Increased mTOR Activation in Idiopathic Multicentric Castleman Disease

**BACKGROUND**

- Idiopathic multicentric Castleman disease (IMCD) is a rare and poorly-understood hematologic disorder characterized by lymphadenopathy, systemic inflammation, cytopenias, and multi-organ dysfunction.
- Two sub-types: TAFRO (thrombocytopenia, anasarca, fibrosis, renal dysfunction and organomegaly) and non-other-specified (NOS).
- Other Castleman types: Unicentric (UCD) and HHV-8-positive MCD.
- 33% of IMCD patients do not respond to silktuximab (the only FDA approved treatment).
- Recent case series demonstrated beneficial responses to mTOR inhibition by sirolimus in 3 patients.
- 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 sample set used to date.
- Controls: 6 reactive lymph nodes without an autoimmune/cancer diagnosis, 5 sentinel nodes from non-metastasized breast cancer.
- Related autoimmune diseases: Autoimmune lymphoproliferative syndrome (ALPS), 5 SLE, and 5 Hodgkin’s lymphoma.
- Immunohistochemistry for pS6 and other mTOR effectors: p4EBP1 and p70S6K.
- Annotational of secondary follicle structures.
- Color deconvolution to find percentages of areas with none, weak, moderate, or 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 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).

**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 expression 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 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).

**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 treated 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 (NCT03393304).

References:

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