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Immune infiltration correlates with *TP53* mutational status in a multi-cohort acute myeloid leukemia study

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- Research support; Kura Oncology, San Diego, CA

Background – I

- Chemotherapy remains the standard of care for most patients with AML, in spite of recent approvals of novel drugs
- We recently identified immune subgroups of AML ('immune-infiltrated' and 'immune-depleted') that predict chemotherapy resistance but also response to flotetuzumab immunotherapy (#O460 at ASH 2019)
  - The genetic drivers of immune infiltration in AML are presently unknown
- *TP53* mutations occur in 8-10% of *de novo* AML cases and are associated with chemotherapy resistance, high risk of relapse and dismal prognosis even after HSCT
- The functional consequences of *TP53* mutation/inactivation on host immune regulation have been largely overlooked in AML
  - The *TP53* mutants studied thus far in AML do not show any evidence of GOF mechanisms (Boettcher S, et al. *Science* 2019)

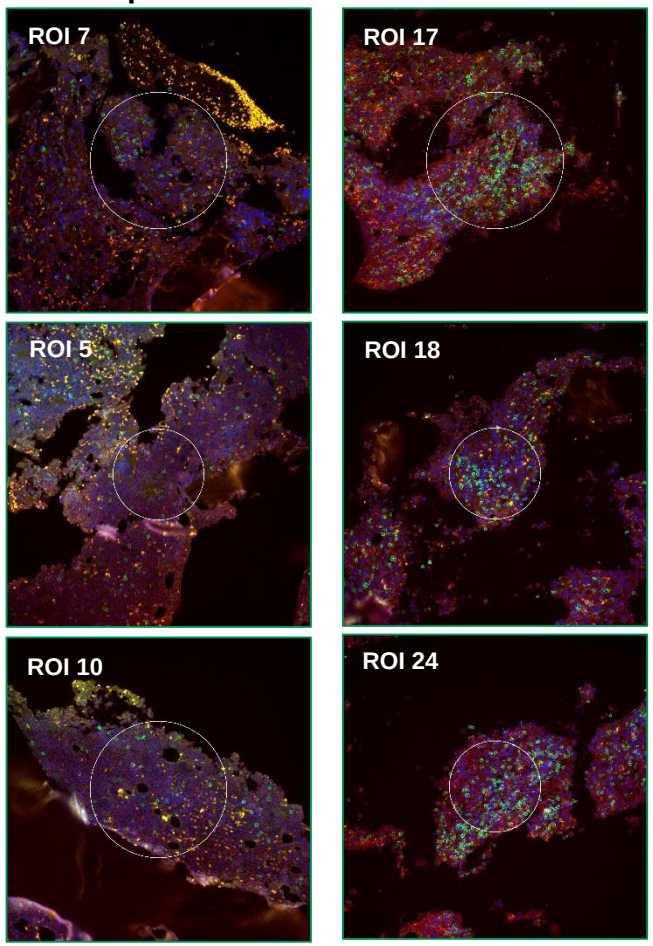
Background – II

- *TP53* suppresses inflammatory responses in mice (Komarova EA, et al. *FASEB J.* 2005)
- *TP53* inactivation in T cells enhances differentiation to Th17 cells and promotes spontaneous autoimmunity in mice (Zhang S, et al. *FASEB J.* 2011)
- *TP53* mutations are enriched in the immune favorable phenotype of breast cancer (ICR4 or Th1-dominant [PD-L1$^+$, PD-1$^+$, IDO1$^+$]) (Hendrickx W, et al. *Oncoimmunology* 2017)
- *TP53* mutational status predicts clinical benefit of PD-1 blockade in lung adenocarcinoma (Dong ZY, et al. *Clin. Cancer Res.* 2017)
  - *TP53*-mutated cases show higher TMB and increased expression of T cell-effector genes and IFN-γ–related genes

Preliminary work - I

A

T cell-poor ROIs | **T cell-rich ROIs**



Blue: Nuclei
Green: CD3
Red: CD123

240 ROIs
(>7,400 data points)

10 BM FFPEs (SAL cohort)

B

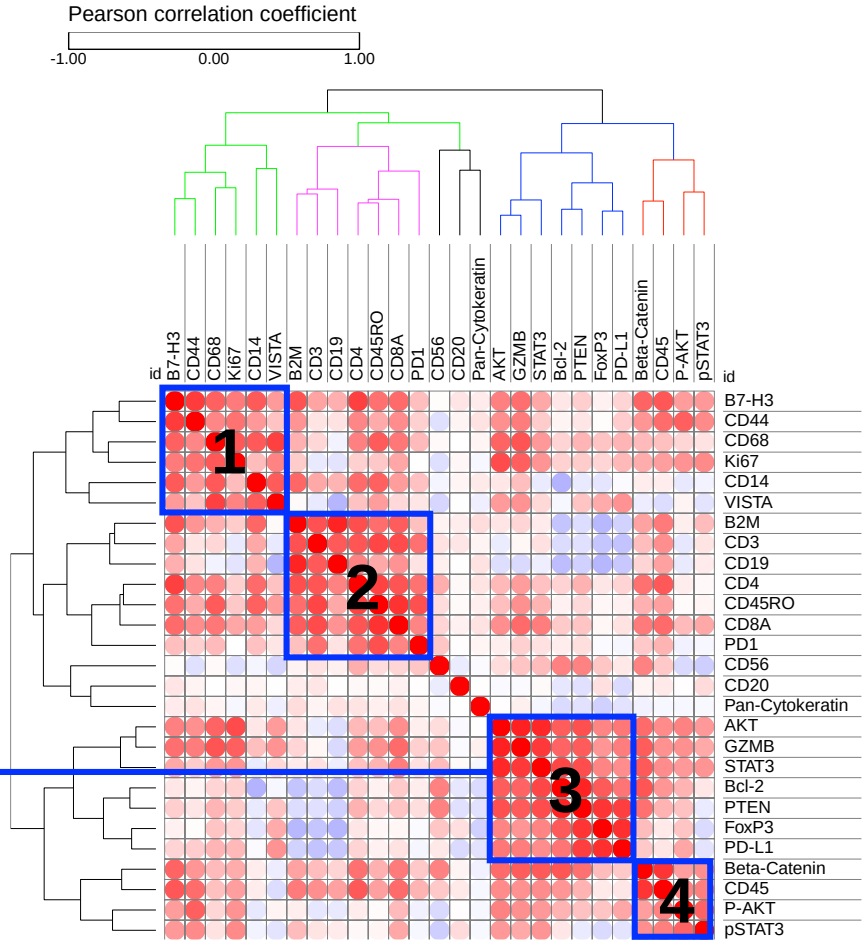
Protein co-localisation profiles

Signature 1 B7H3, CD44, CD68, Ki-67 (MKI67), CD14, VISTA (B7H5)

Signature 2 B2M, CD3, CD19, CD4, CD45RO (PTPRC), CD8A, PD1 (PDCD1)

Signature 3 AKT1, GZMB, STAT3, Bcl-2, PTEN, FoxP3, PD-L1 (CD274)

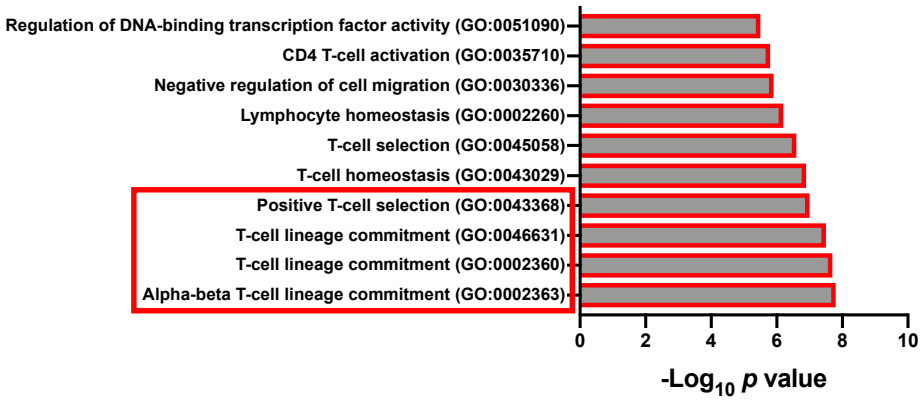
Signature 4 β-catenin, CD45, P-AKT, P-STAT3



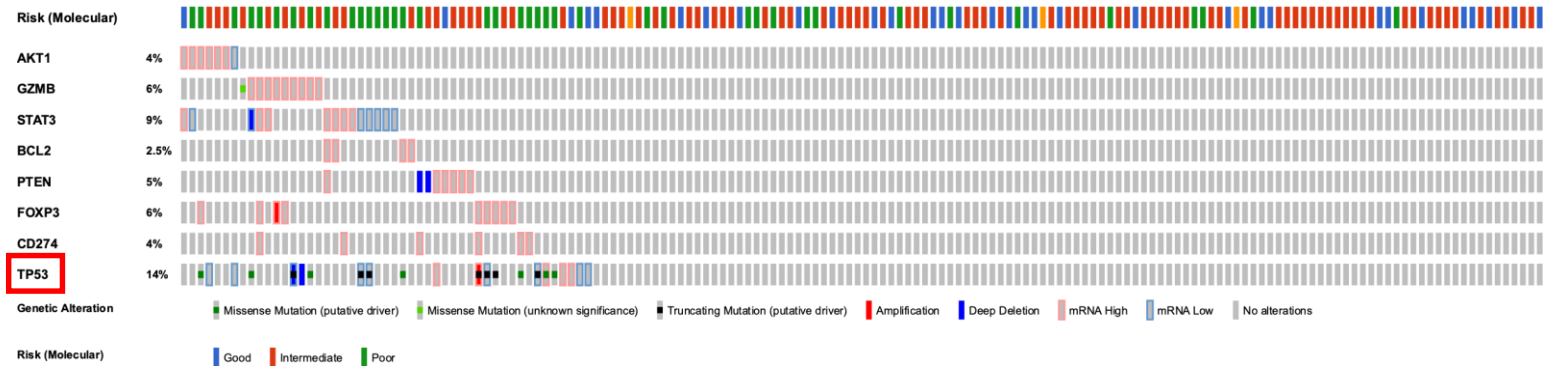
Vadakekolathu J, *et al*. bioRxiv 2019; DOI: 10.1101/702001. Under revision.

Preliminary work - II

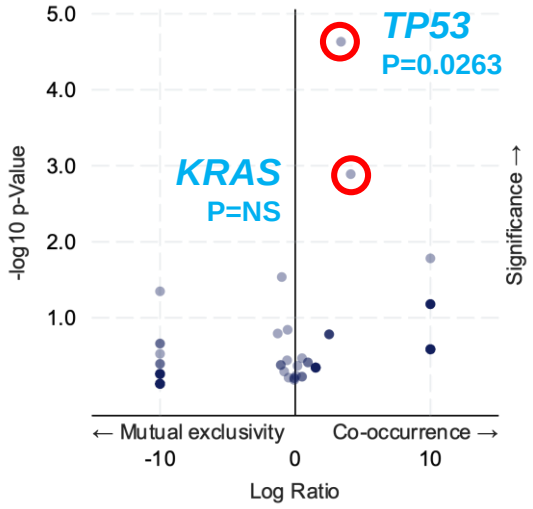
A Gene ontology (GO) biological processes



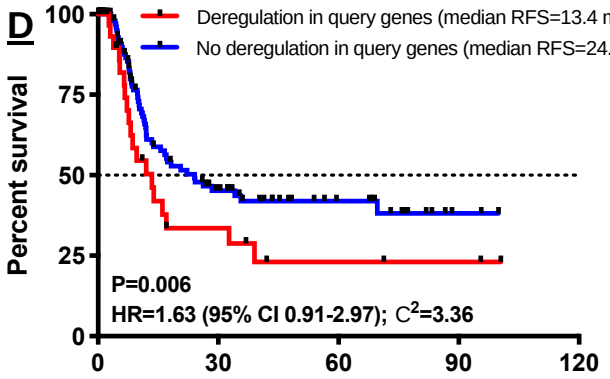
B Deregulated in 42 (26%) of 162 sequenced TCGA cases (cBioPortal)



C Mutation enrichment

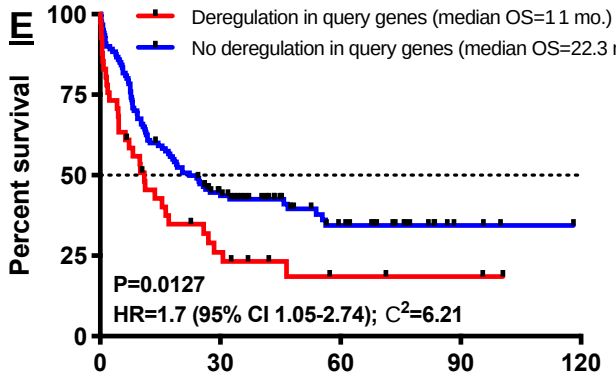


D



Number at risk					
Not deregulated	113	33	15	3	1
Deregulated	40	8	4	3	1

E



Number at risk					
Not deregulated	120	45	19	4	1
Deregulated	41	10	4	3	1

Vadakekolathu J, *et al*. bioRxiv 2019; DOI: 10.1101/702001. Under revision.

Graphical ‘cohorts and methods’

A

***In silico* analyses (TCGA-AML + Beat AML Master Trial®)**

Discovery cohort (TCGA)
(147 non-promyelocytic AMLs;
13 with *TP53* mutation; 9%)

Validation cohort (Beat AML)
(140 non-promyelocytic AMLs;
17 with *TP53* mutation; 12%)

Immune gene and biological activity signatures (n=45)
(computed as in Danaher P, *et al*. JITC 2017 and 2018)

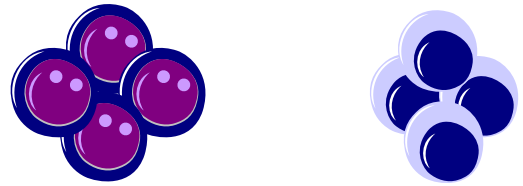
Correlation with prognostic molecular lesions (*TP53* mutational status, *NPM1* mutational status, *FLT3*-ITD status, CHIP-defining mutations) and clinical outcomes (Cox PH)

B

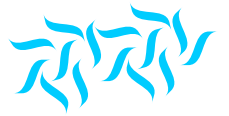
Primary AML blasts

TP53-mutated (n=36);
n=38 *TP53* mutations

TP53-wt (n=24)



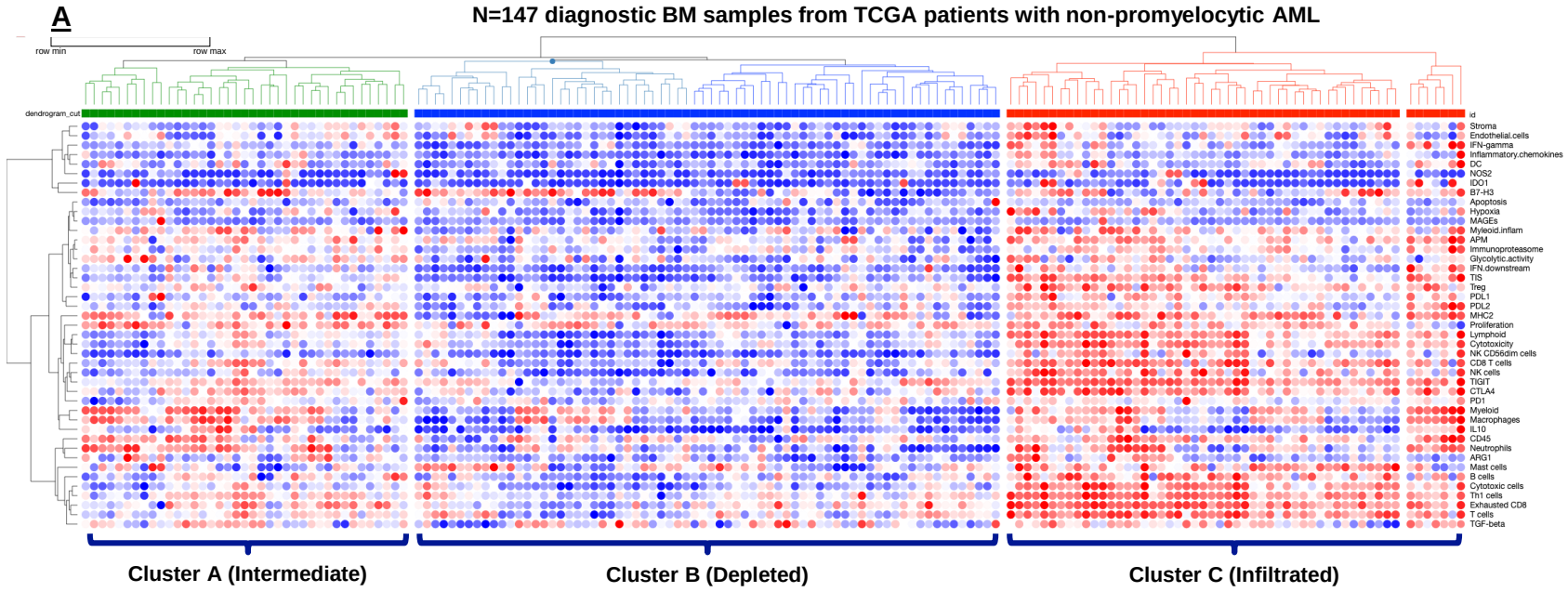
RNA extraction

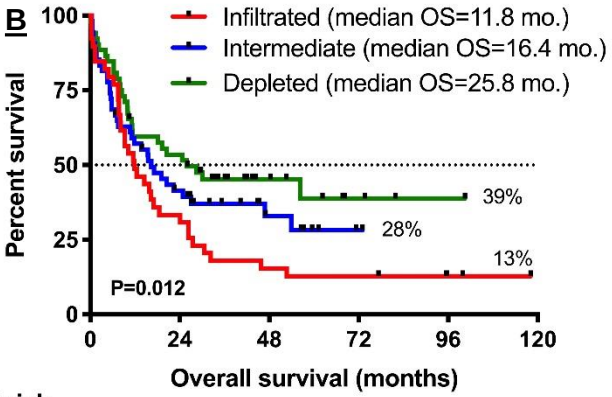


Targeted Immune GEP
(IO360™ PanCancer Panel) - RUO

DE genes
GO ontologies (METASCAPE)
Network analysis

Immune subtypes predict outcomes



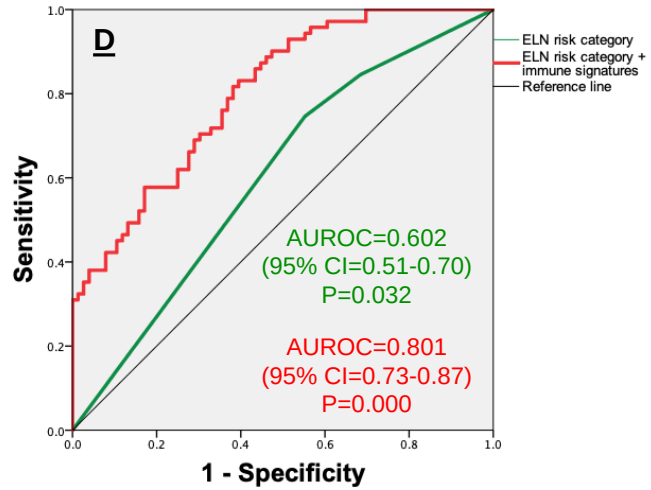


Number at risk

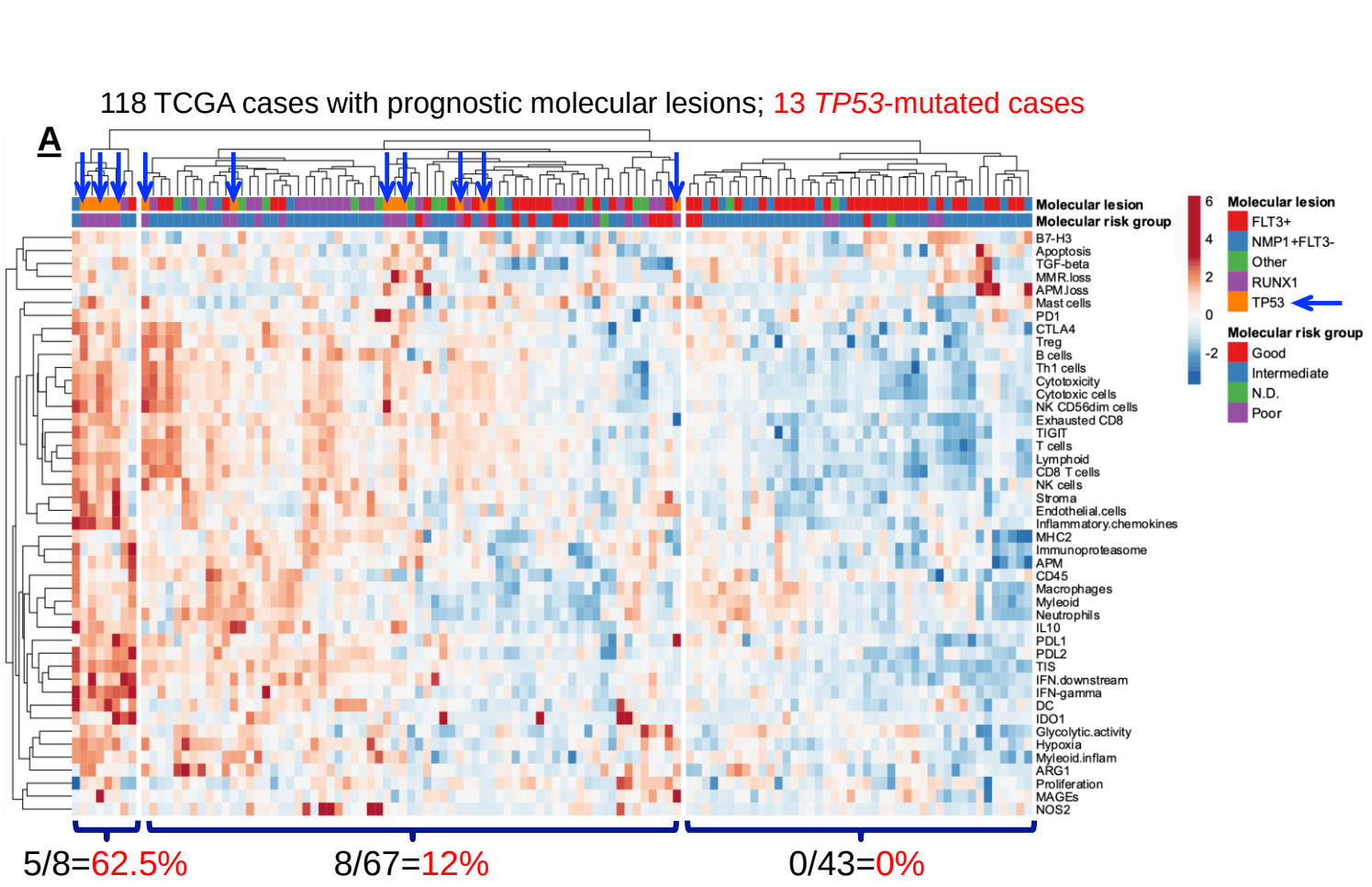
Depleted	52	45	32	27	15	5
Intermediate	54	42	32	20	12	3
Infiltrated	39	32	22	13	8	1

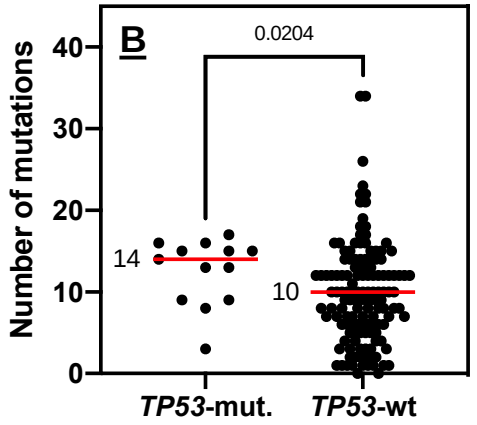
C

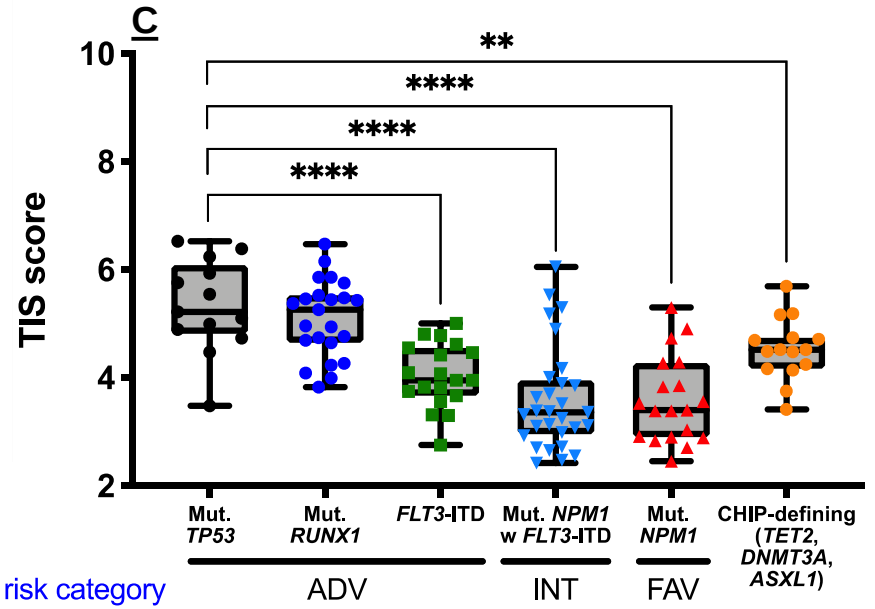
Cox PH Model (Overall Survival)	Wald	*P* value
Inflammatory chemokine score	6.444	0.011
IDO1 score	4.890	0.027
IFN-γ score	4.391	0.036
B7H3 score	4.021	0.045
ELN cytogenetic risk	3.881	0.049



‘Hot’ TIME in *TP53*-mutated AML







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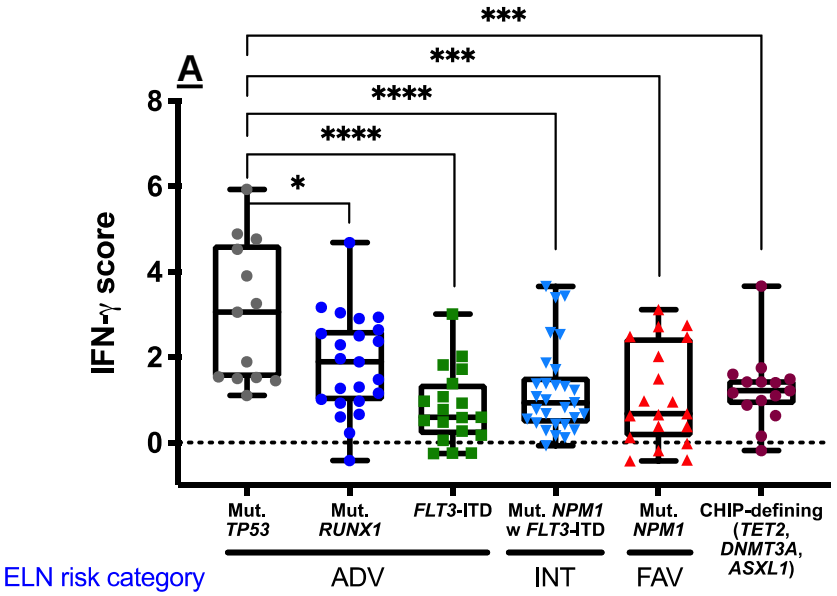
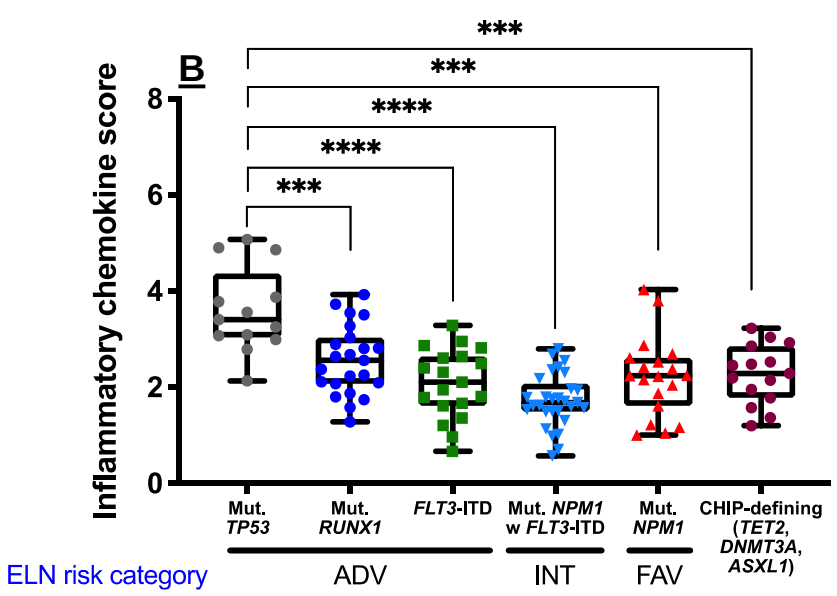
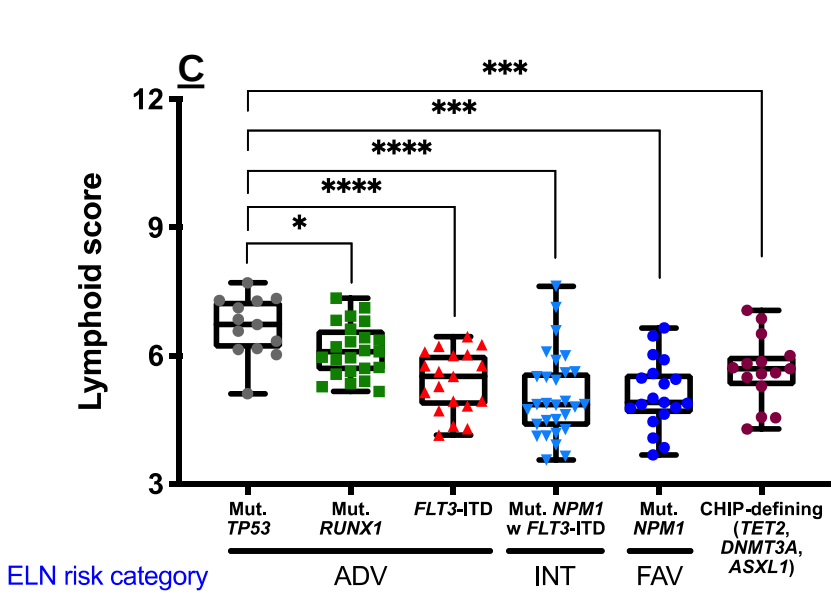
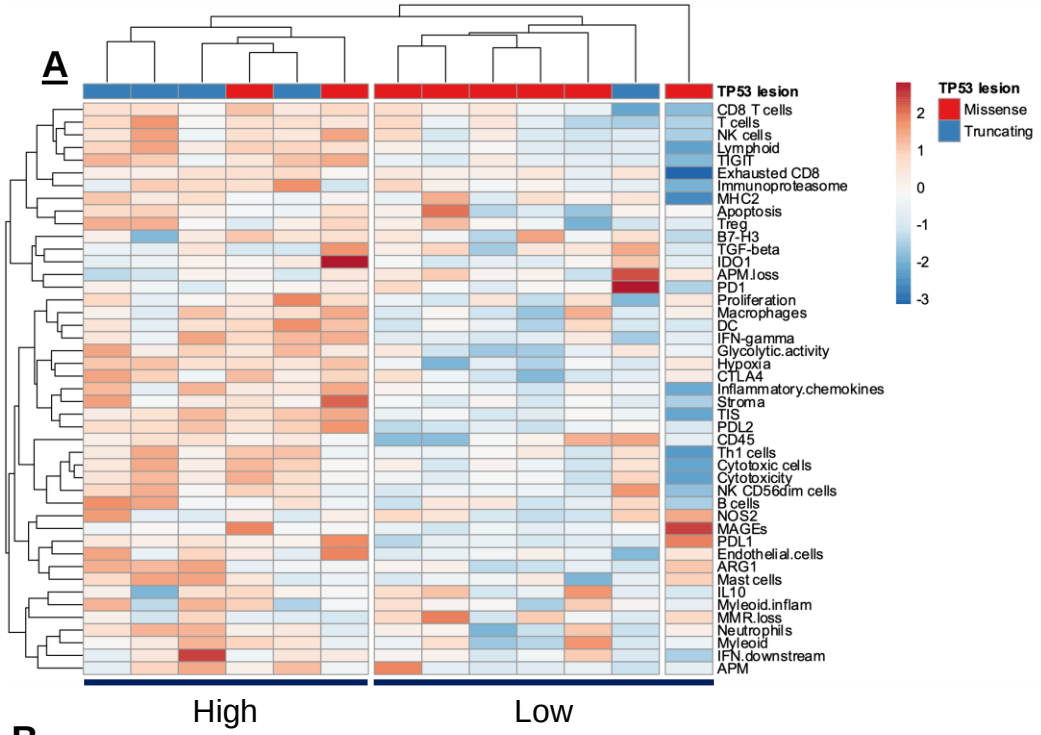




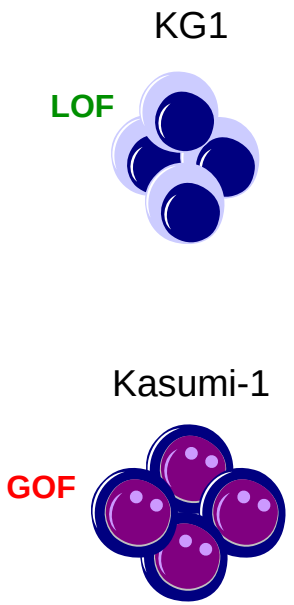


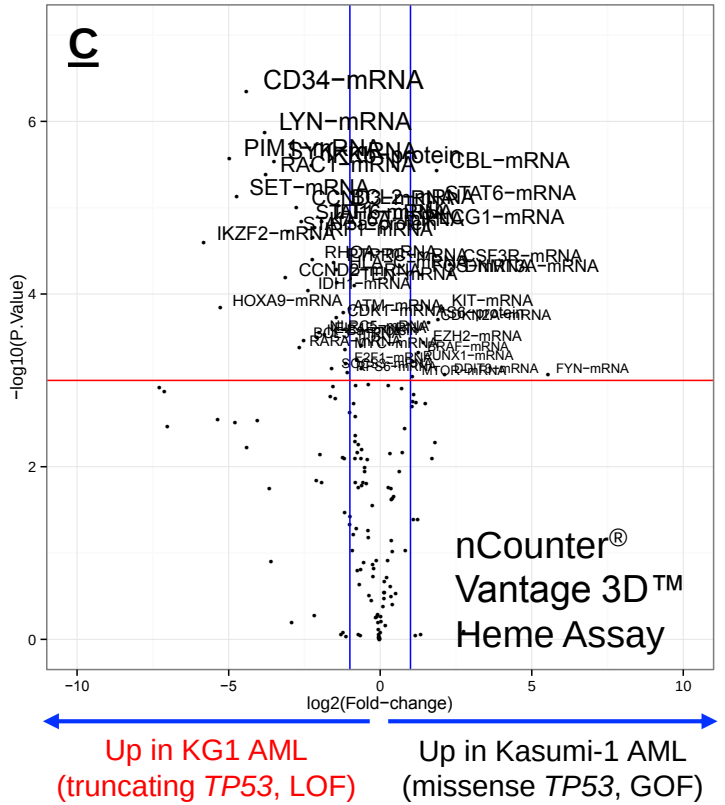
Immune scores in *TP53* LOF/GOF AML



B

	TP53 mutation	
	Truncating	Missense
Immune infiltration		
High	4	2
Low	1	6





Up in KG1 AML (truncating *TP53*, LOF)

Up in Kasumi-1 AML (missense *TP53*, GOF)

Cancer Cell Line Encyclopedia (CCLE)

KG1	p.?	Splice site
Kasumi-1	p.R248Q	Missense

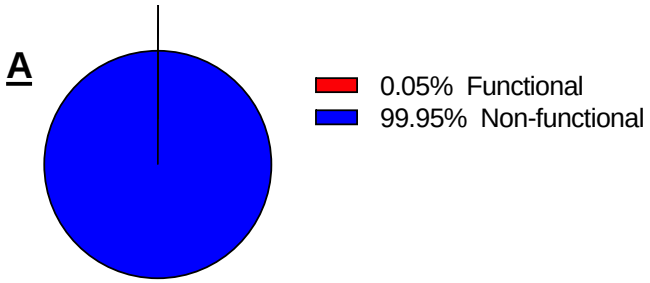
https://portals.broadinstitute.org/ccle

IFN and inflammation pathway molecules (up in KG1)	
HGF	*IL2RA*
CIITA	*STAT1*
PIM1	*IRF1*
	OSM
	LCK
	LYN

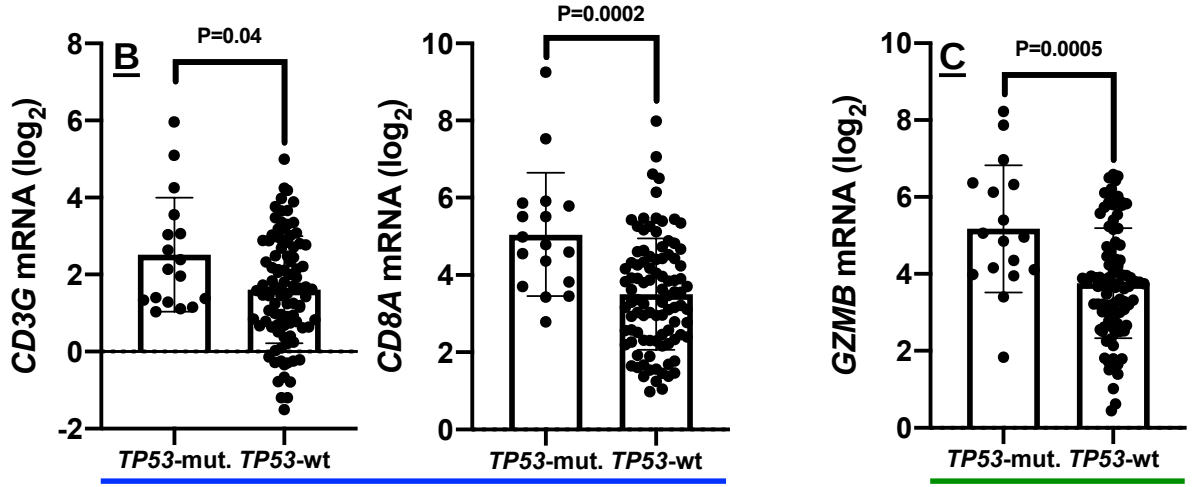
Beat AML Master Trial® *TP53*-mutated AML

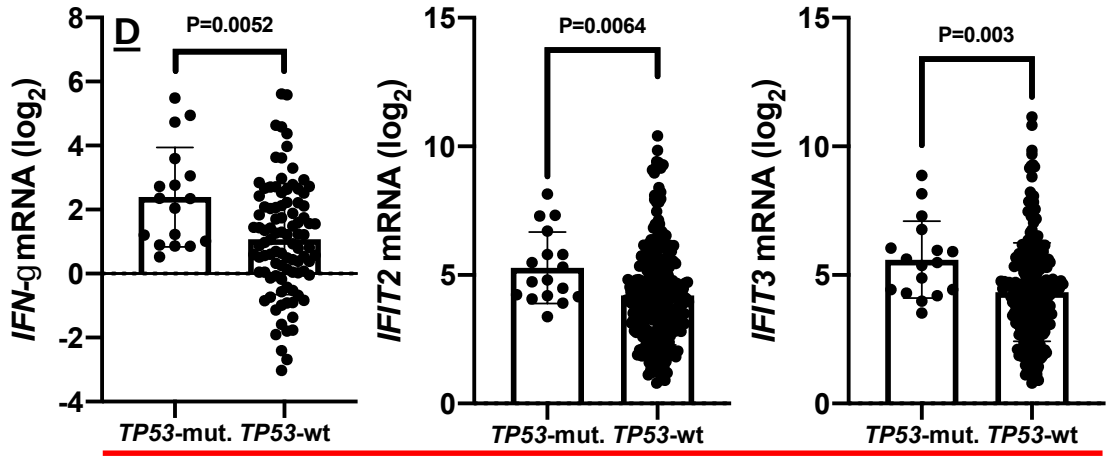
140 non-promyelocytic AML tested for *TP53* mutation (n=17 positive; 12%).

RNA-sequencing data accessed through www.vizome.org.



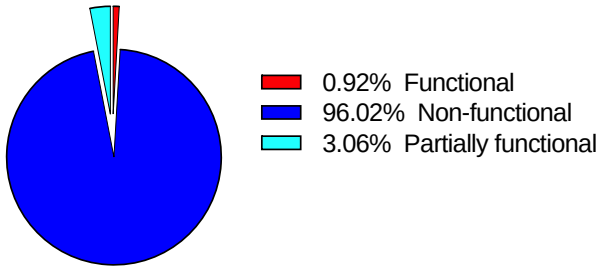
6 mutations not found in the IARC *TP53* database





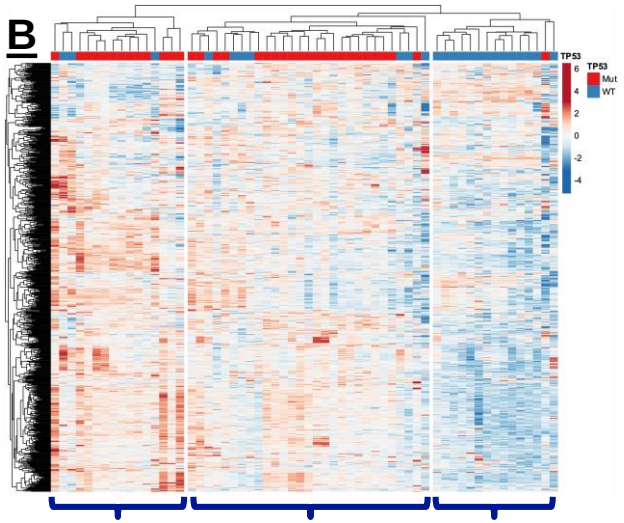
Immune GEP in *TP53*-mutated AML BMs

A



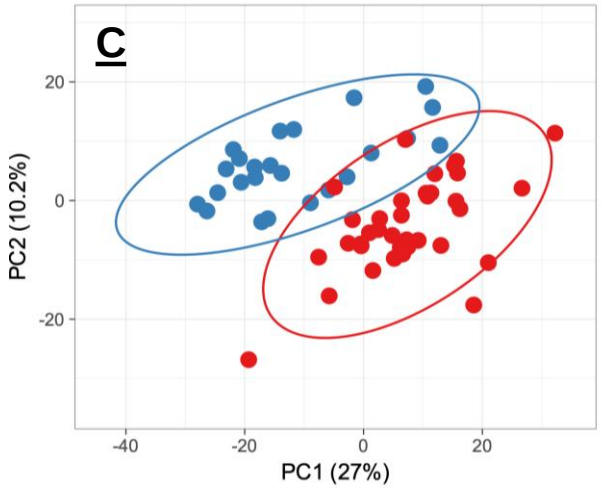
6 mutations not found in the IARC *TP53* database

B



Mut.=13/16 (81%) 22/29 (76%) 1/15 (7%)

C

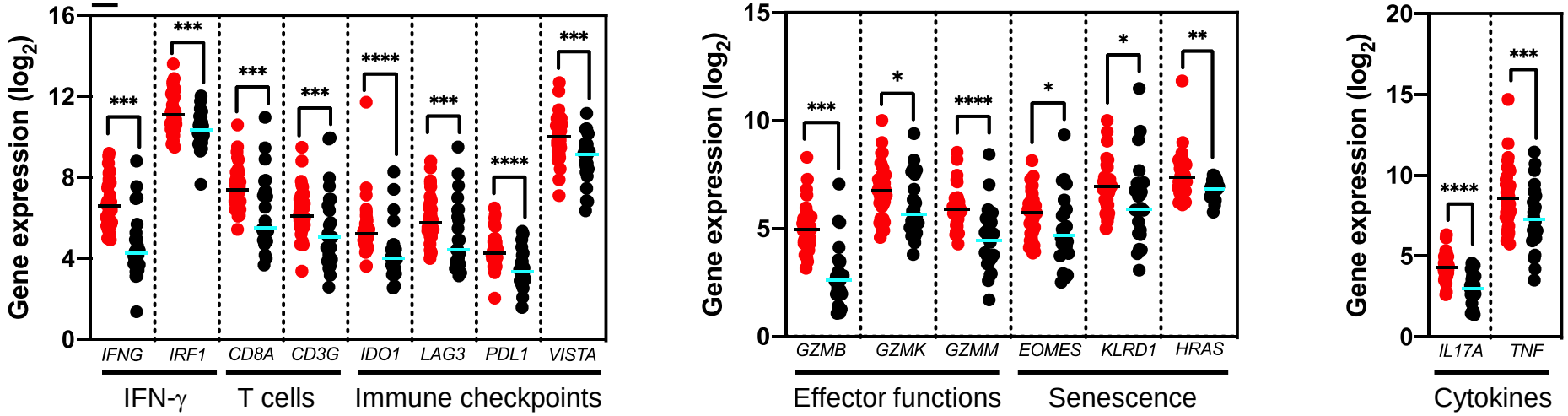


TP53-mutated AML
TP53-wt AML

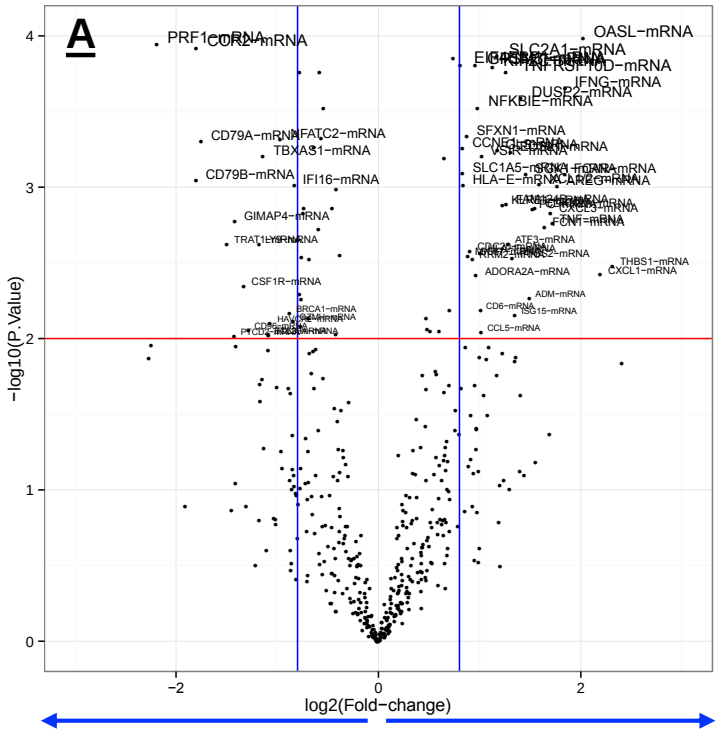
D

TP53-mutated AML
TP53-wt AML

*P<0.05
**P<0.01
***P<0.001
****P<0.0001



DE genes in *TP53*-mutated AML BMs



Up in *TP53*-wt AML (n=24 cases)

Up in *TP53*-mut. AML (n=36 cases)

IFN and inflammation pathway (up in *TP53*-mut. AML)

IL-33
IL-6
IFN-γ

OASL
RIPK2
TNFAIP3
CSF1
PTGER4

Top 25 DE genes (log-FC >1.6)

B

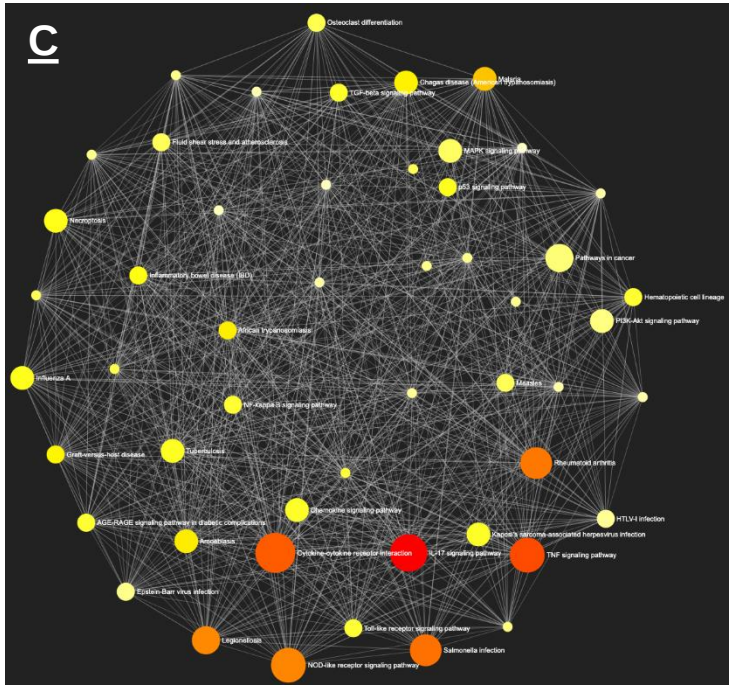
ID	Log FC	P value
THBD	3.32856775	2.35×10^{-10}
IL33	2.76524296	4.49×10^{-6}
CCL3/L1	2.73524144	1.02×10^{-6}
IL6	2.50824515	5.30×10^{-5}
THBS1	2.31796505	0.00330311
CXCL8	2.30207249	3.31×10^{-5}
RIPK2	2.20959349	2.17×10^{-12}
CXCL1	2.19815369	0.00376795
CSF1	2.04674703	2.98×10^{-5}
OASL	2.028931	0.00010347
PTGER4	1.89650468	1.54×10^{-9}
IFNG	1.85193487	0.00022149
DUSP5	1.84974391	1.54×10^{-7}
FCAR	1.84846687	0.00082265
BBC3	1.83044505	2.45×10^{-6}
CXCL2	1.81007542	6.23×10^{-5}
AREG	1.77348512	0.00097531
MYCT1	1.74250168	9.78×10^{-5}
TNF	1.72575676	0.0017361
CXCL3	1.70533983	0.00147901
TNFAIP3	1.66978448	1.47×10^{-5}
FCN1	1.64769014	0.00183543
SMAD5	-1.7042361	1.24×10^{-8}
CD79A	-1.7430675	0.00049201
CCR2	-1.7946521	0.00011963
CD79B	-1.794843	0.00089026
NT5E	-1.8445725	1.63×10^{-5}
GIMAP6	-2.0200277	3.95×10^{-5}
CD19	-2.1700794	4.43×10^{-5}
PRF1	-2.1827182	0.00011301
CXCR2	-2.2465728	7.37×10^{-5}
IL11	-3.2501286	3.18×10^{-13}
MS4A1	-3.3935679	1.06×10^{-8}
VTCN1	-3.8084555	4.99×10^{-21}
MARCO	-3.9024322	3.51×10^{-6}
CX3CR1	-5.0519174	8.85×10^{-9}

Up in *TP53*-mutated AML

Up in *TP53*-wt AML

Enrichment analysis

C



KEGG	FDR
IL-17 signaling pathway	1.97×10^{-9}
TNF signaling pathway	6.45×10^{-9}
Cytokine-cytokine receptor	1.70×10^{-8}
Salmonella infection	5.41×10^{-8}
Rheumatoid arthritis	7.61×10^{-8}
NOD-like receptor signaling	1.83×10^{-7}

Conclusions

- *TP53*-mutated cases (TCGA-AML and Beat-AML) show higher levels of T-cell infiltration, immune checkpoints and IFN-γ signaling compared with AML subgroups with other risk-defining molecular lesions
- *TP53*-mutated primary AML BMs are enriched in IL-17, TNF and IFN signaling molecules and show higher levels of T-cell infiltration and immune checkpoints relative to *TP53*-wt primary AML BMs
- Whether *TP53*-mutated AML can be amenable to respond to T-cell targeting immunotherapies remains to be determined

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