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Feature

TNF-α: The shape of small molecules to come?

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In 2020, the anti-tumor necrosis factor (TNF) monoclonal antibody Humira® generated US$165.8 billion in cumulative sales and snatched the crown for the industry's most successful drug from Lipitor (atorvastatin). TNF-α is a major component in beneficial and disease-related inflammation and TNF-α-inhibitor biologics have gained widespread use in autoimmune diseases, such as rheumatoid arthritis (RA). Many more diseases could benefit from TNF-α inhibitors, such as Alzheimer's disease (AD) or major depression. However, the nature of TNF-α-inhibitor biologics prohibits central nervous system (CNS) applications. Moreover, high drug production costs and pricing, together with antidrug immune reactions and insufficient patient coverage, argue for the development of small-molecule drugs. Recently, drug-like orally available small molecules were described with high activity in animal disease models with activities comparable to those of antibodies.

Keywords: Protein/protein interaction; TNF-a; TNF-a receptor; Small molecule; Antibody; Antagonist; Structure-based design; Symmetry disruption; Inflammation

TNF-α is a pleiotropic cytokine that is involved in inflammatory processes in the body.[1] Upon infection, macrophages release TNF-α and alert other immune cells, causing inflammation. TNF-α can induce fever, apoptotic cell death, cachexia, and inflammation, inhibit tumorigenesis and viral replication, and respond to sepsis via IL-1 and IL-6-producing cells. TNF-α is dysregulated in autoimmune diseases, such as psoriasis, RA, ankylosing spondylitis, and inflammatory bowel disease (IBD). Autoimmune diseases can be treated by several TNF-α inhibitors belonging to different classical biotech drug classes: mAbs (e.g., Remicade® and Humira®), or receptor fusion proteins (e.g., Enbrel®) working as decoys competing for TNF receptor (TNFR) binding. TNF-α is also upregulated in AD, cancer, asthma, and major depression.

Increasing evidence points to a crucial role of the immune system in AD and Parkinson's disease (PD). Abnormal glial activation in patients with neurodegenerative diseases is a hallmark of AD.[2] Modulation of the neuroinflammatory response could be a therapeutic strategy for treating neurodegenerative diseases.[3,4] For example, a large, retrospective case-control study of electronic health records from 56 million unique adult patients found that treatment with a TNF-blocking agent was associated with a lower risk for AD in patients with RA, psoriasis, and other inflammatory diseases, which are mediated, in part, by TNF and for which a TNF blocker is an approved treatment.[5] Specifically, activation of TNFR2 signaling, either by directly targeting TNFR2 via TNFR2 agonists or by blocking TNFR1 signaling with TNFR1-selective antagonists,

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appears a promising strategy for AD therapy.[6] However, blood–brain barrier (BBB) penetration of the drug would be a requirement for its efficacy.

TNF-α was discovered in 1975 and belongs to a large superfamily comprising more than 50 transmembrane proteins.[7] Membrane-bound TNF-α (mTNF-α) is cleaved to yield soluble TNF-α (sTNF-α) by the metalloprotease TNF-converting enzyme (TACE). TNF-α signals through binding to two receptors, TNFR1, found on all cells, and TNFR2, found mostly on immune cells. mTNF-α is mainly expressed on monocytes/macrophages, where it induces cell–cell contact by interacting with other cell surface receptors. sTNF-α binding to TNFR1 activates the nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) inflammation pathways or the caspase cascade (Fig. 1).

TNF-α is a homotrimer in the membrane-bound and soluble form, as are both its receptors. sTNF-α binds primarily to TNFR1 and has an important role in inflammatory immune responses.[8] By contrast, mTNF-α interacts primarily with TNFR2 and mediates effects that are both overlapping and opposing to those of sTNF-α.[7] Interestingly, TNFR2/mTNF-α promotes bi-directional signaling in target cells and mTNF-α-expressing cells.[9] In the tumor microenvironment, crosstalk between mTNF-α and TNFR2 has an important role in tumor progression.

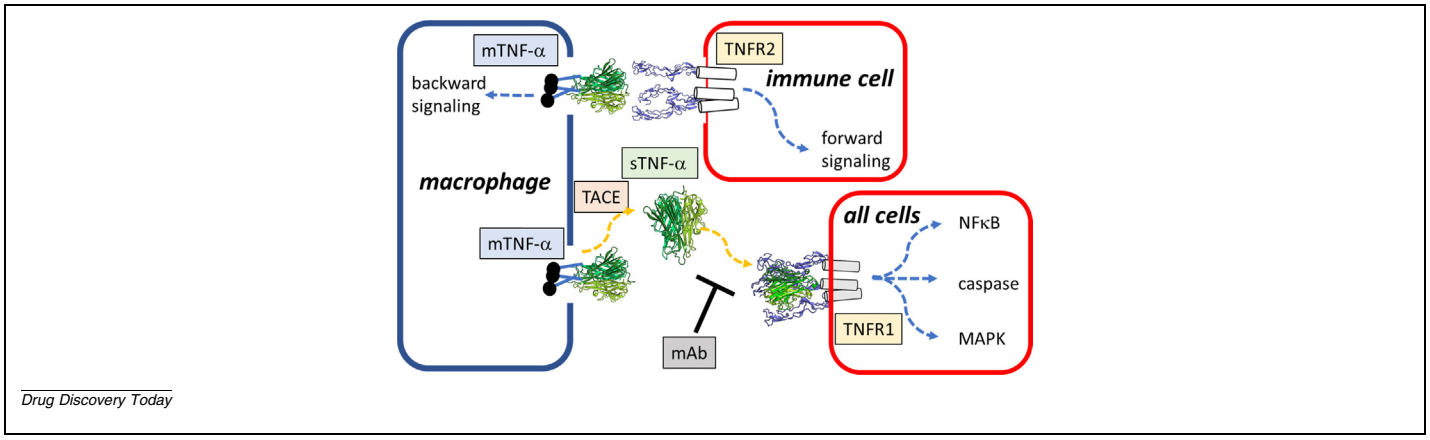

FIG. 1
Simplified schematic view of tumor necrosis factor (TNF)-α biology. Abbreviations: mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; mTNF, membrane-bound TNF; NF-κB, nuclear factor κB; sTNF, soluble TNF; TACE, TNF-converting enzyme; TNFR, TNF receptor.

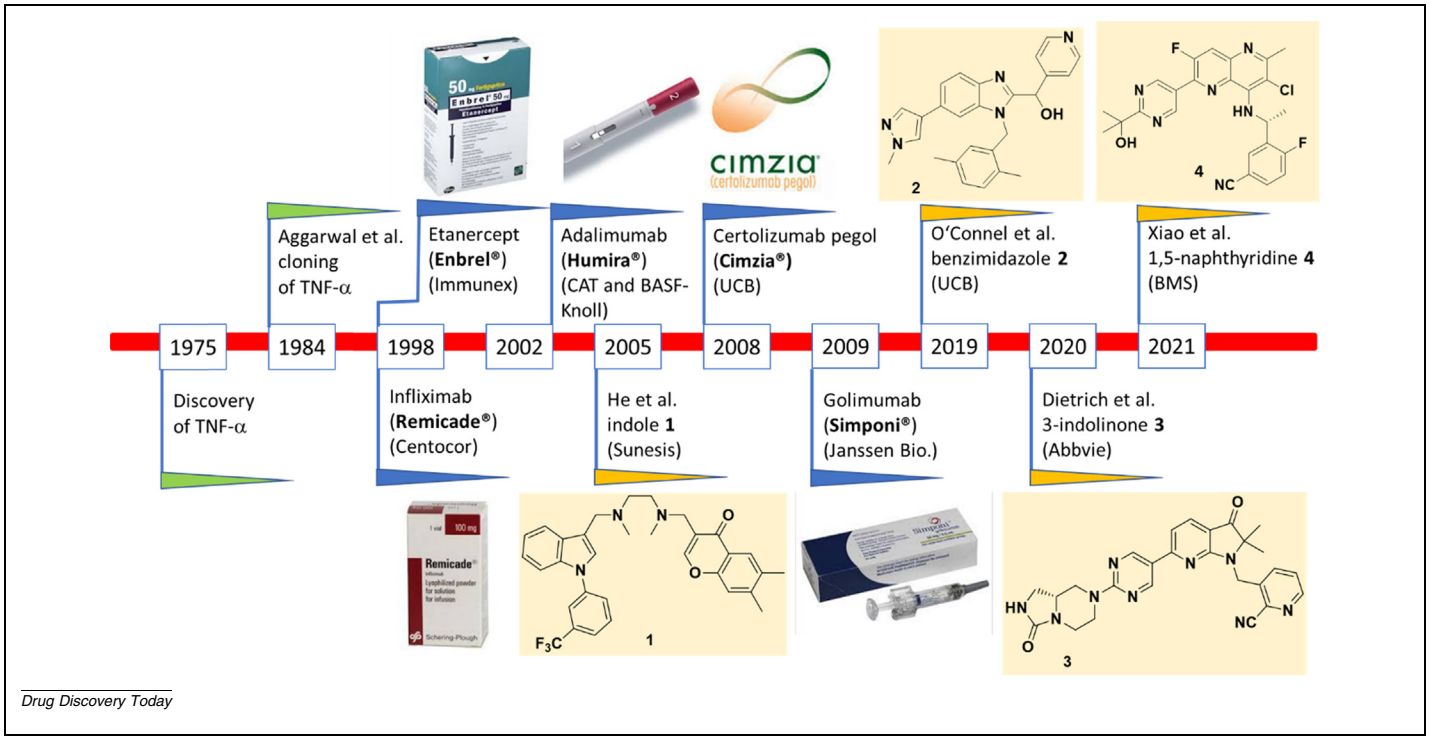

FIG. 2
Milestones of tumor necrosis factor (TNF)-α-directed biologics and small molecules. Abbreviation: BMS, Bristol Myers Squibb.

The TNF-α/TNFR2 interaction is characterized by an interface that measures more than 2500 Å^2, as determined by several co-crystal structure analyses [e.g., Protein Data Bank (PDB) ID: 3ALK], exhibiting a strong sub-nM affinity, and is challenging to antagonize by small molecules.[10] However, antibodies can efficiently bind and antagonize such large interfaces, as proven by multiple marketed antibodies, such as infliximab, adalimumab, golimumab, and certolizumab pegol or the protein etanercept, and several recent biosimilars thereof (Fig. 2). Biologics are more difficult to produce, expensive, cause immune reactions, are not orally bioavailable, are unable to cross the BBB, work only with a subset of patients, and, in some patients, even lead to aggravation of the disease. By contrast, small molecules are orally bioavailable, nonimmunogenic, can be designed to enter the brain, and are cost-effective to produce, particularly for chronic indications. Thus, different companies have embarked on the challenge to discover small molecules acting as TNF-α inhibitors (Fig. 2). The generally pursued strategy involves designing molecules binding to TNF-α to antagonize the interaction with its receptors.

Pioneering work by He *et al.* of Sunesis in 2005 described a small molecule (**1**) that promotes subunit disassembly of the TNF-α trimer, thereby inhibiting receptor binding.[11] However, numerous attempts to improve the drug-like properties of the original molecule failed. In 2019, O'Connell *et al.* of UCB disclosed 2-hydroxymethyl benzimidazoles (**2**) that destabilized the symmetrical TNF-α trimer into a nonsymmetrical conformation, allowing the TNF-α trimer to recruit only two of the three copies of TNFR1, leading to an incompetent TNF–TNFR1 signaling complex.[12] Further studies based on co-crystal structure analysis and biochemical assays showed that the inhibitors reduced the binding affinity of TNF to the third TNFR1 molecule.[13] The distorted inhibitor-bound TNF-α trimer forms a complex with a dimer of TNFR1 molecules. A model has been proposed of TNF-α signaling based on TNF–TNFR1 clusters, which are disrupted by small-molecule inhibitors. Recently, scientists from Abbvie and Bristol Myers Squibb (BMS) disclosed the discovery of potent orally bioavailable molecules (**3** and **4**) displaying *in vivo* efficacy in animal models of inflammation comparable to a TNF-α antibody.[14,15] The Abbvie group described a two-phase approach, including a nuclear magnetic resonance (NMR)-based fragment-based drug discovery (FBDD) effort leading to the discovery of the isoquinoline core **5** disrupting the protein–protein interactions (PPIs) through allosteric desymmetrization of TNF-α observed from high-resolution crystal structures (Fig. 3).

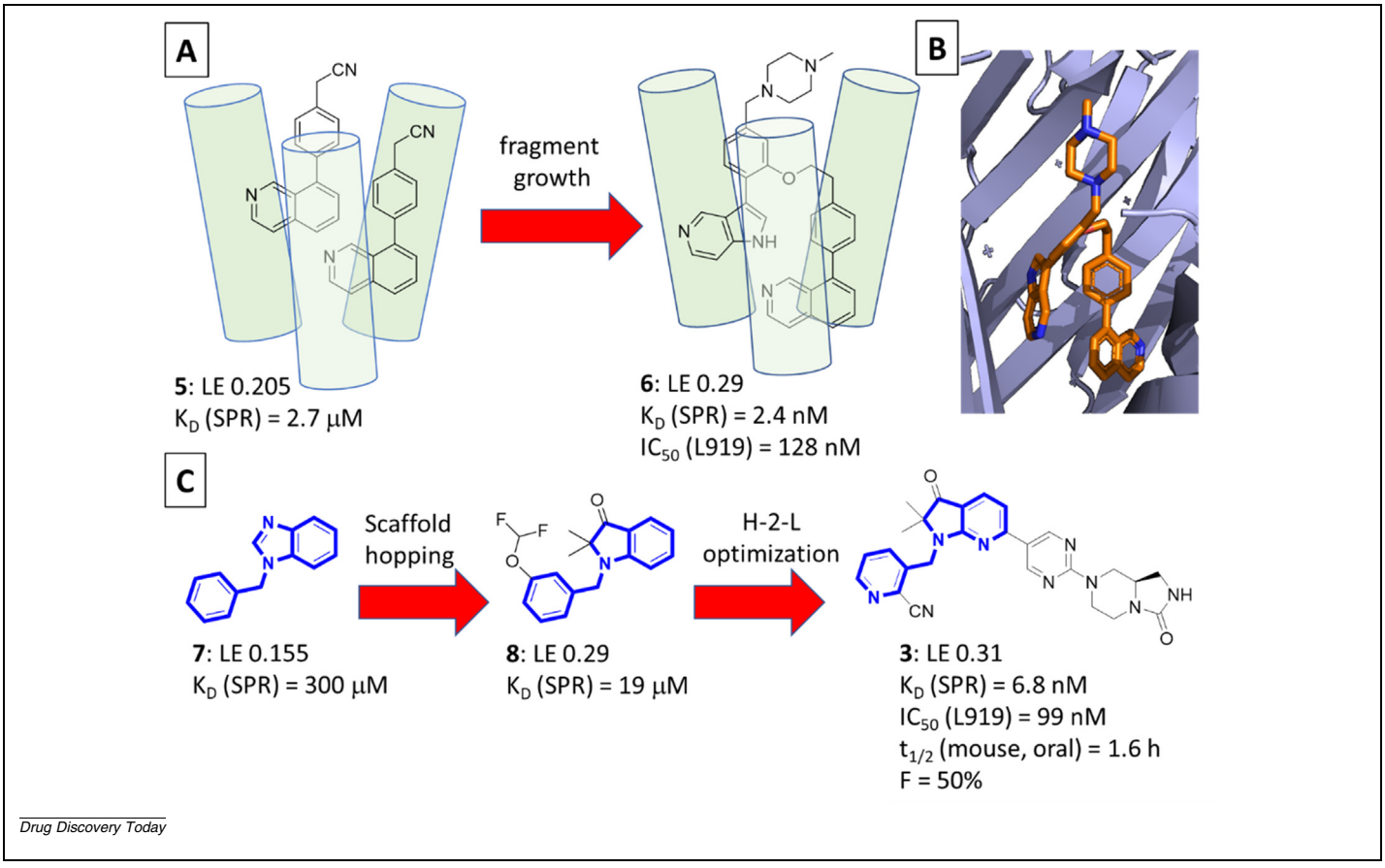

FIG. 3

Fragment evolution of compound **3** from Abbvie. **(a)** isoquinoline fragment **5** was grown to **6** [schematically shown in the center of the tumor necrosis factor (TNF)-α trimer cylinder] with increased binding affinity and cellular activity while keeping ligand efficacy high. **(b)** Crystal structure of **6** in the TNF-α trimer shown as a blue cartoon (PDB ID: 6X82). **(c)** An alternative benzimidazole fragment **7** was transformed to **8** by scaffold hopping, followed by hit-to-lead optimization of activity and pharmacokinetics/pharmacodynamics properties, resulting in lead compound **3**.

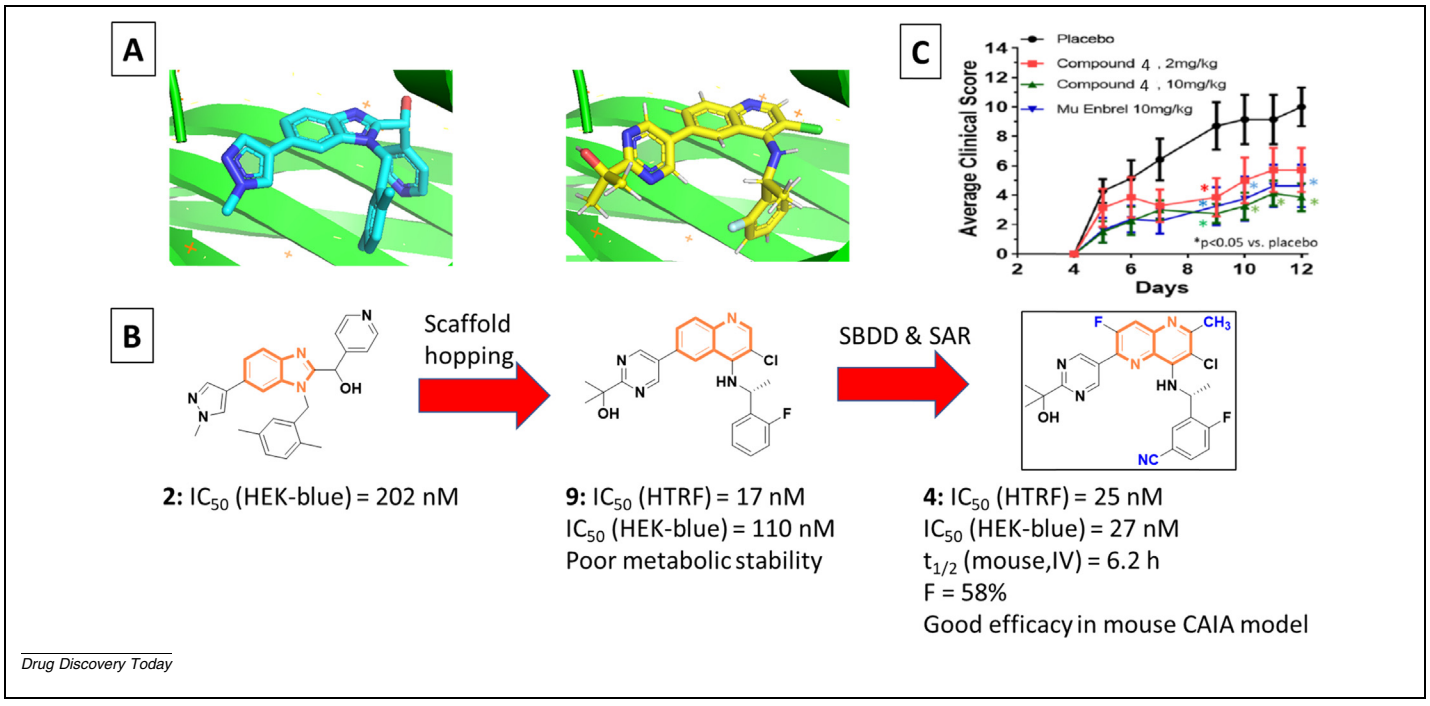

FIG. 4

Fragment progression of compound **4** from Bristol Myers Squibb (BMS). **(a)** Crystal structure of **2** (PDB ID: 6OP0, cyan) and **9** (PDB ID: 7JRA, yellow) with the TNF-α trimer shown as a green cartoon. **(b)** Original benzimidazole fragment **2** was converted to **9** via scaffold hopping, followed by further optimization of biological and pharmacokinetics/pharmacodynamics activity to obtain the lead compound **4**. **(c)** **4** reduced the clinical score and the levels of inflammatory cytokines and leukocyte cell surface receptors in a dose-dependent manner in a several-day-old mouse model, at a 10 mg/kg dose, similar to mouse Enbrel. Abbreviations: SAR, structure–activity relationship; SBDD, structure-based drug design.

Fragment growing by combining two isoquinolines led to compound **6**, with potent low nM TNF-α affinity. Although **6** could not be optimized further, *de novo* design and optimization during the second phase of the alternative fragment benzimidazole **7** via 3-indolinone **8** with improved binding efficiency and drug-like properties, guided by a mix of biophysical and cell-based assays, resulted in the 3-indolinone-based compound **3**. The compound was found to be selective to other TNF family members, including CD40L, TNFβ, and TWEAK. Excitingly, the lead compound displayed oral bioavailability and *in vivo* efficacy in a mouse paw swelling model comparable to that seen with a TNF-α antibody. Inspired by the research by UCB, the BMS group performed a scaffold-hoping approach starting from the reported benzimidazole-based scaffold **2** and achieved a novel quinoline compound **9**, with better activity; then, guided by structure-based drug design, finally obtained the optimized compound **4** (Fig. 4a,b). Compound optimization was guided by a high-throughput human cell-based reporter gene assay (HEK-Blue) and by a homogeneous time-resolved fluorescence (HTRF) assay to determine the binding of compounds to TNF-α. The use of a reporter cell assay from the start addressed stability and protein-binding issues, combined with efficacy, helping to drive the activity of the initial quinoline scaffold. The key to obtain activity in the series was a H/Cl exchange in position 3 of the quinoline: whereas H was inactive in the HEK-Blue assay, the 3-Cl derivative showed promising double-digit nM activity ('magic chlorine'). X-ray structures of compound (**9**) with TNF-α helped to determine the binding interactions and to further optimize the compounds (Fig. 4a). A switch from quinoline to naphthyridine increased the electrostatics of the pi-stacking interaction with Tyr135. Further optimization of the different substituents resulted in compound **4** with low nM affinity and cellular activity, and good metabolic stability in mouse. Finally, pharmacological *in vivo* activity was shown in a collagen antibody-induced arthritis mouse model (Fig. 4c).

The UCB group developed a conformational selective mAb able to sense the presence of small molecules bound to sTNF-α in complex biological samples.[16] This could become an important tool as an engagement biomarker in future clinical studies, and could also speed up trials and help prove correlations of target engagement with efficacy.

TNF-α inhibitors are not only important and efficacious drugs, but also generate substantial revenue. In 2018, Humira® was the top-selling prescription drug globally. The global anti-inflammatory biologics market size is expected to reach US $150 billion by 2027. A major share of the market is covered by anti-TNF agents. With the exciting recent discovery of truly drug-like small-molecule TNF-α antagonists, it remains to be seen how such compounds will perform in clinical trials and if they are able to replace mAbs in this important market traditionally occupied by 'biologics'.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Holbrook, J. Holbrook J, Lara-Reyna S, Jarosz-Griffiths H, McDermott M. Tumour necrosis factor signalling in health and disease. F1000Res 2019;8:1–12.

[2] Z. Fan, A.A. Okello, D.J. Brooks, P. Edison, Longitudinal influence of microglial activation and amyloid on neuronal function in Alzheimer's disease, Brain 138 (12) (2015) 3685–3698.

[3] B. Decourt, D.K. Lahiri, M.N. Sabbagh, Targeting tumor necrosis factor alpha for Alzheimer's disease, Curr Alzheimer Res 14 (4) (2017) 412–425.

[4] R. Chang, K.L. Yee, R.K. Sumbria, Tumor necrosis factor α Inhibition for Alzheimer's Disease, J Cent Nerv Syst Dis 9 (2017) 1–5.

[5] M. Zhou, R. Xu, D.C. Kaelber, M.E. Gurney, Tumor Necrosis Factor (TNF) blocking agents are associated with lower risk for Alzheimer's disease in patients with rheumatoid arthritis and psoriasis, PLoS ONE 15 (3) (2020) 1–23.

[6] N. Ortí-Casañ, Y. Wu, P.J.W. Naudé, P.P. De Deyn, I.S. Zuhorn, U.L.M. Eisel, Targeting TNFR2 as a novel therapeutic strategy for Alzheimer's disease, Front Neurosci 13 (49) (2019) 1–8.

[7] R.M. Locksley, N. Killeen, M.J. Lenardo, The TNF and TNF receptor superfamilies: integrating mammalian biology, Cell 104 (4) (2001) 487–501.

[8] M. Grell, H. Wajant, G. Zimmermann, P. Scheurich, The type 1 receptor (CD120a) is the high-affinity receptor for soluble tumor necrosis factor, Proc Natl Acad Sci U S A 95 (2) (1998) 570–575.

[9] Y. Qu, G. Zhao, H. Li, Forward and reverse signaling mediated by transmembrane tumor necrosis factor-alpha and TNF receptor 2: potential roles in an immunosuppressive tumor microenvironment, Front Immunol 8 (2017) 1–7.

[10] Y. Mukai, T. Nakamura, M. Yoshikawa, Y. Yoshioka, S. Tsunoda, S. Nakagawa, et al., Solution of the structure of the TNF-TNFR2 complex, Sci Signal 3 (2010) 1–11.

[11] M.M. He, A.S. Smith, J.D. Oslob, W.M. Flanagan, A.C. Braisted, A. Whitty, et al., Small-molecule inhibition of TNF-alpha, Science 310 (2005) 1022–1025.

[12] J. O'Connell, J. Porter, B. Kroeplien, T. Norman, S. Rapecki, R. Davis, et al., Small molecules that inhibit TNF signalling by stabilising an asymmetric form of the trimer, Nat Commun 10 (1) (2019) 1–12.

[13] D. McMillan, C. Martinez-Fleites, J. Porter, D. Fox 3rd, R. Davis, P. Mori, et al., Structural insights into the disruption of TNF-TNFR1 signalling by small molecules stabilising a distorted TNF, Nat Commun 12 (1) (2021) 1–12.

[14] J.D. Dietrich, K.L. Longenecker, N.S. Wilson, C. Goess, S.C. Panchal, S.L. Swann, et al., Development of orally efficacious allosteric inhibitors of TNFα via fragment-based drug design, J Med Chem 64 (1) (2021) 417–429.

[15] H.Y. Xiao, N. Li, J.J. Duan, B. Jiang, Z. Lu, K. Ngu, et al., Biologic-like in vivo efficacy with small molecule inhibitors of TNFα identified using scaffold hopping and structure-based drug design approaches, J Med Chem 63 (23) (2020) 15050–15071.

[16] D.J. Lightwood, R.J. Munro, J. Porter, D. McMillan, B. Carrington, A. Turner, et al., A conformation-selective monoclonal antibody against a small molecule-stabilised signalling-deficient form of TNF, Nat Commun 12 (1) (2021) 1–10.

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