【S-12】

The Nonhuman Primate in Preclinical Development of Biotechnology Derived Therapeutics

John C. Kapeghian, Ph.D.

D.A.B.T., Navigator Consulting, Preclinical Services,
Charles River Laboratories, Inc.

The nonhuman primate has become the default "species of choice" for the nonclinical development of biological therapeutics primarily for two reasons: 1) the therapeutic target is often expressed at levels comparable to humans and "monitorable" within the confines of IND-enabling toxicology studies, and 2) the phylogenetic similarities of nonhuman primates to humans reduces the potential for immunogenicity. Although many species of nonhuman primate are used in safety evaluation studies, the nonclinical safety of biologics have been typically tested in young adult rhesus and cynomolgus monkeys. The chimpanzee is important in selected types of disease and research programs; however, it is rarely used in toxicology studies due to strict protection as a highly-threatened species. Essentially all nonhuman primates used for nonclinical research are now purpose-bred, and are imported from off-shore colonies; there are also, however, domestic breeding sources within the U.S. for laboratory use of these species. Due to recent supply shortages and increased cost of rhesus monkeys, the cynomolgus monkey, a closely-related macaque, became the primary nonhuman primate species used in nonclinical studies within the U.S. Both the rhesus and cynomolgus are Old World species and have been well-characterized from a "historical data perspective"to allow relatively low numbers of animals to be used in toxicology test designs, and often, these animals provide the only relevant nonclinical safety data prior to dosing humans with biological therapeutics. For this reason, pharmacokinetic/pharmacodynamic, immunologic, and clinical pathology endpoints are routinely modeled in macaques for comparable monitoring in human trials, often with correlative predictive value. The similarities in genetic, physiologic, and pharmacologic responses between humans and macaques used in nonclinical safety evaluation of biologic therapeutics add to the rising importance of the rationale for the use of nonhuman primates in this role.

The Nonhuman Primate in Preclinical Development of Biotechnology-Derived Therapeutics

John C. Kapeghian, Ph.D., D.A.B.T.
Preclinical Services
Charles River Laboratories, Inc.

Nonhuman Primates in Biologics Safety Evaluation

Presentation Outline:

- Empirical Rationale
- Historical Use of Nonhuman Primates
- Species and Sources
- Typical Program/Study Designs
- Clinical Relevance
- Future Considerations

Why Use Nonhuman Primates?

- Therapeutic Targets of Biologics Often Similarly Expressed in Primates
- Phylogenetic "Closeness" Reduces Immunogenicity Potential
- Immunogenicity when present, may have unique relevance to humans

Types of Biological Therapeutic Products Studied in Primates

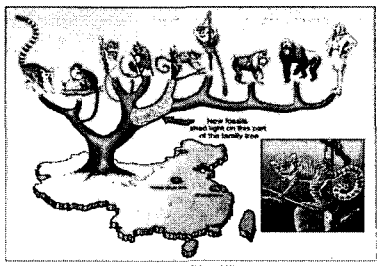
- Interferon's & Various Cytokines
- Growth Hormones - Replacement Therapy
- Colony Stimulating Factors/Hematopoietics
- Monoclonal Antibodies – Immune targets, Antigens expressed in cancer, Angiogenesis
- Gene Therapy
- *Oligonucleotides/siRNAs*
- Vaccines
- Stem Cells

What Therapeutic Areas are Primates Used in Safety Evaluation?

- Oncology
- Ophthalmology ⟶ Only Lab Species with Macula
- Immunomodulation
- Diabetes
- CNS Diseases
- Aids

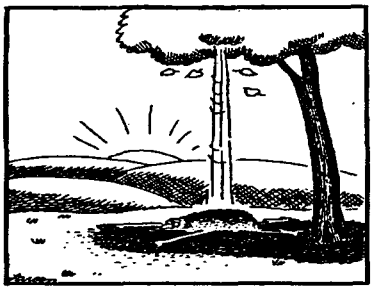
The Evolutionary Tree:

Are we really cousins?

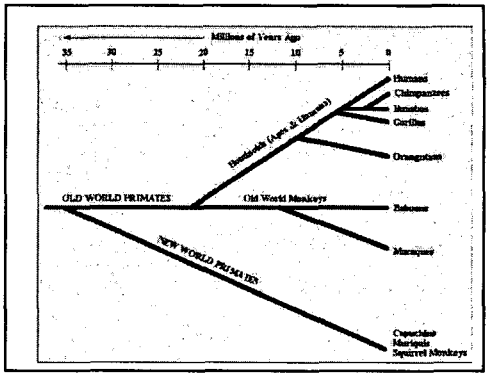


Graphics by Mark A. Klingler, Carnegie Museum of Natural History

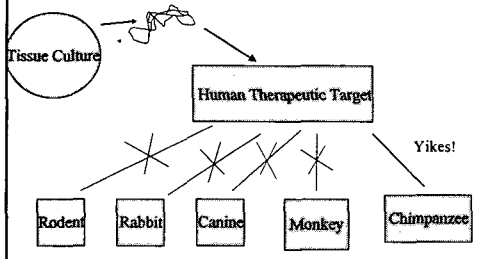
Modern Day Beginnings



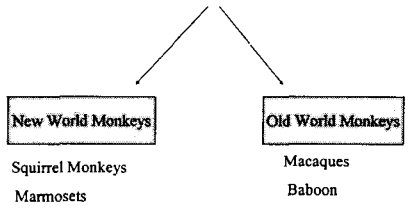
The Dawn of Man



What Species to Use?

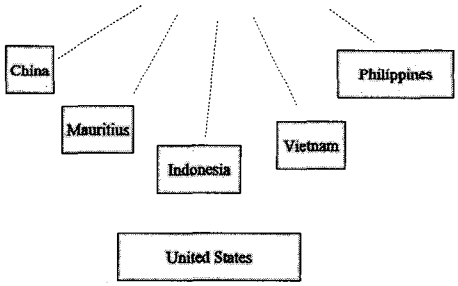


Which Monkey To Choose?



- Genus *Macaca*
- Barbary Macaque, *Macaca sylvanus*
- Lion-tailed Macaque, *Macaca silenus*
- Sunda Pig-tailed Macaque or Beruk, *Macaca nemestrina*
- Northern Pig-tailed Macaque, *Macaca leonina*
- Mentawai Macaque or Bokkoi, *Macaca pagensis*
- Moor Macaque, *Macaca maura*
- Booted Macaque, *Macaca ochreata*
- Tonkean Macaque, *Macaca tonkeana*
- Heck's Macaque, *Macaca hecki*
- Gorontalo Macaque, *Macaca nigriscens*
- Celebes Crested Macaque or Black "Ape", *Macaca nigra*
- Crab-eating Macaque or Long-tailed Macaque or Kera, *Macaca fascicularis*
- Stump-tailed Macaque or Bear Macaque, *Macaca arctoides*
- Rhesus Macaque, *Macaca mulatta*
- Japanese Macaque, *Macaca fuscata*
- Toque Macaque, *Macaca sinica*
- Bonnet Macaque, *Macaca radiata*
- Assam Macaque, *Macaca assamensis*
- Tibetan Macaque, *Macaca thibetana*

Sources of Primates



Origin - Source Issues

- India stopped all export of primates for research purposes – created Rhesus shortage
- Availability
  - China is currently largest provider
- Preference
  - Quality, Historical Use, Cost
- World Health Issues
  - SARs, TB, Herpes B-Virus
- Political Stability

Rhesus or Cynomolgus?

- Binding Affinity
- Tissue Cross-Reactivity
- Frequency of Use Within Class
- Efficacy Models
- Known Sensitivity
- Availability
- Cost

Rhesus Vs Cynos

Rhesus

World-wide Shortage Of Rhesus -1990's

Traditionally Used
Slightly Larger than Cynos
Seasonal Menstrual Cycling
Lower Availability
Higher Cost

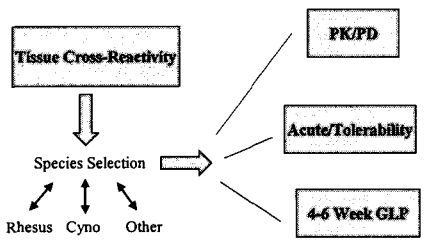
Cynomolgus

Increased Usage Since Mid-70's
Normal Menstrual Cycling
More Readily Available
Lower Cost
Typically Similar in Response

Preclinical Safety Studies Conducted In Macaques: Biological Therapeutic Products

- Immunohistochemistry: Tissue Cross-Reactivity (Monoclonal Antibodies)
- Safety Pharmacology
  - Cardiovascular
  - Respiratory
  - CNS/Neurobehavioral
- Pharmacokinetic Studies
- "Standard" Toxicology Testing
- Reproductive Toxicology

Typical IND-Enabling Program Monoclonal Antibody



Features of a Typical Primate IND-Enabling Study for Biological Therapeutic

- Dosing Regimen – Closely Mimics Clinical Plan
- Route/Mode of Administration: Intravenous infusion (1-4 hr, continuous), intracerebral, intrathecal, s.c., i.m., intranasal, inhalation, intravitreal, peribulbar
- ECGs
- Blood Pressure
- Vital Signs
- Ophthalmology
- Clinical Pathology: Serum Chemistries, Hematology, Coags
- Toxicokinetics
- Immunogenicity: Serum antibody titers, neutralization
- Anatomic Pathology
- Tissue Localization/Gene Expression

Design Considerations

- Ty pically Low n per group
  - 3/sex/group (Main); 2/sex/group (Recovery)
- An imals weigh 2-5 Kg; 3-5 yrs of age
- P restudy/Longitudinal comparisons more important than statistical differences from control
- In dividual Animal Evaluation Crucial
- An imals Typically Mixed: adult/juvenile
- S erum LDH & CPK –Esse ntially worthless for cardiotox
- S erum Troponins – Invaluable for suspected cardiotox
- Rep rotox –vagin al swabbing necessary, gestation (155-165 days)
  - Low conception (25%), spontaneous abortion (17%), single off-spring
- A lways Stick with Same Macaque Species, Lab, Source

Known Laboratory, Species, Origin Differences in Macaque Studies

- Inter-Laboratory:
  - Chow, Fed vs Fasted, Housing, Cage Changing
- Inter-Species:
  - Macaque sensitivity to thrombosis: anti-CD40 ligand
  - Metabolism of Xenobiotics
- Inter-Origin:
  - Red cell size (smaller in Indonesian vs Chinese Cynos)
  - Differences in lymphocyte subpopulations as per cell surface markers (via FACs): Indonesian vs Chinese Cynos
  - Parasite Load, Viral status, Background Histopath

Clinical Relevance

- Infusion reactions "First time effects" can be monitored in primates
- Immunogenic Toxicity – rhThrombopoietin immunogenicity & bone marrow toxicity
- Neuroanatomy & physiology of the brain more similar to human
- Primate retina has macula
- Teratogenicity – thalidomide
- Immune Function
- Naturally require vitamin C

Issues Unique to Primates

- Herpes B-Virus
- Stereotypy
- Handling
- Heterogeneity
- Susceptibility to Tuberculosis
- Pathogenic Viruses: SRV, STLV-1, SIV
- Parasitism
- CITES Permit Required for Intl Shipment

Future Considerations

- Supply
- Cost
- Quality
- Immunological Competence
- Regulatory Pressures
- Humane Care Priorities

Summary

- Nonhuman Primates Well-Suited for Studying Biological Therapeutics
- Macaques are now Species of Choice
- Cynomolgus Monkey is Most Widely Used
- End-points Have Clinical Relevance
- Still Man is Man; Monkey is Monkey yet someday the twain shall meet . . . .

Someday . . . ?