Increased mTOR Activation in Idiopathic Multicentric Castleman Disease

Daniel J. Arenas[1], Katherine Floess[1], Dale Kobrin[1], Maya Srkalovic[1], Jasira Ziglar[1], Johnson Khor[1], Sophia A.T. Parente[1], Sheila Pierson[1], Daniel Martinez[2], Gerald Wertheim[3], Joseph Burr[1], David Teachey[3], David C Fajgenbaum[1]

[1]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Department of Pathology, Children's Hospital of Philadelphia, Philadelphia, PA [3]Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA

BACKGROUND

- Idiopathic multicentric Castleman disease (iMCD) is a rare and poorly-understood hematologic disorder characterized by lymphadenopathy, systemic inflammation, cytopenias, and multi-organ dysfunction.[1]
- Two sub-types: TAFRO (thrombocytopenia, anasarca, fibrosis, renal dysfunction and organomegaly) and non-otherwise-specified (NOS)
- Other Castleman types: Unicentric (UCD) and HHV-8-positive MCD.
- 33% of iMCD patients do not respond to siltuximab (the only FDA approved treatment).[2]
- Recent case series demonstrated beneficial responses to mTOR inhibition by sirolimus in 3 patients.[3]
- Activation of the mTOR pathway, including mTORC1 and mTORC2, has been demonstrated in a number of lymphoproliferative disorders.

OBJECTIVE

- Compare mTOR activation in iMCD compared to multiple types of controls.
- Characterize mTOR activation in other types of Castleman disease and related lymphoproliferative disorders.
- Identify specific cell types that manifest mTOR hyperactivation.

METHODS

- Lymph-node samples: 20 iMCD-TAFRO, 6 iMCD-NOS, 8 HHV-8+ MCD, 7 UCD. Largest CD lymph node tissue sampleset used to date.
- Controls: 6 reactive lymph nodes without an autoimmune/cancer diagnosis. 5 sentinel nodes from non-metastasized breast cancer.
- Related diseases: 4 Autoimmune lymphoproliferative syndrome (ALPS), 5 SLE, and 5 Hodgkin's lymphoma.
- Immunohistochemistry for pS6 and other mTOR effectors: p4EBP1 and p70S6K. Annotation of secondary follicle structures. Color deconvolution to find percentages of areas with none, weak, medium, and strong pS6+ staining.
- Compositional analysis to compare proportion of stained-areas across groups (centrometric log-rate transformation of the data).
- Random effect model to combine effect sizes (standardized mean differences) from different mTOR effectors.
- Co-immunofluorescence studies on 4 iMCD samples to identify which cell types are pS6+.
- Gene set enrichment analysis (GSEA) on serum proteomics data from 90 iMCD patients and 44 healthy controls. Test hypothesis of increased mTOR activation in iMCD.

RESULTS

pS6 is significantly increased in iMCD-TAFRO: Analysis of 10 iMCD lymph node samples and 5 sentinel nodes showed increased pS6 staining in the interfollicular space (p=0.003) (Fig 1).

pS6-expresson is consistent across MCD and trends lower in UCD: pS6 staining was similar across clinical subtypes of iMCD as well as HHV-8+ MCD (Fig 2A). In UCD, there was a trend towards decreased staining in the mantle zone (p=0.065) and interfollicular space (p=0.089).

pS6-expression in iMCD is similar to ALPS and increased compared to other lympho-proliferative disorders: pS6 expression in iMCD is similar to ALPS (a well-known responder to mTOR inhibition). iMCD ps6-expression was significantly higher than SLE (p=0.032) and HL (p=0.019), suggesting that the mTOR activation observed in iMCD is not simply due to lymphoproliferation.

Other mTOR effectors and read-outs of mTOR activation are also elevated in iMCD: p4EBP1 and p70s6K were also elevated in iMCD compared to controls (Fig 3A-3C). Effect size synthesis, of the standardized mean difference, confirms the result (Fig 3D).

Multiple cell populations in the interfollicular space express pS6: We sought to identify a cell type harboring the increased mTOR activation. Most pS6-positive cells were of hematopoietic lineage (CD45+) and T-cells represented only a small proportion. Macrophages and plasma cells made up the largest proportion of pS6-positive cells (Fig 4).

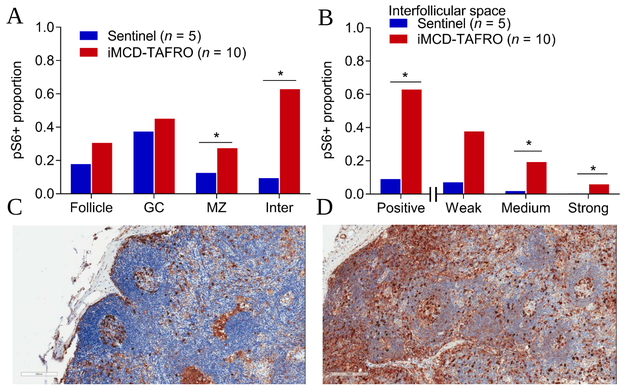

Fig. 1. pS6 staining of iMCD-TAFRO vs. controls. (A) Across lymph node structures. pS6 expression was significantly increased in iMCD versus sentinel lymph nodes. (B) Staining-intensity proportions in the inter-follicular space. (C,D) Representative images of sentinel nodes and iMCD-TAFRO, respectively.

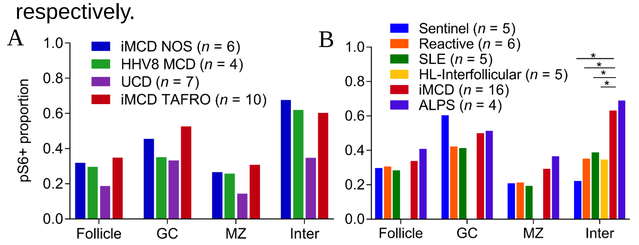

Fig. 2. Comparison of pS6 staining across CD subtypes and other lymphoproliferative diseases. Staining was similar across the multi-centric types but trended lower for UCD. pS6 expression was similar for iMCD and ALPS, but higher than other inflammatory lymphadenopathies, SLE and HL.

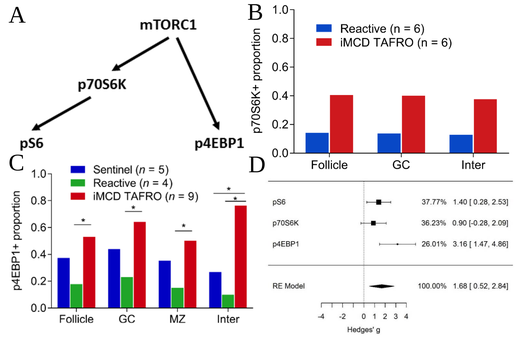

Fig. 3. Other mTORC1 effectors, p4EBP1 and p70S6K, are also elevated in iMCD. (A-C). It was important to rule out a potential novel bypass of mTORC1 to phosphorylate S6. Synthesis of the standardized mean differences, by random effects model, shows comparable effects for all three mTOR effectors and a significant combined effect size (D).

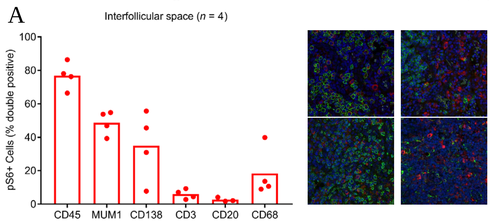

Fig. 4. Identification of cell types with increased mTOR activation. (A) Co-IF for different cell markers. A majority of pS6-positive cells were of the hematopoietic lineage (CD45+). Only a small fraction were T cells (CD3+).

CONCLUSIONS

- **Characterization of mTOR hyperactivation in iMCD through the first systematic investigation of lymph node tissue in CD. Increased activation in the interfollicular space; not limited to one cell type.**
- **The level of mTOR activation is similar to ALPS, a disease well known to respond to mTOR-inhibition.**
- **Unicentric Castleman disease trended towards lower mTOR activation compared to iMCD.**
- **mTOR activation in iMCD is likely not simply a result of lymphoproliferation.**
- **Findings led to the establishment of a clinical trial of sirolimus in iMCD (NCT03933904).**

David Fajgenbaum, MD, MBA, MSc
Executive Director, Castleman Disease Collaborative Network (www.cdcn.org)
Assistant Professor of Medicine, Division of Translational Medicine/Human Genetics, University of Pennsylvania
DavidFa@mail.med.upenn.edu | 215-614-0936

Conflict of Interest Disclosure: DF has received research funding from Janssen Pharmaceuticals. No other authors have anything to disclose.

References:

1. Fajgenbaum DC, Uldrick TS, Bagg A, et al. International, evidence-based consensus diagnostic criteria for HHV-8–negative/idiopathic multicentric Castleman disease. Blood. Prepublished online January 13, 2017; DOI 10.1182/blood-2016-10- 746933.
2. van Rhee, Frits, et al. "Siltuximab for multicentric Castleman's disease: a randomised, double-blind, placebo-controlled trial." The lancet oncology 15.9 (2014): 966-974.
3. Fajgenbaum, David C., et al. "Identifying and targeting pathogenic PI3K/AKT/mTOR signaling in IL-6-blockade-refractory idiopathic multicentric Castleman disease." The Journal of clinical investigation (2019).