

Janus kinase inhibitors

Mechanisms of action

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SUMMARY

The Janus kinase family of enzymes are associated with cytokine receptors on the surface of cells. They are part of the Signal Transducer and Activation of Transcription pathway which is involved in inflammatory and immune responses.

An abnormality in the pathway can cause abnormal proliferation of cells. Possible outcomes include polycythaemia vera, leukaemia and lymphoma.

Inhibiting Janus kinase can reduce immune responses. This can lead to improvements in autoimmune conditions such as rheumatoid arthritis.

Ruxolitinib and baricitinib mainly inhibit Janus kinase 1 and 2. Tofacitinib inhibits Janus kinase 1 and 3.

As Janus kinase inhibitors alter the immune response they increase the risk of serious infections. There is a possibility that they may also increase the risk of cancer.

Introduction

Many diseases related to the immune system involve abnormal production of cytokines, a group of proteins which enable cells to signal each other. For example, the cytokine interleukin-2 stimulates the production of T cells. After a cytokine binds to its receptor, an enzyme called Janus kinase (JAK) contributes to the processes within the cell to produce an immune or inflammatory response. Inhibiting this enzyme may be beneficial in some haematological malignancies and autoimmune diseases including rheumatoid arthritis.

Janus kinase takes its name from the Roman god Janus. As Janus had two faces he could look in two directions and so statues of Janus were often placed at gateways or doors. Janus kinase has two domains and is located at the entry to cells.

Janus kinase-Signal Transducer and Activation of Transcription signalling

Cytokines such as interferons, interleukins and colony stimulating factors play a critical role in cell proliferation and differentiation, metabolism, haematopoiesis, host defence, apoptosis and immunoregulation.¹ Cytokines function by binding to specific receptors on cell membranes. There are two large subgroups of cytokine-receptor interactions that cause signal transduction via the Janus kinase-Signal Transducer and Activation of Transcription (JAK-STAT) pathway.² This pathway is a crucial intracellular conduit by which many cytokines interact with their receptors (see Fig.):

- type I receptors bind several interleukins, colony stimulating factors and hormones such as erythropoietin, prolactin and growth hormone
- type II receptors bind interferons and interleukin-10 related cytokines.³

Janus kinases

The Janus kinases are part of the tyrosine kinase group of enzymes (Table 1). At present, four important members of the Janus kinase family have been identified. They all selectively interact with the intracellular parts of the receptors. Janus kinase 1 and Janus kinase 2 are involved in a broad range of functions including host defence, haematopoiesis, neural development and growth. In contrast, Janus kinase 3 and tyrosine kinase 2 have a narrower role in the immune response.² Janus kinase 3 is predominantly expressed in haematopoietic cells and is critical for signal transduction of interleukins integral to lymphocyte activation, function and proliferation.

As well as the functional part of the Janus kinase molecule, there is a similar part which is thought to be inactive. This 'second face' is known as the pseudokinase domain.

Signal Transducer and Activation of Transcription

The family of transcription factors includes Signal Transducer and Activation of Transcription (STAT) 1-5a, 5b and 6. Activation of the Janus kinases leads to phosphorylation of receptor chains and formation

of docking sites for the STATs.⁴ After phosphorylation the STATs translocate to the nucleus where they bind to DNA and regulate gene expression.¹ They can both activate and repress gene transcription (Fig.).⁴

Janus kinase abnormalities

Animal models have shown that the JAK-STAT pathways and the cytokines using these pathways play a critical role in the pathogenesis of autoimmunity, allergy, asthma and haematopoietic disorders. Any mutations which cause a gain or loss of function of JAK-STAT, and variations in the genes encoding cytokines and their receptors, are associated with a significant increase in immune-mediated disorders (Table 1).

V617F is an activating mutation in the pseudokinase domain of Janus kinase 2. Activating this normally inactive domain produces kinase activity which can lead to proliferation of haematopoietic cells.^{5,6} This causes polycythaemia vera and other myeloproliferative processes.

Alteration of the receptors associated with Janus kinases can contribute to diseases such as leukaemia and myelofibrosis. For example, loss-of-function mutations in the interleukin-2 receptor-Janus kinase 3 signalling pathway are responsible for some cases of severe combined immunodeficiency syndrome.

Sometimes cells abnormally secrete cytokines. This can lead to persistent activation of Janus kinases. Examples of this autocrine cytokine secretion include secretion of interleukin-13 in primary B cell lymphoma and Hodgkin lymphoma. Interleukins-6 and -10 activate Janus kinases in activated B cell-like lymphomas. This secretion leads to increased survival of malignant cells.

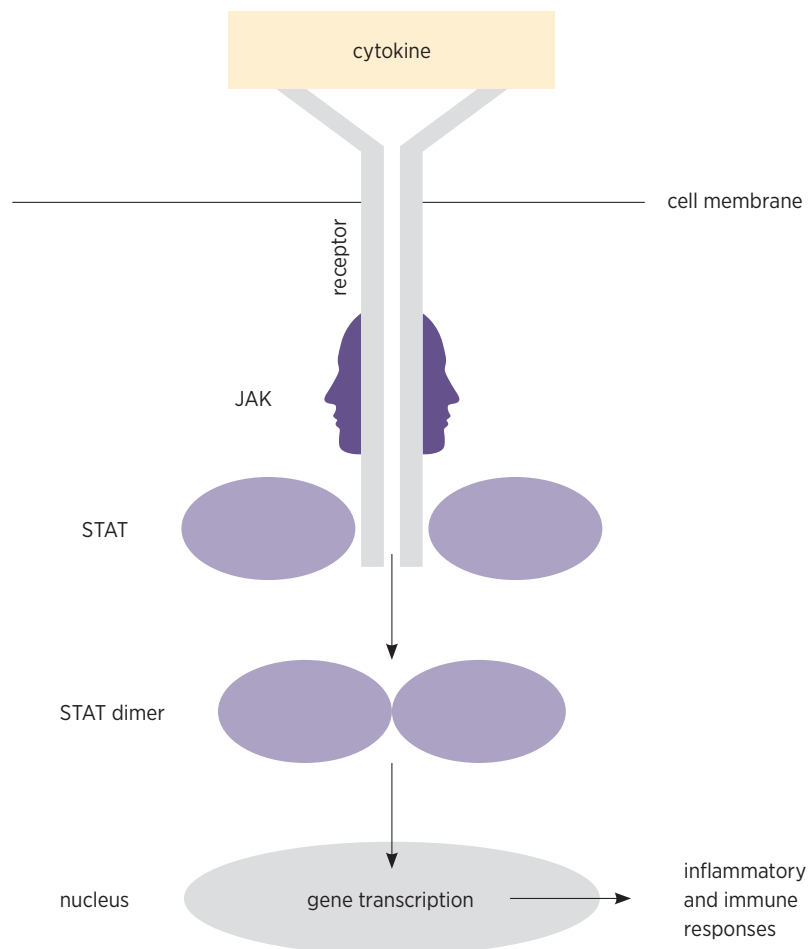
Sometimes Janus kinases can be involved in changing the activity of a gene without binding to DNA. For example, the Janus kinase 2 V617F mutation that is associated with myelofibrosis can directly phosphorylate chromatin targets in the nucleus. This exerts an effect on gene transcription that is independent of STATs.

Janus kinase inhibition

Inhibiting Janus kinase interrupts the JAK-STAT pathway. One effect of this inhibition in myelofibrosis is a significant reduction in splenomegaly with overall improvement in associated symptoms.⁴

Janus kinase inhibition has been widely studied in rheumatoid arthritis as there is overproduction of interleukin-6, interleukin-12, interleukin-15, interleukin-23, granulocyte-macrophage colony stimulating factor and interferons.⁷ The cytokine

Fig. Signalling through the JAK-STAT pathway



JAK Janus kinase STAT Signal Transducer and Activation of Transcription

receptors are therefore very prominent in driving autoimmunity particularly through Janus kinase 1 and 3. Inhibition of Janus kinase 1 and 3 will inhibit signalling and therefore suppress immune responses.

Due to the critical role of Janus kinases in host defence, autoimmunity and haematological cancers, they have become an attractive target for therapeutics for a variety of disorders (Table 2).

Ruxolitinib

Ruxolitinib is a Janus kinase 1 and 2 inhibitor that selectively targets myeloproliferative disorders involving the gain of function mutation in Janus kinase 2 (V617F mutation). It reduces splenomegaly and systemic symptoms, and improves overall survival in myelofibrosis. It is also being studied in rheumatoid arthritis and skin psoriasis.⁸

Ruxolitinib is largely eliminated by hepatic cytochrome P450 3A4 metabolism, warranting care when choosing

Table 1 Cytokines associated with Janus kinases and the results of their mutations

Janus kinases	Associated cytokines	Associated diseases	
		Loss of function mutations	Gain of function mutations
Janus kinase 1	interleukin-2, 4, 6, 7, 9, 10, 11, 13, 15, 19, 20, 21, 22, 24, 26, 29 oncostatin M leukaemia inhibitory factor granulocyte colony stimulating factor tumour necrosis factor	-	T and B cell acute lymphoblastic leukaemia acute myeloid leukaemia acute myeloid leukaemia with severe congenital neutropenia activated B cell-like diffuse large B cell lymphoma
Janus kinase 2	erythropoietin growth hormone prolactin thrombopoietin oncostatin M leukaemia inhibitory factor granulocyte colony stimulating factor leptin tumour necrosis factor interleukin-3, 5, 6, 13 interferon gamma	-	polycythaemia vera essential thrombocytosis myelofibrosis Hodgkin lymphoma Down syndrome-associated B cell acute lymphoblastic leukaemia primary mediastinal B cell lymphoma
Janus kinase 3	interleukin-2, 4, 7, 9, 15, 21	severe combined immunodeficiency	megakaryoblastic leukaemias
Tyrosine kinase 2	interleukin-10, 12, 22, 26, 29 interferon alfa/beta/omega interleukin-28 alfa/beta	primary immunodeficiency	

other drugs for patients. Starting doses are generally lower for patients with renal impairment.

Baracitinib

Baracitinib is also an inhibitor of Janus kinase 1 and 2. It has shown clinical efficacy in patients with severely active rheumatoid arthritis resistant to other treatments.

Tofacitinib

Tofacitinib is principally an inhibitor of Janus kinase 1 and 3. It also inhibits Janus kinase 2 to some extent, but has very little effect on tyrosine kinase 2. There is some evidence that it may have an effect in patients with rheumatoid arthritis that has not responded to other therapies.

Adverse effects of Janus kinase inhibition

As Janus kinase inhibitors alter the immune response, there is an increased risk of serious bacterial, fungal, mycobacterial and viral infections including opportunistic infections like tuberculosis and

non-disseminated herpes zoster. This can be attributed to a reduction of natural killer cells as a consequence of Janus kinase 1 and Janus kinase 3 inhibition.³ There is also a potentially increased risk of cancer as a result of blocking the action of interferons and natural killer cells, as these play an important role in tumour surveillance. Unresolved concerns about safety led the European Medicines Agency to conclude that the benefits of tofacitinib did not outweigh the potential harms.⁹

Erythropoietin and colony stimulating factor activate Janus kinase 2. Anaemia, neutropenia and thrombocytopenia may therefore be consequences of Janus kinase 2 inhibition.

Future developments

As Janus kinase inhibitors block cytokines they are being studied in diseases such as psoriasis, inflammatory bowel disease, transplantation and systemic lupus erythematosus. There is a potential role for an inhibitor of Janus kinase 1 and 2 like tofacitinib in asthma and

allergy as these conditions are associated with T-helper lymphocytes and the action of interleukin-4, which will require Janus kinase 1 and 2 for signalling.

Ruxolitinib and tofacitinib are non-specific JAK inhibitors as they act on more than one kinase. There are several trials investigating whether selective Janus kinase inhibitors have better safety with comparable efficacy. <

Paul Kubler is a member of medical advisory groups for Reckitt Benckiser, Eli Lilly and AbbVie, and is a principal investigator for UCB, Bristol-Myers Squibb and Ardea. He is an external advisor for the Therapeutic Goods Administration, and chair of the Australian Prescriber Editorial Executive Committee.

Table 2 Janus kinase inhibitors

Drug	Janus kinase inhibition		
	1	2	3
ruxolitinib *	✓	✓	-
baricitinib	✓	✓	-
tofacitinib	✓	✓	✓
pacritinib	-	✓	-
momelotinib	✓	✓	-

* currently available in Australia

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Undergraduate student prize 2014

Congratulations to Veronica Ho, the winner of the 2014 pre-registration student prize, sponsored by *Australian Prescriber*, and awarded by the Australian and New Zealand Association for Health Professional Educators (ANZAHPE).

Veronica is a third-year student in the Bachelor of Medicine, Bachelor of Surgery program at the University of New South Wales. Her project was 'Online testable concept maps for learning about pathogenesis of disease'. She developed an online learning tool where students could test their knowledge of the pathogenesis of a disease by dragging and dropping missing pieces of information into a visual representation of the disease.

A manuscript describing her study has been accepted for publication by Medical Education.



Veronica Ho accepting the *Australian Prescriber* sponsored prize from Professor Gary Rogers, President of ANZAHPE