# Molecular Phenotypes in Systemic Sclerosis Enable Patient Stratification and Precision Medicine

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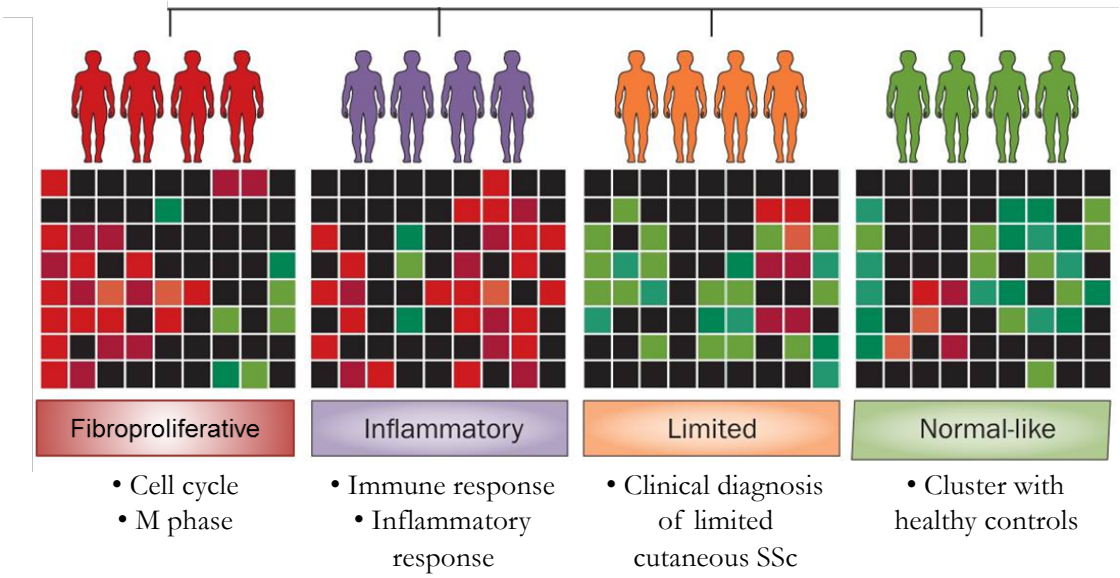
# Global View of Gene Expression in SSc Tissues




- Identified in multiple cohorts of SSc patients
- Identified in multiple end-target tissues
- Driving pathways are conserved across cohorts

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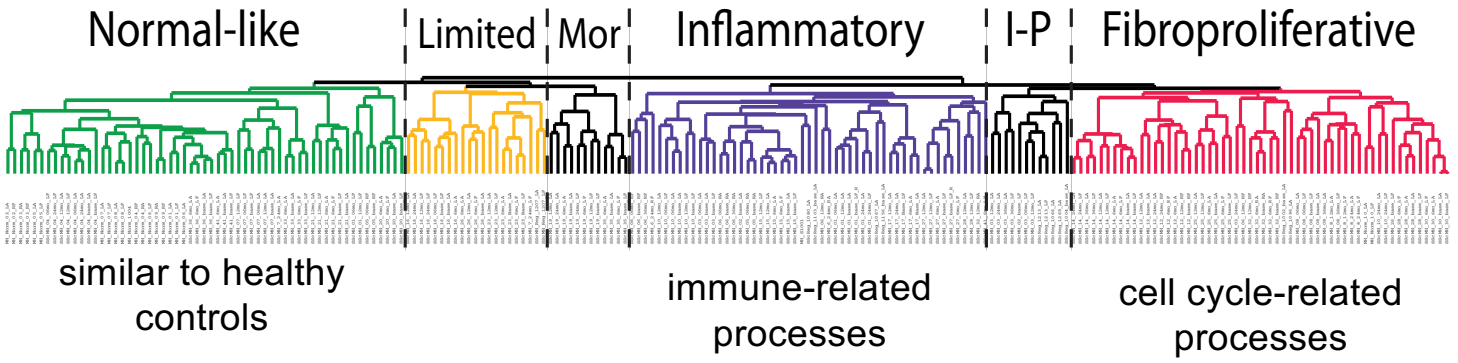
Milano et al. 2008 PLoS One
Graphic modified from Varga J, Hinchcliff M. *Nat Rev Rheumatol* (2014)

# Gene Expression in SSc Skin is Dominated by Intrinsic Gene Expression Subsets




- Subsets based on gene expression profiling in skin
- Found in multiple independent skin cohorts

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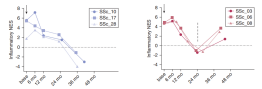
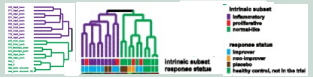
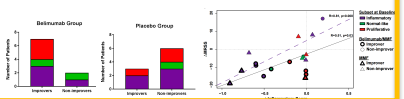
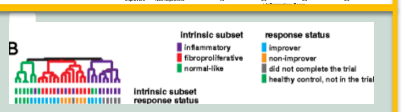
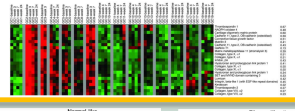
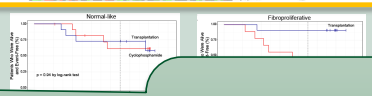
Milano et al. *PLoS ONE* (2008)
Pendergrass et al. *J. Invest. Dermatol. (*2012)
Hinchcliff et al. *J. Invest. Dermatol*. (2013)
Johnson et al. PLoS ONE (2015)
Franks et al. Arthritis. Rheum (2019), *In Press*

# Gene Expression Subsets Identify Patients that Improve with Therapy.

| Trials Analyzed | Primary Results | |
|---|---|---|
| Mycophenolate Mofetil (MMF) Hinchcliff M, *et al.* (2013) | Improvers are most often inflammatory while non-improvers are most often fibroproliferative. |   |
| Mycophenolate Mofetil (MMF) Hinchcliff M, Toledo D, *et al.* (2018) | Inflammatory signature decreases with MMF therapy and rebounds upon cessation, mirroring macrophage counts and CCL2. |  |
| Abatacept Chakravarty EF, *et al.* (2015) | 4 of 5 SSc patients who improved with abatacept were inflammatory. |  |
| Belimumab (with background MMF) Gordon J, *et al.* (2017) | Improvement coincides with a decrease in inflammatory signature and movement to the normal-like subset. |  |
| Nilotinib Gordon J, *et al.* (2015) | Activation of the TGFβ pathway (which spans the inflammatory and fibroproliferative subsets) was important for improvement. |   |
| Fresolimumab Rice L, *et al.* (2015) Taroni *et al.* (2016) | Activation of the TGFβ pathway (which spans the inflammatory and fibroproliferative subsets) was important for improvement. |  |
| SCOT (Scleroderma Cyclophosphamide or Transplantation) Sullivan *et al.* NEJM (2018) Franks JM, *et al* (In prep) | Fibroproliferative patients see improved long-term event-free survival with transplantation compared to cyclophosphamide treatment. |  |

Improvers typically associated with the inflammatory subset

Improvers associated with activation of a specific pathway, e.g. TGF-b

Improvers most likely to be fibroproliferative

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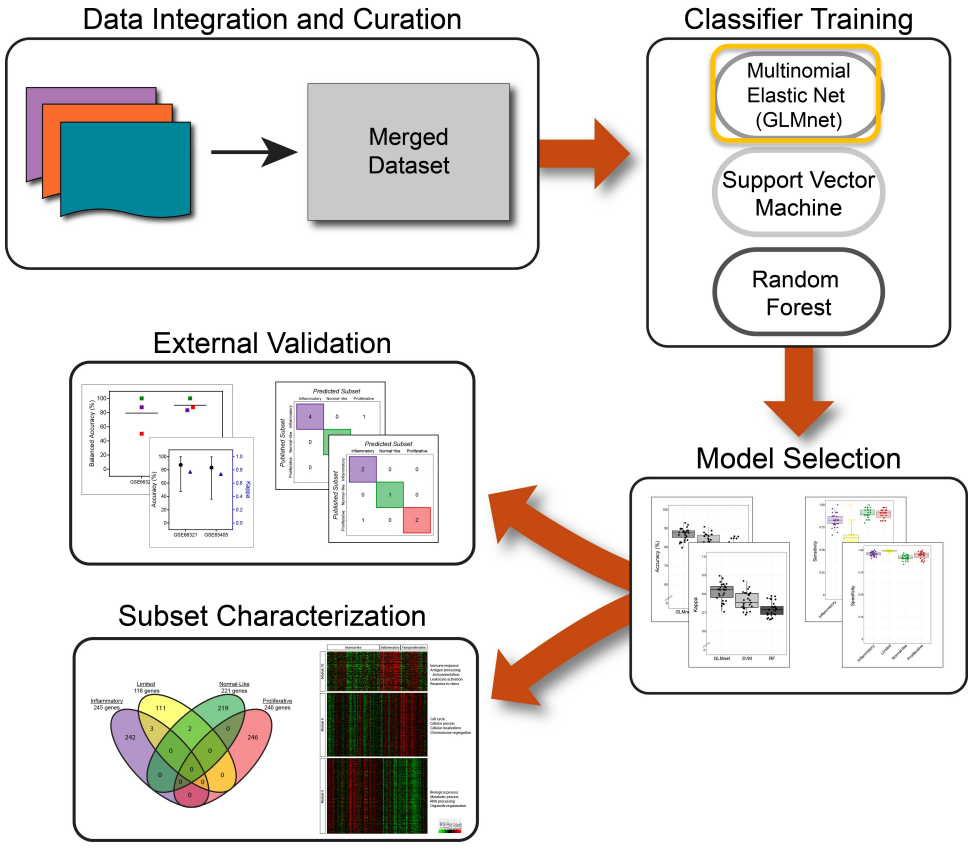
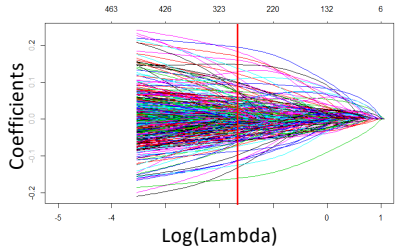
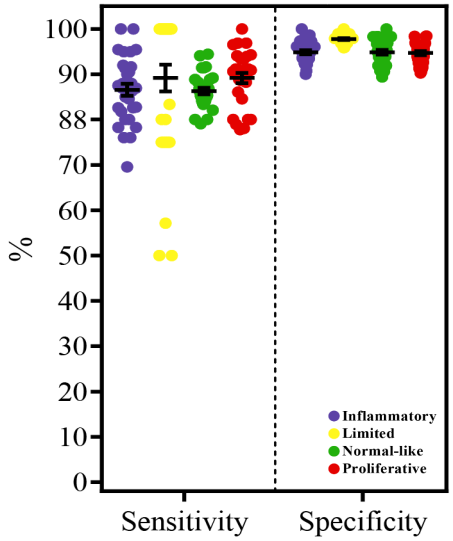
# Development of a SSc Subset Classifier Using Machine Learning




**Repeated 3-fold cross-validation**




Cross-fold Validation Accuracy **>0.89**

External Validation Accuracy **>0.85**

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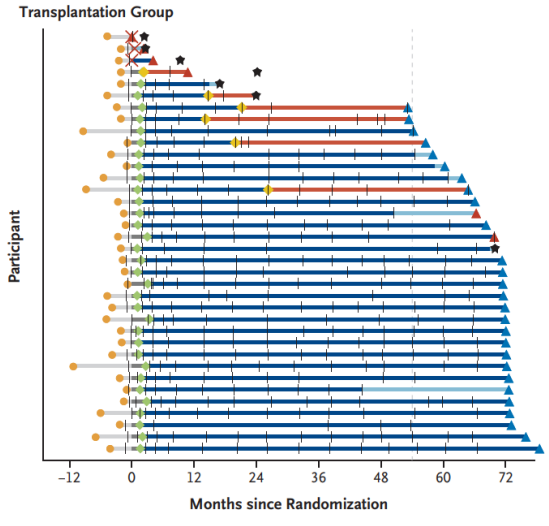
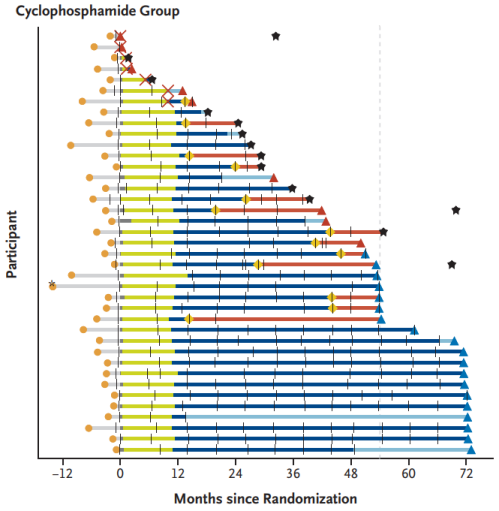
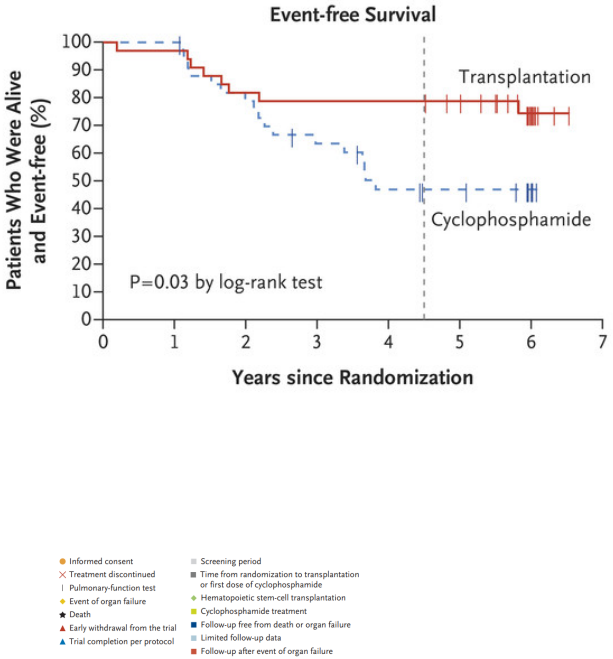
Franks JM, et al. 2019 Arthritis Rheum

# SCOT: Scleroderma Cyclophosphomide or Transplantation

- Patients with severe scleroderma were randomized to receive either 12 doses of cyclophosphamide or myeloablative hematopoietic stem cell transplant
- Participants were followed for 5+ years and evaluated for event-free survival
- Events: respiratory, cardiac, or renal failure; death
- Patients who received a transplant had significantly increased EFS







Sullivan K, et al. *NEJM*. (2018)

# Gene Expression Analyses of PBMC Samples from SCOT Trial Participants

- Gene expression analyses were performed on 63/75 per-protocol (PP) patients defined as the participants who received at least nine doses of cyclophosphamide or received a HSCT.
  - 30 HSCT, 33 CYC (Per-protocol)
- 229 RNA samples analyzed by DNA microarray
  - 63 SSc patients analyzed over six timepoints: Baseline, 8, 14, 20/26, 38, 48/54 months
- Analyzed for , intrinsic subtype, differential expression, cell-type deconvolution, and association with covariates.

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Franks JM, Martyanov et al. 2020 Ann Rheum Dis

# Clinical Characteristics of Patients in the SCOT Gene Expression Cohort

| Gene Expression Dataset - Per-Protocol | | | |
|---|---|---|---|
| | Cyclophosphamide | HSCT | p value |
| | n=33 | n=30 | |
| **Sex** | | | |
| **Female - no. (%)** | 23 (69.7) | 15 (50.0) | 0.1292 |
| **Male - no. (%)** | 10 (30.3) | 15 (15.0) | |
| | | | |
| **Race** | | | |
| **White - no. (%)** | 25 (75.8) | 23 (76.7) | 0.8512 |
| **Black/African-American - no. (%)** | 4 (12.1) | 2 (6.7) | |
| **Asian - no. (%)** | 1 (3.0) | 2 (6.7) | |
| **Multiple/Other/Unknown - no. (%)** | 3 (9.1) | 3 (10.0) | |
| | | | |
| **Age - Med. (IQR)** | 47.0 (41.0, 53.0) | 45.0 (37.0, 49.75) | 0.3893 |
| | | | |
| **MRSS - Med. (IQR)** | 29.5 (22.0, 38.5) | 27.75 (20.62, 34.5) | 0.3018 |
| **FVC - Med. (IQR)** | 74.0 (60.0, 87.0) | 76.0 (69.25, 80.75) | 0.6694 |
| **DLCO - Med. (IQR)** | 50.69 (45.85, 56.63) | 54.36 (49.74, 59.0) | 0.1666 |
| **SHAQ - Med. (IQR)** | 1.375 (0.5938, 1.938) | 1.125 (0.75, 1.625) | 0.2841 |

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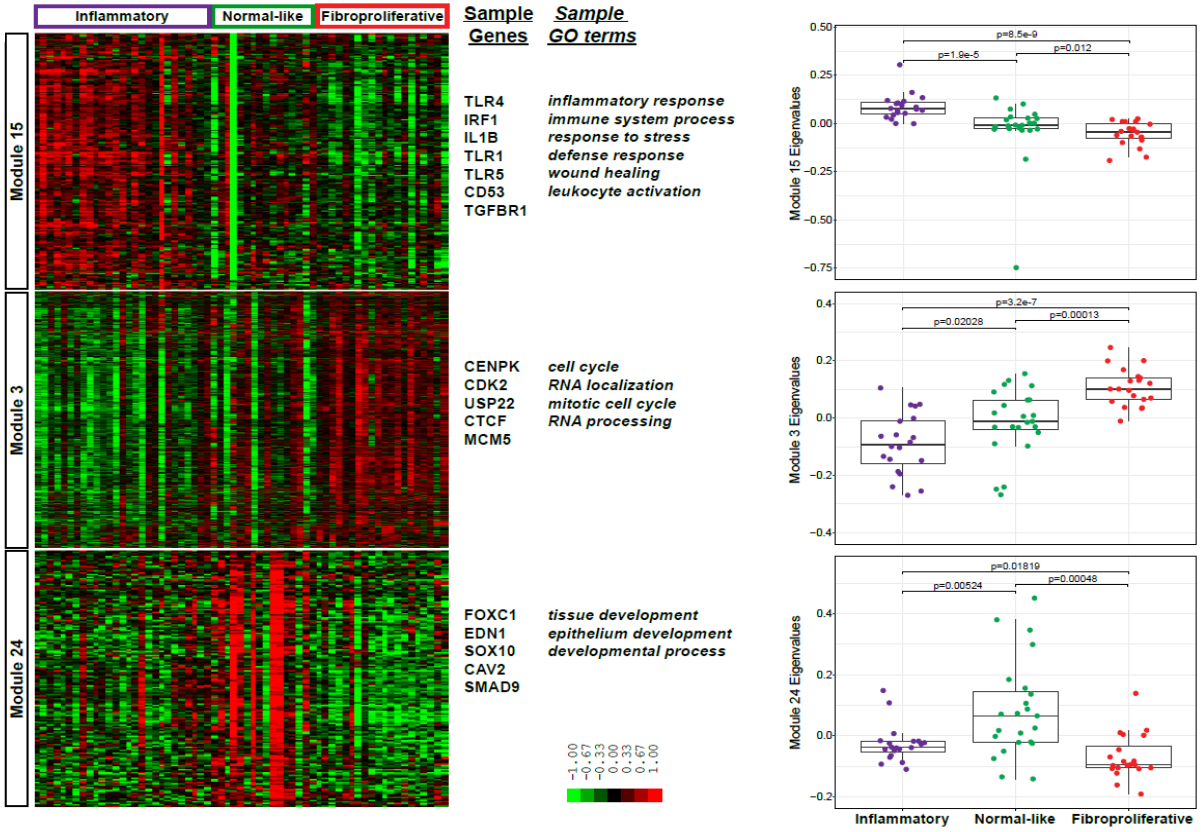
# Intrinsic Gene Expression Subsets in the SCOT Participant PBMC Gene Expression Data Mirror Previous Observations

- Gene expression from DNA microarrays; applied classifier to determine subsets at baseline
- Identified gene expression modules associated intrinsic gene expression subsets in PBMCs using WGCNA.
- Asked the question, are there prognostic differences between subsets for these treatments?

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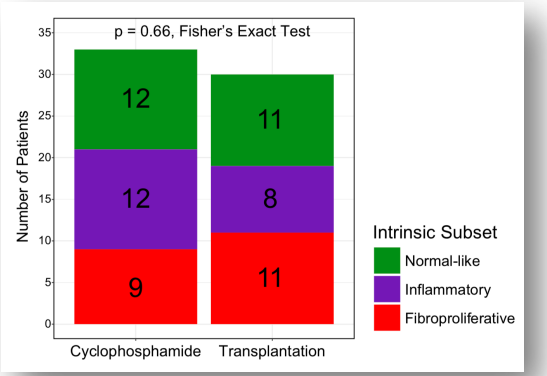
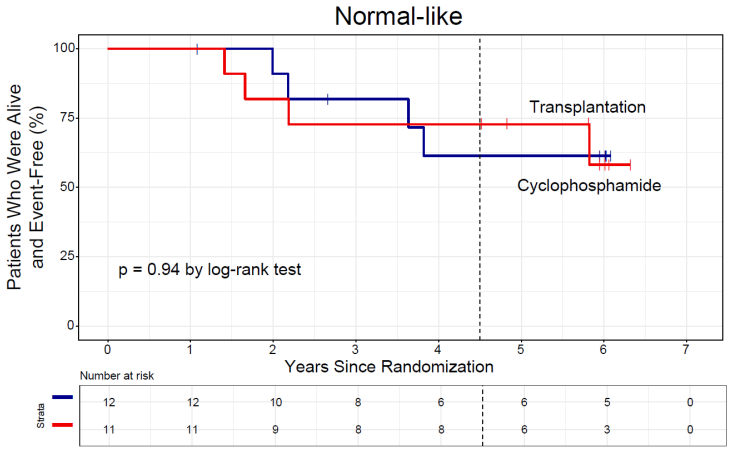
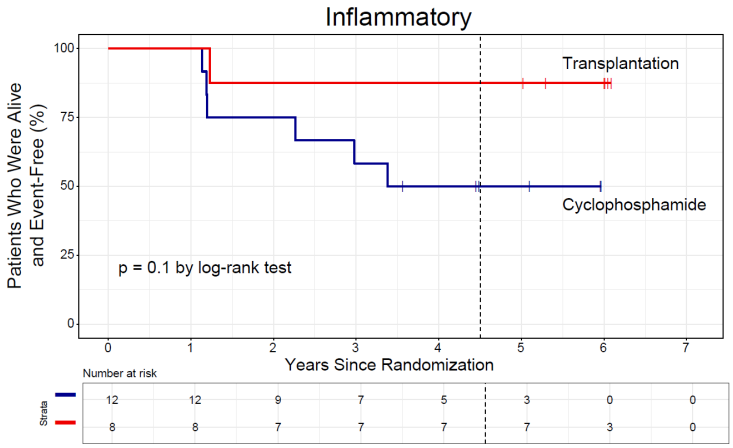
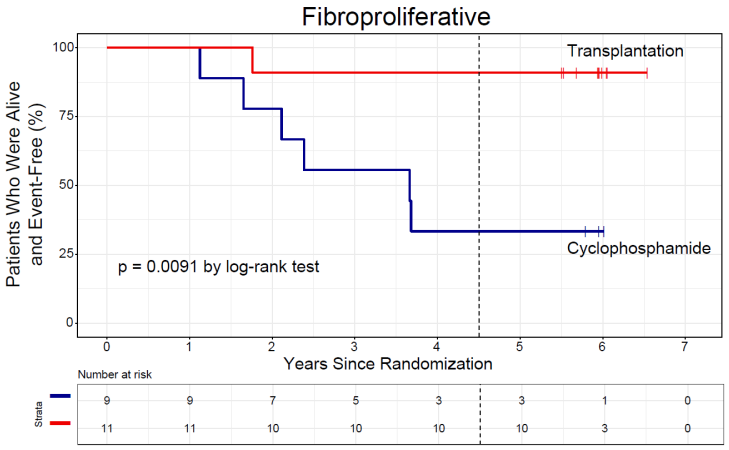
# SCOT Event-Free Survival Stratified by Subset

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# SCOT Conclusions

- The HSCT arm of the SCOT trial showed substantially larger changes in gene expression compared to CYC arm.
- Participants assigned to the fibroproliferative subset, who tend not to improve on immunosuppressive therapy (e.g. mycophenolate mofetil or abatacept), were the most likely to benefit from HSCT compared to CYC ($p=0.0091$)
- Participants assigned to the normal-like subset did not show benefit from HSCT treatment over CYC ($p=0.94$)
- Inflammatory patients show a trend toward improvement in HSCT which did not reach statistical significance ($p=0.1$)

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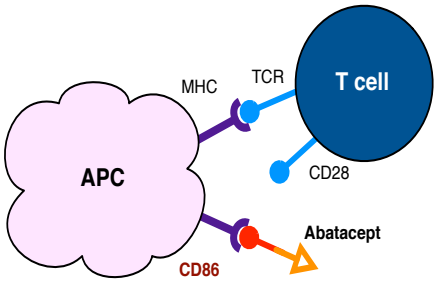
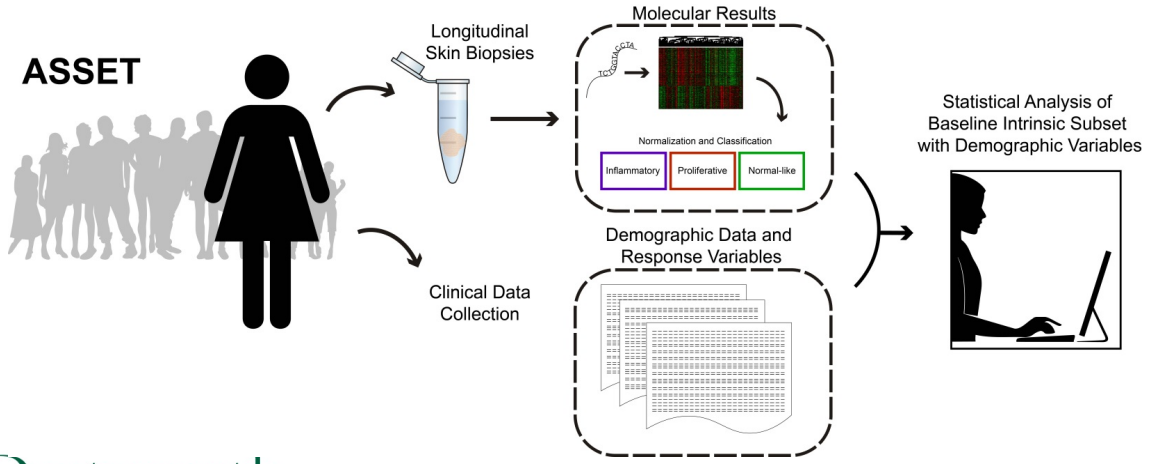
# ASSET Study & Intrinsic Subsets

- Summary of pilot study by Chakravarty et al. 2015
- Preliminary analyses in the ASSET Trial
  - Randomized control trial of Abatacept to treat dcSSc
  - Longitudinal skin biopsies collected
  - RNA-seq for gene expression analyses

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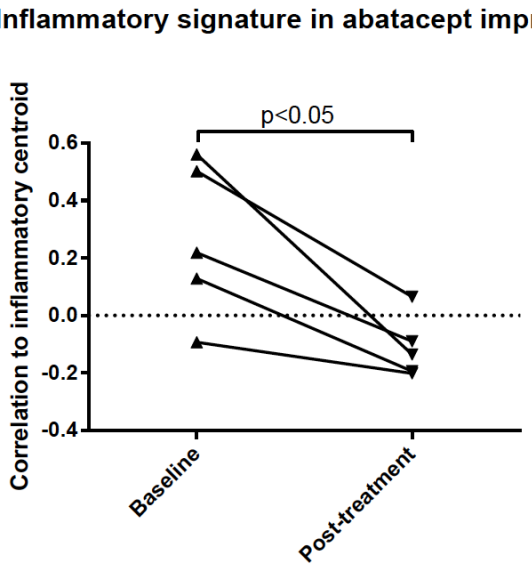
# Abatacept Pilot Study: Inflammatory subset shows clinical response in skin

| Patient | Baseline | Post-treatment |
|---|---|---|
| Imp1 | I | I |
| Imp2 | I | P |
| Imp3 | I | L |
| Imp4 | NL | P |
| Imp5 | I | NL |
| NonImp1 | NL | NL |
| Plb1 | NL | NL |
| Plb2 | I | I |

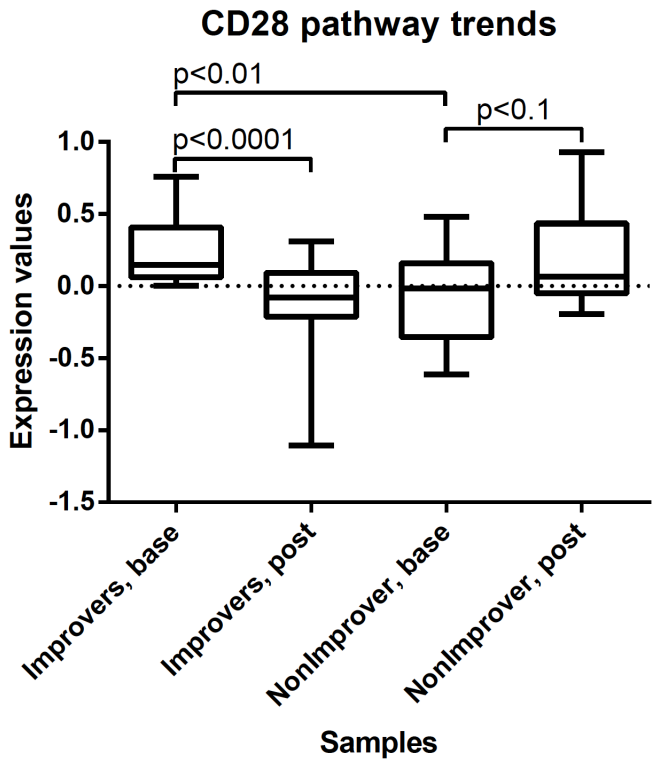


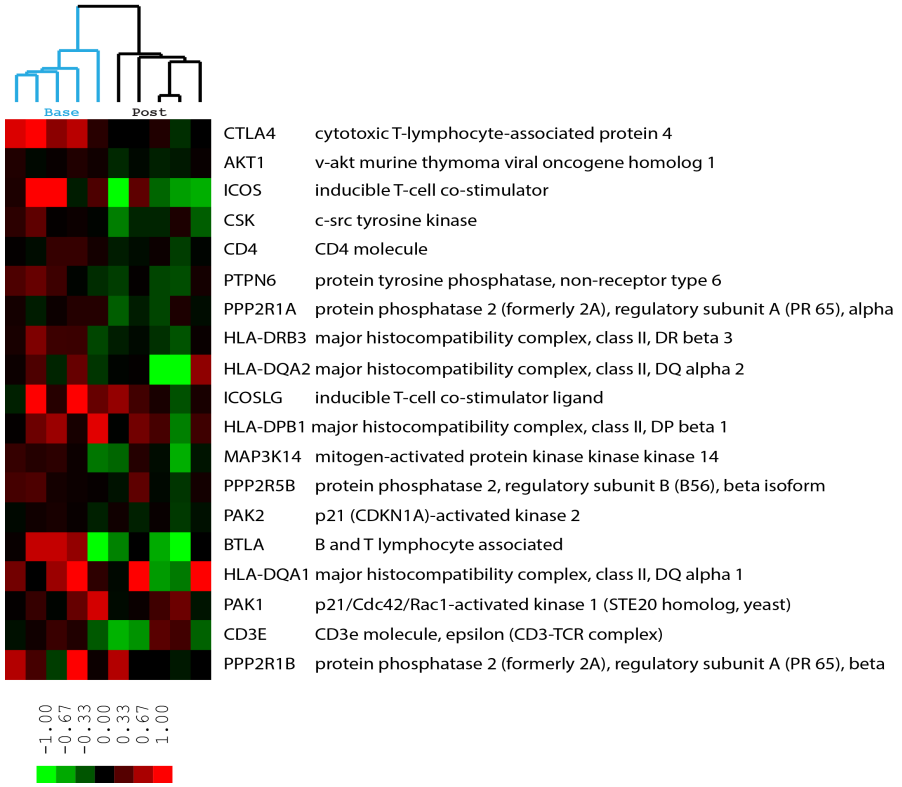

- 4/5 improvers are inflammatory at baseline
- 4/5 improvers show a significant decrease in the inflammatory gene signature (p=0.014)
- Inflammatory patients show a trend towards greater MRSS improvement at 24 weeks vs. normal-like

  (−13.5 ± 3.1 vs. –4.5 ± 6.4, p = 0.067)

Chakravarty et al. ART 2015

# Abatacept: Expression of genes regulated by CD28 show a decrease in improvers

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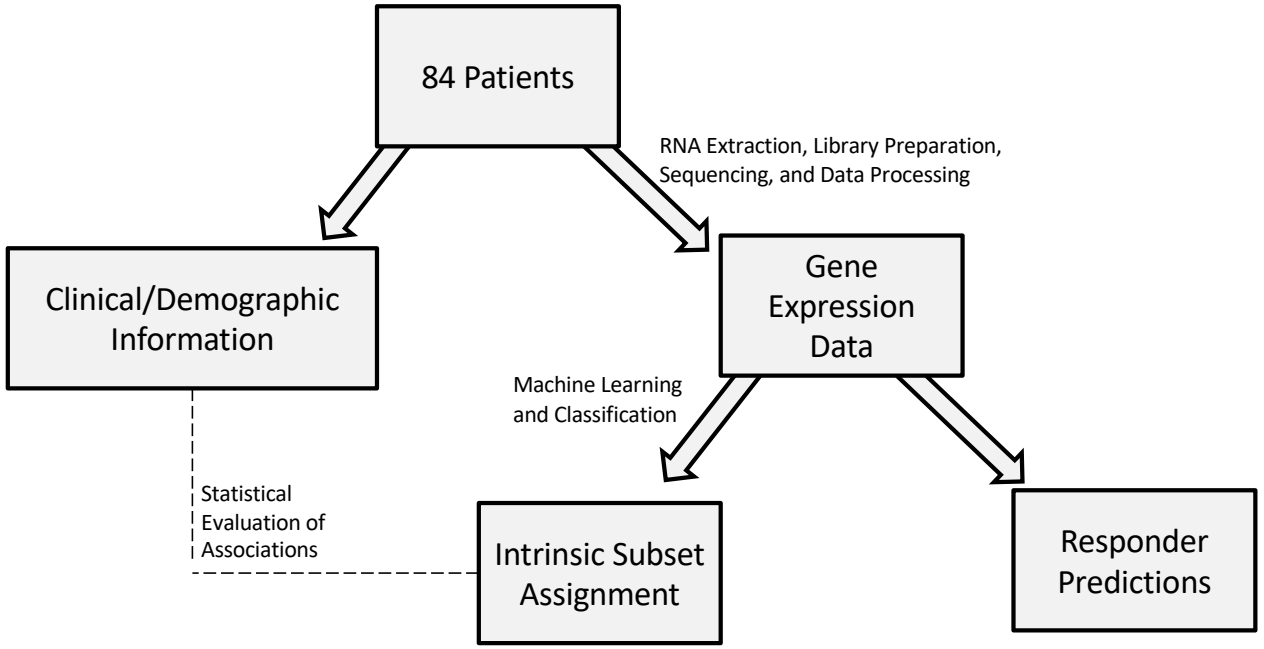
Chakravarty et al. ART 2015

# ASSET Trial Analysis Overview

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Khanna et al. Arthritis Rheum. 2020

# Intrinsic Subsets & Treatment Arm

Subsets as assigned at baseline

| | Inflammatory | Normal-like | Proliferative |
|---|---|---|---|
| **Abatacept** | 19 | 16 | 8 |
| **Placebo** | 14 | 17 | 10 |

Fisher's Exact Test: p-0.6112

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# Clinical outcomes by intrinsic subset in the ASSET trial

**Hypothesis:** Participants in the inflammatory subset will be the most likely to show improvement in MRSS with abatacept treatment.

- Change in mRSS over 12 months was significantly different between the abatacept and placebo treatment arms for the inflammatory ($p<0.001$) and normal-like ($p=0.03$) subsets.
- No difference in mRSS for the fibroproliferative subset of patients ($p=0.47$)
- For FVC% predicted, the fibroproliferative subset showed a numerical increase in FVC% in the abatacept arm ($p=0.19$) while all other groups showed decreases in FVC%.
- All subsets showed decreases in HAQ-DI in the abatacept arm not observed in the placebo arm, with the most robust changes occurring in the inflammatory ($p=0.09$) and normal-like ($p=0.06$) subsets.

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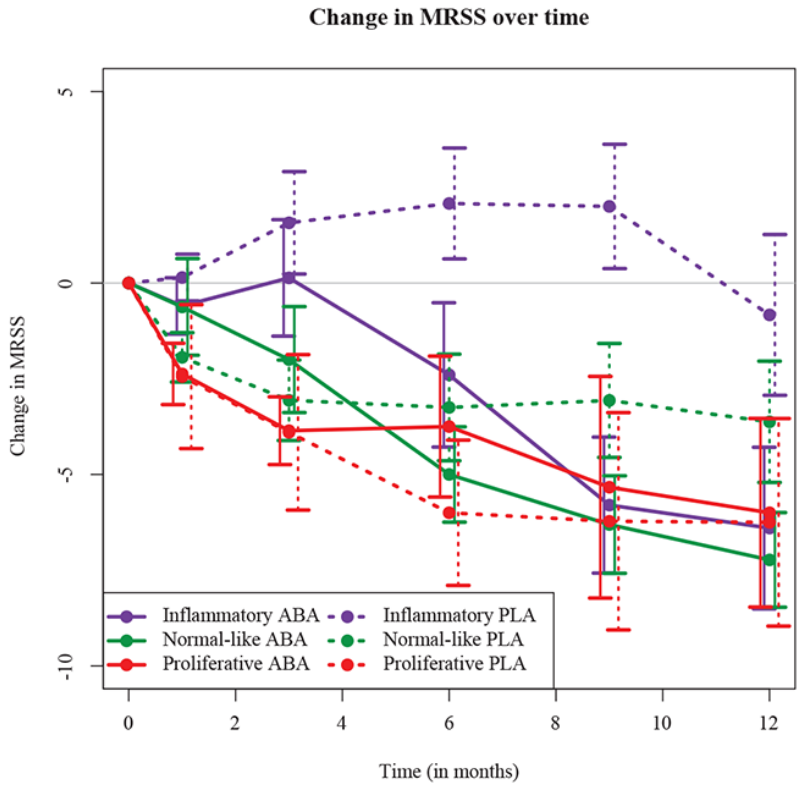
# Clinical outcomes by intrinsic subset in the ASSET trial

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Khanna et al. Arthritis Rheum. 2020

# Conclusions

- Abatacept resulted in a marked divergence in mRSS change for the inflammatory subset with little impact on the fibroproliferative subset.

- Patients who improve on abatacept have the high expression of the *Costimulation by the CD28 family* pathway.

- Degree of pathway expression is correlated change in MRSS. Higher pathway expression results in a larger change in MRSS.

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