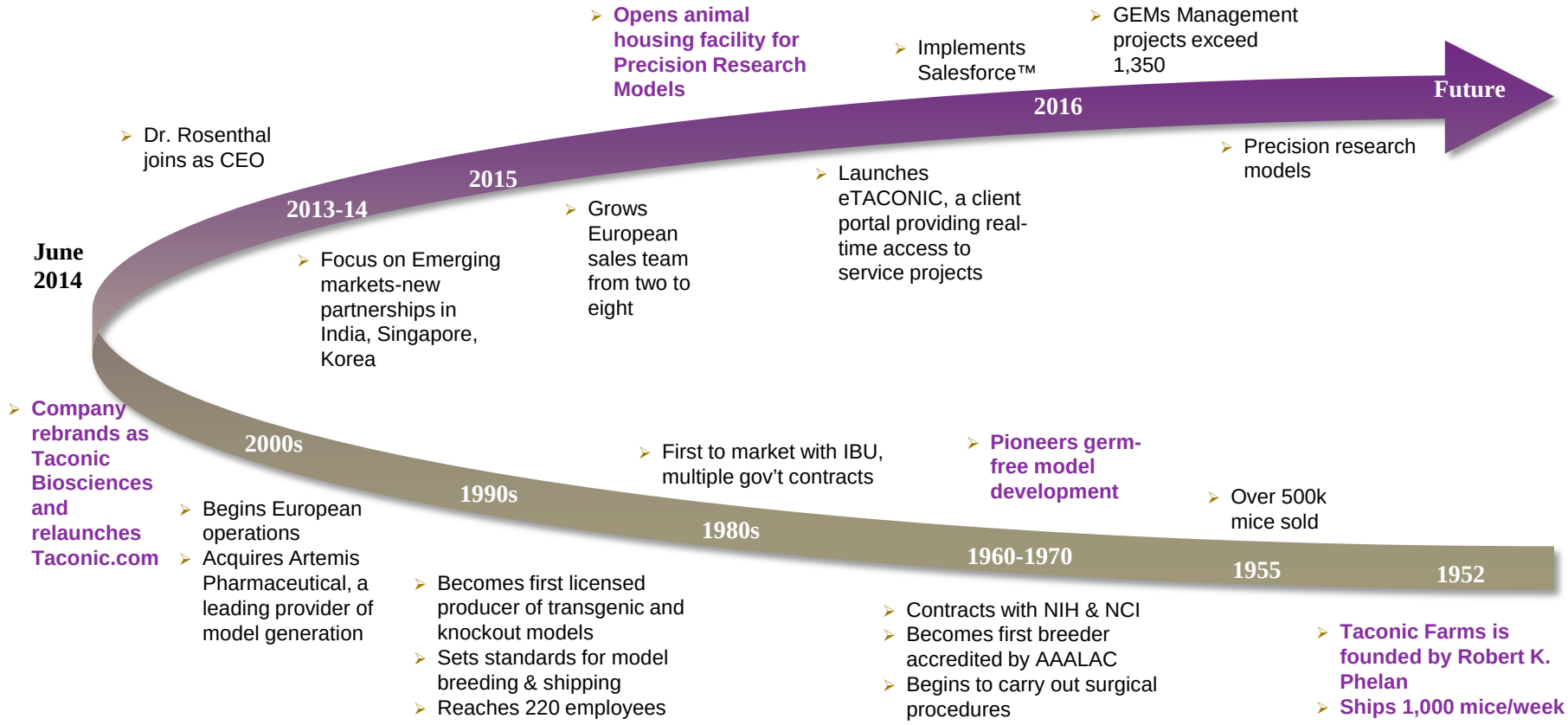
Human TNFα Transgenic Mouse Model of Spontaneous Arthritis

Dr.Paul Volden
Application Scientist

Presented by Dr.Krishna Bhagavatula,Ph.D.
Country Manager-India
Taconic Biosciences

Brief Company History



US Facilities

TACONIC

Corporate Headquarters, Hudson, NY
- 12k square feet
- Administration, Product Strategy, Customer Service, Business Technology
- 75 employees, including field sales
- All global sites have AAALACi accreditation

Germantown Facility, Germantown, NY
- 219k square feet
- 135k cages; MPF, RF, EF, DF & GF health standards
- GEMs Management, GEMs Models, Traditional, Spontaneous Mutants
- Gnotobiotics, Embryology
- 248 employees

Isolator Breeding Services, Albany, NY
- 30k square feet
- 9000 cages, multiple health standards
- GEMs Management, Precision Research Models, surgical services
- 32 employees

Production Facility, Cambridge City, Indiana
- 29k square feet
- 27k cages; MPF, RF, EF health standards
- GEMs Management, GEMs Models, Gnotobiotics Traditional, Spontaneous Mutants
- 40 employees

Scientific Services, University of Albany, Rensselaer, NY
- 10,000 square feet
- GEMs Management, Laboratory Services
- 37 Employees

European Facilities

GEMs Design Services, Cologne, Germany

34k square feet

6,000 cages; MPF, EF health standards

GEMs Design, Bioinformatics

89 employees

Production Facility, Hallingore, Denmark
- 13k square feet
- 6,000 cages, MPF health standard
- Gnotobiotics
- 10 employees

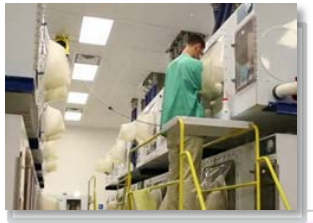
Building Strong Customer Relationships

TACONIC

	Pharma	Academic	Biotech	CRO	Government / Institute
Representative Customers	Bristol-Myers Squibb, Novartis, Lilly, Bayer, Merck, novo nordisk, Pfizer, Lupin, Glenmark	Harvard Medical School, MIT, Dana-Farber Cancer Institute, NYU, University of Maryland, thsti	Genentech, MacroGenics, Ionis, Champions Oncology, Takeda, Biogen, Jubilant LifeSciences, Sanofi, Aurigene, Biological E. Limited	BioReliance, Envigo, charles river, WuXi AppTec, MPI Research, Covance, Syngene, Vimta, Advinus	FDA, NIH National Institutes of Health, The Michael J. Fox Foundation for Parkinson's Research, OmniRat, Karolinska Institutet, folkehelseinstituttet, Cure Alzheimer's Fund, Inception Sciences, leidos
Products	GEMs Management GEMs Design Traditional Spontaneous	GEMs Design GEMs Management GEMs Models Traditional Spontaneous	GEMs Management Traditional Spontaneous GEMs Design	GEMs Models	GEMs Management GEMs Design

High Value Products and Services

TACONIC



Scientific Services

- **GEMs Design:** Consult with customer to develop custom model concepts
- **GEMs Management:** Develop and implement complex client breeding plans, including licensing, breeding, testing, and distribution

Commercial GEMs Models

- **Humanized Models:** Proprietary research models providing highly-translatable results to clinical trials
- **rasH2**: Therapeutic compound carcinogenicity testing
- **Specialty Models & Repositories**: Models for specific applications--neurology, metabolic, and immunology

Traditional Models

- **Traditional Models:** High quality, well-defined, and inbred & outbred mice and rats
- **Spontaneous Mutants:** Research models portfolio with diverse phenotypes exhibiting specific natural mutations

Commercial GEMs Portfolio

Portfolio of proprietary research models that provide highly-translatable results to human clinical trials

- 20 + years experience breeding and selling these complex and challenging models. Recognized as a leader in Genetically Engineered Models
- Exclusive in-licensing relationship for key products, including NOG and rasH2
- Customer base includes CRO, pharma, biotech & academic
- Areas of therapeutic focus: carcinogenicity, ADME, metabolic and cardiovascular disease, diabetes, endocrinology, neuro, respiratory, oncology, immuno-oncology, and immunology
- Over 4,500 unique models, including a large repository of cyro-preserved models available through hour EZ cohort program

- rasH2 Mouse Model
  - The transgenic mouse alternative to lifetime rodent bioassays for Carcinogenicity Studies
- Immune System Engrafted Models
  - Advanced hu-models for Oncology, Immunology, Imuno-oncololgy and Infectious disease
- NOG Portfolio
  - Oncology, Infectious Disease and Immunology research models
- tADMET
  - An advanced portfolio of genetically engineered models and services for translational research

Traditional Models Portfolio

Taconic Biosciences is committed to providing high quality, precisely defined inbred, outbred, and hybrid mice and rats to biomedical researchers worldwide.

- 60+ years of experience in breeding and distributing traditional laboratory research models
- Rat and mouse models for numerous research applications, including toxicology, pharmacology, immunology, oncology, and various other disease related research disciplines.
- Taconic B6 model is used in many long-running pharma studies
- Multiple health standards to meet researchers' needs, including germ-free mice for microbiome research
- Taconic B6 mouse serves as the genetic background for key GEMs models and are also utilized in GEMs management programs

Spontaneous Mutant Models Portfolio

Models with diverse phenotypes carrying specific natural mutations. The genetic line is stabilized via breeding strategies, for high genetic integrity. Models are often highly immunodeficient, making them amenable to human tumor engraftment

- Lower-cost alternative to NOG mouse for later stage trials or studies that do not require a high specificity
- Customers include CRO, pharma, biotech, govt. and academic
- May be cross bred to other animal backgrounds to obtain multiple mutations
- Models are maintained across multiple health standards
- Therapeutic areas of focus: oncology & immunology
- Gold standard for oncology studies for 30+ years

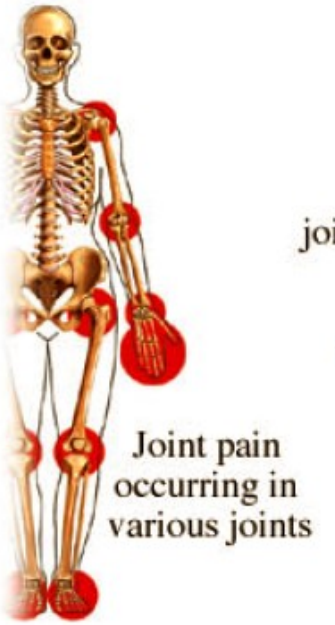
Human TNFα Transgenic Mouse Model of Spontaneous Arthritis

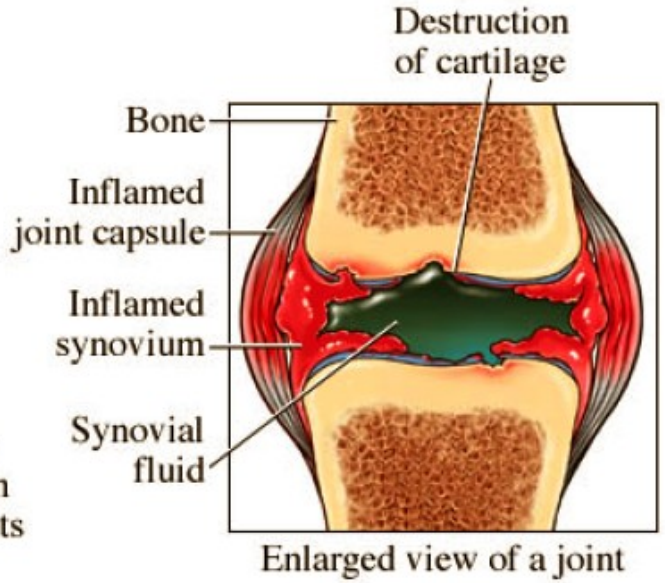
Model #1006

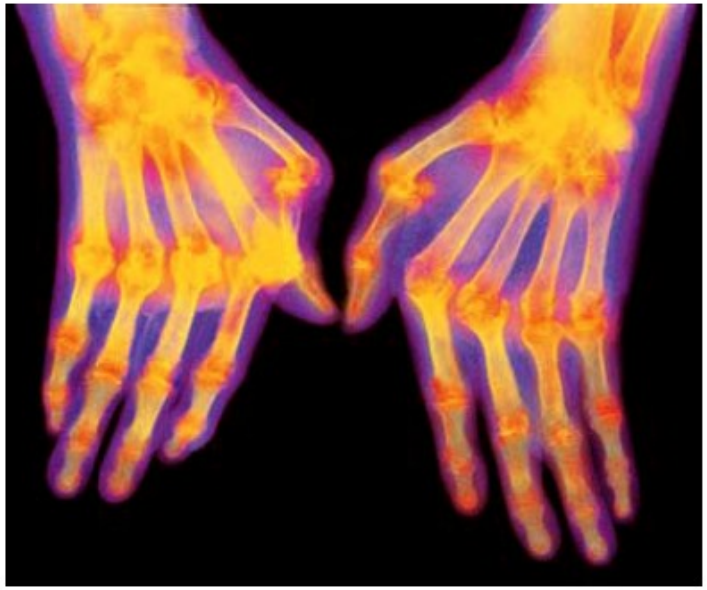
Tumor Necrosis Factor α (TNF-α) Random Transgenic:

- Overview of TNF-α ***/*** Rheumatoid Arthritis
- Background on the model
- How the model has advanced the field
- Modern applications

Rheumatoid Arthritis

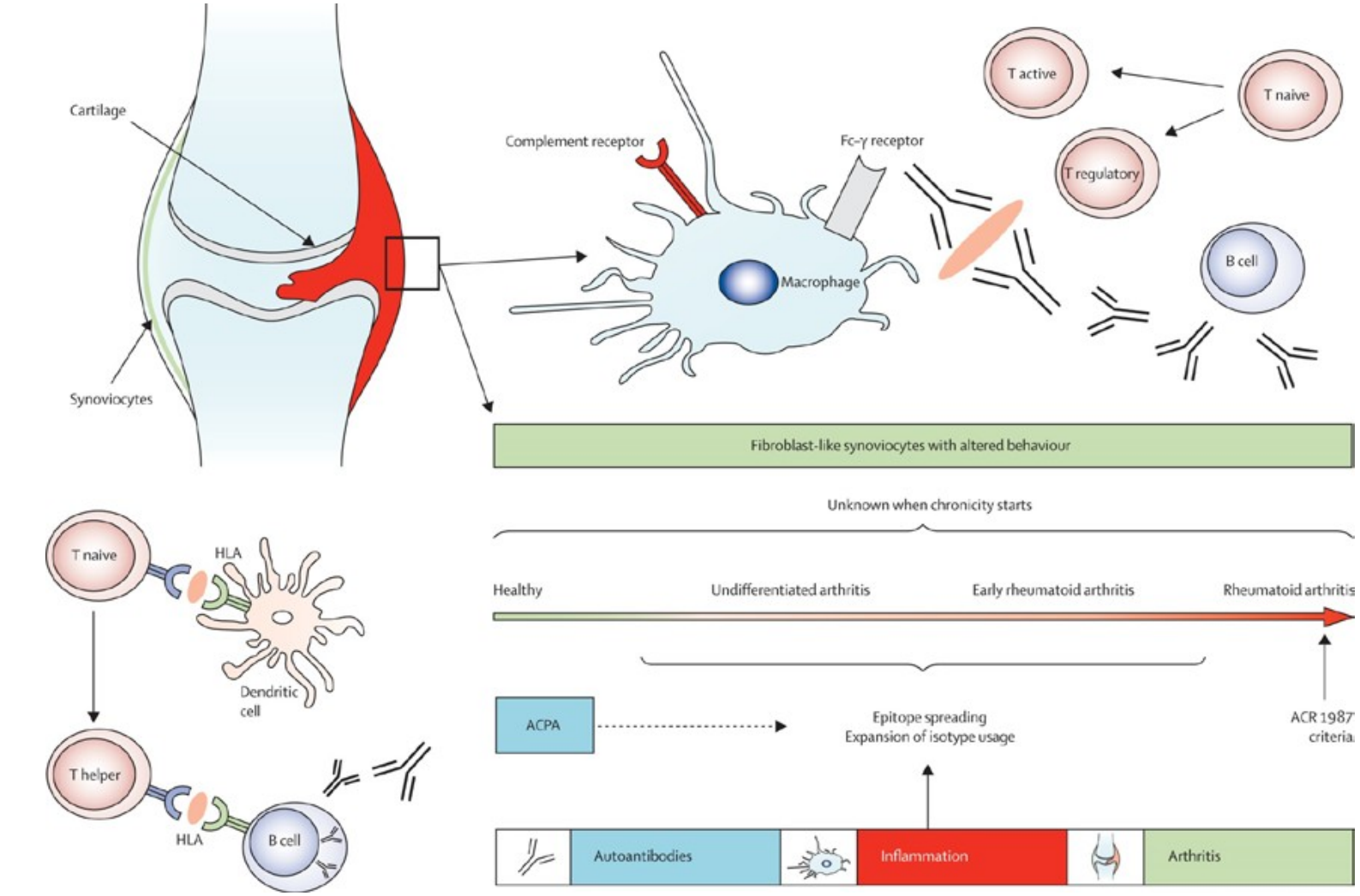






Infliximab (Remicade®)
Etanercept (Enbrel®)
Anakinra (Kineret®)
Adalimumab (Humira®)
Certolizumab Pegol (Cimzia™)

Rheumatoid Arthritis : Pathogenesis



PRECLINICAL MODELS FOR RA

TACONIC

Categories	Induction principle	Examples	Inciting agents/genetic alteration	Species
Genetically engineered	Deliberate manipulation of one or more genes encoding proteins that regulate the immune response	HLA-B27 transgenic	Human leukocyte antigen (HLA) B27 (a major histocompatibility complex (MHC) class 1 molecule) and human β2-microglobulin	Rat
		HLA-DR transgenic	Human leukocyte antigen, D-related (a MHC class II molecule)	Mouse
		IL-1ra knockout	Interleukin-1 receptor antagonist	Mouse
		K/BxN	Human T-cell receptor (KRN) and a human MHC class II molecule	Mouse
		TNF-α transgenic	Tumor necrosis factor-α	Mouse
Induced	Administration of an exogenous material	Adjuvant-induced arthritis (AIA)	Lipoidal amine	Rat
			Mycobacterium tuberculosis	Rat
			Pristane	Mouse, rat
		Collagen-induced arthritis (CIA)	Type II collagen (bovine, porcine, and rodent)	Mouse, rat
		Bacterial cell wall-induced arthritis	Bacterial cell wall peptidoglycan (polysaccharide): *Lactobacillus* sp., *Streptococcus* sp. (SCW)	Rat
Spontaneous		MRL/lpr	MRL/Mpj-lpr/lpr	Mouse

Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2011, Article ID 569068, 21 pages
doi:10.1155/2011/569068

SIMILARITIES & DIFFERENCES

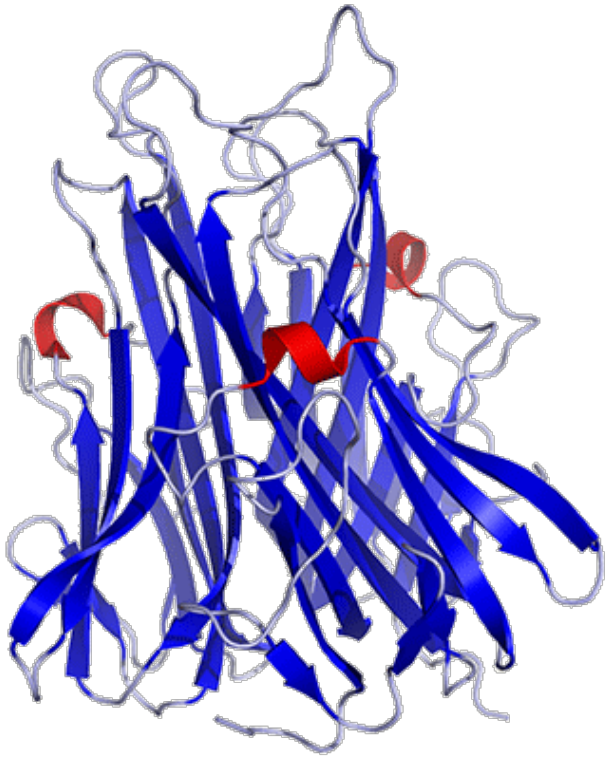
TACONIC

Animal model	Similarities to RA	Differences from RA
CIA in mice	Symmetric joint involvement, peripheral joints affected, synovitis, cartilage and bone erosions, inflammatory cell infiltrate, pannus formation, erythema, edema, genetically regulated by MHC and non-MHC genes	Formation of antibodies to collagen, greater incidence in males, periostitis, poor responses to NSAIDs, not characterized by exacerbations and remissions
CIA in rats	Higher susceptibility in females, symmetric joint involvement, peripheral joints affected, synovial hyperplasia, inflammatory cell infiltrate, genetically regulated by MHC and non-MHC genes, production of rheumatoid factor	Not characterized by exacerbations and remissions
PGIA in mice	Development of polyarthritis, presence of rheumatoid factor, deposition of immune complexes in the joint, persistent joint inflammation	Development of ankylosing spondylitis, not characterized by exacerbations and remissions
AIA in rats	Symmetric joint involvement, inflammatory cell infiltrate, cartilage degradation, synovial hyperplasia, genetic linkage, T cell dependence	Damage to cartilage less severe than in RA, bone destruction more prominent; no rheumatoid factor produced, gastrointestinal tract and skin affected
SCW-induced arthritis in mice	Characterized by exacerbations and remissions	None specified in publications
Polyarticular SCW-induced arthritis in rats	Symmetric joint involvement, synovial hyperplasia, inflammatory cell infiltration, relapsing inflammation	No rheumatoid factor produced
Monarticular SCW-induced arthritis in rats	Characterized by exacerbations and remissions	None specified in publications
STIA in mice	Inflammatory cell infiltrate, synovial hyperplasia, pannus formation, cartilage destruction	None specified in publications
K/BxN-Tg mice	Symmetrically affects small peripheral joints	Distal interphalangeal joints often affected, no systemic manifestations, no production of rheumatoid factor, arthritis does not remit
Human TNF–Tg mice	Synovial hyperplasia, presence of an inflammatory cell infiltrate, pannus formation, cartilage destruction, and bone resorption	No production of rheumatoid factor

* RA = rheumatoid arthritis; CIA = collagen-induced arthritis; MHC = major histocompatibility complex; NSAIDs = nonsteroidal antiinflammatory drugs; PGIA = proteoglycan-induced arthritis; AIA = adjuvant-induced arthritis; SCW = streptococcal cell wall; STIA = serum transfer–induced arthritis; Tg = transgenic; TNF = tumor necrosis factor.

ARTHRITIS & RHEUMATISM
Vol. 62, No. 8, August 2010, pp 2192–2205
DOI 10.1002/art.27503

Tumor Necrosis Factor α (TNF-α):

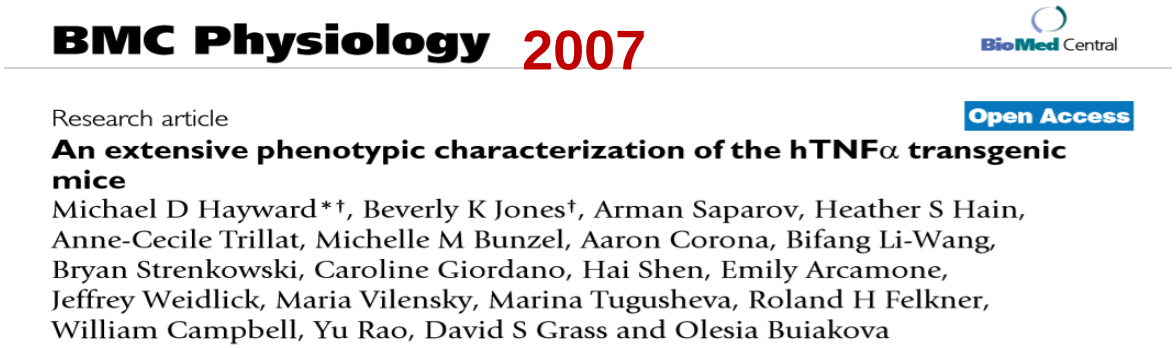


- A cell signaling protein (cytokine) involved in systemic inflammation
- Involved in the acute phase response
- Produced primarily by activated macrophages (also many other cells)
- Hyper-production of TNF plays a central role in rheumatoid arthritis **(RA)**

TNFα: overview

Cell Source	Inducers	Inhibitors	Cell Target	Primary Effects on Each Target
Mononuclear phagocytes, T cells, B cells, NK cells, vascular endothelial cells, keratinocytes, smooth muscle cells, mast cells, neutrophils, astrocytes, glial cells.	Lipopolysaccharide, zymosan, phorbol esters, ultraviolet light, viral infection, allogenic B cells, protozoa, and other microorganisms. Cytokines and other endogenous mediators (TNF-a, IL-1, IFN-g, IFN-a, GM-CSF, IL-2, TGF-b, substance P, platelet activating factor).	Prostaglandins, corticosteroids, IL-4, IL-6, TGF-b	Mononuclear phagocytes Neutrophils, eosinophils Endothelial cells Hypothalamus Liver Muscle, fat Thymocyte	Activation (Inflammation and Infection) Activation (Inflammation) Activation (Inflammation, coagulation) Fever Acute phase reactants (serum ameloid A protein) Catabolism (cachexia) Costimulator

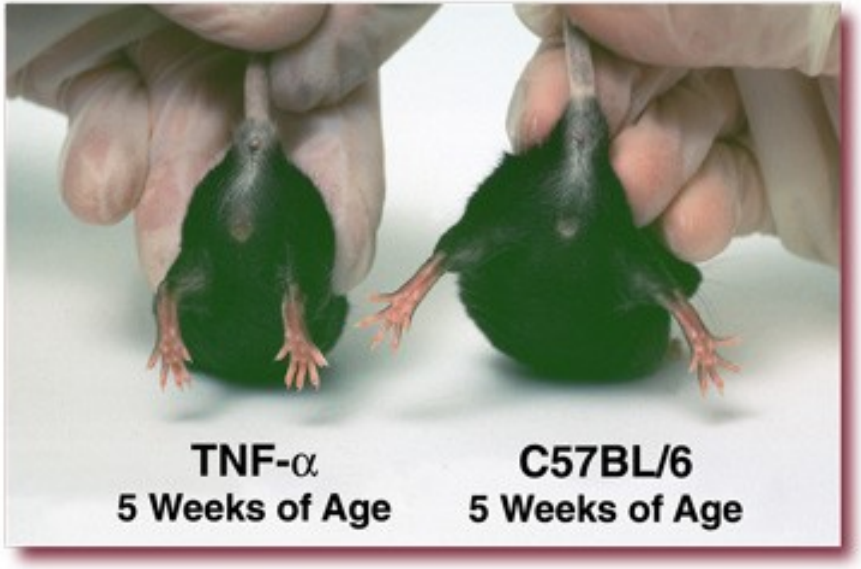
Origin of the TNFα transgenic model



- The TNFα transgenic mice were generated using a construct that contains a 2.8 kb fragment of the human TNFα gene, including the entire coding region and promoter, fused to the human β-globin 3' untranslated region (UTR) that replaces the endogenous 3'UTR of the human TNFα gene
  - Designed to model dysregulated human TNF□expression
- This transgenic line was produced by pronuclear microinjection of B6SJLF2 hybrid zygotes
- The animals have been backcrossed for over 21 generations onto the C57BL6/NTac genetic background

Progressive arthritis in the TNFα mice

- **The TNFα mice develop inflammatory arthritis spontaneously**
- **Ideal for screening new small molecules and biologics for the treatment of arthritis**



Experimental procedures

- **Treatment was initiated when mice were 5 weeks old following the randomization of the experimental mice into groups of 10 mice based on their body weights**
- **Treatment was given through i.p. injection of 100 µl of working concentration of Humira freshly prepared just before each dosing**
- **Doses of 0.25, 1, 10 and 25 mg/kg Humira were used**
- **The arthritis disease progression in the experimental animals was monitored by clinical scoring twice weekly.**
- **After giving total 22 doses to each animal, the study was terminated when the animals reached 15 weeks old**
  - Paws were fixed in 10% buffered formalin for histology analysis

Arthritis clinical assessment criteria

Maximum 24 scores were given to each mouse.

The sum score of all 4 paws from each mouse will be used for graphing and statistical analysis

✓20 digits: score 0 or 0.2 for each digit (maximum 4 scores)

✓0 = normal

✓0.2 = one or more swollen joints

✓4 paws: score 0 or1 or 2 (maximum 8 scores)

✓0 = normal

✓1 = noticeable swollen

✓2 = severe swollen

✓2 wrists: score 0 or 1 or 2 (maximum 4 scores)

✓0 = normal

✓1 = noticeable swollen

✓2 = severe swollen

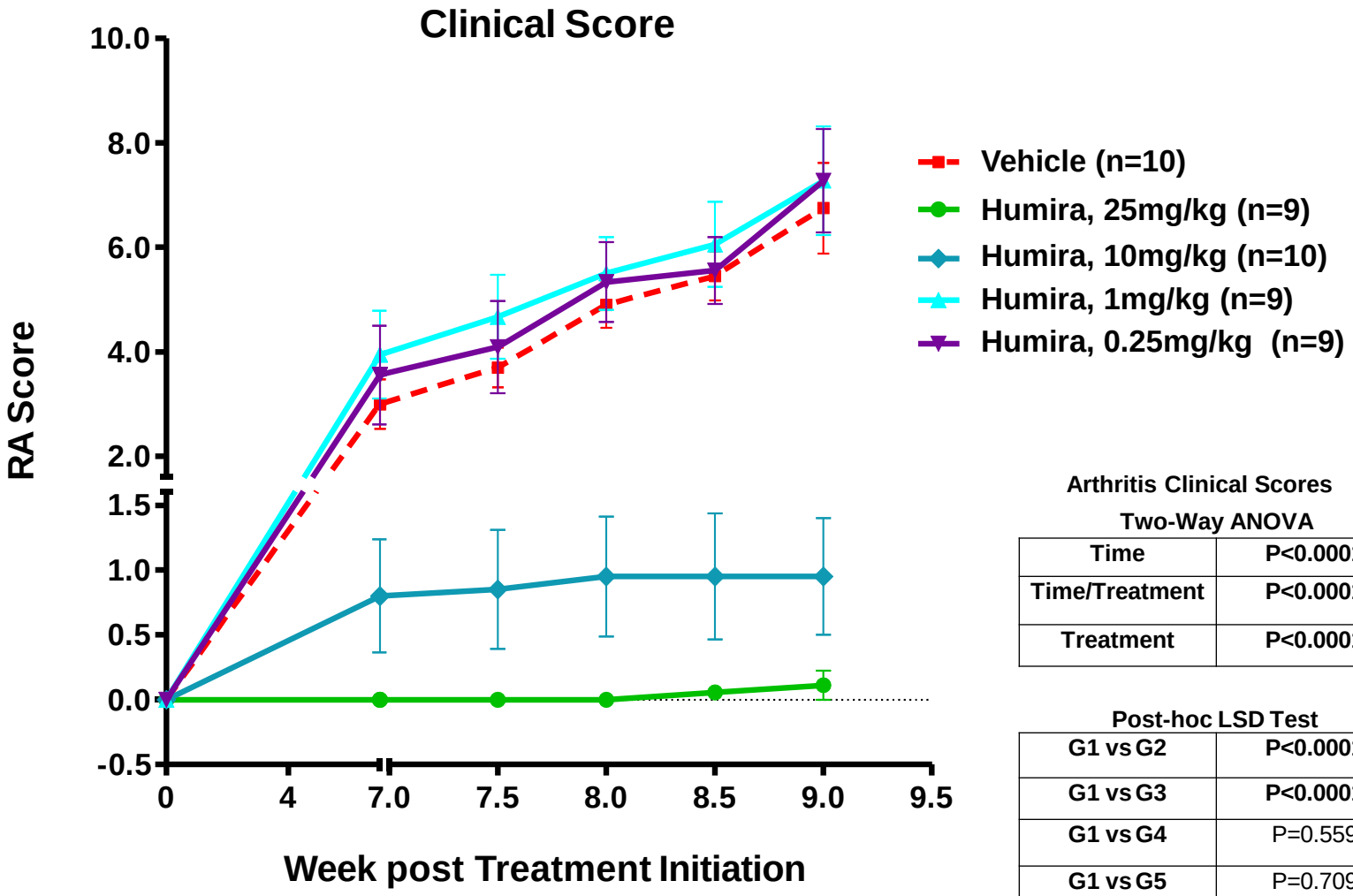
✓2 ankles: score 0 or 2 or 4 (maximum 8 scores)

✓0 = normal

✓2 = noticeable swollen

✓4 = severe swollen with stiffness of ankle joint

Dose dependent effect of treatment on clinical progression of arthritis



Arthritis Clinical Scores

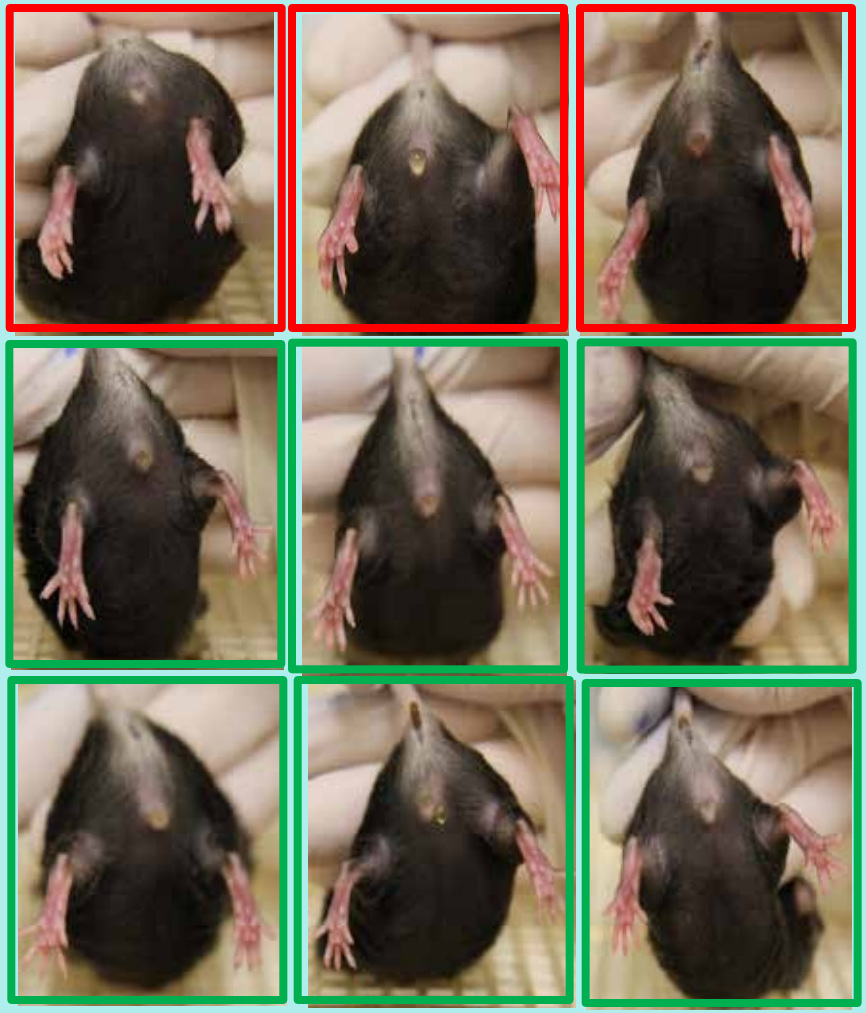
Two-Way ANOVA

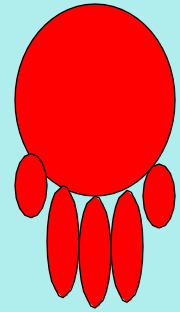
Time	**P<0.0001**
Time/Treatment	**P<0.0001**
Treatment	**P<0.0001**

Post-hoc LSD Test

G1 vs G2	**P<0.0001**
G1 vs G3	**P<0.0001**
G1 vs G4	P=0.559
G1 vs G5	P=0.709

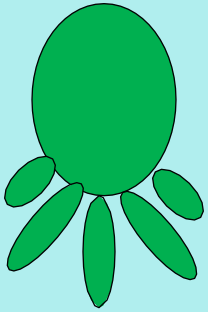
Clinical manifestation of arthritis in treated and untreated animals





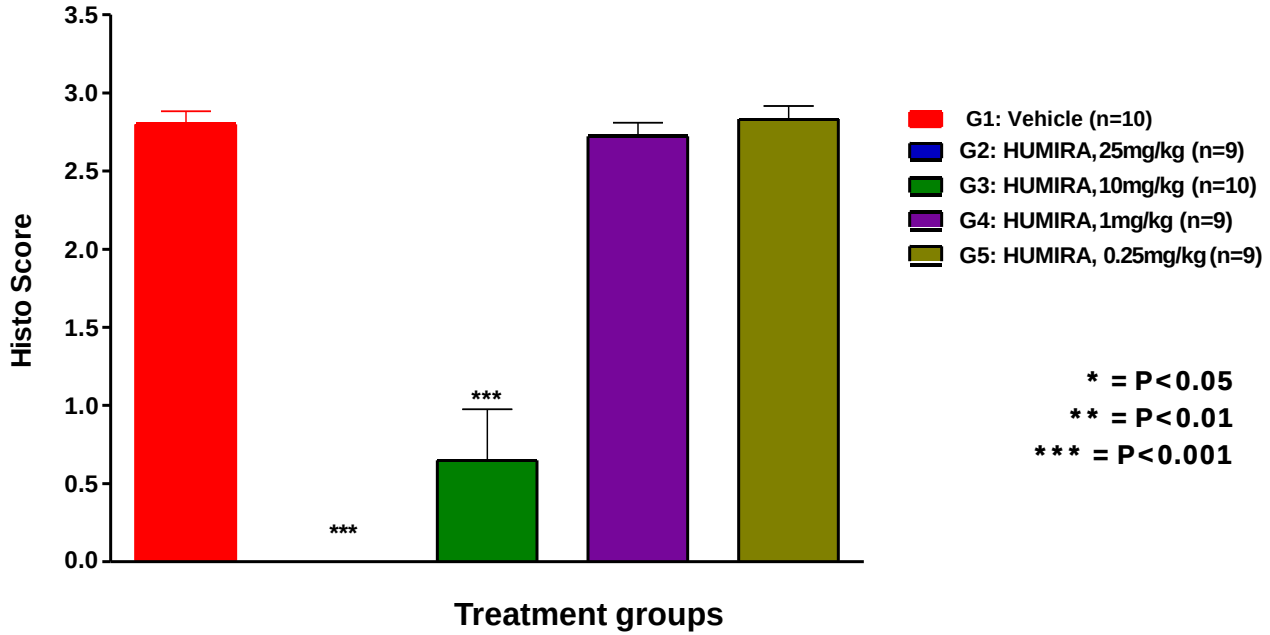
Vehicle

Humira
10 mg/kg



Humira
25 mg/kg

Histopathology scores of front and rear paw joints

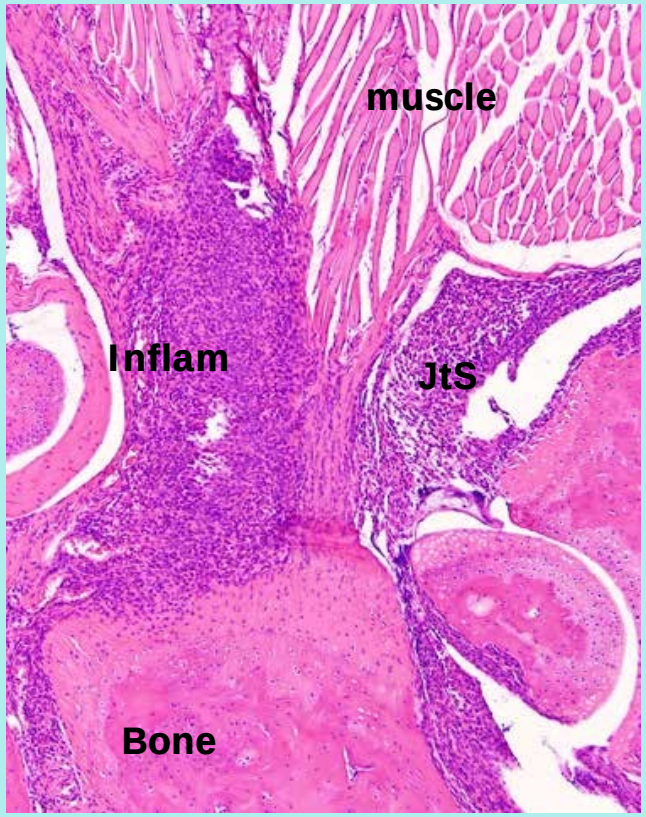


One way ANOVA
P<0.0001

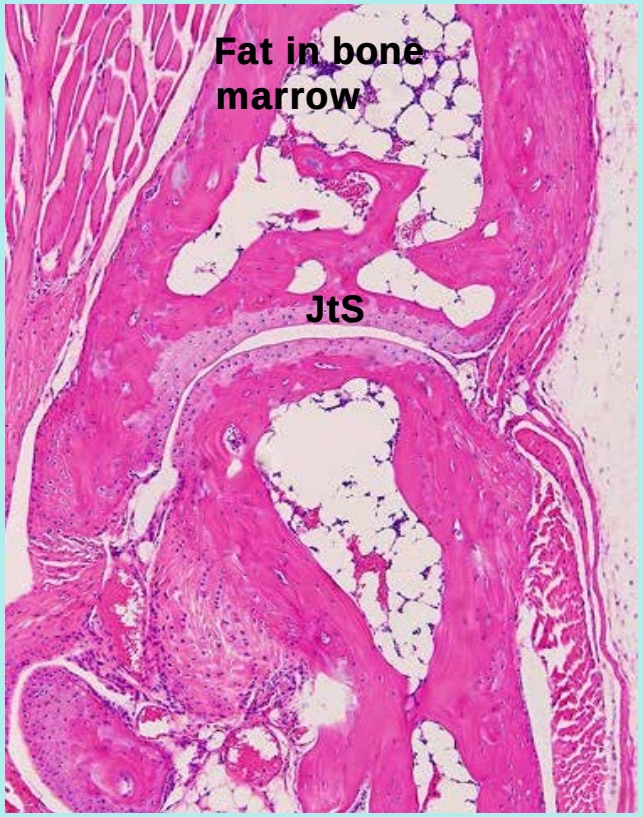
Post Hoc Analysis:
Dunnett's Multiple Comparison Test
G1 vs G2: P<0.001 G1 vs G3: P<0.001
G1 vs G4: ns G1 vs G5: ns

- **Grade 0: no lesions**
- **Grade 1: minimal to mild leukocyte infiltration**
- **Grade 2: moderate leukocyte infiltration**
- **Grade 3: severe leukocyte infiltration, often much of the joints spaces were filled with abundant exudate, inflammatory lesions**

Representative histopathology of ankles from experimental mice

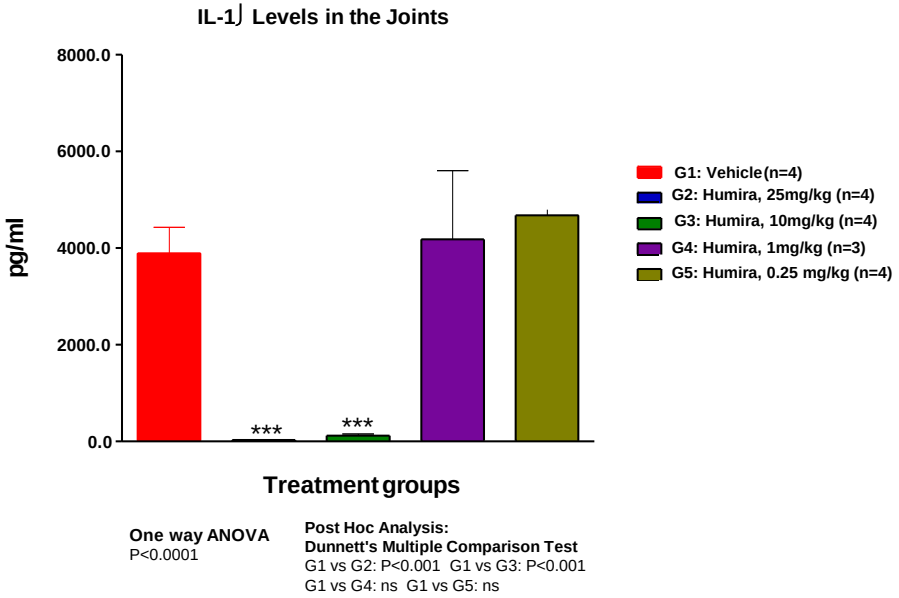


Inflamed ankle joint, 100x, # 560 (non-treated)



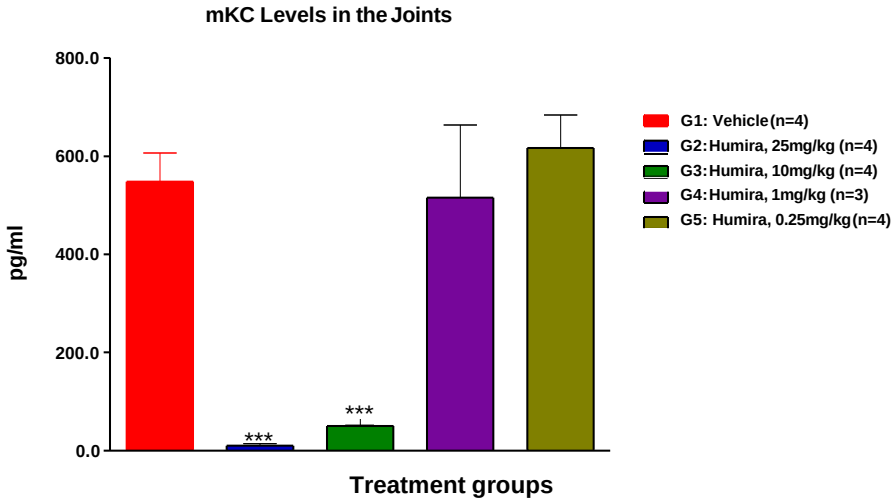
Normal ankle joint, 100x, # 561 (25mg/kg HUMIRA treated)

Paw tissue pro-inflammatory cytokines: IL-1β and m



One way ANOVA
P<0.0001

Post Hoc Analysis:
Dunnett's Multiple Comparison Test
G1 vs G2: P<0.001 G1 vs G3: P<0.001
G1 vs G4: ns G1 vs G5: ns



One way ANOVA
P<0.0001

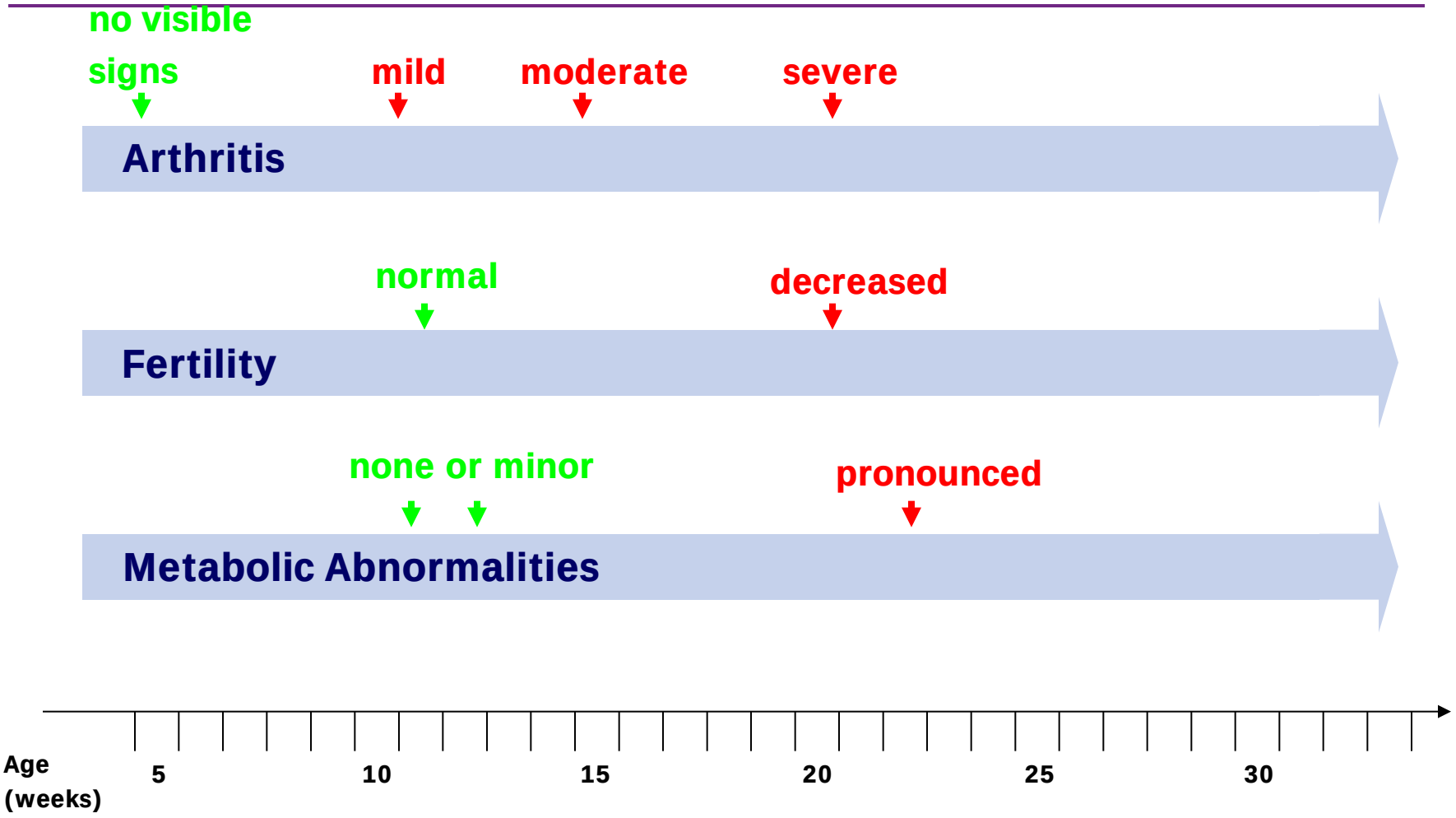
Post Hoc Analysis:
Dunnett's Multiple Comparison Test
G1 vs G2: P<0.001 G1 vs G3: P<0.001
G1 vs G4: ns G1 vs G5: ns

*** = P<0.05**
**** = P<0.01**
***** = P<0.001**

Experimental considerations

- **Males often preferred**
  - Males have earlier onset and more severe disease phenotype
- **Age at study initiation**
  - To see best therapeutic effect, start study with young mice
  - If wish to see efficacy against advanced disease, start with older mice
  - Inflammation seen first; this can be reversed
  - As the disease progresses, bone and tissue remodeling occurs, which may not be reversible
- **Readouts**
  - Clinical score, histopathology and cytokine measurements all relevant
  - Understand time course of cytokine induction and pick relevant timepoints

Other physiological consequences of constitutive human TNFα expression



Summary

- **Model**
  - Spontaneous, no immunization
  - Paw swelling, Clinical score, histopathology and cytokine measurements
  - Similar results at two different sites

- **Advantages**
  - Highly reproducible
  - 100% incidence of disease
  - Highly similar to human RA

- **Suitable for**
  - Anti-TNF compounds
  - Biologic drugs & small molecules in relevant pathway

Contact: Paul.Volden@taconic.com

Krishna.Bhagavatula@Taconic.com

Our partner in India: Vivo BioTech
Kalyan@vivobio.com
bd@vivobio.com

STP-I Society of Toxicologic Pathology - India

& Everyone