from bulk of tumor, not invasion
- Shorter doubling times than original growth in human
- Less necrosis, better blood supply
- Difficult to maintain animals due to infection risks
- Host directed therapies (angiogenesis, immune modulation) may not be applicable
  - Human vs. murine effects
  - Ability to mimic the human tumor microenvironment is limited

Other Efficacy Models

- Orthotopic animal models: Tumor cell implantation in target organ
  - Metastatic disease models
- Transgenic Animal Models
  - P53 or other tumor suppressor gene knockout animals
  - Endogenous tumor cell development
  - May be of high value for mAb therapies
- Three-dimensional co-culture models
  - Reconstitution of the tumor microenvironment
- Low passage xenograft tumors
  - Direct implantation from patients to animals

Nonclinical Safety Studies

- Safety pharmacology
- Pharmacokinetic and toxicokinetics studies
- Genotoxicity studies
- Reproductive toxicity studies
- Carcinogenicity studies
- Formal toxicology studies
  - Single dose toxicity studies
  - Repeated dose toxicity studies
- Excellent references
  - Anticancer Drug Development Guide, 2nd edition, BA Teicher and PA Andrews, editors, Humana Press, Totowa, NJ, 2004
  - For oncology agents, FDA Guidance for Industry, S9 Nonclincial evaluation for anticancer pharmaceuticals, March 2010

Nonclinical Toxicology Studies in Oncology

- GLP Toxicology is expected
  - Use the same route and formulation
  - Use the approximate clinical schedule
- For small molecules, general toxicology testing ususally includes rodents and non-rodents (i.e., dogs)
  - Non-human primates for biologicals
- Assessment of the potential to recover from toxicity should be provided
- Embryofetal toxicity studies of oncology agents should be available when marketing application is submitted
- Genotoxicity studies not essential for clinical trials in advanced cancer
  - Perform to support marketing
- Carcinogenicity studies not warranted for advanced cancer

-- S9 Guidance for Industry, 2010

Treatment Schedules to Support Initial Oncology Trials

(S9 Guidance for Industry, March 2010)

Clinical Schedule	Nonclinical Treatment Schedule *
Once every 3-4 wks	Single dose
Daily for 5 days every 3 wks	Daily for 5 day
Daily for 5-7 days, alternating wks	Daily for 5-7 days, alternating wks (2-dose cycles)
Once a week for 3 wks, 1 wk off	Once a week for 3 weeks
Two or three times a week	Two or three times a week for 4 wks
Daily	Daily for 4 wks
Weekly	Once a week for 4-5 doses

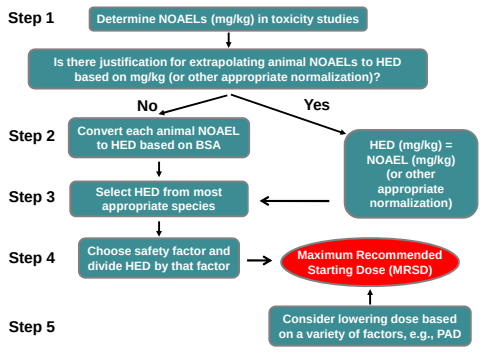
Maximum Recommended Starting Dose (MRSD) for FIH Trials

- Step 1: Determination of the No Observed Adverse Effect Level (NOAEL)
- Step 2: Conversion of NOAEL to Human Equivalent Dose (HED)
- Step 3: Selection of the most appropriate animal species
- Step 4: Application of a safety factor to determine MRSD
- Step 5: Compare MRSD with pharmacologically active dose (PAD)

-- FDA Guidance for Industry July 2005

Selection of MRSD

(FDA Guidance 2005)



Step 1: Determination of No Observed Adverse Effect Level (NOAEL)

- NOAEL Definition
  - The highest dose level that does not produce a significant increase in adverse effects in comparison to the control group
  - Not the same as the no observed effect level
- Review all available data in all species tested
- Adverse events can be overt toxicities, surrogate laboratory markers, or exaggerated PD effects
  - Adverse effects defined as events that are considered unacceptable if produced by the initial dose in a Phase I clinical trial

-- FDA Guidance for Industry July 2005

Step 2: Convert Animal Dose to Human Equivalent Dose (HED)

- Normalization of toxic dose levels across species often based upon body surface area
  - Deviations from BSA normalization must be justified
- Animal dose in mg/kg is converted to mg/m^2 and reconverted to mg/kg
  - Many cancer treatments are dosed based on BSA (mg/m^2)

-- FDA Guidance for Industry July 2005

HED Calculation

$$HED\ (mg/kg) = \frac{Animal\ Km}{Human\ Km} \times Animal\ Dose\ (mg/kg)$$

- Km: mg/kg to mg/m^2 conversion factor
  - Adult human = 37
  - Child (20 kg) = 25
  - Dog = 20
  - Mouse = 3
  - Rat = 6
  - Cynomolgus, rhesus or stumptail monkey = 12

-- FDA Guidance for Industry July 2005

Exceptions to BSA Scaling

- Weight based (mg/kg) scaling
  - Oral therapies limited by local toxicities
  - Exposure parameters that scale by weight predict toxicity
    - Example Cmax for antisense molecules
  - Proteins administered IV with Mr > 100,000
- Other scaling factors
  - Alternate routes of administration (e.g. topical, intranasal, subcutaneous, intramuscular
    - Normalize to area of application or to mg
  - Administration into anatomical compartments with limited outside distribution (e.g. intrathecal, intravesical, intraocular, or intrapleural)
    - Normalize to compartmental volumes

Step 3: Most Appropriate Species Selection

- After the NOAEL from all toxicology studies are converted to HED, then the MRSD must be derived from the most appropriate species
- By default, use the most sensitive species, but must also consider…
  - Pharmacokinetic ADME differences
  - Class pharmacodynamic effects
  - Agent pharmacology, receptor cross reactivity, etc
- Example
  - Phosphorothioate antisense DLT in humans and monkeys is complement activation
  - Does not occur in rodents

-- FDA Guidance for Industry July 2005

Step 4: Application of a Safety Factor

- Applied to the HED derived from the NOAEL from the most appropriate species
- Divide the HED by the safety factor to determine the MRSD
- By default, a safety factor = 10 is recommended
  - May raise or lower with justification

Altering the Safety Factor

- Increasing the safety factor
  - Steep dose response curve
  - Severe toxicities anticipated
  - Non-monitorable toxicity
  - Toxicities without premonitory signs
  - Variable bioavailability
  - Irreversible toxicity
  - Unexplained mortality
  - Large PK variability
  - Non-linear PK
  - Inadequate dose-response data
  - Novel therapeutic target
  - Animal models with limited utility
- Decreasing the safety factor
  - Requires highest quality toxicology data
  - Well characterized class of drugs
  - If NOAEL is based on toxicity studies of longer duration than the proposed clinical trial

Step 5: Adjustments Based on the Pharmacologically Active Dose

- If a robust estimate of the pharmacologically active dose (PAD) is available from preclinical studies
- Convert to HED and compare to the MRSD
- If PAD < MRSD consider decreasing the starting dose

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 ?

- In the wake of the Tegenero FIH disaster, new recommendations exist for starting dose selection in Europe
  - EMEA Guidelines, 2007
- MABEL: minimal anticipated biological effect level
  - The anticipated dose level leading to a minimal biological effect level in humans
  - Consider differences in sensitivity for the mode of action across species
- Consider selection of starting doses based upon reduction from the MABEL, not NOAEL dose

Calculation of MABEL

(EMEA Guidelines, 2007)

- MABEL calculations should utilize all in vitro and in vivo information from PK/PD experiments, including…
  - Target binding and receptor occupancy data in target cells in vitro in human and animals
  - Concentration-response curves in vitro in target human cells and dose/exposure-response in vivo in relevant animals
  - Exposures at pharmacological doses 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
- If NOAEL method gives a different estimation, use the lowest value unless otherwise justified

The Biomarker Hypothesis

(adapted from N Dracopoli)

- Increase probability of technical and registrational success
  - Predictive toxicology
  - Early proof of mechanism of action
  - Deeper PK/PD exploration
  - Precise determination of biologically effective dose
- Permit focused clinical studies with higher probability of demonstrating clinical benefit
  - Adaptive trial designs
  - Prospective screening of patients for enrollment in clinical trials
- Enable more cost-effective delivery of healthcare
  - Personalized medicine
  - Value-based pricing

Biomarkers in Drug Development

- **Pharmacodynamic/Mechanism of Action Biomarkers**
  - Inform about a drug's pharmacodynamic actions
  - Most relevant to early development
    - Dose and schedule selection
    - Define pharmacological behavior in patients
    - Goal: Improve efficiency of early development
- **Predictive Biomarkers**
  - Identify patients who will/will not respond to treatment
  - Most relevant to mid/late development
    - Basis for stratified/personalized medicine
    - Develop co-diagnostic biomarker assays
    - Goal: Enrich treatment population to maximize benefit

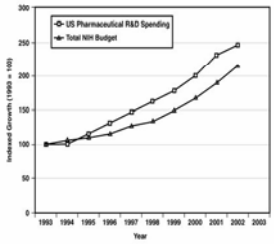
An Oncology Example: How Preclinical Studies Can Drive Clinical Drug Development

Why New Strategies for Oncology Drug Development are Needed

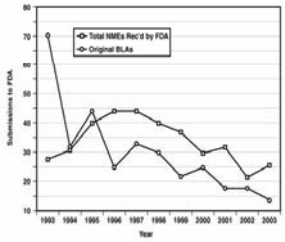
- Poor efficiency of historical oncology drug development efforts
  - Yet costs continue to rise
  - Oncologic diseases face specific challenges
- Modern treatments are molecularly targeted in contrast to conventional cytotoxic chemotherapy
  - Previously, mechanism of action was irrelevant to clinical trial design
- Emphasis on biomarkers and individualized drug therapies

FDA's Critical Path Report 2004: Innovation or Stagnation?

•**Biomedical Research Spending 1993 - 2000**

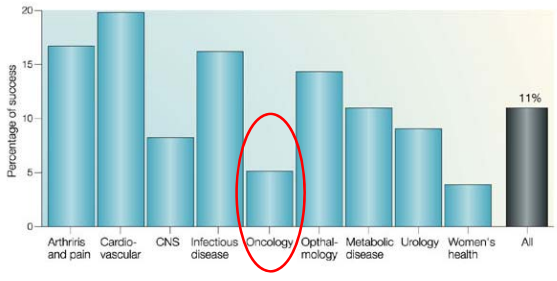


•**New NDA and BLA FDA Submissions 1993 - 2000**



•*-- Challenge and Opportunity on the Critical Path*
• *to New Medical Products, FDA, March 2004*

Clinical Success by Therapeutic Area



Nature Reviews | Drug Discovery

•*-- Kola and Landis, Nature Rev Drug Discov 2004*

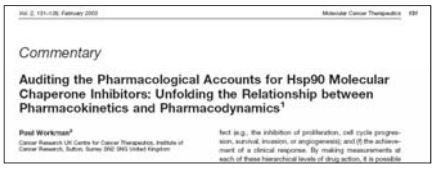
Characteristics of Molecularly Targeted Therapies *(adapted from Paoletti 2005)*

Characteristic	Cytotoxic Agents	Targeted Agents
Discovery	Cell based, empirical	Receptor based screen, rationale
Mechanism	Often unknown	Basis for screening
Pharmacological Effect	Cytotoxic	Cytostatic
Specificity	Non-selective	Selective
Dose and schedule	Pulsed, cyclical at MTD	Continuous, at tolerable dose
Development Strategy	Biomarkers for decision making is rare	Biomarkers for PD/MofA and patient selection

Our Strategy

- **Pharmacological Audit Trail (PhAT)** evaluation in preclinical and early clinical trials
- **Model-based Drug Development** approach initiated during preclinical stages
- Novel **Translational Phase I FIH study** designs with formal biomarker-defined endpoints

The Pharmacological Audit Trail



Commentary

Auditing the Pharmacological Accounts for Hsp90 Molecular Chaperone Inhibitors: Unfolding the Relationship between Pharmacokinetics and Pharmacodynamics[1]

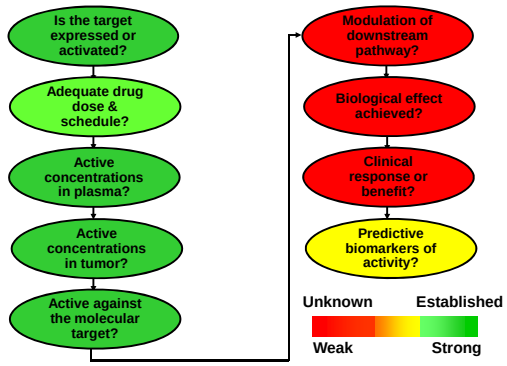
-- Paul Workman, Mol Cancer Therap 2003 and Current Pharmaceut Design 2003

The Pharmacological Audit Trail

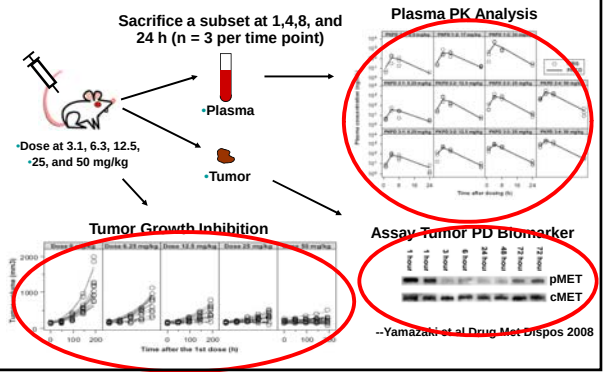
- A series of sequential questions or benchmarks to evaluate in early drug development
  - Likelihood of failure decreases as each successive benchmark is addressed
- Stepwise approach to proof of principle
  - Modulation of the intended target results in clinical benefit
- Organize strategic thinking about early development assets
  - Allows for critical decision making based upon biomarker and clinical endpoints
- Applies equally to preclinical and early clinical development

The Pharmacological Audit Trail (PhAT)

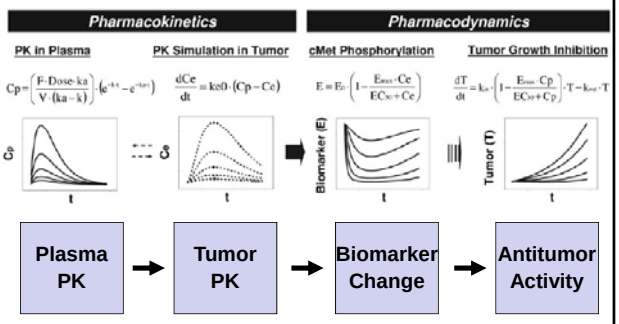
(modified from Workman et al, Mol Cancer Therap 2003)



Requirements for Preclinical PK-PD Modeling: Example cMET Inhibition



Unified Preclinical PK-PD Biomarker Model Prior to Clinical Testing



(Yamazaki et al Drug Met Dispos 2008)

PK-PD Model-Based Drug Development

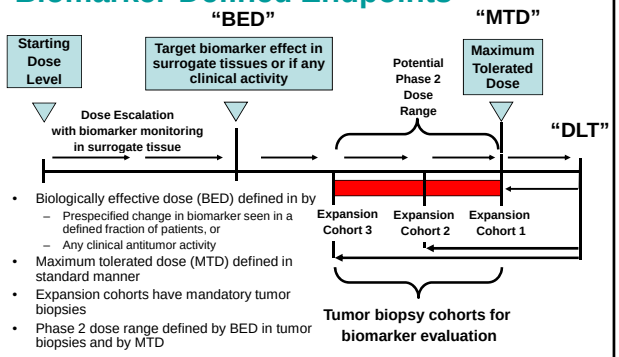
- Model-Based Drug Development
  - Preclinical PK/PD/biomarker models with direct relevance to clinical setting
- Requires extensive resource investment preclinical pharmacology studies
  - Discovery Research -- Clinical Pharmacology
  - Biomarkers -- Clinical/Transl Medicine
- Essential for evaluation of the PhAT benchmarks in first-in-human Phase 0 or 1 clinical trials

But how do we incorporate this approach into our early development clinical trial designs?

A New Approach

- Translational Phase I study with Biomarker Defined Endpoints
  - A new study design for targeted oncology agents
- PD/MOA biomarkers are formal study endpoints
  - Biologically effective dose (BED): biomarker defined
  - Maximum tolerated dose (MTD): toxicity defined
  - Recommended Phase 2 dose range: toxicity and biomarker defined
- Allows for the objective evaluation of the PhAT benchmarks

Translational Phase I Study with Biomarker-Defined Endpoints



- Biologically effective dose (BED) defined in by
  - Prespecified change in biomarker seen in a defined fraction of patients, or
  - Any clinical antitumor activity
- Maximum tolerated dose (MTD) defined in standard manner
- Expansion cohorts have mandatory tumor biopsies
- Phase 2 dose range defined by BED in tumor biopsies and by MTD

New Phase I Study Design Requirements

- Validated/qualified PD/MOA biomarker assay
  - Robust and reproducible
- Measurable signal in normal and malignant tissues
  - Surrogate tissues: skin, buccal mucosa, PBMC, etc.
  - Tumor biopsies
- Prestudy definition of a positive biomarker signal
  - What change is associated with antitumor activity?
- Phase I centers and study support staff comfortable with tissue biopsies

Example

Phase 1 Pharmacokinetic (PK) and Pharmacodynamic (PD) Study of JNJ-26854165 (Serdemetan*) in Patients with Advanced Refractory Solid Tumors

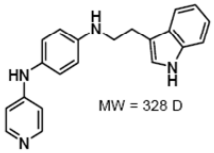
Josep Tabernero[1], Luc Dirix[2], Patrick Schöffski[3], Andrés Cervantes[4], Jose Antonio Lopez-Martin[5],Jaume Capdevila[1], José Baselga[1], Ludy van Beijsterveldt[6], Brett Hall[6], Hans Winkler[6], Silvija Kraljevic[6], Janine Arts[6], Sen Hong Zhuang[6]

[1]Vall d' Hebron University Hospital, Barcelona, Spain; [2]AZ Sint Augustinus, Wilrijk, Belgium; [3]University Hospitals Leuven, UZ Gasthuisberg, Belgium; [4]Hospital Clínico Universitario de Valencia, Spain; [5]Hospital Universitario 12 de Octubre, Madrid, Spain; [6]Ortho Biotech Oncology Research and Development, a Division of Johnson & Johnson Pharmaceuticals, Beerse, Belgium

JNJ-26854165: Serdemetan, A Novel Oral Anticancer Agent

- Designed to modulate p53 expression
- Increased p53 levels lead to:
  - Apoptosis
  - Senescence
  - Cell cycle arrest
  - Block of angiogenesis & metastasis

Chemical structure of JNJ-26854165



Study Design

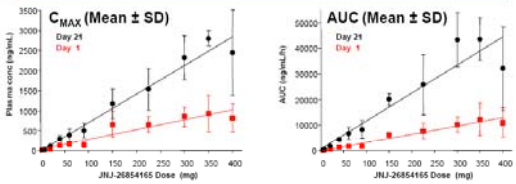
- Standard 3 + 3 patient Phase I dose escalation design
  - Toxicity defined endpoints: Dose limiting toxicity (DLT) and Maximum tolerated dose (MTD)
- Continuous PK-PD monitoring
  - Pharmacokinetics: standard PK profiling, drug-drug interaction (DDI) profile and food intake effect
  - Pharmacodynamic (PD) activity
    - Sequential and skin biopsies in all patients for IHC for p53, Ki67, TUNEL
    - Selected tumor biopsies for IHC (similar to skin)
    - Plasma: MIC-1 (p53 response gene product), CK18 (apoptosis); LC/MS: proteomics and metabolomics
  - Anti-tumor activity

Actual Dose Escalation Cohorts

Cohort No.	Dose (mg QD)	Number of patients treated (evaluated*)	DLT
C 1	4	4 (3)	None
C 2	8	3	None
C 3	20	3	None
C 4**	40	4 (3)	None
C 5	60	4	None
C 6	90	4	None
C 7	150	4	None
C 8	225	7	None
C 9	300	7	1 (Gr 3 QTc)
C 10	350	4 (3)	None
C 11	400	3	2 (Gr 3 QTc, Gr 3 rash)

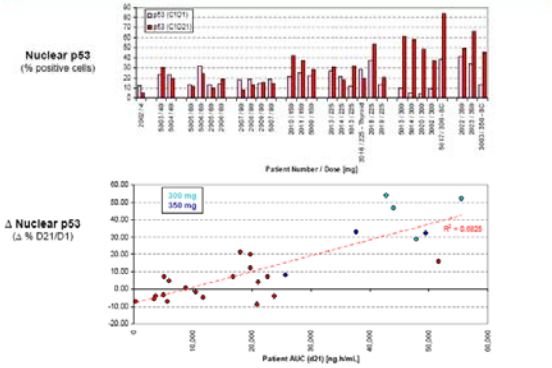
*Subjects evaluated for DLT determination
**Drug Drug Interaction (DDI) cohort

Clinical Pharmacokinetics

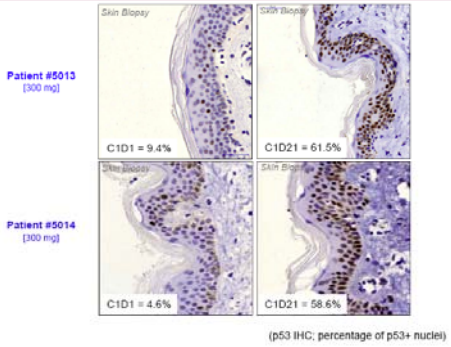


- Dose proportional (linear) PK on Day 1, Day 2 (not shown) and Day 21
- No difference between liquid (up to 225 mg) and solid-dose (from 300 mg)
- Limited inter-individual variability
- No relevant food effect (not shown), no DDI at 40 mg
- C_{MAX} and AUC_{24h} values are 3- to 4-time higher at steady state than after single dose indicating an effective $T_{1/2}$ of about 50 hr

Pharmacodynamics – p53 induction in skin

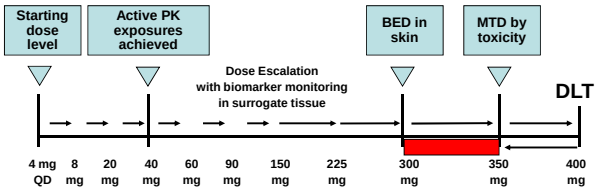


Pharmacodynamics – p53 induction in skin



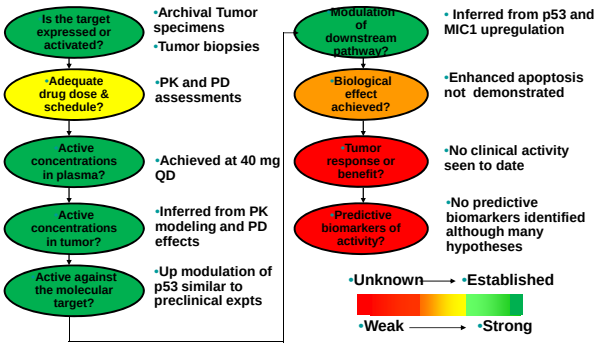
(p53 IHC; percentage of p53+ nuclei)

JNJ-26854165 Phase I Study



- Active PK plasma concentrations achieved based upon **animal studies**
- Biologically effective dose (BED) defined in by p53 change in skin biopsies
- **DLT and MTD defined by toxicity endpoints**
- Range of potential Phase 2 doses to be explored in expansion cohorts with tumor biopsies

PhAT Phase I Evaluation of JNJ-26854165 (Serdemetan)



Confidential

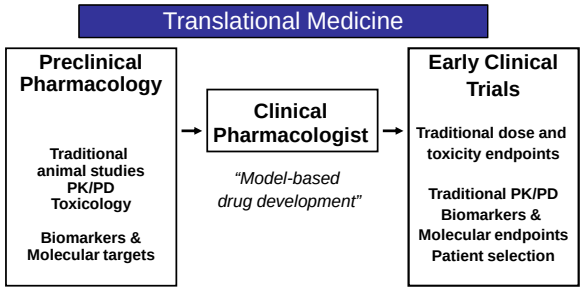
Phase I Study of JNJ-26854165 (Serdemetan)

- Study accrual is completed
- This trial was not originally designed with formal biomarker-defined endpoints, but clinical trial data matches well with this study design
- Further work on this class of agents is ongoing

Conclusions

- PK-PD model-based drug development is the cornerstone for our early development strategy
  - Requires substantial investments in preclinical testing
- The Pharmacological Audit Trail can help organize strategic thinking for the early development of molecularly targeted therapies
- Novel study designs are required for the optimal implementation of this strategy
  - Example: Translational Phase I study with biomarker-defined endpoints
- It is a great time to be working in oncology drug development!!

And Finally….



What is the biggest secret about drug development?

"It is all Clinical Pharmacology!!"