Development of a 10 color flow cytometric assay to assess binding of a monoclonal antibody (VB421) against IGF 1R in Peripheral Blood Mononuclear Cells (PBMCs) from patients with Thyroid Eye Disease

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Introduction

Thyroid Eye Disease (also known as Graves' ophthalmopathy, is a debilitating autoimmune disorder that occurs in patients with Graves' Disease in which inflammation in the muscle and fat tissue behind the eyes results in proptosis, diplopia, redness, pain, and swelling, leading to photosensitivity, blurred vision, and in serious cases, blindness The mechanistic underpinnings of TED involve a complex interaction between autoantibody mediated stimulation of Thyroid Stimulating Hormone Receptor (and Insulin like growth factor 1 receptor (IGF 1 R) signaling in orbital fibroblasts that cause orbital tissue inflammation and expansion Current therapies include corticosteroids and teprotumumab, as well as surgical intervention to prevent vision loss VB 421 L onigutamab) is a high affinity (KD 50 pM monoclonal antibody directed against IGF 1 R that induces rapid and efficient receptor internalization VB 421 is being developed as a potential treatment for TED To support clinical development of VB 421 a multi color flow cytometric assay was developed to monitor the binding of VB 421 to IGF 1 R on the surface of human Peripheral Blood Mononuclear Cells (As VB 421 induces rapid IGF 1 R internalization upon binding, a traditional receptor occupancy assay format is less feasible Therefore, this assay is designed to detect both total IGF 1 R (free IGF 1 R and IGF 1 R/VB 421 complex) as well as free IGF 1 R This assay utilizes two anti IGF 1 R antibodies that bind different IGF 1 R epitopes and do not compete 1 H 7 competes with VB 421 while 33255 does not Together these two antibodies allow the assay to distinguish between unbound and total IGF 1 R This format was qualified using a cell line that constitutively expresses IGF 1 R at high levels (A 549 This assay will be used to monitor total and free amounts of IGF 1 R on live CD 3 Total T Cells, CD 4 T Cells, CD 8 T Cells, CD 19 B Cells, and Myeloid Cells expressing both CD 11 b and CD 16 after administration of VB 421 This assay format has the potential to be applied to other situations where target receptor binding leads to rapid internalization and loss of receptor binding epitopes.

Assay Principle

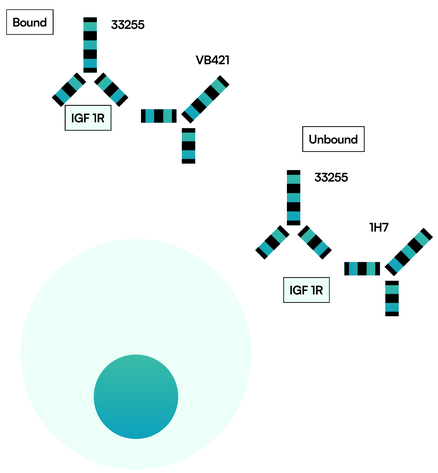

Figure 1 Healthy Donor PBMCs are isolated, frozen, and stored in Liquid Nitrogen (Vapor Phase) Upon thawing, PBMCs are counted, and treated with 0 5 µ g/mL VB 421 followed by viability dye, and surface staining using competitive 1 H 7 and noncompetitive 33255 clones to VB 421 Cells are enumerated by Bound 33255 Unbound 1 H 7 and Total 33255 1 H 7 as well as mean fluorescence intensity (MFI).

Multi Color Staining Distinguishes Distinct PBMC Populations

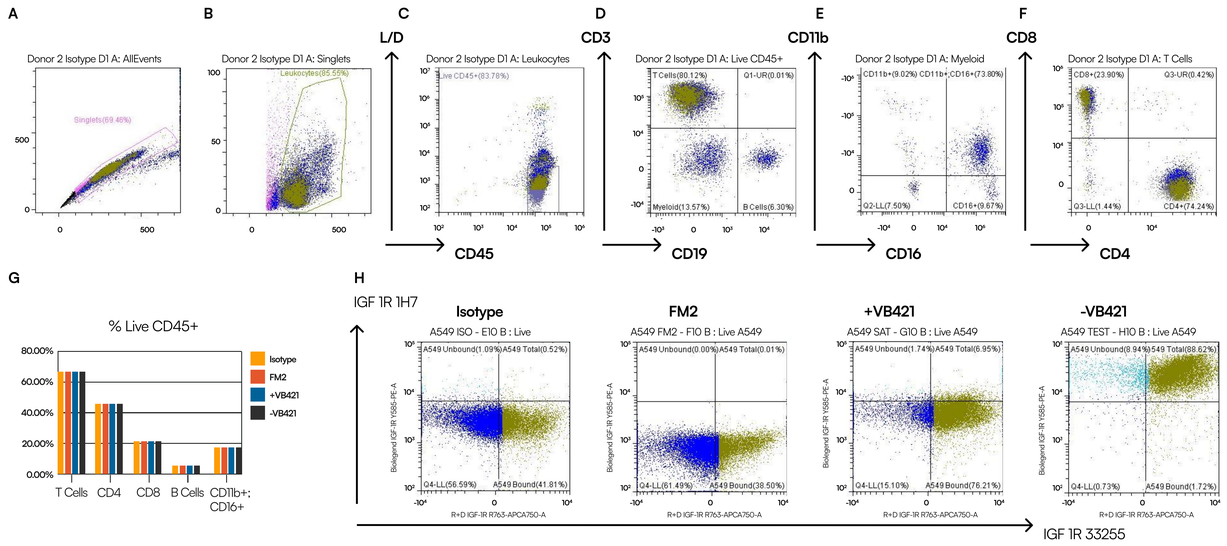

Figure 2 Healthy PBMCs are first gated on Singlets (followed by FSC/SSC (and Live CD 45 ++(T and B cells are then distinguished within Live CD 45 by CD 3 /CD 19 (and Myeloid Cells are distinguished from CD 3 /CD 19 Cells by CD 11 b/CD 16 (Within CD 3 cells, CD 4 and CD 8 T cells are distinguished by CD 4 and CD 8 (Consistency of cell populations across groups (3 in 6 replicates) is evident (A 549 cells (ATCC CCL 185 are used as positive controls for IGF 1 R staining (H).

Representative IGF 1R Gating

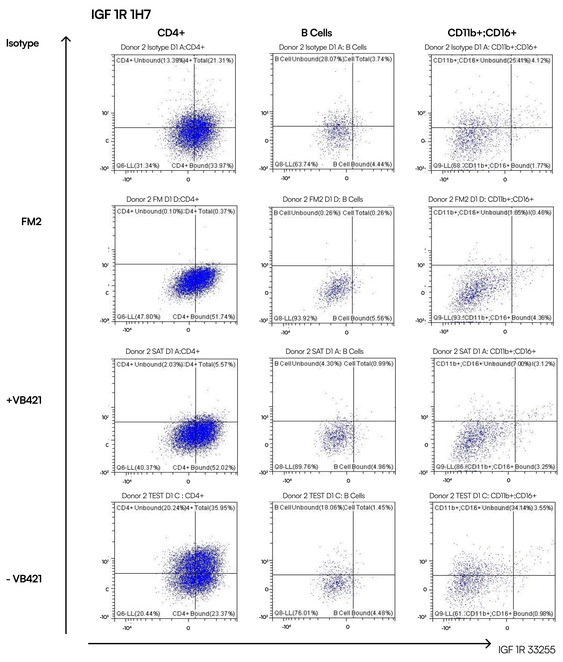

Figure 3 Representative IGF 1 R Gating Following Following gating as described in Figures 2 A F, Bound, Unbound, and Total IGF 1 R are distinguished by 1 H 7 (vs 33255 (AlexaFluor® 750 A clear reduction in IGF 1 R signal is observed with VB 421 treatment in CD 4 B Cells, and CD 11 b+ CD 16 FM 2 control includes all surface stains except anti IGF 1 R clones 1 H 7 and 33255.

Conclusions

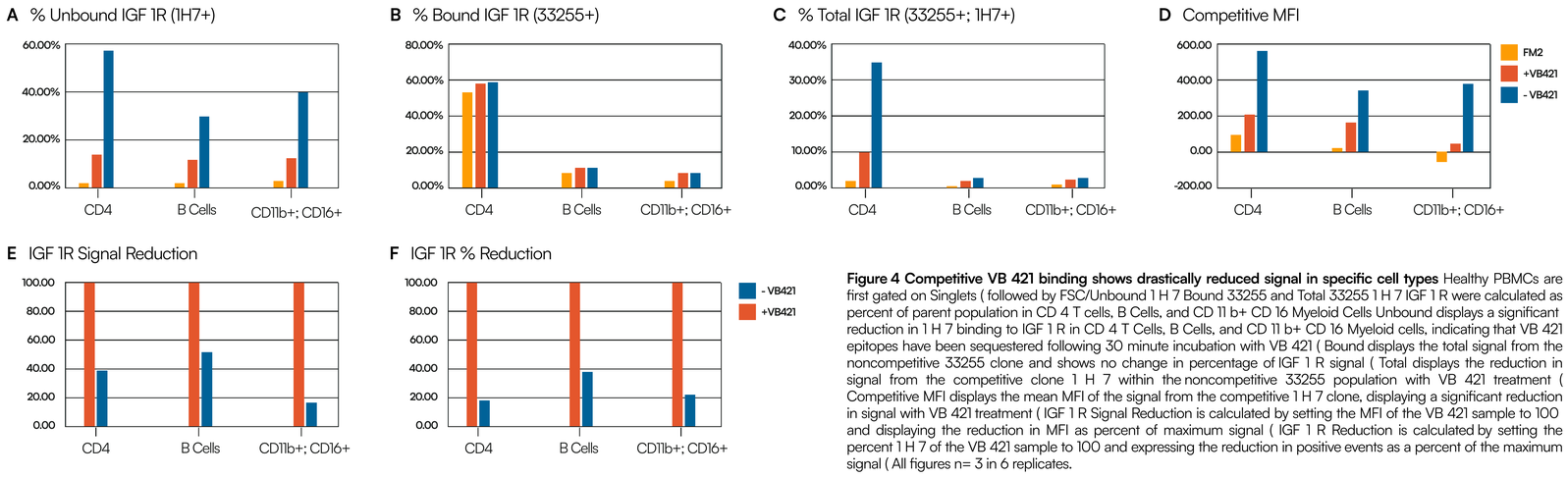

Figure 4 Competitive VB 421 binding shows drastically reduced signal in specific cell types Healthy PBMCs are first gated on Singlets (followed by FSC/Unbound 1 H 7 Bound 33255 and Total 33255 1 H 7 IGF 1 R were calculated as percent of parent population in CD 4 T cells, B Cells, and CD 11 b+ CD 16 Myeloid Cells Unbound displays a significant reduction in 1 H 7 binding to IGF 1 R in CD 4 T Cells, B Cells, and CD 11 b+ CD 16 Myeloid cells, indicating that VB 421 epitopes have been sequestered following 30 minute incubation with VB 421 (Bound displays the total signal from the noncompetitive 33255 clone and shows no change in percentage of IGF 1 R signal (Total displays the reduction in signal from the competitive clone 1 H 7 within the noncompetitive 33255 population with VB 421 treatment (Competitive MFI displays the mean MFI of the signal from the competitive 1 H 7 clone, displaying a significant reduction in signal with VB 421 treatment (IGF 1 R Signal Reduction is calculated by setting the MFI of the VB 421 sample to 100 and displaying the reduction in MFI as percent of maximum signal (IGF 1 R Reduction is calculated by setting the percent 1 H 7 of the VB 421 sample to 100 and expressing the reduction in positive events as a percent of the maximum signal (All figures n= 3 in 6 replicates.

Conclusions

This assay is designed to detect a reduction in both MFI and of population in specific leukocyte subsets by using a novel combination of VB 421 competitive and noncompetitive antibodies against IGF 1 R This assay has reliably shown precision and accuracy with regard to five cell types (T Cells, CD 4 CD 8 B Cells, and CD 11 b+ CD 16 Myeloid cells) With respect to IGF 1 R signal, a significant reduction in both MFI and of parent population is seen in the competitive 1 H 7 antibody with 30 minutes of VB 421 treatment, while no significant difference is seen in the noncompetitive 33255 clone, indicating the specificity of the detection system in specific cell populations A 549 cells were used as positive controls for high levels of IGF 1 R, as total IGF 1 R levels are low in healthy PBMCs, indicating that in TED patients, this assay is sufficient to detect high levels of IGF 1 R, as well as detect a reduction due to VB 421 treatment This assay format has the potential to be applied to other situations where target receptor binding leads to rapid internalization and loss of receptor binding epitopes.

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