MIFlowCyt-Compliant Items

Tviblindi algorithm identifies branching developmental trajectories of human B cell development and describes abnormalities in RAG and WAS patients

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Requirement	Please Include Requested Information
1.1. Purpose	To describe human B cell development in the bone marrow compartment using CyTOF data and computational framework.
1.2. Keywords	B cell development, mass cytometry, trajectory inference, CD73, RAG-1, WAS
1.3. Experiment variables	n=3 healthy donor (HD) buffy coat (BC) samples – B cell enriched n=2 HD peripheral blood (PB) samples – B cell enriched n=5 HD bone marrow (BM) samples – B cell enriched n=1 RAG-1 mut.BM sample n=1 Wiskott-Aldrich syndrome (WAS) BM sample n=1 WAS PB sample
1.4. Organization name and address	CLIP, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University, Prague, Czech Republic V Uvalu 84 Prague 150 06 Czech Republic
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1.6. Date or time period of experiment	Since 2020
1.7. Conclusions	We developed, validated and presented a comprehensive set of tools for investigation of B-cell development in the bone marrow compartment.
1.8. Quality control measures	All in-house mass cytometry antibodies were validated and titrated on relevant control cells EQ Four Element Calibration Beads (Standard BioTools) were used for normalization
2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description	
2.1.1.2. Biological sample source description	Healthy donor or patient (RAG-1 or WAS) bone marrow or peripheral blood RAG-1 BM: RAG1 compound heterozygot c.256_257delAA (p.Lys86ValfsX33), c.2210G>A (p.Arg737His), female, age 1y WAS BM and PB: hemizygot c.397G>A, male, age 1.5 months

	HD (PB and BC): adult healthy donors HD (BM): pediatric patients with excluded hematological disease or immunological disorder (n=4) or from fully recovered patient 1 year after successful B-cell precursor leukemia therapy (n=1)
2.1.1.3. Biological sample source organism description	Human
2.1.2.2. Environmental sample location	
2.3. Sample treatment description	Samples were stained according to MaxPar protocols (Standard BioTools), either fresh or thawed for 1 min in 37°C water bath and rested for 30 min in RPMI medium at 37°C if previously cryopreserved (in fetal bovine serum containing 10% DMSO in liquid nitrogen).
2.4. Fluorescence reagent(s) description	Metal-tagged monoclonal antibodies
3.1. Instrument manufacturer	Standard BioTools
3.2. Instrument model	Helios instrument (CyTOF 6.7.1014 software) and CyTOF XT
3.3. Instrument configuration and settings	Prepared for acquisition according to the manufacturer's recommendation
4.1. List-mode data files	Repository identifier: https://github.com/tomas-kalina/B-cell-developmentFCS
4.2. Compensation description	No compensation
4.3. Data transformation details	
4.4.1. Gate description	See supplementary Figure 1,2
4.4.2. Gate statistics	
4.4.3. Gate boundaries	