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NEUROLOGICAL UPDATE

Some recent advances in multiple sclerosis

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Abstract In this article, we review some of the key advances in multiple sclerosis (MS) over the last 3 years. Significant progress has been made in understanding the genetics and pathogenesis of MS. The classification of MS phenotypes has been revised and the landscape of therapeutics is rapidly evolving. We provide a practical summary of the main developments for the practising neurologist.

Keywords Multiple sclerosis · Genetics · Classification · MRI · Therapy

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system. Until recently, it was thought to be a predominantly T cell mediated autoimmune disease. Little was known of the genetic factors resulting in susceptibility beyond long known human leucocyte antigen (HLA) associations. Few effective disease-modifying therapies had been developed. The clinical classification had not been updated for nearly 20 years. In a rapidly changing landscape, we have reviewed some key areas of progress in these areas over the last 3 years. The review is inevitably selective and does not cover a number of areas recently reviewed elsewhere including imaging [1], environmental risk factors [2] and pathology [3].

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Genetics

Up until the last decade, there had been relatively slow advances in the understanding of the genetics of MS since the finding of the importance of variants in HLA genes of the major histocompatibility complex (MHC) in the early 1970s [4]. Since then there has been a revolution led by large genome-wide association studies (GWASs) [5]. The latest study from the International MS Genetics Consortium (IMSGC) [6] used a specially constructed gene chip (ImmunoChip), designed to look in detail at loci with significant genome-wide associations to at least one autoimmune disease, with lighter coverage of other regions with some evidence of association. The genome of nearly 30,000 patients and over 50,000 controls were analysed. Of the 97 statistically independent single nucleotide polymorphisms (SNPs) identified, 48 were new, more or less doubling the known non-MHC associations of MS. There was significant overlap with SNPs associated with other autoimmune diseases, and the majority were within 50 kb of genes with immunological function.

This, however, still falls some way short of identifying specific genetic variants relevant to the disease. Farh et al. [7] looked in more detail at SNPs identified in GWASs in MS as well as other autoimmune diseases. They developed a fine mapping algorithm, which they termed probabilistic identification of causal SNPs (PICS), to estimate the probability that SNPs identified were causal rather than merely associated by linkage to nearby relevant SNPs. The

results would suggest that only around 5 % of SNPs identified were causal and that, of these, around 90 % were in non-coding sequences, particularly clustering around sites associated with stimulus-specific immune cell activation.

The IMSGC used a large network of known protein interactions to demonstrate that genes identified by GWAS are more likely to fall within specific networks or pathways, predominantly concerned with immune function [8]. Incorporating such protein-interaction-network-based pathway analysis allowed identification of five plausible MS susceptibility candidates (B cell lymphoma 10, CD48, v-rel reticuloendotheliosis viral oncogene homologue, TNF-receptor-associated factor 3 and TEC protein tyrosine kinase). The GWASs have, therefore, opened up a whole new field of research into genetic and subtle epigenetic influences. The hope is that these techniques will yield new insights into the pathophysiology of MS, and in time, result in novel approaches to therapy.

Antibodies

The influence of B lymphocytes has been underappreciated for many years in the understanding of pathological processes in MS. This is changing. Rituximab, a chimeric anti-CD20 monoclonal antibody, highly effectively depletes B cells. In relapsing-remitting MS, it significantly reduces gadolinium-enhancing lesions and clinical relapses [9]. B cells are likely to influence MS via a variety of mechanisms including antigen presentation, cytokine production and establishment of ectopic lymphoid follicles within the CNS, as well as via antibody production. Searching for autoreactive antibodies specific to MS has largely proved unrewarding. Srivastava et al. [10] purified and enriched IgG from patients with MS using a column containing the membrane protein fraction from human brain. The enriched IgG was then immunoprecipitated with human brain tissue lysate and the identified brain antigens were separated using sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. One of the resulting spots on the gel was shown to be KIR4.1, a glial membrane associated potassium channel.

The authors found KIR4.1 antibodies in 47 % of 397 patients with multiple sclerosis compared to 1 % of 329 people with other neurological conditions, and in none of 59 healthy controls. They further elegantly showed that binding of serum from patients with MS co-localised in brain slices with binding from purified anti-KIR4.1 antibodies, whereas this was not shown with serum from neurological controls. The antibodies seemed to bind, as one might expect in vivo, to one of the extracellular domains of the KIR4.1 molecule. They demonstrated pathological effects of these antibodies when injected intrathecally in mice. The study did not confirm that the antibodies are pathogenic in humans and interestingly, they only found evidence of intrathecal production in two of 19 patients where serum and CSF were available.

Subsequent studies have, however, failed to reproduce these findings. Using enzyme-linked immunosorbent assay (ELISA), Brickshawana et al. only found anti-KIR4.1 in three of 286 serum samples from MS patients compared to two of 208 control samples antibodies [11]. Also, using an ELISA similar to the original study, Nerrant et al. found anti-KIR4.1 antibodies in 7.5 % of 286 MS patients' sera compared to 4.3 % of 46 sera from patients with other neurological diseases and 4.4 % of 45 healthy controls [12]. The disparity could represent differences in the size, glycosylation or conformation of the KIR4.1 epitopes used in the assays [13, 14] although the subsequent studies clearly cast some doubt over the validity of the original result. Although the pathological role (if any) of anti-KIR4.1 antibodies in MS still needs to be confirmed, the study provides further impetus to study of B cell mechanisms in MS and of B cell targeted therapies.

Classification of the types of MS

In 2014, the International Advisory Committee on Clinical Trials of MS published their revised classification of the clinical subtypes of MS [15]. The latest classification takes into account MRI activity (gadolinium-enhancing lesions and new or unequivocally enlarging T2 lesions) as well as clinical relapses. It also includes the entity of clinically isolated syndrome (CIS) which has emerged since the group's original publication in 1996. The new classification is pragmatic and aims to bring clarity to the subtypes of MS which will aid selection for clinical trials and treatment decisions for physicians. The original classification is now embedded in clinical practice and there are no major changes to the nomenclature other than the eradication of the progressive-relapsing subtype. MS patients are now classified as either: relapsing-remitting MS (RRMS) or progressive MS (Fig. 1). Progressive MS is further divided into either primary progressive or secondary progressive. Each phenotype is defined as either active (clinically or radiologically) or inactive.

The committee advised that patients with relapsing MS should have a clinical assessment and MRI brain at least yearly, although this could represent a significant burden for some radiology departments. Annual spinal cord MRI was not recommended unless there is clinical evidence of spinal cord activity. Patients with progressive MS should have an annual clinical assessment but no recommendation was given for the frequency of MRI scanning in this group.

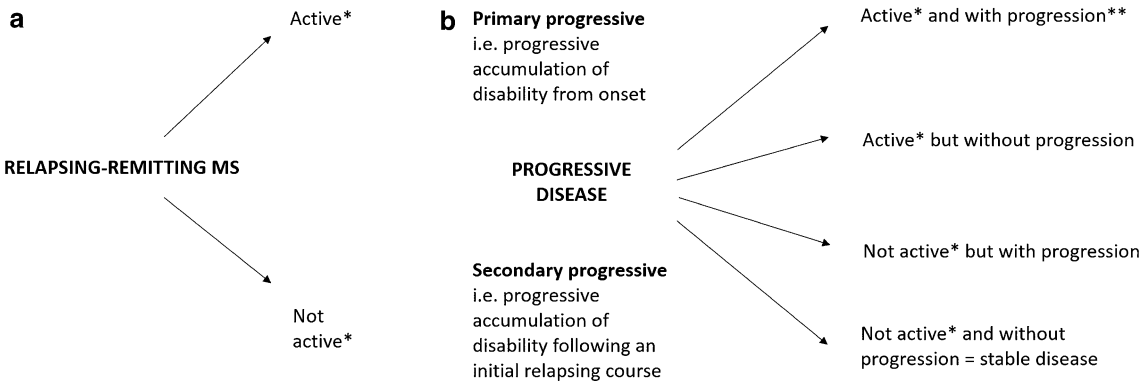

Fig. 1 Adapted from the 2013 multiple sclerosis phenotype descriptions by Lublin et al. [15] for **a** relapsing-remitting disease and **b** progressive disease. *Activity determined by clinical relapses assessed at least annually and/or MRI activity (contrast-enhancing lesions; new and unequivocally enlarging T2 lesions). **Progression measured by clinical evaluation, assessed at least annually

Also, progressive disease should (slightly confusingly) be classified as either having progressed or not progressed in the last year. For example, a patient with primary progressive MS who has not had any relapses or evidence of clinical disease progression in the last year, but has gadolinium-enhancing lesions on MRI scan, would be 'primary progressive, active but without progression'.

The group makes some important points regarding the terminology used in clinical trials relating to increases in Expanded Disability Status Scale (EDSS) scores. 'Sustained worsening' is used as a clinical trials outcome, referring to a worsening of the EDSS score that persists for a specified period of time (usually 3 or 6 months). It is often interpreted to mean disability progression, although it may not accurately reflect the clinical picture, as some functional systems may have improved and others worsened, to generate an overall increase in EDSS score. The group offers the term 'confirmed worsening' in place of sustained, to reflect that disability can improve [15]. The terms disease or disability progression are often used when a worsening of EDSS is observed but they do not distinguish the accumulation of disability due to relapses from the onset of the progressive phase of the illness. The group sensibly suggests using the term 'worsening' for patients with relapsing disease and reserving 'progression' only for those in the progressive phase of MS with an increasing EDSS independent of relapse activity [15].

Current therapeutics

The range of disease-modifying therapies (DMTs) available to neurologists for use in relapsing-remitting MS has expanded significantly in recent years. Currently, 12 products are licenced by the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA). New products/formulations licenced since 2013 are: dimethyl fumarate (Tecfidera), alemtuzumab (Lemtrada), pegylated interferon-beta (Plegridy) and glatiramer acetate (Copaxone) 40 mg. Therapies vary widely in their efficacy, side effect profiles and safety monitoring requirements.

For patients who prefer the safety profile of the original DMTs, new formulations of interferon-beta and glatiramer acetate have been developed to reduce the frequency of injections. Interferon beta-1a (Avonex) is now available as a pegylated product (Plegridy), which has equivalent efficacy to the non-pegylated version but only requires one subcutaneous injection per fortnight [16]. Glatiramer acetate is now available at 40 mg given three times a week, compared to the previous daily dose of 20 mg. Interferon-beta is perceived as a safe, albeit moderately effective treatment. However, cases of thrombotic microangiopathy associated with interferon-beta have emerged in recent years. In 2014, Hunt et al. [17] reported four MS patients treated for years with interferon-beta who all presented with renal failure, severe hypertension and microangiopathic haemolytic anaemia. In the UK, 13 cases were reported and a European review has been triggered [18].

Dimethyl fumarate (Tecfidera) was originally used in the treatment of psoriasis and has been shown in clinical trials to reduce the annualised relapse rate in MS by approximately 50 % [19, 20]. The main side effects are flushing and gastrointestinal upset, which usually resolve in the first few months. However, progressive multifocal leukoencephalopathy (PML) has been reported in three patients treated with dimethyl fumarate who were not previously immunosuppressed [21]. In October 2015, the EMA recommended that patients are monitored throughout treatment with 3 monthly full blood counts and that treatment is suspended if lymphocyte counts are persistently below 0.5×10^9/L for greater than 6 months [21]. Following the EMA guidance, however, details of a fourth

case of PML have emerged [Hughes, S, Medscape Medical News, Neurology 2015]. The patient is reported to have been previously treated with natalizumab but this was stopped 2 years prior to dimethyl fumarate. Worryingly, the patient had a persistent lymphopaenia of 0.6×10^9/L. Although the risk of PML in dimethyl fumarate treatment of MS remains very low, the fourth case raises questions about of what level of lymphopaenia should be tolerated and whether JCV antibody testing should be checked prior to treatment.

For patients treated with the anti-α4-integrin antibody natalizumab, the risk of PML has been further defined by McGuigan et al. [22]. The authors recommend risk stratification based on anti-JCV antibody index, duration of treatment and prior immunosuppressant use. In patients receiving natalizumab for more than 2 years, with a high anti-JCV antibody index, the risk of PML can be as high as 1 in 113 [22]. In February 2016, the EMA updated its guidance on PML risk reduction with similar recommendations for risk stratification based on anti-JCV antibody index testing [23]. The recommendations state that all patients treated with natalizumab should have an yearly MRI brain scans and those at high risk should be scanned every 3–6 months. A recent and ongoing study suggests that increasing the interval between natalizumab infusions to 6 or even 8 weeks may reduce the risk of PML without adversely affecting efficacy although the results have not yet reached statistical significance [Zhovtis Ryerson L, Kister I, Foley J et al., ECTRIMS 2015, abstract 57].

Whether to switch from natalizumab to an alternative therapy, and how to make the transition, is a difficult decision for both patient and physician. Following cessation of natalizumab treatment, no rebound of disease activity (i.e. higher disease activity compared to pre-natalizumab), was observed in the phase II and III natalizumab studies [24]. A retrospective study of 375 patients who stopped treatment with natalizumab in Denmark also suggests that rebound activity is not a major concern [25]. The majority of these patients switched to fingolimod (65 %), 10 % to interferon-beta/copaxone, 9 % to mitoxantrone, 8 % resumed natalizumab, 4.5 % to other treatments and 2.7 % had no treatment. On average, the relapse rate increased in the first 3 months after discontinuation, but did not reach the same level as in the time period before natalizumab treatment. After 3 months, the relapse rate subsequently decreased [25].

The RESTORE study looked at the effect of natalizumab treatment interruption on disease activity in 175 patients and whether this could be ameliorated by an alternative therapy [26]. All patients were relapse-free for 1 year and had no gadolinium-enhancing lesions on MRI brain. Patients were randomised 1:1:2 to natalizumab, placebo or an alternate immunomodulatory therapy (immediate interferon beta-1a or glatiramer acetate or monthly methylprednisolone started after 3 months). Radiological evidence of disease activity was seen in 29 % of patients after 12 weeks (all had discontinued natalizumab). Clinical relapses occurred in 19 % of patients off natalizumab and 4 % on natalizumab. Neither interferon-beta, glatiramer acetate, nor methyl prednisolone reduced relapses compared to placebo but the numbers in each group were small. One brain abscess occurred in a patient who received methyl prednisolone.

Fingolimod is currently being used in patients who are JCV antibody positive as a follow-on treatment after natalizumab, however, it does carry its own risk of PML. Three cases of PML are reported with fingolimod treatment in patients who have not previously received natalizumab [27]. In patients with prior natalizumab treatment, 17 cases of PML have been reported [27]. This suggests 'carry-over' PML following natalizumab and raises the question of washout periods following natalizumab. There is also an argument to be made for checking CSF for JC virus (by PCR) after natalizumab treatment before starting another therapy. Current clinical practice for switching from natalizumab to an alternative therapy varies widely and further evidence is needed to establish safe, definitive protocols. In December 2015, the EMA published recommendations for a baseline MRI scan prior to fingolimod to check for PML. Also, medical evaluation of the skin is now recommended before starting fingolimod as basal cell carcinomas have been associated with treatment.

The lymphocyte-depleting monoclonal antibody alemtuzumab was approved for use in relapsing-remitting MS in 2013 and 2014 by the EMA and FDA, respectively. It is highly efficacious (reduces relapses by approximately 50 % compared to treatment with interferon-beta) but has well documented autoimmune side effects [28, 29]. Despite the requirement for rigorous monitoring, it has proved an useful drug in patients with active relapsing-remitting MS. Standard treatment involves two courses given a year apart and pregnancy can be considered 4 months after the second annual course making it an useful drug in young women. There have been no cases of PML on treatment with alemtuzumab, except for one person who, in retrospect, had developed first symptoms of PML whilst on another disease-modifying therapy (personal communication from Prof. Coles, University of Cambridge, UK).

Vitamins

There is a long history of putative alternative treatments for multiple sclerosis, often championed beyond the mainstream of MS research. These treatments go through the cycle of initial (sometimes chance) observation, often "a

posteriori" rationale for possible mode of action, enthusiastic and vociferous support (especially from patient groups), neurological scepticism/caution and finally waning enthusiasm, either based on evidence of lack of efficacy or arrival of the next hope. Examples from relatively recent history could include the "Cari Loder" regimen [30], low dose naltrexone [31] and angioplasty for "cerebrospinal venous insufficiency" [32], which failed to stand up to the scrutiny of a placebo controlled trial [33]. It remains to be seen if Vitamin D and biotin will stand the test of time.

Vitamin D supplementation at least has a reasonable a priori rationale behind it, with the known association of low Vitamin D levels with increased risk of the development of MS [34, 35]. Small randomised studies have shown no consistent therapeutic benefit [36–40]. Røsjø et al. [41], in this journal, looked at the effects of vitamin D3 supplementation (20,000 IU per week) on eleven markers of systemic inflammation in 68 RR patients enrolled in a (negative) Norwegian trial [42], originally designed to investigate effects on bone health. Despite mean serum vitamin D levels being double in the treatment arm, there were no significant differences in any of the inflammatory markers measured, whereas there was an effect of immunomodulatory therapy (mostly interferon-beta). Pihl-Jensen and Frederiksen [43] found seasonally adjusted vitamin D levels to be lower in patients with optic neuritis compared to those with established MS but were unable to account for possible vitamin D supplementation in the MS group. In contradiction to an earlier study [44], there was no correlation between vitamin D levels and severity of optic neuritis as judged by visual acuity, contrast sensitivity or retinal nerve fibre thickness. Some neurologists may continue to recommend vitamin D supplementation on the grounds that it is cheap and safe (probably, at least up to 5000 IU per day) but should also counsel their patients on the current slender evidence of efficacy.

High dose biotin has been studied as a treatment for progressive multiple sclerosis by a group from Paris [45]. They studied 23 patients with progressive MS treated in an open label trial with maximum doses of 200–600 mg a day and reported improvements in a disparate group of measures in 21 of them. They postulate an effect of high dose biotin in boosting biotin-dependent carboxylases involved in Krebs cycle, helping to reverse an energy deficit in demyelinated axons. Such a small open label observational study clearly needs to be interpreted very cautiously, although the same group is conducting double blinded placebo controlled trials. The results are currently unpublished but have been presented [Tourbah, Lebrun-Frenay, Edan et al., ECTRIMS 2015, abstract 233]. They showed some improvement in EDSS or 25 foot timed walk in 13/103 progressive MS patients treated with biotin 600 mg a day but in none of 51 patients treated with placebo. Given the current paucity of treatments for progressive MS, it will be of particular interest whether these results can be confirmed in further trials and by other groups.

Simvastatin

Whilst awaiting the results of further biotin trials, there is at least one agent that has been shown to have a beneficial effect on secondary progressive MS. In a placebo controlled phase 2 trial, Chataway et al. gave simvastatin 80 mg or placebo to 140 patients with SPMS randomised 1:1 and followed them for 2 years [46]. The primary outcome measure was the rate of whole brain atrophy, with additional secondary outcomes both MRI (new or enlarging T2 lesions) and clinical: EDSS, multiple sclerosis functional composite scale (MSFC), multiple sclerosis impact scale-29 (MSIS-29) and relapse frequency. A statistically significant 43 % reduction in the accumulation of atrophy was found in the simvastatin group. All the clinical measures deteriorated in both groups, as might be expected in progressive disease. However, there were significant differences in favour of the simvastatin group in change in EDSS and MSIS-29 (but not MSFC). Whether the result was due directly to effects on vascular comorbidity (there was as expected a significant fall in serum cholesterol in the active arm) or anti-inflammatory or cell protective effects of simvastatin remains to be shown. A statistically significant result is of course not necessarily the same as a clinically significant one (a slight reduction in the worsening of EDSS over time is still some way from preventing further deterioration or even reversing it). This reasonably small trial does not support indiscriminate administration of high dose simvastatin to all patients with progressive MS. It does, however, show the way for further trials in progressive MS. In the MS-SMART study, the simvastatin trial methodology is currently being further tested in exploratory studies repurposing other agents (fluoxetine, amiloride and riluzole) that may have neuroprotective properties [47].

The future

Further therapeutic agents will make it into the clinic over the next few years. Ocrelizumab, another anti-CD20 monoclonal antibody targeting B cells appears to be efficacious and well tolerated, reducing relapses by nearly 50 % compared to interferon-beta in two phase 3 trials (OPERA I and OPERA II) although the trials have yet to be published [Hauser SL, Comi GC, Hartung H-P et al., ECTRIMS 2015, abstract 190]. In a separate phase 3 study

(ORATORIO), it also appeared to be effective in primary progressive MS [M Montalban X, Hemmer B, Rammohan K et al., ECTRIMS 2015, abstract 228] reducing clinical progression, as well as the rate of atrophy, although in a primary progressive cohort with moderately active disease in terms of gadolinium-enhancing lesions on MRI.

Daclizumab is a humanised anti-CD25 (interleukin 2 receptor) antibody, which has recently been shown to be more effective than interferon-beta in relapsing-remitting MS in the phase III DECIDE trial [48]. The study showed a 45 % reduction in annualised relapse rate in daclizumab treated patients compared to those receiving interferon beta-1a (annualised relapse rate 0.22 vs. 0.39, $p < 0.001$). A significant effect on MRI disease activity was also seen with a reduction in the number of new or newly enlarged T2 lesions by 54 % in the daclizumab group. The study demonstrated an increased risk of infection, cutaneous events and liver derangement in the daclizumab group. The mechanism of action of daclizumab is intriguing. Initial studies were based on the hypothesis that lymphocytes in MS patients are chronically activated and dependent on high affinity IL-2R signalling [49]. However, rather than a change in T cell function, an expansion of CD56bright NK cells has been observed, which in vitro limits the survival of activated T cells by a contact dependent mechanism [49].

Minocycline is a tetracycline antibiotic which shows promise in reducing the risk of conversion from CIS to multiple sclerosis. A phase III study of 143 people with CIS and at least two T2 lesions on MRI, randomised patients to either minocycline 100 mg twice daily or placebo. The results were presented at ECTRIMS but are yet to be published [Metz LM, Li D, Traboulsee A et al., ECTRIMS 2015, abstract 227]. The risk of conversion to MS by 6 months was 61.4 % in the placebo group and 34.0 % in the minocycline group. At 6 months, there was an absolute risk reduction of MS of 27.4 % and relative risk reduction of 44.6 %. At 12 months, the absolute risk reduction was 25.1 %, the relative risk reduction was 37.6 %, and the NNT was 4 ($p = 0.002$). It should be noted, however, that more patients in the placebo group had two or more gadolinium-enhancing lesions and also spinal cord onset.

Epstein-Barr virus (EBV) remains a key research interest with future trials planned to study the effect of famciclovir in MS patients [50]. The development of a vaccine to prevent EBV infection is also on the horizon. If a vaccine is licenced, future trials can interrogate whether preventing EBV infection could prevent the development of MS.

Compliance with ethical standards

Conflict of interests Dr. McCarthy has obtained funding to attend a scientific meeting from Biogen Idec and was an investigator on the phase II and III alemtuzumab trials. Dr. Thorpe has obtained funding to attend scientific meetings from Biogen Idec, GlaxoSmithKline, Merck Serono, Novartis Pharmaceuticals and Teva UK. He has received a lecturing fee from Biogen Idec.

References

1. Rocca MA, Messina R, Filippi M (2013) Multiple sclerosis imaging: recent advances. J Neurol 260:929–935
2. Belbasis L, Bellow V, Evangelou E, Ioannidis JP, Tzoulaki I (2015) Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses. Lancet Neurol 14:263–273
3. Dendrou CA, Fugger L, Friese MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15:545–558
4. Jersild C, Svejgaard A, Fog T (1972) HL-A antigens and multiple sclerosis. Lancet 299:1240–1241
5. Sawcer S, Franklin RJM, Ban M (2014) Multiple sclerosis genetics. Lancet Neurol 13:700–709
6. International Multiple Sclerosis Genetics Consortium (IMSGC) (2013) Advances in genetics of MS: Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet 45:1353–1360
7. Farh KKH, Marson A, Zhu J et al (2015) Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature 518:337–343
8. International Multiple Sclerosis Genetics Consortium (IMSGC) (2013) Network-based multiple sclerosis pathway analysis with GWAS data from 15,000 cases and 30,000 controls. Am J Hum Genet 92:854–865
9. Hauser SL, Waubant E, Arnold DL et al (2008) B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 358:676–88108
10. Srivastava R, Aslam M, Kalluri SR, Schirmer L, Buck D, Tackenberg B et al (2012) Potassium channel KIR4.1 as an immune target in multiple sclerosis. N Engl J Med 367:115
11. Brickshawana A, Hinson SR, Romero MF et al (2014) Investigation of the KIR4.1 potassium channel as a putative antigen in patients with multiple sclerosis: a comparative study. Lancet Neurol 13:795–806
12. Nerrant E, Salsac C, Charif M et al (2014) Lack of confirmation of anti-inward rectifying potassium channel 4.1 antibodies as reliable markers of multiple sclerosis. Mult Scler 20:1699–1703
13. Hemmer B (2015) Antibodies to the inward rectifying potassium channel 4.1 in multiple sclerosis: different methodologies—conflicting results? Mult Scler 21:537–539
14. Filippi M, Rocca MA, Lassmann H (2014) KIR4.1: another misleading expectation in multiple sclerosis? Lancet Neurol 13:753–755
15. Lublin FD, Reingold SC, Cohen JA et al (2014) Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 83:278–286
16. Calabresi PA, Kieseier BC, Arnold DL et al (2014) Pegylated interferon beta-1a for relapsing-remitting multiple sclerosis (ADVANCE): a randomised, phase 3, double-blind study. Lancet Neurol 13:657–665
17. Hunt D, Kavanagh D, Drummond I, Weller B, Bellamy C, Overell J, Evans S, Jackson A, Chandran S (2014) Thrombotic microangiopathy associated with interferon beta. N Engl J Med 370:1270–1271
18. Medicines and Healthcare products Regulatory Agency (2014) Interferon-beta: risk of thrombotic microangiopathy and risk of nephrotic syndrome. https://www.gov.uk/drug-safety-update/interferon-beta-risk-of-thrombotic-microangiopathy-and-risk-of-nephrotic-syndrome. Accessed 20th Jan 2016

19. Fox RJ, Miller DH, Phillips JT et al (2012) Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med 367:1087–1097
20. Gold R, Kappos L, Arnold DL et al (2012) Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med 367:1098–1107
21. European medicines agency (2015) Updated recommendations to minimise the risk of the rare brain infection PML with Tecfidera. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2015/10/news_detail_002423.jsp&mid=WC0b01ac058004d5c1. Accessed 20th January 2016
22. McGuigan C, Craner M, Guadagno J, Kapoor R, Mazibrada G, Molyneux P, Nicholas R, Palace J, Pearson OR, Rog D, Young CA (2015) Stratification and monitoring of natalizumab-associated progressive multifocal leukoencephalopathy risk: recommendations from an expert group. J Neurol Neurosurg Psychiatry. doi:10.1136/jnnp-2015-311100. (Published Online First: 22 October 2015)
23. EMA confirms recommendations to minimise risk of brain infection PML with Tysabri. http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Tysabri_20/Opinion_provided_by_Committee_for_Medicinal_Products_for_Human_Use/WC500202394.pdf. Accessed 22nd Mar 2016
24. O'Connor PW, Goodman A, Kappos L, Lublin FD, Miller DH, Polman C, Rudick RA, Aschenbach W, Lucas N (2011) Disease activity return during natalizumab treatment interruption in patients with multiple sclerosis. Neurology 76:1858–1865
25. Sorensen PS, Koch-Henriksen N, Petersen T, Ravnborg M, Oturai A, Sellebjerg F (2014) Recurrence or rebound of clinical relapses after discontinuation of natalizumab therapy in highly active MS patients. J Neurol 261:1170–1177
26. Fox RJ, Cree BA, De Sèze J, Gold R, Hartung HP et al (2014) MS disease activity in RESTORE: a randomized 24-week natalizumab treatment interruption study. Neurology 82:1491–1498
27. New recommendations to minimise risks of the rare brain infection PML and a type of skin cancer with Gilenya. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2015/12/news_detail_002447.jsp&mid=WC0b01ac058004d5c1. Accessed 23rd Mar 2016
28. Cohen JA, Coles AJ, Arnold DL et al (2012) Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. Lancet 380:1819–1828
29. Coles AJ, Fox E, Vladic A et al (2012) Alemtuzumab more effective than interferon beta-1a at 5-year follow-up of CAMMS223 clinical trial. Neurology 78:1069–1078
30. Wade DT, Young CA, Chaudhuri KR, Davidson DLW (2002) A randomised placebo controlled exploratory study of vitamin B-12, lofepramine, and L-phenylalanine (the "Cari Loder regime") in the treatment of multiple sclerosis. J Neurol Neurosurg Psychiatry 73:246–249
31. Sharafaddinzadeh N, Moghtaderi A, Kashipazha D, Majdinasab N, Shalbafan B (2010) The effect of low-dose naltrexone on quality of life of patients with multiple sclerosis: a randomised placebo-controlled trial. Mult Scler 16:964–969
32. Zamboni P, Galeotti R, Menegatti E et al (2009) A prospective open-label study of endovascular treatment of chronic cerebrospinal venous insufficiency. J Vasc Surg 50:1348–1358
33. Siddiqui AH, Zivadinov R, Benedict RHB et al (2014) Prospective randomized trial of venous angioplasty in MS (PREMiSe). Neurology 83:441–444
34. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A (2006) Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 296:2832–2838
35. Ascherio A, Munger KL (2007) Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. Ann Neurol 61:504–513
36. Stein MS, Liu Y, Gray OM, Baker JE, Kolbe SC, Ditchfield MR, Egan GF, Mitchell PJ, Harrison LC, Butzkueven H, Kilpatrick TJ (2011) A randomized trial of high-dose vitamin D2 in relapsing remitting multiple sclerosis. Neurology 77:1611–1618
37. Mosayebi G, Ghazavi A, Ghasami K, Jand Y, Kokhaei P (2011) Therapeutic effect of vitamin D3 in multiple sclerosis patients. Immunol Invest 40:627–639
38. Shaygannejad V, Janghorbani M, Ashtari F, Dehghan H (2012) Effects of adjunct low-dose vitamin D on relapsing-remitting multiple sclerosis progression: preliminary findings of a randomized placebo-controlled trial. Mult Scler Int 2012:452541
39. Soilu-Hanninen M, Aivo J, Lindstrom BM et al (2012) A randomised, double blind, placebo controlled trial with vitamin D3 as an add on treatment to interferon beta-1b in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry 83:565–571
40. Aivo J, Lindsrom BM, Soilu-Hanninen M (2012) A randomised, double-blind, placebo-controlled trial with vitamin D3 in MS: subgroup analysis of patients with baseline disease activity despite interferon treatment. Mult Scler Int 2012:802796
41. Røsjø E, Steffensen LH, Jørgensen L, Lindstrøm JC, Šaltytė Benth J, Michelsen AE, Aukrust P, Ueland T, Kampman MT, Torkildsen Ø, Holmøy T (2015) Vitamin **D** supplementation and systemic inflammation in relapsing-remitting multiple sclerosis. J Neurol 262:2713–2721
42. Steffensen LH, Jørgensen L, Straume B, Mellgren SI, Kampman MT (2011) Can vitamin D supplementation prevent bone loss in persons with MS? A placebo-controlled trial. J Neurol 258:1624–1631
43. Pihl-Jensen G, Frederiksen JL (2015) 25-Hydroxyvitamin D levels in acute monosymptomatic optic neuritis: relation to clinical severity, paraclinical findings and risk of multiple sclerosis. J Neurol 262:1646–1654
44. Malik MT, Healy BC, Benson LA, Kivisakk P, Musallam A, Weiner HL, Chitnis T (2014) Factors associated with recovery from acute optic neuritis in patients with multiple sclerosis. Neurology 82:2173–2179
45. Sedel F, Papeix C, Bellanger A, Touitou V, Lebrun-Frenay C, Galanaud D, Gout O, Lyon-Caen O, Tourbah A (2015) High doses of biotin in chronic progressive multiple sclerosis: a pilot study. Mul Scler Relat Disord 4:159–169
46. Chataway J, Schuerer N, Alsanousi A et al (2014) Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. Lancet 383:2213–2221
47. ClinicalTrials.gov (2015) MS-SMART: multiple sclerosis-secondary progressive multi-arm randomisation trial. https://clinicaltrials.gov/ct2/show/NCT01910259. Accessed 20th Jan 2016
48. Kappos L, Wiendl H, Selmaj K, Arnold DL, Havrdova E et al (2015) Daclizumab HYP versus interferon beta-1a in relapsing multiple sclerosis. NEJM 373:1418–1428
49. Bielekova B, Catalfamo M, Reichert-Scrivner S, Packer A, Cerna M, Waldmann TA, McFarland H, Henkart PA, Martin R (2006) Regulatory CD56 super(bright) natural killer cells mediate immunomodulatory effects of IL-2R α-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci 103:5941–5946
50. Giovannoni G (2015) Help us find out if we can treat multiple sclerosis with antiviral drugs. https://www.crowdacure.com/projects/researching-the-role-of-viruses-in-causing-multiple-sclerosis. Accessed 20th Jan 2016