Prediction of Immunotherapy Treatment Outcome

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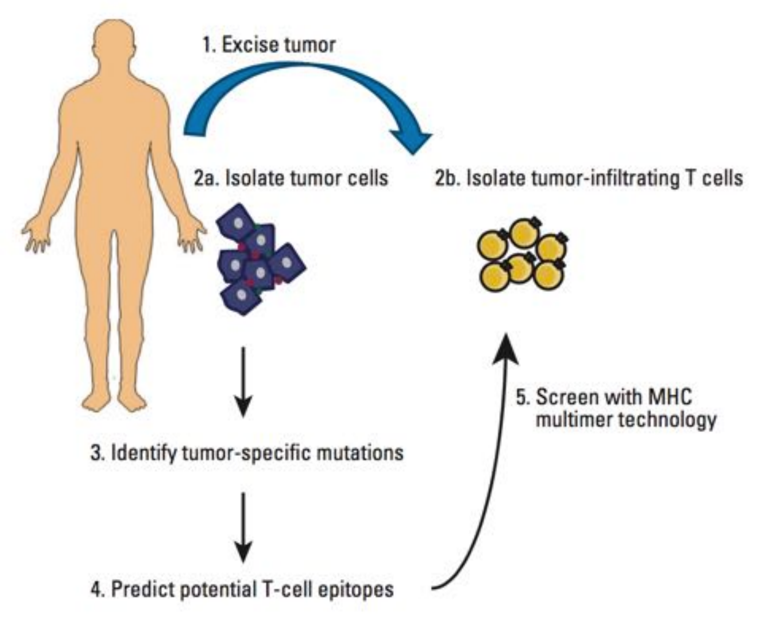
Today's topics

1. Prediction of immune targets in tumor cells
2. Characterization of immune phenotype
3. Correlating phenotype with clinical outcome
4. Integrating targets/phenotype

Clinical application

Sequence-driven identification of neoepitopes in metastatic melanoma



Predicted 448 potential HLA binders

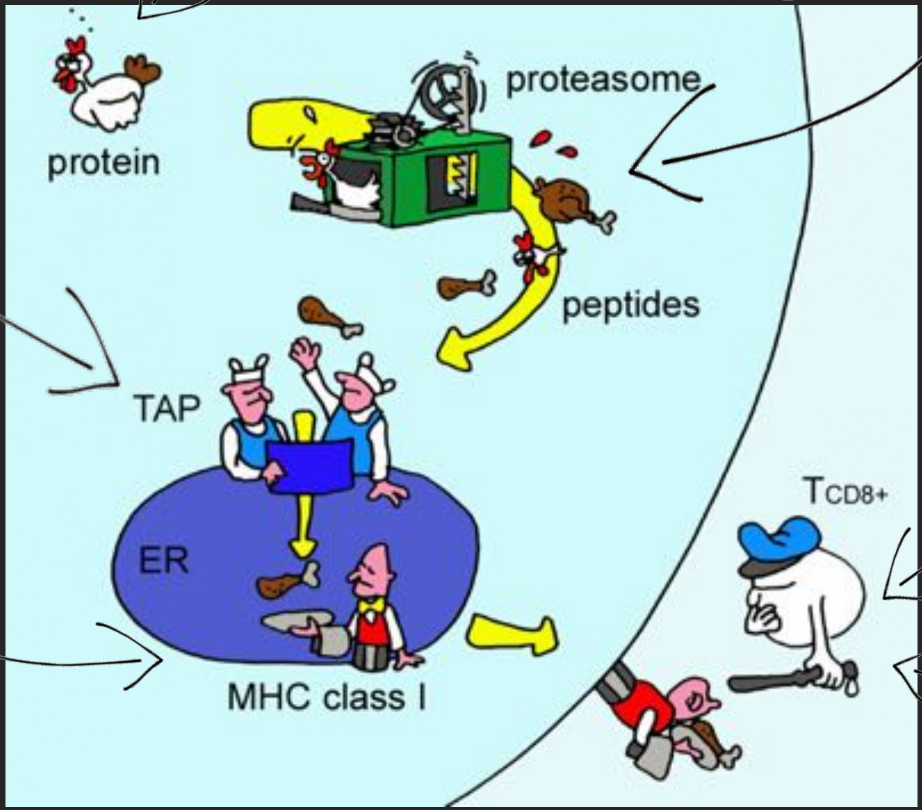
Minor T cell response against **1 antigen**

Dominant T cell response against **1 antigen**

Why only two epitopes??

van Rooij et al. JCO 2013

What predictions did they include in their model?



Estimate of protein expression

Prediction of proteasomal cleavage

(Prediction of TAP transport)

Prediction of MHC binding

Prediction of pMHC:TCR interaction

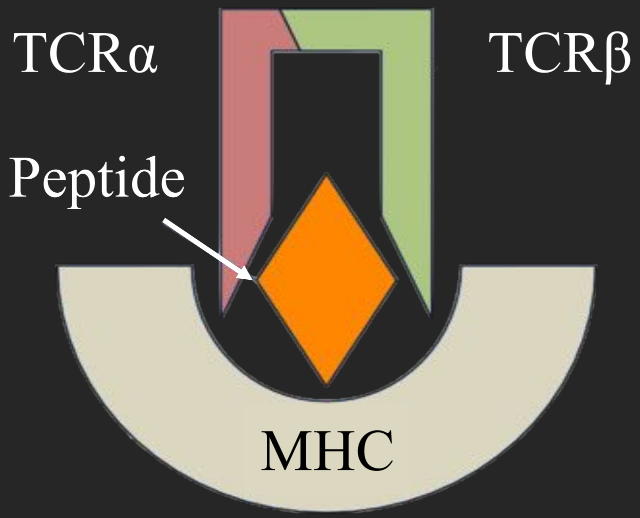
Regulation of T cell response

TCR-p ction

Essentially just one more rate-
receptor-ligand interaction

Response is dependent on TCR re e,
which is highly diverse due to soma hyper
mutation (10^8 – 10^15)

Unpredictable from DNA
central and peripheral sele



Prediction of response

Biomarkers for immune competence / therapy response

* Mutational load of the tumor

* Quantity, ratio, location, and anergy of CD8+ T cells and CD4+ T cells

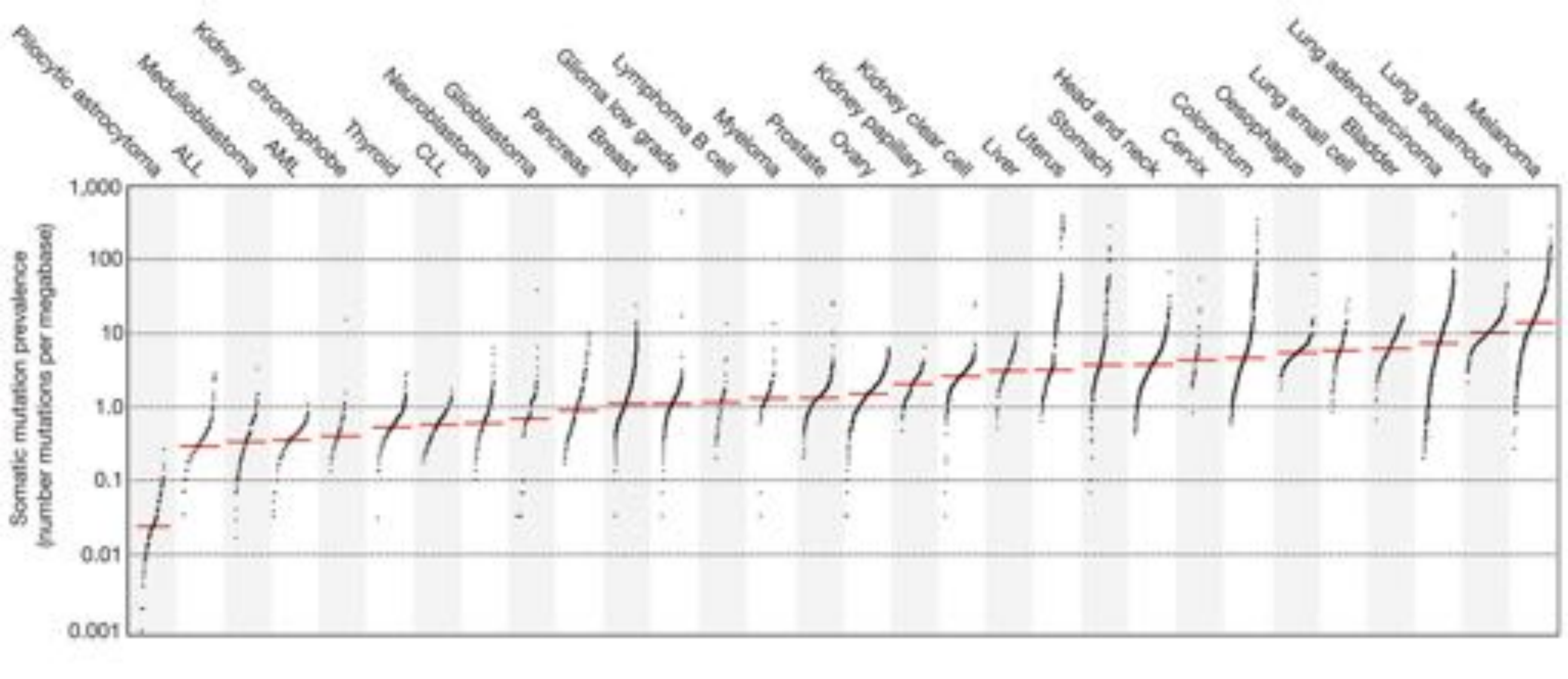
* Molecular markers associated with effector inhibitory mechanisms (ARG1, NOS2, IDO1, IDO2, NOX2, PD-L1, PD-L2, IL-10)

* Quantity of suppressive immune subsets (Myeloid-derived suppressor cells, Tregs, etc.)

Chang et al. Cancer Immunol Immunoth 2013
Gnjatic et al. J Immunoth Cancer 2017

Mutational load of the tumor

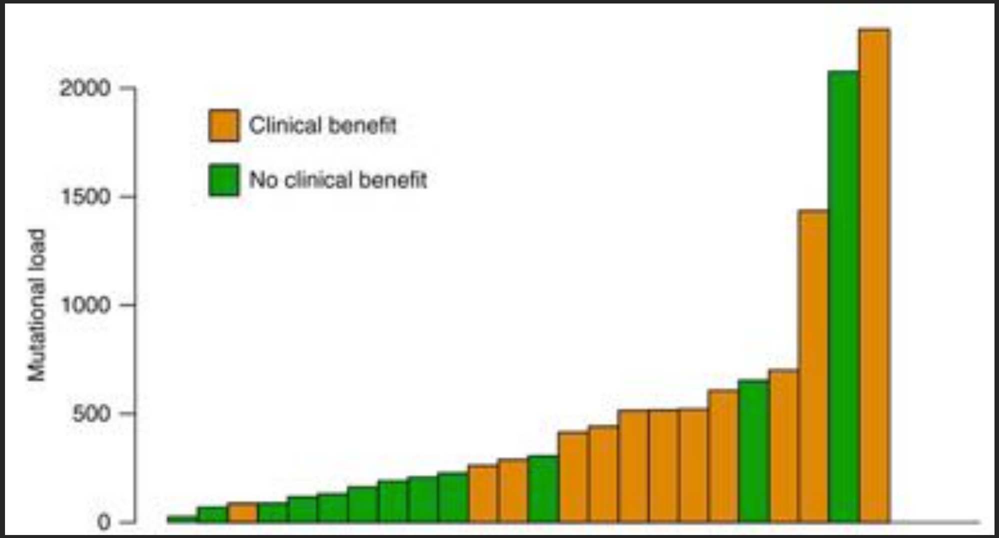
Varies among cancers, and among individual tumors



Alexandrov et al. Nat. 2013

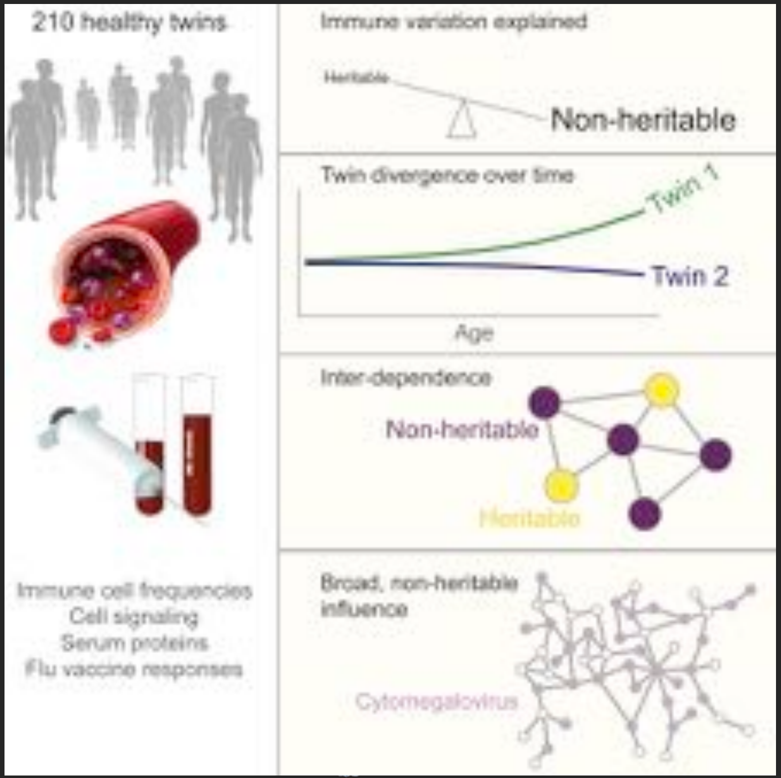
Mutational load of the tumor

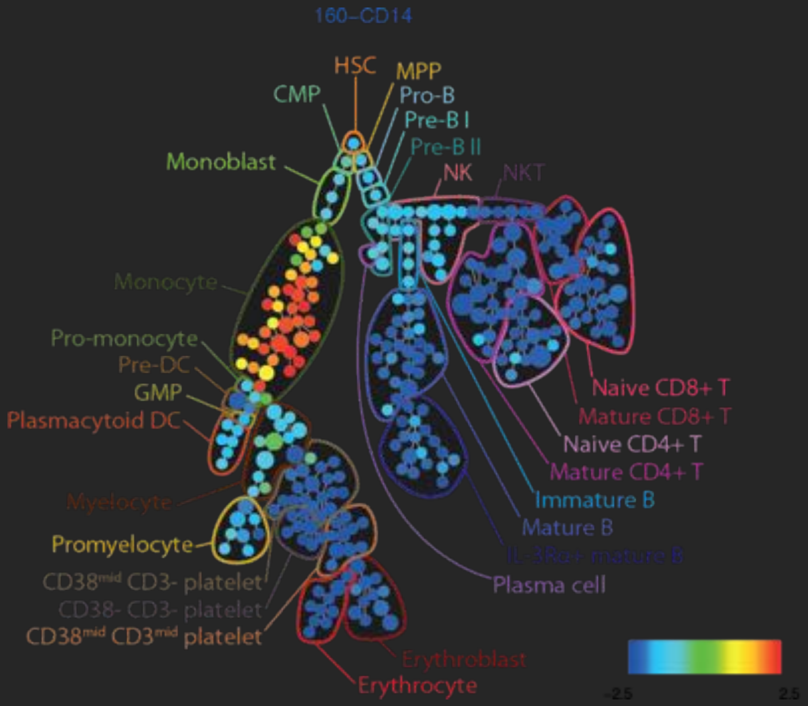
Mutational load predict clinical benefit of adoptive T cell therapy in melanoma



Lauss M, Donia M, et al. Nat. Comm. 2017

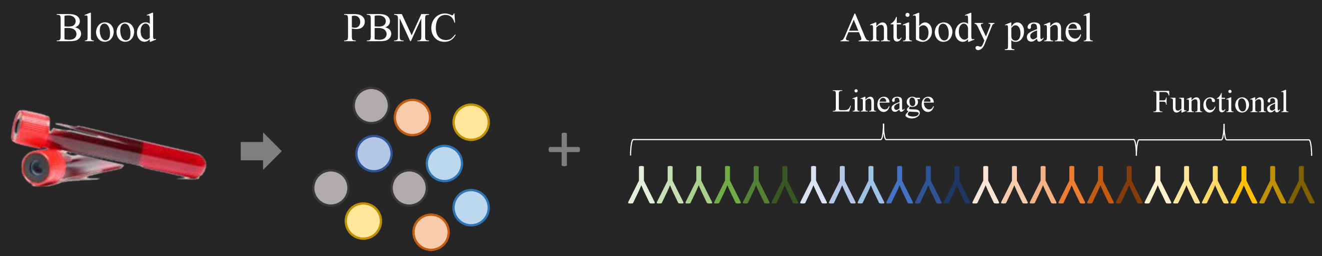
The tumor is only half of the system





Brodin et al. Cell 2015

Profiling the immune system using single cell cytometry



Blood

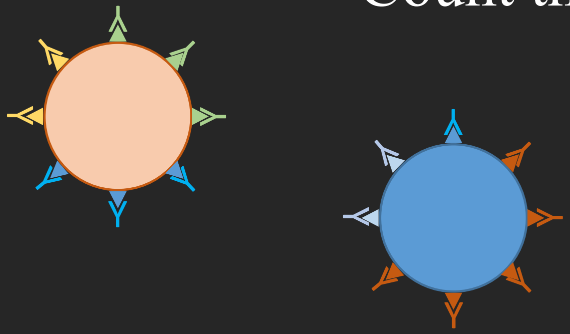
PBMC

Antibody panel

Lineage

Functional

Count the antibodies!



Profiling the immune system using single cell cytometry

Flow cytometry

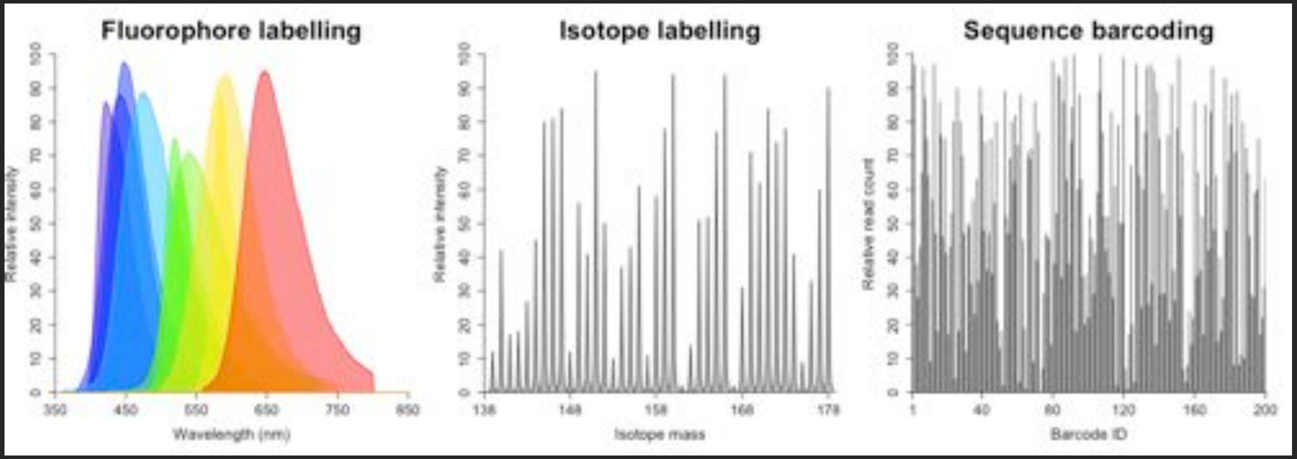
Antibodies labelled with fluorescent molecules

Mass cytometry

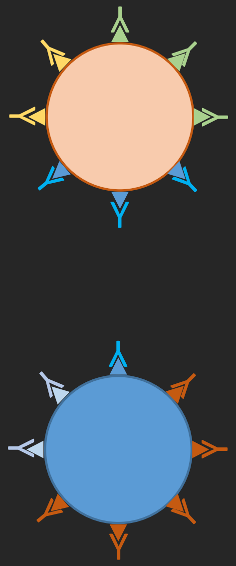
Antibodies labelled with metal isotopes

Sequence-based cytometry

Antibodies labelled with sequence barcodes

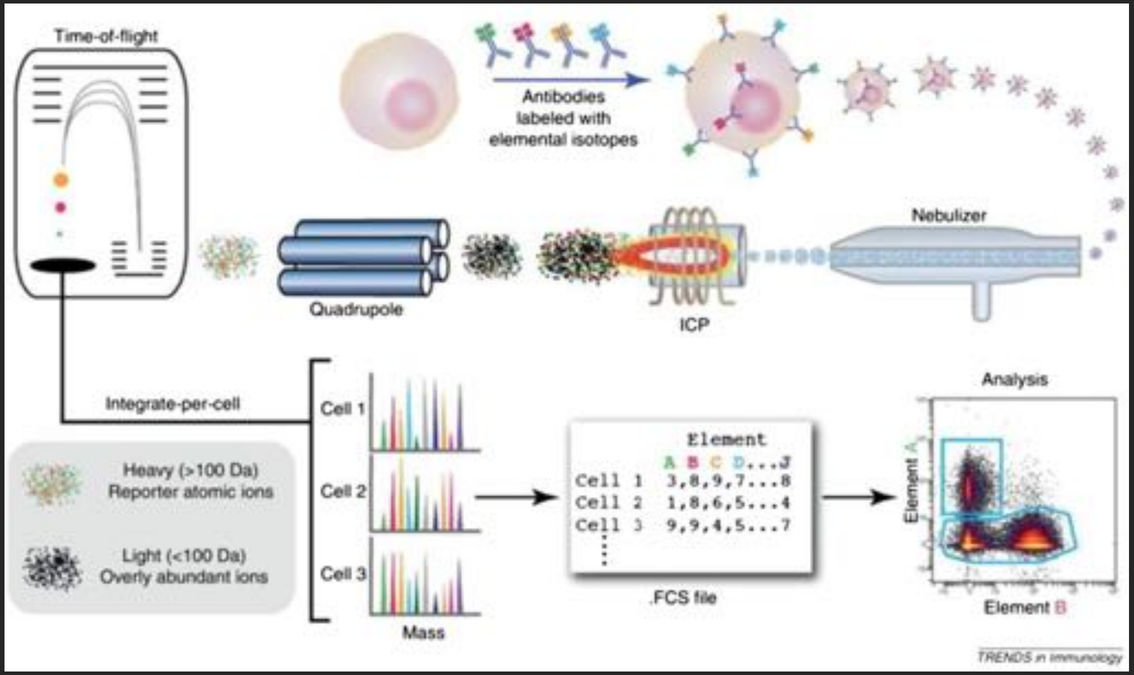


Profiling the immune system using single cell cytometry



	Protein 1	Protein 2	Protein 3	Protein 4	Protein 5
Cell 1	2	3	3	0	0
Cell 2	0	0	1	5	2

Mass cytometry

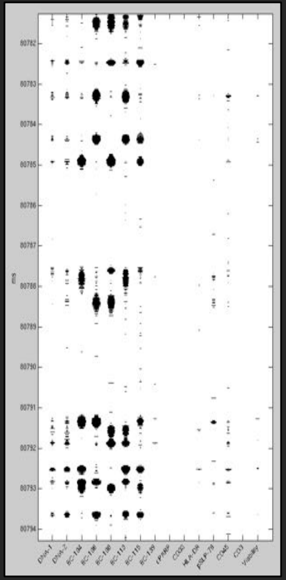


Bendall et al., Trends Immunol. 2013

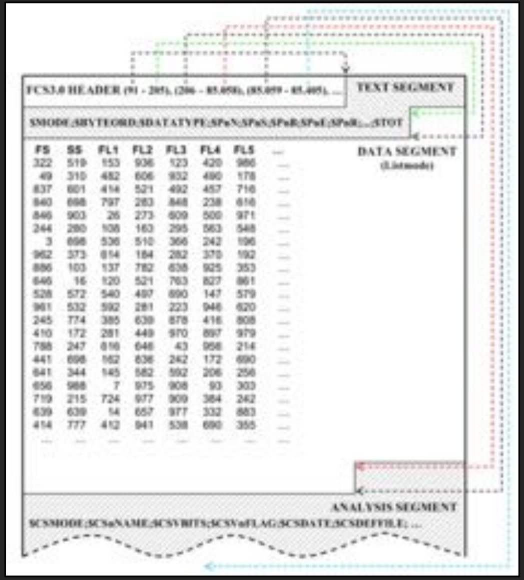
Mass cytometry

Structure of the data

Raw output:
"Rain" plot

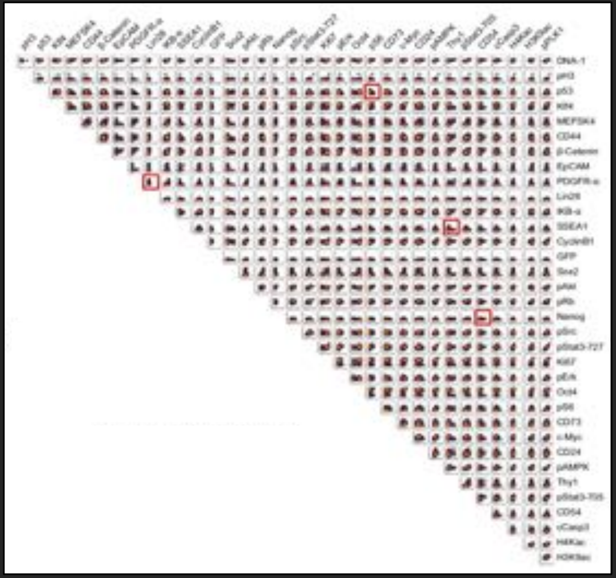


Processed output:
FCS file



FS	SS	FL1	FL2	FL3	FL4	FL5	...
322	519	153	936	123	420	986	...
49	310	482	606	932	490	178	...
837	601	414	521	492	457	716	...
840	698	797	283	848	238	616	...
846	903	26	273	609	500	971	...
244	280	108	163	295	563	548	...
3	698	536	510	366	242	196	...
962	373	614	184	282	370	192	...
886	103	137	782	638	925	353	...
646	16	120	521	763	827	861	...
528	572	540	497	690	147	579	...
961	532	592	281	223	946	620	...
245	774	385	639	878	416	808	...
410	172	281	449	970	897	979	...
788	247	616	646	43	958	214	...
441	698	162	636	242	172	690	...
641	344	145	582	592	206	256	...
656	988	7	975	908	93	303	...
719	215	724	977	909	384	242	...
639	639	14	657	977	332	883	...
414	777	412	941	538	690	355	...
...	...	...	...	...	...	...	...

Interpretation:
Biaxial gating



Mass cytometry

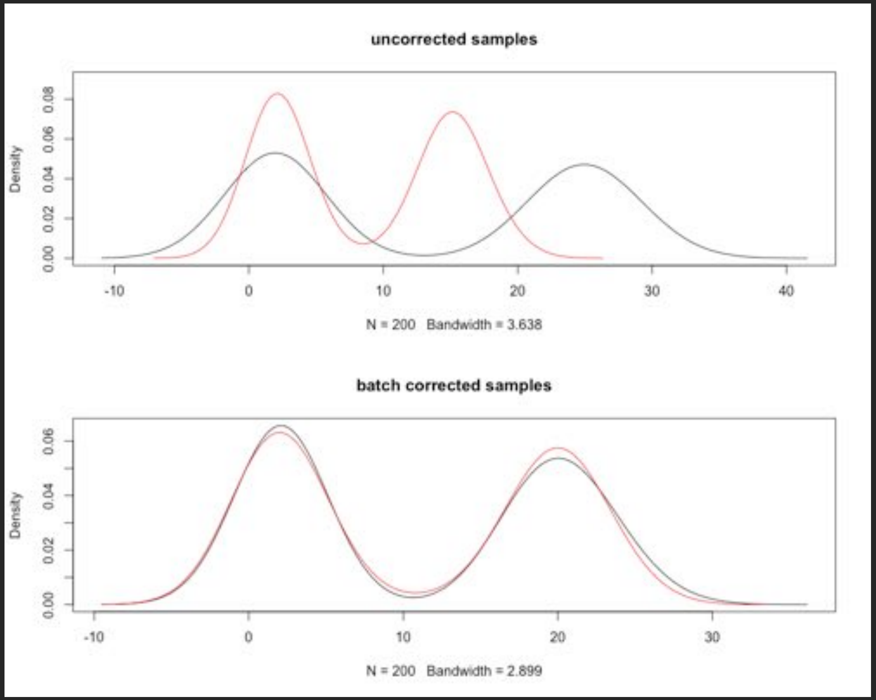
What comes out of the machine:

* A matrix of 1,000,000+ observations
* 40+ variables is measured for each
* Cells are of unknown type and state
* Figure out what they are
* Compare across conditions
* …And the data is noisy!

Analyzing cytometry data

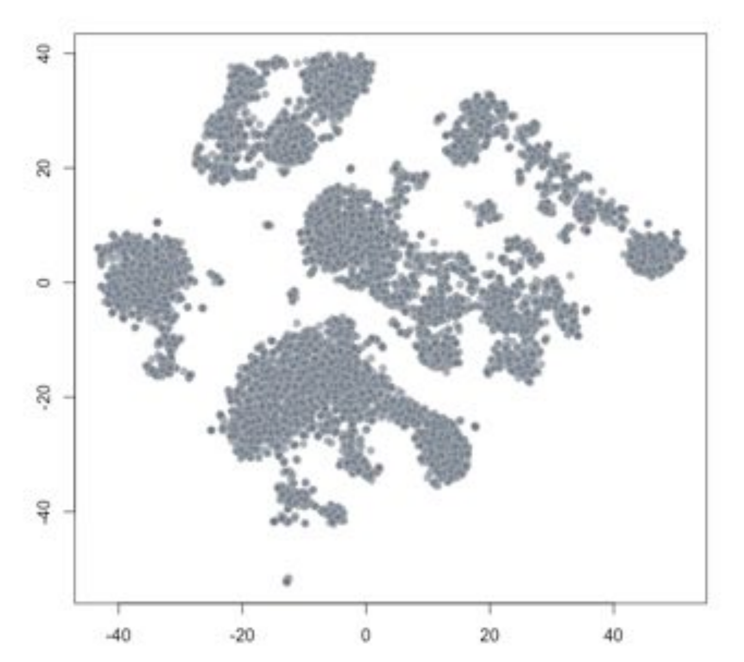
Data pre-processing steps

* Normalization
* Transformation
* Batch correction
* Removing normalization beads, doublets, debris, and other junk



Analyzing cytometry data

Visualizing the data
(dimensionality reduction)



Analyzing cytometry data

Cell subset detection (clustering)

One example: agglomerative hierarchical clustering

First, we calculate the distance between each cell (vector of protein expression values)

$$d(\vec{u},\vec{v}) = \|\vec{u}-\vec{v}\| = \sqrt{(u_1-v_1)^2+(u_2-v_2)^2\ldots(u_n-v_n)^2}$$

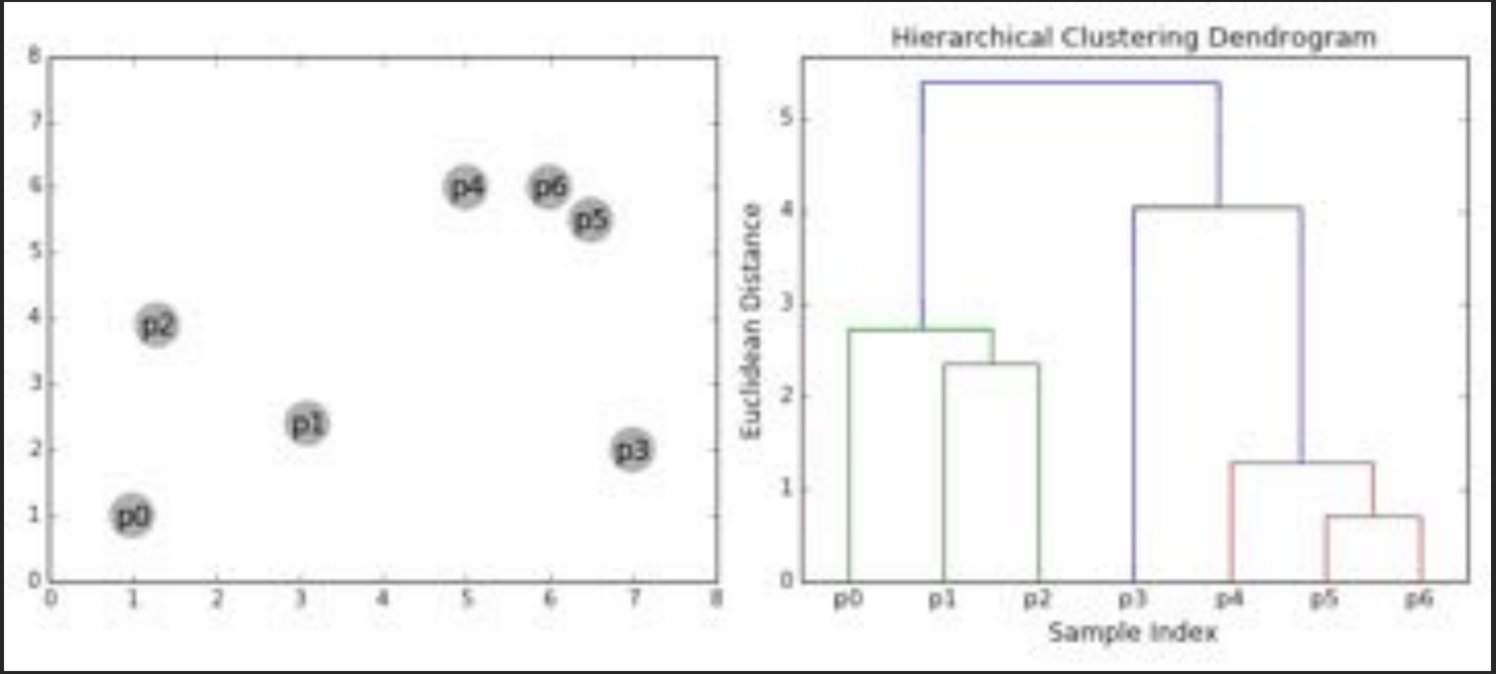
Analyzing cytometry data

Cell subset detection (clustering)

One example: agglomerative hierarchical clustering

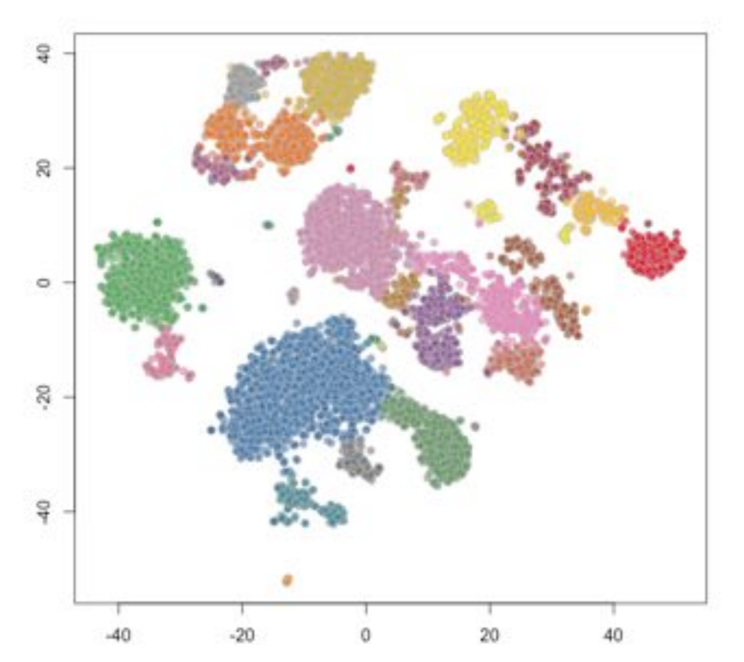
Then, we iteratively combine cells that are closest to each other.

Analyzing cytometry data



Analyzing cytometry data

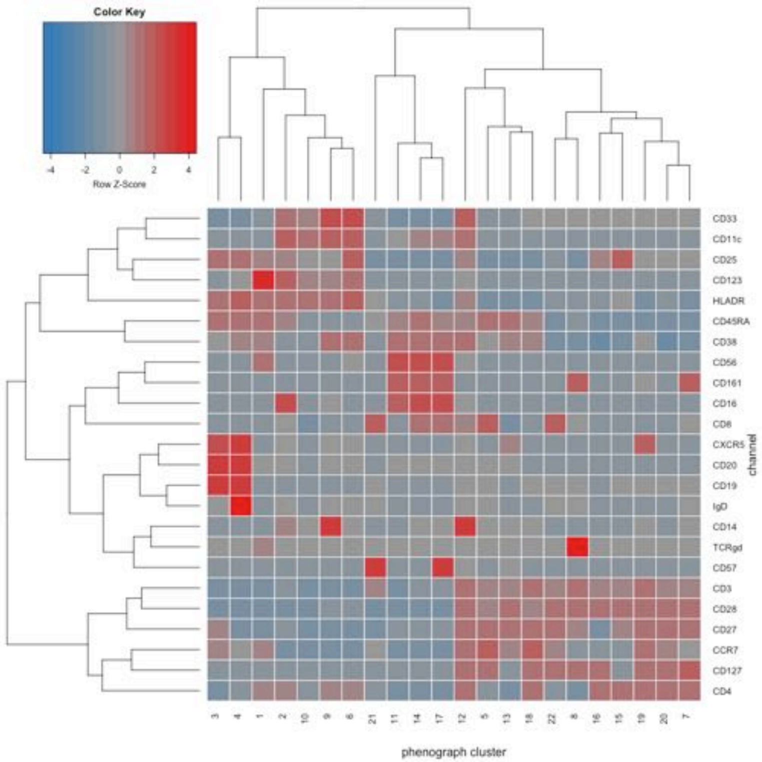
Color dimensionality reduced plot by cluster



Analyzing cytometry data

What cell types constitute the clusters?

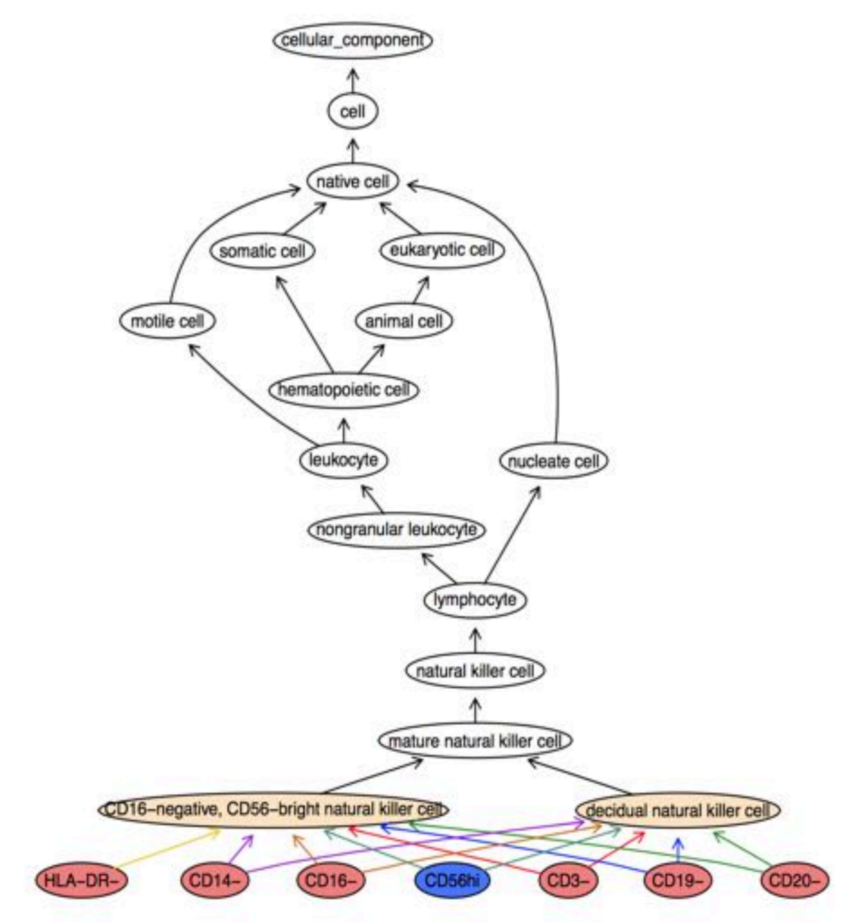
Visualizing cluster marker expression



Analyzing cytometry data

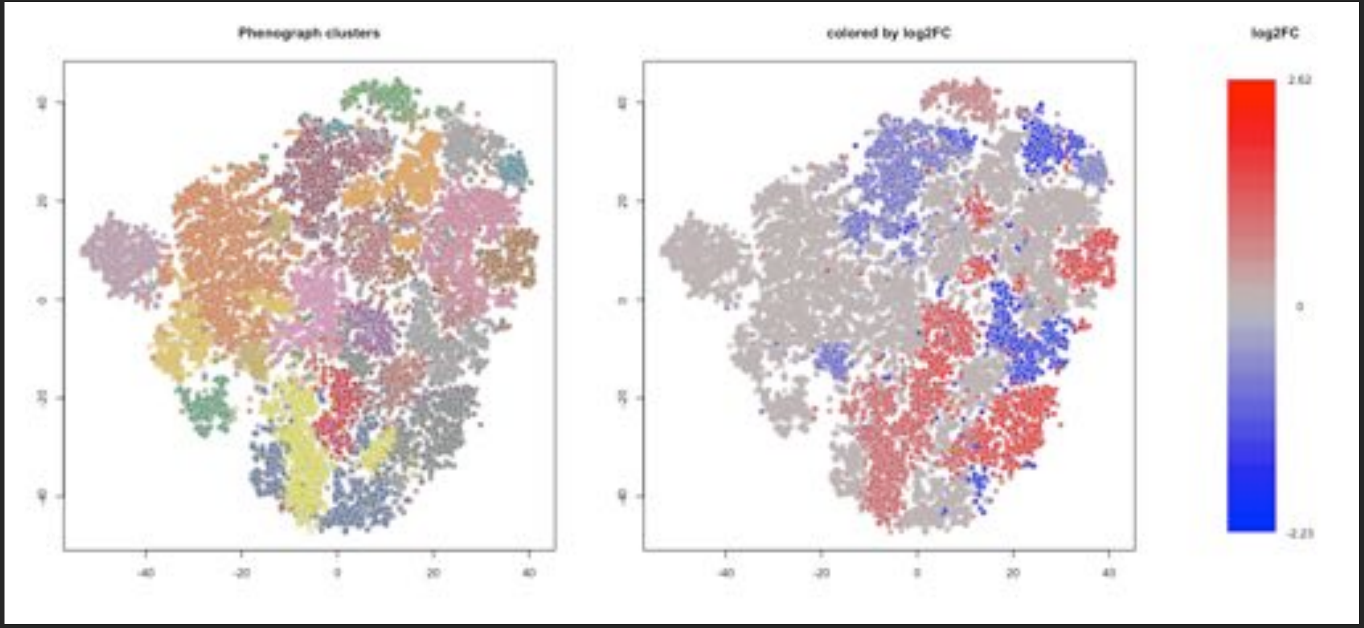
Assigning population label to clusters: reverse querying the cell ontology database

…but in the end: ask an expert



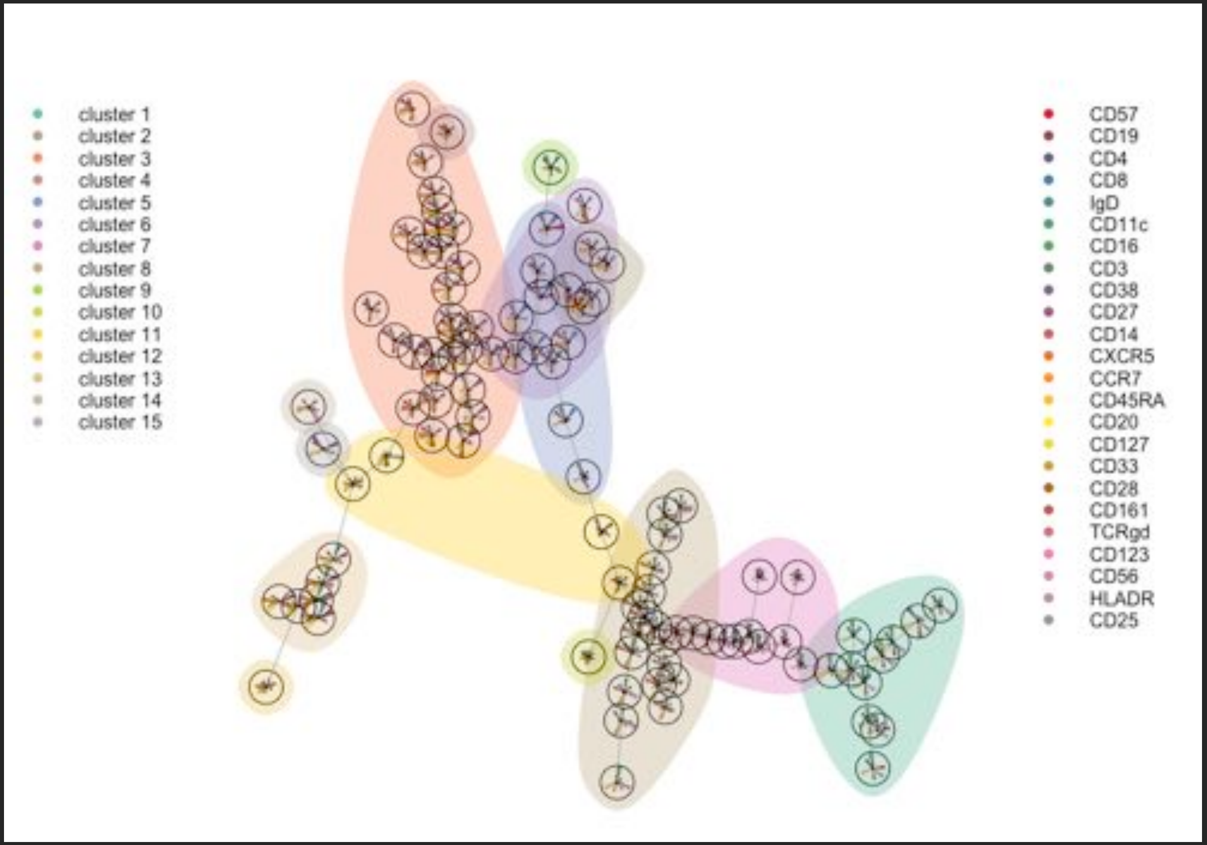
Analyzing cytometry data

Differential abundance of cells / proteins



Analyzing cytometry data

Elucidating cellular hierarchies using self-organizing maps (SOM) and minimum spanning trees (MST)



Analyzing cytometry data

Result

The immunophenotype for each patient, which we can then proceed to correlate to clinical outcome

HOWEVER!

We measure tumor antigens, immune phenotypes, immune cell reactivity, etc. *in parallel* – not in an integrated manner.

Questions?