Kv1.3 Expression on Effector Memory T-Cells in Sporadic Inclusion Body Myositis: Potential for Targeted Immunotherapy with Dalazatide

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Introduction

- Sporadic Inclusion Body Myositis (sIBM) is a progressive and disabling muscle disease that afflicts patients over the age of 45 year
- Estimated prevalence is 51.3 per million over the age of 50 years
- Prevalence of sIBM is likely to increase over the next few decades as the percentage of population above the age of 65 years will double by 2030
- IBM remains greatly underdiagnosed and little is known about differences in disease phenotype, association with other diseases, and rates of progression within those diagnosed with IBM
- sIBM remains a disease without cure and treatment and treatment trials of most conventional immunosuppressive agents have failed to date
- Recently two separate groups reported immunophenotypic changes in circulating and muscle-based lymphocytes of patients with sIBM, with the development of an apoptotic-resistant immunophenotype and development of a Natural Killer (NK) cells-dominant T-cell leukemic phenotype (TGL)
- Both groups described the same cells but looked at different cell markers; Pandya looked at $CD28^{null}$ status while Greenberg evaluated $CD57^{+}$ cells. It is well known that **$CD4^{+}$ and $CD8^{+}$ cells that lose CD28 expression start expressing CD57 on the cell surface**
- **$CD8^{+}CD28^{null}CD57^{+}$** are known to express Kv1.3 channels on the cell surface, a marker of T Effector Memory Cell (T_{EM})
- **Dalazatide** is a potent and specific inhibitor of Kv1.3 channels and shown effective in reducing the pathology in experimental autoimmune encephalitis (EAE) and in human patients with psoriasis (phase 1)

Objectives

The purpose of this study is

1. to demonstrate Kv1.3+ immune cells in muscles from patients with sIBM
2. to quantitate Kv1.3+ immune cells in muscles from patients with sIBM
3. Provide pre-clinical data for a phase 1 study of dalazatide in sIBM

Design and Methods

- Three unstained slides prepared from formalin-fixed paraffin-embedded blocks from 16 patients was sent to HistoTox in Boulder, CO
- Slides were stained with hematoxylin and eosin (H&E) and by routine immunohistochemical (IHC) methods for CD3 (BD Biosciences mouse monoclonal) and voltage-gated potassium channel (Kv1.3) (Alomone APC101 rabbit anti-Kv1.3)
- Slides were scanned (Aperio AT2 whole slide scanner) and analysis performed with whole slide imaging software (Aperio Image Scope) by a pathologist blinded to the biopsy diagnosis

Muscle Biopsy Samples Demographics Table

Sample	Disease	Age	Sex
UCI0102	IBM	76	M
UCI0103	IBM	61	F
UCI0104	IBM	72	M
UCI0105	IBM	54	M
UCI0113	IBM	55	F
UCI0106	Dermatomyositis	57	F
UCI0107	Dermatomyositis	45	F
UCI0108	Dermatomyositis	53	F
UCI0120	Dermatomyositis	40	M
UCI0116	Necrotizing myopathy	73	M
UCI0117	Necrotizing myopathy	64	F
UCI0118	Necrotizing myopathy	63	F
UCI0119	Necrotizing myopathy	67	F
UCI0114	Normal control	55	M
UCI0115	Normal control	63	M
UCI0121	Normal control	36	M

Results

Figure 1: H&E staining shows fiber size variability and many small muscle fibers with rimmed vacuoles

Figure 2: H&E staining shows endomysial lymphocytic inflammation, muscle fiber invasion of non-necrotic fibers and muscle fiber variability

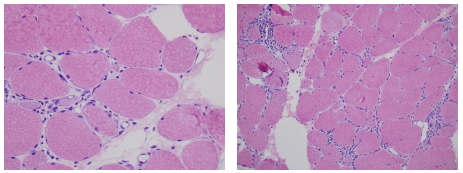


Figure 3: UCI0103 (Patient with IBM) Site 5; CD3 IHC, 200x. Inflammatory cells expressing CD3 (black arrows) are numerous and obscure view of the affected myocyte.

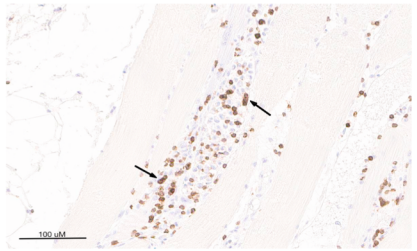
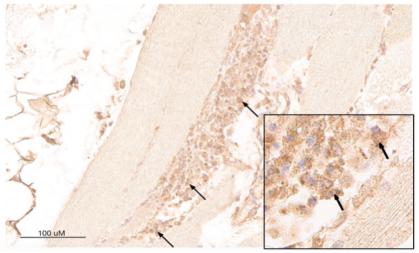


Figure 4: UCI0103 (Patient with IBM) Site 5; Kv1.3 IHC, 200x. Inset 400x. Cells expressing Kv1.3 (examples indicated by black arrows) are abundant at this site. There is increased background staining of surrounding tissues.



Results

Figure 5: CD3 immunostaining shows many CD3+ mononuclear cells surrounding muscle fibers. The CD3+ cells also staining strongly positive for Kv1.3

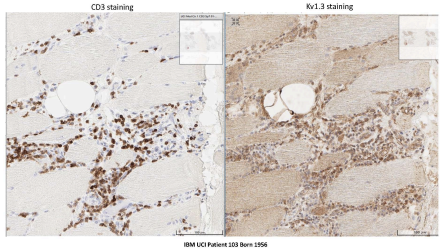


Figure 6: Group average total cell count of CD3+ and KV1.3+ cells A) per site in muscle biopsies and B) in whole muscle biopsies

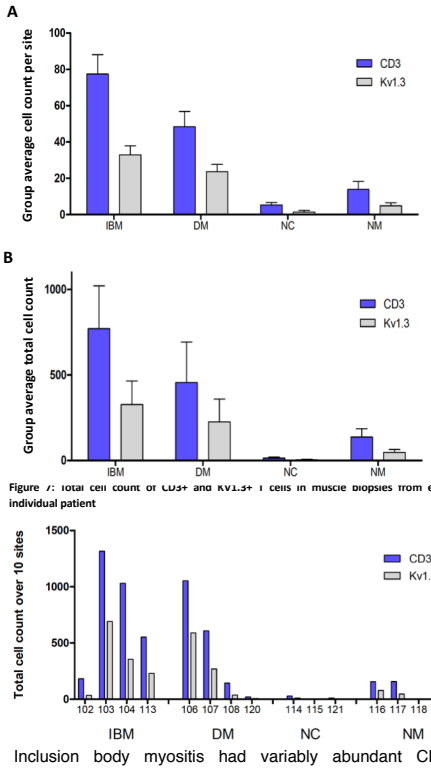
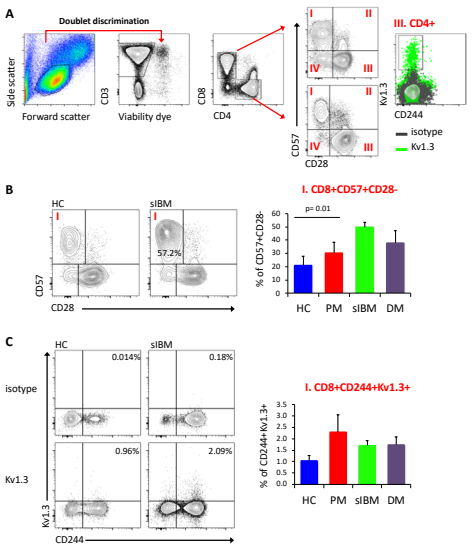


Figure 7: Total cell count of CD3+ and KV1.3+ T cells in muscle biopsies from each individual patient

- Inclusion body myositis had variably abundant CD3+ T-lymphocytes present within the perimysium and infiltrating the endomysium/myocytes
- Kv1.3 co-localization ranged from 22.4% to 53.1%
- In DM, CD3-expressing T-cells were primarily present surrounding blood vessels and multi focally extending into the interstitium/perimysium
- Kv1.3 co-localization ranged from 28.3% to 64.3%
- Kv1.3 is expressed in a small subset of circulating CD8+ T cells that express CD57 or CD244, but not CD28.

Figure 8: Interrogation of Kv1.3 expression on human PBMCs by flow cytometry. (A) the gating strategy used to determine the expression levels of Kv1.3 on CD4+ and CD8+ T cells. (B) The frequency of CD8+CD57+CD28- cells in patients. (C) The frequency of CD8+CD244+Kv1.3+ cells in patients. Isotype staining is shown to determine the specificity of the Kv1.3+ antibody.



Conclusions and Next Steps

- Patient biopsy samples from IBM and DM showed variable amount of Kv1.3+ immune cells
- These immune cells co-localized with CD3+ lymphocytes
- These Kv1.3+ cells represent a potential opportunity to treat IBM with dalazatide
- Next step would be to estimate the distribution and frequency of $CD4^{+}CD28^{+}CD57^{+}Kv1.3^{+}$ and $CD8^{+}CD28^{+}CD57^{+}Kv1.3^{+}$ lymphocytes in peripheral blood in patients with sIBM and compare to other disease controls and normal healthy controls
- Compare the frequency of Kv1.3+ cells in peripheral blood to the frequency of Kv1.3+ cells in skeletal muscle
- Plan a phase 1 trial of dalazatide in sIBM

References

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3. **Greenberg SA**, Pinkus JL, Amato AA, Kristensen T, Dorfman DM. Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. Brain 2016;139:1348-60.