Trends in Immunology

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Opinion

VAV1 as a putative therapeutic target in autoimmune and chronic inflammatory diseases

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The guanine nucleotide exchange factor (GEF) VAV1, a previously 'undruggable' protein integral to T/B lymphocyte antigen–receptor signaling, promotes actin polymerization, immunological synapse formation, T cell activation and differentiation, and cytokine production. With the development of novel modalities for targeting proteins, we hypothesize that interventions targeting VAV1 will have therapeutic potential in T and T/B cell-mediated autoimmune and chronic inflammatory diseases. This opinion is supported by recent CRISPR-Cas9 studies showing VAV1 as a key positive regulator of T cell receptor (TCR) activation and cytokine production in primary human CD4$^+$ and CD8$^+$ T cells; data demonstrating that loss/suppression of VAV1 regulates autoimmunity and inflammation; and promising preclinical data from T and T/B cell-mediated disease models of arthritis and colitis showing the effectiveness of selective VAV1 targeting via protein degradation.

VAV1: new options for targeting a previously 'undruggable' immune target

Targeted therapies, including **biologic therapies** (see Glossary) and small-molecule inhibitors, are now standard of care for many **autoimmune and chronic inflammatory diseases**. Despite advances over the past 25 years, considerable unmet needs remain, including treatment failure, drug safety/tolerability, glucocorticoid dependency, and a lack of targeted therapies for certain conditions. In the autoimmune and chronic inflammatory disease treatment landscape, oral small molecules, which largely target intracellular protein tyrosine kinases (PTKs), such as Janus kinases [1], present certain advantages over injectable biologics, including penetration of the cell membrane, low immunogenicity, and convenient administration. Many other intracellular proteins, such as those lacking a targetable (active/allosteric) binding site or that work through protein–protein/DNA interactions, might represent clinically interesting targets but have classically been considered 'undruggable' [2]. One such molecule is VAV1, a multidomain protein with a dual function as both a **GEF** and a scaffolding protein involved in GEF-independent signaling pathways [3].

Vav1$^{-/-}$ mouse data suggest a key role for VAV1 in T/B lymphocyte function and antigen–receptor signaling, particularly in T cells [4,5]. Recently, genome-wide CRISPR-Cas9 screening identified VAV1 as a key positive regulator of T cell activation/function in human Jurkat T cells and primary human CD4$^+$ and CD8$^+$ T cells, all of which are receptive to TCR-mediated signaling [6–8], potentially rendering it a useful target in certain human immune-mediated diseases, including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and multiple sclerosis (MS). While VAV1 is crucial for thymocyte development [4,5], we focus here on its role in mediating TCR and B cell receptor (BCR) signaling in mature T/B cells, because disruption of VAV1 GEF-dependent/independent signaling pathways in effector lymphocytes in an adult immune system is likely more relevant to VAV1 as a potential therapeutic target. We also consider what is known about VAV1 in autoimmune/inflammatory disease

Highlights

Mammalian dual-function guanine nucleotide exchange factor (GEF) VAV1 regulates T/B cell receptor signaling, T cell activation, T helper cell differentiation, cytokine production, actin polymerization, and cytoskeleton reorganization via GEF-dependent and -independent pathways.

Recent CRISPR-Cas9 screening data and animal models (e.g., rodent) of autoimmune and chronic inflammatory disease confirm VAV1 as a positive T cell regulator and support its potential as a candidate therapeutic target in T and T/B cell-mediated diseases.

To date, VAV1 has been considered 'undruggable'. Directly targeting GEF domains is challenging, and approaches to inhibit GEF activity, leaving scaffolding functions intact, may lead to inadequate attenuation of VAV1 activity.

Preclinical studies exploring a new modality of attenuating the dual function of VAV1 through protein degradation show promise in animal models of some autoimmune/inflammatory diseases.

Significance

Despite the availability of multiple advanced targeted biologics and oral small molecules to treat certain autoimmune and chronic inflammatory diseases, considerable unmet needs remain, prompting the search for new drug targets. Novel mechanisms of action that target the dual catalytic and scaffolding functions of the GEF VAV1 might optimally 'drug' VAV1 and, therefore, have broad therapeutic potential in T and T/B cell-mediated diseases.

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pathophysiology and hypothesize that interventions targeting VAV1 have the potential to regulate T and T/B cell-mediated autoimmunity and chronic inflammation.

What is VAV1?

Identified as a human oncogene [9], *VAV1* encodes a GEF that is expressed predominantly in human hematopoietic cells, including T and B cells, monocytes, natural killer (NK) cells, granulocytes, and dendritic cells, whereas family members *VAV2* and *VAV3* are more ubiquitously expressed[I]. The protein structure of VAV1 has been reviewed previously [3,10]. However, the multidomain structure of VAV1 is critical to its activation and function, with different domains regulating VAV1 GEF-dependent and -independent activity and subsequent downstream signaling [3].

Regulation of VAV1 activity

Lymphocyte activation through the TCR and BCR leads to rapid tyrosine phosphorylation of VAV1, releasing intrinsic autoinhibition of the protein [3]. Recent *in vitro* analyses in human Jurkat T cells showed that VAV1 activation is influenced by multiple upstream PTKs and scaffolding proteins and complex phosphorylation dynamics involving multiple tyrosine residues on different VAV1 domains, which may differentially regulate its catalytic and noncatalytic functions [11,12]. For example, in T cells, transmembrane linker for activation in T cells (LAT) and Src homology (SH)2-domain-containing leukocyte protein of 76 kDa (SLP76) form a key scaffold for VAV1, facilitating its proximity to receptor-associated PTKs (Figure 1) [13]. At the BCR, VAV1 interacts in a complex that includes a corresponding scaffolding protein (SLP65) and Bruton's tyrosine kinase

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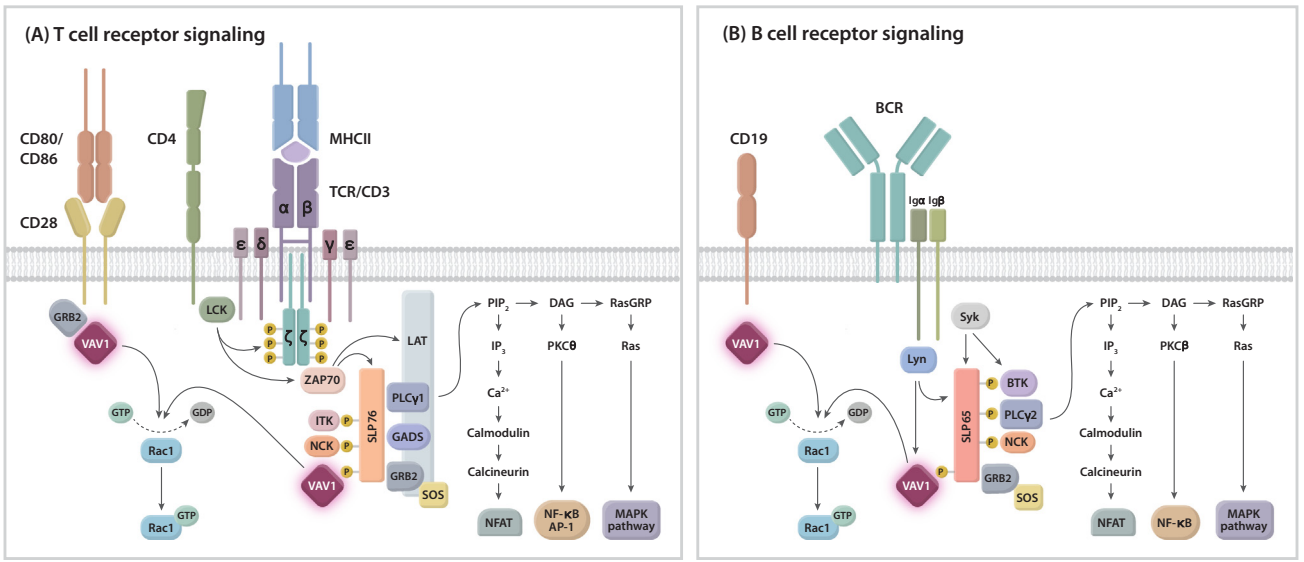

Figure 1. The role of VAV1 in antigen receptor signaling. VAV1 is a key component of antigen–receptor signaling complexes associated with (A) the T cell receptor (TCR)/CD3 (reviewed in [13]) and (B) B cell receptor (BCR; reviewed in [14]). In T cells, Src homology (SH)2-domain-containing leukocyte protein of 76 kDa (SLP76) is recruited to transmembrane linker for activation in T cells (LAT) via its SH2 domain and the LAT/SLP76 complex acts as a critical scaffold for other proteins in the TCR proximal signaling complex, including VAV1. VAV1 interacts with other proteins via SH2 and proline-rich-region/SH3 domains [122–125]. In B cells, VAV1 interacts in a signaling complex that includes a scaffolding protein homologous to SLP76, SLP65 (also known as B cell linker), Bruton's tyrosine kinase (BTK), Grb2, and phospholipase-gamma (PLCγ)2 [32,118,126]. Assembly of these protein complexes activates downstream events, including phosphorylation of PLCγ1/2; activation of signaling pathways mediated by Ca^{2+}, protein kinase C (PKC), and p38 mitogen-activated protein kinase (MAPK); and regulation of transcription factors, including nuclear factor of activated T-cells (NFAT), nuclear factor kappa B (NF-κB), and activator protein-1 (AP-1) [13]. The guanine nucleotide exchange factor (GEF)-independent 'scaffolding' functions of VAV1 appear to depend on its participation in these antigen receptor–proximal signaling complexes. The primary GEF-dependent function of VAV1 is to activate Rac/Rho family GTPases [26]. In both T and B cells, co-stimulation through CD28 and CD19, respectively, are crucial for optimal phosphorylation of VAV1 and activation of downstream signaling pathways [3]. The exact mechanism for co-receptor activation of VAV1 remains to be fully determined.

(BTK), a key component of BCR signaling [14]. Co-stimulation of T and B cells through CD28 and CD19, respectively, is also essential for optimal activation of VAV1 [3].

Target validation in human T cells

Our understanding of VAV1 function (Box 1) derives largely from older studies in knockout mice (phenotypes are reviewed extensively elsewhere [3]). *Vav1*$^{-/-}$ mice have severe T cell developmental defects, including significant reductions in thymocyte and peripheral T cell numbers [4,5]. They also show functional defects in mature T/B cells, such as impaired TCR/BCR signaling (Box 1), and in other hematopoietic lineages (neutrophils, NK cells, dendritic cells, and mast cells), including crawling/migration defects in neutrophils [3]. Although *Vav1*$^{-/-}$ germline knockouts have some utility, defects in thymocyte development may disrupt normal function of adult T cells to the extent that they obscure the role of VAV1 in mediating T/B cell function in mature effector lymphocytes. Furthermore, there are no inducible Vav1 knockouts to better mimic the therapeutic targeting of VAV1, and the 'undruggable' nature of VAV1 has limited investigation of complete functional attenuation of the protein in adult mice.

These limitations, together with possible interspecies differences, restrict our ability to predict the outcome of therapeutically targeting VAV1 in humans. However, VAV1-deficient human Jurkat T cells (J.Vav1) demonstrate defective TCR signaling, shown via impairment of Ca^{2+} mobilization and **interleukin (IL)-2** upregulation [15], supporting the importance of VAV1 in human T cells. Moreover, unbiased genome-wide CRISPR-Cas9-based screens identified VAV1 as a key

Box 1. Key defects in *Vav1*$^{-/-}$ T and B lymphocytes

T lymphocytes

Vav1$^{-/-}$ mice exhibit severe T cell developmental defects with significant reductions in the number of $CD4^+/CD8^+$ double-positive thymocytes, $CD4^+$ and $CD8^+$ single-positive thymocytes, and $CD4^+$ and $CD8^+$ single-positive peripheral splenic T cells [4,5]. By contrast, T cell development is normal in *Vav2*$^{-/-}$ [110], *Vav3*$^{-/-}$, and *Vav2*$^{-/-}$/*3*$^{-/-}$ knockouts [5], suggesting a critical, nonredundant role for Vav1 in T cells, although loss of Vav3 exacerbates the Vav1$^{-/-}$ phenotype [5]. *Vav1*$^{-/-}$ mice also demonstrate impaired production of IL-2 and T cell proliferation following activation through the TCR complex [4,5].

In response to TCR/CD28 co-stimulation, *Vav1*$^{-/-}$ $CD4^+$ Th cells show deficient expression of the **Th2** cytokine IL-4 (but not IL-5/IL-13) and c-Maf, a Th2-specific transcription factor that regulates IL-4 expression [108]. By contrast, expression of the **Th1** cytokine IFNγ and Th1-specific transcription factor T-bet are enhanced even under Th2 polarizing conditions [108].

Multiple defects in TCR-mediated signaling in Vav1-deficient T-cells have been reported, including Ca^{2+} mobilization [4,15,17], PLCγ1 phosphorylation [17], inositol 1,4,5-trisphosphate production [17], activation of Tec family kinases Akt and Itk [17], mitogen-activated protein kinase (MAPK) pathway activation [18], actin reorganization, F-actin polymerization, and TCR clustering [4,5], formation of peptide-specific conjugates with APCs, and activation of integrin lymphocyte function-associated antigen 1 (LFA-1) [27,28].

Vav1-deficient T cells demonstrate impaired activation of certain transcription factors, including NFAT, nuclear factor-kappa B (NF-κB), and activator protein-1 (AP-1), all of which regulate the *Il2* promoter [4,15].

B lymphocytes

Vav1$^{-/-}$ mice have normal numbers of pro-B, pre-B, and immature B cells in the bone marrow and mature splenic and lymph node B cells, although severely reduced numbers of innate-like B-1a cells in the peritoneal cavity are reported [4,5]. While B cell development appears to be relatively normal, *Vav1*$^{-/-}$ mice display defects in T cell-dependent B cell responses, immunoglobulin (Ig)G1/2b/3 class switching, BCR-mediated Ca^{2+} mobilization, and B cell proliferation in peripheral B cells, even when recombinant IL-4 is added [5,110].

While *Vav1*$^{-/-}$ mice do not present developmental defects in the B cell compartment, Vav1 deficiency exacerbates B cell developmental defects in mice lacking Btk, a key component of BCR signaling. Compared with *Btk*$^{-/-}$ single knockouts, *Vav1*$^{-/-}$*Btk*$^{-/-}$ double-knockout mice exhibit more severe reductions in mature splenic B cells, IgM production, IgG class switching, Ca^{2+} mobilization, and B cell proliferation [121].

Glossary

Actin reorganization and F-actin polymerization: reversible process in which actin monomers assemble into an actin nucleus followed by elongation, where additional monomers are rapidly added to each end of the actin nucleus to form an actin 'filament'.
Autoimmune and chronic inflammatory diseases: heterogenous spectrum of disorders characterized by loss of self-tolerance, chronic tissue-specific and systemic inflammation, and long-term tissue damage.
Biologic therapy: treatments derived from living organisms (e.g., vaccines, proteins); in this case, monoclonal antibodies or antibody fragments that act on circulating cytokines and cell surface receptors of the immune system.
Common variable immunodeficiency (CVID): rare condition characterized by hypogammaglobulinemia secondary to T cell abnormalities (T-CVID).
Diapedesis: migration of cells across the vascular wall into target tissues.
Drug retention rate: percentage of patients remaining on treatment over time.
GEF 'trap' assay: *in vitro* assay in VAV1-deficient Jurkat T cells transfected with fluorescent-tagged SLP76, dominant-negative Rac/Rho GTPases, and VAV GEFs; used to visualize the co-localization of different Rac/Rho GTPases with physiologically activated VAV isoforms in SLP76 clusters in intact cells using confocal microscopy.
Guanine nucleotide exchange factor (GEF): promotes the exchange of GDP for GTP on downstream target Rac/Rho GTPases, switching them from an inactive (GDP-bound) to an active (GTP-bound) state.
Immunological synapse: stable interface between lymphocytes (e.g., T cells) and target cells (e.g., APCs), including receptors responsible for adhesion and signal transduction; requires actin polymerization and cytoskeleton reorganization to form.
Integrins: transmembrane proteins involved in the adhesion of cells to each other or to the extracellular matrix.
Interleukin (IL)-2: pleiotropic cytokine crucial to the differentiation and regulation of proinflammatory and anti-inflammatory T cells.
Nuclear factor of activated T-cells (NFAT): transcription factor family

positive regulator of TCR activation/function [6–8]. Specifically, genome-scale perturbation demonstrated that loss of VAV1 prevented: (i) TCR-mediated activation in Jurkat T cells, as measured by upregulation of CD69 [8], a classical early lymphocyte activation marker and key surface protein involved in activated T cell egress from the lymph nodes [16]; (ii) TCR-mediated cytokine secretion, such as IL-2 and interferon-gamma (IFNγ), in primary human $CD4^+$ and $CD8^+$ T cells, respectively [6]; and (iii) TCR-mediated T cell proliferation [7]. Taken together, these data further validate the importance of VAV1 in TCR-mediated activation and T cell effector function.

GEF-independent (scaffolding) functions

Mouse *Vav1*$^{-/-}$ thymocytes and splenic T-cells and human J.Vav1 T-cells show multiple defects in TCR-mediated signaling, e.g. impaired Ca^{2+} mobilization and transcription factor activation (Box 1) [4,15,17,18]. Considering its participation in the TCR complex, much of this activity likely depends on the scaffolding function of VAV1 (Figure 1) [17–19]. For example, lymph node T cells isolated from mice expressing GEF-inactive Vav1 (*Vav1*$^{L334A/K335A}$ or *Vav1*$^{AA/AA}$) showed normal TCR-mediated Ca^{2+} flux and activation of **nuclear factor of activated T-cells (NFAT)** *in vitro* [20]. Conversely, human VAV1-deficient J.Vav1 T cells transfected with VAV1 N-terminal truncation mutants that retain GEF activity did not interact with phospholipase C-gamma (PLCγ)1 and showed defective downstream Ca^{2+} mobilization [21], calmodulin binding, and NFAT activation *in vitro* [22]. Similarly, biochemical data showed impaired downstream phosphorylation of PLCγ1 in T cell lines deficient in the scaffolding proteins LAT, SLP76, or Tec family kinase ITK [23–25]. Together, these data suggest that loss/dysfunction of any of these scaffolding proteins, including VAV1, destabilizes the TCR proximal signaling complex, disrupting downstream events, such as the activation of PLCγ1-dependent pathways.

GEF-dependent (catalytic) functions

The downstream effects of the primary GEF-dependent function of VAV1 (to activate Rac/Rho GTPases [26]) include **actin reorganization and F-actin polymerization**, **TCR clustering** [4,5], activation of **integrin**-mediated cell adhesion, **immunological synapse** formation between T cells and antigen-presenting cells (APCs) [27–29], and chemokine-mediated cell migration [30,31]. VAV1 also regulates cytoskeletal reorganization in B cells [32].

Contrary to the historical belief that VAV1 activates multiple Rac/Rho GTPases, most biochemical data suggest that VAV1 preferentially acts as a GEF for Rac1, with limited activation of other Rac/Rho family members, including RhoA, Cdc42, and Ras [11,26,33]. *In vitro* **nucleotide-exchange assays** showed that VAV1 mediates activation of Rac1 by an order of magnitude greater than its activation of RhoA or Cdc42 [33]. Therefore, it is likely that VAV1 does not bind with high affinity to GTPases other than Rac1. Indeed, a recent study using a novel *in vitro* **GEF 'trap' assay** showed obvious co-localization of VAV1 with Rac1 but not with any other GTPases, whereas VAV2 co-localized with a much broader range of GTPases, including Rac2, RhoG, and Cdc42 [34], supporting the notion that VAV1 preferentially activates Rac1.

Actin polymerization and cytoskeleton remodeling are necessary for many of the key functions of T lymphocytes, including immunological synapse formation, integrin-mediated adhesion, chemokine responses, cell migration, and extravasation into peripheral tissues [35]. The importance of Rac1 in actin polymerization is evidenced by GEF-dependent actin defects in *Vav1*$^{-/-}$ mice, which are sufficient to block thymocyte development [4,5]. Furthermore, mice expressing the *Vav1*$^{AA/AA}$ GEF-inactive form demonstrated severely impaired Rac1 activation, actin polymerization, T cell–APC conjugate formation, and integrin activation [20]. Moreover, reconstitution of *Vav1*$^{-/-}$ mice with a constitutively active Rac1 mutant, but not a RhoA mutant, overcame their thymocyte developmental block, restoring pre-T cell differentiation and thymocyte numbers, thereby

involved in TCR-mediated early gene transcription (e.g., *IL2*).

Nucleotide-exchange assay: *in vitro* system using purified, crystallized complexes of VAV1 fragments containing the VAV1 catalytic GEF domains and Rac1, Cdc42, and RhoA GTPases; assesses the rate of VAV1-mediated GDP-GTP exchange on Rac/Rho proteins using fluorescence spectroscopy.

Primary treatment failure: lack of response (i.e., persistent signs and symptoms of disease) despite drug treatment.

Proteolysis-targeting chimera (PROTAC) degraders: small molecules comprising two ligands joined by a linker molecule; one ligand recruits the target protein by binding to a distinct binding pocket and the second recruits and binds E3 ubiquitin ligase leading to ubiquitination of the target and its subsequent removal from the cell using the ubiquitin-proteosome system of the body.

Regulatory T cells (Tregs): subset of $CD4^+$ Th cells characterized by the production of anti-inflammatory cytokines (e.g., IL-10 and TGFβ) and expression of the Treg-specific transcription factor FoxP3; involved in immunosuppression and immune tolerance.

Secondary treatment failure: loss of response to drug treatment over time.

T cell polarization: in response to activating stimuli, such as antigen binding of the TCR, T cells undergo rapid reorganization of the actin cytoskeleton to form distinct 'poles' (e.g., the immunological synapse at one end of the cell and the distal-pole complex, a complex of other cell-surface receptors, at the opposite side of the cell).

T cell receptor (TCR) clustering: following T cell activation through antigen receptor binding, multiple TCR molecules cluster at the cell membrane at the center of the immunological synapse.

T helper (Th) 1 cells: $CD4^+$ Th cell subset characterized by the production of proinflammatory Th1 cytokine IFNγ and expression of Th1-specific transcription factor Tbet; involved in type 1 immune responses (intracellular bacteria/protozoa/viruses) and autoimmunity.

T helper (Th) 2 cells: $CD4^+$ Th cell subset characterized by the production of proinflammatory Th2 cytokines IL-4, IL-5, and IL-13, and expression of Th2-specific transcription factors GATA3 and c-Maf; involved in type 2 immune

demonstrating the dominant role of Rac1 in VAV1 GEF-dependent TCR signaling and T cell function [36,37].

Consistent with its pivotal role in actin polymerization downstream of the TCR, VAV1 is activated in response to chemokines, such as C-X-C motif chemokine ligand 12 (CXCL12), involved in cytoskeletal reorganization [31,38,39]. For instance, CXCL12 promotes actin polymerization, **T cell polarization**, and cell migration, events that were blocked *in vitro* by a dominant-negative form of VAV1 ($VAV1^{L213Q}$) overexpressed in human peripheral blood lymphocytes (PBLs) [31]. *In vitro* evidence also indicates that CXCL12-mediated integrin activation in PBLs is dependent on VAV1-dependent activation of Rac1 [29].

Given the importance of Rac1 to actin polymerization, if, as we hypothesize, VAV1 preferentially mediates its GEF function through Rac1 activation in response to TCR signaling, then many of the major defects observed in VAV1-deficient T cells, including in immunological synapse formation, integrin adhesion, and cell migration, might be attributable to deficiencies in actin polymerization and cytoskeleton remodeling (Figure 2).

VAV1 in autoimmune and chronic inflammatory diseases

Although its precise role in human disease remains to be clinically validated, multiple lines of evidence implicate VAV1 in autoimmune and chronic inflammatory diseases (Table 1 [40–71]), supporting its candidacy as a putative therapeutic target.

VAV1 expression and pathway activation

Few studies have directly evaluated *VAV1* expression in humans and the significance of its expression levels is unclear. For example, compared with healthy controls, VAV1 mRNA was increased in immune cells of the inflamed gut mucosa from patients with IBD [63] and in skin biopsies from patients with psoriasis [69], but was significantly reduced in peripheral blood mononuclear cells from patients with MS [47]. Given that VAV1 is ubiquitously expressed across immune cells, its downstream functions may not be regulated by the extent of *VAV1* expression. Rather, VAV1 phosphorylation and subsequent pathway activation following TCR ligation may be a better indication of VAV1 activity and pathogenic function in diseased tissues.

VAV1 gene variants

Relative to wild-type (WT), rodents harboring the $Vav1^{R63W}$ variant exhibit reduced susceptibility to experimental autoimmune encephalomyelitis (EAE) [51,54] and pristane-induced arthritis [43]. This variant, which is associated with reduced VAV1 scaffolding functions (Ca^{2+} mobilization) but normal [51] or even hyperactive [72] GEF activity, is also associated with increased numbers of anti-inflammatory $CD4^+$ $FoxP3^+$ **regulatory T cells (Tregs)** in blood, lymph nodes, and spleen, relative to WT [72], as well as reduced IFNγ and IL-17A production by autoreactive $CD4^+$ T cells [51]. These data suggest that GEF-independent functions are important for VAV1-mediated inflammation and proinflammatory cytokine production. Moreover, $Vav1^{R63W}Themis\text{-}T^{-/-}$ mice, which carry both the $Vav1^{R63W}$ variant and a T cell-conditional knockout of *Themis* (a gene required for T cell development and effector functions [73]), exhibit pronounced attenuation of EAE symptoms [including reduced IFNγ, IL-17A, and tumor necrosis factor (TNF) production and T cell infiltration of the central nervous system (CNS)] compared with either variant alone [50]. Given that Themis associates with the TCR and enhances TCR signaling, as evidenced from reduced NFAT nuclear translocation in $Themis^{-/-}$ $CD4^+$ T cells [73], the reduced susceptibility to EAE in $Vav1^{R63W}Themis\text{-}T^{-/-}$ mice versus $Vav1^{R63W}$ mice suggests cooperativity between VAV1 and other TCR-associated proteins in autoimmune pathogenesis. Somewhat paradoxically, $Vav1^{R63W}$ is also linked to increased susceptibility to IBD in rats [64] and myasthenia gravis in mice [66].

responses (extracellular helminths and venoms) and allergy.
T helper (Th) cells: $CD4^+$ T cells that have a crucial role in regulating cells of the adaptive and innate immune systems.
Treat-to-target: strategy in which therapy is monitored regularly and tailored to achieve a specific outcome (e.g. disease remission).

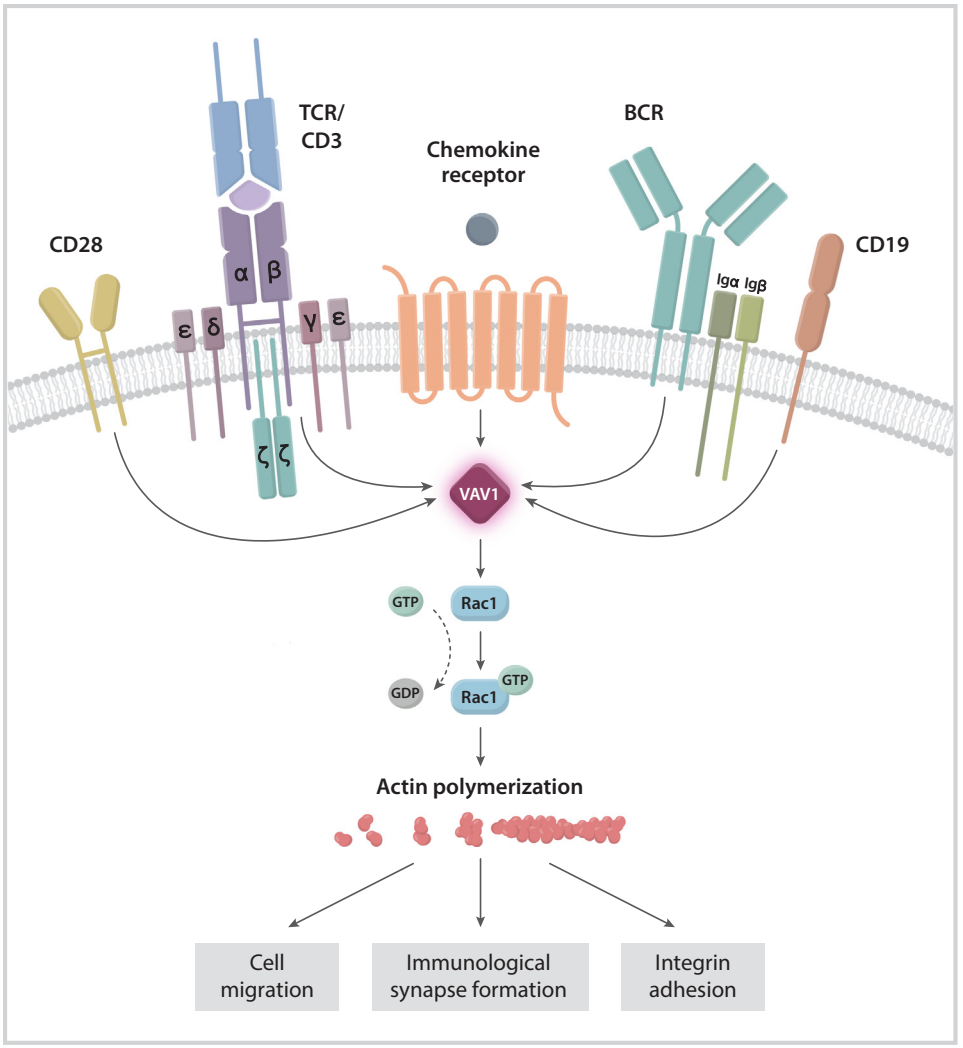

Figure 2. Core guanine nucleotide exchange factor (GEF)-dependent functions of VAV1. Activation of VAV1 in response to stimulation through the T cell receptor (TCR) and B cell receptor (BCR), co-stimulation through their corresponding co-receptors CD28 and CD19, respectively, or through chemokine receptors promotes the exchange of GDP for GTP on downstream target Rac/Rho family GTPases, switching them from an inactive (GDP-bound) to active (GTP-bound) state. Historically, it has been suggested that VAV1 activates multiple Rac/Rho GTPases. However, available biochemical data suggest that VAV1 preferentially acts as a GEF for Rac1, with limited activation of other family members, including RhoA, Cdc42, or Ras [11,26,33]. Rac1 activation downstream of VAV1 is essential for actin reorganization, F-actin polymerization, and cytoskeleton remodeling [4,5,20,36], which is in turn pivotal for many of the key functions of T lymphocytes, including formation of the immunological synapse, integrin-mediated cell adhesion, chemokine responses, cell migration, and extravasation into secondary lymphoid organs and other peripheral tissues [35]. If, indeed, VAV1 largely mediates its GEF function through Rac1 activation, then we could hypothesize that defects in actin polymerization and cytoskeleton remodeling underlie many of the defects observed in VAV1-deficient cells.

Gene variants corresponding to *Vav1*^{R63W} have not been identified in humans; nevertheless, VAV1 single-nucleotide polymorphisms (SNPs) and haplotypes are associated with increased susceptibility to RA [41–43], relapsing/remitting MS [48,54], myasthenia gravis [65], and psoriasis [69]. Together, these findings suggest that human SNPs/haplotypes exist that affect VAV1 function and predispose individuals to disease. However, these gene association studies do not

Table 1. Published studies of the role of VAV1 in autoimmune and chronic inflammatory disease in patients (and/or healthy volunteers) and animal models of disease[a,b]

Species; disease/model	Key findings	Refs
RA/animal models of arthritis		
Mouse: adaptive and innate models of arthritis in *Vav1*$^{-/-}$ versus WT mice	*Vav1*$^{-/-}$ mice had reduced susceptibility to antigen (methylated BSA)-induced arthritis versus WT mice, showing reductions in: inflammation, synovial thickening, cartilage degradation; T cell proliferation and joint infiltration by CD4^{+} T cells, neutrophils, and macrophages; expansion of CD4^{+} T cell subsets and proinflammatory cytokines (IFNγ, TNF, IL-6, IL-17A, and IL-1β) in the knee joint. In a zymosan A-induced arthritis model, disease progression (joint swelling and hyperplasia) was similar in *Vav1*$^{-/-}$ and WT mice	[40]
Human: weighted gene co-expression network analysis	Gene network analysis was used to screen for targets and potential drug mechanisms in RA. Four RA-related modules were identified, encompassing immune and infection related processes. Hub genes with high connectivity in the gene regulation and pathway networks included *VAV1*, various *HLA* genotypes, *BTK*, *BLNK*, *SYK*, *CD4*, and *IL2RG*, among others	[41]
Human: SNPs in patients with RA	Evaluated association between VAV1 SNPs rs2546133 and rs2617822 and RA. A significantly higher frequency of rs2546133 T versus C alleles was observed in patients with RA (*N* = 422) versus healthy controls (*N* = 338). No difference in rs2617822 genotypes was observed. Patients with VAV1 rs2546133 T and rs2617822 G alleles had an increased frequency of extra-articular manifestations	[42]
Human: patients with RA Rat: DA.BN-25 congenic rats carrying *Vav1*^{R63W}	MS-associated haplotype rs2546133-rs2617822 CA was associated with RA (*N* = 11 475) versus controls (*N* = 5870) Arthritis severity was reduced in DA.BN-25 congenic rats carrying the *Vav1*^{R63W} polymorphism versus arthritis-susceptible DA rats in a pristane but not CIA model	[43]
Mouse: C57BL/6J WT and *hFcγRIIA*-transgenic mice Human: patients with RA	hFcγRIIA, expressed in myeloid cells, promotes inflammation; hFcγRIIA polymorphisms are associated with increased risk of RA. In transgenic mice expressing *hFcγRIIA*, blocking hFcγRIIA signaling inhibited arthritis and reduced ROS production through inhibition of Vav1. In infiltrating cells from synovial fluid from patients with RA (*N* = 7), SYK was constitutively associated with hFcγRIIA	[44]
Mouse: model of collagen-induced arthritis (CIA)	SAP expression in T cells but not B cells was essential for the development of CIA and production of collagen-specific antibodies. Increased tyrosine phosphorylation of Vav1 was seen in T cell hybridoma (BI-141) cells transfected with WT *SAP* but not mutant *SAP*^{R78A}	[45]
Mouse: innate model of arthritis in WT, *Vav1*$^{-/-}$, and *PLCγ2*$^{-/-}$ mice	In a K/BxN serum-transfer mouse model of arthritis, *Vav1*$^{-/-}$ and *PLCγ2*$^{-/-}$ mice were protected against inflammation, cellular infiltration of the joint, osteoclast recruitment, and cartilage and bone erosion compared with WT mice, showing decreased production of proinflammatory cytokines IL-1, IL-6, and TNFα	[46]
MS/experimental autoimmune encephalomyelitis (EAE)		
Mouse: *Vav1*^{R63W}*Themis-T*$^{-/-}$ mice	*Vav1*^{R63W}*Themis-T*$^{-/-}$ mice carry the *Vav1*^{R63W} polymorphism and a T cell conditional knockout of *Themis*, a TCR-associated protein that enhances TCR signaling. *Vav1*^{R63W}*Themis-T*$^{-/-}$ mice demonstrated pronounced attenuation of EAE symptoms, including reduced IFNγ, IL-17A, and TNF production, and T cell infiltration of the CNS, compared with mice carrying either mutation alone	[50]
Human: patients with MS	VAV1 mRNA was significantly reduced in PBMCs from untreated patients with MS (*N* = 54) compared with healthy controls (*N* = 23), including in patients with relapsing-remitting MS and progressive MS, regardless of whether patients were in remission or relapsing	[47]
Human: patients with MS	Systems medicine approach, using blood samples from newly diagnosed relapsing–remitting MS (NDP; *N* = 11), patients taking disease-modifying therapy (*N* = 47) and age/sex-matched healthy controls (*N* = 92), showed that TCR and BCR signaling pathways were main pathways over-represented in NDP. VAV1, among other proteins, was involved in these pathways	[48]
Mouse: MOG-induced EAE in *Vav1*^{R63W} knock-in model	Compared with WT mice, *Vav1*^{R63W} mice demonstrated reduced susceptibility to development of EAE, including lower production of proinflammatory effector cytokines (IFN-γ, IL-17, and GM-CSF) by autoreactive CD4^{+} T cells	[51]
Mouse: Ezh2-deficient *Ezh2f/f Mx1-Cre* mice	Mice deficient in methyltransferase Ezh2 showed compromised integrin-dependent cell migration of dendritic cells and lower disease scores in MOG-induced model of EAE. Ezh2 forms cytosolic complexes with Vav1 and talin1 in mouse dendritic cells	[52]
Mouse: *Ifnar1*$^{-/-}$ mice	Signaling through IFNAR on macrophages inhibited Rac1 activation and ROS generation, suppressed NLRP3 inflammasome (which regulates innate immune system and inflammatory signaling) and reduced EAE pathogenicity. Treatment of bone marrow-derived macrophages with IFNβ *in vitro* reduced cytosolic Vav1 protein and suppressed activation of Rac1	[53]

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Table 1. (continued)

Species; disease/model	Key findings	Refs
Human: patients with MS Rat: DA.BN-*Eae4* and LEW.BN-*Eae4* congenic rats	Susceptibility to EAE in rats was localized to *Vav1* and R63W polymorphism. Analysis of seven human cohorts (*N* = 12 735) showed an association between haplotype rs2546133-rs2617822 CA in VAV1 and increased risk of MS, as well as increased *VAV1* mRNA and Th1 cytokines TNFα and IFNγ in PBMCs and CSF mononuclear cells from patients with MS (*N* = 417) versus healthy controls (*N* = 143)	[54]
Mouse: myelin proteolipid protein$_{139–151}$ model of EAE	Inhibition of transmethylation downregulated TCR signaling in primary mouse T cells, including methylation of Vav1, and CD4$^+$ T cell activation *in vitro,* and protected mice against development of EAE (no direct link between Vav1 and EAE reported)	[55]
Mouse: *Vav1*$^{-/-}$ versus WT mice	Compared with WT, *Vav1*$^{-/-}$ mice were resistant to MOG-induced EAE due to impaired priming and expansion of antigen-specific CD4$^+$ and CD8$^+$ lymph node T cells. Production of IL-2, IL-4, and IFNγ also impaired in MOG-induced *Vav1*$^{-/-}$ but not in WT mice	[49]
Systemic lupus erythematosus		
Human: case-control association study in North America	In 3716 individuals of Hispanic descent and 4867 of European descent, two NCF2 SNPs [rs17849502 (H389Q); rs13306575 (R395W)] were independently associated with SLE. H389Q and R395W mutations are hypothesized to destabilize docking of NCF2 to VAV1 via its ZF and DH domains, altering structure and function of NADPH oxidase complex in neutrophils	[58]
Mouse: C57BL/6 congenic B6.Sle1 model of SLE	In mice, Sle1 interval caused loss of immune tolerance leading to production of ANA. Primary T and B cells, taken from a mouse model of SLE before onset of disease, formed T/B cell couples less frequently and retained polarity less efficiently in response to low-affinity stimulation, which was also associated with decreased Vav1 and actin recruitment	[59]
Human: patients with SLE and healthy controls	Genotype analysis showed significantly increased risk of childhood-onset SLE (*N* = 663 versus 879 controls) and adult-onset SLE (*N* = 4578 versus 3910 controls) and a single NCF2 SNP rs17849502 (H389Q). Computational modeling suggested that H389Q mutation affects interaction of NCF2 with VAV1 ZF (C1) domain, altering function of NADPH oxidase complex	[56]
Human: isolated T cells from patients with SLE and healthy controls	Higher amounts of SYK protein and protein phosphorylation detected in CD4$^+$ and CD8$^+$ T cells from patients with SLE (*N* = 58) versus healthy controls (*N* = 52). Immunoprecipitation studies showed association between SYK and VAV1 that was approximately ten-fold higher in SLE versus normal T cells. VAV1 phosphorylation in response to anti-CD3 stimulation was more intense in SLE versus normal T cells	[57]
Mouse: BI-141 T-cell line expressing Ly108 isoforms	Ly108-1, an isoform of Ly108/NTB-A, a self-ligating member of SLAM family expressed on T and B cells, is strongly linked to SLE susceptibility in mice. In CD4$^+$ T-cells, all Ly108 isoforms tested mediated tyrosine phosphorylation of Ly108 itself and Vav1	[60]
Mouse: MRL/Mp$^{lpr/lpr}$ and MRL/Mp$^{+/+}$ mice	MRL/Mp$^{lpr/lpr}$ mice develop symptoms similar to human SLE, including autoantibody production, vasculitis, and glomerulonephritis, and have a generalized nonmalignant lymphoproliferative disorder. CD4$^+$/CD8$^+$ double-negative T cells accumulate in spleen and lymph nodes. Vav1 was constitutively phosphorylated in *lpr/lpr* versus +/+ mice and did not respond to TCR activation	[61]
IBD		
Human: patients with Crohn's disease	Immunofluorescence showed increased phosphorylated PAK1 and reduced apoptosis in macrophages from gut mucosa of patients with Crohn's disease (*N* = 10) versus uninflamed controls (*N* = 8). Peptidoglycan significantly increased phosphorylated VAV1, PAK2, and PAK1, as well as Rac1-GTP in RAW264.7 macrophages *in vitro*. 6-thioguanine partially ablated macrophage phagocytosis in human PBMCs	[62]
Human: biopsies from patients with IBD and CD4$^+$ T cells purified from human PBMCs	VAV1 mRNA increased in intestinal lamina propria mononuclear cells from patients with IBD (*N* = 11) versus non-inflamed controls (*N* = 7). B-0N, a designer 6-thio-GTP analog optimized for Rac1 blockade, induced stronger T cell apoptosis, increased Rac1 inhibition in primary peripheral blood T cells and intestinal lamina propria immune cells, and was associated with decreased toxicity	[63]
Rat: BN Themis1-deficient rats (BN$^{Themis1-/-}$)	*BN*$^{Themis1-/-}$ impairs Treg function and predisposes rats to spontaneous IBD. This deficiency appears to depend on *Vav1*^{R63W} polymorphism; introduction of 117-kb interval of Lewis rat *Vav1*^{R63} variant restored Treg function and protected *BN*$^{Themis1-/-}$ rats from spontaneous IBD. This suggests an epistatic interaction between Vav1 and Themis, at least in this animal model	[64]
Myasthenia gravis		
Mouse: EAMG induced in a *Vav1*^{R63W} knock-in model	*Vav1*^{R63W} polymorphism increased susceptibility to EAMG, as evidenced from a higher AChR loss, increased frequency of antigen-specific CD4$^+$ T cells, and increased production of proinflammatory cytokines (IFNγ, IL-17A, and GM-CSF) by antigen-specific CD4$^+$ T cells relative to controls	[66]

Table 1. (continued)

Species; disease/model	Key findings	Refs
Human: patients with EOMG	Genetic study to identify candidate loci for susceptibility to EOMG: eight of 34 candidate genes were identified in exploration phase (EOMG *N* = 384; matched controls *N* = 384) and confirmed in a replication study (EOMG *N* = 1177; controls *N* = 814). Allele frequency differences were found in four novel loci, including *VAV1*	[65]
Periodontitis		
Human: patients with periodontitis	Regulatory network model for human gingival interactome was used to analyze expression data from clinically healthy (*N* = 70) or periodontitis-affected (*N* = 243) gingival sites to identify master regulator genes that may be driving gingival disease or sustaining gingival pathology. *VAV1* was one of six regulons enriched for more than ten pathways	[67]
Human: genome network analysis	Multiple data sources and computational methods were used to identify 21 genes as being involved or potentially involved in periodontitis, including *VAV1*, proinflammatory cytokines, chemokines, and other genes involved in immune signaling	[68]
Psoriasis		
Human: patients with psoriasis	Gene network analysis identified four psoriasis and atherosclerosis common immune-related genes (*SELP, CD93, IL2RG,* and *VAV1*) that showed optimal diagnostic value (AUC >0.8). Positive expression of *VAV1* was found in skin biopsies from patients with psoriasis (*N* = 27). *VAV1* expression positively correlated with chemokine receptors, interferons, NK cell and TCR signaling genes	[69]
Autoimmune diabetes		
Rat: *Lyp/Lyp and Lyp/+* and +/+ BioBreeding (BB) rats	*Lyp/Lyp* rats develop lymphopenia and T cell-mediated autoimmune diabetes. Expression of p95vav (*Vav1*) mRNA and protein was increased in thymus and bone marrow of *Lyp/Lyp* rats compared with $Lyp/^{+}$ and $^{+/+}$ rats (*N* = 6 each)	[70]
Autoimmune hepatitis		
Human: patients with autoimmune hepatitis	Kupffer cells from patients with autoimmune hepatitis (*N* = 21) had higher VAV1 and p21-activated kinase 1 protein expression versus NAFLD controls (*N* = 7), *P* <0.05. A correlation between increased VAV1 and PAK1 and advanced disease was reported but data were not shown	[71]

[a]A systematic search of the PubMed database using prespecified search terms ('VAV1 protein, human' OR 'Vav1 protein, mouse' OR 'Vav1 protein, rat' OR 'Proto-Oncogene Proteins c-vav' OR VAV1 OR Vav1 OR p95Vav) was carried out on August 7, 2023 and produced 1379 results; a follow-up search was carried out on May 14, 2024 to identify any relevant articles published subsequent to the original search date. Search restrictions included: articles published from 1989 (date of first published paper on VAV1/p95Vav) to present; English language publications only. Only articles that reported on VAV1 in autoimmune or chronic inflammatory disease are included.
[b]Abbreviations: AChR, acetylcholine receptor; ANA, anti-nuclear antibodies; AUC, area under the curve; BLNK, B cell linker; BN, Brown-Norway; BTK, Bruton's tyrosine kinase; CSF, cerebrospinal fluid; CNS, central nervous system; DA, dark Agouti; DH, Dbl homology; EAMG, experimental autoimmune myasthenia gravis; EOMG, early-onset myasthenia gravis; GM-CSF, granulocyte-macrophage colony stimulating factor; H, histidine; hFcγRIIA, human IgG Fc receptor IIA; HLA, human leukocyte antigen; IFNγ, interferon-gamma; IFNAR, interferon-α/β receptor; IL, interleukin; IL2RG, interleukin-2 receptor gamma; LEW, Lewis rats; M-CSF, macrophage colony-stimulating factor; MOG, myelin oligodendrocyte glycoprotein; NAFLD, non-alcoholic fatty liver disease; NCF2, neutrophil cytosolic factor 2; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3; p38, p38 mitogen-activated kinase; PLCγ2, phospholipase-gamma 2; PBMC, peripheral blood monocytic cells; Q, glutamine; R, arginine; ROS, reactive oxygen species; SAP, SLAM-associated protein; SLAM, signaling lymphocyte activation molecule; SYK, spleen tyrosine kinase; TNFα, tumor necrosis factor-alpha; W, tryptophan; WT, wild-type; ZF, zinc finger.

elucidate the impact of various SNPs/haplotypes on VAV1 function in the context of autoimmune disease, making their clinical relevance difficult to interpret.

Mouse models

In a mouse model of antigen (methylated bovine serum albumin)-induced arthritis (AIA), $Vav1^{-/-}$ mice showed reduced signs of disease (inflammation, synovial thickening, and cartilage degradation), T cell proliferation, and joint infiltration by $CD4^{+}$ T cells, neutrophils, and macrophages compared with WT mice with AIA [40]. Expansion of $CD4^{+}$ T cell subsets producing IFNγ, TNF, IL-4, and IL-17A, and production of proinflammatory cytokines (IFNγ, TNF, IL-6, IL-17A, and IL-1β) in the knee joint, observed following disease onset in WT AIA mice, were also significantly reduced in $Vav1^{-/-}$ mice [40]. A similarly reduced disease phenotype was seen in $Vav1/2/3^{-/-}$ mice (compared with WT mice with AIA) but not in $Vav2/3^{-/-}$ mice [40], again suggesting a dominant role for VAV1 in T cell differentiation and function. Vav1 deficiency did not result in B cell abnormalities in this model, suggesting that B cell development is less dependent on VAV1 signaling [40].

In contrast to the AIA model, studies in innate immune models of arthritis show inconsistent outcomes. In a zymosan A-induced arthritis model, disease progression (joint swelling and hyperplasia) was observed in both *Vav1*$^{-/-}$ and WT mice [40], whereas, in a K/BxN serum-transfer model of arthritis, *Vav1*$^{-/-}$ mice were protected against inflammation, bone erosion, and neutrophil infiltration of the joint compared with WT mice [46].

Furthermore, compared with WT mice, *Vav1*$^{-/-}$ mice were resistant to myelin oligodendrocyte glycoprotein (MOG)-induced EAE due to impaired priming and expansion of antigen-specific CD4^{+} and CD8^{+} lymph node T cells [49]. Similar to the AIA model, production of IL-2, IL-4, and IFNγ was impaired in MOG-induced *Vav1*$^{-/-}$ mice, but not in WT mice, although APC function and migration of T cells/macrophages into the CNS were normal [49], again suggesting a role for VAV1 in T cell priming and differentiation, as well as expansion of **T helper (Th) cells** subsets. While genetic attenuation of VAV1 appears to suppress autoimmune/inflammatory disease activity and ameliorate disease progression and structural damage, it is not possible to draw definitive conclusions, and these animal data remain to be rigorously validated in human diseases.

The evolving need for new treatments in autoimmune/inflammatory diseases

The development of targeted therapies was a major milestone in improving treatment for certain autoimmune and chronic inflammatory diseases, including IBD, RA, spondyloarthritis, and psoriatic disorders. Multiple highly efficacious drugs with different mechanisms of action (MOA) are now available for many conditions (Figure 3) [74–76].

Although **drug retention rates** vary by MOA and disease indication, **primary** and **secondary treatment failure** are the most common reasons for switching therapy [77–81], exacerbated by comorbidities [77,79]. Moreover, management of conditions such as systemic lupus erythematosus (SLE) relies heavily on older therapies and is associated with chronic glucocorticoid toxicity, persistent disease activity, cumulative organ damage, and poor long-term survival [82]. Positive clinical trial data have been reported for the biologics belimumab [83] and anifrolumab [84] (which target BlyS/BAFF and IFNα/β, respectively) and both were recently approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of SLE. Additionally, positive clinical trial data in SLE have recently been reported for the oral TYK2 inhibitor deucravacitinib [85]. Despite such progress, a substantial unmet need remains for additional enhanced targeted therapies in SLE [82] and other conditions, including rheumatological disorders [86,87] and IBD [88].

Treatment guidelines for many conditions recommend that glucocorticoids be used only at the lowest effective dose as short-term, adjunctive therapy, and are tapered or discontinued when clinically feasible [89–92]. Nevertheless, despite the wide availability of targeted therapies and considerable, often severe, side effects that affect multiple organs [93], glucocorticoids are still widely used to manage refractory disease [82,94]. Although relatively safe [76,95,96], many immunomodulators, including biologics (e.g., TNF and IL-6 blockers) and oral JAK inhibitors, have shown an increased risk of infections, including serious and opportunistic infections [76,95,96]. Certain infections, such as herpes zoster, TB reactivation, and candidiasis, are associated with specific drug classes, namely, JAK inhibitors [95,96], TNF blockers [95,96], and IL-17 blockers, respectively [97]. Thus, the risk of infection must be considered with any new immunomodulatory agent.

In mice, both Vav1 and Btk have been implicated in Dectin-1-mediated macrophage phagocytosis of *Candida albicans* and *Btk*$^{-/-}$ and *Vav1*$^{-/-}$ mice appear more susceptible to *C. albicans* infection compared with WT mice [98]. Furthermore, in a small number of patients, low *VAV1* expression was putatively linked to **common variable immunodeficiency (CVID)** [99–101].

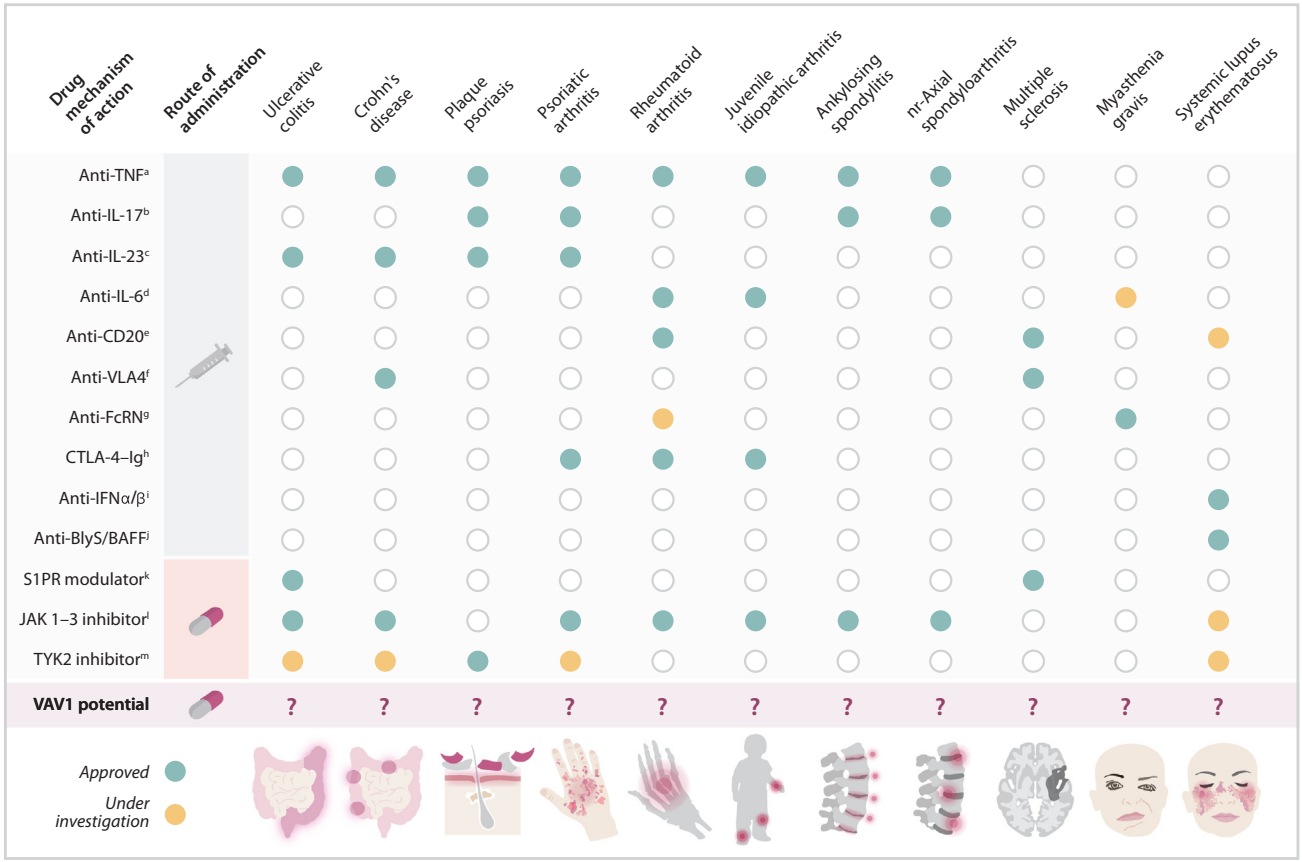

Figure 3. Mechanisms for treating autoimmune and chronic inflammatory disease. Major drug mechanisms of action (MOA) that have been approved (green circles) by the US Food and Drug Administration (FDA[iv]) and/or the European Medicines Agency (EMA[v]) for the treatment of autoimmune and chronic inflammatory disorders are shown by therapeutic indication and are based on the most recent prescribing information. MOA that are 'under investigation' (yellow circles) for specific conditions and have shown positive Phase 2/3 trial results and/or have strong evidence of off-label use within a given indication are also shown (this is not an exhaustive list covering every autoimmune/chronic inflammatory condition). Interventions targeting VAV1 have the potential to act across multiple T and T/B cell-mediated conditions. [a]Anti-tumor necrosis factor (TNF): adalimumab, certolizumab, etanercept, golimumab, and infliximab. Certolizumab is not approved for juvenile idiopathic arthritis (JIA) or ulcerative colitis (FDA/EMA), or Crohn's disease (EMA). Etanercept is not approved for Crohn's disease or ulcerative colitis (FDA/EMA). Golimumab is not approved for Crohn's disease or plaque psoriasis (FDA/EMA), and is only approved in ulcerative colitis among patients with inadequate response/intolerance to prior treatment. Infliximab is not approved for JIA. [b]Anti-interluekin (IL)-17: bimekizumab, brodalumab, ixekizumab, and secukinumab. In the USA, bimekizumab is only approved for plaque psoriasis. Brodalumab is only approved for plaque psoriasis (FDA/EMA). Only secukinumab is approved for JIA (EMA). [c]Anti-IL-23: ustekinumab inhibits IL-12 as well as IL-23 signaling by binding to their common p40 subunit and blocking interaction with the IL-12 receptor β1; guselkumab, mirikizumab, rizankizumab, and tildrakizumab inhibit the p19 subunit of IL-23. Rizankizumab is approved for psoriasis (FDA/EMA), psoriatic arthritis (PsA; EMA), and Crohn's disease (EMA). Guselkumab is approved for psoriasis (FDA/EMA) and PsA (EMA). Tildrakizumab is only approved for psoriasis (FDA/EMA). Mirikizumab is only approved for ulcerative colitis (FDA/EMA). [d]Anti-IL-6: sarilumab binds IL-6 and tocilizumab binds soluble and membrane-bound IL-6 receptors. Only tocilizumab is approved for the treatment of both polyarticular and systemic JIA (FDA/EMA). [e]Anti-CD20: rituximab is approved for severe RA and has been used off-label for the treatment of systemic lupus erythematosus (SLE). Ocrelizumab is approved for multiple sclerosis. [f]Anti-alpha4/beta7-integrin (VLA4): natalizumab; not approved for Crohn's disease by the EMA. [g]Anti-neonatal fragment crystallizable (Fc) receptor (FcRN): nipocalimab. [h]Cytotoxic T lymphocyte-associated antigen-immunoglobulin fusion protein (CTLA-4–Ig): abatacept. [i]Anti-interferon alpha and beta receptor subunit 1 (IFNα/β): anifrolumab; approved for treatment of adult patients with active autoantibody-positive SLE despite standard therapy. [j]Anti-B lymphocyte stimulator/B cell activating factor (BlyS/BAFF): belimumab; approved as add-on therapy in adult patients with active, autoantibody-positive SLE with high disease activity despite standard therapy. [k]Sphingosine 1-phosphate receptor (S1PR) modulators: fingolimod, siponimod, ozanimod, and ponesimod. Only ozanimod is approved for ulcerative colitis (EMA). [l]Janus kinase (JAK) inhibitors: filgotinib, baricitinib, upadacitinib, and tofacitinib. Only tofacitinib (FDA/EMA) and baricitinib (EMA) are approved for JIA. Only upadacitinib is approved for Crohn's disease (FDA/EMA). Only tofacitinib and upadacitinib are approved for axial spondyloarthritis (EMA). Baricitinib is not approved for psoriatic arthritis. [m]Tyrosine kinase 2 (TYK2) inhibitor: deucravacitinib.

This may have implications for clinical interventions targeting VAV1, particularly given the increased risk of infections associated with CVID [102]. Although a definitive causal link between increased risk of candidiasis, or other opportunistic infections, and the attenuation of VAV1

remains to be determined, these data suggest the need for vigilance and monitoring for specific opportunistic infections with new agents targeting VAV1.

For many conditions, a **treat-to-target** approach is now recommended [90–92] and the availability of multiple agents and drug MOA facilitates such a personalized treatment approach. Additionally, recent studies indicate the therapeutic potential of combining advanced targeted therapies. For example, the Phase 2, randomized, double-blind, controlled VEGA trial (NCT03662542[ii]) in patients with ulcerative colitis reported a higher clinical response at Week 12 (primary endpoint; intention-to-treat analysis) and similar safety outcomes in response to combined therapy with the TNF blocker golimumab plus the IL-23-p19 blocker guselkumab versus either treatment alone [103]. However, additive toxicity of combined agents may increase the risk of adverse effects and the long-term risks/benefits must be determined. Thus, new agents that simultaneously and safely target multiple inflammatory pathways would be welcome (see Clinician's corner).

Key figure

Proposed model for VAV1-mediated regulation of the adaptive immune system

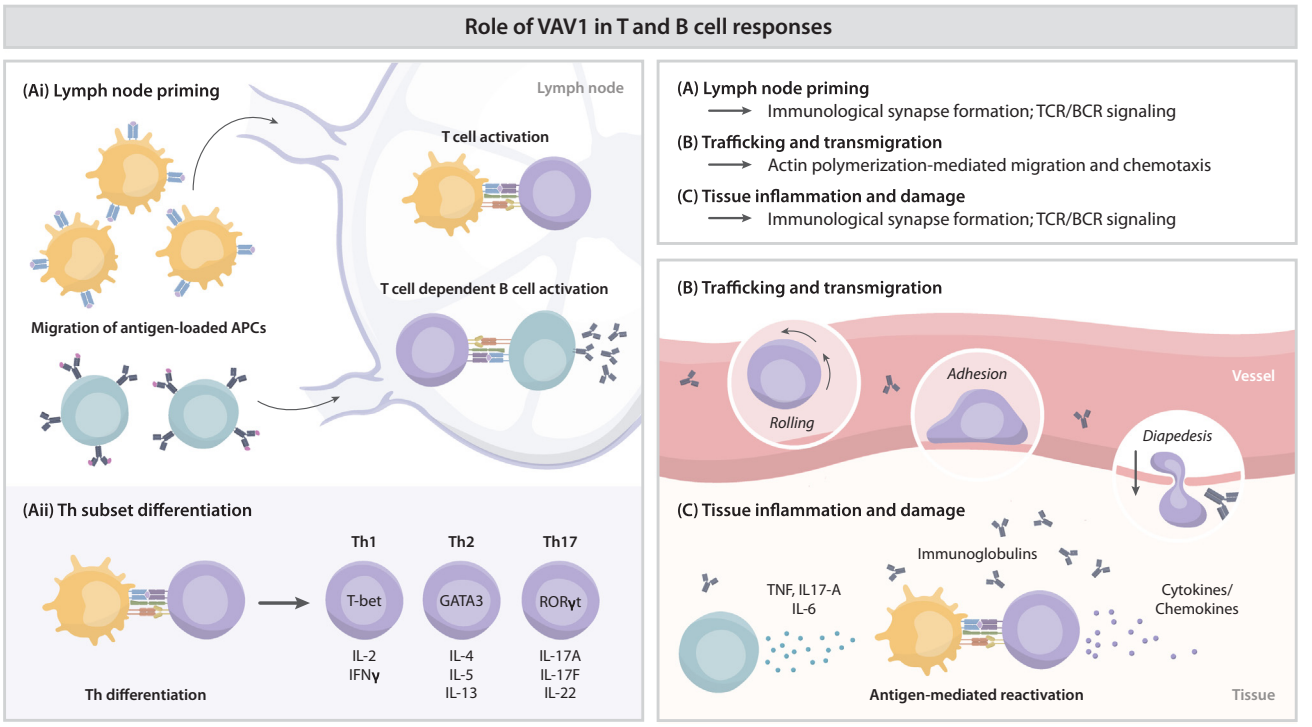

Figure 4. (A) Lymph node priming and T helper (Th) cell differentiation. VAV1 is a positive regulator of both T cell receptor (TCR) and B cell receptor (BCR) signaling. VAV1 facilitates T cell–antigen presenting cell (APC) conjugate formation (i.e., formation of the immunological synapse), TCR-mediated T cell activation, interleukin (IL)-2 production [4–6,109], differentiation of $CD4^+$ Th cell subsets, and production of subset-specific proinflammatory cytokines [108]. (B) Trafficking and transmigration of cells. The primary function of the guanine nucleotide exchange factor (GEF) activity of VAV1, at least in T cells, appears to be the activation of the Rac1 small GTPase [11,26,33], which regulates downstream actin polymerization and cytoskeletal reorganization necessary for immunological synapse formation, integrin-mediated cell adhesion, cell migration, and diapedesis (i.e., migration across the vascular endothelium into target tissues) [35]. (C) Tissue inflammation and damage. VAV1 could have a key role in inflammation-mediated tissue damage and progression of certain autoimmune and/or chronic inflammatory diseases, via antigen-mediated reactivation of self-reactive T cells and B cells, and the production of autoreactive antibodies and proinflammatory cytokines [e.g., IL-6, IL-17A, and tumor necrosis factor (TNF)].

VAV1 as a putative therapeutic target

Considering that *VAV1* expression is highly restricted to hematopoietic cells, we postulate that interventions targeting VAV1 could directly impact proinflammatory responses while avoiding collateral damage to non-immune cells, as reported with other T cell modulators (e.g., azathioprine and calcineurin inhibitors) [104,105]. Azathioprine, an immunosuppressant used for decades to treat certain autoimmune diseases and chronic inflammation, such as Crohn's disease, broadly establishes the precedent of targeting VAV1/Rac1 signaling. For instance, *in vitro*, the azathioprine metabolite 6-Thio-GTP bound recombinant Rac1 and suppressed Rac1 activation in isolated primary human $CD4^+$ T cells from healthy volunteers [106]. Furthermore, azathioprine (and its metabolites/analogs) has been shown to induce significant apoptosis of peripheral blood $CD4^+$ T cells from patients with Crohn's disease, mediated through binding of its metabolite 6-Thio-GTP to Rac1 [63,107]. Of note, as a purine analog, azathioprine may interfere with DNA and RNA synthesis, presenting liabilities that may be avoided with more VAV1-selective therapies.

Figure 4 (Key figure) proposes a model for three areas of adaptive immunity where VAV1 is essential and where targeting it might successfully regulate an overactive immune system. First, based on data from *Vav1*$^{-/-}$ mouse models [4,5,108] and human CRISPR-Cas9 screening [6,109] showing a key role for VAV1 in TCR and CD28 signaling and cytokine production (discussed above), targeting VAV1 might affect the priming, expansion, and differentiation of Th subsets and associated cytokines in peripheral lymphoid organs (Figure 4A). Reductions in immunoglobulin (Ig)-class switching and impaired BCR-mediated signaling and B cell proliferation in *Vav1*$^{-/-}$ mice [5,110] also suggest that targeting VAV1 would regulate BCR-mediated antibody responses or T cell-mediated support of B cell responses. Second, it appears that the primary GEF-dependent function of VAV1 is to preferentially activate Rac1 [11,26,33,34], a key regulator of actin polymerization and cytoskeleton reorganization (at least in T cells) [4,5]. This points to a role for VAV1 in not only immunological synapse formation, but also, as mentioned, cell adhesion, rolling, and **diapedesis** (Figure 4B). This is relevant because it hypothetically suggests that targeting VAV1 would help regulate trafficking and transmigration of T cells and, potentially, other VAV1-expressing cell types to sites of inflammation and disease. Finally, we hypothesize that therapeutic targeting of VAV1 in diseased tissues might block antigen-mediated reactivation of self-reactive T cells and the secretion of proinflammatory cytokines driving inflammation, tissue damage, and disease progression (Figure 4C). As discussed, mouse models of arthritis and EAE indicate that loss/suppression of VAV1 might restrict tissue-specific autoimmunity, slowing disease onset/progression [40,46,49,51]. Thus, targeting VAV1 may lead to attenuation of key pathogenic immune cell subsets across both arms of the adaptive immune system.

Options for VAV1-based therapeutic interventions

Despite being a preclinically validated target for T and T/B cell-mediated diseases, such as AIA and EAE [40,49], the dual GEF-dependent and -independent functions of VAV1 have remained 'undruggable'. Thiopurine metabolites/analogs interfere with GEF function, but do not directly or selectively target VAV1 [63,106,107]. Directly targeting GEF active domains, such as those found in VAV1, has proven challenging for drug discovery; the design of small-molecule modulators that target large multidomain proteins lacking clearly defined binding pockets is difficult [2]. Preliminary reports of the genetic sequence and crystal structure of a VAV1 allosteric inhibitor[iii] suggest that targeting GEF function allosterically is possible, but further research is required. Release of autoinhibition is an absolute requirement for VAV1 activation [3]. Thus, any agent designed to bind VAV1 through the domains constituting its catalytic (GEF) function would require VAV1 to be activated to make binding sites available. Moreover, therapeutic approaches that interfere exclusively with GEF function might, hypothetically, preserve VAV1 scaffolding functions,

Clinician's corner

Therapeutic options for autoimmune and chronic inflammatory diseases remain limited. Many drugs, the effect of which is based on blocking a single protein (e.g., anti-TNF or anti-IL-17A agents) or signaling pathway (e.g., JAK inhibitors), show insufficient efficacy and/or are associated with side effects.

Innovative therapeutic approaches that are targeted, yet able to block several proinflammatory mediators simultaneously, would be useful additions to the therapeutic armamentarium, having the potential to break through the 'therapeutic ceiling' in the treatment of autoimmune and chronic inflammatory diseases.

Targeted agents that block both GEF-dependent and -independent functions of VAV1 have the potential to block multiple processes relevant to autoimmune inflammatory responses, including activation of cells of both the adaptive and innate immune systems, formation of immunological synapses between T cells and APCs, and migration of immune cells to inflamed tissue.

Preclinical data in animal model systems for arthritis and colitis show that inactivation of VAV1 can attenuate certain autoimmune and chronic inflammatory processes.

New data suggest that it might be possible to inhibit the expression or function of VAV1 using therapeutic approaches including RNA interference, designer thiopurine analogs, and molecular glue degrader strategies.

resulting in inadequate attenuation of the target. Given these limitations, interventions that seek to eliminate total protein function may be more effective compared with active site inactivation.

Which therapeutic modalities might target VAV1 expression/function? Building on the activity of azathioprine, more specific designer thiopurine analogs may offer new avenues for VAV1/Rac1 pathway blockade [63], but are yet to be tested in clinical trials. Another emerging modality is targeted VAV1 protein degradation, which might be used to harness the ubiquitin-proteosome system in the body, a natural process that regulates protein quality and homeostasis. Novel techniques for degrading and eliminating disease-causing proteins are being investigated [111]. Recent progress toward modulators of previously 'undruggable' proteins [2] has been made by using strategies, such as protein–protein interaction disruptors or **proteolysis targeting chimera (PROTAC) degraders**, to, for example, modulate the GEF Son of sevenless homolog 1 [112]. A PROTAC degrader requires moieties capable of binding simultaneously to the target protein through a distinct binding pocket and to an E3 ubiquitin ligase, leading to ubiquitination of the former and its subsequent removal from the cell [111]. While effective, this modality poses challenges for proteins such as VAV1, which lack obvious binding pockets.

Notably, molecular glue degraders (MGDs) are oral small molecules that can bind to, and induce structural changes in, the surface of E3 ligase complexes, driving interaction with, and subsequent ubiquitination of, novel protein targets and earmarking them for degradation [111]. Recent data on a VAV1-targeting MGD, MRT-6160, which is in development for autoimmune and inflammatory diseases, such as IBD and RA, report successful and specific degradation of VAV1 *in vitro* and *in vivo*, as well as *in vitro* attenuation of TCR/BCR-mediated activation in primary human T/B lymphocytes, and inhibition of T cell proliferation, and cytokine production in a dose-dependent manner [113,114]. *In vivo*, orally administered MRT-6160 demonstrates promising preclinical activity in mouse models of T and T/B cell-mediated disease, inhibiting disease progression in collagen-induced arthritis [113] and colitis [114]. These studies also report that MRT-6160 inhibits disease progression concomitant with reductions in: IL-17$^+$ and TNF$^+$ CD4$^+$ T cells [114,115]; proinflammatory cytokines (IL-6 and TNF) in the serum [113] or diseased tissues [114,115]; autoantibody production [113]; and expression of key markers of disease severity [113,114].

Finally, advances in RNA interference (e.g., antisense oligonucleotides and gene therapy) offer the potential to target *VAV1* mRNA expression and block protein translation [116], although this remains to be investigated. However, selectivity for *VAV1* (particularly over *VAV2/3*), dosing flexibility, and treatment accessibility are important considerations.

Concluding remarks

Although the efficacy and safety of any treatment modality targeting VAV1 remain to be determined in humans (see Outstanding questions), we hypothesize that such interventions may have therapeutic potential to regulate T and T/B cell-mediated autoimmunity and chronic inflammation for certain pathologies. Loss/mutation of Vav1 can successfully abrogate inflammation, tissue damage, and disease progression in mouse models of arthritis and EAE [40,46,49,51]. However, the use of germline knockouts (where disruption of early T cell development might impact mature effector lymphocyte function), and the lack of inducible systems or other methods to target the protein in mature animals, clearly limit extrapolation of these data to adult human diseases. Certain preclinical studies in models of arthritis [113] and colitis [114] have provided promising exploratory results supporting the total attenuation of VAV1 function through protein degradation as one possible therapeutic approach. Nevertheless, the effectiveness of these MOA in humans must be established in randomized controlled clinical trials before targeting VAV1 can be considered a viable option for treating certain autoimmune or inflammatory conditions.

Outstanding questions

Is VAV1 a viable clinical target in autoimmune and/or chronic inflammatory diseases? While there is strong evidence of a key role for VAV1 in antigen receptor signaling, and involvement in T and T/B cell-mediated pathology, the efficacy of agents targeting VAV1 must be determined in randomized controlled clinical trials.

Can targeting VAV1 be safe? All available drugs with potent immunomodulatory capabilities have some increased risk of infection, including serious and opportunistic infections, and an inherent, albeit small, risk of malignancy, particularly in the presence of certain risk factors (older age and smoking). The risk of serious and opportunistic infections, such as *C. albicans*, as well as other adverse outcomes in response to agents targeting VAV1 must be investigated.

How will targeting VAV1 affect proinflammatory cytokine production in patients? The exact role of VAV1 in Th1/Th2/Th17 differentiation and cytokine production requires further investigation.

Will targeting VAV1 affect other hematopoietic cells? The risk:benefit ratio of targeting VAV1 in different cell types must be carefully determined. Many autoimmune/chronic inflammatory disorders are heterogenous in etiology; to date, there is no 'one size fits all' approach in terms of effective drug MOA. Agents that target multiple aspects of the adaptive and innate immune systems could have broader than expected therapeutic benefits.

Will targeting VAV1 show efficacy in other immune-mediated conditions? Although a detailed review was beyond the scope of this article, research shows a pivotal role for VAV1 in oncology [117]. BTK, an essential component of BCR signaling that physically associates with VAV1 [118], is a major therapeutic target in oncology and an investigational target in autoimmune disease [119]. Studies also link VAV1, as a positive regulator of T cell function, with allograft survival [120]. The efficacy/safety of targeting VAV1 in patients with cancer or those undergoing transplantation remains to be fully elucidated.

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Declaration of interests

M.F.N. is a consultant for Boehringer Ingelheim and SciRhom, served as an advisor to Carpmaels & Ransford LLP and PPM, and has appeared on advisory boards for Nimbus Therapeutics, AbbVie, Takeda, Janssen, Pentax, Tr1x, MSD, Pfizer, and Monte Rosa Therapeutics. He has research funding from the Deutsche Forschungsgemeinschaft (TRR241, FOR2458) and IZKF Erlangen. L.J.B. is a co-founder of ImmVue Therapeutics, and a consultant for Pfizer and Monte Rosa Therapeutics. She has research funding from the NIH and the US–Israel Binational Research Foundation.

Resources

[i]www.proteinatlas.org

[ii]https://clinicaltrials.gov/

[iii]www.rcsb.org/structure/6NFA

[iv]www.fda.gov/

[v]www.ema.europa.eu/en/homepage

References

1. Zarrin, A.A. *et al.* (2021) Kinase inhibition in autoimmunity and inflammation. *Nat. Rev. Drug Discov.* 20, 39–63
2. Xie, X. *et al.* (2023) Recent advances in targeting the 'undruggable' proteins: from drug discovery to clinical trials. *Signal Transduct. Target. Ther.* 8, 335
3. Bustelo, X.R. and Dossil, M. (2018) Vav family. In *Encyclopedia of Signaling Molecules* (Choi, S., ed.), pp. 5892–5906, Springer
4. Fischer, K.D. *et al.* (1998) Vav is a regulator of cytoskeletal reorganization mediated by the T-cell receptor. *Curr. Biol.* 8, 554–562
5. Fujikawa, K. *et al.* (2003) Vav1/2/3-null mice define an essential role for Vav family proteins in lymphocyte development and activation but a differential requirement in MAPK signaling in T and B cells. *J. Exp. Med.* 198, 1595–1608
6. Schmidt, R. *et al.* (2022) CRISPR activation and interference screens decode stimulation responses in primary human T cells. *Science* 375, eabj4008
7. Shifrut, E. *et al.* (2018) Genome-wide CRISPR screens in primary human T cells reveal key regulators of immune function. *Cell* 175, 1958–1971
8. Shang, W. *et al.* (2018) Genome-wide CRISPR screen identifies FAM49B as a key regulator of actin dynamics and T cell activation. *Proc. Natl. Acad. Sci. U. S. A.* 115, E4051–E4060
9. Katzav, S. *et al.* (1989) vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. *EMBO J.* 8, 2283–2290
10. Rodríguez-Fdez, S. and Bustelo, X.R. (2019) The Vav GEF family: an evolutionary and functional perspective. *Cells* 8, 465
11. Barreira, M. *et al.* (2014) The C-terminal SH3 domain contributes to the intramolecular inhibition of Vav family proteins. *Sci. Signal.* 7, ra35
12. Barreira, M. *et al.* (2018) New insights into the Vav1 activation cycle in lymphocytes. *Cell. Signal.* 45, 132–144
13. Shah, K. *et al.* (2021) T cell receptor (TCR) signaling in health and disease. *Signal Transduct. Target. Ther.* 6, 412
14. Tanaka, S.B.Y. (2020) B cell receptor signaling. In *B Cells in Immunity and Tolerance* (Wang, J.-Y., ed.), pp. 23–36, Springer
15. Cao, Y. *et al.* (2002) Pleiotropic defects in TCR signaling in a Vav-1-null Jurkat T-cell line. *EMBO J.* 21, 4809–4819
16. Bankovich, A.J. *et al.* (2010) CD69 suppresses sphingosine 1-phosophate receptor-1 (S1P1) function through interaction with membrane helix 4. *J. Biol. Chem.* 285, 22328–22337
17. Reynolds, L.F. *et al.* (2002) Vav1 transduces T cell receptor signals to the activation of phospholipase C-gamma1 via phosphoinositide 3-kinase-dependent and -independent pathways. *J. Exp. Med.* 195, 1103–1114
18. Reynolds, L.F. *et al.* (2004) Vav1 transduces T cell receptor signals to the activation of the Ras/ERK pathway via LAT, Sos, and RasGRP1. *J. Biol. Chem.* 279, 18239–18246
19. Zugaza, J.L. *et al.* (2002) Structural determinants for the biological activity of Vav proteins. *J. Biol. Chem.* 277, 45377–45392
20. Saveliev, A. *et al.* (2009) Function of the nucleotide exchange activity of vav1 in T cell development and activation. *Sci. Signal.* 2, ra83
21. Knyazhitsky, M. *et al.* (2012) Vav1 oncogenic mutation inhibits T cell receptor-induced calcium mobilization through inhibition of phospholipase Cγ1 activation. *J. Biol. Chem.* 287, 19725–19735
22. Li, S.Y. *et al.* (2013) The N-terminal 20-amino acid region of guanine nucleotide exchange factor Vav1 plays a distinguished role in T cell receptor-mediated calcium signaling. *J. Biol. Chem.* 288, 3777–3785
23. Schaeffer, E.M. *et al.* (1999) Requirement for Tec kinases Rlk and Itk in T cell receptor signaling and immunity. *Science* 284, 638–641
24. Yablonski, D. *et al.* (1998) Uncoupling of nonreceptor tyrosine kinases from PLC-gamma1 in an SLP-76-deficient T cell. *Science* 281, 413–416
25. Zhang, W. *et al.* (1999) Functional analysis of LAT in TCR-mediated signaling pathways using a LAT-deficient Jurkat cell line. *Int. Immunol.* 11, 943–950
26. Crespo, P. *et al.* (1997) Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature* 385, 169–172
27. Ardouin, L. *et al.* (2003) Vav1 transduces TCR signals required for LFA-1 function and cell polarization at the immunological synapse. *Eur. J. Immunol.* 33, 790–797
28. Krawczyk, C. *et al.* (2002) Vav1 controls integrin clustering and MHC/peptide-specific cell adhesion to antigen-presenting cells. *Immunity* 16, 331–343
29. García-Bernal, D. *et al.* (2005) Vav1 and Rac control chemokine-promoted T lymphocyte adhesion mediated by the integrin alpha4beta1. *Mol. Biol. Cell* 16, 3223–3235
30. Laufer, J.M. *et al.* (2019) Chemokinereceptor CCR7 triggers an endomembrane signaling complex for spatial Rac activation. *Cell Rep.* 29, 995–1009
31. Vicente-Manzanares, M. *et al.* (2005) Control of lymphocyte shape and the chemotactic response by the GTP exchange factor Vav. *Blood* 105, 3026–3034
32. Löber, J. *et al.* (2020) Vav family proteins constitute disparate branching points for distinct BCR signaling pathways. *Eur. J. Immunol.* 50, 1912–1928

33. Rapley, J. *et al.* (2008) Crucial structural role for the PH and C1 domains of the Vav1 exchange factor. *EMBO Rep.* 9, 655–661
34. Fray, M.A. *et al.* (2020) Vav2 lacks Ca(2+) entry-promoting scaffolding functions unique to Vav1 and inhibits T cell activation via Cdc42. *J. Cell Sci.* 133, jcs238337
35. Dupre, L. *et al.* (2015) T lymphocyte migration: an action movie starring the actin and associated actors. *Front. Immunol.* 6, 586
36. Gomez, M. *et al.* (2000) Control of pre-T cell proliferation and differentiation by the GTPase Rac-l. *Nat. Immunol.* 1, 348–352
37. Corre, I. *et al.* (2001) Analysis of thymocyte development reveals that the GTPase RhoA is a positive regulator of T cell receptor responses in vivo. *J. Exp. Med.* 194, 903–914
38. Okabe, S. *et al.* (2005) Stromal cell-derived factor-1alpha/CXCL12-induced chemotaxis of T cells involves activation of the RasGAP-associated docking protein p62Dok-1. *Blood* 105, 474–480
39. Dios-Esponera, A. *et al.* (2015) Positive and negative regulation by SLP-76/ADAP and Pyk2 of chemokine-stimulated T-lymphocyte adhesion mediated by integrin α4β1. *Mol. Biol. Cell* 26, 3215–3228
40. Conde, J. *et al.* (2021) Distinct roles of Vav family members in adaptive and innate immune models of arthritis. *Biomedicines* 9, 695
41. Li, X. *et al.* (2020) Promising targets and drugs in rheumatoid arthritis: a module-based and cumulatively scoring approach. *Bone Joint Res.* 9, 501–514
42. Pawlik, A. *et al.* (2020) VAV1 gene polymorphisms in patients with rheumatoid arthritis. *Int. J. Environ. Res. Public Health* 17, 3214
43. Guerreiro-Cacais, A.O. *et al.* (2017) VAV1 regulates experimental autoimmune arthritis and is associated with anti-CCP negative rheumatoid arthritis. *Genes Immun.* 18, 109
44. Ben Mkaddem, S. *et al.* (2014) Shifting FcγRIIA-ITAM from activation to inhibitory configuration ameliorates arthritis. *J. Clin. Invest.* 124, 3945–3959
45. Zhong, M.C. and Veillette, A. (2013) The adaptor molecule signaling lymphocytic activation molecule (SLAM)-associated protein (SAP) is essential in mechanisms involving the Fyn tyrosine kinase for induction and progression of collagen-induced arthritis. *J. Biol. Chem.* 288, 31423–31436
46. Cremasco, V. *et al.* (2008) Vav/phospholipase Cgamma2-mediated control of a neutrophil-dependent murine model of rheumatoid arthritis. *Arthritis Rheum.* 58, 2712–2722
47. Malhotra, S. *et al.* (2018) Circulating EZH2-positive T cells are decreased in multiple sclerosis patients. *J. Neuroinflammation* 15, 296
48. Pazhouhandeh, M. *et al.* (2018) A systems medicine approach reveals disordered immune system and lipid metabolism in multiple sclerosis patients. *Clin. Exp. Immunol.* 192, 18–32
49. Korn, T. *et al.* (2003) Vav1-deficient mice are resistant to MOG-induced experimental autoimmune encephalomyelitis due to impaired antigen priming. *J. Neuroimmunol.* 139, 17–26
50. Marrocco, R. *et al.* (2024) Positive regulation of Vav1 by Themis controls CD4 T cell pathogenicity in a mouse model of central nervous system inflammation. *Cell. Mol. Life Sci.* 81, 161
51. Kassem, S. *et al.* (2016) A natural variant of the t cell receptor-signaling molecule vav1 reduces both effector T cell functions and susceptibility to neuroinflammation. *PLoS Genet.* 12, e1006185
52. Gunawan, M. *et al.* (2015) The methyltransferase Ezh2 controls cell adhesion and migration through direct methylation of the extranuclear regulatory protein talin. *Nat. Immunol.* 16, 505–516
53. Inoue, M. *et al.* (2012) Interferon-β therapy against EAE is effective only when development of the disease depends on the NLRP3 inflammasome. *Sci. Signal.* 5, ra38
54. Jagodic, M. *et al.* (2009) A role for VAV1 in experimental autoimmune encephalomyelitis and multiple sclerosis. *Sci. Transl. Med.* 1, 10ra21
55. Lawson, B.R. *et al.* (2007) Inhibition of transmethylation down-regulates CD4 T cell activation and curtails development of autoimmunity in a model system. *J. Immunol.* 178, 5366–5374
56. Jacob, C.O. *et al.* (2012) Lupus-associated causal mutation in neutrophil cytosolic factor 2 (NCF2) brings unique insights to the structure and function of NADPH oxidase. *Proc. Natl. Acad. Sci. U. S. A.* 109, E59–E67
57. Krishnan, S. *et al.* (2008) Differential expression and molecular associations of Syk in systemic lupus erythematosus T cells. *J. Immunol.* 181, 8145–8152
58. Armstrong, D.L. *et al.* (2015) Systemic lupus erythematosus-associated neutrophil cytosolic factor 2 mutation affects the structure of NADPH oxidase complex. *J. Biol. Chem.* 290, 12595–12602
59. Sinai, P. *et al.* (2014) T/B-cell interactions are more transient in response to weak stimuli in SLE-prone mice. *Eur. J. Immunol.* 44, 3522–3531
60. Zhong, M.C. and Veillette, A. (2008) Control of T lymphocyte signaling by Ly108, a signaling lymphocytic activation molecule family receptor implicated in autoimmunity. *J. Biol. Chem.* 283, 19255–19264
61. Mimura, T. *et al.* (1997) Constitutive tyrosine phosphorylation of the vav proto-oncogene product in MRL/Mp-lpr/lpr mice. *J. Immunol.* 158, 2977–2983
62. Jing, Y. *et al.* (2018) Peptidoglycan suppresses phagocytic activities and apoptosis of macrophages in colonic mucosa tissues of Crohn's disease patients and in vitro. *Med. Sci. Monit.* 24, 3382–3392
63. Atreya, I. *et al.* (2016) Designer thiopurine-analogues for optimised immunosuppression in inflammatory bowel diseases. *J. Crohns Colitis* 10, 1132–1143
64. Pedros, C. *et al.* (2015) An epistatic interaction between Themis1 and Vav1 modulates regulatory T cell function and inflammatory bowel disease development. *J. Immunol.* 195, 1608–1616
65. Avidan, N. *et al.* (2014) VAV1 and BAFF, via NFκB pathway, are genetic risk factors for myasthenia gravis. *Ann. Clin. Transl. Neurol.* 1, 329–339
66. Bernard, I. *et al.* (2018) A natural variant of the signaling molecule Vav1 enhances susceptibility to myasthenia gravis and influences the T cell receptor repertoire. *Front. Immunol.* 9, 2399
67. Sawle, A.D. *et al.* (2016) Identification of master regulator genes in human periodontitis. *J. Dent. Res.* 95, 1010–1017
68. Zhan, Y. *et al.* (2014) Prioritization of candidate genes for periodontitis using multiple computational tools. *J. Periodontol.* 85, 1059–1069
69. Liu, S. *et al.* (2023) The SELP, CD93, IL2RG, and VAV1 genes associated with atherosclerosis may be potential diagnostic biomarkers for psoriasis. *J. Inflamm. Res.* 16, 827–843
70. Bieg, S. (1999) Differential expression of p95vav in primary lymphoid tissue of BB rats congenic for the lymphopenia gene. *Autoimmunity* 30, 37–42
71. Lin, R. *et al.* (2016) Altered function of monocytes/macrophages in patients with autoimmune hepatitis. *Mol. Med. Rep.* 13, 3874–3880
72. Colacios, C. *et al.* (2011) The p.Arg63Trp polymorphism controls Vav1 functions and Foxp3 regulatory T cell development. *J. Exp. Med.* 208, 2183–2191
73. Prasad, M. *et al.* (2021) Themis regulates metabolic signaling and effector functions in CD4(+) T cells by controlling NFAT nuclear translocation. *Cell. Mol. Immunol.* 18, 2249–2261
74. Kerschbaumer, A. *et al.* (2023) Efficacy of synthetic and biological DMARDs: a systematic literature review informing the 2022 update of the EULAR recommendations for the management of rheumatoid arthritis. *Ann. Rheum. Dis.* 82, 95–106
75. Webers, C. *et al.* (2023) Efficacy and safety of biological DMARDs: a systematic literature review informing the 2022 update of the ASAS-EULAR recommendations for the management of axial spondyloarthritis. *Ann. Rheum. Dis.* 82, 130–141
76. Avedillo-Salas, A. *et al.* (2023) The efficacy and safety of biologic drugs in the treatment of moderate-severe Crohn's disease: a systematic review. *Pharmaceuticals (Basel)* 16, 1581
77. Vilarrasa, E. *et al.* (2015) ORBIT (Outcome and Retention Rate of Biologic Treatments for Psoriasis): a retrospective observational study on biologic drug survival in daily practice. *J. Am. Acad. Dermatol.* 74, 1066–1072
78. Bhushan, V. *et al.* (2021) Real-life retention rates and reasons for switching of biological DMARDs in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *Front. Med. (Lausanne)* 8, 708168
79. Rashid, N. *et al.* (2016) Rates, factors, reasons, and economic impact associated with switching in rheumatoid arthritis patients newly initiated on biologic disease modifying anti-rheumatic drugs in an integrated healthcare system. *J. Med. Econ.* 19, 568–575

80. Sartini, A. *et al.* (2019) Retention rate, persistence and safety of adalimumab in inflammatory bowel disease: A real-life, 9-year, single-center experience in Italy. *Dig. Dis. Sci.* 64, 863–874
81. Pombo-Suarez, M. *et al.* (2023) After JAK inhibitor failure: to cycle or to switch, that is the question - data from the JAK-pot collaboration of registries. *Ann. Rheum. Dis.* 82, 175–181
82. Arnaud, L. and Tektonidou, M.G. (2020) Long-term outcomes in systemic lupus erythematosus: trends over time and major contributors. *Rheumatology (Oxford)* 59, v29–v38
83. Stohl, W. *et al.* (2017) Efficacy and safety of subcutaneous belimumab in systemic lupus erythematosus: A fifty-two-week randomized, double-blind, placebo-controlled study. *Arthritis Rheumatol.* 69, 1016–1027
84. Morand, E.F. *et al.* (2020) Trial of anifrolumab in active systemic lupus erythematosus. *N. Engl. J. Med.* 382, 211–221
85. Morand, E. *et al.* (2023) Deucravacitinib, a tyrosine kinase 2 inhibitor, in systemic lupus erythematosus: A phase II, randomized, double-blind, placebo-controlled trial. *Arthritis Rheumatol.* 75, 242–252
86. Winthrop, K.L. *et al.* (2023) Unmet need in rheumatology: reports from the Advances in Targeted Therapies meeting, 2022. *Ann. Rheum. Dis.* 82, 594–598
87. Lindqvist, J. *et al.* (2022) Unmet needs in rheumatoid arthritis: a subgroup of patients with high levels of pain, fatigue, and psychosocial distress 3 years after diagnosis. *ACR Open Rheumatol.* 4, 492–502
88. Reves, J. *et al.* (2021) Unmet needs in inflammatory bowel disease. *Curr. Res. Pharmacol. Drug Discov.* 2, 100070
89. Fanouriakis, A. *et al.* (2019) 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann. Rheum. Dis.* 78, 736–745
90. Gossec, L. *et al.* (2020) EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. *Ann. Rheum. Dis.* 79, 700–712
91. Ramiro, S. *et al.* (2023) ASAS-EULAR recommendations for the management of axial spondyloarthritis: 2022 update. *Ann. Rheum. Dis.* 82, 19–34
92. Smolen, J.S. *et al.* (2023) EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. *Ann. Rheum. Dis.* 82, 3–18
93. Yasir, M. *et al.* (2023) *Corticosteroid Adverse Effects*, StatPearls Publishing
94. Moore, M.N. and Wallace, B.I. (2021) Glucocorticoid and opioid use in rheumatoid arthritis management. *Curr. Opin. Rheumatol.* 33, 277–283
95. Sepriano, A. *et al.* (2023) Safety of synthetic and biological DMARDs: a systematic literature review informing the 2022 update of the EULAR recommendations for the management of rheumatoid arthritis. *Ann. Rheum. Dis.* 82, 107–118
96. Frisell, T. *et al.* (2023) Safety of biological and targeted synthetic disease-modifying antirheumatic drugs for rheumatoid arthritis as used in clinical practice: results from the ARTIS programme. *Ann. Rheum. Dis.* 82, 601–610
97. Davidson, L. *et al.* (2022) Risk of candidiasis associated with interleukin-17 inhibitors: a real-world observational study of multiple independent sources. *Lancet Reg. Health Eur.* 13, 100266
98. Strijbis, K. *et al.* (2013) Bruton's tyrosine kinase (BTK) and Vav1 contribute to Dectin1-dependent phagocytosis of *Candida albicans* in macrophages. *PLoS Pathog.* 9, e1003446
99. Capitani, N. *et al.* (2010) Impaired TH2 response in patients with Vav1-deficient common variable immunodeficiency with T-cell defects. *J. Allergy Clin. Immunol.* 126, 671–675
100. Capitani, N. *et al.* (2012) Vav1 haploinsufficiency in a common variable immunodeficiency patient with defective T-cell function. *Int. J. Immunopathol. Pharmacol.* 25, 811–817
101. Paccani, S.R. *et al.* (2005) Defective Vav expression and impaired F-actin reorganization in a subset of patients with common variable immunodeficiency characterized by T-cell defects. *Blood* 106, 626–634
102. Pescador Ruschel, M.A. and Vaqar, S. (2023) *Common Variable Immunodeficiency*, StatPearls Publishing
103. Feagan, B.G. *et al.* (2023) Guselkumab plus golimumab combination therapy versus guselkumab or golimumab monotherapy in patients with ulcerative colitis (VEGA): a randomised, double-blind, controlled, phase 2, proof-of-concept trial. *Lancet Gastroenterol. Hepatol.* 8, 307–320
104. de Boer, N.K.H. *et al.* (2018) Thiopurines in inflammatory bowel disease: new findings and perspectives. *J. Crohns Colitis* 12, 610–620
105. Farouk, S.S. and Rein, J.L. (2020) The many faces of calcineurin inhibitor toxicity-what the FK? *Adv. Chronic Kidney Dis.* 27, 56–66
106. Poppe, D. *et al.* (2006) Azathioprine suppresses ezrin-radixin-moesin-dependent T cell-APC conjugation through inhibition of Vav guanosine exchange activity on Rac proteins. *J. Immunol.* 176, 640–651
107. Tiede, I. *et al.* (2003) CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. *J. Clin. Invest.* 111, 1133–1145
108. Tanaka, Y. *et al.* (2005) Impaired IL-4 and c-Maf expression and enhanced Th1-cell development in Vav1-deficient mice. *Blood* 106, 1286–1295
109. Schmidt, R. *et al.* (2024) Base-editing mutagenesis maps alleles to tune human T cell functions. *Nature* 625, 805–812
110. Tedford, K. *et al.* (2001) Compensation between Vav-1 and Vav-2 in B cell development and antigen receptor signaling. *Nat. Immunol.* 2, 548–555
111. Tsai, J.M. *et al.* (2024) Targeted protein degradation: from mechanisms to clinic. *Nat. Rev. Mol. Cell Biol.*, Published online April 29, 2024. https://doi.org/10.1038/s41580-024-00729-9
112. Zhou, C. *et al.* (2022) Discovery of the first-in-class agonist-based SOS1 PROTACs effective in human cancer cells harboring various KRAS mutations. *J. Med. Chem.* 65, 3923–3942
113. Cartwright, A. *et al.* (2023) A VAV1-directed molecular glue degrader, MRT-6160, reduces joint inflammation in a collagen-induced arthritis autoimmune disease model. *Arthritis Rheumatol.* 75, 132–133
114. Cartwright, A. *et al.* (2024) P164 MRT-6160, a VAV1-directed molecular glue degrader, inhibits disease progression and inflammation in a T-cell transfer model of colitis. *J. Crohns Colitis* 19, i469
115. Cartwright, A. *et al.* (2024) Tu1727 MRT-6160, a VAV1-directed molecular glue degrader, inhibits disease progression in a t-cell transfer mediated murine colitis model concomitant with reduced calprotectin expression. *Gastroenterology* 166, S1396
116. Jadhav, V. *et al.* (2024) RNA interference in the era of nucleic acid therapeutics. *Nat. Biotechnol.* 42, 394–405
117. Shalom, B. *et al.* (2023) Unraveling the oncogenic potential of VAV1 in human cancer: lessons from mouse models. *Cells* 12, 1276
118. Guinamard, R. *et al.* (1997) The SH3 domain of Bruton's tyrosine kinase interacts with Vav, Sam68 and EWS. *Scand. J. Immunol.* 45, 587–595
119. Alu, A. *et al.* (2022) BTK inhibitors in the treatment of hematological malignancies and inflammatory diseases: mechanisms and clinical studies. *J. Hematol. Oncol.* 15, 138
120. Wisniewska, M. *et al.* (2021) VAV1 gene polymorphism is associated with kidney allograft rejection. *Transplant. Proc.* 53, 1528–1531
121. Betzler, A.C. *et al.* (2022) Differential requirement of Vav proteins for Btk-dependent and -independent signaling during B Cell development. *Front. Cell Dev. Biol.* 10, 654181
122. Katzav, S. *et al.* (1994) The protein tyrosine kinase ZAP-70 can associate with the SH2 domain of proto-Vav. *J. Biol. Chem.* 269, 32579–32585
123. Tuosto, L. *et al.* (1996) p95vav associates with tyrosine-phosphorylated SLP-76 in antigen-stimulated T cells. *J. Exp. Med.* 184, 1161–1166
124. Barda-Saad, M. *et al.* (2010) Cooperative interactions at the SLP-76 complex are critical for actin polymerization. *EMBO J.* 29, 2315–2328
125. Ye, Z.S. and Baltimore, D. (1994) Binding of Vav to Grb2 through dimerization of Src homology 3 domains. *Proc. Natl. Acad. Sci. U. S. A.* 91, 12629–12633
126. Wienands, J. *et al.* (1998) SLP-65: a new signaling component in B lymphocytes which requires expression of the antigen receptor for phosphorylation. *J. Exp. Med.* 188, 791–795