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# New Strategies in Multiple Sclerosis Therapy

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## Abstract

Multiple Sclerosis (MS) is the first non traumatic neurological disease among the young. Several clinical and preclinical observations have led to define it as a chronic autoimmune demyelinating neurodegenerative disease of the central nervous system. Although the MS etiopathogenesishas not been yet clarified, it is clear that it is a multifaced disease in which genetic as well environmental factors combine and define the disease onset and course.

Although some few studies have been focused on developing remyelination strategies, the main strength to fight the disease progression has been given in counteracting the detrimental immune system activation.Therefore we are going to give an overview of the strategies for modulating the immune system in MS patients which has been reached thanks to the improvement in the knowledge of the immune system cellular and molecular functions. In this short review, we deal with monoclonal antibodies which has been developed to deplete leukocytes and to counteract their activation as well as with the attempt of developing possible vaccines to target antigen-specific inflammation processes. Other strategies to modulate immune system function, e.g. stem cells, or to sustain remyelination processes, e.g. anti-LINGO-1, are not discussed in below.

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## Introduction

Multiple Sclerosis (MS) is as a chronic demyelinating disease of the central nervous system (CNS) primarily triggered by the activation of immune system. The repeated immune system activation can lead to a progressive irreversible lost in myelin and both axon and neuron damage which are mainly responsible for the neurodegeneration. MS affects more than 2 million individuals worldwide [1]while the incidence of the disease is increasing due to both the diagnostic tool improvement and environmental reasons [2]. Among young adultsMS is the most frequently diagnosed non-traumatic disability neurological disease, it occurs 2-3 times more frequently in women with this ratio that seems to have increased over time [3]. MS signs occur most likely between the second and the fourth decade of life although over 50 years of age as well as underage individuals can be affected[4, 5].

Although MS etiopathogenesis has not been defined, itis clear that both genetic [6,7] and environmental factors [8,9] contribute to the development and progression of this multifaced autoimmune disease.

From a clinical point of viewMS is characterized by heterogenous manifestation of signs and symptomsdue to the dissemination of lesions in time and space at any level of the neuraxis. The length and intensity of disease presentation are variable with double vision, weakness in arms or legs, fatigue, difficulties with concentration or memory and bludderd is function being the most frequently reported symptoms.

Although the high variability of the clinical presentation and course, four main disease types can be recognized. Therelapsing-remitting MS (RRMS), which affects 80-85% of MS patients, characterized by recurrent differently enduring attacks that can resolve completely or almost completely, whether treated or not. The incomplete recovery from repeated relapses has been usually considered responsible for the cumulative deficit which characterizes the general clinical deterioration. Within a decade, approximately half of RRMS patients evolve towards a secondary progressive MS form (SPMS)[10]which begins when the symptoms and disability start worsening even without developing new relapses and no relevant changes reported along with neuroradiological examination.

Approximately 10% of MS patients experience the Primary Progressive MS (PPMS)form defined as progression without previous relapses. Occasionally relapses are superimposed on progressive disease in the progressive-relapsing MS form (PRMS), which represents 5% of patients with MS [11].

Other two clinical conditions have recently been defined: the so called "Radiologically Isolated Syndrome (RIS)" [12,13] and the"Clinically Isolated Syndrome (CIS)"[14,15] which are highly suggestive of the risk of developing MS.

The damaged Blood Brain Barrier (BBB) is the pathogenic mechanism that allows cells to infiltrateand it is evident with Gadolinium (Gd) enhancement at Magnetic Resonance Imaging (MRI) during active disease phases. In MS patient brain as well as in CNS of the disease animal model, i.e. Experimental Autoimmune Encephalomyelitis (EAE), the presence of immune cells contribute to define MS as a disease mediated by anti-myelin antigen pathogenic T-cells [16-17]. The immune cells overcome the BBB, penetrate the CNS and the auto-reactive T-cells are involved in the damage to myelin sheaths, neurons and axons.Nevertheless, this is a too restricted view of the immune system attack, since other immune system components

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have been considered to be involved in the CNS damage such as the B-cells and the antibodies they release.

Although the role of B-cells in MS is controversial the presence of oligoclonal bands (OCB) (mainly immunoglobulins)is one of the exams required for MS differential diagnosis and the intrathecal IgG production can be detected in early,as well as in chronic, disease while the deposition of immunoglobulin/complement in MS lesions has also been observed [18]. Moreover cerebro spinal fluid (CSF)-derived plasma cells produce autoantibodies specific to myelin [19]. Nevertheless, during demyelination these specific autoantibodies can be detected in only a few MS patients [20]leaving the presence of OCB in the CSF still unexplained.

Besides lymphocytes other immune system cells seem contribute to disease onset and progression in different ways. In acute lesions, macrophages and activated microglia are the most numerically relevant components of the inflammation process responsible for the release of proteolytic enzymesand the production of reactive oxygen species [21]. On the other hand, mast cells were also described in MS patient brains [22] and later within demyelinated lesions [23, 24] while neutrophil phenotype and functions have been recently demonstrated to be affected by the disease stage while their role has been more extensively studied in EAE [25-28].

Despite MS is a complex and not completely defined disease, the main research forces have been driven towards the comprehension of the immune activation mechanisms and regulation, while the attempt toaffect and regulate the activation of the immune system compartment has led to the clinical viability of various effective modifying disease compounds and immunomodulating/immunosuppressive strategies.

Therefore besides the already largely used disease modifying therapies such as steroids, beta interferon and glatiramer acetate, new biological drugs such as monoclonal antibodies, tolerogenic vaccines, stem cells and other less developed approaches (e.g. vitamin D, helmints) are actually available or under consideration in clinical trials. We have limited our work to provide a short overview about the monoclonal antibody and tolerogenic vaccine leaving aside the huge field of the stem cell therapeutic potential as well as other still scientifically interesting attempts for regulating the immune system in MS. Moreover we completely miss to deal with the few strategies which are developing to sustain remyelination processes in the CNS.

## Monoclonal Antibodies

The monoclonal therapeutic antibody era began in 1975 with the technical innovation of the mouse hybridoma producing antibodies [29]. Since then, several strategies have been evolved to improve antibody bioavailability reducing their immunogenicity [30]. The monoclonal therapeutic antibodies can be considered as potentially high precisesurgeons which can specifically operate the pathogenic cells and molecules involved in the disease without undesired off-target effects, although side effects have always to be considered.

Several monoclonal antibodies which are actually under consideration for MS therapy arealready available or proposed as therapeutic approach in oncology or other autoimmune disorders.

## Targeting Lymphocytes

As previously mentioned, for a long time T-cells have been considered the main players in sustained CNS lesions. Therefore,to limit their harmful entrance into the CNS was one of the first target in the antibody-based therapy which took advantage from the knowledge regarding the interaction of the immune cells with the vascular endothelial cells needed to effectively extravasate to the CNS. The first anti-T cell monoclonal antibody was designed to target the α4β1 integrin heterodimer, also termed the very late antigen-4 (VLA-4), which is present on the surface of T-cells and which, interacting with vascular cell adhesion molecule-1; (VCAM-1), enables the leukocytes to firmly adhere to the blood vessel wall and to subsequently migrate through the BBB into the CNS [31]. Natalizumab, a humanized immunoglobulin IgG4 antibody targeting the α4-chain, was the first selective adhesion molecule inhibitor limiting the immune cell adhesion to the endothelium of the BBB, reducing the migration into the brain' parenchyma. In clinical practice subcutaneous (s.c.) dose of Natalizumab is used to prevent relapses and to slow down the disability worsening in patients with MS relapses or which got unsatisfactory response to other classical pharmacological therapy [32]. Natalizumab has been approved in 2004 by FDA and the treatment schedule suggests a use no longer than two years in patients which are serum positive for JCV infection. Infect patients under Natalizumab treatment there is an increasing risk of reactivation of this neurotropic virus in anti-JCV positive MS patients with the consequence development of progressive multifocal leukoencephalopathy (PML), a progressive demyelinating damage which may lead to death or to severe neurological disabilities in surviving individuals. Commonly experienced Natalizumab adverse effects are fatigue, allergic reactions and elevated blood levels of liver enzymes.

Another α4β1 antagonist, termed SB-683669, was also considered on different clinical trials on RRMS because of its lower half-life compared to Natalizumab showing its safety although its incomplete effect on totally preventing leukocyte access to the subarachnoid space [33, 34].

Alemtuzumab is a humanized IgG1 antibody which specifically recognizes CD52 whose function remains uncertain, although it may serve to promote cell-cell adhesion or may be involved in T-cell migration and costimulation[35-37]. CD52 has been identified on the surface of mature lymphocytes, monocytes, DC and granulocytes [38-40], althoughAlemtuzumabtreatment differentially affects circulating T and B-cell depletion and recovery, with the B-cells recover faster than the T-cell ones[41] while alterations in T-cell subsets and properties have been observed, e.g the increase in regulatory T (Treg) cell percentage [42,43].

The efficacy of Alemtuzumab in MS patients has been proved in several clinical studies showing its greater effectiveness compared to sub cutaneous IFN-beta administration. Nevertheless, an increased risk of potentially serious side effects such as autoimmune reaction towards thyroid gland, the anti-glomerular basement antibody disease (Goodpasture syndrome) and the immune thrombocytopenic purpura have also been reported [41,44]. Alemtuzumabhas been proposed as a therapy for RRMS and it was approved in Europe in September 2013, while it has not been approved in the US yet.

Although MSis commonly perceived as a T-cell driven disease, the presence of OCB in the CSF as well as the ectopic lymphoid follicles in the meninges of SPMS patients has led to considered B-cells a possible target in MS therapy. Drawing from B-cell based malignancy and

autoimmune disorder therapy, anti B-cell monoclonal antibodies has been tested even in the treatment of RR and SPMS patients.

B-cells express different membrane bound molecules that make possible staging their maturation. One of them is CD20 which is present on the majority of B-cells, except on lymphoid progenitor cells in the bone marrow and mature plasma cells [45]. Rituximab, an IgG1 anti-CD20 antibody, has been administrated in few MS clinical trials up to now as it is reported on the NIH clinical trial web site. Nevertheless, the preliminary results of this studies report that Rituximab affects disease progression in younger PPMS patients, particularly those with inflammatory lesions in a subgroup analysis (NCT00087529) [46]. Moreover a single course of Rituximab has reduced inflammatory brain lesions and clinical relapses for 48 weeks, suggesting B-cell involvement in the pathophysiology of RRMS [47, 48]. Intrathecal (i.t.) Rituximab administration is under consideration to increase the level of the drug in the CNS thus leading to a more effective disruption of the meningeal ectopic lymphoid follicles and hopefullyreducing also the cortical lesions.Clinical trials evaluating i.t. release safety are ongoing and the investigators are alsoevaluating its effect on the quantity of meningeal lesions on MRI, changes in biomarkers of inflammatory activity and neuronal injury in the CSF (NCT01212094). Although Rituximab has generally been well tolerated, the patients may experience sustained B-cell depletion and secondary hypogammaglobulinemia while, in rare cases, even PML when Rituximab was co-administered with other chemotherapeutic drugs for other indications, e.g. hematological malignancies [49], suggesting the clinician to carefully select and screen patients for anti-JCV antibody titer before considering Rituximab treatment option.

The use of chimeric antibodies in clinical practicecan be responsible for potential immunogenicity risks, therefore a new generation of anti-CD20 antibodies have been developed.

Ocrelizumab and Ofatumumab are both humanized IgG1antibody which differently recognized CD20 [50]. Ocrelizumab binds to a different but overlapping epitope compared to Rituximab [51] while Ofatumumab recognizes a more membrane proximal epitope depleting even Rituximab-resistant B-cells *in vitro* [52,53].Both antibodies have been tested in B-cell malignancy and clinical trials have also been performed for safety and efficacy in immune mediated disease [52,54,55]. They are currently under consideration for the treatment of MS patients.

Ocrelizumab infusion has been compared to IFN-beta treatment (OPERA I and II)in RRMS patients demonstrating it is safe and no PML nor opportunistic infections have been reported while its use reduces BBB leakage attack (i.e. Gd-enhancing MRI lesions) and disease relapse[56]. Moreover at the last ECTRIMS 2015 (European Comittee for the Treatment and Research in Multiple Sclerosis) meeting the exiting results obtained during the ORATORIO study in PPMS patients have been presented (ORATORIO trial) [57].

On the other hand, even the three different intravenous (i.v.) Ofatumumab doses administered to RRMS patients demonstrate Ofatumumab safety with no increase in the number of serious adverse events and a decrease in the number of new MRI lesions [58]. Moreover the safety of sub cutaneous (s.c.) Ofatumumab formulation is also under consideration in a second clinical trial in RRMS patients (NCT01457924).While no official data have not been publishedyet, actually at the ECTRIMS meeting 2014 results demonstrate that the most common Ofatumumab adverse effect was injection-related reactions while only a few serious adverse events were observed in the highest dose group. Neither PML nor infections were recorded and Ofatumumab reduced the cumulative number of new T1 Gd-enhancing lesions for each Ofatumumab dose regimen [59].

Besides CD20, another relevant target to deplete circulating B-cells is CD19. They were first designed to overcome the unresponsiveness to Rituximab of certain B-cell malignancy.

MEDI-551 is a humanized IgG1 monoclonal antibody originated by modification of another fucosylated anti-CD19 antibody. MEDI-551 results in binding characteristics that are favorable forB-cells depletion at lower concentrations than Rituximab [60]. On the NIH web site only one clinical study on MS patients is present which started in 2012 (NCT01585766), it is still ongoing while no recruiting new patients. It aims to ascertainthe safety and tolerability of ascending doses of i.v. or s.c. administration of the antibody in confirmed relapsing patients (RRMS, SPMS, PRMS, and CIS) while the effects of pharmacokinetics, pharmacodynamics and the immunogenicity of MEDI-551 are also going to be considered.

## Targeting Chemokines and Cytokines

Besides directly affecting the immune cell compartment another potential therapeutic target are the concern chemokines and cytokines which regulate immune cell functions.

Interleukin (IL)-2 (IL-2) is mainly produced by activated T-cells playing a relevant role in T-cell proliferation and differentiation by binding to CD25 (IL-2Rα) a component of the heterotrimeric high affinity IL-2 binding receptor which is present on recently activated T-cells.

The monoclonal IgG1 antibody Daclizumab selectively binds to the IL2Rα chain masking the IL-2 binding site. It was first designed to block adult T-cell leukemia (ATL) and subsequently it has been also used in clinical practice for transplantation and refractory uveitis treatment [61,62].

The use of Daclizumab in MS originates from the idea that the auto-reactive $CD25^{+}$ T-cell have a relevant role in the disease and theycan be blockedto limit the disease effects. Nevertheless, Daclizumabhas showed to exert also other anti-inflammatory properties since its administration increasesboth the circulating number of $CD56^{bright}$NK and their immunoregulatory properties as well as the dendritic cell cytokine release and functional profile [63]. In several clinical trials Daclizumab safety, well tolerability and effectiveness have been proven and no specific serious adverse events or PML cases have been reported while herpes infection is similarly distributed between placebo- and Daclizumab-treated patients [64-66]. Daclizumabi.v. infusion efficacy has been showed by the reduction in contrast enhancing lesions and improvement of the clinical condition of active RRMS and SPMS patients [67, 68] while s.c. drug release had radiological benefits in an add-on therapy with IFN-betatreatment increasing in CD56brightNK cell number that well correlated with the clinical and radiological signs [61, 65, 66]. A different new formulation of Daclizumab, called Daclizumab high-yield process (DAC-HYP),was recently usedin clinical trials. DAC-HYP was designed to present a different glycosylation pattern of the same amino acid sequence of Daclizumab. DAC-HYP safety and efficacy as monotherapy treatment has been proved in highly active RRMS patients [69] while the positive

radiological and clinical effectshave led to the extension of the study to further assess theimmunogenicity potential of a prolonged DAC-HYP administration. Moreoverthe results regarding the superiority of DAC-HYP to IFNbeta-1a treatment in preventing relapse and slowing clinical decline has been recently published [70].

On activation by pathogens, naïve CD4 T-cells undergo differentiation into different Th subsets classically defined as Th1 and Th2 based on their cytokine production profile. Nevertheless, this paradigm has been disrupted by the discovery of Th17 [71] which have a crucial role in both MS and EAE [72,73], whose commitment, differentiation and activation are driven by IL-6, TGFβ and IL-23 [74] and which are mainly characterized by IL-17 release.

Therefore targeting IL-17 both directly or thought its receptor was the first attempt to affect Th17 function. Secukinumab, a fully humanized IgG1, selectively neutralizes IL-17A and it was first tested in other immune mediated diseases and approved in psoriasis treatment [75-77]. A couple of studies have been performedto assess the safety of i.v. administration of Secukinumab in relapsing MS patients (NCT01874340 and NCT01051817) and its efficacy by monitoring MRI scans. While the first studyhas been formally completed without publishing the results, the preliminary data presented at the ECTRIMS 2013 have showed a significant reduction in cumulative new Gd-enhancing lesion number in Secukinumab treated patients with the raw data recently presentedon the NIH web site.

On the other hand targeting IL-23 and IL-6 which sustain Th17 activation could be an alternative option to affect Th17. Ustekinumab (formally called CNTO 1275) is a humanized IgG1κmonoclonal antibody to IL12/IL23p40 and it has been mainly tested in psoriasis treatment [78]. Actually one clinical trial is registered at the NIH (NCT00207727) for defining the safety and efficacy of different doses of the drug subcutaneously administered to RRMS patients. Raw data are actually available while only a study designpaper was published indicating Ustekinumab did not show significant changes in the number of new Gd-enhanced T1 weighted images of brain [79].

While Dalizumab targets T-cell, Tabalumab and MOR113 have been designed to affect B-cell and monocyte biological functionsrespectively.

Tabalumab (LY2127399) is a fully humanized IgG4 monoclonal antibody which prevents B-cell activating factor (BAFF), a B-cell maturation and maintenance factor [80, 81], from binding to its receptor[82, 83]. Tabalumab treatment has already been shown to transiently reduce circulating B-cell frequency in rheumatoid arthritis patients[82 84, 85]while only one clinical study has been completed on RRMS (NCT00882999).

Granulocyte macrophage-colony stimulating factor (GM-CSF) regulates the properties of the granulocyte and macrophage lineage cells during host defence and inflammatory reactions. It promotes the differentiation and pathogenicity of proinflammatory Th17-cells [86-88] and it is essential in EAE development [89, 90] while some evidences support its role even in MS[91].

Therefore, an anti-GM-CSF antibody could represent a therapeutic approach in MS treatment.MOR103 is a fully humanized IgG1antibody which blocks the interaction between GM-CSF and its receptor. Three doses of MOR103have been recently tested for safety and efficacy in RR and SPMS patients (NCT01517282) and the study results have been published this year. MOR103 was no responsible for severe adverse effects while a few new or enlarging lesions could be retrieved in all treated group of patientseven if noevidence for MOR103 immunogenicity was found [92].

**Other Immunoregulatory Approaches: Tolerogenic Vaccines**

Besides specific immune cell depletion and function modulation strategies, other therapeutic attempts have been carried out to teach the immune system to be self-tolerant instead of self-aggressive: the tolerogenic vaccines. Unlike classical immunogenic vaccines the tolerogenic ones require that the administered antigens are recognized in non-activated, non-inflammatory environmentto developa long-lasting memory of antigen-specific Treg and to induce anergy of auto-reactive naïve.

The rationale for MS patient vaccination is based on infectious agent molecular mimicry hypothesis which sustain that infection agents etiologically instigate MS by the induction of a T-cell-mediated immunity towards infective epitopes that are also cross-reactive to myelin epitopes while the infectious agents may also persist and hide in the CNS thus sustaining epitope spreading over time increasing myelin-reactive clone number and inducing additional neurological damage [93].

Tolerogenic vaccination is currently carried out in pre-clinical settings and its clinical application is limited to early phase clinical trials [93].

Two different approaches have been used for MS patient's vaccination: using and manipulating epitopes and peptides which are claimed to be pathogenic in MS or peripheral blood cells (PBMC).

One promising tolerogenic vaccine is the GM-CSF-neurogenic fusion protein (GM-CSF-NAg) generated by the fusion of GM-CSF to the major encephalitogenic epitopes derived from myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP). The main target of this vaccination is the dendritic cell compartment to induce tolerance due to an efficient antigen non-activating presentation.

The injection of GM-CSF-NAgin threerodentmodels of EAE representingmonophasic (Lewis), chronic-progressive (C57BL/6), and relapsing-remittingcourses (SJL) of MShas beendemonstrated to prevent EAE or to slow down disease progression whenever it was administered before EAE induction or at EAE onset [93]. Moreover GM-CSF-Nag could be effective even when it was administered under pro-inflammatory conditions *in vivo* [94].

Another vaccination approach is based on the use of a mixture of 4 human MBP peptides selected on the basis of the immunodominant epitopes (ATX-MS-1467) identified in HLA DRB1*1501 and DQB1*0602 MS patients.In a preclinical study, administration of ATX-MS-1467 before EAE induction in Lewis rats delayed EAE onset and reduced its severity. This evidence correlated with an increased production of anti-inflammatory cytokines and with a reduction ofCNS inflammatory cell infiltration [95].ATX-MS-1467 has been already tested in a phase I clinical trial (NCT01097668) aiming to assess vaccine safety and biological activity and to follow the course of MS in HLA DRB1*15 positive RRMS patients. As result, no safety issues have emerged and a significant improvement

in MS patient visual-sight has been observed. Moreover a phase II study (NCT01973491) has been started for evaluating ATX-MS-1467 clinical and biological effects in RRMS patients as well as assessing its efficacy on immune tolerance [96].

Another attempt in designing MBP-based tolerogenic approach was the synthesis of a MBP peptide (MBP8298) which is immunodominant in MBP-specific T-cells as well as in auto-antibodies isolated mainly from HLA-DR2+ and DR4+patients [97-99]. MBP8298 has been tested in a phase II trial for RRMS as well asin three phase III trials for SPMS without improvement in the clinical course although a significant delay in clinical progression was found in people with a specific HLA aplotype[100].

One limitation of tolerogenic vaccination is that it needs prolonged treatments with high doses of the autoantigensto have a therapeutic effect, indeed the maintenance of immune tolerance is strictly correlated to the retention of the antigen.

An innovative approach to overcome this problem is based on the use of polymeric biodegradable lactic-glycolic acid nanoparticles (PLGA-NP) which can be loaded with antigenic proteins and immune-regulatory adjuvants for Treg function, guaranteeing their slow and sustained release. This method has been used in a pre-clinical study involving EAE C57BL/6 mice subcutaneouslyinjected with PLGA-NP loaded with MOG 35-55and recombinant IL-10 according to a prophylactic vaccination schedule or a therapeutic one. Loaded PLGA-NP were able to decrease EAE severity without affecting the disease onset while the clinical amelioration was associated with a reduction in T-cell infiltration, a mild myelin damage at spinal cord level and a decreased in vitro secretion of IL-17 and IFN-γ by spleen lymphocyte stimulated by MOG 35-55 suggesting an inhibition in the autoantigen-specific Th1 and Th17 response. Interestingly biodistribution analysis evidenced that a significant amount of PLGA-NP was detectable in the animal brain indicating this new tolerogenic approach might act directly at the level of the disease target organ [101].

Besides these molecular attempts in MS vaccination, MS patient autologous PBMCwere also used after being pulsed with different numbers of immunodominant myelin peptides (MOG, MBP and PLP) in the presence of the chemical cross-linker 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.

PBMC isolated from MS patients by leukapheresis and chemically coupled with seven myelin peptides have been used in a first clinical trial [ETIMS, NCT01414634] performed on RRMS and SPMS patients. The infusion was well-tolerated and it has been observed no disease relapses and a decrease in the antigen specific T-cell response during the first 3 months after treatment [103]. Although the infusion of modified PBMC showed to be safe, a second clinical trial was performed confirming safety and suggesting there was no increase in auto-antibodies and no induction of new auto-antibody reactivity in MS patients [104].

## Conclusion

The increasing knowledge in the immune system element cross talk and regulation together with the advance in the traetment of other immune related diseases have provided more effective and promising weapons for fighting MS. Nevertheless the use of these more sofisticated and target driven strategies need to prove their consistent and enduring efficacy while recording possibile traetment related adverse effects over time is mandatory before their extensive use in clinical practise.

## Competing Interests

The authors declare they have no competing interests.

## Author Contribution's

RR designs the manuscript, writes the monoclonal section and contributes to the paper correction, MG writes the clinical section of the paper, EB writes the tolerogenic vaccine section and corrects the paper proof.

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