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BRIEF REPORT

Differences in JAK Isoform Selectivity Among Different Types of JAK Inhibitors Evaluated for Rheumatic Diseases Through In Vitro Profiling

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Objective. The selectivity of JAK inhibitors (Jakinibs) forms the basis for understanding their clinical characteristics; however, evaluation of selectivity is hampered by the lack of comprehensive head-to-head studies. Our objective was to profile in parallel Jakinibs indicated or evaluated for rheumatic diseases for their JAK and cytokine selectivity in vitro.

Methods. We analyzed 10 Jakinibs for JAK isoform selectivity by assaying their inhibition of JAK kinase activity, binding to kinase and pseudokinase domains, and inhibition of cytokine signaling using blood samples from healthy volunteers and using isolated peripheral blood mononuclear cells (PBMCs) from patients with rheumatoid arthritis and from healthy donors.

Results. Pan-Jakinibs effectively suppressed kinase activity of 2 to 3 JAK family members, whereas isoform-targeted Jakinibs possessed varying degrees of selectivity for 1 or 2 JAK family members. In human leukocytes, Jakinibs predominantly inhibited the JAK1-dependent cytokines interleukin-2 (IL-2), IL-6, and interferons (IFNs). In PBMCs from patients with rheumatoid arthritis compared with healthy controls, inhibition of these cytokines was more pronounced, and some cell-type and STAT isoform differences were observed. Novel Jakinibs demonstrated high selectivity: the covalent Jakinib ritlecitinib showed 900- to 2,500-fold selectivity for JAK3 over other JAKs and specific suppression of IL-2-signaling, whereas the allosteric TYK2 inhibitor deucravacitinib inhibited IFNα signaling with high specificity. Interestingly, deucravacitinib targeted the regulatory pseudokinase domain and did not affect JAK in vitro kinase activity.

Conclusion. Inhibition of JAK kinase activity did not directly translate into cellular inhibition of JAK/STAT signaling. Despite differences in JAK selectivity, the cytokine inhibition profiles of currently approved Jakinibs were highly similar, with preference for JAK1-mediated cytokines. Novel types of Jakinibs showed narrow cytokine inhibition profile specific for JAK3- or TYK2-mediated signaling.

INTRODUCTION

JAK kinases are effective therapeutic targets in rheumatic and other inflammatory diseases. The JAK family of 4 nonreceptor tyrosine kinases (JAK1–3, TYK2) transduce signals from multiple cytokines, hormones, and growth factors to regulate cell growth, differentiation, and immune responses. JAKs are structurally conserved with characteristic 2 kinase or kinase-like domains: the catalytically active C-terminal tyrosine kinase domain (JH1) and the regulatory pseudokinase domain (JH2). Almost all JAK

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inhibitors (Jakinibs) are type I inhibitors, which engage the ATP-binding pocket of JH1 in active conformation and thereby disrupt the catalytic activity. The dual regulatory function (negative and positive) of JH2 is well-established in JAK family members, and JH2 is known to bind ATP but shows low or is devoid of catalytic activity (for review, see ref. 1).

Pathogenic signaling in rheumatic diseases is driven by the aberrant cytokine milieu with elevated levels of proinflammatory cytokines, many of which signal through the JAK/STAT pathway (for review, see refs. 2,3). Currently 5 Jakinibs (tofacitinib, baricitinib, peficitinib, filgotinib, and upadacitinib) have been approved for the treatment of 1 or multiple rheumatic diseases. Five more Jakinibs (deucravacitinib, decernotinib, itacitinib, ritlecitinib, and brepocitinib) are currently being evaluated or have previously been evaluated in clinical phase II or III trials. The first approved Jakinibs for rheumatic diseases (tofacitinib, baricitinib, and peficitinib) are pan-Jakinibs that target multiple JAKs (4).

Subsequently, the development of Jakinibs has focused on JAK- or pathway-selective Jakinibs, with the aim of obtaining desired disease specificity and/or avoiding JAK2 inhibition and hematopoietic effects in inflammatory indications. The first selective JAK1-targeting Jakinibs, filgotinib and upadacitinib, together with the atopic dermatitis indicated drug abrocitinib, have recently been approved for use in clinics. Furthermore, novel mechanistic approaches for JAK inhibition are entering the field of rheumatic diseases. For example, the first JH2-targeting inhibitor deucravacitinib and the first covalent inhibitor ritlecitinib are in phase II and III clinical trials (5,6), and several novel inhibitor types are at preclinical stage.

Information on inhibitor selectivity forms the basis for understanding differences in the efficacy and safety of Jakinibs, but the evaluation has been hampered by the lack of comprehensive head-to-head studies and assay-to-assay variation in individual studies (7,8). Currently, in vitro efficacy and selectivity data are only available for individual or a small panel of 3 or 4 Jakinibs (9–11). Here, we performed in vitro head-to-head profiling of Jakinibs that are either approved or have been clinically evaluated for the treatment of 1 or multiple rheumatic diseases. We analyzed these 10 Jakinibs (Table 1) for JAK isoform selectivity by assaying their 1) inhibition of catalytic activity, 2) binding to kinase and regulatory pseudokinase domains, 3) inhibition of cytokine signaling in peripheral blood from healthy volunteers, and 4) inhibition percentages of cytokine-induced STAT phosphorylation at clinically relevant Jakinib concentrations in vitro in isolated peripheral blood mononuclear cells (PBMCs) from patients with rheumatoid arthritis (RA) and from healthy donors.

MATERIALS AND METHODS

Additional details are described in the Supplementary Methods, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42547. Briefly, we selected 10 rheumatic disease–evaluated Jakinibs (Table 1 and Supplementary Table 1, available at https://onlinelibrary.wiley.com/doi/10.1002/art.42547) for protein- and cell-based efficacy and selectivity analyses. Recombinant human JAK proteins were expressed in insect cells using the Bac-to-Bac expression system and purified using affinity and size-exclusion chromatography.

We determined the effects of inhibitors on the catalytic activity of recombinant JAK JH2 and JH1 proteins using the LANCE Ultra kinase assay (PerkinElmer) at reaction conditions recommended by the manufacturer. Assays were performed at physiologic ATP (1 m*M*), and inhibitors were assessed at concentrations ranging from 0.1 n*M* to 100 μ*M*. We included vehicle controls (H_2O for ritlecitinib, DMSO for other inhibitors) in all experiments. After mixing all reaction components, we detected phosphorylation of peptide substrate by measuring fluorescence resonance energy transfer (at 320 nm/665 nm excitation/emission) in 5-minute intervals for 30 minutes using the EnVision Multilabel Plate Reader (PerkinElmer). The concentration giving half-maximal inhibition (IC_{50}) and fold-IC_{50} values were calculated. Results are presented as averages of triplicate samples and representative of 2–4 individual experiments. One-way analysis of variance (ANOVA) with Dunnett's multiple comparisons post hoc test was used to assess the significant differences in inhibition of kinase activity between the JAK family members.

We assessed the binding of inhibitors to JAK JH1 and JH2 in fluorescence polarization assays using the Bodipy FL–labeled ATP pocket binder as a tracer. Jakinibs were assessed at concentrations ranging from 0.1 n*M* to 100 μ*M*, and the level of inhibitor bound to the JH1 and JH2 ATP-binding pocket was detected by measuring fluorescence polarization (at 480 nm/535 nm excitation/emission) using the EnVision Multilabel Plate Reader (PerkinElmer). We calculated the IC_{50} of tracer binding and the dissociation constant (K_d) of ligand binding. Results are presented as averages of 3–6 individual reactions.

For effects on cytokine signaling in T cells and monocytes, we collected human peripheral blood from 3 healthy volunteers who gave informed consent for the study. All research with human subjects was carried out in accordance with the ethics principles of the Declaration of Helsinki and in accordance with protocols approved by the Tampere University Hospital or Helsinki University Hospital Ethics Committees. Blood was incubated with Jakinibs (concentrations from 0.1 n*M* to 10 μ*M*) or vehicle controls (DMSO or H_2O) followed by stimulation with interferon-α (IFNα), IFNγ, interleukin-2 (IL-2), IL-6, or granulocyte–macrophage colony-stimulating factor (GM-CSF). After samples were fixed and red blood cells were lysed, cells were permeabilized with methanol and stored at −80°C until analysis. Samples were fluorescent barcoded in sets of 18 samples using Pacific Orange and Pacific Blue NHS esters, and each sample set was stained for surface markers (CD3, CD4, CD33) and pSTATs (1, 3, and 5). (Supplementary Table 2, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42547) We analyzed samples on FACSAria Fusion (BD).

Table 1. Top clinical status of JAK inhibitors for rheumatic diseases, JAK selectivity, and pseudokinase-binding affinity*

Inhibitor	Top clinical status in rheumatic diseases	JAK selectivity (activity fold-IC_{50})				Binding (K_d, nM) to pseudokinase domain			
		JAK1	JAK2	JAK3	TYK2	JAK1	JAK2	JAK3	TYK2
pan-JAK inhibitors									
Baricitinib	Approved: RA	3.0	1.0	70	34	ND	ND	ND	ND
Peficitinib	Approved (Japan, Korea): RA	1.0	2.3	1.1	10	2539	ND	ND	2870
Tofacitinib	Approved: RA, PsA, JIA, AS	1.0	1.4	1.9	60	ND	ND	ND	ND
JAK1-targeted inhibitors									
Filgotinib	Approved (Europe): RA	1.0	1.9	264	11	ND	ND	ND	ND
Upadacitinib	Approved: RA, AS, PsA, nonradiographic axial SpA	1.0	2.4	136	194	ND	ND	ND	ND
Itacitinib	Phase II: RA, SSc	1.0	39	5161	941	ND	ND	ND	ND
JAK3-targeted inhibitors									
Ritlecitinib	Phase II: RA	192	361	1.0	4056	ND	ND	ND	ND
Decernotinib	Phase III: RA†	2.3	4.1	1.0	244	ND	ND	ND	ND
TYK2 or JAK1/TYK2-targeted inhibitors									
Deucravacitinib	Phase III: PsA	ND	ND	ND	ND	<1	27	946	<1
Brepocitinib	Phase II: SLE, PsA	1.0	1.0	101	3.1	ND	ND	ND	ND

* The fold concentration giving half-maximal inhibition (fold-IC_{50}) for JAK selectivity was calculated based on detected IC_{50} (Supplementary Figure 1) of JAK inhibitors (Jakinibs) for the catalytic activity of JAK family members. K_d = dissociation constant; RA = rheumatoid arthritis; PsA = psoriatic arthritis; JIA = juvenile idiopathic arthritis; AS = ankylosing spondylitis; PsA = psoriatic arthritis; SpA = axial spondyloarthritis; SSc = systemic sclerosis; SLE = systemic lupus erythematosus; ND = inhibition or binding not detected.
† Development presumably discontinued (no recent reports).

We calculated IC_{50} values for each cell type, cytokine, and pSTAT combination in which the cytokine stimulation–induced pSTAT signal increased by ≥50%, which is considered the threshold for substantial stimulation and the level in which inhibition can be reliably measured. Results are presented as averages of 3 individual experiments. ANOVA with Dunnett's multiple comparisons post hoc test was used to assess the differences in cytokine signaling pIC_{50} between Jakinibs. Wilcoxon's signed rank test was used to compare cell-type effects in inhibition of IL-6–pSTAT3 and STAT-originated effects in inhibition of IL-6 and IFNγ signaling.

Inhibition percentages for Jakinibs at clinically relevant concentrations (see Supplementary Methods, available at https://onlinelibrary.wiley.com/doi/10.1002/art.42547) were determined in vitro in isolated PBMCs from 6 RA patients and from 3 age- and sex-matched healthy donors who gave an informed consent for the study. Frozen and revived PBMCs were incubated with clinically relevant concentrations of Jakinibs or vehicle control, followed by incubation with phosphate buffered saline or cytokines IFNα, IFNγ, IL-2, IL-6, or GM-CSF. After samples were fixed with paraformaldehyde, cells were permeabilized with methanol and stored at –80°C until analysis. Samples were fluorescent barcoded in sets of 15 samples using Pacific Orange and Pacific Blue NHS esters, and each sample set was stained with surface markers (CD3, CD4, CD33) and either pSTAT1 (IFN-stimulated samples), pSTAT3 (IL-6–stimulated samples), or pSTAT5 (IL-2–and GM-CSF–stimulated samples). The samples were analyzed on CytoFLEX S (Beckmann Coulter).

Inhibition percentages were calculated for each cell type, cytokine, and pSTAT combination in which the cytokine stimulation–induced pSTAT signal increased by ≥50%. IL-2 stimulated only a minor population of CD4+ T cells, and thus inhibition percentage was calculated from ratio of pSTAT5-positive cells. ANOVA with Tukey's multiple comparisons post hoc test was used to assess differences in inhibition percentage among cell type, cytokine, and pSTAT combinations. Wilcoxon's signed rank test was used to compare cell-type effects in inhibition of IL-6–pSTAT3 and IFNα–pSTAT1 in isolated PBMCs, and paired *t*-tests with multiple comparisons correction was used to compare inhibition percentage in PBMCs from healthy controls versus RA patients.

RESULTS

JAK selectivity of Jakinibs and binding to pseudokinase domain. Inhibition profiles of Jakinibs on the catalytic activity of JAKs was assessed using recombinant JH2–JH1 tandem domain constructs. Most of the Jakinibs effectively suppressed kinase activity of 2–3 JAK family members (Table 1). Pan-Jakinibs targeted JAK1, JAK2, and JAK3 with high potency (IC_{50} <10 n*M*) except baricitinib, which was less effective toward JAK3 (Table 1 and Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42547). TYK2 was poorly inhibited by pan-Jakinibs.

The JAK1-targeted inhibitors inhibited numerically most potently JAK1, as indicated by the IC_{50} values in Supplementary

Figure 1. However, itacitinib was the only clearly JAK1-selective inhibitor (39-fold over JAK2), whereas filgotinib and upadacitinib also targeted JAK2 (2-fold selectivity for JAK1 over JAK2) (Table 1). Notably, JAK3 and TYK2 were not potently inhibited by JAK1-targeted Jakinibs. JAK3-targeted decernotinib showed modest selectivity for JAK3, whereas JAK1/TYK2–targeted brepocitinib inhibited the activity of JAK1, JAK2, and TYK2 (Table 1).

All of the pan-JAK and JAK1-targeted Jakinibs that we analyzed, as well as decernotinib and brepocitinib, are type I inhibitors, which reversibly compete with ATP for binding to the JH1 domain. Covalent JAK3-targeted ritlecitinib and allosteric deucravacitinib differed from type I inhibitors in their JAK inhibition profiles. Ritlecitinib was highly selective for JAK3 (900- to 2,500-fold selectivity over other JAKs), whereas TYK2-targeted deucravacitinib did not inhibit activity of any recombinant JAK in vitro and was the only inhibitor to bind at (sub)nanomolar-scale affinity to the regulatory pseudokinase domains (TYK2, JAK1, to lesser extent JAK2) (Table 1).

Cytokine selectivity of Jakinibs. Inhibition of cytokine signaling in T cells and monocytes was assessed in whole blood using cytokines representing canonical JAK1/JAK3 (IL-2), JAK1/JAK2/TYK2 (IL-6), JAK1/TYK2 (IFNα), JAK1/JAK2 (IFNγ), and JAK2/JAK2 (GM-CSF) signaling pathways and using STAT phosphorylation as a readout. Inhibition by the Jakinibs varied from pan-cytokine to highly specific inhibition of IL-2 or IFNα, and significant differences among the Jakinibs were evident for each cytokine (Supplementary Table 3, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42547).

Next, we assessed the inhibition percentages of cytokine-induced STAT phosphorylation for clinically relevant concentrations of each Jakinib in vitro in isolated PBMCs from 6 RA patients, who had active disease despite traditional treatment, and from age- and sex-matched healthy controls (Supplementary Table 4, available at https://onlinelibrary.wiley.com/doi/10.1002/art.42547). Cytokine signaling by IL-2, IL-6 (only in CD4+ T cells), IFNα, and IFNγ was more prone to inhibition by Jakinibs in cells from RA samples compared with that shown in cells from healthy control samples (Supplementary Figure 2, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42547).

In PBMCs from RA patients, pan-JAK and JAK1-targeted Jakinibs showed similar inhibition profiles at clinically applied doses, but the inhibition levels varied (Figure 1). These Jakinibs mainly inhibited signaling mediated by JAK1-dependent cytokines IL-2 and IFNα, whereas inhibition of JAK2-dependent GM-CSF was weak, and differences occurred in tendency for IFNγ and IL-6 inhibition (Figure 1; statistical significance in Supplementary Table 5, available on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42547).

The novel Jakinibs, ritlecitinib and deucravacitinib, demonstrated high specificity in inhibition of JAK/STAT–mediated cytokine signaling. Ritlecitinib was highly selective for JAK1/JAK3–mediated IL-2-signaling, and deucravacitinib inhibited JAK1/TYK2–mediated IFNα signaling with high efficacy and specificity (Figure 1).

Effects of cell types and STAT isoforms on inhibition of cytokine signaling by Jakinibs. IL-6 stimulated STAT3 phosphorylation (in whole blood and in isolated PBMCs), and IFNα stimulated STAT1 (in isolated PBMCs) in both CD4+ T cells and monocytes, enabling the assessment of the cell-type–specific effects of Jakinibs. Interestingly, inhibition of IL-6–pSTAT3 signaling was more effective in CD33+ monocytes than in CD4+ T cells (Figures 2A and E), whereas inhibition of IFNα was not cell-type dependent (Figure 2F). IL-6 also induced the phosphorylation of STAT1 and STAT5 (in addition to its canonical isoform STAT3) in CD4+ T cells, and IFNγ induced STAT3 and STAT5 phosphorylation in addition to its canonical isoform STAT1 in CD33+ monocytes. Inhibition of the canonical STAT family members by Jakinibs was weaker compared with that shown in other stimulated STAT family members (Figures 2B and C).

DISCUSSION

The clinical response to Jakinibs is the summed effect of several parameters. In addition to disease and patient-derived factors, the binding affinities and enzymatic inhibition of Jakinibs translate into effects on cellular signaling. Selectivity data are presently available for individual or a small panel of 3–4 Jakinibs. However, variations in selectivity and potency outcomes among individual studies make their comparison practically impossible. Our present study was designed to provide a comprehensive in vitro analysis of direct JAK-related effects (JAK selectivity and cytokine inhibition profiles in cells from heathy controls and RA patients) in a comparison of 10 Jakinibs that have been evaluated for the treatment of rheumatic diseases.

Selectivity of Jakinibs can be viewed from 2 perspectives. Highly specific inhibitors can achieve more predictable responses and have less undesired side effects by having reduced off targets, whereas a wider inhibitory range can increase clinical efficacy (but possibly also side effects) by inhibiting several targets. The panel of Jakinibs that we analyzed included 5 globally approved Jakinibs for the treatment of RA (namely, the pan-Jakinibs tofacitinib, baricitinib, and peficitinib and the JAK1-targeted inhibitors filgotinib and upadacitinib) and 5 inhibitors that are or have been in clinical-stage evaluation for rheumatic disease(s).

In the domain-binding analysis, almost all Jakinibs bound exclusively to the JH1 domains (data not shown); the only

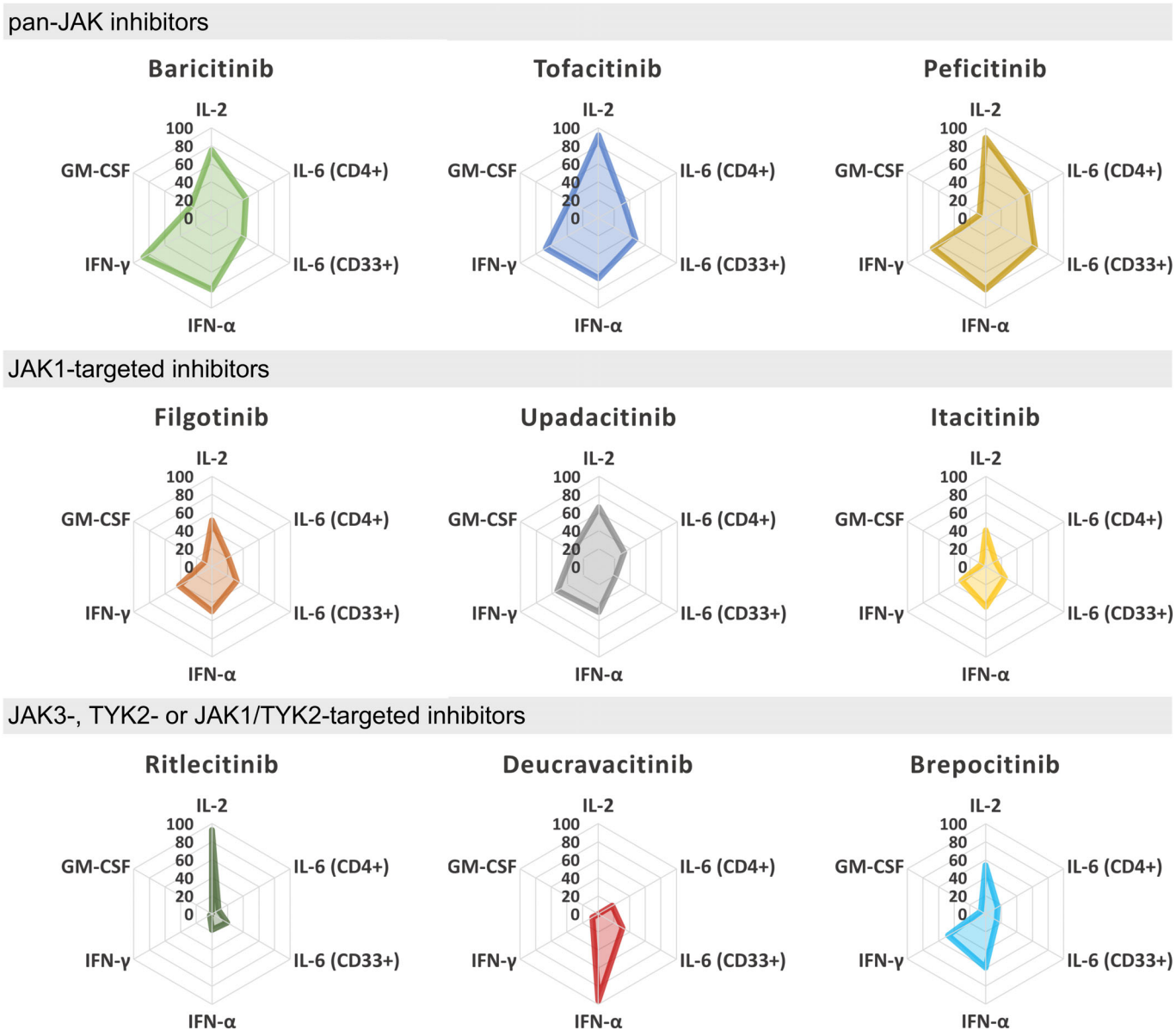

Figure 1. Cytokine inhibition profiles of JAK inhibitors (Jakinibs) in immune cells from patients with rheumatoid arthritis (RA). The indicated inhibition percentages of cytokine-induced STAT phosphorylation for clinically relevant concentrations of Jakinibs were calculated based on measured pSTAT levels after incubation with Jakinib and subsequent cytokine stimulation in isolated, frozen, and revived peripheral blood leukocyte populations. Interleukin-2 (IL-2) and interferon-α (IFNα) inhibition results were measured in CD4+ T cells, and IFN-γ and granulocyte–macrophage colony-stimulating factor (GM-CSF) inhibition results were measured in CD33+ monocytes.

exception in this pattern was deucravacitinib, which bound only to JH2. The binding affinity to the kinase domain generally correlated well with kinase activity inhibition in vitro. However, our data indicate that, although the RA-indicated Jakinibs inhibited the in vitro activity of multiple JAKs, cytokine inhibition in human blood leukocytes was the most potent for JAK1-dependent cytokines IL-2, IL-6, and interferons, aligning with previous reports (9–11). Significant differences in cellular cytokine IC_{50} levels were observed between these Jakinibs; however, when inhibition at clinically relevant concentration was considered, the cytokine inhibition profiles were comparable, aligning with the similarly observed overall clinical efficacy and safety results.

JAK2 inhibition with effects on hematopoiesis is considered an undesired characteristic for Jakinibs indicated for inflammatory diseases; thus, the development of selective Jakinibs has been aimed at increasing JAK1/JAK3/TYK2 selectivity over JAK2 in inflammatory indications such as RA and other rheumatic diseases. Although most Jakinibs indicated for RA effectively suppressed JAK2 catalytic activity in vitro, the tendency for inhibition of JAK2-mediated GM-CSF signaling in human monocytes was

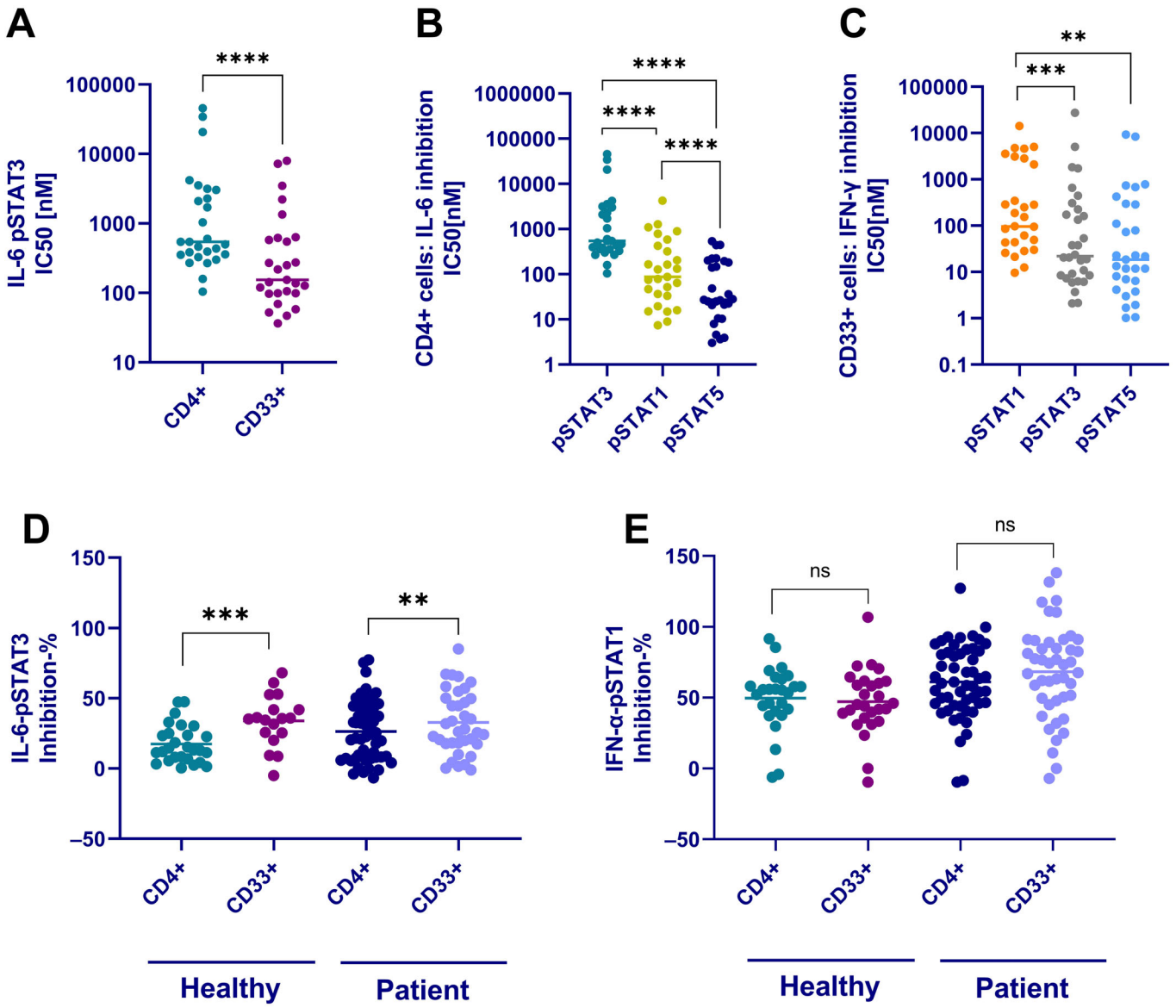

Figure 2. Cell-type–dependent and pSTAT-dependent variations in inhibition of cytokine signaling by Jakinibs. **A–C**, Concentrations giving half-maximal inhibition (IC_{50}) of Jakinibs for IL-6 signaling using pSTAT3 readout in CD4+ T cells and in CD33+ monocytes (**A**); for IL-6 signaling using pSTAT3, pSTAT1, and pSTAT5 readouts in CD4+ T cells (**B**), and for IFNγ signaling using pSTAT3, pSTAT1, and pSTAT5 readouts in CD33+ monocytes (**C**). **D** and **E**, Inhibition percentage at clinically relevant concentrations of Jakinibs for IL-6 signaling using pSTAT3 readout in CD4+ T cells and in CD33+ monocytes from healthy donors or RA patients (**D**) and for IFNα signaling using pSTAT1 readout in CD4+ T cells and in CD33+ monocytes from healthy donors or RA patients (**E**). Dots in **A–C** are IC_{50} data for JAKinibs from 3 individual experiments. Dots in **D** and **E** are inhibition percentages of Jakinibs from 3 (healthy control cells) or 6 (RA cells) individual measurements. Lines represent the median value. **** = $P < 0.0001$; *** = $P < 0.0002$; ** = $P < 0.0021$; ns = not significant, using Wilcoxon's signed rank test.

low. The underlying reason for the difference between inhibition of enzymatic activity and cellular inhibition of JAK2 remains unknown.

Ritlecitinib and deucravacitinib use distinct JAK-targeting mechanisms compared with the reversible type I Jakinibs. Ritlecitinib couples covalently to a unique Cys^{909} in the kinase domain ATP-binding pocket of JAK3 (12), whereas deucravacitinib targets the ATP-binding pocket in the regulatory pseudokinase domain (5). These novel Jakinibs showed high specificity: ritlecitinib inhibited JAK3 activity and JAK1/JAK3–mediated IL-2 signaling at high selectivity, and deucravacitinib inhibited JAK1/TYK2–mediated IFNα signaling with high specificity, even though it did not inhibit TYK2 catalytic activity in vitro (Supplementary Figure 1). Our findings indicate a unique mechanism of action for deucravacitinib that requires a full-length kinase and/or other cellular components for inhibition of TYK2-mediated signaling.

Alternative approaches in JAK inhibitor development, besides the type I ATP-competitive inhibitors, may provide

improved precision and safety in treatment of rheumatic diseases. In a phase II trial, ritlecitinib decreased RA disease activity without inducing anemia, neutropenia, or lipoprotein changes; however, additional studies are needed to confirm its efficacy and safety (12). In a phase II clinical trial of psoriatic arthritis, deucravacitinib demonstrated good efficacy and an improved safety profile with no observed tuberculosis, herpes zoster, or opportunistic infections, changes in lipid levels, or changes in other laboratory parameters associated with JAK1–3 inhibition (13). The emergence of allosteric inhibitors that do not directly impair catalytic activity highlights the importance of a versatile methodology for assessing efficacy and selectivity. The intriguing potential of novel Jakinibs also emphasizes the need for detailed understanding of molecular regulation of JAK kinases to guide the development of isoform-selective Jakinibs. Ritlecitinib is currently in phase III clinical trials for alopecia areata, its main indication, whereas deucravacitinib most recently obtained approval from the US Food and Drug Administration without black box warnings for the treatment of psoriasis, is in phase III trials for psoriatic arthritis, and is in phase II trials for systemic lupus erythematosus and inflammatory bowel disease.

Our results also point to the possible relevance of cell-type–originated differences within Jakinibs. Jakinibs suppressed IL-6 signaling (100 ng/ml IL-6) more effectively in monocytes than in CD4+ T cells. A similar difference has been observed with upadacitinib when using 400 ng/ml of IL-6 (14) but not in assays using 10–30 ng/ml of IL-6 (9,10). The underlying mechanism for the cell-type difference in IL-6 inhibition is not known, but it may be influenced by cell-type differences in the expression of signaling proteins or in drug elimination processes (e.g., via interaction with drug transporters) (15). These results, together with less effective inhibition of canonical STAT over secondary STATs in IL-6 and IFNγ signaling and the lack of an inhibitory effect of deucravacitinib on TYK2 kinase activity, indicate clear differences between Jakinibs that cannot be explained by direct inhibition of kinase activity.

Our study has some limitations. First, the analysis focused on direct effects of Jakinibs on JAK kinases and JAK/STAT signaling in immune cells of healthy donors and RA patients, and thus the off-target effects or the effects on immune cell activation or proliferation of Jakinibs were outside the scope of this study. Second, the in vitro evaluation of the Jakinibs could enable only predictive conclusions on the inhibitory effects of Jakinibs in vivo. JAK/STAT signaling has multiple functions in the pathogenesis of RA; for example, tofacitinib inhibits cellular metabolism ex vivo in RA synovial samples (16). Future studies should compare the effects of Jakinibs on these parameters and on T cell activation and proliferation.

In conclusion, our study provides directly comparable data on the effects and characteristics of Jakinibs and significantly adds to our understanding about the unique inhibition mechanisms of deucravacitinib and high cytokine specificity of deucravacitinib and ritlecitinib compared with other Jakinibs evaluated in rheumatic diseases. Results from late-stage clinical trials of these emerging Jakinibs will confirm whether their specificity is translated into appropriate clinical efficacy and/or improved safety.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Virtanen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Virtanen, Silvennoinen.
Acquisition of data. Virtanen, Palmroth, Liukkonen, Kurttila.
Analysis and interpretation of data. Virtanen, Haikarainen, Isomäki, Silvennoinen.

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