Receptor Tyrosine Kinase and Tyrosine Kinase Inhibitors: New Hope for Success in Multiple Sclerosis Therapy

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ABSTRACT

Receptor tyrosine kinases (RTKs) are essential components of signal transduction pathways that mediate cell-to-cell communication and their function as relay points for signaling pathways. They have a key role in numerous processes that control cellular proliferation and differentiation, regulate cell growth and cellular metabolism, and promote cell survival and apoptosis. Recently, the role of RTKs including TCR, FLT-3, c-Kit, c-Fms, PDGFR, ephrin, neurotrophin receptor, and TAM receptor in autoimmune disorder, especially rheumatoid arthritis and multiple sclerosis has been suggested. In multiple sclerosis pathogenesis, RTKs and their tyrosine kinase enzymes are selective important targets for tyrosine kinase inhibitor (TKI) agents. TKIs, compete with the ATP binding site of the catalytic domain of several tyrosine kinases, and act as small molecules that have a favorable safety profile in disease treatment. Up to now, the efficacy of TKIs in numerous animal models of MS has been demonstrated, but application of these drugs in human diseases should be tested in future clinical trials.

INTRODUCTION

Multiple sclerosis (MS) is an autoantigen-specific T lymphocytes disease characterized by the inflammation, oligodendrocyte depletion, and destruction of the myelin sheath that surrounds neuronal axons in the central nervous system (CNS), a mechanism that leads to the formation of MS plaques in the brain and spinal cord. The sclerotic plaques are the main pathological hallmark of the MS neurodegeneration, which are associated with reactive astrogliosis and oligodendrocytes destruction accompanied by loss of myelin and axonal damage.

In this autoimmune disorder, the
immune system cells play a pivotal role in the pathogenesis of disease. Both CD4+ and CD8+ T lymphocytes have been indicated in MS lesions, with CD4+ T cells predominating in acute lesions, whereas CD8+ T cells are observed more often in chronic lesions. There are data linking CD4+ T cells secreting IL-17, termed Th helper (Th) 17 cells, and IFN-γ-secreting Th1 cells with the pathogenesis of MS and its animal model, experimental autoimmune encephalomyelitis (EAE). Activated myelin-reactive Th1/Th17 cells are present in the blood and cerebrospinal fluid (CSF) of MS patients, which promote blood-brain barrier (BBB) disruption, demyelination, and neurodegeneration. Moreover, a T-cell dependent, macrophage-mediated, autoimmune attack on constituents in the normal myelin sheath underlies the disease.

In the brain lesions of MS patients, macrophage could contribute to demyelination directly by phagocytosis of myelin antigens. Macrophage also contributes to demyelination indirectly by stimulating immune cells infiltration and inflammation in the CNS. For example, macrophages produce pro-inflammatory cytokines, including TNF-α, and therefore are implicated in the MS pathogenesis.

Macrophage and Th1/Th17 cells are infiltrated in the CNS and mediate inflammation, in part, through microglia activation. The release of mediators by activated microglia, which are harmful for oligodendrocytes, proceeds demyelination in MS. Astrocytes, a significant component of the BBB, produce neurotoxins and neurotrophins and are handled as one of the immune effector cells in the CNS. Astrocytes could potentially promote MS pathogenesis in several ways, especially through unrestrained proliferation of astrocytes that result in astrogliosis, a scarring process in MS that concludes axonal regeneration and remyelination. Indeed, interactions of the infiltrated immune cells along with activated resident cells (microglia and astrocytes) play a pivotal role in neuroinflammatory processes by releasing various kinds of noxious factors, such as matrix metalloproteinases (MMPs) and pro-inflammatory cytokines. It has been demonstrated that MMPs play a pivotal role in the immunopathogenesis of MS, in part through the disruption of the BBB and the recruitment of inflammatory cells into the CNS. Moreover, MMPs can also enhance the cleavage of myelin basic protein (MBP) and the demyelination process.

Therefore, inhibiting resident cells, macrophage, and Th1/Th17 activation attenuates disease in animal models of MS, and this amelioration is accompanied by a reduction in inflammatory mediators in the CNS. On the other hand, the family of receptor tyrosine kinases (RTKs) has been implicated in signaling of these cells and demonstrated as important players during demyelination in both animal models of MS and in the human disease. Finally, selective inhibition of this enzyme would be a new strategy in treatment of an inflammatory disease as MS.

RECEPTOR TYROSINE KINASES AND MS

Receptor tyrosine kinases (RTKs), a family of cell-surface receptors that transduce signals to polypeptide and protein hormones, cytokines, and growth factors, are key regulators of critical cellular processes. They have a key role in numerous processes that affect cell proliferation and differentiation and cell migration and cell cycle control, as well as regulate cell growth and modulation of cellular metabolism and promote cell survival and apoptosis. In the human genome, 58 RTKs have been identified that fall into 20 families; however, for normal CNS function and neuroinflammatory disease progression type III (PDGFR, CSFR, Kit, FLT-3 receptor family), type VI (PTK7/CCK4), type VII (neurotrophin receptor/Trk family), type XI (TAM receptor family), and type XIII (ephrin receptor family) RTKs are more important.

In MS diseases pathogenesis, multiple signal transduction pathways have been implicated by RTKs and non-RTKs, including TCR and p38 MAPK signaling in T cell, FLT-3 in myeloid cells, c-Kit signaling in mast cell, macrophage c-fms activation, TAM family of receptor tyrosine kinases in oligodendrocytes, and platelet-derived growth factor (PDGF) in astrocytes.

TCR. The success of several T-cell–targeted therapies in MS reinforces the importance of the role of the T cell in MS pathogenesis. The T-cell immune response is started upon engagement of the TCR and co-receptor, CD4 or CD8. TCR/co-receptor engagement stimulates the activation of signaling pathways that, in combination with signals from co-stimulator molecules and cytokine receptors, direct the outcome of the response. Activation of the src-family kinases Lck (LSTRA cell kinase) is central to the initiation of TCR signaling pathways. Lck is one of the human src families of non-transmembrane protein tyrosine kinases, which is required for ZAP70 activation and T cell signaling. It phosphorylates the CD3 and TCR ζ-chain, so that ZAP-70 is recruited and activated, and finally conducts T-cell activation by starting nuclear factor of activated T cells and nuclear factor-κB (NF-κB) dependent transcription of cytokine genes. Therefore, Lck expression is restricted to lymphoid cells, and selective inhibition of Lck is expected to offer a new therapy for the treatment of T cell-mediated autoimmune disorders.

Another signaling pathway in T cells is MAP kinase. MAPKs (ERK, JNK, and p38 MAPK) are activated by several cellular stresses, as well as in response to inflammatory cytokines. The ERK’s function is the control of cell division, and suppressors of these enzymes are...
being explored as anticancer agents. The molecular mechanisms mediated by p38 MAPK signaling cascade have been defined. Activation of p38 MAPK cascade increases the release of inflammatory cytokines, such as IL-17 production in Th17 cells and IFN-γ by CD4+ Th1 cells and CD8+ T-effectors cells. These pro-inflammatory cytokines are able to play an adverse role in the target organs of several autoimmune diseases, such as MS.\textsuperscript{20,24,25} Kremenetsov et al\textsuperscript{26} recognized this signaling pathway as a central player in MS and its principal animal model, EAE. It is indicated that p38 MAPK activation is required for the development and progression of both chronic and relapsing-remitting forms of EAE. In addition, Noubade et al\textsuperscript{20} demonstrated that the regulation of p38 MAPK activity specifically in T cells is sufficient to modulate EAE severity.

**FLT-3.** FMS-like tyrosine-3 (FLT-3) is expressed on hematopoietic and dendritic cells (DCs), which, upon binding to FLT-3 ligand (FL), promoted cell proliferation, differentiation, and survival.\textsuperscript{27} In brain, FLT-3 is expressed on two categories of cells that may have the competency to present antigen: one category is DCs and other category may be microglia. Activated microglia can produce neurototoxic pro-inflammatory cytokines including IL-1β and TNF-α.\textsuperscript{28} Additionally, it is reported that FLT-3 inhibition in murine microglia blocks IFN-γ-induced expression of MHC class II and CD86, and LPS-induced secretion of IL-6. These findings revealed that FLT-3 is involved in microglial cells tendency to respond to environmental signals for acting as antigen presenting cells and mediating CNS inflammation.\textsuperscript{29}

Recent data revealed that the mononuclear phagocyte system, specifically DCs, plays multiple fundamental roles in the development of MS and EAE. In MS patients, DCs are plentifully present in brain lesions, and show an altered phenotype and function. Hesske et al. demonstrate that during EAE functionally distinct DC subpopulations are present in the CNS. At peak of EAE, the majority of DCs included of a CD11b+F4/80+ inflammatory DC phenotype.\textsuperscript{30} Moreover, DCs pathologically influence the auto-reactive T and B cells effector function.\textsuperscript{31}

The significance of FLT-3 in contributing to EAE is revealed by data showing that the onset of EAE is delayed in mice lacking FLT-3.\textsuperscript{32} Moreover, investigations show that inhibition of FLT-3 signaling induces apoptosis in both mouse and human DCs, and thus could be a possible target for immune suppression.\textsuperscript{33} Whartenby et al. demonstrated that targeted inhibition of FLT-3 with a selective FLT-3 inhibitor remarkably improved the course of established EAE, which was found to modulate the maturation of DCs but had no direct effect on T cells.\textsuperscript{34} It is showed that treatment with FL is not capable of recruiting stimulatory/inflammatory DCs into the CNS, whereas production of granulocyte-macrophage colony-stimulating factor (GM-CSF) leading to recruitment of DCs populations. This is an important point indicating that the improvement in EAE of knockout mice is not necessarily a result of receptor binding but possibly and indirect effect.\textsuperscript{35} Accordingly, these data suggest that FLT-3 might be recommended as therapeutic target on microglia and DCs, in order to mitigate CNS inflammation in EAE and MS.\textsuperscript{23,34}

**c-Fms.** The c-fms is a receptor for M-CSF (also known as CSF1), a major growth factor for macrophage. c-fms is expressed at low levels on monocytes, and its expression significantly increases during differentiation to macrophages. The proliferation, differentiation, survival, and activation of macrophages are regulated by M-CSF/c-fms interaction. Therefore c-fms is crucial for the growth and differentiation of the monocyte-macrophage lineage. Moreover, M-CSF/c-fms signaling plays a key role in macrophage accumulation in tissues during inflammation and also regulates the production of cytokines by macrophages.\textsuperscript{35} In vivo, it was demonstrated that mice deficient in M-CSF have fewer macrophages than wild-type mice.\textsuperscript{36,38} Moreover, M-CSF is found in the brain whose receptor is expressed by microglia. Investigations indicate a pivotal role for M-CSF in brain development and normal functioning as well as in several disease processes involving neuroinflammation.\textsuperscript{19} Interestingly, this is demonstrated that M-CSF is up-regulated in various neurological diseases, including MS and EAE development.\textsuperscript{40,41} There is little evidence for the role of c-fms specifically in EAE/MS as knockout studies have not been done. The only study that directly implicates c-Fms in EAE using the c-fms inhibitor, K20227, by Uemura et al.,\textsuperscript{42} revealed that the Ki20227 suppresses EAE.

**PDGFR.** In MS pathogenesis, microglia/macroglia activation and astrocyte reactivity are important components of the lesion environment that can impact remyelination. Astrocyte reactivity persists throughout demyelination and its activity is characterized by early proliferation depends on signaling mediated by PDGFRs.\textsuperscript{42,43} PDGF as a potent glial cell mitogen is up-regulated in peripheral blood leukocytes in EAE. The T, NK, NKT and monocytes/macrophages cells express PDGF. The PDGF production by lymphocytes may have potential therapeutic value when activating or modulating T-cell responses in demyelinating diseases.\textsuperscript{44} PDGF binds to and induces homo- or hetero-dimerization of PDGF-Rα or PDGF-Rβ. PDGF-Rα is a well-established marker for oligodendrocyte precursor cells (cells producing the myelin membrane around the axons in the CNS) and treatment with PDGF promotes oligodendrocyte maturation and remyelination, while there may be deleterious effects of PDGF. PDGF stimulates the
activities of MAPK in protein kinase C independent and dependent manners. The involvement of MAPK/ERK in P2YR-mediated astrogliosis has been disclosed in vitro. The proliferation of astrocytes induced by traumatic injury and their modulation by pharmacological ligands suggests the involvement of the MAPK/ERK1-2 and PI3K/Akt-pathways in astrogial proliferation. Thus PDGFR signaling could contribute to MS pathogenesis by promoting astrocyte proliferation and consequently astrogliosis. The tyrosine kinases MAPK/ERK and PDGFR are thus involved in key aspects of MS pathogenesis and may have potential as drug targets in the treatment of MS.

**Ephrin receptor.** The ephrin and ephrin-related receptors have been implicated in mediating developmental events, particularly in the nervous system. The ephrin-related transmembrane tyrosine kinases constitute the largest known family of receptor-like tyrosine kinases, with two identified subfamilies (EphA and EphB), which have a role in the regulation of neuronal development, cell migration and angiogenesis. EphA4/-/- mice displayed an abnormal CNS vascular structure in both the cerebral cortex and the spinal cord, with disorganized branching and a 30-percent smaller diameter. The EphA4 receptor tyrosine kinase is a main regulator of axonal growth and astrocyte reactivity and is a conceivable inflammatory mediator. It is demonstrated that after spinal cord injury in wild-type mice, EphA4 expression was remarkably up-regulated on activated astrocytes, which were mainly associated with blood vessels. Moreover, following injury in EphA4/-/-, spinal cord, astrocytes were not as tightly associated with blood vessels as the wild-type astrocytes. In uninjured EphA4/-/- mice, the BBB was normal, but it showed a prolonged leakage following spinal cord injury. These findings support a role for EphA4 in CNS vascular formation and development along with an additional role in BBB repair. In recent study, Munro et al demonstrated that EAE was induced in EphA4 knockout mice and exhibited a markedly less severe clinical course than wild type mice, with a lower maximum disease score and a slightly later onset of clinical symptoms. Moreover, EphA4 knockout mice showed a decreased axonal pathology. Recently, it is revealed that blocking of EphA4 in wild type mice by administration of soluble EphA4 (EphA4-Fc) as a decoy receptor following EAE induction produced a delay in onset of symptoms. These results are consistent with a noninflammatory, CNS specific, deleterious effect of EphA4 during neuroinflammation that results in axonal pathology.

Amyotrophic lateral sclerosis (ALS), which is described by the progressive degeneration of motor neurons, is one of the most common neuromuscular disorders, but there is just one approved therapy, riluzole, which it delays the onset of ventilator-dependence or tracheostomy in selected patients and may increase survival. A study in *Nature Medicine* showed that EphA4 can modulate motor neuron degeneration and disease progression in ALS. In humans with ALS, EphA4 expression inversely correlates with disease onset and survival, and loss-of-function mutations in EphA4 are affiliated with long survival. This indicates that EphA4 generically modulates the vulnerability of motor neurons to axonal degeneration and may display a new target for therapeutic intervention.

**VEGFR.** The active lesions in MS are characterized by BBB breakdown, up-regulation of adhesion molecules on capillary endothelial cells, and perivascular inflammation, suggesting that altered vessel permeability and activated endothelial cells are involved in the pathogenesis of the disease. VEGF mediates multiple aspects of blood vessel physiology including permeability, regulation of growth, and inflammation. To investigate a possible relationship between expression of VEGF and CNS autoimmune disease, Proescholdt et al examined VEGF expression in MS plaques and showed VEGF expression was consistently up-regulated in both acute and chronic MS plaques. Also during the course of EAE, VEGF-positive cells with astrocytic morphology increased in the spinal cord during the development of EAE and were found that the inflammatory cells such as T cells, macrophages and activated glia, are able to produce VEGF. In addition, intracerebral infusion of VEGF in animals which have been previously immunized with MBP induced an inflammatory response in the brain. These results suggest that overexpression of VEGF may aggravate the inflammatory response in CNS autoimmune diseases by inducing focal BBB breakdown and inflammatory cells migration into the lesions. Moreover, an increased expression of VEGF is associated with demyelinated lesions in both MS and EAE, implicating changes in vasculature as a potential component of CNS plaque formation. Seabrook et al demonstrated glial expression of VEGF and glial and blood vessel expression of the pro-angiogenic receptor VEGFR2 on chronic active human MS plaque.

**TAM receptor.** TAM family of RTKs (TYRO3, AXL and MERTK) plays pivotal roles in the processes of cell survival and proliferation, modulation of the immune response, and the removal of dead cells from tissue. Disruption of these processes has been shown to be central to both the initial development, and the subsequent clinical course of demyelinating diseases. All three receptors (TYRO3, AXL and MERTK) and their ligands, Gas6 (growth arrest-specific gene 6) and protein S, are expressed in myelin-producing cell, oligodendrocytes in the CNS. Recent studies have shown that Gas6-dependent TAM receptor signaling is an important modulator of oligodendrocyte...
survival and microglial phenotype both in vitro and in vivo. In 2009, Binder and Kilpatrick proposed that TAM family has been implicated as important players during demyelination in both animal models of MS and in the human disease. During a demyelinating challenge, dysfunctional TAM receptor signaling could lead to a ‘vicious cycle of cell death, reduced phagocytosis, and detrimental immune hyper-activation.

In the nervous system, Axl and its ligand Gas6 are expressed on multiple cell types. Axl functions in dampening the immune response, regulating cytokine secretion, clearing apoptotic cells and debris and maintaining cell survival. Axl is up-regulated in various disease states, such as cuprizone toxicity-induced model of demyelination and in MS lesions, suggesting that it plays a main role in disease pathogenesis. Weinger et al showed that up-regulation of soluble Axl and Mer RTKs negatively associates with Gas6 in MS lesions. While in normal tissue Gas6 significantly affiliated with soluble Axl and Mer, there was a negative correlation in established MS lesions between Gas6 and soluble Axl and Mer. Moreover, it is demonstrated that augmented levels of soluble Axl and Mer were correlated with increased levels of mature ADAM17 and ADAM10, as well as furin, proteins that are connected with solubilization of Axl and Mer. Therefore, soluble Axl and Mer function both as decoy receptors are blocking of Gas6 binding to membrane-bound receptors. These findings establish Gas6 as an important regulator of both CNS demyelination and remyelination. Furthermore, in MS lesions, dysregulation of protective Gas6 receptor signaling may prolong lesion activity in MS lesions.

In another study, Weinger et al showed that Axl alleviates EAE disease progression and suggesting that Axl has functions in the recruitment of microglia/macrophages as well as in the clearance of debris following demyelination. Accordingly, these data provide further support that administration of the Axl ligand Gas6 could be a therapeutic strategy for immune-mediated demyelinating diseases. In addition, Ma et al suggested that the MERTK gene is a novel risk gene for MS predisposition and polymorphisms in the receptor tyrosine kinase MERTK gene are associated with MS susceptibility.

**Neurotrophin receptor.** The neurotrophin receptor family of RTKs includes tyrosine kinase A, B and C (trkA, trkB and trkC) receptors, which respond to nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin3 (NT3), respectively. They are associated primarily with proliferative and migration effects in neural systems. While mature neurotrophins exhibit neuroprotective roles via TrkA, TrkB and TrkC, the pro-forms of neurotrophins show totally different biological effects that may induce apoptotic cell death of neurons by triggering p75NTR (p75 neurotrophin receptor)-sortilin signaling cascades. The receptor for NGF encompasses a p75NTR and a TrkA subunit. TrkA-mediated rescue from apoptosis is correlated with MAPK activation. Simultaneously, activation of TrkA in oligodendrocytes resulted in suppression of c-jun kinase activity initiated by p75. Therefore TrkA-mediated rescue involves not only activation of survival signals but also concurrent suppression of a death signal by p75. In an animal study, Valdo et al showed that at the edge of chronic active MS lesions, selective NGFRp75 was prominent on reactive astrocytes, while throughout the lesion, NGFRp75 was expressed on microglia/macrophages and the vast majority of mature or precursor oligodendrocytes did not express NGFRp75. Consequently, expression of NGF receptors in active MS lesions indicates a critical role for NGF in regulating the autoimmune response at both immune and glial cell levels.

It is recently shown that human immune cells are capable of producing the BDNF, which can prevent axonal and neuronal damage after various pathological insults. BDNF imported into the CNS by immune cells would thus be an attractive candidate for mediating neuroprotective effects in MS. In MS lesions, BDNF is primarily present in immune cells (T cells, macrophages/microglia), and reactive astrocytes and the number of BDNF immunopositive cells correlated with lesional demyelinating activity. The BDNF receptor gp145trkB is found in neurons in the immediate vicinity of MS plaques as well as in reactive astrocytes within the lesion, but not in immune cells. On the contrary another findings by De Santi et al revealed that gp145trkB is mainly expressed on T cell lines from MS patients and that the BDNF/gp145trkB axis is involved in the regulation of peripheral T-cell apoptosis in MS. These data suggest that BDNF and its receptor gp145trkB are involved in immune-mediated neuroprotective interactions in MS and support the concept that immune cells produce both damaging and protective factors in MS lesions.

**c-Kit.** The receptor tyrosine kinase c-Kit (also called CD117 and stem cell factor receptor) is a 145 kD transmembrane protein, which acts as a key controller receptor for a number of cell types, including mast cells, astrocytes, hematopoietic stem cells, and lineage progenitor cells. In most lineages, c-Kit is down-regulated during cell development, except for mast cells. In mast cells, high expression of c-kit is maintained during development and its signaling is essential for mast cell development. Recent researches demonstrated that mast cells have had pathogenic role in the development of autoimmune diseases, including EAE. It is shown that mast cell-deficient c-Kit mutant Kit (W/ W-) mice are protected against EAE, suggesting a
detrimental role for mast cells in this disease. As Piconese et al. observed, MOG-induced chronic EAE was exacerbated in Kit(W-sh/W-sh) compared with Kit(+/+ ) mice. In addition, Kit(W-sh/W-sh) mice showed more inflammatory foci in the CNS and increased T-cell showed more inflammatory foci in the CNS and increased T-cell response against myelin. However, in some another research an immunoregulatory function of mast cells has recently been suggested. Obtained data by Li at al indicate that mast cells responsiveness is not required in the pathogenesis of inflammatory demyelination in the CNS and that, in the absence of mast cells, increased MCP-1, CCR2, IL-17, IFN-γ, CD44, and other inflammatory molecules may be responsible for increased severity of EAE.

**TYROSINE KINASE INHIBITORS**

Protein tyrosine kinases (PTKs) regulate proliferation, differentiation, and signaling processes in the cells of the immune system. Uncontrolled signaling from RTKs and intracellular tyrosine kinases can lead to inflammatory responses. RTKs are drug targets in many types of disease specially cancer and autoimmune disease. Many diseases result in genetic changes or abnormalities that either alters the abundance, activity, cellular distribution and regulation of RTKs. Drugs that modify the dysregulated functions of these receptors fall into two categories. One group is often described as “biologics,” which block the activation of RTKs directly or by chelating the cognate ligands, while the second are small molecules designed to inhibit the tyrosine kinase activity directly. Thus, inhibitors that block the activity of tyrosine kinases and the signaling pathways may provide a useful basis for drug development. Recently, it was demonstrated that TKIs have immunomodulatory effects on immune cells implicated in autoimmune disorders. TKIs are a class of chemotherapy medications that block, or inhibit, the enzyme tyrosine kinase. In this category, imatinib was the first to be introduced into clinical oncology, and it was then followed by the drugs sorafenib, dasatinib, sunitinib, nilotinib, gefitinib, erlotinib, bosutinib, lapatinib, pazopanib, and regorafenib. Although they share the same mechanism of action (it is a competitive ATP inhibitor at the catalytic binding site of tyrosine kinase), they differ from each other in the spectrum of targeted kinases, pharmacokinetics, and substance-specific adverse side effects. Besides the hematological side effects of most of TKIs (e.g., anemia, neutropenia, and thrombopenia), the most common extra-hematologic adverse side effects are edema, nausea, vomiting, diarrhea, and hypothyroidism. With variations from drug to drug, TKIs cause skin toxicity, including folliculitis, in more than 50 percent of patients. Among the TKIs that are commercially accessible, the agents that target EGFR (gefitinib and erlotinib) display the broadest spectrum of side effects on skin and hair, including folliculitis, paronychia, facial erythema, facial hair growth, and varying forms of frontal alopecia. In contrast, folliculitis is not common during administration of sorafenib and sunitinib, which target PDGFR, VEGFR, FLT-3, and others, whereas both agents have been associated with subungual splinter hemorrhages. Periorbital edema is a common side effect of imatinib. Though TKIs entirely appear to be a well tolerated drug class, cardiac toxicity with congestive heart failure is under investigation in patients receiving imatinib and sunitinib, though this perceived side effect might be more related to patient selection.

**Imatinib mesylate.** Imatinib (originally STI571) is a drug used to treat certain types of cancer. It is currently marketed by Novartis as Gleevec or Glivec as its mesylate salt, imatinib mesylate. Imatinib mesylate, an orally administered 2-phenylaminopyrimidine derivative, is formulated in hard capsules or tablets as a salt (imatinib methane sulfonate or mesylate, molecular weight: 589.7). Each tablet contains 100mg or 400mg of imatinib free base. The hard capsule is dosed at 100mg of base. In 2011, Gleevec was approved by the United States Food and Drug Administration (FDA) to treat 10 different cancers. This drug is a selective protein tyrosine kinase inhibitor, which was expanded to inhibit BCR- Abl kinase activity in CML and c-Kit express in gastrointestinal stromal tumors (GIST). In addition to BCR- Abl and c-Kit, two other tyrosine kinase receptors inhibited by imatinib are the c-fms and PDGFR receptors. Imatinib also blocks the activity of the tyrosine kinases, such as Lck, MAPK and FLT-3. Research has shown that imatinib has immunomodulatory effects and anti-proliferative activity on various cells types so that imatinib can act on normal cells of immune system and modulate the differentiation, proliferation, activation, and function of these cells, including T lymphocytes, macrophage, and DC. Imatinib directly inhibits TCR/Abl tyrosine kinase signaling pathway and therefore could significantly reduce ZAP70 and LAT tyrosine phosphorylation in response to T-cell activation through the TCR and LCK. In addition, imatinib can diminish the levels of activated NF-κB and change phosphorylation or protein levels of Lck and ERK1/2 during T-cell stimulation. Along with modulatory effect of imatinib on T-cell functions and activation, imatinib decreases IL-2, IL-17, and IFN-γ production by activated T-cells and also inhibits release of granzyme B by CD8+ T lymphocytes. It was demonstrated that imatinib inhibits antigen-specific IFN-γ production of both CD4+ and CD8+ T-effector cells at therapeutically relevant concentrations, while T cells stay responsive. Interestingly, the decrease of IFN-γ production was not due to the lack of T-cell viability. Therefore, imatinib inhibits T-cell
response, but does not effect T-cell apoptosis, so long-term imatinib administration in high doses might have an effect on T cells in immune system. Leder et al. showed that the effector T cells are modulated rather than suppressed because the cytokolytic functions of cytotoxic T cells were not changed. These findings provide evidence for a therapeutically relevant modulation of T-cell effector functions via imatinib. Agosti et al. demonstrated that treatment of normal mice with imatinib, along with deficit in pro T and pro B cell development, also inhibited astrocytes and mast cell proliferation. Crespo et al. revealed that imatinib inhibited astrocyte proliferation mediated by the PDGFR, a process involved in the typical astrogliosis of MS. Moreover, imatinib can inhibit SCF-induced c-Kit phosphorylation and downstream activation of MAPK pathways in mast cell accompanied by a decreased production of TNF-α, IL-6 and GM-CSF pro-inflammatory cytokines. Hence, the receptor tyrosine kinases might be considered as therapeutic targets for imatinib to alleviate cytokine-mediated disorders during CNS inflammation in EAE and MS, and to inhibit recruitment of inflammatory cells to the CNS by reducing the TNF-α, IL-1β, and IL-6 secretion. Recently, Moawad showed that histologic grade and TNF-α level in mouse model of MS would be strongly and inversely correlated in attenuating MS effectively by imatinib therapy.

Imatinib is able to inhibit IL-6 and TNF-α secretion by macrophages, as well as reducing delayed hypersensitivity in mice. In addition, it has an inhibitory effect on macrophage proliferation and development induced by both M-CSF and GM-CSF through decreasing c-fms signaling and functional activity. In the presence of therapeutic concentrations of imatinib, human monocyte-derived DCs generated a reduced expression of MHC class I and II, CD1a, and co-stimulatory molecules (e.g., CD40 and CD80), and also decreased secretion of cytokines, resulting in an impaired ability of DCs to elicit primary T-cell responses. The modulatory effects of imatinib were along with down-regulation of nuclear localized RelB protein. These data revealed that imatinib can inhibit the differentiation and function of DCs, which is in part mediated through the NF-κB signal transduction pathway.

The TKI imatinib with immunomodulatory and anti-inflammatory properties can attenuate CIA, EAE, glomerulonephritis and autoimmune diabetes in animal models. In MS and its animal model, it is demonstrated that imatinib can be effective in attenuation of inflammatory process in CNS. Crespo et al. in 2011 showed imatinib can diminish the development of EAE and treat established disease in a rat model of MS. These data were supported by Azizi et al. that showed imatinib has potential therapeutic effects on EAE by attenuation in the severity and delay in the onset of EAE in C57BL/6 mice. In the only clinical trial in humans, it was found that imatinib ameliorated neurologic deficits in a rare case of simultaneous association of missed MS and chronic myeloblastic leukemia. Although, very low influence into the CNS of humans with accurate BBB has been reported for imatinib, however the surveys about imatinibs influence on CNS cells is still controversial. Recent prosperities in animal models treatment to clarify the therapeutic effect of imatinib on EAE has been achieved with regard to the fact that the BBB is compromised as a result of the disease pathology, and dissociation of the BBB allowing access to imatinib via serum ingredients. In addition it is suggested that BBB is a target for imatinib, as Chi et al. suggested that in one side, imatinib reduces BBB disruption and stroke volume by targeting PDGFR-α signaling, following an experimentally induced ischemic stroke. Here they demonstrate that PDGFR-α signaling is a pivotal regulator of BBB integrity during neuroinflammation.

On the other side, MMP-2 can be expressed in CNS by resident cells such as reactive astrocytes and seems to play a key role in BBB disruption which facilitates immune cell migration into the CNS. Moreover the over expression of MMP-2 is associated with fragmentation of MBP, degrading the myelin sheath, and damaging axons. Recently Azizi et al. showed that imatinib inhibited MMP-2 expression and activity in glioblastoma and astrocytoma cell lines. In another study Schultz et al. showed that MMP-2 expression was suppressed in the presence of imatinib in HNSCC cell lines due to inhibition of receptor tyrosine kinases c-kit. Finally, Down-regulation of MMP-2 by imatinib could inhibit BBB disruption and migration ability of inflammatory cells to CNS, and therefore imatinib should be discussed as a potentially effective treatment for MS. In consistent with this data, Adzemovic et al. revealed that imatinib augments BBB integrity in EAE rat model accompanied by reduced CNS inflammation, modulating the peripheral immune response and especially reduced T-cell recruitment. This phenomenon was supported by down-regulation of the CCR2 in CNS and lymph nodes, and by modulation of the peripheral immune response towards an anti-inflammatory phenotype.

Interestingly, imatinib alleviated neuroinflammation, even when the treatment was initiated after the clinical manifestation of the EAE. Sunitinib. Sunitinib (marketed as Sutent by Pfizer) is an orally available small-molecule multi-kinase inhibitor. This agent potently inhibits the VEGFR, PDGFR, FLT-3 and c-Kit in addition to other kinases in biochemical and cell-based assays. Sunitinib was approved by the FDA for the treatment of renal cell...
carcinoma and imatinib-resistant GIST. Sunitinib may also be used to treat a rare type of pancreatic cancer called a neuroendocrine tumour.

Information regarding the direct effects of sunitinib on brain-derived neurons is confined. In a study by Son et al., it is revealed that the injection of sunitinib reduces pathologic autophagic vacuoles formation in the brains of the APP/PS1 double transgenic AD mouse model. In a same study, the increase in pathologic vacuole formation in the human neuroblastoma cell line by amyloid beta was reduced by sunitinib. On the other side, sunitinib has been shown to arouse autophagy in the neuronal-like PC12 cell line, an effect that is mediated by inhibition of the mTOR signaling pathway. Moreover, examination of cultured neurons derived from the Tg2576 mouse model of AD reveals that injection of sunitinib reduces in pathologic vacuole formation in this regions and within the brain parenchyma. Whartenby et al. demonstrated that inhibition of FLT-3 signaling by lestaurtinib would thus produce an inhibition of DC-induced stimulation of T cells, thereby inhibiting autoimmune responses. Skarica et al. indicate that in vivo administration of lestaurtinib that targets DCs to mice with EAE led to a decrease in CNS infiltration of pathogenic Ag-specific T cells. Other results also showed a decrease in production of TNF-α, IL-6, and IL-23 by DCs as well as a decrease in co-stimulatory molecules expression. Moreover it is demonstrated that levels of phospho-Stat1, Stat3, Stat5, and NF-kB, which are signaling molecules that have been implicated in these pathways, were decreased. In another study, DeBoy et al. revealed that treatment of activated microglia with FLT-3 inhibitor, lestaurtinib, results in a dose-dependent decrease of surface expression of MHCII and CD86 and secretion of IL-6. These data display that peripheral DCs are the primary target but that microglia is also modestly affected by lestaurtinib, as numbers and activation states of the cells in the CNS are decreased after therapy. Therefore, targeted inhibition of FLT-3 in DCs and microglia by lestaurtinib significantly improved the course of established disease in EAE, suggesting a potential avenue for treating MS disease.

Sorafenib. Sorafenib (Nexavar) is a small molecular inhibitor of several tyrosine kinases (VEGFR, c-KIT and PDGFR) and Raf kinases. In addition, sorafenib is a unique agent in targeting the MAP kinase pathway. Sorafenib is approved for the treatment of primary kidney cancer and advanced primary liver cancer.

Massard et al. in 2006 suggested that sorafenib, is probably able to penetrate the BBB. In a research using MS animal model, Crespo et al. tested the therapeutic efficacy of two TKI: sorafenib and GW2580, an orally bioavailable inhibitor of c-fms kinase. GW2580 is a relatively specific inhibitor of c-fms that can attenuate autoimmune arthritis in mice, and also completely inhibited human c-fms kinase in vitro. Crespo et al. showed that sorafenib and GW2580 can each effectively treat EAE. Sorafenib abrogated PDGF-induced proliferation of astrocytes, whereas both GW2580 and sorafenib suppressed TNF-α production by macrophages. In another study Conway et al. showed that GW2580 inhibited LPS-induced TNF-α production in mice, in contrast to effects on monocytes and macrophages in vitro. The ability of GW2580 to chronically inhibited CSF-1 signaling through c-fms kinase in normal immune cells in vivo makes GW2580 a useful target in assessing the role of c-fms kinase in normal and disease processes as MS.

Ki20227, a novel quinoline-urea derivative is a c-fms TKI which may be candidate as a drug for the treatment of human MS. Ki20227 can inhibit c-fms, KDR, c-Kit, and PDGF-Rβ but does not inhibit other kinases, such as FLT-3, epidermal growth factor receptor, or c-Src. Uemura et al. investigated whether Ki20227 has suppressive effects upon EAE and indeed found that this drug significantly reduced the severity of EAE both preventively and therapeutically. Remarkably, Ki20227 treatments inhibited the turn-over/ expansion of myeloid cells stimulated by the immunization and subsequent MOG-specific T cell responses in MS animal model.

The TKIs of VEGFRs are ATP-mimetic proteins that bind to the ATP-binding catalytic site of the tyrosine kinase domain of VEGFRs, resulting in blockade of intracellular signaling. Several of these agents as Cediranib (AZD2171) and semaxinib
(SU5416) are currently in different phases of clinical development. Roscoe et al investigated the functional contribution of VEGF in acute and chronic EAE by treating immunized mice with semaxinib, a potent and selective inhibitor of VEGFR2. In an acute status, semaxinib treatment produced a significant clinical improvement versus vehicle controls, with less demyelination and cellular infiltration in the spinal cord. In addition, treated animals had significantly fewer blood vessels than controls, and significantly reduced laminin abnormalities. There was no improvement in clinical score or tissue pathology, and no difference in vessel number, when semaxinib was administered during the chronic disease.

As mentioned mast cells actively participate in the pathogenesis of MS, in part because they secrete large amounts of various mediators that sustain the inflammatory network. Masitinib (AB1010), a selective oral TKI, effectively inhibits the survival, migration and activity of mast cells. Vermesch et al demonstrated that masitinib has a positive effect on primary progressive MS (PPMS) or relapse-free secondary progressive MS (rSPMS) patients, as evidenced by an improvement in MSFC scores relative to baseline, compared with a worsening MSFC score in patients receiving placebo. These data suggest that masitinib has therapeutic benefit to PPMS and SPMS patients and could therefore represent an innovative avenue of treatment for this disease.

CONCLUSION

In MS pathogenesis the tyrosine kinases are involved in key aspects and may have potential as drug targets in the treatment of this disease. RTK inhibitors can exert numerous effects on multiple cell types, affecting immune responsiveness and inflammatory processes. Several reports represent that these agents have direct effects on inflammatory mediators and processes in the brain and periphery immune system. In addition, TKIs can be administered orally to the patients and comparatively limited adverse effects have been reported for these immunomodulatory drugs than usual drugs used for MS treatment. Up To now, the efficacy of some TKIs in animal models of MS has been demonstrated, but application of these drugs in human should be tested in future clinical trials.

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