RA-MAP Project

Towards an improved understanding of immune function and response in rheumatoid arthritis

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RA-MAP

- UK flagship Rheumatoid Arthritis (RA) Consortium funded through MRC/ABPI Initiative
- Industry-Academic Partnership
- To improve understanding of the human immune system in RA
- Focus is on investigating clinical and **biological predictors** of disease outcomes in RA patients

Background

- Compelling evidence implicates dysregulated immune function in the pathogenesis (origin and progression) of RA
  - Includes genetic predisposition, serologic abnormalities, synovial histology and response to immune targeted therapies
  - Little is understood of immune dysregulation that leads to the disease we recognise as RA and the immune status that defines remission
- In RCTs, inflammation and damage are measured, but not directly immune dysfunction or immunological tolerance
- Hinder the development of immune-modulated drugs
  - Immunological tolerance can take weeks to develop
  - Drugs may not have anti-inflammatory properties
  - Short term clinical outcomes in RCTs may not truly reflect their long-term effect or impact

Challenges

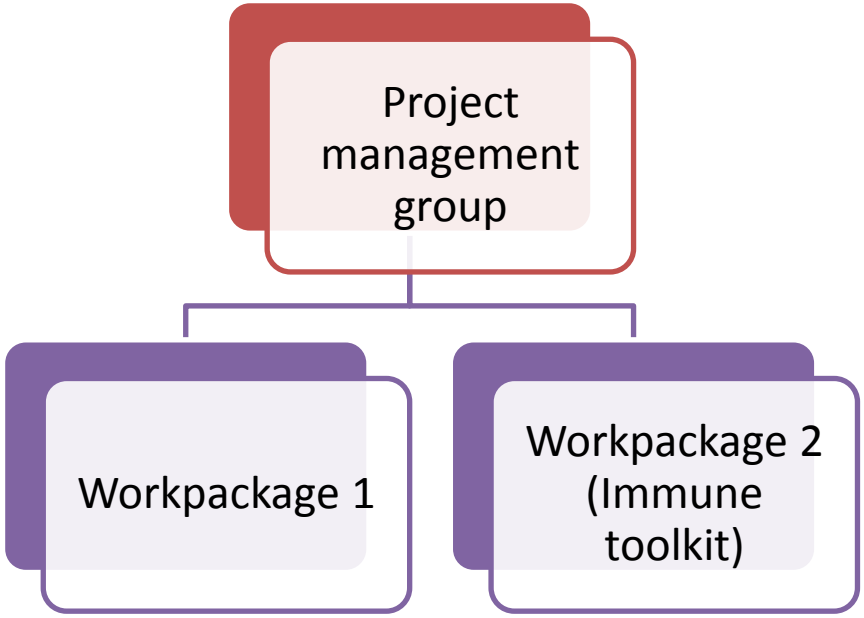
- Define RA in terms of immune dysregulation
- Identification of up-stream immunological biomarkers that relate to disease impact (e.g. early erosion modification) and response to therapy
- Facilitate rapid decision making to deliver specific therapeutic targets for patient benefit
- Develop cell-based and molecular assays (i.e. immune toolkit) that can be used for longitudinal monitoring of patients and inform on the impact of interventions

RA-MAP Strategic Goals

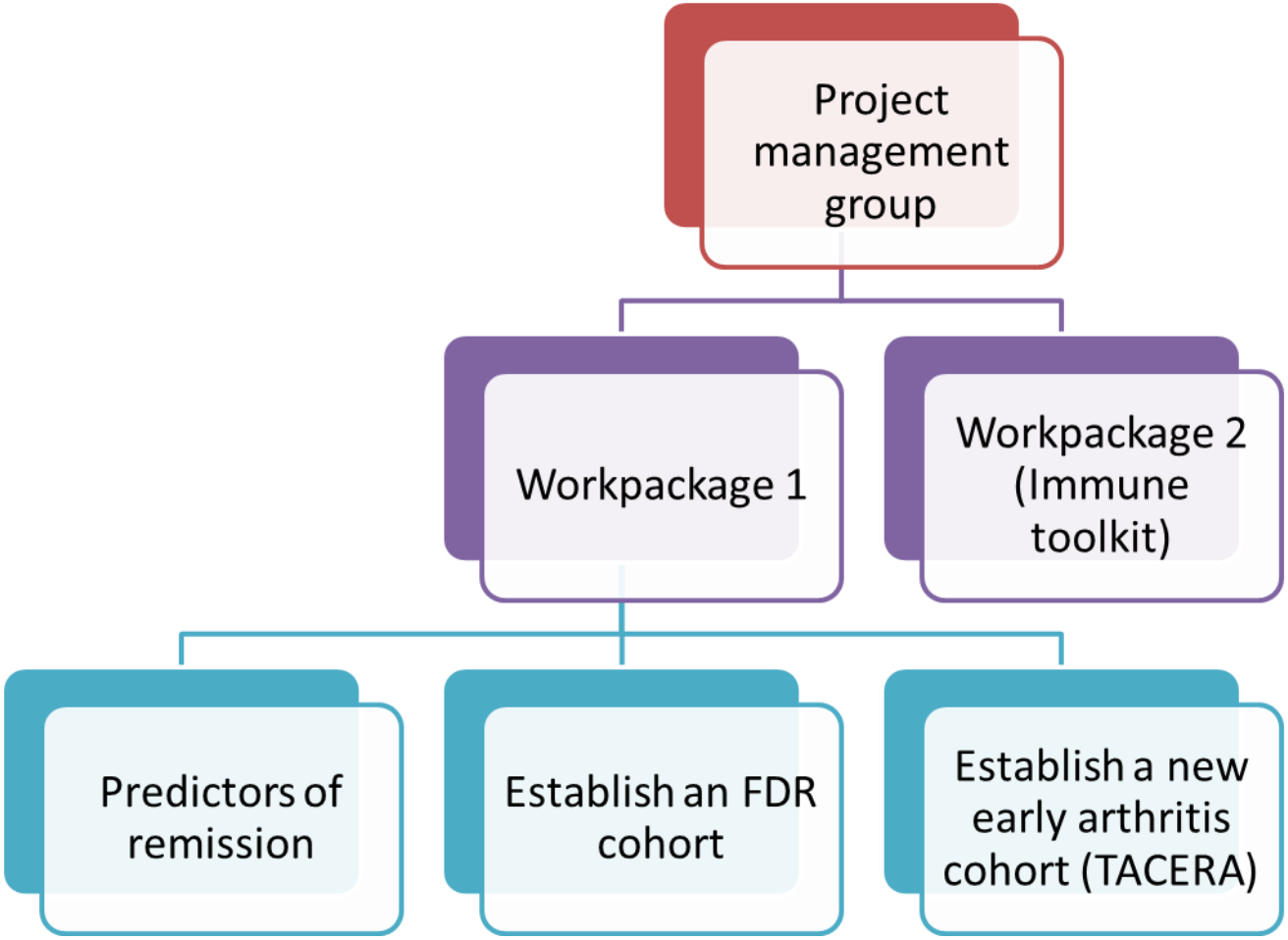
1. **Define fundamental immunological mechanisms characterising early stages of rheumatoid arthritis. Exploiting this knowledge of mechanisms to:**
   - ❖ **Predict therapeutic responsiveness**
   - ❖ **Predict prognosis and remission**
   - ❖ **Predict downstream damage**
   - ❖ **Inform therapeutic decision making (personalized medicine)**
   - ❖ **Provide novel biomarkers for use in clinical trials**
2. **Identify predictors for clinical remission, and define true immunological remission states**
3. **Characterise 'pre-RA' state according to putative levels of risk in asymptomatic populations, and define aberrant immune signatures associated with high risk**

➢**TOWARDS A CURE FOR RHEUMATOID ARTHRITIS**

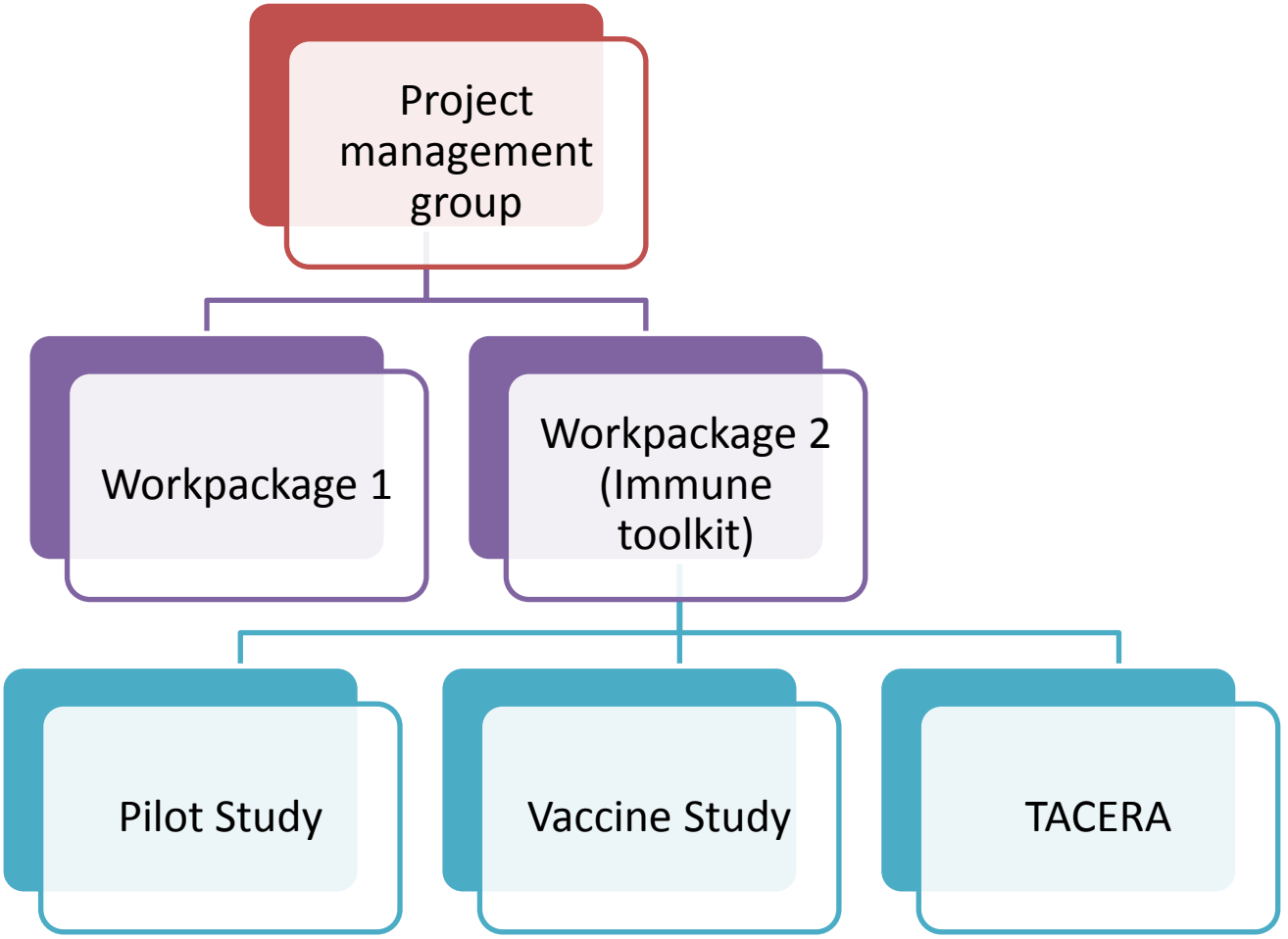
Consortium Structure



Work Package 1

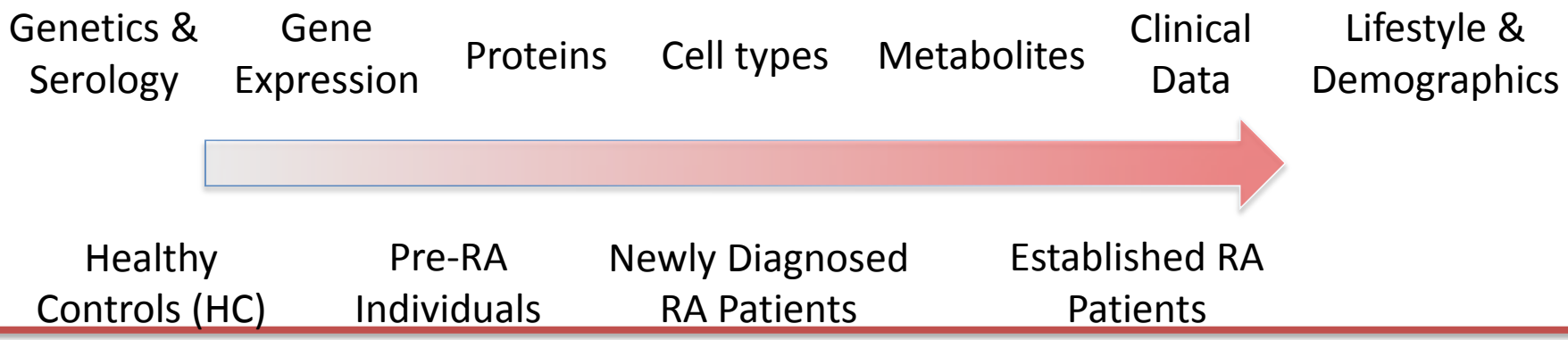


Work Package 2



RA-MAP Strategy

- Two complementary work packages
  - WP1 (Clinical Science) informs WP2 (Basic Science)
- Staged and iterative
- Expansive and ambitious
  - Data collected
  - Populations looked at
  - Questions being asked



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Study Design

Plan of Investigation

WP1

- Clinical predictors of clinical remission and characterise disease course
  - Pooled data from **control** arms of 18 (19) RCT trials (over 3000 subjects)
  - Clinical data from TACERA **inception** cohort (274 patients)
    (visits every 3 mths, up to a max of 18 mths follow-up)
- Determine or define appropriate clinical phenotypes that can be used to investigate associations with biological immunological markers
  - Clinical outcomes from TACERA
  - Extremes of clinical phenotype
  - Derived "endotypes"
  - Based on composite disease activity measures (DAS28-CRP, SDAI)
  - Preliminary data on biological markers (e.g. cell based markers)

Allows a better understanding of the heterogeneity in the disease due to clinical factors and the use of a cleaner "down-stream" manifestation of immune dysregulation

Plan of Investigation

WP2 (Pilot Study – 50 HC and 50 RA)

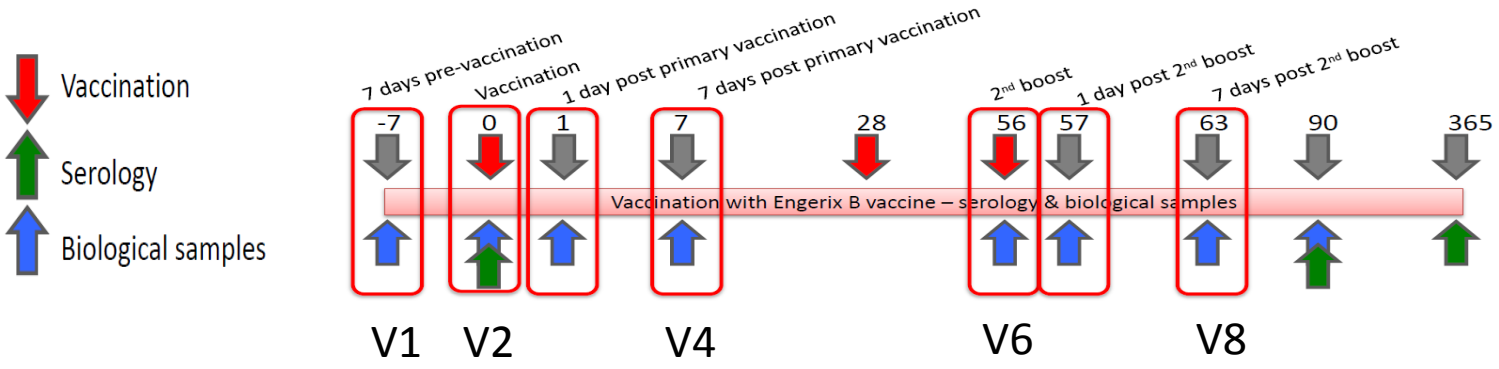
- Investigate differences between HC (pre-Engerix B vaccination) and RA subjects (DMARD naïve) in cell subsets, whole blood, PBMC, urine and sera using gene expression (mRNA and microRNA), flow cytometry and metabolomics
  - Can we identify immunological signals and with what technology?
- Investigate whether any differences seen in whole blood can be seen to the same extent in separated leucocyte cell subsets (CD4, CD8, CD14) (gene expression)
  - Do we need to focus on all of these?
- Refine FACS panels to include only markers that differentiate between HC and RA

Preliminary molecular and cell-based understanding of what may have changed in newly diagnosed "untreated" RA patients. Pilot also allows the refinement of protocols and the more efficient future use of resources

Plan of Investigation

WP2 (Vaccine Study of 50 HC)

- Investigate immune response in HC challenged with HB vaccine using the biological data
  - Characterise marker profiles over time in HC undergoing a **normal** immune response
  - Investigate whether immunological marker profiles (at baseline and over time) differ between those with a high HBV antibody response vs low response (at 90 days)



To study immune dysfunction in RA we need to understand a normal immune system in terms of (i) response to novel antigens; (ii) activation (switching on); (iii) memory; and (iv) quiescence (switching off)

Plan of Investigation

WP2 (TACERA – 274 RA)

- Baseline immunological markers associated with being in remission (defined by SDAI or DAS28-CRP) or other clinical or patient-reported outcomes at 6 months
- Baseline markers associated with derived WP1 “endotypes”
- How 6-mth change in biomarkers (or biomarker signatures) associate with clinical outcomes
- Defining true biological/subclinical remission state(s)

Identifying molecular signatures of early RA disease course and towards translating this information to robust, validated assays of immune phenotype and function that can be used in routine monitoring and decision making

Statistical Methods

Addressing the Questions

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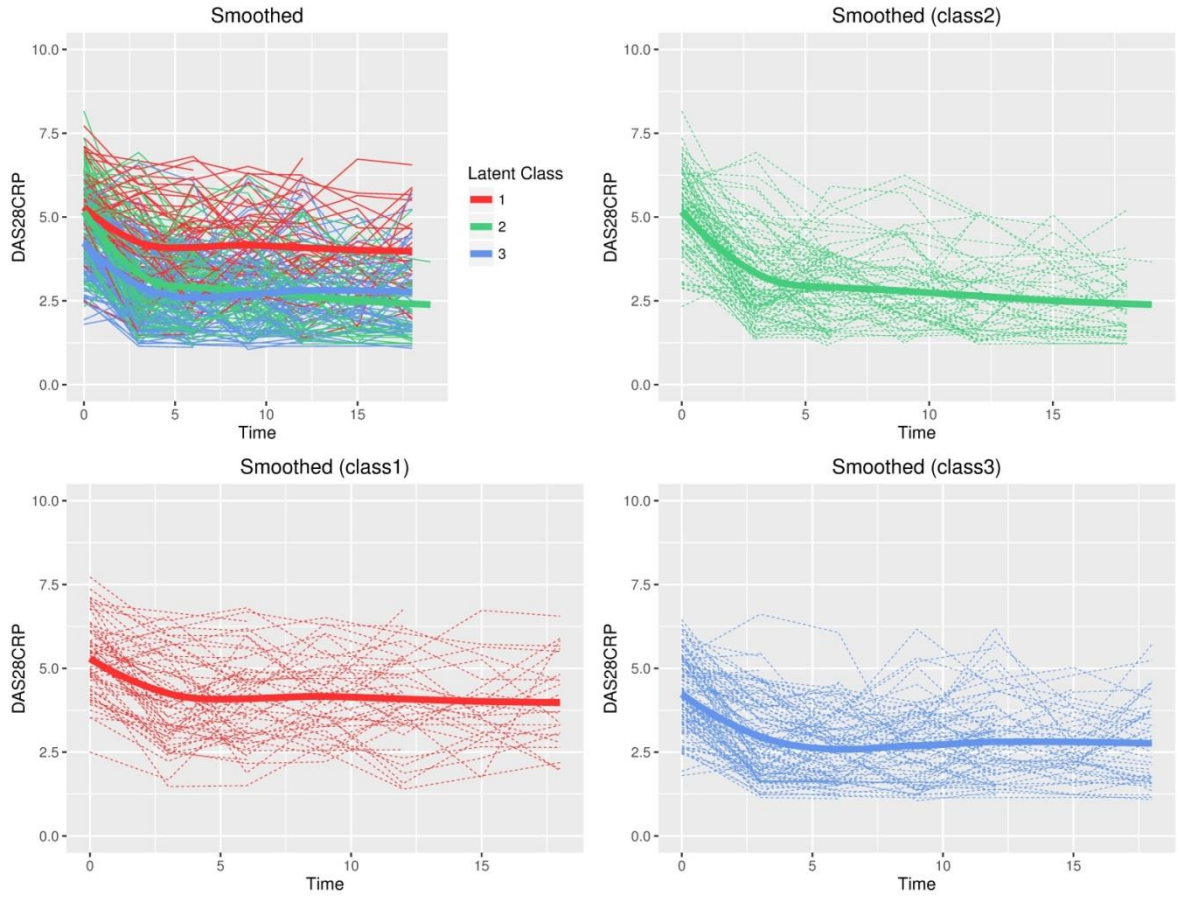
Modelling Remission and Characterising Disease Course

- For investigating DAS28-ESR remission at 6 months and characterising DAS28-ESR over time, logistic regression models and longitudinal models were fitted to the Pooled Trial data separately for MTX-naïve and not MTX-naïve at entry
  - Logistic regression for MTX-naïve identified age at entry, gender, ethnicity, baseline disease activity, steroid use, functional health associated with 6 month remission
  - Latent class mixed models (random intercept and random slope) for MTX-naive identified 3 latent subgroups ; Functional disability (HAQ) at baseline was associated with class membership; Disease duration, ethnicity, steroid use and time-varying HAQ were associated with mean level of disease activity in some of these "latent trajectory" subgroups
  - Handled missing data through multiple imputation (MICE)
  - Other issues concerned model and variable selection, multiple testing and validation
- Similar analyses were repeated on the TACERA data but now for SDAI and DAS28-CRP (not DAS28-ESR)
  - Latent Class Mixed Models again identified 3 latent trajectories for both SDAI and DAS28-CRP

LCMM for DAS28-CRP in TACERA



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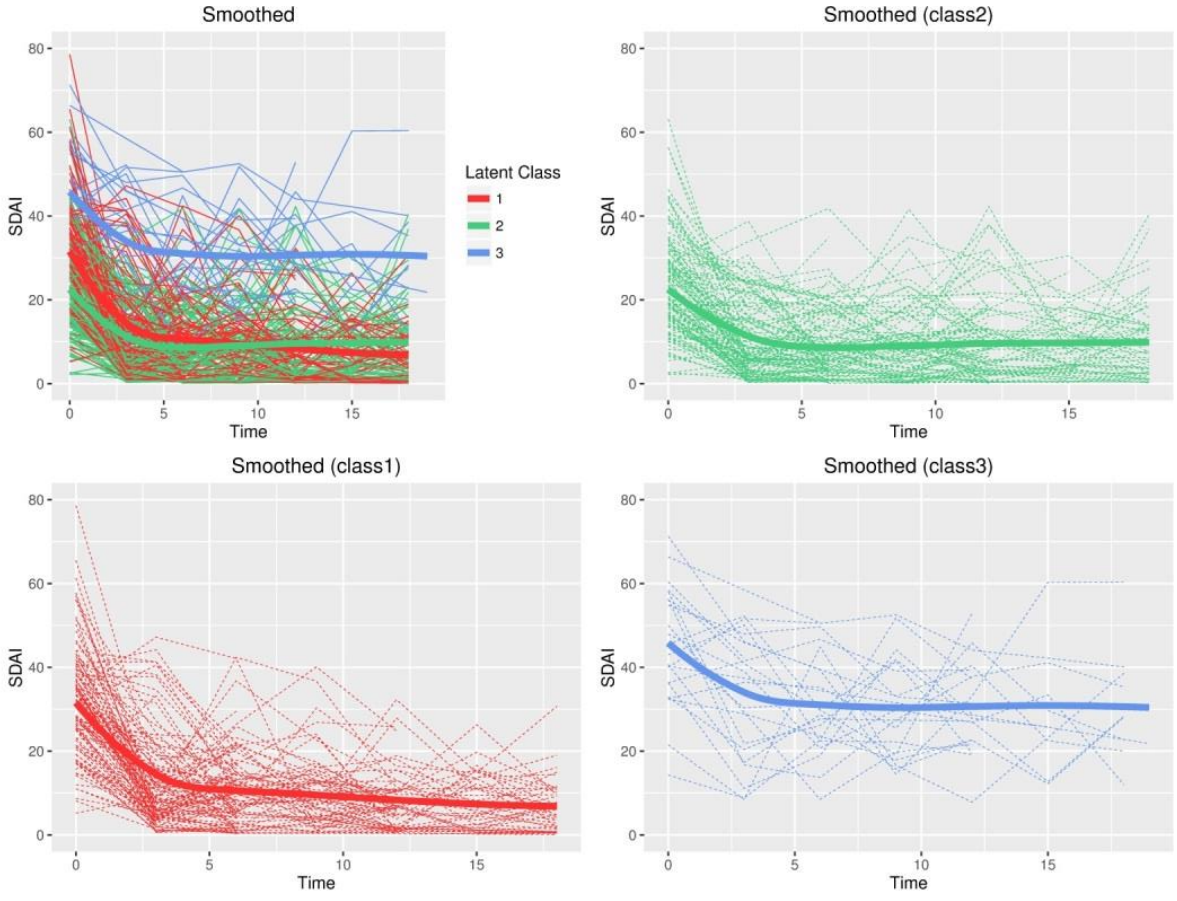
LCMM for SDAI in TACERA



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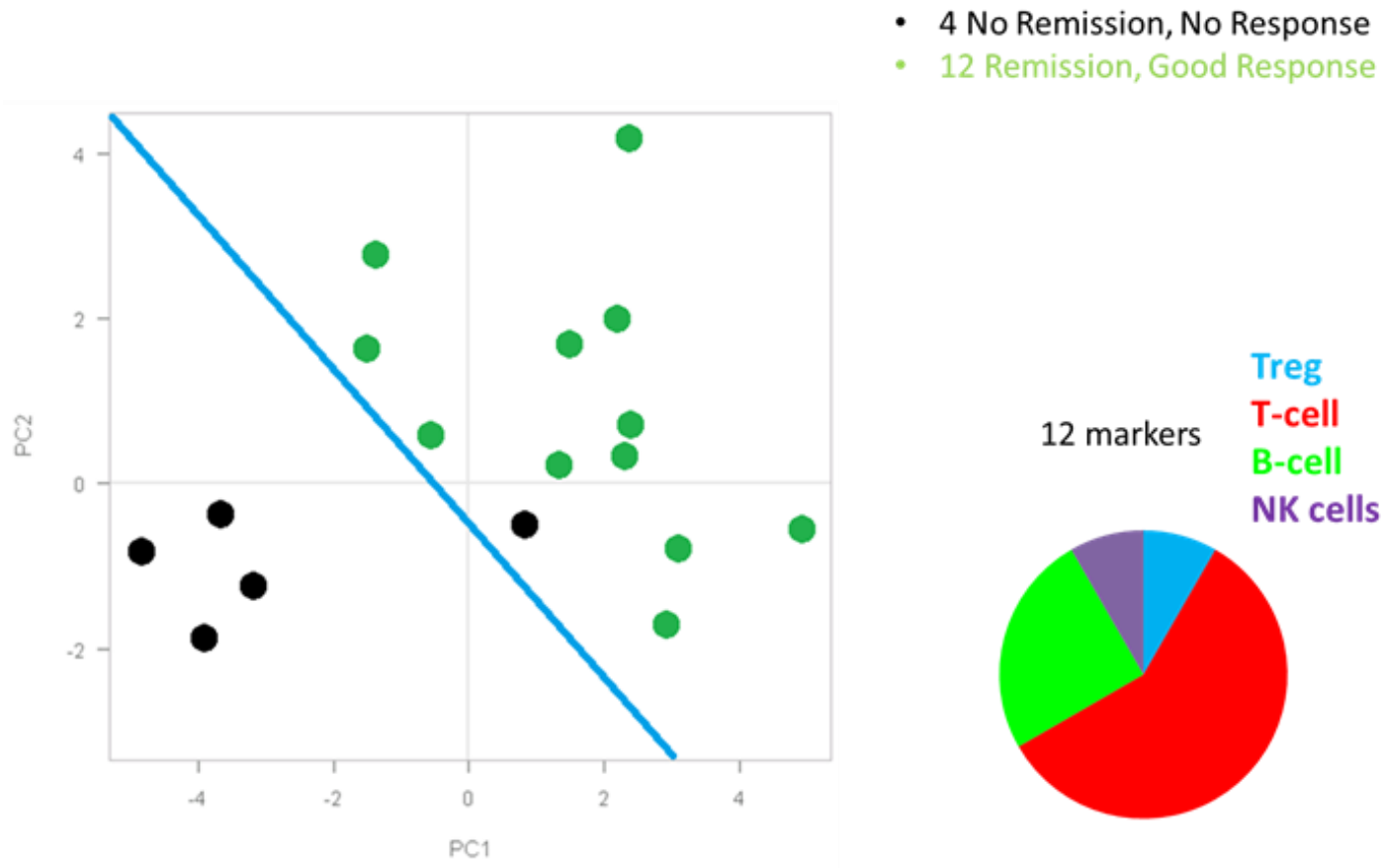
Defining Response

- Our first look at defining response was based on the first 70 patients from TACERA (40 of which had some flow cytometry data available)
- We investigated various definitions of 6-month response using DAS28-CRP
  - Remission (DAS28-CRP < 2.6 at 6mths)
  - EULAR response (ΔDAS28-CRP and DAS28-CRP at 6mths)
  - ΔDAS28-CRP response (ΔDAS28-CRP > 3.2 reduction)
  - Low Disease Activity (DAS28-CRP < 3.2 at 6mths)
  - Plus combinations (Remission/EULAR response)
- Then related these to flow markers
- Interested in scalability (to all 274) and number of markers identified
- Frequencies and projections
- T-tests for determining whether any statistically significant differences in mean level of markers between the groups defined by response
- Supervised clustering/classification methods such as partial least squares and random forest

Defining Response

- Except for ΔDAS28-CRP response all others appeared to be scalable
- EULAR response, LDA and combination of Remission/EULAR produced more statistically significant findings with t-tests (caveat – multiple testing)
- Partial least square method appears to have reasonable discriminatory power. Random forest not very successful in prediction
- However, later discussion led to the decision to use SDAI response at 6 months and the latent trajectory endotypes
  - SDAI at 6 month important to clinician (short term response)
  - Latent trajectories describe the overall patterns over time (longer term)
  - Decision has implications on choice of samples to send for further protein analyses



PLS using FACS for investigating definition of response using DAS28-CRP

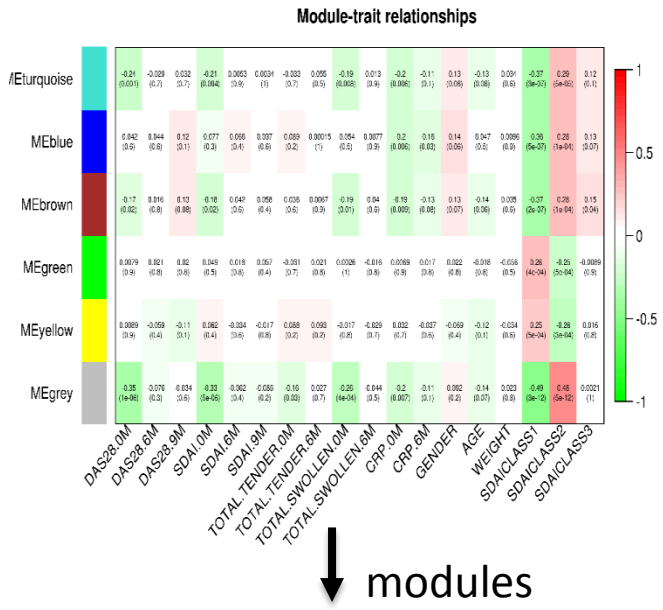
Basic Science Questions of WP2

- Statistical approaches adopted typically are platform-specific
- Questions from WP2 can typically be formulated in terms of single contrast hypotheses for biomarkers
- Gene expression analyses (mRNA and micro RNA)
  - Linear (LIMMA) or negative binomial models with FDR corrections (after QC and normalisation steps) for DE
  - Multivariate analysis techniques: Clustering, dimension reduction and supervised classification for stratification and developing gene expression signatures
  - Pathway analysis: GSVA, WGCNA, Ingenuity for gene set enrichment
  - Graphical tools: volcano plots, heat maps and bi-plots

WGCNA can be used in a supervised manner to generate modules from DE genes for analysis

Gargalovic, P. S., Imura, M., Zhang, B., Gharavi, N. M., Clark, M. J., Pagnon, J., ... & Nelson, S. F. (2006). Identification of inflammatory gene modules based on variations of human endothelial cell responses to oxidized lipids. *Proceedings of the National Academy of Sciences*, *103*(34), 12741-12746.

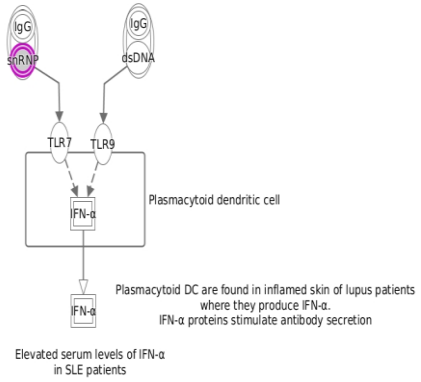
Diff exp, CD14 contrasts = trajectory 1 versus 2 (corrected for gender, age) = 166 genes



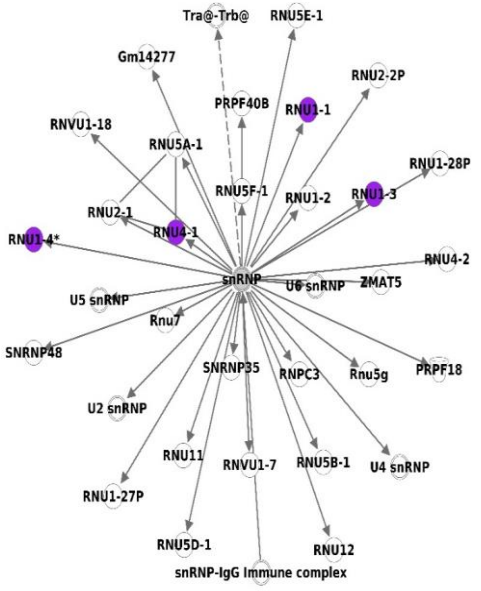
modules

Ingenuity pathway analysis

- Over enrichment analysis
- Pathway visualisation



green module



RNU family of genes are involved in SLE

These are differentially expressed between 1 and 2 trajectories and are co expressed (green module)

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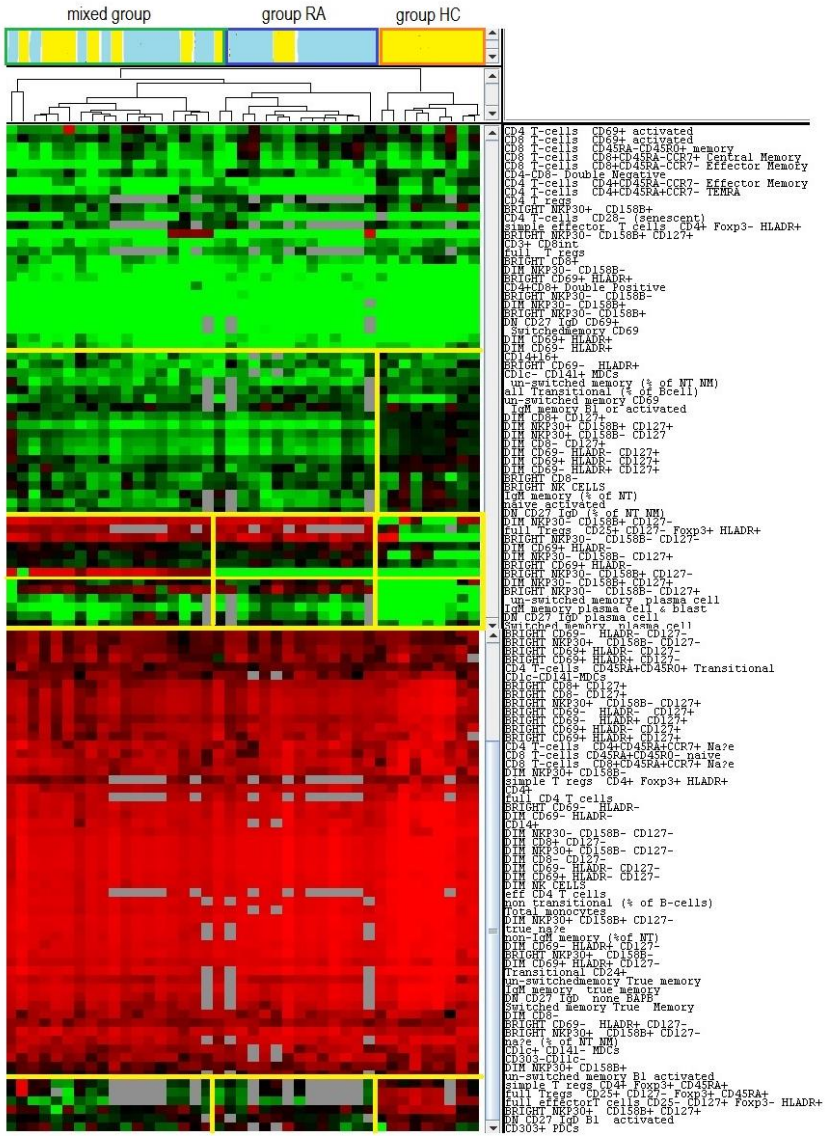
Basic Science Questions of WP2

- FACS analyses (T cell, NK cell, B cell, Mcyte/DC, Treg panels)
  - T-test and Mann-Whitney U test
  - Clustering and PLS regression (account for confounders)
- Metabolomics (NMR spectra)
  - PCA and PLS-DA or PLS-R
  - Genetic algorithm for variable selection



Hierarchical clustering analysis

Grouping :

Pure HC group

"Pure" RA group

Mixed HC + RA group

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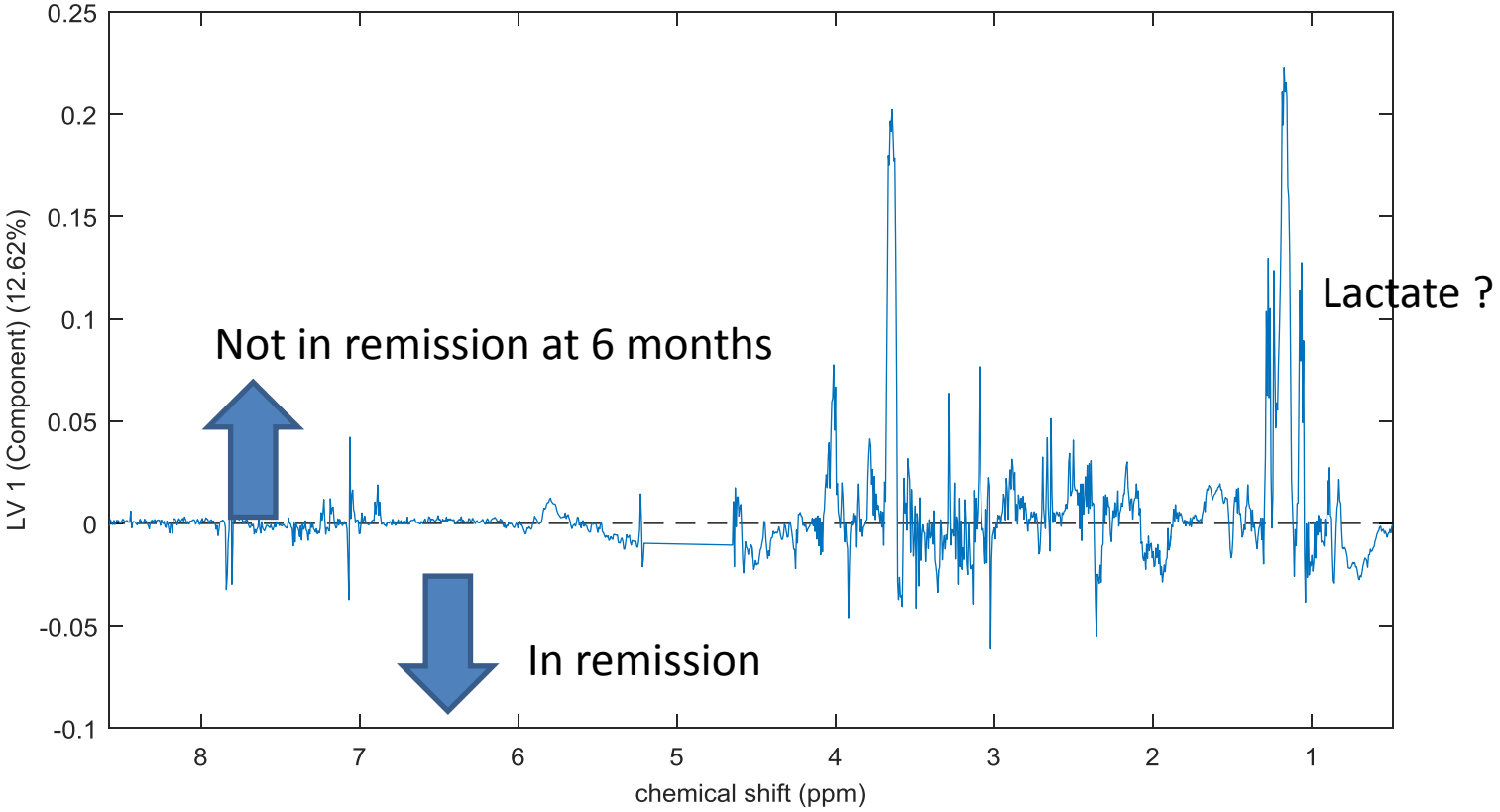

PLSDA of baseline serum with samples group with SDAI less than 3 at 6 months or not
Loading for LV 1

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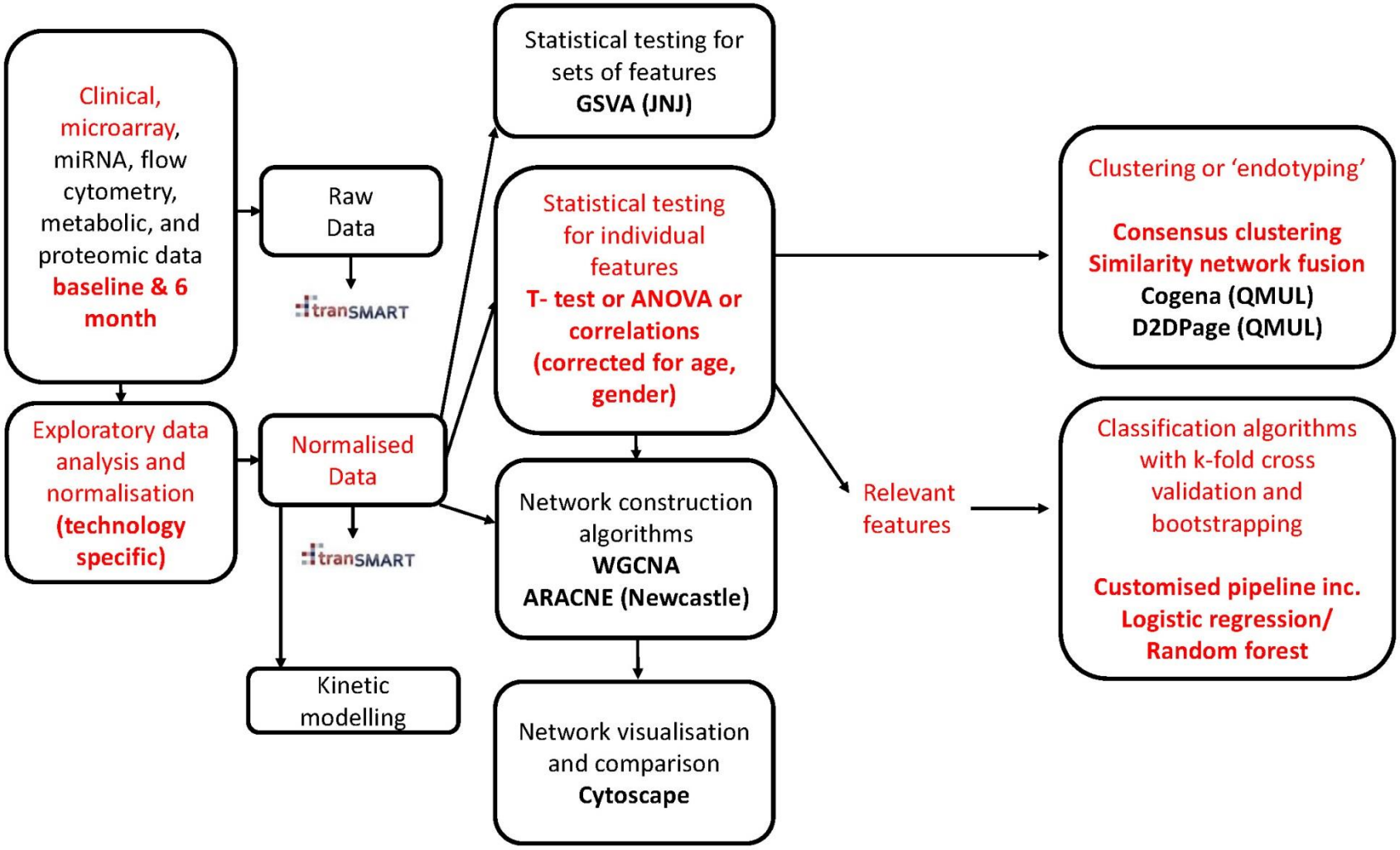
Issues

- Quality control
- Normalisation/Gating and Binning
- Detecting and handling batch effects
- Multiple testing
- Validation

Schema



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Where to next?

- Statistical analyses of the biological data is still at an early stage
- As yet to look at characterising biological marker profiles over time in the Vaccine study for HC
- Proteomics at baseline and 6 months has yet to be done on a subset of TACERA RA patients
- Heading towards a more integrative analysis rather than analysing each technology separately

Conclusions

- RA-MAP is an ambitious project to try and improve the understanding of the human immune system in RA
- The strategy adopted is broad ranging with respect to the populations looked at (HC, pre-RA, early RA and established RA), the type of data collected and technologies used, and the questions asked (and hopefully answered)
- At present, we have gone some ways in identifying clinical predictors and defining appropriate outcomes
- More to be done with regards to the biological correlates of disease outcomes
- A more integrative analysis/systems approach is an end goal in order to better understand how each of the different levels of aggregation play a role in immune dysregulation
- Thus leading to the future development of an immune toolkit

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