GM-CSF Pathway Signature Identified in Temporal Artery Biopsies of Patients With Giant Cell Arteritis

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Abstract
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BACKGROUND

- Giant Cell Arteritis (GCA) is a type of large vessel vasculitis that can cause blindness and aortic aneurysms[1]
- A significant unmet medical need remains in GCA, as current treatment options are limited
  - Relapse increases corticosteroids (CS) exposure and toxicity
  - Tocilizumab blockade of IL-6R significantly reduces relapses; however, ~45% of patients in the GiACTA trial did not achieve a sustained remission following weekly or bi-weekly tocilizumab dosing for 52 wks[2]
- Macrophages/dendritic cells (DCs) and T helper (T_H1/T_H17) lymphocytes are major contributing cell types in GCA pathogenesis[1]
- Granulocyte-macrophage colony stimulating factor (GM-CSF) may contribute to GCA pathogenesis by promoting the actions of key cell types involved by stimulating giant cell formation[3,4]
  - GM-CSF produced primarily by CD4+ T helper T_H1 and T_H17 cells can stimulate conventional DCs and promote differentiation of monocyte-derived DCs
  - GM-CSF may drive DCs to program naïve CD4+ cells to T_H1, T_H17, T_H follicular and T_HGM[5] phenotypes characterized by the expression of IFNγ, IL-17, IL-21 and GM-CSF respectively
  - GM-CSF may promote the proliferation and migration of vascular endothelial cells, thus contributing to angiogenesis
- GM-CSF mRNA expression has been reported in GCA lesions[6] and in peripheral blood mononuclear cells of symptomatic GCA patients[7]
- Mavrilimumab (KPL-301), a human monoclonal antibody targeting the GM-CSF receptor alpha subunit, (GM-CSF-Rα) is currently under investigation for GCA (Phase 2; NCT03827018)

OBJECTIVES

- To examine expression of GM-CSF cytokine and receptor in GCA arteries compared to control arteries at the mRNA and protein level
- To explore the GM-CSF-Rα signaling pathway associated transcriptomic signature in GCA arteries compared to control arteries
- To measure the effect of mavrilimumab on genes relevant to GCA pathophysiology in *ex vivo* GCA artery cultures

METHODS

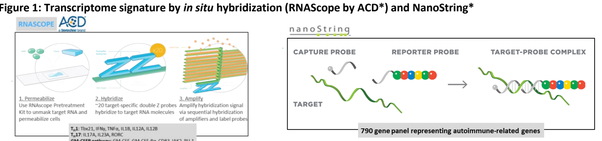
mRNA detection:

- RNA from fresh human GCA and control temporal artery biopsies (TAB) (n=10 each) was analyzed for GM-CSF and GM-CSF-Rα by qRT-PCR using Taqman assays. Data were normalized to housekeeping gene GUSb and expressed as fold change with respect to average of control samples
- Fresh GCA and control TAB (n=7 each) were sectioned, embedded in Matrigel®, and cultured in supplemented RPMI medium for 5 days. RNA was isolated and expression of relevant genes analyzed by qRT-PCR using Taqman™ assays; data were normalized to housekeeping gene GUSb and expressed as fold change with respect to average of control samples
- Two different platforms, RNAScope® (RS) and NanoString® (NS), were used to assay mRNA expression of multiple genes in commercially sourced FFPE sections of GCA (n=18) and control (n=5 for RS + 5 for NS) TAB (Figure 1)
  - RNAScope *in situ* hybridization to detect 15 mRNA transcripts associated with T_H1, T_H17, and GM-CSFR pathway. Expression score was calculated as RS score (dots/cell; Table 1) multiplied by % cells positive (> 1 dot/cell)
  - NS analysis with Autoimmune Discovery panel (~800 genes including those associated with T_H1, T_H17, and GM-CSFR pathway) using nSolver software to perform data analysis

Table 1: RS Score

RS score	Scoring criteria
0	No staining or <1 dot/10 cells
1	1-3 dots/cell
2	4-9 dots/cell
3	10-15 dots/cell
4	>15 dots/cell

Figure 1: Transcriptome signature by *in situ* hybridization (RNAScope by ACD®) and NanoString®



Immunohistochemical (IHC) staining for protein expression:

- TAB were fixed in 4% PFA in PBS, embedded in OCT, frozen at −80°C, sectioned at 10 microns thickness, and incubated at 4°C overnight with anti-GM-CSF followed by secondary Ab

***Ex vivo* culture of temporal arteries:**

- Control or GCA temporal arteries (n=7 each) were sectioned (0.8-1 mm), embedded in Matrigel and cultured in supplemented RPMI medium
- Each artery sample was cultured in the presence of placebo or mavrilimumab (20 mcg/ml) for 5 days prior to RNA isolation and qRT-PCR analysis and normalized as mentioned above

RESULTS

- GM-CSF and GM-CSF-Rα mRNA expression as detected by qRT-PCR was elevated in fresh GCA arteries compared to controls (Figure 2A)
- *Ex vivo* cultures of fresh GCA and control artery samples demonstrated increased mRNA expression of GM-CSF-Rα pathway-associated genes such as GM-CSF and PU.1 and immune cell infiltration, activation and inflammation-associated genes such as CD3ε, CD83, HLA-DRA and TNFα (Figure 2B)
- Transcriptomic analysis showed elevated mRNA expression of genes associated with GM-CSF-Rα pathway such as STAT5 and transcription factor PU.1 and downstream inflammatory cell types such as CD83 and HLA-DRA in GCA arteries compared to controls as examined by NS and RS (Figure 2C-2F)
- Increased GM-CSF protein in GCA arteries compared to control arteries where GM-CSF protein was not detected further demonstrated presence of this pathway in GCA (Figure 2G)
- mRNA expression of T_H1 associated genes was increased in GCA biopsies compared to control, consistent with previously published[8] data showing increased presence of T_H1 cells in GCA (Figure 3)

Figure 2: GCA arteries exhibit increased expression of genes associated with GM-CSF-Rα pathway and downstream cell types (mRNA and protein) which indicates activation of GM-CSF-Rα pathway in disease relevant tissue regions of GCA

Figure 2A. Fresh TAB

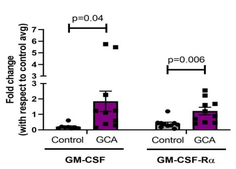


Figure 2B. Cultured fresh TAB

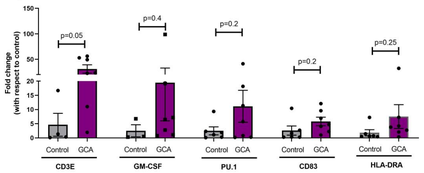


Figure 2C. RNAScope

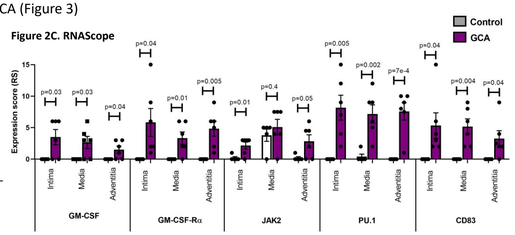


Figure 2D. RNAScope

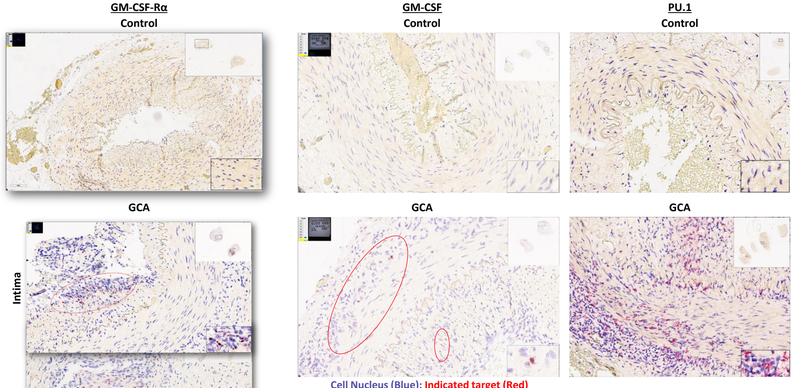


Figure 2E. Nanostring

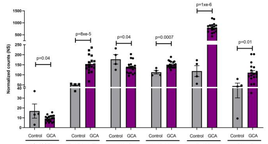


Figure 2F. Differential Expression of Genes in GCA vs. Control

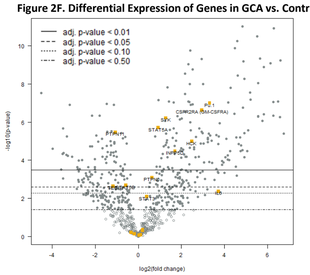


Figure 2G. Immunohistochemistry

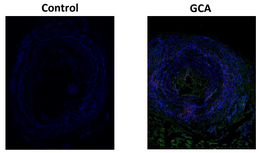


Figure 3: mRNA expression of multiple genes associated with T_H1 was elevated in GCA arteries consistent with previous findings

Figure 3A. RNAScope

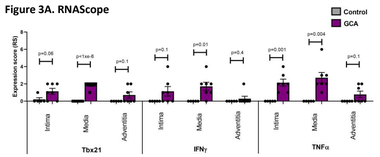


Figure 3B. Nanostring

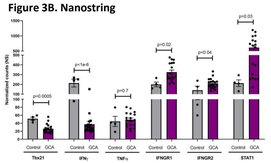


Figure 3C. qRT-PCR (Cultured TAB)

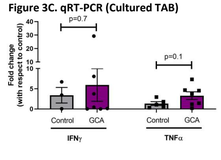


Figure 4: mRNA expression of multiple genes associated with T_H17 was lower than expected in GCA arteries potentially due to CS treatment of contributing patients

Figure 4A. RNAScope

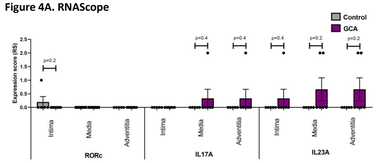


Figure 4B. Nanostring

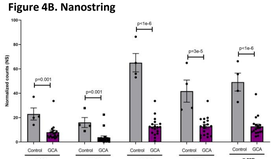
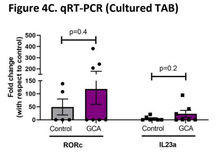
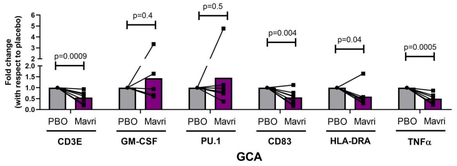


Figure 4C. qRT-PCR (Cultured TAB)



- In contrast, mRNA expression of T_H17 associated-genes and CS-responsive genes (IL17A, IL23A) was lower than expected in GCA biopsies suggesting patients may have been treated with CS prior to biopsy (T_H17 cells particularly sensitive to CS[8,9]) (Figure 4)
  - Increase in GM-CSF pathway signature in biopsies of potentially CS-treated patients could suggest GM-CSF pathway in GCA pathology is less affected by CS and indicates potential therapeutic opportunity in GCA independent of CS
- Treatment of *ex vivo* cultures of GCA arteries with mavrilimumab suppressed expression of these genes indicating the biological effect of mavrilimumab on genes relevant to GCA pathophysiology (Figure 5)

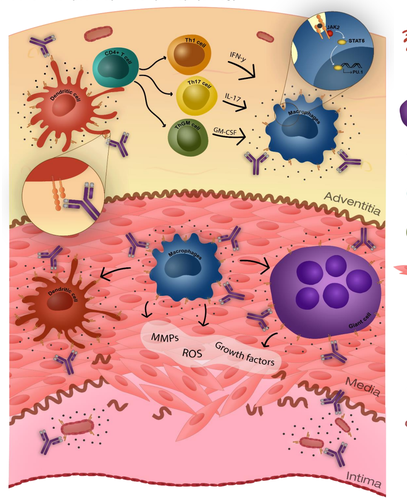
Figure 5 – Mavrilimumab suppresses the increased expression of genes associated with immune cell infiltration, inflammation and GM-CSF pathway in cultured GCA arteries



CONCLUSIONS

- Increased GM-CSF and T_H1 pathway transcriptomic signatures were observed in GCA arteries by independent analytical techniques
- GM-CSF protein expression in GCA biopsies is consistent with increased transcriptomic signature
- Increased expression of genes associated with immune cell infiltration, inflammation, and GM-CSF-Rα pathway signaling in cultured GCA arteries is suppressed by mavrilimumab
- These data implicate the GM-CSF pathway in GCA pathophysiology and support confidence in rationale for targeting the GM-CSF pathway in GCA (Figure 6)

Figure 6: GM-CSF pathway in GCA pathophysiology



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DISCLOSURES:
* Images Courtesy of ACD/NanoString
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