Human TNFα Transgenic Mouse Model of Spontaneous Arthritis

Heidelberg Pharma

Taconic

Smart Solutions To Improve Human Health

HEIDELBERG PHARMA AT A GLANCE



- CRO situated in Ladenburg, near Heidelberg, Germany
- 45 employees, 2000 m^2 of lab space
- Core competence: pre-clinical profiling of small molecules and biologicals
- Focus: cancer, inflammatory & autoimmune diseases
- Services: Explorative pharmacology, drug-metabolism and pharmacokinetics (DMPK), molecular biology
- Standard models, customized experimental design, new solutions

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INFLAMMATION & AUTOIMMUNITY



Mechanistic models

- Thioglycolate induced peritonitis
- LPS-induced cytokine release (IL-2; -4; -5; -6; -10, MCP-1; IL-12p70; IFNg and TNFa)
- Anti-CD3- induced cytokine release (Il-2; -4; -5; -6; -10, MCP-1; IL-12p70; IFNg and TNFa)
- DTH (delayed type hypersensitivity) model with KLH (keyhole limpet hemocyanin)

Autoimmune disease models

- Experimental Autoimmune Encephalitis (EAE, Multiple Sclerosis) in SJL/J mice
- Collagen - Induced Arthritis (CIA) in DBA/1 mice
- Diabetes (DIO model)

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Syngenic models

- Syngenic models using s.c., i.p. or i.v. application:
  Leukemia, lung, colon, testicular teratoma and melanoma

Standard xenograft models

- Several subcutaneous xenograft models are established:
  Glioma, Stomach, Cervix, Ovary, Pancreas, Colon, Kidney, Lung, Breast, Prostate, Bladder

Orthotopic xenograft models

- Luciferase transfected cell lines suitable for Bioimaging. Implantation sites: Caecum, Pancreas, Prostate, Kidney in development

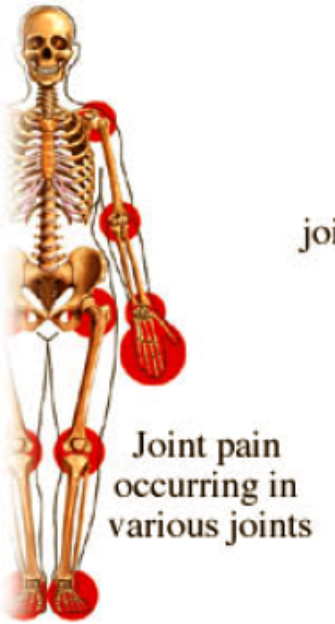
Metastasis models using human cell lines

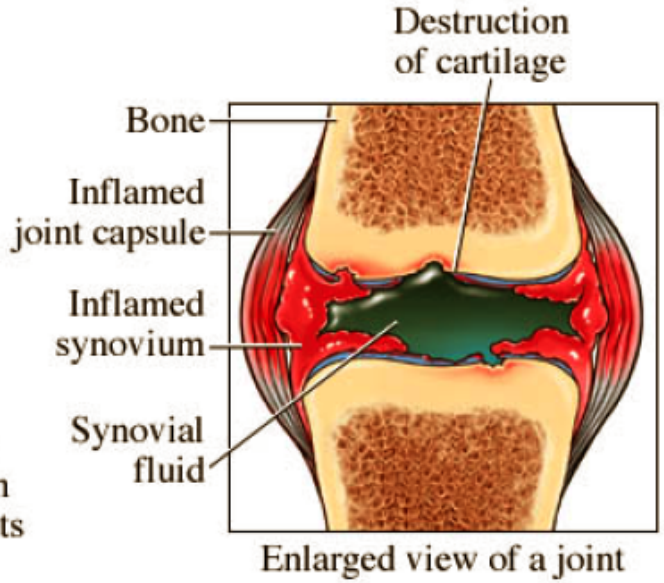
- Left-ventricular inoculation of luciferase transfected cell lines suitable for Bioimaging.

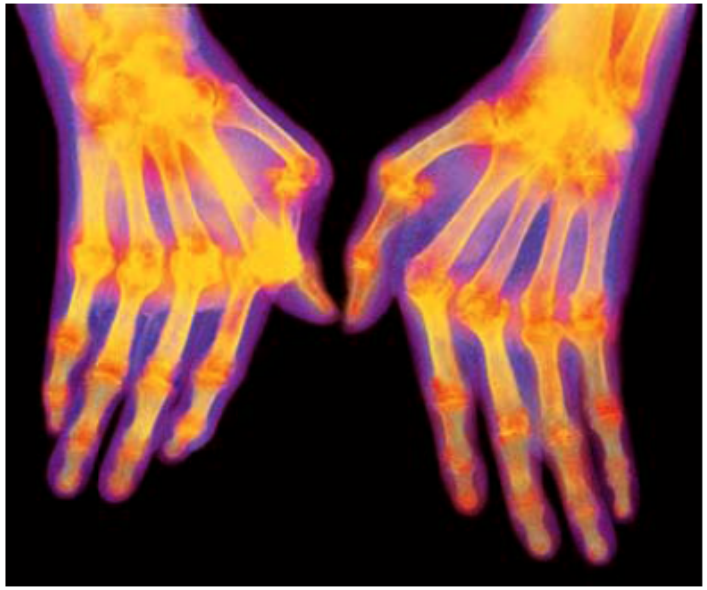
Models to evaluate bispecific Antibodies

- Implantation of a mixture of cancer cell lines with human peripheral blood mononuclear cells (hPBMCs)

Rheumatoid Arthritis



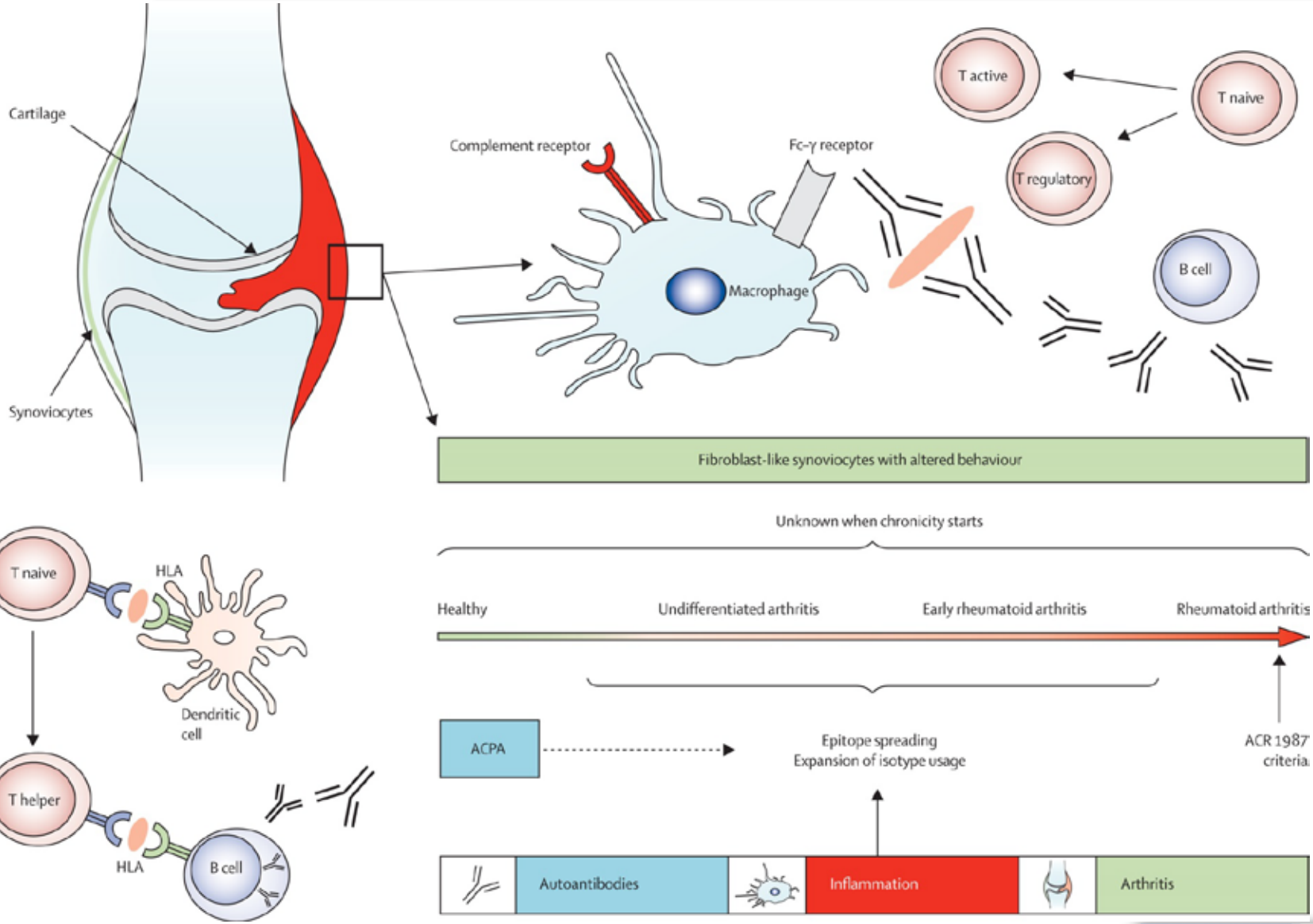




Infliximab (Remicade®)
Etanercept (Enbrel®)
Anakinra (Kineret®)
Adalimumab (Humira®)
Certolizumab Pegol (Cimzia™)

Rheumatoid Arthritis : Pathogenesis





PRECLINICAL MODELS FOR RA



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

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SIMILARITIES & DIFFERENCES

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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.

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Origin of the TNFa transgenic model

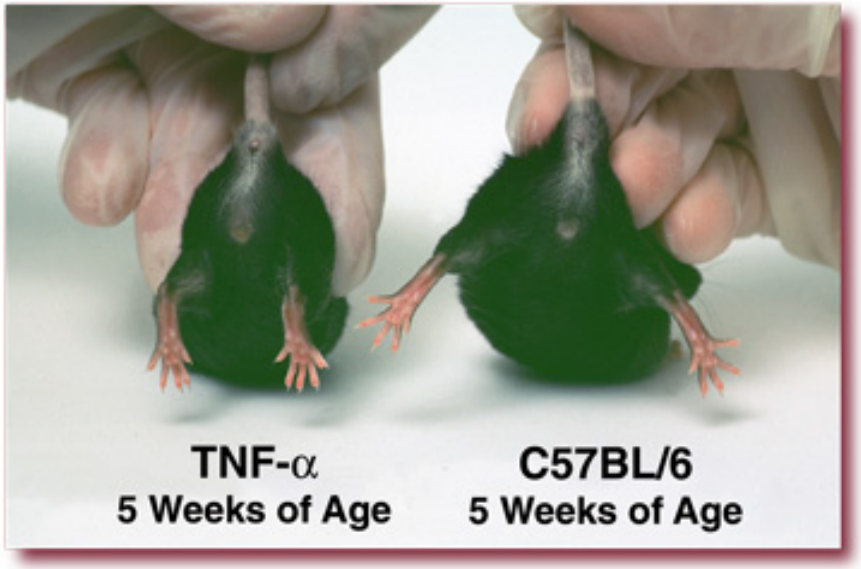


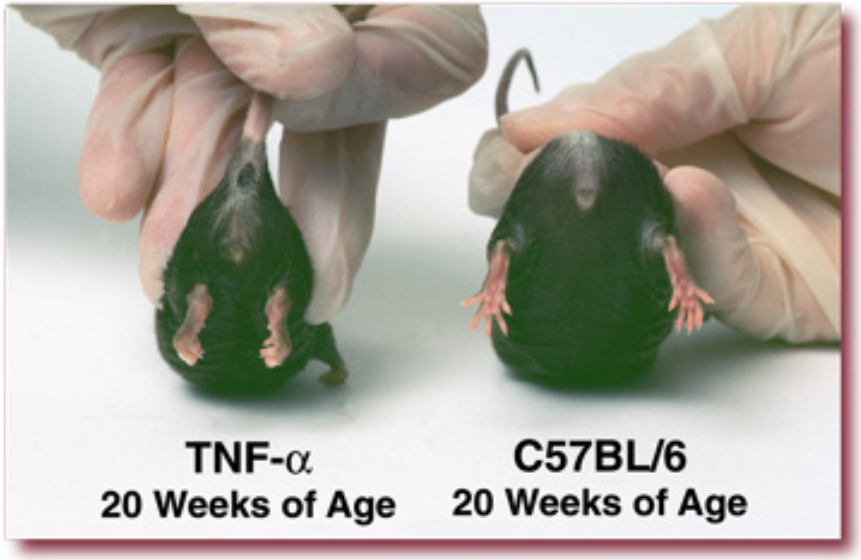
- **The TNFα transgenic mice were generated using a construct that contains a 2.8 kb fragment of the human TNFα gene, including the entire coding region and promoter, fused to the human β-globin 3' untranslated region (UTR) that replaces the endogenous 3'UTR of the human TNFα gene**
  - Designed to model dysregulated human TNFa 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 TNFa mice



- **The TNFa 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

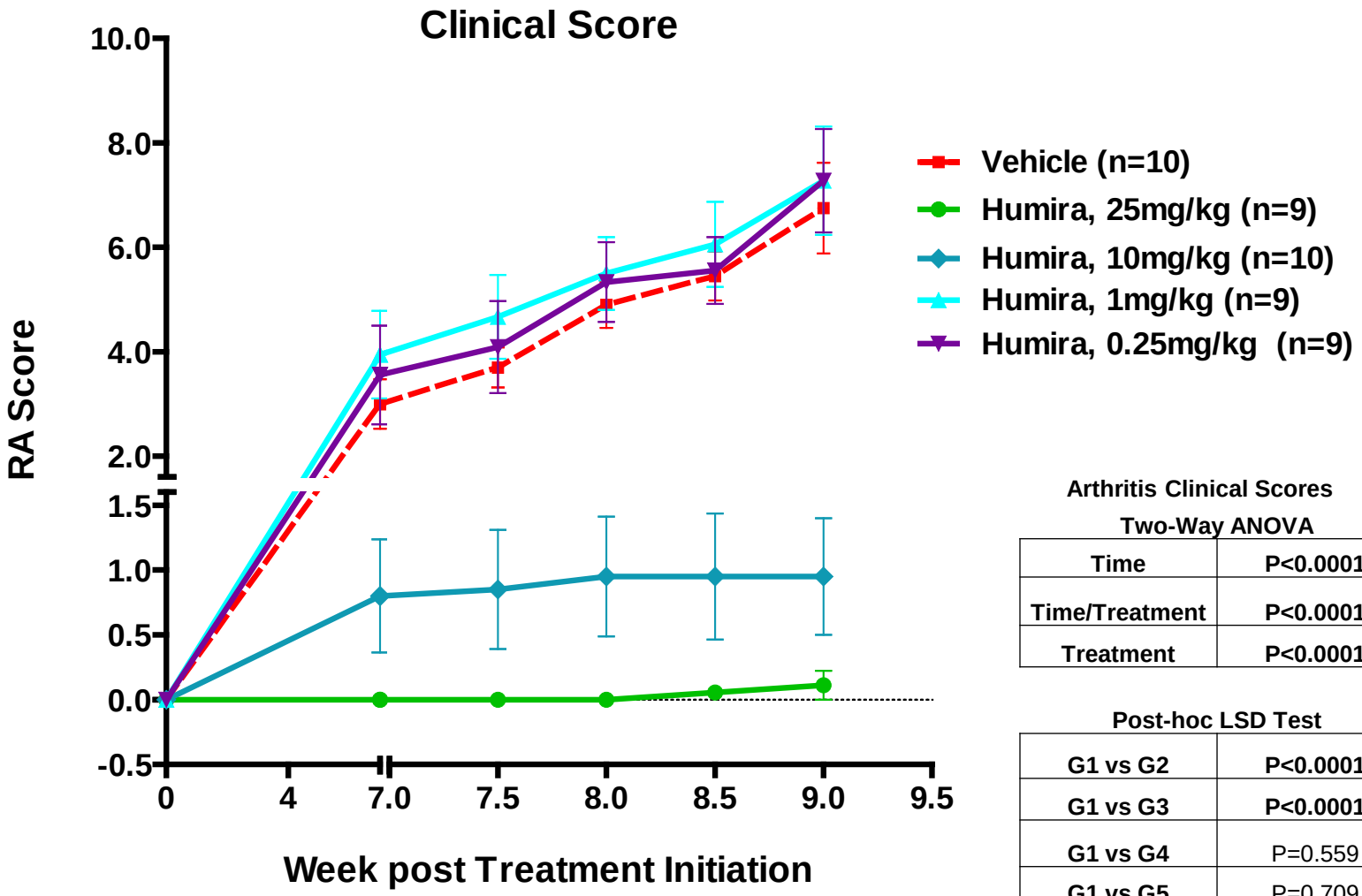


Maximum 24 scores were given to each mouse.

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

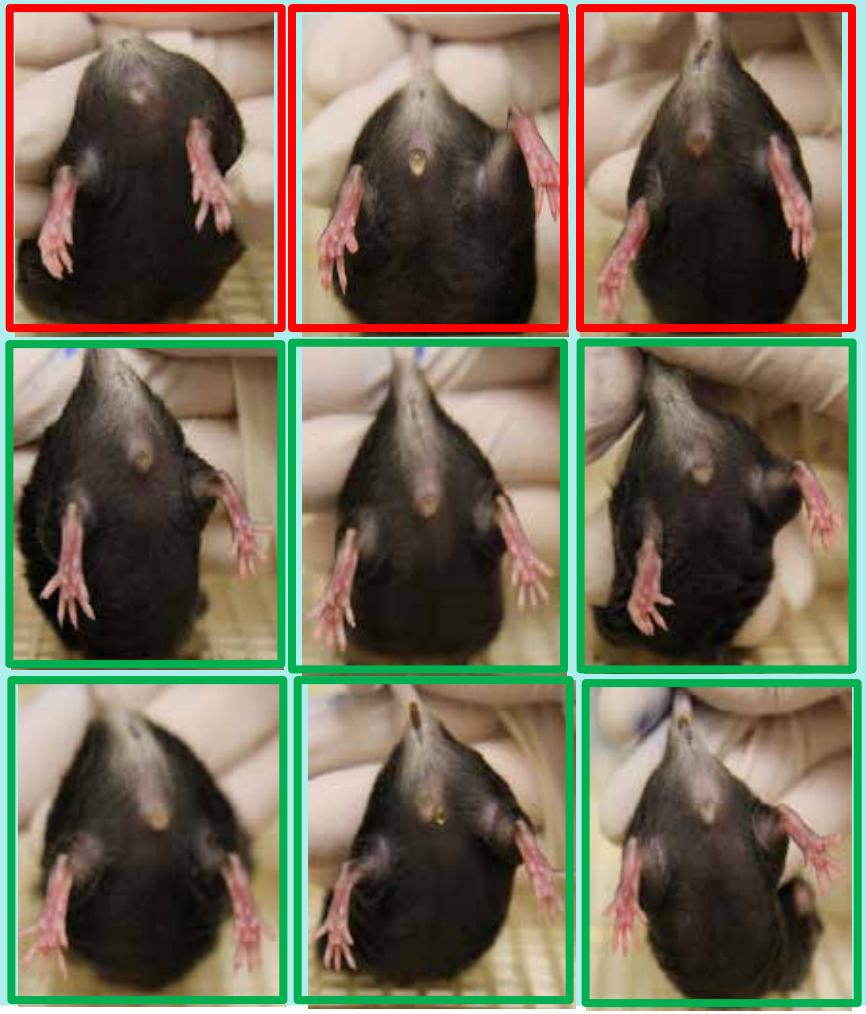
Two-Way ANOVA

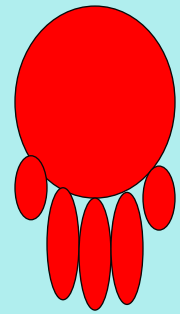
Time	**P<0.0001**
Time/Treatment	**P<0.0001**
Treatment	**P<0.0001**

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

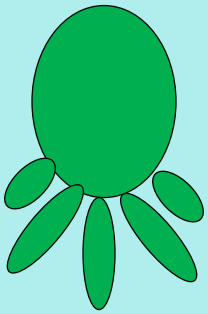
Clinical manifestation of arthritis in treated and untreated animals





Vehicle

Humira
10 mg/kg



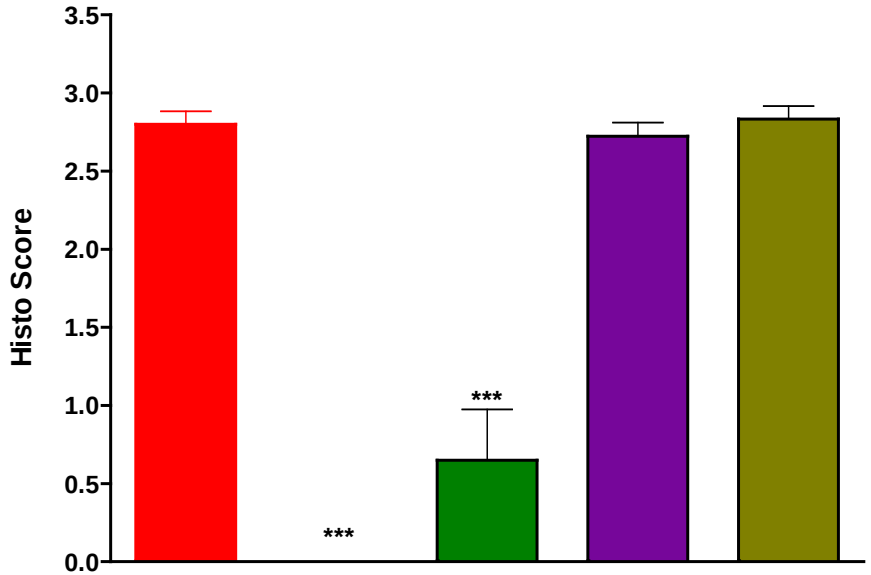
Humira
25 mg/kg

Histopathology grading of joint lesions



- **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**

Histopathology scores of front and rear paw joints



G1: Vehicle (n=10)
G2: HUMIRA, 25mg/kg (n=9)
G3: HUMIRA, 10mg/kg (n=10)
G4: HUMIRA, 1mg/kg (n=9)
G5: HUMIRA, 0.25mg/kg (n=9)

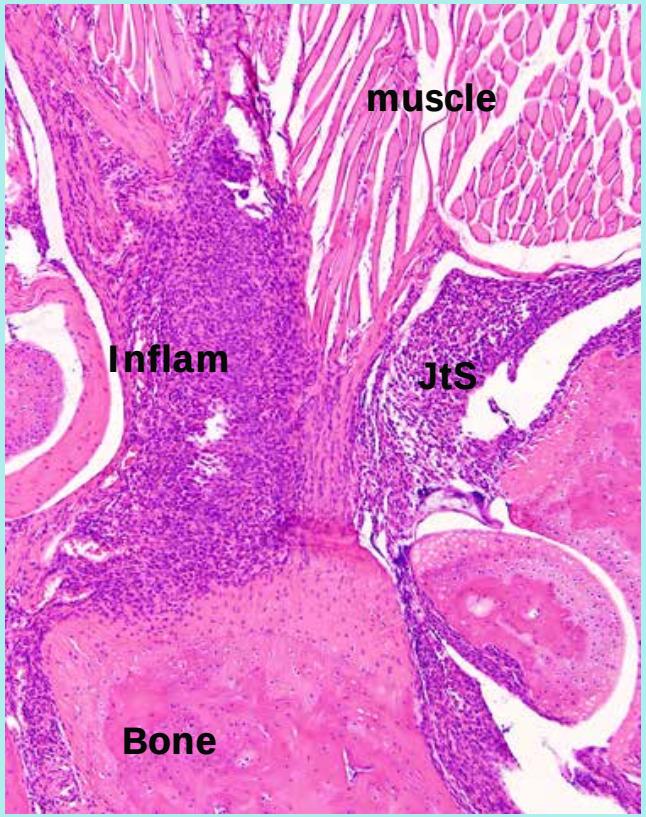
* = P<0.05
** = P<0.01
*** = P<0.001

One way ANOVA
P<0.0001

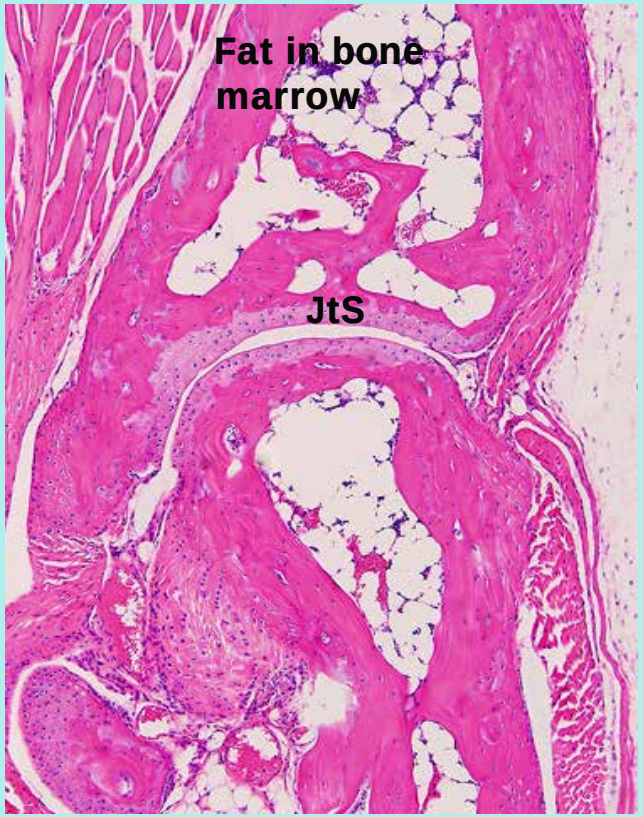
Post Hoc Analysis:
Dunnett's Multiple Comparison Test
G1 vs G2: P<0.001
G1 vs G3: P<0.001
G1 vs G4: ns
G1 vs G5: ns

- **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

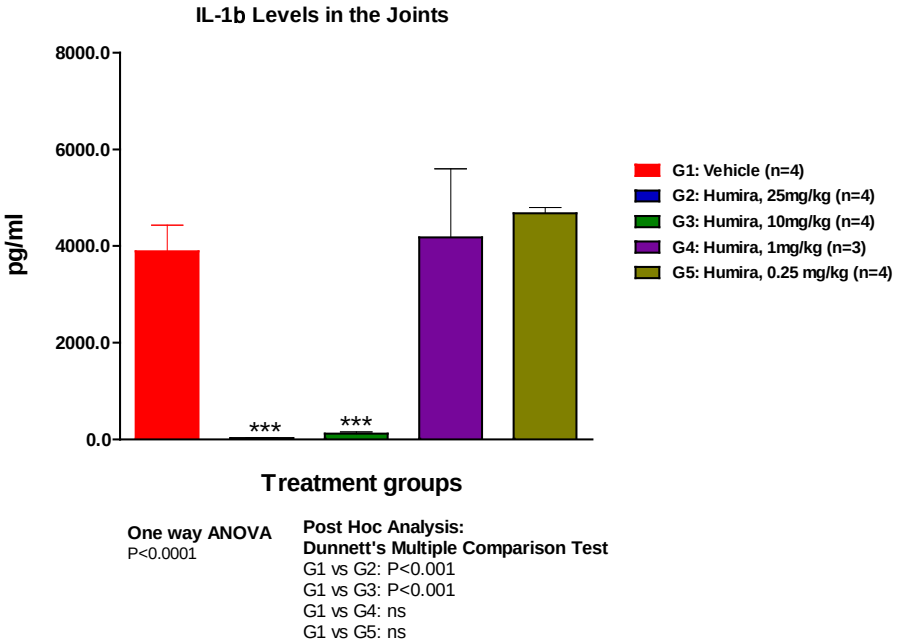


Inflamed ankle joint, 100x, # 560 (non-treated)



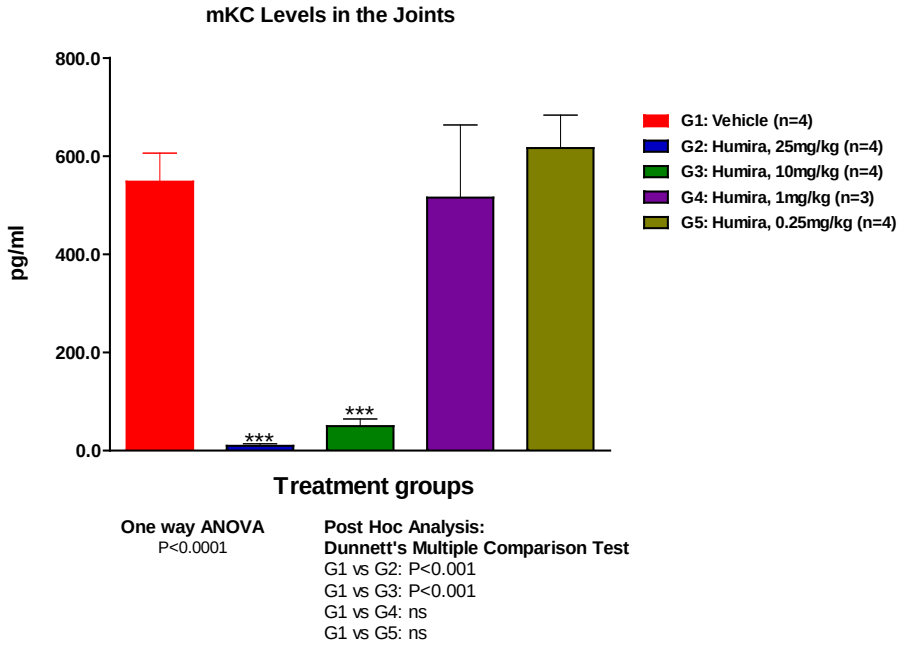
Normal ankle joint, 100x, # 561 (25mg/kg HUMIRA treated)

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



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**

Study at Heidelberg Pharma



Animals

- Age at delivery 5 weeks
- Age at start of experiment 6 weeks

Treatment Groups (n=8)

- Vehicle
- Humira 5 mg/kg ip
- Humira 10 mg/kg ip
- Schedule 2x weekly

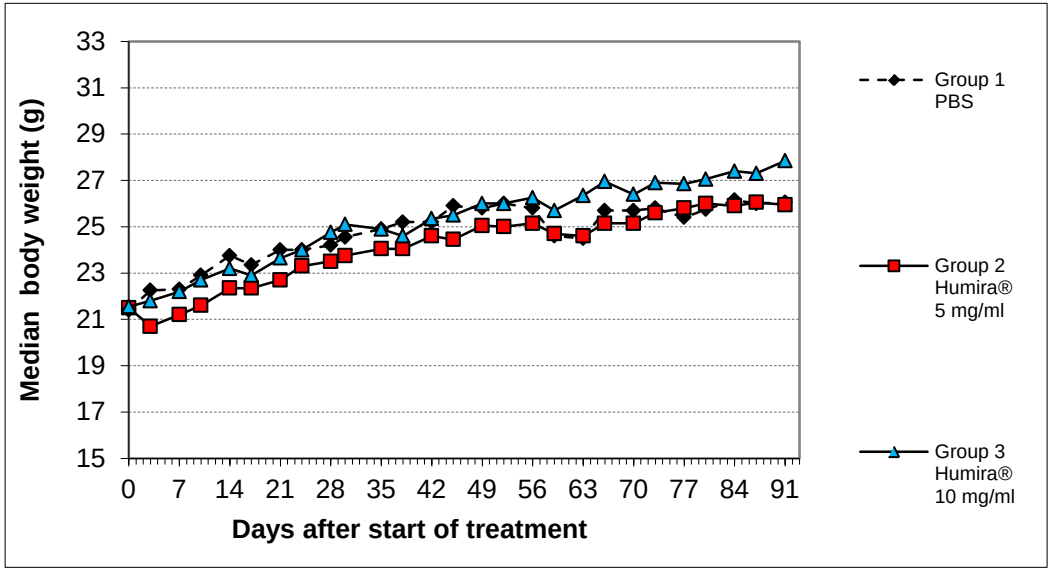
Readouts

- Clinical Score
- Paw swelling
- Histology : Paws fixed and embedded

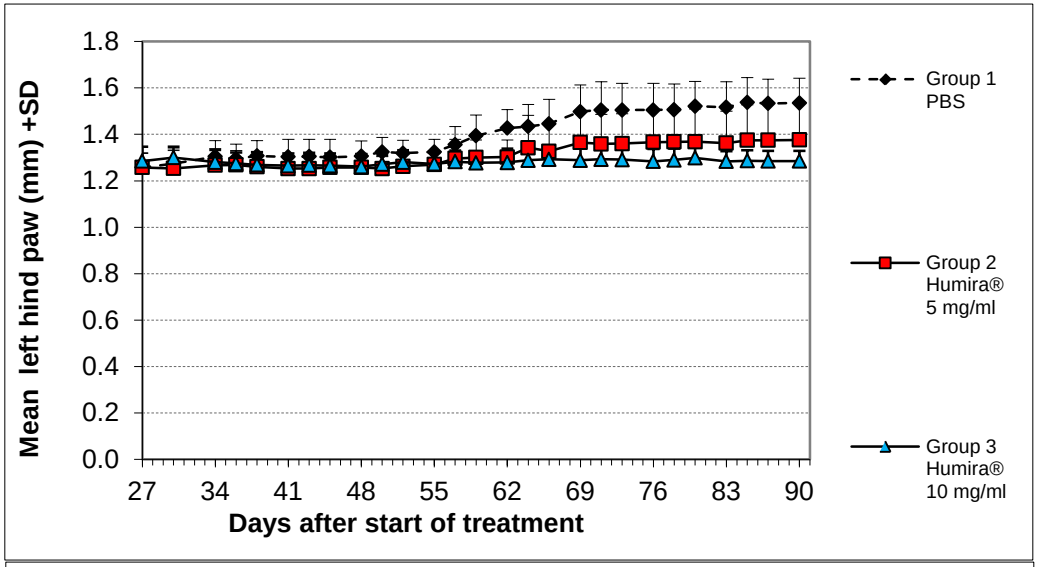
Body Weight

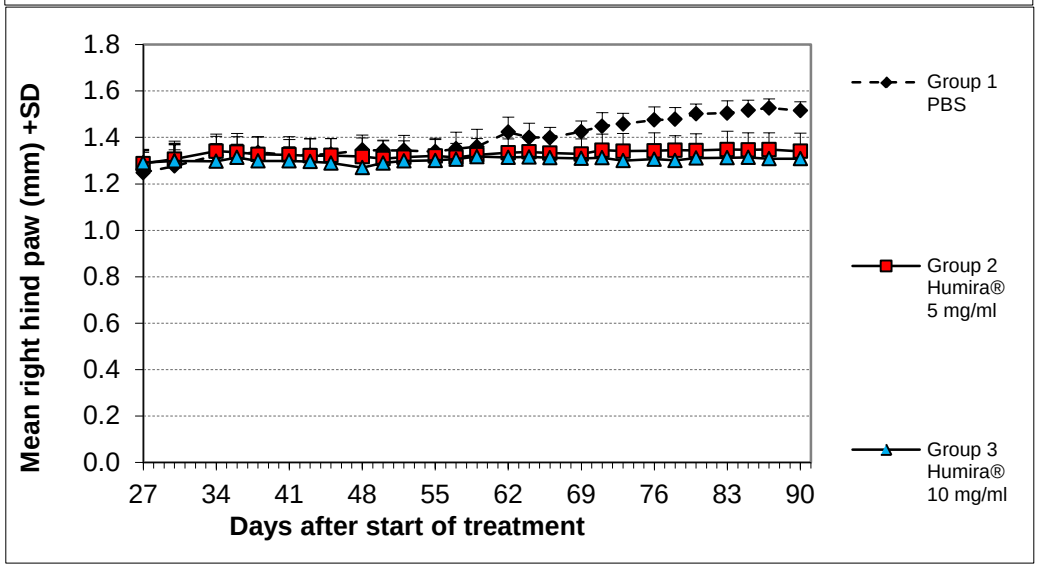


- **Steady increase in body weight**
- **No effect on body weight by treatment with Humira**



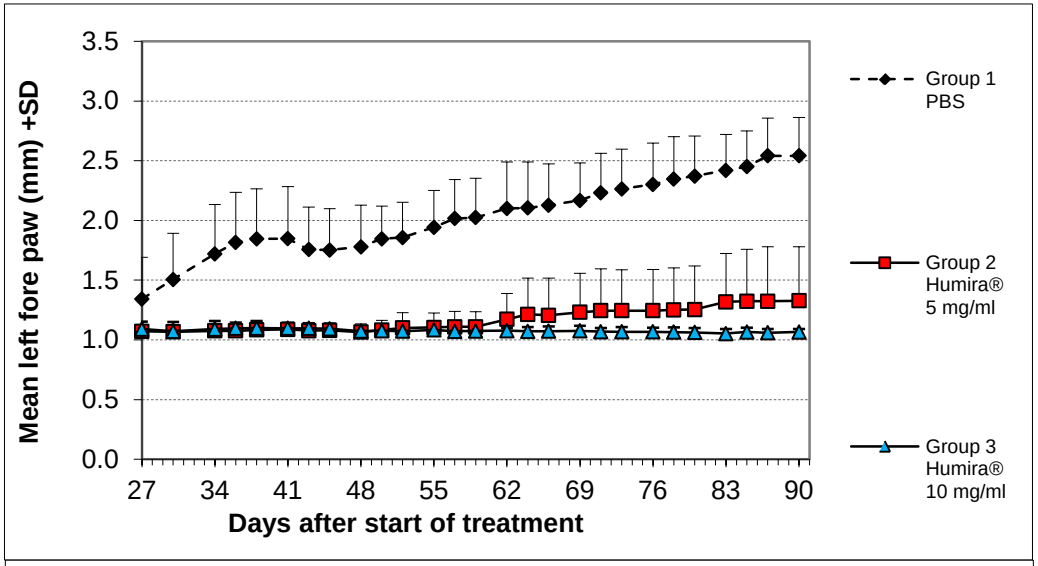
Paw swelling

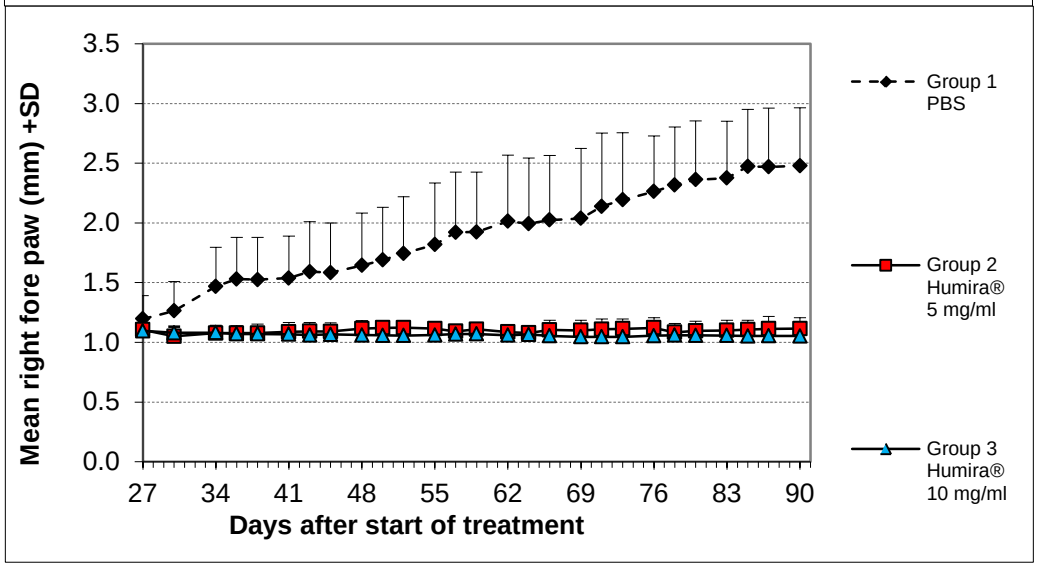




- **Only minor swelling in hind paws**
- **Humira effectively inhibited paw swelling**

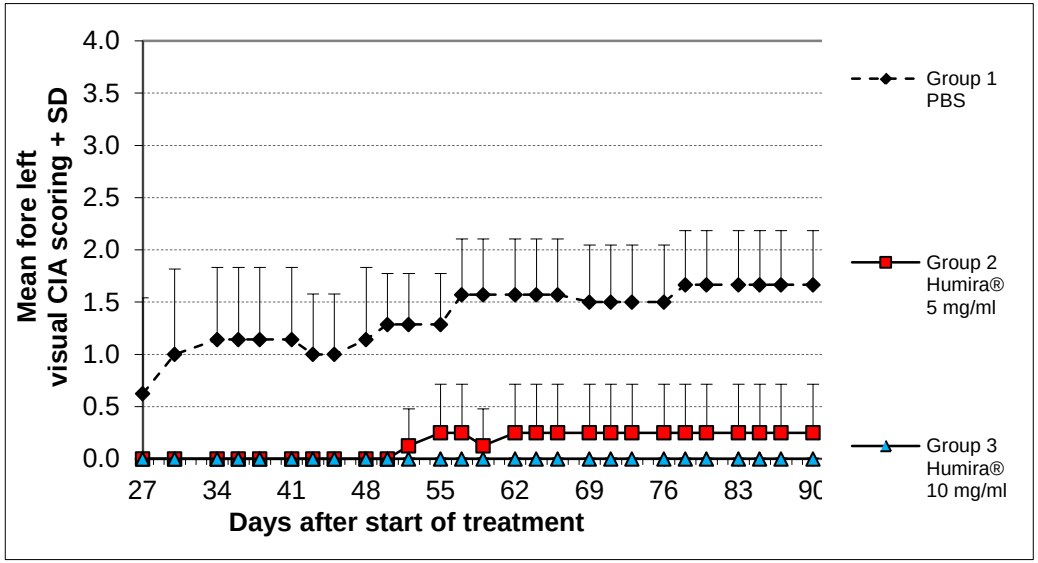
Paw swelling

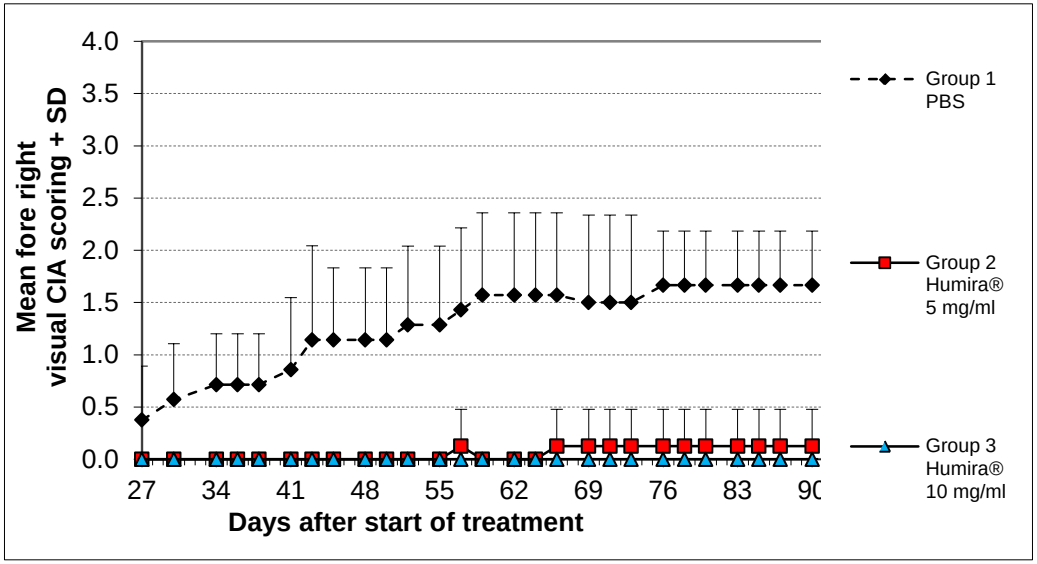




- **Similar swelling in both fore paws**
- **Humira effectively inhibited paw swelling**

Clinical Score





- **Similar development of disease in both fore paws**
- **Humira effectively inhibited progression of disease**

Scores:
0: No evidence of erythema and swelling
1: Erythema and mild swelling confined to the mid-foot (tarsals) and ankle joint
2: Erythema and mild swelling extending from the ankle to the mid-foot
3: Erythema and moderate swelling extending from the ankle to the metatarsal joints
4: Erythema and severe swelling encompass the ankle, foot, and digits

(acc. to Current Protocols in Immunology, 15.5.11)

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

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



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Backup



INVOLVEMENT OF IMMUNE SYSTEM



	Mouse CIA	Rat CIA	Rat AIA	Mouse PGIA	Mouse SCW-induced arthritis	Rat polyarticular SCW-induced arthritis	Rat monarticular SCW-induced arthritis	Mouse STIA	K/BxN-transgenic mouse	Human TNF–transgenic mouse
Antigen	Type II collagen	Type II collagen	Hsp65, peptide 180-186	Proteoglycan	SCW components	PG-PS 10S	PG-PS 100P	G6PI-specific autoantibodies	G6PI	Human TNF
Monocyte/ macrophages	Yes	Yes	Yes	Yes	Yes	Yes, starting during acute phase (~5 days after injection)	Yes (after initial neutrophil phase, a few days after IA injection)	Yes	Yes	Yes
Dendritic cells	Yes	NR	Yes	NR; B cells are dominant APCs	NR	NR	NR	NR	Yes	NR
Granulocytes	Yes	Yes	Yes	Yes	NR	NR	Yes (during initial and reactivation response)	Yes	Yes	Yes
T cells	Yes, CD4+, type II collagen reactive, mainly during induction	Yes	Yes, synovial	Yes, CD4+ T cells	Yes (only during reactivation phase), main role for CD4+ T cells	Yes, only during chronic phase	Yes (during reactivation phase)	No (can increase severity but are not crucial for disease induction)	Yes, autoreactive to G6PI	No
B cells, antibody production	Yes, production of complement fixing type II collagen–reactive antibodies	Yes, production of antibodies to type II collagen	Yes	Yes, autoantibodies required for initiation of disease	SCW-specific antibodies are detected	Yes, only during chronic phase; minimal/no antibody response to PG-PS	NR	No (G6PI-specific autoantibodies crucial, B cells in recipient mice not crucial)	Yes, produce G6PI-specific autoantibodies	No
NK cells	Dampen CIA	NR	NR	NR	NR	NR	NR	NR	NR	NR
Complement	Yes	Yes	No	Yes	NR	Yes	Yes	Yes	Yes	NR
Specific MHC	No	Yes	No	No	NR	No	NR	No	Yes	Influences severity of arthritis

* CIA = collagen-induced arthritis; AIA = adjuvant-induced arthritis; PGIA = proteoglycan-induced arthritis; SCW = streptococcal cell wall; STIA = serum transfer–induced arthritis; TNF = tumor necrosis factor; PG-PS = peptidoglycan–polysaccharide; G6PI = glucose-6-phosphate isomerase; IA = intraarticular; NR = not reported; APCs = antigen-presenting cells; NK = natural killer.

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INVOLVEMENT OF CYTOKINES



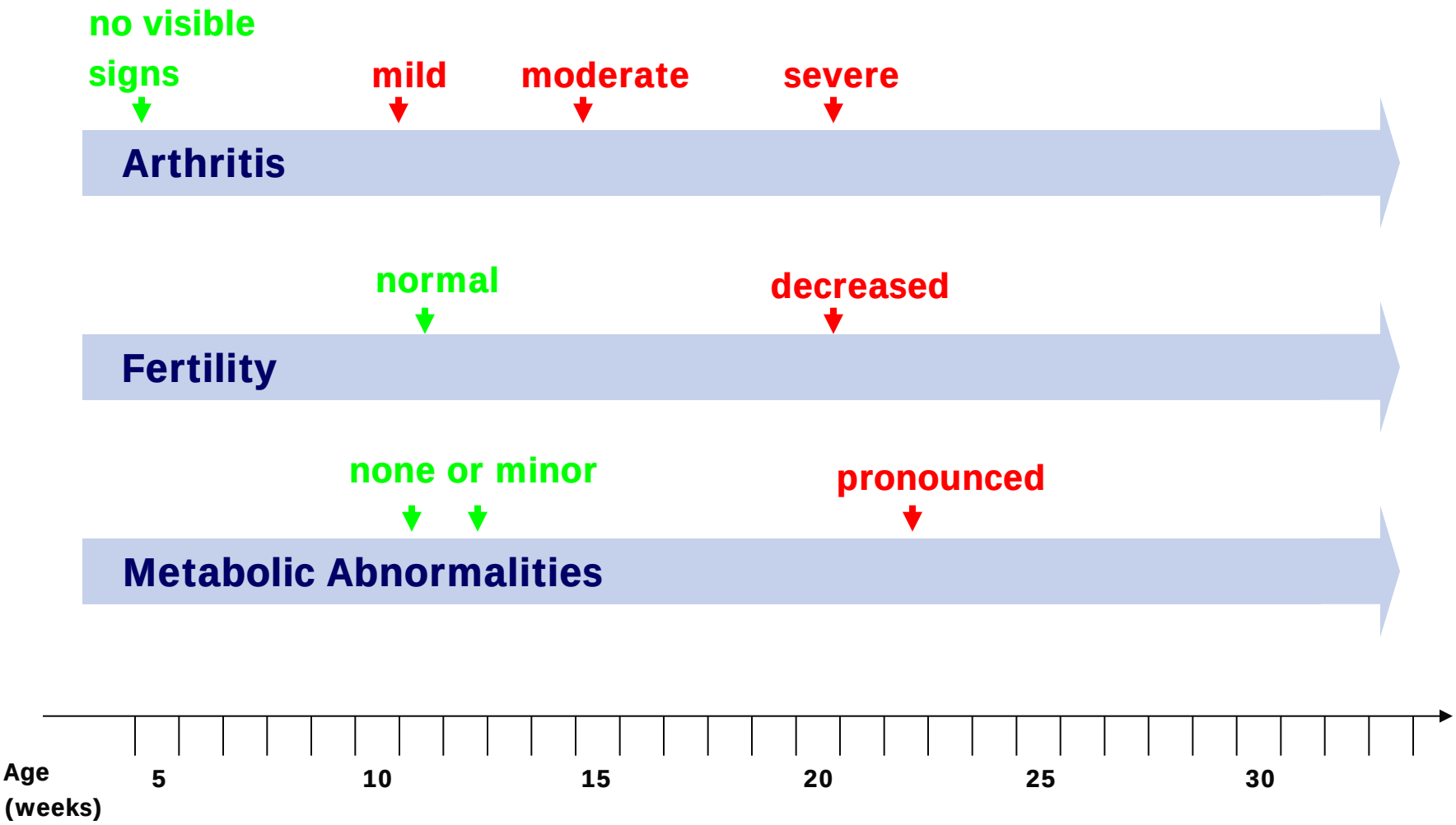
Table 3. Involvement of cytokines in animal models of arthritis*

	Mouse CIA	Rat CIA	Rat AIA	Mouse PGIA	Mouse SCW-induced arthritis	Rat polyarticular SCW-induced arthritis	Rat monarticular SCW-induced arthritis	Mouse STIA	K/BxN-Tg mice	Human TNF–Tg mice
TNFα	Yes	Yes	Yes, detectable 4 days post-injection	Yes	Yes/negative, minor role, only expressed during initial joint swelling	Yes	Yes, during reactivation	Yes/negative, varying results	No	Yes
IL-1β	Yes	Yes	Yes, detectable 4 days post-injection	Yes	Yes, involved in cartilage breakdown and inflammatory cell influx	Yes	Yes, during reactivation	Yes	Yes	Yes
IL-4	No (can dampen inflammation)	No (can dampen inflammation)	Not until later stage (can dampen response)	No (can dampen inflammation)	No	Yes	Yes	No	Yes	NR
IL-6	Yes	Yes	Yes, detectable 4 days post-injection	Yes	Yes	Yes	Yes	No	No	No
IL-10	No (can dampen inflammation)	No (can dampen inflammation)	No (can dampen response)	No (can dampen inflammation)	No (can dampen inflammation by influencing TNF levels)	No	No	NR	NR	NR
IL-12	No (protects from inflammation)	NR	NR	Yes	Yes	NR	NR	No	No	NR
IL-17	Yes	Yes	Yes	No	Yes, required to switch from an acute to a chronic reaction	Yes	Yes	NR	NR	NR
IL-21	Yes	NR	Yes	NR	NR	NR	NR	NR	Yes	NR
IL-23	Yes	Yes	NR	NR	Yes, chronic stage	NR	NR	NR	NR	NR
IL-32	Yes	NR	NR	NR	NR	NR	NR	NR	NR	NR
IFNγ	Contradictory findings, possible role in regulating T cells	Yes	Yes	Yes	NR	Yes/no, conflicting reports	No	No (can dampen response)	NR	NR
MCP-1	Yes	Yes, recruitment of monocytes, plays role in development of arthritis	Yes	Yes	NR	NR	Yes (during reactivation phase, up-regulated via IL-4)	NR	NR	NR
MIP-1α	Yes	Yes	Yes	Yes	NR	NR	Yes (reactivation phase)	NR	NR	NR
MIP-2	Yes	Yes	NR	Yes	NR	NR	Yes (reactivation phase)	NR	NR	NR

* CIA = collagen-induced arthritis; AIA = adjuvant-induced arthritis; PGIA = proteoglycan-induced arthritis; SCW = streptococcal cell wall; STIA = serum transfer–induced arthritis; Tg = transgenic; TNFα = tumor necrosis factor α; IL-1β = interleukin-1β; NR = not reported; IFNγ = interferon-γ; MCP-1 = monocyte chemotactic protein 1; MIP-1α = macrophage inflammatory protein 1α.

Other physiological consequences of constitutive human TNFa expression



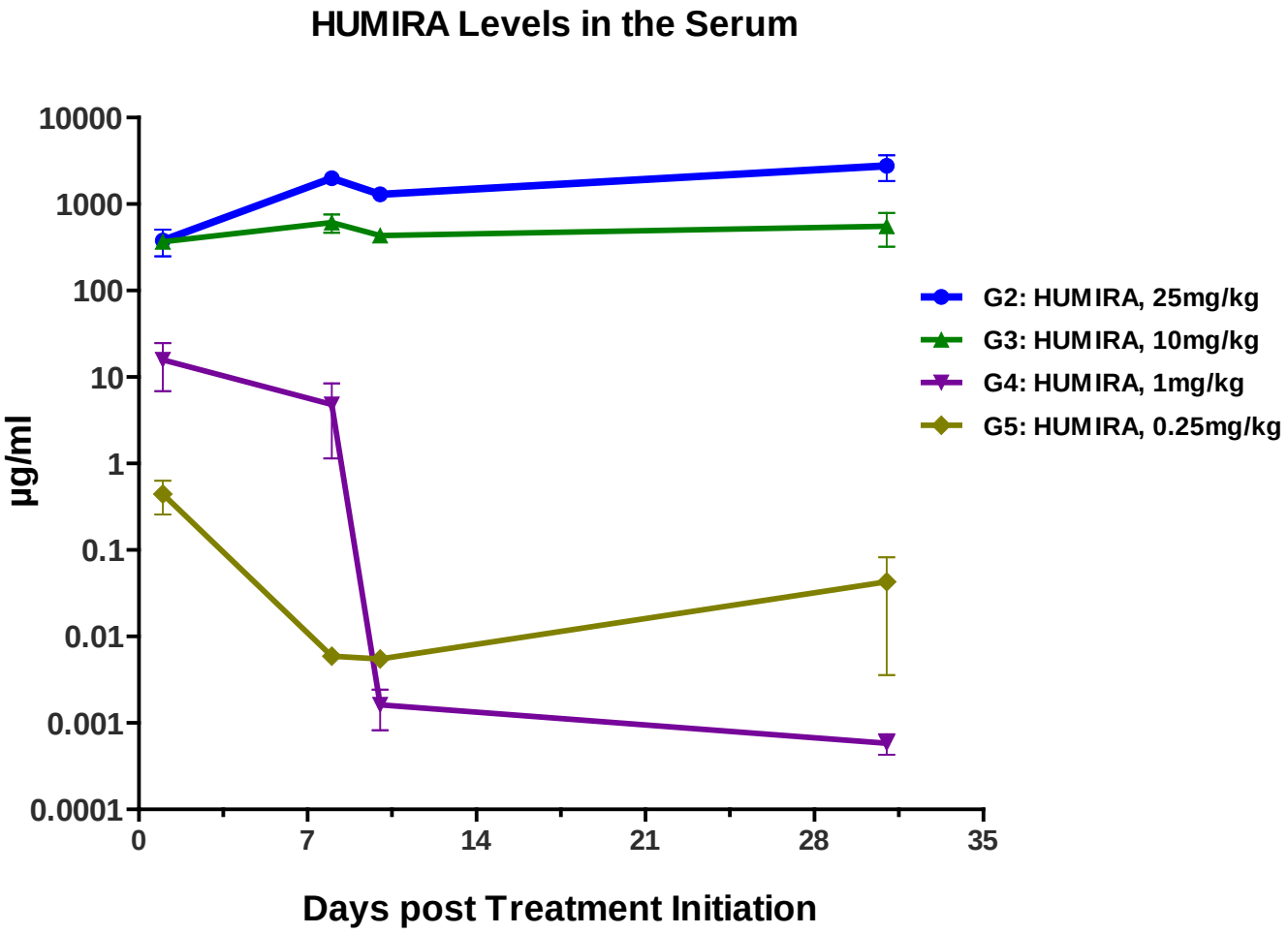


TNFa: overview



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

Humira levels in mouse serum during the study



Proof of concept study with Humira



- **Humira (adalimumab) is a biologic drug approved for the treatment of arthritis**
  - Recombinant human IgG1 monoclonal antibody
  - Mechanism of action involves binding to TNFa to block signaling

Experimental groups



Group #	Treatment	Dosing Schedule	Route/Volume	Concentration
1	Placebo	Twice weekly	i.p./100µl	1:10 dilution in PBS
2	HUMIRA-022512E	Twice weekly	i.p./100µl	25mg/kg
3	HUMIRA-022512E	Twice weekly	i.p./100µl	10mg/kg
4	HUMIRA-022512E	Twice weekly	i.p./100µl	1mg/kg
5	HUMIRA-022512E	Twice weekly	i.p./100µl	0.25mg/kg

Experimental considerations



- **Group size**
  - Minimum group size = 8. Recommended group size = 10.
- **Readouts**
  - Clinical score, histopathology and cytokine measurements all relevant
  - Understand time course of cytokine induction and pick relevant timepoints
- **Immunogenicity and efficacy**
  - Biologic drugs can induce an immune response in mice
  - Important to monitor drug concentrations over course of study
  - May need to use progressively higher concentrations to preserve efficacy
  - Humira has low immunogenicity and thus decrease in effective concentrations is not a big concern over typical study