

The current status of Castleman disease (CD) research

Background

- **Castleman disease** includes a group of disorders including **unicentric Castleman disease (UCD)**, **human herpesvirus-8 (HHV-8) associated multicentric Castleman disease (HHV8-associated MCD)**, and **HHV-8-negative/idiopathic MCD (iMCD)** that share common lymph node histopathological changes but have different etiologies, mechanisms, and treatments.
- iMCD can be subdivided into **iMCD-TAFRO** (thrombocytopenia, anasarca, fever, reticulin fibrosis, organomegaly) and **iMCD-NOS** (not otherwise specified), which often presents with thrombocytosis and hypergammaglobulinemia.
- The etiology and pathogenesis of HHV8-associated MCD is well understood: uncontrolled infection of lymph node plasmablasts with HHV-8 leads to an IL-6 driven cytokine storm that can be effectively treated with anti-CD20 therapy.
- **The etiology and pathogenesis of UCD and iMCD is poorly understood.**
- The CDCN underwent a crowdsourcing effort in 2013 to determine the key research questions that need to be answered to achieve the CDCN's vision of a world where CD patients' lives are not limited in length or quality by CD.
- The below overarching research questions were identified as well as a number of specific research questions that have been investigated over the last 8 years. See below for updates so that you're fully informed when you contribute to AIM 2021.

How is Castleman disease diagnosed?

- The CDCN established **evidence-based international diagnostic criteria** for iMCD in 2017 (1) and for UCD in 2020 (2) that rely on clinical, laboratory, radiological, and pathological findings and exclusion of disorders that can mimic iMCD and UCD.
- **No sensitive or specific diagnostic markers (blood tests, lymph node stains, etc.) currently exist for iMCD or UCD;** this leads to some diagnostic uncertainty in the majority of patients.

What is the cause of the lymphoproliferation in UCD and the immune system hyperactivation/cytokine storm in iMCD?

- Data that have emerged over the last several years suggest that **UCD is a clonal neoplastic process of stromal cell origin** (3).
- A recent study using **next-generation sequencing of UCD lymph node tissue revealed somatic platelet-derived growth factor receptor Beta (PDGFRb) mutations** in nearly 20% of cases (4). In vitro functional experiments have confirmed that the mutation is a gain of function mutation that confers proliferation and survival advantages. Further investigation of PDGFRb and other genetic alterations that may be involved in UCD are underway.
- **The trigger for the cytokine storm in iMCD is unknown.** An acute viral infection, germline genetic mutations, somatic mutations, and pathologic auto-antibodies have all been proposed as potential etiologies.
- Vircapseq did not identify evidence of an acute viral infection in iMCD lymph node tissue (5). A follow up study is currently underway.
- Several small **studies have identified genetic alterations in individual patients with iMCD** that may be related (FAS gene mutation (6); somatic MEK2P128L mutation and germline RUNX1G60C mutation (7); somatic DNMT3A L295Q mutation and amplifications of ETS1, PTPN6, and TGFBR2 (8)). Given that these mutations were only reported in single patients, these may all be red herrings. **A whole exome sequencing and HLA typing study of constitutional DNA from 300 iMCD patients is currently planned** (9). Targeted genetic sequencing of lymph node tissue is currently planned to look for somatic mutations that may cause iMCD (10).
- **A study searching for autoantibodies that may be involved in pathogenesis is underway** (11). A retrospective literature review found that at least 20% of Castleman disease patients have auto-antibodies in their blood (12).

What type of immune cells are involved in UCD and iMCD?



- **Lymph node stromal cells seem to be key components of UCD pathogenesis**; the effectiveness of surgical excision for treating UCD suggests that the pathological driver is present in the lymph node.
- **It is unclear which immune cells are involved in iMCD pathogenesis.**
- A recent study found increased proportions of **activated CD4+ and CD8+ T cells** as well as increased numbers of **inflammatory monocytes** in circulation of iMCD-TAFRO patients (13). Similar immune cell profiling has not been performed in iMCD-NOS.
- A recent study found increased expression of **CXCL13, the most up-regulated cytokine in iMCD**, by lymph node stromal cells, suggesting that they may be involved in iMCD pathogenesis as well.
- A pathogenic role of **B cells and plasma cells in iMCD** is supported by the increased numbers of lymph node plasma cells and hypergammaglobulinemia observed in some patients with iMCD-NOS, as well as the response by a portion of these patients to B cell depletion with rituximab (14).
- **Immunohistochemistry and flow cytometry studies are underway** to help determine the active cell types and cell pathways in UCD and iMCD lymph nodes (15). An **in situ hybridization study of Castleman disease lymph nodes is also in progress** to pinpoint the cells responsible for producing specific cytokines (16).

What cellular signaling pathways are activated in the immune cells involved in UCD and iMCD?



- The cellular signaling pathways involved in UCD are not known, but may be related to **PDGFRb**.
- Immunohistochemistry studies found **increased activation of the mTOR pathway in lymph node tissue from patients with iMCD** and flow cytometry studies found **increased sensitivity to mTOR pathway activation in circulating immune cells from iMCD patients** upon stimulation with a specific cytokine (17, 18, 19).
- **Early evidence also points toward involvement of the JAK pathway in iMCD.** JAK tyrosine kinases (JAK1, JAK2) are key regulators of cell proliferation and activation downstream of the receptors for IL-6 and a number of other cytokines. JAK1 and JAK2 lead to activation of mTOR and STAT3, two pathways reported to be involved in iMCD pathogenesis (20).

What factors/cytokines are released by the immune cells involved in UCD and iMCD?

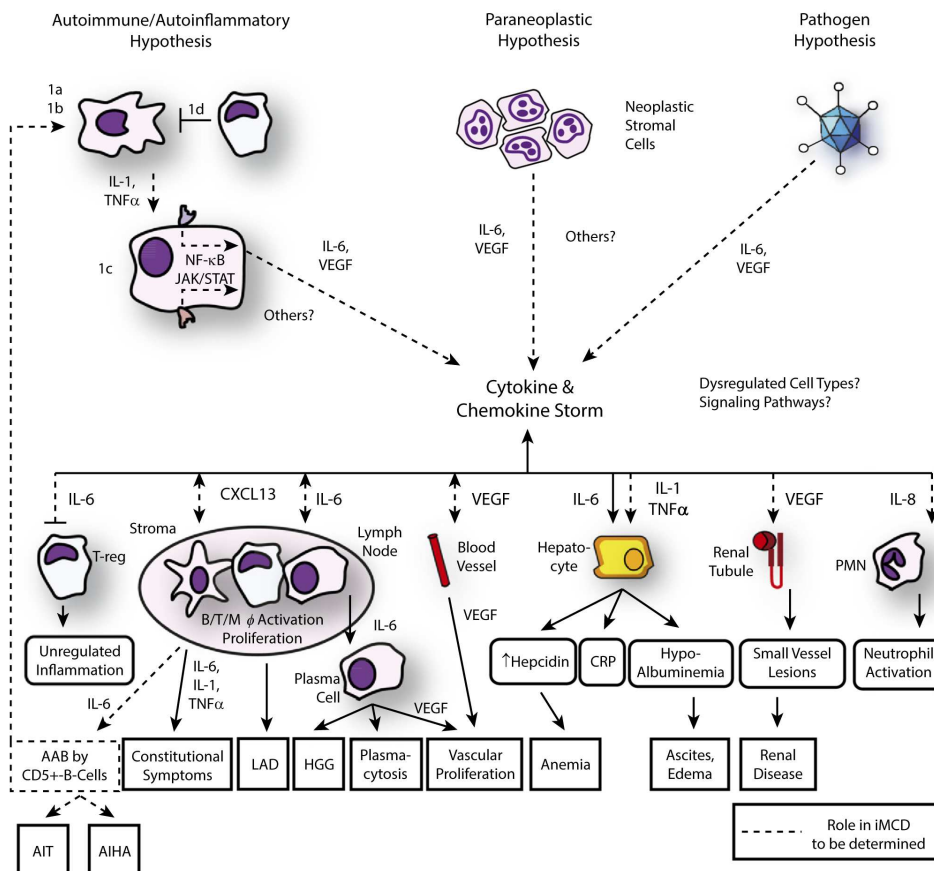


- Most UCD patients do not have elevated circulating cytokines or other factors; rare UCD patients have elevated circulating IL-6 levels.
- **IL-6 is a critical disease driver in a large portion of iMCD patients** as demonstrated by abrogation of iMCD symptoms with anti-IL-6 therapy (21, 22). However, IL-6 is not universally elevated in iMCD, and approximately one-half of iMCD patients do not respond to IL-6 inhibition (23), suggesting that other cytokines are involved in those patients.
- **VEGF is an angiogenic protein that may also be implicated in iMCD pathogenesis.** VEGF was found to be elevated in 16/20 published iMCD cases reporting VEGF levels (24), and similar results were found in another cohort of 17 cases (25). Elevated VEGF may account for the eruptive cherry hemangiomas, capillary leak syndrome, and lymph node hypervascularity seen in iMCD (26).
- A previous study was performed examining serum proteins in patients with iMCD during disease flare. This study found that **CXCL13, a chemokine that directs migration of B cells in lymph nodes, was the most elevated cytokine in flare compared to remission** (27). Subsequent studies are looking for differences in expressions of various proteins after treatment with siltuximab (28).
- **IL-1 β has been proposed as a possible contributor to iMCD pathogenesis.** IL-1 β inhibition has been effective in two documented cases of patients with iMCD refractory to anti-IL-6 therapy (29, 30). IL-1 β is upstream of IL-6 and VEGF and leads to IL-6 production through activation of NF- κ B. What cellular signaling pathways are activated in the immune cells involved in UCD and iMCD?

What treatments are most effective at stopping UCD and iMCD?

- A systematic literature review found a variety of treatments (corticosteroids, cytotoxic chemotherapy, anti-IL-6 therapy, immunomodulatory) are used with variable effectiveness in patients with iMCD.
- A group of international experts reviewed the existing data and published **treatment guidelines for iMCD (32) and UCD (33)**.
- **Surgical excision is the recommended first line treatment for UCD.**
- **IL-6 inhibition is the recommended first line treatment for iMCD.** Approximately one-third of iMCD patients do not respond to recommended first-line treatment with IL-6 signaling blockade (34). There are **few treatment options for patients who do not respond to treatment with anti-IL-6 therapy**. Cytotoxic chemotherapy is commonly administered in these patients with good efficacy but a harmful side effect profile.
- **Three documented cases of iMCD have been successfully treated with the mTOR inhibitor sirolimus (35).** Sirolimus inhibits four aspects of iMCD that CDCN research has identified: T cell activation, B cell activation, VEGF production, and mTOR activation. A **clinical trial** at the University of Pennsylvania and the University of Arkansas for Medical Sciences is currently evaluating sirolimus in anti-IL-6 refractory patients (NCT03033904) (36).
- An in-depth analysis of available clinical trial data was performed to identify characteristics that may predict which patients will respond to siltuximab (37). The study found **eight baseline laboratory parameters that were significantly different between responders and nonresponders**: albumin, immunoglobulin G (IgG), immunoglobulin A, C reactive protein (CRP), fibrinogen, hemoglobin, sodium, and triglycerides.
- A **natural history study of Castleman disease patents (ACCELERATE)** is ongoing (38).

For more information, please refer to the following reviews of Castleman disease:



Dispenzieri A, Fajgenbaum DC. Overview of Castleman disease. *Blood*. 2020; 135(16):1352-1364.

Fajgenbaum DC. Novel insights and therapeutic approaches in idiopathic multicentric Castleman disease. *Blood*. 2018;132(22):2323-2330.