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Emerging principles of cytokine pharmacology and therapeutics

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Abstract

Cytokines are secreted signalling proteins that play essential roles in the initiation, maintenance and resolution of immune responses. Although the unique ability of cytokines to control immune function has garnered clinical interest in the context of cancer, autoimmunity and infectious disease, the use of cytokine-based therapeutics has been limited. This is due, in part, to the ability of cytokines to act on many cell types and impact diverse biological functions, resulting in dose-limiting toxicity or lack of efficacy. Recent studies combining structural biology, protein engineering and receptor pharmacology have unlocked new insights into the mechanisms of cytokine receptor activation, demonstrating that many aspects of cytokine function are highly tunable. Here, we discuss the pharmacological principles underlying these efforts to overcome cytokine pleiotropy and enhance the therapeutic potential of this important class of signalling molecules.

Cytokines, such as interleukins and interferons, regulate diverse aspects of physiology including immunity, development, growth and tissue repair[1]. Given the central role of cytokines in health and disease, many attempts have been made to harness the power of cytokines for therapeutic use.

Although cytokine antagonists have found success in clinical contexts such as autoimmunity, there are few examples of cytokine receptor agonists that have been successfully deployed in the clinic, despite the plethora of cytokines that are encoded in the human genome[2]. This is due, in large part, to the complex biology of natural cytokines, which often promote countervailing effects on multiple cell types. Although this pleotropic activity is crucial for ensuring homeostasis and regulating the natural biological functions of cytokines, it has impeded clinical development by limiting efficacy and contributing to dose-limiting toxicity[3].

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Competing interests

K.C.G. is the founder of Synthekine Therapeutics. R.A.S., C.R.G. and K.C.G. are inventors on patents relating to molecules discussed in this Review that are being commercialized.

New strategies are needed to realize the full potential of cytokines as therapeutic agents, and the past 5 years have seen a resurgence of interest in both academia and biotech to tailor cytokine function using a wide range of protein engineering approaches. By applying insights from structural biology, medicinal chemistry and classical receptor pharmacology, mechanism-based protein engineering strategies that enable the systematic 'tuning' of cytokine receptor activity have been developed. These principles form the basis of a new field of cytokine pharmacology.

In this Review, we highlight the principles of cytokine receptor pharmacology that these efforts have illuminated and discuss how these principles can enable the clinical translation of new and improved cytokine-based therapeutics. We primarily focus on strategies to directly modulate how cytokines engage and activate their receptors, and describe notable examples that highlight key concepts in cytokine receptor pharmacology (TABLES 1 and 2). A comprehensive discussion of all cytokine-based therapeutics currently in preclinical or clinical studies is beyond the scope of this Review, and this topic has recently been thoroughly covered elsewhere[4,5].

Overview of cytokine biology

Cytokines are secreted growth factors that initiate signalling on target cells by engaging the extracellular domains (ECDs) of cell surface receptors to compel receptor dimerization, in a similar manner to other single-pass transmembrane receptors such as `receptor tyrosine kinases` (RTKs)[6–8] (FIG. 1a). Although early studies suggested that cytokine receptors were pre-dimerized on the plasma membrane[9], more recent experiments using single-molecule tracking experiments on cells with physiological expression levels of receptors show clear ligand-dependent dimerization[10]. This dimerization of receptor ECDs brings together the intracellular receptor-associated `Janus kinases` (JAKs), which dimerize via their pseudokinase domains to position the kinase domains into an appropriate orientation and proximity for signalling[11]. The dimerized JAKs *trans*-phosphorylate each other as well as tyrosine residues on the receptor intracellular domains, enabling the recruitment of transcription factors known as signal transducer and activator of transcription (STAT) proteins[12]. Upon receptor binding, STAT proteins are in turn phosphorylated by the receptor-bound JAKs, triggering STAT dimerization, nuclear translocation and transcriptional activation[13,14] (FIG. 1a). Despite the apparent simplicity of this signalling cascade, the human genome encodes ~40 distinct cytokine receptors, 4 JAKs and 7 STATs, resulting in a complex signalling system capable of eliciting a wide range of diverse biological outcomes[15] (FIG. 1b).

Structural and functional studies into the mechanism of cytokine receptor signalling have shown that most cytokines contain both high-affinity and low-affinity receptor binding sites[16,17] (FIG. 1a). This has led to a two-step model of receptor activation in which the cytokine first engages the high-affinity receptor subunit, before recruiting a second receptor subunit from elsewhere on the cell membrane via the low-affinity binding site[10,18] (FIG. 1a). This sequential, asymmetric binding can be exploited by mechanism-based engineering strategies to modulate cytokine activity. In many cases, the high-affinity receptor subunit is a cytokine-specific private receptor that dictates cellular specificity as well as the dose

sensitivity, or EC_{50}, of the cytokine[16] (FIG. 1c). By contrast, low-affinity receptor subunits are often shared between multiple cytokines, and primarily influence the efficiency of complex assembly and, in turn, the maximal strength (E_{max}) and duration of receptor signalling[17,19] (FIG. 1c).

Cytokine pleiotropy, which describes the ability of cytokines to act on multiple cell types both within the immune system and on peripheral tissues, is both a critical homeostatic property of natural cytokines and may be the major barrier to their clinical translation[15] (FIG. 1b). Some cytokines, such as type 1 interferons, act on all nucleated cells[20], whereas others, such as type 3 interferons, act more selectively on a restricted subset of cell types and tissues[21]. Critically, individual cytokines can often elicit divergent functional responses on different cell types. For example, interleukin-2 (IL-2) acts on both conventional effector T cells as well as immunosuppressive `regulatory T cells` (T_{reg} cells)[22]. To regulate these pleotropic effects, cells naturally differ in cytokine sensitivity based on expression of receptors and signalling pathway components[15]. In combination with localized cytokine secretion, this differential sensitivity can create gradients within tissues that spatially restrict signalling activation. These parameters are actionable from a protein engineering standpoint by modifying how cytokines interact with their receptors.

Cytokine-based therapeutics

Cytokine antagonists have achieved remarkable clinical success, for example in blocking the pro-inflammatory actions of tumour necrosis factor-α (TNFα), IL-6, IL-17 and IL-23 (REF.[23]). By comparison, immunomodulatory cytokines or cytokine receptor agonists have largely fallen short of attaining widespread adoption as therapeutics beyond limited experimental uses in academic medical centres[2,24]. The first cytokines clinically approved in the United States were the pro-inflammatory type 1 and type 2 interferons, including IFNα2b in 1986 (hairy cell leukaemia) and IFNγ in 1991 (chronic granulomatous disease)[25]. Despite the striking immunostimulatory and antiviral effects of these interferons in preclinical models, their use has been restricted to a narrow range of diseases and patient populations, due in large part to highly unfavourable safety profiles[2,22].

Similarly, the cytokine IL-2 was discovered as a potent T cell growth factor in 1976 (REFs.[26,27]) and was subsequently shown to promote striking antitumour immune responses in the context of melanoma and renal cell carcinoma[28–30]. Although recombinant IL-2 was eventually clinically approved for use in renal cell carcinoma in 1992 and metastatic melanoma in 1996, systemic administration of IL-2 induces a durable response in a small percentage of patients (8–10%) while also eliciting severe and sometimes fatal adverse effects, limiting its broader clinical application[28,29]. More recently, modified IL-2 variants with reduced affinity for IL-2Rα (CD25) and other modifications such as PEGylation or antibody fusions have also progressed to clinical trials[31,32]. Although these are still ongoing, current evidence suggests that these approaches have not substantially improved the efficacy or tolerability of IL-2 treatment.

In addition to interferons and IL-2, several other immunomodulatory cytokines have progressed to clinical trials including IL-7, IL-10, IL-12, IL-18, IL-21 and IL-22, but have so

far failed to achieve US Food and Drug Administration (FDA) approval[5]. This is largely due to deleterious pro-inflammatory side effects arising from the extensive `cellular pleiotropy` of these molecules. Indeed, the most successful cytokine drugs to date have been erythropoietin (EPO), thrombopoietin (TPO), granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF), all regulators of haematopoiesis whose receptors are expressed on a far more restricted subset of cell types[33,34]. Thus, whereas some natural cytokines with narrow biological functions and few target cell types may be suitable drugs without modification, most cytokines are highly pleiotropic and likely require substantial alterations to endow them with drug-like properties.

Recent efforts to reduce toxicity of cytokine-based therapies have largely focused on selective targeting of wild-type cytokines to disease tissues through various approaches including local cytokine injection[35], injection of cytokine-producing oncolytic viruses[36,37], adoptive transfer of cytokine-producing cells[38,39], administration of pro-cytokines that require processing from tumour-associated enzymes for activity[40] and tissue targeting of natural cytokines using binding proteins or antibodies that promote enrichment on cell types or tissues of interest including tumours[41–47] (BOx 1). Although these techniques can help limit the cellular pleiotropy of cytokines and mitigate off-target toxicity on peripheral tissues, additional approaches are needed to address the issue of `functional pleiotropy` resulting from the ability of cytokines to activate multiple cell types within a given tissue, or to activate multiple signalling pathways within a given cell type[48] (FIG. 1b). Critically, such functional pleiotropy can limit therapeutic efficacy, for example by simultaneously inducing both pro-inflammatory and anti-inflammatory gene expression programmes[49].

The clinical history of cytokines emphasizes the need for new pharmacologic approaches that exploit the natural regulatory mechanisms of cytokine receptor signalling, in order to exert precise control over cytokine function and unlock their full therapeutic potential.

Concepts in receptor pharmacology and their application to cytokines

Insights from GPCR medicinal chemistry

The most extensively characterized and therapeutically exploited family of cell signalling receptors are G-protein-coupled receptors (GPCRs)[50]. GPCRs are seven-pass transmembrane proteins and are the targets of many small-molecule drugs including opioid analgesics, antihistamines and antipsychotics[51]. Over the past several decades, extensive study of GPCR signalling using medicinal chemistry approaches has illuminated many foundational principles of receptor pharmacology[51]. Although the mechanism of signalling by GPCRs differs substantially from that of cytokine receptors, these insights provide a powerful conceptual framework for inspiring and understanding recent advances in cytokine pharmacology described below.

As with cytokine receptors, GPCRs induce intracellular signalling following binding to an extracellular ligand[52]. In the case of GPCRs, extracellular ligand binding within pockets of the seven-pass transmembrane bundle triggers a conformational change at intracellular binding sites to alter interaction with downstream proteins such as `G proteins` and β-arrestins[53,54]. Ligands that activate receptor signalling to the same extent as the endogenous

ligand are defined as full agonists, those that inhibit the action of endogenous ligands are called antagonists and those that inhibit basal signalling are referred to as inverse agonists[55]. Agonists can further be subdivided into ‘super agonists’, which stimulate GPCR signalling with greater potency than endogenous ligands, and ‘partial agonists’, which induce submaximal signalling even at saturating ligand concentrations.

GPCR partial agonists such as buspirone (serotonin 5-HT_{1A} receptor agonist) and buprenorphin (μ-opioid receptor agonist) have shown clinical success in the treatment of anxiety and chronic pain, respectively[55]. The defining feature of partial agonists is that they induce submaximal responses even at saturating concentrations, and therefore have lower signalling E_{max} independent of their receptor affinity. As a result, partial agonists can achieve therapeutically desired submaximal responses across a wide range of ligand concentrations — bypassing clinical challenges related to the narrow therapeutic windows offered by many full agonists. In addition, because partial agonists often retain high affinity for their receptor at the same binding site as endogenous ligands, in some contexts these compounds can compete with endogenous agonists to partially suppress receptor signalling.

Similar to cytokine receptors, GPCRs are also capable of simultaneously activating multiple intracellular signalling pathways including heterotrimeric G proteins, β-arrestin and kinases[50]. Pharmacological studies of various GPCR families have identified agonists that, relative to the endogenous ligand, activate some downstream signalling pathways but not others. These ligands are termed ‘functionally selective’ or ‘biased’ agonists, and in theory provide a powerful way of decoupling desirable and undesirable cellular responses downstream of a given receptor[54]. Although the full therapeutic implications of biased GPCR agonists are yet to be determined, the first G-protein-biased GPCR agonist was recently approved for clinical use[56] with many more currently being investigated.

Insights from natural cytokines

Given that the structural mechanism of cytokine signalling differs substantially from that of GPCRs, it has historically been unclear whether cytokine receptors could be pharmacologically modulated in analogous ways. However, examples from natural cytokine ligands have suggested that cytokine receptor super agonists, partial agonists and biased agonists are indeed possible.

In particular, studies into the biology of type 1 interferons have demonstrated that cytokines can elicit distinct agonist profiles through the same receptor complex[57]. Type 1 interferons comprise 16 members, all of which signal through a heterodimeric receptor consisting of interferon-α/β receptor subunit 1 (IFNAR1) and IFNAR2. Notably, the different type 1 interferon subtypes can elicit a wide range of functional responses, exhibiting varying degrees of antiviral and anti-proliferative activities, despite all signalling primarily through the transcription factors STAT1/2 (REF.[58]). Structural studies of type 1 interferon receptor complexes have revealed that although the geometry of these receptor complexes is highly conserved between ligands, relative differences in IFNAR1 binding affinity across type 1 interferons result in differences in ligand–receptor complex stability[59–61]. As a result, interferon paralogues display varying levels of signalling strength and potency, ultimately yielding differences in gene expression responses[62].

Another key feature of cytokine biology is the existence of shared receptor subunits, such as common γ-chain (γ_c), gp130 and IL-10Rβ, which can engage multiple cytokine ligands in the context of different private receptor chains[17]. Moreover, in some cases, such as in the IL-4 and IL-6 family cytokines, individual ligands can act through multiple different receptor complexes, eliciting a distinct signalling response depending on which receptor type is engaged[19,63]. The highly modular nature of cytokine receptor pairing and ligand engagement greatly enhances the pleiotropy and redundancy of these natural systems, and highlights the importance of receptor subunit composition in dictating cytokine function[64].

Tunable parameters in cytokine pharmacology: receptor affinity, geometry and composition

The structural and mechanistic studies of natural cytokine systems described above have established that cytokine function is influenced by which receptors they bind, their affinity for those receptors and the structure of the resulting signalling complexes[65]. However, the vast majority of natural cytokine systems access only a narrow range of the possible signalling outputs that may be feasible using engineered ligands. Below, we discuss recent efforts to pharmacologically modulate cytokine function by targeting three key parameters: receptor affinity, receptor geometry and receptor composition (FIG. 2a). Although we cover each of these classes separately, they are not mutually exclusive, and the simultaneous modulation of multiple parameters may further increase the cell type or functional selectivity of cytokine receptor agonists. Ultimately, the goal of cytokine pharmacology is to tune cytokine receptor signalling with the aim of reducing cellular and functional pleiotropy, thereby enhancing the therapeutic effects of these important signalling molecules.

Receptor affinity

Despite the functional diversity observed across type 1 interferons, most cytokine receptors have only one natural ligand, indicating that these systems fail to sample the full range of possible signalling behaviours. Guided by structures of cytokine signalling complexes, the affinity of cytokines for their receptors has been systematically altered and the effects of these engineered ligands have been studied in various biological contexts. Although the potential functionality of affinity-engineered cytokines is diverse, they generally fall into five, non-mutually exclusive classes: super agonists, partial agonists, cell type selective agonists, biased agonists and decoy-resistant agonists.

Super agonists.—Super agonists are ligands that have increased affinity for their receptor, and therefore enhanced signalling effects relative to the endogenous ligand. Given the importance of receptor affinity in controlling dose sensitivity and downstream signalling, super agonists can be used to increase cytokine potency, particularly on cell types which naturally have low levels of receptor expression. To increase the affinity of cytokines for their receptors, in vitro evolution techniques have been used to screen large numbers of mutant cytokines for binding to cytokine receptor ECDs. This approach has now been used to engineer super agonist variants for various cytokines including IFNα[66], IFNλ[67], IL-2 (REFs.[68,69]), IL-4 (REF.[70]), IL-6 (REF.[71]), IL-10 (REFs.[72,73]), IL-13 (REF.[74]) and IL-22 (REF.[75]).

Interest in enhancing the affinity of cytokines for their receptors was sparked by the observation that differences in the anti-proliferative effect of type 1 interferons correlate with their affinity for IFNAR1 (REF.[76]). Using phage display to enhance the affinity of IFNα2 for binding to IFNAR1, an IFNα2 variant with selectively increased anti-proliferative activity was identified[66], with minimal effect on antiviral function. Although these affinity-matured IFNα variants have not yet progressed to clinical studies, this work was critical in demonstrating that receptor affinity is sufficient to determine the balance of anti-proliferative and antiviral functions of interferons and highlights the power of protein engineering to systematically interrogate the linkage between receptor binding and downstream signalling.

Unlike the type 1 interferons which engage a dimeric receptor, IL-2 acts through a trimeric receptor complex comprising the shared signalling receptors IL-2Rβ and γ_c, as well as the unique non-signalling receptor IL-2Rα, which confers biological specificity[77]. In addition to increasing the local concentration of IL-2 at the cell surface, IL-2Rα also induces an allosteric switch in the C helix of IL-2 that enhances binding to IL-2Rβ (REFs.[18,78]). Using yeast surface display to select for IL-2 mutants with increased affinity for IL-2Rβ, IL-2 variants with core-stabilizing mutations that adopt this enhanced receptor binding conformation independently of IL-2Rα were identified[68]. These high-affinity IL-2 variants, called ‘super-2’, were less dependent on expression of IL-2Rα and showed selectively enhanced activity on natural killer cells, which naturally express high levels of IL-2Rβ. Consistent with an increased affinity for IL-2Rβ, super-2 showed a reduced EC_{50} relative to wild-type IL-2, with no change in E_{max} (FIG. 1c). More recently, computational approaches have been used to enhance IL-2 affinity for its receptors either by mutation[79] or de novo protein design[80] to similar effect. Both super-2 (MDNA-11) and de novo designed IL-2Rα-independent IL-2 (NL-201) variants have now entered phase I/II clinical trials for treatment of various tumour types (NCT05086692, NCT04659629).

IL-10 is an immunoregulatory cytokine that exerts both anti-inflammatory and immunostimulatory effects. A PEGylated version of wild-type IL-10 (pegilodecakin) has progressed to phase III clinical trial for pancreatic cancer but failed to show efficacy in that context[81]. Using a yeast display-based directed evolution approach, IL-10 variants with enhanced affinity for the IL-10 receptor subunit IL-10Rβ were recently engineered[72,73]. These ‘super-10’ variants showed a reduced EC_{50} on multiple cell types, but also elicited a higher E_{max} for both STAT1 and STAT3, consistent with IL-10Rβ binding being limiting for IL-10 receptor assembly and signalling. This enhanced E_{max} was particularly evident on cells with low IL-10Rβ expression, including T cells and natural killer cells, suggesting that these super-10 variants may provide a means of selectively enhancing the immunostimulatory and antitumour effects of IL-10.

Partial agonists.—The fact that type 1 interferons can elicit diverse functional responses despite signalling through the same receptor complex suggests that the amplitude of a particular cytokine signal can have a profound influence on the functional responses[60,82]. In some cases, improved efficacy and reduced toxicity may occur at submaximal signalling levels, as is the case with many GPCR ligands[55]. The development of cytokine receptor partial agonists, which elicit submaximal cellular responses even at saturating ligand

concentrations and, in turn, elicit functional profiles distinct from those of the corresponding natural cytokine, has therefore been pursued.

One prominent example of a cytokine that promotes both beneficial and counterproductive effects is IL-2, which drives the proliferation of cytotoxic $CD8^+$ T cells, but also induces an exhaustion phenotype that limits efficacy in the context of cancer immunotherapy[83]. Using a super-2 variant with enhanced affinity for IL-2Rβ as a starting point, additional mutations were incorporated into IL-2 that reduced affinity for γ_c, the limiting component in the assembly of the IL-2 receptor signalling complex[84]. These IL-2 variants acted as high-affinity partial agonists, displaying reduced maximal signalling at saturating ligand concentrations (FIG. 3a).

Subsequently, one of these variants, called H9T, was developed and its ability to modulate $CD8^+$ T cell functions was characterized[85]. Remarkably, H9T promoted $CD8^+$ T cell expansion while retaining cells in a $TCF1^+$ stem-like state in vitro, in part by promoting a distinct metabolic profile in these cells compared with wild-type IL-2 (FIG. 3a). Moreover, culturing $CD8^+$ T cells with H9T ex vivo enhanced their antitumour activity following adoptive transfer, demonstrating that partial agonists can alter key functional properties of pleiotropic cytokines.

IFNγ is a pro-inflammatory and cytotoxic cytokine that mediates immune responses against intracellular pathogens and cancer. In particular, the ability of IFNγ to enhance tumour immunosurveillance by increasing expression of MHC class I on cancer cells has garnered substantial clinical interest. However, clinical trials using IFNγ in cancer have generally shown little to no efficacy. This is due, in part, to the extremely short half-life of IFNγ in humans, but potentially also because the pro-inflammatory functions of IFNγ are balanced by the simultaneous induction of inhibitory ligands such as PDL1, which limit antitumour T cell responses[86]. This functional pleiotropy is crucial for the ability of endogenous IFNγ to protect bystander cells from excessive T cell killing but may also limit the therapeutic efficacy of IFNγ administration. Using a crystal structure of the IFNγ receptor complex, engineered variants of IFNγ with altered receptor stoichiometry were engineered[87]. Functionally, these variants acted as partial agonists with reduced maximal STAT1 activation. Transcriptional profiling of these partial agonists on diverse tumour types revealed that some were capable of upregulating MHC class I expression without the accompanying induction of PDL1, demonstrating that these two opposing functions can be decoupled, enhancing the therapeutic potential of this key cytokine.

Cell type selective agonists.—An important source of cytokine pleiotropy is their capacity to act on multiple cell types, resulting in off-target toxicity or counterproductive cellular responses[64]. Although in many cases cells naturally differ in their cytokine sensitivity due to differential receptor expression levels, efforts to exploit this property have historically been limited by the fact that cytokine therapeutics are often administered systemically, preventing any control of local cytokine concentrations. To overcome this, various ligand engineering strategies have been employed to narrow the cell type specificity of therapeutically relevant cytokines. Below we discuss several examples of engineered partial cytokine receptor agonists that exploit natural differences in response thresholds

between immune cell types, thereby overcoming the cellular pleiotropy associated with natural cytokines (FIG. 2b).

As a quintessential pleotropic cytokine, IL-2 has been the focus of intense work to alter receptor interactions and enhance cell type selectivity. Although IL-2 was first described as a potent driver of T cell proliferation[27], mice deficient in IL-2 or its receptors were found to develop systemic autoimmunity[88–91]. This paradox was resolved by the discovery of a critical role for IL-2 in T_{reg} cell development and function[92]. Interest in cell type selective IL-2 receptor agonists was further ignited by the discovery of antibodies that redirected IL-2 activity towards particular cell types, as well as subsequent structural studies that elucidated their mechanism of action[93,94]. This was followed by a series of studies on IL-2 mutants with reduced affinity for IL-2Rβ, which showed enhanced dependence on IL-2Rα for activity[95,96]. As IL-2Rα is most highly expressed on T_{reg} cells, these agonists selectively drove anti-inflammatory T_{reg} cell functions with greatly reduced effects on natural killer cells and conventional T cells, a finding with significant implications for the potential use of IL-2 to treat autoimmune disease.

Recently, it was discovered that T_{reg} cell selectivity of IL-2 could also be achieved by modulating affinity for γ_c, without altering affinity for IL-2Rα or IL-2Rβ (REF.[97]). In particular, one such agonist that expanded T_{reg} cells in vivo with greatly reduced activity on effector $CD8^+$ T cells was identified. Mechanistic characterization of this variant uncovered that this cell type selectivity depended, in part, on high IL-2Rα expression on T_{reg} cells, as well as reduced expression of the negative feedback regulator suppressor of cytokine signalling 1 (SOCS1). Interestingly, these same partial agonists also show specificity for antigen-stimulated T cells, such as tumour-infiltrating T cells, which also express high levels of IL-2Rα. One of these partial agonists, STK-012, has recently entered a phase I clinical trial analysing its safety and tolerability alone or in combination with pembrolizumab across various solid tumour types (NCT05098132).

An alternative strategy for endowing IL-2 with cell type selectivity has involved the use of anti-IL-2 antibodies that modulate the interaction between the cytokine and its receptor subunits[93,94]. One notable example is ‘CD25-mimobodies’, which disrupt the binding of IL-2 to IL-2Rα, resulting in preferential activation of $IL\text{-}2R\alpha^{lo}/IL\text{-}2R\beta^{hi}$ cells such as effector $CD8^+$ T cells and natural killer cells[98,99]. Based on structural insights into one of these antibodies in complex with IL-2, the CD25 mimobody was grafted onto wild-type IL-2 in a single polypeptide. The resulting fusion protein, called NARA1leukin, selectively expands and prevents exhaustion in effector $CD8^+$ T cells in vivo, eliciting antitumour responses in multiple mouse tumour models[99]. A similar antibody–cytokine fusion approach has also been employed using the anti-IL-2 antibody JES6–1, which increases dependency on IL-2Rα and, in turn, endows IL-2 with pronounced T_{reg} cell selectivity in vitro and in vivo[93,100].

IL-4 is a key driver of type 2 immunity that engages two different receptor complexes; a ‘type I’ complex composed of IL-4Rα and γ_c as well as a ‘type II’ complex made up of IL-4Rα and IL-13Rα1 (REF.[101]). Expression of these receptor subunits differs by cell type, with lymphocytes predominantly expressing γ_c, non-haematopoietic cells primarily

expressing IL-13Rα1 and myeloid cells expressing both γ_c and IL-13Rα1 (REFs.[102–104]). Using structures of the type I and type II complexes, super agonists that preferentially bound γ_c or IL-13Rα1 were generated[70]. These 'superkines' selectively acted on cells expressing low levels of the targeted receptor, indicating that affinity maturation can also be used to target cytokines towards particular cell types based on expression of different receptor complexes.

IL-10 is a master regulator of inflammatory responses that is best known for its ability to suppress inflammatory cytokine production and antigen presentation by monocytes and macrophages[105]. However, IL-10 can also drive immunostimulatory effects, including the production of IFNγ and granzyme B by $CD8^+$ T cells, which limits the therapeutic efficacy of IL-10 in the context of autoimmune disease[106]. By first solving the cryo-electron microscopy structure of the IL-10 receptor complex, IL-10 variants with reduced affinity for IL-10Rβ were rationally designed[72] (FIG. 3b). By exploiting natural differences in IL-10Rβ expression levels across immune cell populations, these variants functioned as weak partial agonists on T cells, B cells and natural killer cells, but as full agonists on monocytes and macrophages. Due to this myeloid selectivity, these variants retained the ability to suppress systemic inflammation in vivo, but no longer stimulated cytotoxic T cell functions, thereby decoupling these opposing roles of IL-10 signalling (FIG. 3b).

Unlike IL-2 and IL-10, which balance pro-inflammatory and anti-inflammatory functions, IL-12 induces a robust pro-inflammatory response by activating STAT4 signalling and IFNγ production in both T cells and natural killer cells[107]. The dual function of IL-12 ensures efficient killing of virally infected and transformed cells but can produce severe toxicity when administered systemically in the context of cancer immunotherapy. Previously, the antitumour activity of IL-12 has been attributed to actions on T cells[108] whereas the toxic side effects have predominantly been ascribed to natural killer cells[109]. Structure-based design was applied to engineer versions of IL-12 with reduced binding to IL-12Rβ1 that preferentially activated T cells with reduced activity on natural killer cells due to cell type-dependent differences in IL-12Rβ1 expression[110] (FIG. 3c). As a result, these IL-12 partial agonists retained potent antitumour activity in a mouse tumour model, without the systemic toxicity associated with wild-type IL-12 treatment.

Biased agonists.—Another major source of cytokine pleiotropy is the capacity of cytokine receptors to activate multiple downstream signalling pathways and STAT transcription factors[15] (FIG. 1b). In many cases, these distinct signalling outputs can promote opposing biological responses, such as cellular homeostasis and survival versus inflammation, the simultaneous activation of which can dampen or cancel out the desired therapeutic effects of cytokines administered therapeutically[49]. By analogy to GPCRs, functionally selective/biased cytokine receptor agonists represent a promising avenue for decoupling these distinct signalling responses and isolating or enhancing the therapeutically desired response.

A natural case of a biased agonism through a cytokine receptor was uncovered when genetic analysis of `Diamond–Blackfan anaemia` revealed a disease-associated mutation in the cytokine EPO (R150Q)[111]. Functional characterization of EPO R150Q revealed that this

mutant has a reduced affinity for the EPO receptor (EPOR), resulting in less activation of STAT1 and STAT3 relative to wild-type EPO, despite similar activation of STAT5. In this case, however, the biased agonism resulted in disease, as the loss of STAT1 and STAT3 signalling contributed to the development of anaemia.

An engineered cytokine-biased agonist with enhanced therapeutic properties was recently demonstrated for IL-22, a cytokine that activates multiple countervailing signalling pathways[112]. IL-22 is a lymphocyte-derived cytokine that primarily acts on epithelial cells to induce STAT1, STAT3 and STAT5 transcriptional responses[112]. Notably, IL-22-mediated activation of STAT3 drives key tissue protective and regenerative functions of IL-22, whereas STAT1 activation drives pro-inflammatory effects including the induction of chemokines. Although the tissue protective and regenerative aspects of IL-22 function have garnered substantial clinical interest in the context of autoimmune disease[113], the pro-inflammatory functions of IL-22 can actually contribute to disease pathology in several disease models[114]. Using the structure of the IL-22 receptor complex, IL-22 variants were designed with reduced affinity for the shared receptor IL-10Rβ. Critically, several of these variants retained the capacity to activate STAT3, but no longer activated STAT1 or STAT5 (REF.[75]) (FIG. 3d). Mechanistic interrogation of these biased agonists revealed that they induce greatly reduced tyrosine phosphorylation of the IL-22Rα receptor intracellular domain, which differentially impacted the recruitment of STAT1/5 and STAT3 due to their distinct modes of receptor engagement[76]. Critically, these STAT3-biased IL-22 variants promoted tissue protection and repair in vivo in the context of acute pancreatitis and intestinal radiation damage, but no longer drove local or systemic inflammation characteristic of wild-type IL-22.

Decoy-resistant agonists.—Another potential limiting factor in the efficacy of cytokine therapeutics is the presence of natural antagonists, such as soluble decoy receptors[115]. One example of this occurs with the immunostimulatory IL-1 family cytokine IL-18, which is inhibited by the decoy receptor IL-18BP (REF.[116]). Although IL-18 elicits potent stimulatory effects on both natural killer cells and $CD8^+$ T cells, clinical trials of recombinant IL-18 in cancer have shown little to no efficacy[117]. The decoy receptor IL-18BP was reported to be upregulated in human tumours and limits the antitumour actions of IL-18 (REF.[118]). To overcome this, directed evolution was employed to generate an IL-18 variant that no longer binds IL-18BP but retains binding to the signalling receptor IL-18R1. Unlike wild-type IL-18, this decoy-resistant IL-18 (DR-18) elicited robust antitumour immune responses in multiple mouse tumour models, representing a promising new approach for cancer immunotherapy[118]. DR-18 (ST-067) is currently being evaluated in a phase I/II clinical trial aimed at evaluating safety and clinical activity in multiple cancer types (NCT04787042).

Receptor geometry

GPCR partial and biased agonists exert their effects, in part, through the stabilization of specific receptor conformational states, yielding signalling responses distinct from that of the fully activated receptor[50]. By contrast, extensive structural studies of cytokine receptor complexes have revealed a striking convergence of receptor geometries, suggesting that

natural cytokines may be limited in their ability to induce alternate receptor conformations. Using engineered receptor binding proteins as surrogate ligands, agonists that engage cytokine receptors in distinct geometries have been generated. These studies have revealed that modulating cytokine receptor topology can substantially alter downstream signalling and functional responses, opening an entirely new avenue for the pharmacological control of cytokine function (FIG. 2a).

Diabodies.—One approach to modulate cytokine receptor geometry has been to leverage antibody fragments targeting cytokine receptor ECDs, which can be combined in different ways to yield a diverse set of receptor orientations. Although traditional monoclonal antibodies against cytokine receptors can act as agonists in some cases[119,120], the flexible linker between the fragment antigen-binding (Fab) and the fragment crystallizable (Fc) regions makes enforcement of specified receptor geometries difficult. To minimize flexibility and distance between receptors, researchers have used covalently linked dimeric antibody variable fragments, called diabodies, as surrogate ligands[121]. Importantly, diabodies possess two receptor binding sites and can induce receptor dimers with a wide range of possible inter-dimer distances and orientations (FIG. 4a). As a proof of concept, screening diabodies targeting EpoR identified several ligands that activate EpoR signalling with varying degrees of STAT activation ranging from partial agonism to antagonism[122]. Structural characterization of several diabody–EpoR complexes revealed that these diabodies engage EpoR subunits in orientations very distinct from the EPO-bound receptor, and that differences in signalling amplitude correlated with differences in receptor topology.

Nanobody-based surrogate cytokines.—Although diabodies can effectively reorient receptor geometries to produce distinct signalling outcomes, they require appropriate pairing of heavy and light chains, making the generation of heterodimeric agonists difficult. Single-domain fragments derived from the variable regions of heavy chain only antibodies (VHHs), also known as nanobodies, encode specificity within a single binding site enabling the generation of heterodimeric agonists through expression of tandem nanobodies (FIG. 5a). Furthermore, VHHs can be raised to multiple different epitopes on the receptor ECDs, providing structural diversity that could translate into functional diversity when paired as dimers. This approach was applied to generate surrogates of IL-2 (IL-2Rβ/γ_c) and type I interferon (IFNAR1/IFNAR2), which displayed diverse pharmacological properties based on nanobody binding epitope and fusion orientation[123] (FIG. 5b,c). A subset of surrogate IL-2R agonists supported STAT5 signalling with reduced ERK and AKT activity, suggesting that natural signal pathways can also be uncoupled by synthetic nanobody-based ligands. The highly modular nature of nanobody agonists makes them ideal as a system to explore the functional diversity of altered receptor geometry. That VHH modules can be ‘mixed and matched’ and tested in high throughput begins to position cytokine engineering alongside traditional small-molecule medicinal chemistry screening approaches, representing a potential alternative to the structure-based engineering of endogenous cytokines described above.

DARPins.—One drawback of antibody-based approaches is flexibility between receptor binding proteins that prevents precise control over geometric orientation[122]. In order to

overcome this, an alternative cytokine receptor module based on a synthetic designed ankyrin repeat protein (DARPin) scaffold was developed[124,125]. Using DARPins, the inter-dimer distance, angle and orientation can be systematically altered in a stepwise fashion, enabling precise control over receptor topology (FIG. 4a,b). Studies employing a panel of engineered homodimeric DARPins targeting EpoR revealed that stepwise alterations in dimer distance and angle yielded corresponding reductions in signalling output, resulting in a wide range of agonist profiles including full, partial and biased agonism[125]. Functional analysis of these engineered DARPins showed that they induced differential effects on proliferation and differentiation over the course of haematopoiesis. Thus, engineered DARPins represent a promising platform for exerting precise pharmacologic control of cytokine signalling therapeutically. However, as synthetic protein scaffolds, a full understanding of the safety profile and potential for immunogenicity of DARPin-based ligands requires further investigation.

Receptor composition

Synthekines.—Cytokine receptors are typically composed of a unique receptor that is specific for a given cytokine and a shared receptor that engages multiple cytokines[65]. Generally, expression of shared receptors is broad whereas expression of unique receptors is more restricted. However, given the pairing between unique and shared receptors, only a fraction of possible receptor dimers are engaged by natural ligands. The ability to engage non-natural receptor pairs has the potential to generate new downstream signalling and narrow the cell type specificity by limiting signalling to cells which express both receptor subunits.

Initial studies using chimeric cytokine receptors found that non-natural cytokine pairs were capable of inducing STAT signalling[126,127]. To determine whether this was amenable to endogenous cytokine receptors, dominant negative (DN) cytokine mutants that only engage a single receptor subunit were linked, generating synthetic cytokines (synthekines) that engage non-natural receptor combinations (FIG. 6a). These synthekines elicited distinct signalling responses relative to their parental cytokines and induced unique gene expression programmes in peripheral blood mononuclear cells. More recently, the VHH agonist platform has been used to generate synthetic heterodimers between IL-2Rβ and IL-10Rβ that elicited a STAT5 signalling profile on T cells and natural killer cells. Although the signalling properties of this non-natural receptor complex appear to be similar to an attenuated IL-2, the full extent of its functional properties is not yet known. The combinatorial possibility of synthekines indicates an unexplored space of new cytokine ligands with distinct cell type specificity and downstream signalling.

Cytokine chimeras.—An alternative approach for assembling non-natural cytokine receptor complexes is the use of chimeric cytokine proteins, in which receptor binding sites from one cytokine can be grafted onto another cytokine. This approach has been successfully used to develop the chimeric cytokine IC7, in which the LIFR binding site from the cytokine LIF was grafted onto IL-6, replacing one of its gp130 binding sites[128]. The engineered IC7 therefore signals through a heterodimer of LIFR and gp130, equivalent to the natural cytokine CNTF, but in a manner dependent on expression of IL-6Rα rather than

CNTFR. By eliciting an IL-6Rα-restricted CNTF-like signal, IC7 demonstrated beneficial metabolic effects in vivo, including improved glucose tolerance and the prevention of hepatic steatosis[129]. Thus, IC7 represents a promising potential strategy for the treatment of type 2 diabetes and highlights the potential for chimeric cytokines to enhance therapeutic function by engaging non-natural receptor complexes.

Future directions and ongoing challenges

De novo protein design

A promising avenue for the development of future cytokine receptor agonists is the use of de novo protein design to complement the various combinatorial ligand engineering strategies described above. A de novo computational approach to design IL-2 and IL-15 analogues that signal independently of IL-2Rα/IL-15Rα has recently been employed[80]. These synthetic analogues, called ‘neoleukins’, comprised a distinct structural topology relative to their natural cytokine counterparts, displayed enhanced stability and improved efficacy in mouse tumour models, and are now being tested in a phase I clinical trial for multiple cancer types alone or in combination with immune checkpoint blockade (NCT04659629).

With the rapid advances currently being made in artificial intelligence and machine learning-based protein structure prediction, it appears likely that many new and even more sophisticated de novo designed cytokines will be developed. Future work may yield new types of synthetic ligands with entirely distinct structural topologies capable of modulating receptor geometry and composition in ways that have not been possible thus far. Potential other advantages of de novo cytokines include enhanced stability as well as potential ease of manufacturing relative to their natural counterparts. The major potential downside, however, is the possibility of increased risk of immunogenicity arising from non-human amino acid sequences (discussed in more detail below).

Pharmacokinetic/pharmacodynamic properties

It is also important to note that significant advances are being made towards improving the pharmacokinetic and pharmacodynamic properties of cytokines, which will also be critical determinants of their clinical success going forward (BOX 1). In particular, improvements in half-life extension via Fc fusions[129] or PEGylation[130], as well as in the local production of cytokines via engineered T cells[131] or ‘masked’ cytokines[40], have the potential to greatly improve cytokine safety and efficacy, especially in combination with the partial, biased and cell type selective cytokine variants described above. It is worth noting, however, that such modifications can themselves influence cytokine signalling activity, for example through steric hindrance arising from high molecular weight polyethylene glycol (PEG) moieties[132]. As protein engineering strategies become more complex and multi-faceted, it will be important to maintain a mechanistic understanding of how each modification influences both pharmacokinetic/pharmacodynamic and receptor activation.

Experimental models

A central consideration for the development of future cytokine receptor agonists will be which experimental models should be used to screen and test for novel activities. In vitro

cell culture can be a valuable first step, but such experiments fail to capture the complex cellular interactions experienced in vivo. For example, it is clear that cellular responsiveness to cytokines can be dynamically regulated during disease progression or in response to treatment through changes in receptor expression, internalization or the induction of negative feedback mediators such as SOCS proteins and tyrosine phosphatases[15]. Given previous findings that receptor expression and internalization rates are key determinants of the extent of partial and biased cytokine receptor agonism exerted by engineered ligands[12,14,25,75], it will be important to interpret altered signalling and functional effects of engineered cytokines in a context-dependent manner. Moreover, combining these preclinical studies with new approaches in single-cell sequencing and spatial transcriptomics will provide even greater insight into cellular specificity and heterogeneity of responses. Ultimately, however, as with all drug classes, experiments in mouse models have limited power in predicting efficacy in humans. New advances in human patient-derived organoid cultures containing diverse immune cell populations may therefore also be particularly helpful in assessing new cytokine-based drugs going forward.

Immunogenicity

The potential for immunogenicity is an important consideration for all protein therapeutics, and engineered cytokines are no exception. Therapy-induced immune responses can limit both efficacy, due to drug neutralizing antibodies, and safety, due to induction of inflammatory responses. An additional risk for human protein-based drugs is the potential for the development of cross-neutralizing antibodies, which both block the action of the drug and inhibit the function of the corresponding endogenous protein, as has been observed for EPO[133] and CNTF[134]. Several parameters are known to influence the risk of protein drug immunogenicity, including deviation from natural sequence, a propensity for aggregation and degradation, and the presence of post-translational modifications such as glycosylation or PEGylation which can reduce immunogenicity by masking potential epitopes[135]. Particular caution must therefore be paid to non-human proteins, such as de novo designed cytokines, DARPins and nanobodies, which may be more likely to elicit immunogenicity due to their fully divergent amino acid sequences. However, these modalities will likely not have the concern of inducing cross-neutralizing antibodies that inhibit the function of endogenous cytokines, and their improved thermostability may limit the extent of degradation in vivo and, thereby, reduce their immunogenicity. For mutant cytokines, efforts to minimize the number of mutations while assessing potential neo-epitopes using databases such as NetMHC[136] will be particularly important, as will close monitoring for the presence of cross-neutralizing antibodies induced during or after treatment. Importantly, for several of the engineered cytokines discussed here, single point mutations were sufficient to confer the desired partial, biased or cell type selective agonism. Ultimately, relatively few mutant cytokines have entered the clinic thus far, and the extent to which immunogenicity will be limiting for the safety and efficacy of this drug class remains to be seen.

Outlook

The purification, cloning and recombinant expression of cytokines in the 1970s, 1980s and 1990s led to tremendous clinical interest in the use of cytokines as drugs. Except for a

few cases in which exogenous cytokine supplementation provides benefit, the clinical utility of cytokines has been greatly limited by the complex pleiotropy and redundancy inherent in their biology. However, the increasing availability of structural information for cytokine receptor complexes is now enabling the design of novel cytokine receptor agonists capable of modulating cytokine receptor affinity, geometry and composition. Functional profiling of these new ligands has revealed fundamental insights into the mechanisms of cytokine signalling, and has also generated new therapeutic molecules that may overcome many of the limitations associated with the administration of wild-type cytokines.

It is important to note that the pharmacologic approaches described here exploit natural underlying differences in cytokine function and cell type specificity. Although extensive research over the past several decades has revealed tremendous insight into cytokine biology, there is still much to learn regarding how the combination and timing of diverse cytokine signals contribute to their function in both physiological and disease contexts. By integrating insights from basic immunology together with physical chemistry, and innovative structure-based protein design strategies, the emerging field of cytokine pharmacologically is poised to finally unleash the full potential of cytokine-based therapeutics.

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Glossary

Receptor tyrosine kinases (RTKs)
Cell surface receptors with intrinsic tyrosine kinase activity

Janus kinases (JAKs)
A family of cytosolic tyrosine kinases that associate with cytokine receptor intracellular domains

EC_{50}
The ligand concentration that induces half-maximal response

***E* max**
The maximal response elicited by saturating ligand concentrations

Regulatory T cells (T_{reg} cells)
subpopulation of immunosuppressive T cells important for maintaining self-tolerance and homeostasis

Cellular pleiotropy
Pleiotropy arising from the ability of one molecule to stimulate multiple distinct cell types or tissues

Functional pleiotropy

Pleiotropy arising from the ability of one molecule to activate multiple signalling pathways and, thereby, induce multiple functional responses on a target cell type

G proteins
Guanine nucleotide binding proteins that mediate signal transduction in a GTP-dependent manner

Diamond–Blackfan anaemia
A genetic disorder resulting in reduced production of red blood cells

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Box 1 |

Engineering strategies to enhance cytokine bioavailability and biodistribution

In addition to strategies aimed at directly modulating cytokine receptor signalling, various approaches are being developed to enhance the pharmacokinetic and pharmacodynamic properties of cytokine therapeutics (reviewed elsewhere[4,5]). In addition to pleiotropy, another limitation of cytokines as drugs is their extremely short half-life in vivo. A common approach for extending the protein half-life is through fusion of the cytokine to the fragment crystallizable (Fc) domain of IgG, which significantly extends the serum half-life due to both increasing the hydrodynamic radius to reduce renal clearance and also enabling serum retention via binding to the neonatal Fc receptor (FcRn)[139]. Clinical trials studying efficacy of Fc-fused versions of interleukin-2 (IL-2) (NCT04857866), IL-7 (NCT03687957), IL-12 (NCT0442029) and IL-15 (NCT04250155) are currently underway[4,5]. Another frequently used half-life extension approach is the covalent addition of polyethylene glycol (PEG) conjugates, which increases the hydrodynamic radius to reduce renal clearance. PEGylated versions of IFNα, IL-2 and IL-10 have previously been studied clinically, albeit with limited success[5]. Additional PEGylated cytokines including IFNλ, IL-12 and IL-15 are currently undergoing clinical or preclinical evaluation (for example, NCT04967430).

In addition to half-life extension, approaches for altering the biodistribution of cytokines by targeting them to disease tissues such as tumours are also being extensively investigated (reviewed elsewhere[4,5]). For example, ‘immunocytokines’ comprising cytokines fused to antibodies targeting tumour or cell type-specific proteins are being explored for several cytokines including IFNα, IFNγ, IL-2, IL-12, IL-15 and IL-21 (REF.[5]). Another approach involves the conditional activation of cytokines at target tissues, such as through the use of cytokines fused to protease cleavable auto-inhibitory domains, which are only activated in the presence of proteases that are upregulated in tumour microenvironments[40]. Preclinical studies have shown promising results for protease-activatable versions of IFNα, IL-12 and IL-15, but these have not yet reached clinical trials. Finally, a promising strategy for the targeted delivery of cytokines is through their incorporation into engineered T cells, which can be programmed to deliver cytokines of interest only upon antigen engagement[131]. Several of these so-called ‘cytokine armoured CARs’ are currently being investigated in preclinical and clinical trials for the targeted delivery of IL-12, IL-15 and IL-18 to tumours (for example, NCT04684563).

It is important to note that these strategies of half-life extension and tissue targeting can be combined with approaches to modulate receptor signalling via altered receptor affinity, geometry and composition. Indeed, innovative combinations of all of these strategies will likely be necessary to ultimately realize the full potential of cytokine-based therapeutics.

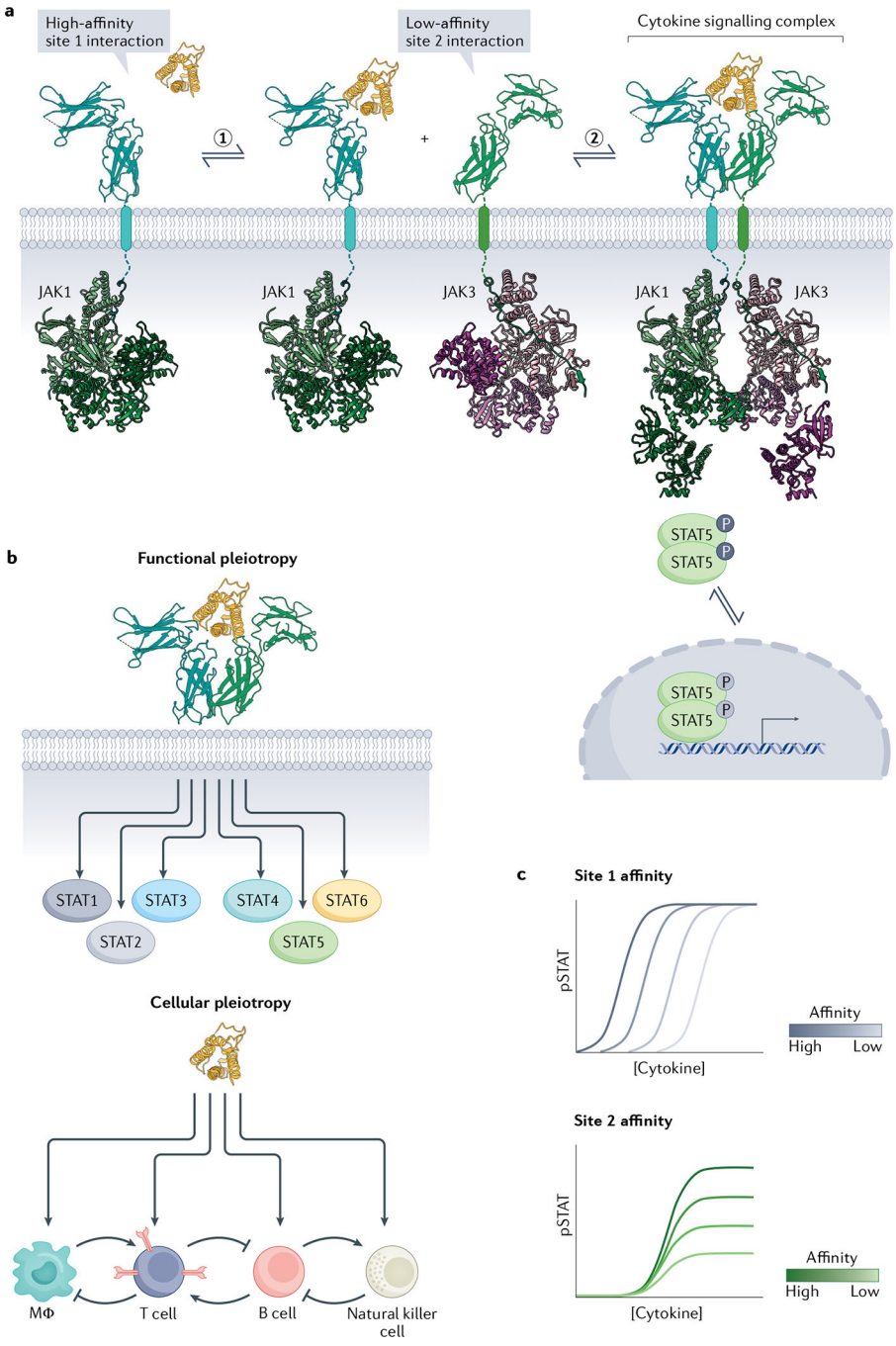

Fig. 1 |. Mechanism of cytokine signalling and sources of pleiotropy.

a | Model of the two-step activation mechanism common among cytokine receptors, in which the soluble cytokine ligand (yellow) first engages a high-affinity receptor subunit (cyan) via site 1, enabling recruitment of the low-affinity receptor subunit (green) from elsewhere on the cell surface. Formation of the ternary cytokine receptor complex enables the activation and transphosphorylation of the intracellular Janus kinases (JAKs) (green, purple), triggering the phosphorylation and nuclear translocation of the signal transducer and activator of transcription (STAT) transcription factors. **b** | Many cytokines exhibit functional

and/or cellular pleiotropy due to the activation of multiple downstream transcription factors or due to their ability to signal on diverse cell types such as macrophages (cyan), T cells (lilac), B cells (red) or natural killer cells (white). **c** | Modulation of cytokine receptor affinity at site 1 alters the dose sensitivity (EC_{50}) of STAT activation, whereas modulation of site 2 alters the maximal strength (Emax). Graphs show the extent of STAT phosphorylation (pSTAT) as a function of cytokine concentration.

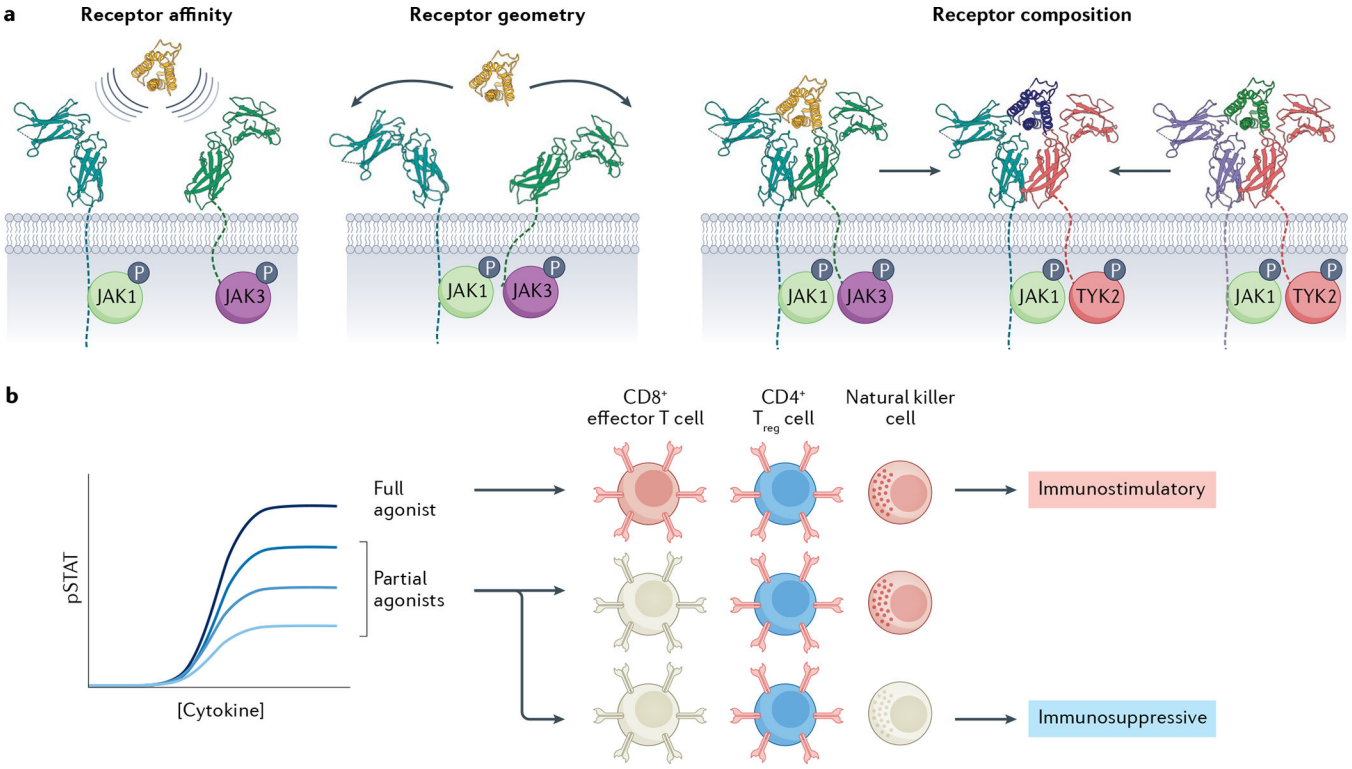

Fig. 2 |. Tunable parameters in cytokine receptor pharmacology.

a | Model highlighting the three parameters of cytokine receptor signalling that can be controlled through cytokine engineering to influence cytokine function, including receptor affinity, receptor geometry/orientation and receptor subunit composition. **b** | Cytokine partial agonists, which exhibit a lower signalling maximal strength (E_{max}) relative to their natural cytokine counterparts, can overcome cytokine pleiotropy by exploiting differential signalling response thresholds across cell populations. For example, interleukin-2 (IL-2) partial agonists can selectively exert immunosuppressive activity by preferentially expanding $CD4^+$ regulatory T cells (T_{reg} cells) (blue) over effector $CD8^+$ T cells or natural killer cells (red). JAK, Janus kinase; pSTAT, signal transducer and activator of transcription (STAT) phosphorylation.

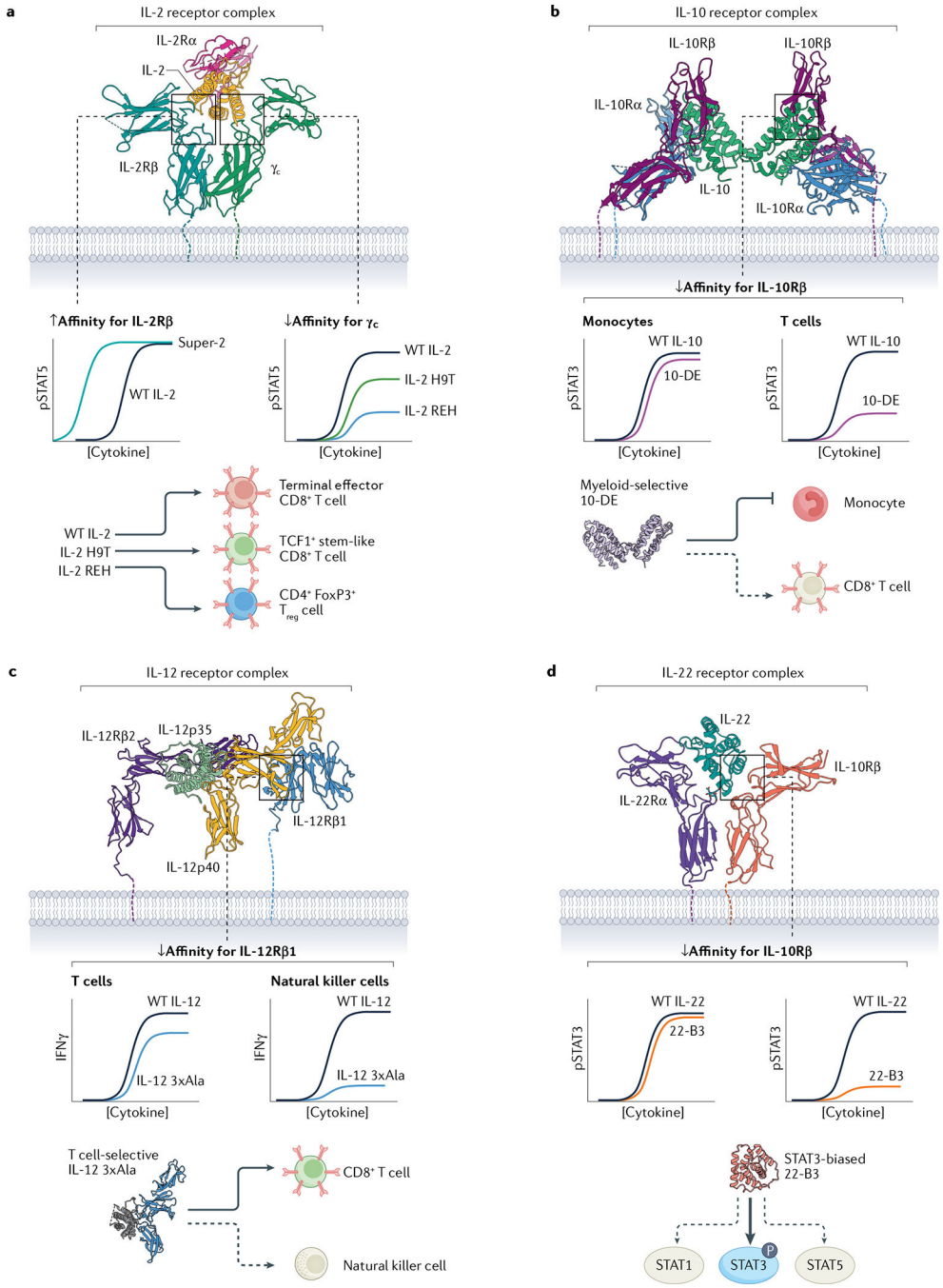

Fig. 3 |. Tuning cytokine function by controlling receptor affinity.

a | Crystal structure of the interleukin-2 (IL-2) receptor complex [PDB:2B5I] showing IL-2 (yellow), IL-2Rα (pink), IL-2Rβ (cyan) and common γ-chain (γ_c) (green). Protein engineering strategies that increase the affinity of IL-2 for IL-2Rβ yielded super-2, a more potent IL-2 variant with reduced dose sensitivity (EC_{50}). By contrast, reducing the affinity of IL-2 for γ_c yielded partial agonists, such as H9T, which preferentially polarize $CD8^+$ T cells towards a $TCF1^+$ stem-like state. **b** | Cryo-electron microscopy structure of the IL-10 receptor complex [PDB:6×93] showing IL-10 (green), IL-10Rα (blue) and IL-10Rβ

(purple). Structure-based design of IL-10 variants with reduced affinity for IL-10Rβ yielded myeloid selective variants, such as 10-DE, which suppress pro-inflammatory myeloid activity but no longer stimulate IFNγ production by T cells. **c** | Cryo-electron microscopy model of the IL-12 receptor complex [EMD:21645] showing IL-12p35 (green), IL-12p40 (yellow), IL-12Rβ1 (blue) and IL-12Rβ2 (purple). Structure-based design of IL-12 variants with reduced affinity for IL-12Rβ1 retains activity on $CD8^+$ effector T cells without stimulating natural killer cell functions. **d** | Crystal structure of the IL-22 receptor complex [PDB:6WEO] showing IL-22 (cyan), IL-22Rα (purple) and IL-10Rβ (orange). Structure-based design of IL-22 variants with reduced affinity for IL-10Rβ, such as 22-B3, yielded biased agonists that selectively activate STAT3 but not STAT1 or STAT5 in epithelial cells. pSTAT, signal transducer and activator of transcription (STAT) phosphorylation; T_{reg} cells, regulatory T cells; WT, wild type.

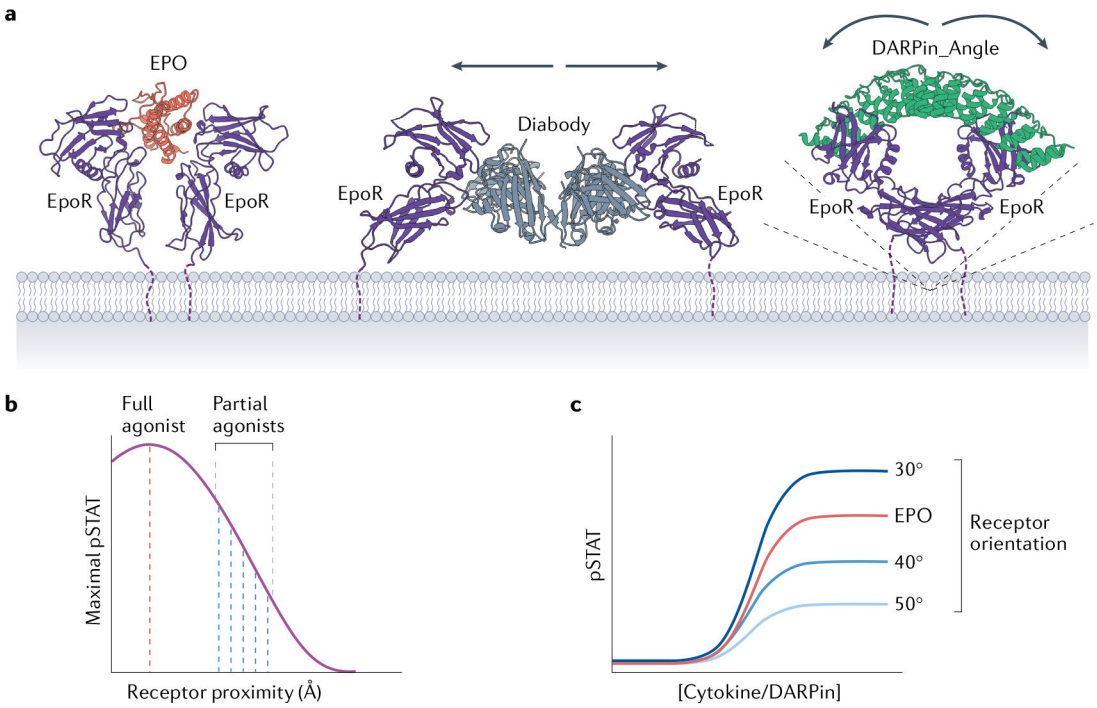

Fig. 4 |. Tuning cytokine function by controlling receptor geometry.

a | Crystal structures of the erythropoietin (EPO) receptor (EpoR) in complex with EPO [PDB:1CN4] (left), a diabody [PDB:4Y5Y] (middle) and a homodimeric DARPin [PDB:6MOI] (right). **b** | Modulation of receptor distance, such as through diabodies, can tune the strength of downstream signal transducer and activator of transcription (STAT) activation. **c** | Modulation of receptor angle, such as through dimeric DARPins, yields varying degrees of partial agonism which can differentially impact downstream biological functions. pSTAT, STAT phosphorylation.

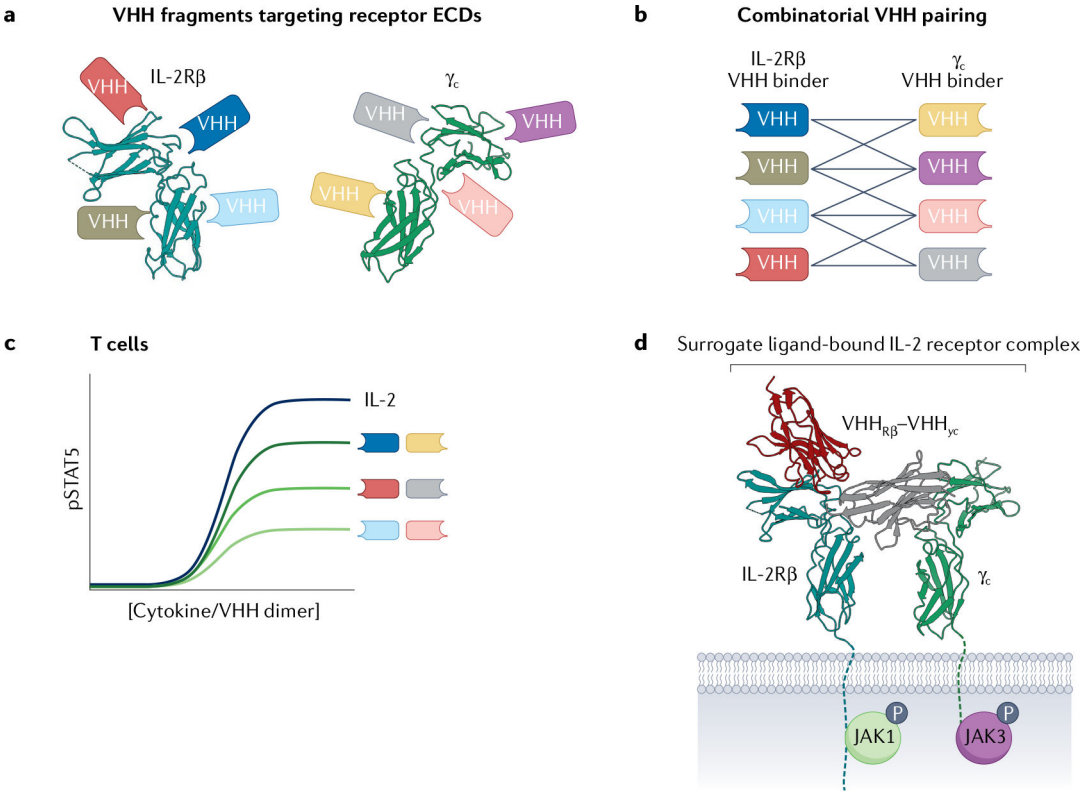

Fig. 5 |. Nanobody-based surrogate cytokines.

a | Single-domain antibody fragments (nanobodies) can be raised against various distinct epitopes on cytokine receptors, such as IL-2Rβ (cyan) and common γ-chain (γ_c) (green). **b** | Nanobodies targeting different cytokine receptors can be paired in a combinatorial manner, providing a panel of potential surrogate cytokines with various receptor binding sites and orientations. **c** | Different nanobody pairings can elicit distinct signalling profiles, such as varying levels of partial or biased agonism. **d** | Structural model of a heterodimeric nanobody pairing capable of engaging the interleukin-2 (IL-2) receptor and initiating signal transduction, with IL-2Rβ (cyan), γ_c (green) and nanobodies (grey and red; respectively [PDB:7S2S] and [PDB:7S2R]). ECD, extracellular domain; JAK, Janus kinase; pSTAT, signal transducer and activator of transcription (STAT) phosphorylation; VHH, variable region of heavy chain only antibody.

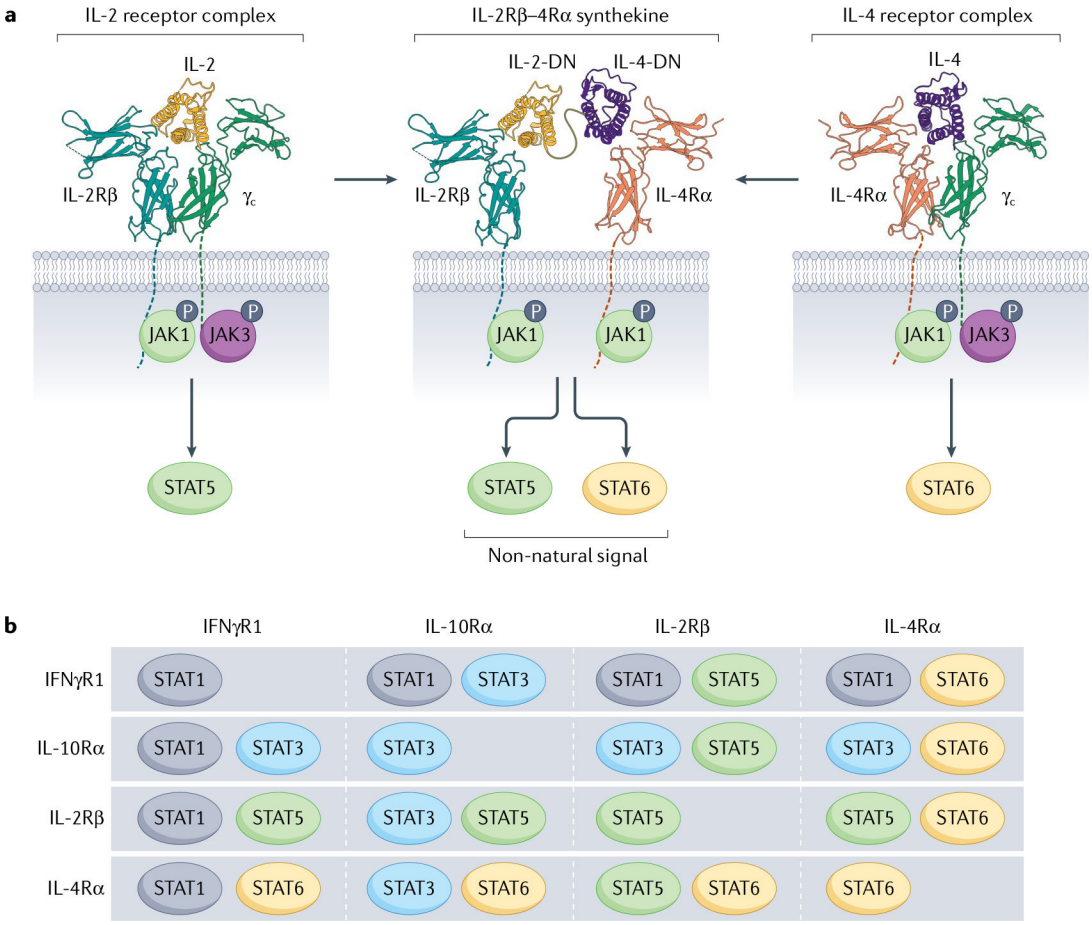

Fig. 6 |. Tuning cytokine function by controlling receptor composition.

a | A ‘synthekine’ comprising dominant negative (DN) versions of interleukin-2 (IL-2) (yellow) and IL-4 (purple) linked together through a flexible linker can recruit an unnatural receptor dimer comprising IL-2Rβ (cyan) and IL-4Rα (orange), to elicit a signal transducer and activator of transcription (STAT) activation signal distinct from that of the IL-2 receptor [PDB:2B5I] (left) or the IL-4 receptor [PDB:3BPL] (right) alone. **b** | Synthekines that pair different cytokine receptor subunits in non-natural combinations can enable the selective activation of a wide range of STAT pairings, theoretically enabling the customized activation of distinct transcriptional programmes. γ_c, common γ-chain; JAK, Janus kinase.

Table 1

Examples of engineered cytokines and their therapeutic applications

Engineered cytokine	Engineering strategy	Agonist profile	Application	Biological effects	Refs.
IL-2 (super-2)	Increased affinity for IL-2Rβ	Super agonist	Cancer	Increased potency, reduced dependence on CD25 (IL-2Rα) expression	68
IL-2 (V69A Q74P)	Increased affinity for IL-2Rα	Super agonist	Cancer	Potent IL-2R agonist; can be combined with mutations that ablate IL-2Rβ/γ_c binding to generate T_{reg} cell selective IL-2R antagonists	69,137
IL-2 (F42A)	Reduced affinity for IL-2Rα	Full agonist	Cancer	Natural killer cell selective IL-2R agonist with reduced binding to IL-2Rα	138
IL-2 (NARA1leukin)	Antibody fusion that reduces affinity for IL-2Rα	Full agonist	Cancer	Selective expansion of effector $CD8^+$ T cells and natural killer cells	99
IL-2 (N88D, FcMut24)	Reduced affinity for IL-2Rβ	Partial agonist	Autoimmunity	Selectively activates T_{reg} cells but not effector T cells	95,96
IL-2 (H9T)	Increased affinity for IL-2Rβ, reduced affinity for γ_c	Partial agonist	Cancer	Preferentially expands $TCF1^+$ stem-like T cells ex vivo	85
IL-2 (REH, REK)	Reduced affinity for γ_c	Partial agonist	Autoimmunity/ cancer	Selectively expands T_{reg} cells/ antigen-stimulated T cells; currently in phase I clinical trials as STK-012	97
IL-2/15 (neo-2/15)	No affinity for IL-2Rα/IL-15Rα	Full agonist	Cancer	Reduced selectivity for T_{reg} cells compared with wild-type IL-2; currently in phase I clinical trials as NL-201	80
IL-4 (super-4)	High affinity for γ_c	Super agonist	Cancer	Increased potency and B cell/T cell activation	70
IL-10 (10-DE)	Reduced affinity for IL-10Rβ	Partial agonist	Autoimmunity, chronic inflammation	Retains anti-inflammatory effects on myeloid cells Reduced induction of IFNγ by T cells	72
IL-10 (super-10)	Increased affinity for IL-10Rβ	Super agonist	Autoimmunity, cancer	Increased potency, enhanced STAT1/3 activation on T cells	72,73
IL-12 (3xAla)	Reduced affinity for IL-12Rβ1	Partial agonist	Cancer	Selectively stimulates IFNγ induction by T cells over natural killer cells	110
IL-18 (DR-18)	Reduced affinity for IL-18BP	Full agonist	Cancer	Bypasses suppression by decoy receptor IL-18BP	118
IL-22 (22-B3)	Reduced affinity for IL-10Rβ	Biased agonist	Autoimmunity, chronic inflammation, GVHD	Retains STAT3-mediated tissue protective and regenerative functions No longer induces STAT1-mediated tissue inflammation	75
IFNγ (GIFN3)	Reduced affinity for IFNGR2	Partial agonist	Cancer	Decouples induction of MHC class I from PDL1 on tumour cells	87
CNTF/IL-6 (IC7-Fc)	Chimeric cytokine of IL-6 and LIF	Full agonist	Diabetes/ metabolic syndrome	Exerts CNTF-like signal restricted to IL-6Rα-expressing cells	128,129

Fc, fragment crystallizable; γ_C, common γ-chain; IL-2, interleukin-2; STAT, signal transducer and activator of transcription; T_{reg} cells, regulatory T cells.

Table 2

Approaches for surrogate cytokine ligands and synthekines

Ligand modality	Engineering method	Targets tested	Advantages	Limitations	Ref.
Diabodies	Single-chain scFv dimer, derived from antibody sequences	EpoR	Reorients receptor dimers in distinct geometries	Geometry cannot be precisely controlled/ altered	122
Dimeric DARPins	DARPin scaffolds selected against target receptors via yeast display	EpoR	Enables precise control over receptor geometry/orientation	Synthetic proteins with increased potential for immunogenicity	125
Bispecific nanobodies	Single-domain VHHs derived from camelid antibodies	IL-2Rβ/α_c IL-2Rβ/IL-10Rβ IFNAR1/IFNAR2	Modular, VHHs with distinct specificities can be combined and screened	Geometry cannot be precisely controlled	123

EpoR, erythropoietin (EPO) receptor; γ_C, common γ-chain; IFNAR1, interferon-α and β receptor subunit 1; VHH, variable region of heavy chain only antibody.