



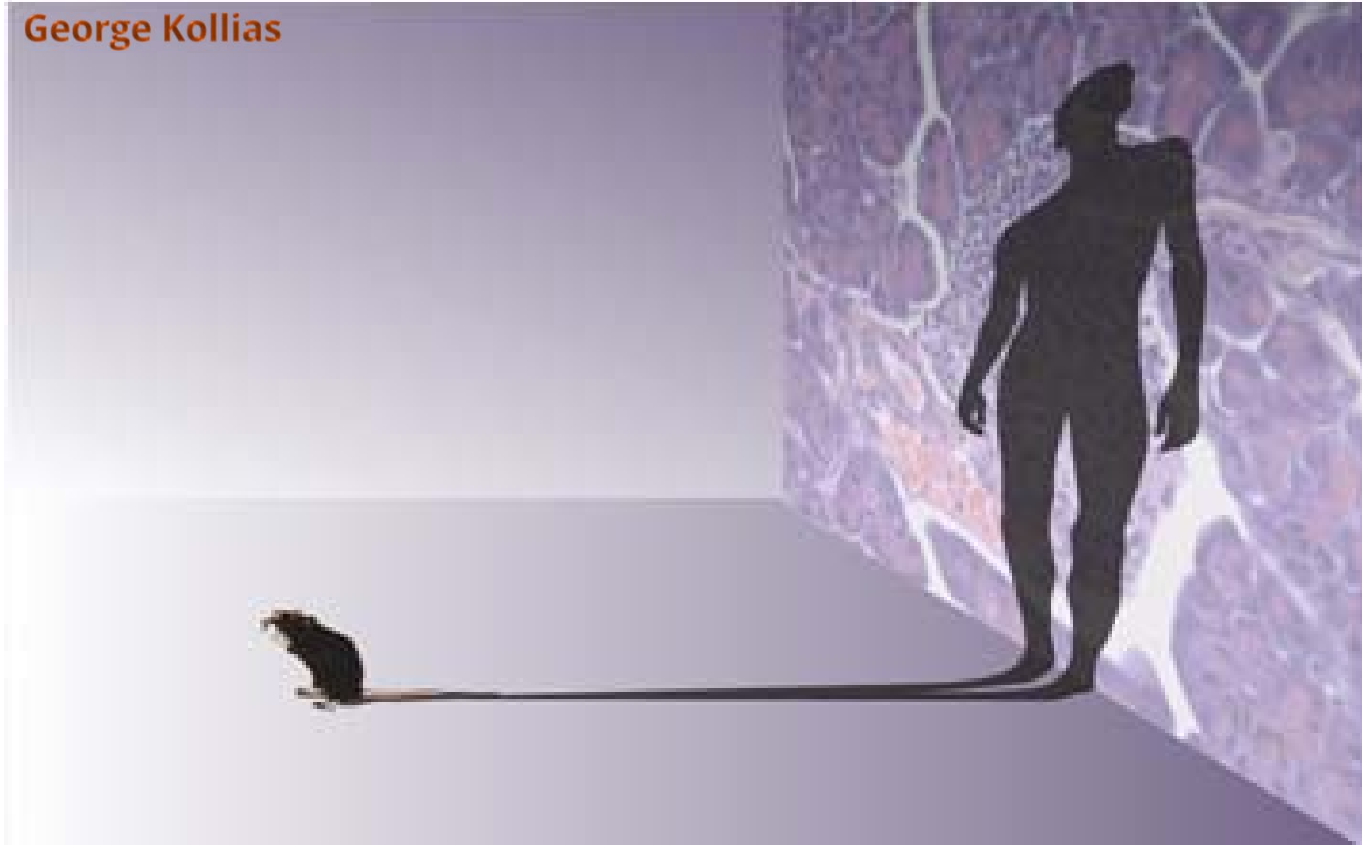
"ALEXANDER FLEMING"  
Biomedical Sciences Research Center

# Contribution of Disease Modelling to Precision Medicine Initiatives



National and Kapodistrian  
UNIVERSITY OF ATHENS

George Kollias



INFRAFRONTIER / IMPC Stakeholder Meeting

November 14-16, 2017



Athens, Royal Olympic Hotel



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## PERSPECTIVE

# Animal models in an age of personalized medicine

Personalized medicine is based on intraspecies differences. It is axiomatic that small differences in genetic make-up can result in dramatic differences in response to drugs or disease. To express this in more general terms: in any given complex system, small changes in initial conditions can result in dramatically different outcomes. Despite human variability and intraspecies variation in other species, nonhuman species are still the primary model for ascertaining data for humans. We call this practice into question and conclude that human-based research should be the primary means for obtaining data about human diseases and responses to drugs.

**KEYWORDS:** complexity • evolution • personalized medicine • prediction • species variation

The observation that humans responded differently to drugs formed the basis for the field of personalized medicine [1,2]. These are cases of intraspecies differences and can be traced back to differences in genetic make-up. Some examples include copy number variants, SNPs, gene regulation and epigenetics.

The age of personalized medicine has come on the heels of changes in physics that include

genes for the receptors have evolved differently since splitting from our common ancestor, each species uses the receptors in different ways [3,301].

We examine the practice of using models, specifically animal models used in drug development but also including animal models of disease, that have far more differences between the model and the complex system being modeled than the intraspecies differences exploited by

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## Executive summary

### Prediction

- In medical science, in order for a practice, test or modality to be considered predictive it must be found to have a very high probability of obtaining the correct answer. Occasional correlation is not synonymous with prediction.

### Intraspecies differences

- Personalized medicine is based on differences in drug and disease responses between individual humans, despite the genetic similarity of these humans.

### Complex systems

- Humans and animals are examples of complex systems, thus extrapolating results of drug testing and disease response among species is expected to be problematic.

### Interspecies differences

- Empirically, we find that extrapolating results of drug testing and disease response among species is problematic, and in fact, does not reach the level of probability necessary to be considered predictive.

### Evolution

- Differences among species in genes, gene regulation and expression, and the networks the genes and proteins operate in explain the empirically observed low probability that any two species will respond the same to drugs and disease.



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## PRECISION MEDICINE

## Animal-based studies will be essential for precision medicine

AT AN INSPIRING WHITE HOUSE CEREMONY ON 25 FEBRUARY 2016, PRESIDENT OBAMA celebrated the 1-year anniversary of the launch of the Precision Medicine Initiative (PMI), which promises to usher in a transformation of medical practice. A collaborative effort between government, academia, and industry, directed and led by the National Institutes of Health (NIH), the PMI envisages invoking an individual's molecular profile and other phenotypic descriptors combined with environmental exposures and lifestyle behaviors to guide therapies more targeted and cost-effective than current "one-size-fits-all" strategies. The extent to which massive amounts of genomic and other data can expose statistically relevant and clinically actionable results will be tested in a planned >1 million-person cohort. Although we very much support this audacious plan, it is important to note that no matter how large a cohort, statistical power will never be sufficient to address by data analysis alone every observation that emerges or to drive to mechanism each human finding. In this context, the PMI will benefit greatly from integrative informatics and innovative animal-based research and validation studies that leverage existing networks of biological knowledge to create a new taxonomy of disease and to accelerate the successful incorporation of precision medicine into mainstream clinical practice.

Francis Collins, the NIH Director, and his team have made tremendous progress in planning and preparing for the implementation of PMI during the last year since the official announcement of the vision for the PMI Cohort Program ([www.nih.gov/news/health/sep2015/od-17.htm](http://www.nih.gov/news/health/sep2015/od-17.htm)). This vision was informed by numerous public workshops of the PMI Working Group (completed September 2015) to resolve the challenges to building and launching a cohort with \$130 million in funding opportunities awarded this year ([www.nih.gov/precision-medicine-initiative-cohort-program/program-components](http://www.nih.gov/precision-medicine-initiative-cohort-program/program-components)). As part of these discussions, numerous elements, including genomics, electronic health records, participant-provided data, sensors, and mobile health technologies, have been proposed as essential to generate the data required to drive precision medicine. However, the critical role of experimental studies in genetic model organisms, for which there are enormous extant data sets that continue to accrue, has not been fully considered, especially in four areas that are key to the successful deployment of the PMI.

**Gene variant interpretation.** We are able to provide a confident interpretation of the clinical relevance for only a vanishingly small proportion of variants in human populations. Preclinical and codicinal studies using animal models strategically designed to reflect the genomic variation observed in cohort participants will be necessary to define downstream functional consequences and discriminate causal from correlative factors at relevant efficiency. New genome-editing technologies [for example, CRISPR (clustered regularly interspaced short palindromic repeats)] now enable the efficient derivation of precision disease models incorporating patient-specific genetic variants as a means of recapitulating essential aspects of human disease in zebrafish, mouse, rats, pigs, and other organisms. Indeed, the study of patient-derived avatars to define disease pathogenicity will fine-tune the diagnostic precision inherent in the PMI and accelerate the discovery of new therapeutic targets. The NIH can capitalize on recent technological advances in animal modeling by completing current efforts to functionally annotate key model organism genomes, such as the Knockout Mouse Project, which is part of the International Mouse Phenotyping Consortium ([www.mousephenotype.org](http://www.mousephenotype.org)), and by expanding the nascent Pilot Center program in Precision Disease Modeling (<http://grants.nih.gov/grants/guide/pa-files/PAR-14-280.html>). Ultimately, disease penetrance, pleiotropy, and even higher-order gene-gene interactions will be accessible in such systems. Further, a commitment to the comprehensive investigation of variants found in traditionally underserved populations would contribute to inclusivity by clarifying the roles of variants that are rare or absent in majority ethnicities.

**Incorporating "-omic" data.** Effectively linking the unprecedented amounts of genomic, metabolomic, and other -omic data with environmental, behavioral, and lifestyle information

- Gene Variant interpretation
- Incorporating '-omic' Data
- Environmental exposures
- Integrative *in vivo* modelling

# Data Interpretation Matters !



2013

## Genomic responses in mouse models poorly mimic human inflammatory diseases

Junhee Seok<sup>a,1</sup>, H. Shaw Warren<sup>b,1</sup>, Alex G. Cuenca<sup>c,1</sup>, Michael N. Mindrinos<sup>a</sup>, Henry V. Baker<sup>c</sup>, Weihong Xu<sup>a</sup>, Daniel R. Richards<sup>d</sup>, Grace P. McDonald-Smith<sup>e</sup>, Hong Gao<sup>a</sup>, Laura Hennessy<sup>f</sup>, Celeste C. Finnerty<sup>g</sup>, Cecilia M. López<sup>c</sup>, Shari Honari<sup>f</sup>, Ernest E. Moore<sup>h</sup>, Joseph P. Minei<sup>i</sup>, Joseph Cuschieri<sup>j</sup>, Paul E. Bankey<sup>k</sup>, Jeffrey L. Johnson<sup>h</sup>, Jason Sperry<sup>l</sup>, Avery B. Nathens<sup>m</sup>, Timothy R. Billiar<sup>l</sup>, Michael A. West<sup>n</sup>, Marc G. Jeschke<sup>o</sup>, Matthew B. Klein<sup>j</sup>, Richard L. Gamelli<sup>p</sup>, Nicole S. Gibran<sup>j</sup>, Bernard H. Brownstein<sup>q</sup>, Carol Miller-Graziano<sup>k</sup>, Steve E. Calvano<sup>r</sup>, Philip H. Mason<sup>e</sup>, J. Perren Cobb<sup>s</sup>, Laurence G. Rahme<sup>t</sup>, Stephen F. Lowry<sup>r,2</sup>, Ronald V. Maier<sup>j</sup>, Lyle L. Moldawer<sup>c</sup>, David N. Herndon<sup>g</sup>, Ronald W. Davis<sup>a,3</sup>, Wenzhong Xiao<sup>a,t,3</sup>, Ronald G. Tompkins<sup>t,3</sup>, and the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program<sup>4</sup>

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2015

## Genomic responses in mouse models greatly mimic human inflammatory diseases

Keizo Takao<sup>a,b</sup> and Tsuyoshi Miyakawa<sup>a,b,c,1</sup>

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<sup>b</sup>Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan; and <sup>c</sup>Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-1192, Japan

Edited by Ruslan Medzhitov, Yale University School of Medicine, New Haven, CT, and approved June 11, 2014 (received for review January 31, 2014)

The use of mice as animal models has long been considered essential in modern biomedical research, but the role of mouse models in research was challenged by a recent report that genomic responses in mouse models poorly mimic human inflammatory diseases. Here we reevaluated the same gene expression datasets used in the previous study by focusing on genes whose expression levels were significantly changed in both humans and mice

to the stimulus would generally decrease the sensitivity to detect the responses shared by the disorders and their models. For this reason, we excluded such genes from our analysis. Second, we compared each of the conditions in a single mouse study independently with the human reference conditions. Mouse studies, such as GSE7404 and GSE19668, included multiple conditions or gene sets. For example, GSE19668 contains multiple datasets

Ça a commencé comme ça ...

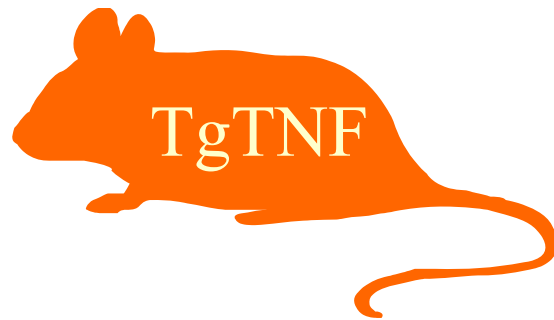
*Celine, 1932*

***'Anything found to be true of E.  
coli must also be true of elephants.'***

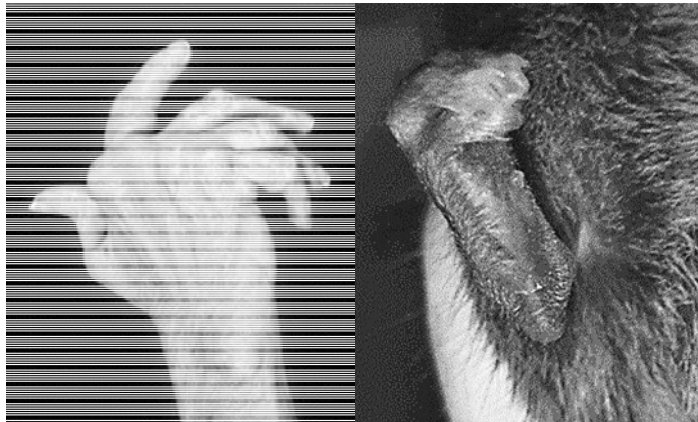
*Jacques Monod & Francois Jacob,  
Cold Spring Harbor Symposia, 1961*



# Modeling Rheumatoid Arthritis: The Human TNF Transgenic mouse



Human TNF transgenic mouse  
model of spontaneous arthritis that  
closely resembles the human  
pathology



The EMBO Journal vol.10 no.13 pp.4025–4031, 1991

## Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis

1991

Jeanne Keffer, Lesley Probert, Haris Cazlaris,  
Spiros Georgopoulos, Evangelos Kaslaris<sup>1</sup>,  
Dimitris Kioussis<sup>2</sup> and George Kollias

Laboratory of Molecular Genetics, Hellenic Pasteur Institute, 127,  
Av. Vas. Sofias, Athens 115 21, <sup>1</sup>Pathology Department, Aghia Sofia  
Children's Hospital, Athens 115 27, Greece, and <sup>2</sup>Laboratory of Gene  
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Communicated by F.Grosveld

lipopolysaccharide (LPS), a major inducer of inflammation, transcription from the TNF gene is augmented 3-fold while steady-state TNF mRNA levels are increased by 50-fold or more (Beutler *et al.*, 1986). In addition, TNF production is further regulated by LPS at the translational level (Han *et al.*, 1990). Deregulated production of TNF in humans is thought to contribute to the development of diseases such as cancer-associated cachexia (Olioff *et al.*, 1987), endotoxic shock (Beutler *et al.*, 1985), graft versus host disease (Piguet *et al.*, 1987), autoimmunity (Held *et al.*, 1990) and

ARTHRITIS & RHEUMATISM Volume 36  
Number 12, December 1993, pp. 1681–1690  
© 1993, American College of Rheumatology

1993

1681

## TREATMENT OF RHEUMATOID ARTHRITIS WITH CHIMERIC MONOCLONAL ANTIBODIES TO TUMOR NECROSIS FACTOR $\alpha$

MICHAEL J. ELLIOTT, RAVINDER N. MAINI, MARC FELDMANN, ALICE LONG-FOX,  
PETER CHARLES, PETER KATSIKIS, FIONULA M. BRENNAN, JEAN WALKER, HANNY BIJL,  
JOHN GHAYEB, and JAMES N. WOODY

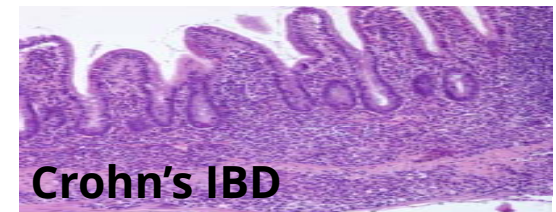
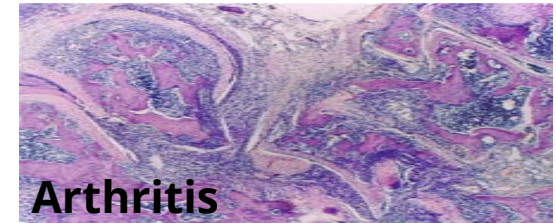
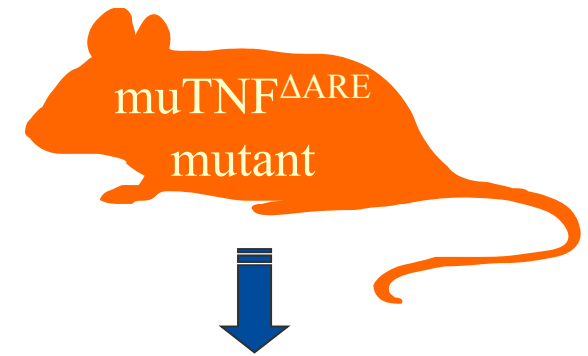
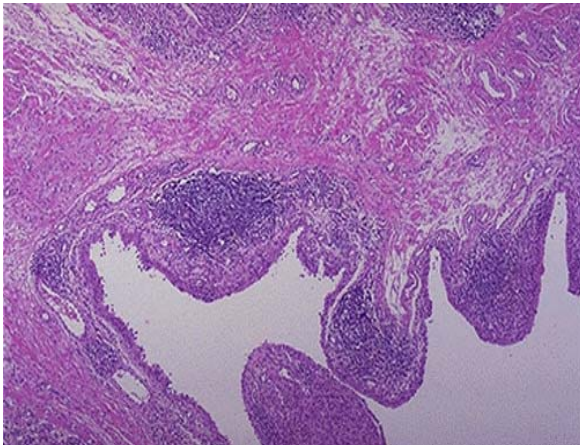
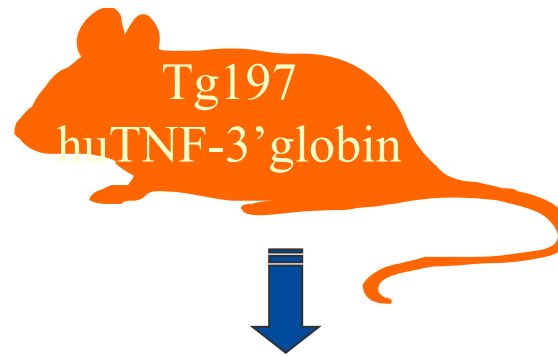
**Objective.** To evaluate the safety and efficacy of a chimeric monoclonal antibody to tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) in the treatment of patients with rheumatoid arthritis (RA).

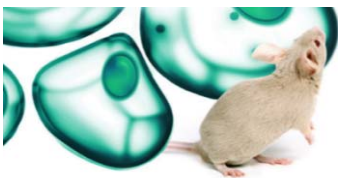
**Methods.** Twenty patients with active RA were treated with 20 mg/kg of anti-TNF $\alpha$  in an open phase

to 5 ( $P < 0.001$ ) over the same period, and in the other major clinical assessments. Serum C-reactive protein levels fell from a median of 39.5 mg/liter at study entry to 8 mg/liter at week 6 ( $P < 0.001$ ), and significant decreases were also seen in serum amyloid A and interleukin-6 levels.

# The Tg197 and TNF<sup>ΔARE</sup> MODELS OF TNF-MEDIATED DISEASE

(EMBO J. 1991; Immunity 1999)





# TgA86 (tmTNF<sup>mu</sup>): a Novel Model of Spondyloarthritis

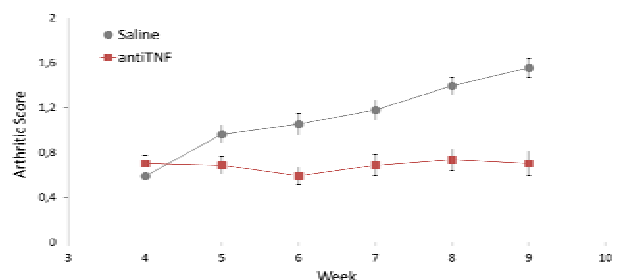
TgA86 (Alexopoulou et al. Eur. J. Immunol 1997)

- Overexpression of mouse tmTNF
- No severe systemic disease
- Peripheral and axial pathology
  - Spontaneous development of mild arthritis
  - Spontaneous development of spondylitis



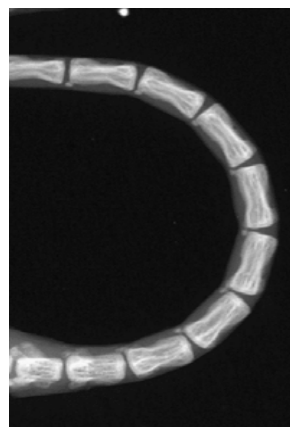
- **Axial pathology**
  - Tail bending
  - Tail ankylosis
- **Peripheral pathology**
  - Arthritis

## Arthritis

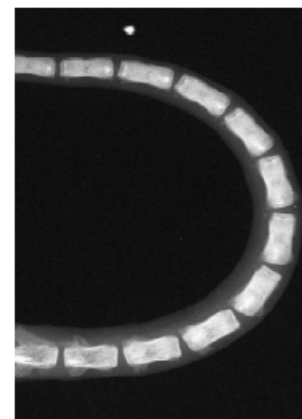


## Spondylitis

WT



TgA86



## X-ray signs of spondylitis:

- **Inflammation:** soft tissue swelling
- **bone erosion:** loss of small connecting bones between vertebrae
- **new bone formation:** brighter appearance of denser bones, loss of bar-bell shape and rectangular shapes of vertebrae

Karagianni et al., (unpublished)

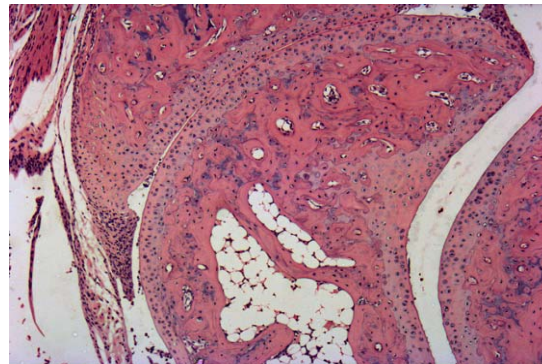
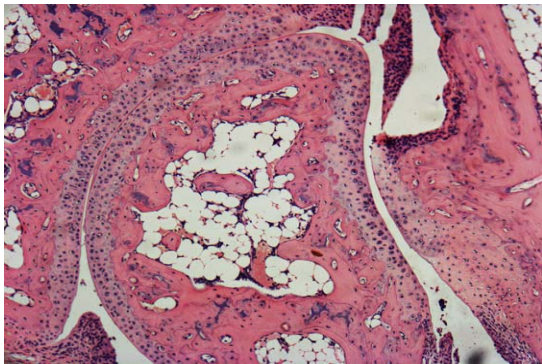


# TgTNF arthritis develops in the absence of mature T- and B-cells

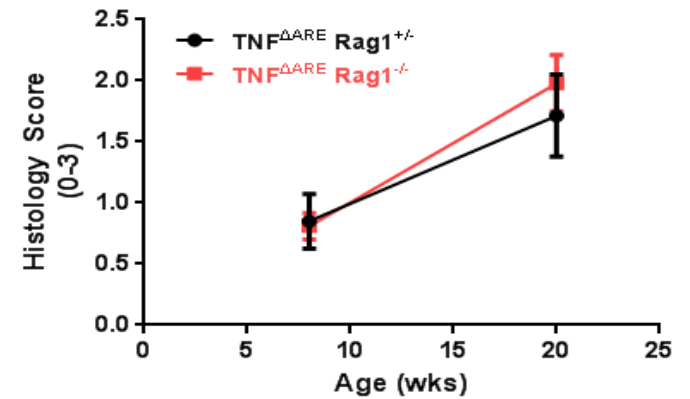
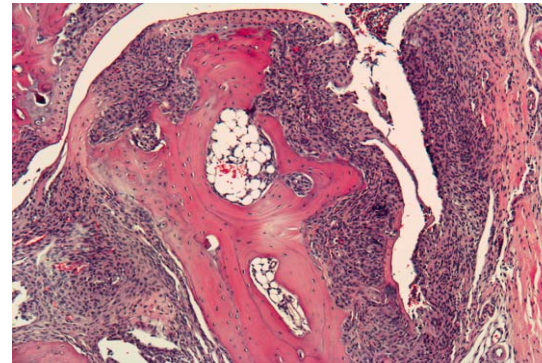
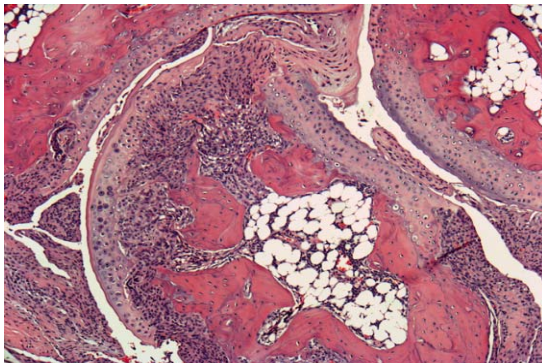
**TNF $\Delta$ ARE**

**TNF $\Delta$ ARE Rag1<sup>-/-</sup>**

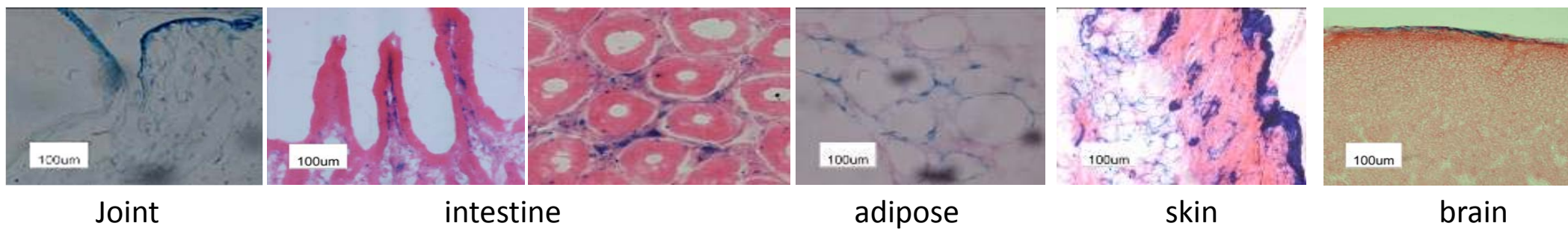
wk  
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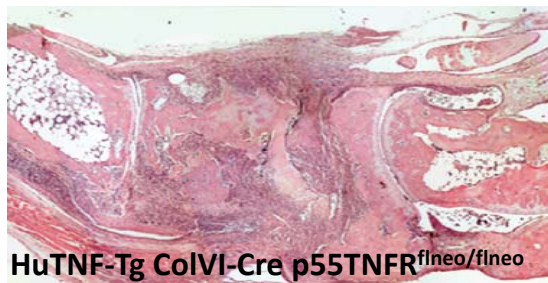
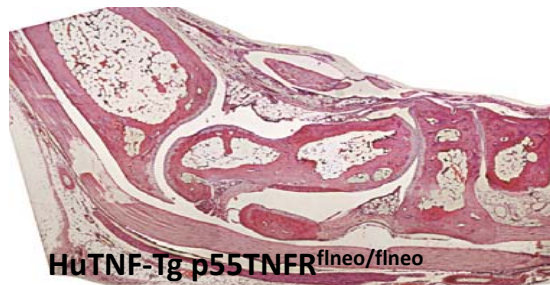
wk20



# Tissue specificity of the CollagenVI-Cre mouse



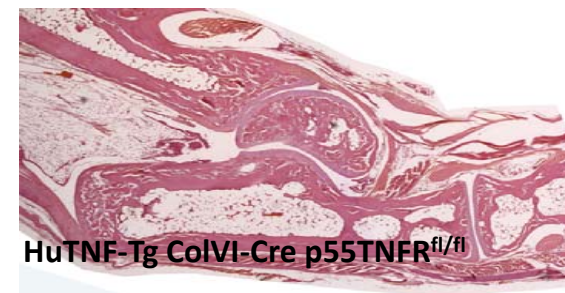
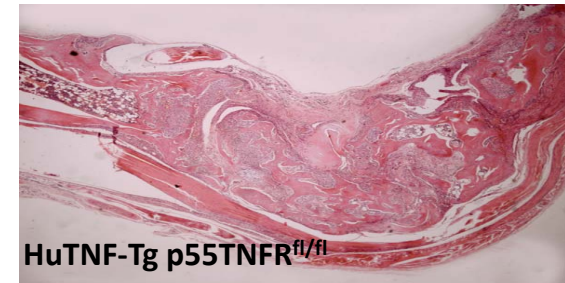
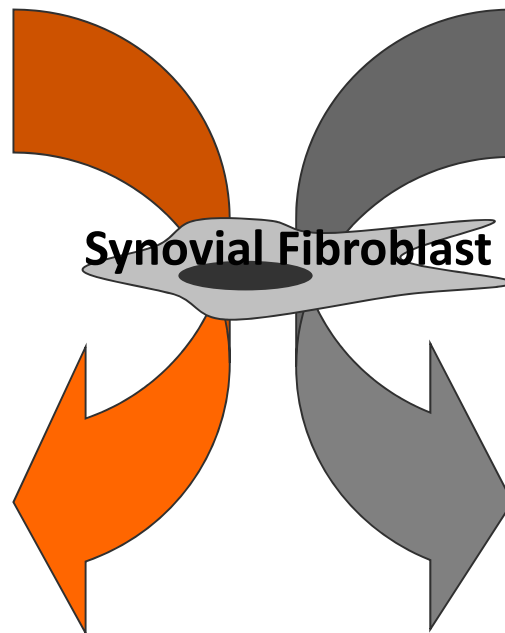
# TNFR1 on Synovial Fibroblasts is both SUFFICIENT and NECESSARY to orchestrate full TNF-driven destructive arthritis



**SUFFICIENT**

Armaka et al., J. Exp. Med. (2008)

TNF / TNFR

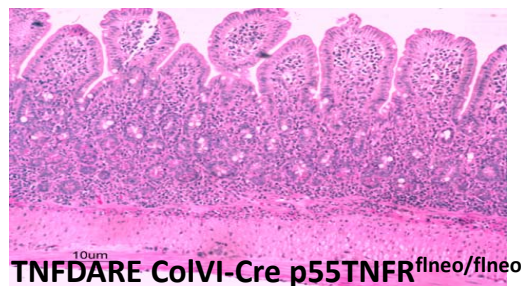
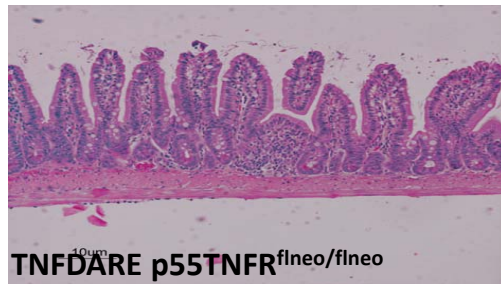


**NECESSARY**

Armaka et al., (unpublished)

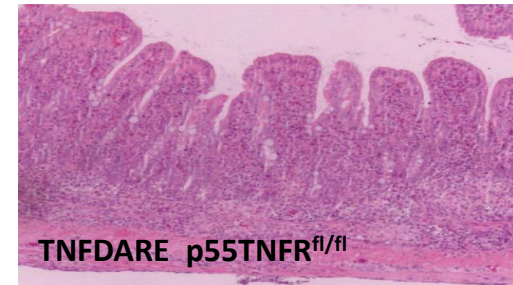
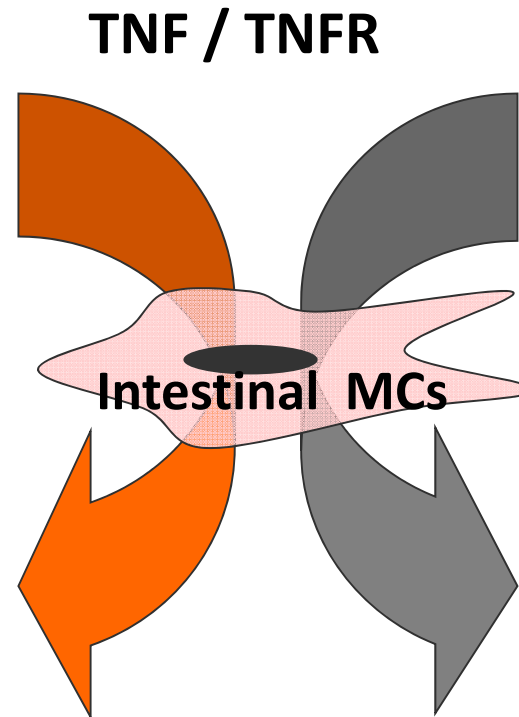


# TNFR1 on IMCs is both SUFFICIENT and NECESSARY to orchestrate TNF-driven Crohn-like pathology



## SUFFICIENT

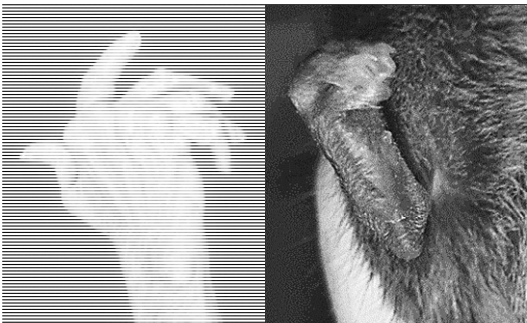
Armaka et al., J. Exp. Med. (2008)



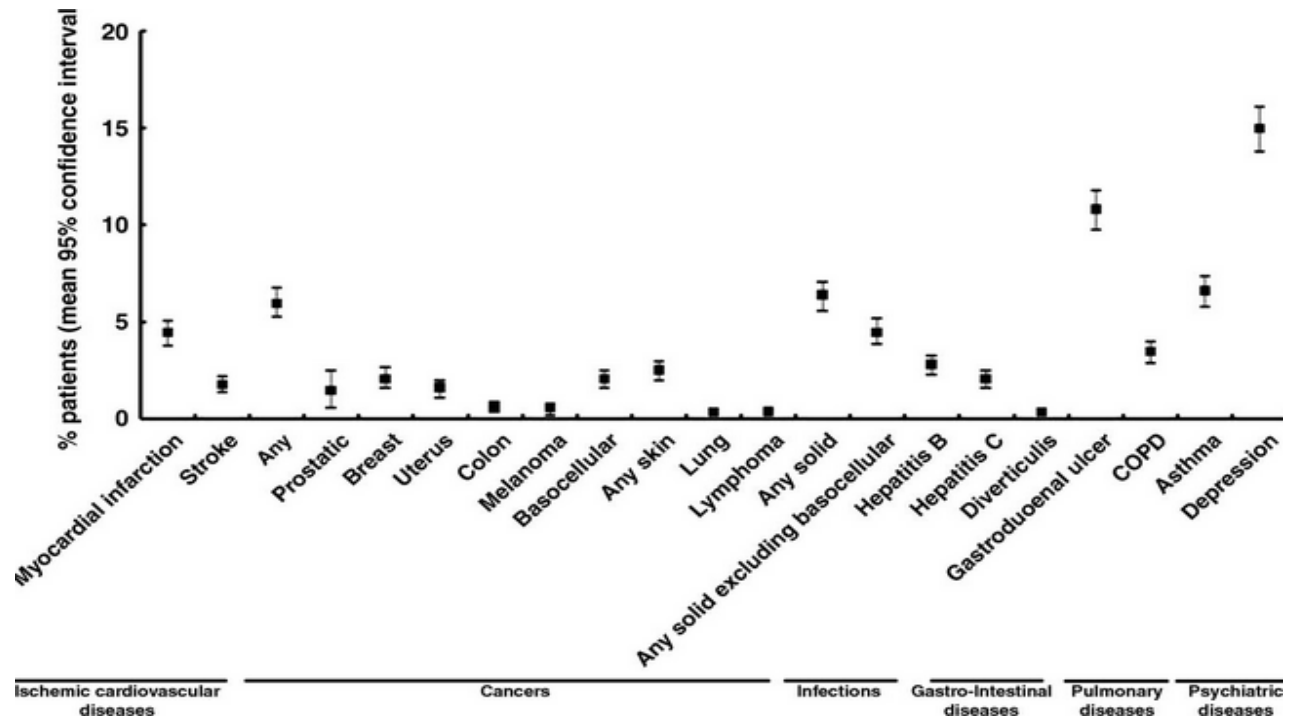
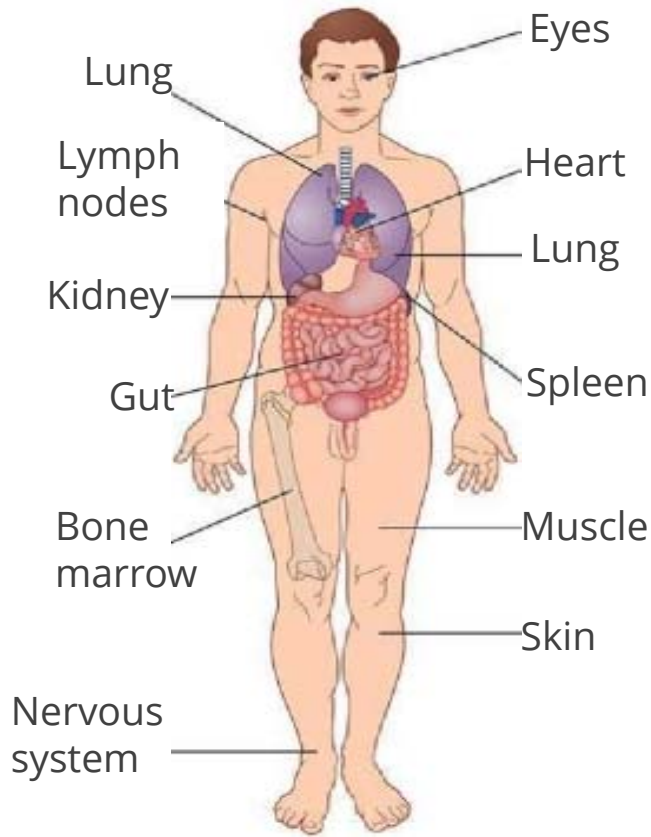
## NECESSARY

Armaka et al., (unpublished)





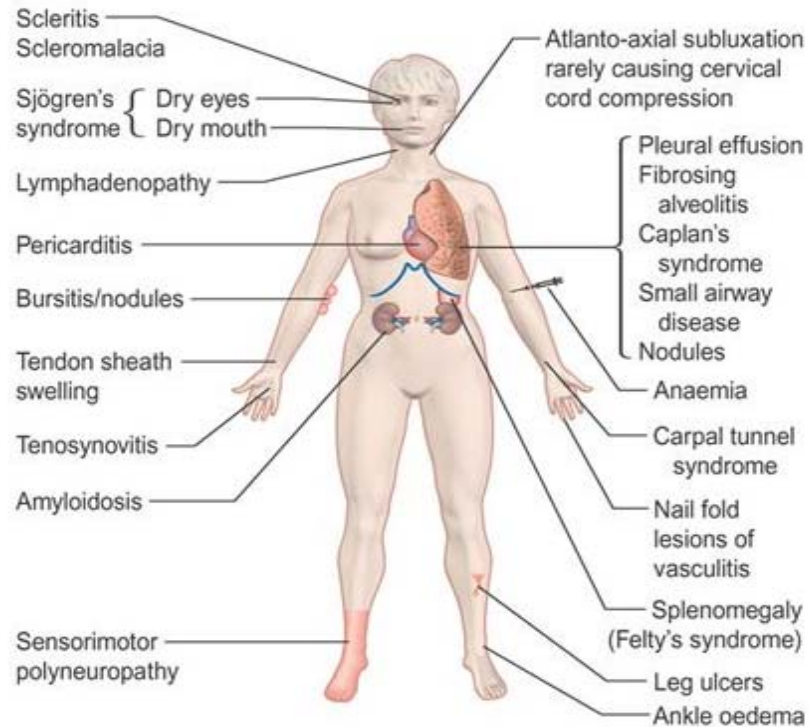
# CHRONIC JOINT DISEASES AND COMORBIDITIES



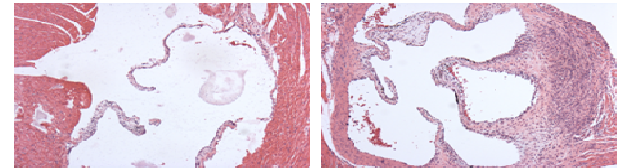
Molto et al., Ann Rheum Dis. 2016 Jun;75(6):1016-23



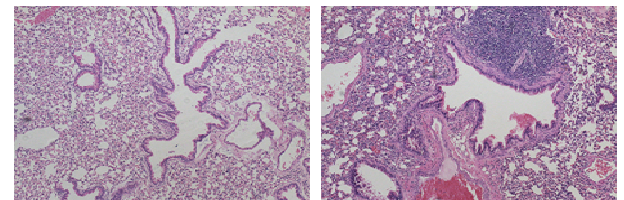
# TNF- $\Delta$ ARE: A mouse model of spondyloarthropathies



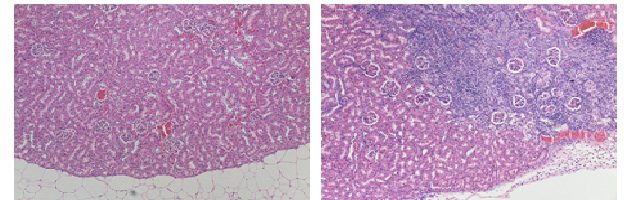
Aortic valves



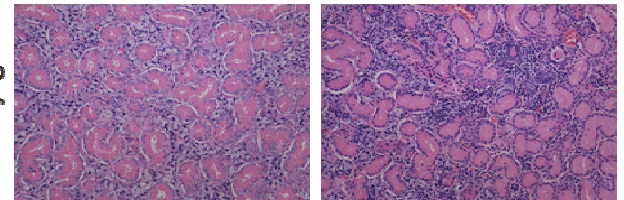
Lung



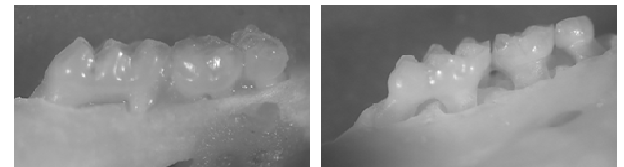
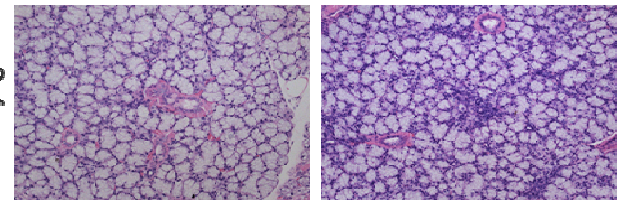
Kidney



Submandibular Salivary gland



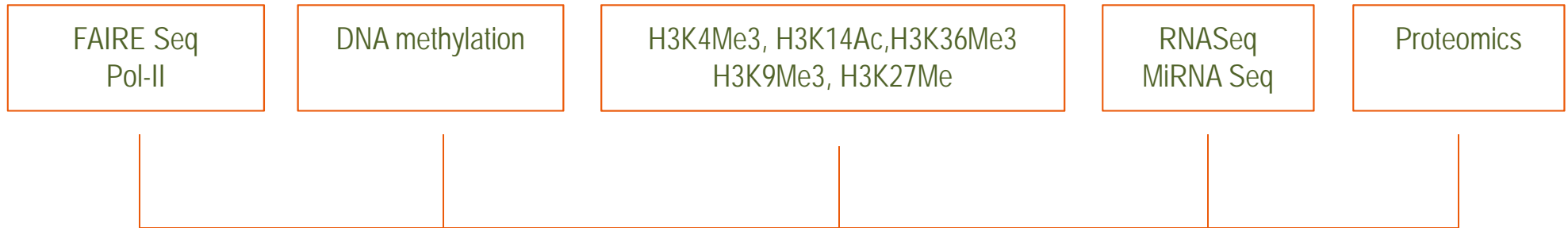
Maxilla



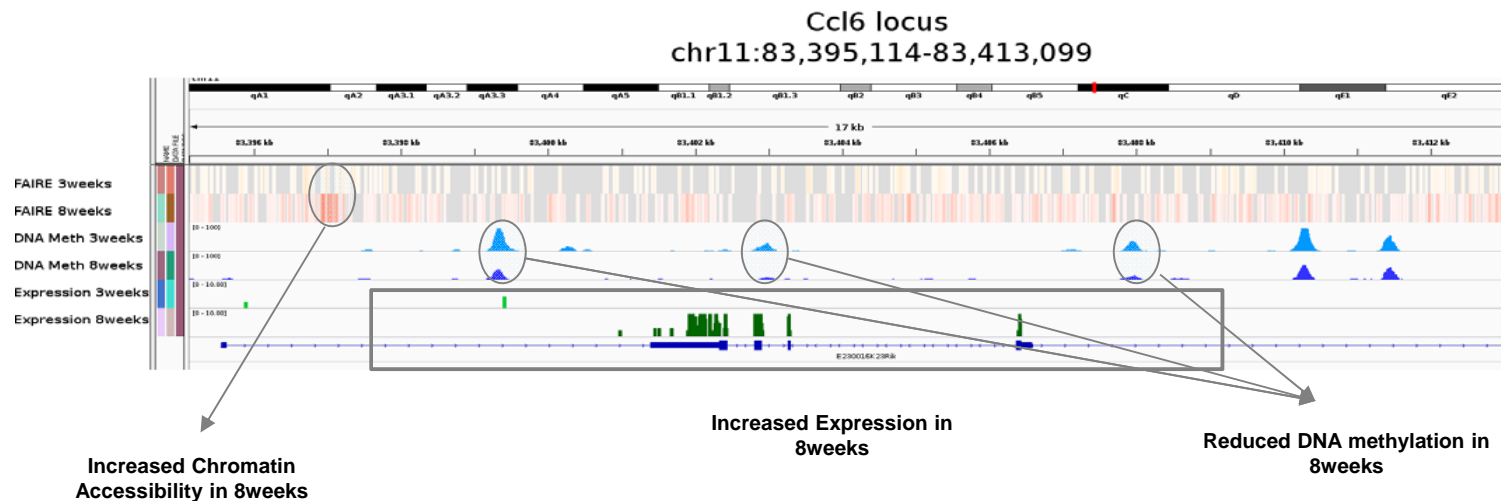
**Comorbidities** uncovered through  
**Infrafrontier** phenotyping pipeline  
(collaboration with German Mouse Clinic).



# A mouse ENCODE approach for arthritogenic SFs

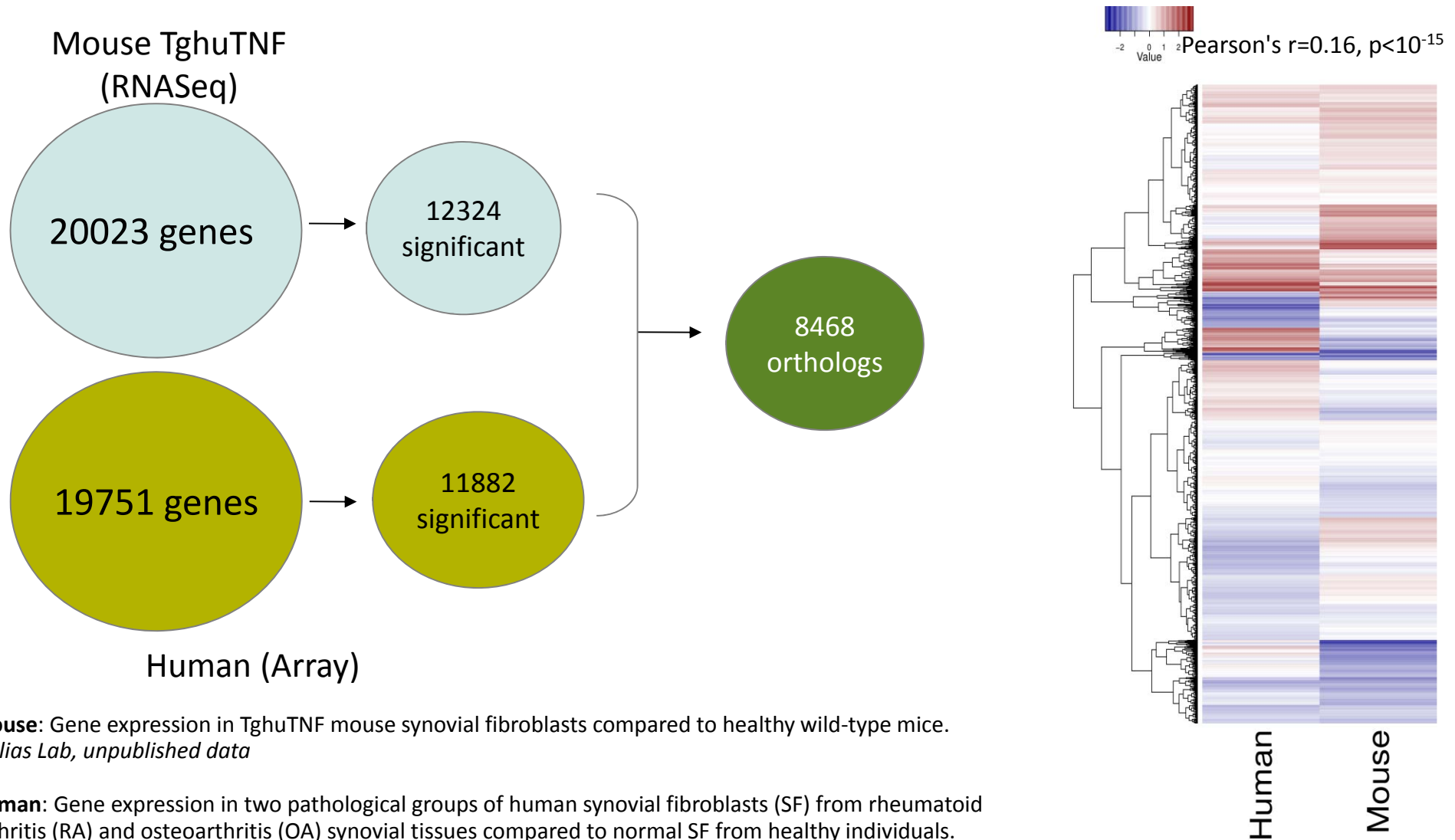


Three stages of disease progression



Ntougkos V, Chouvardas P et al., (A&R, 2017)

## A human-mouse comparison: Gene Level



**Mouse:** Gene expression in TghuTNF mouse synovial fibroblasts compared to healthy wild-type mice.  
*Kollias Lab, unpublished data*

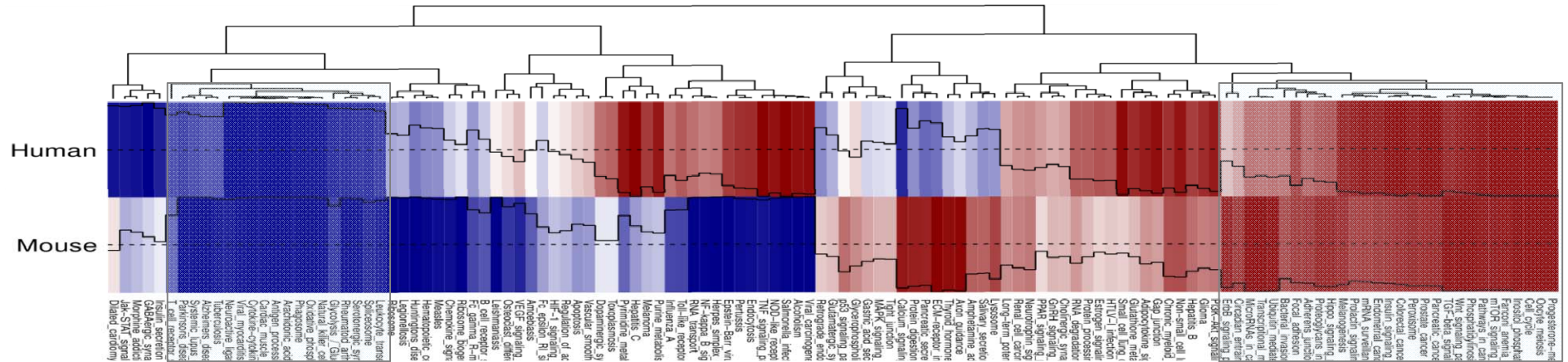
**Human:** Gene expression in two pathological groups of human synovial fibroblasts (SF) from rheumatoid arthritis (RA) and osteoarthritis (OA) synovial tissues compared to normal SF from healthy individuals.  
*Del Rey MJ et al. Ann Rheum Dis* **2012** Feb;71(2):275-80.

Ntougkos V, Chouvardas P et al., (A&R, 2017)

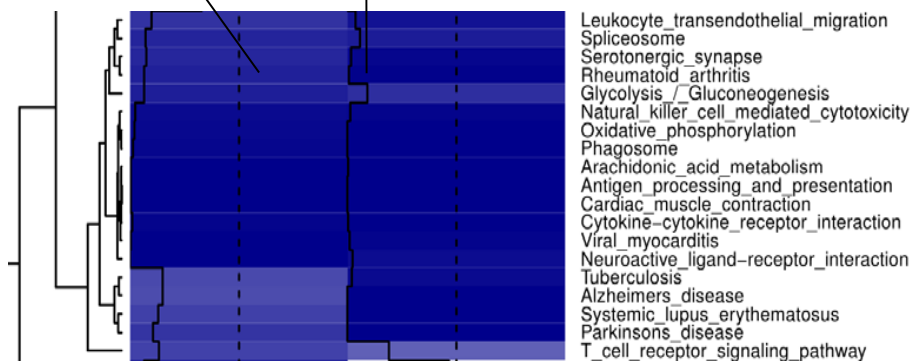


# A human-mouse comparison: Pathway Level

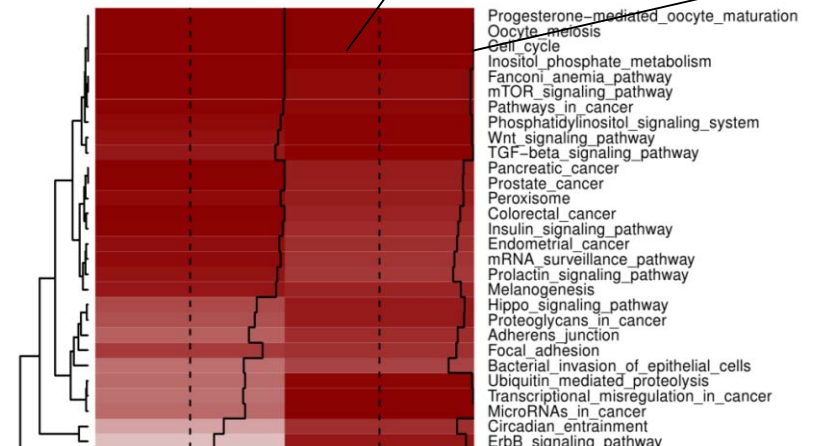
Side-by-side analysis of KEGG pathway enrichments in human and mouse RASF/HSF samples reveals pathways with very similar profiles



Pearson's  $r=0.38$ ,  $p<10^{-9}$

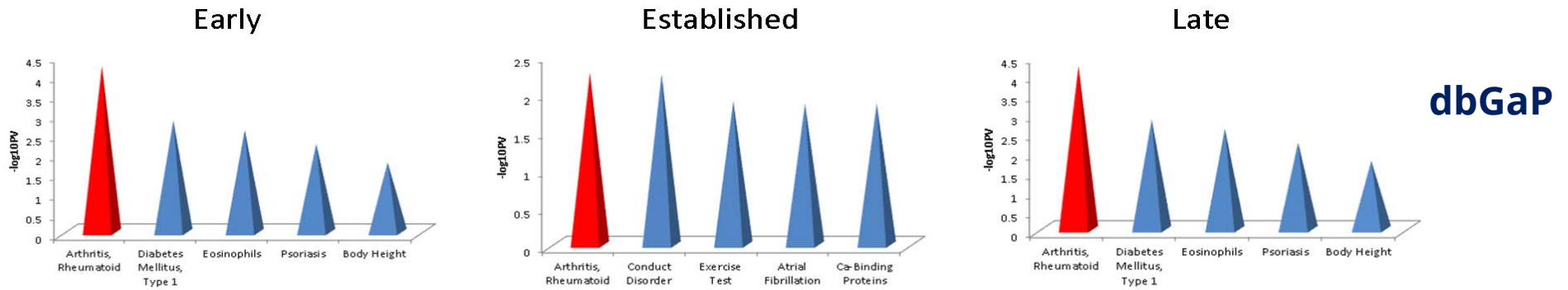


Pathways enriched in deregulated genes



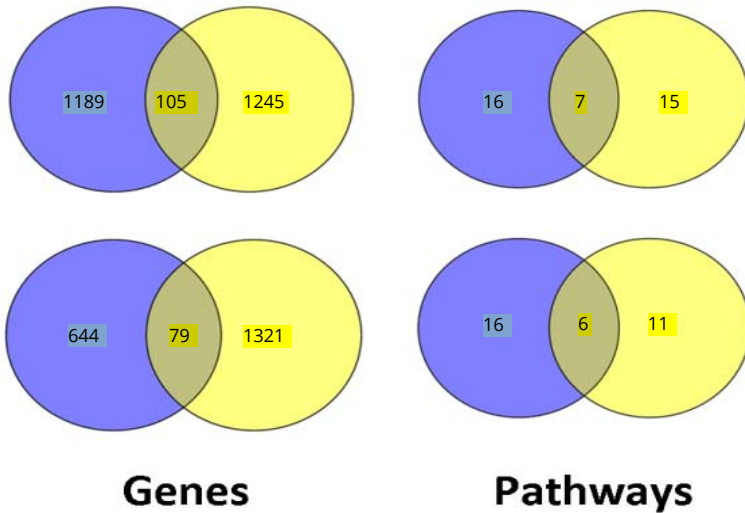
Pathways depleted in deregulated genes

# Genomic Responses of Mouse Synovial Fibroblasts During Tumor Necrosis Factor-Driven Arthritogenesis Greatly Mimic Those in Human Rheumatoid Arthritis



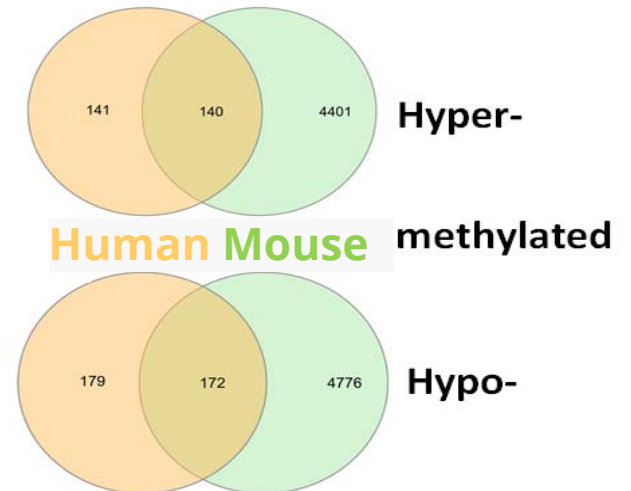
dbGaP

## Mouse Human



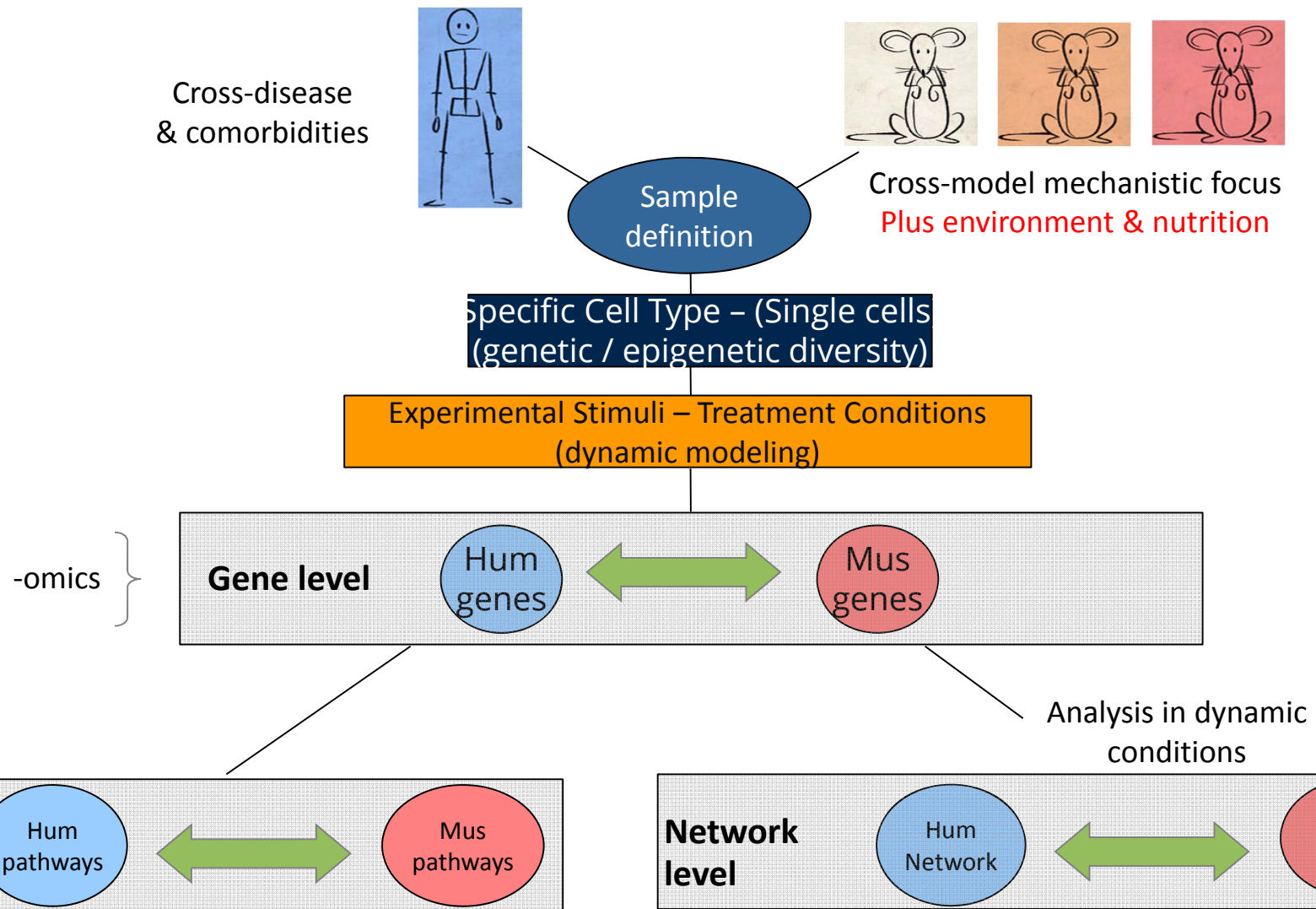
Upregulated

Downregulated



Ntougkos V, Chouvardas P et al., (A&R, 2017)

# Human – mouse comparison pipeline



# OF ANIMALS AND MEN



## *Species do differ !*

- *Unity in Biology but also diversity and descent by modification*
- *Size, metabolic rates, sensory systems, stress, cognitive functions*
- *Life expectancy, reproductive rate, diets, microbiomes, pathogens*

## *... but animal model research is essential*

- *To reproduce the cause and biology underlying complex disease*
- *To tweak the system with genetics and ensure safety for human*
- *To experiment with new treatments before they are ready for the clinic*
- *To understand essential biological mechanisms: the last 10 Nobel in Medicine and multiple landmark discoveries involved studies in animals.*



# Rethink, Resolve, Rationalize, Ruminare, Reflect



- Consider **multi-layered pathways** and disease **heterogeneity** (select your model based on target biology)
- **Do not blame** your animal model (blame your choice!)
- Support conclusions by **evidence** (not statistical magic!)
- **Know your animal model** (target biology, pathways, cellular mechanisms, comorbidities)
- Establish **disease-specific** primary/dominant **causalities**
- **Design** preclinical experiments **more rigorously** (avoid noise by genetic background, balance for gender and environment, and **standardize** induction and treatment protocols)
- **Standardize** design, analyses and **publication of research**
- **Standardize** statistics, **interpretations** and extrapolations

# Animal models in the era of Precision Medicine



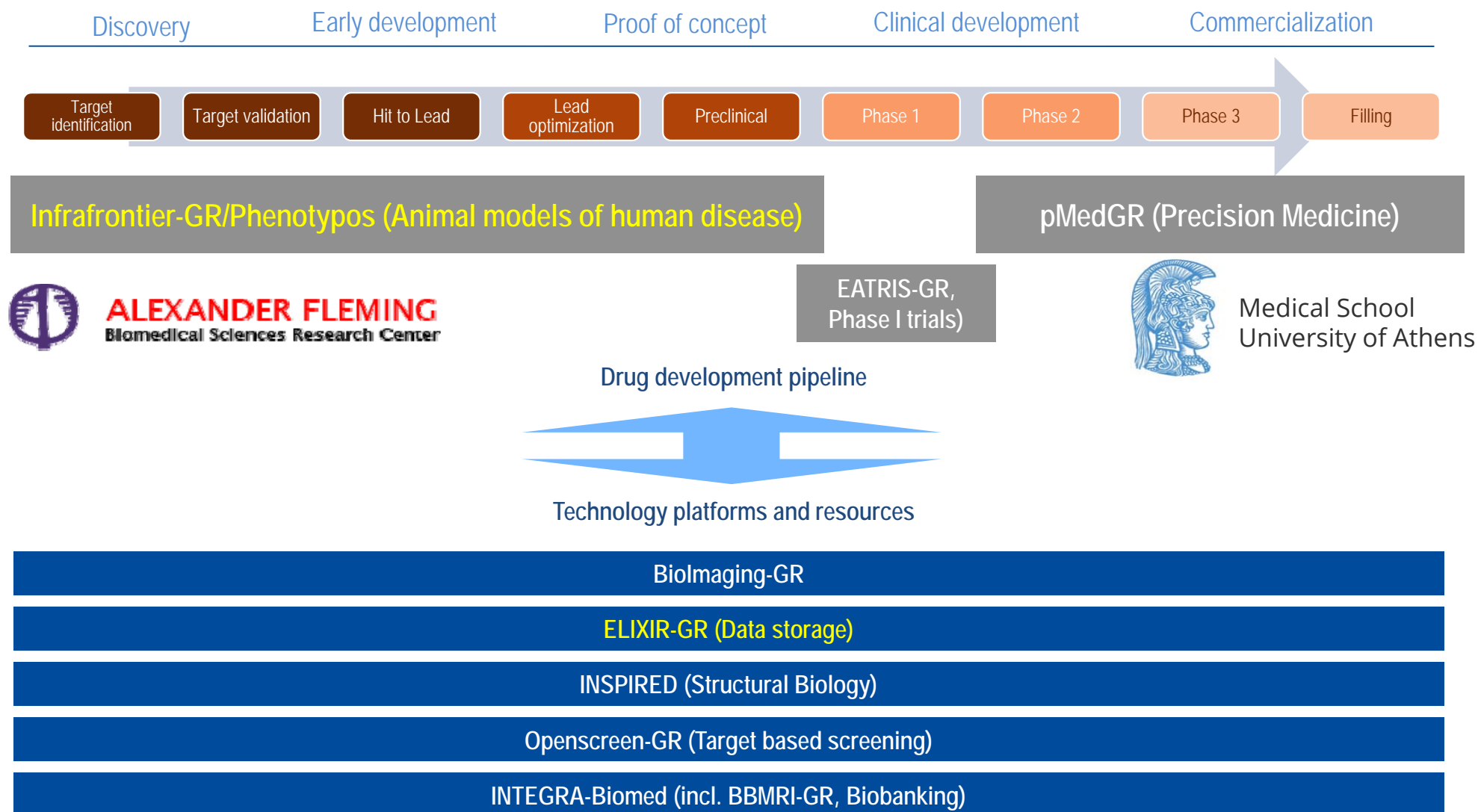
Towards next generation animal models that target personalized phenotypes through:

- Mouse models for **mechanistic understanding of complex diseases** and **biomarker development**
- **“Pathogenesis Maps”** aligning animal models to the different subsets of human disease
- **“Mouse Avatars”** (e.g. PDX) and **Humanized Mice** for personalized drug efficacy studies
- **“Co-clinical trials”** - real time integration of mouse and human data to guide therapeutic approaches in ongoing clinical trials
- Prompt and efficient **“human to mouse to human”** discoveries.





# The National RoadMap for Research Infrastructures





# Thanks to Sponsors



European  
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Commission

Horizon 2020  
European Union funding  
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RIGHT



I-DCC

International Data Coordination Centre



MARIE CURIE ACTIONS

EURO-RA



INFRAFRONTIER  
mouse disease models



FOR RHEUMATOID ARTHRITIS



European network of excellence



Scaffolds for Alternative Delivery



The  
Danish Council for  
Strategic Research



Thank you!