Human TNFα Transgenic Mouse Model of Spontaneous Arthritis
HEIDELBERG PHARMA AT A GLANCE

- CRO situated in Ladenburg, near Heidelberg, Germany
- 45 employees, 2000 m² 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
Mechanistic models

- Thioglycolate induced peritonitis
- LPS-induced cytokine release (IL-2; -4; -5; -6; -10, MCP-1; IL-12p70; IFNγ and TNFα)
- Anti-CD3- induced cytokine release (IL-2; -4; -5; -6; -10, MCP-1; IL-12p70; IFNγ and TNFα)
- 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)
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

Fibroblast-like synoviocytes with altered behaviour

Unknown when chronicity starts

Healthy, Undifferentiated arthritis, Early rheumatoid arthritis, Rheumatoid arthritis

ACPA

Epitope spreading, Expansion of isotype usage

Autoantibodies, Inflammation, Arthritis
<table>
<thead>
<tr>
<th>Categories</th>
<th>Induction principle</th>
<th>Examples</th>
<th>Inciting agents/genetic alteration</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetically engineered</td>
<td>Deliberate manipulation of one or more genes encoding proteins that regulate the immune response</td>
<td>HLA-B27 transgenic</td>
<td>Human leukocyte antigen (HLA) B27 (a major histocompatibility complex (MHC) class I molecule) and human β2-microglobulin</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLA-DR transgenic</td>
<td>Human leukocyte antigen, D-related (a MHC class II molecule)</td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-1ra knockout</td>
<td>Interleukin-1 receptor antagonist</td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K/BxN</td>
<td>Human T-cell receptor (KRN) and a human MHC class II molecule</td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF-α transgenic</td>
<td>Tumor necrosis factor-α</td>
<td>Mouse</td>
</tr>
<tr>
<td>Induced</td>
<td>Administration of an exogenous material</td>
<td>Adjuvant-induced arthritis (AIA)</td>
<td>Lipoidal amine</td>
<td>Rat</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Mycobacterium tuberculosis</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pristane</td>
<td>Mouse, rat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collagen-induced arthritis (CIA)</td>
<td>Type II collagen (bovine, porcine, and rodent)</td>
<td>Mouse, rat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacterial cell wall-induced arthritis</td>
<td>Bacterial cell wall peptidoglycan (polysaccharide): Lactobacillus sp., Streptococcus sp. (SCW)</td>
<td>Rat</td>
</tr>
<tr>
<td>Spontaneous</td>
<td></td>
<td>MRL/lpr</td>
<td>MRL/Mpj-lpr/lpr</td>
<td>Mouse</td>
</tr>
<tr>
<td>Animal model</td>
<td>Similarities to RA</td>
<td>Differences from RA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIA in mice</td>
<td>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</td>
<td>Formation of antibodies to collagen, greater incidence in males, peristosis, poor responses to NSAIDs, not characterized by exacerbations and remissions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIA in rats</td>
<td>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</td>
<td>Not characterized by exacerbations and remissions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGIA in mice</td>
<td>Development of polyarthritis, presence of rheumatoid factor, deposition of immune complexes in the joint, persistent joint inflammation</td>
<td>Development of ankylosing spondylitis, not characterized by exacerbations and remissions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIA in rats</td>
<td>Symmetric joint involvement, inflammatory cell infiltrate, cartilage degradation, synovial hyperplasia, genetic linkage, T cell dependence</td>
<td>Damage to cartilage less severe than in RA, bone destruction more prominent; no rheumatoid factor produced, gastrointestinal tract and skin affected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCW-induced arthritis in mice</td>
<td>Characterized by exacerbations and remissions</td>
<td>None specified in publications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyarticular SCW-induced arthritis in rats</td>
<td>Symmetric joint involvement, synovial hyperplasia, inflammatory cell infiltration, relapsing inflammation</td>
<td>No rheumatoid factor produced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monarticular SCW-induced arthritis in rats</td>
<td>Characterized by exacerbations and remissions</td>
<td>None specified in publications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIA in mice</td>
<td>Inflammatory cell infiltrate, synovial hyperplasia, pannus formation, cartilage destruction</td>
<td>None specified in publications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K/BxN-Tg mice</td>
<td>Symmetrically affects small peripheral joints</td>
<td>Distal interphalangeal joints often affected, no systemic manifestations, no production of rheumatoid factor, arthritis does not remit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human TNF- Tg mice</td>
<td>Synovial hyperplasia, presence of an inflammatory cell infiltrate, pannus formation, cartilage destruction, and bone resorption</td>
<td>No production of rheumatoid factor</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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.
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 TNFα expression

This transgenic line was produced by pronuclear microinjection of B6SJ LF2 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.
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 or 1 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

Clinical Score

Week post Treatment Initiation

RA Score

Vehicle (n=10)
Humira, 25mg/kg (n=9)
Humira, 10mg/kg (n=10)
Humira, 1mg/kg (n=9)
Humira, 0.25mg/kg (n=9)

Arthritis Clinical Scores
Two-Way ANOVA

<table>
<thead>
<tr>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time/Treatment</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Post-hoc LSD Test

<table>
<thead>
<tr>
<th>G1 vs G2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 vs G3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>G1 vs G4</td>
<td>0.559</td>
</tr>
<tr>
<td>G1 vs G5</td>
<td>0.709</td>
</tr>
</tbody>
</table>
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

- **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

*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

\* = \( P < 0.05 \)  
\** = \( P < 0.01 \)  
\*** = \( P < 0.001 \)
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

IL-1β Levels in the Joints

- G1: Vehicle (n=4)
- G2: Humira, 25mg/kg (n=4)
- G3: Humira, 10mg/kg (n=4)
- G4: Humira, 1mg/kg (n=3)
- G5: Humira, 0.25 mg/kg (n=4)

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

mKC Levels in the Joints

- G1: Vehicle (n=4)
- G2: Humira, 25mg/kg (n=4)
- G3: Humira, 10mg/kg (n=4)
- G4: Humira, 1mg/kg (n=3)
- G5: Humira, 0.25mg/kg (n=4)

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

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

- Similar swelling in both fore paws
- Humira effectively inhibited paw swelling
• 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
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E-Mail: a.pahl@hdpharma.com
Web: www.heidelberg-pharma.com
### INVOLVEMENT OF IMMUNE SYSTEM

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Mouse CIA</th>
<th>Rat CIA</th>
<th>Rat AIA</th>
<th>Mouse PGIA</th>
<th>Mouse SCW-induced arthritis</th>
<th>Rat polyarticular SCW-induced arthritis</th>
<th>Rat monarticular SCW-induced arthritis</th>
<th>Mouse STIA</th>
<th>K/BnN-transgenic mouse</th>
<th>Human TNF-transgenic mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte/macrophages</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>SCW components</td>
<td>PG-PS 10S</td>
<td>PG-PS 100P</td>
<td>G6PI-specific autoantibodies</td>
<td>G6PI</td>
<td>Yes</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Yes</td>
<td>NR</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>Yes (after initial neutrophil phase, a few days after IA injection)</td>
<td>NR</td>
<td>NR</td>
<td>Yes</td>
<td>NR</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>NR (during initial and reactivation response)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>T cells</td>
<td>Yes, CD4+, type II collagen reactive, mainly during induction</td>
<td>Yes, synovial</td>
<td>Yes, CD4+ T cells</td>
<td>Yes (only during reactivation phase), main role for CD4+ T cells</td>
<td>Yes, only during chronic phase</td>
<td>Yes (during reactivation phase)</td>
<td>No (can increase severity but are not crucial for disease induction)</td>
<td>Yes, autoreactive to G6PI</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>B cells, antibody production</td>
<td>Yes, production of complement fixing type II collagen reactive antibodies</td>
<td>Yes</td>
<td>Yes, autoantibodies required for initiation of disease</td>
<td>SCW-specific antibodies are detected</td>
<td>Yes, only during chronic phase; minimal/no antibody response to PG-PS</td>
<td>NR</td>
<td>No (G6PI-specific autoantibodies crucial, B cells in recipient mice not crucial)</td>
<td>Yes, produce G6PI-specific autoantibodies</td>
<td>No</td>
<td>No</td>
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<tr>
<td>NK cells</td>
<td>Dampen CIA</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Complement-specific MHC</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Mouse CIA</th>
<th>Rat CIA</th>
<th>Rat AIA</th>
<th>Mouse PGIA</th>
<th>Mouse SCW-induced arthris</th>
<th>Rat polyarticular SCW-induced arthritis</th>
<th>Rat monarcticular SCW-induced arthritis</th>
<th>Mouse STIA</th>
<th>K/Bln-Tg mice</th>
<th>Human TNF-Tg mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, detectable 4 days post-injection</td>
<td>Yes</td>
<td>Yes/negative, minor role, only expressed during initial joint swelling</td>
<td>Yes, during reactivation</td>
<td>Yes, during reactivation</td>
<td>Yes/negative, varying results</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, detectable 4 days post-injection</td>
<td>Yes</td>
<td>Yes, involved in cartilage breakdown and inflammatory cell influx</td>
<td>Yes, during reactivation</td>
<td>Yes, during reactivation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>IL-4</td>
<td>No (can dampen inflammation)</td>
<td>No (can dampen inflammation)</td>
<td>No until later stage (can dampen response)</td>
<td>No (can dampen inflammation)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>NR</td>
</tr>
<tr>
<td>IL-6</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, detectable 4 days post-injection</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>IL-10</td>
<td>No (can dampen inflammation)</td>
<td>No (can dampen inflammation)</td>
<td>No until later stage (can dampen response)</td>
<td>No (can dampen inflammation)</td>
<td>No</td>
<td>No</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>IL-12</td>
<td>No (protects from inflammation)</td>
<td>NR</td>
<td>NR</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>No</td>
<td>NR</td>
<td>No</td>
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<tr>
<td>IL-17</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes, required to switch from an acute to a chronic reaction</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
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<td>IL-21</td>
<td>Yes</td>
<td>NR</td>
<td>Yes</td>
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<td>Yes</td>
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<tr>
<td>IL-23</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>Yes, chronic stage</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>IL-32</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<td>NR</td>
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<tr>
<td>IFNγ</td>
<td>Contradictory findings, possible role in regulating T cells</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>Yes/no, conflicting reports</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Yes</td>
<td>Yes, recruitment of monocytes, plays role in development of arthritis</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>Yes (during reactivation phase, up-regulated via IL-4)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>MIP-1α</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>Yes (reactivation phase)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>MIP-2</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>Yes</td>
<td>NR</td>
<td>Yes (reactivation phase)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

* CIA = collagen-induced arthritis; AIA = adjuvant-induced arthritis; PGIA = proteoglycan-induced arthritis; SCW = streptococcal cell wall; STIA = serum transfer-induced arthritis; Tg = transgene; 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 TNFα expression

- **Arthritis**
  - No visible signs
  - Mild, Moderate, Severe

- **Fertility**
  - Normal, Decreased
  - None or Minor, Pronounced

- **Metabolic Abnormalities**

Age (weeks): 5, 10, 15, 20, 25, 30
## TNFα: overview

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Inducers</th>
<th>Inhibitors</th>
<th>Cell Target</th>
<th>Primary Effects on Each Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mononuclear phagocytes, T cells, B cells, NK cells, vascular endothelial cells, keratinocytes, smooth muscle cells, mast cells, neutrophils, astrocytes, glial cells.</td>
<td>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).</td>
<td>Prostaglandins, corticosteroids, IL-4, IL-6, TGF-b</td>
<td>Mononuclear phagocytes</td>
<td>Activation (Inflammation and Infection)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neutrophils, eosinophils</td>
<td>Neutrophils, eosinophils</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Endothelial cells</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypothalamus</td>
<td>Hypothalamus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Muscle, fat</td>
<td>Muscle, fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thymocyte</td>
<td>Thymocyte</td>
</tr>
</tbody>
</table>
Humira levels in mouse serum during the study

HUMIRA Levels in the Serum

Days post Treatment Initiation

- G2: HUMIRA, 25mg/kg
- G3: HUMIRA, 10mg/kg
- G4: HUMIRA, 1mg/kg
- G5: HUMIRA, 0.25mg/kg
• **Humira (adalimumab) is a biologic drug approved for the treatment of arthritis**
  - Recombinant human IgG1 monoclonal antibody
  - Mechanism of action involves binding to TNFα to block signaling
**Experimental groups**

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