

# JAK inhibitors selectivity: New opportunities, better drugs?

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## Abstract

Cytokines act as communication tools of the immune system, serving critical functions in many biological responses and shape the immune response. When cytokines production or their biological activity goes awry, the homeostatic balance of the immune response is altered with the development of several pathologies such as autoimmune and inflammatory disorders. Cytokines bind to specific receptors on cells, triggering the activation of intracellular enzymes known as Janus kinases (JAKs). The JAKs family is comprised of four members, JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2), which are critical for intracellular cytokine signalling. Since the mid 2010s multiple JAK inhibitors have been approved for inflammatory and haematological indications. Currently, approved JAK inhibitors have demonstrated clinical efficacy; however, improved selectivity for specific JAKs is likely to enhance safety profiles, and different strategies have been employed to accomplish enhanced JAK selectivity. In this update, we discuss the background of JAK inhibitors, current approved indications and adverse effects along with new developments in this field. We address the issue of JAK selectivity and its relevance in term of efficacy, and describe new modalities of JAK targeting, as well as new aspects of JAK inhibitors action.

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## [H1] Introduction

Cytokines have a critical role in the pathogenesis of a diverse range of inflammatory and autoimmune diseases. The development of biologic therapies targeting extracellular cytokines and their receptors revolutionized the treatment of several autoimmune and allergic diseases including rheumatoid arthritis (RA), atopic dermatitis and inflammatory bowel disease (IBD), validating cytokines as relevant drug targets. Despite their overall success, biologics have not been entirely successful, with a substantial portion of patients not achieving long-term remission, thereby creating the need for alternative therapeutic strategies including targeting intracellular signalling. JAKs are a family of phosphotransferases originally identified in the early 90's, they possess tandem kinase domains making them plausible targets to target signalling of numerous cytokines (Figure 1) [1]. Many of these cytokines have important roles in the pathogenesis of immune-mediated diseases thus providing the rationale for JAK inhibition as a therapeutic approach. [2-6]

The JAK family consists of four members, JAK1, JAK2, JAK3 and TYK2 and individual JAKs have selectivity for different cytokine receptor subunits [7, 8]. The binding of cytokines to their cognate receptors results in enzymatic activation of JAKs and subsequent phosphorylation of their substrates, including the signal transducer and activator of transcription (STAT) family of transcription factors. The STAT family consists of seven mammalian members: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. Selective binding of cytokines to their cognate receptors allows preferential recruitment of different STATs. The combination of four JAKs and seven STATs results in a complex web of signals, ultimately regulating various cellular functions. [9].

The N-terminus of JAKs consist of FERM and SH2-like domains, which bind to the Box1 and 2 cytoplasmic regions of cytokine receptors, thereby mediating JAK-cytokine interactions and conferring functional specificity of JAKs in cytokine signalling [10] (Figure 2) JAKs contain two kinase domains: an active tyrosine kinase domain (JAK homology 1 (JH1)) and a catalytically-inactive pseudokinase domain (JAK homology 2 (JH2)) The role of the pseudokinase domain was recognized by mutational analysis but also thanks to the identification of the pseudokinase gain-of-function mutations underlying myeloproliferative neoplasms (MPNs) [11, 12].] Researchers defined the basic

65 concepts of JAK-STAT signalling within just a few years. However, understanding of JAK activation at  
66 the molecular and atomic levels remained elusive until 2022, when researchers uncovered the  
67 structure of a full-length JAK1 in complex with the intracellular domain of a cytokine receptor [13].  
68 The crystal structure showed that JAKs pseudokinase domains facilitate receptor dimerization and  
69 had a critical multifunctional role in regulating JAK activity. [14, 15][13, 16]. As mentioned below,  
70 the adenosine triphosphate (ATP) binding pocket of the pseudokinase domain, which is critical for  
71 JAK activation, was then considered a potential target for selective drug development is

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73 The first JAK inhibitor, ruxolitinib was approved by the FDA in 2011, followed by tofacitinib in 2012  
74 [17, 18]. Since then, a dozen JAK inhibitors have been approved, providing additional possibilities to  
75 treat rheumatologic, dermatologic, gastroenterological and oncologic disease, as well as COVID-19.  
76 The use of JAK inhibitors to treat these diseases often shows similar or superior efficacy compared  
77 to biologics . Alongside the first-generation JAK inhibitors, which unselectively target several JAKs,  
78 new molecules with increased selectivity for one of the JAKs have been developed and here are  
79 described as second-generation inhibitors.

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81 In this Review, we briefly cover the biology of JAKs and history of JAK inhibitors, their selectivity and  
82 safety, which has fuelled consideration about the pros and cons of these agents. STATs also have a  
83 critical role in the regulation of immune and non-immune cell functions but due to the paucity of  
84 molecules available to inhibit their functions effectively, we do not include discussion of STAT  
85 inhibitors in this Review. New molecules with greater selectivity for single JAKs have already been  
86 approved with the goal of improving safety profiles while maintaining efficacy We also discuss some  
87 of the more recently developed allosteric inhibitors that do not target the kinase domain. We focus  
88 on enzyme-specific drugs, but also discuss future prospects, including the possibility of developing  
89 tissue or organ-specific JAK inhibitors and other modalities that target JAKs to treat immune-  
90 mediated diseases

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92 [H1] The evolving specificity of JAK inhibitors

93 Thanks to the increased knowledge and mechanistic understanding of their activation, multiple JAK  
94 inhibitors have been approved for the treatment of a wide range of inflammatory and autoimmune  
95 diseases as well as for the treatment of MPNs [19]. The issue of JAK inhibitor selectivity stems from  
96 the need to have drugs that are effective and safe, limiting the predictable adverse effects of

97 cytokine and growth factor inhibition while maintaining therapeutic efficacy . The original first-  
98 generation JAK inhibitors targeted several JAKs. The development of second-generation JAK  
99 inhibitors aimed to increase selectivity towards one JAK isoform, particularly sparing JAK2, thereby  
100 reducing the risk of haematological adverse events. The quest for higher selectivity is also desirable  
101 for tailoring therapies to various diseases or disease conditions with distinct cytokine profiles.  
102 Approved drugs, such as monoclonal antibodies that specifically target a cytokine or a cytokine  
103 receptor have demonstrated that,] IL-6 (and downstream JAK1 signalling) is important in the  
104 pathogenesis of RA, IL-12 and IL-23 (and downstream JAK2 and TYK2 signalling) in IBD, psoriasis and  
105 psoriatic arthritis, IL-4 and IL-13 (and downstream JAK1, TYK2 and JAK2 signalling) in atopic  
106 dermatitis, and type I interferons (and downstream JAK1 and TYK2 signalling) in systemic lupus  
107 erythematosus. Blocking these pathways is therefore a viable treatment option in these diseases.  
108 JAK inhibitors inherently lack cytokine specificity; inhibiting one JAK isoform can affect multiple  
109 cytokine pathways, which can lead to both beneficial or undesirable effects depending on the  
110 context. For example, by targeting various signals downstream of a single cytokine, such as IL-2,  
111 pan-JAK inhibitors can influence both pro-inflammatory events like expansion of cytotoxic T cells as  
112 well as regulatory pathways (such as regulatory T cell development) [20] Hence, one rationale for  
113 developing inhibitors with a higher JAK isoform selectivity is to enable a more accurate prediction  
114 of the biological and clinical outcomes .

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116 The first-generation and second-generation JAK inhibitors target the ATP binding pocket in the JAK  
117 kinase domain. The highly conserved structure of the ATP pocket poses challenges to obtain high  
118 selectivity, nevertheless, researchers have exploited the subtle structural differences in the ATP  
119 pocket to develop molecules with a higher affinity for one enzyme than others in the family [21].  
120 Examples of JAK inhibitors that target the ATP binding pocket in the kinase domain include  
121 abrocitinib and ritlecitinib. Importantly, some inhibitors such as filgotinib and upadacitinib are  
122 relatively more selective than tofacitinib or baricitinib but are not completely selective [22]. The  
123 clinical effects of first-generation and second-generation JAK inhibitors are quite similar, though  
124 differences between individual drugs exist, and the distinction between first-generation and  
125 second- generations reflect historical development For convenience, in this Review, the newer  
126 allosteric inhibitors that target the regulatory pseudokinase domain are referred to as third-  
127 generation JAK inhibitors. Research over the past 30 years provides a reliable model of the  
128 regulation of JAKs and their domains, this is critical not only to understand the JAK-STAT cascade

129 but also for the design of better drugs that could inhibit specific pathways. There are now drugs in  
130 development with increased selectivity for one of the JAKs or compounds with specificity for target  
131 tissues (topical or inhaled JAK inhibitors).

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## 133 [H2] First-generation JAK inhibitors

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135 As mentioned above, first-generation JAKinibs are orthosteric, ATP-competing inhibitors and  
136 generally display limited selectivity for a specific JAK. In this Review, we summarise the affected  
137 JAKs, indications and doses of the approved first-generation and second-generation ATP-  
138 competitive JAK inhibitors (Table 1). In addition, we summarize the phase III clinical trials of first-  
139 generation JAK inhibitors in the different clinical indications with their respective primary endpoints.

## 140 [H3] First-generation JAK inhibitors mode of action

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142 From a structural standpoint, ruxolitinib, tofacitinib and baricitinib share the pyrrolo-pyrimidine  
143 moiety, [23] which has an important role in making hydrogen bonds with the hinge region in the  
144 ATP binding pocket of the JAK kinase domain [24]. Additionally, ruxolitinib and baricitinib share a  
145 unique pyrazole moiety directly linked to the pyrrolo-pyrimidine moiety [23]. From a  
146 pharmacological standpoint, these three drugs inhibit JAK1 as well as JAK2, albeit tofacitinib is less  
147 effective than ruxolitinib and baricitinib at JAK2 inhibition in immune cells. Tofacitinib also inhibits  
148 JAK3, whereas ruxolitinib and baricitinib are much weaker inhibitors of JAK3 [24].

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150 In addition to inhibition of JAK enzyme phosphorylation, first-generation JAK inhibitors have  
151 reported off-target effects. In 2020, the first artificial intelligence predictions of off-target effects  
152 were made whilst identifying potential therapeutic targets for the treatment of SARS coronavirus 2  
153 [25]. A study searching for compounds that can inhibit clathrin-mediated endocytosis, showed that  
154 baricitinib targets the numb-associated kinase family. In vitro studies suggest that members of the  
155 numb-associated kinase family, including AAK1 and GAK, are involved in protection against viral  
156 infection of cells [25]. Other off-target effects identified by machine learning approaches are based  
157 on ligand similarity including attenuation of pulmonary vascular remodelling, modulation of  
158 response to hepatitis C virus, and hypomagnesemia [26].

## 159 [H3] Approved applications of first-generation JAK inhibitors

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Ruxolitinib was the first JAK inhibitor approved by the FDA for the treatment of patients with myelofibrosis in November 2011 (Figure 3). The association between the JAK2 V617F gain-of-function mutation (in the pseudokinase domain) and clinical features of myelofibrosis such as anaemia, splenomegaly and increased risk of transformation to acute myeloid leukaemia made JAK2 an ideal target for treating myelofibrosis [27]. The authorization for myelofibrosis came from two phase III randomised clinical trials, where ruxolitinib reduced spleen size by at least 35%, ameliorated debilitating symptoms, improved quality of life and had a favourable safety profile [28, 29]. In 2014 and 2019, respectively, ruxolitinib received approval for two more pathologies: polycythemia vera and graft versus host disease (GVHD) [30, 31]. Tofacitinib was first assessed for the prevention of renal transplant rejection [32] but was the pioneer of JAK inhibitors approved for rheumatological and gastroenterological conditions, with the first approval received in 2012 for RA (Figure 3). The phase III ORAL program assessing tofacitinib treatment in patients with RA who had an inadequate response to methotrexate or biological DMARDs (bDMARDs) led to the FDA approval of tofacitinib 5mg twice a day for patients with moderate to severe active disease and inadequate response to methotrexate. In these clinical studies, tofacitinib treatment resulted in greater clinical and radiological benefits than treatment with placebo in patients who failed – for efficacy or safety reasons – at least one conventional synthetic or bDMARD [33-35]. Tofacitinib showed non-inferiority to the TNF inhibitor adalimumab. The use of tofacitinib was then extended to psoriatic arthritis (PsA), polyarticular juvenile idiopathic arthritis (JIA) and ankylosing spondylitis (AS). Moreover, the OCTAVE program, involving patients with ulcerative colitis with inadequate response to conventional therapies or TNF inhibitors, showed the superiority of tofacitinib compared to placebo in inducing and maintaining up to 52 weeks clinical remission and mucosal healing [36] [

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Baricitinib was first approved for RA by the EMA in 2017 (2mg and 4mg per day) and by the FDA in 2018 (2mg only) (Figure 3). The phase III program included four randomized clinical trials that involved patients with RA with an inadequate response to both methotrexate and, bDMARDs or naive to methotrexate [37-40]. Overall, baricitinib met the primary endpoint, showing superiority to placebo in clinical outcomes (ACR20 response) in all patients enrolled, and to adalimumab in patients with an inadequate response to methotrexate; moreover, the radiographic progression was slower in baricitinib-treated patients than with placebo-treated patients [38, 41]. In April 2023, baricitinib (4mg) received approval for the treatment of polyarticular JIA in children  $\geq 2$  years. The

192 JUVE-BASIS trial demonstrated that in patients with various forms of JIA (including polyarticular JIA,  
193 extended oligoarticular JIA, enthesitis-related arthritis JIA, and juvenile psoriatic arthritis) who has  
194 an inadequate response or intolerance to standard therapy , the time to flare was remarkably longer  
195 with baricitinib than with placebo [42]. Notably, after FDA emergency use approval, baricitinib is  
196 now the only JAK inhibitor approved for COVID-19 in hospitalized adult patients requiring oxygen  
197 therapy [43]. Another first-generation JAK inhibitor, peficitinib, is currently approved for RA in  
198 Japan, Taiwan and South Korea only. The phase III clinical trials of peficitinib (RAJ3 and RAJ4)  
199 demonstrated treatment with peficitinib (100mg or 150mg) was superior to placebo treatment at  
200 week 12, in terms of the ACR20 response [44, 45] .

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## 202 [H2] Second-generation JAK inhibitors

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204 As mentioned above, first-generation JAK inhibitors, which include four compounds approved  
205 through extensive phase 3 clinical programmes (Supplemental Table 2) inhibited more than one JAK  
206 with similar potency. Therefore, a new set of inhibitors with improved selectivity toward a specific  
207 JAK isoform (such as JAK1, JAK3 or TYK2) known as second-generation inhibitors were developed  
208 and several other compounds are currently in phase II or III trials (Table 2I).

## 209 [H3] Second generation JAK inhibitors mode of action

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211 First-generation JAK inhibitors were followed by the development of a new set of inhibitors with  
212 improved selectivity toward a specific JAK isoform (such as JAK1, JAK3 or TYK2) known as second-  
213 generation inhibitors. Two of the main reasons to develop a more selective JAK inhibitor were to  
214 target a specific set of cytokines while sparing inhibition of JAK2 to avoid undesirable  
215 haematopoietic effects including inhibition of red blood cell development and actions of  
216 erythropoietin [46], or effects on megakaryocytes and platelets development dependent on  
217 thrombopoietin [47]. Mechanistic characterization and preclinical studies of JAK1 inhibitors, as well  
218 as the aforementioned first-generation JAK inhibitors have demonstrated that second-generation  
219 compounds have a profile of cytokine inhibition that is consistent with inhibition and the respective  
220 roles of these JAK isoforms in cytokine receptor signalling [48-50]. Importantly, all JAK1 inhibitors,  
221 regardless of their selectivity towards other JAK proteins in biochemical assays, display equivalent  
222 inhibition of JAK1-dependent cytokine pathways [48, 50, 51] .

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### [H3] Approved applications of second-generation JAK1 selective inhibitors

The second-generation JAK1 inhibitors , abrocitinib, filgotinib and upadacitinib are approved for the treatment of various inflammatory diseases [49, 52, 53]. Upadacitinib shows some JAK1 selectivity but, at clinically used doses, JAK2 seems to be inhibited as well [22, 51]. This JAK inhibitor was first approved in August 2019 for moderate-severe active RA [54] . The extensive phase III SELECT program included patients with inadequate response to methotrexate or other conventional synthetic DMARDs or bDMARDs and demonstrated the superiority of upadacitinib (15mg) over placebo, as monotherapy over methotrexate, and over two bDMARDs: adalimumab (in methotrexate- inadequate response patients) and abatacept (in TNF inhibitor inadequate response patients) [55-60]. The trials met the primary endpoints including the ACR20 response and more stringent outcomes as requested by the EMA, including low disease activity or remission . Two studies confirmed the effect of upadacitinib on structural damage, as evaluated with radiographic outcome measures, in patients naive to treatment and in methotrexate-inadequate responders. Along with RA, three more rheumatological indications were added : PsA, AS and non-radiographic axial spondylarthritis. In 2022, upadacitinib was approved for adult and adolescent patients with atopic dermatitis and for two gastroenterological conditions, ulcerative colitis and Crohn’s disease.

Filgotinib was approved by the EMA and Pharmaceuticals and Medical Devices Agency but not by the FDA, for RA (in 2020) and ulcerative colitis (in 2021) . The phase III FINCH program included three studies involving patients with RA who has an inadequate response to TNF inhibitors or methotrexate or who were naive to methotrexate treatment; the primary endpoint was the percentage of patients achieving an ACR20 response at week 12 (FINCH 1 and FINCH 2, in conventional synthetic bDMARDs experienced patients) or week 24 (FINCH 3, including patients with no or limited exposure to methotrexate) [61-63]. The studies demonstrated the superiority of filgotinib versus placebo and the non-inferiority to adalimumab [61-63]. In the SELECTION study, filgotinib (200mg) induced clinical remission in a greater percentage of patients with moderately to severely active ulcerative colitis compared with placebo after 10 weeks (induction study) and 58 weeks of treatment (maintenance study) [64]. Abrocitinib was approved by the FDA for the treatment of atopic dermatitis in September 2021. The approval was based on a large phase III clinical program with abrocitinib in adults and adolescents . In moderate-severe atopic dermatitis,

255 abrocitinib (100 and 200mg) in combination with topical steroids or as a monotherapy showed  
256 superiority to placebo in combination with topical corticosteroids, with a greater percentage of  
257 patients achieving the endpoints [65-67]. Moreover, in patients who showed a clinical response  
258 after 12 weeks of open label treatment, abrocitinib (200mg and 100mg) reduced the risk of flares  
259 at week 40 compared with placebo [68]. In the JADE COMPARE trials, abrocitinib 100 and 200mg  
260 with placebo or dupilumab (currently the gold standard and most prescribed drug in atopic  
261 dermatitis treatment) were assessed. The primary endpoints were investigator global assessment  
262 (IGA) and eczema area and severity index (EASI)-75 responses at week 12 and Peak Pruritus  
263 Numerical Rating Scale (PP-NRS4) at week 2 and a 90% or better improvement in Eczema Area and  
264 Severity Index (EASI-90) at week 4 [69, 70]. Both doses showed significantly greater reductions in  
265 signs and symptoms of moderate-to-severe atopic dermatitis than placebo at weeks 12 and 16 but  
266 only abrocitinib 200mg, showed a superior itch response at week 2 compared with dupilumab. [69,  
267 70].

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269 [H3] Approved applications of second-generation JAK2 selective inhibitors

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271 Given the importance of JAK2 in haematologic malignancies, selective JAK2 inhibitors have also  
272 been developed for the treatment of MPNs, particular MPNs caused by mutations in JAK2, but also  
273 other cancers. Three JAK inhibitors, namely, fedratinib, pacritinib (which also inhibits Fms-like  
274 tyrosine kinase 3) and momelotinib (which also inhibits activin A receptor type 1 and increases iron  
275 availability) are approved for the treatment of myelofibrosis and hydroxyl urea resistant  
276 polycythemia vera. These drugs can reduce various clinical symptoms such as splenomegaly and  
277 fatigue [71-73].

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279 [H3] Approved applications of second-generation JAK3 selective inhibitors

280 To date, ritlecitinib is the only approved JAK3 selective inhibitor, this drug was approved in 2023 by  
281 the EMA and FDA for the treatment of severe alopecia areata in adults and adolescents  $\geq 12$  years  
282 [74]. Ritlecitinib inhibits JAK3 by binding in an irreversible fashion to cysteine 909 in this molecule;  
283 this residue is adjacent to the ATP pocket, and hence ritlecitinib functions as a competitive ATP  
284 compound. Cysteine 909 is unique to JAK3 and other JAKs instead contain a serine residue at this  
285 position [21]. In addition to JAK3, TEC kinases also contain a conserved cysteine in proximity to the  
286 ATP pocket; hence, ritlecitinib also inhibits five TEC kinase family members [21, 75]. Notably,

287 decernotinib is also a JAK3 selective inhibitor; however, the clinical use of this agent was limited by  
288 drug-drug interactions. Moreover, JAK1 displays a lower affinity for ATP than JAK3, which partly  
289 explains why several ATP competitive reversible JAK1-selective inhibitors have been developed  
290 whereas no other JAK3 inhibitors have reached the clinical stage.

## 291 [H2] The next generation: allosteric JAK inhibitors

292 In the absence of stimulation, the pseudokinase domain interacts with the kinase domain,  
293 preventing the conformational dynamics needed for kinase activation [14, 15]. In an active state,  
294 JAK extends to an open conformation where the pseudokinase domain forms dimers between the  
295 neighbouring JAKs (Figure 2). The resulting close apposition enables transphosphorylation of the  
296 kinase domains, leading to their activation and initiation of the signalling cascade [13, 16]. This  
297 knowledge led to the possibility to target the pseudokinase domain in allosteric fashion. (also known  
298 as Type III inhibitors) or remote from the ATP binding site of the pseudokinase domain (Type IV  
299 inhibitors). Both type III and type IV allosteric inhibitors have been exploited for JAK inhibition based  
300 on the important regulatory role of the pseudokinase domain [76]. Notably, the allosteric inhibitors  
301 already approved or under development (Table 3) have a higher specificity than ATP-competitive  
302 kinase-domain targeting inhibitors and correlate with less adverse events [77-79].

## 303 [H3] Allosteric inhibitors mode of action

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305 , Deucravacitinib, a drug that binds with high-affinity to the pseudokinase ATP pocket of TYK2, is, as  
306 of 2024, the only approved allosteric inhibitor . Deucravacitinib also binds to JAK1 and, to a lesser  
307 extent, JAK2 [51, 80-84]. Despite sub-nanomolar affinity to TYK2 and JAK1, and nanomolar affinity  
308 to JAK2, this drug preferentially inhibits TYK2-mediated cytokine pathways. The mode-of-action of  
309 deucravacitinib remains elusive, but this drug is postulated to stabilize kinase-pseudokinase domain  
310 interactions, an interaction that results in in TYK2 autoinhibition. The latter hypothesis is supported  
311 by in vitro enzymatic activity studies in which deucravacitinib did not inhibit recombinant TYK2  
312 pseudokinase-kinase recombinant protein indicating the need of other signaling molecules for the  
313 inhibitory effect. One would assume that if deucravacitinib induces intramolecular inhibition  
314 (stabilization of inhibitory interaction between the kinase and pseudokinase domains), it would  
315 induce inhibition of enzymatic activity of TYK2 kinase-pseudokinase protein. Thus, the absence of  
316 this inhibition suggests that other pathway components are needed for the inhibitory effect. [51].

317 Deucravacitinib might, upon binding to the TYK2 pseudokinase domain, prevent TYK2 from  
318 assuming a conformation required for transphosphorylation of another JAK and subsequent  
319 pathway activation [16]. Given the quest for better selectivity with preservation of efficacy, the  
320 interest in allosteric inhibition of JAKs has increased in the past decade and several investigational  
321 allosteric inhibitors are currently being evaluated in clinical trials.

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323 Other allosteric TYK2 inhibitors including zasocitinib, are being investigated in plaque psoriasis and  
324 psoriatic arthritis. ESK-001 is being evaluated in plaque psoriasis, non-infectious uveitis and systemic  
325 lupus erythematosus . VTX958 is in phase II trials for plaque psoriasis and achieved the study's  
326 primary endpoint showing a statistically significant proportion of patients experienced a 75%  
327 reduction in the Psoriasis Area and Severity Index (PASI75) at week 16 compared with placebo.  
328 Nonetheless, development of VTX958 for psoriasis as well as for PsA has ceased, but is continuing  
329 for Crohn's disease . Interestingly, another allosteric type IV inhibitor (VVD-118313) also binds to  
330 JAK1 and TYK2 pseudokinase domains but selectively inhibits only JAK1-mediated signalling. VVD-  
331 118313 is an investigational compound that covalently binds to an allosteric cysteine present in JAK1  
332 and TYK2, a site analogous to the myristoyl pocket of ABL kinase [79]. In ABL, this pocket is the target  
333 of asciminib, an ABL-inhibitor approved to treat Philadelphia chromosome-positive chronic myeloid  
334 leukaemia [85]. VVD-118313 inhibits JAK1-mediated transphosphorylation [79] and modelling data  
335 suggests that this compound perturbs the conformation of a linker between kinase and  
336 pseudokinase domains, destabilizing transactivation and resulting in loss of JAK1 phosphorylation  
337 [16]. Proof of VVD-118313 mediated loss of JAK1 phosphorylation though, is still lacking. The fact  
338 that two allosteric inhibitors target the same JAKs (JAK1 and TYK2) but show different inhibition  
339 profiles demonstrate that cytokine signalling specificity can be obtained via allosteric JAK targeting.  
340 However, this disparity also illustrates that in vitro JAK-selectivity profiles do not always translate  
341 into the expected cellular effects.

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343 [H3] Approved applications for allosteric inhibitors

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345 The efficacy and safety of deucravacitinib in psoriasis was assessed by comparison to placebo or  
346 apremilast as active comparators (POETYK PSO-1 and POETYK PSO-2) [86, 87]. The co-primary  
347 endpoints,  $\geq 75\%$  reduction from baseline in PASI75 and a Physician's Global Assessment (PGA) score  
348 of 0 or 1 at week 16, were met in the two phase III clinical trials. Specifically, a higher percentage of

349 patients treated with deucravacitinib (6mg) met the primary endpoints compared with those  
350 treated with placebo or apremilast, confirming the superiority of the TYK2 inhibitor [78, 79]. These  
351 results led to the approval of deucravacitinib by the EMA and FDA in 2023 for adult patients with  
352 moderate to severe plaque psoriasis who are eligible for systemic therapy or phototherapy.

### 353 [H1] Adverse effects and events

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355 Owing to the wide range of signalling pathways downstream of cytokines and growth factors that  
356 are affected by JAK inhibitors, some adverse effects are predictable, although the underlying  
357 mechanism of some adverse events remain unknown . JAKs work in pairs and knowledge of the  
358 hierarchy of one enzyme over the other is still incomplete. Therefore, understanding the exact  
359 contribution of inhibition of each kinase in the pair in specific cells, tissues or cell state is not an easy  
360 task. Certain adverse effects are common to JAK inhibitors, regardless of the drug selectivity profile,  
361 and other factors such as ethnicity and underlying disease could explain different incidence rates.  
362 Notably, JAK inhibitors are quickly absorbed and reach peak plasma concentrations within 1 to 2  
363 hours [88]. Conversely, these drugs are also rapidly cleared, predominantly by the liver and kidneys.  
364 This rapid clearance often requires twice daily administration or extended-release formulations.  
365 This regime could be seen as a problem leading to compliance issues but also as a safety net in case  
366 of severe adverse events. Deucravacitinib has a longer half-life ranging between 10 and 16 hours  
367 and can be administered once daily [89] Interestingly, covalent binding of JAK inhibitors to the kinase  
368 domain might not result in a prolonged pharmacokinetic profile, as observed in the case of  
369 ritlecitinib targeting of JAK3, as this kinase is rapidly resynthesized.

### 370 [H2] Infections

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372 While severe and opportunistic infections occur in patients treated with JAK inhibitors; as well as  
373 those exposed to other immunosuppressive therapies, a specific adverse effect of JAK inhibitors is  
374 reactivation of varicella zoster virus infection. This effect is not surprising, given the role of  
375 interferons in the antiviral response, and is common to all available JAK inhibitors. Sparing JAK2 and  
376 JAK3, however, could result in a better safety profile. In fact, the JAK1 preferential drug, filgotinib,  
377 has a two-fold lower incidence of herpes zoster reactivation [90]. In a randomized controlled trial in  
378 patients with inflammatory diseases including RA, PsA, psoriasis, spondylarthritis and IBD , network  
379 metaanalysis found only baricitinib (4mg daily), tofacitinib (10mg twice a day) and upadacitinib (30mg

380 daily) were associated with a higher incidence of herpes zoster compared with placebo, with odd  
381 ratios (95% CI) of 3.46 (1.38-8.67), 1.96 (1.01-3.78), and 3.25 (1.50-7.02), respectively. Conversely,  
382 no difference emerged with other compounds (filgotinib, peficitinib and decernotinib), other  
383 dosages compared with placebo and between different JAK inhibitors [91]. Overall, these data  
384 should be interpreted with caution in the absence of direct comparison between different  
385 molecules. Zoster reactivation is also less common with deucravacitinib compared to the other JAK  
386 inhibitors [92].

387

## 388 [H2] Haematological changes

389

390 Common  $\gamma$ -chain cytokines signal via JAK1, JAK2 and JAK3. These cytokines have a role in the  
391 development, proliferation, and function of adaptive immune cells including B and T lymphocytes,  
392 and NK cells, and hence a transient and reversible reduction in lymphocyte number is common  
393 following JAK inhibitor therapy [42]. Some cytokines have a more selective JAK signalling profile. For  
394 example, granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating  
395 factor signal through JAK2 heterodimers-coupled receptors. These cytokines are critical for  
396 myelopoiesis, and a reduction in neutrophil count can occur following JAK inhibition, depending on  
397 the JAK inhibitor selectivity profile [51, 93]. JAK2 mediates signalling downstream of the  
398 erythropoietin receptor, and reduction of haemoglobin levels can occur following treatment with  
399 first-generation JAK inhibitors and to a lesser extent with upadacitinib, as well as with the JAK2  
400 inhibitor, fedratinib. However, the decline in haemoglobin levels is generally not clinically relevant,  
401 with less than 1% of patients with RA showing meaningful changes with tofacitinib [94, 95]. As  
402 expected, JAK inhibitors that spare the JAK2 signalling pathway (filgotinib, abrocitinib and  
403 ritlecitinib) are not associated with a decrease in haemoglobin [60, 61, 63, 65, 66, 96]. In the SELECT  
404 EARLY study, a higher incidence of anaemia occurs with 30mg upadacitinib compared with the lower  
405 dose of 15mg (4.1 percent vs. 2.6 percent). By contrast, in the FINCH programme, filgotinib  
406 treatment led to increased levels of haemoglobin compared with placebo treatment. These findings  
407 suggest that changes in haemoglobin levels might serve as a surrogate marker of JAK 2 inhibition  
408 [60, 61, 63]. Treatment with most JAK inhibitors leads to a modest, and transient reduction in  
409 platelet count, regardless of JAK selectivity [97].

410

## 411 [H2] Change in lipid profile

412 The lipid profile can be affected by the inflammatory state and by drugs targeting inflammatory  
413 pathways; this is particularly relevant in pathological conditions known to be associated with excess  
414 cardiovascular risk [98]. Changes in total, low density lipoprotein and high-density lipoprotein  
415 cholesterol commonly occur in patients treated with JAK inhibitors targeting JAK1, but not with  
416 ritlecitinib, fedratinib and abrocitinib [65, 74, 99]. Indeed, cytokines that signal via JAKs are involved  
417 in cholesterol metabolism . For instance, type I interferons reduce the biosynthesis of cholesterol  
418 which, in turn, affects interferon-mediated responses to viral infection and promotes the  
419 accumulation of cholesterol in lipid droplets [100]. Similarly, IL-6 affects cholesterol levels by  
420 decreasing the hepatic synthesis of lipids, stimulating lipid uptake, and increasing lipolysis [101].  
421 Therefore, an increase in cholesterol levels when targeting IL-6 and interferons, which both signal  
422 via JAK1, is not surprising.

## 423 [H2] Cardiovascular and thromboembolic risk

424

425 Neoplastic, cardiovascular and thromboembolic risks are associated with all commercially available  
426 JAK inhibitors (with the exception of deucravacitinib). These adverse events are unlikely to be  
427 explained by the inhibition of a single JAK. A safety signal emerged from the phase IIIb and IV ORAL  
428 Surveillance study. The study showed non-inferiority of tofacitinib 5mg twice a day and 10mg twice  
429 a day compared with two TNF inhibitors (adalimumab and etanercept) in the incidence of major  
430 cardiovascular events (MACEs) and malignancies, with tofacitinib overtaking the upper limit of the  
431 confidence interval [102]. Specifically, the incidence rate of MACEs with tofacitinib 5mg twice a day  
432 and TNF inhibitors in the ORAL Surveillance was 0.91 (0.67–1.21) and 0.73 (0.52–1.01) per 100  
433 patient-years, respectively [108]. In The ENTRACTE study comparing anti-IL-6 receptor with TNF  
434 inhibitors in a similar population of patients with RA, the incidence rate of MACEs with tocilizumab  
435 and TNF inhibitors was 1.82 (1.46–2.24) and 1.7 (1.35-2.10) per 100 patient-years , respectively  
436 [103]. The long-term observation of two cohorts of patients with RA treated mainly with csDMARDs  
437 (~60% with methotrexate and only 10% with bDMARDs), showed an incidence rate of 2.8-3.1 per  
438 100 patient-years of MACEs [104, 105] . Data from the German RABBIT register suggest that the  
439 incidence rate of MACEs with JAK inhibitors is similar to that of bDMARDs and lower than that of  
440 csDMARDs. Among 14,203 treatment courses, 145 MACEs were recorded, accounting for an overall  
441 incidence rate of 0.73 per 100 patient-years without any significant difference between JAK  
442 inhibitors and other RA treatments: incidence rate of 0.68 (0.47-0.95) for JAK inhibitors, 0.62 (95%

443 CI 0.45-0.83) for TNF inhibitors, 0.76 (95% CI 0.53-1.06) for bDMARDs with other mode of action and  
444 incidence rate of 0.95 (95% CI 0.68-1.29) for csDMARDs [106]. There are higher incidence rates in  
445 male patients, individuals with cardiovascular risk (regardless of treatment), those older than 65  
446 years and in those with previous cardiovascular events [106]. A Korean national database analysis  
447 including 111,334 patients followed-up for a mean time of 5.23 year showed an incidence rate ratio  
448 for cardiovascular disease of 0.91 (95% CI 0.90–0.92) in patients treated with JAK inhibitors or  
449 bDMARDs compared with patients treated only with csDMARDs (1.17 vs 1.3 per 100 patient years).  
450 These data suggest that optimal disease control could better control cardiovascular risk [107]. Real  
451 world data from large national registries or administrative data did not confirm increased risk of  
452 MACEs in patients with RA treated with JAK inhibitors (mainly tofacitinib and baricitinib) matched  
453 with patients treated with TNF inhibitors or bDMARDs, even in patients with cardiovascular risk  
454 factors such as those enrolled in the ORAL Surveillance study [106, 108-116].

455  
456 Conversely, data on thromboembolic risk remain controversial. Most real-world evidence suggests  
457 no excess of venous thromboembolism (VTE) in patients treated for up to five years. However, some  
458 data indicate a potential increase in thromboembolic events, particularly primary pulmonary  
459 embolism [116-123]. In the Swedish registry, the incidence of VTE was higher in patients treated  
460 with baricitinib or tofacitinib than in those treated with TNF inhibitors, and pulmonary embolism,  
461 rather than deep vein thrombosis, accounted for the increased risk [123]. Long-term extension  
462 studies with tofacitinib, baricitinib, upadacitinib and filgotinib in RA as well as the post-hoc analysis  
463 of tofacitinib and upadacitinib in RA, PsA, AS and atopic dermatitis patients did not show any  
464 increased risk of MACEs or VTE [124-128]. Interestingly, the more selective second-generation JAK  
465 inhibitors do not seem to be associated with any increased incidence of MACEs, VTE or malignancies  
466 [128, 129]. Notably, MACEs and VTE were reported almost exclusively in patients with  
467 rheumatologic conditions, suggesting a role for the underlying disease [130]. Moreover, patients  
468 with RA who were at greatest risk were those carrying cardiovascular risk factors or with previous  
469 atherosclerotic cardiovascular disease along with smoking and increased age [131-133]. Indeed, RA  
470 and atherosclerosis share many common inflammatory pathways that contribute to the progression  
471 of both atherosclerotic plaque and synovial hyperplasia, affect the arterial wall in both the disease  
472 and modulating traditional risk factors such as dyslipidaemia and metabolic syndrome [98]. Whether  
473 JAK inhibitor administration is actually associated with an increased risk of cardiovascular events  
474 has yet to be defined, and a mechanistic explanation of this possible association is also lacking.

475 Interestingly, in a phase II, placebo-controlled trial with baricitinib in patients with recent onset type  
476 1 diabetes — a disease known to be associated with increased cardiovascular risk — the insulin-  
477 secreting function of  $\beta$ -cells was preserved. Moreover, baricitinib improved the glycaemic control  
478 as assessed by continuous glucose monitoring and allowed patients to use lower doses of insulin  
479 [134].

480

481 Notably in patients with ulcerative colitis, known to be at risk of thrombotic events, tofacitinib did  
482 not increase the risk of MACEs or VTE compared with TNF inhibitors [135]. Concerns of excess  
483 thromboembolic events first arose in the RA clinical trial program, where six cases of excess  
484 thromboembolic events were reported in patients treated with baricitinib and none in those treated  
485 with placebo [136]. Moreover, analysing 14 real-world datasets including 9,013 baricitinib-treated  
486 patients propensity score-matched with 7,606 TNF inhibitor-treated patients found that risk of VTE  
487 increased with baricitinib versus TNF inhibitors [122]. Notably, in patients with myeloproliferative  
488 disorders carrying a high risk of thromboembolic events treatment with ruxolitinib, another JAK1  
489 and JAK2 inhibitor, reduced the risk of thromboembolic events, supporting the contribution of the  
490 underlying disease to the occurrence of certain adverse events [137]. Safety data regarding events  
491 of special interest (including cardiovascular, thromboembolic and neoplastic risk) should be put into  
492 the correct context and taken into consideration for patients who are candidates or already being  
493 treated with JAK inhibitors. Particularly, the warnings and prescribing restrictions issued by the EMA  
494 following its review of ORAL surveillance data and registrational trials of all JAK inhibitors should be  
495 considered.

496

## 497 [H2] Neoplastic risk

498

499 From a mechanistic point of view, it remains unknown whether selectivity of JAK inhibition could  
500 be associated with lower neoplastic risk. A first signal of a possible carcinogenetic effect came with  
501 the report of an increased risk of non-melanoma skin cancer (NMSC) in patients with myelofibrosis  
502 and polycythemia vera treated with ruxolitinib. In a cohort of 564 patients (188 treated with  
503 ruxolitinib) the hazard ratio for NMSC was 2.69 (95% CI, 1.03-7.02) with a higher risk of squamous  
504 cell carcinoma, hazard ratio of 3.24 (95% CI, 1.45-7.22) [138]. A large dataset of patients developed  
505 NMSC following treatment with ruxolitinib suggesting an aggressive phenotype of this cancer that  
506 was recurrent, metastatic and associated with a high mortality rate [139]. For ruxolitinib, a warning

507 of a potential increased risk of NMSC is given. An analysis of the World Health Organization  
508 pharmacovigilance database published in 2022 showed a disproportionality signal for melanoma  
509 and NMSC with tofacitinib, ruxolitinib, and to a lesser extent, baricitinib, suggesting an additional  
510 risk of skin cancer with the use of JAK inhibitors. However, the pharmacovigilance analysis did not  
511 report if patients had previously been treated with other immunosuppressive drugs, which can also  
512 promote cancer development, which is an important consideration.[140]. A metanalysis of 18  
513 randomised clinical trials with biologic and targeted synthetic DMARDs in psoriasis and PsA showed  
514 a higher incidence rate of NMSC, but not melanoma, in patients treated with JAK inhibitors [141].  
515 Increased susceptibility to skin cancers in patients possibly treated with phototherapy for psoriasis,  
516 however, cannot be neglected[141].

517  
518 A safety signal concerning the risk of incident malignancies emerged with the ORAL Surveillance  
519 study [102]. In the cardiovascular risk factor-enriched population enrolled in the ORAL Surveillance,  
520 the incidence of cancer and NMSC, was almost twice as high among patients treated with tofacitinib  
521 compared with those treated with a TNF inhibitor, with an incidence of 4.2 and 2.9%, respectively,  
522 and a hazard ratio of 1.48 (95% CI, 1.04 to 2.09) [102, 124-126, 128]. The most reported cancer  
523 being lung cancer, which is known to have a higher incidence in patients with RA, particularly in  
524 patients older than 65 years, smokers and those with a history of lung disease; interestingly,  
525 geographical location in North America was also associated with an increased neoplastic risk [142].  
526 The post-hoc analysis of the study showed a stronger interaction between treatment duration and  
527 the emergence of cancer, with malignancy-free survival starting to diverge after 18 months of  
528 treatment. Notably, real-world evidence does not confirm the excess of neoplastic risk with JAK  
529 inhibitors compared with TNF inhibitors or other biologic DMARDs [107, 108, 113, 114, 116, 143,  
530 144].

531

## 532 [H1] Characterizing selectivity

533

534 Current selectivity classification of JAK inhibitors is largely based on in vitro assays using  
535 recombinant kinases. Other methods of measuring JAK inhibitor selectivity are available (Box 1) .  
536 Notably, the half maximal inhibitory concentration (IC50) obtained with these assays might vary  
537 substantially. Assessing the selectivity with in vitro biochemical assays is conceptually  
538 straightforward when similar assays are used to measure the inhibition of the different JAK isoform

539 domains (recombinant kinase, kinase or pseudokinase) with any given drug . The biochemical  
540 measurements combined with structural information are critically important in JAK inhibitor  
541 development, especially for increased selectivity and potency [145]. However, activity-based  
542 biochemical assays are not suitable for detecting drugs that do not directly alter intramolecular  
543 enzymatic activity. For example, the allosteric inhibitor, deucravacitinib, binds tightly to the ATP  
544 binding pocket in the pseudokinase domains of TYK2 and JAK1, but shows very weak binding to JAKs  
545 kinase domains [51, 81]. Experimental conditions and recombinant proteins have a critical role in  
546 these assays. For example, the concentration of ATP, activation state, domain content and  
547 posttranslational modifications are important factors that can influence the readouts. Substantial  
548 inter-assay variability thereby could occur in the measured IC50s and selectivity measures of JAK  
549 inhibitors are best compared when obtained in a single study under the same, or comparable,  
550 conditions. Biochemical in vitro analyses, however, do not necessarily directly translate into cellular  
551 effects [146, 147].

552

553 Assessment of JAK inhibitors in cellular assays, such as measuring the inhibition of STAT  
554 phosphorylation in response to a cytokine stimulation is more complex. JAK signalling downstream  
555 of type 1 and type 2 cytokine receptors requires dimerization of the JAKs, with five known  
556 combinations (JAK1 and JAK2, JAK1 and JAK3, JAK1 and TYK2, JAK2 and TYK2 and JAK2 and JAK2) .  
557 The specific role or requirement of activity for a given JAK protein, to phosphorylate tyrosine  
558 residues on the intracellular domain of the cytokine receptors as well as substrates recruited to the  
559 phosphorylated receptors, could vary depending on the cytokine receptor being considered. Indeed,  
560 the cytokine receptors couple to seven STATs and their differential activation in cytokine receptors  
561 (and by individual JAKs) and distinct biological functions further complicate the assessment. The  
562 reason for the differences in JAK inhibition selectivity is not fully understood but the FERM and SH2  
563 domains of JAK isoforms are important modules regulating the interaction with the intracellular  
564 domains of cytokine receptors that are diverse in their primary sequences [148]. The level of direct  
565 involvement that the FERM and SH2 domains have in regulating the kinase domain of JAKs upon  
566 cytokine binding to its cognate receptor is unclear [148]. Additionally, the precise usage of a given  
567 JAK with respect to different cytokine receptors might differ among different cells or activation  
568 states. Cytokine inhibition studies have demonstrated that the approved and late-stage clinical JAK  
569 inhibitors in inflammatory diseases predominantly inhibit JAK1-mediated cellular signalling, except  
570 for ritlecitinib (specific for JAK3-dependent cytokines) and deucravacitinib (specific for TYK2-

571 mediated signalling) [48, 50, 51, 82]. When considering the selectivity of JAK inhibitors and their  
572 effect on cytokine signalling in vivo, the complexity increases further.

573

574 Importantly, although JAK inhibition results in many downstream effects, measurement of the  
575 putative relevant STAT phosphorylation is commonly used as a readout for JAK inhibition. However,  
576 which STAT to measure to obtain the most reliable readout is not always obvious, as STAT usage  
577 could also differ amongst cells and activation states. Moreover, STATs are not the only downstream  
578 mediators of signalling. Another complication is that cellular responses are the combined result of  
579 various cytokines at different levels over time and therefore assessing the contribution of inhibiting  
580 individual cytokine pathways to the therapeutic responses of JAK inhibitors is difficult.

581

582 In vivo responses of target engagement can be measured in animals. Most importantly though, is  
583 assessment of consequences in patients – do putative selective JAK inhibitors target the desired  
584 JAKs and avoid others as evidenced by biological readouts? This unanswered question is perhaps  
585 the greatest challenge. With limitations, some studies have indeed shown that, when coupled with  
586 their in vivo pharmacokinetic profiles, JAK inhibitors modulate cytokine pathways in a variable  
587 fashion [22, 50]. Importantly, in clinical practice, JAK inhibitor elimination, metabolism and potential  
588 drug-drug interactions can influence the administered doses (for those drugs with multiple dosages  
589 approved) especially for patients with renal and hepatic impairment. In conclusion, one should bear  
590 in mind that, as no established criteria for selectivity exist, each company defines the selectivity  
591 'label' of their compounds based on their best understanding of the available data.

592

593 [H1] Organ selectivity: a safer approach?

594

595 The attempt to minimize adverse effects and increase the efficacy of JAK inhibition has also led to  
596 the development of JAK inhibitors with different formulations, capable of selectively targeting  
597 inflamed tissues. Achieving organ selectivity with oral administration remains an unsurmountable  
598 obstacle, with possibly the only exception being JAK inhibitors that target inflammation in the  
599 gastrointestinal tract. Perhaps the most obvious strategy is the topical use of JAK inhibitors for  
600 various dermatological conditions but inhaled JAK inhibitors are also under consideration.  
601 Furthermore, transdermal or injectable delivery of JAK inhibitors are being considered.

## 602 [H2] Topical JAK inhibitors

603

604 Ruxolitinib (1.5% cream) is the first topical JAK inhibitor approved for short-term and non-  
605 continuous chronic treatment of mild-to-moderate atopic dermatitis. Many of the cytokines  
606 involved in the pathogenesis of atopic dermatitis signal through the JAK and STAT pathways . Both  
607 in acute and chronic lesions, type 2 cytokines including IL-4, IL-13, IL- 31 but also IFN- $\gamma$ , IL-12 and IL-  
608 23, orchestrate the local infiltration of innate and adaptive immune cells [149]. Keratinocyte-derived  
609 alarmins such as IL-25, IL-33 and thymic stromal lymphopoietin also signal through JAK and STAT ,  
610 as well as IL-22, inducing the expression of proinflammatory genes in keratinocytes and resulting in  
611 proliferation and epidermal acanthosis [149]. In two phase III clinical trials, a higher percentage of  
612 patients achieved the primary endpoint of an IGA score of 0 or 1 (with more than 2% improvement  
613 from baseline) following treatment with 0.75% or 1.5% ruxolitinib cream compared with vehicle  
614 treatment after 8 weeks. Notably, ruxolitinib treatment led to a marked control of itch reported  
615 within 12 hours [150]. Delgocitinib is another JAK inhibitor approved for atopic dermatitis, albeit  
616 only in Japan. In the phase III study, 0.5% ointment twice-daily was effective and well tolerated in  
617 adult patients with moderate-severe atopic dermatitis [151]. The improvement of modified EASI  
618 (mEASI) was greater with the drug than with the vehicle treatment, was detectable already after 4  
619 weeks and persisted until 28 weeks of follow-up [152]. The same formulation of ruxolitinib indicated  
620 for atopic dermatitis has been approved by the FDA and EMA for the treatment of nonsegmental  
621 vitiligo in adult and adolescent patients. The rationale for topical use of JAK inhibitors in vitiligo came  
622 from the evidence that IFN- $\gamma$  is the main cytokine responsible for melanocyte damage and is  
623 involved in the skin homing of autoreactive T cells; other JAK and STAT-coupled cytokines are locally  
624 or systemically involved in the pathogenesis of vitiligo, including IL-6, IL-15, IL-22, and IL-23 [153].  
625 Four phase II and three phase III clinical trials assessed the efficacy and safety profile of topical  
626 ruxolitinib. These studies showed clinically relevant skin re-pigmentation through 52 weeks  
627 compared with the vehicle control, particularly for facial lesions, perhaps due to differences in hair  
628 follicle distribution [154].

629

630 Preliminary data from small studies and case reports suggest that treatment with 2% tofacitinib  
631 ointment, or 1% ruxolitinib shows promise in the treatment of alopecia areata in both adult and  
632 paediatric patients [155]. The bioavailability of ruxolitinib and tofacitinib after topical application is  
633 relatively low and oral administration associated adverse effects are not expected. [156, 157]. A few

634 local adverse effects including pruritus and acne have been reported, mostly with ruxolitinib [154].  
635 Finally, a phase II clinical trial involving patients with psoriasis receiving topical brepocitinib one or  
636 two times a day at different concentrations did not demonstrate a greater PASI response at week  
637 12 compared with vehicle [158]. As generic versions of JAK inhibitors become available, their use in  
638 many conditions is likely to expand and, in principle, could be used in place of steroids. Conditions  
639 that are potential treatable with topical JAK inhibitors include alopecia areata, vitiligo and psoriasis,  
640 pathologies such as chronic hand eczema, Lichen planus and the allergic reaction to poison ivy  
641 exposure. Of particular importance with respect to dermatological conditions, is the impact of JAK  
642 inhibitors on itch. Soluble factors that promote itch include JAK-STAT dependent cytokines such as  
643 IL-4, IL-13, and IL-31, which are known to drive itch independently of histamine pathways [159] .  
644 Notably, neurons express cytokine receptors that have been successfully targeted with biologics.  
645 Similarly, JAK inhibitors, have potent anti-itch effects and both ruxolitinib and abrocitinib are  
646 approved for the treatment of atopic dermatitis and are in the late stage of clinical assessment for  
647 prurigo nodularis and in chronic pruritus of unknown origin [160].

648

## 649 [H2] Transdermal delivery

650

651 Another possible application under investigation is the transdermal delivery of JAK inhibitors.  
652 Tofacitinib was the first JAK inhibitor tested for transdermal delivery in a rat model of Freund's  
653 Complete Adjuvant-induced arthritis; administering the drug through transdermal patch or  
654 microneedle array resulted in 24% and 95% drug release, and 12% and 85% drug permeation,  
655 respectively, in 4 hours. There was a notable decrease in synovial hyperplasia, cartilage and bone  
656 erosion as well as circulating cytokine levels [161]. As injectable steroids are often used  
657 therapeutically for pain in joints, bursae, and tendon, using injectable JAK inhibitors might also be  
658 effective. Findings from clinical trials have shown that patients with RA treated with JAK inhibitors  
659 achieved greater improvements in pain compared with TNF inhibitors [162] . Multiple cytokines  
660 regulated by the JAK and STAT pathway seem to have roles in mechanisms of pain [163] ;  
661 understanding these mechanisms is clearly an important area of research .

## 662 [H2] Inhaled JAK inhibitors

663

664 Another area of interest is the development of tissue-specific JAK inhibitors for respiratory diseases.  
665 Most of the cytokines responsible for the pathogenesis of atopic dermatitis are also involved in the  
666 pathogenesis of type 2 (IL-4, IL-5, IL-9, IL-13 and TSLP) and non-type 2 asthma (IL-6, IL-9, IL-13, GM-  
667 CSF, type I and II interferons) [164]. Some of these cytokines have already been targeted with  
668 monoclonal antibodies, therefore, providing a strong rationale for the use of JAK inhibitors in the  
669 treatment of asthma, particularly for inhaled drugs with chemical structures that facilitate lung  
670 retention and limited systemic exposure. The inhaled JAK inhibitors under development, their  
671 discovery and characterization, are reviewed elsewhere [165]. Some compounds have been already  
672 tested in preclinical, animal models. The inhalable compound iJak-381, is designed to inhibit JAK1 in  
673 the lung and was tested in animal models of asthma [166]. The compound inhibited the IL-13, JAK1  
674 and STAT6 signalling axis and normalized lung influx of both eosinophils and neutrophils in mice  
675 exposed to ovalbumin or allergens (*Aspergillus*, *Alternaria* and *Dermatophagoides farinae*).  
676 Moreover, in guinea pigs immunized with ovalbumin, iJak-381 reversed lung inflammation in a dose-  
677 dependent manner [166]. LAS194046, a JAK1 and JAK3 compound tested in an ovalbumin-induced  
678 airway inflammation model, inhibited eosinophil and neutrophil infiltration and improved lung  
679 function in mice treated one hour before allergen challenge [167]. A preliminary study with  
680 tofacitinib aerosol showed that the nebulization of the drug to house dust mite-challenged mice  
681 reduced the eosinophil count and protein concentration in the bronchoalveolar lavage fluid [168].  
682 Intranasal administration of tofacitinib was also effective in a mouse model of chronic rhinosinusitis  
683 with nasal polyposis, inducing pronounced reduction in both eosinophilic infiltrate and polyp-like  
684 lesions [169]. Another compound, the JAK3 and JAK1 inhibitor R507, was tested in a model of  
685 tracheal allografts to prevent chronic obliterative airway disease [170]. Airway obliterative disease  
686 is a risk factor for mortality in lung transplant recipients and has pathological features common to  
687 human obliterative bronchiolitis [171]. Both oral and aerosol R507 administration abolished the  
688 airway lumen obliteration induced by inflammatory cell infiltration and fibroproliferation and  
689 resulted in repair of the damaged epithelial surface; after the aerosol administration, plasma levels  
690 of R507 were 10-fold lower compared with systemic treatment [170]. Data examining the use of  
691 inhaled JAK inhibitors in humans are still scarce. Preliminary data from a phase II randomized,  
692 placebo-controlled clinical trial in 25 patients with severe COVID-19 requiring oxygen  
693 supplementation demonstrated that one-week administration (once-a-day inhalation) of pan-JAK  
694 inhibitor nezulcitinib improved oxygen saturation and reduced mortality (5%, versus 33% in the  
695 placebo-treated group) [172]. A larger phase II study, however, carried out in 205 patients with

696 severe COVID-19 failed with a similar percentage of patient treated with nezulcitinib or placebo  
697 meeting the primary and secondary endpoints (including number of respiratory failure-free days  
698 and mortality at day 28) [173].

699

700 Two phase I randomized clinical trials assessed the effect of two different JAK inhibitors in patients  
701 with asthma. The first one demonstrated that 10-days treatment with four ascending doses of the  
702 preferential JAK1 inhibitor GDC-0214 reduced a biomarker of airway inflammatory infiltrate  
703 including fractional exhaled nitric oxide, in a dose-dependent manner, with no effect on circulating  
704 eosinophils, in patients with mild asthma [174]. In the second study, also involving patients with  
705 mild asthma (n=48), inhaled GDC-4379 decreased clinical fractional exhaled nitric oxide and  
706 inflammatory biomarkers, CCL17 and eosinophils, this effect correlated with the plasma  
707 concentrations of the drug [175].

708 [H1] Other modalities of JAK inhibition?

709

710 Besides interfering directly with the enzymatic activity of JAKs, alternative strategies to interfere  
711 with the pathway are also currently been considered to achieve selectivity. These alternatives  
712 include the development of chemically modified small interfering RNA (siRNA) to silence JAK  
713 expression as well as assessing the efficacy of metabolites (or metabolites derivatives) with cysteine  
714 modifier properties and anti-inflammatory effects.

715

716 [H2] siRNAs as novel JAK inhibitors

717

718 Selectivity of most second-generation JAK inhibitors is not absolute. An alternative way to obtain  
719 specificity for a given JAK family member is modulation of mRNA level via RNA interference-based  
720 approaches, where double-stranded ribonucleic acids recognize and pair with specific mRNA  
721 sequences, leading to silencing of the corresponding protein product. Currently, siRNA therapeutics  
722 exist in the clinic, both for rare and common diseases, but none target JAK-STAT signalling.  
723 Preclinical investigations demonstrate effective silencing of expression of given JAK family members  
724 and suppression of JAK-mediated signalling by JAK1-specific, JAK3-specific, and JAK2-targeted  
725 synthetic siRNAs in vitiligo, IBD and breast cancer models [176-178]. Silencing JAK1 expression, for  
726 example, effectively inhibited JAK1-mediated cytokine signalling ex vivo in human skin biopsies, in

727 vivo in a mouse vitiligo model and in vitro in primary immune cells [177, 178]. Although, evaluating  
728 the translation of high JAK1-selectivity observed at protein or mRNA level to inhibition of specific  
729 cytokine signalling is important. The effect of chemically modified siRNAs was long-lasting; for  
730 example in a mouse vitiligo model, a single subcutaneous injection of JAK1-selective lead siRNA  
731 si3033 induced a therapeutic effect for up to five weeks [177]. Although si3033 demonstrated local  
732 retention at the injection site, this molecule also accumulates in clearance organs, including the  
733 liver, spleen, and kidney. This accumulation in clearance organs has raised concerns on systemic  
734 effects, which need to be further investigated in appropriate animal or cell models. The clinical value  
735 of these early findings employing a novel angle for JAK-inhibition is difficult to predict. High  
736 specificity could result in a beneficial safety profile in comparison to existing JAK inhibitors, but  
737 safety and efficacy need to be carefully assessed in preclinical and clinical experiments.

## 738 [H2] Itaconate as a JAK inhibitor

739

740 Itaconate is a krebs cycle-derived metabolite that has anti-inflammatory effects [179] . It inhibits  
741 the production of proinflammatory molecules, including cytokines, by macrophages stimulated in  
742 vitro with lipopolysaccharide [179] . Itaconate can also suppress glycolysis as well as oxidative  
743 phosphorylation in T cells and affect the balance between T helper 17 and T regulatory cells [180].  
744 As itaconate is a highly polar molecule, the synthesis and use of derivatives such as dimethyl  
745 itaconate or 4-octyl itaconate have shown efficacy in animal models of sepsis and psoriasis [180,  
746 181]. Mechanistically, itaconate inhibits succinate dehydrogenase [182]. Itaconate and its  
747 derivatives can also suppress pro-inflammatory macrophages via inhibition of JAK1 [181] .  
748 Interestingly, itaconate seems to interact with JAKs in an allosteric fashion very similar to VVD-  
749 118313 by covalently interacting with cysteine 816 in the pseudokinase domain of mouse JAK1  
750 (residue C817 in human JAK1) and inhibiting its kinase activity [181]. Interestingly, another  
751 derivative, 4-methyl itaconate (also termed SCD-153), possess greater skin and cell penetration  
752 compared to itaconate analogues 4-methyl itaconate and dimethyl itaconate. A study has assessed  
753 the use of this derivative in vitro and in vivo for the treatment of alopecia areata [183]. Whether  
754 itaconate derivatives influence macrophage polarization or T cell differentiation remains unclear ,  
755 but studies suggest such compounds hold promise in the treatment of inflammatory diseases.

## 756 [H1] Conclusions

757

758 An important unmet need in rheumatology is the development of therapies for patients who are  
759 refractory or unresponsive to biologic drugs [184]. JAK inhibitors have now been in the clinic for  
760 over 10 years and have provided an important therapeutic option for patients with autoimmune  
761 and inflammatory diseases. With clear evidence of JAK inhibitor efficacy, often superior to biologics,  
762 the focus now is on obtaining higher selectivity for disease-relevant JAKs. Current clinical  
763 information on efficacy and safety are mainly derived from pan-JAK (first-generation) and second-  
764 generation JAK1-selective JAK inhibitors. Although many of the adverse effects are also common  
765 with biologics, JAK inhibitors carry a black box warning for patients over 65 years of age, who smoke  
766 or have cardiovascular, thromboembolic or cancer risks. The adverse effects of JAK inhibitors have  
767 raised considerable attention, understanding the actual risk level of adverse events when using JAK  
768 inhibitors is an important consideration for clinicians . However, these concerns do not seem to  
769 apply to all the pathologies for which JAK inhibitors have been approved.

770 Clinical response to JAK inhibitors is a sum effect of several parameters. In addition to disease and  
771 patient-derived factors, drug dosing and characteristics such as binding affinities and enzymatic  
772 inhibition of the kinases as well as drug pharmacokinetics, affect cellular signalling . Furthermore,  
773 cytokine receptor levels might vary depending on cell-type and this can directly influence the the  
774 role of individual JAKs . In vitro assessment of selectivity shows differences between first-generation  
775 and second-generation JAK inhibitors in both binding affinity and kinase inhibition. However, these  
776 differences do not seem to directly translate to biological effects or clinical efficacy .

777  
778 First-generation and second-generation JAK inhibitors are traditional ATP competitive compounds,  
779 where the conserved structure of the ATP binding pocket poses challenges in obtaining high  
780 selectivity. Alternative approaches in JAK inhibitor development besides the type I ATP-competitive  
781 inhibitors, such as type II and allosteric inhibitors, could provide improved precision, and safety, in  
782 the treatment of autoimmune and inflammatory diseases. Overall, JAK1-selective agents seem to  
783 have some advantages in limiting some of the adverse effects observed with the first-generation  
784 JAK inhibitors. Deucravacitinib, via its ability to target the pseudokinase domain, has a greater  
785 degree of selectivity inhibiting only few cytokines including IL-12, IL-23 and type I interferons.  
786 Importantly, deucravacitinib does not carry the black box warning associated with other JAK  
787 inhibitors. Ritlecitinib is a covalent JAK3 kinase domain inhibitor that has shown high selectivity for  
788 JAK3 compared with other agents. However, although highly specific inhibitors can achieve more  
789 predictable responses and have less undesired adverse effects, a wider inhibitory range might result

790 in better clinical efficacy as several cytokines are inhibited simultaneously, which could be  
791 particularly relevant in inflammatory diseases.

792

793 Selectivity is not the only way to achieve effective JAK inhibition in inflammatory diseases. A  
794 successful approach at achieving clinical response, whilst diminishing long-term adverse events, was  
795 in the treatment of ulcerative colitis with tofacitinib and upadacitinib, where an induction phase  
796 with a higher dose was followed by a maintenance therapy with a lower dose. Exploring if this  
797 regimen could be employed in other pathologies would be interesting. Nonetheless, JAK specificity  
798 clearly can provide crucial advantages in the clinic as exemplified by the diminished incidence of  
799 zoster reactivation with filgotinib, better safety profile of deucravacitinib and efficacy of ritlecitinib  
800 in alopecia areata. One can envision that availability of specific inhibitors for all JAKs in future could  
801 allow precise tailoring of therapy, and possibly combination therapies as tested in cancer  
802 indications, based on the specific disease or a phase of the disease and its associated cytokine  
803 profile. To achieve this goal, structural and mechanistic information of JAK regulation downstream  
804 of cytokine receptor activation is still needed and could help in identifying additional drug targets .  
805 The final word on the relevance and impact of selective JAK inhibition must wait for more real-world  
806 data from current and JAK inhibitors currently under development.

## 807 References

- 808 1. Russell, S.M., et al., *Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid*  
809 *development*. Science, 1995. 270(5237): p. 797-800.
- 810 2. Firmbach-Kraft, I., et al., *tyk2, prototype of a novel class of non-receptor tyrosine kinase genes*.  
811 *Oncogene*, 1990. 5(9): p. 1329-36.
- 812 3. Wilks, A.F., et al., *Two novel protein-tyrosine kinases, each with a second phosphotransferase-related*  
813 *catalytic domain, define a new class of protein kinase*. Mol Cell Biol, 1991. 11(4): p. 2057-65.
- 814 4. Silvennoinen, O., et al., *Structure of the murine Jak2 protein-tyrosine kinase and its role in interleukin*  
815 *3 signal transduction*. Proc Natl Acad Sci U S A, 1993. 90(18): p. 8429-33.
- 816 5. Johnston, J.A., et al., *Phosphorylation and activation of the Jak-3 Janus kinase in response to*  
817 *interleukin-2*. Nature, 1994. 370(6485): p. 151-3.
- 818 6. Witthuhn, B.A., et al., *Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in*  
819 *lymphoid and myeloid cells*. Nature, 1994. 370(6485): p. 153-7.
- 820 7. Villarino, A.V., et al., *SnapShot: Jak-STAT Signaling II*. Cell, 2020. 181(7): p. 1696-1696 e1.
- 821 8. Macchi, P., et al., *Mutations of Jak-3 gene in patients with autosomal severe combined immune*  
822 *deficiency (SCID)*. Nature, 1995. 377(6544): p. 65-8.
- 823 9. Darnell, J.E., Jr., I.M. Kerr, and G.R. Stark, *Jak-STAT pathways and transcriptional activation in*  
824 *response to IFNs and other extracellular signaling proteins*. Science, 1994. 264(5164): p. 1415-21.
- 825 10. Yamaoka, K., et al., *The Janus kinases (Jaks)*. Genome Biol, 2004. 5(12): p. 253.
- 826 11. Raivola, J., T. Haikarainen, and O. Silvennoinen, *Characterization of JAK1 Pseudokinase Domain in*  
827 *Cytokine Signaling*. Cancers (Basel), 2019. 12(1).

- 828 12. Hammaren, H.M., et al., *Janus kinase 2 activation mechanisms revealed by analysis of suppressing*  
829 *mutations*. J Allergy Clin Immunol, 2019. 143(4): p. 1549-1559 e6.
- 830 13. Glassman, C.R., et al., *Structure of a Janus kinase cytokine receptor complex reveals the basis for*  
831 *dimeric activation*. Science, 2022. 376(6589): p. 163-169.
- 832 14. Lupardus, P.J., et al., *Structure of the pseudokinase-kinase domains from protein kinase TYK2 reveals*  
833 *a mechanism for Janus kinase (JAK) autoinhibition*. Proc Natl Acad Sci U S A, 2014. 111(22): p. 8025-  
834 30.
- 835 15. Shan, Y., et al., *Molecular basis for pseudokinase-dependent autoinhibition of JAK2 tyrosine kinase*.  
836 Nat Struct Mol Biol, 2014. 21(7): p. 579-84.
- 837 16. Caveney, N.A., et al., *Structural basis of Janus kinase trans-activation*. Cell Rep, 2023. 42(3): p.  
838 112201.
- 839 17. FDA. *Drug Approval Package*. 2011 [cited 2024 July 24, 2024]; Available from:  
840 [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2011/202192Orig1s000Approv.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202192Orig1s000Approv.pdf).
- 841 18. FDA. *Drug Approval Letter*. 2012; Available from:  
842 [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2012/203214s000lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203214s000lbl.pdf).
- 843 19. Castelo-Soccio, L., et al., *Protein kinases: drug targets for immunological disorders*. Nat Rev Immunol,  
844 2023. 23(12): p. 787-806.
- 845 20. Ghoreschi, K., et al., *Modulation of innate and adaptive immune responses by tofacitinib (CP-*  
846 *690,550)*. J Immunol, 2011. 186(7): p. 4234-43.
- 847 21. Telliez, J.B., et al., *Discovery of a JAK3-Selective Inhibitor: Functional Differentiation of JAK3-Selective*  
848 *Inhibition over pan-JAK or JAK1-Selective Inhibition*. ACS Chem Biol, 2016. 11(12): p. 3442-3451.
- 849 22. McInnes, I.B., et al., *Comparison of baricitinib, upadacitinib, and tofacitinib mediated regulation of*  
850 *cytokine signaling in human leukocyte subpopulations*. Arthritis Res Ther, 2019. 21(1): p. 183.
- 851 23. Coricello, A., et al., *Inside Perspective of the Synthetic and Computational Toolbox of JAK Inhibitors:*  
852 *Recent Updates*. Molecules, 2020. 25(15).
- 853 24. Clark, J.D., M.E. Flanagan, and J.B. Telliez, *Discovery and development of Janus kinase (JAK) inhibitors*  
854 *for inflammatory diseases*. J Med Chem, 2014. 57(12): p. 5023-38.
- 855 25. Stebbing, J., et al., *COVID-19: combining antiviral and anti-inflammatory treatments*. Lancet Infect  
856 Dis, 2020. 20(4): p. 400-402.
- 857 26. Faquetti, M.L., et al., *Identification of novel off targets of baricitinib and tofacitinib by machine*  
858 *learning with a focus on thrombosis and viral infection*. Sci Rep, 2022. 12(1): p. 7843.
- 859 27. Ostojic, A., R. Vrhovac, and S. Verstovsek, *Ruxolitinib: a new JAK1/2 inhibitor that offers promising*  
860 *options for treatment of myelofibrosis*. Future Oncol, 2011. 7(9): p. 1035-43.
- 861 28. Verstovsek, S., et al., *A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis*. N Engl J  
862 Med, 2012. 366(9): p. 799-807.
- 863 29. Harrison, C., et al., *JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis*. N  
864 Engl J Med, 2012. 366(9): p. 787-98.
- 865 30. Vannucchi, A.M., et al., *Ruxolitinib versus standard therapy for the treatment of polycythemia vera*.  
866 N Engl J Med, 2015. 372(5): p. 426-35.
- 867 31. Przepiorka, D., et al., *FDA Approval Summary: Ruxolitinib for Treatment of Steroid-Refractory Acute*  
868 *Graft-Versus-Host Disease*. Oncologist, 2020. 25(2): p. e328-e334.
- 869 32. Changelian, P.S., et al., *Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor*.  
870 Science, 2003. 302(5646): p. 875-8.
- 871 33. Fleischmann, R., et al., *Placebo-controlled trial of tofacitinib monotherapy in rheumatoid arthritis*. N  
872 Engl J Med, 2012. 367(6): p. 495-507.
- 873 34. Fleischmann, R., et al., *Efficacy and safety of tofacitinib monotherapy, tofacitinib with methotrexate,*  
874 *and adalimumab with methotrexate in patients with rheumatoid arthritis (ORAL Strategy): a phase*  
875 *3b/4, double-blind, head-to-head, randomised controlled trial*. Lancet, 2017. 390(10093): p. 457-468.
- 876 35. Burmester, G.R., et al., *Tofacitinib (CP-690,550) in combination with methotrexate in patients with*  
877 *active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: a*  
878 *randomised phase 3 trial*. Lancet, 2013. 381(9865): p. 451-60.

- 879 36. Sandborn, W.J., et al., *Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis*. N Engl  
880 J Med, 2017. 376(18): p. 1723-1736.
- 881 37. Taylor, P.C., et al., *Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis*. N Engl J Med,  
882 2017. 376(7): p. 652-662.
- 883 38. Dougados, M., et al., *Baricitinib in patients with inadequate response or intolerance to conventional  
884 synthetic DMARDs: results from the RA-BUILD study*. Ann Rheum Dis, 2017. 76(1): p. 88-95.
- 885 39. Fleischmann, R., et al., *Baricitinib, Methotrexate, or Combination in Patients With Rheumatoid  
886 Arthritis and No or Limited Prior Disease-Modifying Antirheumatic Drug Treatment*. Arthritis  
887 Rheumatol, 2017. 69(3): p. 506-517.
- 888 40. Smolen, J.S., et al., *Patient-reported outcomes from a randomised phase III study of baricitinib in  
889 patients with rheumatoid arthritis and an inadequate response to biological agents (RA-BEACON)*.  
890 Ann Rheum Dis, 2017. 76(4): p. 694-700.
- 891 41. van der Heijde, D., et al., *Structural damage progression in patients with early rheumatoid arthritis  
892 treated with methotrexate, baricitinib, or baricitinib plus methotrexate based on clinical response in  
893 the phase 3 RA-BEGIN study*. Clin Rheumatol, 2018. 37(9): p. 2381-2390.
- 894 42. Ramanan, A.V., et al., *Baricitinib in juvenile idiopathic arthritis: an international, phase 3, randomised,  
895 double-blind, placebo-controlled, withdrawal, efficacy, and safety trial*. Lancet, 2023. 402(10401): p.  
896 555-570.
- 897 43. FDA. *Emergency Use Authorization (EUA) for the emergency use of baricitinib for the treatment of  
898 COVID-19*. 2022; Available from: <https://www.fda.gov/media/143822/download>.
- 899 44. Tanaka, Y., et al., *Efficacy and safety of peficitinib (ASP015K) in patients with rheumatoid arthritis and  
900 an inadequate response to conventional DMARDs: a randomised, double-blind, placebo-controlled  
901 phase III trial (RAJ3)*. Ann Rheum Dis, 2019. 78(10): p. 1320-1332.
- 902 45. Takeuchi, T., et al., *Efficacy and safety of peficitinib (ASP015K) in patients with rheumatoid arthritis  
903 and an inadequate response to methotrexate: results of a phase III randomised, double-blind,  
904 placebo-controlled trial (RAJ4) in Japan*. Ann Rheum Dis, 2019. 78(10): p. 1305-1319.
- 905 46. Witthuhn, B.A., et al., *JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated  
906 and activated following stimulation with erythropoietin*. Cell, 1993. 74(2): p. 227-36.
- 907 47. Tortolani, P.J., et al., *Thrombopoietin induces tyrosine phosphorylation and activation of the Janus  
908 kinase, JAK2*. Blood, 1995. 85(12): p. 3444-51.
- 909 48. Dowty, M.E., et al., *Janus kinase inhibitors for the treatment of rheumatoid arthritis demonstrate  
910 similar profiles of in vitro cytokine receptor inhibition*. Pharmacol Res Perspect, 2019. 7(6): p. e00537.
- 911 49. Vazquez, M.L., et al., *Identification of N-cis-3-[Methyl(7H-pyrrolo[2,3-d]pyrimidin-4-  
912 yl)amino]cyclobutylpropane-1-sulfonamide (PF-04965842): A Selective JAK1 Clinical Candidate for the  
913 Treatment of Autoimmune Diseases*. J Med Chem, 2018. 61(3): p. 1130-1152.
- 914 50. Traves, P.G., et al., *JAK selectivity and the implications for clinical inhibition of pharmacodynamic  
915 cytokine signalling by filgotinib, upadacitinib, tofacitinib and baricitinib*. Ann Rheum Dis, 2021. 80(7):  
916 p. 865-875.
- 917 51. Virtanen, A., et al., *Differences in JAK Isoform Selectivity Among Different Types of JAK Inhibitors  
918 Evaluated for Rheumatic Diseases Through In Vitro Profiling*. Arthritis Rheumatol, 2023. 75(11): p.  
919 2054-2061.
- 920 52. Parmentier, J.M., et al., *In vitro and in vivo characterization of the JAK1 selectivity of upadacitinib  
921 (ABT-494)*. BMC Rheumatol, 2018. 2: p. 23.
- 922 53. Van Rompaey, L., et al., *Preclinical characterization of GLPG0634, a selective inhibitor of JAK1, for the  
923 treatment of inflammatory diseases*. J Immunol, 2013. 191(7): p. 3568-77.
- 924 54. FDA. *Drug Approval Package*. 2019; Available from:  
925 [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2019/211675Orig1s000Approv.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/211675Orig1s000Approv.pdf).
- 926 55. Burmester, G.R., et al., *Safety and efficacy of upadacitinib in patients with rheumatoid arthritis and  
927 inadequate response to conventional synthetic disease-modifying anti-rheumatic drugs (SELECT-  
928 NEXT): a randomised, double-blind, placebo-controlled phase 3 trial*. Lancet, 2018. 391(10139): p.  
929 2503-2512.

- 930 56. Genovese, M.C., et al., *Safety and efficacy of upadacitinib in patients with active rheumatoid arthritis refractory to biologic disease-modifying anti-rheumatic drugs (SELECT-BEYOND): a double-blind, randomised controlled phase 3 trial*. *Lancet*, 2018. 391(10139): p. 2513-2524.
- 931
- 932
- 933 57. Fleischmann, R., et al., *Upadacitinib Versus Placebo or Adalimumab in Patients With Rheumatoid Arthritis and an Inadequate Response to Methotrexate: Results of a Phase III, Double-Blind, Randomized Controlled Trial*. *Arthritis Rheumatol*, 2019. 71(11): p. 1788-1800.
- 934
- 935
- 936 58. Rubbert-Roth, A., et al., *Trial of Upadacitinib or Abatacept in Rheumatoid Arthritis*. *N Engl J Med*, 2020. 383(16): p. 1511-1521.
- 937
- 938 59. Smolen, J.S., et al., *Upadacitinib as monotherapy in patients with active rheumatoid arthritis and inadequate response to methotrexate (SELECT-MONOTHERAPY): a randomised, placebo-controlled, double-blind phase 3 study*. *Lancet*, 2019. 393(10188): p. 2303-2311.
- 939
- 940
- 941 60. van Vollenhoven, R., et al., *Efficacy and Safety of Upadacitinib Monotherapy in Methotrexate-Naive Patients With Moderately-to-Severely Active Rheumatoid Arthritis (SELECT-EARLY): A Multicenter, Multi-Country, Randomized, Double-Blind, Active Comparator-Controlled Trial*. *Arthritis Rheumatol*, 2020. 72(10): p. 1607-1620.
- 942
- 943
- 944
- 945 61. Genovese, M.C., et al., *Effect of Filgotinib vs Placebo on Clinical Response in Patients With Moderate to Severe Rheumatoid Arthritis Refractory to Disease-Modifying Antirheumatic Drug Therapy: The FINCH 2 Randomized Clinical Trial*. *JAMA*, 2019. 322(4): p. 315-325.
- 946
- 947
- 948 62. Combe, B., et al., *Filgotinib versus placebo or adalimumab in patients with rheumatoid arthritis and inadequate response to methotrexate: a phase III randomised clinical trial*. *Ann Rheum Dis*, 2021. 80(7): p. 848-858.
- 949
- 950
- 951 63. Westhovens, R., et al., *Filgotinib in combination with methotrexate or as monotherapy versus methotrexate monotherapy in patients with active rheumatoid arthritis and limited or no prior exposure to methotrexate: the phase 3, randomised controlled FINCH 3 trial*. *Ann Rheum Dis*, 2021. 80(6): p. 727-738.
- 952
- 953
- 954
- 955 64. Feagan, B.G., et al., *Filgotinib as induction and maintenance therapy for ulcerative colitis (SELECTION): a phase 2b/3 double-blind, randomised, placebo-controlled trial*. *Lancet*, 2021. 397(10292): p. 2372-2384.
- 956
- 957
- 958 65. Silverberg, J.I., et al., *Efficacy and Safety of Abrocitinib in Patients With Moderate-to-Severe Atopic Dermatitis: A Randomized Clinical Trial*. *JAMA Dermatol*, 2020. 156(8): p. 863-873.
- 959
- 960 66. Simpson, E.L., et al., *Efficacy and safety of abrocitinib in adults and adolescents with moderate-to-severe atopic dermatitis (JADE MONO-1): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial*. *Lancet*, 2020. 396(10246): p. 255-266.
- 961
- 962
- 963 67. Eichenfield, L.F., et al., *Efficacy and Safety of Abrocitinib in Combination With Topical Therapy in Adolescents With Moderate-to-Severe Atopic Dermatitis: The JADE TEEN Randomized Clinical Trial*. *JAMA Dermatol*, 2021. 157(10): p. 1165-1173.
- 964
- 965
- 966 68. Blauvelt, A., et al., *Abrocitinib induction, randomized withdrawal, and retreatment in patients with moderate-to-severe atopic dermatitis: Results from the JAK1 Atopic Dermatitis Efficacy and Safety (JADE) REGIMEN phase 3 trial*. *J Am Acad Dermatol*, 2022. 86(1): p. 104-112.
- 967
- 968
- 969 69. Bieber, T., et al., *Abrocitinib versus Placebo or Dupilumab for Atopic Dermatitis*. *N Engl J Med*, 2021. 384(12): p. 1101-1112.
- 970
- 971 70. Reich, K., et al., *Efficacy and safety of abrocitinib versus dupilumab in adults with moderate-to-severe atopic dermatitis: a randomised, double-blind, multicentre phase 3 trial*. *Lancet*, 2022. 400(10348): p. 273-282.
- 972
- 973
- 974 71. Harrison, C.N., et al., *Janus kinase-2 inhibitor fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study*. *Lancet Haematol*, 2017. 4(7): p. e317-e324.
- 975
- 976
- 977 72. Oh, S.T., et al., *ACVR1/JAK1/JAK2 inhibitor momelotinib reverses transfusion dependency and suppresses hepcidin in myelofibrosis phase 2 trial*. *Blood Adv*, 2020. 4(18): p. 4282-4291.
- 978
- 979 73. Mesa, R.A., et al., *Pacritinib versus best available therapy for the treatment of myelofibrosis irrespective of baseline cytopenias (PERSIST-1): an international, randomised, phase 3 trial*. *Lancet Haematol*, 2017. 4(5): p. e225-e236.
- 980
- 981

- 982 74. King, B., et al., *Efficacy and safety of ritlecitinib in adults and adolescents with alopecia areata: a*  
983 *randomised, double-blind, multicentre, phase 2b-3 trial*. *Lancet*, 2023. 401(10387): p. 1518-1529.
- 984 75. Xu, H., et al., *PF-06651600, a Dual JAK3/TEC Family Kinase Inhibitor*. *ACS Chem Biol*, 2019. 14(6): p.  
985 1235-1242.
- 986 76. Lu, X., J.B. Smail, and K. Ding, *New Promise and Opportunities for Allosteric Kinase Inhibitors*. *Angew*  
987 *Chem Int Ed Engl*, 2020. 59(33): p. 13764-13776.
- 988 77. Nogueira, M., L. Puig, and T. Torres, *JAK Inhibitors for Treatment of Psoriasis: Focus on Selective TYK2*  
989 *Inhibitors*. *Drugs*, 2020. 80(4): p. 341-352.
- 990 78. Morand, E., et al., *TYK2: an emerging therapeutic target in rheumatic disease*. *Nat Rev Rheumatol*,  
991 2024. 20(4): p. 232-240.
- 992 79. Kavanagh, M.E., et al., *Selective inhibitors of JAK1 targeting an isoform-restricted allosteric cysteine*.  
993 *Nat Chem Biol*, 2022. 18(12): p. 1388-1398.
- 994 80. Thorarensen, A., et al., *ATP-mediated kinome selectivity: the missing link in understanding the*  
995 *contribution of individual JAK Kinase isoforms to cellular signaling*. *ACS Chem Biol*, 2014. 9(7): p. 1552-  
996 8.
- 997 81. Wroblewski, S.T., et al., *Highly Selective Inhibition of Tyrosine Kinase 2 (TYK2) for the Treatment of*  
998 *Autoimmune Diseases: Discovery of the Allosteric Inhibitor BMS-986165*. *J Med Chem*, 2019. 62(20):  
999 p. 8973-8995.
- 1000 82. Chimalakonda, A., et al., *Selectivity Profile of the Tyrosine Kinase 2 Inhibitor Deucravacitinib*  
1001 *Compared with Janus Kinase 1/2/3 Inhibitors*. *Dermatol Ther (Heidelb)*, 2021. 11(5): p. 1763-1776.
- 1002 83. Burke, J.R., et al., *Autoimmune pathways in mice and humans are blocked by pharmacological*  
1003 *stabilization of the TYK2 pseudokinase domain*. *Sci Transl Med*, 2019. 11(502).
- 1004 84. Zhou, Y., et al., *Novel Small Molecule Tyrosine Kinase 2 Pseudokinase Ligands Block Cytokine-Induced*  
1005 *TYK2-Mediated Signaling Pathways*. *Front Immunol*, 2022. 13: p. 884399.
- 1006 85. Wylie, A.A., et al., *The allosteric inhibitor ABL001 enables dual targeting of BCR-ABL1*. *Nature*, 2017.  
1007 543(7647): p. 733-737.
- 1008 86. Armstrong, A.W., et al., *Deucravacitinib versus placebo and apremilast in moderate to severe plaque*  
1009 *psoriasis: Efficacy and safety results from the 52-week, randomized, double-blinded, placebo-*  
1010 *controlled phase 3 POETYK PSO-1 trial*. *J Am Acad Dermatol*, 2023. 88(1): p. 29-39.
- 1011 87. Strober, B., et al., *Deucravacitinib versus placebo and apremilast in moderate to severe plaque*  
1012 *psoriasis: Efficacy and safety results from the 52-week, randomized, double-blinded, phase 3 Program*  
1013 *fOr Evaluation of TYK2 inhibitor psoriasis second trial*. *J Am Acad Dermatol*, 2023. 88(1): p. 40-51.
- 1014 88. Dowty, M.E., et al., *The pharmacokinetics, metabolism, and clearance mechanisms of tofacitinib, a*  
1015 *janus kinase inhibitor, in humans*. *Drug Metab Dispos*, 2014. 42(4): p. 759-73.
- 1016 89. Catlett, I.M., et al., *First-in-human study of deucravacitinib: A selective, potent, allosteric small-*  
1017 *molecule inhibitor of tyrosine kinase 2*. *Clin Transl Sci*, 2023. 16(1): p. 151-164.
- 1018 90. Sunzini, F., I. McInnes, and S. Siebert, *JAK inhibitors and infections risk: focus on herpes zoster*. *Ther*  
1019 *Adv Musculoskelet Dis*, 2020. 12: p. 1759720X20936059.
- 1020 91. Xu, Q., L. He, and Y. Yin, *Risk of herpes zoster associated with JAK inhibitors in immune-mediated*  
1021 *inflammatory diseases: a systematic review and network meta-analysis*. *Front Pharmacol*, 2023. 14:  
1022 p. 1241954.
- 1023 92. Mease, P.J., et al., *Efficacy and safety of selective TYK2 inhibitor, deucravacitinib, in a phase II trial in*  
1024 *psoriatic arthritis*. *Ann Rheum Dis*, 2022. 81(6): p. 815-822.
- 1025 93. Strober, B., et al., *Deucravacitinib in moderate-to-severe plaque psoriasis: Pooled safety and*  
1026 *tolerability over 52 weeks from two phase 3 trials (POETYK PSO-1 and PSO-2)*. *J Eur Acad Dermatol*  
1027 *Venereol*, 2024.
- 1028 94. Schulze-Koops, H., et al., *Analysis of haematological changes in tofacitinib-treated patients with*  
1029 *rheumatoid arthritis across phase 3 and long-term extension studies*. *Rheumatology (Oxford)*, 2017.  
1030 56(1): p. 46-57.
- 1031 95. Kay, J., et al., *Changes in selected haematological parameters associated with JAK1/JAK2 inhibition*  
1032 *observed in patients with rheumatoid arthritis treated with baricitinib*. *RMD Open*, 2020. 6(3).

- 1033 96. King, B., et al., *Integrated Safety Analysis of Ritlecitinib, an Oral JAK3/TEC Family Kinase Inhibitor, for*  
1034 *the Treatment of Alopecia Areata from the ALLEGRO Clinical Trial Program*. *Am J Clin Dermatol*, 2024.  
1035 25(2): p. 299-314.
- 1036 97. Winthrop, K.L., *The emerging safety profile of JAK inhibitors in rheumatic disease*. *Nat Rev Rheumatol*,  
1037 2017. 13(4): p. 234-243.
- 1038 98. Skeoch, S. and I.N. Bruce, *Atherosclerosis in rheumatoid arthritis: is it all about inflammation?* *Nat*  
1039 *Rev Rheumatol*, 2015. 11(7): p. 390-400.
- 1040 99. Pardanani, A., et al., *Safety and Efficacy of Fedratinib in Patients With Primary or Secondary*  
1041 *Myelofibrosis: A Randomized Clinical Trial*. *JAMA Oncol*, 2015. 1(5): p. 643-51.
- 1042 100. York, A.G., et al., *Limiting Cholesterol Biosynthetic Flux Spontaneously Engages Type I IFN Signaling*.  
1043 *Cell*, 2015. 163(7): p. 1716-29.
- 1044 101. Hashizume, M., et al., *Overproduced interleukin 6 decreases blood lipid levels via upregulation of very-*  
1045 *low-density lipoprotein receptor*. *Ann Rheum Dis*, 2010. 69(4): p. 741-6.
- 1046 102. Ytterberg, S.R., et al., *Cardiovascular and Cancer Risk with Tofacitinib in Rheumatoid Arthritis*. *N Engl*  
1047 *J Med*, 2022. 386(4): p. 316-326.
- 1048 103. Giles, J.T., et al., *Cardiovascular Safety of Tocilizumab Versus Etanercept in Rheumatoid Arthritis: A*  
1049 *Randomized Controlled Trial*. *Arthritis Rheumatol*, 2020. 72(1): p. 31-40.
- 1050 104. Agca, R., et al., *Cardiovascular Event Risk in Rheumatoid Arthritis Compared with Type 2 Diabetes: A*  
1051 *15-year Longitudinal Study*. *J Rheumatol*, 2020. 47(3): p. 316-324.
- 1052 105. Raadsen, R., et al., *In RA patients without prevalent CVD, incident CVD is mainly associated with*  
1053 *traditional risk factors: A 20-year follow-up in the CARRE cohort study*. *Semin Arthritis Rheum*, 2023.  
1054 58: p. 152132.
- 1055 106. Meissner, Y., et al., *Risk of major adverse cardiovascular events in patients with rheumatoid arthritis*  
1056 *treated with conventional synthetic, biologic and targeted synthetic disease-modifying antirheumatic*  
1057 *drugs: observational data from the German RABBIT register*. *RMD Open*, 2023. 9(4).
- 1058 107. Ahn, S.S., et al., *Cancers and cardiovascular diseases in patients with seropositive rheumatoid arthritis*  
1059 *treated with JAK inhibitors, biologics and conventional synthetic DMARDs*. *Clin Exp Rheumatol*, 2023.  
1060 41(9): p. 1908-1916.
- 1061 108. Khosrow-Khavar, F., et al., *Tofacitinib and risk of cardiovascular outcomes: results from the Safety of*  
1062 *TofAcitinib in Routine care patients with Rheumatoid Arthritis (STAR-RA) study*. *Ann Rheum Dis*, 2022.  
1063 81(6): p. 798-804.
- 1064 109. Frisell, T., et al., *Safety of biological and targeted synthetic disease-modifying antirheumatic drugs*  
1065 *for rheumatoid arthritis as used in clinical practice: results from the ARTIS programme*. *Ann Rheum*  
1066 *Dis*, 2023. 82(5): p. 601-610.
- 1067 110. Bower, H., et al., *Comparative cardiovascular safety with janus kinase inhibitors and biological*  
1068 *disease-modifying antirheumatic drugs as used in clinical practice: an observational cohort study*  
1069 *from Sweden in patients with rheumatoid arthritis*. *RMD Open*, 2023. 9(4).
- 1070 111. Song, Y.K., et al., *Cardiovascular risk of Janus kinase inhibitors compared with biologic disease-*  
1071 *modifying antirheumatic drugs in patients with rheumatoid arthritis without underlying*  
1072 *cardiovascular diseases: a nationwide cohort study*. *Front Pharmacol*, 2023. 14: p. 1165711.
- 1073 112. Yoshida, S., et al., *Safety of JAK and IL-6 inhibitors in patients with rheumatoid arthritis: a multicenter*  
1074 *cohort study*. *Front Immunol*, 2023. 14: p. 1267749.
- 1075 113. Mok, C.C., et al., *Safety of the JAK and TNF inhibitors in rheumatoid arthritis: real world data from*  
1076 *the Hong Kong Biologics Registry*. *Rheumatology (Oxford)*, 2024. 63(2): p. 358-365.
- 1077 114. Uchida, T., et al., *Comparison of risks of cancer, infection, and MACEs associated with JAK inhibitor*  
1078 *and TNF inhibitor treatment: a multicentre cohort study*. *Rheumatology (Oxford)*, 2023. 62(10): p.  
1079 3358-3365.
- 1080 115. Popa, C.D., et al., *Therapy with JAK inhibitors or bDMARDs and the risk of cardiovascular events in*  
1081 *the Dutch rheumatoid arthritis population*. *Rheumatology (Oxford)*, 2023.
- 1082 116. Min, H.K., et al., *Risk of cancer, cardiovascular disease, thromboembolism, and mortality in patients*  
1083 *with rheumatoid arthritis receiving Janus kinase inhibitors: a real-world retrospective observational*  
1084 *study using Korean health insurance data*. *Epidemiol Health*, 2023. 45: p. e2023045.

- 1085 117. Desai, R.J., et al., *Risk of venous thromboembolism associated with tofacitinib in patients with*  
1086 *rheumatoid arthritis: a population-based cohort study*. *Rheumatology (Oxford)*, 2021. 61(1): p. 121-  
1087 130.
- 1088 118. Gau, S.Y. and H.C. Chang, *Janus kinase inhibitor and the risk of venous thromboembolism in*  
1089 *rheumatoid arthritis patients-A global federated health network analysis*. *Semin Arthritis Rheum*,  
1090 2024. 65: p. 152369.
- 1091 119. Hoisnard, L., et al., *Risk of major adverse cardiovascular and venous thromboembolism events in*  
1092 *patients with rheumatoid arthritis exposed to JAK inhibitors versus adalimumab: a nationwide cohort*  
1093 *study*. *Ann Rheum Dis*, 2023. 82(2): p. 182-188.
- 1094 120. Tong, X., et al., *Cardiovascular risk in rheumatoid arthritis patients treated with targeted synthetic*  
1095 *and biological disease-modifying antirheumatic drugs: A multi-centre cohort study*. *J Intern Med*,  
1096 2023. 294(3): p. 314-325.
- 1097 121. Song, Y.J., et al., *Risk of venous thromboembolism in Korean patients with rheumatoid arthritis*  
1098 *treated with Janus kinase inhibitors: A nationwide population-based study*. *Semin Arthritis Rheum*,  
1099 2023. 61: p. 152214.
- 1100 122. Salinas, C.A., et al., *Evaluation of VTE, MACE, and Serious Infections Among Patients with RA Treated*  
1101 *with Baricitinib Compared to TNFi: A Multi-Database Study of Patients in Routine Care Using Disease*  
1102 *Registries and Claims Databases*. *Rheumatol Ther*, 2023. 10(1): p. 201-223.
- 1103 123. Molander, V., et al., *Venous thromboembolism with JAK inhibitors and other immune-modulatory*  
1104 *drugs: a Swedish comparative safety study among patients with rheumatoid arthritis*. *Ann Rheum*  
1105 *Dis*, 2023. 82(2): p. 189-197.
- 1106 124. Cohen, S.B., et al., *Long-term safety of tofacitinib up to 9.5 years: a comprehensive integrated analysis*  
1107 *of the rheumatoid arthritis clinical development programme*. *RMD Open*, 2020. 6(3).
- 1108 125. Taylor, P.C., et al., *Safety of baricitinib for the treatment of rheumatoid arthritis over a median of 4.6*  
1109 *and up to 9.3 years of treatment: final results from long-term extension study and integrated*  
1110 *database*. *Ann Rheum Dis*, 2022. 81(3): p. 335-343.
- 1111 126. Winthrop, K.L., et al., *Integrated safety analysis of filgotinib in patients with moderately to severely*  
1112 *active rheumatoid arthritis receiving treatment over a median of 1.6 years*. *Ann Rheum Dis*, 2022.  
1113 81(2): p. 184-192.
- 1114 127. Mease, P., et al., *Incidence of venous and arterial thromboembolic events reported in the tofacitinib*  
1115 *rheumatoid arthritis, psoriasis and psoriatic arthritis development programmes and from real-world*  
1116 *data*. *Ann Rheum Dis*, 2020. 79(11): p. 1400-1413.
- 1117 128. Burmester, G.R., et al., *Safety profile of upadacitinib over 15 000 patient-years across rheumatoid*  
1118 *arthritis, psoriatic arthritis, ankylosing spondylitis and atopic dermatitis*. *RMD Open*, 2023. 9(1).
- 1119 129. Hordinsky, M., et al., *Efficacy and safety of ritlecitinib in adolescents with alopecia areata: Results*  
1120 *from the ALLEGRO phase 2b/3 randomized, double-blind, placebo-controlled trial*. *Pediatr Dermatol*,  
1121 2023. 40(6): p. 1003-1009.
- 1122 130. Bieber, T., et al., *A Review of Safety Outcomes from Clinical Trials of Baricitinib in Rheumatology,*  
1123 *Dermatology and COVID-19*. *Adv Ther*, 2022. 39(11): p. 4910-4960.
- 1124 131. Charles-Schoeman, C., et al., *MACE and VTE across upadacitinib clinical trial programmes in*  
1125 *rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis*. *RMD Open*, 2023. 9(4).
- 1126 132. Dougados, M., et al., *Impact of cardiovascular risk enrichment on incidence of major adverse*  
1127 *cardiovascular events in the tofacitinib rheumatoid arthritis clinical programme*. *Ann Rheum Dis*,  
1128 2023. 82(4): p. 575-577.
- 1129 133. Charles-Schoeman, C., et al., *Risk of major adverse cardiovascular events with tofacitinib versus*  
1130 *tumour necrosis factor inhibitors in patients with rheumatoid arthritis with or without a history of*  
1131 *atherosclerotic cardiovascular disease: a post hoc analysis from ORAL Surveillance*. *Ann Rheum Dis*,  
1132 2023. 82(1): p. 119-129.
- 1133 134. Waibel, M., et al., *Baricitinib and beta-Cell Function in Patients with New-Onset Type 1 Diabetes*. *N*  
1134 *Engl J Med*, 2023. 389(23): p. 2140-2150.

- 1135 135. Kochar, B.D., et al., *Comparative Risk of Thrombotic and Cardiovascular Events with Tofacitinib and*  
1136 *Anti-TNF Agents in Patients with Inflammatory Bowel Diseases*. *Dig Dis Sci*, 2022. 67(11): p. 5206-  
1137 5212.
- 1138 136. Taylor, P.C., et al., *Cardiovascular Safety During Treatment With Baricitinib in Rheumatoid Arthritis*.  
1139 *Arthritis Rheumatol*, 2019. 71(7): p. 1042-1055.
- 1140 137. Greenfield, G., et al., *The ruxolitinib effect: understanding how molecular pathogenesis and*  
1141 *epigenetic dysregulation impact therapeutic efficacy in myeloproliferative neoplasms*. *J Transl Med*,  
1142 2018. 16(1): p. 360.
- 1143 138. Lin, J.Q., et al., *A 10-year retrospective cohort study of ruxolitinib and association with nonmelanoma*  
1144 *skin cancer in patients with polycythemia vera and myelofibrosis*. *J Am Acad Dermatol*, 2022. 86(2):  
1145 p. 339-344.
- 1146 139. Rampotas, A., et al., *Outcomes and characteristics of nonmelanoma skin cancers in patients with*  
1147 *myeloproliferative neoplasms on ruxolitinib*. *Blood*, 2024. 143(2): p. 178-182.
- 1148 140. Jalles, C., et al., *Skin cancers under Janus kinase inhibitors: A World Health Organization drug safety*  
1149 *database analysis*. *Therapie*, 2022. 77(6): p. 649-656.
- 1150 141. Krzysztofik, M., et al., *Risk of Melanoma and Non-Melanoma Skin Cancer in Patients with Psoriasis*  
1151 *and Psoriatic Arthritis Treated with Targeted Therapies: A Systematic Review and Meta-Analysis*.  
1152 *Pharmaceuticals (Basel)*, 2023. 17(1).
- 1153 142. Curtis, J.R., et al., *Malignancy risk with tofacitinib versus TNF inhibitors in rheumatoid arthritis: results*  
1154 *from the open-label, randomised controlled ORAL Surveillance trial*. *Ann Rheum Dis*, 2023. 82(3): p.  
1155 331-343.
- 1156 143. Westermann, R., et al., *Cancer risk in patients with rheumatoid arthritis treated with janus kinase*  
1157 *inhibitors: a nationwide Danish register-based cohort study*. *Rheumatology (Oxford)*, 2024. 63(1): p.  
1158 93-102.
- 1159 144. Huss, V., et al., *Cancer risks with JAKi and biological disease-modifying antirheumatic drugs in*  
1160 *patients with rheumatoid arthritis or psoriatic arthritis: a national real-world cohort study*. *Ann*  
1161 *Rheum Dis*, 2023. 82(7): p. 911-919.
- 1162 145. Miao, Y., et al., *Functional and Structural Characterization of Clinical-Stage Janus Kinase 2 Inhibitors*  
1163 *Identifies Determinants for Drug Selectivity*. *J Med Chem*, 2024. 67(12): p. 10012-10024.
- 1164 146. Elwood, F., et al., *Evaluation of JAK3 Biology in Autoimmune Disease Using a Highly Selective,*  
1165 *Irreversible JAK3 Inhibitor*. *J Pharmacol Exp Ther*, 2017. 361(2): p. 229-244.
- 1166 147. Rudolf, A.F., et al., *A comparison of protein kinases inhibitor screening methods using both enzymatic*  
1167 *activity and binding affinity determination*. *PLoS One*, 2014. 9(6): p. e98800.
- 1168 148. Ferrao, R. and P.J. Lupardus, *The Janus Kinase (JAK) FERM and SH2 Domains: Bringing Specificity to*  
1169 *JAK-Receptor Interactions*. *Front Endocrinol (Lausanne)*, 2017. 8: p. 71.
- 1170 149. Guttman-Yassky, E., et al., *The role of Janus kinase signaling in the pathology of atopic dermatitis*. *J*  
1171 *Allergy Clin Immunol*, 2023. 152(6): p. 1394-1404.
- 1172 150. Papp, K., et al., *Efficacy and safety of ruxolitinib cream for the treatment of atopic dermatitis: Results*  
1173 *from 2 phase 3, randomized, double-blind studies*. *J Am Acad Dermatol*, 2021. 85(4): p. 863-872.
- 1174 151. Suehiro, M., et al., *Real-world efficacy of proactive maintenance treatment with delgocitinib*  
1175 *ointment twice weekly in adult patients with atopic dermatitis*. *Dermatol Ther*, 2022. 35(7): p.  
1176 e15526.
- 1177 152. Nakagawa, H., et al., *Delgocitinib ointment, a topical Janus kinase inhibitor, in adult patients with*  
1178 *moderate to severe atopic dermatitis: A phase 3, randomized, double-blind, vehicle-controlled study*  
1179 *and an open-label, long-term extension study*. *J Am Acad Dermatol*, 2020. 82(4): p. 823-831.
- 1180 153. Custurone, P., et al., *Role of Cytokines in Vitiligo: Pathogenesis and Possible Targets for Old and New*  
1181 *Treatments*. *Int J Mol Sci*, 2021. 22(21).
- 1182 154. Rosmarin, D., et al., *Two Phase 3, Randomized, Controlled Trials of Ruxolitinib Cream for Vitiligo*. *N*  
1183 *Engl J Med*, 2022. 387(16): p. 1445-1455.
- 1184 155. Abduelmula, A., et al., *Management of Alopecia Areata With Topical JAK Inhibitor Therapy: An*  
1185 *Evidence-Based Review*. *J Cutan Med Surg*, 2023. 27(1): p. 73-75.

- 1186 156. Gong, X., et al., *Pharmacokinetics of Ruxolitinib in Patients with Atopic Dermatitis Treated With Ruxolitinib Cream: Data from Phase II and III Studies*. Am J Clin Dermatol, 2021. 22(4): p. 555-566.
- 1187
- 1188 157. Purohit, V.S., et al., *Systemic Tofacitinib Concentrations in Adult Patients With Atopic Dermatitis Treated With 2% Tofacitinib Ointment and Application to Pediatric Study Planning*. J Clin Pharmacol, 2019. 59(6): p. 811-820.
- 1189
- 1190
- 1191 158. Landis, M.N., et al., *Efficacy and safety of topical brepocitinib cream for mild-to-moderate chronic plaque psoriasis: a phase IIb randomized double-blind vehicle-controlled parallel-group study*. Br J Dermatol, 2023. 189(1): p. 33-41.
- 1192
- 1193
- 1194 159. Misery, L., et al., *Basic mechanisms of itch*. J Allergy Clin Immunol, 2023. 152(1): p. 11-23.
- 1195 160. Auyeung, K.L. and B.S. Kim, *Emerging concepts in neuropathic and neurogenic itch*. Ann Allergy Asthma Immunol, 2023. 131(5): p. 561-566.
- 1196
- 1197 161. Li, Y., et al., *Development and evaluation of tofacitinib transdermal system for the treatment of rheumatoid arthritis in rats*. Drug Dev Ind Pharm, 2021. 47(6): p. 878-886.
- 1198
- 1199 162. Taylor, P.C., et al., *Achieving Pain Control in Rheumatoid Arthritis with Baricitinib or Adalimumab Plus Methotrexate: Results from the RA-BEAM Trial*. J Clin Med, 2019. 8(6).
- 1200
- 1201 163. Simon, L.S., et al., *The Jak/STAT pathway: A focus on pain in rheumatoid arthritis*. Semin Arthritis Rheum, 2021. 51(1): p. 278-284.
- 1202
- 1203 164. Georas, S.N., et al., *JAK inhibitors for asthma*. J Allergy Clin Immunol, 2021. 148(4): p. 953-963.
- 1204 165. Zak, M., H.S. Dengler, and N.S. Rajapaksa, *Inhaled Janus Kinase (JAK) inhibitors for the treatment of asthma*. Bioorg Med Chem Lett, 2019. 29(20): p. 126658.
- 1205
- 1206 166. Dengler, H.S., et al., *Lung-restricted inhibition of Janus kinase 1 is effective in rodent models of asthma*. Sci Transl Med, 2018. 10(468).
- 1207
- 1208 167. Calbet, M., et al., *Novel Inhaled Pan-JAK Inhibitor, LAS194046, Reduces Allergen-Induced Airway Inflammation, Late Asthmatic Response, and pSTAT Activation in Brown Norway Rats*. J Pharmacol Exp Ther, 2019. 370(2): p. 137-147.
- 1209
- 1210
- 1211 168. Younis, U.S., et al., *Preformulation and Evaluation of Tofacitinib as a Therapeutic Treatment for Asthma*. AAPS PharmSciTech, 2019. 20(5): p. 167.
- 1212
- 1213 169. Joo, Y.H., et al., *Therapeutic Effects of Intranasal Tofacitinib on Chronic Rhinosinusitis with Nasal Polyps in Mice*. Laryngoscope, 2021. 131(5): p. E1400-E1407.
- 1214
- 1215 170. Deuse, T., et al., *The Selective JAK1/3-Inhibitor R507 Mitigates Obliterative Airway Disease Both With Systemic Administration and Aerosol Inhalation*. Transplantation, 2016. 100(5): p. 1022-31.
- 1216
- 1217 171. Arjuna, A., et al., *An update on current treatment strategies for managing bronchiolitis obliterans syndrome after lung transplantation*. Expert Rev Respir Med, 2021. 15(3): p. 339-350.
- 1218
- 1219 172. Singh, D., et al., *A phase 2 multiple ascending dose study of the inhaled pan-JAK inhibitor nezulcitinib (TD-0903) in severe COVID-19*. Eur Respir J, 2021. 58(4).
- 1220
- 1221 173. Belperio, J., et al., *Efficacy and safety of an inhaled pan-Janus kinase inhibitor, nezulcitinib, in hospitalised patients with COVID-19: results from a phase 2 clinical trial*. BMJ Open Respir Res, 2023. 10(1).
- 1222
- 1223
- 1224 174. Braithwaite, I.E., et al., *Inhaled JAK inhibitor GDC-0214 reduces exhaled nitric oxide in patients with mild asthma: A randomized, controlled, proof-of-activity trial*. J Allergy Clin Immunol, 2021. 148(3): p. 783-789.
- 1225
- 1226
- 1227 175. Chen, H., et al., *Effects of inhaled JAK inhibitor GDC-4379 on exhaled nitric oxide and peripheral biomarkers of inflammation*. Pulm Pharmacol Ther, 2022. 75: p. 102133.
- 1228
- 1229 176. Bousoik, E., et al., *Combinational silencing of components involved in JAK/STAT signaling pathway*. Eur J Pharm Sci, 2022. 175: p. 106233.
- 1230
- 1231 177. Tang, Q., et al., *Rational design of a JAK1-selective siRNA inhibitor for the modulation of autoimmunity in the skin*. Nat Commun, 2023. 14(1): p. 7099.
- 1232
- 1233 178. Clement, F., et al., *Therapeutic siRNAs Targeting the JAK/STAT Signalling Pathway in Inflammatory Bowel Diseases*. J Crohns Colitis, 2022. 16(2): p. 286-300.
- 1234
- 1235 179. Hoofman, A. and L.A.J. O'Neill, *The Immunomodulatory Potential of the Metabolite Itaconate*. Trends Immunol, 2019. 40(8): p. 687-698.
- 1236

- 1237 180. Bambouskova, M., et al., *Electrophilic properties of itaconate and derivatives regulate the*  
1238 *IkappaBzeta-ATF3 inflammatory axis*. *Nature*, 2018. 556(7702): p. 501-504.
- 1239 181. Runtsch, M.C., et al., *Itaconate and itaconate derivatives target JAK1 to suppress alternative*  
1240 *activation of macrophages*. *Cell Metab*, 2022. 34(3): p. 487-501 e8.
- 1241 182. Lampropoulou, V., et al., *Itaconate Links Inhibition of Succinate Dehydrogenase with Macrophage*  
1242 *Metabolic Remodeling and Regulation of Inflammation*. *Cell Metab*, 2016. 24(1): p. 158-66.
- 1243 183. Tsai, J., et al., *Topical SCD-153, a 4-methyl itaconate prodrug, for the treatment of alopecia areata*.  
1244 *PNAS Nexus*, 2023. 2(1): p. pgac297.
- 1245 184. Winthrop, K.L., et al., *Unmet need in rheumatology: reports from the Advances in Targeted Therapies*  
1246 *meeting, 2022*. *Ann Rheum Dis*, 2023. 82(5): p. 594-598.
- 1247 185. Choy, E.H., *Clinical significance of Janus Kinase inhibitor selectivity*. *Rheumatology (Oxford)*, 2019.  
1248 58(6): p. 953-962.

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1252 Author contributions

1253 The authors contributed equally to all aspects of the article and reviewed the manuscript before  
1254 submission.

1255 Competing interests

1256 The authors declare no competing interests. I am not sure if here we should mention that Olli  
1257 Silvennoinen is a scientific advisory board member and co-founder of Ajax Therapeutics Inc

1258 Jean-Baptist Telliez is an employee of Pfizer Inc.

1259 Key Points

- 1260 • JAK inhibitors show equivalent or superior efficacy compared with biologic DMARDs in  
1261 several autoimmune and inflammatory diseases.
- 1262 • The use of JAK inhibitors has been hampered by adverse events, which could be linked to  
1263 the JAKs that are blocked.
- 1264 • Assessment of individual JAK inhibitors selectivity is still a matter of debate as different  
1265 assays can yield different results.
- 1266 • Selective JAK inhibitors are generally equally effective, but improved, long term safety has  
1267 not been fully established.
- 1268 • Tissue-targeted JAK inhibitors could circumvent the problem of adverse effects resulting  
1269 from systemic administration.
- 1270 • Alternative strategies for JAK inhibition with small interfering RNAs (siRNAs) or metabolites  
1271 derivatives are an exciting area of development.

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1274 Table 1: Target JAK(s), indication(s) and dose(s) of the approved first and second-generation non-  
1275 allosteric JAK inhibitors.

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<b>DRUG</b>	<b>TARGET</b>	<b>INDICATION</b>	<b>APPROVED DOSE</b>
Ruxolitinib	JAK1 and JAK2	Myelofibrosis Polycythemia vera GVHD	5-20 mg twice a day <sup>a</sup> 10 mg twice a day 10 mg twice a day
Tofacitinib	JAK1, JAK3 and JAK2	RA PsA AS polyarticular JIA	5 mg twice a day or 11 mg daily 5 mg twice a day or/11 mg daily 5 mg twice a day 3.2-5 mg twice a day

		Ulcerative colitis	5 <sup>c</sup> - 10 mg twice a day
Baricitinib	JAK1 and JAK2	RA	2-4 mg daily <sup>b</sup>
		COVID-19	4 mg daily
		Alopecia areata	4 mg daily
		JIA	2-4 mg daily <sup>b</sup>
Peficitinib	pan-JAK	RA	150 mg daily
Upadacitinib	JAK1	RA	15 mg daily
		PsA	15 mg daily
		AS	15 mg daily
		axSpA	15 mg daily
		Atopic dermatitis	15 mg daily
		Ulcerative colitis	15-30 <sup>d</sup> or 5 mg daily
		Crohn's disease	15-30 <sup>d</sup> or 45 mg daily
polyarticular JIA	3, 4 or 6 <sup>e</sup> mg twice a day		
juvenile PsA	3, 4 or 6 <sup>e</sup> mg twice a day		
Filgotinib	JAK1	RA	100-200 mg daily <sup>f</sup>
		Ulcerative colitis	200 mg daily
Abrocitinib	JAK1	Atopic dermatitis	100-200 mg daily <sup>g</sup>
Ritlecitinib	JAK3	Alopecia areata	50 mg daily
Fedratinib	JAK2	Myelofibrosis	400 mg daily
Pacritinib	JAK2	Myelofibrosis	200 mg twice a day
Momelotinib	JAK2	Myelofibrosis	150-200 mg daily <sup>h</sup>
Delgocitinib	pan-JAK	Atopic dermatitis	0.5% (topical)

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<sup>a</sup>the dose depends on platelet count

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<sup>b</sup> the 2 mg dosage is recommended for patients  $\geq 65$  years old, those with eGFR 30-<60 mL/min/1.73m<sup>2</sup> in patients at high risk of cardiovascular, thromboembolic or neoplastic events and in children aged from 2 to <9 years or weighing less than 30 kg; baricitinib 2 mg can be considered in adults and children  $\geq 9$  years with sustained control of disease. In US 2 mg is the only approved dose

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<sup>c</sup> the dose of 5 mg twice a day is indicated in the maintenance after 16 weeks induction with 10 mg twice a day

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<sup>d</sup> the doses of 15 or 30 mg daily are indicated in the maintenance after 12 weeks induction with 45 mg daily<sup>e</sup> the dose Depends on the patient's weight

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<sup>f</sup>the 100 mg dosage is recommended for patients  $\geq 65$  years old, those with eGFR 30-<15 mL/min/1.73m<sup>2</sup>, and patients at high risk of cardiovascular, thromboembolic or neoplastic events

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<sup>g</sup>high risk of cardiovascular, thromboembolic or neoplastic events

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<sup>h</sup>the 150 mg dosage is recommended in patients with liver impairment (Child -Pugh grade C)

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AS, ankylosing spondylitis; COVID-19=coronavirus disease 19; GVHD, graft-versus-host disease; JIA, juvenile idiopathic arthritis; axSpA, axial spondyloarthritis; PsA, psoriatic arthritis; RA,rheumatoid arthritis.

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Table 2: Phase II and phase III clinical trials with second-generation non-allosteric JAK inhibitors not yet approved

Drug	Target	Indication	Stage of development	NCT number
GLPG3667	TYK2 <sup>a</sup>	Systemic lupus erythematosus	Phase II	NCT05856448 <sup>d</sup>
		Dermatomyositis	Phase II	NCT05695950 <sup>d</sup>
Ivarmacitinib	JAK1	Ulcerative colitis	Phase III	NCT05181137 <sup>d</sup>
		Crohn's disease	Phase II	NCT003677648 <sup>e</sup>
		Atopic dermatitis	Phase II and III	NCT04717310 <sup>d</sup>
		axSpA	Phase III	NCT04875169 <sup>e</sup>
		AS	Phase III	NCT05324631 <sup>d</sup>
		RA	Phase III	NCT04481139 <sup>e</sup>
		Psoriatic arthritis	Phase II	NCT03254966 <sup>e</sup>
		Vitiligo	Phase III	NCT04333771 <sup>e</sup>
		Alopecia areata	Phase III	NCT04957550 <sup>d</sup>
Povorcitinib	JAK1	Hidradenitis suppurativa	Phase II and III	NCT06212999 <sup>d</sup>
				NCT05620823 <sup>d</sup>
				NCT05620836 <sup>d</sup>
				NCT04476043 <sup>e</sup>
				NCT03569371 <sup>e</sup>
		Vitiligo	Phase III	NCT03607487 <sup>e</sup>
				NCT06113471 <sup>d</sup>
				NCT06113445 <sup>d</sup>
				NCT04818346 <sup>e</sup>
				NCT05936567 <sup>d</sup>
Chronic urticaria	Phase II	Asthma	NCT05851443 <sup>d</sup>	
		Prurigo nodularis	NCT05061693 <sup>d</sup>	
		Crohn's disease	NCT05688852 <sup>d</sup>	
		PsA	NCT05715125 <sup>d</sup>	
		Itacitinib	JAK1 <sup>a</sup>	GVHD
Ulcerative colitis	Phase II			NCT03627052 <sup>f</sup>
Systemic sclerosis	Phase II			NCT04789850 <sup>d</sup>
Plaque psoriasis	Phase II			NCT01634087 <sup>e</sup>
Hemophagocytosis lymphohistiocytosis	Phase II			NCT05063110 <sup>d</sup>
KL130008	JAK1 <sup>a</sup>	Alopecia areata	Phase II	NCT05496426 <sup>d</sup>
Ifidancitinib	JAK1 <sup>b</sup>	Alopecia totalis and universalis	Phase II	NCT03315689 <sup>e</sup>
		Alopecia areata	Phase II	NCT03551821 <sup>e</sup> , NCT03354637 <sup>f</sup>
		Vitiligo	Phase II	NCT03468855 <sup>e</sup>
Ropsacitinib	JAK2 <sup>c</sup>	Ulcerative colitis	Phase II	NCT04209556 <sup>f</sup>
		Plaque psoriasis	Phase II	NCT03895372 <sup>e</sup>

		Hidradenitis suppurativa	Phase II	NCT04092452 <sup>e</sup>
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1296 <sup>a</sup>Inhibitor also binds JAK2, <sup>b</sup> Inhibitor also binds JAK3, <sup>c</sup> Inhibitor also binds TYK2

1297 <sup>d</sup>Study is active, <sup>e</sup>Study is completed, <sup>f</sup>Study has been terminated or withdrawn

1298 Table 3 Current stage of development of allosteric JAK inhibitors

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Drug	Target	Indication	Stage of development	NCT number
Deucravacitinib	TYK2 <sup>a</sup>	Psoriasis	Approved	
		Psoriatic arthritis	Phase III	NCT04908202 <sup>c</sup> NCT04908189 <sup>c</sup>
		Palmoplantar pustulosis	Phase III	NCT05710185 <sup>c</sup>
		Systemic lupus erythematosus	Phase III	NCT05620407 <sup>c</sup> NCT05617677 <sup>c</sup>
		Alopecia areata	Phase II	NCT05556265 <sup>c</sup>
		Cutaneous Lupus erythematosus	Phase II	NCT04857034 <sup>c</sup>
		Lupus nephritis	Phase II	NCT03943147 <sup>d</sup> NCT03934216 <sup>d</sup>
		Ulcerative colitis	Phase II	NCT04877990 <sup>d</sup> NCT04613518 <sup>d</sup>
		Crohn's disease	Phase II	NCT04877990 <sup>d</sup> NCT03599622 <sup>c</sup>
Zasocitinib	TYK2	Psoriatic arthritis	Phase II	NCT05153148 <sup>d</sup>
		Psoriasis	Phase III	NCT06088043 <sup>c</sup> NCT06108544 <sup>c</sup>
VTX958	TYK2	Psoriasis	Phase II	NCT05655299 <sup>e</sup> , NCT05715125 <sup>c</sup>
		Crohn's disease	Phase II	NCT05688852 <sup>c</sup>
		Psoriatic arthritis	Phase II	NCT05715125 <sup>c</sup>
ESK-001	TYK2	Psoriasis	Phase II	NCT05600036 <sup>d</sup>
		Non-infectious uveitis	Phase II	NCT05953688 <sup>c</sup>
		Systemic lupus erythematosus	Phase II	NCT05966480 <sup>c</sup>

1301 <sup>a</sup>Inhibitor also binds JAK1 and to lesser extent JAK2, <sup>b</sup>Inhibitor also binds TYK2

1302 <sup>c</sup>Study is active, <sup>d</sup>Study is completed, <sup>e</sup>Study has been terminated

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In this Review, the authors provide an update on new developments in Janus kinase (JAK) inhibitor disease indications and adverse effects. The issues of selectivity and efficacy of JAK inhibitors are discussed and new routes for administration of JAK inhibitors explored.

### **[bH1] Box 1 How to assess specificity of JAK inhibitors?**

JAK-selectivity of inhibitors is assessed using biochemical activity- and binding-based assays using recombinant proteins. Prediction of effects on cytokine signalling based on JAK-selectivity is challenging as JAKs function in pairs and the functional role for a given JAK isoform varies between cytokine receptors. Thereby cell-based specificity assessments of JAK-inhibitors are important.

#### **[bH2] Biochemical activity-based assessment**

- Measures kinase activity by either conversion of ATP to ADP or phosphorylation of peptide substrate
- Assessment uses recombinant kinase or kinase or pseudokinase domains
- Highly sensitive for alterations in assay conditions: up to 50-100-fold variance in inhibitory concentrations (IC50) can occur for a given JAK inhibitor complicating inter-study comparisons [24, 32, 48, 52, 53]
- ATP concentration affects the results considerably as orthosteric JAK inhibitors are ATP-competitive inhibitors [80]. Mechanistic information on JAK-selectivity best obtained from assays at Km ATP (affinity of kinase for ATP) but assays at physiological [ATP] (1-5 mM) describes better the selectivity in cells
- Influenced by the amount and characteristics of the recombinant protein and the peptide substrate

#### **[bH2] Biochemical binding-based assessment**

- Detects affinity of a drug to target protein(s)
- Assessment uses recombinant kinase or pseudokinase domains
- Domain content, activation state and posttranslational modifications of recombinant protein can influence the results
- Binding is a valid approximation for inhibition of kinase activity [147] but the results of the assay might not directly translate into cellular effects

#### **[bH2] Cell-based assessment**

- Typically measures the inhibition of STAT phosphorylation in response to cytokine stimulation in whole blood or isolated peripheral blood mononuclear cells
- Drugs tend to bind to plasma proteins leading to apparent weaker inhibition in whole blood compared with a plasma-free environment [50]
- Results can be affected by stimulation and inhibition methods, cell-type and read-out used [22, 48, 50, 51]
- Therapeutic response, a result of the inhibition of various cytokines at different levels over time, is influenced by the selectivity profile, dose, pharmacokinetics, cell type, cytokine or inflammatory environment and patient genetics[185].

1345 Figure legends

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1347 Figure 1. Function and inhibition of Janus kinases. JAKs work in pairs and mediate signalling of  
1348 many cytokines and hormones . Activated JAKs phosphorylate STATs, which dimerize, translocate  
1349 in nucleus and regulate transcription of their target genes . JAK inhibitors target JAKs and suppress  
1350 JAK-mediated cytokine signalling. JAK inhibitors are either non-selective (the first-generation  
1351 inhibitors) or selective to a given JAK family member (second-generation and third-generation  
1352 inhibitors). Abbreviations: LIF, leukemia inhibitory factor; OSM, oncostatin M; EPO, erythropoietin;  
1353 TPO, thrombopoietin; GH, growth hormone; GM-CSF, granulocyte macrophage colony-stimulating  
1354 factor; TSLP, thymic stromal lymphopoietin; IFN, interferon.

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1356 Figure 2. Structure of JAK2 and inhibitor target sites. A Activated JAKs have an open  
1357 conformation. The majority of the current JAK inhibitors bind the kinase ATP pocket (shown in  
1358 magenta) but also the pseudokinase adenosine triphosphate (ATP) pocket (shown in cyan),  
1359 cysteine JAK1 C817 or TYK2 C838 (shown in blue) are also targets sites. B JAKs are multidomain  
1360 proteins comprising of FERM (the band-4.1, ezrin, radixin, and moesin domain; in yellow) and SH2  
1361 (Src homology 2; in purple) domains that mediate receptor interactions, regulatory pseudokinase  
1362 domain (shown in green) and active kinase domain (shown in turquoise). C Inactive JAKs form a  
1363 closed structure. The first and second-generation inhibitors, for example, tofacitinib (shown in  
1364 magenta) target the ATP pocket of the kinase domain, whereas allosteric inhibitors target the  
1365 pseudokinase domain. Deucravacitinib (shown in cyan) binds the ATP-pocket of the pseudokinase  
1366 domain, and the investigational inhibitor VVD-118313 target cysteine JAK1 C817/TYK2 C838  
1367 (shown in blue) close to the so-called myristoyl pocket. The figure was prepared by superimposing  
1368 Alphafold prediction for human JAK1 (Uniprot P23458; open structure) or human TYK2 (Uniprot  
1369 P29597; closed structure) with JAK1 crystal structure in complex with tofacitinib (PDB 3EYG) and  
1370 TYK2 crystal structure in complex with deucravacitinib (6NZP) using UCSF ChimeraX.

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1372 Figure 3. Timeline of JAK inhibitor development. JAKs were discovered in the early 90's, followed  
1373 by the initial JAK inhibition studies few years later. Tofacitinib was the first JAK inhibitor to enter  
1374 clinical trials, whereas ruxolitinib was the first JAK inhibitor approved for clinical use. The timeline  
1375 highlights the initial approvals dates of various JAK inhibitors. Currently 14 JAK inhibitors have  
1376 obtained approval by various regulatory agencies including by FDA, EMA and or Japan's  
1377 Pharmaceuticals and Medical Devices Agency (PMDA).

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