Case Studies of Non-Clinical Safety Assessment for Biopharmaceuticals in the USA

Shawn M. Heidel, D.V.M., Ph.D.
Eli Lilly and Company

Drug Evaluation Forum
August 10, 2007
Shibuya, Japan

Outline

- Homologues ("Surrogates")
  - Guidance
  - Considerations and Challenges
  - Case Studies
- Carcinogenicity
  - Goals and Science
  - Guidance
  - Case Studies: Alternatives to 2-year Bioassays
- Conclusions

ICH S6 Guidance

- "A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies)."
- "When no relevant species exists, the use of ….. homologous proteins should be considered…..While useful information may also be gained from the use of homologous proteins, it should be noted that the production process, range of impurities/contaminants, pharmacokinetics, and exact pharmacological mechanism(s) may differ between the homologous form and the product intended for clinical use."

Comparability of the homologue with the Clinical Candidate is Critical

- Characterize pharmacology
  - Literature – what's known about the target in the test species compared to humans?
  - In vitro binding – similar affinity or neutralization?
  - Functional Assays
    - In vitro cells
    - In vivo bioassays (if possible and relevant)
  - Similar tissue distribution (tissue cross-reactivity for mAbs)
- Pharmacokinetics
- Fc activity important and similar?

Challenges - Homologues

- A Second Test Article!
  - Decision must be made early in development
    - Sometimes, not possible to make a homologue
    - If possible, months to years needed to develop construct, clones, manufacture material, characterize pharmacology, establish bioanalytical support
    - May be immunogenic, thus limiting usefulness
- How do you interpret the data?
  - No 'validation' that homologue is predictive of human toxicities
  - What if findings are different from the clinical candidate in an appropriate toxicology species?
  - How do we extrapolate safety margins to the clinical candidate?

Regulatory Challenges

- No common criteria for what's expected
  - How much comparison with the clinical candidate is enough?
  - Expectations for analytical characterization and does this need to be comparable?
  - Do all aspects of testing need to be GLP?
- Can studies with homologue replace studies with the clinical candidate?
  - Developmental and reproductive testing
  - What if the results are more severe than with the clinical candidate?
- Are negative findings meaningful?
  - It's not your clinical candidate, so does the data impact risk assessment?

Homologues Have Been Used to Support Registration

- Infliximab (Remicade®) (anti-TNF)
- Efalizumab (Raptiva ®) (anti-CD11a)
- In both cases, there wasn't an appropriate species for the clinical candidate
  - Chimpanzees were the only pharmacologically responsive species, but they are not acceptable for toxicity testing due to humane reasons

Case Study 1

Use of a Homologous Antibody for Pre-clinical Safety Assessment

Background

Monkey (Clinical Candidate)

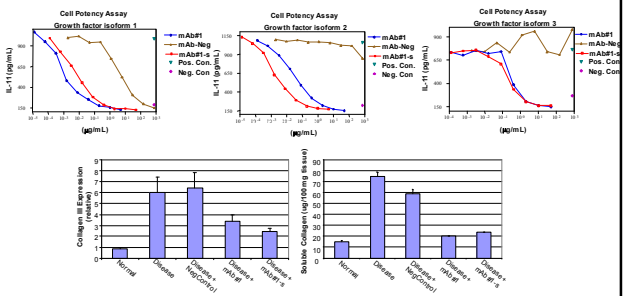
- IgG4
- Designed against series of growth factors
- Diminished effector activity intended
- High immunogenicity in rats

Rodent (Homologue)

- IgG1
  - Change in isotype may affect activity
- Must show broad based growth factor inhibition
- Murine homologue for efficacy studies
- Extensive toxicity studies in mouse models

In vitro and in vivo analysis

mAb#1 = clinical candidate; mAb#1-s = homologue (=surrogate)



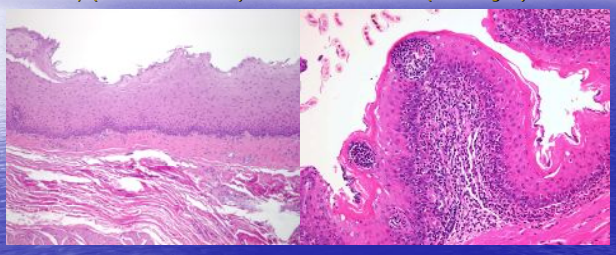
The Clinical Candidate and Homologue are Similar

Utility of this Homologue in Toxicology Studies

- The mouse homologue was used in 28 day repeat toxicology studies in mice
  - Toxicity observed with histopathology
  - This data resulted in an initial clinical hold
- The human product was utilized in repeat dose toxicology studies in NHP
  - Histopathology was not consistent with that seen in the mouse
  - Physiology of the target tissue is comparable in NHP and humans, but not in the mouse

Histopathology (physiological differences)

Monkey (Clinical Candidate) Rodent (homologue)



Which one is the predictive species?

Case Study 2

Use of a Homologous Antibody for Pre-clinical Safety Assessment

Background

- Sponsor had a small molecule and antibody development program against the same target
- Toxicology studies with the small molecule resulted in a cardiac toxicity
- Prior to the conduct of GLP studies with antibody clinical candidate, sponsor conducted a rodent homologue antibody study to determine if cardiac toxicity occurred with the antibody
- The potency of the clinical candidate was greater than 10-fold that of the rodent homologue Ab, in vitro
- The homologue and clinical candidate inhibited the intended signaling pathway in the target tissue to approximately the same extent, in vivo

Outcome: Cyno and Rodent Studies

Monkey (Clinical Candidate)
- Study Design: 5 weekly doses
- Outcome: NOAEL not established based on cardiac toxicity and other target organ toxicities at all doses.

Rodent (homologue)
- Study Design: 5 weekly doses
- Outcome: NOAEL at the highest dose tested, no evidence for cardiac toxicity

Toxicity Observed with the Clinical Candidate, but Not the Homologue

Case Study 3

Tegenero: When a Homologue was More Predictive than the Clinical Candidate

Background

- TGN1412
  - Humanized IgG4
  - Binds to C28, activates T cells without need for TCR pre-activation
  - Polyclonal T cell expansion and activation (human cells)
  - Concentration-dependent IL-2 production (human cells)
- JJ316 (murine homologue)
  - Mouse IgG1 anti-rat CD28 mAb
  - Binds to C28, activates T cells without need for TCR pre-activation
  - Polyclonal T cell expansion and activation (murine cells)
  - Th2 response (IL-4, -5, -10)

Species Differences

- Homologue (JJ316) led to a quick, dramatic polyclonal stimulation and lymphocytosis in rats
  - Intended superagonist pharmacology
- In contrast, TGN1412 stimulated only mild T cell expansion with delayed kinetics in cynomolgus monkeys.
  - This is a major difference from the intended pharmacology
- Other agonistic T cell antibodies (i.e OKT3) have shown cytokine release in humans but not NHP, raising this as a potential concern for TGN1412.
  - Previous experience suggested Cynomolgus Monkey was not an appropriate species

Cynomolgus Monkey was NOT an appropriate species

Relative potency of TGN1412

- Cynomolgus monkey not similar to human

Summary of *in vitro* activation and proliferation responses of human and Cynomolgus macaque lymphocytes to immobilised TGN1412

	TGN1412 evoked activation	TGN1412 evoked proliferation	IL-2 evoked activation	IL-2 evoked proliferation	TGN1412+IL-2 evoked activation	TGN1412+IL-2 evoked proliferation
Human PBMC	++++	++++	–	–	Could not be tested*	Could not be tested*
Macaque PBMC	++	–	–	–	+++	+++

*: TGN1412 stimulates activation, IL-2 secretion and proliferation when given alone.

In initial *in vitro* assays, in which PBMC from Cynomolgus macaques were stimulated with immobilised TGN1412, cells did not undergo a proliferative response. Early indications are that Cynomolgus macaque PBMC are activated by TGN1412 but do not undergo proliferation. However, when exogenous human IL-2 was added to cultures of Cynomolgus macaque PBMC stimulated with immobilised TGN1412 then a strong proliferative response was observed. No proliferative response was observed following the addition of human IL-2 alone to Cynomolgus macaque PBMC cultures.

Expert Scientific Group on Phase 1 Clinical Trials Final Report, November 2006

Outcome: Rodent, Cyno and Clinical Studies

Monkey (Clinical Candidate)
- Minimal or no CD28-mediated "superagonist" T cell activation and proliferation in monkeys
- NOAEL = 50 mg/kg
- FIH dose set off of this NOAEL

Rodent (Homologue)
- 5 mg/kg - rapid, dramatic polyclonal stimulation and lymphocytosis in rats – this is intended superagonist pharmacology
- 0.5 mg/kg - NOEL

Clinic
- First dose of 0.1 mg/kg led to cytokine release syndrome in all healthy volunteers
- Homologue data in rodents more relevant

Does TGN1412 Imply Inadequacy of ICH S6?

- TGN1412 event is an unfortunate outlier and should not warrant a complete redesign of nonclinical development program requirements for novel biologics
  - Nonclinical antibody testing paradigms that have been developed over the last decade have proven to be adequate to support the determination of 'Safe Use Conditions' for most clinical trials
  - Need to consider the literature and experience with biopharmaceuticals that have similar pharmacology
- The data from T-cell superagonists does not mean that homologues should become a standard in the battery of all nonclinical studies with biopharmaceuticals

Summary: Homologues

- Case-by-case considerations of the clinical candidate should determine the potential value of a homologue
  - The science drives the hypotheses and the appropriate toxicology studies
- Findings with a homologue must be carefully considered with respect to the clinical candidate
  - When results differ across species or when compared to similar molecules in the literature, use the best scientific rationale for decision making

Carcinogenicity Testing

Alternatives to 2-year Rodent Bioassays and a 2-year study with a homologue

Why do Carcinogenicity Testing?

- Provide a Science Based Risk Assessment for Cancer Potential in Patients
  - Genetic Toxicity is not of concern for most biopharmaceuticals
  - The 'concern' for some biopharmaceuticals is their potential mitogenicity or demonstrated immunosuppression
  - Does the pharmacology indicate some risk and the need for an assessment?

Looks easy, but it's very difficult !

The Carcinogenicity "Starting Point"

- Relevance of 2-yr rodent bioassays for human risk assessment is controversial, even for NCEs
  - Approximately 50% of NCEs tested are positive in a rodent bioassay
  - Most positives are nongenotoxic
  - Some nongenotoxic rodent carcinogens have little/no relevance to humans; saccharin, beta blockers, dopamine agonists, etc
  - "…most carcinogenicity findings…attributable to hormonal or immunosuppressive mechanisms or exaggerated pharmacologic actions…"
    - Jacobs and Jacobson-Kram, 2004, Tox Sci

Issues for Carcinogenicity Assessments of Biopharmaceuticals

- 2-yr rodent bioassays frequently not possible
  - No pharmacology
  - Immunogenicity
  - Technical challenges, e.g. daily Injections
- Homologue/surrogate studies
  - Cross species issues
    - Biology comparison? Pharmacology (epitope)? Dose selection?
  - Should only be done when scientifically appropriate
    - Same pathway in rodents as in humans

ICH S1A Guidance

- Carcinogenicity studies not needed for:
  - Pharmaceuticals with clinical dosing < 3/6 months
  - Life expectancy of the indication is < 2 - 3 years
- "Carcinogenicity studies not generally needed for *endogenous* substances given essentially as *replacement therapy*, particularly where there is *previous clinical experience* with similar products"
- "Although not usually necessary, carcinogenicity studies….should be considered for the other biotechnology products noted above…"

http://www.ich.org/MediaServer.jser?@_ID=489&@_MODE=GLB

ICH S6 Guidance

- "Standard carcinogenicity bioassays are *generally inappropriate* for biopharmaceuticals"
- "Product-specific assessment of carcinogenic potential *may* still be needed depending on duration of clinical dosing, patient population, and/or biological activity (e.g., growth factors, immunosuppressive agents, etc.)"
- A standard carcinogenicity bioassay should be *considered* if "…the product is biologically active and non-immunogenic in rodents and other studies have not provided sufficient information to allow an assessment of carcinogenic potential…"

Case Study 4

Insulin Analogues: The Utility of Comparative Data for Carcinogenicity Assessments

CPMP: Insulin Analogue -Specific PTC

- "..a thorough assessment of carcinogenic potential is indicated for *all* new insulin analogues."
- "…activity profile…*in vitro and in vivo*…" using native human insulin and AspB10 insulin as references should be considered.
- "If in vitro tests and/or repeated dose toxicity studies reveal evidence for *increased mitogenicity* or other effects which are cause for concern…further *in vivo carcinogenicity* testing should be considered.."

http://www.emea.eu.int/pdfs/human/swp/037201en.pdf

What's Known

- Normal human insulin (HI) will cause tumors in rats
  - Within a year of dosing high doses
  - Threshold for tumorigenicity

Alternative Carcinogenicity Strategy for Insulin Analogues

- New insulin analogues should be evaluated for in vitro and in vivo mitogenicity compared to HI
  - Pharmacology studies (e.g., HI and IGF-1 receptor binding, activation, kinetics)
    - Minimal set of ligands: HI, Asp(B10), IGF-1
  - Repeat-dose toxicity studies (at least 6-mo)
    - Reference compound: HI
    - Positive control: Asp(B10)
    - Include cellular proliferation markers for target tissues (e.g., mammary and PCNA/BrdU/Ki67 etc.)

Recently Approved Insulin Analogues

Drug/ Approval Date	Insulin Class	Relative Mitogenicity (in vitro)	HI Reference Used	Positive Control	Longest Rodent Study
Levemir / 2005	Basal	< HI	HI, NPH		6 mos[1]
Lantus / 2000	Basal	> HI	NPH	Asp(B10)	24 mos[1]
Apidra 2004	Fast (meal time)	≈ HI	HI, Humalog, Novolog	Asp(B10)	12 mos[1]
Novolog 2000	Fast	≤ HI	HI		12 mos[1]

[1]Mammary tumors or hyperplasia vs vehicle control, but not vs HI reference

Insulin Conclusions

- Comparators are powerful tools for assessing carcinogenicity risk
- Not all carcinogenicity assessments require a 2-year rodent bioassay
  - If low mitogenic potential, then 6-month studies are sufficient
  - In 2-year studies, a threshold exists for tumorigenicity of hormones and the risk assessment is dependent on cross-species pharmacology comparisons and the Margin of Safety compared to the therapeutic clinical exposure

Case Study 5

An Alternative to a 2-Year Carcinogenicity Assessment for a "Differentiation Factor"

"Differentiation Factor"

- Vast majority of reports in the literature and internal data indicated either inhibition or no effect on human tumor cell proliferation in vitro
  - Justification for no carcinogenicity assessment?
- FDA agreed that traditional rodent carcinogenicity studies were not appropriate or scientifically justified for this product
  - Product does not persist for greater than 3 months
  - Fast clearance with little systemic exposure
  - Primarily a differentiation factor, not a growth factor, based on in vitro tumor cell line proliferation data

"Differentiation Factor" (cont.)

- FDA agreed on a non-traditional approach to assess possible effects on tumor growth at sites distant from the implant site
- Carcinogenicity assessment done in short term assays in vitro and in vivo
  - mRNA receptor expression - assess in human tumor cell lines
  - In vitro proliferation – evaluate several human tumor cell lines based on receptor mRNA expression
  - Tumor Xenograft Model - evaluate effect of product, as it is intended to be used, on growth of human tumor cell lines (selected based on tumor type and receptor mRNA expression), in a nude mouse human tumor xenograft model

Results of Nonclinical Studies to Assess Tumor "Promotion" – In Vitro

- PCR screening of receptors in human tumor cell lines
  - 21 tumor cell line evaluated for expression of multiple components of receptor complex
  - 10/21 appeared to express the receptor complex
- Effect on in vitro proliferation of human cancer cells lines
  - 11 tumor cell lines (including the 10 receptor positive cells) evaluated for capacity to enhance proliferation in vitro
  - 10/11 showed no enhanced growth and 1/11 showed inhibition

Results of Nonclinical Studies to Assess Mitogenicity – In Vivo

- Effects on growth of human tumor xenografts in nude mice
  - 8 tumor cell lines expressing receptor complex assessed in nude mouse tumor xenograft model
    - Included colon carcinoma, melanoma, epidermoid carcinoma, pancreatic carcinoma, and glioblastoma cell lines
  - No growth enhancement and no metasteses

There are alternatives to 2-year studies to evaluate potential effect on tumor Growth

Case Study 6

2-year Carcinogenicity Studies with Rodent Homologues to hGH

Growth Hormone - Background

- Identical in amino acid sequence to endogenous human growth hormone (hGH)
- Administered as replacement therapy (not supra-physiologic)
- Post-marketing surveillance from patients given hGH has not indicated an increased risk of tumors
  - J Pediatr 1997: S32-36
- Substantial data in patients with Acromegaly indicates no increased risk for tumor formation after years of supra-physiologic levels of hGH
  - hGH up to 40- to 80-fold higher than normal physiologic levels

Carcinogenicity Results with Growth Hormone Homologues and Risk Assessment


Recombinant Rat and Mouse Growth Hormones: Risk Assessment of Carcinogenic Potential in 2-Year Bioassays in Rats and Mice

- Negative for tumors in both rats and mice
- Was this study necessary given the historical data in humans?
- If either of these studies that used homologues were positive, would it change the risk assessment for hGH?
  - Clinical data suggests no risk for tumors

Alternative Model Conclusions

- The appropriate hypotheses to be tested should determine the appropriate study(ies)
  - There may not be a need for any additional studies (if pharmacology is well characterized in the literature and does not indicate a cause for concern for neoplasia)
- There are experimental alternatives to a 2-year rodent bioassay
  - Justify the approach
  - 2-year studies with the rodent homologue should not be an expectation

Immunosuppressives

Case Studies 7 & 8:
Kineret®, Amevive®

Carcinogenicity Assessment of Immunosuppressives

- The 'cause for concern' is decreased immune surveillance, but potency must be considered
  - Immunomodulators will not have the same concern as potent immunosuppressives (i.e. cyclosporine)
- There is no agreement on potential tests to use for carcinogenic assessments of immunosuppressives

Kineret ®

- IL-1 receptor antagonist for the treatment of rheumatoid arthritis
- Natural amino acids
  - Produced in E. Coli
- Single methionine residue added at the end of native IL-1Ra

Kineret® Data

- Full set of in vitro and in vivo genotoxicity studies completed (relevance?)
  - Negative in all tests
- Carcinogenicity assessment: no 2-year studies based on the case for no cause for concern
  - Kineret binding to IL-1 does not cause any signal transduction
  - No tumors or cell proliferation noted in the 6-month rat study
  - No evidence of immunosuppression in toxicity studies
  - No tumors in transgenic mice overproducing IL-1ra

Kineret® Label

Immunosuppression

- The impact of treatment with Kineret® on active and/or chronic infections and the development of malignancies is not known.

Malignancies (clinical experience - most important data?)

- The observed rates and incidences (malignancies) were similar to those expected for the population studied.

Carcinogenesis, Mutagenesis, and Fertility

- Kineret has not been evaluated for its carcinogenic potential in animals. Using a standard in vivo in vitro battery of mutagenesis assays, Kineret did not induce gene mutations in either bacteria or mammalian cells. In rats and rabbits, Kineret at doses of up to 100-fold greater than the human dose had no adverse effects on male or female fertility.

Amevive®

- CD-2:Fc fusion protein for the treatment of psoriasis
  - Designed to inhibit T-lymphocyte activation
- Natural amino acids
- Produced in CHO cells

Amevive® Data

- Studies up to 44-weeks in cynomolgus monkeys
  - Lymphomas observed
  - All animals were positive for lymphocryptovirus (LCV), which can lead to B-cell lymphomas when animals are immune suppressed.
- Carcinogenicity assessment: no 2-year studies
  - Lack of pharmacology in rodents

Amevive® Label

Malignancies (clinical experience - most important data?)

- AMEVIVE® may increase the risk of malignancies. Some patients who received AMEVIVE in clinical studies developed malignancies. In preclinical studies, animals developed B cell hyperplasia, and one animal developed a lymphoma. AMEVIVE® should not be administered to patients with a history of systemic malignancy. Caution should be exercised when considering the use of AMEVIVE® in patients at high risk for malignancy. If a patient develops a malignancy, AMEVIVE® should be discontinued.

Carcinogenesis, Mutagenesis, and Fertility

- The role of AMEVIVE® in the development of the lymphoid malignancy and the hyperplasia observed in non-human primates and the relevance to humans is unknown. Immunodeficiency-associated lymphocyte disorders (plasmacytic hyperplasia, polymorphic proliferation, and B-cell lymphomas) occur in patients who have congenital or acquired immunodeficiencies including those resulting from immunosuppressive therapy.

Carcinogenicity Conclusions

- Carcinogenicity assessments need to be based on a scientific cause for concern
  - Consider pharmacology, data from chronic studies, patient population
  - Alternative approaches are useful and justified in many cases
  - A 2-year rodent bioassay may not be the best assessment
- Potency of immunosuppression is an important consideration
  - Clinical data is the most relevant and important data
  - Potential for lymphomas in patients cannot be assumed
  - Immunosuppressives have labels that indicate the potential risk, even in the absence of nonclinical data

Overall Conclusions for Biopharmaceuticals

- The appropriate studies must be considered on a case-by-case basis
  - Risk assessment needs be based on the best science and not on completion of a standard list of studies
- 2-year carcinogenicity assessments should not be an expectation
  - Sponsors need to justify their approach
  - Consider alternatives
  - Simply referencing a statement from ICH-S6 is not sufficient
- Use of homologues for toxicology testing should not be an expectation for all programs

Acknowledgements

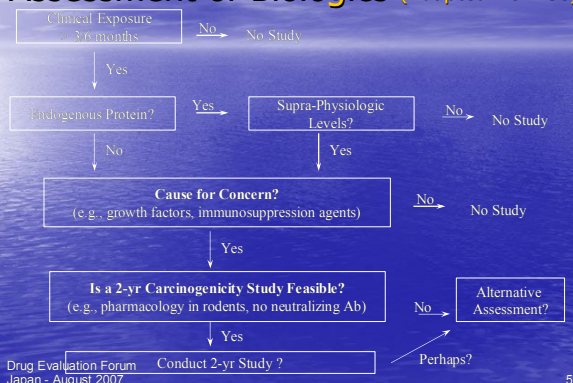
- BioSafe Leadership Committee
  - Laura Andrews, Jeanine Bussiere, Joy Cavagnaro, Maggie Dempster, Jim Green, Shawn Heidel, Christopher Horvath, Art Levin, Mark Rogge, Jennifer Sims, Randy Soltys, Jay Stoudemire, Tim Terrell, George Treacy, Garvin Warner
- Case Studies
  - Laura Andrews, Jeanine Bussiere, Richard Byrd, Christopher Horvath, Michael Leach, Anja Stauber, Garvin Warner

Backup and Informational Slides

Carcinogenicity Risk

- Can risk be quantified in the absence of a 2-year carcinogenicity study?
  - Many potent immunosuppressives cause lymphoproliferation in shorter term toxicology studies
  - Labels are similar regardless of whether a formal carcinogenicity test was completed
  - If the risk is identified in the label, should patient monitoring be used to determine the actual risk?

Decision Tree for Carcinogenic Assessment of Biologics (Adapted from S6)



Guidance for Carcinogenicity Assessment of Biological Drugs

- ICH S1A: Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals
  - Step 4: November 1995
- ICH S6: Safety Studies for Biotechnological Products
  - Step 4: July 1997
- FDA Draft Guidance: Development of PTH for the Prevention and Treatment of Osteoporosis
  - April 2000
- CPMP: PTC on the Carcinogenic Potential of Insulin Analogues
  - February 2001