Soluble ligands as drug targets

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Abstract | Historically, the main classes of drug targets have been receptors, enzymes, ion channels and transporters. However, owing largely to the rise of antibody-based therapies in the past two decades, soluble protein ligands such as inflammatory cytokines have become an increasingly important class of drug targets. In this Review, we analyse drugs targeting ligands that have reached clinical development at some point since 1992. We identify 291 drugs that target 99 unique ligands, and we discuss trends in the characteristics of the ligands, drugs and indications for which they have been tested. In the last 5 years, the number of ligand-targeting drugs approved by the FDA has doubled to 34, while the number of clinically validated ligand targets has doubled to 22. Cytokines and growth factors are the predominant types of targeted ligands (70%), and inflammation and autoimmune disorders, cancer and ophthalmological diseases are the top therapeutic areas for both approved agents and agents in clinical studies, reflecting the central role of cytokine and/or growth factor pathways in such diseases.

Drug targets
Pharmacological targets, such as proteins, that mediate the desired therapeutic effect of a drug. This Review focuses on endogenous soluble protein ligands, such as cytokines, as drug targets.

Cytokines
Small proteins involved in cell signalling, including interleukins, interferons, tumour necrosis factors, chemokines and lymphokines, but not hormones or growth factors.

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In the twentieth century, drug discovery largely involved the identification of small molecules that exert their therapeutic effects by interacting with the binding sites of endogenous small-molecule ligands such as neurotransmitters or hormones on target proteins. Reflecting this, drug targets fell mainly into one of four classes of proteins: receptors, enzymes, ion channels and transporters. However, while small molecules remain the predominant therapeutic modality among approved agents[1–5], advances in molecular and cellular biology, genomics and pharmacology in the past few decades have enabled the development of several other therapeutic modalities, including full-length monoclonal antibodies (mAbs), decoy receptors, antibody fragments and mimetics, oligonucleotide therapies and gene therapies[6]. Antibody-based therapies in particular have dramatically changed the landscape of drug development; more than 80 mAbs have been approved since the pioneering mAb muromonab-CD3 was approved by the FDA in 1986, and three of the top ten best-selling drugs in 2018 and 2019 were ligand-targeting mAbs[7–9].

The expansion in the types of biopharmaceutical agents has broadened the types of targets of approved drugs and drugs in clinical trials because characteristics of these agents, such as their target specificity and size, enable the effective modulation of target types that are far less tractable for small molecules. In particular, soluble protein ligands such as cytokines and growth factors can be readily targeted with mAbs, fusion proteins and other modalities such as aptamers to achieve a therapeutic goal such as blockade of the interaction between protein ligands and their receptors — a goal that is far more challenging to achieve with small-molecule drugs. Protein ligands have been successfully targeted by many drugs since the first FDA approval of the ligand-targeting agents etanercept and infliximab in 1998, including cytokines such as tumour necrosis factor (TNF) and growth factors such as vascular endothelial growth factor A (VEGFA).

Therapeutic mAbs and their targets have been investigated, often with a focus on specific indications — predominantly cancer or inflammatory diseases — and other modalities such as gene therapies and their targets have also been reviewed[10–12]. However, to our knowledge, a comprehensive study of the endogenous soluble ligands targeted by all therapeutic modalities has not been published. Here we present an analysis of soluble ligands as a class of drug targets based on approved agents and agents in clinical trials between 1992 and 2020 (see BOX 1 for details of the origin of the dataset and analysis), including the types of ligands being targeted and the characteristics of the drugs and their indications. Furthermore, we discuss strategic issues such as the pros and cons of different ligand-targeting therapeutic modalities and why targeting ligands versus their receptors might be preferable in different disease contexts.

Overview of ligand-targeting drugs

The FDA has approved 34 new molecular entities (NMEs) that target soluble ligands since the first such agents — etanercept and infliximab — were approved in 1998 (FIG. 1). There is a trend towards an increasing number of ligand-targeting drugs being approved, with more than half of the NMEs gaining approval in the last

Box 1 | **Dataset and analysis**

	Approved ligand-targeting drugs	Novel investigational agents
Data sources	• DrugBank dataset • Drugs@FDA: FDA-approved drugs	• CenterWatch clinical trials dataset • Literature reviews • AdisInsight database
Datasets	FDA-approved ligand-targeting drugs	Investigational ligand-targeting agents in clinical trials
	1998–2020 Beginning from first ligand-targeting drug approved by the FDA	1992–2020 Beginning from study start date of first clinical trial of ligand-targeting drug registered in ClinicalTrials.gov
Validation of drug–target interactions	• Google Scholar • Patent applications • PubMed • Industry press releases • Conference abstracts	• Google Scholar • Patent applications • PubMed • Industry press releases • Conference abstracts
Final results	**34** FDA-approved drugs acting on **22** novel ligands, including **3** combinations	**257** novel investigational agents acting on **77** novel ligands, including **22** combinations

The overall dataset, which is provided in Supplementary Table 1, comprises FDA-approved agents as well as investigational drugs that target soluble ligands.

The dataset for FDA-approved ligand-targeting drugs is composed of a manually selected subset from the updated 2018 dataset[2] of the study by Rask-Andersen et al. on drug–target interactions of approved agents that affect targets that are encoded by the human genome[130] (see the left side of the figure). The original study, which extends from 1982, was based on the DrugBank database[131], with further updates and verifications from the Drugs@FDA website and monthly reports. The ligand-targeting subset spans from 1998, when the first drugs targeting ligands were approved, to 15 June 2020.

The dataset of investigational ligand-targeting agents in clinical trials is also a manually selected subset from the updated 2018 dataset[2] based on the study by Rask-Andersen et al. on the druggable genome and evaluation of drug targets in clinical trials[1] (see the right side of the figure). The original study was based on the CenterWatch Drugs in Clinical Trials Database (DCTD; not currently accessible), which was established in 1994, and subsequent updates from the CenterWatch Weekly Pipeline. Additionally, literature reviews and the publicly accessible pages from the AdisInsight database were used to identify potential investigational agents that target ligands. Investigational agents verified to have an NCT (National Clinical Trials) number in ClinicalTrials.gov were included in the dataset. In addition, there were 27 agents that had literature describing trials but an NCT number could not be found. ClinicalTrials.gov was launched to the public in 2000 owing to the requirements of the FDA Modernization Act of 1997 and is the registry of US clinical trials information. Registration requirements of trials expanded in 2007 after the FDA Amendments Act was passed and hence the database has grown significantly since that time. Our subset of investigational agents targeting ligands spans from 1992, the first year in which we identify one of the ligand-targeting agents began a registered clinical trial, to 15 June 2020. As the registration of clinical trials has been inconsistent, particularly in the decades preceding and immediately following the turn of the century, our dataset may underestimate the number of ligand-targeting agents and clinical trials that were conducted during that time frame. Furthermore, retrospective registration of clinical trials could also result in underestimates in our dataset as some trials may not be registered yet, especially for the years 2018–2020.

The drug–target interactions were manually validated using sources including PubMed, Google Scholar, industry press releases and websites, conference abstracts and patent applications. The drug–target mechanism of action was carefully researched to exclude targets that induce pleiotropic or other unknown effects. As the focus is on innovation of agents and targets, biosimilars were excluded from the dataset. Trial information for interventional studies with the primary purpose of treatment was obtained from ClinicalTrials.gov for each agent. The NCT number, which signifies one record per unique study protocol, was used to distinguish individual clinical trials. The 'study start date' field was used to identify when studies of novel agents and targets began as well as when trial phases were initiated. To assess whether an agent was still being actively investigated, the status of its clinical trials was first reviewed, and it was considered active if it was involved in a trial that was designated as 'active' or expected to start. Additionally, industry press releases and websites were used to distinguish whether an agent was still in active development or had been discontinued. The classification of endogenous ligands was determined with use of the IUPHAR/BPS Guide to Pharmacology[128].

6 years and the highest number of approvals in the last 2 years, with six agents approved in 2018 and five agents approved in 2019.

In total, we identify 291 ligand-targeting agents that are approved or have been in clinical trials since 1992, which target a total of 99 unique ligands, with 84 ligands targeted individually and 25 different combinations of ligand targets. At present, 119 investigational ligand-targeting agents are currently in clinical trials or apparently still in active development, while the development of 138 agents that reached clinical trials has been discontinued (FIG. 1b). There are 22 clinically validated ligand targets — that is, ligands that are targeted by FDA-approved drugs — and 77 further novel ligand targets of investigational agents that have reached clinical trials. Of these, 52 ligands are the targets of agents currently in clinical development, while clinical development of agents for 25 of the ligand targets has been discontinued (FIG. 1c).

The number of ligand-targeting agents entering clinical trials has increased substantially over the past two decades, with eight agents entering trials between 1990 and 1999, 115 entering trials between 2000 and 2009 and 168 entering trials between 2010 and 2020 (FIG. 2a). Furthermore, the number of agents that bind multiple soluble ligands has increased substantially in the last decade (36 agents) in comparison with the previous two decades (10 agents). Such agents may bind multiple ligands through an epitope that is common to closely related proteins (for example, luspatercept, a decoy receptor that functions through binding growth/differentiation factor 8 (GDF8; also known as myostatin) and GDF11 of the transforming growth factor-β superfamily[13]) or may be designed to bind unrelated ligands (for example, faricimab, a bispecific mAb that binds to angiopoietin 2 (ANGPT2) and VEGFA[14]). While the number of ligand targets that have been investigated in trials has ranged from a total of 58 between 2000 and 2009 to 35 between 2010 and 2019, the number of unique combinations of ligand targets has increased 1.5-fold in the last decade to a total of 15 (FIG. 2b), and the

Growth factors
Proteins that regulate cellular proliferation and/or differentiation; examples include vascular endothelial growth factor and members of the fibroblast growth factor family.

Interleukin
A cytokine that promotes the development and differentiation of T and B lymphocytes and haematopoietic cells.

opportunities for multitargeting are discussed further in the following sections.

More than 5,700 clinical trials for ligand-targeting agents have been initiated since 1992 (FIG. 2c), with ~30% of trials initiated involving novel agents and ~70% involving approved agents. The number of trials initiated for novel agents increased between 2010 and 2019 compared with 2000–2009, reaching 142 in 2018, including 46 phase III trials. Most FDA-approved ligand-targeting agents are for the treatment of inflammatory and autoimmune disorders (FIG. 2d), while cancer is an increasingly popular area for agents in earlier stages of clinical development.

Which ligands are being targeted?

Cytokines constitute 50% (11 cytokines) of the 22 ligands targeted by FDA-approved drugs and 40% (31 cytokines) of the 77 novel ligands for which agents are in development (FIG. 3a,b). Growth factors are the second-largest group and constitute 27% of the ligands targeted by FDA-approved drugs and 31% of the novel ligand targets. The other ~30% of ligands targeted by approved and investigational agents can be classified in a range of categories, including neuropeptides such as calcitonin gene-related peptide (CGRP), inflammatory mediators in the complement pathway[15] and other signalling molecules such as sclerostin (SOST) (FIG. 3c).

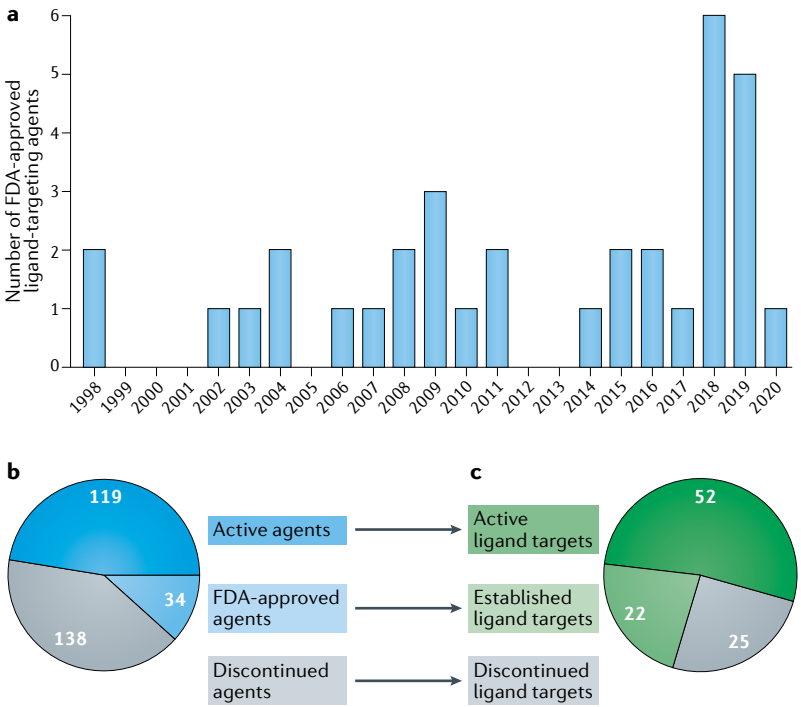


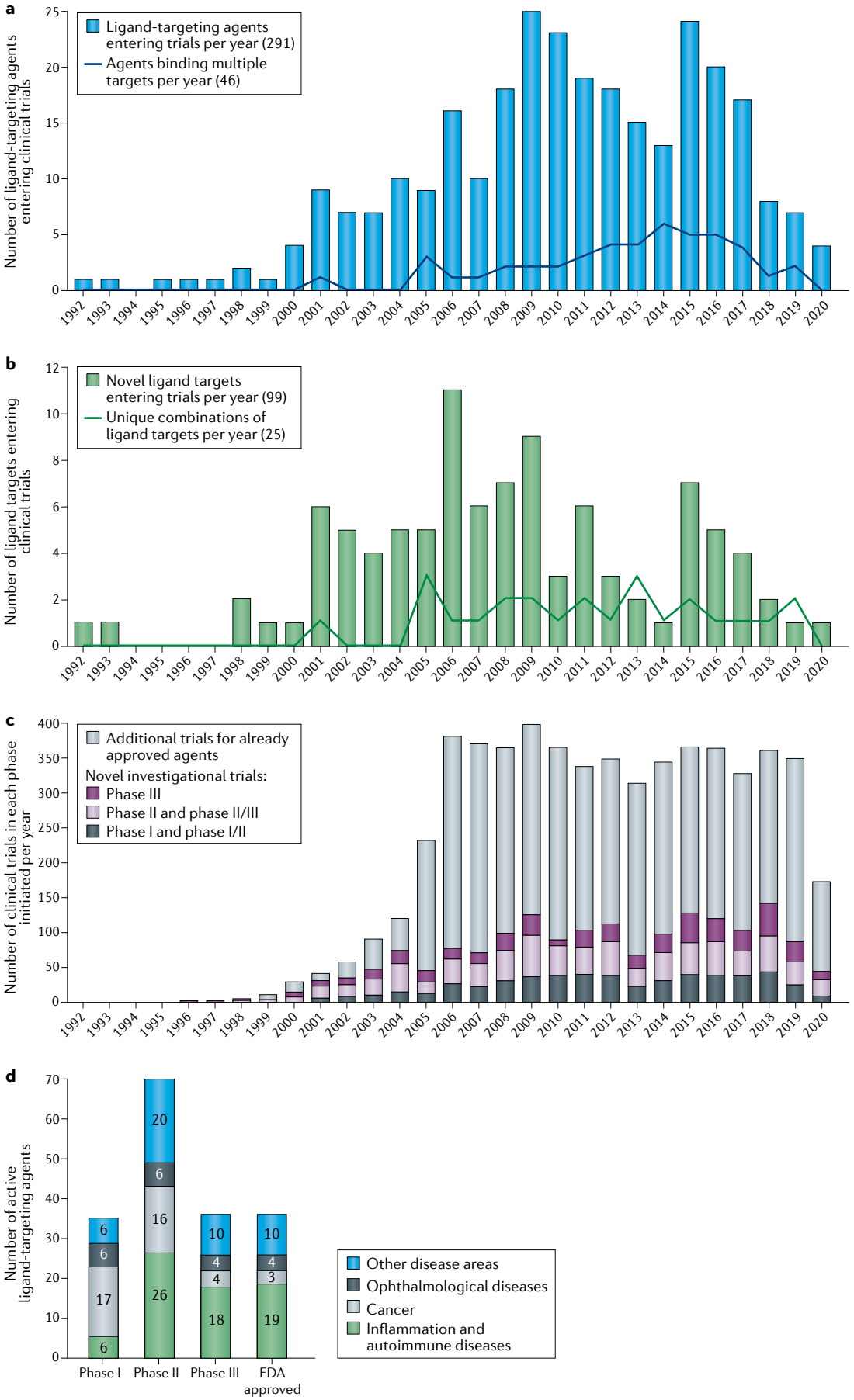
Fig. 1 | **Overview of ligand-targeting drugs and therapeutic targets. a** | Number of agents that target ligands approved by the FDA per year. In total, 34 ligand-targeting drugs have been approved by the FDA since 1998. **b** | A total of 291 ligand-targeting agents were identified through the analysis described in BOX 1, of which 34 are FDA-approved agents, 119 are currently in clinical trials or apparently still in active clinical development and 138 have been discontinued. **c** | A total of 99 unique ligands were identified, of which 22 are targeted by FDA-approved drugs, 52 are targeted by agents in clinical development and 25 are the targets of agents for which clinical development has been discontinued. The data are to 15 June 2020.

Selected examples from the major ligand classes based on the number of FDA-approved agents and/or the number of drugs in clinical trials are shown in TABLE 1, and the FDA-approved ligand-targeting agents and their approved indications are shown in TABLE 2.

TNF has been one of the most targeted ligands (FIG. 3d). Of the 32 agents that target TNF, 25 agents target it exclusively, of which 5 have been approved, and 7 drugs target two unique combinations that include it. This reflects its position at the apex of the proinflammatory cytokine cascade, which makes it a key target for inflammatory disease treatment[16]. Anti-TNF drugs were initially developed to treat rheumatoid arthritis and Crohn's disease, with two of these — the decoy receptor etanercept and the full-length mAb infliximab — both gaining FDA approval in 1998. Since then, three additional anti-TNF drugs have been approved (TABLE 2), and this class of drugs has been investigated in many other autoimmune diseases, gaining approval for indications such as ankylosing spondylitis, ulcerative colitis, juvenile idiopathic arthritis and psoriasis.

VEGFA, a key regulator of normal and pathological angiogenesis[17], has also been a heavily targeted ligand, with 29 agents, of which 5 have been approved. There are currently 18 active novel agents targeting it in clinical trials — the most for any ligand (FIG. 3d). Initially, research on VEGFA as a drug target focused on its role in tumour angiogenesis, and bevacizumab, a full-length mAb that binds to VEGFA, became the first drug against this target to reach the market when it was approved in 2004 for treatment of colorectal cancer in combination with chemotherapy. It is now approved for treatment of multiple cancers, as both a single agent and in combination with chemotherapy. VEGFA was also found to be important in eye disorders characterized by ocular neoangiogenesis, such as neovascular age-related macular degeneration (AMD) and macular oedema[18]. This has led to the approval of several drugs targeting VEGFA for treatment of such disorders, beginning with the aptamer pegaptanib for treatment of AMD in 2004 (discussed further later).

The interleukin-17 (IL-17) pathway has also been a popular target, because of its role in inflammatory and autoimmune diseases such as psoriatic diseases[19,20] (FIG. 3d). IL-17A is part of a family of six cytokines (IL-17A–IL-17F), and can exist as a homodimer or as a heterodimer with IL-17F. Of the 20 agents that target IL-17A (FIG. 3d), 8 target just IL-17A, while 12 target 4 unique combinations, including 6 drugs that target IL-17A plus TNF. Two agents that target IL-17A have been approved in the last 5 years: secukinumab and ixekizumab in 2015 and 2016, respectively. IL-17 is produced by T helper 17 (T_H17) cells that are primarily regulated by IL-23, which has also been a popular target for treatment of psoriasis and other immunoinflammatory diseases. IL-23, a heterodimeric cytokine composed of the subunits IL-23p19 and IL-12p40, is the interleukin with the greatest number of approved agents targeting it — three full-length mAbs that target the IL-23p19 subunit (guselkumab, tildrakizumab and risankizumab, approved in 2017, 2018 and 2019, respectively) and one full-length mAb that targets the


a
Number of ligand-targeting agents entering clinical trials
Ligand-targeting agents entering trials per year (291)
Agents binding multiple targets per year (46)
b
Number of ligand targets entering clinical trials
Novel ligand targets entering trials per year (99)
Unique combinations of ligand targets per year (25)
c
Number of clinical trials in each phase initiated per year
Additional trials for already approved agents
Novel investigational trials:
Phase III
Phase II and phase II/III
Phase I and phase I/II
d
Number of active ligand-targeting agents
Phase I
Phase II
Phase III
FDA approved
Other disease areas
Ophthalmological diseases
Cancer
Inflammation and autoimmune diseases

◀ Fig. 2 | **Therapeutic agents and targets entering clinical trials. a** | The blue bars represent the total number of therapeutic agents targeting ligands (291 agents) that have entered clinical trials each year beginning in 1992, which is the first year in which we identify one of the agents beginning a registered clinical trial, and extends to 15 June 2020. The dark blue line indicates the subset of agents that bind multiple targets that have entered clinical trials. The 'study start date' field from ClinicalTrials.gov was used to determine when an agent entered trials. Owing to the possible inconsistent registration of clinical trials in ClinicalTrials.gov during the decade preceding and the years immediately following the turn of the century, this may be an under-representation of all the ligand-targeting drugs and trials (see BOX 1 for details). **b** | The green bars indicate the number of unique ligand targets that entered clinical trials per year, while the darker green line represents the number of novel target combinations that entered clinical studies. The year a target ligand (or combination) entered trials is derived from when the earliest therapy targeting that ligand (or combination) entered clinical trials. The data include 99 unique ligands and 25 combinations. **c** | More than 5,700 clinical trials of ligand-targeting drugs have been registered since 1992. Approximately 30% involve novel investigational agents entering trials, while ~70% are additional studies involving the 34 approved agents (grey) after they had already been approved. The number of trials in each phase initiated per year is shown using the study start date from ClinicalTrials.gov. The data include clinical trials that have a designated phase, are listed as an interventional study type and have the primary purpose of treatment. **d** | The major therapeutic categories being treated by active agents. Inflammation and autoimmune diseases, cancer and ophthalmological diseases are three major disease groups being treated by both approved agents and agents in development. Inflammation and autoimmune diseases are the predominant areas for approved agents, while cancer is an increasingly popular area for agents in earlier stages of development. The data include all active agents and the highest phase achieved for a specified disease area is selected to prevent redundancies (agents in multiple trials for similar indications). Two agents have FDA approval in multiple disease areas and investigational agents may be in trials for more than one disease area. The data are to 15 June 2020.

p40 subunit and consequently also binds and inhibits IL-12 (ustekinumab, approved in 2009).

Although many approved drugs targeting ligands are indicated for treatment of immuno-inflammatory disorders, reflecting the role of the ligands in the immune system (FIG. 2d), there are a growing number of examples in other therapeutic areas. For example, SOST is a secreted protein that has recently been clinically validated as a target for treatment of osteoporosis, leading to the FDA approval of the full-length mAb romosozumab in 2019 (REF.[21]). SOST negatively regulates the formation of mineralized bone matrix and bone mass, and so inhibition of SOST results in increased spine and hip bone mineral density[22]. In another recent example, CGRPα, the protein product of *CALCA*, has been validated with the FDA approval of three CGRP-specific full-length mAbs for the treatment of migraines: fremanezumab and galcanezumab in 2018 and eptinezumab in 2020 (REFS[23–25]). These mAbs also bind to CGRPβ (encoded by *CALCB*), which has 90% structural similarity to CGRPα[26]. CGRPα, the principal form of CGRP in the nervous system, contributes to the pain of a migraine attack through its roles in vasodilation, neurogenic inflammation and peripheral sensitization, and antagonism of the CGRP pathway has been shown to ameliorate migraine pain as well as to offer the potential to prevent migraine attacks[27].

Classes of ligand-targeting drugs

Among the approved ligand-targeting drugs, approximately three-quarters (25 of 34) are full-length mAbs, with the remainder made up of four decoy receptors, three antibody fragments, one nanobody and one aptamer (FIG. 4a). These drugs all bind to a specific soluble ligand (or ligands) and sequester it (or them), thereby modulating the targeted pathway(s) by inhibiting the ligand–receptor interaction(s) and subsequent signalling.

There is greater diversity in the novel agents in clinical trials. Overall, nearly two-thirds of the 257 biopharmaceutical agents in clinical trials are antibody-based drugs and derivatives, including 151 full-length mAbs, 8 antibody fragments such as Fabs and single-chain variable regions (scFvs) and 6 single-domain antibodies such as nanobodies (FIG. 4b). The other classes in the dataset include decoy receptors, engineered protein scaffolds, gene therapies, therapeutic vaccines and various types of oligonucleotide therapies. Therapeutic antibodies and decoy receptors, which have become well-developed classes of agents over time, have the highest number of active agents (FIG. 4c) as well as the greatest number of drugs in phase III trials, further indicating the maturity and continued interest in these therapeutic modalities. In the following sections, we discuss the characteristics of the major therapeutic modalities in the dataset in more depth.

Monoclonal antibodies. As already noted, mAbs have become established as a key therapeutic modality in the past two decades — a success that has been based on the specificity, stability and adaptability of the antibody framework[28] (FIG. 5a). Therapeutic mAbs can be distinguished by several characteristics, including the technology from which the mAb is derived, the immunoglobulin G (IgG) subtype and the engineering strategies applied to the mAb[29]. Here we focus briefly on aspects that are most relevant to ligand-targeting mAbs.

Immunogenicity, leading to the presence of anti-drug antibodies that compromise drug efficacy and safety, is a major challenge for therapeutic mAbs as well as other biological therapies[30]. The pioneering therapeutic mAbs in the 1980s were derived from mice, and produced human anti-mouse antibody responses owing to the non-human sequences in the constant regions[28]. This led to the development of successive generations of technology to reduce the extent of foreign-derived sequences in the antibody: chimeric mAbs with foreign variable regions and human constant regions, humanized mAbs that are human except for foreign complementarity-determining regions and, most recently, fully human mAbs[31] (FIG. 5b). The first fully human mAb, adalimumab, was approved in 2002, and since then mAbs that are fully human have become increasingly popular. Of the 25 approved full-length ligand-targeting mAbs in the dataset, 13 (52%) are humanized, 10 (40%) are fully human and 2 (8%) are chimeric. The 151 novel ligand-targeting mAbs that are in clinical trials also have a high proportion of humanized or fully human mAbs; 72 are humanized (48%), 68 are human (45%) and 3 are chimeric (1%) (data were not obtainable for six agents and an additional two older agents are full mouse constructs).

The choice of IgG subtype may be important depending on the intended therapeutic application of a mAb, as IgG subtypes can differentially interact with the five

activating Fcγ receptors (FcγRs), the single inhibitory receptor FcγRIIb and the C1q component of the complement cascade[28,32,33]. Binding to activating FcγRs can stimulate immune responses and evoke antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis and/or complement-dependent cytotoxicity (CDC)[32], whereas binding with the inhibitory FcγRIIb can dampen immune responses[34]. The IgG1 subclass is the most prevalent in our dataset, with 17 (68%) of the approved ligand-targeting full-length mAbs and 81 (53%) of the agents in clinical trials. IgG2 frameworks (three mAbs) and IgG4 frameworks (three mAbs) and combinations (two mAbs) have also been used in approved ligand-targeting mAbs. However, for investigational agents, IgG4 subtypes (34 mAbs; 23%) are more than twice as common as IgG2 subtypes (17 mAbs; 11%) (data were not obtainable for 19 agents).

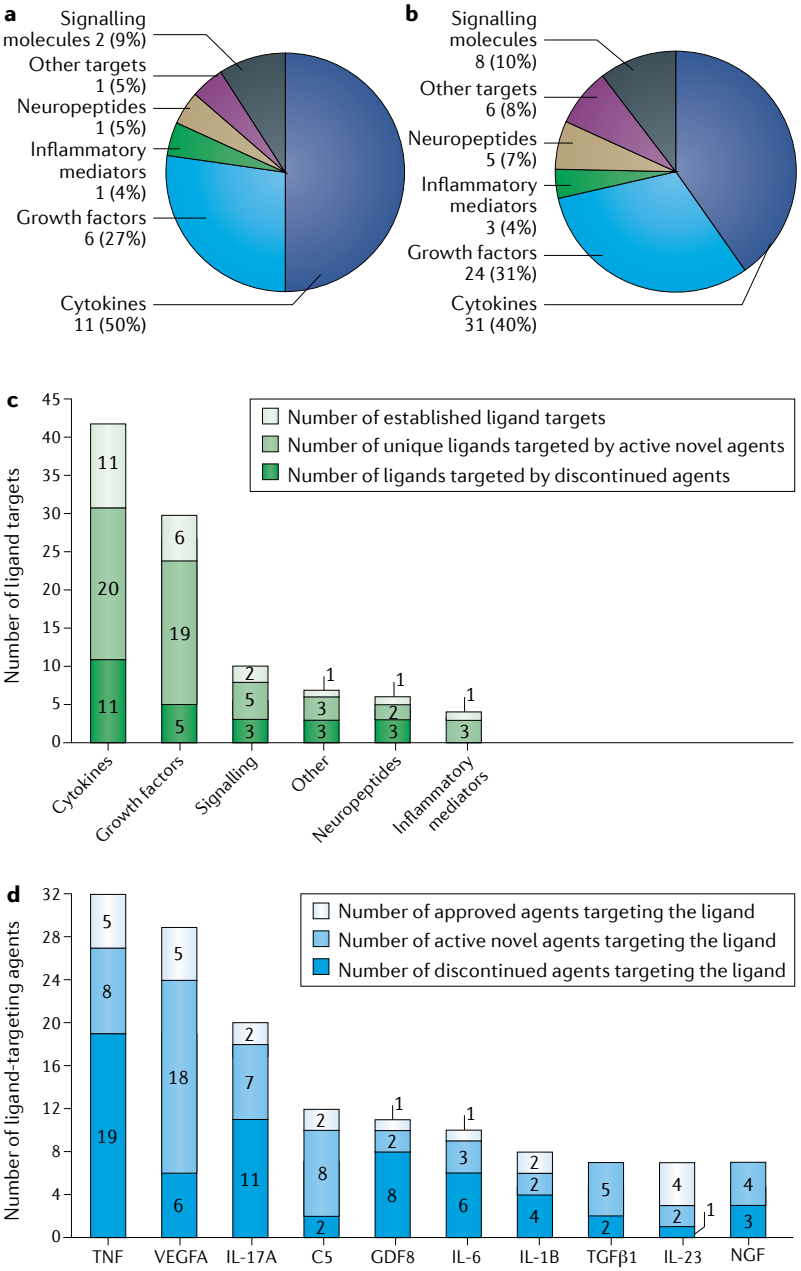


Fig. 3 | **Classes of the target ligands. a** | The 22 unique soluble ligands that have been clinically established as targets by FDA-approved agents are separated into different classes. Cytokines include interleukins, interferons and chemokines in parts **a**, **b** and **c**. **b** | The 77 unique ligands that are targeted by novel investigational agents in clinical trials are separated into different classes. **c** | The different classes of the 99 unique ligands that have been targeted by all agents are separated into the number of ligands that have been clinically established as targets by FDA-approved drugs, the number of ligands that are being targeted by therapeutic agents in active development and the number of ligands that are targeted by agents that are now discontinued. **d** | Ten selected ligands that are targeted by the greatest number of agents in the dataset are shown with the number of FDA-approved agents that target the ligand, the number of active agents that target the ligand and the number of discontinued agents that previously targeted that ligand in clinical trials. GDF8, growth/differentiation factor 8; IL, interleukin; TGFβ1, transforming growth factor-β1; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A.

Decoy receptors. The second most common class of agents among the approved ligand-targeting drugs (four drugs; 12%) and the agents in clinical trials (22 drugs; 9%) are decoy receptors, also known as Fc-fusion proteins, which incorporate the binding domains from endogenous ligand receptors to trap the ligand (FIG. 5c). The fusion of the receptor binding moiety with the Fc region helps to extend the half-life of the drug, although the large size (~150 kDa) can limit tissue penetration. The first such agent to reach the market was the TNF-receptor–Fc-fusion protein etanercept, which received approval by the FDA for treatment of rheumatoid arthritis in 1998, and has since been approved for treatment of several other inflammatory diseases (TABLE 2).

Three other decoy receptors have been approved so far. Rilonacept, an engineered dimeric fusion protein consisting of the ligand-binding domains of the extracellular portions of the human IL-1 receptor component (IL-1RI) and IL-1 receptor accessory protein (IL-1RAcP) linked to the Fc portion of human IgG1, was approved in 2008 for the treatment of a group of rare autoinflammatory diseases known as cryopyrin-associated

Table 1 | **Selected examples of ligand targets from different ligand classes**

Ligand target	Number of approved agents (names)	Number of agents in clinical trials	First year in trials	First year approved	Number of active/ completed trials
Cytokines (42 targets, 146 agents)					
TNF	5 (etanercept, infliximab, adalimumab, certolizumab, golimumab)	32	1992	1998	202/705
IL-1A and IL-1B	1 (rilonacept)	3	2005	2008	6/20
IL-1B	1 (canakinumab)	5	2003	2009	22/64
IL-12 and IL-23 (p40 subunit)	1 (ustekinumab)	2	2003	2009	33/54
TNFSF11	1 (denosumab)	3	2002	2010	79/83
TNFSF13B	1 (belimumab)	4	2002	2011	19/56
IL-6	1 (siltuximab)	10	2004	2014	23/28
IL-5	2 (mepolizumab, reslizumab)	2	2001	2015	16/46
IL-17A[a]	2 (secukinumab, ixekizumab)	20	2008	2015	86/131
IL-23 (p19 subunit)	3 (guselkumab, tildrakizumab, risankizumab)	5	2009	2017	66/49
IFNγ	1 (emapalumab)	3	2002	2018	5/5
GDF8	1 (luspatercept[b])	11	2004	2019	17/23
IL-13	–	11	2006	–	13/50
Growth factors (30 targets, 89 agents)					
VEGFA	5 (pegaptanib, bevacizumab, ranibizumab, brolucizumab, aflibercept[c])	29	1998	2004	632/1429
FGF23	1 (burosumab)	1	2008	2018	9/10
ANGPT2	–	9	2005	–	14/35
NGF	–	8	2006	–	14/36
Signalling molecules and other targets (17 targets, 33 agents)					
IgE Fc region	1 (omalizumab)	5	1999	2003	28/108
SOST	1 (romosozumab)	3	2006	2019	1/25
VWF	1 (caplacizumab)	2	2006	2019	2/7
HTT	–	3	2015	–	6/1
Inflammatory mediators in the complement pathway (4 targets, 17 agents)					
C5	2 (eculizumab, ravulizumab)	11	2000	2007	55/57
Neuropeptides (6 targets, 11 agents)					
CGRPα and CGRPβ	3 (fremanezumab, galcanezumab, eptinezumab)	3	2011	2018	17/29

Ligand targets selected on the basis of the number of approved agents and/or the number of drugs in clinical trials that target them. Numbers include ligand targets that are in combinations as well. All 34 ligand-targeting drugs that had been approved by 15 June 2020 are included; see TABLE 2 for further details on these. ANGPT2, angiopoietin 2; CGRP, calcitonin gene-related peptide; GDF8, growth/differentiation factor 8; HTT, huntingtin; IFNγ, interferon-γ; IgE, immunoglobulin E; IL, interleukin; SOST, sclerostin; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A; VWF, von Willebrand factor. [a]IL-17A exists as a homodimer or as a heterodimer with IL-17F; secukinumab and ixekizumab bind to IL-17A. [b]Luspatercept binds GDF8 and GDF11 of the transforming growth factor-β superfamily. [c]Aflibercept binds VEGFA, VEGFB and PGF.

periodic syndromes[39]. Aflibercept, which contains the second binding domain of the VEGFR1 receptor and the third domain of the VEGFR2 receptor to bind isomers of the VEGF family[40], was approved for treatment of wet AMD in 2011. It was also approved in a combination treatment for colorectal cancer in 2012 (REF.[41]), for treatment of diabetic macular oedema and macular oedema following retinal vein occlusion in 2014 and for treatment of diabetic retinopathy in 2015. Luspatercept is a fusion protein that contains the extracellular domain of human activin receptor type IIB (ActRIIB) that has been modified to reduce activin binding[13]. It inhibits select ligands of the transforming growth factor-β superfamily to reduce aberrant SMAD2/3 signalling and promote late-stage erythropoiesis and was approved in 2019 for treatment of adult patients with β-thalassaemia and for treatment of anaemia in adults with myelodysplastic syndromes in 2020. Fifteen decoy receptors are currently in clinical trials, and more than half (ten agents) of these began trials in the last 5 years, indicating continued industry interest in this class (FIG. 4c).

Table 2 | **FDA-approved ligand-targeting drugs and their indications**

Drug name (modality)	Targeted ligand	Indication(s)
Etanercept (fusion protein)	TNF	Rheumatoid arthritis (1998), polyarticular juvenile idiopathic arthritis[a] (1999), psoriatic arthritis (2002), ankylosing spondylitis (2003), plaque psoriasis (2004), paediatric plaque psoriasis (2016)
Infliximab (mAb)	TNF	Crohn's disease[a] (1998), rheumatoid arthritis (1999), ankylosing spondylitis (2004), psoriatic arthritis (2005), ulcerative colitis (2005), paediatric Crohn's disease[a] (2006), plaque psoriasis (2006), paediatric ulcerative colitis[a] (2011)
Adalimumab (mAb)	TNF	Rheumatoid arthritis (2002), psoriatic arthritis (2005), ankylosing spondylitis (2006), Crohn's disease (2007), juvenile idiopathic arthritis[a] (2008), plaque psoriasis (2008), ulcerative colitis (2012), paediatric Crohn's disease[a] (2014), hidradenitis suppurativa[a] (2015), uveitis[a] (2016)
Certolizumab pegol (antibody fragment)	TNF	Crohn's disease (2008), rheumatoid arthritis (2009), ankylosing spondylitis (2013), psoriatic arthritis (2013), plaque psoriasis (2018), non-radiographic axial spondyloarthritis (2019)
Golimumab (mAb)	TNF	Rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis (2009), ulcerative colitis (2013), moderate to severe rheumatoid arthritis (2013), adult active ankylosing spondylitis and psoriatic arthritis (2017)
Omalizumab (mAb)	IgE Fc region	Severe asthma (2003), chronic idiopathic urticaria (2014)
Pegaptanib (aptamer)	VEGFA	Neovascular AMD (2004)
Bevacizumab (mAb)	VEGFA	Metastatic colorectal cancer (2004), NSCLC, in combination (2006), breast cancer, in combination but revoked (2008), glioblastoma[a] (2009), renal cell carcinoma[a] (2009), metastatic cervical cancer (2014), recurrent ovarian cancer[a], in combination (2014), advanced ovarian cancer, in combination (2016, 2018), recurrent glioblastoma (2017)
Ranibizumab (antibody fragment)	VEGFA	Neovascular AMD (2006), macular oedema (2010), diabetic macular oedema (2012), diabetic retinopathy (2015, 2017), myopic choroidal neovascularization (2017)
Aflibercept (fusion protein)	VEGFA, VEGFB and PGF	Neovascular AMD (2011), colorectal cancer (2012), diabetic macular oedema and macular oedema following retinal vein occlusion (2014), diabetic retinopathy (2015)
Brolucizumab (antibody fragment)	VEGFA	Neovascular AMD (2019)
Eculizumab (mAb)	C5	PNH[a] (2007), aHUS[a] (2011), generalized myasthenia gravis[a] (2017), neuromyelitis optica[a] (2019)
Ravulizumab (mAb)	C5	PNH[a] (2018)
Rilonacept (fusion protein)	IL-1A and IL-1B	CAPS, FCAS, MWS[a] (2008)
Canakinumab (mAb)	IL-1B	CAPS, FCAS, MWS[a] (2009), juvenile idiopathic arthritis[a] (2013), TRAPS, HIDS, MKD, FMF[a] (2016)
Ustekinumab (mAb)	IL-12 and IL-23 (p40 subunit)	Psoriasis (2009), psoriatic arthritis (2013), Crohn's disease (2016), plaque psoriasis (2017), ulcerative colitis (2019)
Denosumab (mAb)	TNFSF11	Osteoporosis (2010), bone loss in patients with prostate or breast cancer undergoing hormone ablation therapy (2011), bone loss in men with osteoporosis (2012), giant cell tumour of bone[a] (2013), hypercalcaemia in malignancy[a] (2013), glucocorticoid-induced osteoporosis (2018)
Belimumab (mAb)	TNFSF13B	Systemic lupus erythematosus (2011)
Siltuximab (mAb)	IL-6	Multicentric Castleman disease[a] (2014)
Mepolizumab (mAb)	IL-5	Severe asthma (2015), EGPA[a] (2017), paediatric eosinophilic asthma (2019)
Reslizumab (mAb)	IL-5	Severe asthma (2016)
Secukinumab (mAb)	IL-17A[b]	Plaque psoriasis (2015), ankylosing spondylitis and psoriatic arthritis (2016)
Ixekizumab (mAb)	IL-17A[b]	Plaque psoriasis (2016), psoriatic arthritis (2017), ankylosing spondylitis (2019)
Guselkumab (mAb)	IL-23(p19 subunit)	Plaque psoriasis (2017)
Tildrakizumab (mAb)	IL-23(p19 subunit)	Plaque psoriasis (2018)
Risankizumab (mAb)	IL-23(p19 subunit)	Plaque psoriasis (2019)
Emapalumab (mAb)	IFNγ	Haemophagocytic lymphohistiocytosis[a] (2018)
Burosumab (mAb)	FGF23	X-linked hypophosphataemia[a] (2018)
Galcanezumab (mAb)	CGRPα	Migraine disorders (2018)
Fremanezumab (mAb)	CGRPα	Migraine disorders (2018)
Eptinezumab (mAb)	CGRPα	Migraine disorders (2020)
Caplacizumab (nanobody)	VWF	Thrombotic thrombocytopenic purpura[a] (2019)
Romosozumab (mAb)	SOST	Osteoporosis (2019)
Luspatercept (fusion protein)	GDF8 and GDF11	β-Thalassaemia anaemia[a] (2019), anaemia in adults with myelodysplastic syndromes[a] (2020)

The year of approval is shown in brackets. aHUS, atypical haemolytic uraemic syndrome; AMD, age-related macular degeneration; CAPS, cryopyrin-associated periodic syndromes; CGRPα, calcitonin gene-related peptide-α; EGPA, eosinophilic granulomatosis polyangiitis; FCAS, familial cold autoinflammatory syndrome; FMF, familial Mediterranean fever; GDF, growth/differentiation factor; HIDS, hyperimmunoglobulin D syndrome; IFNγ, interferon-γ; IgE, immunoglobulin E; IL, interleukin; mAb, monoclonal antibody; MKD, mevalonate kinase deficiency; MWS, Muckle–Wells syndrome; NSCLC, non-small-cell lung cancer; PNH, paroxysmal nocturnal haemoglobinuria; SOST, sclerostin; TNF, tumour necrosis factor; TRAPS, tumour necrosis factor receptor-associated periodic syndrome; VEGF, vascular endothelial growth factor A; VWF, von Willebrand factor. [a]Approved orphan designation; identified through the FDA Search Orphan Drug Designations and Approvals website. [b]IL-17A exists as a homodimer or as a heterodimer with IL-17F; secukinumab and ixekizumab bind to IL-17A.

Antibody fragments and single-domain antibodies. Although full-length mAbs have successfully been developed to treat a variety of disorders, their size (~150 kDa) can impede the desired tissue penetration[42]. Various strategies have been pursued to develop antibody-based drugs with reduced size to address this issue, including antibody fragments and nanobodies. However, antibody fragments have short half-lives as they are preferentially cleared via lysosomal degradation in addition to renal filtration[28], which could result in more frequent dosing requirements. This may be undesirable for chronic therapies, but can be addressed by modifications such as pegylation, as with the TNF-targeted antibody fragment certolizumab pegol[28].

Roughly 6% of the antibody therapies (18 agents) in our total dataset consist of fragment constructs including Fab, $(Fab)_2$, scFv and single-domain antibodies such as nanobodies (FIG. 5b). Antibody fragment therapies were clinically established in 1998 with the FDA approval of abciximab, and the first ligand-targeting Fabs ranibizumab, certolizumab pegol and brolucizumab were approved in 2006, 2008 and 2019, respectively[43] (see later for further discussion of the rationale for pursuing fragments for these targets).

Nanobodies are constructs engineered from the unique antibodies produced by members of the Camelidae family (camels and llamas), which are composed solely of a V_{HH} domain where the target recognition module contains a single variable domain[44]. In 2019, the FDA approved the first nanobody-based medicine, caplacizumab, which targets von Willebrand factor (VWF) for treatment of the rare disease acquired thrombotic thrombocytopenic purpura[45]. The drug has a short half-life, but patients undergo plasmapheresis every day, and so this is not expected to be a major barrier to its use.

Aptamers and spiegelmers. Aptamers and spiegelmers are nucleic acid polymers that fold into distinct 3D structures that bind specifically and with high affinity to a defined target, including ligands, ions or small molecules[46]. They are essentially nucleotide analogues of antibodies that could have several advantages, including smaller size, rapid chemical production and lack of immunogenicity[47]. Spiegelmers are a mirror-image

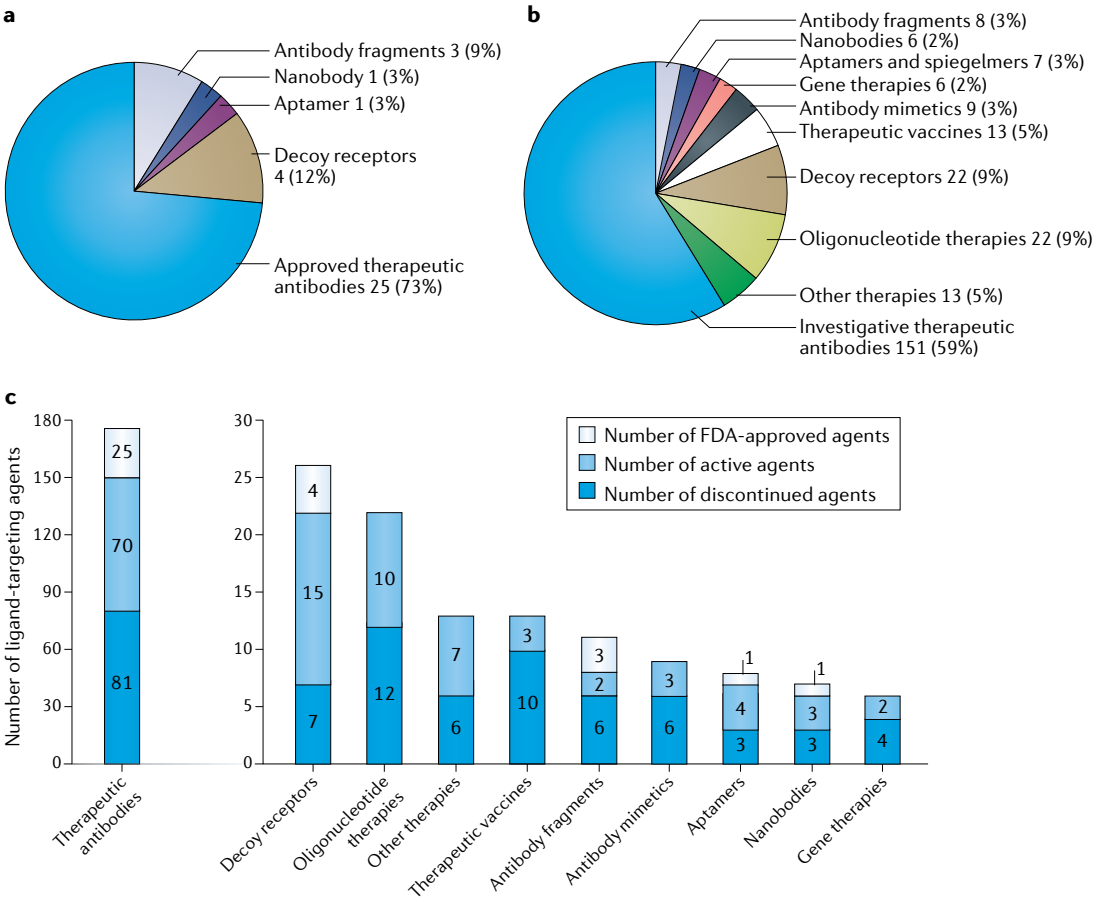


Fig. 4 | **Classes of ligand-targeting agents. a**,**b** | The 34 FDA-approved therapies (part **a**) and 257 investigational agents (part **b**) were identified and classified by the different therapeutic platforms. Antibody therapies including full-length monoclonal antibodies and antibody fragments such as nanobodies, Fabs and single-chain variable regions are shown in blue tones. Other platforms include aptamers and spiegelmers, gene therapies, antibody mimetics, therapeutic vaccines, decoy receptors, oligonucleotide therapies such as antisense oligonucleotides and anti-microRNAs, and other therapies. The number of agents and the percentage in each category are shown. **c** | The 291 therapeutic agents in the dataset classified by their different platforms and separated by the number of approved agents, the number of active agents and the number of discontinued agents in each platform.

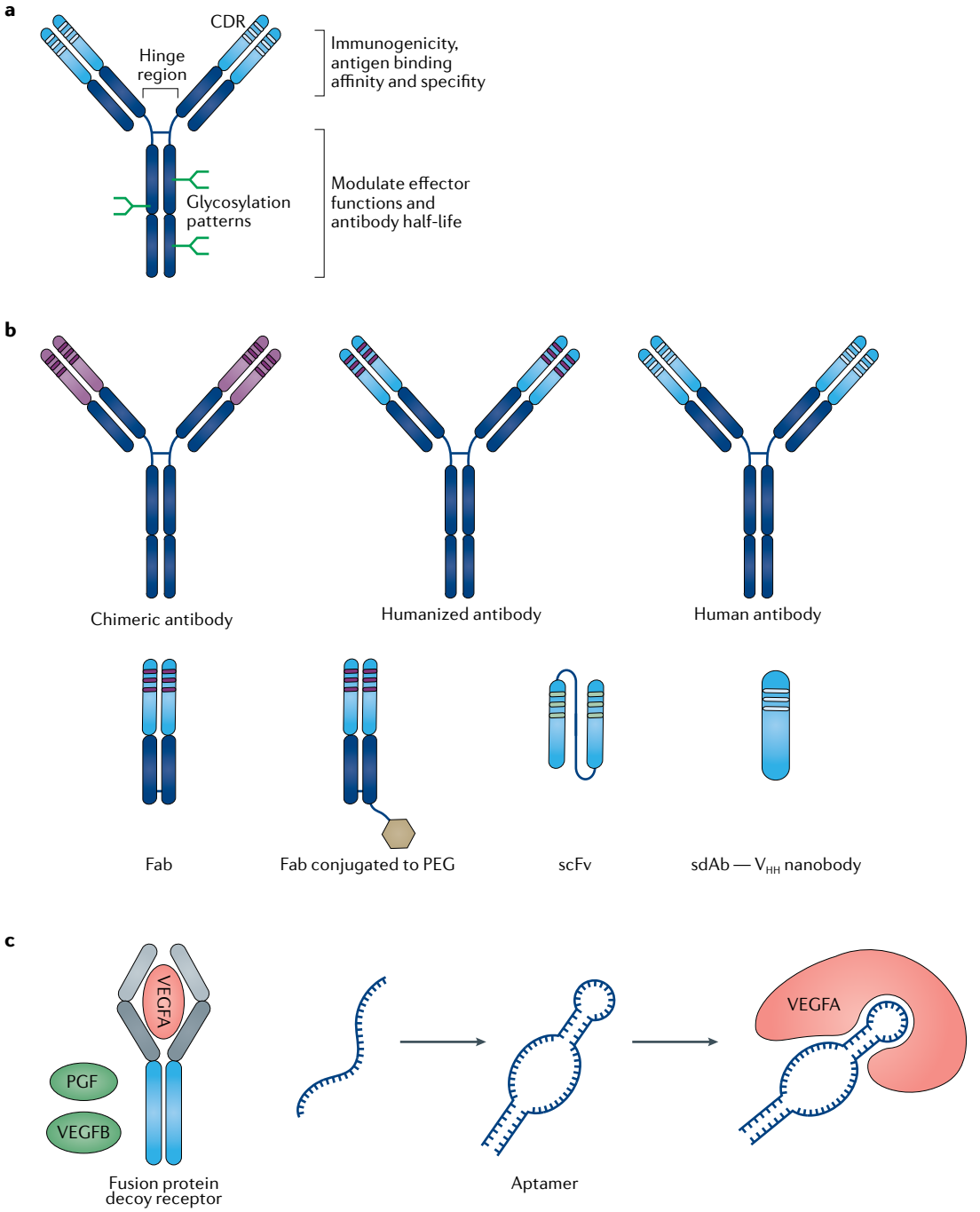


Fig. 5 | **Established therapeutic platforms for ligand-targeting drugs. a** | Structural components and functional activities of antibody therapies. The antibody is constructed of variable (light blue) regions that contain three complementarity-determining regions (CDRs; or hypervariable regions) that together form the target-binding paratope, which binds to an antigen epitope. Modifications in the variable region can affect immunogenicity, antigen-binding affinity and specificity. The Fc region and glycosylation patterns modulate effector functions and antibody half-life. Specific amino acid alterations in this region or alteration of the glycosylation patterns can induce or suppress effector functions and alter antibody circulating times. **b** | Antibody engineering used in FDA-approved ligand-targeting agents. The first row illustrates the progression in strategies to address immunogenicity issues, from chimeric full-length antibodies, shown with purple variable regions and CDRs to indicate material from other species (2 approved agents) to humanized antibodies with non-human CDRs (12 approved agents) to fully human antibodies (10 approved agents). The second row shows antibody fragment constructs for approved agents: one humanized Fab (ranibizumab); one humanized Fab conjugated with polyethylene glycol (PEG) for increased half-life (certolizumab pegol); one single-chain variable region (scFv; brolucizumab); and one single-domain antibody (sdAb) — a V_{HH} fragment derived from the heavy-chain regions from camelids and called a 'nanobody' (caplacizumab). **c** | Decoy receptors are the second most common therapeutic platform among approved ligand-targeting agents; there are four approved decoy receptors, including aflibercept (shown), which targets vascular endothelial growth factor A (VEGFA), VEGFB and PGF. One ligand-targeting aptamer has been approved: pegaptanib is initially a single RNA strand that folds into a specific 3D structure that binds and inhibits VEGFA.

configuration of aptamers composed of non-natural L-nucleotides, which makes them highly resistant to degradation by nucleases[48].

The RNA aptamer pegaptanib, which binds to VEGFA, is the first and to date only aptamer approved by the FDA[49]. Pegaptanib was approved for treatment of wet AMD in 2004, and was the first treatment that could effectively slow disease progression; however, it has since been superseded by other VEGFA-targeted therapies, as discussed further later. There are two active aptamers and two spiegelmers that are currently in phase II trials. Recent reviews indicate that there are also many aptamers in preclinical development[50,51], suggesting that this platform may attract continued therapeutic interest.

Engineered protein scaffolds. Several types of engineered protein scaffolds, also known as antibody mimetics, can bind to antigens and ligands in a similar fashion to antibodies and have been developed with the aim of addressing some of the limitations of mAbs (for recent reviews on antibody mimetics, see[52,53]). Their advantages could include higher affinity, greater stability and solubility, and inexpensive large-scale production. Furthermore, as with antibody fragments, the smaller sizes of mimetics could enable an increased rate of diffusion and more efficient penetration into the target tissues. However, similarly to antibody fragments, antibody mimetics can also have problems such as immunogenicity and short plasma half-life[52].

The nine antibody mimetics that target ligands in our dataset represent a rich cross section of different scaffold designs, including designed ankyrin repeat proteins (DARPins), affibodies and avimers. Two of the three agents in active clinical trials are DARPins, which are artificial protein scaffolds based on ankyrin repeat domains that mediate a broad range of protein interactions[54]. They are highly stable and soluble, and their small size (14–18 kDa) could enable greater tissue penetration than full-length mAbs[53]. The anti-VEGFA DARPin abicipar pegol[55] has shown positive effects in phase III trials for treatment of neovascular AMD, although recently the FDA declined to approve this agent. The DARPin agent MP0250 is currently in phase I/II trials. The other mimetic in clinical development is an affibody, which is in phase II trials. Affibodies are small (~6-kDa) constructs that consist of three α-helices that hold the variable domains that are responsible for epitope recognition and for binding to a range of proteins[56].

Gene therapies. Gene therapies — which deliver a therapeutic gene to target cells using a vector such as an adeno-associated virus (AAV) — are becoming established as a treatment modality after years of setbacks[57]. Most such therapies involve the delivery of a functional copy of a defective gene, typically for the treatment of rare monogenic disorders. However, there are six investigational therapies in our dataset for which the mode of action involves the delivery of a gene to express a protein that targets a ligand. The two currently active gene therapies are both in phase I trials; one is an AAV vector which encodes the anti-VEGF agent aflibercept and has been optimized for intravitreal delivery and strong protein expression[58], while the other is an AAV vector that carries a gene that encodes a soluble anti-VEGF protein[59]. Eye disorders have been one of the areas in which gene therapies in general have been successful in recent years, and in the case of VEGFA-targeted agents for treatment of AMD, such agents could circumvent the need for repeated intravitreal injections, which is a limitation of current therapies[60].

Therapeutic vaccines. There are 13 therapeutic vaccines in our dataset that contain an antigen–carrier construct that, on delivery, evokes antibodies to a specific ligand target to achieve a therapeutic effect[61]. However, the extent of recent developments in this class of agents is limited compared with other classes, possibly due to issues with lack of efficacy in various indications[62,63]. Three ligand-targeting vaccines are in active trials, including an EGF-targeted immunotherapy known as EGF-PTI[64] that is in two phase II trials, although three phase III trials of this agent were terminated by the sponsor because of enrolment issues. Ten agents have been discontinued, with most of the agents not progressing past phase I or phase I/II trials.

Oligonucleotide therapies. All the agent classes discussed so far directly target the ligand protein. However, it is also possible to affect the production of the ligand protein with various platforms, including antisense oligonucleotides (ASOs)[65], small interfering RNAs (siRNAs)[66] and microRNA-based agents[67].

ASOs, which can bind with high specificity to the mRNA for a protein and promote its degradation, are the most developed of these platforms, with several ASOs having gained FDA approval, although none of these target ligands. There are 11 ASOs in our dataset that modulate the expression of endogenous ligands, with 6 currently active in trials. The most advanced agent is ISIS-HTTRx, which targets the huntingtin (*HTT*) gene for treatment of Huntington disease and is in phase III trials[68].

There are also three small siRNA-based therapies that silence the expression of genes coding for ligands by exploiting the RNA interference pathway. One of the first siRNA drugs to begin clinical trials was the anti-VEGFA agent bevasiranib in 2004, although its development was terminated following a phase III trial owing to poor efficacy in reducing vision loss[69]. Two siRNAs which have targets that are not ligands have recently received FDA approval, and of the ligand-targeting siRNAs currently in clinical trials, the most advanced is the angiotensinogen targeting agent ALN-AGT[70] for treatment of hypertension, which is in phase I trials.

Finally, six agents in the dataset affect the expression of ligand targets by targeting regulatory microRNAs. One of the agents that has progressed the furthest in clinical development is cobomarsen, which targets miR-155 (which subsequently targets FGF7 (REF.[71])) and is in phase II trials for treatment of haematological cancers[72].

Strategic issues in targeting ligands

When we noted in 2014 that soluble ligands were becoming one of the largest categories of potentially novel drug targets in clinical trials, there were 11 established ligand

targets for FDA-approved drugs and 37 ligand targets being investigated in clinical trials[1]. The number of established ligand targets has doubled in the past 5 years to a total of 22, while potentially novel targets in clinical trials have increased twofold to 77 ligand targets in our analysis. Ligands now constitute 10% of novel targets in clinical trials and are currently the third-largest target class for therapeutic agents targeting human protein products, after enzymes and receptors[2].

There are several factors that contribute to the increasing importance of soluble ligands as drug targets. First, a key factor is the growing understanding of the role of the immune system in many diseases and the tractability of cytokines as therapeutic targets for agents such as mAbs. Second, it may be easier to reach ligands than their receptors. For example, a receptor target expressed in a solid tumour can be inaccessible owing to challenges related to tissue distribution and penetration of the therapeutics[73], while ligands that are involved in cancers may be more readily targetable. A third factor is that it may be easier to map epitopes on protein ligands in comparison with transmembrane receptors owing to the complications inherent in resolving tertiary membrane-bound structures. Epitope characterization helps elucidate the mechanism of action by which an antibody and its target interact and can be beneficial in selecting potential therapeutic candidates, as well as for intellectual property claims[74]. Fourth, some proteins have membrane-bound forms that then become soluble via cleavage; for example, TNF (discussed later), TNFSF13B (also known as BAFF)[75] or FASLG[76]. The different forms of soluble ligand, cleaved transmembrane protein and also possibly both together in a complex may have different roles in normal physiology and disease progression, potentially presenting opportunities to more specifically intervene in disease processes by targeting a particular form. In this section, we consider such strategic issues in the development of ligand-targeting drugs.

Targeting ligands versus receptors. Inflammatory mediators such as cytokines often have complex signalling pathways, with the potential to signal through more than one type of receptor complex, and/or redundant actions if the receptor can interact with more than one cytokine. Consequently, there could be disease scenarios where it may be beneficial to target the ligand rather than the receptor (or vice versa). For example, clinical trials with the IL-17A-targeting mAb ixekizumab showed improved signs and relief of symptoms in patients with rheumatoid arthritis[77], whereas trials of the IL-17R-targeting mAb brodalumab, which inhibits all signalling by all members of the IL-17 family, showed no response[78,79]. It has been speculated that this non-response may be a result of the additional inhibition of signalling by the cytokine IL-17E (also known as IL-25) by brodalumab[80].

An interesting example beyond the immune system in which the benefits of blocking the ligand versus the receptor are currently unclear is in the treatment of migraines with the four recently approved therapeutic mAbs. Fremanezumab, galcanezumab and eptinezumab are humanized mAbs that bind CGRPα and CGRPβ, while erenumab is a fully human mAb that targets the CGRP receptor[23,24,81]. Erenumab blocks only the canonical CGRP receptor and does not affect actions of CGRP mediated by other CGRP-binding receptors[27], which could limit the side effect profile. However, the effects mediated by the interaction of CGRP with other receptors, such as amylin 1 (AMY1), have not been fully elucidated yet and they may also be involved in migraine pathology, which might mean that targeting the CGRP receptor rather than CGRP could have lower efficacy[27]. In this case, the relative merits of ligand or receptor targeting have yet to be determined, and a deeper understanding of the CGRP pathways in connection with migraines and other effects, assessments of genetic factors such as possible individual polymorphisms that influence therapeutic response and analyses of the comparative efficacy of these agents are needed to establish the appropriate agent given the circumstances.

Opportunities to target multiple ligands. Approximately 16% of the dataset (46 agents) are agents that bind more than one soluble ligand, with 25 unique combinations of ligands being targeted. As mentioned previously (FIG. 2a,b), increases in the number of agents targeting more than one ligand as well as the number of unique combinations of ligands can be seen in the last decade in comparison with previous years. The agents include 26 bispecific or multispecific agents that are designed to bind multiple unrelated targets or bind different epitopes on related proteins. These constructs include 16 full-length mAbs and four antibody fragments, three antibody mimetics, two decoy receptors that contain multiple domains to target unrelated proteins and one peptide–Fc-fusion protein. Another 19 agents bind more than one protein because one epitope is common to more than one protein, such as in closely related members of a family. This includes 14 decoy receptors, four full-length mAbs and one peptide–Fc-fusion protein. Additionally, there is one combination (or mixture) that contains two separate monospecific agents (we did not include three agents considered bispecific as the second target was human serum albumin to extend the half-life).

Bispecific antibodies became validated as a therapeutic class in the USA with the 2014 FDA approval of blinatumomab for treatment of acute lymphoblastic leukaemia[82], although it is a bispecific T cell engager that does not target soluble ligands. Although bispecific constructs that target cell-surface ligands and receptors primarily for treatment of cancer are a large portion of the bispecific antibody pipeline, they will not be further discussed here (see REFS[83–85] for reviews). However, nearly 30% of the 26 multispecific ligand-targeting therapies are currently in clinical trials for treating cancer. Multispecific antibodies are able to address the multifactorial nature of complex diseases, and in particular cancer, where therapeutic resistance can emerge, as cancer cells have the ability to downregulate targeted pathways, upregulate alternative pathways and generate crosstalk between pathways, which can lead to resistance and reduction of antibody efficacy[83]. This appears to occur primarily for antibody therapies where a single epitope of a target in one specific pathway is targeted.

Indeed, as tumour angiogenesis is known to play a crucial role in tumour growth, seven bispecific therapies for oncological applications target either VEGFA plus DLL4 to enhance antitumour effects while limiting VEGFA-induced vascular sprouting[86] or VEGFA plus ANGPT2 for dual inhibition of the VEGFA–VEGFR and ANGPT2–TIE2 signalling pathways.

Advances in engineering technology and the ability to effectively and safely target more than one ligand has combined with increased knowledge of disease pathways and also clinical experience from single-target agents to better identify and effectively utilize therapies. Additionally, the development of monotherapies targeting multiple targets can be less complex than the development of multiple drug products in combination, as well as contributing to reducing production costs. Furthermore, using multiple paratopes on multispecific antibodies can promote prolonged serum half-life, improved tissue distribution and local enrichment[87].

Platform choices may influence therapeutic effects in different contexts. For some popular ligand targets, several strategies have been applied. For example, there are five anti-TNF agents that have been approved by the FDA: three full-length mAbs (infliximab, adalimumab and golimumab), one TNF receptor–Fc-fusion protein (etanercept) and one pegylated Fab (certolizumab pegol). All of these agents are successful anti-inflammatory agents that target TNF; however, each platform may confer different attributes, and the choice of platform may affect the efficacy for specific indications.

Infliximab, adalimumab, golimumab and certolizumab pegol have all been approved for treatment of inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis[88], whereas etanercept has been shown to not be effective for IBDs[89]. The primary mechanism of action of anti-TNF agents is to bind and neutralize the proinflammatory cytokine TNF, predominantly the soluble form. However, the agents that contain an Fc domain also bind to tmTNF, the transmembrane-bound precursor to soluble TNF, albeit to a lesser degree[90]. Fc-mediated interactions with tmTNF appear to play a key role in IBD treatment: they induce apoptosis in immune cells through reverse signalling in tmTNF-expressing cells[90,91], they promote cell lysis via ADCC and CDC[92], and they downregulate proinflammatory mediators[93,94]. TNF inhibitors also decrease intestinal permeability[95], which minimizes mucosal inflammation, and induce regulatory macrophages[96], which promotes mucosal healing. Certolizumab, however, does not contain the Fc portion of IgG1 and has been shown to be unable to induce ADCC, CDC, regulatory macrophages or apoptosis via reverse signalling[97]. Nevertheless, it has been shown to be effective for induction of clinical remission and response in patients with Crohn's disease[98], which could be because certolizumab induces a direct cytotoxic effect on tmTNF-expressing cells by a non-apoptotic mechanism[99]. Etanercept, which also has an Fc region, is unique in that it binds and neutralizes members of the lymphotoxin family, possibly due to the fusion protein construct retaining the ligand-binding specificity of its parent receptor TNFR2 (REF.[100]). Etanercept inhibits lymphotoxin-α_3, which is a key cytokine in the regulation of the mucosal immune system, and it is suspected that this inhibitory action contributes to the ineffectiveness of etanercept in Crohn's disease and it may even slightly increase the risk of developing IBD[88].

All five of these anti-TNF agents have been approved for treatment of rheumatoid arthritis. TNF is important in the pathogenesis of rheumatoid arthritis owing to its ability to induce the production of other proinflammatory cytokines and chemokines and facilitate the destruction of underlying articular cartilage and subchondral bone[101,102]. Lymphotoxin-α_3 and TNF both mediate proliferation and proinflammatory cytokine secretion of rheumatoid arthritis synovial fibroblasts, and as etanercept blocks both of these cytokines, it may allow better control of inflammation and synovial proliferation in the treatment of rheumatoid arthritis[103]. There have not been any direct head-to-head comparison trials between anti-TNF biologics, although systematic reviews and meta-analyses have found comparable effectiveness and safety between the five biologics[104,105].

Another popular target for which several platforms have been applied is VEGFA. There are currently five FDA-approved agents that target soluble VEGFA: the pegylated RNA aptamer pegaptanib for treatment of neovascular AMD, the full-length mAb bevacizumab for treatment of various cancers and off-label use for ophthalmic indications, the Fab ranibizumab and the scFv brolucizumab for ophthalmic indications, and the recombinant fusion protein aflibercept for ophthalmic indications and colorectal cancer[106].

For this target, ophthalmic indications such as wet AMD have been a focus of optimization strategies. The eye is an immunoprivileged site and the blood–retinal barrier prevents free entry from the circulation, so all of the therapeutic agents are administered locally through intravitreal injections. It was anticipated that the molecular size of the agent could be relevant in improving the delivery of the agents, which encouraged the development of antibody fragments[107,108]. However, despite their structural differences, bevacizumab, ranibizumab and aflibercept all appear to have comparable efficacy and safety, although aflibercept may be superior in patients with initial lower visual acuity[109,110]. Brolucizumab, the smallest agent so far and the most recently approved, has higher tissue penetrance than the other antibody fragment ranibizumab and has shown longer anti-VEGFA suppression[111] as well as more favourable anatomical outcomes in comparison with aflibercept[112], but the clinical relevance remains to be established.

However, current treatment paradigms with all of the approved agents involve regular intravitreal injections, which are associated with complications and possible side effects as well as posing a significant burden for the patient and the caregiver[113]. Of the nine antiangiogenic VEGFA inhibitors currently in clinical trials for treatment of AMD, two are gene therapies, which may provide sustained anti-VEGFA levels in the retina following a single injection and improve patient adherence and comfort.

Investigating alternative delivery routes. Even with the success of therapeutic antibodies, there are still difficult-to-reach sites of action and previously undruggable targets that could be pursued with continued engineering developments[114]. Advances in therapeutic platforms along with emerging delivery and administration options can facilitate more effective and safer disease management and possibly access privileged areas, for example, behind the blood–brain barrier (BBB). Investigational recombinant proteins have been designed that cross the BBB by binding to transferrin receptors, which transport the therapeutic agent across the BBB and enable it to bind to a target in the central nervous system[115]. Intranasal administration of agents, where drug absorption is through the olfactory and trigeminal routes, has also been explored to cross the BBB[116]. Methods to effectively deliver therapies to mucosal tissues are also being investigated. For example, there are three anti-TNF agents — a nanobody, a decoy receptor and a mAb — with an oral administration route for treatment of Crohn's disease and ulcerative colitis in clinical trials, of which the nanobody V565 is the most advanced, having reached phase II trials[117]. An inhaled formulation of a high-affinity antibody fragment, abrezekimab, that targets IL-13 was well tolerated in a phase I safety trial, and is proposed to provide a more rapid onset of action at lower doses than subcutaneous systemic administration for the treatment of asthma[118].

Repurposing approved drugs for new indications. It is evident that the repurposing of approved drugs for new indications is common; more than half of the 34 approved drugs that target ligands have been approved for additional indications. Furthermore, more than 70% of the initiated clinical trials in our dataset are studies of agents after they have gained FDA approval (FIG. 2c).

One factor in the high number of clinical trials of approved ligand-targeting drugs for new indications is the role of some of the most targeted ligands in immune pathways that are involved in several inflammatory disorders. For example, since the pioneering approvals for treatment of rheumatoid arthritis and Crohn's disease, TNF-targeted agents have also been approved for other indications, including psoriasis, psoriatic arthritis, ulcerative colitis, ankylosing spondylitis and hidradenitis suppurativa. Similar trends can be seen with other popular cytokine targets, and in some cases these efforts are expanding into new therapeutic areas. For example, inflammation has been suggested to play a role in a psychiatric illnesses such as major depressive disorder[119,120], and the effects of various cytokine-targeted drugs on depressive symptoms have been investigated in a number of trials[121].

There may also be a substantial number of rare diseases in which a ligand is a promising target; indeed, nearly 45% of the approved drugs targeting ligands have had additional approvals for a rare disease indication, with ten of them having initial approval for treatment of a rare disease. Furthermore, nearly 15% of the novel agents in clinical trials are being investigated as treatments for rare diseases.

Finally, given emerging understanding of the involvement of the immune system in patients with severe COVID-19 (REF.[122]), including high levels of inflammatory cytokines such as IL-6, IL-17, CSF2, interferon-γ, TNF and IL-2 in some patients[123], there has been intense recent interest in repurposing various ligand-targeting agents for treatment of COVID-19. Several approved ligand-targeting agents are in clinical trials involving patients with COVID-19, including the IL-6-targeting drug siltuximab (NCT04329650 and NCT04330638), the VEGFA-targeting drug bevacizumab (NCT04305106, NCT04344782 and NCT04275414), the C5-targeting drugs ravulizumab (NCT04369469 and NCT04390464) and eculizumab (NCT04346797), the interferon-γ-targeting drug emapalumab (NCT04324021) and the IL-1B-targeting drug canakinumab (NCT04362813 and NCT04365153). Additionally, several investigational agents that had entered clinical trials for other indications are now in COVID-19 studies, including three CSF2-targeting agents, gimsilumab (NCT04351243), lenzilumab (NCT04351152) and TJ003234 (NCT04341116); two C3-targeting agents, AMY-101 (NCT04395456) and APL-9 (NCT04402060); the C5-targeting agent IFX-1 (NCT04333420); and the IL-6-targeting agent clazakizumab (NCT04343989, NCT04348500, NCT04351724, NCT04363502 and NCT04381052).

Drugs that target ligands are economically successful. Therapeutic agents that target ligands have proved financially successful. From 2010 to 2018, the number of unique therapeutic antibodies in the top 200 performing drugs based on US retail sales rose from 2 to 29, and roughly half of these drugs have consistently targeted ligands[124]. Furthermore, in the last 3 years, ligand-targeting drugs have been responsible for nearly 20% of US sales among the top 200 drugs, and they account for more than 50% of all therapeutic antibody sales[124]. According to a recent analysis of the financial performance of agents modulating the top 20 drug targets, 24 FDA-approved drugs that target 10 cytokines plus the growth factors VEGFA and VEGFB had more than US$250 billion in cumulative sales from 2011 to 2015 (REF.[125]). The cytokine TNF was the most valuable target, with sales generating more than US$163 billion dollars from 2011 to 2015 (REF.[125]). Recently approved drugs that target ligands are also expected to generate large financial returns, such as the IL-23-targeted mAbs guselkumab and risankizumab, which are forecast to have sales in excess of US$3.4 billion by 2022 (REF.[126]) and US$3.2 billion by 2024 (REF.[127]), respectively.

Outlook

Our analysis highlights the increased number of ligand-targeting drugs approved in the past decade compared with the previous one, as well as substantial interest in agents for novel ligand targets in clinical trials. Given the increasing body of evidence that inflammation is involved in many diseases beyond typical inflammatory disorders such as rheumatoid arthritis and the position of ligand-targeting drugs at the forefront of anti-inflammatory therapies, there will be continued

interest in this class of agents. In this respect, there is vast territory to explore, as there are more than 580 endogenous peptides according to the IUPHAR/BPS Guide to Pharmacology, with more than 140 of these identified as specific immunopharmacology ligands that have curated immunological data associated with them[128]. Furthermore, the potential to target ligands also continues to be expanded by technological accomplishments such as bispecific constructs, antibody fragments and innovative engineering approaches such as sweeping antibodies[129]. In conclusion, while ligands as a type of drug target were previously categorized as 'miscellaneous' or 'other'[5], they now merit consideration as a distinct and expanding group of targets for a range of biopharmaceutical agents that can exert their effects through single targets or in selected combinations.

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1. Rask-Andersen, M., Masuram, S. & Schiöth, H. B. The druggable genome: evaluation of drug targets in clinical trials suggests major shifts in molecular class and indication. *Annu. Rev. Pharmacol. Toxicol.* **54**, 9–26 (2014).
2. Attwood, M. M., Rask-Andersen, M. & Schiöth, H. B. Orphan drugs and their impact on pharmaceutical development. *Trends Pharmacol. Sci.* **39**, 525–535 (2018).
3. Hopkins, A. L. & Groom, C. R. The druggable genome. *Nat. Rev. Drug Discov.* **1**, 727–730 (2002).
4. Overington, J. P., Al-Lazikani, B. & Hopkins, A. L. How many drug targets are there? *Nat. Rev. Drug Discov.* **5**, 993–996 (2006).
5. Santos, R. et al. A comprehensive map of molecular drug targets. *Nat. Rev. Drug Discov.* **16**, 19–34 (2017).
6. Kesik-Brodacka, M. Progress in biopharmaceutical development. *Biotechnol. Appl. Biochem.* **65**, 306–322 (2017).
7. Urquhart, L. Top drugs and companies by sales in 2018. *Nat. Rev. Drug Discov.* **18**, 245–245 (2019).
8. Urquhart, L. Top companies and drugs by sales in 2019. *Nat. Rev. Drug Discov.* **19**, 228–228 (2020).
9. Lu, R.-M. et al. Development of therapeutic antibodies for the treatment of diseases. *J. Biomed. Sci.* **27**, 1 (2020).
10. Sullivan, L. A. & Brekken, R. A. The VEGF family in cancer and antibody-based strategies for their inhibition. *MAbs* **2**, 165–175 (2010).
11. Shim, H. One target, different effects: a comparison of distinct therapeutic antibodies against the same targets. *Exp. Mol. Med.* **43**, 539–549 (2011).
12. Giacca, M. & Zacchigna, S. VEGF gene therapy: therapeutic angiogenesis in the clinic and beyond. *Gene Ther.* **19**, 622–629 (2012).
13. Suragani, R. N. V. S. et al. Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. *Nat. Med.* **20**, 408–414 (2014).
14. Regula, J. T. et al. Targeting key angiogenic pathways with a bispecific CrossMAb optimized for neovascular eye diseases. *EMBO Mol. Med.* **8**, 1265–1288 (2016).
15. Ricklin, D., Mastellos, D. C., Reis, E. S. & Lambris, J. D. The renaissance of complement therapeutics. *Nat. Rev. Nephrol.* **14**, 26–47 (2018).
16. Monaco, C., Nanchahal, J., Taylor, P. & Feldmann, M. Anti-TNF therapy: past, present and future. *Int. Immunol.* **27**, 55–62 (2015).
17. Apte, R. S., Chen, D. S. & Ferrara, N. VEGF in signaling and disease: beyond discovery and development. *Cell* **176**, 1248–1264 (2019).
18. Amadio, M., Govoni, S. & Pascale, A. Targeting VEGF in eye neovascularization: what's new?: a comprehensive review on current therapies and oligonucleotide-based interventions under development. *Pharmacol. Res.* **103**, 253–269 (2016).
19. Bartlett, H. S. & Million, R. P. Targeting the IL-17–T_H17 pathway. *Nat. Rev. Drug Discov.* **14**, 11–12 (2015).
20. Hawkes, J. E., Yan, B. Y., Chan, T. C. & Krueger, J. G. Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. *J. Immunol.* **201**, 1605–1613 (2018).
21. FDA. FDA approves new treatment for osteoporosis in postmenopausal women at high risk of fracture. http://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-osteoporosis-postmenopausal-women-high-risk-fracture (2019).
22. Suen, P. K. & Qin, L. Sclerostin, an emerging therapeutic target for treating osteoporosis and osteoporotic fracture: a general review. *J. Orthop. Translat.* **4**, 1–13 (2016).
23. Hoy, S. M. Fremanezumab: first global approval. *Drugs* **78**, 1829–1834 (2018).
24. Lamb, Y. N. Galcanezumab: first global approval. *Drugs* **78**, 1769–1775 (2018).
25. Dhillon, S. Eptinezumab: first approval. *Drugs* **80**, 733–739 (2020).
26. Edvinsson, L. in *Calcitonin Gene-Related Peptide (CGRP) Mechanisms: Focus on Migraine* (eds Brain, S. D. & Geppetti, P.) 121–130 (Springer, 2019).
27. Edvinsson, L. The CGRP pathway in migraine as a viable target for therapies. *Headache J. Head Face Pain* **58**, 33–47 (2018).
28. Goulet, D. R. & Atkins, W. M. Considerations for the design of antibody-based therapeutics. *J. Pharm. Sci.* **109**, 74–103 (2019).
29. Igawa, T. et al. Engineering the variable region of therapeutic IgG antibodies. *MAbs* **3**, 243–252 (2011).
30. Pineda, C., Castañeda Hernández, G., Jacobs, I. A., Alvarez, D. F. & Carini, C. Assessing the immunogenicity of biopharmaceuticals. *BioDrugs* **30**, 195–206 (2016).
31. Chames, P., Regenmortel, M. V., Weiss, E. & Baty, D. Therapeutic antibodies: successes, limitations and hopes for the future. *Br. J. Pharmacol.* **157**, 220–233 (2009).
32. Brezski, R. J. & Georgiou, G. Immunoglobulin isotype knowledge and application to Fc engineering. *Curr. Opin. Immunol.* **40**, 62–69 (2016).
33. Sondermann, P. & Szymkowski, D. E. Harnessing Fc receptor biology in the design of therapeutic antibodies. *Curr. Opin. Immunol.* **40**, 78–87 (2016).
34. Tridandapani, S. et al. Regulated expression and inhibitory function of FcγRIIb in human monocytic cells. *J. Biol. Chem.* **277**, 5082–5089 (2002).
35. Jiang, X.-R. et al. Advances in the assessment and control of the effector functions of therapeutic antibodies. *Nat. Rev. Drug Discov.* **10**, 101–111 (2011).
36. Rother, R. P., Rollins, S. A., Mojcik, C. F., Brodsky, R. A. & Bell, L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. *Nat. Biotechnol.* **25**, 1256–1264 (2007).
37. Kennedy, P. J., Oliveira, C., Granja, P. L. & Sarmento, B. Monoclonal antibodies: technologies for early discovery and engineering. *Crit. Rev. Biotechnol.* **38**, 394–408 (2018).
38. Sheridan, D. et al. Design and preclinical characterization of ALXN1210: a novel anti-C5 antibody with extended duration of action. *PLoS ONE* **13**, 0195909 (2018).
39. Dubois, E. A., Rissmann, R. & Cohen, A. F. Rilonacept and canakinumab. *Br. J. Clin. Pharmacol.* **71**, 639–641 (2011).
40. Stewart, M. W. Aflibercept (VEGF Trap-eye): the newest anti-VEGF drug. *Br. J. Ophthalmol.* **96**, 1157–1158 (2012).
41. Perkins, S. L. & Cole, S. W. Ziv-aflibercept (Zaltrap) for the treatment of metastatic colorectal cancer. *Ann. Pharmacother.* **48**, 93–98 (2014).
42. Shah, D. K. & Betts, A. M. Antibody biodistribution coefficients. *MAbs* **5**, 297–305 (2013).
43. Bates, A. & Power, C. A. David vs. Goliath: the structure, function, and clinical prospects of antibody fragments. *Antibodies* **8**, 28 (2019).
44. Bannas, P., Hambach, J. & Koch-Nolte, F. Nanobodies and nanobody-based human heavy chain antibodies as antitumor therapeutics. *Front Immunol* **8**, 1603 (2017).
45. FDA. FDA approved caplacizumab-yhdp. http://www.fda.gov/drugs/resources-information-approved-drugs/fda-approved-caplacizumab-yhdp (2019).
46. Haβel, S. K. & Mayer, G. Aptamers as therapeutic agents: has the initial euphoria subsided? *Mol. Diagn. Ther.* **23**, 301–309 (2019).
47. Nimjee, S. M., White, R. R., Becker, R. C. & Sullenger, B. A. Aptamers as therapeutics. *Annu. Rev. Pharmacol. Toxicol.* **57**, 61–79 (2017).
48. Ali, M. H., Elsherbiny, M. E. & Emara, M. Updates on aptamer research. *Int. J. Mol. Sci.* **20**, 2511 (2019).
49. Ng, E. W. M. et al. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat. Rev. Drug Discov.* **5**, 123–132 (2006).
50. Maier, K. E. & Levy, M. From selection hits to clinical leads: progress in aptamer discovery. *Mol. Ther. Methods Clin. Dev.* **5**, 16014 (2016).
51. Morita, Y., Leslie, M., Kameyama, H., Volk, D. E. & Tanaka, T. Aptamer therapeutics in cancer: current and future. *Cancers* **10**, 80 (2018).
52. Yu, X., Yang, Y.-P., Dikici, E., Deo, S. K. & Daunert, S. Beyond antibodies as binding partners: the role of antibody mimetics in bioanalysis. *Annu. Rev. Anal. Chem.* **10**, 293–320 (2017).
53. Simeon, R. & Chen, Z. In vitro-engineered non-antibody protein therapeutics. *Protein Cell* **9**, 3–14 (2018).
54. Plückthun, A. Designed ankyrin repeat proteins (DARPins): binding proteins for research, diagnostics, and therapy. *Annu. Rev. Pharmacol. Toxicol.* **55**, 489–511 (2015).
55. Stahl, A. et al. Highly potent VEGF-A-antagonistic DARPins as anti-angiogenic agents for topical and intravitreal applications. *Angiogenesis* **16**, 101–111 (2013).
56. Frejd, F. Y. & Kim, K.-T. Affibody molecules as engineered protein drugs. *Exp. Mol. Med.* **49**, e306 (2017).
57. Goswami, R. et al. Gene therapy leaves a vicious cycle. *Front. Oncol.* **9**, 297 (2019).
58. Grishanin, R. et al. Preclinical evaluation of ADVM-022, a novel gene therapy approach to treating wet age-related macular degeneration. *Mol. Ther.* **27**, 118–129 (2019).
59. Jiang, D. J., Xu, C. L. & Tsang, S. H. Revolution in gene medicine therapy and genome surgery. *Genes* **9**, 575 (2018).
60. Guimaraes, T. A. C., de, Georgiou, M., Bainbridge, J. W. B. & Michaelides, M. Gene therapy for neovascular age-related macular degeneration: rationale, clinical trials and future directions. *Br. J. Ophthalmol.* https://doi.org/10.1136/bjophthalmol-2020-316195 (2020).
61. Anguela, X. M. & High, K. A. Entering the modern era of gene therapy. *Annu. Rev. Med.* **70**, 273–288 (2019).
62. Hollingsworth, R. E. & Jansen, K. Turning the corner on therapeutic cancer vaccines. *NPJ Vaccines* **4**, 1–10 (2019).
63. Nakagami, H. & Morishita, R. Recent advances in therapeutic vaccines to treat hypertension. *Hypertension* **72**, 1031–1036 (2018).
64. Rosell, R. et al. Pathway targeted immunotherapy: rationale and evidence of durable clinical responses with a novel, EGF-directed agent for advanced NSCLC. *J. Thorac. Oncol.* **11**, 1954–1961 (2016).
65. Bennett, C. F., Baker, B. F., Pham, N., Swayze, E. & Geary, R. S. Pharmacology of antisense drugs. *Annu. Rev. Pharmacol. Toxicol.* **57**, 81–105 (2017).
66. Setten, R. L., Rossi, J. J. & Han, S. The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discov.* **18**, 421–446 (2019).
67. Rupaimoole, R. & Slack, F. J. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat. Rev. Drug Discov.* **16**, 203–222 (2017).
68. Leavitt, B. et al. Discovery and early clinical development of ISIS-HTTRx, the first HTT-lowering drug to be tested in patients with Huntington's disease (PL01.002). *Neurology* **86**, PL01.002 (2016).
69. Chakraborty, C., Sharma, A. R., Sharma, G., Doss, C. G. P. & Lee, S.-S. Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine. *Mol. Ther. Nucleic Acids* **8**, 132–143 (2017).
70. Uijl, E. et al. A3941 long-lasting small interfering RNA targeting angiotensinogen induces a robust and durable antihypertensive effect. *J. Hypertens.* **36**, e17 (2018).
71. Neilsen, P. M. et al. Mutant p53 drives invasion in breast tumors through up-regulation of miR-155. *Oncogene* **32**, 2992–3000 (2013).

72. Querfeld, C. et al. Preliminary results of a phase 1 trial evaluating MRG-106, a synthetic microRNA antagonist (LNA antimiR) of microRNA-155, in patients with CTCL. *Blood* **128**, 1829–1829 (2016).
73. Kim, J. W. & Cochran, J. R. Targeting ligand–receptor interactions for development of cancer therapeutics. *Curr. Opin. Chem. Biol.* **38**, 62–69 (2017).
74. Sandercock, C. G. & Storz, U. Antibody specification beyond the target: claiming a later-generation therapeutic antibody by its target epitope. *Nat. Biotechnol.* **30**, 615–618 (2012).
75. Nakayamada, S. & Tanaka, Y. BAFF- and APRIL-targeted therapy in systemic autoimmune diseases. *Inflamm. Regen.* **36**, 6 (2016).
76. Matsumoto, H. et al. Membrane-bound and soluble Fas ligands have opposite functions in photoreceptor cell death following separation from the retinal pigment epithelium. *Cell Death Dis.* **6**, e1986 (2015).
77. Genovese, M. C. et al. A phase II randomized study of subcutaneous ixekizumab, an anti-interleukin-17 monoclonal antibody, in rheumatoid arthritis patients who were naive to biologic agents or had an inadequate response to tumor necrosis factor inhibitors. *Arthritis Rheumatol.* **66**, 1693–1704 (2014).
78. Martin, D. A. et al. A phase Ib multiple ascending dose study evaluating safety, pharmacokinetics, and early clinical response of brodalumab, a human anti-IL-17R antibody, in methotrexate-resistant rheumatoid arthritis. *Arthritis Res. Ther.* **15**, R164 (2013).
79. Pavelka, K. et al. A study to evaluate the safety, tolerability, and efficacy of brodalumab in subjects with rheumatoid arthritis and an inadequate response to methotrexate. *J. Rheumatol.* **42**, 912–919 (2015).
80. Beringer, A., Noack, M. & Miossec, P. IL-17 in chronic inflammation: from discovery to targeting. *Trends Mol. Med.* **22**, 230–241 (2016).
81. FDA. FDA approves novel preventive treatment for migraine. http://www.fda.gov/news-events/press-announcements/fda-approves-novel-preventive-treatment-migraine (2019).
82. Przepiorka, D. et al. FDA approval: blinatumomab. *Clin. Cancer Res.* **21**, 4035–4039 (2015).
83. Labrijn, A. F., Janmaat, M. L., Reichert, J. M. & Parren, P. W. H. I. Bispecific antibodies: a mechanistic review of the pipeline. *Nat. Rev. Drug Discov.* **18**, 585–608 (2019).
84. Brinkmann, U. & Kontermann, R. E. The making of bispecific antibodies. *MAbs* **9**, 182–212 (2017).
85. Mullard, A. Bispecific antibody pipeline moves beyond oncology. *Nat. Rev. Drug Discov.* **16**, 666–668 (2017).
86. Jimeno, A. et al. A first-in-human phase 1a study of the bispecific anti-DLL4/anti-VEGF antibody navicixizumab (OMP-305B83) in patients with previously treated solid tumors. *Invest. New Drugs* **37**, 461–472 (2019).
87. Egan, T. J. et al. Novel multispecific heterodimeric antibody format allowing modular assembly of variable domain fragments. *MAbs* **9**, 68–84 (2016).
88. Levin, A. D., Wildenberg, M. E., Brink, V. D. & R, G. Mechanism of action of anti-TNF therapy in inflammatory bowel disease. *J. Crohns Colitis* **10**, 989–997 (2016).
89. Sandborn, W. J. et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* **121**, 1088–1094 (2001).
90. Mitoma, H., Horiuchi, T., Tsukamoto, H. & Ueda, N. Molecular mechanisms of action of anti-TNF-α agents – comparison among therapeutic TNF-α antagonists. *Cytokine* **101**, 56–63 (2018).
91. Kirchner, S., Holler, E., Haffner, S., Andreesen, R. & Eissner, G. Effect of different tumor necrosis factor (TNF) reactive agents on reverse signaling of membrane integrated TNF in monocytes. *Cytokine* **28**, 67–74 (2004).
92. Scallon, B. J., Moore, M. A., Trinh, H., Knight, D. M. & Ghrayeb, J. Chimeric anti-TNF-α monoclonal antibody cA2 binds recombinant transmembrane TNF-α and activates immune effector functions. *Cytokine* **7**, 251–259 (1995).
93. Xin, L. et al. Dual regulation of soluble tumor necrosis factor-α induced activation of human monocytic cells via modulating transmembrane TNF-α-mediated 'reverse signaling'. *Int. J. Mol. Med.* **18**, 885–892 (2006).
94. Ringheanu, M. et al. Effects of infliximab on apoptosis and reverse signaling of monocytes from healthy individuals and patients with Crohn's disease. *Inflamm. Bowel Dis.* **10**, 801–810 (2004).
95. Toedter, G. et al. Genes associated with intestinal permeability in ulcerative colitis: changes in expression following infliximab therapy. *Inflamm. Bowel Dis.* **18**, 1399–1410 (2012).
96. Vos, A. C. W. et al. Regulatory macrophages induced by infliximab are involved in healing in vivo and in vitro. *Inflamm. Bowel Dis.* **18**, 401–408 (2012).
97. Olesen, C. M., Coskun, M., Peyrin-Biroulet, L. & Nielsen, O. H. Mechanisms behind efficacy of tumor necrosis factor inhibitors in inflammatory bowel diseases. *Pharmacol. Ther.* **159**, 110–119 (2016).
98. Yamazaki, H. et al. Certolizumab pegol for induction of remission in Crohn's disease. *Cochrane Database Syst. Rev.* https://doi.org/10.1002/14651858.CD012893.pub2 (2019).
99. Ueda, N. et al. The cytotoxic effects of certolizumab pegol and golimumab mediated by transmembrane tumor necrosis factor α. *Inflamm. Bowel Dis.* **19**, 1224–1231 (2013).
100. Tracey, D., Klareskog, L., Sasso, E. H., Salfeld, J. G. & Tak, P. P. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol. Ther.* **117**, 244–279 (2008).
101. Guo, Q. et al. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res.* **6**, 15 (2018).
102. Brennan, F. M. & McInnes, I. B. Evidence that cytokines play a role in rheumatoid arthritis. *J. Clin. Invest.* **118**, 3537–3545 (2008).
103. Calmon-Hamaty, F., Combe, B., Hahne, M. & Morel, J. Lymphotoxin α stimulates proliferation and pro-inflammatory cytokine secretion of rheumatoid arthritis synovial fibroblasts. *Cytokine* **53**, 207–214 (2011).
104. Devine, E. B., Alfonso-Cristancho, R. & Sullivan, S. D. Effectiveness of biologic therapies for rheumatoid arthritis: an indirect comparisons approach. *Pharmacotherapy* **31**, 39–51 (2011).
105. Gartlehner, G., Hansen, R. A., Jonas, B. L., Thieda, P. & Lohr, K. N. The comparative efficacy and safety of biologics for the treatment of rheumatoid arthritis: a systematic review and metaanalysis. *J. Rheumatol.* **33**, 2398–2408 (2006).
106. Yang, S., Zhao, J. & Sun, X. Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. *Drug Des. Devel. Ther.* **10**, 1857–1867 (2016).
107. Gaudreault, J., Fei, D., Rusit, J., Suboc, P. & Shiu, V. Preclinical pharmacokinetics of ranibizumab (rhuFabV2) after a single intravitreal administration. *Invest. Ophthalmol. Vis. Sci.* **46**, 726–733 (2005).
108. Gaudreault, J. et al. Preclinical pharmacology and safety of ESBA1008, a single-chain antibody fragment, investigated as potential treatment for age related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **53**, 3025–3025 (2012).
109. Schmid, M. K. et al. Efficacy and adverse events of aflibercept, ranibizumab and bevacizumab in age-related macular degeneration: a trade-off analysis. *Br. J. Ophthalmol.* **99**, 141–146 (2015).
110. Zhang, Y., Chioreso, C., Schweizer, M. L. & Abràmoff, M. D. Effects of Aflibercept for neovascular age-related macular degeneration: a systematic review and meta-analysis of observational comparative studies. *Invest. Ophthalmol. Vis. Sci.* **58**, 5616–5627 (2017).
111. Sharma, A., Kumar, N., Kuppermann, B. D., Loewenstein, A. & Bandello, F. Brolucizumab: is extended VEGF suppression on the horizon? *Eye* **34**, 424–426 (2020).
112. Dugel, P. U. et al. HAWK and HARRIER: phase 3, multicenter, randomized, double-masked trials of brolucizumab for neovascular age-related macular degeneration. *Ophthalmology* **127**, 72–84 (2020).
113. Bordet, T. & Behar-Cohen, F. Ocular gene therapies in clinical practice: viral vectors and nonviral alternatives. *Drug Discov. Today* https://doi.org/10.1016/j.drudis.2019.05.038 (2019).
114. Carter, P. J. & Lazar, G. A. Next generation antibody drugs: pursuit of the 'high-hanging fruit'. *Nat. Rev. Drug Discov.* **17**, 197–223 (2018).
115. Neves, V., Aires-da-Silva, F., Corte-Real, S. & Castanho, M. A. R. B. Antibody approaches to treat brain diseases. *Trends Biotechnol.* **34**, 36–48 (2016).
116. Erdő, F., Bors, L. A., Farkas, D., Bajza, Á. & Gizurarson, S. Evaluation of intranasal delivery route of drug administration for brain targeting. *Brain Res. Bull.* **143**, 155–170 (2018).
117. Crowe, J. S. et al. Preclinical development of a novel, orally-administered anti-tumour necrosis factor domain antibody for the treatment of inflammatory bowel disease. *Sci. Rep.* **8**, 4941 (2018).
118. Burgess, G. et al. Randomized study of the safety and pharmacodynamics of inhaled interleukin-13 monoclonal antibody fragment VR942. *EBioMedicine* **35**, 67–75 (2018).
119. Miller, A. H. & Raison, C. L. Are anti-inflammatory therapies viable treatments for psychiatric disorders?: Where the rubber meets the road. *JAMA Psychiatry* **72**, 527–528 (2015).
120. Goldsmith, D., Rapaport, M. & Miller, B. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol. Psychiatry* **21**, 1696–1709 (2016).
121. Wittenberg, G. M. et al. Effects of immunomodulatory drugs on depressive symptoms: a mega-analysis of randomized, placebo-controlled clinical trials in inflammatory disorders. *Mol. Psychiatry* **25**, 1275–1285 (2020).
122. Vabret, N. et al. Immunology of COVID-19: current state of the science. *Immunity* https://doi.org/10.1016/j.immuni.2020.05.002 (2020).
123. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497–506 (2020).
124. McGrath, N. A., Brichacek, M. & Njardarson, J. T. A graphical journey of innovative organic architectures that have improved our lives. *J. Chem. Educ.* **87**, 1348–1349 (2010).
125. Oprea, T. I. et al. Unexplored therapeutic opportunities in the human genome. *Nat. Rev. Drug Discov.* **17**, 317–332 (2018).
126. Malik, A. & Urquhart, L. EvaluatePharma World Preview 2018, outlook to 2024. http://info.evaluategroup.com/WP2018-EPV.html (2018).
127. Urquhart, L. FDA new drug approvals in Q2 2019. *Nat. Rev. Drug Discov.* **18**, 575–575 (2019).
128. Harding, S. D. et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res.* **46**, D1091–D1106 (2018).
129. Igawa, T., Haraya, K. & Hattori, K. Sweeping antibody as a novel therapeutic antibody modality capable of eliminating soluble antigens from circulation. *Immunol. Rev.* **270**, 132–151 (2016).
130. Rask-Andersen, M., Almén, M. S. & Schiöth, H. B. Trends in the exploitation of novel drug targets. *Nat. Rev. Drug Discov.* **10**, 579–590 (2011).
131. Wishart, D. S. et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.* **46**, D1074–D1082 (2018).

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Author contributions
M.M.A., J.J., M.R.-A. and H.B.S participated in research design and wrote or contributed to the writing of the manuscript. M.M.A. and J.J analysed the data.

Competing interests
M. R.-A. has performed consulting services for Olink Proteomics. The other authors declare no competing interests.

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