

NOX-inflicted oxidative stress in neurodegeneration and inflammation

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To know that we know what we know, and to know that we do not know
what we do not know, that is true knowledge.

ABSTRACT

Further understanding of mechanisms that promote neuronal decay in neurodegenerative diseases may pave the way for new therapies. Aberrant activation of the reactive oxygen species (ROS)-generating enzyme NADPH oxidase 2 (NOX2) in myeloid cells is suggested to contribute to neurodegeneration in experimental models. However, its exact role in human disease is not known. We aimed to define the impact of NOX2 activity on neurodegeneration and identify potential therapeutic strategies for NOX2-inflicted pathologies. To this end, we examined single nucleotide polymorphisms (SNPs) that affect the magnitude of NOX2-derived ROS formation in the context of the neurodegenerative and neuroinflammatory diseases multiple sclerosis (MS), Guillain-Barré syndrome (GBS), and Parkinson's disease (PD). Furthermore, we investigated the NOX2-inhibitory potential of inhibitors of Bruton's tyrosine kinase (BTKi), which regulates myeloid cell activation. We identified two SNP alleles (rs4673 A and rs1049254 G in *CYBA*, encoding the NOX2 subunit p22^{phox}) that were associated with reduced NOX2-derived ROS production. In MS, these low-ROS alleles heralded reduced disease severity and a markedly delayed onset of secondary progressive MS (**paper I**). Patients with GBS carrying low-ROS alleles were less likely to require assisted ventilation during the acute phase and experienced a rapid recovery of motor function (**paper II**). Furthermore, an analysis of clinical milestone cumulation in idiopathic PD revealed that patients with low-ROS alleles showed a reduced rate of disease progression (**paper III**). In **paper IV**, we demonstrated that BTKi effectively blocked activation of NOX2 in myeloid cells in response to surface receptor stimulation. This translated into potentiated natural killer cell-mediated clearance of malignant cells in the presence of immunosuppressive myeloid cells *in vitro* and *in vivo*. In conclusion, our results suggest that NOX2-derived ROS may contribute to neuronal death in MS, GBS, and PD. This implies that NOX2 might serve as a generic driver of neurodegeneration and invites research on its role in additional neurodegenerative diseases. The NOX2-inhibitory potential of BTKi makes them conceivable candidates to target myeloid immunosuppression in both hematological and solid cancers, as well as to alleviate other NOX2-dependent pathologies.

Keywords: NADPH oxidase, NOX2, oxidative stress, neurodegeneration, multiple sclerosis, Guillain-Barré syndrome, Parkinson's disease

SAMMANFATTNING PÅ SVENSKA

Neurodegenerativa sjukdomar kännetecknas av nervcellsdöd i det centrala och/eller perifera nervsystemet. Dessa sjukdomar fortskrider ofta över tid och ger symtom som återspeglar den del av nervsystemet som påverkas. Ökad förståelse för de mekanismer som ger upphov till neurodegeneration kan bana väg för nya terapier. Denna avhandling avser att belysa om NOX2 (NADPH-oxidas typ 2), ett enzym som uttrycks av myeloida immunceller som finns bland annat i nervsystemet, kan bidra till neurodegeneration.

NOX2-enzymet bildar reaktiva syremetaboliter (*reactive oxygen species*, ROS) som utgör en försvarsmekanism mot mikroorganismer i immunsystemets myeloiska celler såsom monocytter, makrofager och granulocyter. Okontrollerad produktion av ROS (oxidativ stress) kan skada närliggande celler, och ROS har visats bidra till skador på nervceller i provrörsexperiment och i djurförsök. NOX2-enzymets roll för nervcellsdöd i mänskliga sjukdomar är dock ofullständigt känd.

Vi studerade normala varianter av NOX2-DNA som påverkar hur mycket ROS som produceras av myeloiska celler. Vi undersökte sedan hur sådan genvariation kunde kopplas till sjukdomsförlopp vid de neurodegenerativa och neuroinflammatoriska sjukdomarna multipel skleros (MS), Guillain-Barrés syndrom (GBS) och Parkinsons sjukdom (PD). Vi använde sjukjournaler med detaljerade beskrivningar av sjukdomsförloppet med flera decenniers uppföljningstid. Därmed kunde vi belysa hur dessa sjukdomar förlöper hos patienter med hög eller låg ROS-produktion från NOX2-enzymet.

Vi fann två genvarianter av NOX2, som kunde kopplas till minskad ROS-produktion från myeloiska celler (låg-ROS-alleler). Vid MS var dessa låg-ROS-alleler associerade med lindrigare sjukdomsförlopp och fördröjd debut av progressiva neurologiska symtom (**arbete I**). Patienter med GBS som bar låg-ROS-alleler hade minskat behov av respiratorvård i den akuta fasen och återhämtade motorisk funktion snabbare (**arbete II**). Vid PD fann vi att patienter med låg-ROS-alleler uppvisade långsammare sjukdomsprogression (**arbete III**). Dessa resultat, som bör verifieras i större studier, tyder på att farmakologisk hämning av NOX2 skulle kunna minska eller förhindra neurodegeneration.

Vi utvärderade NOX2-hämmande egenskaper hos inhibitorer av Brutons tyrosinkinase (BTKi). Dessa substanser används vid blodcancer för att hindra tillväxt av canceromvandlade B-celler men påverkar även aktivering av myeloiska celler. I **arbete IV** fann vi att BTKi effektivt blockerade aktivering av NOX2 i myeloiska celler. NOX2-enzymets ROS-produktion undertrycktes av BTKi vid koncentrationer som motsvarar dem som uppmäts vid behandling av patienter med blodcancer.

Avhandlingsarbetet utmynnar i hypotesen att ROS som producerats av NOX2-enzymet kan bidra till nervcellsöd vid MS, GBS och PD. Resultaten motiverar studier av NOX2-enzymets roll vid andra neurodegenerativa sjukdomar. Fyndet att BTKi, som passerar blod-hjärnbarriären, effektivt hämmar NOX2-medierad ROS-produktion föranleder studier av dessa substansers potentiellt nervskyddande egenskaper *in vivo*.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. **Törnell A**, Kiffin R, Haghghi S, Mossberg N, Andersen O, Hellstrand K, Martner A. Impact of *CYBA* genotypes on severity and progression of multiple sclerosis.
Eur J Neurol. 2022;29(5):1457–64.
- II. **Törnell A**, Lagerstrom N, Mossberg N, Kiffin R, Farman H, Lycke J, Andersen O, Axelsson M, Hellstrand K, Martner A. *CYBA* allelic variants are associated with severity and recovery in Guillain-Barré syndrome.
J Peripher Nerv Syst. 2023;28(3):407–14.
- III. **Törnell A**, von Below D, Levander D, Nissbrandt H, Bergquist F, Hellstrand K, Martner A. Gene variants entailing increased enzymatic ROS formation may accelerate long-term progression in idiopathic Parkinson’s disease.
In manuscript.
- IV. **Törnell A**, Waldenström J, Kiffin R, Akhiani AA, Thorén FB, Hellstrand K, Martner A. Bruton’s tyrosine kinase activates the NOX2/ROS axis to drive myeloid immunosuppression in cancer.
Submitted.

Additional publications not included in this thesis:

1. Einarsdottir S, Martner A, Waldenstrom J, Nicklasson M, Ringlander J, Arabpour M, **Törnell A**, Grauers Wiktorin H, Nilsson S, Bittar R, Nilsson M, Lisak M, Veje M, Friman V, Al-Dury S, Bergstrom T, Ljungman P, Brune M, Hellstrand K, Lagging M. Deficiency of SARS-CoV-2 T-cell responses after vaccination in long-term allo-HSCT survivors translates into abated humoral immunity. *Blood Adv.* 2022;6(9):2723–30.
2. **Törnell A**, Grauers Wiktorin H, Ringlander J, Arabpour M, Nilsson MR, Nilsson S, Kiffin R, Lindh M, Lagging M, Hellstrand K, Martner A. Rapid cytokine release assays for analysis of severe acute respiratory syndrome coronavirus 2-specific T cells in whole blood. *J Infect Dis.* 2022;226(2):208–16.
3. Einarsdottir S, Martner A, Nicklasson M, Wiktorin HG, Arabpour M, **Törnell A**, Vaht K, Waldenstrom J, Ringlander J, Bergstrom T, Brune M, Hellstrand K, Ljungman P, Lagging M. Reduced immunogenicity of a third COVID-19 vaccination among recipients of allogeneic hematopoietic stem cell transplantation. *Haematologica.* 2022;107(6):1479–82.
4. Wiktorin HG, Einarsdottir S, **Törnell A**, Arabpour M, Issdisai N, Waldenstrom J, Ringlander J, Lindh M, Lagging M, Hellstrand K, Martner A. COVID-19 vaccine-induced adverse events predict immunogenicity among recipients of allogeneic haematopoietic stem cell transplantation. *Haematologica.* 2022;107(10):2492–5.
5. Al-Dury S, Waern J, Waldenstrom J, Alavanja M, Saed HH, **Törnell A**, Arabpour M, Grauers Wiktorin H, Einarsdottir S, Ringlander J, Ringstrom G, Hellstrand K, Martner A, Lagging M. Impaired SARS-CoV-2-specific T-cell reactivity in patients with cirrhosis following mRNA COVID-19 vaccination. *JHEP Rep.* 2022;4(7):100496.

6. Martner A, Grauers Wiktorin H, **Törnell A**, Ringlander J, Arabpour M, Lindh M, Lagging M, Nilsson S, Hellstrand K. Transient and durable T cell reactivity after COVID-19.
Proc Natl Acad Sci U S A. 2022;119(30):e2203659119.
7. **Törnell A**, Grauers Wiktorin H, Ringlander J, Arabpour M, Nilsson S, Lindh M, Laggin M, Hellstrand K, Martner A. Induction and subsequent decline of S1-specific T cell reactivity after COVID-19 vaccination.
Clin Immunol. 2023;248:109248.
8. **Törnell A***, Blick E*, Al-Dury S, Grauers Wiktorin H, Waern J, Ringlander J, Einarsdottir S, Lindh M, Hellstrand K, Lagging M, Martner A. Presence of MDSC associates with impaired antigen-specific T cell reactivity following COVID-19 vaccination in cirrhotic patients.
Front. Immunol. 2023;14:1287287.
*Authors contributed equally
9. Einarsdottir S, Waldenstrom J, **Törnell A**, Ringlander J, Stenback JB, Malmstrom S, Hellstrand K, Martner A, Lagging M. Impaired T-cell response to mRNA vaccination heralds risk of COVID-19 in long-term allogeneic hematopoietic stem cell transplantation survivors.
Haematologica. 2024;109(1):303–7.
10. Paul S, Kaya M, Johnsson O, Grauers Wiktorin H, **Törnell A**, Arabpour M, Hellstrand K, Martner A. Targeting murine metastatic cancers with cholera toxin A1-adjuvanted peptide vaccines.
Hum Vaccin Immunother. 2025;21(1):2455240

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ABBREVIATIONS

| | |
|---------------|-------------------------------------|
| ALS | Amyotrophic lateral sclerosis |
| BTK | Bruton's tyrosine kinase |
| CD | Cluster of differentiation |
| CLL | Chronic lymphocytic leukemia |
| CNS | Central nervous system |
| CSF | Cerebrospinal fluid |
| DAMP | Damage-associated molecular pattern |
| DMT | Disease-modifying therapy |
| EBV | Epstein-Barr virus |
| Fc γ R | Fc γ receptor |
| FPR | Formyl peptide receptor |
| GBS | Guillain-Barré syndrome |
| HD | Huntington's disease |
| HLA-DR | Human leukocyte antigen-DR |
| IL | Interleukin |
| iPD | Idiopathic Parkinson's disease |
| LPS | Lipopolysaccharide |
| MAPK | Mitogen-activated protein kinase |
| MDSC | Myeloid-derived suppressor cell |
| MHC | Major histocompatibility complex |

| | |
|----------------|--|
| MS | Multiple sclerosis |
| MSSS | Multiple sclerosis severity score |
| M1 | Classically activated macrophage |
| M2 | Alternatively activated macrophage |
| NET | Neutrophil extracellular trap |
| NF- κ B | Nuclear factor-kappa B |
| NK cell | Natural killer cell |
| NOX | NADPH oxidase |
| PAMP | Pathogen-associated molecular pattern |
| PBMC | Peripheral blood mononuclear cell |
| PD | Parkinson's disease |
| PI3K | Phosphatidylinositol 3-kinase |
| PMA | Phorbol 12-myristate 13-acetate |
| PPMS | Primary progressive multiple sclerosis |
| PRR | Pattern recognition receptor |
| ROS | Reactive oxygen species |
| RRMS | Relapsing-remitting multiple sclerosis |
| NfL | Neurofilament light chain |
| SNP | Single nucleotide polymorphism |
| SOD | Superoxide dismutase |
| SPMS | Secondary progressive multiple sclerosis |

| | |
|-----|-----------------------------|
| TAM | Tumor-associated macrophage |
| TLR | Toll-like receptor |
| TNF | Tumor necrosis factor |

PREFACE

Neurodegenerative diseases like multiple sclerosis, Parkinson's disease, and Alzheimer's disease pose a substantial healthcare burden due to their debilitating symptoms and lack of curative therapies. In most cases, the underlying cause is unknown. Moreover, these diseases are typically diagnosed only after significant nerve damage has already occurred, and the mechanisms driving continued nerve cell death are poorly understood. This hinders the development of new therapies that prevent further destruction of nerves. Chronic inflammation, which is mediated in part by aberrant activation of innate immune cells, has emerged as a hallmark of neurodegenerative diseases. In these conditions, dysregulated immune activation may aggravate disease by damaging host tissue through sustained inflammatory responses. However, the detailed mechanisms that maintain inflammatory activation and propagate cell damage require further investigation. This thesis explores the nerve cell toxicity inflicted by the enzyme NADPH oxidase 2 (NOX2), which, under normal circumstances, eliminates pathogens by the release of toxic reactive oxygen species. Elucidating the role of NOX2 in inflammation-associated neurotoxicity may provide insight into targetable mechanisms to reduce destructive effects of chronic inflammation in neurodegenerative diseases.

INTRODUCTION

THE IMMUNE SYSTEM

A fundamental aspect of survival is the ability of an organism to defend itself against invading pathogens such as viruses, bacteria, fungi, and parasites. The first line of defense consists of physical and chemical barriers (skin, mucosal surfaces, etc.) that prevent the entry of pathogens. If that fails, for example, in the event of a wound, a tightly coordinated network of cells and molecules, collectively representing the immune system, is mobilized to fight the infection. Most immune cells originate from hematopoietic stem cells in the bone marrow, where they develop and mature in a process called hematopoiesis to form functional immune cells in blood, lymphoid organs, and tissues. This development, or differentiation, follows two main branches: the myeloid lineage and the lymphoid lineage. Myeloid immune cells comprise basophils, eosinophils, neutrophils, mast cells, monocytes, and macrophages, while T cells, B cells, and natural killer (NK) cells make up the lymphocytes. Dendritic cells may originate from either lymphoid or myeloid progenitors (Figure 1) (1).

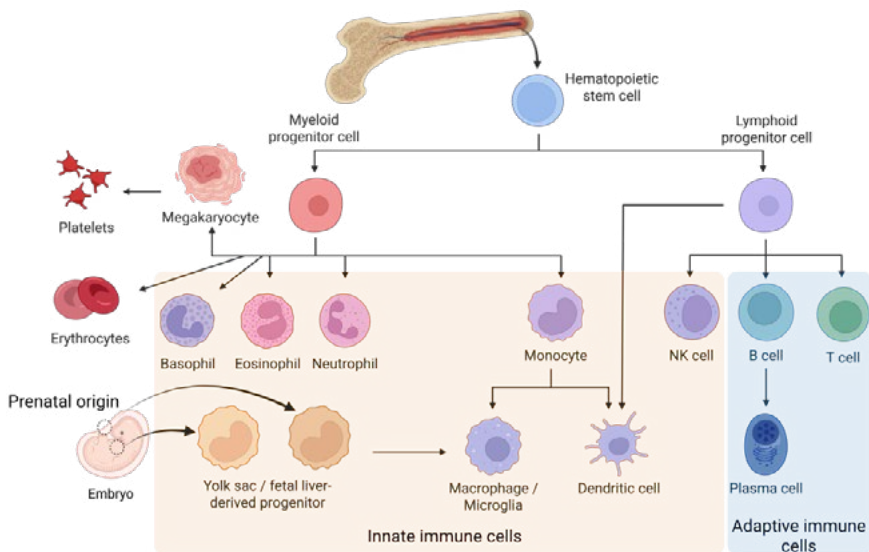


Figure 1. Immune cell development.

Immune cells are functionally classified into two main groups, innate and adaptive (Figure 1). The innate immune cells, as their name implies, are endowed with inherent ability to detect and eliminate a wide variety of pathogens. They also aid in the recruitment and activation of adaptive immune cells. In contrast, adaptive immune cells recognize and respond specifically to unique antigens originating, for example, from a pathogen. Although the adaptive immune response develops more slowly than the innate response, it is highly effective and precise once activated (1).

Box 1: A textbook example. Bacteria that enter a wound are recognized by tissue-resident macrophages through pattern recognition receptors that bind common structures shared by pathogens. They respond by phagocytosing and killing the bacteria, while also signaling to recruit more immune cells, particularly neutrophils that possess multiple mechanisms for pathogen elimination. Antigen-presenting cells (APC) such as dendritic cells engulf the bacteria and travel to lymph nodes to present bacterial antigens to naive T cells. Only T cells bearing a T cell receptor that specifically matches the presented antigen will interact with and become activated by the APC. Meanwhile, B cells that recognize bacterial antigens via their B cell receptor internalize the antigen and present it on their surface. Activated helper T cells specific to the same antigen can interact with the B cell and provide signals that facilitate B cell activation. Activated T and B cells will rapidly multiply to generate a large number of antigen-specific effector cells to combat the infection. Helper T cells secrete cytokines that coordinate and enhance the activity of other immune cells. Cytotoxic T cells eliminate infected or malignant host cells. B cells can differentiate into plasma cells that produce antibodies that bind the bacterial antigens, which facilitates their elimination.

MYELOID CELLS

The focus of this thesis is on myeloid cells, and more specifically monocytes, macrophages, and the macrophage-like cells microglia, which reside in the central nervous system (CNS).

Cell surface receptors

Innate immune cells express an array of surface and intracellular receptors to facilitate recognition and elimination of pathogens and damaged cells, as well as signaling and interaction with other cells. Among these are Fc receptors, toll-like receptors (TLR), and formyl peptide receptors (FPR) (Figure 2).

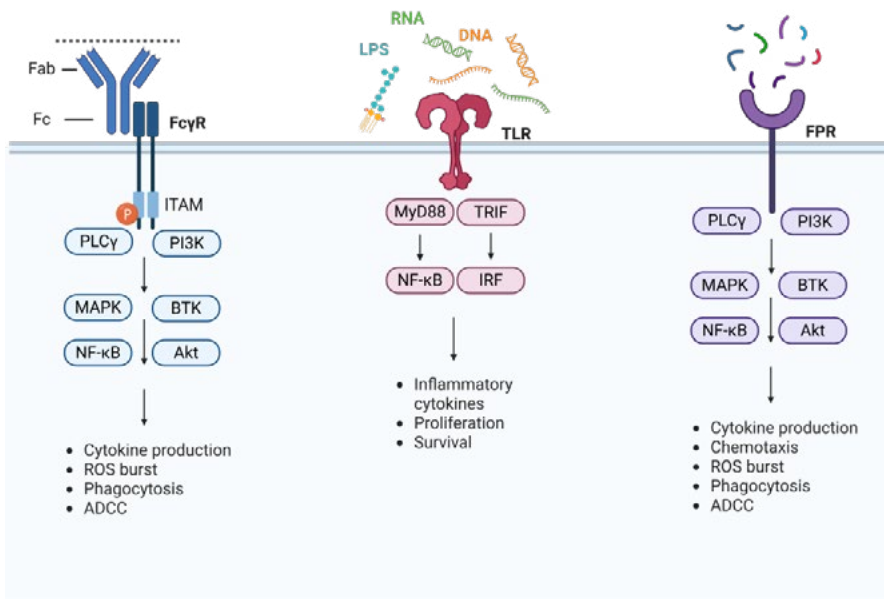


Figure 2. Pro-inflammatory pathways of Fc γ R, TLR, and FPR.

Fc γ -RECEPTORS

Most innate immune cells and some adaptive immune cells express Fc γ receptors (Fc γ R), which bind the constant Fc region of IgG antibodies, opposite to the variable regions (Fab) that interact with an antigen. There are six Fc γ Rs expressed in distinct patterns on human immune cells. While B cells only express the inhibitory Fc γ RIIb and NK cells only express the activating

Fc γ RIIIa, most other immune cells maintain a balance between activating and inhibitory Fc γ Rs. Monocytes and macrophages express the three activating receptors Fc γ RI, Fc γ RIIa, and Fc γ RIIIa, along with the inhibitory receptor Fc γ RIIb. Except for Fc γ RI, all Fc γ R have low binding affinity for IgG and, therefore, only initiate a signal response upon interaction with IgG in multimeric immune complexes or on opsonized cells. Once multiple activating Fc γ Rs are cross-linked, signaling in immune cells is initiated by phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs). This leads to recruitment and activation of multiple key inflammatory mediators, including phosphatidylinositol 3-kinases (PI3K), phospholipase C gamma 1 (PLC γ), and Bruton's tyrosine kinase (BTK). The ensuing cellular response varies by cell type and environment and includes antibody-dependent cellular cytotoxicity (ADCC), release of inflammatory cytokines, phagocytosis, and formation of toxic reactive oxygen species (ROS) (2).

TOLL-LIKE RECEPTORS

TLRs are a family of pattern recognition receptors (PRRs) that detect conserved motifs of pathogens (pathogen-associated molecular patterns, PAMPs) or damaged host cells (damage-associated molecular patterns, DAMPs). There are ten human TLRs (TLR1–TLR10) expressed on the plasma membrane and on intracellular membranes of immune cells, fibroblasts, and epithelial cells. TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are surface expressed and primarily recognize bacterial components, such as lipopolysaccharide (LPS) from Gram-negative bacteria that binds TLR4. Intracellular endosomal TLRs recognize nucleic acids from pathogens and host cells, including double-stranded RNA (TLR3), single-stranded viral RNA (TLR7, TLR8), and unmethylated CpG DNA motifs from bacteria and viruses (TLR9). Upon TLR engagement, a signaling cascade is initiated through myeloid differentiation primary response 88 (MyD88)- or TIR domain containing adaptor protein inducing interferon- β (TRIF)-dependent pathways, leading to activation of nuclear factor-kappa B (NF- κ B) and interferon regulatory factors (IRF), respectively, which are key transcription factors central to the inflammatory response. These signaling pathways also influence additional signaling cascades, including the mitogen-activated protein kinase (MAPK) signaling pathways. As a result, TLR engagement drives the release of inflammatory cytokines and promotes immune cell activation, proliferation, and survival (3).

FORMYL PEPTIDE RECEPTORS

FPRs are cell surface-bound, G protein-coupled PRRs. They sense formylated peptides stemming from bacteria and mitochondria but also recognize a variety of non-formylated peptides, lipids, and proteins. The three types of human FPRs (FPR1, FPR2, FPR3) are highly expressed by myeloid cells such as monocytes, macrophages, and neutrophils, as well as by microglia. FPR engagement initiates either pro- or anti-inflammatory signaling, depending on receptor type, ligand, and cell type (4). FPR1 primarily binds short, N-formylated peptides of bacterial or mitochondrial origin and triggers pro-inflammatory effector functions such as chemotaxis, cytokine production, and neutrophil degranulation, along with the formation and release of ROS (4-6). These functions are effectuated through signal transduction mediators such as PI3K/Akt, phospholipase C gamma 1, Ras family GTPases, and MAPK-dependent cascades. FPR2 exhibits lower affinity for short formylated peptides than FPR1 but instead binds a broader range of ligands, including longer formylated and non-formylated peptides as well as other molecules (5). FPR2 signaling is highly context-dependent, where some ligands, such as lipoxin A4, promote anti-inflammatory responses by suppressing NF- κ B and MAPK signaling, whereas other ligands, such as β -amyloid peptide 42 and serum amyloid A, induce pro-inflammatory pathways (7). FPR3 remains the least characterized of the three receptors. Few ligands have been identified for FPR3, and its function is not fully elucidated (4).

Monocytes & macrophages

Monocytes circulate the bloodstream under homeostatic conditions and migrate into tissues mainly in response to an inflammatory insult, where they differentiate into macrophages or dendritic cells (8). Immune cells are identified and phenotypically defined based on their expression pattern of cell surface proteins. Human monocytes are typically characterized by CD14 and CD16 expression. There are two main monocyte subsets: classical monocytes (CD14^{high}, CD16⁻), which are most abundant and exhibit strong migratory potential, and non-classical monocytes (CD14^{low}, CD16⁺), which patrol the vasculature and support endothelial cells. Additionally, intermediate monocytes (CD14⁺, CD16⁺) represent a subset thought to be in transition between classical and non-classical monocytes. These cells are associated with inflammatory responses in certain conditions (8,9).

Macrophages are functionally similar to monocytes but reside in tissues and possess tissue-specialized functions. Traditionally, they were believed to

originate primarily from circulating monocytes migrating into tissues. However, recent evidence suggests that most tissue-resident macrophages originate from the yolk sac or fetal liver-derived progenitors and maintain their populations through self-renewal. Nonetheless, bone marrow-derived monocytes can differentiate into macrophages, particularly during inflammation, to replenish tissue-resident populations (10).

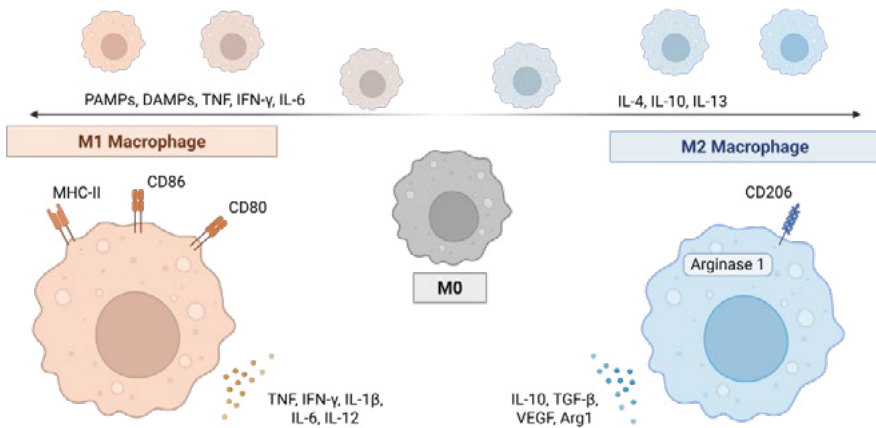


Figure 3. Macrophage polarization.

Monocytes and macrophages express a plethora of surface proteins, such as PRRs and integrins, to sense their surroundings. The recognition of DAMPs, PAMPs, cytokines, and chemokines is crucial in determining their activation state. Traditionally, macrophages were classified into two distinct states upon activation: classically activated (M1) or alternatively activated (M2). However, it is now recognized that macrophages exist along a continuum between these two states, with multiple distinct subtypes displaying overlapping or mixed functional properties. M1-like macrophages are characterized by high expression of surface markers such as CD80, CD86, and the antigen-presenting molecule major histocompatibility complex (MHC) class II, while M2-like macrophages typically express CD206 and arginase 1 (Arg1). The initial phase of inflammation promotes M1-like polarization through the presence of TLR ligands and the release of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF), and interferon- γ (IFN- γ). These factors enhance microbicidal functions such as phagocytosis and ROS formation and trigger the release of inflammatory cytokines and chemokines to attract immune cells. The main role of M2-like macrophages is to prevent excessive host damage by resolving inflammation

and to promote tissue healing. M2-like polarization is favored in the presence of anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 (Figure 3) (10).

In homeostasis, macrophages maintain a balance between M1- and M2-like phenotypes, which allows them to support immune surveillance and tissue remodeling and repair while preventing excessive inflammation. In cancer, however, tumor-derived signals often drive macrophages toward an immunosuppressive M2-like phenotype. Still, tumor-associated macrophages (TAMs) often exhibit mixed or intermediate phenotypes (11).

Neutrophils

Neutrophils are the most abundant white blood cell, constituting up to 70% of leukocytes in circulation. Identification of neutrophils is based on their morphology and high expression of surface markers such as CD15 and CD16, and lack of HLA-DR expression. The primary function of neutrophils is phagocytosis and killing of microbes. Originating in the bone marrow, neutrophils are short-lived and constantly replenished. With an approximate lifespan of only 12–24 hours in circulation, neutrophils are fully functional when exiting the bone marrow and require no further differentiation (12).

Like other myeloid cells, neutrophils detect pathogens through PRRs and utilize FcγRs and complement receptors to eliminate opsonized targets. During inflammation, they are rapidly recruited from the vasculature to the inflamed tissue. Neutrophils employ several mechanisms to eliminate pathogens, including phagocytosis, formation of ROS, granule release, and the formation of neutrophil extracellular traps (NETs). They phagocytose extracellular pathogens and damaged host cells, trapping them in intracellular phagosomes. The phagosomes fuse with intracellular granules that deliver antimicrobial enzymes and peptides. In addition, the NADPH oxidase 2 (NOX2) complex assembles on the phagosomal membrane generating ROS that contribute to microbial killing by inducing oxidative damage to macromolecules. Both ROS and granules can also be released extracellularly, for example, in situations where the pathogens are too large to be phagocytosed. NETs are networks primarily composed of DNA that are released into the extracellular space to trap microbes. When NETs are released, some granule components with antimicrobial effector functions are also expelled to allow the killing of trapped microbes. Due to the non-specific nature of these mechanisms, neutrophil activation may result in bystander tissue damage, contributing to inflammation-associated pathology (12).

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) repress immune responses to avoid uncontrolled inflammation and consequent host damage. MDSCs consist of suppressive immature myeloid cells and myeloid progenitors and expand under stressed conditions such as inflammation, sepsis, and cancer. The main phenotypes are granulocytic and monocytic MDSCs, although referring to these cells collectively as MDSCs has recently been questioned due to their functional heterogeneity. Phenotypically, human monocytic MDSCs are defined by expression of CD11b and CD14, but low expression of HLA-DR, while granulocytic MDSCs express CD11b and CD15, but not CD14 (13).

Under normal conditions, circulating MDSCs are rare and require expansion and activation to mount a response. MDSC expansion is induced by growth factors, including granulocyte/macrophage colony-stimulating factor, macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and vascular endothelial growth factor. Expansion is largely dependent on the activation of signal transducer and activator of transcription 3. Once expanded, immunosuppressive features are activated by a variety of mediators, such as IL-6, IL-1, TNF, TGF- β , and prostaglandin E2. MDSCs exert immunosuppressive functions through several pathways, including depletion of L-arginine by arginase 1, production of nitric oxide and ROS, along with secretion of immunosuppressive cytokines such as TGF- β and IL-10. T and NK cells are highly susceptible to MDSC-induced suppression. Thus, MDSCs and TAMs are assumed to contribute to tumor immune evasion (13).

NATURAL KILLER CELLS

NK cells are innate, circulating lymphocytes that specialize in eliminating stressed host cells, such as virus-infected or malignant cells. Expression of CD56 and lack of CD3 expression characterize NK cells, and NK cell subsets can be defined based on the levels of CD56 and CD16 expression (14).

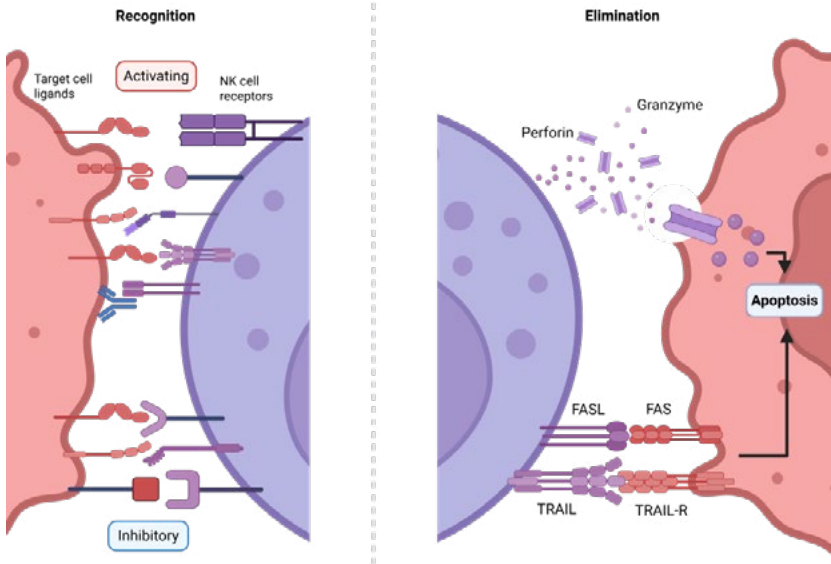


Figure 4. NK cell cytotoxicity.

NK cells express a diverse array of inhibitory and activating receptors. When encountering target cells, their cytotoxic response is determined by the balance of activating and inhibitory signals they receive. Cells normally present antigens on MHC class I, enabling recognition by antigen-specific cytotoxic T cells. Normal MHC class I expression also serves as an inhibitory signal to NK cells, preventing autoimmunity. Malignant and virus-infected cells often downregulate MHC class I to evade T cell-mediated immunity, making them more susceptible to NK cell-mediated killing. NK cells also recognize opsonized targets through the activating receptor Fc γ RIIIa, enabling antibody-dependent cellular cytotoxicity. NK cells eliminate target cells by releasing granules, where perforin facilitates the delivery of granzymes that trigger target cell apoptosis. Alternatively, NK cells induce apoptosis by interacting with the ligands TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FASL) (Figure 4). In addition, activated NK cells secrete cytokines that potentiate immune responses (14).

B LYMPHOCYTES

The production of antibodies is carried out by B cells, which represent the humoral branch of the adaptive immune system. B cells develop in the bone marrow and enter the circulation as mature naive B cells that continuously migrate between secondary lymphoid tissues in search of their specific antigen. B cells are identified by the surface expression of CD19 and CD20 (15).

B cell activation is initiated when the B cell receptor (BCR), a membrane-bound form of immunoglobulin, binds its specific antigen with high affinity. However, full activation and clonal expansion of B cells typically require additional signals provided by helper T cells, unless the antigen itself delivers exceptionally strong stimulatory signals. In T cell-dependent B cell activation, a helper T cell that has been previously activated by an APC recognizes the same antigen presented by the B cell on MHC class II. This interaction provides essential co-stimulatory signals through direct cell-cell contact (e.g., CD40-CD40L interaction) and the secretion of cytokines, ultimately driving B cell activation and clonal expansion.

Following activation, B cells undergo somatic hypermutation and ensuing affinity maturation in structures called germinal centers. These processes refine the antigen-binding regions of the immunoglobulin genes, resulting in a B cell repertoire with high antigen affinity. The activated B cell can undergo class-switch recombination from its IgM/IgD isotype to produce antibodies of either the IgG, IgA, or IgE isotype, each of which has a distinct function. Activated B cells mature into either memory B cells that are readily activated upon re-exposure to antigen, or plasma cells, which secrete large amounts of antibodies (15).

CELLS OF THE NERVOUS SYSTEM

The nervous system is typically divided into two intricately connected components, the CNS, which includes the brain and spinal cord, and the peripheral nervous system. The CNS consists of five main cell types: neurons that transmit signals; oligodendrocytes that provide axons with a protective and functional myelin sheath; microglia, the resident innate immune cells of the CNS; astrocytes that possess a broad range of neuron-supportive functions; and ependymal cells that produce and regulate cerebrospinal fluid (CSF).

Neurons

Most cells communicate over long distances by the release of soluble factors such as hormones, chemokines, and cytokines, but neurons rapidly relay signals in a fully targeted manner via electric impulses. In simplified terms, neurons consist of a soma, which contains the cell nucleus, and two types of extensions: dendrites that receive afferent signals and axons that propagate efferent signals (Figure 5) (16).

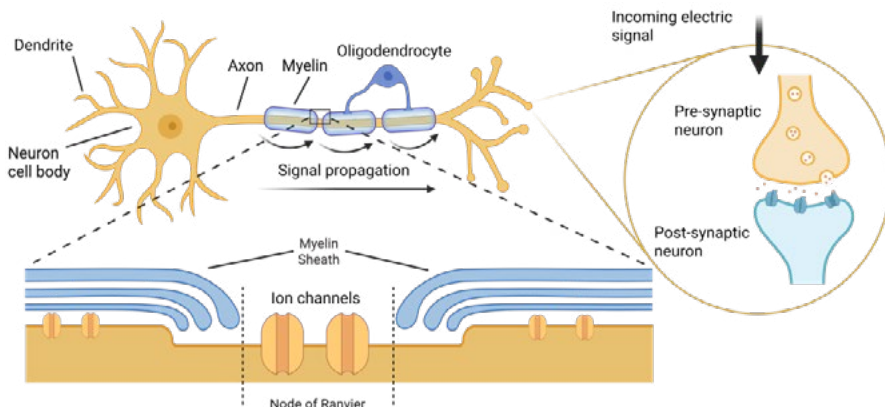


Figure 5. Neuronal structure and communication.

Neuronal signaling is propagated along the axon through sequential opening and closing of ion channels that traffic ions, mainly sodium and potassium, across the axonal plasma membrane. This membrane depolarization activates proximal ion channels to further propagate the signal. Upon depolarization, ion channels enter a refractory state during which they are prevented from immediate reactivation, which allows unidirectional signal transduction (17).

Neurons communicate with other neurons and tissues primarily by converting the electrical signal into a chemical signal, which is transmitted across a small gap, the synaptic cleft. An incoming electric signal, or action potential, triggers the release of neurotransmitters through exocytosis of intracellular vesicles from the presynaptic neuron. These neurotransmitters diffuse across the synaptic cleft and engage receptors on the postsynaptic cell, inducing a functional response that can be excitatory or inhibitory depending on the type of neurotransmitter and receptor involved (18). In certain reflex pathways and brain regions, neurons instead employ electrical synapses, where ions pass directly between neurons through gap junctions, enabling fast communication (Figure 5) (19).

Oligodendrocytes & Schwann cells

Oligodendrocytes originate from oligodendrocyte progenitor cells and populate the CNS, while Schwann cells are present in the peripheral nervous system and derive from neural crest cells. Unlike mature CNS neurons, oligodendrocyte progenitor cells retain proliferative ability in adulthood, allowing them to replenish the oligodendrocyte pool, although this capacity declines with age and in neurodegenerative diseases. Oligodendrocytes and Schwann cells share most functions, although Schwann cells retain higher plasticity and can dedifferentiate and proliferate in response to injury. In contrast, fully differentiated oligodendrocytes do not dedifferentiate, and remyelination in the CNS relies on oligodendrocyte progenitor cells (20,21).

A main function of oligodendrocytes and Schwann cells is to form a myelin sheath, primarily surrounding larger axons. Schwann cells myelinate only one axon, while oligodendrocytes may myelinate multiple axons simultaneously. A key feature of myelination is the presence of nodes of Ranvier, which are small, unmyelinated gaps along the axon. Myelin acts as an insulator, allowing depolarization to occur only at the nodes as opposed to continuously along a non-myelinated axon. Consequently, action potentials jump from node to node, resulting in faster signal propagation (Figure 5) (20,21).

Schwann cells in the peripheral nervous system are regularly replaced, whereas mature oligodendrocytes in the CNS are long-lived. Injury and disease can cause significant demyelination, which may lead to axonal loss, making remyelination essential for restoring function. In the event of infection or injury, Schwann cells and oligodendrocytes are also immunologically active and respond to cytokines. These cells possess some ability to present antigens and provide co-stimulatory signals to immune cells. Furthermore, they affect the

microenvironment by promoting activities such as extracellular matrix remodeling and angiogenesis (20,21).

Microglia

Microglia are CNS-resident innate immune cells that share many features with peripheral tissue-resident macrophages. General aspects of macrophage function and the M1/M2-like phenotype are covered in the section *Monocytes & Macrophages*, while this section highlights functions specific to microglia. Like most tissue-resident macrophages, microglia are not derived from hematopoietic stem cells but instead populate the CNS from the yolk sac in early embryonic development. The colony-stimulating factor 1 receptor is required for microglial differentiation from yolk sac erythromyeloid precursors and for their survival in the adult CNS, along with IL-34. Differentiated microglia retain proliferative ability but exhibit low turnover in homeostatic conditions due to their long lifespan. Typically, microglia are identified by their expression of ionized calcium-binding adaptor molecule 1, but since this can also be expressed by peripheral macrophages, additional markers, such as triggering receptor expressed on myeloid cells 2, may be needed for more precise annotation (22).

In homeostasis, microglia display a ramified morphology with long processes that they utilize to survey the CNS. Neuronal signaling and crosstalk are partly regulated by microglia through formation, pruning, and elimination of synapses influenced by complement proteins. In response to injury or infection, microglia undergo reactive microgliosis characterized by morphological changes to an amoeboid shape, proliferation, and release of inflammatory mediators. Activated microglia exhibit functional states ranging from pro-inflammatory M1-like to anti-inflammatory M2-like phenotypes depending on environmental cues. In response to inflammatory conditions and neural damage, monocytes from the periphery may infiltrate the CNS and differentiate into microglia-like cells. These cells exhibit functions that overlap with resident microglia but tend to have a shorter lifespan. Microglia and astrocytes engage in immunological crosstalk by releasing chemokines and cytokines, which shapes the overall response (22).

Astrocytes

The most abundant non-neuronal cell in the CNS is the astrocyte. Expression of glial fibrillary acidic protein is a common determinant of astrocytes but is often used in combination with functional markers such as S100 calcium-

binding protein β since glial fibrillary acidic protein is also expressed on other glial cells (23). Astrocytes derive from neural progenitor cells and populate all parts of the CNS. As a major component of the blood-brain barrier, astrocytes regulate its formation and permeability and monitor the perivascular space for inflammatory mediators (24).

Within the CNS, astrocytes support neuronal function through multiple mechanisms. Extracellular K^+ levels influence axon membrane potential and, as a result, neuronal excitability. Astrocytes regulate extracellular K^+ levels via uptake through specific K^+ channels. Additionally, astrocytes regulate synaptic signaling by uptake of the excitatory neurotransmitter glutamate from the synaptic cleft, allowing recycling to the pre-synaptic neuron. Dysfunctional astrocytic glutamate uptake can cause excitotoxicity, resulting in neuronal loss, and has been implicated in neurological diseases. Neuronal energy metabolism is supported by astrocytic release of lactate, which is utilized as an energy substrate by neurons (24).

Astrocytes express cytokine receptors and PRRs, and respond to cytokines, ROS, and TLR agonists with inflammatory activation. Like microglia, activated astrocytes exhibit altered morphology and function. NF- κ B-dependent transcriptional programs are initiated in response to inflammatory insult, resulting in the release of cytokines and chemokines, as well as CNS infiltration of peripheral immune cells due to increased blood-brain barrier permeability. Alternative astrocyte activation may lead to the release of anti-inflammatory and neurotrophic factors (23,24).

NEUROLOGICAL DISEASES

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS, leading to progressive physical and cognitive disability. The number of people suffering from MS worldwide was recently estimated at 2.8 million, making it one of the most common neurological disorders among young adults. MS creates a substantial societal cost in part due to the low average age at diagnosis, which is estimated at 32 years. The prevalence of MS is greater among females, with a ratio of 3:1 versus males. However, males may experience more rapid disease progression (25,26).

MS causes focal lesions of demyelination in both white and gray matter, which can be visualized with magnetic resonance imaging. Identification of MS magnetic resonance imaging lesions in individuals without characteristic MS symptoms is termed radiologically isolated syndrome. The presence of such lesions substantially increases the risk of developing MS in the following years and may represent a pre-clinical stage of MS. Clinically isolated syndrome represents a single demyelinating attack that does not fulfill the criteria for MS diagnosis; however, many cases of clinically isolated syndrome evolve into MS (25). A definite MS diagnosis can be established using the McDonald diagnostic criteria, which utilize clinical features in combination with radiological assessment. Fluid biomarkers can also aid in diagnosis (27).

Most patients present with relapsing-remitting MS (RRMS), which comprises acute episodes of neurological dysfunction that are often reversible, although residual symptoms may accumulate over time. RRMS can develop into secondary progressive MS (SPMS), which is characterized by fewer acute episodes but increasing irreversible disability and cognitive decline. The diagnosis of transition to SPMS is thus a poor prognostic factor for overall disability and survival. Despite that RRMS and SPMS are clinically defined as distinct phases and are characterized by certain types of lesions (gadolinium-enhancing lesions in early relapsing MS and slowly expanding lesions in progressive MS), mechanisms related to progressive MS are increasingly recognized to be present already in the early relapsing stage. Approximately 10–15% of patients present with progressive disease at onset (primary progressive MS, PPMS). The time from MS onset to SPMS transition is highly

variable and factors of relevance to this variability are insufficiently understood (Figure 6) (25).

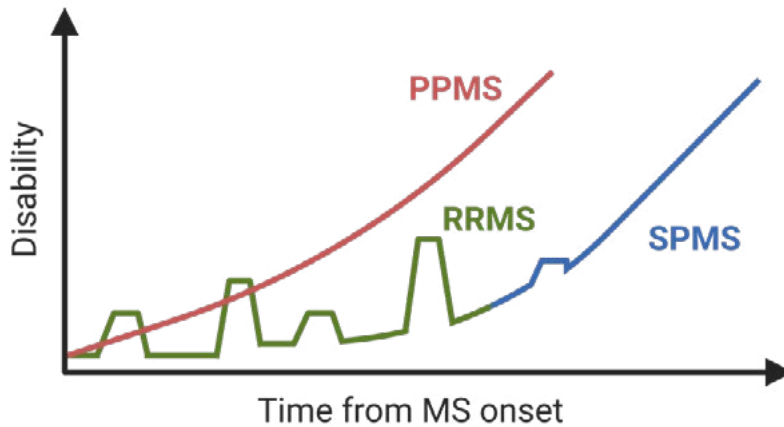


Figure 6. The course of MS.

The cause of MS remains largely unknown, but genetic polymorphisms, particularly in *HLA* genes, have been associated with MS risk. In addition, environmental factors are associated with MS risk, in particular, Epstein-Barr virus (EBV) infection (25). It has been reported that nearly all MS patients have undergone EBV infection, compared with approximately 90–95% of the general population (28). A prospective study of US military personnel showed that one out of 801 patients with MS were seronegative for EBV at MS onset and that 34/35 of individuals who were seronegative at first sampling seroconverted before being diagnosed with MS (29). These findings were supported by a second study of patients with MS that identified the presence of antibodies against EBV nuclear antigen 1 which cross-reacted with alpha-crystallin B chain, a protein expressed by oligodendrocytes. Additionally, EBV nuclear antigen 1- and alpha-crystallin B chain-reactive T cells were identified in patients with MS (30). While these findings support that EBV infection is a risk factor for developing MS, the lack of detectable genomic EBV in white

matter lesions from MS patients may argue against the hypothesis that EBV contributes directly to brain pathology (31).

A wide range of disease-modifying therapies (DMTs) are approved for use in MS, most of which target peripheral immune cells (Figure 7). DMTs include antibodies against CD20 that deplete B cells, sphingosine-1-phosphate receptor (S1PR) modulators that prevent lymphocyte egress from lymph nodes, and anti- $\alpha 4\beta 1$ integrin antibodies that block CNS infiltration of peripheral immune cells. These therapies reduce the frequency and severity of acute neuroinflammatory episodes, but evidence of an impact on long-term disability accumulation is uncertain. However, the prevalence of transition from RRMS to SPMS has reportedly decreased over the last decades for partly unknown reasons that may involve more widespread use of DMTs (32).

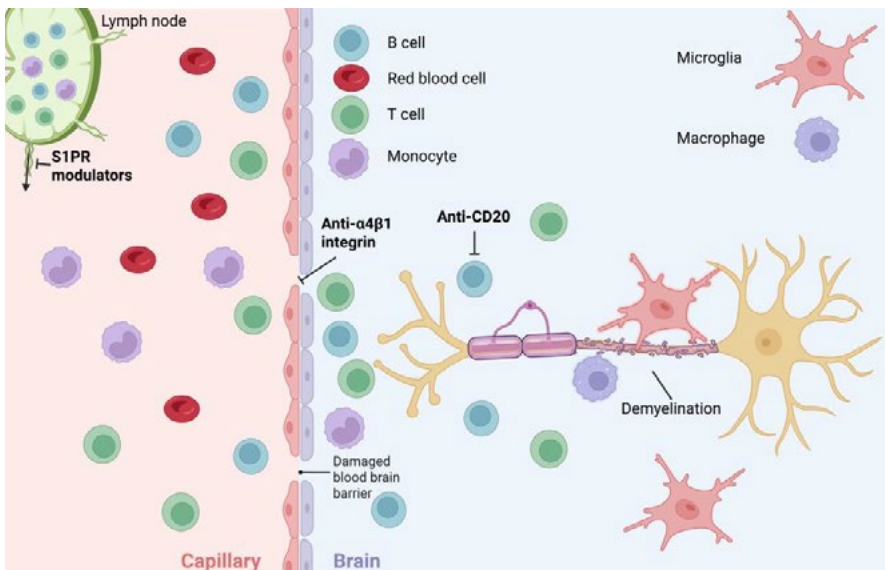


Figure 7. MS pathology and therapies.

Guillain-Barré syndrome

Guillain-Barré syndrome (GBS) is a rare inflammatory neuropathy affecting peripheral nerves that is thought to result, in part, from molecular mimicry between pathogen antigens and neuronal structures leading to antibody-mediated auto-reactivity. Approximately 1–2 per 100,000 persons develop GBS annually. Patients with GBS typically present with numbness or weakness and develop ascending symmetrical weakness that may progress to tetraparesis. However, multiple distinct subtypes with different clinical presentations exist, defined by para- or tetraparetic involvement of motor dysfunction, sensory dysfunction, ataxia, and in some cases, decreased consciousness. The initial course of GBS comprises rapid progression over days to weeks, after which patients reach a plateau phase and subsequently mostly recover over the course of months to years. Mechanical ventilation in the acute phase is required in 20% of cases. Although estimates vary, even with current therapies, approximately 5% of patients succumb to the disease and another 20% experience lasting disability of variable severity. GBS is most often monophasic, but some patients experience recurrent episodes. Risk factors for severe GBS include advanced age, axonal GBS variant, and *Campylobacter jejuni* infection preceding GBS onset (Figure 8) (33).

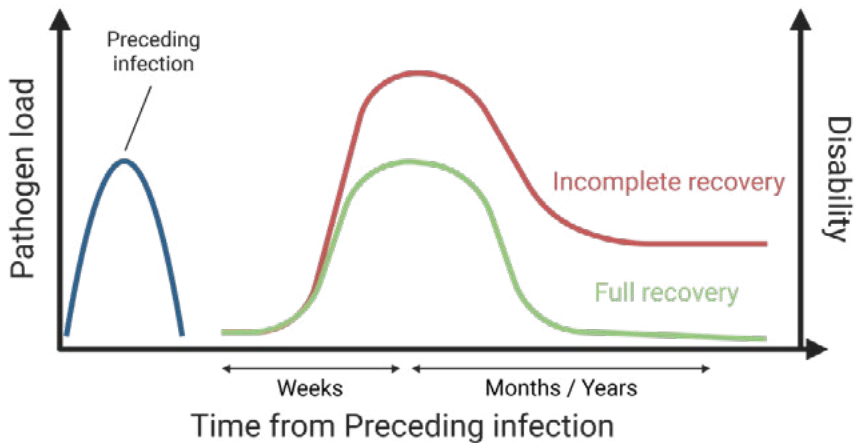


Figure 8. GBS disease course.

GBS may result from molecular mimicry, as evidenced by the presence of cross-reactive antibodies triggered by preceding infections, resulting in immune-mediated destruction of peripheral nerves. Following infection with *Campylobacter jejuni*, autoreactive antibodies targeting gangliosides at nodes of Ranvier or the motor nerve terminal have been identified, though specific

autoantibodies are not always found. Commonly identified antecedent infections include, for example, *Campylobacter jejuni* and *Mycoplasma pneumoniae*, but GBS has also been associated with other immunological insults, such as trauma, malignancy, other infections, and, in rare cases, vaccinations. Diagnosis is initially challenging due to the diverse clinical manifestations, but is assisted by analysis of CSF, nerve conduction studies to confirm peripheral nerve involvement and identify subtype, and serological testing for antecedent infections. Lack of continued disease progression four weeks after onset further supports GBS diagnosis. GBS was traditionally classified as demyelinating or axonal, determined by electrophysiology, although mixed variants are common, suggesting that the immune response may be partly non-specific or target multiple antigens (33).

GBS is treated with intravenous immunoglobulin or plasma exchange. The treatment options have remained unchanged since their introduction more than 30 years ago. The rationale for using these therapies is non-specific inhibition or removal of immune mediators, and are considered equally efficacious. Thus, no mechanism-specific therapy has yet been successfully implemented in clinical practice (33). This highlights an unmet need for patients who die from GBS or suffer lasting disability. Promising results of the anti-C1q antibody ANX005 were recently presented (Annexon Biosciences, 2024-06-04 and 2024-12-16), demonstrating a 2.4-fold reduction in the Guillain-Barré disability scale score, meeting the trial's primary endpoint (34,35). All current or recently completed clinical trials of new DMTs for GBS target complement or antibodies.

Parkinson's disease

Over ten million people are affected by Parkinson's disease (PD) globally, making it the second most common neurodegenerative disorder after Alzheimer's disease (36). Incidence is projected to double by 2040, presenting a major societal challenge. Since the risk of developing PD increases with age, increasing life expectancy is likely contributing to the rising incidence, and additional research is needed to establish the relative contributions of genetic, environmental, and demographic factors. Males are at higher risk of developing PD, although this varies by race, ethnicity, genetic background, and environment (37,38).

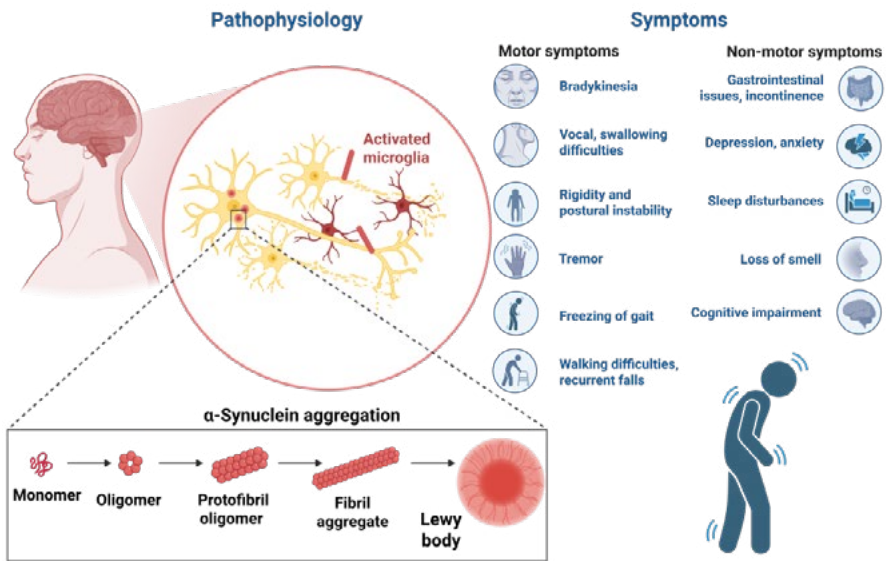


Figure 9. PD pathophysiology and symptoms.

Patients typically present with characteristic symptoms of motor dysfunction such as bradykinesia, tremor, rigidity, and postural instability. However, increasing evidence suggests a prodromal phase marked by non-motor symptoms such as constipation, sleep disturbances, and depression. Autonomic dysfunction, vocal irregularities, hallucinations, and other psychiatric issues are common symptoms during progression of PD, and some patients develop dementia in the later disease stages (Figure 9). Females are assumed to more frequently develop certain motor symptoms, while cognitive decline is more prominent in men. Mutations in genes such as *SNCA*, *PRKN*, *LRRK2*, *GBA*, and *PINK1* are linked to familial forms of PD, but most cases

are sporadic with no known genetic cause. However, genetic variants and environmental factors like exposure to pesticides may increase the risk of developing idiopathic PD (iPD). Clinical diagnosis of iPD is based on symptoms and treatment response, although a definite diagnosis of iPD can only be determined based on post-mortem neuropathological examination. PD mostly progresses slowly over decades, and early-stage patients do not exhibit significantly increased mortality compared to healthy individuals (39).

PD causes degeneration of dopaminergic neurons in the substantia nigra pars compacta, but non-dopaminergic neurons in other brain regions can also be affected. Despite extensive research, the cause of neurodegeneration is not fully elucidated, but dysfunctional mitochondria, protein homeostasis, autophagy, cellular transport, and inflammation are thought to contribute (39). Misfolding and aggregation of the protein α -synuclein is considered a major driver of disease due to the presence of insoluble fibrillar α -synuclein aggregates in the extracellular space and in Lewy bodies, which are intracellular inclusion bodies. α -synuclein is predominantly expressed in neurons, particularly in dopaminergic neurons of the substantia nigra (Figure 9). Fibrillar α -synuclein is neurotoxic and may propagate pathology through a prion-like mechanism, where misfolded aggregates spread between neurons and promote further aggregation. Additionally, extracellular α -synuclein can activate glial cells, including microglia and astrocytes, triggering neuroinflammation and contributing to neuronal dysfunction and degeneration (Figure 9). Mutations in the gene encoding α -synuclein, which cause familial PD, may promote α -synuclein aggregation and increase aggregate toxicity (38,40-43).

Currently, only symptomatic treatments are available for PD and include neurotransmitter precursors, dopamine agonists, and deep brain stimulation. These therapies temporarily restore neuronal signaling, but extensive neurodegeneration results in ineffective symptom relief, and the underlying neurodegeneration is unaffected by any currently available therapy (38). Promising DMTs under investigation include transplantation of dopamine-producing neurons derived from induced pluripotent stem cells and antibodies targeting α -synuclein aggregates for elimination (44,45). However, the benefits of anti- α -synuclein antibodies on long-term disease progression remain uncertain (45).

THE NOX2 ENZYME

The family of NOX enzymes comprises seven isoforms, NOX1–5 and the dual oxidases DUOX1 and DUOX2. NOX2, which is the focus of this thesis, is a multicomponent enzyme consisting of a membrane-bound dimer of gp91^{phox}, also known as NOX2, and p22^{phox}, along with four cytosolic subunits: p40^{phox}, p47^{phox}, p67^{phox}, and Rac. Tissue distribution and the catalytic subunit vary between NOX isoforms. p22^{phox} is required for the activity of NOX1–4. Upon NOX2 activation, which is initiated by phosphorylation of p47^{phox}, the cytosolic subunits translocate to the membrane to assemble a functional enzyme (Figure 10) (46).

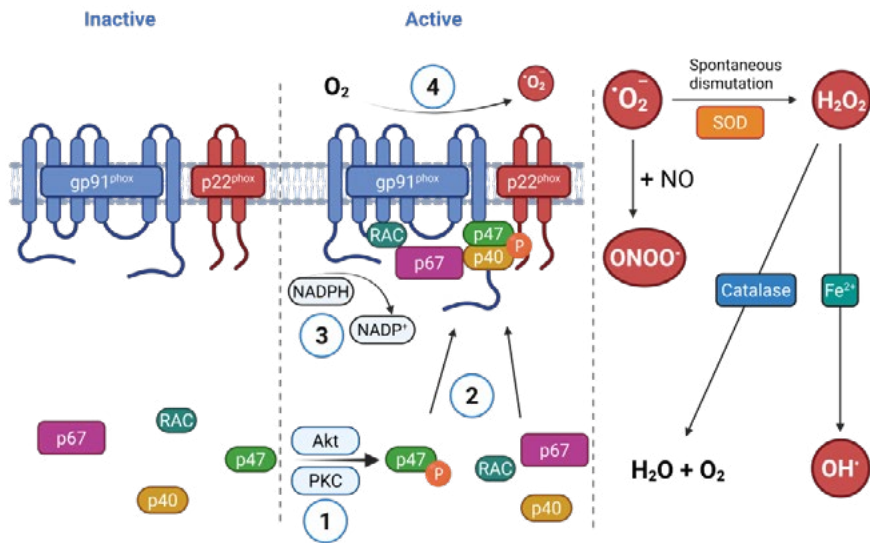


Figure 10. Structure and activation of NOX2, and the potential fates of generated superoxide.

The only known function of the NOX enzymes is to transfer an electron from NADPH across membranes to molecular oxygen, resulting in the formation of ROS in the form of superoxide (O₂⁻), or hydrogen peroxide (H₂O₂) in the case of NOX4 and DUOX1/2. O₂⁻ mainly reacts with molecules close to its source due to its high propensity to donate an unpaired electron and because its polarity prevents passage through lipid membranes. O₂⁻ is rapidly converted to hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD) or through spontaneous dismutation. O₂⁻ may also react with nitric oxide (NO) to form

the highly reactive and toxic peroxynitrite (ONOO⁻). H₂O₂ is less reactive and readily passes membranes, making it potentially toxic over longer distances. H₂O₂ is neutralized by antioxidants such as glutathione that is enzymatically recycled between its oxidized and reduced forms. Catalase catalyzes the conversion of H₂O₂ into water and molecular oxygen. In the presence of ferrous iron (Fe²⁺), H₂O₂ may undergo the Fenton reaction, forming highly toxic hydroxyl radicals (OH[•]) (Figure 10) (47).

NOX2 is densely expressed by myeloid cells such as monocytes, neutrophils, and tissue-resident macrophages, including microglia. NOX2 localizes to the phagosomal membrane where it aids in degradation of ingested pathogens or damaged cells. Additionally, myeloid cells release ROS extracellularly through plasma membrane-bound NOX2 to combat extracellular pathogens or regulate redox homeostasis (48). Functional NOX2 is critical to the defense against invading microbes, as evidenced by frequent and severe bacterial and fungal infections in patients with genetic NOX2 deficiency, such as chronic granulomatous disease (49). In addition, NOX2-derived ROS are ascribed a role in cell signaling by oxidizing specific protein residues and in suppression of innate and adaptive immunity (48). Under inflammatory conditions, Akt and protein kinase C (PKC) have been shown to phosphorylate p47^{phox}, leading to NOX2 activation (Figure 10) (50,51).

Single nucleotide polymorphisms (SNPs) are genetic variations in a single nucleotide at a specific location of the genome. Variants of SNPs may influence transcription, translation, or protein function. In contrast to mutations, which are rare and largely random genetic variants often linked to disease, SNPs represent normal genetic diversity that occur at a frequency of > 1%. The SNPs rs4673 and rs1049254 are non-synonymous variants in *CYBA* which encodes the p22^{phox} subunit of NOX1–4. The approximate estimated positions of the amino acid substitutions within the protein are shown in Figure 11 (52,53). In human neutrophils, the rs4673 A allele was associated with reduced formation of NOX2-derived ROS in response to stimulation with the protein kinase C activator phorbol 12-myristate 13-acetate (PMA) (54), although a second study did not replicate these findings (55). Studies on the impact of rs4673 on ROS formation in cell lines have also yielded partially incongruent results. One study found reduced TNF-induced NOX2 activity in the endothelial cell line HPMEC-ST1.6R carrying the rs4673 A allele (56), while myeloid HL-60 cells transfected with *CYBA* cDNA carrying the rs4673 A allele exhibited increased PMA-induced ROS production compared to cells transfected with *CYBA* homozygous for the G allele (54-57). Analysis of

rs1049254 and rs1049255 in *CYBA*, in PMA-stimulated EBV-transformed B cells, indicated that cells carrying the rs1049254 G and the rs1049255 T allele produced less ROS than cells carrying rs1049254 A and rs1049255 C (58). Taken together, these previous studies suggest that the rs4673 A allele and the rs1049254 G allele might associate with reduced capacity to form ROS in primary myeloid cells. However, the impact of these gene variants may depend on cell type and stimulus.



Figure 11. Approximate locations of the resulting amino acid changes from rs4673 and rs1049254.

OXIDATIVE STRESS, NEUROINFLAMMATION, & NOX2

Normal biological processes such as oxidative phosphorylation in mitochondria generate ROS continuously. ROS are potentially harmful to surrounding tissue but are detoxified by enzymes such as SOD and catalase, as well as through enzymatic recycling of antioxidants such as glutathione. Imbalance between ROS generation and antioxidant defense, either due to excessive ROS generation or antioxidant deficiency, leads to oxidative stress. Oxidative stress encompasses oxidation of macromolecules such as DNA, proteins, and lipids, ultimately causing cellular dysfunction and death (59).

Due to their high energy demand, neurons generate a considerable amount of ROS as a natural byproduct of oxidative phosphorylation and are therefore considered vulnerable to disruptions in ROS homeostasis. Oxidative stress may damage electron transport chain proteins and impair mitochondrial trafficking within axons, causing energy failure (59,60). Thus, mitochondrial stress is thought to contribute to cell death in multiple neurodegenerative diseases (59). Furthermore, the high iron content in the CNS may amplify sensitivity to ROS through the aforementioned Fenton reaction, in which ferrous iron reacts with H_2O_2 to generate hydroxyl radicals (OH^\bullet). Iron content in the CNS increases with age, suggesting a potential link between aging and neurodegenerative diseases (61). The presence of oxidative stress in neurodegenerative diseases has inspired use of antioxidants as DMTs to reduce levels of ROS. However, antioxidants have been largely ineffective in clinical settings. Thus, research focus has instead shifted toward limiting ROS production at its source (46).

Neuroinflammation has emerged as a hallmark of neurodegenerative diseases, with increasing evidence suggesting its role in promoting neurodegeneration (62). The following chapter reviews the role of inflammation and NOX2-mediated oxidative stress in MS, GBS, PD, and related neurodegenerative diseases.

Inflammation in multiple sclerosis

MS was traditionally regarded as an autoimmune disease in which autoreactive T and B cells, alongside infiltrating macrophages and monocytes, contribute to CNS demyelination. In recent years, at least two processes have been implicated in the pathophysiology of MS. In early stages, T cells and B cells are recognized as key drivers of high-grade inflammation during relapses, which are characterized by blood-brain barrier disruption and subsequent immune infiltration into active inflammatory demyelinating lesions (63). This view is supported by the efficacy of therapies that deplete B cells or prevent CNS infiltration of peripheral adaptive immune cells, which significantly reduce the severity and frequency of relapses. However, their limited impact on long-term disease progression suggests the involvement of additional mechanisms contributing to this aspect of disease (32). Indeed, long-term disease progression is closely linked to the appearance and expansion of chronic active lesions, also known as smoldering lesions (64). These lesions consist of a hypoactive demyelinated core, surrounded by a rim of chronically active microglia, which overexpress inflammatory mediators including NOX2 (65,66).

Oxidized lipids and DNA are enriched in MS lesions, especially at the lesion edge and in adjacent normal-appearing white matter, suggesting ongoing oxidative injury (67,68). Notably, oxidized lipids and DNA have been found to co-localize with activated microglia (68). *In vitro* studies suggest that microglial toxicity toward oligodendrocytes is mediated by NOX2-derived ROS (69). Experimental autoimmune encephalomyelitis—a widely used mouse model of MS induced by immunization with myelin antigens—has provided further insight into the role of NOX2 in MS. Induction of experimental autoimmune encephalomyelitis in NOX2-deficient mice resulted in markedly reduced disease severity, neurodegeneration, and release of inflammatory cytokines compared with wild-type mice (70,71). However, while these findings suggest a role for NOX2 in disease progression, a causal link between NOX2 activation and long-term disease progression in MS patients has yet to be established.

Inflammation in Guillain-Barré syndrome

The pathophysiology of GBS is complex and may vary by subtype. In the acute phase, lymphocytes and myeloid cells infiltrate nerve fibers and spinal roots and thus may contribute to nerve injury (33,72). Notably, low-grade neuroinflammation may persist for months to years after clinical recovery (72). B cells likely contribute to the pathogenesis and/or pathophysiology of GBS, as evidenced by the strong association between GBS and presence of autoreactive antibodies (73). In addition, T cells have long been assumed to contribute to GBS pathophysiology since infiltrating CD4⁺ T helper cells and CD8⁺ cytotoxic T cells are found in the endoneurium of GBS lesions (74). Their role in tissue destruction remains elusive, although a recent study has demonstrated presence of autoreactive CD4⁺ and CD8⁺ T cells targeting myelin antigens in patients with demyelinating GBS (75). Historically, complement proteins were primarily implicated in axonal GBS, while macrophage-mediated destruction of myelin was thought to contribute to demyelinating variants. However, complement activation is present in models of both axonal and demyelinating GBS (76-78). The presence of complement deposits at nerve fibers in demyelinating GBS, along with the association between plasma complement levels and clinical outcome in patients with various subtypes, support that complement activation may contribute to pathology in multiple forms of human GBS (79,80).

Macrophages may promote injury in axonal and demyelinating variants of GBS (81). Proinflammatory M1-like macrophages are recruited to lesions early in the acute phase, where they are assumed to contribute to nerve damage by releasing inflammatory cytokines, chemokines, and toxic mediators. This results in the recruitment of additional immune cells and direct tissue damage. In contrast, anti-inflammatory M2-like macrophages dominate in the recovery phase, where they are thought to limit excessive inflammation and support tissue repair and remyelination (82). Promoting the transition from pro-inflammatory to anti-inflammatory macrophages reduced severity in an animal model of GBS, an effect reportedly dependent on activation of the antioxidant-coordinating transcription factor nuclear factor erythroid 2-related factor 2 (83). SNPs in genes related to increased inflammatory signaling and macrophage recruitment are associated with increased severity but not incidence of GBS (84,85). Lesion-infiltrating macrophages may be activated by immune complexes and DAMPs, leading to NOX2 activation and subsequent ROS formation (3,86). Markers of oxidative stress are elevated in patients with GBS compared with healthy controls, and some markers correlate

with disease severity (87-90). However, direct evidence of oxidative damage to neurons in GBS is lacking. NOX2 has been implicated in the pathophysiology of peripheral neuropathies, and the ROS neutralizing enzymes SOD and catalase were shown to alleviate symptoms in an animal model of GBS (91). Taken together, macrophage-driven inflammation and release of ROS through NOX2 may contribute to GBS pathology, but additional evidence is needed.

Inflammation in Parkinson's disease

Although neuroinflammation has long been recognized in PD pathophysiology (92), research has primarily focused on elucidating the pathology of aggregated α -synuclein and strategies for its neutralization or clearance. However, increasing evidence suggests an interplay between inflammation, protein aggregation, dysfunctional cellular homeostasis, and other factors contributing to neurodegeneration (93). Peripheral immune cells may be involved in the pathophysiology of PD. T cells infiltrate the brain of patients with PD and contribute to neurodegeneration in animal models (92,94). Furthermore, circulating monocytes from patients with PD display increased activation in response to inflammatory insult, and fibrillar α -synuclein induces inflammatory activation of monocytes (95,96).

Among immune cells, microglia are particularly implicated in the pathophysiology of PD. The number of activated microglia is markedly elevated in the brain of patients with PD, particularly at sites of neurodegeneration (97). Microglia may exhibit either a classically activated M1-like or an alternatively activated M2-like phenotype. Presence of alternatively activated microglia in PD may be protective through the release of neurotrophic factors or through the engulfment and degradation of aggregated α -synuclein (98,99). However, α -synuclein can also trigger microglial inflammatory activation. *In vitro* stimulation of microglia with α -synuclein induces the release of inflammatory cytokines. Cytokine formation is increased upon stimulation with fibrils formed from α -synuclein containing PD-causing mutations compared with the wild-type protein (100). Additional studies report microglial inflammatory responses upon stimulation with α -synuclein *in vitro* and in animal models of PD. Responses include morphological changes, release of inflammatory cytokines, and ROS formation. Multiple pathways have been implicated in microglial activation in PD animal models or following α -synuclein stimulation, including signaling through the surface receptors TLR1, TLR2, TLR4, CD11b, P2X7 receptor, as

well as mechanisms related to phagocytosis and leucine-rich repeat kinase 2-dependent signaling (100-107). The large number of pathways implicated may depend on differences in microglial origin, the type of response measured, and the aggregation state of α -synuclein.

There is ample evidence of oxidative stress in PD. Oxidized macromolecules are found in the nigrostriatal pathway of patients with PD, but also in other brain regions, and markers of oxidative stress are elevated in the periphery (108). The source of oxidative stress in PD is undetermined but may include dysregulated mitochondrial respiration and/or NOX2 activation. In accordance, aggregated α -synuclein induces microglial ROS formation *in vitro* through stimulation of CD11b, P2X7 receptor, and TLR4, potentially depending on aggregation state (101,104,105). Inflammatory microglia and aggregated α -synuclein may act bi-directionally, as NOX2-derived ROS contribute to α -synuclein aggregation (109). Furthermore, NOX2 promotes neurodegeneration in multiple animal models of PD (106,110-113). A model of chronic neuroinflammation induced by systemic LPS administration aims to mimic symptoms of PD by inducing progressive loss of dopaminergic neurons, α -synuclein pathology, and motor deficit (114,115). In this model, microglial NOX2 is drastically elevated early in the disease course followed by induction of neuronal NOX2, both of which remain elevated (111). Genetic NOX2 deficiency prevented loss of dopaminergic neurons, and treatment with a NOX2 inhibitor for two weeks before or after onset of motor deficit prevented further neurodegeneration for up to 17 months post LPS treatment (116,117). These findings suggest that NOX2 perpetrates progressive inflammatory neurodegeneration in a self-propagating vicious cycle that may be halted by NOX2 inhibition. Microglial and neuronal NOX2 activation is elevated in the brain of patients with PD, but whether NOX2 activation in PD is a cause or consequence of neurodegeneration is unknown (112).

Related neuroinflammatory diseases

Inflammation is a salient feature of several additional progressive neurodegenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), as well as during neuroinflammatory events such as stroke and traumatic brain injury. Despite their distinct causes and clinical manifestations, these conditions may converge on the presence of oxidative stress and activated microglia at sites of nerve damage, with elevated levels of NOX2 (118).

A model of cerebral ischemia revealed that NOX2 expression in neurons was elevated early after injury, while microglial NOX2 expression was increased at later time points (119,120). Inhibition or genetic depletion of NOX2 was neuroprotective in these models (119,121,122). Similarly, in a model of traumatic brain injury an early increase in NOX2 activity was observed in neurons, while a delayed and persistent increase was observed in pro-inflammatory microglia up to one year after traumatic brain injury (123-127). Neuroprotection in these models was conferred by NOX2 inhibition or genetic depletion (124,126,128).

NOX2 activation is markedly elevated in the brain of patients with Alzheimer's disease, and increased NOX2 activity correlates with cognitive decline (129,130). Aggregates of the characteristic misfolding protein amyloid- β induce microglial NOX2 activation *in vitro* (131). Inhibition or genetic depletion of NOX2 ameliorated symptoms in *in vivo* models of Alzheimer's disease (132,133). Similarly, evidence of oxidative stress was found in the brain, CSF, and plasma of patients with HD and correlated with estimates of cognitive decline (134-136). NOX2 inhibition in an animal model of HD reduced inflammatory signaling, increased antioxidant signaling, and improved motor function (137).

Markers of oxidative stress are also elevated in the brain, spinal cord, and CSF in patients with ALS (138-142). Mice overexpressing SOD1 exhibit ALS-like symptoms, resembling those in humans with familial ALS caused by SOD1 mutations. NOX2 expression is elevated in the spinal cord of patients with ALS and SOD1-overexpressing mice, and reduced NOX2 activity in peripheral blood neutrophils is associated with prolonged survival in ALS patients (143,144). In mice overexpressing SOD1, a genetic *Nox2* deficiency led to reduced markers of oxidative stress, reduced neurodegeneration, and prolonged survival (143). Selective expression of mutant SOD1 in neurons is

reportedly insufficient to induce motor impairment in mice, while selective microglial expression causes neurotoxicity *in vitro* (145-147).

BRUTON'S TYROSINE KINASE IN B CELL MALIGNANCIES AND IMMUNE REGULATION

B cell malignancies

B cell malignancies include B cell leukemias, B cell lymphomas, and plasma cell dyscrasias. These diseases arise from different stages of B cell differentiation and maturation (148). In most cases, B cell malignancies are caused by genetic aberrations although infections, environmental factors, and immune dysfunction, including dysregulated antigen responses, have been implicated (149-160). Among leukemias, chronic lymphocytic leukemia (CLL) is the most common variant, typically characterized by the uncontrolled expansion of mature malignant B cells in blood, lymph nodes, and bone marrow. The global incidence of new CLL cases 2019 was estimated at 1.3 per 100,000 persons with slightly higher prevalence in males (149).

B cell malignancies are treated with chemotherapy and, increasingly, with targeted therapies that selectively or specifically eliminate B cells. CD20 is commonly expressed by malignant B cells, especially in leukemias and lymphomas, which has translated into the use of anti-CD20 antibodies to facilitate engagement of innate immune cells. Opsonized cells are eliminated through NK cell-mediated antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis by phagocytic cells, and direct antibody-induced apoptosis (161).

Bruton's tyrosine kinase in B cells

BTK is required for B cell receptor signaling and plays a crucial role in B cell proliferation and survival. It is often dysregulated in malignant B cells, leading to uncontrolled proliferation (162). Inhibition of BTK induces apoptosis in B cells. Multiple BTK inhibitors (BTKi) are approved for treatment of B cell malignancies, and additional candidates are currently being evaluated in clinical trials (162). Second- and third-generation BTKi are modified for increased specificity to reduce off-target toxicity, and some are developed for reversible BTK-binding to limit the emergence of acquired resistance due to binding site mutations (162).

Bruton's tyrosine kinase in myeloid cell activation

BTK was first explored in B cells but is now recognized to play a role as an activating kinase also in other hematopoietic cells, including monocytes, macrophages, and MDSCs (163,164). Upon stimulation of myeloid cell surface receptors, BTK is phosphorylated to initiate the activation of multiple inflammatory mediators, including NF- κ B, PI3K/Akt, and the NLRP3 inflammasome. Downstream effector functions mediated by BTK activation include phagocytosis and secretion of inflammatory cytokines (165).

Inflammation, including the dampening of lymphocyte-mediated immunity against malignant cells, is recognized as a hallmark of cancer. MDSCs and certain subsets of NOX2-expressing TAMs are frequently recruited to the tumor microenvironment. In addition, MDSCs are often present at high levels in the circulation of patients with hematological malignancies. These cells facilitate immune evasion of malignant cells in part by extracellular release of NOX2-derived ROS, which results in suppression of ROS-sensitive T and NK cells (50,166). A recent study reported that high levels of MDSCs in patients with CLL were associated with poor prognosis. Additionally, treatment with the BTKi ibrutinib reduced the numbers of MDSCs in CLL patients (167). The effects of BTK inhibition on myeloid cells have prompted efforts to repurpose BTKi as immunomodulatory therapies aimed at reducing myeloid-driven immunosuppression. In response to BTK inhibition, immunosuppression and tumor growth were reduced in pre-clinical models of solid tumors. BTKi therapy was particularly efficacious in combination with inhibition of the Programmed cell death protein 1/Programmed cell death ligand 1 immune checkpoint axis. No clear benefit has yet been established in patients, although clinical trials have mainly evaluated BTKi in difficult to treat relapsed/refractory or metastatic cancer (163,168-171). BTKi are also being evaluated for the treatment of autoimmune and autoinflammatory conditions, including MS (172-174). Beneficial non-B cell-dependent effects of BTK inhibition have been suggested to involve inhibition of Fc receptor and TLR signaling (168), but the exact mechanism by which BTK inhibition limits immunosuppression and autoinflammation remains unknown.

As BTK mediates activation of Akt, which phosphorylates p47^{phox} to initiate assembly and subsequent activation of NOX2, BTK may be involved in NOX2 activation in response to surface receptor stimulation (50,165). Granulocytes isolated from CLL patients receiving ibrutinib displayed reduced formation of ROS in response to stimulation with bacteria and fungi *ex vivo* (175-178).

NOX2-derived ROS are crucial in the defense against extracellular pathogens. Thus, deficiency of inflammatory signaling in myeloid cells may partly explain the increased frequency of infections observed in patients treated with BTKi (179). However, the mechanisms underlying the interaction between BTK and NOX2 and its potential implications for anti-neoplastic immunity remain incompletely understood.

AIM

The overarching goal of this work was to determine the impact of NOX2-derived reactive oxygen species in inflammatory pathologies with a focus on identifying targetable mechanisms of relevance to long-term outcomes in neurological diseases. The aims for each paper are listed below:

- Paper I:** To determine the impact of *CYBA* polymorphisms at rs1049254 and rs4673 on reactive oxygen species formation from monocytes and rate of disease progression in multiple sclerosis.
- Paper II:** To determine the impact of *CYBA* polymorphisms at rs1049254 and rs4673 on severity of and recovery from Guillain-Barré syndrome.
- Paper III:** To determine the impact of *CYBA* polymorphisms at rs1049254 and rs4673 on long-term disease progression in Parkinson's disease.
- Paper IV:** To evaluate the effect of Bruton's tyrosine kinase inhibition on NOX2 activation and thus provide conceivable NOX2-reductive therapies.

METHODS

STUDY PARTICIPANTS

Paper I

Paper I included two cohorts of patients with MS who were recruited at Sahlgrenska University Hospital. The first cohort comprised 43 patients examined between 1996–1997, with a second cohort of 60 patients examined after 2005. In addition, the study included 108 healthy individuals recruited as controls for the two MS cohorts. All patients fulfilled the Poser diagnostic criteria for MS (180). Review of medical records determined the year of MS onset, the type of MS (PPMS, RRMS, or SPMS) and the expanded disability status scale score at the time of evaluation, and the year of conversion from RRMS into SPMS in accordance with guidelines (181,182). The expanded disability status scale score was converted to the multiple sclerosis severity score to account for disease duration at the time of evaluation, as described (183). Detailed patient characteristics including use of DMTs are presented in Table 1 of **paper I**. Written informed consent was obtained from all participants. Ethical approval was attained from the regional ethical review board of Gothenburg (approval numbers S 196-96, Ad 361-96, and 222-04).

Paper II

In **paper II**, 121 patients with GBS treated at the Department of Neurology, Sahlgrenska University Hospital from 1989 to 2014 were analyzed. Patients were subject to routine diagnostic evaluation at hospital admission, including screening for antecedent infection, nerve conduction studies, electromyography, and analysis of serum and CSF. All study patients fulfilled the Asbury criteria for GBS (184,185). Review of medical records confirmed GBS diagnosis and recorded requirement of assisted ventilation, acute phase motor dysfunction, severity scoring according to the Guillain-Barré disability scale, and time to regained motor function. Detailed patient characteristics including antecedent infection, GBS subtype, and treatment are presented in Table 1 of **paper II**. Patients or their relatives gave written or oral informed consent prior to study admission. The study was approved by the regional ethical review board of Gothenburg (Dnr 222-04, 650-16, and 2021-03471).

Paper III

Paper III included 196 patients with iPD. The study participants were recruited between 2000–2012 among individuals with a diagnosis of PD who visited either of three outpatient clinics in Gothenburg, Falköping, or Skövde. At study inclusion, the participants gave a blood sample and provided demographic and disease-related information. Review of medical records determined the year of motor symptom onset. For an exploratory subset, constituting 95 of the 196 patients, the time from onset of motor symptoms to the occurrence of 21 clinical milestones characteristic of PD progression was determined through screening of medical records. The included milestones and their definitions are described in **paper III**. Detailed patient characteristics are presented in Table 1 of the same paper. Ethical approval was obtained from the regional ethical review board of Gothenburg (L011-99). The ethics approval was amended in 2023 to include review of medical records to determine clinical milestones (2023-04748-02). Study participants who were alive in 2023 were included in the extended medical records review upon additional written informed consent, while participants deceased at this point were included by default.

Paper IV

In **paper IV** blood samples collected from two patients with X-linked agammaglobulinemia, and from a patient with CLL, were included. The patients with X-linked agammaglobulinemia were receiving IgG supplementation at the time of sampling. The patient with CLL was asymptomatic, classified as Binet stage A, and was not receiving treatment for CLL. All patients provided written informed consent, and the study was approved by the regional ethical review board of Gothenburg (Dnr 312-13, 737-17, 2023-06740-02).

GENOTYPING

For **papers I–III**, we utilized predesigned TaqMan genotyping assays to determine SNP genotype at rs4673 and rs1049254 within the *CYBA* gene in genomic DNA isolated from study participants. These assays utilize real-time quantitative PCR with probes conjugated to fluorescent molecules that bind the target sequence only if a specific SNP genotype is present. Probe binding during amplification facilitates fluorescence. The relative fluorescence between the probes in a sample after amplification is used to determine the

genotype (Figure 12). The PCR was run on a 7500 Fast Real-Time PCR machine (Applied Biosystems). Genotypes were determined using the 7500 SDS software version 1.4 (Applied Biosystems). The genomic DNA used as a template was isolated from tissue samples using a high-throughput DNA extraction device (MagnaPure LC, Roche Molecular Systems) with a Total NA (serum, plasma, blood) kit or a DNEasy Blood & Tissue Kit (Qiagen).

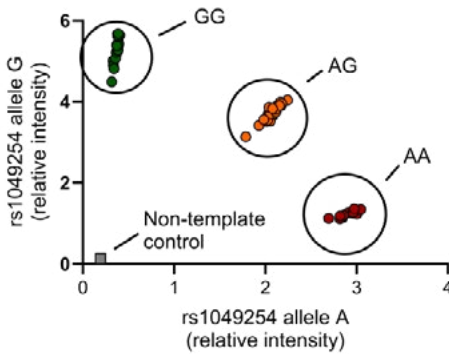


Figure 12. Allelic discrimination. Example of allelic discrimination data, including controls without DNA template. Each dot represents DNA from one individual.

CELL CULTURE ASSAYS

Leukocyte isolation and purification

Peripheral blood mononuclear cells (PBMCs) constitute T cells, B cells, NK cells, monocytes, and dendritic cells. This cell fraction is separated from granulocytes and erythrocytes in blood by utilizing differences in cell density. In **papers I** and **IV**, PBMCs were isolated from healthy blood donor buffy coats or peripheral blood collected from study participants by dextran sedimentation and density gradient centrifugation. In **paper IV**, specific cell types were further purified using methods that rely on targeted labeling of cells with magnetic beads, which allows separation by magnetic retention.

Measurement of reactive oxygen species

In **papers I** and **IV**, we utilized two assays to measure NOX2-derived ROS formation in myeloid cells. ROS were measured in response to surface receptor-dependent and receptor-independent stimuli.

CHEMILUMINESCENCE-BASED $O_2^{\cdot-}$ MEASUREMENT

The first ROS assay is based on measuring chemiluminescence emitted upon the reaction between isoluminol and $O_2^{\cdot-}$, which is catalyzed by horseradish peroxidase. Isoluminol cannot pass through lipid membranes and the assay therefore reflects formation of extracellularly generated NOX2-derived ROS (186). Chemiluminescence intensity indicates the current rate of ROS formation and is measured continuously during an experiment, after which the area under the chemiluminescence intensity curve over time determines the total ROS formed.

AMPLEX ULTRA RED-BASED H_2O_2 MEASUREMENT

The second assay measures H_2O_2 , which is rapidly formed from generated $O_2^{\cdot-}$ through spontaneous or enzymatic dismutation. In the presence of horseradish peroxidase, the Amplex Ultra Red reagent reacts with H_2O_2 to form a stable fluorescent molecule that can be detected by emission at 590 nm upon excitation at 540 nm. Fluorescence intensity indicates H_2O_2 concentration and is measured at an end point to determine the total H_2O_2 formed. The rate of H_2O_2 formation is determined by the change in fluorescence intensity over time when fluorescence is measured continuously.

Flow cytometry

Flow cytometry enables analysis of protein expression and other cell characteristics with single-cell resolution. Cells in suspension are aligned single file and exposed to one or more lasers. Characteristics of light scatter provide information about cell size and granularity, while multiple fluorescent probes are simultaneously detected by combinations of different wavelength lasers and filtered emission detectors. The probes are often fluorescent molecules conjugated to antibodies that target a protein of interest. Alternative probes include molecules that bind stressed or dying cells. Flow cytometry is extensively used in immunology research, where the expression pattern of proteins can specify cell type and function. In **paper IV**, flow cytometry was used to determine factors such as the fraction of dead target cells after co-culture with effector cells, cell proliferation, and protein phosphorylation. A detailed description of panels and gating strategy is presented in **paper IV**.

MEASUREMENT OF NEUROFILAMENT LIGHT CHAIN

Neurofilament light chain (NfL) has emerged as a biomarker for axonal decay. NfL is detectable in CSF and serum (sNfL) although serum levels are lower. Highly sensitive assays such as Single Molecule Array (Simoa) allow quantification of NfL even at the low concentrations found in serum. For **paper II**, 76 patients provided serum samples for analysis of sNfL. Samples were analyzed using the Simoa NF-light™ Advantage Kit on an HD-X Analyzer (Quanterix, Billerica, MA) as described (187).

MELANOMA MOUSE MODEL

In **paper IV**, the anti-neoplastic effect of NOX2 inhibition by ibrutinib was determined in a model of NK cell-sensitive metastatic melanoma. In this model, murine B16F10 melanoma cells are injected intravenously into the tail vein of mice. The malignant cells are allowed to engraft for three weeks, after which the mice are sacrificed and lungs are removed. The black color of melanoma cells allows for visual enumeration of metastatic foci on the lungs under a light microscope. Malignant cells in this model are highly controlled by NK cells, which have been shown to be sensitive to NOX2-mediated immunosuppression (188,189). Thus, the model is suitable to study effects of NOX2 inhibition on anti-neoplastic NK cell immunity *in vivo*. Mice were treated with the BTKi ibrutinib or vehicle daily for five days starting the day prior to tumor cell inoculation. Experiments were conducted in wild-type mice as well as in mice lacking the catalytic subunit of NOX2 or in mice depleted of NK cells to elucidate if observed effects were NOX2- and/or NK cell-dependent.

All illustrations were created with BioRender.com.

RESULTS & DISCUSSION

Elucidating the cause of neuronal decay in neurodegenerative diseases may facilitate development of new therapies. The studies presented in this thesis aimed to identify targetable mechanisms that impact long-term progression of neurodegenerative diseases. NADPH oxidase 2 (NOX2)-derived reactive oxygen species (ROS) contribute to immunosuppression in cancer, preventing the immune-mediated elimination of malignant cells. Despite this, clinically approved, safe, and effective NOX2 inhibitors require further development. We therefore evaluated the NOX2 inhibitory properties of Bruton's tyrosine kinase inhibitors (BTKi) and their potential efficacy in relieving immunosuppression.

CYBA GENOTYPES INFLUENCE NEUROINFLAMMATION AND NEURODEGENERATION

Oxidative stress has long been believed to contribute to neurodegeneration. However, the source of oxidative stress in neurodegeneration is not known and may include ROS produced enzymatically or as a byproduct of other cellular processes, including cellular respiration (59). Inflammatory activation of monocytes and macrophages, including microglia, entails activation of NOX2, leading to the extracellular release of ROS. Increased NOX2 activation is observed in experimental models of neurodegenerative diseases, wherein inhibition or genetic depletion of NOX2 is neuroprotective to suggest a possible causative role of NOX2. Elevated NOX2 activity is also assumed to contribute to neurodegeneration in patients. However, the inflammatory response and subsequent NOX2 activation observed in patients is also suggested to be a response to tissue injury and dying neurons rather than a cause of neurotoxicity (46,118,190). Cellular interactions within the central nervous system (CNS) are difficult to study over time in living patients. Thus, the role of NOX2 in human neurodegeneration may not be fully deciphered without studying the effects of pharmacological intervention.

We aimed to determine the potential impact of NOX2 activity on neurodegeneration and neuroinflammation by analyzing single nucleotide

polymorphisms (SNPs) (rs1049254 and rs4673 in *CYBA*) that influence the capacity of myeloid cells to generate NOX2-derived ROS. This is advantageous for multiple reasons. First, the mechanism is relatively straightforward; the only known function of NOX enzymes is to generate ROS, meaning that there is no evidence of p22^{phox} interacting with proteins or molecules unrelated to the formation of NOX-derived ROS. Thus, it is unlikely that other mechanisms are impacted by changes in the protein sequence, as opposed to, for example, kinases with a multitude of phosphorylation targets. Second, the functional effects of the SNPs under investigation have been documented by us and others in several studies (Figure 13). Lastly, SNPs are germline-encoded and therefore not a consequence of disease, unlike measurements of NOX2 activity in patients. Outstanding questions include whether observed effects reflect direct ROS-induced toxicity or alterations in ROS signaling.

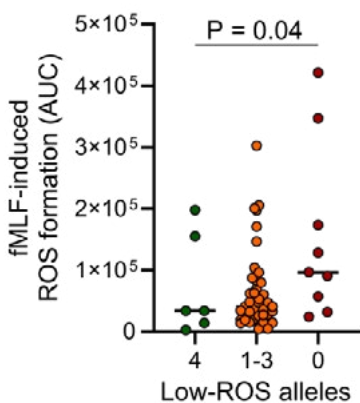


Figure 13. *CYBA* genotype influences fMLF-induced ROS formation. ROS formation from human PBMCs was measured by chemiluminescence upon stimulation with the N-formylated peptide fMLF. Low-ROS alleles denote the number of rs4673 A and rs1049254 G alleles. Statistics by linear regression.

Multiple sclerosis

Genetic influences on multiple sclerosis (MS) incidence have been extensively studied (191). Factors that determine disease progression may be unrelated to the cause of disease and have potentially been undervalued in the search for therapies that prevent progression of disability. Additionally, the primary endpoint of most clinical trials in MS was efficacy in terms of reducing the frequency of relapse, which may not reflect mechanisms that promote progressive neurodegeneration and ensuing disability (32). MS progression occurs over years to decades and long follow-up is therefore required to elucidate aspects of disease progression (25).

We studied the impact of variation at rs1049254 and rs4673 on outcome in two unique cohorts of patients with MS who were followed for up to 49 years from disease onset. The rate of disease progression was assessed by the disease duration-adjusted multiple sclerosis severity score (MSSS) and by the time from MS onset to the diagnosis of progression as defined by secondary progressive MS (SPMS). In the larger cohort alone (cohort 2, n=60) and in the two cohorts combined (n=103) we found a significant impact of *CYBA* genotype on MSSS and time to onset of SPMS. MSSS was lower among patients with genotypes at rs1049254 (G allele) and rs4673 (A allele) that were associated with reduced capacity to generate NOX2-derived ROS (low-ROS alleles) (Figure 14A). The low-ROS alleles were also associated with delayed time to develop SPMS. The effects were pronounced in cohort 2, where patients carrying at least one low-ROS allele at rs1049254 or rs4673 experienced a delayed median time to SPMS by over 20 years compared with patients carrying no low-ROS alleles (Figure 14B).

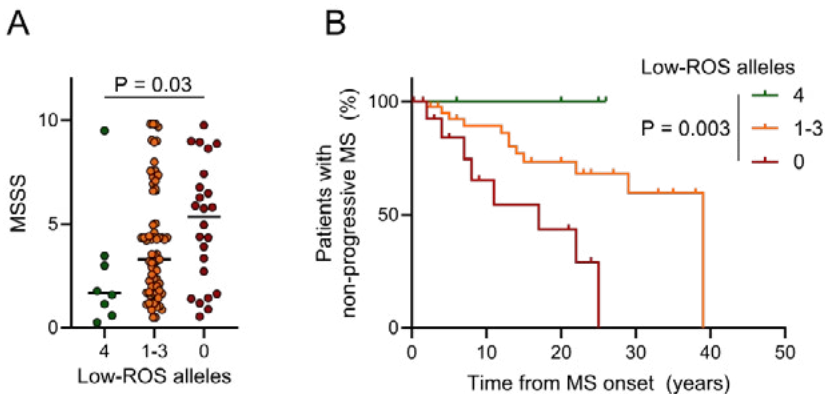


Figure 14. *CYBA* alleles associate with MS severity and progression. (A) MS severity determined by MSSS and (B) progression determined by transition to SPMS. Statistics by (A) linear regression and (B) log rank test for trend.

These effects remained significant when adjusting for potential confounding factors. We also found that MS patients were slightly but significantly less likely to carry low-ROS alleles compared with healthy controls. Although this has not been reported in previous genome-wide association studies of MS, one study found that two SNPs in other NOX2 subunits were significantly associated with MS risk (192-194). We did not find significant effects of *CYBA* genotype on MS progression in cohort 1 alone. This may be due to the smaller

sample size but might also reflect that patients in cohort 1 were evaluated approximately ten years prior to those in cohort 2. Thus, patients in cohort 1 generally experienced more severe disease and had not received DMT, although the impact of genotypes found in cohort 2 remained significant when only untreated patients were analyzed.

Our findings support that NOX2⁺ microglia at the rim of slowly expanding lesions might contribute to neurotoxicity through release of ROS, thereby driving long-term progression. We did not find a correlation between *CYBA* genotypes and the frequency of relapses. This suggests that NOX2 activity might not contribute to the occurrence of relapses. However, NOX2-derived ROS generated by microglia or infiltrating macrophages and monocytes during relapses may promote nerve damage to increase residual disability.

Our results should be validated in additional patient cohorts. However, studies of long-term disease progression in the modern era may be complicated by the wide range of disease-modifying therapies (DMTs) in use and the lack of a standardized treatment protocol. Although most approved DMTs have not demonstrated clinical efficacy in preventing progressive disease in individual clinical trials, the overall outcome for patients with MS has improved in recent years for partly unknown reasons (32,195). This could be due in part to the effects of DMTs on disease progression that are not captured in the short time frame of clinical trials. Taken together, our findings may, with due reservation, point to NOX2-inhibitory therapy in MS to halt disease progression.

Guillain-Barré syndrome

With current therapies, most patients who develop Guillain-Barré syndrome (GBS) recover fully, although the disease remains deadly for approximately 5% of patients and a large portion live with lasting disability. The approved therapies, intravenous immunoglobulin and plasma exchange, improve outcome but GBS therapy has remained unchanged for decades (33). New therapies are required to further improve outcome, but little is known about the detailed mechanisms that cause GBS and propagate the destruction of nerves.

GBS is considered an autoimmune disease, though the specific roles of immune cell subsets in the pathogenesis remain unknown and may vary by subtype. The presence of oxidative stress and potentially autoreactive myeloid cells in GBS coheres with results obtained in neurodegenerative diseases (59,62,81,87-90), despite that GBS does not involve the gradual, progressive nerve cell degeneration characteristic of neurodegenerative diseases, but rather

constitutes an acute condition that typically stabilizes within weeks followed by a recovery phase (33). Antioxidants were shown to improve the outcome of experimental autoimmune neuritis, which mimics GBS (196). However, little is known about the potential contribution of NOX2-derived ROS to GBS pathophysiology.

We analyzed the potential impact of *CYBA* genotypes on clinical outcome. GBS severity was evaluated by need for assisted ventilation in the acute phase, while recovery was evaluated by the time from disease onset to regained motor function and by the normalization of elevated serum neurofilament light chain (sNfL) levels. GBS patients carrying low-ROS alleles were found to be at reduced risk of requiring assisted ventilation (Figure 15A). Furthermore, low-ROS alleles were associated with faster recovery of motor function (Figure 15B), reappearance of normal sNfL levels, and reduced risk of persisting disability.

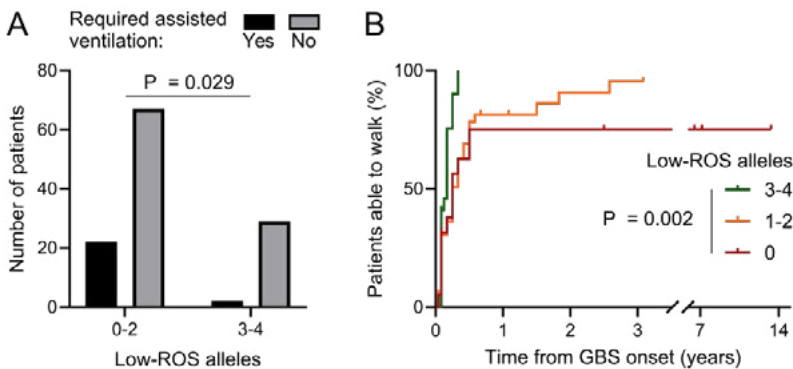


Figure 15. *CYBA* genotype associates with severity of and recovery from GBS. (A) Requirement of assisted ventilation in the acute phase and (B) time to regained ability to walk 10 m independently among patients distinguished by *CYBA* genotype. Statistics by (A) Fisher's exact test and (B) log rank test for trend.

The impact of *CYBA* genotypes on motor function recovery was pronounced in older patients. This may be due in part to inflammaging, which encompasses dysregulated innate immunity that increases with age (197). Immune dysregulation might lead to the inability to resolve GBS-induced inflammation, leading to prolonged or increased activation of NOX2. The observed effect of *CYBA* genotype on acute severity suggests that patients carrying high-ROS alleles experience increased acute phase nerve damage. The impact on long-term recovery may thus result from increased early-phase

damage since severity in the acute phase predicts incomplete recovery (33). However, delayed recovery may also reflect persistent neuroinflammation, which has previously been observed in GBS patients (72). Persistent inflammation could potentially contribute to continued tissue damage and impeded healing. Serum NfL levels remained elevated for several months in some patients, which supports ongoing nerve injury. However, the half-life of sNfL is estimated at several weeks (198). Thus, prolonged elevation might reflect decline from high levels during the acute phase without additional axonal degeneration.

Our observations suggest that NOX2-derived ROS may contribute to GBS pathology. We speculate that NOX2⁺ myeloid cells infiltrate neural tissues in response to a triggering inflammatory event that releases damage-associated molecular patterns (DAMPs) and/or pathogen-associated molecular patterns (PAMPs). In the acute phase, NOX2-derived ROS released by activated infiltrating monocytes, classically activated M1-like macrophages, and neutrophils may contribute to nerve injury. During the recovery, persistent nerve damage might be contributed by NOX2-derived ROS generated by macrophages that are chronically activated by DAMPs released from damaged neurons. The impact of *CYBA* genotypes on GBS outcome requires validation in additional cohorts, and the exact mechanisms underlying the associations need investigation in experimental models of GBS. It is proposed that NOX2-inhibitory therapy might benefit patients with GBS through improved recovery and by avoiding long-term disability.

Parkinson's disease

Parkinson's disease (PD) entails progressive loss of dopaminergic neurons with ensuing disability of motor and non-motor functions. Therapies such as levodopa efficiently alleviate symptoms in early stages, but no available therapy consensually prevents long-term disease progression (39). To uncover novel targets for treating long-term progression of PD, we aimed to determine the potential role of NOX2-derived ROS in promoting neurodegeneration and consequent disease progression. Overall survival was determined for 196 patients with idiopathic PD (iPD), and disease progression was defined by the occurrence of 21 clinical milestones characteristic of PD in an exploratory subset of 95 patients. The milestones aimed to reflect multiple aspects of PD, such as motor fluctuations and dysfunction, autonomic dysfunction, psychiatric and cognitive issues, and requirement of physical or societal assistance.

We did not find differences in age at onset or death between patients discriminated by genotypes at rs1049254 or rs4673. The impact of *CYBA* genotypes on the time from diagnosis until occurrence of milestones was analyzed by individual milestones and by using a composite measure of all milestones. The composite measure was construed as the number of milestones reached over time from the onset of motor symptoms. All milestones, including death, were given equal weight.

Most of the included milestones also occur with normal aging. Thus, we designed a refined composite measure to reduce the potential impact of normal aging, which excluded events after 75% of events had occurred within each milestone. This design retains the impact of each milestone including those occurring on average later during PD, as opposed to limiting the analysis by age or disease duration. Furthermore, this measure lessens the relative severity of slowly progressing patients who may inevitably reach commonly occurring milestones.

We found that rs1049254 and a combined analysis of rs1049254 and rs4673 significantly impacted a subset of individual milestones as well as the cumulation of all milestones over time (Figure 16). Patients carrying low-ROS *CYBA* alleles thus exhibited delayed occurrence of milestones compared with patients with a high-ROS genotype. The effect of *CYBA* genotype on cumulation of all milestones remained significant when analyzed by the refined composite measure and when adjusted for potential confounders.

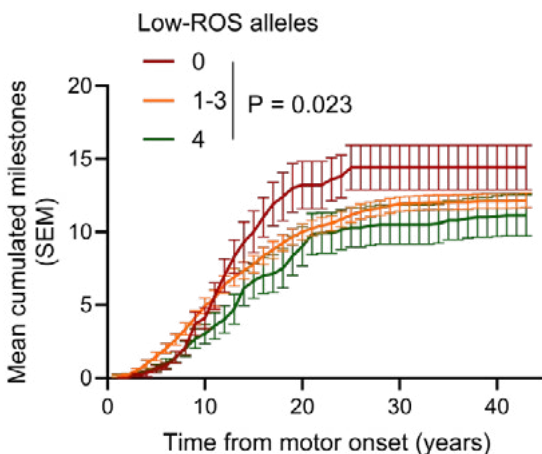


Figure 16. *CYBA* alleles associate with cumulation of PD milestones. Mean number of milestones reached each year from onset of motor symptoms. Statistics by mixed-effects model adjusted for potential confounders.

All milestones are not equally detrimental to the patient. Thus, weighing the milestones based on severity may better reflect disability and neurodegeneration. However, this measure has not been used or validated in other patient cohorts and weighing might introduce bias. The composite measure is sensitive to differences in follow-up as patients who are censored or die early will not cumulate additional milestones. In this cohort, 78/95 patients were followed until death, and there were no differences in follow-up time, age at death, or age at onset between patient groups distinguished by *CYBA* genotypes. Additionally, patients who died early were more likely to experience a rapid cumulation of milestones in the early stages of disease.

The impact of *CYBA* genotypes on progression of iPD aligns with effects of NOX2 inhibition or deletion in experimental models of PD and long-term neurodegeneration, and with the increased NOX2 activity seen in the brain of patients with PD (106,110-113,116,117). What triggers inflammation and increased NOX2 activity in PD is not known. Fibrillar, but not monomeric α -synuclein, has been shown to cause activation of microglia *in vitro*, leading to release of inflammatory cytokines and ROS (100-107). Protein aggregates and DAMPs released from dying neurons may initiate inflammatory microglial activation, resulting in release of NOX2-derived ROS and subsequent neurotoxicity. Neurons subjected to oxidative damage may sustain protein folding deficiency, resulting in protein aggregation. Furthermore, direct oxidation of α -synuclein protein residues can promote misfolding and aggregation (109). Thus, NOX2-derived ROS might propagate a feed-forward mechanism by inflicting cell damage which releases DAMPs, and/or by promoting protein aggregation via oxidation, which further activates microglia.

The results of the initial subset of PD patients will be validated by similar analyses in the remaining patients. Additional validation cohorts with pre-determined end points, which could potentially allow the introduction of milestone weighing, are also warranted. Furthermore, the composite measure should be compared with existing disability scales such as the Movement Disorder Society-Unified Parkinson's Disease Rating Scale.

Is NOX2 a generic contributor to neurodegeneration?

In three neurodegenerative and neuroinflammatory diseases, we have found that patients carrying alleles at *CYBA* that associate with reduced capacity to generate NOX2-derived ROS experience benign disease compared with those carrying high-ROS genotypes. The etiologies of the studied diseases are unrelated, but a common denominator is the proposed involvement of oxidative stress and inflammatory monocytes, macrophages, and microglia in nerve injury.

Oxidized macromolecules and ensuing nerve cell death may be a result of abnormal or persistent NOX2 activation. Myeloid NOX2 is activated upon recognition of DAMPs, which are likely abundant at sites of neurodegeneration. Hence, nerve injury may cause self-propagating NOX2 activation (199). This mechanism is supported by observations in models of traumatic brain injury wherein NOX2 inhibition or deletion limits tissue damage and promotes recovery (124,126,128). Additionally, NOX2 is activated by PAMPs and aggregated proteins. Potential sources of PAMPs include virus-infected cells in MS (200) and bacterial or viral motifs in the pathogenesis of GBS (201). Aggregates of tau, α -synuclein, amyloid- β , and superoxide dismutase 1 are observed in neurodegenerative diseases such as Alzheimer's disease, PD, and amyotrophic lateral sclerosis. The aggregates are commonly shown to initiate inflammatory activation of microglia, and presence of protein aggregates correlates with increased microglial activation in patients (22,202,203).

Our studies of *CYBA* polymorphisms do not strictly reflect microglial NOX2 activity alone. NOX2 is considered a dominant source of enzymatically derived ROS in the CNS under pathological conditions, and it is most highly expressed by myeloid cells (46,53). However, p22^{phox} is a component of additional NOX enzymes (NOX1–4), and we cannot formally exclude contributions beyond NOX2 activity (204). NOX4 is also abundantly expressed in the CNS (46). The chemiluminescence-based assay that was employed to determine ROS formation from cells with different *CYBA* genotypes measures extracellular NOX2-derived ROS; thus, whether rs4673 and rs1049254 impact ROS generated by other NOX enzymes is not completely determined (186). NOX2 is also expressed by cell types other than myeloid cells, including neurons. In fact, both neuronal and microglial NOX2 activation is increased in the brain of patients with PD compared with healthy

controls. Hence, *CYBA* polymorphisms may also affect neuronal NOX2 activity and potential subsequent neurodegeneration (112).

For the observed significant associations between *CYBA* genotypes and clinical outcomes, patients with heterozygous or intermediate genotype groups consistently experienced intermediate clinical outcomes between the extreme genotypes. Furthermore, patients carrying rs4673 AA consistently experienced the most favorable clinical outcomes, while patients carrying rs1049254 AA experienced the most severe disease, which, due to linkage disequilibrium, coincides with presence of four and zero low-ROS alleles at both loci, respectively. Thus, our findings suggest that both rs4673 and rs1049254 impact protein function, since the diametric clinical outcomes would more likely be confined to homozygous genotypes for one SNP if only one SNP impacted protein function. Our annotation is further supported by the fact that the results presented in this thesis show beneficial associations with low-ROS alleles in three diseases wherein oxidative stress and/or NOX2 activation is proposed to contribute to nerve death (65-71,101,104-106,110-113,116,117,196). Still, some ambiguity remains in the scientific community regarding the effect of the rs4673 genotype (54-57).

We propose that NOX2-inflicted oxidative stress might be a common feature of neurodegeneration. Persistent presence of inflammatory triggers may promote chronic inflammation of myeloid cells, initiate NOX2 activation, and prevent resolution of inflammation. Our findings warrant validating studies in MS, GBS, and PD and may point to extended studies of additional neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. In addition, NOX2 should be further investigated in experimental models of neurodegenerative diseases to elucidate factors promoting NOX2 activation, which may include DAMPs, PAMPs, aggregated proteins, or other unidentified factors.

BRUTON'S TYROSINE KINASE MEDIATES TARGETABLE NOX2 ACTIVATION

BTK mediates inflammatory activation of multiple immune cell types, including monocytes, macrophages, microglia, and neutrophils. BTK is required for B cell receptor signaling, which promotes B cell proliferation and survival (165). Thus, BTKi are used in B cell malignancies to control malignant B cells (173,205). The generation of NOX2-derived ROS has been suggested to promote immune evasion in hematological malignancies and in solid cancers (188,206-208). We hypothesized that BTKi may limit the activation of NOX2 in myeloid cells and consequently provide anti-neoplastic efficacy beyond direct targeting of malignant B cells.

We found that BTKi reduced NOX2 activation in response to surface receptor stimulation of monocytes (Figure 17A) but not when cells were exposed to stimuli that bypass surface receptors. BTKi lacked NOX2-inhibitory efficacy in cells from individuals with genetic BTK deficiency. Additionally, receptor-stimulated ROS-burst was blunted in BTK deficient cells. This suggests that BTK is required to mediate surface receptor-triggered NOX2 activation, and that BTKi limit NOX2 activation by on-target inhibition of BTK. In the presence of stimulated monocytes, BTKi improved natural killer (NK) cell viability by limiting ROS-inflicted toxicity (Figure 17B).

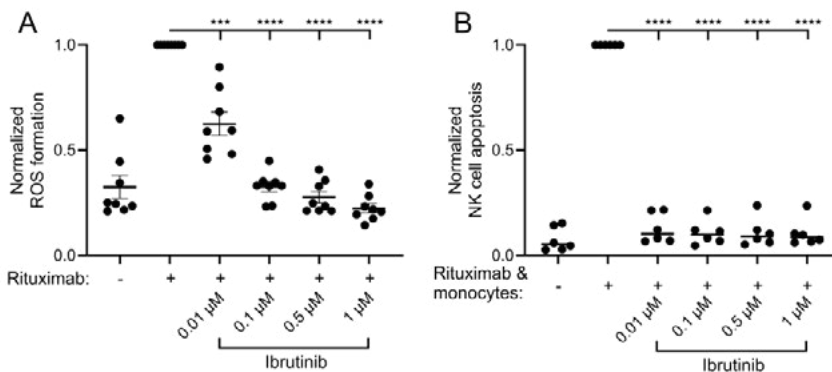


Figure 17. The BTKi ibrutinib reduces IgG-induced ROS formation and rescues NK cells from monocytic immunosuppression. (A) ROS formation from human PBMCs was measured by chemiluminescence upon stimulation with immobilized IgG (Rituximab). (B) NK cell apoptosis in the presence of stimulated monocytes was determined by flow cytometry. Statistics by ANOVA with Holm-Šidák's multiple comparisons test. ***P<0.001, ****P<0.0001.

BTKi-mediated inhibition of NOX2 also enhanced NK cell elimination of malignant cells in the presence of immunosuppressive myeloid cells *in vitro* and *in vivo* (Figure 18). *In vitro*, a similar effect was achieved with the NOX2 inhibitor GSK-2795039 (GSK) and the ROS neutralizer catalase, suggesting an effect dependent on NOX2-derived ROS (Figure 18A). Furthermore, the anti-metastatic effect of BTK inhibition was seemingly dependent on NK cells and NOX2, as ibrutinib lacked therapeutic efficacy in mice depleted of NK cells or lacking functional NOX2 (Figure 18B). These results thus reveal a potential novel anti-neoplastic effect of BTKi that relies on immune regulation rather than direct targeting of malignant cells. In line with our findings, previous studies have demonstrated that granulocytes from patients treated with BTKi generate less ROS in response to stimulation with bacteria and fungi (175-178). Reduced NOX2 activity may additionally explain the increased risk of severe bacterial and fungal infections observed in patients receiving BTKi (179).

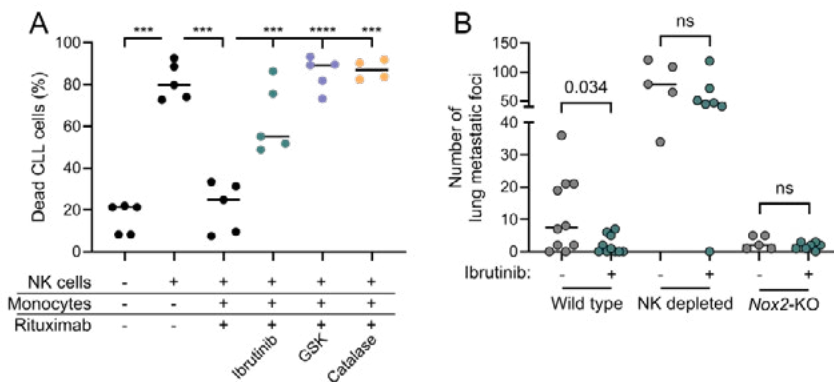


Figure 18. The BTKi ibrutinib reduces myeloid immunosuppression to enhance NK cell elimination of malignant cells *in vitro* and *in vivo*. (A) Elimination of CLL cells in co-culture with NK cells and activated monocytes was determined by flow cytometry. (B) Lung metastatic foci were counted after inoculating mice with B16F10 melanoma cells. Statistics by (A) mixed-effects model with Holm-Šidák’s multiple comparisons test or (B) Mann-Whitney U-test. ***P<0.001, ****P<0.0001.

BTKi have been studied in both hematological malignancies and solid tumors aiming at remodeling myeloid-derived suppressor cells to reduce immunosuppression. These studies demonstrated that BTKi improved tumor clearance in experimental models, particularly in combination with therapy targeting the Programmed cell death protein 1/Programmed cell death ligand 1 immune checkpoint axis (163,168-171), although the exact mechanism

promoting tumor clearance has not been fully elucidated. Our results suggest that BTKi may improve tumor clearance in malignancies where NOX2-induced immunosuppression has been demonstrated. The potential efficacy of BTKi has also been assessed in clinical trials of other inflammatory pathologies. The BTKi tolebrutinib caused a reduction of lesions related to long-term progression in a phase 2 trial of MS (209). In two recent press releases, tolebrutinib was claimed to delay disease progression in SPMS patients in a phase 3 clinical trial (Sanofi, 2024-09-02 and 2024-09-20) (210,211). The mechanism behind the clinical benefit has not been revealed, but may include targeting of inflammatory microglia as these cells are thought to contribute to long-term progression in MS.

CONCLUDING REMARKS

Neurodegenerative diseases lack effective therapies, resulting in an immense burden for the patient, their loved ones, and for society. Despite extensive research, the exact mechanisms that propagate progressive neuronal death have remained elusive. We have demonstrated that patients carrying a *CYBA* genotype entailing reduced capacity to form NOX2-derived ROS were more likely to experience benign disease and/or delayed progression in three neuroinflammatory and neurodegenerative diseases: MS, GBS, and PD. A growing body of research implies a pathological role of NOX2 in models of multiple neurodegenerative diseases. Previous studies also report signs of oxidative damage and aberrant NOX2 activation at sites of neurodegeneration in afflicted patients.

Considering this evidence, we propose that myeloid NOX2 activation under chronic inflammatory conditions might constitute a generic mechanism inflicting oxidative damage upon neurons to promote neurodegeneration. We hypothesize that the initiating factor and localization vary between neurodegenerative diseases, but continued neurodegeneration may be partly propagated by NOX2-derived ROS in all settings. NOX2 activation could be triggered by DAMPs released from injured neurons, PAMPs released from virus-infected cells, or protein aggregates formed in the extracellular space or released from dying neurons. In this environment, NOX2 activation may create a feed-forward mechanism by causing additional oxidative damage to neurons, which leads to further release of DAMPs and oxidation of aggregation-prone proteins.

Notably, there are limitations to this hypothesis, including that results achieved in experimental models might not reflect human disease and that the association between *CYBA* genotypes and neurodegeneration does not prove that ROS are neurotoxic mediators. Further, we cannot exclude the participation by other NOX isoforms for the observed impact on the course of neurodegenerative diseases.

With these reservations, it is proposed that release of NOX2-derived ROS may cause neuronal death in neuroinflammatory and neurodegenerative diseases, as evidenced by associations between *CYBA* SNP alleles and clinical parameters reflecting neuronal death in MS, GBS, and PD. Inhibitors of NOX2, including BTKi, should be further evaluated to halt or reduce neurodegeneration.

FUTURE PERSPECTIVES

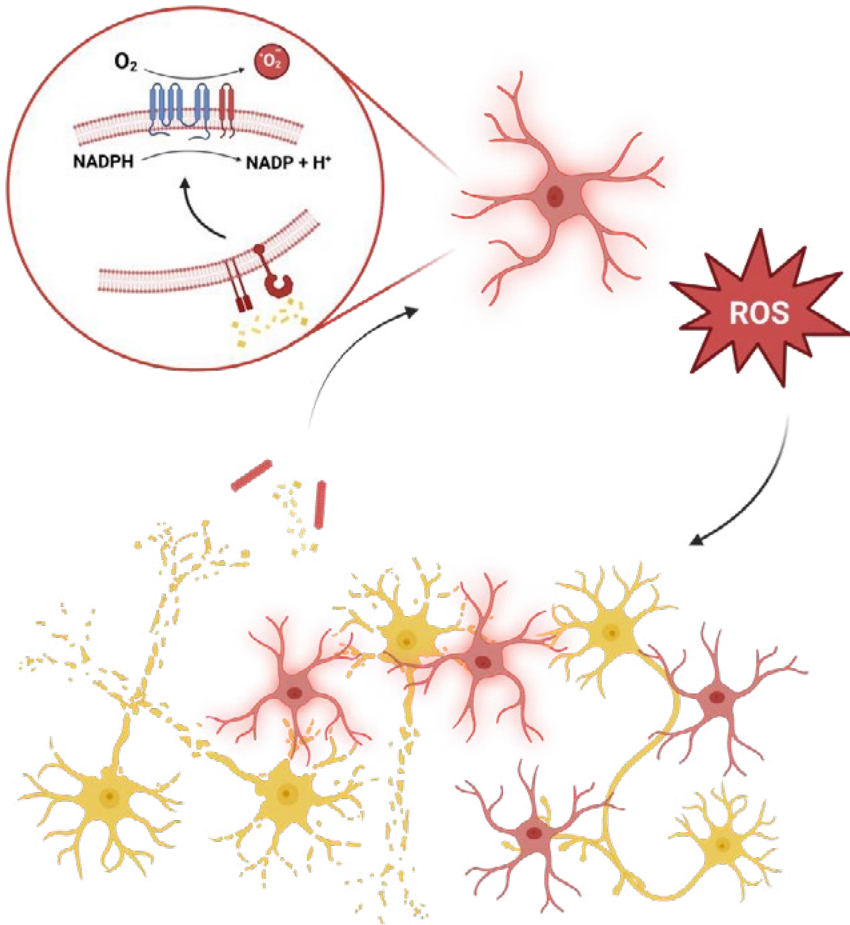
The findings presented in this thesis pose several new and exciting questions. The observed impact of *CYBA* genotypes on outcomes in MS, GBS, and PD can be considered to partly support the accuracy of the findings. Nonetheless, validation in independent cohorts within each disease is necessary to cement the associations. A key strength of the present studies is the long and detailed patient follow-up. Such longitudinal datasets are uncommon and highlight the importance of collecting long-term clinical data in slowly progressing diseases, a practice that should be more frequently implemented.

In addition to replicating the clinical observations, the triggers of NOX2 activation in patients suffering from neurodegenerative diseases should be further elucidated. Potential contributors include DAMPs, PAMPs, aggregated proteins, and other yet to be identified factors. For example, aggregated α -synuclein has been suggested to induce microglial NOX2 activation. However, the molecular pathways underlying α -synuclein-induced NOX2 activation, as well as its contribution to neurodegeneration *in vivo*, remain to be fully characterized.

Our demonstration of the impact of BTKi on NOX2-derived ROS formation, along with their clinical efficacy in MS, provides a rationale for evaluating BTKi in additional neurodegenerative diseases. Their long-standing clinical use supports their safety, and the therapeutic efficacy in MS suggests that sufficient CNS concentrations are achievable. Selectively limiting NOX2 activity in response to inflammatory stimuli, rather than broadly inhibiting NOX2, may preserve physiological ROS signaling pathways that are not directly implicated in disease pathology. The striking impact of the relatively minor differences in ROS formation caused by differences in *CYBA* genotype suggests that complete NOX2 inhibition may not be necessary to achieve meaningful neuroprotection. Retaining some degree of NOX2 activity might be preferable to promote defense against infection, especially in neurodegenerative diseases that mainly affect older people who are more susceptible to severe infections. Future clinical trials of BTKi in neurodegenerative diseases may provide critical evidence of a potential causative role of NOX2 in neurodegeneration.

Immunotherapy holds a great promise in the treatment of cancer. However, immunosuppression by both cancer cells and host cells often limits the full

anti-tumor potential of the immune system. NOX2 is a well-established mediator of immunosuppression by myeloid cells. Therefore, the observed NOX2-inhibitory effect of BTKi suggests that these agents could provide therapeutic benefits by reducing immunosuppression, in particular in combination with therapies that enhance anti-tumor immunity.



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REFERENCES

1. Chaplin DD. Overview of the immune response. *J Allergy Clin Immunol* **2010**;125(2 Suppl 2):S3-23 doi 10.1016/j.jaci.2009.12.980.
2. Bournazos S, Gupta A, Ravetch JV. The role of IgG Fc receptors in antibody-dependent enhancement. *Nat Rev Immunol* **2020**;20(10):633-43 doi 10.1038/s41577-020-00410-0.
3. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol* **2014**;5:461 doi 10.3389/fimmu.2014.00461.
4. He HQ, Ye RD. The Formyl Peptide Receptors: Diversity of Ligands and Mechanism for Recognition. *Molecules* **2017**;22(3) doi 10.3390/molecules22030455.
5. Weiss E, Kretschmer D. Formyl-Peptide Receptors in Infection, Inflammation, and Cancer. *Trends Immunol* **2018**;39(10):815-29 doi 10.1016/j.it.2018.08.005.
6. Lind S, Dahlgren C, Holmdahl R, Olofsson P, Forsman H. Functional selective FPR1 signaling in favor of an activation of the neutrophil superoxide generating NOX2 complex. *J Leukoc Biol* **2021**;109(6):1105-20 doi 10.1002/JLB.2HI0520-317R.
7. Lee C, Han J, Jung Y. Formyl peptide receptor 2 is an emerging modulator of inflammation in the liver. *Exp Mol Med* **2023**;55(2):325-32 doi 10.1038/s12276-023-00941-1.
8. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol* **2017**;17(6):349-62 doi 10.1038/nri.2017.28.
9. Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, *et al.* Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood* **2011**;118(5):e16-31 doi 10.1182/blood-2010-12-326355.
10. Dash SP, Gupta S, Sarangi PP. Monocytes and macrophages: Origin, homing, differentiation, and functionality during inflammation. *Heliyon* **2024**;10(8):e29686 doi 10.1016/j.heliyon.2024.e29686.
11. Pittet MJ, Michielin O, Migliorini D. Clinical relevance of tumour-associated macrophages. *Nat Rev Clin Oncol* **2022**;19(6):402-21 doi 10.1038/s41571-022-00620-6.

12. Burn GL, Foti A, Marsman G, Patel DF, Zychlinsky A. The Neutrophil. *Immunity* **2021**;54(7):1377-91 doi 10.1016/j.immuni.2021.06.006.
13. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* **2009**;9(3):162-74 doi 10.1038/nri2506.
14. Wolf NK, Kissiov DU, Raulet DH. Roles of natural killer cells in immunity to cancer, and applications to immunotherapy. *Nat Rev Immunol* **2023**;23(2):90-105 doi 10.1038/s41577-022-00732-1.
15. Cyster JG, Allen CDC. B Cell Responses: Cell Interaction Dynamics and Decisions. *Cell* **2019**;177(3):524-40 doi 10.1016/j.cell.2019.03.016.
16. Goillard JM, Moubarak E, Tapia M, Tell F. Diversity of Axonal and Dendritic Contributions to Neuronal Output. *Front Cell Neurosci* **2019**;13:570 doi 10.3389/fncel.2019.00570.
17. Kress GJ, Mennerick S. Action potential initiation and propagation: upstream influences on neurotransmission. *Neuroscience* **2009**;158(1):211-22 doi 10.1016/j.neuroscience.2008.03.021.
18. Sudhof TC, Malenka RC. Understanding synapses: past, present, and future. *Neuron* **2008**;60(3):469-76 doi 10.1016/j.neuron.2008.10.011.
19. Vaughn MJ, Haas JS. On the Diverse Functions of Electrical Synapses. *Front Cell Neurosci* **2022**;16:910015 doi 10.3389/fncel.2022.910015.
20. Taveggia C, Feltri ML. Beyond Wrapping: Canonical and Noncanonical Functions of Schwann Cells. *Annu Rev Neurosci* **2022**;45:561-80 doi 10.1146/annurev-neuro-110920-030610.
21. Kuhn S, Gritti L, Crooks D, Dombrowski Y. Oligodendrocytes in Development, Myelin Generation and Beyond. *Cells* **2019**;8(11) doi 10.3390/cells8111424.
22. Gao C, Jiang J, Tan Y, Chen S. Microglia in neurodegenerative diseases: mechanism and potential therapeutic targets. *Signal Transduct Target Ther* **2023**;8(1):359 doi 10.1038/s41392-023-01588-0.
23. Jurga AM, Paleczna M, Kadluczka J, Kuter KZ. Beyond the GFAP-Astrocyte Protein Markers in the Brain. *Biomolecules* **2021**;11(9) doi 10.3390/biom11091361.

24. Lee HG, Wheeler MA, Quintana FJ. Function and therapeutic value of astrocytes in neurological diseases. *Nat Rev Drug Discov* **2022**;21(5):339-58 doi 10.1038/s41573-022-00390-x.
25. Jakimovski D, Bittner S, Zivadinov R, Morrow SA, Benedict RH, Zipp F, Weinstock-Guttman B. Multiple sclerosis. *Lancet* **2024**;403(10422):183-202 doi 10.1016/S0140-6736(23)01473-3.
26. Magyari M, Koch-Henriksen N. Quantitative effect of sex on disease activity and disability accumulation in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **2022**;93(7):716-22 doi 10.1136/jnnp-2022-328994.
27. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, *et al.* Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* **2018**;17(2):162-73 doi 10.1016/S1474-4422(17)30470-2.
28. Pakpoor J, Disanto G, Gerber JE, Dobson R, Meier UC, Giovannoni G, Ramagopalan SV. The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. *Mult Scler* **2013**;19(2):162-6 doi 10.1177/1352458512449682.
29. Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, *et al.* Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science* **2022**;375(6578):296-301 doi 10.1126/science.abj8222.
30. Lanz TV, Brewer RC, Ho PP, Moon JS, Jude KM, Fernandez D, *et al.* Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. *Nature* **2022**;603(7900):321-7 doi 10.1038/s41586-022-04432-7.
31. Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, *et al.* Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. *Brain* **2009**;132(Pt 12):3318-28 doi 10.1093/brain/awp200.
32. Yang JH, Remppe T, Whitmire N, Dunn-Pirio A, Graves JS. Therapeutic Advances in Multiple Sclerosis. *Front Neurol* **2022**;13:824926 doi 10.3389/fneur.2022.824926.
33. Leonhard SE, Papri N, Querol L, Rinaldi S, Shahrizaila N, Jacobs BC. Guillain-Barre syndrome. *Nat Rev Dis Primers* **2024**;10(1):97 doi 10.1038/s41572-024-00580-4.

34. Annexon Announces Positive Topline Results from Pivotal Phase 3 Trial for First-in-Class C1q Blocking Antibody ANX005 in Guillain-Barré Syndrome.
35. Annexon Announces Positive Topline Results from Real-World Evidence Study Comparing ANX005 Treatment to Intravenous Immunoglobulin (IVIg) or Plasma Exchange (PE) in a Matched Patient Cohort for the Treatment of Guillain-Barré Syndrome (GBS).
36. 2025 2025-04-07. Parkinson's Foundation - Statistics. <<https://www.parkinson.org/understanding-parkinsons/statistics#:~:text=Who%20has%20Parkinson%27s%3F%201%20Nearly%20one%20million%20people,PD%20are%20diagnosed%20before%20age%2050.%20More%20items>>. Accessed 2025-04-07.
37. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkman J, *et al.* Parkinson disease. *Nat Rev Dis Primers* **2017**;3:17013 doi 10.1038/nrdp.2017.13.
38. Stocchi F, Bravi D, Emmi A, Antonini A. Parkinson disease therapy: current strategies and future research priorities. *Nat Rev Neurol* **2024**;20(12):695-707 doi 10.1038/s41582-024-01034-x.
39. Bloem BR, Okun MS, Klein C. Parkinson's disease. *Lancet* **2021**;397(10291):2284-303 doi 10.1016/S0140-6736(21)00218-X.
40. Boyer DR, Li B, Sun C, Fan W, Zhou K, Hughes MP, *et al.* The alpha-synuclein hereditary mutation E46K unlocks a more stable, pathogenic fibril structure. *Proc Natl Acad Sci U S A* **2020**;117(7):3592-602 doi 10.1073/pnas.1917914117.
41. Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, *et al.* The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol* **2004**;55(2):164-73 doi 10.1002/ana.10795.
42. Zhao K, Li Y, Liu Z, Long H, Zhao C, Luo F, *et al.* Parkinson's disease associated mutation E46K of alpha-synuclein triggers the formation of a distinct fibril structure. *Nat Commun* **2020**;11(1):2643 doi 10.1038/s41467-020-16386-3.
43. Sun Y, Hou S, Zhao K, Long H, Liu Z, Gao J, *et al.* Cryo-EM structure of full-length alpha-synuclein amyloid fibril with Parkinson's disease familial A53T mutation. *Cell Res* **2020**;30(4):360-2 doi 10.1038/s41422-020-0299-4.

44. Doi D, Magotani H, Kikuchi T, Ikeda M, Hiramatsu S, Yoshida K, *et al.* Pre-clinical study of induced pluripotent stem cell-derived dopaminergic progenitor cells for Parkinson's disease. *Nat Commun* **2020**;11(1):3369 doi 10.1038/s41467-020-17165-w.
45. Pagano G, Taylor KI, Anzures-Cabrera J, Marchesi M, Simuni T, Marek K, *et al.* Trial of Prasinezumab in Early-Stage Parkinson's Disease. *N Engl J Med* **2022**;387(5):421-32 doi 10.1056/NEJMoa2202867.
46. Ma MW, Wang J, Zhang Q, Wang R, Dhandapani KM, Vadlamudi RK, Brann DW. NADPH oxidase in brain injury and neurodegenerative disorders. *Mol Neurodegener* **2017**;12(1):7 doi 10.1186/s13024-017-0150-7.
47. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* **2014**;20(7):1126-67 doi 10.1089/ars.2012.5149.
48. Cipriano A, Viviano M, Feoli A, Milite C, Sarno G, Castellano S, Sbardella G. NADPH Oxidases: From Molecular Mechanisms to Current Inhibitors. *J Med Chem* **2023**;66(17):11632-55 doi 10.1021/acs.jmedchem.3c00770.
49. Yu HH, Yang YH, Chiang BL. Chronic Granulomatous Disease: a Comprehensive Review. *Clin Rev Allergy Immunol* **2021**;61(2):101-13 doi 10.1007/s12016-020-08800-x.
50. Hoyal CR, Gutierrez A, Young BM, Catz SD, Lin JH, Tschlis PN, Babior BM. Modulation of p47PHOX activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. *Proc Natl Acad Sci U S A* **2003**;100(9):5130-5 doi 10.1073/pnas.1031526100.
51. El Benna J, Faust RP, Johnson JL, Babior BM. Phosphorylation of the respiratory burst oxidase subunit p47phox as determined by two-dimensional phosphopeptide mapping. Phosphorylation by protein kinase C, protein kinase A, and a mitogen-activated protein kinase. *J Biol Chem* **1996**;271(11):6374-8 doi 10.1074/jbc.271.11.6374.
52. Zhu Y, Marchal CC, Casbon AJ, Stull N, von Lohneysen K, Knaus UG, *et al.* Deletion mutagenesis of p22phox subunit of flavocytochrome b558: identification of regions critical for gp91phox maturation and NADPH oxidase activity. *J Biol Chem* **2006**;281(41):30336-46 doi 10.1074/jbc.M607191200.

53. Noreng S, Ota N, Sun Y, Ho H, Johnson M, Arthur CP, *et al.* Structure of the core human NADPH oxidase NOX2. *Nat Commun* **2022**;13(1):6079 doi 10.1038/s41467-022-33711-0.
54. Wyche KE, Wang SS, Griendling KK, Dikalov SI, Austin H, Rao S, *et al.* C242T CYBA polymorphism of the NADPH oxidase is associated with reduced respiratory burst in human neutrophils. *Hypertension* **2004**;43(6):1246-51 doi 10.1161/01.HYP.0000126579.50711.62.
55. Schirmer M, Hoffmann M, Kaya E, Tzvetkov M, Brockmoller J. Genetic polymorphisms of NAD(P)H oxidase: variation in subunit expression and enzyme activity. *Pharmacogenomics J* **2008**;8(4):297-304 doi 10.1038/sj.tpj.6500467.
56. Meijles DN, Fan LM, Ghazaly MM, Howlin B, Kronke M, Brooks G, Li JM. p22phox C242T Single-Nucleotide Polymorphism Inhibits Inflammatory Oxidative Damage to Endothelial Cells and Vessels. *Circulation* **2016**;133(24):2391-403 doi 10.1161/CIRCULATIONAHA.116.021993.
57. Shimo-Nakanishi Y, Hasebe T, Suzuki A, Mochizuki H, Nomiyama T, Tanaka Y, *et al.* Functional effects of NAD(P)H oxidase p22(phox) C242T mutation in human leukocytes and association with thrombotic cerebral infarction. *Atherosclerosis* **2004**;175(1):109-15 doi 10.1016/j.atherosclerosis.2004.01.043.
58. Bedard K, Attar H, Bonnefont J, Jaquet V, Borel C, Plastre O, *et al.* Three common polymorphisms in the CYBA gene form a haplotype associated with decreased ROS generation. *Hum Mutat* **2009**;30(7):1123-33 doi 10.1002/humu.21029.
59. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. *Molecules* **2019**;24(8) doi 10.3390/molecules24081583.
60. Jurcau MC, Andronie-Cioara FL, Jurcau A, Marcu F, Tit DM, Pascalau N, Nistor-Cseppento DC. The Link between Oxidative Stress, Mitochondrial Dysfunction and Neuroinflammation in the Pathophysiology of Alzheimer's Disease: Therapeutic Implications and Future Perspectives. *Antioxidants (Basel)* **2022**;11(11) doi 10.3390/antiox11112167.
61. Levi S, Ripamonti M, Moro AS, Cozzi A. Iron imbalance in neurodegeneration. *Mol Psychiatry* **2024**;29(4):1139-52 doi 10.1038/s41380-023-02399-z.

62. Zhang W, Xiao D, Mao Q, Xia H. Role of neuroinflammation in neurodegeneration development. *Signal Transduct Target Ther* **2023**;8(1):267 doi 10.1038/s41392-023-01486-5.
63. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* **2015**;15(9):545-58 doi 10.1038/nri3871.
64. Absinta M, Sati P, Masuzzo F, Nair G, Sethi V, Kolb H, *et al.* Association of Chronic Active Multiple Sclerosis Lesions With Disability In Vivo. *JAMA Neurol* **2019**;76(12):1474-83 doi 10.1001/jamaneurol.2019.2399.
65. Calvi A, Haider L, Prados F, Tur C, Chard D, Barkhof F. In vivo imaging of chronic active lesions in multiple sclerosis. *Mult Scler* **2022**;28(5):683-90 doi 10.1177/1352458520958589.
66. Fischer MT, Sharma R, Lim JL, Haider L, Frischer JM, Drexhage J, *et al.* NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. *Brain* **2012**;135(Pt 3):886-99 doi 10.1093/brain/aws012.
67. van Horssen J, Schreibelt G, Drexhage J, Hazes T, Dijkstra CD, van der Valk P, de Vries HE. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. *Free Radic Biol Med* **2008**;45(12):1729-37 doi 10.1016/j.freeradbiomed.2008.09.023.
68. Haider L, Fischer MT, Frischer JM, Bauer J, Hoftberger R, Botond G, *et al.* Oxidative damage in multiple sclerosis lesions. *Brain* **2011**;134(Pt 7):1914-24 doi 10.1093/brain/awr128.
69. Li J, Baud O, Vartanian T, Volpe JJ, Rosenberg PA. Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. *Proc Natl Acad Sci U S A* **2005**;102(28):9936-41 doi 10.1073/pnas.0502552102.
70. Ravelli KG, Santos GD, Dos Santos NB, Munhoz CD, Azzi-Nogueira D, Campos AC, *et al.* Nox2-dependent Neuroinflammation in An EAE Model of Multiple Sclerosis. *Transl Neurosci* **2019**;10:1-9 doi 10.1515/tnsci-2019-0001.
71. Hu CF, Wu SP, Lin GJ, Shieh CC, Hsu CS, Chen JW, *et al.* Microglial Nox2 Plays a Key Role in the Pathogenesis of Experimental Autoimmune Encephalomyelitis. *Front Immunol* **2021**;12:638381 doi 10.3389/fimmu.2021.638381.

72. Asbury AK, Arnason BG, Adams RD. The inflammatory lesion in idiopathic polyneuritis. Its role in pathogenesis. *Medicine (Baltimore)* **1969**;48(3):173-215 doi 10.1097/00005792-196905000-00001.
73. Notturmo F, Luciani M, Caporale CM, Ciarelli A, Uncini A. Antibodies to ganglioside complexes in Guillain-Barre syndrome: clinical correlates, fine specificity and complement activation. *Int J Immunopathol Pharmacol* **2009**;22(2):437-45 doi 10.1177/039463200902200220.
74. Schmidt B, Toyka KV, Kiefer R, Full J, Hartung HP, Pollard J. Inflammatory infiltrates in sural nerve biopsies in Guillain-Barre syndrome and chronic inflammatory demyelinating neuropathy. *Muscle Nerve* **1996**;19(4):474-87 doi 10.1002/(SICI)1097-4598(199604)19:4<474::AID-MUS8>3.0.CO;2-9.
75. Sukenikova L, Mallone A, Schreiner B, Ripellino P, Nilsson J, Stoffel M, *et al.* Autoreactive T cells target peripheral nerves in Guillain-Barre syndrome. *Nature* **2024**;626(7997):160-8 doi 10.1038/s41586-023-06916-6.
76. Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, *et al.* Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. *J Neurosci* **2007**;27(15):3956-67 doi 10.1523/JNEUROSCI.4401-06.2007.
77. Susuki K, Yuki N, Schafer DP, Hirata K, Zhang G, Funakoshi K, Rasband MN. Dysfunction of nodes of Ranvier: a mechanism for anti-ganglioside antibody-mediated neuropathies. *Exp Neurol* **2012**;233(1):534-42 doi 10.1016/j.expneurol.2011.11.039.
78. Vriesendorp FJ, Flynn RE, Pappolla MA, Koski CL. Complement depletion affects demyelination and inflammation in experimental allergic neuritis. *J Neuroimmunol* **1995**;58(2):157-65 doi 10.1016/0165-5728(95)00006-n.
79. Hafer-Macko CE, Sheikh KA, Li CY, Ho TW, Cornblath DR, McKhann GM, *et al.* Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann Neurol* **1996**;39(5):625-35 doi 10.1002/ana.410390512.
80. Min YG, Ju W, Seo JW, Ha YE, Ban JJ, Kwon YN, *et al.* Serum C3 complement levels predict prognosis and monitor disease activity in Guillain-Barre syndrome. *J Neurol Sci* **2023**;444:120512 doi 10.1016/j.jns.2022.120512.

81. Griffin JW, Li CY, Ho TW, Xue P, Macko C, Gao CY, *et al.* Guillain-Barre syndrome in northern China. The spectrum of neuropathological changes in clinically defined cases. *Brain* **1995**;118 (Pt 3):577-95 doi 10.1093/brain/118.3.577.
82. Kiefer R, Kieseier BC, Stoll G, Hartung HP. The role of macrophages in immune-mediated damage to the peripheral nervous system. *Prog Neurobiol* **2001**;64(2):109-27 doi 10.1016/s0301-0082(00)00060-5.
83. Han R, Xiao J, Zhai H, Hao J. Dimethyl fumarate attenuates experimental autoimmune neuritis through the nuclear factor erythroid-derived 2-related factor 2/hemoxygenase-1 pathway by altering the balance of M1/M2 macrophages. *J Neuroinflammation* **2016**;13(1):97 doi 10.1186/s12974-016-0559-x.
84. Geleijns K, Emonts M, Laman JD, van Rijs W, van Doorn PA, Hermans PW, Jacobs BC. Genetic polymorphisms of macrophage-mediators in Guillain-Barre syndrome. *J Neuroimmunol* **2007**;190(1-2):127-30 doi 10.1016/j.jneuroim.2007.07.008.
85. Hayat S, Ahmad O, Mahmud I, Howlader MZH, Islam Z. Association of matrix metalloproteinase-9 polymorphism with severity of Guillain-Barre syndrome. *J Neurol Sci* **2020**;415:116908 doi 10.1016/j.jns.2020.116908.
86. Akhiani AA, Hallner A, Kiffin R, Aydin E, Werlenius O, Aurelius J, *et al.* Idelalisib Rescues Natural Killer Cells from Monocyte-Induced Immunosuppression by Inhibiting NOX2-Derived Reactive Oxygen Species. *Cancer Immunol Res* **2020**;8(12):1532-41 doi 10.1158/2326-6066.CIR-20-0055.
87. Gumusyayla S, Vural G, Yurtogullari Cevik S, Akdeniz G, Neselioglu S, Deniz O, Erel O. Dynamic thiol-disulphide homeostasis in patients with Guillain-Barre Syndrome. *Neurol Res* **2019**;41(5):413-8 doi 10.1080/01616412.2019.1573955.
88. Kumar KT, Chandrika A, Sumanth KN, Sireesha P, Rao S, Rao A. Free radical toxicity and antioxidants in Guillain-Barre syndrome, a preliminary study. *Clin Chim Acta* **2004**;346(2):205-9 doi 10.1016/j.cccn.2004.03.032.
89. Tang HY, Ho HY, Chiu DT, Huang CY, Cheng ML, Chen CM. Alterations of plasma concentrations of lipophilic antioxidants are associated with Guillain-Barre syndrome. *Clin Chim Acta* **2017**;470:75-80 doi 10.1016/j.cca.2017.05.001.

90. Chang SH, Tian XB, Wang J, Liu MQ, Huang CN, Qi Y, *et al.* Increased Cerebrospinal Fluid Uric Acid Levels in Guillain-Barre Syndrome. *Front Neurol* **2020**;11:589928 doi 10.3389/fneur.2020.589928.
91. Eid SA, Savelieff MG, Eid AA, Feldman EL. Nox, Nox, Are You There? The Role of NADPH Oxidases in the Peripheral Nervous System. *Antioxid Redox Signal* **2022**;37(7-9):613-30 doi 10.1089/ars.2021.0135.
92. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* **1988**;38(8):1285-91 doi 10.1212/wnl.38.8.1285.
93. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. *Nat Rev Immunol* **2022**;22(11):657-73 doi 10.1038/s41577-022-00684-6.
94. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthet V, *et al.* Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest* **2009**;119(1):182-92 doi 10.1172/JCI36470.
95. Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L, de Bernard M. Triggering of inflammasome by aggregated alpha-synuclein, an inflammatory response in synucleinopathies. *PLoS One* **2013**;8(1):e55375 doi 10.1371/journal.pone.0055375.
96. Grozdanov V, Bliederaeuser C, Ruf WP, Roth V, Fundel-Clemens K, Zondler L, *et al.* Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. *Acta Neuropathol* **2014**;128(5):651-63 doi 10.1007/s00401-014-1345-4.
97. Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol* **2003**;106(6):518-26 doi 10.1007/s00401-003-0766-2.
98. Choi I, Zhang Y, Seegobin SP, Pruvost M, Wang Q, Purtell K, *et al.* Microglia clear neuron-released alpha-synuclein via selective autophagy and prevent neurodegeneration. *Nat Commun* **2020**;11(1):1386 doi 10.1038/s41467-020-15119-w.

99. Pranski EL, Dalal NV, Sanford CV, Herskowitz JH, Gearing M, Lazo C, *et al.* RING finger protein 11 (RNF11) modulates susceptibility to 6-OHDA-induced nigral degeneration and behavioral deficits through NF-kappaB signaling in dopaminergic cells. *Neurobiol Dis* **2013**;54:264-79 doi 10.1016/j.nbd.2012.12.018.
100. Grozdanov V, Bousset L, Hoffmeister M, Bliederhaeuser C, Meier C, Madiona K, *et al.* Increased Immune Activation by Pathologic alpha-Synuclein in Parkinson's Disease. *Ann Neurol* **2019**;86(4):593-606 doi 10.1002/ana.25557.
101. Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, *et al.* Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. *Glia* **2013**;61(3):349-60 doi 10.1002/glia.22437.
102. Kim C, Ho DH, Suk JE, You S, Michael S, Kang J, *et al.* Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. *Nat Commun* **2013**;4:1562 doi 10.1038/ncomms2534.
103. Daniele SG, Beraud D, Davenport C, Cheng K, Yin H, Maguire-Zeiss KA. Activation of MyD88-dependent TLR1/2 signaling by misfolded alpha-synuclein, a protein linked to neurodegenerative disorders. *Sci Signal* **2015**;8(376):ra45 doi 10.1126/scisignal.2005965.
104. Hou L, Bao X, Zang C, Yang H, Sun F, Che Y, *et al.* Integrin CD11b mediates alpha-synuclein-induced activation of NADPH oxidase through a Rho-dependent pathway. *Redox Biol* **2018**;14:600-8 doi 10.1016/j.redox.2017.11.010.
105. Jiang T, Hoekstra J, Heng X, Kang W, Ding J, Liu J, *et al.* P2X7 receptor is critical in alpha-synuclein--mediated microglial NADPH oxidase activation. *Neurobiol Aging* **2015**;36(7):2304-18 doi 10.1016/j.neurobiolaging.2015.03.015.
106. Keeney MT, Rocha EM, Hoffman EK, Farmer K, Di Maio R, Weir J, *et al.* LRRK2 regulates production of reactive oxygen species in cell and animal models of Parkinson's disease. *Sci Transl Med* **2024**;16(767):eadl3438 doi 10.1126/scitranslmed.adl3438.
107. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, *et al.* Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J* **2005**;19(6):533-42 doi 10.1096/fj.04-2751com.

108. Sanders LH, Timothy Greenamyre J. Oxidative damage to macromolecules in human Parkinson disease and the rotenone model. *Free Radic Biol Med* **2013**;62:111-20 doi 10.1016/j.freeradbiomed.2013.01.003.
109. Bae EJ, Ho DH, Park E, Jung JW, Cho K, Hong JH, *et al.* Lipid peroxidation product 4-hydroxy-2-nonenal promotes seeding-capable oligomer formation and cell-to-cell transfer of alpha-synuclein. *Antioxid Redox Signal* **2013**;18(7):770-83 doi 10.1089/ars.2011.4429.
110. Hou L, Zhang C, Wang K, Liu X, Wang H, Che Y, *et al.* Paraquat and maneb co-exposure induces noradrenergic locus coeruleus neurodegeneration through NADPH oxidase-mediated microglial activation. *Toxicology* **2017**;380:1-10 doi 10.1016/j.tox.2017.02.009.
111. Tu D, Velagapudi R, Gao Y, Hong JS, Zhou H, Gao HM. Activation of neuronal NADPH oxidase NOX2 promotes inflammatory neurodegeneration. *Free Radic Biol Med* **2023**;200:47-58 doi 10.1016/j.freeradbiomed.2023.03.001.
112. Keeney MT, Hoffman EK, Farmer K, Bodle CR, Fazzari M, Zharikov A, *et al.* NADPH oxidase 2 activity in Parkinson's disease. *Neurobiol Dis* **2022**;170:105754 doi 10.1016/j.nbd.2022.105754.
113. Hernandez MS, Cafe-Mendes CC, Britto LR. NADPH oxidase and the degeneration of dopaminergic neurons in parkinsonian mice. *Oxid Med Cell Longev* **2013**;2013:157857 doi 10.1155/2013/157857.
114. Liu Y, Qin L, Wilson B, Wu X, Qian L, Granholm AC, *et al.* Endotoxin induces a delayed loss of TH-IR neurons in substantia nigra and motor behavioral deficits. *Neurotoxicology* **2008**;29(5):864-70 doi 10.1016/j.neuro.2008.02.014.
115. Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, *et al.* Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* **2007**;55(5):453-62 doi 10.1002/glia.20467.
116. Wang Q, Qian L, Chen SH, Chu CH, Wilson B, Oyarzabal E, *et al.* Post-treatment with an ultra-low dose of NADPH oxidase inhibitor diphenyleneiodonium attenuates disease progression in multiple Parkinson's disease models. *Brain* **2015**;138(Pt 5):1247-62 doi 10.1093/brain/awv034.
117. Qin L, Liu Y, Hong JS, Crews FT. NADPH oxidase and aging drive microglial activation, oxidative stress, and dopaminergic

- neurodegeneration following systemic LPS administration. *Glia* **2013**;61(6):855-68 doi 10.1002/glia.22479.
118. Fiadeiro MB, Diogo JC, Silva AA, Kim YS, Cristovao AC. NADPH Oxidases in Neurodegenerative Disorders: Mechanisms and Therapeutic Opportunities. *Antioxid Redox Signal* **2024**;41(7-9):522-41 doi 10.1089/ars.2023.0002.
 119. Zhang QG, Raz L, Wang R, Han D, De Sevilla L, Yang F, *et al.* Estrogen attenuates ischemic oxidative damage via an estrogen receptor alpha-mediated inhibition of NADPH oxidase activation. *J Neurosci* **2009**;29(44):13823-36 doi 10.1523/JNEUROSCI.3574-09.2009.
 120. Yoshioka H, Niizuma K, Katsu M, Okami N, Sakata H, Kim GS, *et al.* NADPH oxidase mediates striatal neuronal injury after transient global cerebral ischemia. *J Cereb Blood Flow Metab* **2011**;31(3):868-80 doi 10.1038/jcbfm.2010.166.
 121. Jackman KA, Miller AA, De Silva TM, Crack PJ, Drummond GR, Sobey CG. Reduction of cerebral infarct volume by apocynin requires pretreatment and is absent in Nox2-deficient mice. *Br J Pharmacol* **2009**;156(4):680-8 doi 10.1111/j.1476-5381.2008.00073.x.
 122. Tang XN, Zheng Z, Giffard RG, Yenari MA. Significance of marrow-derived nicotinamide adenine dinucleotide phosphate oxidase in experimental ischemic stroke. *Ann Neurol* **2011**;70(4):606-15 doi 10.1002/ana.22476.
 123. Cooney SJ, Bermudez-Sabogal SL, Byrnes KR. Cellular and temporal expression of NADPH oxidase (NOX) isotypes after brain injury. *J Neuroinflammation* **2013**;10:155 doi 10.1186/1742-2094-10-155.
 124. Zhang QG, Laird MD, Han D, Nguyen K, Scott E, Dong Y, *et al.* Critical role of NADPH oxidase in neuronal oxidative damage and microglia activation following traumatic brain injury. *PLoS One* **2012**;7(4):e34504 doi 10.1371/journal.pone.0034504.
 125. Kumar A, Alvarez-Croda DM, Stoica BA, Faden AI, Loane DJ. Microglial/Macrophage Polarization Dynamics following Traumatic Brain Injury. *J Neurotrauma* **2016**;33(19):1732-50 doi 10.1089/neu.2015.4268.
 126. Lu XY, Wang HD, Xu JG, Ding K, Li T. NADPH oxidase inhibition improves neurological outcome in experimental traumatic brain injury. *Neurochem Int* **2014**;69:14-9 doi 10.1016/j.neuint.2014.02.006.

127. Loane DJ, Kumar A, Stoica BA, Cabatbat R, Faden AI. Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation. *J Neuropathol Exp Neurol* **2014**;73(1):14-29 doi 10.1097/NEN.0000000000000021.
128. Dohi K, Ohtaki H, Nakamachi T, Yofu S, Satoh K, Miyamoto K, *et al.* Gp91phox (NOX2) in classically activated microglia exacerbates traumatic brain injury. *J Neuroinflammation* **2010**;7:41 doi 10.1186/1742-2094-7-41.
129. Ansari MA, Scheff SW. NADPH-oxidase activation and cognition in Alzheimer disease progression. *Free Radic Biol Med* **2011**;51(1):171-8 doi 10.1016/j.freeradbiomed.2011.03.025.
130. Shimohama S, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T, *et al.* Activation of NADPH oxidase in Alzheimer's disease brains. *Biochem Biophys Res Commun* **2000**;273(1):5-9 doi 10.1006/bbrc.2000.2897.
131. Bianca VD, Dusi S, Bianchini E, Dal Pra I, Rossi F. beta-amyloid activates the O-2 forming NADPH oxidase in microglia, monocytes, and neutrophils. A possible inflammatory mechanism of neuronal damage in Alzheimer's disease. *J Biol Chem* **1999**;274(22):15493-9 doi 10.1074/jbc.274.22.15493.
132. Malkov A, Popova I, Ivanov A, Jang SS, Yoon SY, Osypov A, *et al.* Abeta initiates brain hypometabolism, network dysfunction and behavioral abnormalities via NOX2-induced oxidative stress in mice. *Commun Biol* **2021**;4(1):1054 doi 10.1038/s42003-021-02551-x.
133. Park L, Anrather J, Zhou P, Frys K, Pitstick R, Younkin S, *et al.* NADPH-oxidase-derived reactive oxygen species mediate the cerebrovascular dysfunction induced by the amyloid beta peptide. *J Neurosci* **2005**;25(7):1769-77 doi 10.1523/JNEUROSCI.5207-04.2005.
134. Sorolla MA, Reverter-Branchat G, Tamarit J, Ferrer I, Ros J, Cabiscol E. Proteomic and oxidative stress analysis in human brain samples of Huntington disease. *Free Radic Biol Med* **2008**;45(5):667-78 doi 10.1016/j.freeradbiomed.2008.05.014.
135. Klepac N, Relja M, Klepac R, Hecimovic S, Babic T, Trkulja V. Oxidative stress parameters in plasma of Huntington's disease patients, asymptomatic Huntington's disease gene carriers and healthy subjects : a cross-sectional study. *J Neurol* **2007**;254(12):1676-83 doi 10.1007/s00415-007-0611-y.

136. Boll MC, Alcaraz-Zubeldia M, Montes S, Rios C. Free copper, ferroxidase and SOD1 activities, lipid peroxidation and NO(x) content in the CSF. A different marker profile in four neurodegenerative diseases. *Neurochem Res* **2008**;33(9):1717-23 doi 10.1007/s11064-008-9610-3.
137. Ibrahim WW, Abdel Rasheed NO. Diapocynin neuroprotective effects in 3-nitropropionic acid Huntington's disease model in rats: emphasis on Sirt1/Nrf2 signaling pathway. *Inflammopharmacology* **2022**;30(5):1745-58 doi 10.1007/s10787-022-01004-z.
138. Shaw PJ, Ince PG, Falkous G, Mantle D. Oxidative damage to protein in sporadic motor neuron disease spinal cord. *Ann Neurol* **1995**;38(4):691-5 doi 10.1002/ana.410380424.
139. Abe K, Pan LH, Watanabe M, Kato T, Itoyama Y. Induction of nitrotyrosine-like immunoreactivity in the lower motor neuron of amyotrophic lateral sclerosis. *Neurosci Lett* **1995**;199(2):152-4 doi 10.1016/0304-3940(95)12039-7.
140. Shibata N, Nagai R, Uchida K, Horiuchi S, Yamada S, Hirano A, *et al.* Morphological evidence for lipid peroxidation and protein glycooxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. *Brain Res* **2001**;917(1):97-104 doi 10.1016/s0006-8993(01)02926-2.
141. Ferrante RJ, Browne SE, Shinobu LA, Bowling AC, Baik MJ, MacGarvey U, *et al.* Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J Neurochem* **1997**;69(5):2064-74 doi 10.1046/j.1471-4159.1997.69052064.x.
142. Smith RG, Henry YK, Mattson MP, Appel SH. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Ann Neurol* **1998**;44(4):696-9 doi 10.1002/ana.410440419.
143. Wu DC, Re DB, Nagai M, Ischiropoulos H, Przedborski S. The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. *Proc Natl Acad Sci U S A* **2006**;103(32):12132-7 doi 10.1073/pnas.0603670103.
144. Marrali G, Casale F, Salamone P, Fuda G, Caorsi C, Amoroso A, *et al.* NADPH oxidase (NOX2) activity is a modifier of survival in ALS. *J Neurol* **2014**;261(11):2178-83 doi 10.1007/s00415-014-7470-0.
145. Pramatarova A, Laganieri J, Roussel J, Brisebois K, Rouleau GA. Neuron-specific expression of mutant superoxide dismutase 1 in

- transgenic mice does not lead to motor impairment. *J Neurosci* **2001**;21(10):3369-74 doi 10.1523/JNEUROSCI.21-10-03369.2001.
146. Massenzio F, Pena-Altamira E, Petralla S, Virgili M, Zuccheri G, Miti A, *et al.* Microglial overexpression of fALS-linked mutant SOD1 induces SOD1 processing impairment, activation and neurotoxicity and is counteracted by the autophagy inducer trehalose. *Biochim Biophys Acta Mol Basis Dis* **2018**;1864(12):3771-85 doi 10.1016/j.bbadis.2018.10.013.
147. Lino MM, Schneider C, Caroni P. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J Neurosci* **2002**;22(12):4825-32 doi 10.1523/JNEUROSCI.22-12-04825.2002.
148. Cuenca M, Peperzak V. Advances and Perspectives in the Treatment of B-Cell Malignancies. *Cancers (Basel)* **2021**;13(9) doi 10.3390/cancers13092266.
149. Jain N, Wierda WG, O'Brien S. Chronic lymphocytic leukaemia. *Lancet* **2024**;404(10453):694-706 doi 10.1016/S0140-6736(24)00595-6.
150. Arruga F, Gyau BB, Iannello A, Vitale N, Vaisitti T, Deaglio S. Immune Response Dysfunction in Chronic Lymphocytic Leukemia: Dissecting Molecular Mechanisms and Microenvironmental Conditions. *Int J Mol Sci* **2020**;21(5) doi 10.3390/ijms21051825.
151. Pagliaro L, Chen SJ, Herranz D, Mecucci C, Harrison CJ, Mullighan CG, *et al.* Acute lymphoblastic leukaemia. *Nat Rev Dis Primers* **2024**;10(1):41 doi 10.1038/s41572-024-00525-x.
152. Troussard X, Maitre E, Paillassa J. Hairy cell leukemia 2024: Update on diagnosis, risk-stratification, and treatment-Annual updates in hematological malignancies. *Am J Hematol* **2024**;99(4):679-96 doi 10.1002/ajh.27240.
153. Sehn LH, Salles G. Diffuse Large B-Cell Lymphoma. *N Engl J Med* **2021**;384(9):842-58 doi 10.1056/NEJMra2027612.
154. Jacobsen E. Follicular lymphoma: 2023 update on diagnosis and management. *Am J Hematol* **2022**;97(12):1638-51 doi 10.1002/ajh.26737.
155. Silkenstedt E, Dreyling M. Mantle cell lymphoma-Update on molecular biology, prognostication and treatment approaches. *Hematol Oncol* **2023**;41 Suppl 1:36-42 doi 10.1002/hon.3149.

156. Lopez C, Burkhardt B, Chan JKC, Leoncini L, Mbulaiteye SM, Ogowang MD, *et al.* Burkitt lymphoma. *Nat Rev Dis Primers* **2022**;8(1):78 doi 10.1038/s41572-022-00404-3.
157. Cheah CY, Seymour JF. Marginal zone lymphoma: 2023 update on diagnosis and management. *Am J Hematol* **2023**;98(10):1645-57 doi 10.1002/ajh.27058.
158. Schaff LR, Grommes C. Primary central nervous system lymphoma. *Blood* **2022**;140(9):971-9 doi 10.1182/blood.2020008377.
159. Malard F, Neri P, Bahlis NJ, Terpos E, Moukalled N, Hungria VTM, *et al.* Multiple myeloma. *Nat Rev Dis Primers* **2024**;10(1):45 doi 10.1038/s41572-024-00529-7.
160. Gertz MA. Waldenstrom macroglobulinemia: 2023 update on diagnosis, risk stratification, and management. *Am J Hematol* **2023**;98(2):348-58 doi 10.1002/ajh.26796.
161. Salles G, Barrett M, Foa R, Maurer J, O'Brien S, Valente N, *et al.* Rituximab in B-Cell Hematologic Malignancies: A Review of 20 Years of Clinical Experience. *Adv Ther* **2017**;34(10):2232-73 doi 10.1007/s12325-017-0612-x.
162. Wen T, Wang J, Shi Y, Qian H, Liu P. Inhibitors targeting Bruton's tyrosine kinase in cancers: drug development advances. *Leukemia* **2021**;35(2):312-32 doi 10.1038/s41375-020-01072-6.
163. Stiff A, Trikha P, Wesolowski R, Kendra K, Hsu V, Uppati S, *et al.* Myeloid-Derived Suppressor Cells Express Bruton's Tyrosine Kinase and Can Be Depleted in Tumor-Bearing Hosts by Ibrutinib Treatment. *Cancer Res* **2016**;76(8):2125-36 doi 10.1158/0008-5472.CAN-15-1490.
164. Rip J, de Bruijn MJW, Appelman MK, Pal Singh S, Hendriks RW, Corneth OBJ. Toll-Like Receptor Signaling Drives Btk-Mediated Autoimmune Disease. *Front Immunol* **2019**;10:95 doi 10.3389/fimmu.2019.00095.
165. Weber ANR, Bittner Z, Liu X, Dang TM, Radsak MP, Brunner C. Bruton's Tyrosine Kinase: An Emerging Key Player in Innate Immunity. *Front Immunol* **2017**;8:1454 doi 10.3389/fimmu.2017.01454.
166. Wu Y, Yi M, Niu M, Mei Q, Wu K. Myeloid-derived suppressor cells: an emerging target for anticancer immunotherapy. *Mol Cancer* **2022**;21(1):184 doi 10.1186/s12943-022-01657-y.

167. Ferrer G, Jung B, Chiu PY, Aslam R, Palacios F, Mazzarello AN, *et al.* Myeloid-derived suppressor cell subtypes differentially influence T-cell function, T-helper subset differentiation, and clinical course in CLL. *Leukemia* **2021**;35(11):3163-75 doi 10.1038/s41375-021-01249-7.
168. Gunderson AJ, Kaneda MM, Tsujikawa T, Nguyen AV, Affara NI, Ruffell B, *et al.* Bruton Tyrosine Kinase-Dependent Immune Cell Cross-talk Drives Pancreas Cancer. *Cancer Discov* **2016**;6(3):270-85 doi 10.1158/2159-8290.CD-15-0827.
169. Ishfaq M, Pham T, Beaman C, Tamayo P, Yu AL, Joshi S. BTK Inhibition Reverses MDSC-Mediated Immunosuppression and Enhances Response to Anti-PDL1 Therapy in Neuroblastoma. *Cancers (Basel)* **2021**;13(4) doi 10.3390/cancers13040817.
170. Sagiv-Barfi I, Kohrt HE, Czerwinski DK, Ng PP, Chang BY, Levy R. Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK. *Proc Natl Acad Sci U S A* **2015**;112(9):E966-72 doi 10.1073/pnas.1500712112.
171. Szklener K, Michalski A, Zak K, Piwonski M, Mandziuk S. Ibrutinib in the Treatment of Solid Tumors: Current State of Knowledge and Future Directions. *Cells* **2022**;11(8) doi 10.3390/cells11081338.
172. Alu A, Lei H, Han X, Wei Y, Wei X. BTK inhibitors in the treatment of hematological malignancies and inflammatory diseases: mechanisms and clinical studies. *J Hematol Oncol* **2022**;15(1):138 doi 10.1186/s13045-022-01353-w.
173. Rozkiewicz D, Hermanowicz JM, Kwiatkowska I, Krupa A, Pawlak D. Bruton's Tyrosine Kinase Inhibitors (BTKIs): Review of Preclinical Studies and Evaluation of Clinical Trials. *Molecules* **2023**;28(5) doi 10.3390/molecules28052400.
174. Kramer J, Bar-Or A, Turner TJ, Wiendl H. Bruton tyrosine kinase inhibitors for multiple sclerosis. *Nat Rev Neurol* **2023**;19(5):289-304 doi 10.1038/s41582-023-00800-7.
175. Prezzo A, Cavaliere FM, Bilotta C, Pentimalli TM, Iacobini M, Cesini L, *et al.* Ibrutinib-based therapy impaired neutrophils microbicidal activity in patients with chronic lymphocytic leukemia during the early phases of treatment. *Leuk Res* **2019**;87:106233 doi 10.1016/j.leukres.2019.106233.
176. Blez D, Blaize M, Soussain C, Boissonnas A, Meghraoui-Kheddar A, Menezes N, *et al.* Ibrutinib induces multiple functional defects in the

- neutrophil response against *Aspergillus fumigatus*. *Haematologica* **2020**;105(2):478-89 doi 10.3324/haematol.2019.219220.
177. Vargas-Blanco DA, Hepworth OW, Basham KJ, Simaku P, Crossen AJ, Timmer KD, *et al.* BTK inhibitor-induced defects in human neutrophil effector activity against *Aspergillus fumigatus* are restored by TNF-alpha. *JCI Insight* **2024**;9(12) doi 10.1172/jci.insight.176162.
 178. Desai JV, Zarakas MA, Wishart AL, Roschewski M, Aufiero MA, Donko A, *et al.* BTK drives neutrophil activation for sterilizing antifungal immunity. *J Clin Invest* **2024**;134(12) doi 10.1172/JCI176142.
 179. Pilmis B, Kherabi Y, Huriez P, Zahar JR, Mokart D. Infectious Complications of Targeted Therapies for Solid Cancers or Leukemias/Lymphomas. *Cancers (Basel)* **2023**;15(7) doi 10.3390/cancers15071989.
 180. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, *et al.* New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* **1983**;13(3):227-31 doi 10.1002/ana.410130302.
 181. Kurtzke JF. Rating Neurologic Impairment in Multiple-Sclerosis - an Expanded Disability Status Scale (Edss). *Neurology* **1983**;33(11):1444-52 doi Doi 10.1212/Wnl.33.11.1444.
 182. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology* **1996**;46(4):907-11 doi 10.1212/wnl.46.4.907.
 183. Roxburgh RHSR, Seaman SR, Masterman T, Hensiek AE, Sawcer SJ, Vukusic S, *et al.* Multiple sclerosis severity score - Using disability and disease duration to rate disease severity. *Neurology* **2005**;64(7):1144-51 doi Doi 10.1212/01.Wnl.0000156155.19270.F8.
 184. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barre syndrome. *Ann Neurol* **1990**;27 Suppl:S21-4 doi 10.1002/ana.410270707.
 185. Criteria for diagnosis of Guillain-Barre syndrome. *Ann Neurol* **1978**;3(6):565-6 doi 10.1002/ana.410030628.
 186. Lundqvist H, Dahlgren C. Isoluminol-enhanced chemiluminescence: a sensitive method to study the release of superoxide anion from

- human neutrophils. *Free Radic Biol Med* **1996**;20(6):785-92 doi 10.1016/0891-5849(95)02189-2.
187. Johnsson M, Farman HH, Blennow K, Zetterberg H, Malmstrom C, Axelsson M, Lycke J. No increase of serum neurofilament light in relapsing-remitting multiple sclerosis patients switching from standard to extended-interval dosing of natalizumab. *Mult Scler* **2022**;28(13):2070-80 doi 10.1177/13524585221108080.
188. Aydin E, Johansson J, Nazir FH, Hellstrand K, Martner A. Role of NOX2-Derived Reactive Oxygen Species in NK Cell-Mediated Control of Murine Melanoma Metastasis. *Cancer Immunol Res* **2017**;5(9):804-11 doi 10.1158/2326-6066.CIR-16-0382.
189. Gorelik E, Wiltrout RH, Okumura K, Habu S, Herberman RB. Role of NK cells in the control of metastatic spread and growth of tumor cells in mice. *Int J Cancer* **1982**;30(1):107-12 doi 10.1002/ijc.2910300118.
190. Zilberter Y, Tabuena DR, Zilberter M. NOX-induced oxidative stress is a primary trigger of major neurodegenerative disorders. *Prog Neurobiol* **2023**;231:102539 doi 10.1016/j.pneurobio.2023.102539.
191. George MF, Briggs FB, Shao X, Gianfrancesco MA, Kockum I, Harbo HF, *et al.* Multiple sclerosis risk loci and disease severity in 7,125 individuals from 10 studies. *Neurol Genet* **2016**;2(4):e87 doi 10.1212/NXG.000000000000087.
192. Cardamone G, Paraboschi EM, Solda G, Duga S, Saarela J, Asselta R. Genetic Association and Altered Gene Expression of CYBB in Multiple Sclerosis Patients. *Biomedicines* **2018**;6(4) doi 10.3390/biomedicines6040117.
193. Baranzini SE, Wang J, Gibson RA, Galwey N, Naegelin Y, Barkhof F, *et al.* Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum Mol Genet* **2009**;18(4):767-78 doi 10.1093/hmg/ddn388.
194. Cotsapas C, Mitrovic M. Genome-wide association studies of multiple sclerosis. *Clin Transl Immunology* **2018**;7(6):e1018 doi 10.1002/cti2.1018.
195. Koch-Henriksen N, Sorensen PS. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol* **2010**;9(5):520-32 doi 10.1016/S1474-4422(10)70064-8.
196. Hartung HP, Schafer B, Heininger K, Toyka KV. Suppression of experimental autoimmune neuritis by the oxygen radical scavengers

- superoxide dismutase and catalase. *Ann Neurol* **1988**;23(5):453-60 doi 10.1002/ana.410230505.
197. Li X, Li C, Zhang W, Wang Y, Qian P, Huang H. Inflammation and aging: signaling pathways and intervention therapies. *Signal Transduct Target Ther* **2023**;8(1):239 doi 10.1038/s41392-023-01502-8.
 198. Fisse AL, Pitarokoili K, Leppert D, Motte J, Pedreiturria X, Kappos L, *et al.* Serum neurofilament light chain as outcome marker for intensive care unit patients. *J Neurol* **2021**;268(4):1323-9 doi 10.1007/s00415-020-10277-9.
 199. Chen SH, Oyarzabal EA, Hong JS. Critical role of the Mac1/NOX2 pathway in mediating reactive microgliosis-generated chronic neuroinflammation and progressive neurodegeneration. *Curr Opin Pharmacol* **2016**;26:54-60 doi 10.1016/j.coph.2015.10.001.
 200. Serafini B, Benincasa L, Rosicarelli B, Aloisi F. EBV infected cells in the multiple sclerosis brain express PD-L1: How the virus and its niche may escape immune surveillance. *J Neuroimmunol* **2024**;389:578314 doi 10.1016/j.jneuroim.2024.578314.
 201. Nyati KK, Prasad KN. Role of cytokines and Toll-like receptors in the immunopathogenesis of Guillain-Barre syndrome. *Mediators Inflamm* **2014**;2014:758639 doi 10.1155/2014/758639.
 202. Parbo P, Ismail R, Hansen KV, Amidi A, Marup FH, Gottrup H, *et al.* Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. *Brain* **2017**;140(7):2002-11 doi 10.1093/brain/awx120.
 203. Vogels T, Murgoci AN, Hromadka T. Intersection of pathological tau and microglia at the synapse. *Acta Neuropathol Commun* **2019**;7(1):109 doi 10.1186/s40478-019-0754-y.
 204. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* **2007**;87(1):245-313 doi 10.1152/physrev.00044.2005.
 205. Hendriks RW, Yuvaraj S, Kil LP. Targeting Bruton's tyrosine kinase in B cell malignancies. *Nat Rev Cancer* **2014**;14(4):219-32 doi 10.1038/nrc3702.
 206. Grauers Wiktorin H, Aydin E, Kiffin R, Vilhav C, Bourghardt Fagman J, Kaya M, *et al.* Impact of Surgery-Induced Myeloid-derived Suppressor Cells and the NOX2/ROS Axis on Postoperative Survival

- in Human Pancreatic Cancer. *Cancer Res Commun* **2024**;4(4):1135-49 doi 10.1158/2767-9764.CRC-23-0447.
207. Aurelius J, Thoren FB, Akhiani AA, Brune M, Palmqvist L, Hansson M, *et al.* Monocytic AML cells inactivate antileukemic lymphocytes: role of NADPH oxidase/gp91(phox) expression and the PARP-1/PAR pathway of apoptosis. *Blood* **2012**;119(24):5832-7 doi 10.1182/blood-2011-11-391722.
208. Grauers Wiktorin H, Aydin E, Hellstrand K, Martner A. NOX2-Derived Reactive Oxygen Species in Cancer. *Oxid Med Cell Longev* **2020**;2020:7095902 doi 10.1155/2020/7095902.
209. Reich DS, Arnold DL, Vermersch P, Bar-Or A, Fox RJ, Matta A, *et al.* Safety and efficacy of tolebrutinib, an oral brain-penetrant BTK inhibitor, in relapsing multiple sclerosis: a phase 2b, randomised, double-blind, placebo-controlled trial. *Lancet Neurol* **2021**;20(9):729-38 doi 10.1016/S1474-4422(21)00237-4.
210. Sanofi. Press Release: Tolebrutinib meets primary endpoint in HERCULES phase 3 study, the first and only to show reduction in disability accumulation in non-relapsing secondary progressive multiple sclerosis. 2024.
211. Sanofi. Press Release: Tolebrutinib demonstrated a 31% delay in time to onset of confirmed disability progression in non-relapsing secondary progressive multiple sclerosis phase 3 study. **2024**.