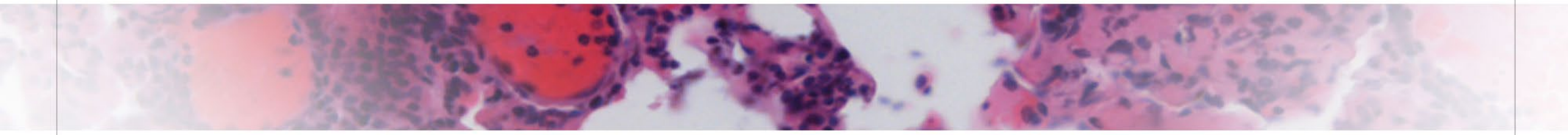
American Society of Hematology
Helping hematologists conquer blood diseases worldwide



An Immune Senescence and Exhaustion-Related RNA Profile Predicts Clinical Outcomes in Acute Myeloid Leukemia

Jayakumar Vadakekolathu[1], Tung On Yau[1], Heidi Altmann[2], Sarah E. Church[3], Hanna Knaus[4], Mark D. Minden[5], Jan Davidson-Moncada[6], Sarah K. Tasian[7], Martin Bornhäuser[2], Ivana Gojo[8], Leo Luznik[8], Sergio Rutella[1,9]

[1]John van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK; [2]Department of Internal Medicine I, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; [3]NanoString Technologies, Inc., Seattle, WA; [4]Internal Medicine I, Medizinische Universität Wien, Austria; [5]Princess Margaret Cancer Centre, Toronto, Canada; [6]MacroGenics, Inc., Rockville, MD; [7]Division of Oncology and Centre for Childhood Cancer Research, Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, PA; [8]Department of Oncology, Johns Hopkins University, Baltimore, MD; [9]Centre for Health, Ageing and Understanding Disease (CHAUD), School of Science and Technology, Nottingham Trent University, Nottingham, UK

Background

- Chemotherapy refractoriness and disease relapse continue to be significant obstacles to therapeutic success in AML
- We have recently identified bone marrow (BM) IFN-γ-related transcriptional profiles that stratify patients with AML into an immune-infiltrated and an immune-depleted subtype, and that refine the accuracy of survival prediction in response to conventional “3+7” chemotherapy beyond that afforded by cytogenetic and molecular prognosticators (Vadakekolathu J, *et al*. *Sci. Transl. Med.* 2020; 12: eaaz0463)
- $CD8^+$ T cells in AML exhibit features of immune exhaustion and senescence (IES), including the upregulation of natural killer (NK) cell-associated transcripts, which persist only in chemotherapy non-responders (Knaus HA, *et al*. *JCI Insight* 2018; 3: e120974)

Senescence and Exhaustion in Anti-Tumor Immunity

- Senescent and exhausted T cells with defective effector functions for tumor immunity are induced by DNA damage, MAPK and STAT1/STAT3 signaling in the tumor microenvironment
- Senescent T cells highly express Tim-3, CD57 and NK receptors, including KLRG1, and remain metabolically active, producing high amounts of TNF-α, IFN-γ and suppressive cytokines (Liu X, et al. *J. Clin. Invest.* 2020; 130: 1073-83)
- Exhausted T cells are characterized by the progressive loss of T-cell function, express high levels of inhibitory receptors (PD1, CTLA4, TIM3, LAG3), co-stimulatory receptors, T cell factor 1 (TCF1) and GZMB, and have impaired cytokine production and replicative capacity (Ghorani E, et al. *Nat. Cancer* 2020; 1: 546-61. Akbar AN, et al, *Nat. Rev. Immunol.* 2011; 11: 289-95)

Questions and Study Aims

- Are senescence and exhaustion intertwined or unrelated molecular programs that compromise immunity?
- Subpopulations of TCF1^{+} precursor exhausted T cells serve as a predictive 'biomarker' for a favorable clinical outcome of checkpoint therapy in melanoma (Siddiqui I, *et al*. Immunity 2019; 50: 195-211)
  - Microenvironmental Immune Senescence and Exhaustion in Acute Myeloid Leukemia Associate with Response to Flotetuzumab, an Investigational CD123 × CD3 Bispecific DART Molecule (Vadakekolathu J, *et a*l. Poster #2878; December 7th, ASH 2020)

- The aim of the current study was to determine whether IES correlate with immune infiltration and with clinical outcomes in treatment-naïve AML

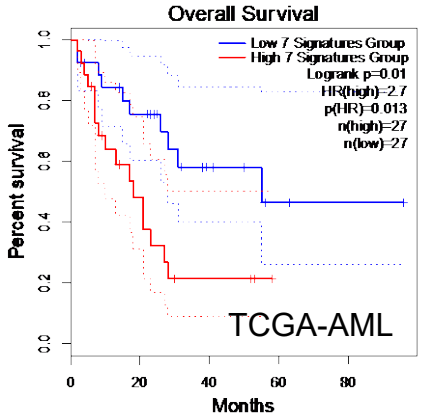
Patients and Methods

Wet-laboratory cohorts					*In silico* cohorts	
	PMCC*	**CHOP^**	**SAL^^**		**Beat AML Master Trial**	**TCGA**
Nr of patients	290	39	38		267	147
Age (y)	52 (18-81)	10 (0.1-20)	52.5 (23-75)		Adult	Adult
Disease status	Onset	Onset	Onset/CR/Relapse		Onset	Onset

- The wet-laboratory AML cohorts used in this study included a total of 367 BM samples from children and adults with AML treated with curative intent. BMs were collected at time of diagnosis, complete remission (CR) and relapse (PMCC, SAL and CHOP series)
- BM RNAs were profiled on the nCounter platform using the PanCancer Immune Profiling Panel (NanoString Technologies, Seattle, WA)
- Immune signature scores and biological activity scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets (Vadakekolathu J, et al. *Sci. Transl. Med.* 2020)

*PMCC = Princess Margaret Cancer Centre, Toronto, Canada
^CHOP = Children's Hospital of Philadelphia, Philadelphia, PA
^^SAL = Studienallianz Leukämie, Dresden, Germany

Identification of an Immune Senescence and Exhaustion (IES) Gene Signature



Gene name	Full Name
B3GAT1 (CD57)	Beta-1,3-Glucuronyltransferase 1
KIR2DL1 (CD158a)	Killer Cell Immunoglobulin-like Receptor 2DL1
KLRC1	Killer Cell Lectin Like Receptor C1
KLRC3	Killer Cell Lectin Like Receptor C3
KLRD1 (CD94)	Killer Cell Lectin Like Receptor D1
KLRF1	Killer Cell Lectin Like Receptor F1
KLRG1	Killer Cell Lectin Like Receptor G1

7-gene immune senescence (IS) signature
(knowledge, prior work)
TCGA-AML and Beat-AML

Top and bottom quartiles

"Immunologic signature" gene sets
(C7; n=4,872) (MSigDB)

GSEA

Leading-edge analysis

"Core" gene set accounting
for the enrichment signal

172 genes
("expanded IS signature")

68 in PCI Panel
61 in IO360 Panel

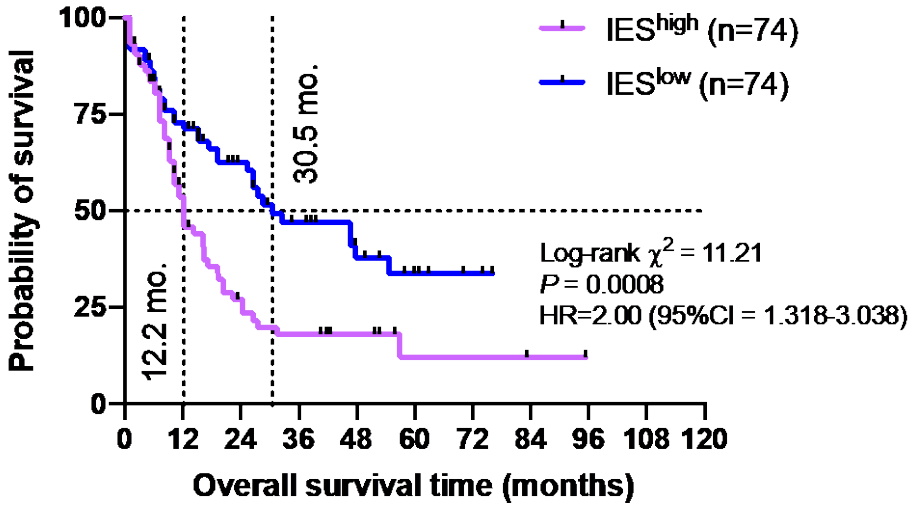
Wet-lab AML cohorts

Rutella S, et al. Unpublished (in collaboration with Johns Hopkins University).

Identification of an Immune Senescence and Exhaustion (IES) Gene Signature

7-gene signature

Prognostic Index estimated by β coefficients multiplied by gene expression values (Wagner S, et al. Blood Advances 2019)

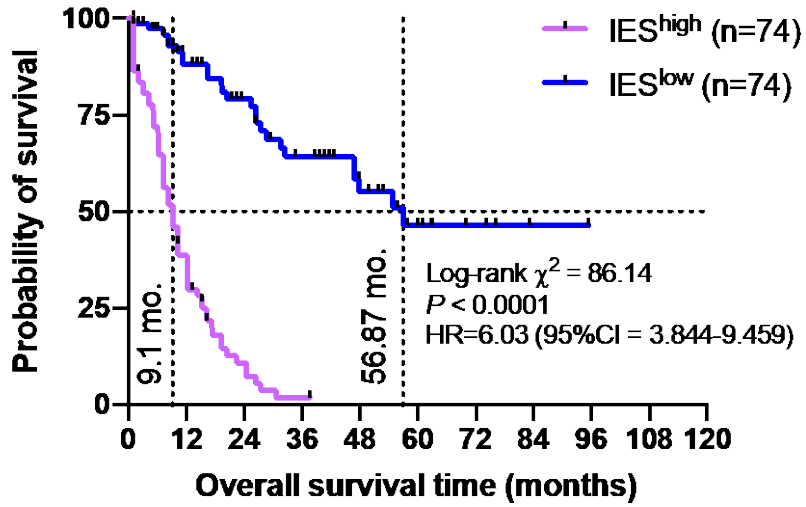


N. at risk

IES^high	74	36	16	11	7	3	2	1	1	1	1
IES^low	75	47	31	20	12	6	2	2	1	1	1

Expanded, 68-gene IES signature

Prognostic Index estimated by β coefficients multiplied by gene expression values (Wagner S, et al. Blood Advances 2019)



N. at risk

IES^high	74	26	6	1	1	1	1	1	1	1	1
IES^low	75	56	41	29	19	9	5	2	1	1	1

ASH 2020 – Rutella S, et al. Unpublished.

IES Gene Signature

Senescence genes
ABCB1
CCL4
DOCK9
DPP4
ETS1
GZMA
GZMK
GZMM
IFNG
KLRB1
KLRC1
KLRD1
KLRG1
KLRK1
LTB
PRF1
SH2D1A
TNFSF8 (CD153)

Exhaustion genes
EOMES
GZMB
ICOS
SLAMF6
TCF7

Cytokines, chemokines and their receptors
CCL5
CCR5
CCR7
CCR9
FLT3LG
IL12RB2
IL18R1
IL18RAP
IL2RB
IL6ST
IL7R

T/NK-cell trafficking
CXCR3
CXCR6

Immune checkpoints
SLAMF3

TCR signaling
CD247
CD3E
CD3G
ITK
LCK
CD3D
ZAP70

Inhibitory receptors
CD48
CTLA4

Costimulatory receptors
CD27
CD28
SLAMF1
TNFSF14 (CD258)

Surface molecules
CD40L
HLA-DOB

Unclassifiable
F2RL1
FYN
IGF2R

T-cell genes
CD2
CD5
CD6
CD7
CD8A
CD8B

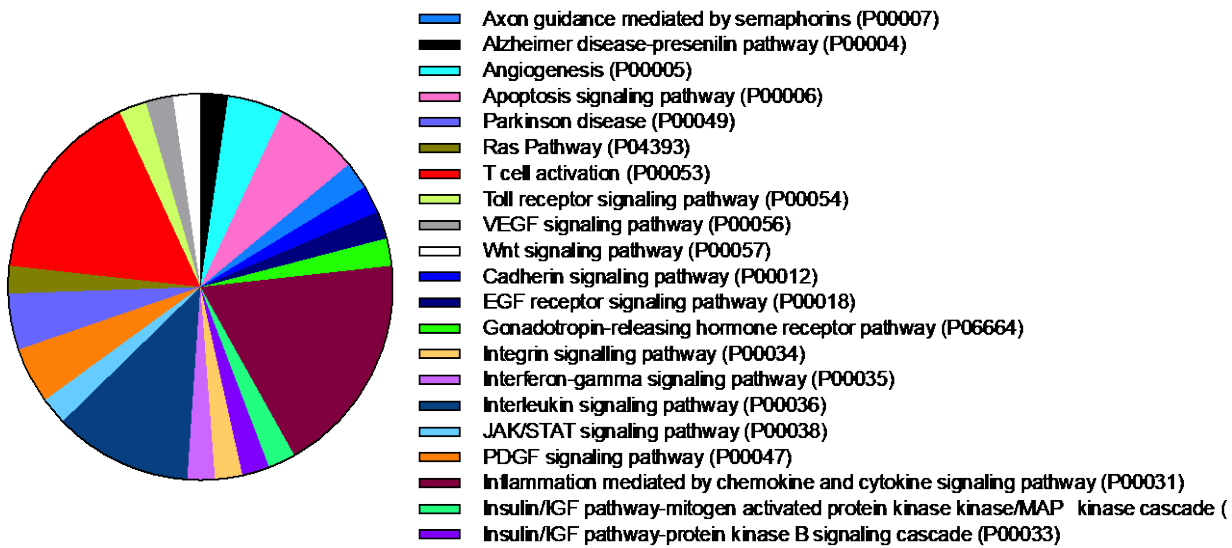
Transcription factors
GATA3
STAT4
TBX21

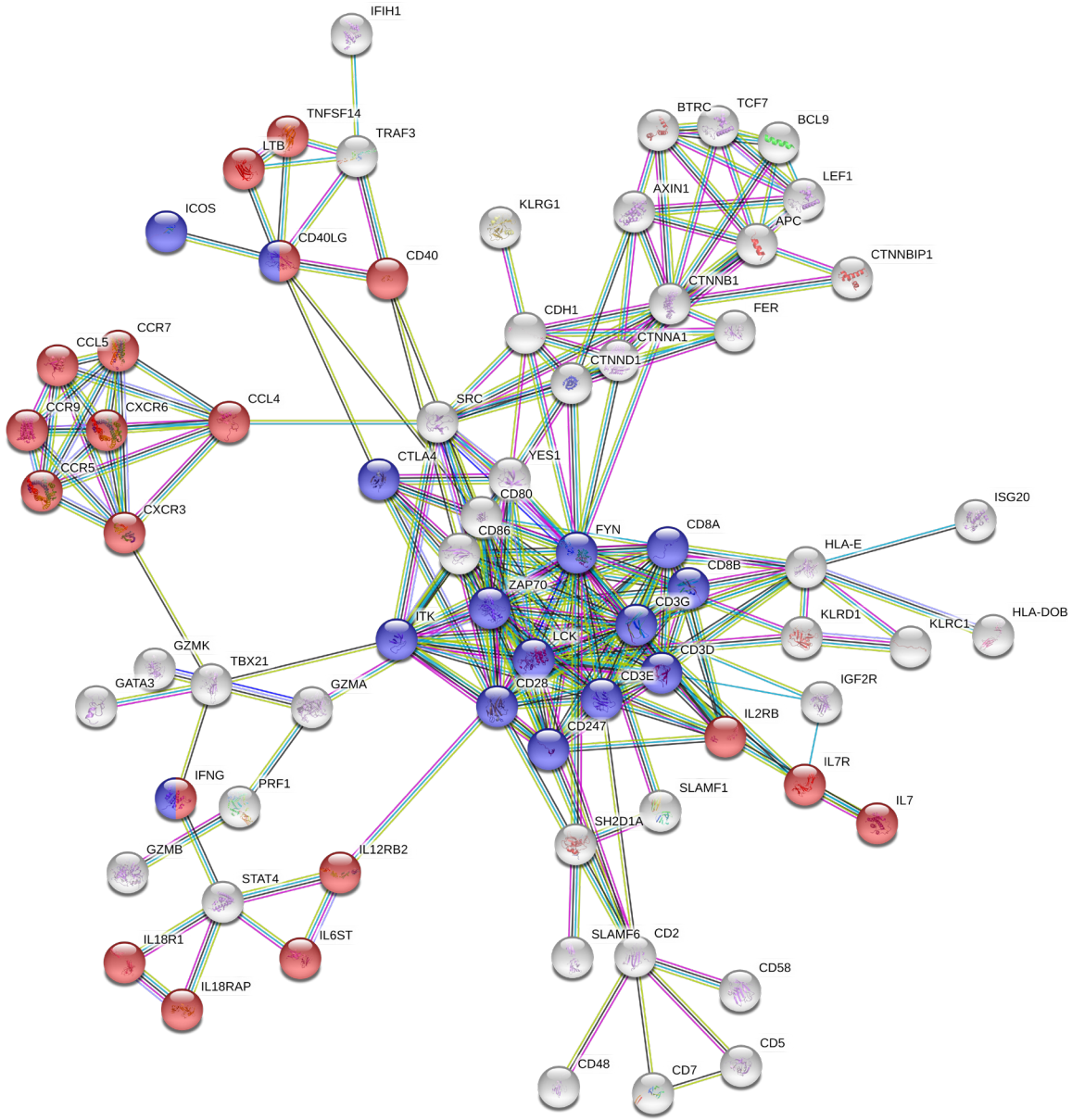
Type I/II IFN response
IL10RA
IFIH1
ISG20

Metabolic reprogramming
TLR1

Manually annotated (Knaus HA, et al. JCI Insight 2018; Kallies A, et al. Nat. Rev. Immunol. 2020; SeneQuest Portal, Gene-to-Senescence Associations)

NTU

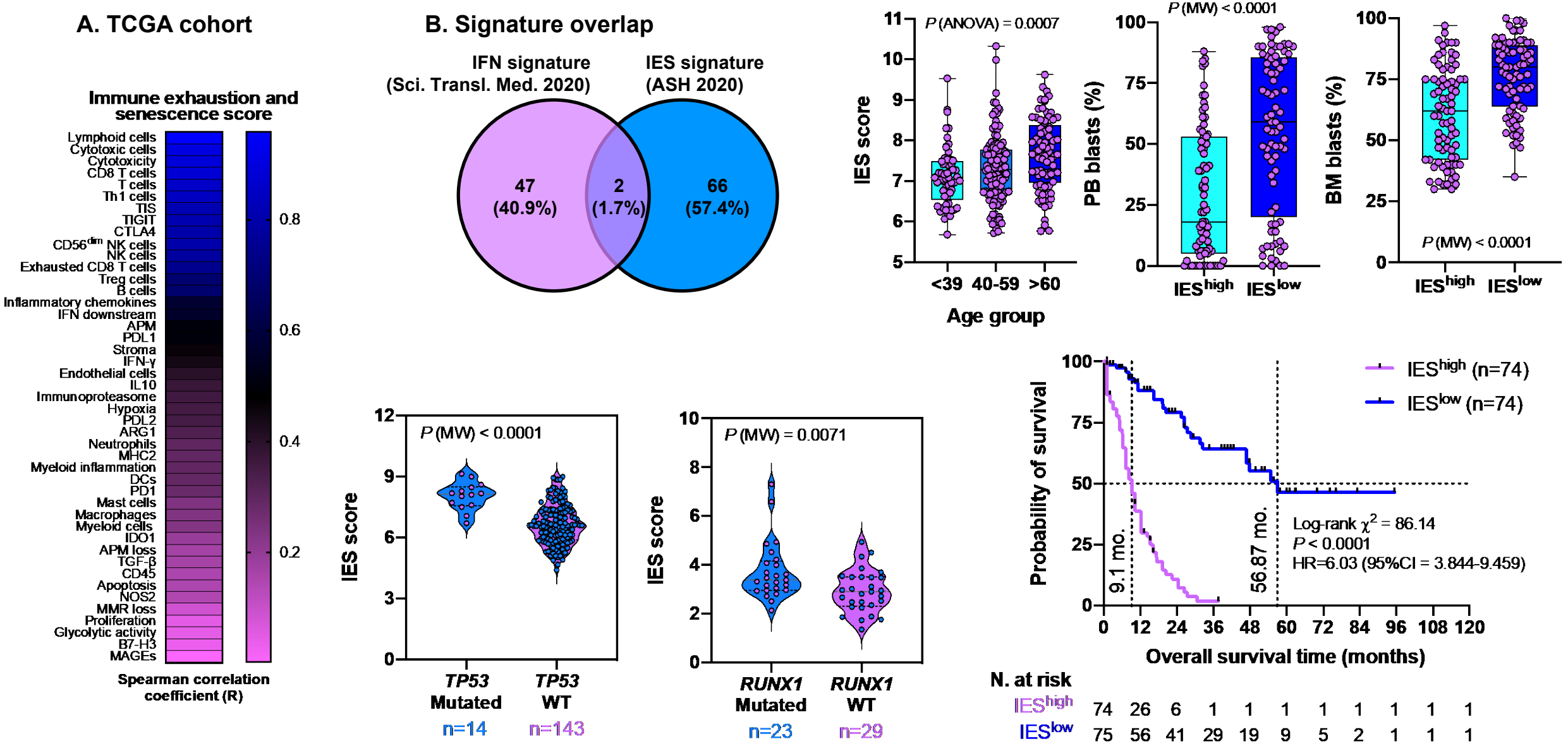

Axon guidance mediated by semaphorins (P00007)
Alzheimer disease-presenilin pathway (P00004)
Angiogenesis (P00005)
Apoptosis signaling pathway (P00006)
Parkinson disease (P00049)
Ras Pathway (P04393)
T cell activation (P00053)
Toll receptor signaling pathway (P00054)
VEGF signaling pathway (P00056)
Wnt signaling pathway (P00057)
Cadherin signaling pathway (P00012)
EGF receptor signaling pathway (P00018)
Gonadotropin-releasing hormone receptor pathway (P06664)
Integrin signalling pathway (P00034)
Interferon-gamma signaling pathway (P00035)
Interleukin signaling pathway (P00036)
JAK/STAT signaling pathway (P00038)
PDGF signaling pathway (P00047)
Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade (
Insulin/IGF pathway-protein kinase B signaling cascade (P00033)



- Cytokine-cytokine receptor interaction
- T cell receptor signaling pathway

ASH 2020 – Rutella S, et al. Unpublished.

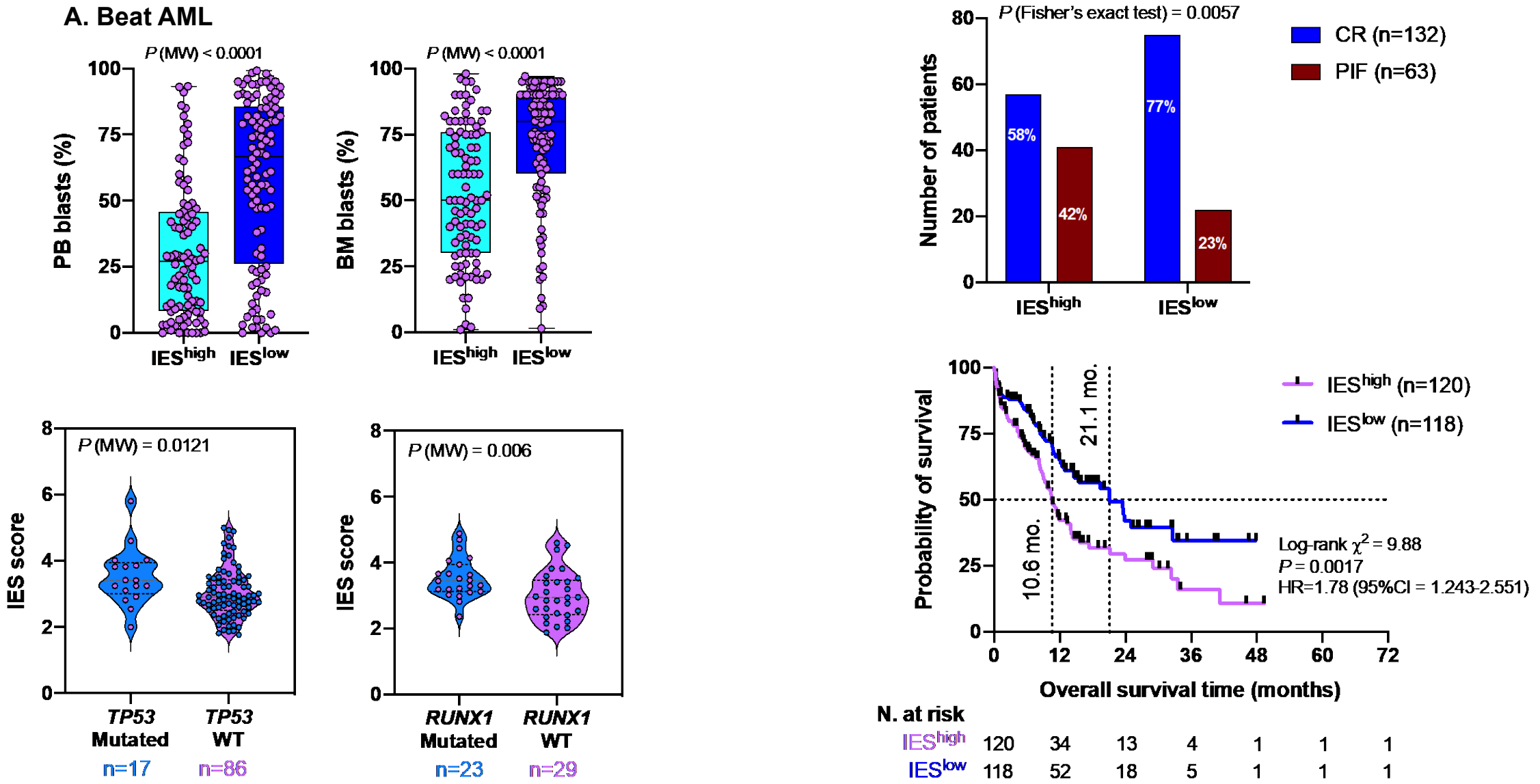
The IES Score Correlates with Immune Infiltration and with AML Prognosis



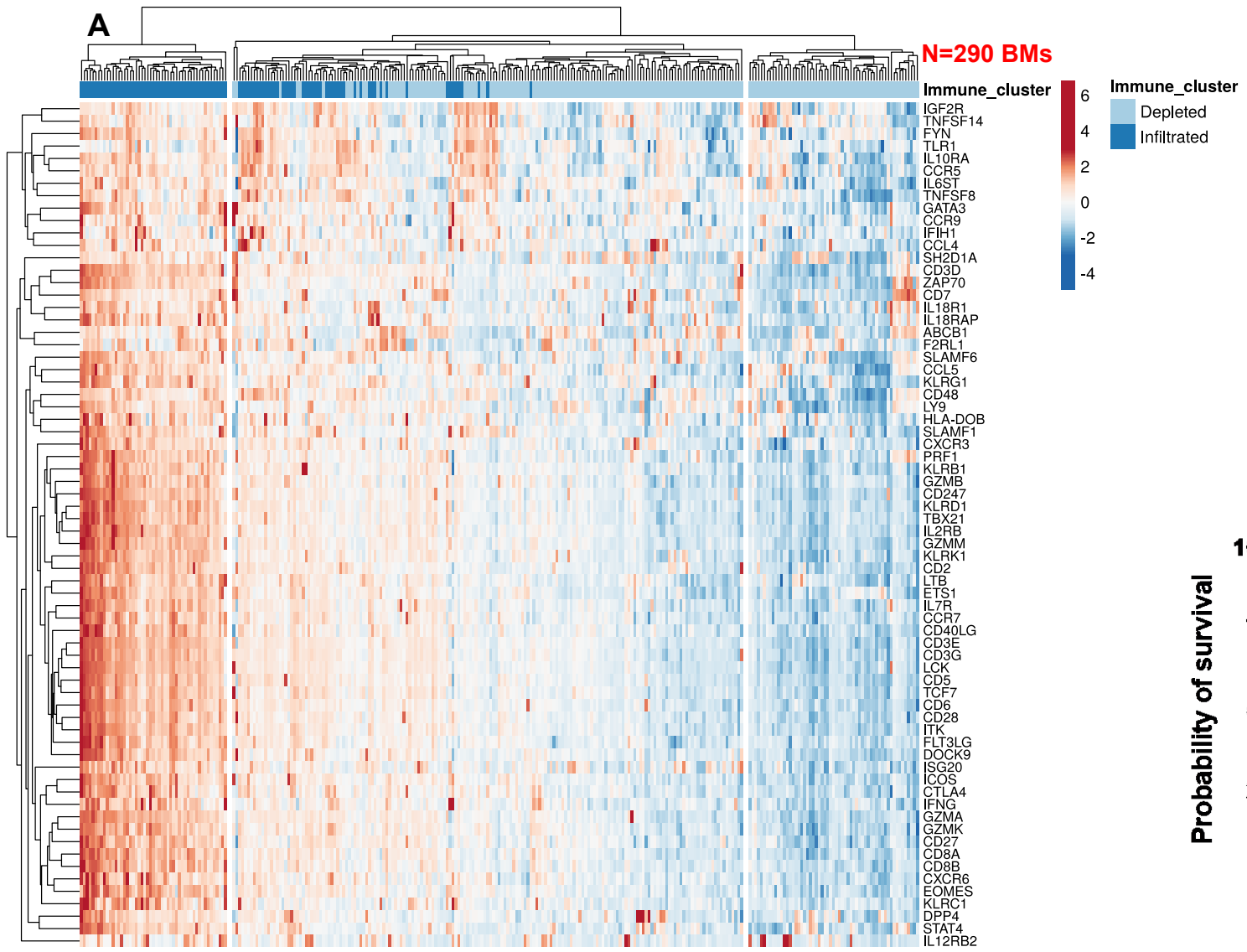
TP53 Mutational Status and Immune Landscapes

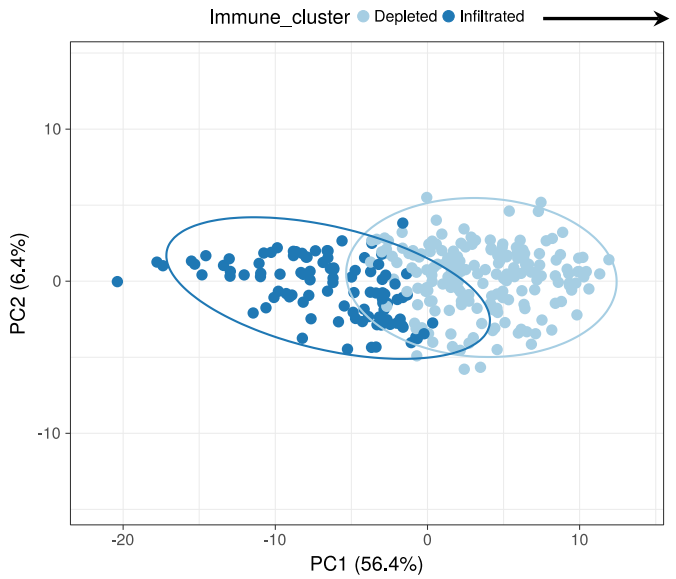
TP53 Abnormalities Correlate with Immune Infiltration and Associate with response to Flotetuzumab Immunotherapy in Acute Myeloid Leukemia (Lai C, *et a*l. Poster #2001; December 6th, ASH 2020)

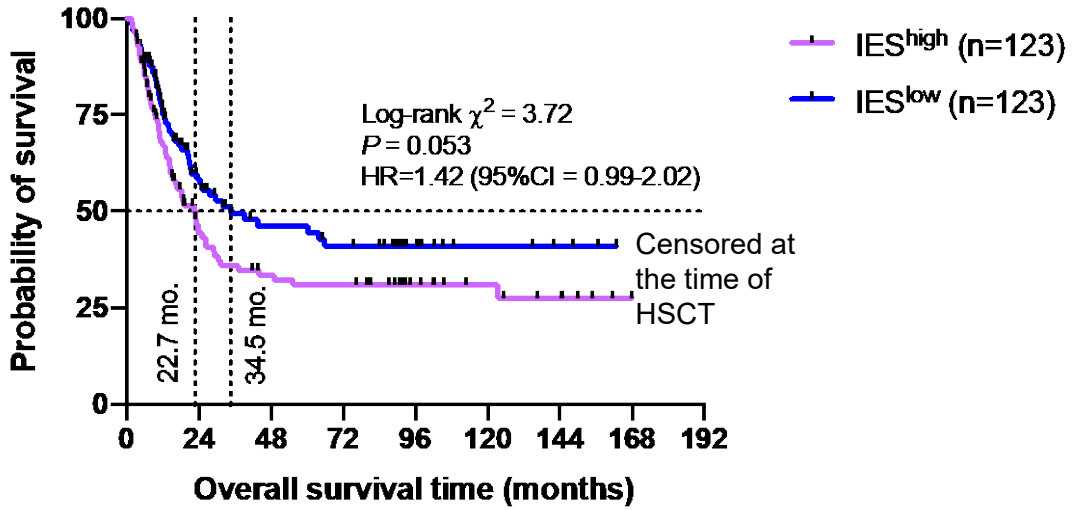
Validation Series □ Beat AML Master Trial



The IES Score Correlates with Immune Infiltration □ PMCC Cohort

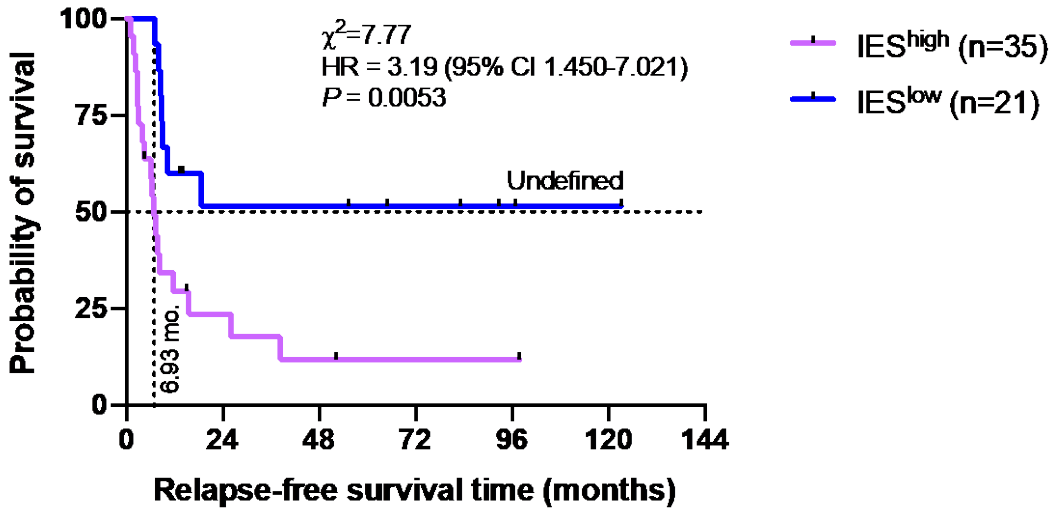


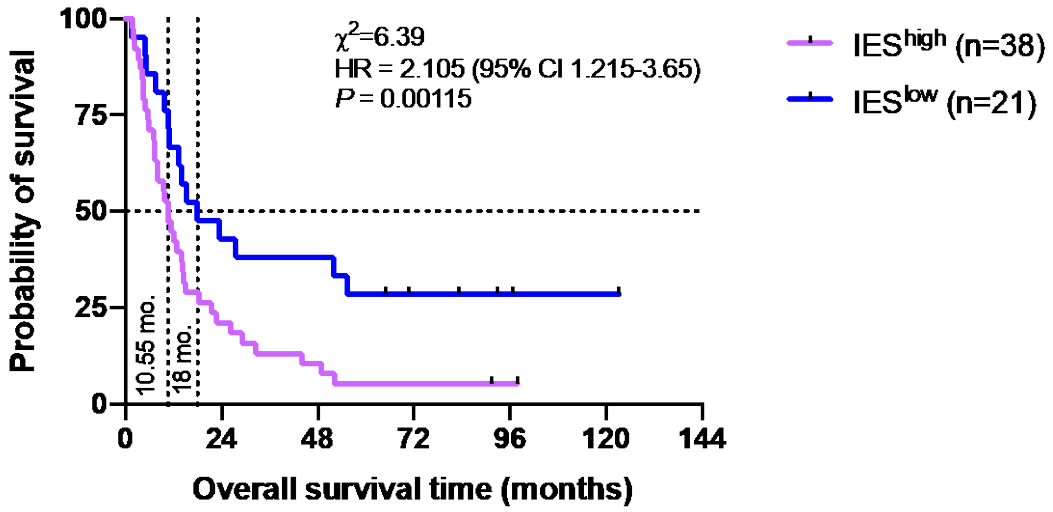




The IES Score Predicts Survival in ELN Adverse Risk

ELN Adverse Risk

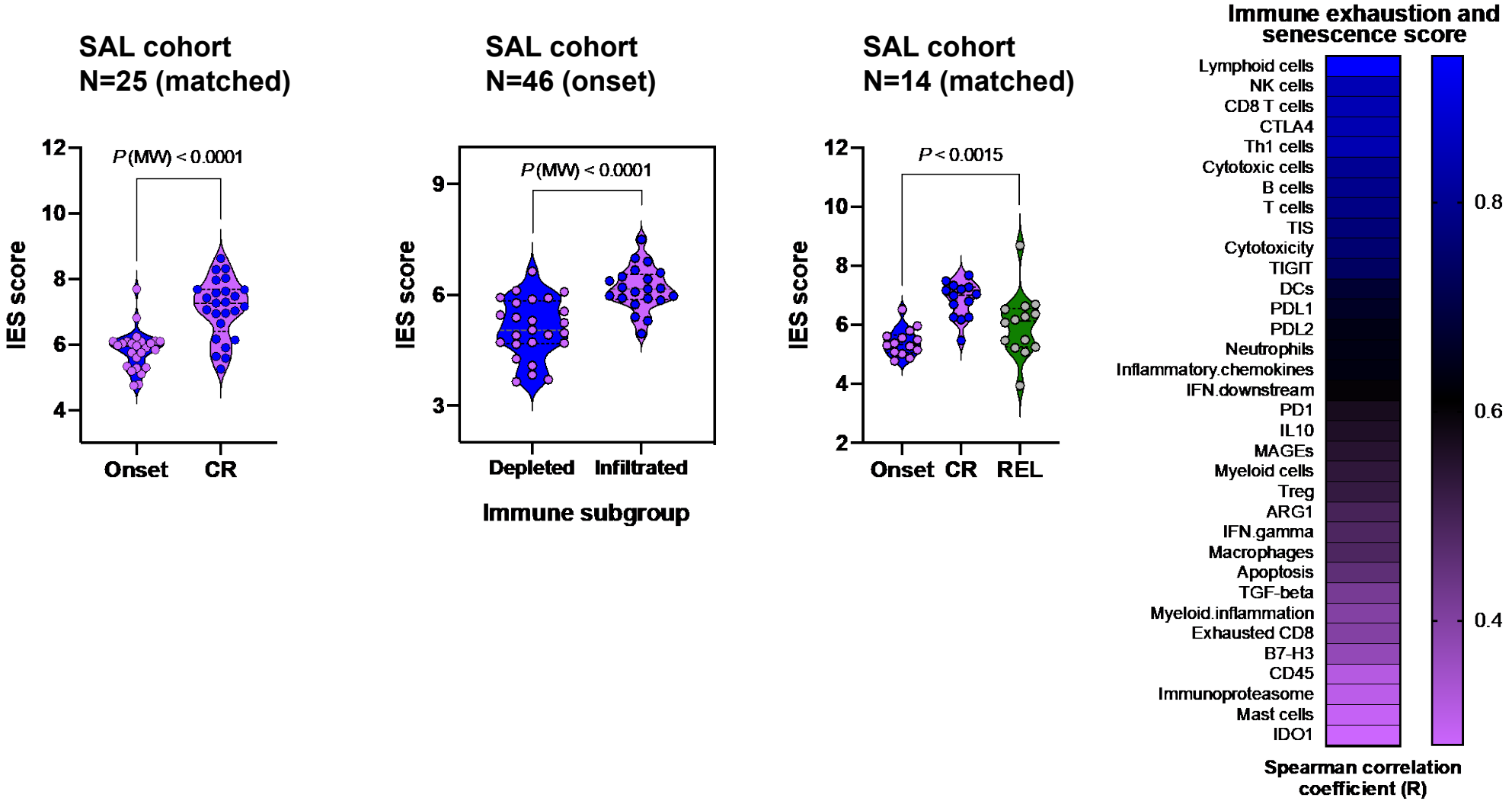




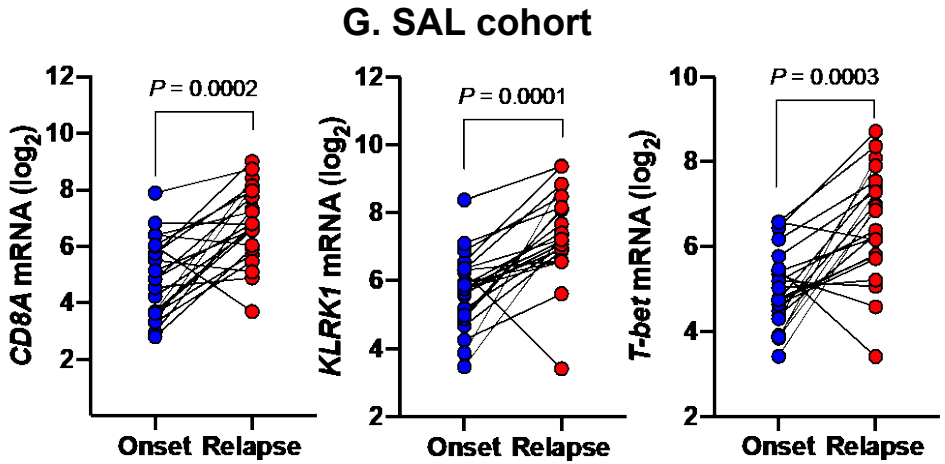
The above findings were validated in independent wet-lab cohorts comprising adults (PMCC series; n=290; SAL series; n=46) and children (CHOP series; n=46) with AML. In the ELN adverse risk subgroup (PMCC cohort), both relapse-free survival (RFS) and OS were significantly shorter in patients with higher than median compared with lower than median IES scores (median RFS time of 6.93 *versus* not reached [log-rank *P*=0.0053], and median OS of 10.5 months *versus* 18 months [log-rank *P*=0.0011], respectively). In contrast, the IES signature score failed to stratify survival in patients with ELN favorable and intermediate risk.

The IES Score Correlates with Immune Infiltration □ SAL Cohort

A



The IES Score Increases in Remissional BM Samples



Finally, a pairwise comparison of matched diagnostic, CR and relapse BM samples (SAL series; n=22 patients and CHOP series; n=40 patients) showed significantly higher IES signature scores at time of CR, congruent with chemotherapy-induced acceleration of IES, and at time of post-chemotherapy relapse compared with disease onset (**Fig. 1F**). Notably, *CD8A*, *TBX21*, a Th1 transcription factor, and markers of NK cells and cytotoxic T lymphocytes, including *KLRK1*, *KLRD1*, *KLRC2*, *GNLY*, and granzymes, were among the top ranked immune genes associated with AML relapse (**Fig. 1G**).

ASH 2020 – Rutella S, et al. Unpublished.

Conclusions

- Patients with immune-infiltrated AML exhibit features of IES, which correlate with adverse-risk molecular lesions (*TP53* and *RUNX1* mutational status), and with chemotherapy refractoriness and shorter survival
- Molecular circuits reflective of IES might also underpin AML relapse after conventional induction chemotherapy
- IES T cells could be functionally rejuvenated by novel immunotherapies being investigated in AML
  - Microenvironmental Immune Senescence and Exhaustion in Acute Myeloid Leukemia Associate with Response to Flotetuzumab, an Investigational CD123 × CD3 Bispecific DART Molecule (Vadakekolathu J, *et a*l. Poster #2878; December 7th, ASH 2020)

Acknowledgements

Co-authors and Collaborators

Nottingham Trent University

Stephen Reeder
Payton Tau
Jayakumar Vadakekolathu

PhD Students
Jenny Ashforth
Melissa Courtney

The Princess Margaret Hospital Foundation
University Health Network

Mark D. Minden
Toronto, Canada

The Children's Hospital of Philadelphia®

Tasleema Patel
Sarah K. Tasian
Philadelphia, PA

Johns Hopkins University

Ivana Gojo
Leo Luznik
Sidney Kimmel Comprehensive Cancer Centre
Baltimore, MD

Universitätsklinikum Carl Gustav Carus Dresden

Heidi Altmann
Martin Bornhäuser
Jörn Meinel
Marc Schmitz
SAL Studienallianz Leukämie
Dresden, Germany

NANOSTRING
Joseph M. Beechem
Alessandra Cesano

Michael Bailey
James Gowen-MacDonald
Thomas Smith

Sarah E. Church
Tressa Hood
Sarah E. Warren
Seattle, WA

MACROGENICS
Patrick Kaminker
Jan K. Davidson-Moncada
John Muth
Rockville, MD

Please email your questions to sergio.rutella@ntu.ac.uk

Funding Sources

الصندوق القطري لرعاية البحث العلمي
Qatar National Research Fund
Member of Qatar Foundation
National Priorities Research Programme, 2016-2020

Nottingham Trent University

HIGHER EDUCATION FUNDING COUNCIL FOR ENGLAND hefce
Mainstream QR funding, 2017-2019

John and Lucille van Geest Foundation

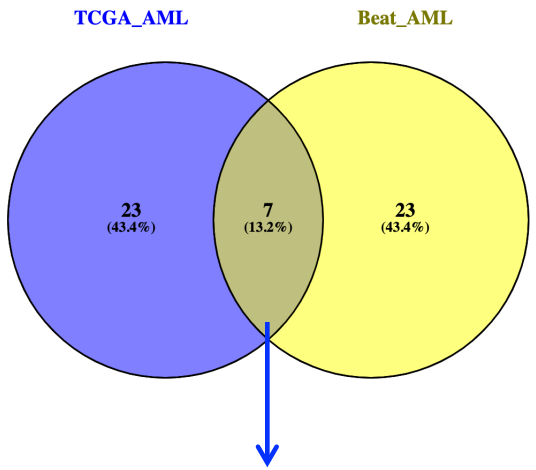
Shared genes associated with survival

Beat AML (ranked by χ^2)

ENO2	20.070838252656436
MCOLN2	14.803805819848602
RASA3	14.003750260434753
ETS1	14.003750260434753
SH3BP5	12.470310438224876
KCNA3	12.470310438224876
NCKAP1	10.336828946454615
STAT4	10.336828946454615
KLF12	10.336828946454615
SESN3	9.670115980276409
BACH2	9.025626779637474
DLG3	8.403361344537815
FYN	8.403361344537815
CD72	8.403361344537815
SLC9A9	7.803319674977429
INPP4B	7.225501770956317
LRIG1	7.225501770956317
WLS	6.669907632474477 5
PTCH1	6.669907632474477 5
TIAM1	6.669907632474477 5
ISG20	6.669907632474477 5
C1orf21	6.669907632474477 5
GBP1	6.136537259531912
ARNTL	6.136537259531912
ARL4C	6.136537259531912
SYNE1	5.625390652128621
IL12RB2	5.625390652128621
TGFBR3	5.136467810264602
KLRD1	5.136467810264602
DOCK9	5.136467810264602

TCGA (ranked by χ^2)

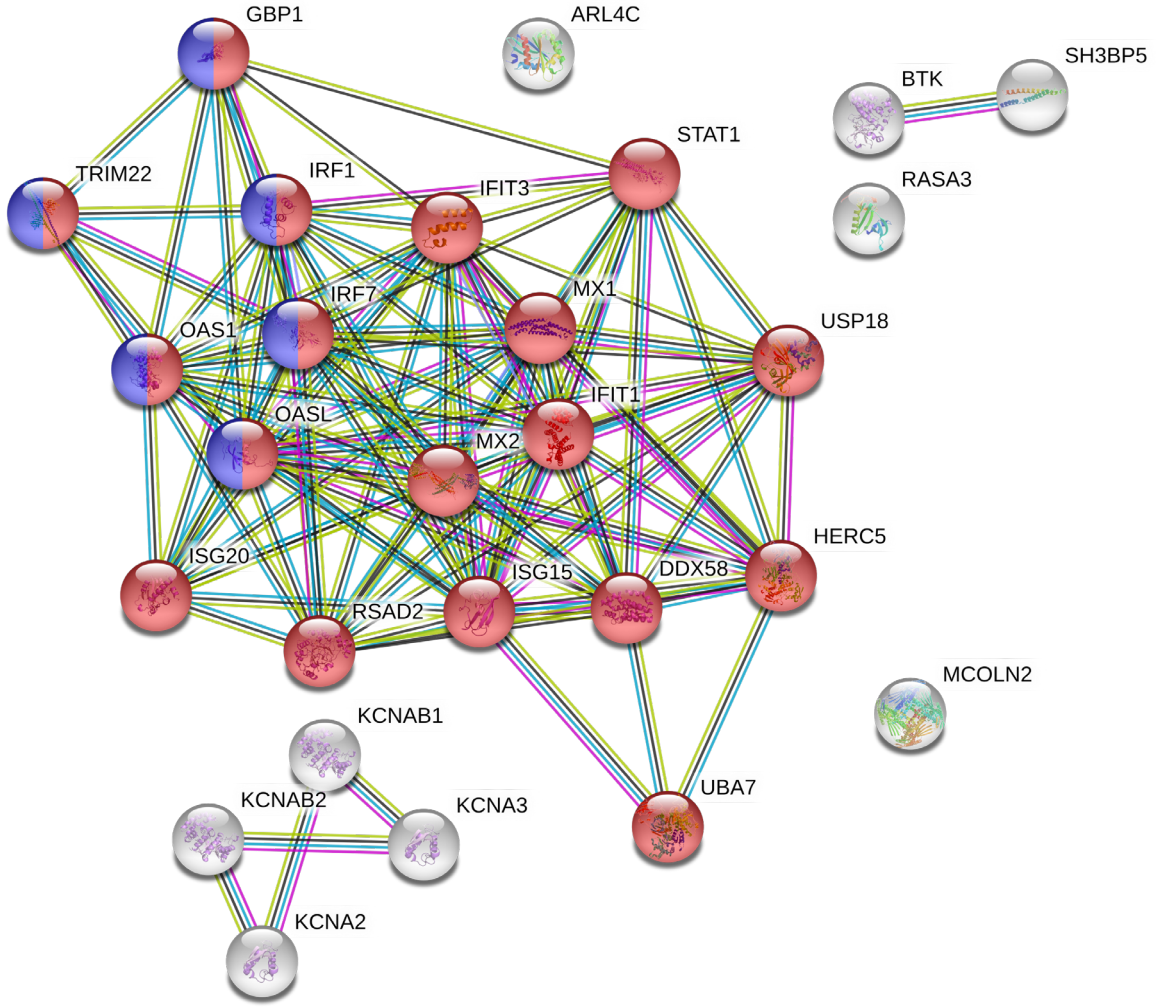
HOPX	6.05901447115447
MCOLN2	6.05901447115447
HS3ST3B1	5.439109698189653
KLRG1	4.155618137008221
USP18	4.155618137008221
MDFIC	3.1266217951199358
CD7	3.0854720706073646
DTX1	3.0854720706073646
EVL	2.610034484315928
CXCR3	2.610034484315928
HLA-DOB	2.610034484315928
RASA3	2.610034484315928
SH3BP5	2.610034484315928
ST8SIA1	2.208919631221047
LAX1	2.174353862630487
TRAT1	1.809703996180595
ABLIM1	1.7784302055510408
ARL4C	1.7784302055510408
GZMB	1.7784302055510408
GBP1	1.7784302055510408
LTB	1.7784302055510408
IGF2R	1.7784302055510408
TNFSF14	1.7784302055510408
CCR9	1.4222635130775894
CHN1	1.4222635130775894
CCL5	1.4222635130775894
IL18RAP	1.4222635130775894
ISG20	1.4222635130775894
KCNA3	1.4222635130775894
NCALD	1.4222635130775894



MCOLN2
RASA3
SH3BP5
ARL4C
GBP1
ISG20
KCNA3

Shared genes associated with survival – pathway analysis

Network interaction analysis



Reactome Pathways

Pathway description	*count in gene set*	*FDR*
HSA-913531		
Interferon signaling	18 of 189	6.17e-28
HSA-909733		
Interferon alpha/beta signaling	12 of 66	6.11e-21
HSA-1169410		
Antiviral mechanism by IFN-stimulated genes	11 of 77	2.73e-18
HSA-1169408		
ISG15 antiviral mechanism	9 of 69	1.56e-14
HSA-168256		
Immune System	20 of 1925	6.65e-14
HSA-877300		
Interferon gamma signaling	6 of 86	4.01e-08

Molecular Function (GO)

GO-term description	*count in gene set*	*FDR*
GO:0005261		
Cation channel activity	6 of 316	0.00093
GO:0005249		
Voltage-gated potassium channel activity	4 of 95	0.0011
GO:0046873		
Metal ion transmembrane transporter activity	6 of 458	0.0016
GO:0022839		
Ion gated channel activity	5 of 329	0.0018
GO:0003725		
Double-stranded RNA binding	3 of 70	0.0023