Lymph Node Transcriptomics in Idiopathic Multicentric Castleman Disease (iMCD) Identifies Pathogenic Mechanisms and Biomarkers Including Increased Clusterin Expression

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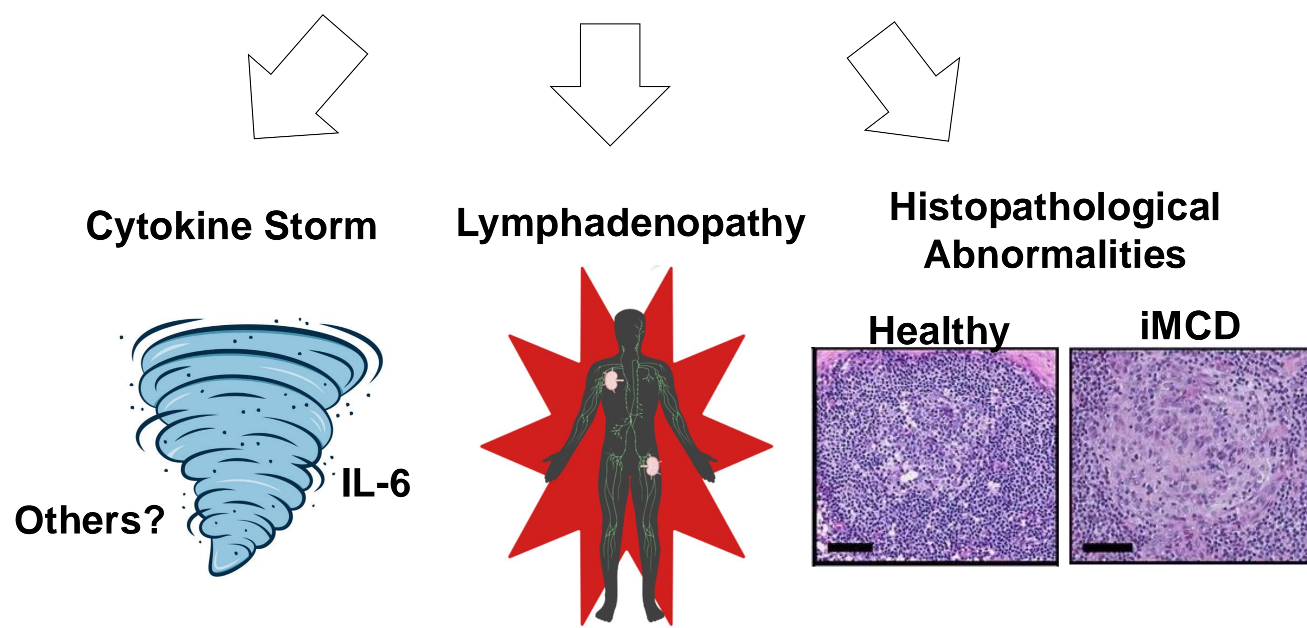
Background

- Idiopathic multicentric Castleman disease (iMCD) is an atypical lymphoproliferative disorder with significant morbidity and mortality and a poorly understood pathophysiology. (**Fig. 1**)
- Defined by a characteristic lymph node (LN) histology, patients can present with a range of symptoms from thrombocytopenia, anasarca, fever, renal dysfunction, and organomegaly (iMCD-TAFRO) to thrombocytosis, hypergammaglobulinemia, and plasmacytosis (iMCD-IPL); and patients not falling into either group are considered iMCD-NOS.
- The molecular mechanisms of iMCD, particularly within these clinical subtypes and in comparison to other inflammatory disorders, has not been elucidated. Additionally, transcriptional programs, pathologic cell types, and distinguishing biomarkers have not been well explored, especially in LN tissue, the site of defining histopathologic changes in iMCD.

Idiopathic Multicentric Castleman Disease (iMCD)



What transcriptional programs, cell types, and biomarkers govern iMCD pathology?

Figure 1: Characteristics of idiopathic multicentric Castleman disease (iMCD). iMCD is an inflammatory disorder characterized by enlarged lymph nodes with associated histopathological abnormalities including prominent follicular dendritic cells and increased vascularity. Patients also exhibit a life-threatening cytokine release syndrome often involving interleukin-6 (IL-6), but the pathology is not fully understood. The goal of this project is to identify genes/gene pathways involved in iMCD pathology in the lymph node.

- We implemented a two-tiered analytical approach using bulk RNA-sequencing followed by targeted gene expression quantification to characterize the LN transcriptome(s) of iMCD clinical subtypes. (**Fig. 2**)

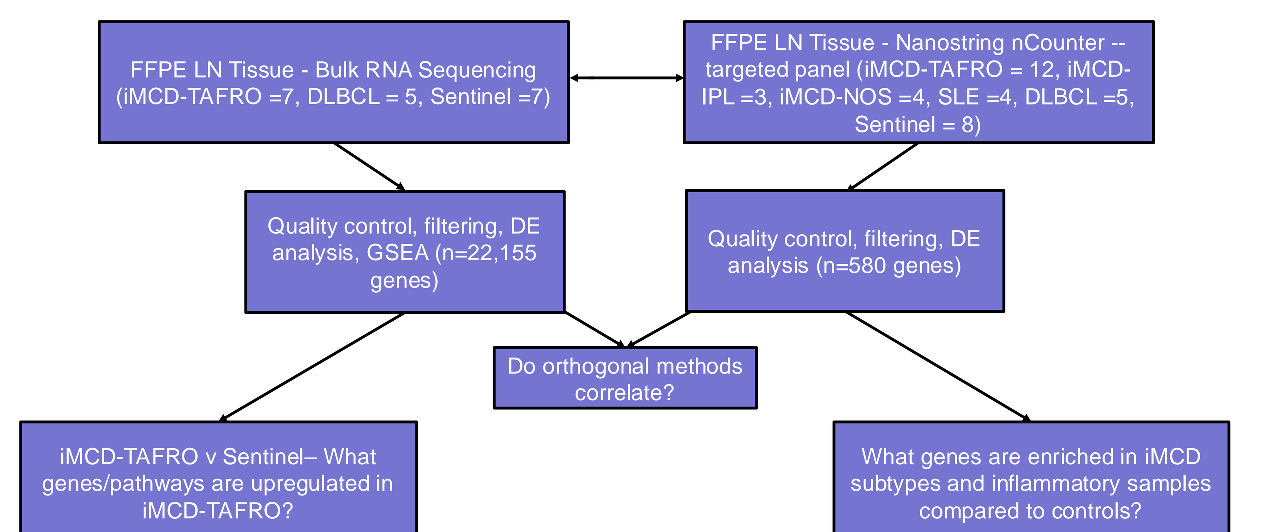


Figure 2: Gene expression workflow. Bulk RNA sequencing of iMCD lymph node tissue, and targeted gene quantification allows to determine gene transcription programs governing pathology in iMCD clinical subtypes compared to healthy controls and other neoplastic and autoimmune phenotypes.

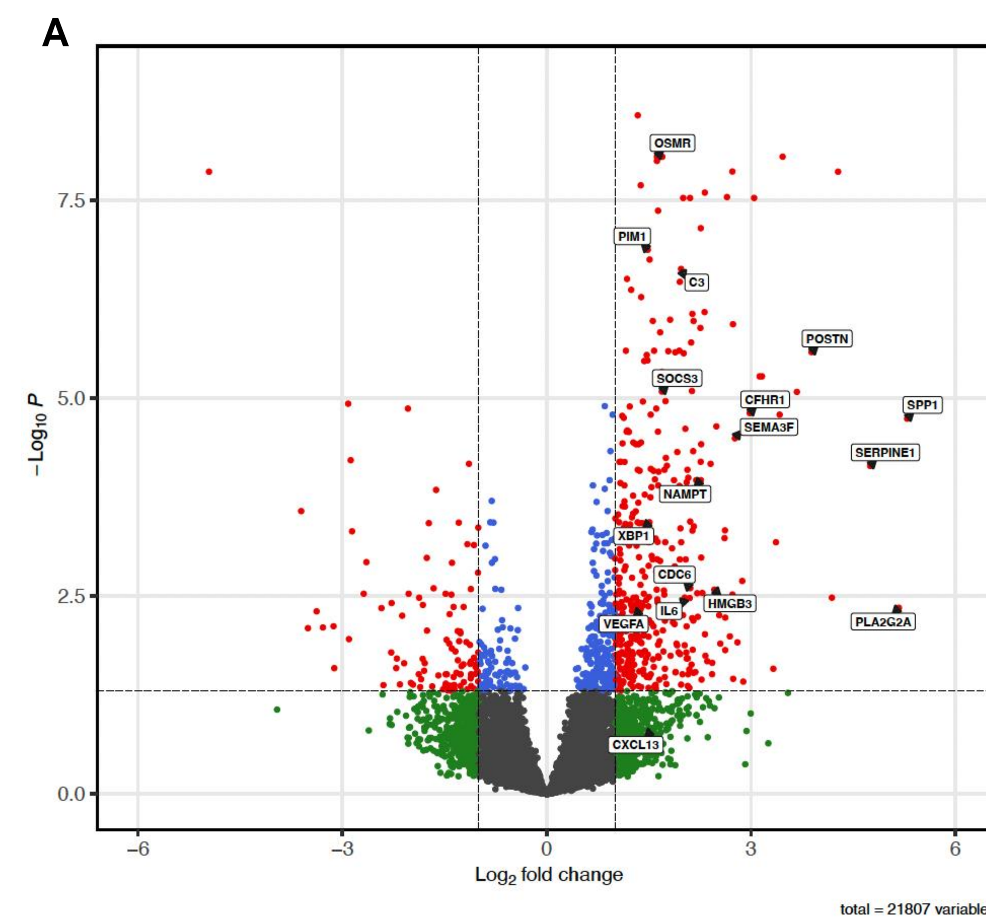
Clinical cohort characteristics

	Sentinel	iMCD-TAFRO
n	7	7
Age, mean (SD)	NA	49.1 (12.01)
Sex M:F	NA	2:5
Location of tissue sampled, n (%)	NA	Auxillary, 4 (67%), Groin, 1 (16.7%), Mediastinal, 1 (16.7%), Not Specified, 1 (16.7%)
Histologic variant, n (%)		
Hyaline vascular	NA	5 (83.3%)
Plasmacytic or mixed	NA	1 (16.7%)
Not Specified	NA	1 (16.7%)
	Sentinel	**iMCD-TAFRO**
n	7	7
Age, mean (SD)	NA	49.1 (12.01)
Sex M:F	NA	2:5

	Sentinel	iMCD-TAFRO	iMCD-IPL	iMCD-NOS	SLE	DLBCL
n	8	12*	3	4	4	5
Age, mean (SD)	NA	44.7 (13.71)	40.9 (12.07)	44.4 (16.67)	NA	NA
Sex M:F	NA	4:8	1:2	3:1	NA	NA
Location of tissue sampled, n (%)	NA	Auxillary, 5 (41.7%), Inguinal, 2 (16.7%), Cervical, 1 (8.3%), Groin, 1 (8.3%), Mediastinal, 1 (8.3%)	Cervical, 2 (66%), Groin, 1 (33%)	Auxillary, 2 (50%), Abdominal, 1 (25%), Cervical, 1 (25%)	NA	NA
Histologic variant, n (%)						
Hyaline vascular	NA	9 (75%)	1 (33%)	2 (50%)	NA	NA
Plasmacytic or mixed	NA	3 (25%)	2 (66%)	2 (50%)	NA	NA

Table 1, Two overlapping cohorts to investigate gene expression from lymph node tissue in iMCD patients compared to healthy controls. Left, bulk RNA sequencing cohort including iMCD-TAFRO, n=7 and Sentinel, n=7. Right, an expanded cohort to investigate iMCD clinical subtype gene expression similarities and differences. This included iMCD-TAFRO, n=12 (including the 7 samples from the RNA sequencing cohort, iMCD-IPL, n=3, iMCD-NOS, n=4, as well as neoplastic and autoimmune comparative groups, DLBCL, n=5, and SLE, n=4.

iMCD-TAFRO (n=7) vs Sentinel (n=7) bulk RNA sequencing identifies enriched proliferative, immune, and cell signaling pathways in iMCD lymph node tissue



B

Hallmark Pathway	Process Category	NES	FDR q-val
Cell Cycle Progression: G2M Checkpoint	Proliferation	1.855	0.043
Unfolded Protein Response	Pathway	1.801	0.044
IL-6 JAK STAT3 Signaling	Immune	1.779	0.036
Cell Cycle Progression: E2F Targets	Proliferation	1.772	0.031
Inflammatory Response	Immune	1.764	0.029
IL-2 STAT5 Signaling	Signaling	1.713	0.051
Angiogenesis	Development	1.713	0.044
Spermatogenesis	Development	1.699	0.045
Cell Cycle Progression: Mitotic Spindle	Proliferation	1.685	0.046
KRAS Signaling Up	Signaling	1.674	0.047
TNF Signaling via NFKB	Signaling	1.660	0.046
Apoptosis	Pathway	1.651	0.048
Hedgehog Signaling	Signaling	1.624	0.055
Coagulation Cascade	Immune	1.607	0.060
Complement Cascade	Immune	1.542	0.089
Androgen Response	Signaling	1.538	0.085
mTORC1 Signaling	Signaling	1.538	0.081
MYC Targets	Proliferation	1.533	0.079

- Overall, 425 genes were upregulated and 108 were downregulated, ($\log_2$FC>±1, adj. *P* <0.05) (**Fig 3A**)
- Several genes (boxed) have been seen in other 'omics based investigations of iMCD including *VEGFA*, *IL6*, and *SOCS3* (**Fig. 3A**)
- Collectively, these DEGs were found to drive several gene pathways related to cell proliferation, immune function, and cell signaling. (**Fig. 3B**)

Figure 3. (**A**) Volcano Plot of differentially expressed genes in iMCD-TAFRO lymph node tissue vs Sentinel lymph nodes. log2 fold change is on the x axis and –log10(adj.P) on the y axis. (**B**) Table of enriched gene pathways in iMCD-TAFRO lymph nodes using gene set enrichment analysis and hallmark pathways. NES = Normalized enrichment score

Cell type deconvolution and drug perturbation screen identifies potential pathologic cell types and targeted treatments in iMCD

A

Cell Type	iMCD TAFRO est. cell number/10000 cells	Sentinel est. cell number/10,000 cells	T test P-value
B cells	2672	3184	0.115
Cycling cells	212	244	0.690
Dendritic cells	67	68	0.986
Erythroid cells	65	3	0.181
Hematopoietic stem cells	23	3	0.274
Innate lymphoid cells	281	176	0.178
Macrophages	42	36	0.805
Mast cells	64	2	0.258
Monocytes	63	0	0.050
Plasma cells	204	15	0.007
Promyelocytes	2	4	0.628
T cells	6282	6203	0.749
pDC	24	61	0.232

B

Perturbagen	z-score (sum)	Mechanism
mitomycin-c	-14.09	Transcriptional inhibitor
AZD-3463	-14.08	ALK inhibitor
tivozanib	-14.04	VEGF inhibitor
dacomitinib	-13.97	Kinase inhibitor
MK-2461	-13.93	c_Met inhibitor
HG-6-64-01	-13.80	B-Raf inhibitor
parthenolide	-13.70	NF-KB inhibitor
clocortolone-pivalate	-13.59	Topical Steroid
resminostat	-13.55	HDAC inhibitor
donitriptan	-13.43	Triptan drug

- Single-cell RNA sequencing data from mesenteric and lung-draining lymph node tissue from 12 deceased organ donors was used to deconvolute the bulk RNA sequencing samples into estimated cell type proportions using the R based software tool, Bisque, which uses a non-negative least-squares regression model.
- Cell type deconvolution highlighted **plasma cells** and **monocytes** in iMCD lymph node tissue **(Table 4A)**
- The LINCS1000 database was used to predict potential reversing therapeutic perturbations. Top differentially expressed genes from the iMCD-TAFRO versus healthy control lymph node comparison (+/- log2FC>1.5, adjusted *P*<0.05) were used to predict reversing drug perturbations **(Table 4B)**.
- Of note, **tivozanib** (VEGF inhibitor) and **parthenolide** (NF-KB inhibitor) were predicted as potential therapies that may be helpful in iMCD.

Figure 4. (A) Cell type deconvolution of bulk RNA sequencing to identify differentially abundant cell populations. **(B)** Drug perturbation screen to identify potential therapies that reverse the DEG signature from iMCD-TAFRO.

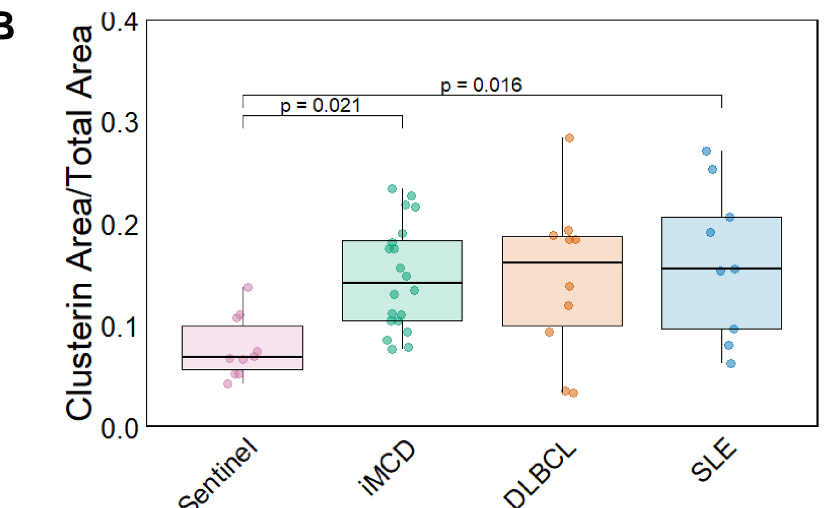
Biomarker discovery and validation

Gene markers upregulated in all iMCD clinical subtypes

A

Gene	log2FC TAF	Padj TAF	log2FC IPL	padj_IPL	log2FC NOS	padj_NOS
XBP1	3.42	7.00E-06	4.91	2.46E-06	1.80	3.84E-03
PRDM1	1.39	7.31E-05	3.40	2.88E-06	1.03	2.58E-03
SPP1	5.03	5.27E-13	3.11	1.67E-02	3.86	6.29E-07
CLU	**1.58**	**4.33E-03**	**1.78**	**1.50E-02**	**1.84**	**2.85E-02**

Immunohistochemistry quantification of clusterin is significantly increased compared to sentinel



Unique differentially expressed genes in iMCD clinical subtypes, C) iMCD-TAFRO, D) iMCD-IPL, and E) iMCD-NOS

C

Gene	Log2FC	Adj. P
PLA2G2A	4.43	1.53E-08
CLEC4E	1.39	1.54E-03
CCR10	2.00	2.79E-02
IL6	1.10	9.84E-03
S100A9	1.67	1.53E-03
LILRA5	1.32	1.57E-03
CCL23	1.46	9.74E-06
IL1RL1	1.55	3.61E-02
CD276	1.31	1.69E-02
CCL20	1.38	9.84E-03
IL8	2.07	2.43E-02
FCGR3A/B	1.73	1.53E-02
FN1	2.02	1.15E-02

D

Gene	Log2FC	Adj. P
IL9	2.948	7.36E-03
KIR3DL1	2.378	2.78E-03
IL18RAP	2.311	4.64E-02
TAL1	1.873	4.62E-02
MAPKAPK2	1.418	1.35E-02
IFNAR2	1.033	4.71E-02

E

Gene	Log2FC	Adj. P
IL7	1.641	2.23E-02
TLR8	1.069	1.35E-03
CX3CR1	1.066	2.85E-02
CLEC7A	1.058	3.70E-02
CISH	1.024	4.45E-02

Figure 5. (A) Differentially expressed genes (DEGs) uniformly upregulated in all iMCD clinical subtypes, iMCD-TAFRO n=12, iMCD-IPL n=3, iMCD-NOS n=4 compared to sentinel lymph nodes. Notably, Clusterin (CLU in bold) was upregulated in all iMCD clinical subtypes, but not in comparative neoplastic (DLBCL) and autoimmune conditions (SLE). **(B)** Immunohistochemistry (IHC) quantification showed increased clusterin in iMCD lymph node tissue compared to sentinel, but was not distinguishing to other disease phenotypes. **Tables C), D), and E)** show unique DEGs in iMCD-TAFRO, iMCD-IPL, and iMCD-NOS respectively.

- Using a targeted gene expression panel (Nanostring nCounter, gene n = 580), we identified gene markers uniformly upregulated across iMCD clinical subtypes (**Fig. 5A**).
- IHC Clusterin was significantly enriched in iMCD compared to sentinel, but was not distinguishing from DLBCL or SLE (**Fig. 5B**).
- Unique DEGs were also identified in each iMCD clinical subtype. These biomarkers require additional investigation and validation (**Fig. 5 C-E**).

Conclusions

1) 425 upregulated and 108 downregulated genes were identified in iMCD lymph node compared to sentinel control samples. These genes drove the enrichment of several gene pathways related to cell proliferation, various immune cell function, and cell signaling.
2) Cell type deconvolution and drug perturbation screening identified plasma cells and monocytes as cell types of interest and VEGF and NF-KB as potential novel therapies in iMCD.
3) Several gene biomarkers were identified that were either uniquely expressed or uniformly upregulated across iMCD clinical subtypes. Immunohistochemistry was used to validate clusterin as a potential differentiating iMCD biomarker. It was significantly upregulated compared to sentinel lymph nodes (P=0.021), but was not significantly different from either DLBCL or SLE.