# Supplementary material 2

## Questionnaire details for comparison and validation of models for RA and CVD in RA

In this study FIMD was applied as a higher level tool of validation. The internal and external validity criteria were used here to closely fit the model with clinical relevance on the basis of fine tuned preclinical data. This purpose of minimizing the translational gap was reached by designing a questionnaire for assessment of the best model for RA according to FIMD. The main eight domains framework was adopted, and the questions were framed using reference. The questions were more focused on RA and associated pathogenic events of clinical disease development and parameters of evaluation.

**Table I. Questionnaire for comparison of models developed for RA**

| Questions for RA Model | SW |
|---|---|
| 1. Epidemiology | 12.5 |
| 1.1 Nature of population (Inbred/Outbred) | 4.16 |
| 1.2 Is the model able to simulate the disease in the relevant age groups (juvenile, adult or aging) | 4.16 |
| 1.3 Is the model able to simulate the disease in different genders | 4.16 |
| 2. Symptomatology and natural history | 12.5 |
| 2.1 Is model able to mimic human disease symptoms? If so, which ones | 4.16 |
| 2.1.1 Inflammatory markers | 1.38 |
| 2.1.2 Immunological markers | 1.38 |
| 2.1.3 Crosslinking markers for cardiovascular complications | 1.38 |
| 2.2 Natural history criteria matching human disease onset | 4.16 |
| 2.2.1 Time of onset | 0.83 |
| 2.2.2 Disease progression | 0.83 |
| 2.2.3 Duration of symptoms | 0.83 |
| 2.2.4 Severity | 0.83 |
| 2.2.5 Metabolic dysbiosis (obesity, TG, TC, LDL, HDL and fat accumulation in stool ) | 0.83 |
| 2.3 Co-Morbid conditions replicated in model similar to human conditions? If yes, which ones | 4.16 |
| 2.3.1 Secondary lesions | 1.38 |
| 2.3.2 Overlap syndrome (digestion of digits) | 1.38 |
| 2.3.3 Extra organ manifestation | 1.38 |
| 3. Biochemical Validation | 12.5 |
| 3.1 Pharmacodynamic biomarkers mimic the pathophysiology of the human disease | 4.16 |
| 3.1.1 Increased inflammatory markers (CRP, ESR, Arthritic Index) | 2.08 |
| 3.1.2 Increased immunological markers (ACCP, IL-6, TNF-α) | 2.08 |
| 3.2 Do these PD markers behave similarly to human? | 4.16 |
| 3.3 Known prognostic markers related to pathophysiology of the disease | 4.16 |
| 3.3.1 Walking disability | 1.04 |
| 3.3.2 Symmetric progression of disease | 1.04 |
| 3.3.3 Increase in secretions | 1.04 |
| 3.3.4 Nodule formation | 1.04 |
| 4. Aetiological Validation | 12.5 |
| 4.1 Is the aetiology of the disease similar to human for Rheumatoid Arthritis? If yes, which one | 6.25 |
| 4.1.1 Cytokine activation (TNF-α, IL-6) | 2.08 |
| 4.1.2 Cell infiltration (ACCP generation) | 2.08 |
| 4.1.3 Radiographic changes | 2.08 |
| 4.2 Is the aetiology of the disease similar to human for RA and co-morbid conditions? If yes, which one | 6.25 |
| 4.2.1 Disability | 2.08 |

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| **4.2.2 Receptor activation (TLR-4)** | **2.08** |
| **4.2.3 Extra organ manifestation** | **2.08** |
| **5. Pharmacological Validation** | **12.5** |
| **5.1 Are effective drugs in humans also effective in this model? If yes, which ones** | **4.16** |
| **5.1.1 Which one (Methotrexate)** | **4.16** |
| **5.2 Are ineffective drugs also ineffective in this model?** | **4.16** |
| **5.3 Have drugs with different mechanisms of action and acting on different pathway been tested in this model? Which ones (aqueous extracts of herbs)** | **4.16** |
| **5.3.1 Test drug 1 (MPTN02)** | **1.38** |
| **5.3.2 Test drug 2 (MPTN01)** | **1.38** |
| **5.3.3 Test drug 3 (MPTN04)** | **1.38** |
| **6. Histological Validation** | **12.5** |
| **6.1 Do the histopathological structures in relevant tissues resemble the ones found in humans? If yes, which ones** | |
| **6.1.1 Histopathology of bone** | **12.5** |
| **7. Endpoint Validation** | **12.5** |
| **7.1 Are the endpoints used in preclinical studies the same or translatable to the clinical endpoints?** | **6.25** |
| **7.1.1 Radiographic changes** | **2.08** |
| **7.1.2 Perceptive changes** | **2.08** |
| **7.1.3 Cell infiltration in histopathology** | **2.08** |
| **7.2 Are the methods used to assess preclinical endpoints comparable to the ones used to assess related clinical endpoints?** | **6.25** |
| **7.2.1 Paw volume** | **1.56** |
| **7.2.2 Walking disability** | **1.56** |
| **7.2.3 Symmetric progression** | **1.56** |
| **7.2.4 Severity of disease** | **1.56** |
| **8. Genetic Validation** | **12.5** |
| **8.1 Does this species also have orthologous genes and /or proteins involved in the human disease?** | **4.16** |
| **8.2 If so, are the relevant genetic mutations or alterations also present in the orthologous genes/proteins?** | **4.16** |
| **8.3 If so, is the expression of such orthologous genes and/ or proteins similar to the human condition?** | **4.16** |

*Note:* **SW – Same weight score**

## FIMD for best model developed for cardiovascular complications in RA

Questionnaire for model of co-morbid CV complications in RA was developed again using the standard format of eight domains as mentioned above. As this model is a combination of higher complexity in terms of cardiovascular complications in RA, the questions were framed according to the symptoms and the pathogenesis involved in both the cases. The groups optimized and validated on all the complexities and disease severity points and the three states were scrutinized. All the models were compared for the questions and were answered individually according to the standard format.

**Table II. Questionnaire for comparison of models developed for cardiovascular complications in RA**

| Questions for RA with CVD Model | |
|---|---|
| 1. Epidemiology | 12.5 |
| 1.1 Nature of population (Inbred/Outbred) | 4.16 |
| 1.2 Is the model able to simulate the disease in the relevant age groups (juvenile, adult or aging) | 4.16 |
| 1.3 is the model able to simulate the disease in different genders | 4.16 |
| 2. Symptomatology and natural history | 12.5 |
| 2.1 Is model able to mimic human disease symptoms? If so, which ones | 4.16 |
| 2.1.1 Inflammatory markers | 1.38 |
| 2.1.2 Immunological markers | 1.38 |
| 2.1.3 Crosslinking markers for cardiovascular complications | 1.38 |
| 2.2 Natural history criteria matching human disease onset | 4.16 |
| 2.2.1 Time of onset | 0.83 |
| 2.2.2 Disease progression | 0.83 |
| 2.2.3 Duration of symptoms | 0.83 |
| 2.2.4 Metabolic dysbiosis | 0.83 |
| 2.2.5 GUT infiltration | 0.83 |
| 2.3 Co-morbid conditions replicated in model similar to human conditions? If yes, which ones | 4.16 |
| 2.3.1 Secondary lesions | 1.04 |
| 2.3.2 Overlap syndrome (digestion of digits) | 1.04 |
| 2.3.3 Extra organ manifestation | 1.04 |
| 2.3.4 Steatosis | 1.04 |
| 3. Biochemical Validation | 12.5 |
| 3.1 Pharmacodynamic biomarkers mimic the pathophysiology of the human disease | 4.16 |
| 3.1.1 Increased inflammatory markers (CRP, ESR, Arthritic Index) | 1.38 |
| 3.1.2 Increased immunological markers (TNF-α, IL-6, ACCP, Hyc) | 1.38 |
| 3.1.3 Increased Atherogenic markers (TG, TC, Cholesterol, Atherosclerotic index) | 1.38 |
| 3.2 Do these PD markers behave similarly to human? | 4.16 |
| 3.3 Known prognostic markers related to pathophysiology of the disease | 4.16 |
| 3.3.1 Walking disability | 0.69 |
| 3.3.2 Symmetric progression of disease | 0.69 |
| 3.3.3 Increase in secretions | 0.69 |
| 3.3.4 Nodule formation | 0.69 |
| 3.3.5 Obesity | 0.69 |
| 3.3.6 Metabolic dysbiosis | 0.69 |
| 4. Aetiological Validation | 12.5 |
| 4.1 Is the aetiology of the disease similar to human for Rheumatoid Arthritis? If yes, which one | 6.25 |
| 4.1.1 Cytokine activation (TNF-α, IL-6) | 1.25 |

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| **4.1.2 Cell infiltration (ACCP generation and Hyc activation)** | **1.25** |
| **4.1.3 fibre length of vistus medialis** | **1.25** |
| **4.1.4 Fibre length of biceps femoris** | **1.25** |
| **4.1.5 Radiographic changes** | **1.25** |
| **4.2 Is the aetiology of the disease similar to human for RA and co-morbid conditions? If yes, which ones** | **6.25** |
| **4.2.1 Disability** | **1.56** |
| **4.2.2 Metabolic dysbiosis (Obesity, TG, TC, LDL, HDL and fat accumulation in stool )** | **1.56** |
| **4.2.3 Receptor activation (TLR-4, NLRP-3)** | **1.56** |
| **4.2.4 Extra organ manifestation** | **1.56** |
| **5. Pharmacological Validation** | **12.5** |
| **5.1 Are effective drugs in humans also effective in this model?** | **4.16** |
| **5.1.1Which one (Methotrexate)** | |
| **5.2 Are ineffective drugs also ineffective in this model?** | **4.16** |
| **5.3 Have drugs with different mechanisms of action and acting on different pathway been tested in this model? Which ones ( aqueous extracts of herbs)** | **4.16** |
| **5.3.1 Test drug 1 (MPTN02)** | **1.38** |
| **5.3.2 Test drug 2 (MPTN01)** | **1.38** |
| **5.3.3 Test drug 3 (MPTN04)** | **1.38** |
| **6. Histological Validation** | **12.5** |
| **6.1 Do the histopathological structures in relevant tissues resemble the ones found in humans? If yes, which ones** | **4.16** |
| **6.1.1 Histopathology of bone** | **4.16** |
| **6.1.2 Histopathology of heart** | **4.16** |
| **6.1.3 Histopathology of vistus medialis and biceps femoris muscle** | **4.16** |
| **7. Endpoint Validation** | **12.5** |
| **7.1 Are the endpoints used in preclinical studies the same or translatable to the clinical endpoints?** | **6.25** |
| **7.1.1 Radiographic changes** | **2.08** |
| **7.1.2 Perceptive changes** | **2.08** |
| **7.1.3 Cellular infiltration in histopathology** | **2.08** |
| **7.2 Are the methods used to assess preclinical endpoints comparable to the ones used to assess related clinical endpoints?** | **6.25** |
| **7.2.1 Paw volume (pain and stiffness)** | **1.04** |
| **7.2.2 Walking disability** | **1.04** |
| **7.2.3 Symmetric progression** | **1.04** |
| **7.2.4 Severity of disease** | **1.04** |
| **7.2.5 Atherogenic biochemical markers (Lipid Profile)** | **1.04** |
| **7.2.6 Obesity and metabolic dysbiosis** | **1.04** |
| **8. Genetic Validation** | **12.5** |
| **8.1 Does this species also have orthologous genes and /or proteins involved in the human disease?** | **4.16** |
| **8.2 If so, are the relevant genetic mutations or alterations also present in the orthologous genes/proteins?** | **4.16** |

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|---|---|
| **8.3 If so, is the expression of such orthologous genes and/ or proteins similar to the human condition?** | **4.16** |

*Noe:* **SW – Same weight score**