Personalized medicine with Pharmacoscopy-guided treatments

Interdisciplinary Technical Journal Club

12.6.2018

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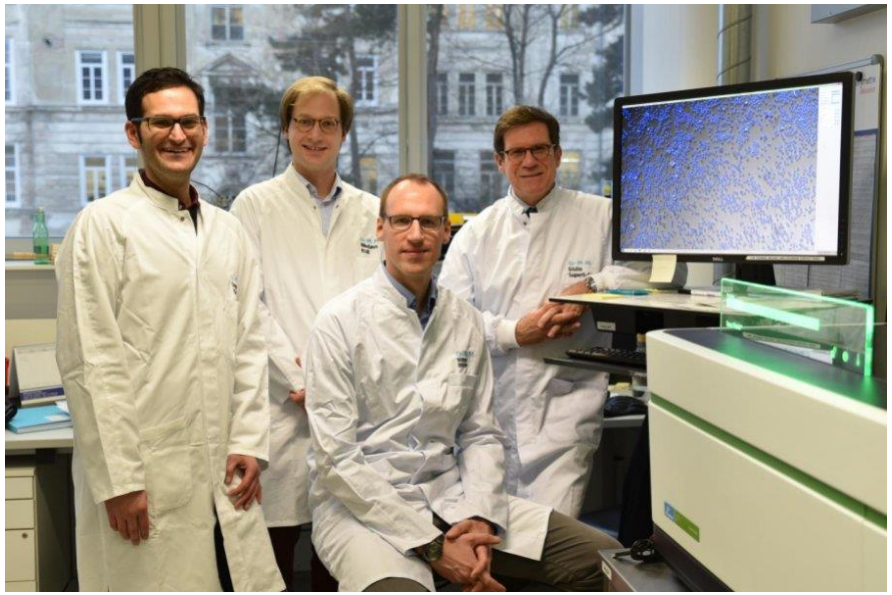
Lab of Alexandre Theocharides

Pharmacoscopy

- Combination of automated microscopy, population-wide single-cell image analysis and novel analysis algorithms
  - Developed at CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences
  - Tested in collaboration with the Medical University of Vienna
  - High statistical power due to large number of events monitored
  - Allows high-troughput screening of co-culture systems
  - Quantification of a variety of cellular parameters



Gregory Vladimer, Nikolaus Krall, Berend Snijder and Giulio Superti-Furga (from left to right) are next to the Pharma-coscopy high-throughput microscope.
Credit: Wolfgang Däuble/CeMM
Sciencedaily.com

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 24 APRIL 2017 | DOI: 10.1038/NCHEMBIO.2360

Global survey of the immunomodulatory potential of common drugs

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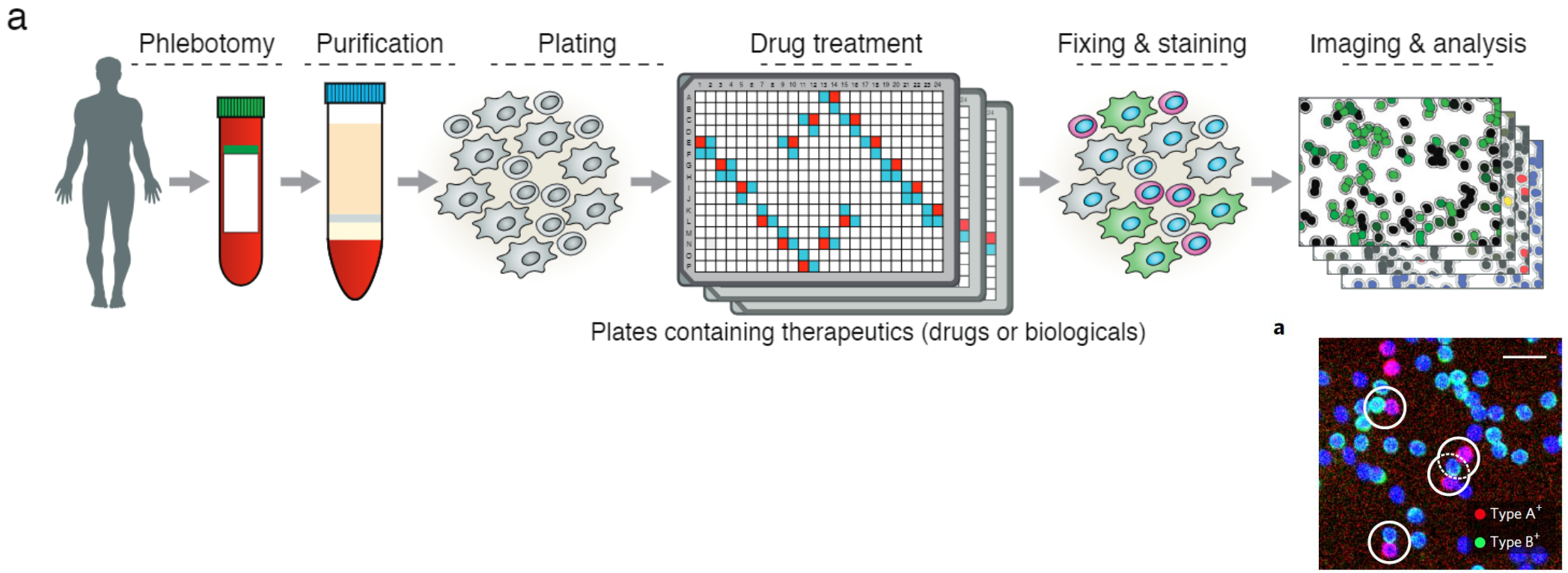
Pharmacoscopy allows to quantitatively study the cell-cell interactions in blood for large drug libraries

- *Ex vivo* population-wide single-cell microscopy of PBMC monolayers
  - "phenotypic" drug screening of 1402 small chemical molecules

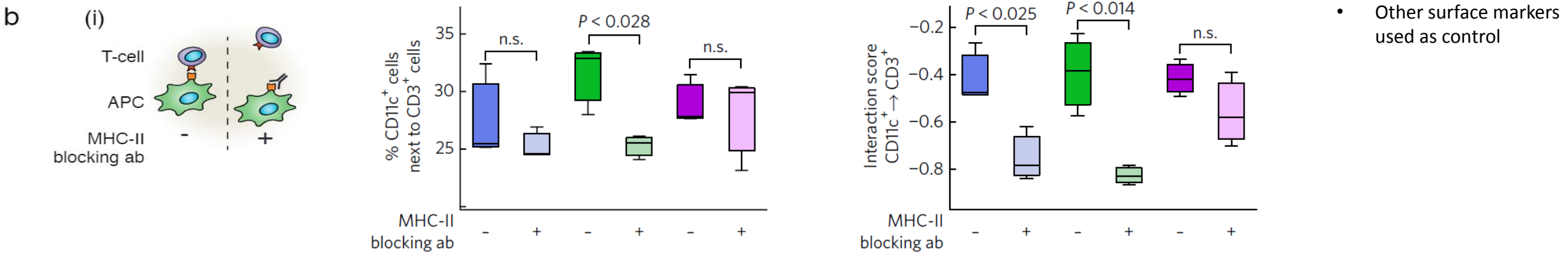
⇒ check for their ability to alter cell-cell interactions among PBMC *ex vivo*

⇒ identify and characterize the immunomodulatory properties of small-molecule drugs



Testing the assay with biologicals that in/decrease cell-cell contacts

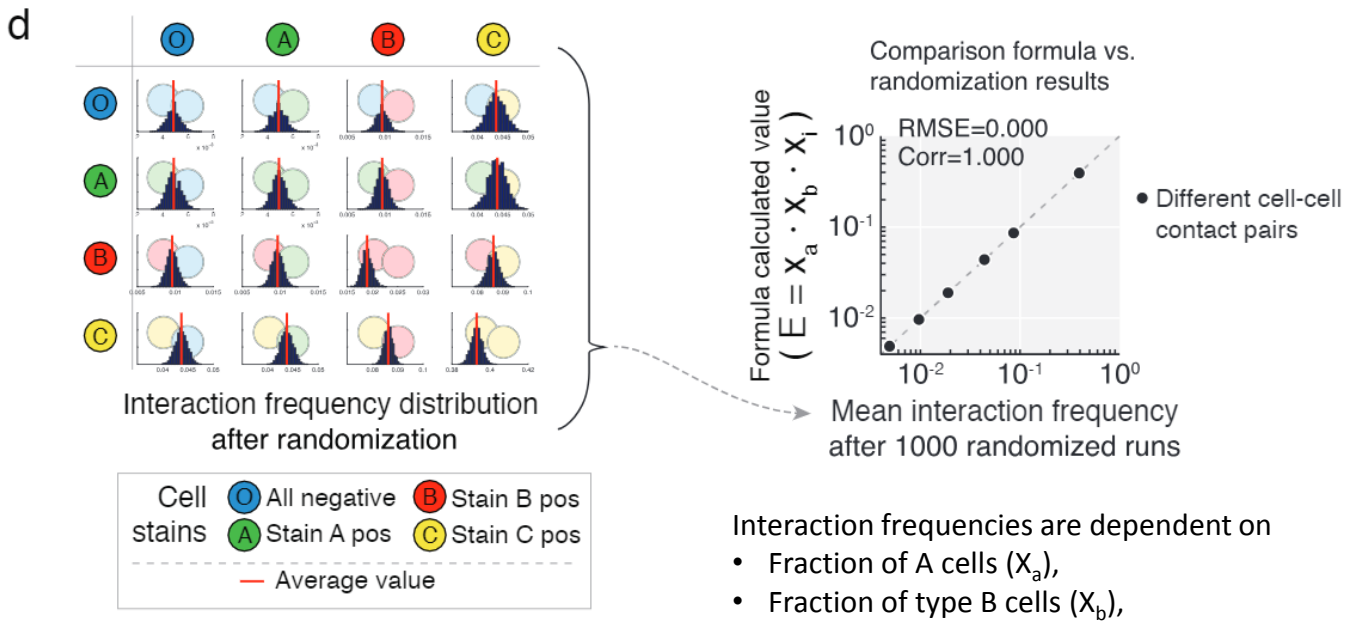
- Treatment of VSV-stimulated PBMC with MHC-II blocking antibody
  - Percentage of CD11c+ dendritic cells that were in direct contact with CD3+ T-cells was reduced from 33% to 25% measured over a total of 124’059 cell-cell contacts
  - Dendritic cell → T-cell interaction score reduced under VSV-stimulated but also naïve conditions (reduced “ag-scanning”?)



- IgG control
- Other surface markers used as control

The interaction score

- The interaction score indicates how much of the observed interaction frequency deviates from what would be expected by random chance occurrence
  - Robust to alterations in relative abundance of either subpopulation
  - Robust to alterations in overall cell density or number of cell-cell contacts
  - Many-to-one cell contacts
  - Gain or loss of cellular subpopulations

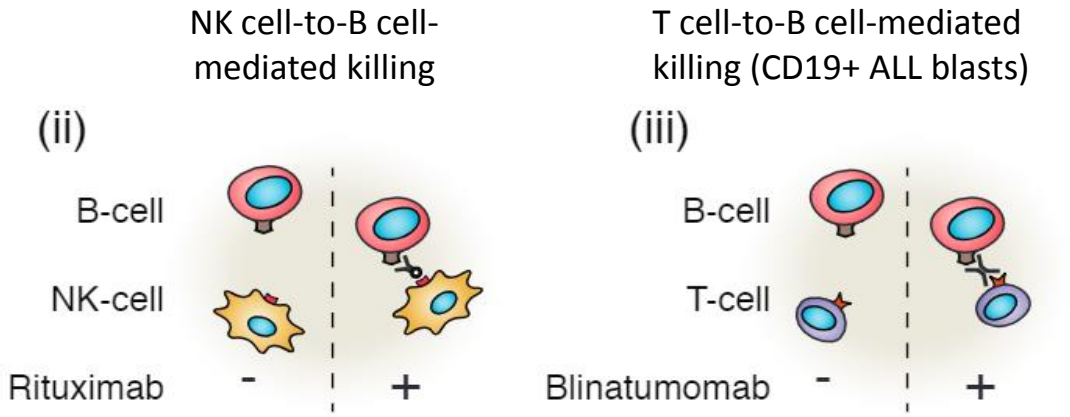


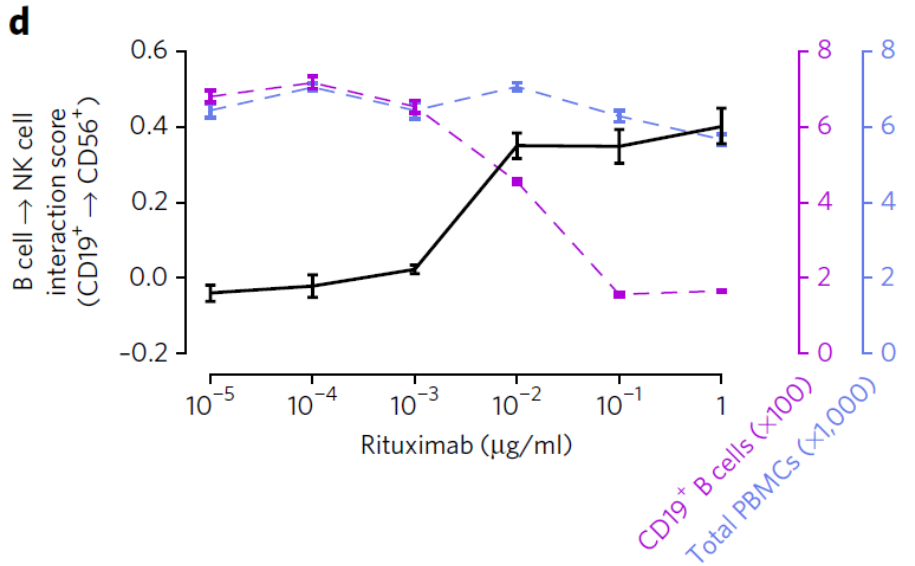
Interaction frequencies are dependent on

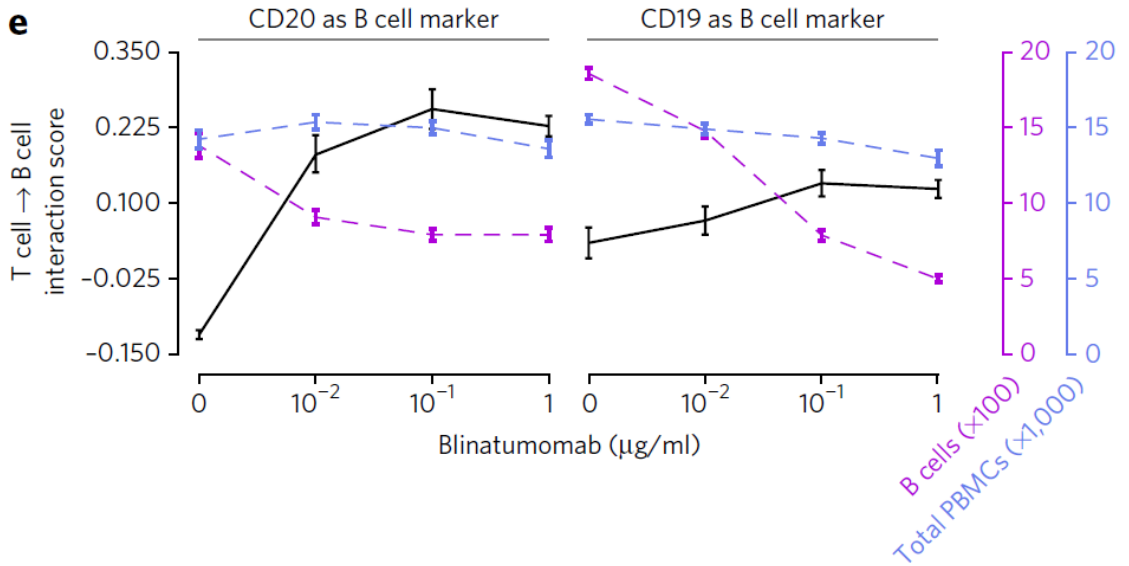
- Fraction of A cells (X_a),
- Fraction of type B cells (X_b),
- and overall clustering index (i.e. fraction of PBMC that contact 1 or more cells, X_i).
- E => fraction of cells that are of type A and interact with type B

Treatment of PBMC with anticancer biologicals with well-defined mechanisms of action

- Dose-dependent increase in the respective interaction score and concomitant loss of target cells
- Interaction score increased even with the reduction of target B cells (score's normalization)





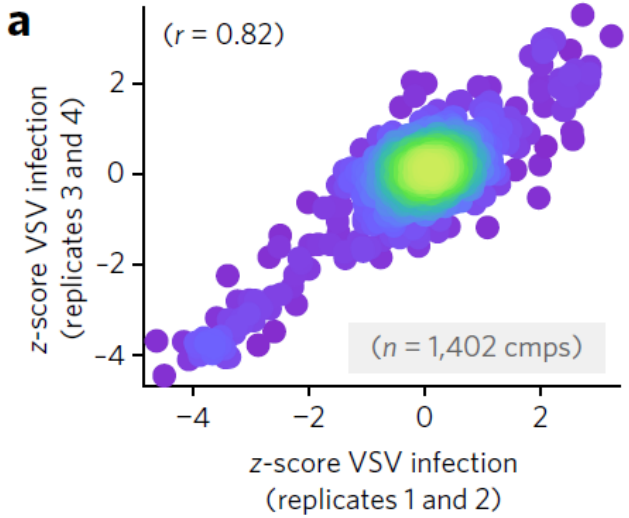


Screening for chemical modifiers of PBMC cell-cell contacts

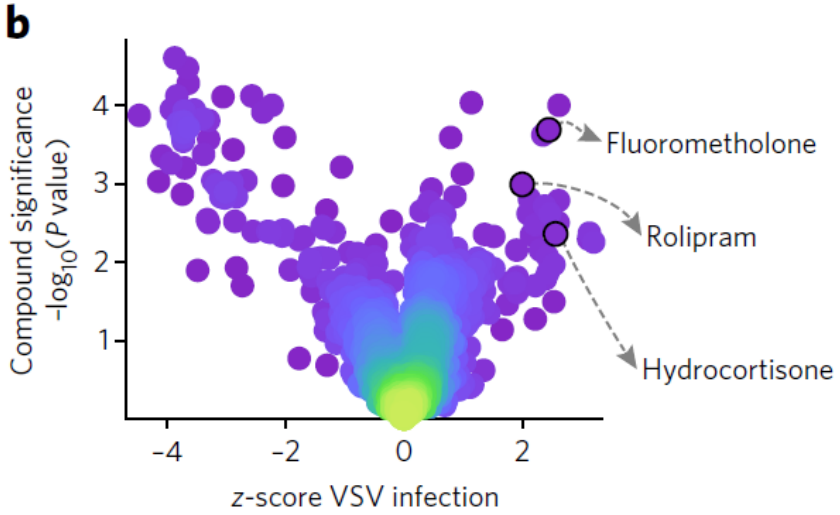
- Leukocyte interactions were screened for 1’402 compounds tested (quadruplicates)
- Analysis of cell-to-cell contacts of 80 million PBMCs
- Immune stimulation with VSV-GFP (to induce higher cell-cell interactions)
- Pairwise combinations of four populations were stained after infection

Screening for chemical modifiers of PBMC cell-cell contacts

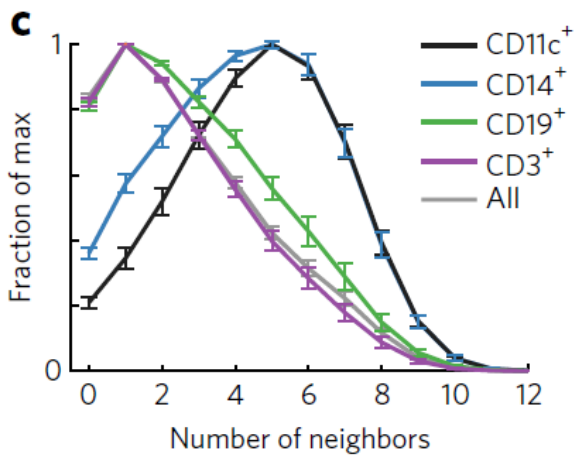
- 80 compounds decreased and 22 compounds increased VSV infection
- Monocytes had higher number of direct neighbours than lymphocytes (analysis of 246 x 10^6 cell contacts)



- High reproducibility of VSV infection
- 1 dot = 1 compound



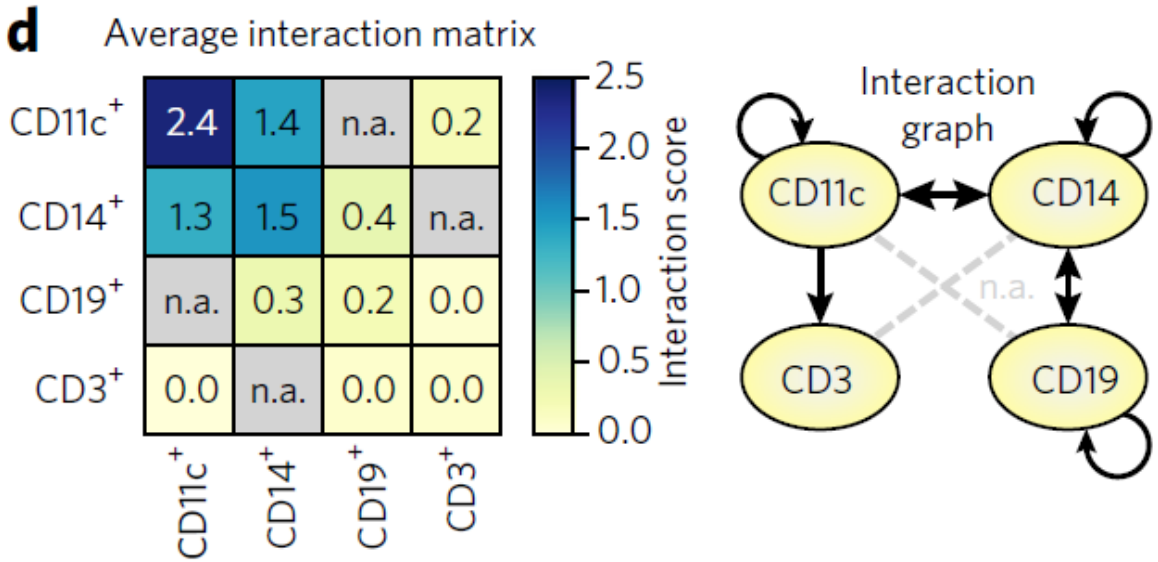
- Average change in VSV infection per compound (z-score normalized) vs. significance
- Anti-inflammatory compounds indicated

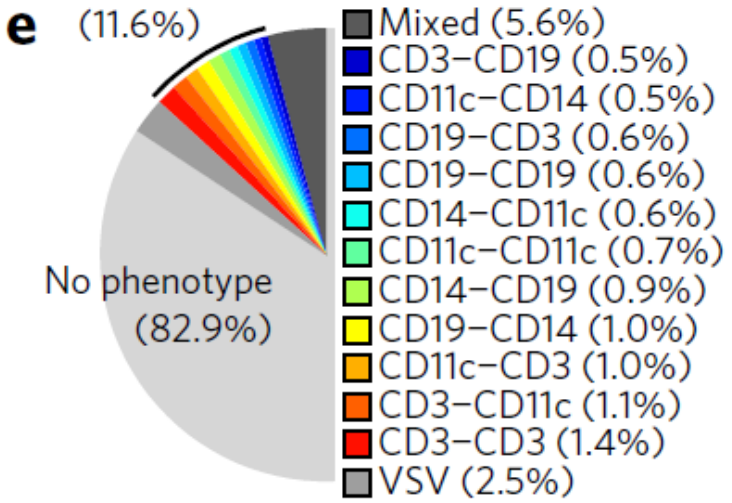


- Distribution of number of direct contacts per cell type normalized to the Max of each distribution

Screening for chemical modifiers of PBMC cell-cell contacts

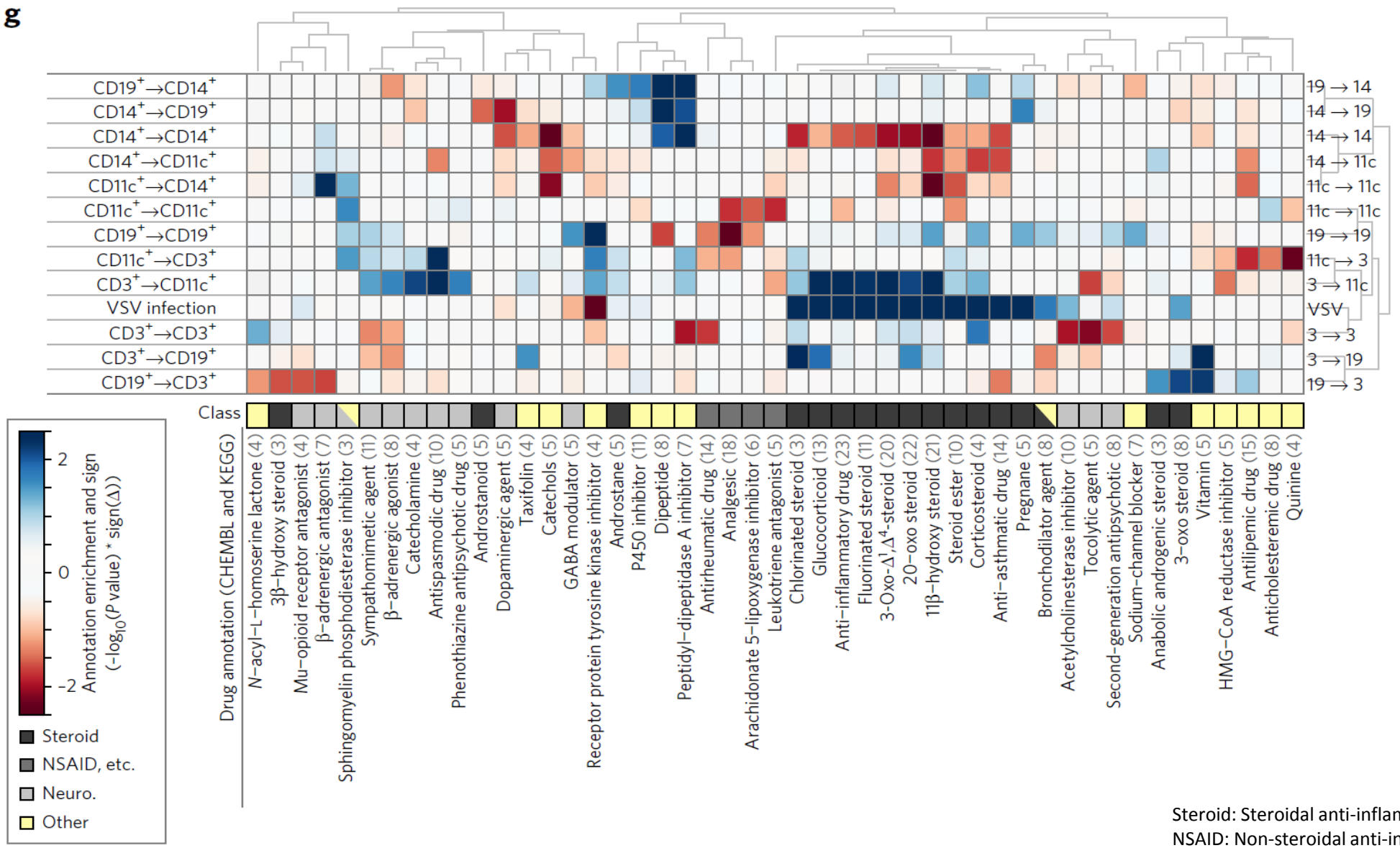
- Highest interaction scores were observed between and among CD11c+ and CD14+ monocytes
- Overall, more compounds (11.6 %) altered only leukocyte cell-cell contacts than those altered by only virus infection (2.5%) at 2 s.d.





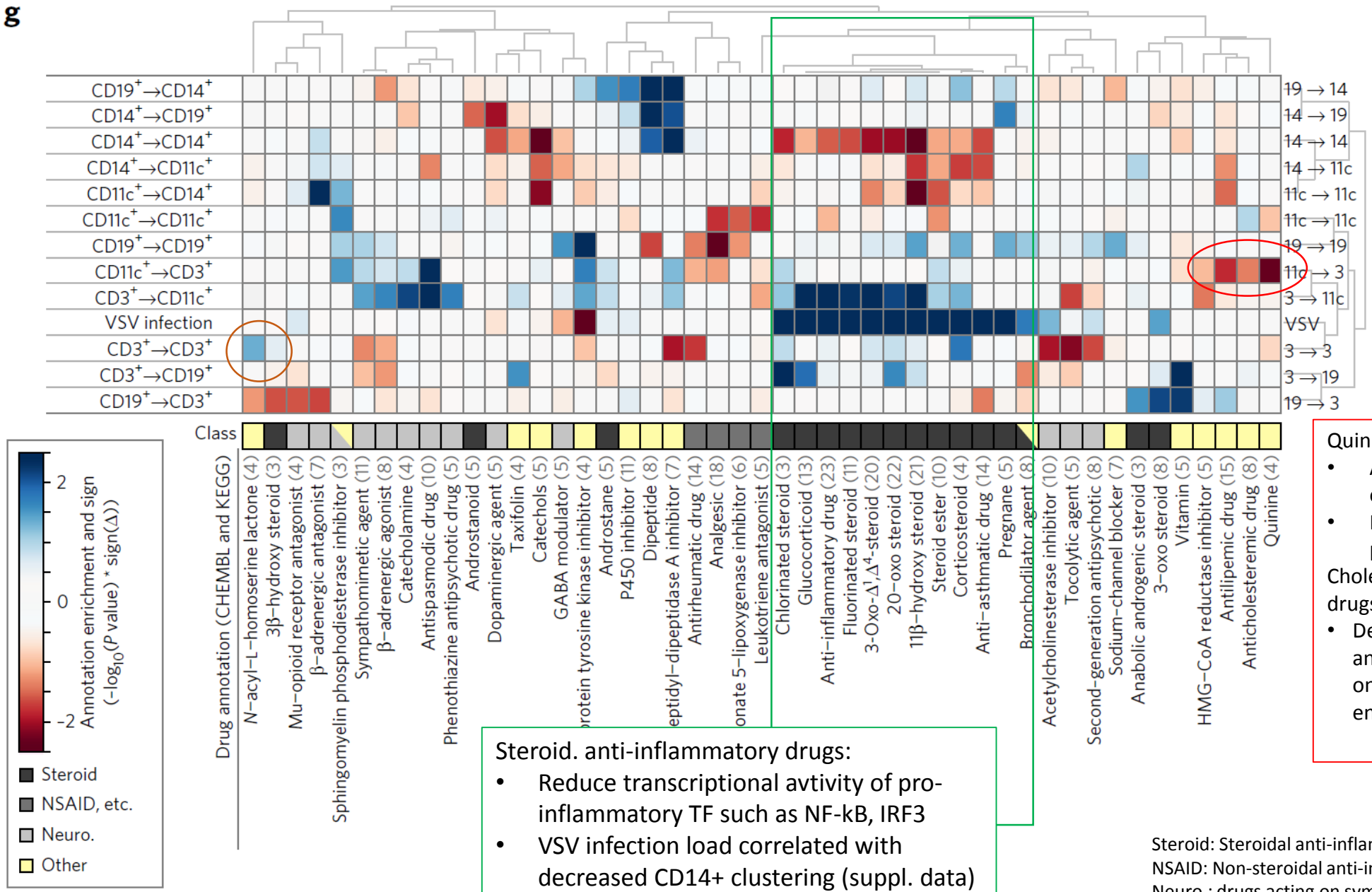
- Percentage of compounds with mixed or unique phenotypes

Enriched drug classes that altered PBMC cell-cell contacts



Steroid: Steroidal anti-inflammatory drugs
NSAID: Non-steroidal anti-inflammatory drugs
Neuro.: drugs acting on sympathetic nervous system

Enriched drug classes that altered PBMC cell-cell contacts



Steroid. anti-inflammatory drugs:

- Reduce transcriptional avtivity of pro-inflammatory TF such as NF-kB, IRF3
- VSV infection load correlated with decreased CD14+ clustering (suppl. data)

Quinine

- Anti-inflammatory effect
- Interfere with MHC presentation

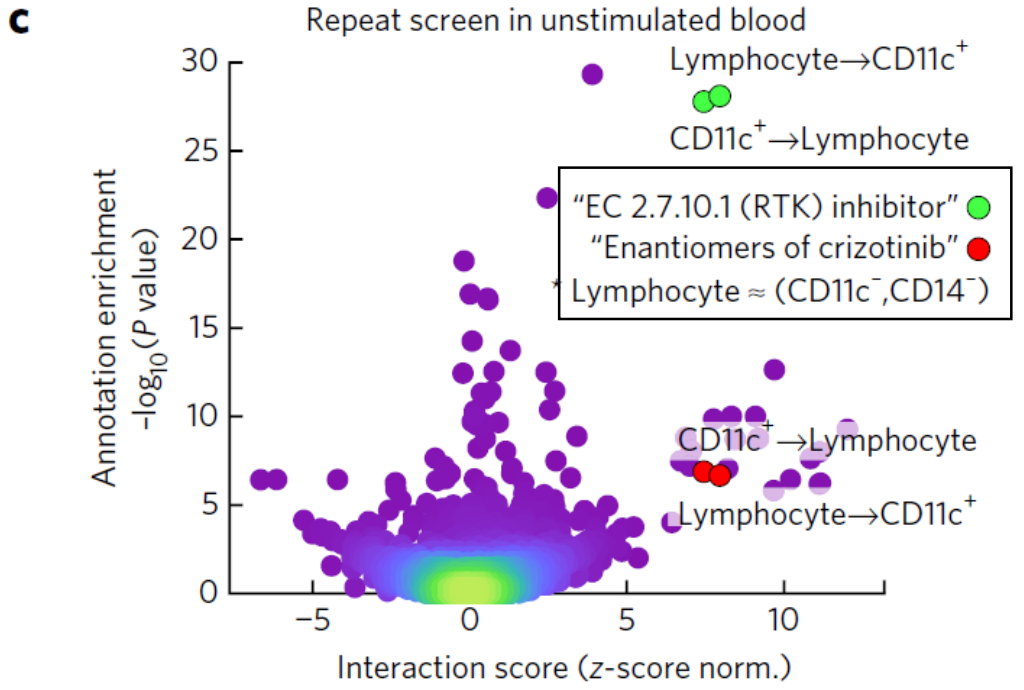
Cholesterol-lowering drugs

- Dependency of MHC-II antigen presentation on cholesterol-enriched lipid rafts

Steroid: Steroidal anti-inflammatory drugs
NSAID: Non-steroidal anti-inflammatory drugs
Neuro.: drugs acting on sympathetic nervous system

Crizotinib increases the interactions between T cells and APCs

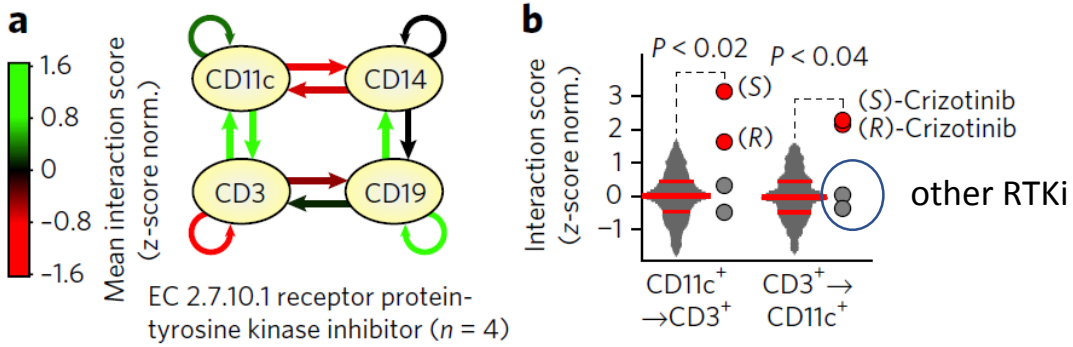
- One of the drugs with previously unknown immunomodulatory properties was the RTKi (inhibitor of the receptor protein tyrosine kinases) Crizotinib
  - Increased interactions between CD11c+ cells and CD3+ c T cells
  - Crizotinib = inhibitor of MET, ALK and ROS1 kinases
  - Observed for both enantiomers
- Repeat of the screen using unstimulated blood
  - Results were reproduced
  - RTKi were strongest-enriched drug class over all cell-cell interactions
  - Significant increase in CD11c+ cells and lymphocytes (i.e. monocyte-n

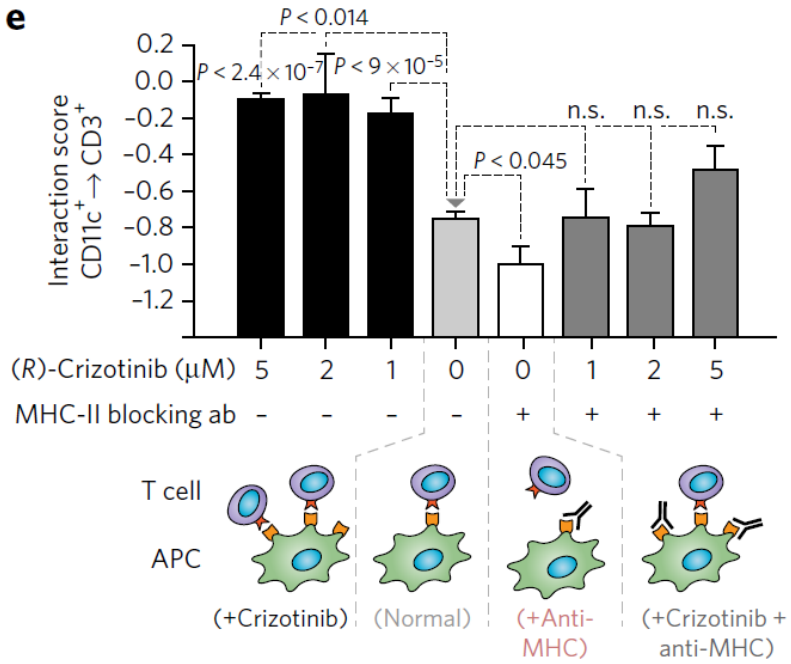


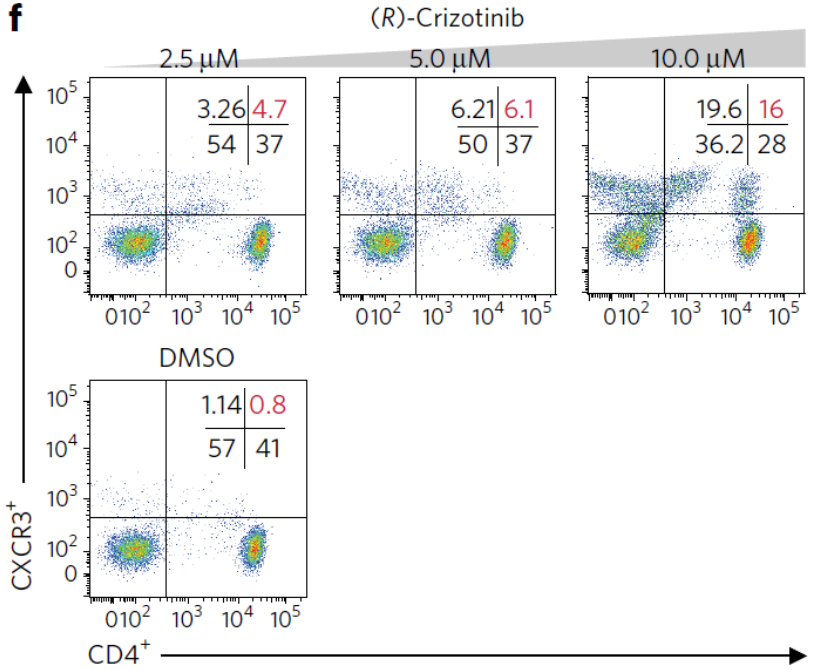
- Dots indicate individual drug annotations and the affected cell-cell interaction

Crizotinib increases T cell interactions with APCs through upregulation of MHC-II

- APC → T-cell interactions depend on MHC-II – TCR interaction



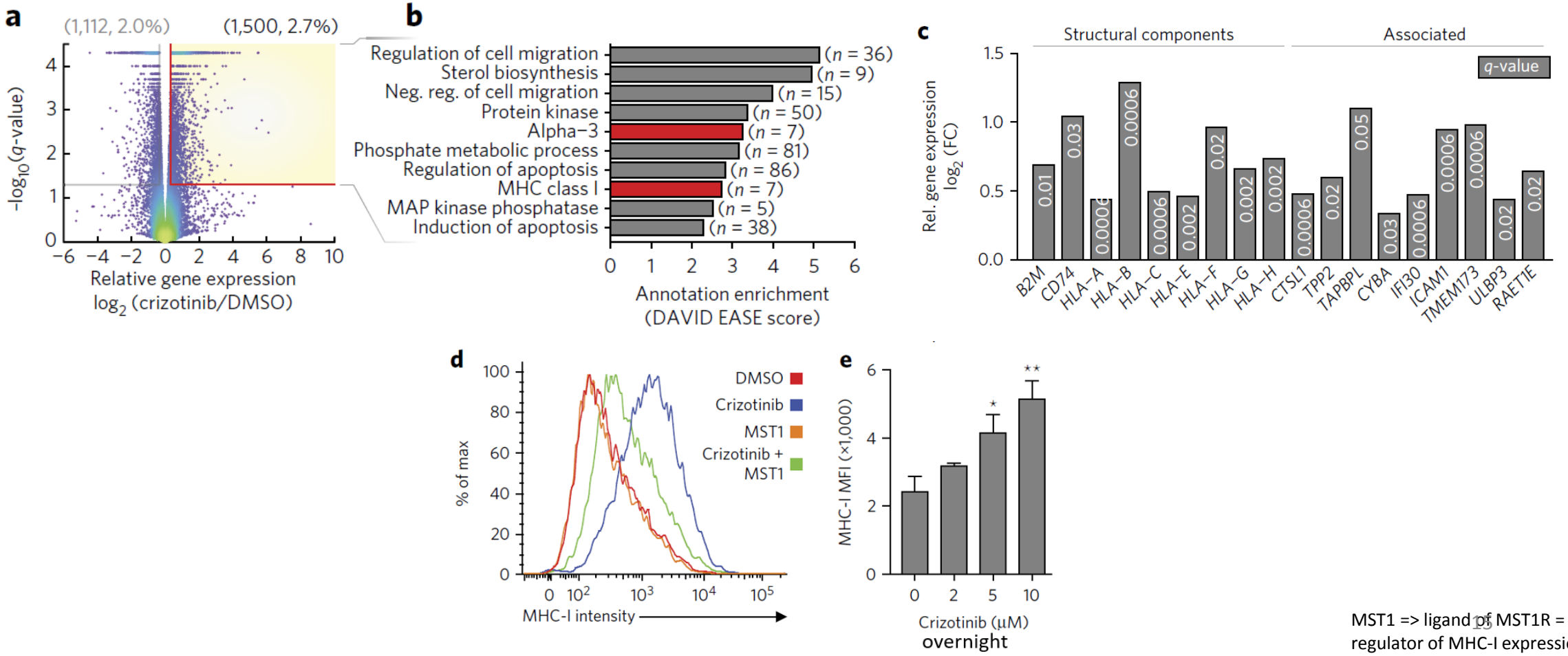




- MHC-II expression assessed on more healthy donors without VSV-infection (*ex vivo* incubation with Crizotinib)
- Crizotinib-induced CD4+ T helper 1 response that is indicative of an inflammatory milieu

Crizotinib drives MHC-I expression in colon cancer cells

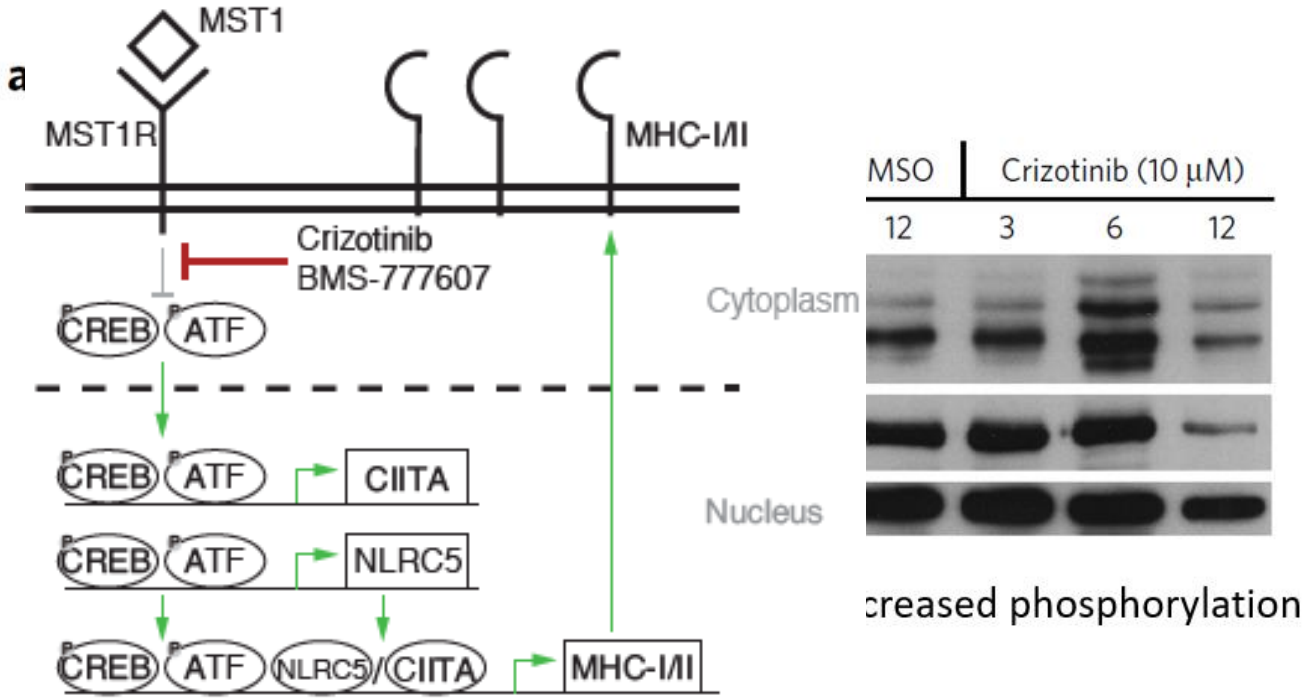
- RNA-seq (2uM crizotinib) and FACS analysis of crizotinib-treated SW480 colon cancer cells

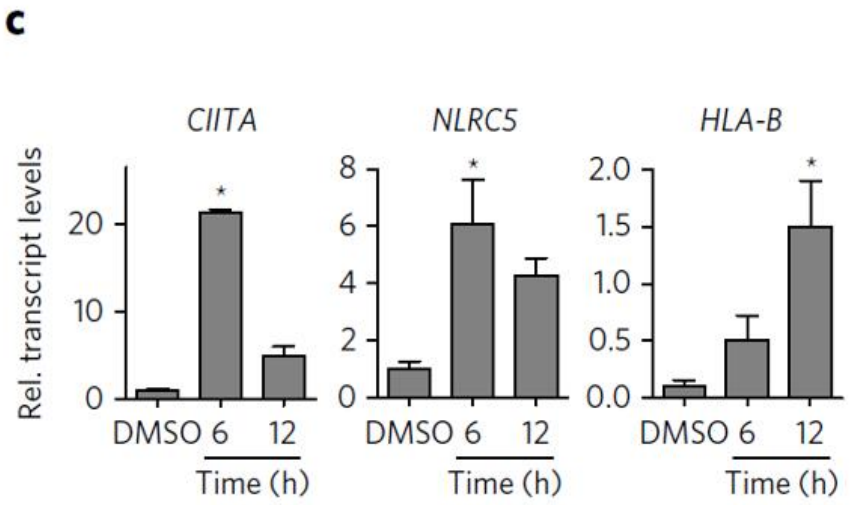


MST1 => ligand of MST1R = neg. regulator of MHC-I expression

Immunomodulatory effect of crizotinib is mediated by MSTR1 inhibition

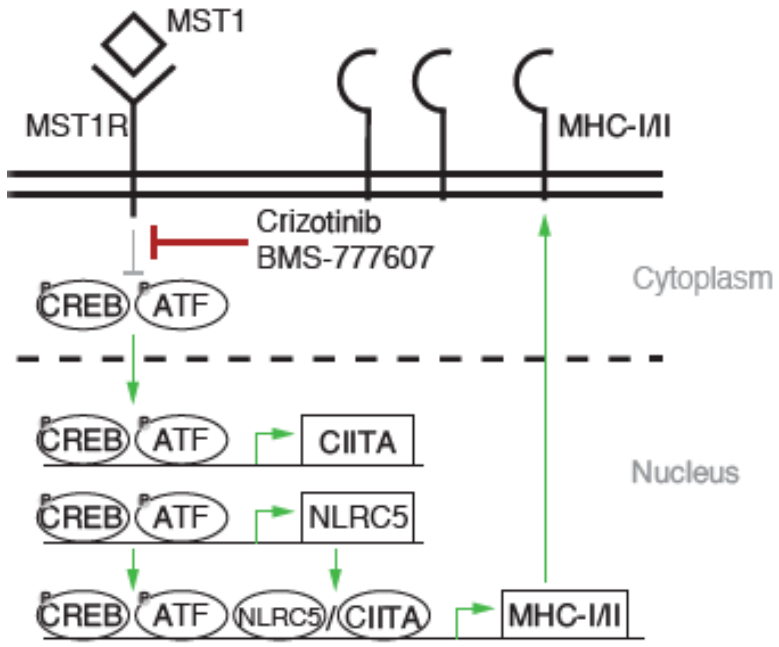
- Analysis for the enrichment of TF-binding sites in the upregulated genes revealed a strong enrichment for CREB and ATF
- CREB and ATF are important TFs for MHC-I/II molecules and regulate and cooperate with the MHC-specific TFs CIITA and NLRC5

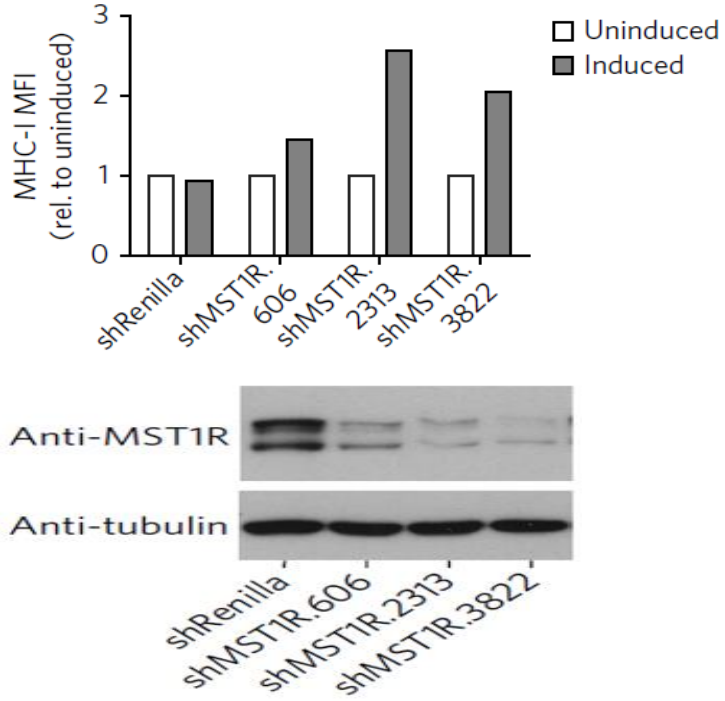




Immunomodulatory effect of crizotinib is mediated by MSTR1 inhibition

- MST1R (macrophage-stimulating 1 receptor) = neg. regulator of MHC expression and immune system upon binding of ligand MST1
- Crizotinib binds and inhibits MSTR1

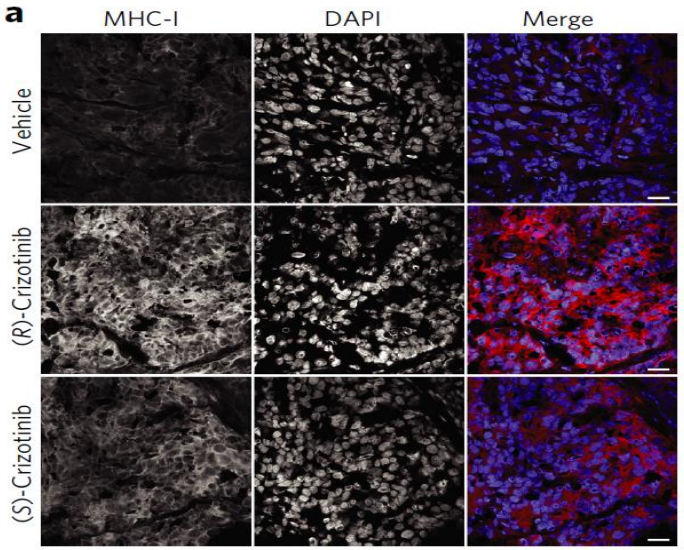


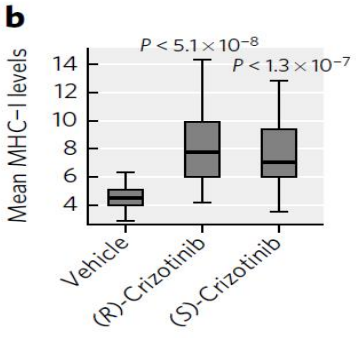


- shRNA against MSTR1

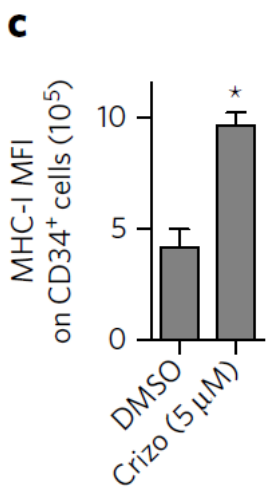
In vivo assessment of the immunomodulatory potential of crizotinib

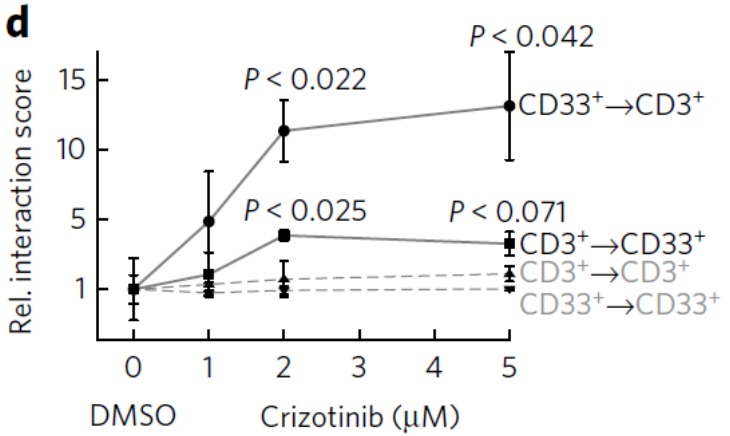
- Crizotinib increases MHC-I levels in xenografted cancer cells or patient-derived peripheral blasts
- Lung cancer mouse models treated with Crizotinib => CD8+ T-cell infiltration in lungs, reduced metastasis
- This may aid an anticancer immune response => e.g. clinical trials with CTLA-4 blockade (ipilimumab) + crizotinib in NSCLC (non-small cell lung cancer) patients





- Crizotinib injected into SCID mice harbouring a SW480 (colon carcinoma cells) xenografted tumor
- 50 mg/kg body weight





- *Ex vivo* treatment with Crizotinib of PBMC of patient with CMML (chronic myelomonocytic leukemia, >70 % CD33+ and CD34+ blast cells in peripheral blood)
- Peripheral blasts
  - Showed twofold increase in MHC-I expression measured by FACS
  - Concentration-dependent increase of T-cell <-> blast interactions

Image-based ex-vivo drug screening for patients with aggressive haematological malignancies: interim results from a single-arm, open-label, pilot study

Berend Snijder, Gregory I Vladimer*, Nikolaus Krall, Katsuhiro Miura, Ann-Sofie Schmolke, Christoph Kornauth, Oscar Lopez de la Fuente, Hye-Soo Choi, Emiel van der Kouwe, Sinan Gültekin, Lukas Kazianka, Johannes W Bigenzahn, Gregor Hoermann, Nicole Prutsch, Olaf Merkel, Anna Ringler, Monika Sabler, Georg Jeryczynski, Marius E Mayerhoefer, Ingrid Simonitsch-Klupp, Katharina Ocko, Franz Felberbauer‡, Leonhard Müllauer, Gerald W Prager, Belgin Korkmaz, Lukas Kenner, Wolfgang R Sperr, Robert Kralovics, Heinz Gisslinger, Peter Valent, Stefan Kubicek, Ulrich Jäger, Philipp B Staber†, Giulio Superti-Furga†*

Lancet Haematol 2017; 4: e595–606

Published Online November 15, 2017 http://dx.doi.org/10.1016/S2352-3026(17)30208-9

See **Comment** page e567

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Pharmacoscopy, a helpful tool for personalized medicine?

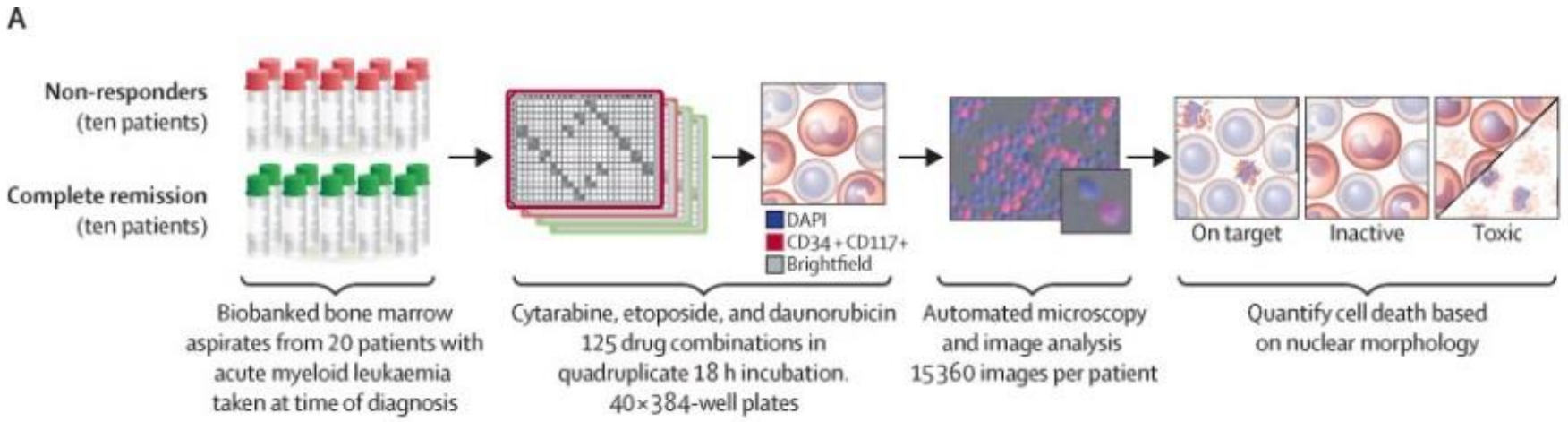
- Problem: cancer genotype vs. cancer phenotype vs. response to therapy
- Aim: investigate clinical impact of pharmacoscopy (PCY) in a small trial
- Single-arm, open-label pilot study with patients that had undergone at least 2 lines of therapy

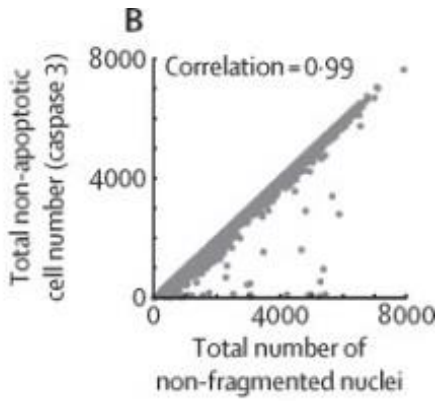
1. Ability of PCY to separate responders from non-responders in retrospective study
    - Cohort of 20 newly diagnosed and untreated patients with acute myeloid leukemia (AML)

2. Prospective pharmacoscopy-guided treatment with 48 patients (17 with treatment) with aggressive hematological malignancies
    - Primary endpoint: Progression-free survival in pharmacoscopy-treated patients vs. their progression-free survival for the most recent regimen (with disease progression)

Can PCY predict clinical response in retrospective study with AML patients?

- Samples from AML patients before first-line remission induction therapy
- Remission induction therapy consists of cytarabine, etoposide, daunorubicin (60% remission rate)
- Drug combination matrix for all 3 drugs tested in mononuclear cells
- On-target effect (CD34+ CD117+ blast fraction), off-target effect (blast-marker-neg. cells) and chemoresistance of blasts determined

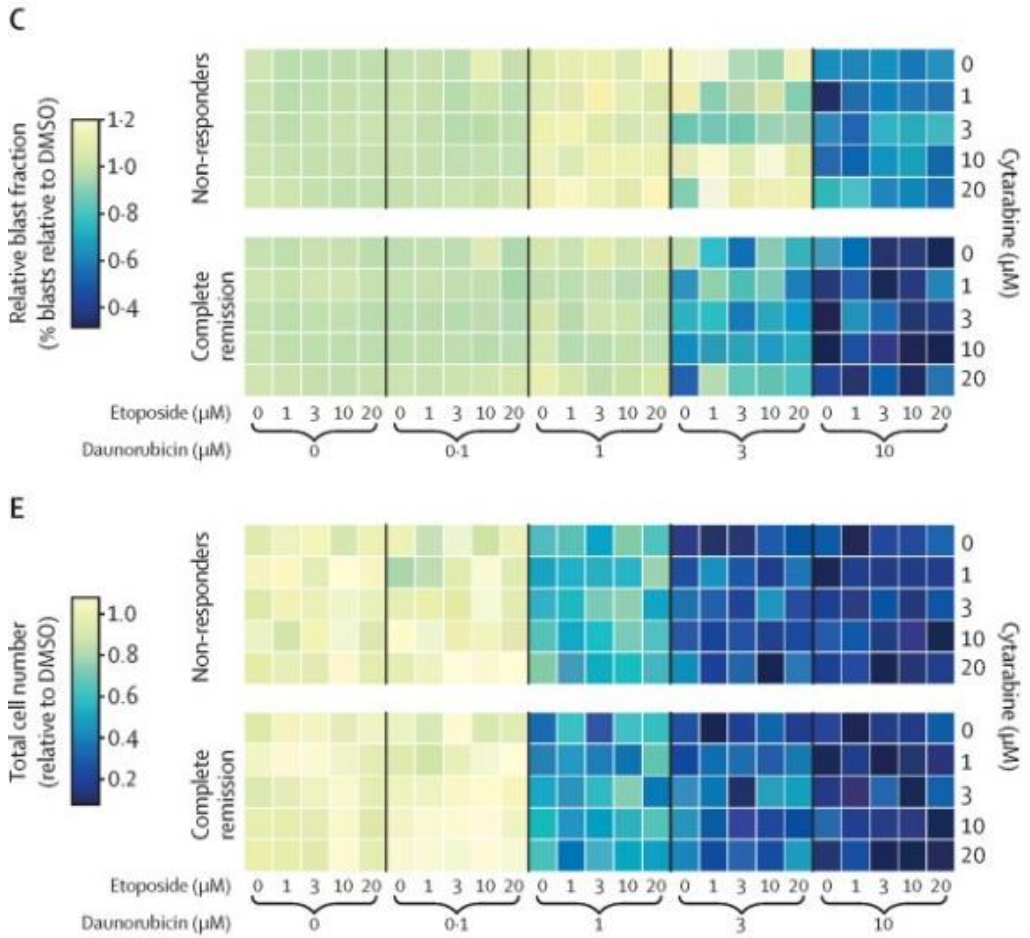


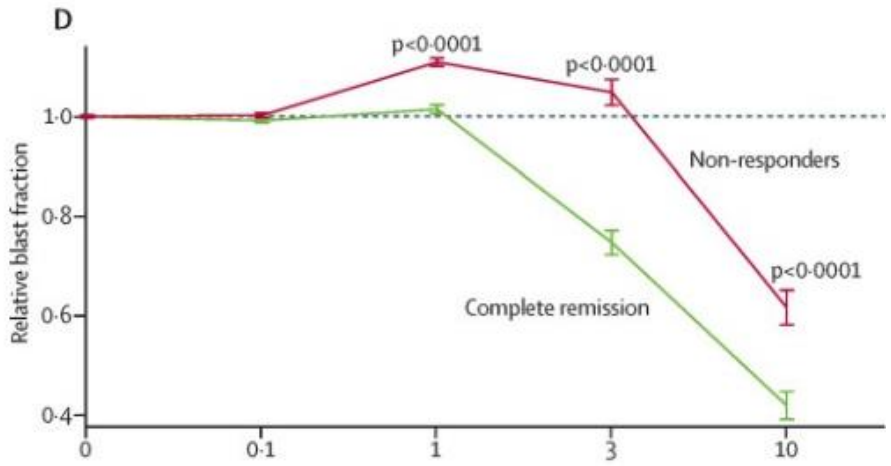


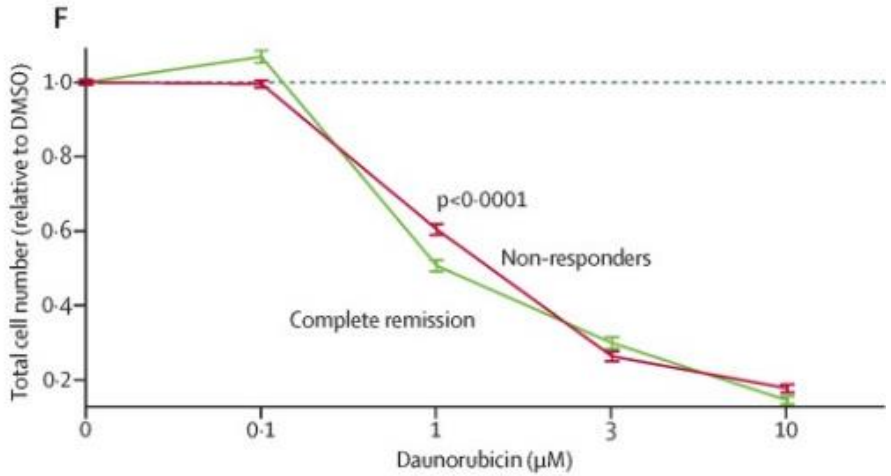
Dot = values of wells from drug-screen (n=3)

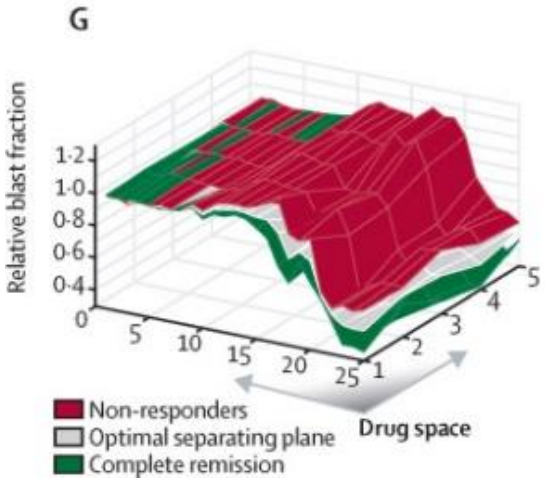
Pharmacoscopy and response to first-line AML therapy

- Relative blast fraction (RBF, specific killing of blast cells) allows to stratify patients according to their clinical response, population-averaged cytotoxicity mesaurements (i.e. total cell death) does not







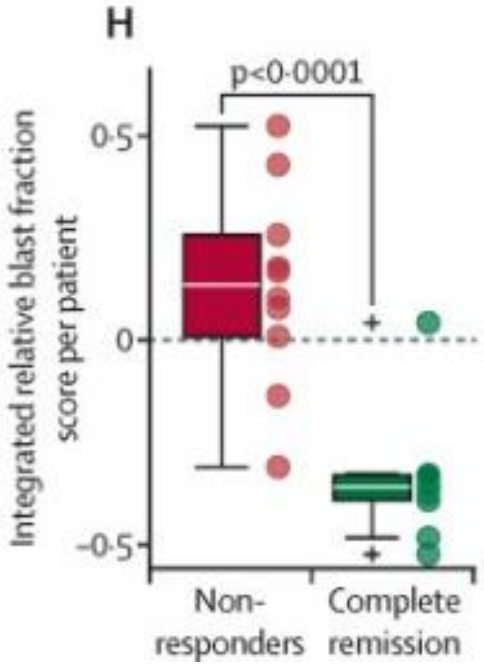


Averaged values

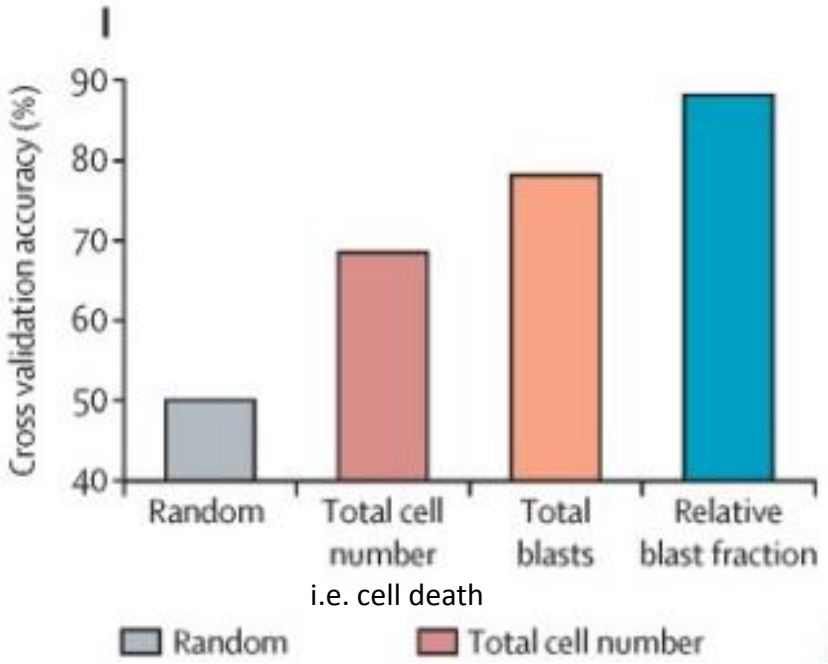
RBF = (averaged fraction blasts after drug treatment) / (averaged fraction blasts treated with DMSO)

Integrated response score for drug sensitivity allowed good separation of responders vs. non-responders

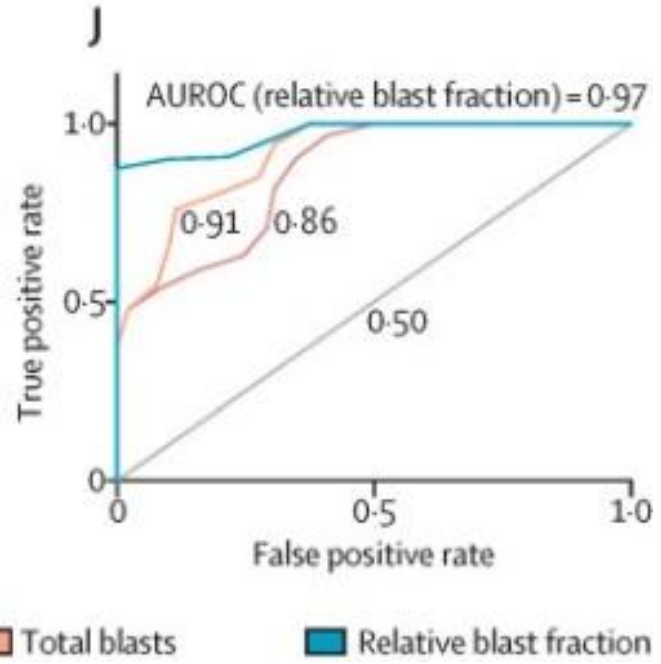
- Cross-validation by leaving out and reclassifying every possible combination of 2 patient samples => Average classification accuracy (CA) of 88.1%



One very strong outlier!

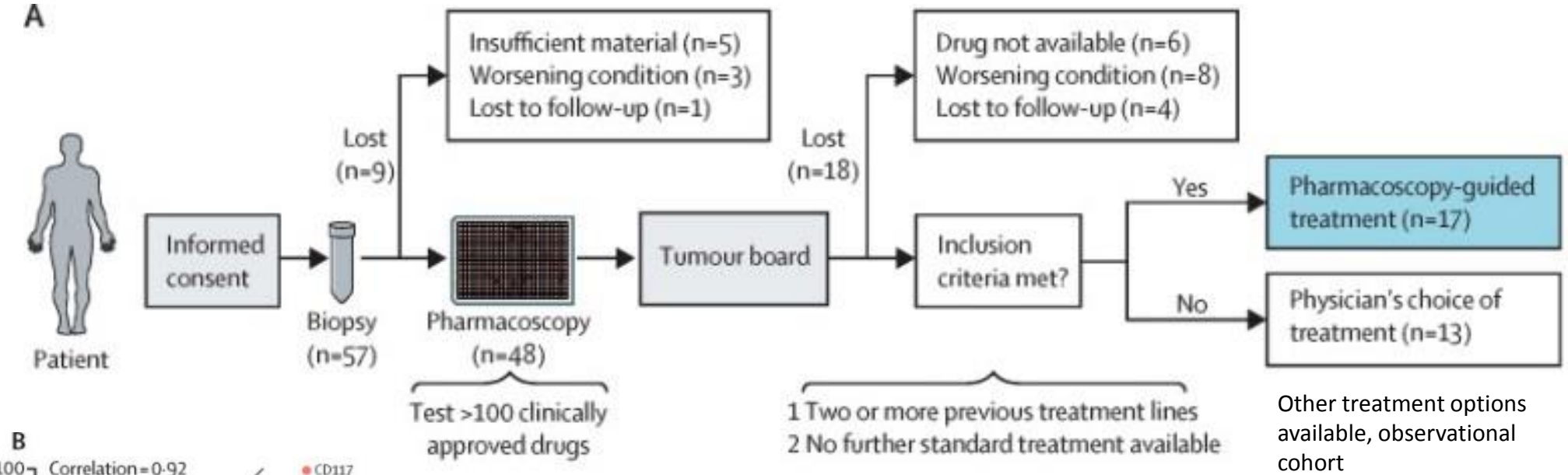


i.e. cell death

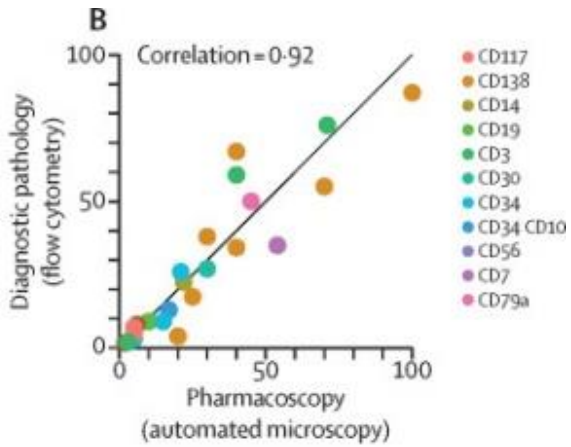


AU = area under the curve
ROC = receiver operating characteristics
Sensitivity vs. 1-specificity

Prospective clinical trial with pharmacoscopy-guided patient treatment



Other treatment options available, observational cohort

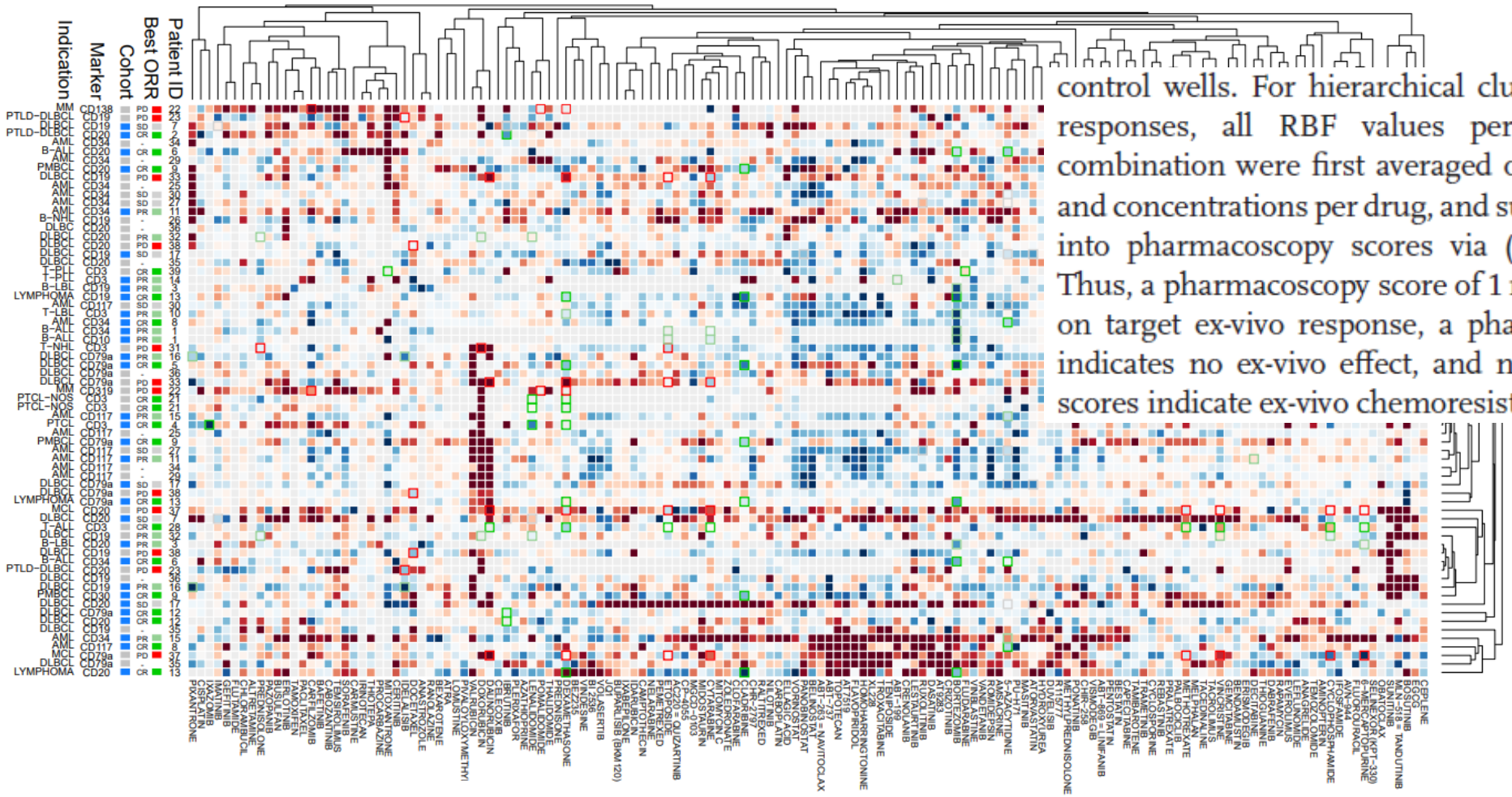


Comparison of fraction "marker-pos. cells"

Prospective clinical trial with pharmacoscopy-guided patient treatment

	Diagnosis	Age (years)	Previous treatment lines	Sample type	Clinical diagnostic mutations	Cell markers used	Pharmacoscopy-guided treatment	Overall response	Progression-free survival (weeks)	Ongoing response
1	B-cell acute lymphoblastic leukaemia	23	5	Peripheral blood	NRAS, CDKN2A	CD10, CD34	Bortezomib	Partial response	5·3	No
2	Diffuse large B-cell lymphoma	69	7	Dissociated lymph node	MYD88, CDKN2A	CD20	Ibrutinib	Complete remission	42·0	No
3	Precursor B-cell lymphoblastic lymphoma	51	3	Pleural effusion	Not determined	CD19, CD20	Obinutuzumab, 6-mercaptopurine, bortezomib	Partial response	12·9	No
4	Peripheral T-cell lymphoma	56	4	Bone marrow	TP53	CD3	Ixazomib, lenalidomide, dexamethasone	Complete remission	22·6	No
5	Diffuse large B-cell lymphoma	29	2	Dissociated lymph node	No alterations detected	CD79a	Bortezomib, cladribine, dexamethasone	Complete remission	34·0	Yes
6	B-cell acute lymphoblastic leukaemia	29	2	Peripheral blood	FLT3, KRAS	CD20, CD34	Bortezomib, azacitidine	Complete remission	37·1	Yes
7	Diffuse large B-cell lymphoma	60	5	Dissociated lymph node	MYD88	CD19, CD20	Imatinib, ibrutinib, lenalidomide, obinutuzumab; fludarabine, cyclophosphamide*	Stable disease	37·3	Yes
8	Acute myeloid leukaemia	72	2	Peripheral blood	NRAS	CD34, CD117	Azacitidine	Complete remission	22·4	No
9	Primary mediastinal large B-cell lymphoma	27	6	Dissociated lymph node	No alterations detected	CD20, CD30, CD79a	Brentuximab vedotin, cladribine	Complete remission	34·7	Yes
10	T-cell lymphoblastic lymphoma	31	4	Peripheral blood	PIK3CA, FBXW7, NOTCH1	CD3	Bortezomib, cyclophosphamide, dexamethasone	Partial response	4·1	No
11	Acute myeloid leukaemia	72	3	Peripheral blood	NPM1, KRAS	CD34, CD117	Decitabine	Partial response	8·4	No
12	Diffuse large B-cell lymphoma	67	3	Lymph node	MYC	CD20, CD79a	Ibrutinib	Complete remission	21·9	Yes
13	Follicular lymphoma grade 3A	63	3	Skin biopsy	TP53	CD19, CD20, CD79a	Bortezomib, cladribine, dexamathasone	Complete remission	19·3	Yes
14	T-cell prolymphocytic leukaemia	40	2	Peripheral blood	No alterations detected	CD3	Venetoclax	Partial response	13·9	No
15	Acute myeloid leukaemia	76	4	Bone marrow	No alterations detected	CD34, CD117	Azacitidine	Partial response	3·6	Yes
16	Diffuse large B-cell lymphoma	53	3	Dissociated lymph node	TP53	CD19, CD79a	Pixantrone, idelalisib, obinotuzumab	Partial response	7·4	Yes
17	Diffuse large B-cell lymphoma	50	3	Bone marrow	TP53	CD19, CD20, CD79a	Azacitidine, panobinostat, atorvastatin	Stable disease	3·3	No

Figure S4



control wells. For hierarchical clustering of ex-vivo drug responses, all RBF values per patient and marker combination were first averaged over technical replicates and concentrations per drug, and subsequently normalised into pharmacoscopy scores via (1−RBF)/max (1−RBF). Thus, a pharmacoscopy score of 1 represents the strongest on target ex-vivo response, a pharmacoscopy score of 0 indicates no ex-vivo effect, and negative pharmacoscopy scores indicate ex-vivo chemoresistance.

RBF > 1 => blast resistant to drug
RBF = 1 => no change
RBF < 1 => blasts sens. to drug

Figure S4. Clustering of pharmacoscopy results per patient, marker, and drug. (A) Clustering of PCY results per drug (columns) and patient sample / marker combination (rows) over all patients. Note that the number and identity of molecular markers used to identify cancer cells for each patient were adapted to clinical diagnostics results and can thus vary per patient. Colors indicate PCY scores, and selected boxes are outlined to indicate the corresponding ORR to that patient/drug combination (see legend).

- Most cases, 139 drugs at 2 concentrations
- 5 technical replicates
- RBF = (averaged fraction blasts after drug treatment) / (averaged fraction blasts treated with DMSO)

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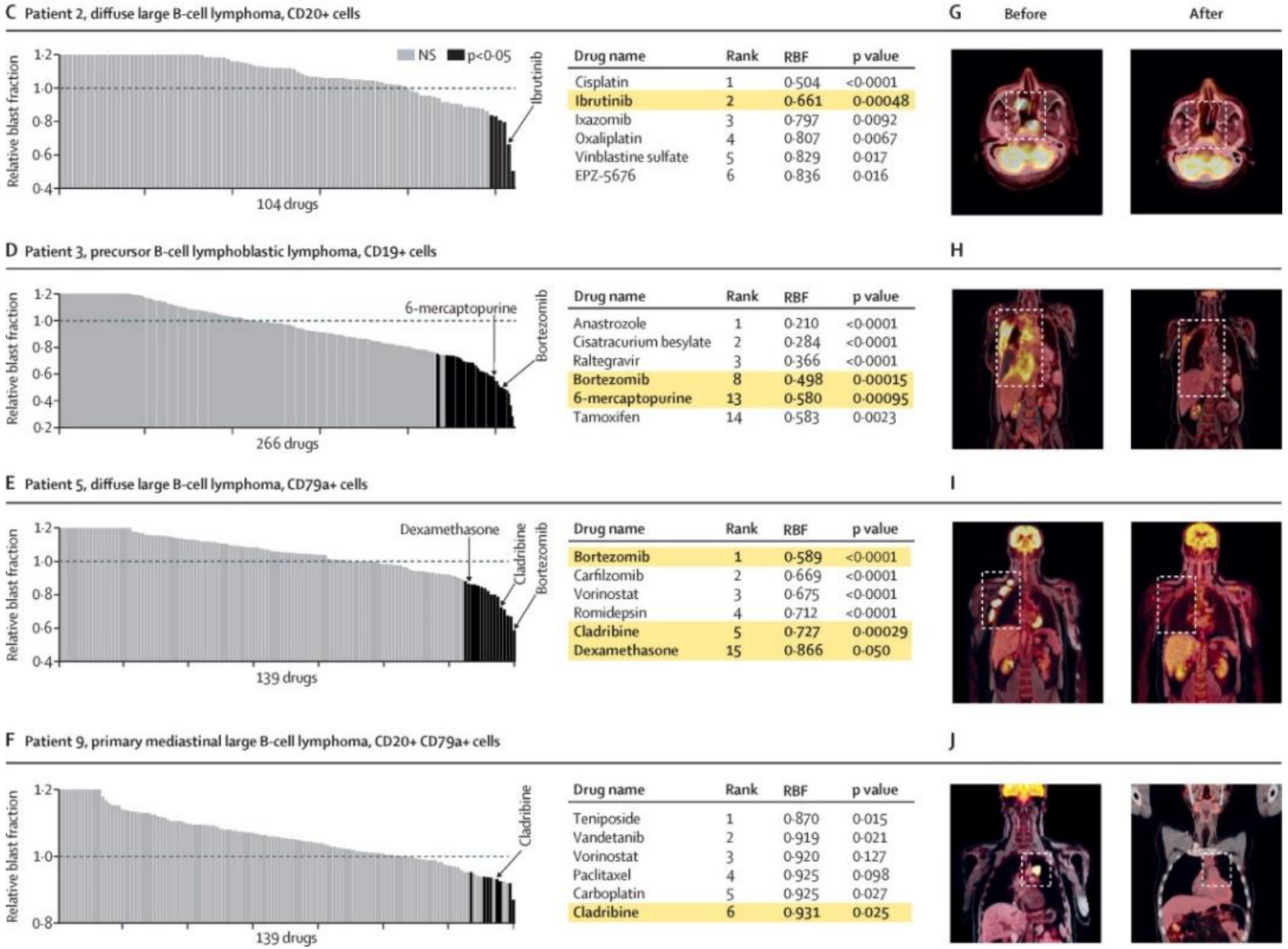
Example of patients with complete or partial response



RBF (relative blast fraction) values capped at 1.2, line = DMSO-control levels, p-values from two-tailed t test against control, before = before last relaps, after = after PC-guided treatment

Patient 2:

- 69-year old man
- Diffuse large B-cell lymphoma
- Relapsed after seven lines of previous treatment
- Resitant against most 104 tested drugs
- Six drugs had significant *ex-vivo* on-target effects
- Cisplatin and oxaliplatin ruled out due to patient's history, age, and comorbidities
- Ibrutinib (Bruton's tyosine kinase inhibitor) second strongest *ex-vivo* efficacy

=> Complete remission (PET-CT on day 49)

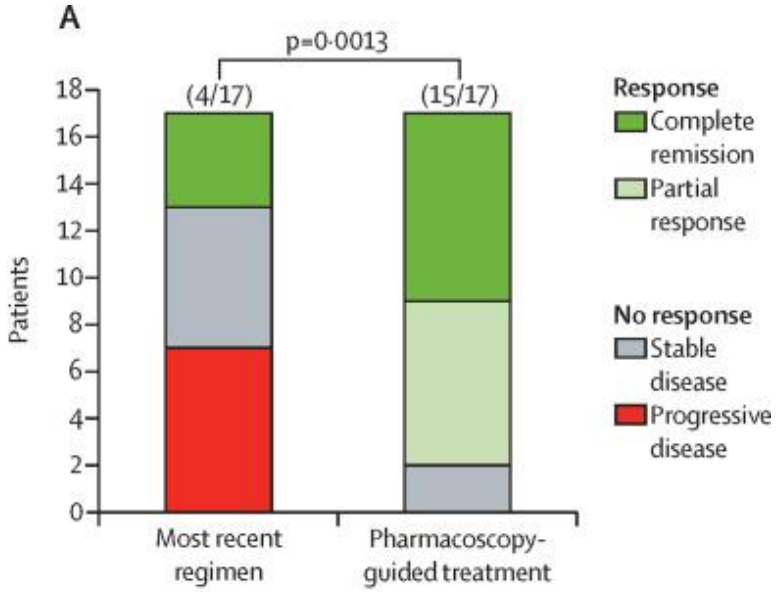
- Ibrutinib <-> *MyD88* mutation

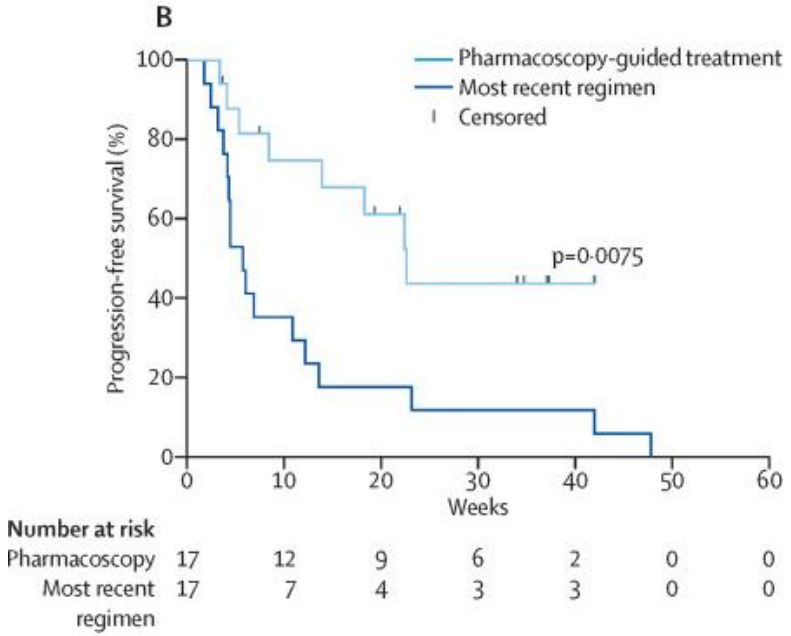
=> Subsequent sequencing: *MyD88* mutation

(Btw, this study also shows that cells for PC can also be isolated from pleural effusion or lymph nodes...)

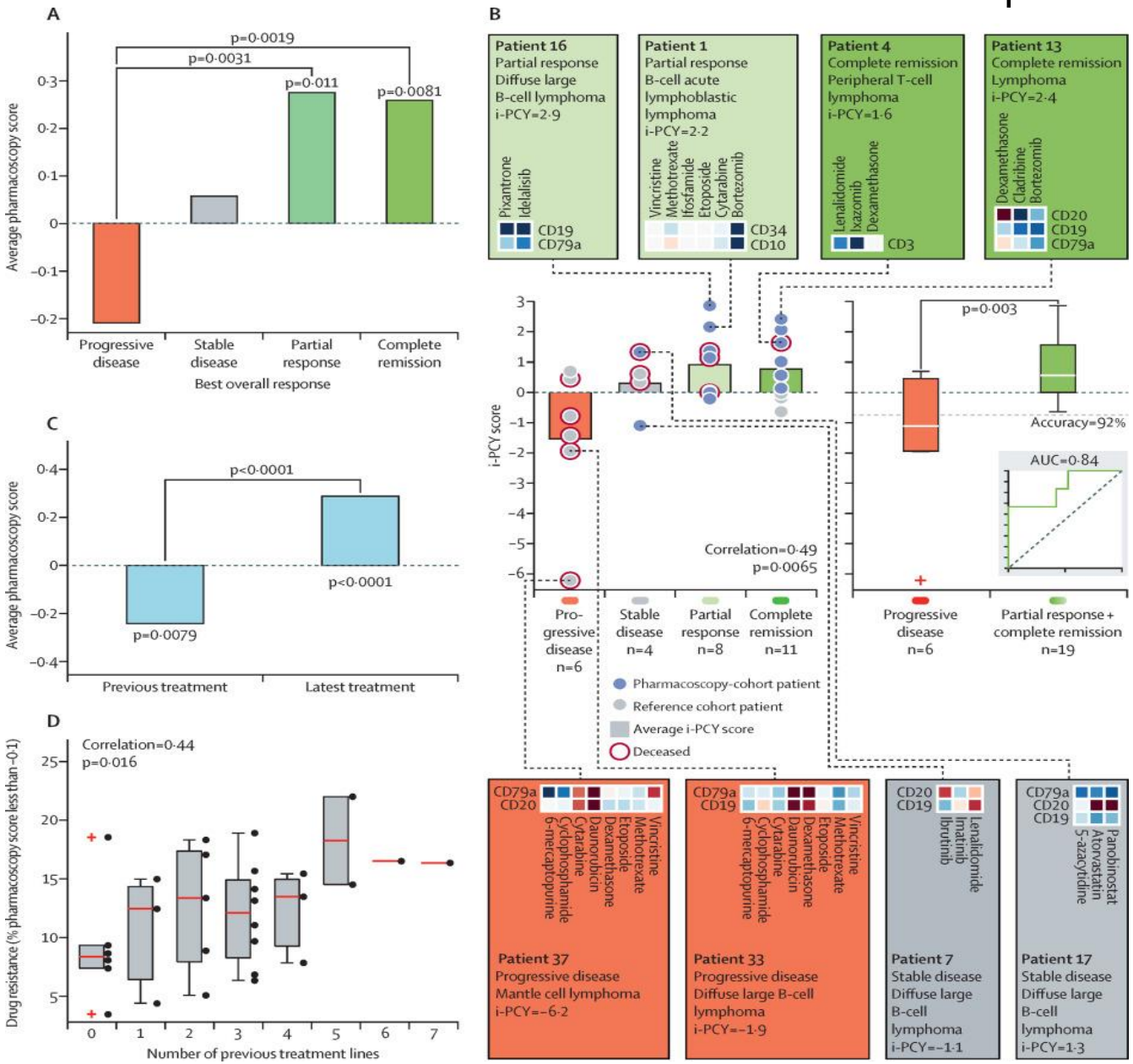
Overall response and progression-free survival is improved in pharmacoscopy-guided treatment

- 88% (15/17) of patients receiving PCY-guided treatment had an overall response compared with 24% of the (4/17) patients with their most recent regimen
- Median progression free survival increased by four times





Is chemoresistance measured by PCY predictive of poor clinical response?



- Correlation between ex vivo-chemoresistance and poor clinical outcome
- Overall, the integrated-PCY score separated progressive disease from patients with response (classif. acc. of 92%)

- A: treatments leading to disease progression had negative PCY scores

  =>all patients have history of failed treatments

  =>check PCY score of these compounds from the failed treatments

  =>negative values indicated chemoresistance: cancer cells live, by-stander cells die (off-target > on-target effect)

  => plotting of <u>average</u> PCY score <u>over all markers</u> and drugs in relation to associated overall response (i.e. all cells)
- C: The treatments to which the patients had relapsed before PCY testing had on average negative PCY scores
- D: Percentage of tested drugs with *ex-vivo* resistance (PCY < 0.1) increased with the number of previous treatment rounds

Summary & Discussion

- Technical feasibility of PCY for patients with aggressive haematological malignancies was proven
  - No randomized control group
  - Small cohort
  - Prospective study in which every patient acted as their own control
  - Feedback to treating physician within 5 days!
- Test-guided treatments lead to significantly longer progression-free survival and improved overall response in patients with various hematological malignancies
- Retrospective study was able to predict clinical response to first line AML treatment with high accuracy
- The same read-out guided selection of treatments in prospective study and predicted both good as well as poor clinical responses

Summary & Discussion

- This study shows that a wide array of working chemotherapeutics already exists, that can kill even multirefractory cancer cells but only if the right drugs are selected at the right time for each individual patient
- PCY is a helpful tool for personalized medicine: choice of therapy / diagnostic tool
  - Complex interplay of various molecular parameters taken into account such as genetic, proteomic and metabolic state of responding cell and their interactions with other cells
  - Assessment of health status of patient
  - Synergy with genomics & proteomics

Thank you for your attention!