Nucleome THERAPEUTICS

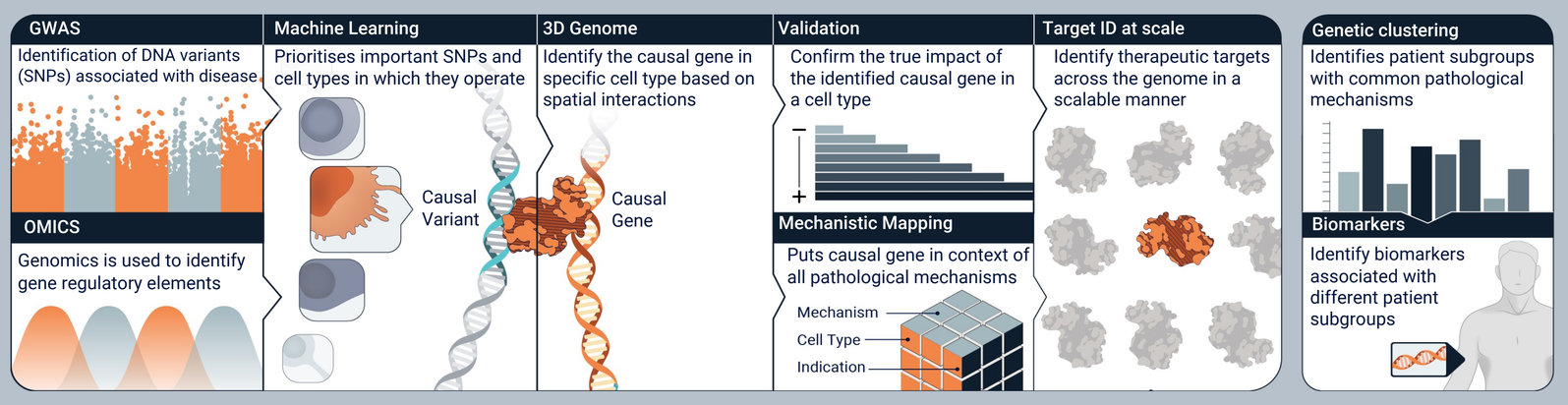
Nucleome website

V-domain Immunoglobulin Suppressor of T cell Activation (VISTA) acts as an activating ligand for monocytes and is upregulated in systemic lupus erythematosus

Tasnia Chowdhury • Kirsty Waddington • Wiktoria Jozefowska • Neil Ashley • Gabriela Pirgova • Chantal Hargreaves • James Heward • Neil Stokes • Rachael Nimmo • Stephen Harrison

Introduction

Nucleome platform unlocks human genetics for drug-discovery

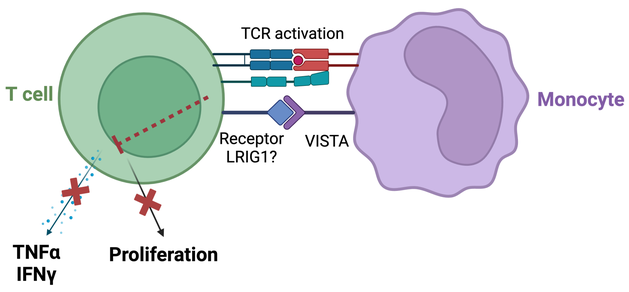


Abbreviations: GWAS – genome wide association studies, SNP – single nucleotide polymorphism

- Genetic evidence increases clinical success of antibody drugs x1.8.[1-2]
- However, GWAS data provides the lowest increase in clinical success, likely due to uncertainty around causal gene identification.
- The precision and scale of Nucleome's platform increases this to x2.8.
- The Nucleome platform has applications beyond target ID e.g. patient selection biomarker identification, drug repurposing and pathological mechanistic mapping.

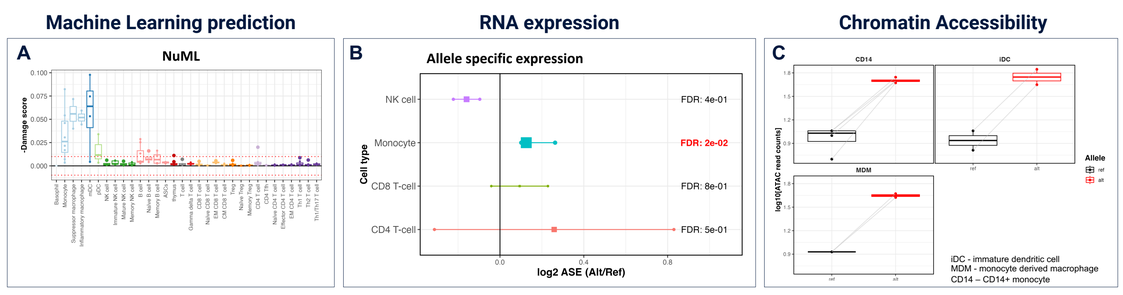
1 Nucleome platform predicted a gain-of-function SNP linked to VISTA is associated with SLE risk

Figure 1



- Systemic lupus erythematosus (SLE) is a chronic autoimmune condition that can affect any organ system, estimated to affect 3.4 million people worldwide.[3]
- VISTA, encoded by the gene *VSIR*, is a negative checkpoint regulator of T cells (Fig. 1).
- Antagonistic antibodies against VISTA have been developed as potential therapeutics for immuno-oncology.
- Literature suggests using VISTA agonists to treat autoimmune conditions, including SLE.[4]
- VISTA's impact on myeloid cells has not been extensively explored.[5]

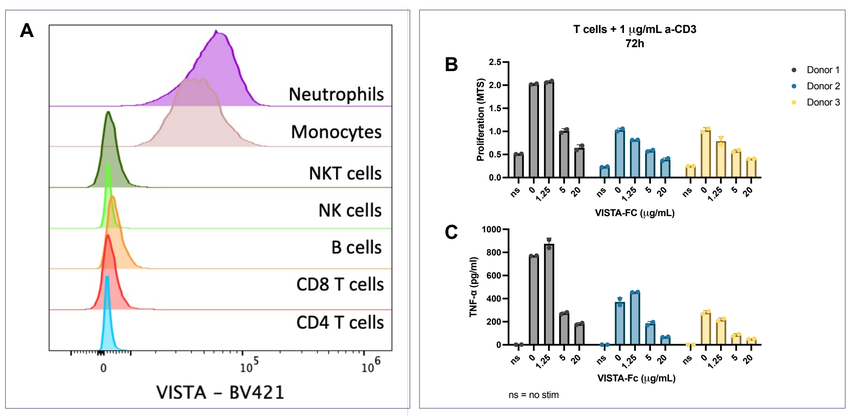
Figure 2



- We have predicted (Fig. 2A) and validated (Fig 2B-C) that a variant associated with risk of SLE increases chromatin accessibility at a putative regulatory element for *VSIR* in monocytes, macrophages and dendritic cells.
- We confirmed increased RNA expression in monocytes (Fig. 2B), and expression quantitative trait loci (eQTL) data (ImmuNexUT) matched the prediction, with increased allele specific expression of this variant in monocytes and mature dendritic cells.
- Our analysis of the genetics predicted an unexpected pro-inflammatory role in myeloid cells suggesting that antagonising VISTA might be beneficial for limiting inflammation in SLE.

2 VISTA is highly expressed on monocytes and acts as a negative regulator of T cell activation

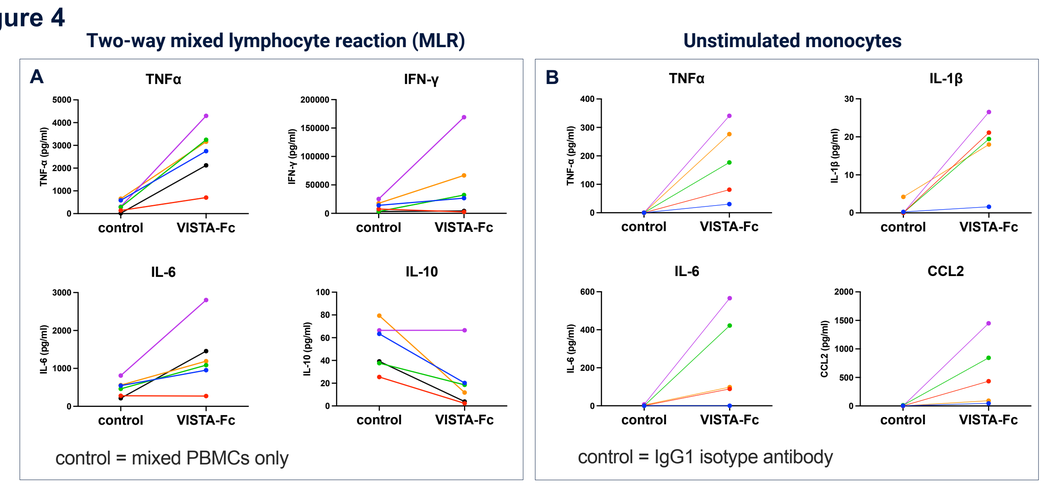
Figure 3



- Monocytes and neutrophils have the highest expression of VISTA amongst white blood cells (n=3 donors) (Fig. 3A).
- To investigate the role of VISTA as a ligand, we used recombinant human VISTA-Fc fusion protein in our functional studies.
- Proliferation (MTS assay, Fig. 3B) and TNFα release (ELISA, Fig. 3C) in primary human T cells stimulated with αCD3 antibody were reduced in a dose-dependent manner with VISTA-Fc treatment after 72 hours.
- These data corroborate the reported inhibitory effects of VISTA on T cells.

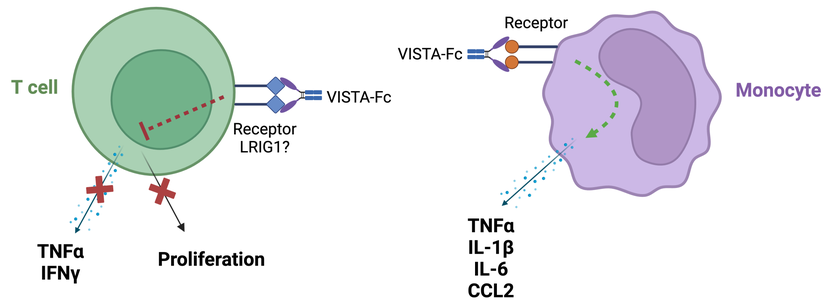
3 VISTA functions as an activating ligand for monocytes

Figure 4



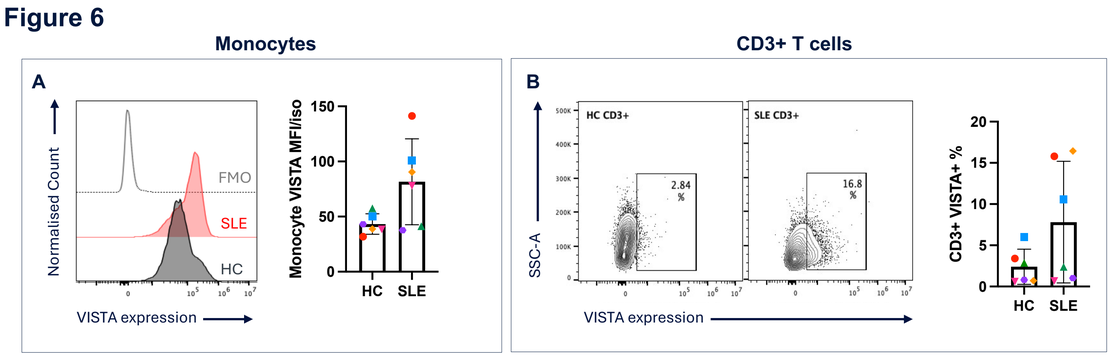
- Pro-inflammatory cytokine release was increased and anti-inflammatory cytokine (IL-10) decreased after culturing peripheral blood mononuclear cells (PBMCs) from two donors in the presence of VISTA-Fc for 4 days (Fig. 4A).
- VISTA-Fc induced pro-inflammatory cytokine release in unstimulated primary human monocytes 18h post-treatment (Fig. 4B), suggesting that monocytes were the cell type driving the inflammatory effect in the mixed cell cultures.
- Our data from these functional studies highlight the divergent effects of VISTA on T cells and monocytes and demonstrates the activatory function of VISTA as a ligand for monocytes (Fig. 5).[6]

Figure 5



4 Expression of VISTA is elevated on SLE patient monocytes and T cells

Figure 6



	Sex	Age	Ethnicity	DMTs
●	F	26	C	HCQ, Mtx
■	F	60	C	HCQ, Pred
▲	F	42	C	Benlysta
◆	M	49	unknown	unknown
⬟	F	32	unknown	none
▼	F	44	unknown	unknown

- VISTA expression was profiled in PBMCs from SLE patients of varied age, sex and treatment status, and compared to age and sex matched healthy controls (HC) (n=6/group).
- VISTA expression was increased on SLE patient monocytes (4 out of 6) (Fig. 6A) and T cells (3 out of 6) (Fig. 6B) compared to healthy controls.
- This demonstrates that VISTA expression is altered in a subset of SLE patients.

Abbreviations: DMT – disease modifying therapy, HCQ – hydroxychloroquine, Pred – prednisolone

Conclusions

- Nucleome Therapeutics' platform predicted and subsequently validated that a SNP in a putative regulatory region for *VSIR* is associated with risk of SLE and increased expression of *VSIR*.
- This indicates that VISTA should be inhibited in SLE, contrary to known roles for VISTA as a checkpoint inhibitor and in vivo studies in murine models that suggest VISTA has a protective role in SLE.
- Mechanistic studies in primary human immune cells were consistent with this prediction and VISTA expression is elevated on monocytes in a subset of SLE patients.
- Our data suggest a novel molecular mechanism for VISTA as an activating ligand, and that antagonising VISTA function on monocytes may be a useful therapeutic strategy for SLE.

References

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Figures 1 and 5 were created with BioRender.com

Contact: tasnia.chowdhury@nucleome.com