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# Dendritic cell Flt3 – regulation, roles and repercussions for immunotherapy

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**Abstract**

Dendritic cells (DCs) are essential for initiating immune responses. Depending on the environment, the type of DC and the way in which it interacts with T cells; these immune responses can be beneficial or detrimental. DCs can be exploited as cellular vectors for vaccines against infection and cancer. The development and maintenance of DCs is dependent on the FMS-like tyrosine kinase 3 (Flt3)/Flt3 ligand (Flt3L) signaling cascade. Flt3 is also one of the most commonly mutated genes in leukemia and as such represents an attractive drug target. In this review,

Flt3 is discussed with a particular focus on DCs. We detail the lifecycle of Flt3, from transcription to degradation, and interrogate recent studies as to how this pathway can be manipulated for immunotherapy, vaccination and treatment of autoimmune disease.

## Introduction

An exciting advance to immunotherapy against infection, cancer and autoimmunity is to exploit the biology of dendritic cells (DCs).[1] DCs are unrivalled in their ability to acquire, process and present antigenic peptide to T cells. Discovered in 1973,[2] they are a functionally and phenotypically heterogenous population of cells that are widely distributed throughout the body. Under steady state conditions, DCs are well-equipped at acquiring and processing antigen but poor at inducing immunity. Interactions between immature DCs and naïve T cells can lead to tolerance, are important in shaping T cell development in the thymus and maintaining T cell populations in the peripheral lymphoid organs. Upon encounter with inflammatory stimuli DCs undergo maturation. Inflammatory stimuli include pathogen associated molecular patterns (PAMPs) which are recognized by pattern recognition receptors (PRRs) on DCs in addition to inflammatory cytokines.[3] Mature DCs have elevated MHC, costimulatory molecules and inflammatory cytokines and migrate to T cell zones of secondary lymphoid organs where they can initiate immune responses. DC interaction with T cells can be a double-edged sword, with DCs capable of not only initiating immune responses against non-self but also initiating autoimmunity.

To manipulate DCs in clinical settings, it is important to understand the major signaling cascade that generates these cells - FMS-like tyrosine kinase 3 (Flt3)/Flt3 ligand (Flt3L). Indeed, years of studies into Flt3 have revealed it is essential in DC development and maintenance and is a tool commonly employed to manipulate the number of DCs *in vitro* and *in vivo*. More recently, focus has shifted to how this pathway can be manipulated for effective immunotherapy. In this review, we discuss the lifecycle of Flt3, including its transcription, intracellular trafficking, signaling and degradation. Understanding the biology of Flt3/Flt3L is important to create new avenues for the clinical manipulation of this pathway and exploitation of DCs. Finally, we highlight recent efforts that exploit Flt3 in immunotherapy and vaccination.

**FMS-like tyrosine kinase 3 (Flt3) receptor**

The *Flt3* gene encodes a type III receptor tyrosine kinase in the same family as macrophage colony-stimulating factor (M-CSF) receptor (M-CSFR), mast/stem cell growth factor receptor (SCFR/c-KIT/CD117) and platelet-derived growth factor receptors A and B (PDGFR-A and -B). These receptors share a similar structure with five extracellular immunoglobulin-like domains, one transmembrane domain, one juxtamembrane domain and two tyrosine kinase domains (**Figure 1**). Flt3 expression is restricted to hematopoietic stem cells and DCs.[4]

**Flt3 ligand (Flt3L)**

The ligand for Flt3 is Flt3 ligand (Flt3L), a type I transmembrane protein in the same family as the ligands for SCFR and M-CSFR. It comprises five extracellular domains, a transmembrane domain and a cytosolic tail (**Figure 1**). There are three main isoforms of Flt3L. The first isoform, found in both humans[5,6] and mice,[7] is cleavable membrane-bound Flt3L which can form soluble Flt3L. Flt3L can also be generated as a soluble protein which lacks a membrane anchor.[5–7] This occurs due to an early stop-codon which is introduced into Flt3L due to exon skipping. The last isoform is membrane-tethered and cannot be cleaved.[8] Similar to soluble Flt3L, membrane-tethered Flt3L occurs due to alternative splicing and results in a protein that lacks the cleavage site. While all forms are biologically active, the abundance of each differs between species. In humans, the most common form is the cleavable membrane-bound Flt3L,[8] whereas in mice the most common form is the membrane-tethered Flt3L.[5,8] It is currently unclear whether the different isoforms perform different functions.

In contrast to the Flt3 receptor, Flt3L mRNA is expressed by most hematopoietic and non-hematopoietic tissues.[5,7] Even so, in healthy individuals serum Flt3L levels are low.[9] The non-hematopoietic sources have not been studied extensively; however, for the hematopoietic source it has been shown that $CD4^+$ T cells secrete Flt3L.[10] When lethally irradiated $Flt3L^{-/-}$ mice are reconstituted with bone marrow from mice lacking T cells ($CD3\varepsilon^{-/-}$) there is a significant reduction in serum Flt3L in comparison to $Flt3L^{-/-}$ mice reconstituted with wild type bone marrow.[10] In particular, $CD4^+$ T cells were found to secrete higher amounts of Flt3L than $CD8^+$ T cells when stimulated *ex vivo* with anti-CD3ε and anti-CD28 antibodies.[10] In T cells, Flt3L is stored

intracellularly and colocalizes with Golgi-resident proteins.[9,11] Trafficking to the cell surface can be induced by cytokines which signal through the common γ receptor chain though the mechanism involved is unclear.[11] The metalloprotease TNFα converting enzyme (TACE/ADAM17) cleaves membrane-bound Flt3L to soluble Flt3L.[12]

Recently, there has been evidence of additional hematopoietic sources of Flt3L during steady-state conditions. Whilst not as highly expressed as in T cells, *Flt3L* is detected in conventional DC (cDC) subset 2 (cDC2) with low levels of soluble Flt3L detected in primary murine cDC2 cultures. The metalloprotease responsible for cleaving cDC2 Flt3L is a disintegrin and metalloproteinase 10 (ADAM10).[13] Mice lacking ADAM10 specifically in DCs have increased surface cDC2 Flt3L, reduced serum Flt3L and significantly reduced splenic cDC2 (defined as $CD11b^+$ $CD4^+$ $ESAM^+$).[13] This is the first paper to provide evidence for differential roles of Flt3L isoforms, with cleaved soluble Flt3L, not membrane-bound or tethered, required for the development and maintenance of $ESAM^+$ cDC2. It is currently unclear why splenic cDC2 preferentially require soluble Flt3L, though it has been suggested this improves accessibility of the ligand.[13]

**The lifecycle of Flt3**

Transcription

*Flt3* is regulated by a number of transcription factors. In DC progenitors, including common myeloid, lymphoid and DC progenitors, the transcription factor PU.1 (*Sfpi1*) promotes *Flt3* expression by directly binding to the *Flt3* promoter.[14] As a result, progenitors lacking this transcription factor have reduced DC developmental potential. *Flt3* expression is also enhanced by the transcription factor homeobox A9 (HOXA9), which has been shown to bind directly to the promoter of *Flt3*.[15] While mice lacking HOXA9 have reduced $Flt3^+$ progenitors, there are no changes to the DC lineage, indeed the number of bone marrow cDC and plasmacytoid DC (pDC) are unaltered.[16] It is unknown if HOXA9 maintains the steady-state level of Flt3 in terminally differentiated DCs. *Flt3* is also enhanced by CCATT/enhancer binding protein alpha (C/EBPα) and Myb in acute myeloid leukemia cells, with their knockdown decreasing *Flt3* expression. [17] DC development is dependent on C/EBPα; however, it is not a requirement for survival or function of terminally

differentiated DCs. Similarly, Myb regulates hematopoiesis and conditional deletion of this transcription factor in haematopoietic stem cells ablates bone marrow cellularity. Reduced *Flt3* on progenitor cells has been observed in *Myb* conditional deletion mice.[18] It is unclear whether C/EBPα and Myb regulate *Flt3* in DCs. In contrast, *Flt3* transcription is repressed by the transcription repressor Hes family BHLH transcription factor 1 (Hes1) which binds to the *Flt3* promoter.[19] Mice lacking Hes1 have impaired T cell development and lack a thymus.[20] It is unclear whether Flt3 is regulated by Hes1 in DCs; however, progenitor cells lacking this transcription factor have a developmental bias towards myeloid and DC development.[20] *Flt3* suppression also occurs due to paired box protein 5 (Pax5), which binds the *Flt3* promoter in pro-B cells.[21] This is critical for B cell development.[21] It is unclear whether Pax5 regulates steady-state expression of *Flt3* in DCs.

Trafficking to the cell surface

Given that endogenous expression of Flt3 in terminally differentiated cells is restricted to DCs, surprisingly little is known about its intracellular biology in this cell type. Most of these studies have been carried out in irrelevant cell lines, often over expressing *Flt3*. Nevertheless, once transcribed and translated the Flt3 receptor behaves similar to other receptor tyrosine kinases (**Figure 2**). It transits to the cell surface as a monomer which is N-linked glycosylated as confirmed by N-glycosidase F (PNGase F) digestion.[22] Once at the cell surface, and bound to the ligand, dimerization occurs. In support of this, when cells are treated with Flt3L, Flt3 can be detected as a species of ~360kDa (representative of 2 monomers bound to the 30kDa Flt3L).[23] Flt3L stimulation promotes Flt3 phosphorylation, indicative of activation.[24]

Flt3/Flt3L signaling

Once activated, Flt3 signals via mitogen-activated protein kinase (MAPK), phosphatidylinositol 3 kinase (PI3K) and signal transducers and activators of transcription 3 (STAT3) pathways (**Figure 3**). Flt3 signaling is also implicated in the indirect regulation of other signaling pathways given that in the absence of Flt3, DCs and their progenitors are more responsive to other growth factors (such as M-CSF and stem cell factor) and can activate additional pathways.[25] The MAPK pathway is implicated in Flt3 signaling in different cell lines overexpressing wild type Flt3. Once stimulated with Flt3L, increased activation of SHC adaptor protein 1 (SHC) and

extracellular signal-related kinase (ERK)1/2 is observed.[26] While this likely occurs in DCs, it has yet to be shown. The PI3K pathway is also implicated in Flt3 signaling. Flt3-mediated PI3K activation has been demonstrated in DCs.[27] Indeed, *in vivo* administration of Flt3L leads to increased phosphorylation of ribosomal protein S6 kinase beta-1 (S6K) in splenic cDCs. Congruent to this, global or DC-specific deletion of phosphatase and tensin homolog (PTEN), an inhibitor of AKT and downstream of PI3K signaling, leads to an increase in the number of DCs suggesting an important role of Flt3L-induced PI3K signaling in DC regulation.[27] Finally, activation of STAT3 is critical for DC development and occurs following Flt3L stimulation.[28] Consequently, mice lacking STAT3 in hematopoietic cells have fewer DCs which cannot be rescued by Flt3L treatment.[28] Similarly, forced expression of STAT3 leads to increased DC developmental potential.[29]

Flt3 degradation

Once activated, Flt3 internalizes and degrades as quickly as 5 and 20 minutes respectively.[23] Internalization of Flt3 from the cell surface is likely regulated by ubiquitination. Much of our current understanding of Flt3 ubiquitination has come from studies in transformed cell lines overexpressing Flt3, with little information for DCs. Nevertheless, these studies have revealed an attractive candidate for Flt3 ubiquitination – the Casitas B-lineage lymphoma (Cbl) family. Cbl was first implicated in Flt3 signaling when their phosphorylation was increased in transformed cell lines stimulated with Flt3L.[30] Studies using c-Cbl and Cbl-b mutant mice have further implicated these E3 ligases in Flt3 regulation. Mice harboring a mutation in the RING domain of c-Cbl (c-Cbl$^{A/-}$) have increased Flt3 expression and signaling in LSK (Lin$^-$ SCA-1$^+$ c-Kit$^+$) cells and develop lethal myeloid leukemia.[31] Increased Flt3 expression and signaling is lost when c-Cbl$^{A/-}$ mice are crossed to Flt3L$^{-/-}$ mice.[31] It is currently unclear as to whether the Cbl family regulates DC development and/or Flt3 expression in DCs. Once internalized, sorting of Flt3 into late endosomes is dependent on late endosomal/lysosomal adaptor and MAPK and mTOR activator 2 (LAMTOR2).[32] As a result, mice lacking LAMTOR2 have increased Flt3 expression and signaling in DCs and increased DC progenitors.[32]

**Flt3 and DC development**

Single cell analysis has revealed the development of terminally differentiated DCs is a continuous process whereby progenitor cells progressively differentiate.[33] DC development is dependent on Flt3/Flt3L signaling. As such, all cells that differentiate into cDCs and pDCs express Flt3.[34,35] Surface expression of Flt3 differs, with the highest expression observed for splenic cDC1 and the lowest for pre-cDC1 and pre-cDC2.[36] It should be noted that not all DCs rely on Flt3. Indeed, Langerhans cells, an epidermal-resident DC, are unaffected by loss of Flt3 signalling,[37] indicating their establishment and maintenance is Flt3-independent. The critical role of Flt3 in cDC and pDC development has been reinforced by studies overexpressing Flt3.[29] Onai and colleagues showed $Flt3^-$ hematopoietic progenitors, such as the megakaryocyte erythrocyte progenitor, MEP, cannot produce cDC or pDC irrespective of Flt3L stimulation. However, upon *Flt3* introduction, these progenitors can give rise to cDC and pDC.[29] Further to this, lethally irradiated mice have reduced cDCs and pDCs following reconstitution with $Flt3L^{-/-}$ bone marrow. Similarly, lethally irradiated $Flt3L^{-/-}$ mice reconstituted with wild type bone marrow have reduced cDCs and pDCs.[10] This not only demonstrates the importance of Flt3 signaling in DC development but also highlights the importance of hematopoietic sources of Flt3L, particularly $CD4^+$ T cells. Further evidence of the critical role of Flt3 in haematopoietic development comes from studies showing an increase in DC and progenitor number when Flt3L is administered to mice[35,38,39] or humans.[40] Other growth factors, such as granulocyte colony-stimulating factor (G-CSF) increase DC numbers; however, they are incapable of expanding DCs to the same extent as Flt3L.[38] Although increased cellularity is not observed in the bone marrow of Flt3L treated mice, there is an increase in the number of B cell and DC precursors and their mobilization.[39] In support of this, when Flt3L-treated and untreated splenocytes are depleted of cDCs and transferred into irradiated recipients there is an increase in DCs originating from Flt3L-treated splenocytes.[39] This is indicative of increased DC progenitors in Flt3L-treated spleens.

A greater understanding of Flt3 signaling on hematopoiesis has been gained from mice lacking *Flt3* (*$Flt3^{-/-}$*)[41] or *Flt3L* (*$Flt3L^{-/-}$*).[42] Initially $Flt3^{-/-}$ mice were described to have no changes in cellularity with the exception of reduced bone marrow B cell progenitors.[41] However, more recent analysis using complex multicolor immunophenotyping has revealed additional decreases in lymphoid[39] and

nonlymphoid[37] DCs. A similar, albeit more severe, phenotype is observed in Flt3L$^{-/-}$ mice. Mice lacking Flt3L$^{-/-}$ have reduced DC progenitors.[42] In addition, significant reductions in lymphoid[25,39,42] and non-lymphoid DCs[37] are observed. Flt3L$^{-/-}$ mice also have reduced natural killer (NK) cells.[42] This indicates Flt3 signaling regulates early progenitor cells involved in the differentiation of a number of lineages. In competitive bone marrow transplantation experiments Flt3$^{-/-}$ bone marrow cells are unable to reconstitute DCs[39] and other lineages[39,41] to the same extent as wild type bone marrow. In addition, by assessing bromodeoxyuridine (BrdU) incorporation into Flt3$^{-/-}$ and wild type splenic DCs, Waskow *et al*. demonstrated that Flt3$^{-/-}$ DCs in the periphery are unable to proliferate to the same extent as their wild type counterparts.[39] Therefore, Flt3 signaling regulates not only the differentiation and mobilization of progenitor DCs, but also plays a role in the homeostatic division of cDCs in peripheral lymphoid organs. More recent analysis suggests that while the earlier stages of cDC1 development require Flt3 signalling, the final stage is independent of this pathway.[36] Similar proportions of cDC1 are generated when purified splenic pre-cDCs (CD11c$^{+}$ MHC II$^{int}$ Flt3+ SiRPα$^{int}$) were cultured *in vitro* with feeder cells with and without Flt3L. This is in contrast to cDC2, that are significantly reduced in the absence of Flt3L.[36] This is surprising given cDC1 express higher Flt3 than cDC2,[36] and suggests Flt3 may play other important roles in cDC1.

**Flt3 and immune responses**

Flt3-mediated DC homeostasis is important for regulating normal immune responses, mainly due to its indirect effect on T cell homeostasis. DC numbers highly correlate with regulatory T cell (Treg) numbers. Flt3L administration increases Tregs and Flt3 depletion reduces Tregs.[43,44] This relationship is observed in humans, with Flt3L treatment causing significant expansion of Tregs.[45] Alterations to T cell compartments are independent of thymic Treg production as Flt3L administration increases splenic Tregs in thymectomized mice.[43] DCs regulate Treg proliferation in the periphery.[43–45] Given the relationship between DCs and Tregs, aberrant DC Flt3 signaling may alter immunosurveillance in cancer.[46]

Serum Flt3L levels, and as a result DC and T cell numbers, vary during different diseased states. Dramatic increases in serum Flt3L is often observed in conditions of decreased progenitors, for example patients undergoing high-dose chemotherapy.[9]

Increased Flt3L is also evident at inflammation sites, such as following *Plasmodium* infection.[47] While CD4$^+$ T cells and cDC2 may be responsible for steady-state levels of Flt3L, this may not be the case in the presence of reduced hematopoietic progenitors, inflammation or infection. Indeed, mast cells, not CD4$^+$ T cells, are a major source of Flt3L during *Plasmodium* infection.[47] Similarly, in cancer, natural killer (NK) cells produce Flt3L at the tumor site.[48] Increases in Flt3L during infection and cancer play important protective roles by expanding DCs capable of presenting exogenous antigen by MHC I (cross-presenting cDC1) and improving CD8$^+$ T cell responses.

**Exploiting Flt3/Flt3L signaling in immunotherapy and vaccination**

There is significant interest in exploiting DCs in tumor immunotherapy. This is because of their excellent ability to capture and present antigen, in addition to trafficking to T cell zones. Unfortunately, harnessing the power of DCs has proved difficult due to their rare number in healthy and malignant tissues. Indeed, assessment of tumor infiltrating immune cells shows DCs to be the rarest cell type.[49,50] Whilst the number of tumor infiltrating DCs (TIDs) may be small, the role they play in tumor clearance is anything but. Cross-presenting cDC1 (CD141$^+$/BDCA3$^+$ in humans and CD103$^+$ in mice) are essential in solid tumor clearance. Mice lacking cross-presenting DCs (*Batf3*$^{-/-}$) have decreased CD8$^+$ T cell recruitment and increased tumor burden following tumor inoculation.[50,51] Similarly, high expression of BDCA3$^+$-related gene expression in melanoma correlates with increased overall patient survival.[48] This dramatic phenotype is due to an increase in CD8$^+$ T cell activation and infiltration.[51] Indeed, while a number of tumor-infiltrating immune cells, such as macrophages, can capture tumor-associated antigen, only cross-presenting TIDs are able to traffic to, and activate, CD8$^+$ T cells in the tumor and at tumor draining lymph nodes.[49,50] The normal number of TIDs is reliant on Flt3L production by NK cells, with a decrease in cross-presenting TIDs in mice lacking NK cells.[48] In agreement with this, the number of NK cells correlates with *Flt3L* gene expression and BDCA3$^+$ DC numbers in human melanoma.[48]

The Flt3/Flt3L signaling cascade is being targeted to manipulate the number of cross-presenting TIDs to improve anti-tumor immune responses. Administration of Flt3L to mice and humans significantly expands cross-presenting TIDs and their

progenitors.[49,50,52,53] This promotes anti-tumor immunity in mice with solid tumors.[53,54] Improved outcomes occur when Flt3L is used together with a stimulatory agent and checkpoint inhibitors. Mice treated with Flt3L in combination with poly I:C (TLR3 agonist), mAb specific for programmed cell death-1 (PD1) with and without irradiation have significantly reduced primary and secondary tumor growth and increased survival.[49,52,55] This phenotype is reliant on cross-presenting $CD103^+$ DC and $CD8^+$ T cells.[49,52] The former is essential for anti-PD1 therapy.[55] Including Flt3L in combination therapy appears promising in clinical trials, with increased tumor regression in B cell lymphoma patients treated with Flt3L, irradiation and poly I:C.[52]

Adoptive T cell transfer (ACT) is an immunotherapy where patients are infused with T cells that are expanded *in vitro*. Importantly, these T cells can be genetically modified to improve their anti-cancer efficacy. For example, chimeric antigen receptor (CAR) T cells are engineered to express receptors specific for tumor antigens. In mice reconstituted with *Batf3*$^{-/-}$ bone marrow, adoptively transferred T cells were unable to migrate to the tumor site.[51] However, once reconstituted with activated DCs, this phenotype was rescued.[51] It is therefore possible that Flt3L, in combination with a TLR agonist, could potentiate ACT. Indeed, increased proliferation of adoptively transferred OT-I cells occurs in B16-OVA bearing mice treated with Flt3L and poly I:C.[49] CAR T cells have been engineered to express and secrete Flt3L.[53] Lai and colleagues demonstrate Flt3L-CAR T cells improve tumor clearance, especially in combination with poly I:C and anti-4-IBB. This is dependent on cDC1, with no significant reduction in tumor size observed in *Batf3*$^{-/-}$ mice. Importantly, Flt3L-CAR T cell therapy increases the host anti-tumor response with an increase in endogenous DC and T cells. In addition, improved host TCR diversity is observed. As a result, when mice are rechallenged with tumors lacking antigen, anti-tumor responses are improved.

Flt3/Flt3L signaling has been exploited for vaccines against infection. Incorporation of Flt3L into vaccines against infectious agents can enhance immunity by increasing DC numbers and infiltration. Following subcutaneous immunization with DC-targeted antigen there is an increase in serum Flt3L alongside alterations in DC composition in skin-draining LNs.[56] Mice treated with Flt3L during immunization display increased antigen uptake, and upon antigen recall, improved $CD4^+$ T cell interferon gamma

(IFNγ) production, $CD4^+$ T cell expansion and increased serum IgG. In addition, Flt3L treatment expands $CD8^-$ $CD24^+$ precursors of cDC1.[57] Mice vaccinated with these DC precursors prior to viral infection have increased specific memory T cell expansion and decreased viral load in comparison to mice vaccinated with cDC1.[57] During early stages of influenza A virus infection, there is a decrease in Flt3L and cDCs, impacting immune outcomes.[58] By treating mice with a Flt3L-encoding plasmid prior to infecting with influenza, Beshara and colleagues exploited the Flt3/Flt3L signaling pathway and effectively increased the number of DC progenitors and lung DCs, in addition to reducing the number of tissue-damaging inflammatory monocytes. This strategy protected mice against secondary bacterial infections.[58]

**Flt3 and autoimmunity**

While increased Flt3/Flt3L signaling may be desirable during infection and cancer, a genome-wide association study of more than 30,000 autoimmune thyroid disease (AITD) cases highlights this pathway can also elicit autoimmune disease.[59] Saevarsdottir and colleagues identified an intron variant of Flt3 impacting AITD, systemic lupus, rheumatoid arthritis (RA) and coeliac disease risk.[59] The intron variant has a premature stop codon predicted to generate a truncated non-functional protein. The reduction in Flt3 correlates with increased serum Flt3L and expansion of myeloid cells, indicative of a positive feedback loop between Flt3 receptor and ligand.[59] This is the first study to detail a germline mutation in *Flt3* leading to increased risk of autoimmunity. Increased Flt3L is a well-established hallmark of autoimmunity. Indeed, RA patients not only exhibit increased serum Flt3L but also significantly increased Flt3L in the synovial fluid of their joints, where most inflammation and tissue damage is observed.[60] It is hypothesized that increased Flt3L exacerbates autoimmunity in RA. In support of this, increased arthritis pathogenesis is observed in mice administered with Flt3L.[60]

In contrast, Flt3/Flt3L signaling can dampen autoimmunity via expansion of Tregs. Flt3L pre-treatment prevents death due to graft-versus-host-disease (GVHD) and protects mice against type 1 diabetes and inflammatory bowel disease.[43,44] In part this is due to increased cDC1s with protection lost when Tregs are depleted.[44] Unsurprisingly, the relationship between Tregs and DCs is also observed in mice

harboring Flt3-ITD mutations, with progressively increased Tregs in $Flt3^{ITD/+}$ and $Flt3^{ITD/ITD}$ mice.[46] Indeed, when Tregs are selectively depleted with diphtheria toxin in $Flt3^{ITD/+}FoxP3^{DTR\text{-}GFP}$ or $Flt3^{+/+}FoxP3^{DTR\text{-}GFP}$ mice, there was faster reconstitution of Tregs in $Flt3^{ITD/+}$ mice.[46]

## Conclusion

Since its discovery, the Flt3/Flt3L signaling cascade has proved to be one of the most important pathways for the development and maintenance of DCs. Recent clinical strategies aim to use this pathway to exploit DCs to shape immune responses for desired outcomes. This is proving particularly useful in cancer treatment as our understanding of tumor infiltrating immune cells and their role in cancer progression and clearance grows. Given the relationship between DCs and Tregs, manipulating the Flt3/Flt3L cascade may also prove useful in the context of autoimmune disease. Currently, clinical strategies rely upon administration of recombinant Flt3L. Improving understanding of the biology of Flt3 in DCs would enable more sophisticated approaches, including the use of small molecule pharmaceuticals. To do this, further understanding of the transcription, trafficking and regulation of Flt3 in DCs, and their progenitors, is required, research that to date, has largely been neglected. Attention to this important aspect of DC biology will reveal novel targets for regulating DCs in healthy and malignant settings.

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## Author contributions

**Kayla R Wilson:** Conceptualization; Writing – original draft; Writing - review & editing. **Jose A Villadangos:** Conceptualization; Supervision; Writing - review & editing. **Justine D Mintern:** Conceptualization; Funding acquisition, Supervision; Writing -review & editing.

## Conflicts of interest

The authors declare no conflicts of interest.

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**Figure Captions**

**Figure 1.** Schematic of the structure of Flt3 and Flt3L. Flt3 comprises five extracellular immunoglobulin (Ig)-like domains at the N-terminus. These are followed by one transmembrane (TM) domain and a juxtamembrane (JM) domain. The C-terminus comprises 2 tyrosine kinase domains. The most common mutation in the *Flt3* gene is the internal tandem duplication (ITD), where an in-frame duplication of exons 14 and 15 is present in the JM domain. Membrane-bound Flt3L comprises of 5 extracellular domains, one TM domain and one cytosolic tail (CT). The arrow indicates cleavage site to form soluble Flt3L.

**Figure 2.** Schematic of the trafficking of Flt3**.** Flt3 is transcribed and translated as a monomer which traffics to the cell surface. During this journey it is glycosylated. Once at the surface, it binds to Flt3L which promotes dimerization and phosphorylation of Flt3. Flt3 internalizes quickly and is degraded by either/or both the lysosomal and proteasomal degradation pathways.

**Figure 3.** Signaling pathways induced by activated Flt3**.** Flt3 signals through 3 pathways –MAPK (pink), STAT3 (blue) and PI3K (green). Dashed arrows indicate pathways that have only been demonstrated using transformed cell lines (not DCs)

608 whereas solid lines indicate pathways confirmed in DCs or DC progenitors.