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Journal Club:

Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq

by I.Tirosh, B.Izar et al. in *Science, 2016*

Petr Nazarov

2018-05-22

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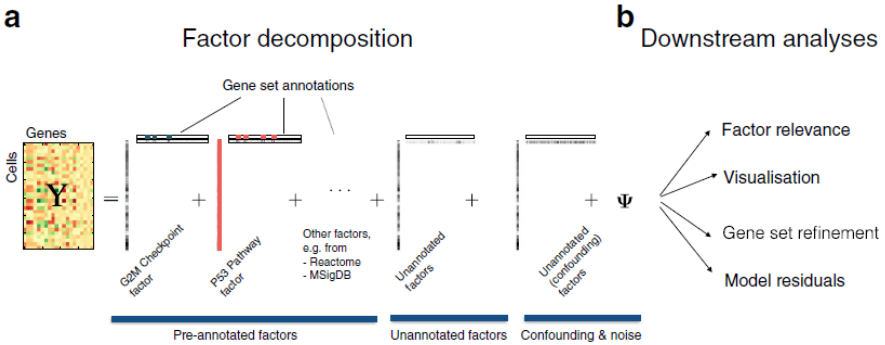
Variants

f-scLVM: scalable and versatile factor analysis for single-cell RNA-seq

Florian Buettner[1,6*], Naruemon Pratanwanich[1], Davis J. McCarthy[1,2], John C. Marioni[1,3,4*] and Oliver Stegle[1,5*]



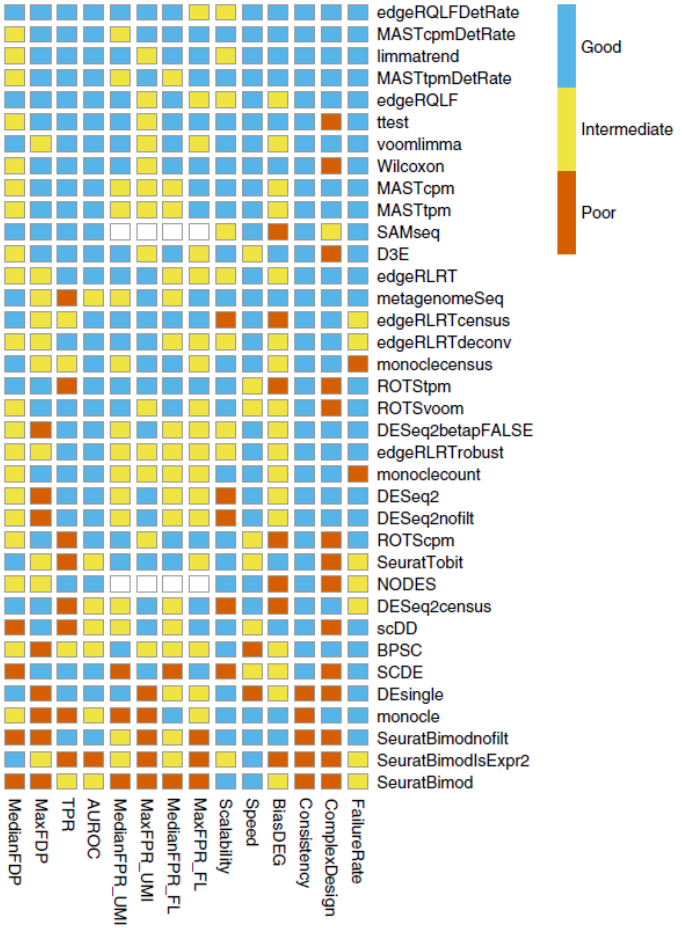
$$Y = \underbrace{\sum_{c=1}^{C} u_c V_c^T}_{\text{cell covariates}} + \underbrace{\sum_{a=1}^{A} p_a R_a^T}_{\text{annotated factors}} + \underbrace{\sum_{h=1}^{H} s_h Q_h^T}_{\text{unannotated factors}} + \Psi.$$

$$p(w_{g,k}|z_{g,k}) = \begin{cases} N(w_{g,k}|0,\ 1/\alpha_k) & \text{if } z_{g,k} = 1 \\ \delta_0(w_{g,k}) & \text{otherwise.} \end{cases}$$

$$p\left(I^n_{g,k}|z_{g,k}\right) = \begin{cases} \text{Bernoulli}\left(I^n_{g,k} = 1|1-\text{FPR}\right) & \text{if } z_{g,k} = 1 \\ \text{Bernoulli}\left(I^n_{g,k} = 1|\ \text{FNR}\right) & \text{otherwise} \end{cases}$$

Bias, robustness and scalability in single-cell differential expression analysis

Charlotte Soneson[1,2] & Mark D Robinson[1,2]



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Paper

RESEARCH ARTICLES

CANCER GENOMICS

Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq

Itay Tirosh,[1*] Benjamin Izar,[1,2,3*†‡] Sanjay M. Prakadan,[1,4,5,6] Marc H. Wadsworth II,[1,4,5,6] Daniel Treacy,[1] John J. Trombetta,[1] Asaf Rotem,[1,2,3] Christopher Rodman,[1] Christine Lian,[7] George Murphy,[7] Mohammad Fallahi-Sichani,[8] Ken Dutton-Regester,[1,2,9] Jia-Ren Lin,[10] Ofir Cohen,[1] Parin Shah,[2] Diana Lu,[1] Alex S. Genshaft,[1,4,5,6] Travis K. Hughes,[1,4,6,11] Carly G. K. Ziegler,[1,4,6,11] Samuel W. Kazer,[1,4,5,6] Aleth Gaillard,[1,4,5,6] Kellie E. Kolb,[1,4,5,6] Alexandra-Chloé Villani,[1] Cory M. Johannessen,[1] Aleksandr Y. Andreev,[1] Eliezer M. Van Allen,[1,2,3] Monica Bertagnolli,[12,13] Peter K. Sorger,[8,10,14] Ryan J. Sullivan,[15] Keith T. Flaherty,[15] Dennie T. Frederick,[15] Judit Jané-Valbuena,[1] Charles H. Yoon,[12,13†] Orit Rozenblatt-Rosen,[1†] Alex K. Shalek,[1,4,5,6,11,16†] Aviv Regev,[1,17,18†‡] Levi A. Garraway[1,2,3,14†‡]

Challenging, but interesting!

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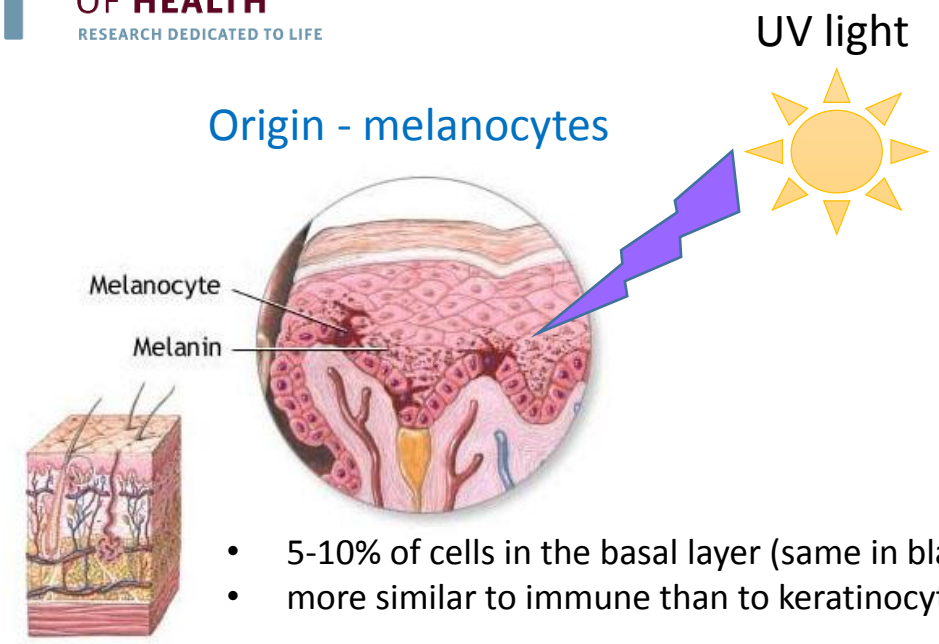
Melanoma

Origin - melanocytes

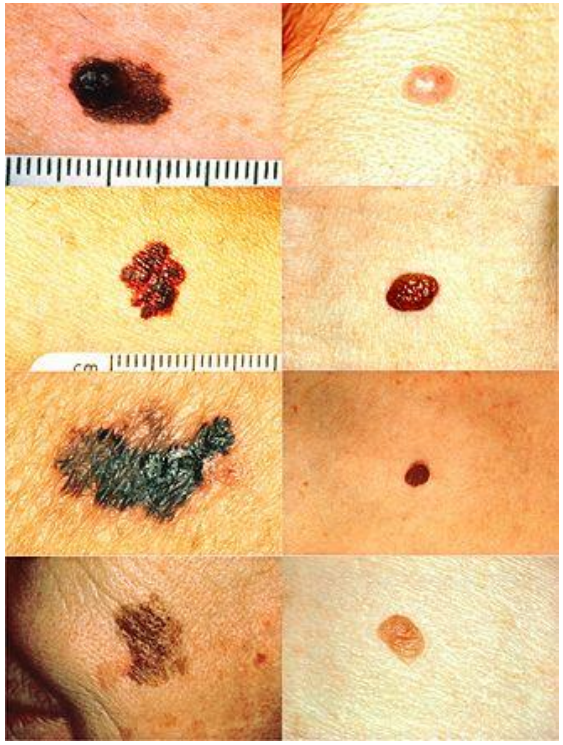


- 5-10% of cells in the basal layer (same in black/white)
- more similar to immune than to keratinocytes

Only 25% of tumors originate from nevi (moles)!

Reminder

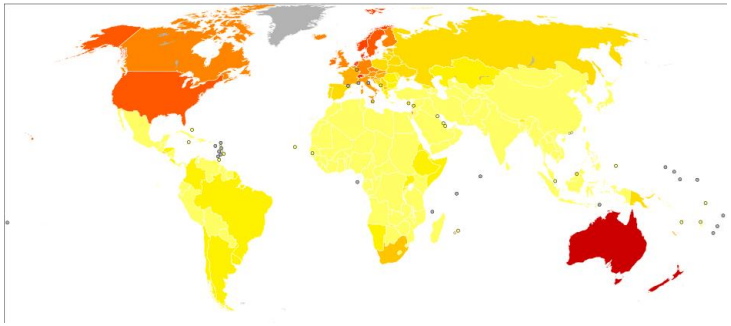
Tumor Normal

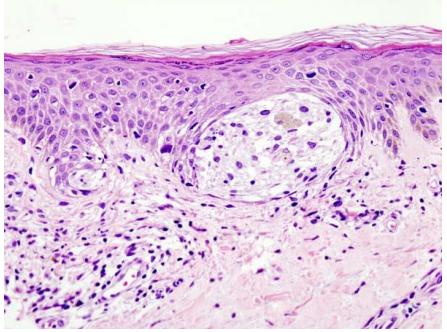


Properties:

- Rapid progression
- Early metastasis
- Highest mutation load
- Immune response +/-
- 5-year survival:
  - 98% when primary
  - 17% after spread

Age-standardized new cases per year





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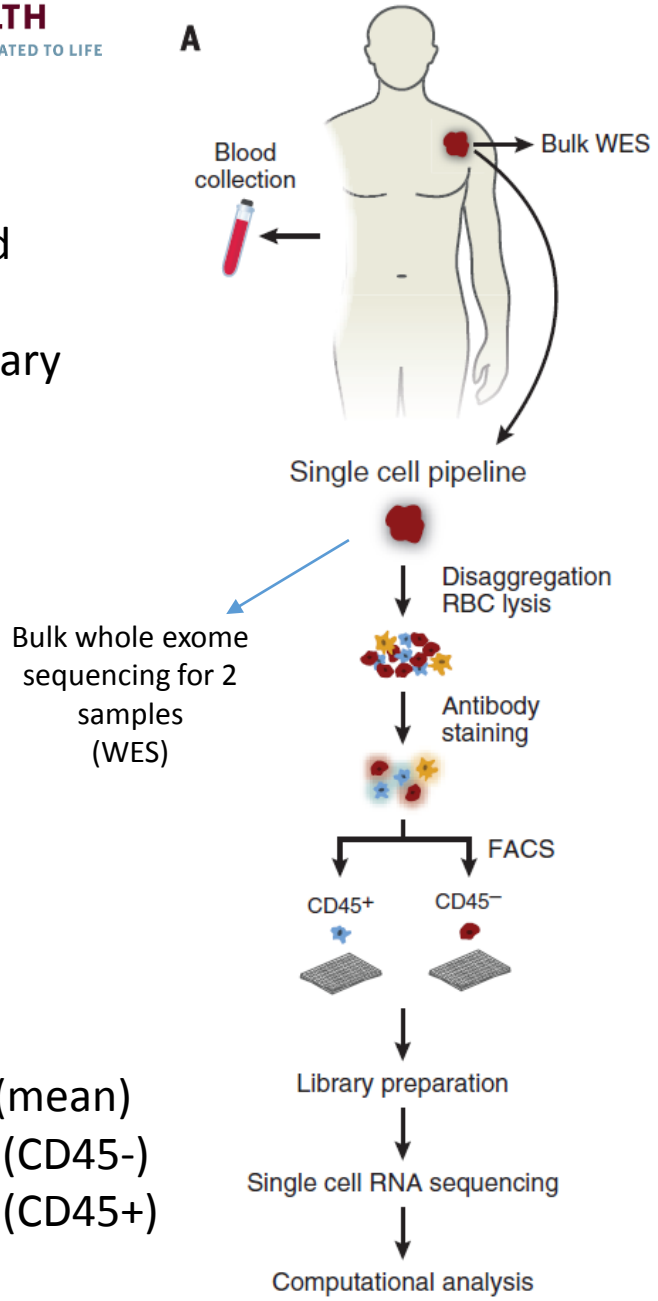
Experiment

Sample collection & sorting

19 tumors:
- 10 lymphoid
- 8 distant
- 1 acral primary



4645 cells:
- 150k reads (mean)
- 4659 genes (CD45-)
- 3438 genes (CD45+)

- Fresh tumors (45 min to cell suspension)
- Cell sorting: gated for viable cells
- Cell sorting separated viable cells to immune and non-immune by CD45

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Experiment

Pipeline for RNA-seq

- Illumina NextSeq 500 instrument using 30bp paired-end reads
- Bowtie for mapping on UCSC hg19
- Expression by RSEM using TPM: $E = \log_2(TPM/10 + 1)$
- Exclude cells with less than 1700 genes detected or expression of housekeeping genes < 3

$$\mathrm{TPM}_i = \frac{X_i}{\widetilde{l}_i} \cdot \left(\frac{1}{\sum_j \frac{X_j}{\widetilde{l}_j}} \right) \cdot 10^6$$

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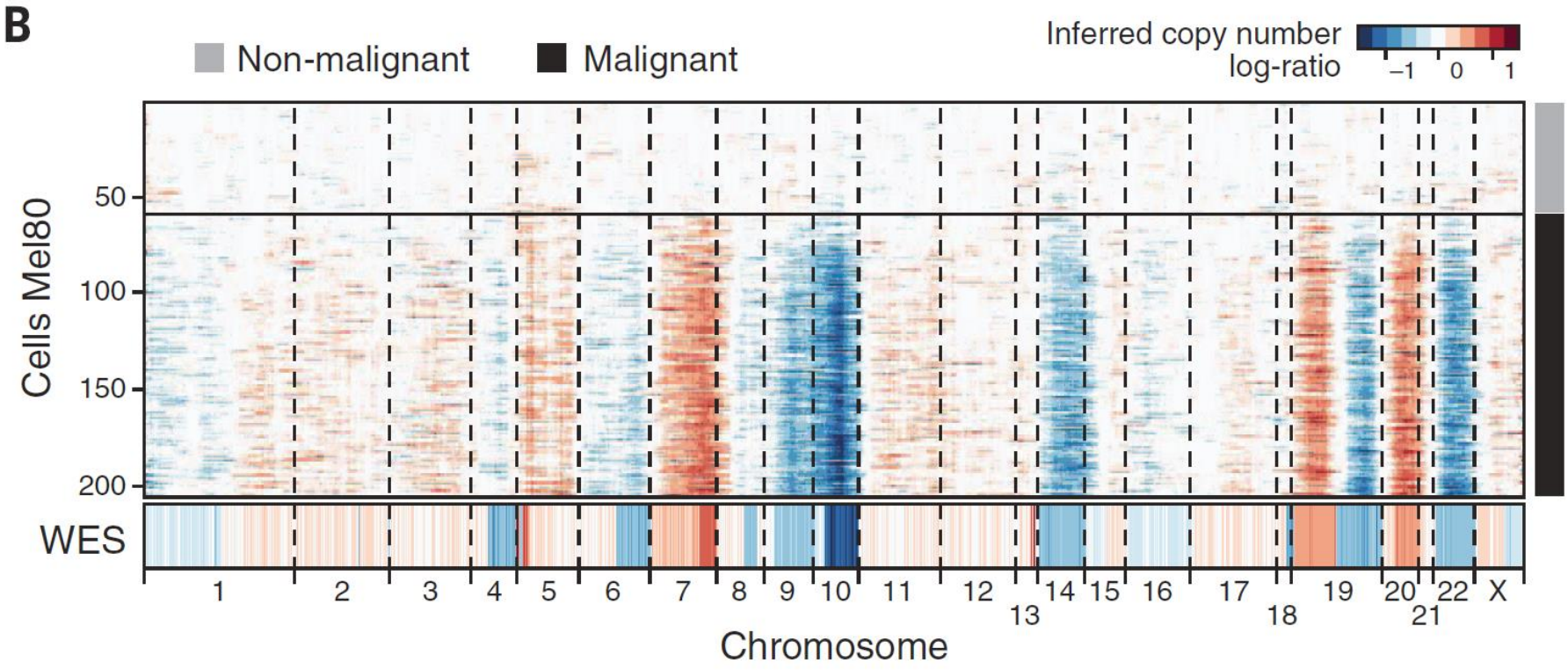
Cell Classification

Malignant / Normal

CNV were estimated from sequencing data: moving average with limited standardized expression b/w -3, 3



SNV allowed separating malignant from non-malignant non-immune cells (CAF)

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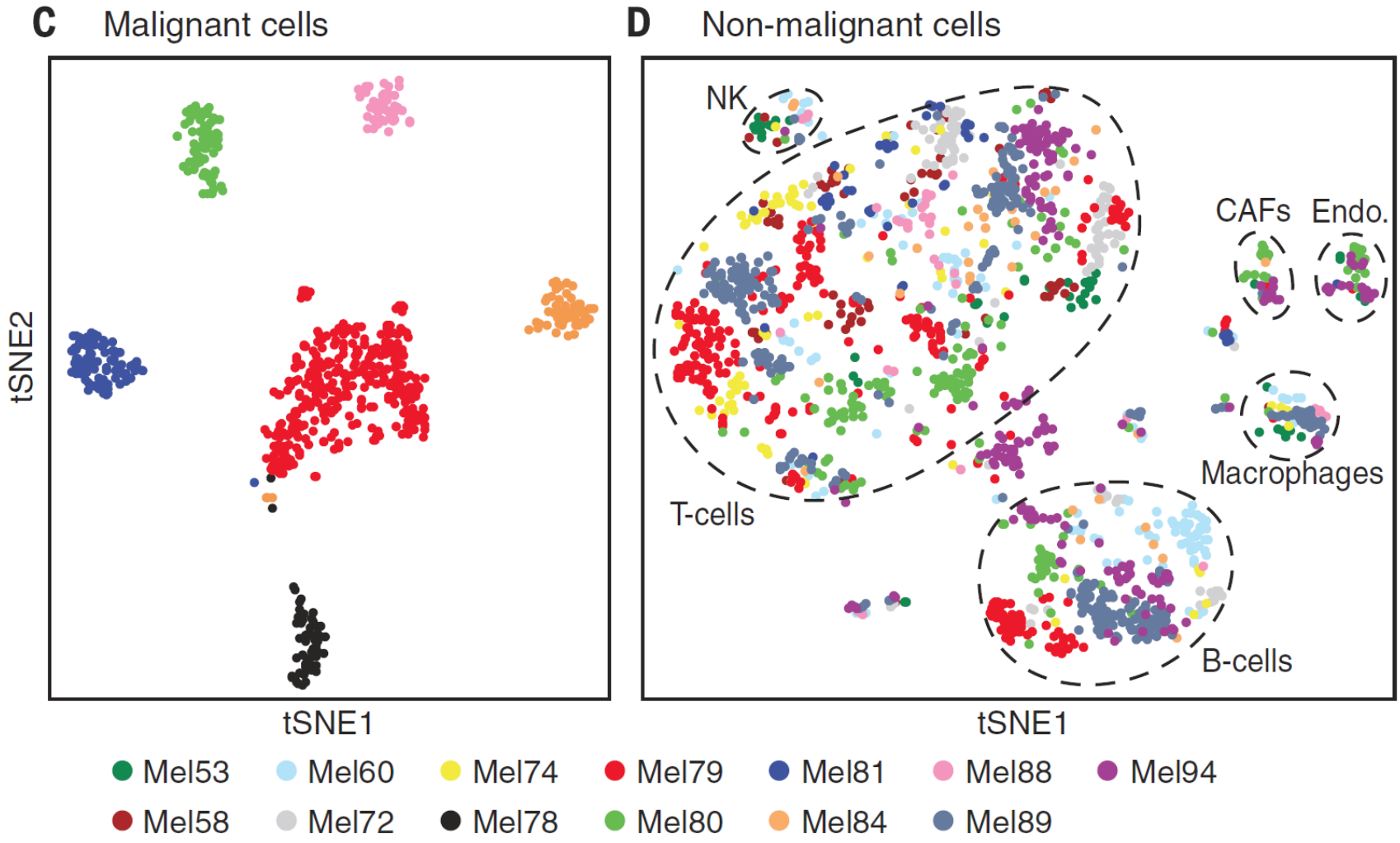
Cell Classification

Inter-tumor heterogeneity



Tumor cells – patient specific. Non-malignant – similar b/w patients

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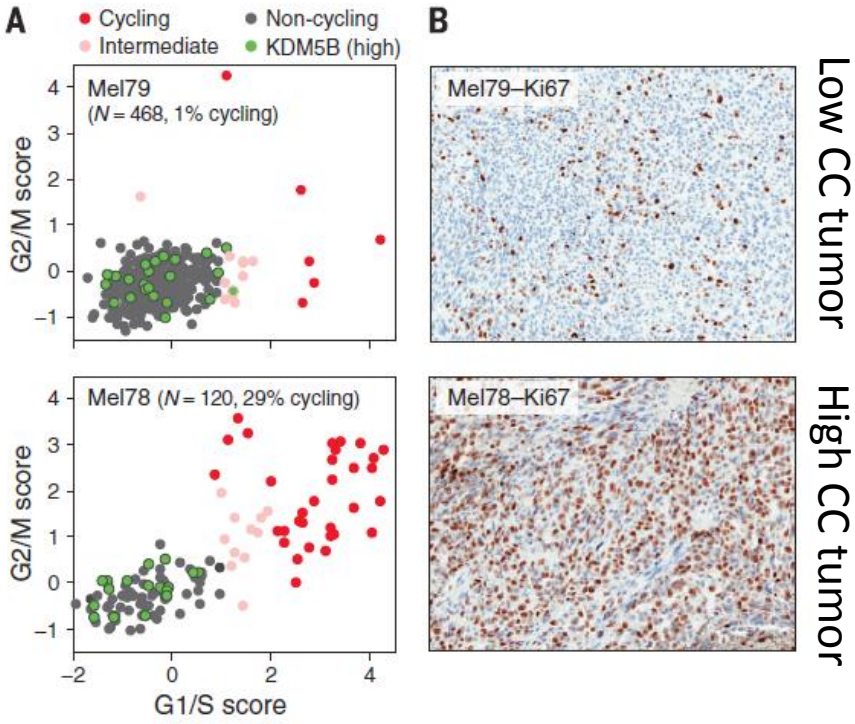
Malignant Cells

Heterogeneity in cell cycle

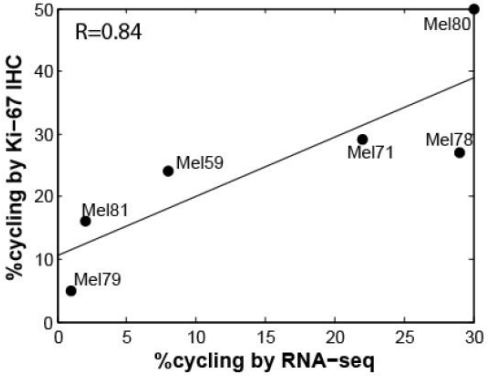
Data → PCA → Interpretation

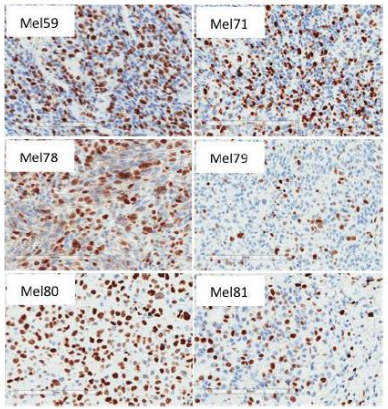
- PC1 was linked to number of reads per sample
- PC2 was linked to cell cycle
- PC3,PC6 segregation treatment-naïve tumor from rest



Gene signature taken from literature resulted in G1/S and G2/M scores

Cells in cell cycle among tumors





Cell cycle status of cells can be identified. Tumors differ in the proliferation speed

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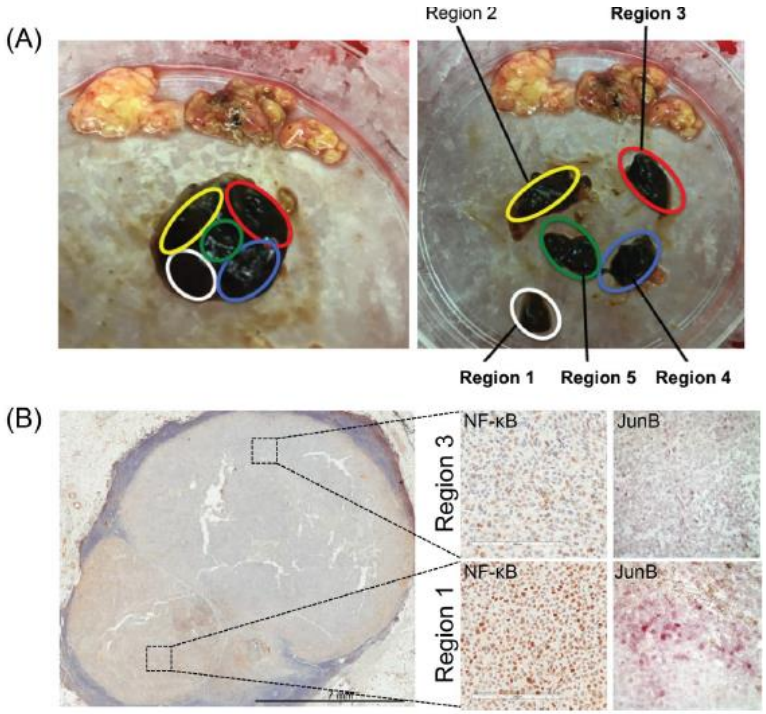
Malignant Cells

Heterogeneity in location

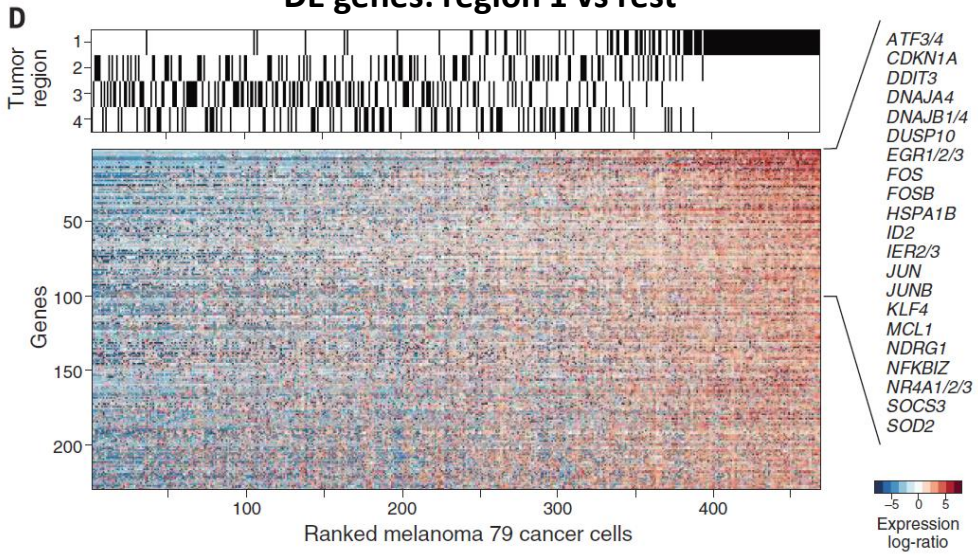
Treatment-naïve tumor 79



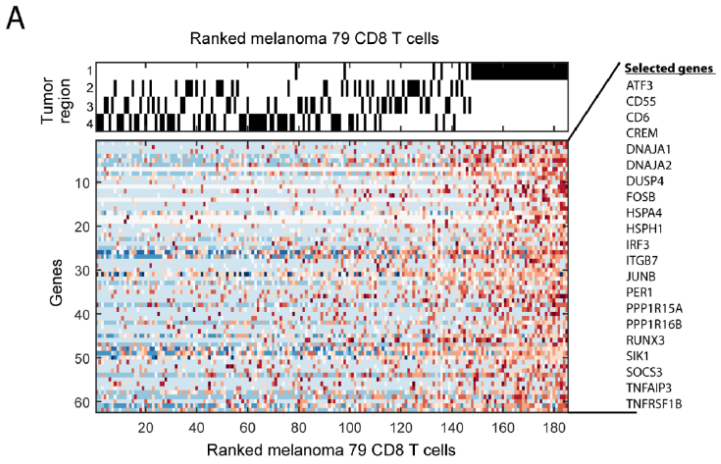
These genes one are also generally coexpressed across melanoma tumors profiled in bulk in TCGA

Cancer cells from different locations express different genes. Interestingly – similar genes observed in immune cells

DE genes: region 1 vs rest



Similar in immune cells:



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Malignant Cells

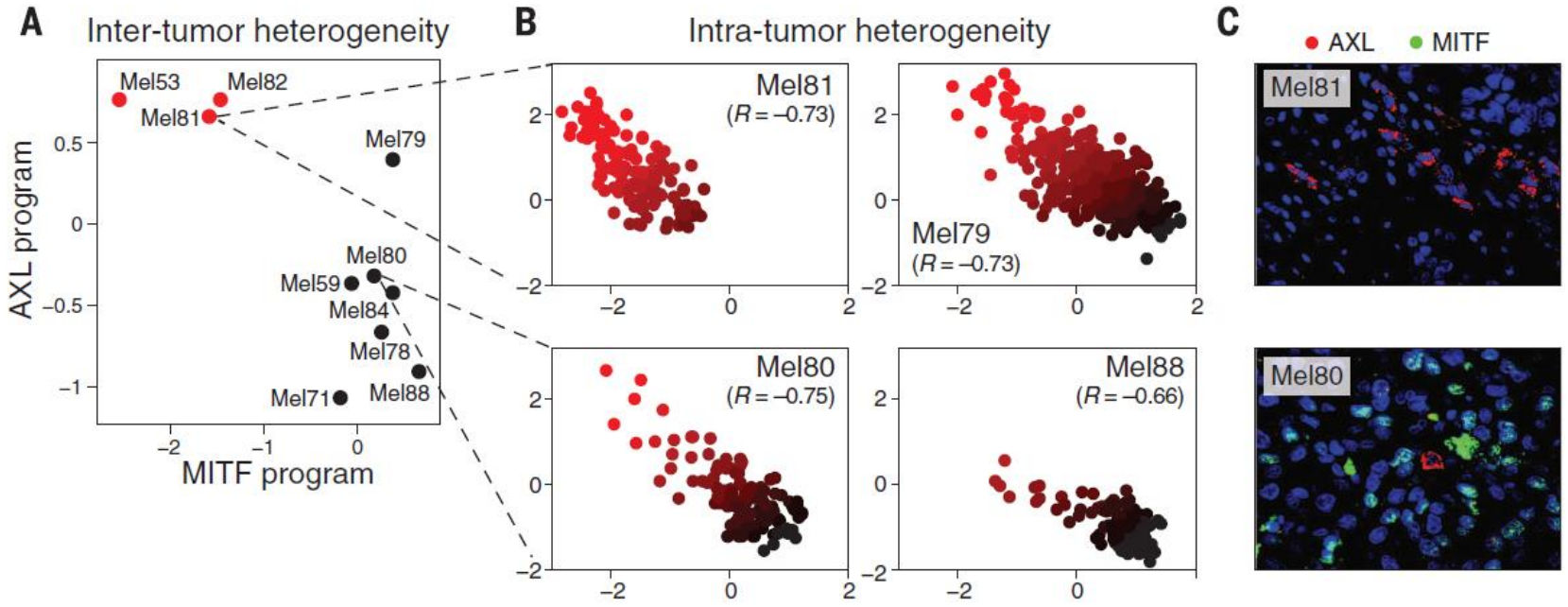
Dormant drug-resistant subpopulation

- PC4-PC5 are highly correlated with MITF (master melanocyte tr. regulator and oncogene)

Hypothesis: there **<u>may be</u>** dormant drug-resistant cells.

MITF ↑↑ TYR, PMEL, MLANA

MITF ↑↓ AX L, NGFR: resistance to RAF/MEK inhibition that blocks proliferation, and activate apoptosis



2 programs. Intratumor heterogeneity was observed, suggesting presence of “dormant” cells

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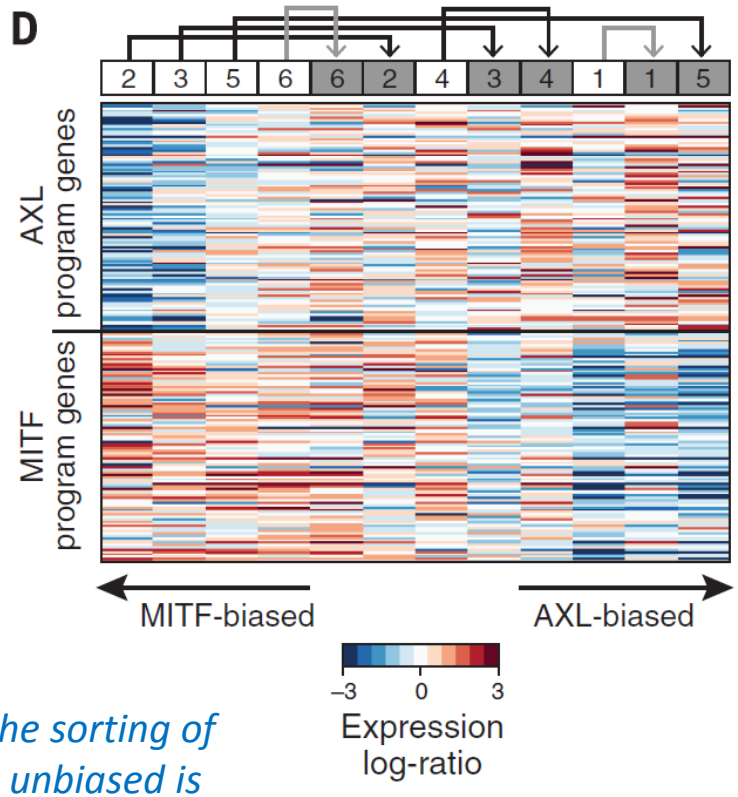
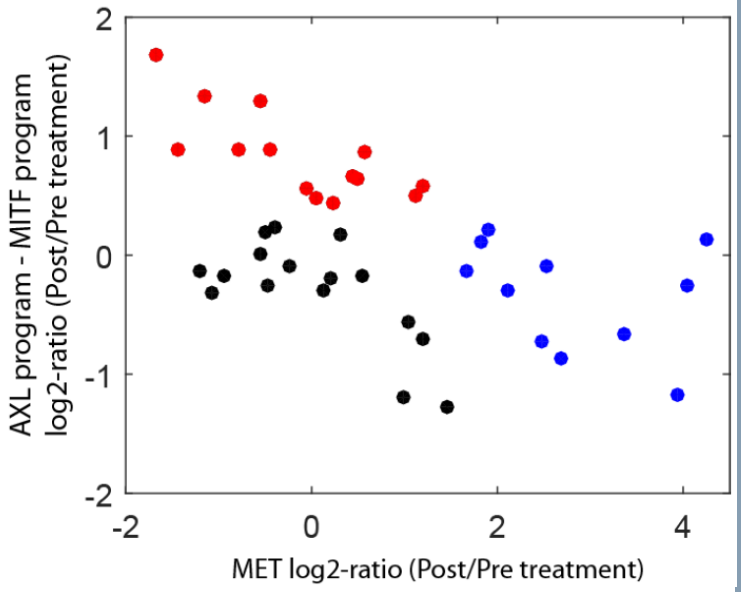
Malignant Cells

Dormant drug-resistant subpopulation: test

6 paired BRAF-mut melanoma biopsies before and after RAF/MEK inhibition: vemurafenib(1) and dabrafenib + trametinib(5).



Hugo et al (2015)Cell



Whether the sorting of samples is unbiased is questionable

- AXL-vs-MITF
- MET Proto-Oncogene, Receptor Tyrosine Kinase (Mesenchymal-epithelial transition)

After treatment, tumors become more “AXL-biased”. AXL-program and MET mutually exclusive

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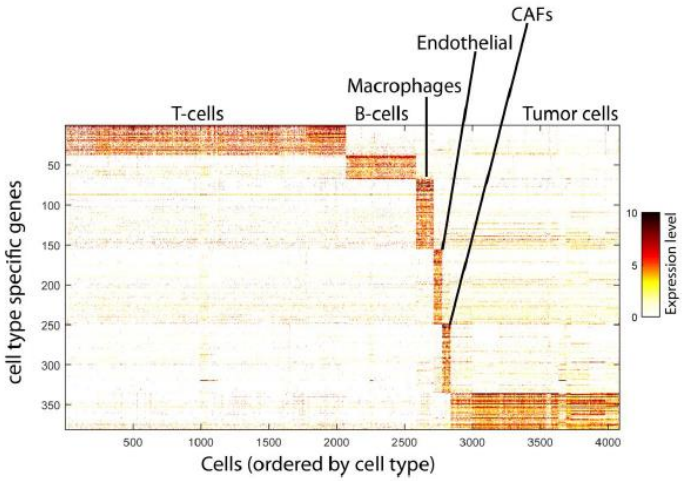
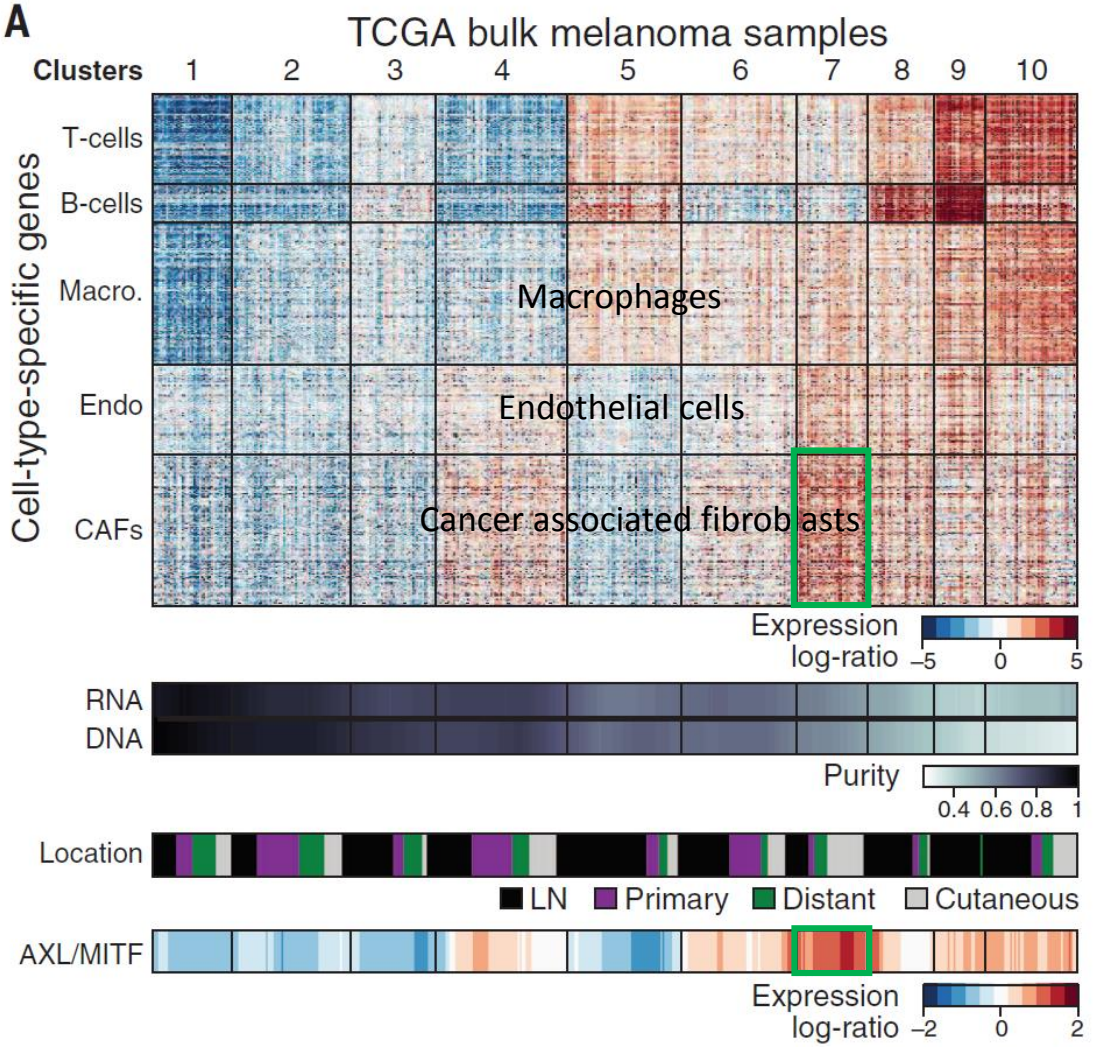
Non-Malignant Cells

Types and environment

Samples → tSNE → Classes → Mean profiles → Composition of public TCGA





- Purity is correlated to expression of NM-cell specific genes
- CAF abundance may be linked to AXL-high over the MITF-high
- Thus it is possible that specific tumor-CAF interactions may shape the melanoma cell transcriptome

Why not vice versa?

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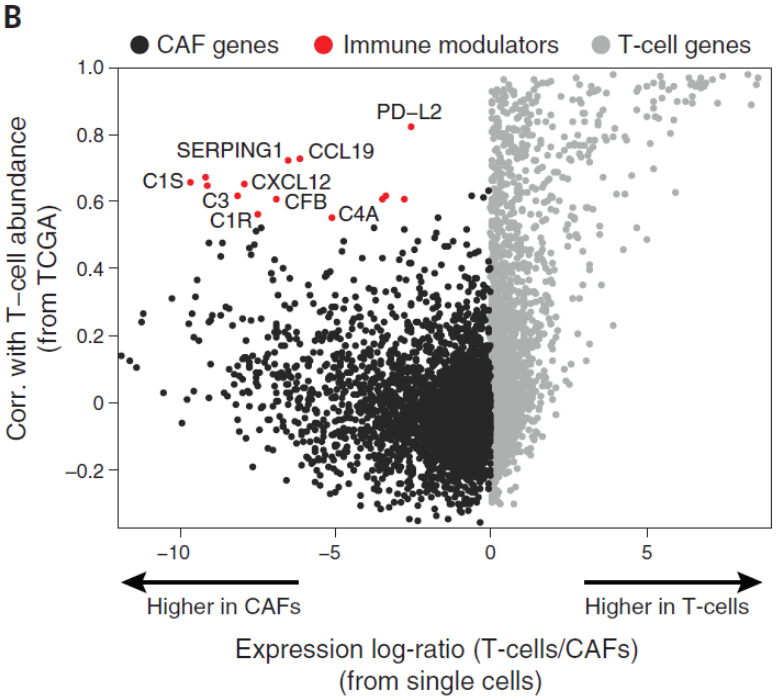
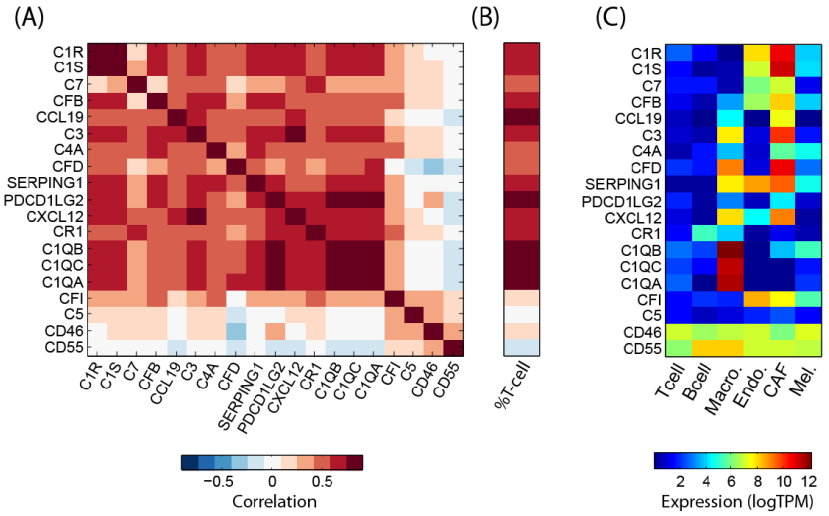
Non-Malignant Cells

Genes expressed by CAF are associated with T-cell abundance



CCL19,CXCL12 - chemotactic (movement stimuli)
PD-L2 – immune modulating
C1S,C1R,C3,C4A,CFB,SERPING1 – complement

Complements the ability of antibodies and phagocytic cells to clear pathogens from an organism



Some genes, up-regulated in CAF, are also linked to higher percentage of T-cells – suggested to be an effect of stroma on immune cells.

A bit strange plot. Why sharp switch?
Again – why genes are “calling for T-cells not vice versa – T-cells induce genes?

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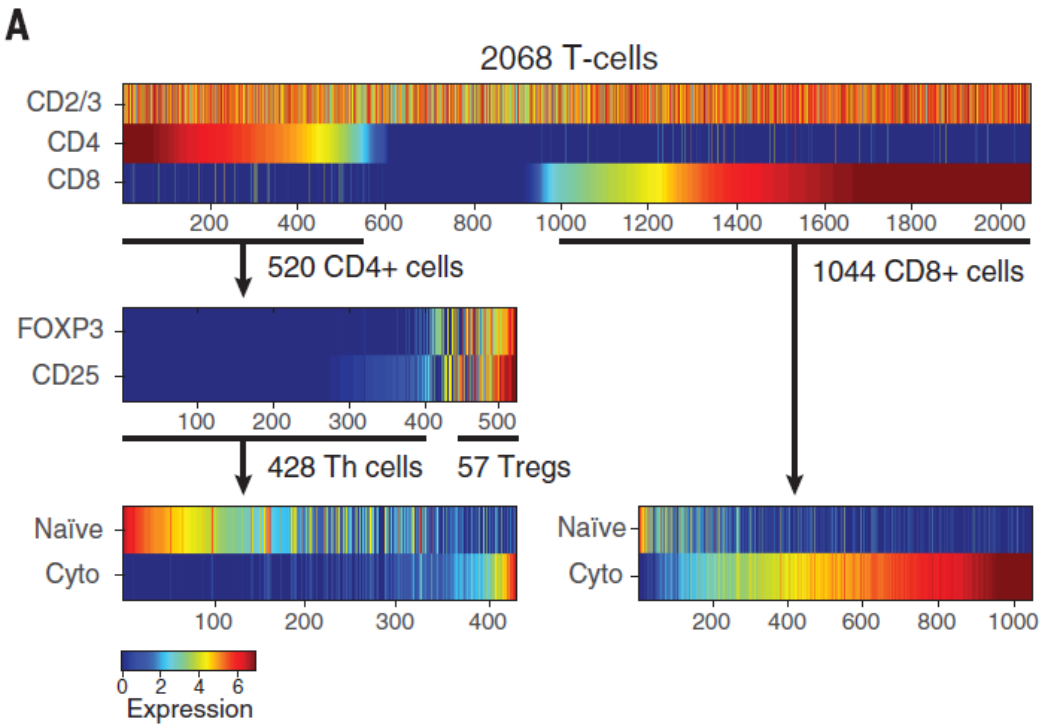
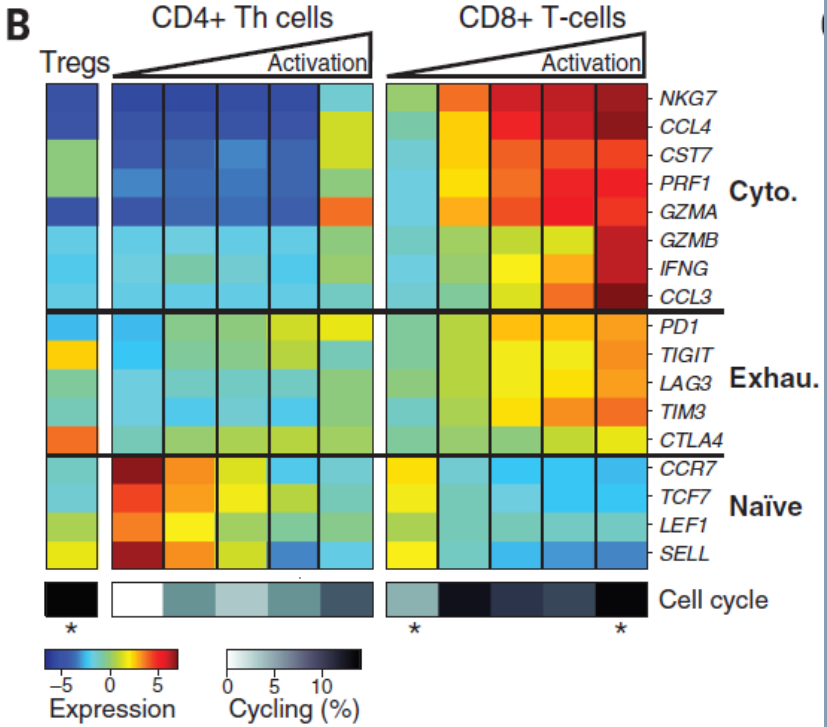
Non-Malignant Cells

T-cells: classification and exhaustion status





T cells can be classified and their exhaustion status measured

helper T cells (CD4+)
cytotoxic T cells (CD8+)
regulatory T cells (CD4+, CD25+, FOXP3+)

Signature of exhaustion → Stratify cells → DEA → Enhanced signature

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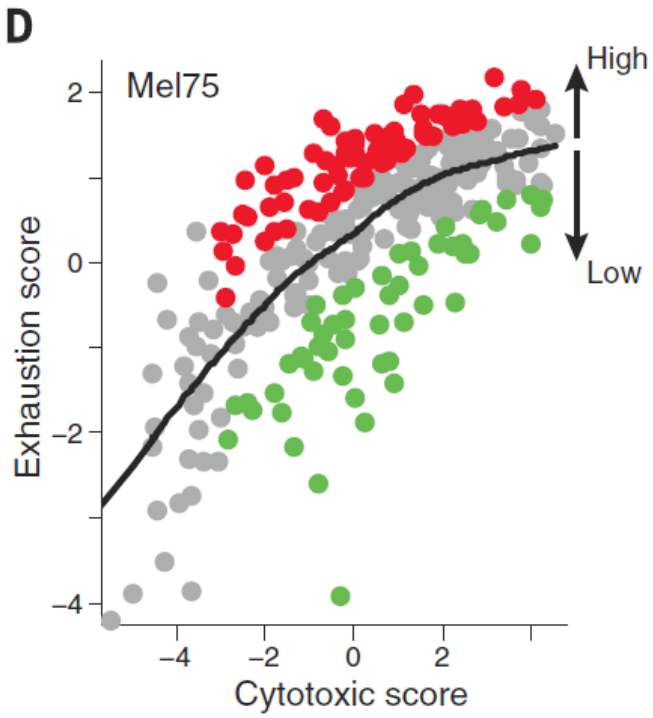
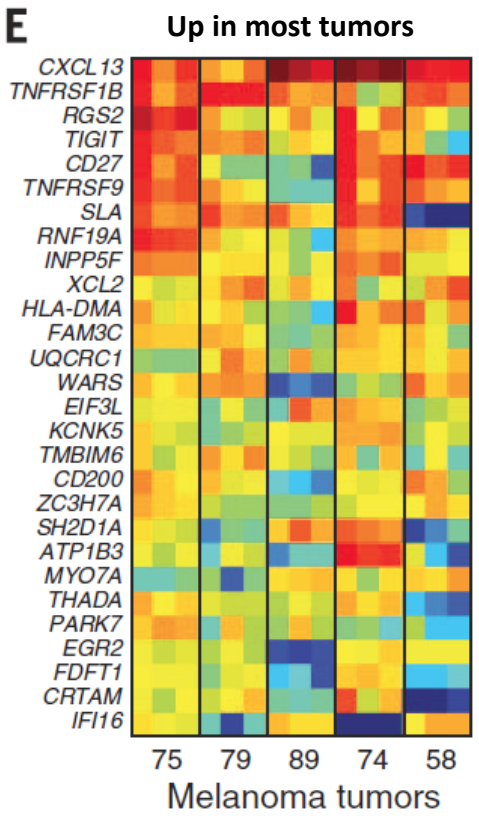
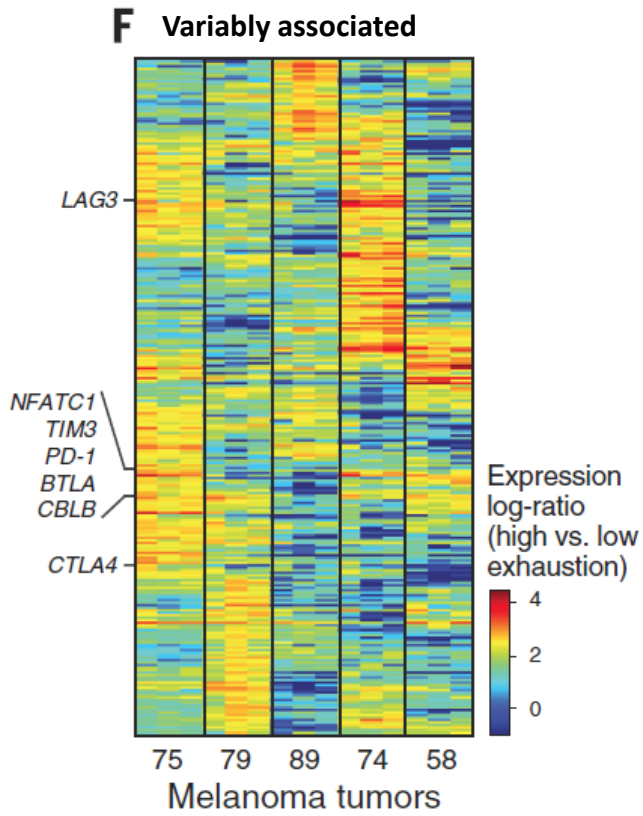
Non-Malignant Cells

T-cells exhaustion status

Signature of exhaustion → Stratify cells → DEA → Enhanced signature







Core exhaustion signature was determined across various tumors

Only 5 tumors considered, so “across various tumors” = “across 5 tumors”…

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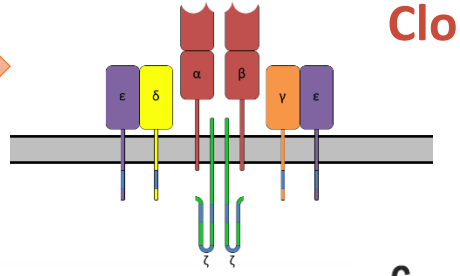
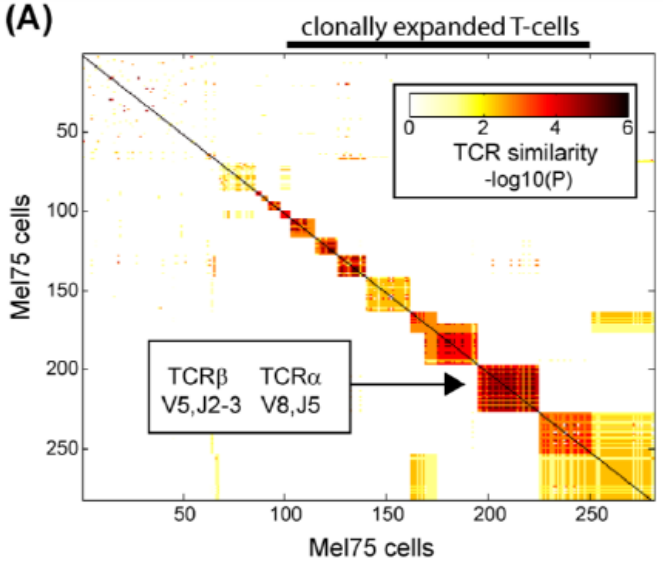
Non-Malignant Cells

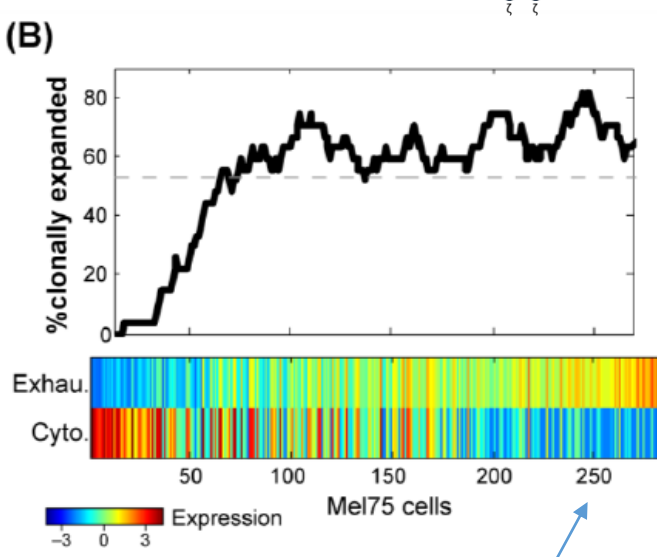
RNA-seq → reads from TCR region → Similarity

T cell receptor

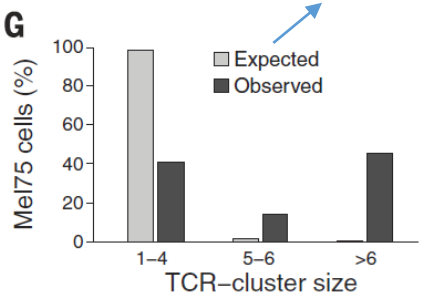


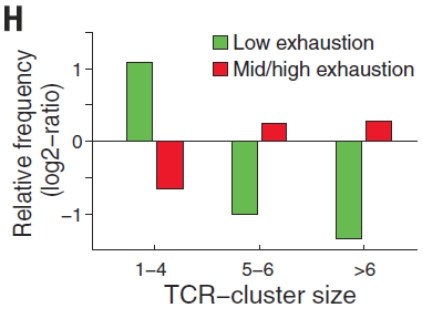
Clonal expansion

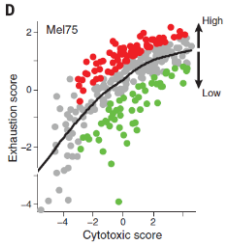




Expected from shuffling TCR across cells







Looks like a negative correlation, while positive correlation reported before

Expanded clones are depleted of non-exhausted cells and enriched for exhausted cells.
scRNA-seq can characterize functionality of variable T cell populations.

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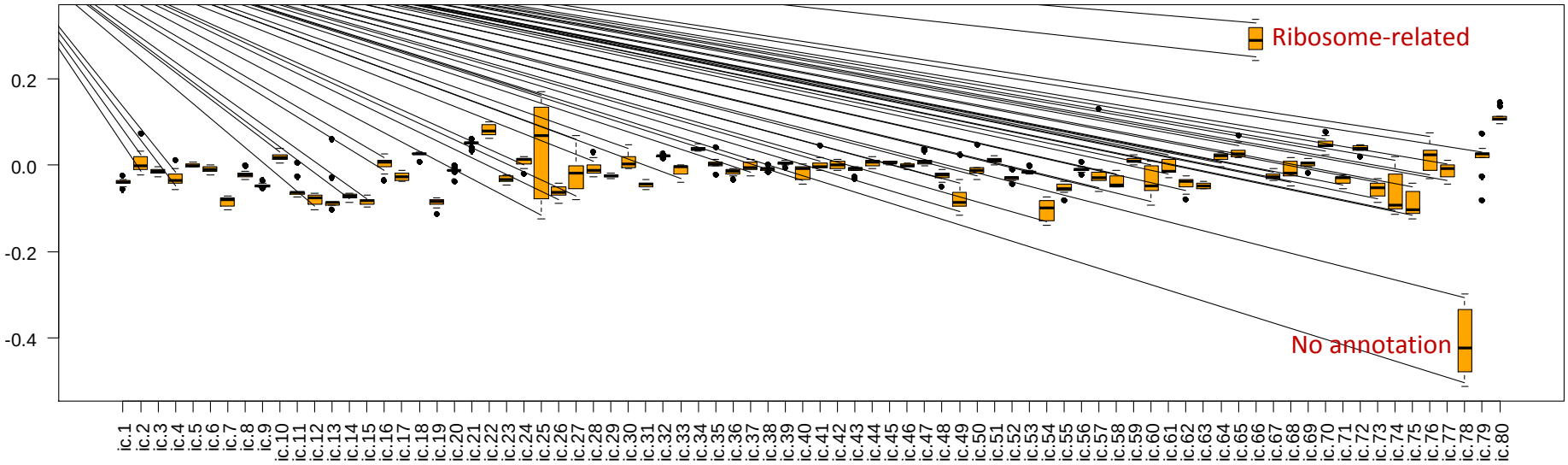
Impact on Our Work

MelanomICA

SKCM RNA-seq → ICA → Metagenes S / Metasamples M → R ← 9 averaged Tirosh profiles

Correlating 80 metagenes with Tirosh profiles



Highest correlation with ribosome related component and a technical component

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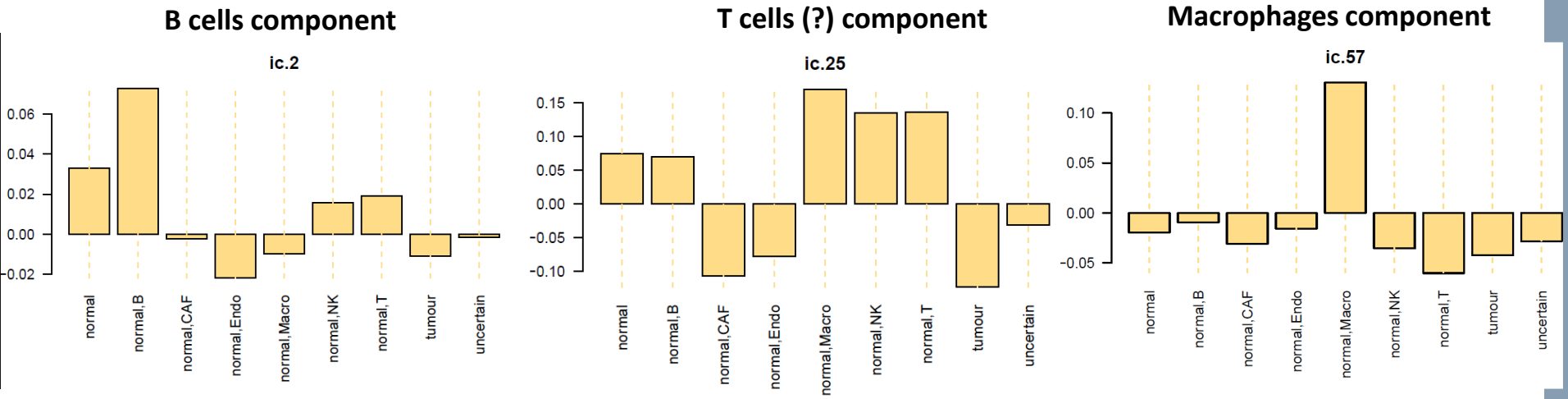
Impact on Our Work

MelanomICA

SKCM RNA-seq → ICA → Metagenes S / Metasamples M → R ← 9 averaged Tirosh profiles

Identified metagenes vs Tirosh profiles



Top correlations were reasonable. But with low absolute value (perhaps can be improved)

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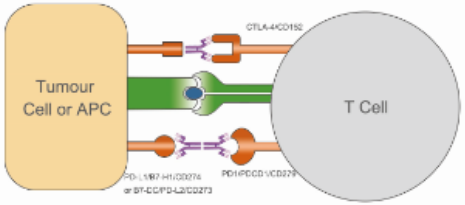
Summary

And Comments

1. Inter and intra-tumor heterogeneity is uncovered
   - tumors are different, stromal cells similar
   - spatial heterogeneity within the same tumor
   - presence of dormant resistant cells

2. Stromal CAF cells can influence proportion of T cells. Tumor cell composition is important!

3. Potential biomarkers are suggested for exhausted and cytotoxic T cells – may help selecting patients for immune checkpoint blockade.



Authors used PCA and were quite lucky to see main effects. ICA would be better.
Some ideas can be used for our work.
In general – I liked the paper. But some statements seems to be quite voluntaristic, esp. with tests used (t-test, permutation test, +/- adjustment, etc). Seems like selecting evidence that fit the theory.

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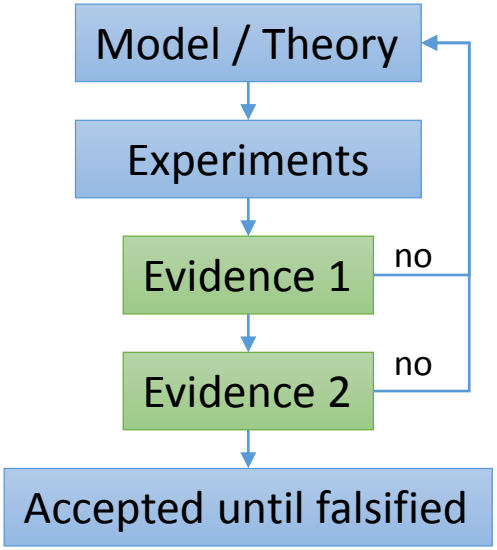
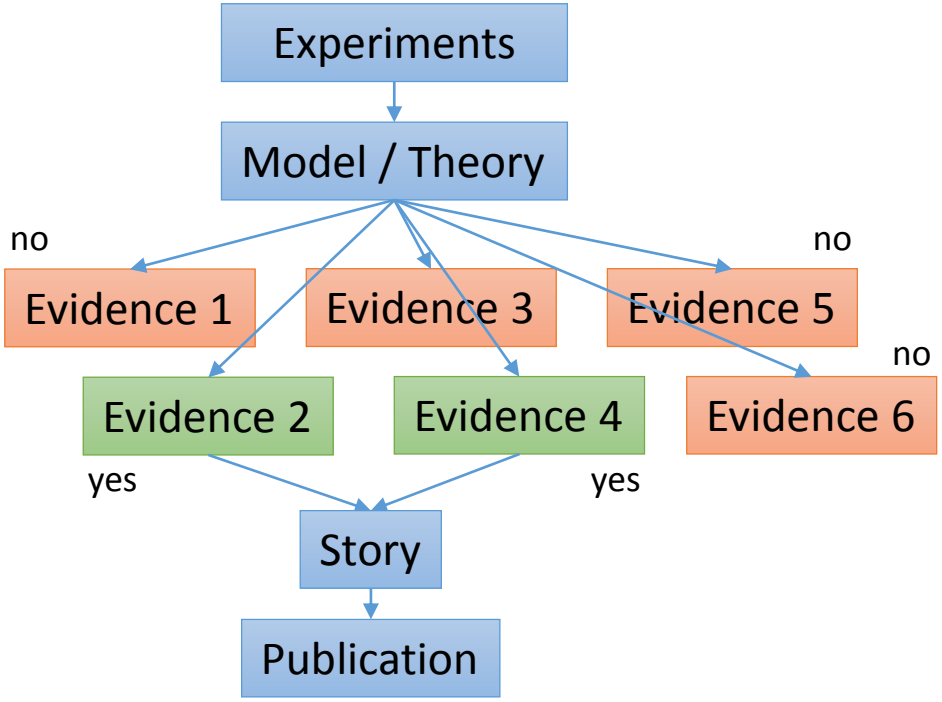
Methods in Research

Classical vs "Modern" Scheme





Only supporting evidences are published

☺ ☹

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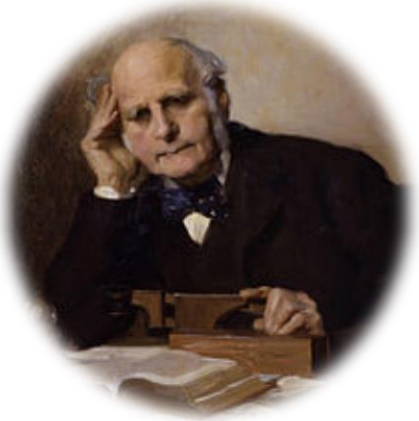
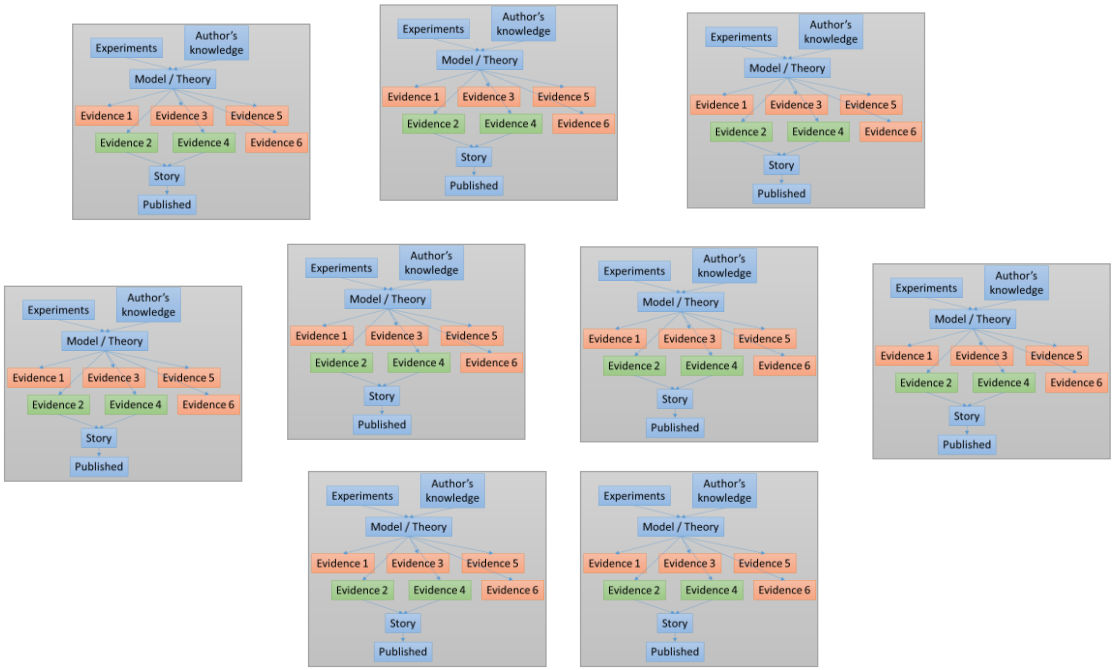
Methods in Research

Some Optimism in Wisdom of the Crowd



Sir Francis Galton



- Irreproducible results are less cited than reproducible
- On average, like in Random Forest, we should go towards the **objective** ☺