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Executive summary

Myasthenia Gravis (MG) is a heterogeneous rare autoimmune neurological disease affecting the neuromuscular junction (NMJ). The molecular events causing and maintaining MG are still unknown and current treatments do not lead to remission and entail considerable side-effects stressing the need for improved therapies

The main scientific and technological results of the project are as follows:

- 1) We developed an European database updated with about 4300 MG patients. All partners from FIGHT-MG agreed to pursue the implementation and use of the database after the end of FIGHT-MG. MG patients have a lower quality of life compared to peers in their respective countries.
- 2) We improved knowledge on the etiology of MG by identifying several genes associated with MG. The study of monozygotic twins revealed that healthy twin had an epigenetic profile very similar to his MG twin, highlighting its high risk to the development of the disease. However, many genes differentially expressed between the healthy and the MG twin could explain the onset of the disease in one of the twins. Involvement of molecules related to infections (IFN type I and TLR) was established. The role of AIRE and VAV1 in the susceptibility to MG was demonstrated. The imbalance between Treg and Th17 cells was clearly demonstrated in MG and EAMG rat model.
- 3) We shed light on pathological mechanisms occurring in MG by visualizing morphological changes in mice immunized with MuSK, by investigating the role of nNOS in MG associated muscle fatigue and by analyzing the satellite cells in muscle from MG patients. Differential expression of miRNAs in MG and EAMG muscle generated large list of deregulated miRNAs.
- 4) We developed new diagnostic and monitoring assays by developing highly sensitive assays for MuSK and AChR, by developing assays for the new autoantigen LRP4 and identifying the pathogenicity of these antibodies and several biomarkers (seric proteins and circulating miRNA). We demonstrated that variants in genes involved in metabolite transport, together with those in TPMT gene, are able to predict the response to azathioprine in MG patients.

5) We developed new efficient therapies by using novel cell-mediated therapies based on immunoregulation, by eliminating pathogenic antibodies, by developing new non-cell based therapies and by targeting epigenetic regulators. To this end, we developed new models of MG.

Altogether, the tasks proposed in the initial project have been successfully achieved. Many of the molecules discovered during FIGHT-MG, namely in the omics study, shed light on the mechanism of MG and could lead to new promising therapeutics, improved diagnostic and finally alleviate the negative impact of the disease on the quality of life of the patients and their families.

In summary, the project has been running according to schedule and significant steps have been achieved towards its final goals. FIGHT-MG has generated a large amount of basic multidisciplinary knowledge, which is being disseminated by the Partners through publications, conference presentations, as well as press releases and videos. The omics approaches (miRNA, transcriptomics, methylome) allowed discovering a very large amount of data that could not be fully explored during the duration of the project time, and will have very likely significant impact in the coming years. Altogether, 57 publications in journals with high impact factors have been accepted or published. Many other manuscripts are in preparation.

Summary description of project context and objectives

Myasthenia Gravis (MG) is a heterogeneous rare autoimmune neurological disease affecting the neuromuscular junction (NMJ). The molecular events causing and maintaining MG are still unknown and current treatments do not lead to remission and entail considerable side-effects stressing the need for improved therapies

The main scientific and technological objectives of the project were as follows:

- 1) **Address the natural course of the disease** by developing the European database and a federated biobank, by defining the disease features in the different subgroups of MG and by evaluating the overall quality of life of the patients.
- 2) **Improve knowledge on the etiology of MG** by identifying new genetic risk factors, analyzing molecular mechanisms that trigger the disease, determining the role of environmental factors and investigating the role of AIRE and VAV1 in the susceptibility of MG.
- 3) **Shed light on pathological mechanisms occurring in MG** by characterizing the pathogenic factors, by visualizing morphological changes in mice immunized with MuSK, by investigating the role of nNOS in MG associated muscle fatigue and by analyzing the regenerative capacity of MG muscle.
- 4) **Develop new diagnostic and monitoring assays** by developing highly sensitive assays, by identifying protective factors in human sera, new diagnostic and monitoring tools as well as genes as predictors of pharmacological responses.
- 5) **Develop new therapies** by establishing the proof of concept for novel cell-mediated therapies based on immunoregulation, by eliminating pathogenic antibodies, by developing new non-cell based therapies and by targeting epigenetic regulators.

Description of the work done and the results

The FIGHT-MG project started in December 2009 and has been running for 4 and half years. The progress achieved in all work-packages within the whole duration of the project is in line with the initial plan. The results obtained will be divided into six parts according to the questions addressed:

- ✚ Natural course of the disease
- ✚ How does MG start?
- ✚ How does MG develop?
- ✚ What happens in the muscle of MG patients?
- ✚ How to improve the diagnostic and monitoring?
- ✚ How to improve therapies in MG?

1. Natural course of the disease

1.1 Database

The EuroMyasthenia MG DB containing clinical and diagnostic data was implemented and included data derived from about 4300 MG patients at the end of the project. It is so far the largest collection of data for Myasthenia Gravis in Europe (Fulvio and Mantegazza 2014). Due to the large number of data, specific efforts have been made to provide data analysis in a quick way. Business Intelligence (BI) softwares are very powerful solutions since these applications can provide interactive analysis of the data; data mining from the EuroMG DB is then performed through a graphical interface to analyze correlations between all data elements. BI has allowed the epidemiological analyses. All the partners of FIGHT-MG agreed to pursue to implement and use the EuroMyasthenia MG DB after the end of FIGHT-MG project.

1.2 Biobank

A biobank describing the available samples from MG patients (cells, DNA, RNA, etc..) and as well as the teams responsible for these samples was set up. Standard Operating Procedures (SOPs) were established for the different preparations of tissues and/or molecules and were distributed among the partners in order to have all teams using the same techniques for collecting and preparing the samples.

1.3 MG monozygotic twin recruitment

Recruitment of monozygotic twins with MG was a real challenge since MG is a rare disease. Therefore MG among monozygotic twin is extremely rare. Thanks to a large collaboration all over the world with neurologists, and associations of patients, we could identify a large number of monozygotic twins (15 couples). A questionnaire was first sent to the twins. Then a homogeneous

group in terms of gender was selected for the epigenetic study. Seven couples were included in the biological study, three concordant, and 4 discordant twin pairs. These twins were invited to participate to the transcriptomic and epigenetic study. The study was done in two steps: a) the first phase to optimize the technical conditions was performed on healthy individuals: transcriptome and methylation profile of the main peripheral blood cell subsets were analyzed. B) The second phase aimed to compare the epigenetic status of the twins was successfully implemented (Parag. 2.4).

1.4 Quality of Life of MG patients

A questionnaire aiming to evaluate and improve the quality of life of MG patients was validated, translated and improved (Maniaol, Brunborg et al. 2010). The questionnaire was sent to the whole population of MG patients in Norway (n=493) as well as to MG patients in parts of the Netherlands that has the same amount of inhabitants as whole Norway. The response rate was higher than 75% in both populations. In summary there were no clinical relevant differences in Health related quality of life (HRQOL) scores between the Dutch and the Norwegian MG cohort. In general, **the MG patients have a lower health status compared to peers in their respective countries.** Female sex, generalized disease, usage of non-steroid drugs and MG patients with a co-morbid psychiatric disorder scored lower on the SF-36 questionnaire, which implies a lower quality of life. AChR positive MG and thymoma MG patients scored lower on the physical parts of the QoL questionnaire than seronegative and MuSK MG patients. The hormonal factors were investigated by considering links between pregnancy and MG onset. Sex hormone appears to play a role in loss of immune tolerance and development of autoimmunity in the female subset of the early onset MG patients, since 22 % of the Norwegian females between 15-45 reported onset related to pregnancy. Finally, the analysis of potential risk factors to MG revealed a correlation between smoking and MG in the Norwegian MG population. Smokers were more prevalent in female young myasthenia patients compared to the general population (Maniaol, Bolding et al. 2013).

Based on this work, a set of guidelines for history taking and follow-up for MG patients was written and posted on the website www.fight-mg.eu. The main purpose is to ensure that Myasthenia Gravis patients get the same standardized medical treatment and follow up, independently of care providers in local or university hospitals.

2. How does MG start?

Myasthenia Gravis (MG) is a multifactorial disease. The onset of the disease is linked to environmental factors that could trigger the disease in individuals that are already predisposed to autoimmunity. As with many autoimmune diseases, MG has a multifactorial etiology, resulting from complex interactions between genetic and environmental factors, including viral infections or exposure to sun that determines the level of Vitamin D.

2.1 Role of Genetics

By performing a genetic analysis of 38 genes, we discovered three highly associated with MG

appearing at early onset (EOMG), and four other ones weakly associated. The first study included around 400 patients, and the second step 1200 MG patients. Altogether 9 cohorts were included in this research provided by 5 partners from FIGHT-MG and 3 collaborators. We confirmed the association of 6 genes with MG, two of them were known (HLA and TNF) and 4 never mentioned in the literature. These genes are CD86, AKAP12, VAV1 and B-cell activating factor (BAFF). Interestingly, the allele frequency at HLA-DRA and TNF- α loci was different in females versus males. The genetic associations to EOMG outside the HLA complex are novel and of interest as VAV1 is a key signal transducer essential for T- and B-cell activation, and BAFF is a cytokine that plays important roles in the proliferation and differentiation of B-cells. Finally, VAV1, BAFF and CD86 share the same signaling pathway, namely nuclear factor-kappa B (NF κ B), thus implicating dysregulation of proinflammatory signaling in predisposition to EOMG. This work has been published (Avidan, Le Panse et al. 2014).

2. 2 Role of viral infections

The exact mechanisms triggering and perpetuating the intra-thymic autoimmune response to AChR are still unknown. Viral infections could play a central role in MG mainly through the induction of dysregulated Toll-like receptor (TLR)-mediated innate immune responses, which can lead to inflammation and adaptive autoimmune response. The role of Toll-like receptors particularly TLR4, was demonstrated. It seems that TLR4 activation may alter the chemokine/cytokine network in MG thymus, favoring immune dysregulation and autoimmunity (Cordiglieri, Marolda et al. 2014). Growing evidence of chronic inflammation, TLR activation, and persistent viral infections in the thymus of MG patients, strongly supports the hypothesis that, in the context of a genetic susceptible background, the intra-thymic innate immune responses to pathogen infections might contribute to MG etiology (Cavalcante, Le Panse et al. 2011) (Cavalcante, Maggi et al. 2011) (Cavalcante, Cufi et al. 2013) .

We showed that viruses, such as EBV might contribute to onset or perpetuation of the intra-thymic autoimmune response in MG, by inducing abnormal B-cell activation and proliferation, as well as survival of autoreactive B cells (Cavalcante, Serafini et al. 2010). Moreover, we showed the presence of latent EBV infection in thymoma tissues from MG patients, suggesting that EBV might be involved in autoimmunity development or maintenance in thymoma-associated MG.

One major mediator produced during infection is interferon type I (IFN-I). We showed that this molecule was highly increased in the thymus and thymoma from MG patients. The increased expression of this molecule could be responsible for the changes observed in the pathological changes of the thymus in MG. Interestingly, the overexpression of IFN-I was found in thymoma-associated MG but not in thymoma without MG. These overexpressions could explain the presence of autoantibodies against IFN-I subtypes in thymoma patients (Cufi, Soussan et al. 2014). These observations suggest that activation of innate immunity pathways leading to IFN-I subtype release plays a central role in AChR sensitization associated with MG.

In addition, we showed that Poly(I:C) that mimics dsRNA from viral infection was able to induce interferon (IFN)- β release by thymic epithelial cells (TECs) (Cufi, Dragin et al. 2013). We demonstrated that IFN- β mediates many changes in the molecules involved in thymic hyperplasia and induces an increased expression of the B-cell activating factor (BAFF) by TECs. These effects have been observed *in vitro* on human TECs or *in vivo* in Poly(I:C)-injected mice. More importantly mice injected with

Poly(I:C) produce antibodies to acetylcholine receptor. This work showed a proof of concept that molecules mimicking viral infections are sufficient to induce autoantibodies to acetylcholine receptor. In addition to scientific publications, this work published in 2013 has been disseminated via Press releases.

Altogether, these data suggest that IFN- β could trigger α -AChR autosensitization, the abnormal recruitment of peripheral cells, and thymic follicular hyperplasia development (Cufi, Dragin et al. 2014).

2.3 Potential role of vitamin D

Vitamin D, or cholecalciferol, is a steroidal hormone whose main function is the regulation of calcium homeostasis, and bone formation and reabsorption through the interaction with the parathyroid glands, kidneys, and bowel. A relationship between vitamin D deficiency and the prevalence of some autoimmune diseases like diabetes, multiple sclerosis, lupus, arthritis has been demonstrated, suggesting a contributory role of vitamin D in the pathophysiology of autoimmune diseases (Berrih-Aknin 2014). In a pilot study, we showed that plasma vitamin D levels are significantly lower in patients with MG compared with healthy controls. In addition, vitamin D has beneficial effects on the autoimmune response and on fatigue score in patients with MG (Askmark, Haggard et al. 2012). A study in another cohort including 100 MG patients in collaboration with Pr Y. Shoenfeld (Tel Hashomer Hospital) revealed that female patients untreated with corticosteroids had low level of Vitamin D compared to age- and sex-matched controls, while there is no difference in males. In addition, patients with corticosteroid did not show abnormal level of vitamin D, probably because most of these patients are supplemented with vitamin D during their corticosteroid treatment.

These studies suggest that, similarly to other Autoimmune Diseases, vitamin D deficiency could affect MG risk and progression and could represent an additional predisposing factor in the development of MG.

2.4 How studies on monozygotic twins can help to understand the contribution of genetics and environment?

Identical twins concordance rate in MG is about 35%, indicating that MG pathogenesis, as in many other autoimmune diseases, comprises genetic, environmental, as well as presumed epigenetic components (Avidan, Le Panse et al. 2013). Environmentally induced methylation changes in specific immune cell subsets could affect the expression and function of genes, and could be involved in the pathogenesis of MG. Studies of monozygotic (MZ) twins offer a unique opportunity to identify genetic factors of underlying disease and to estimate the contribution of epigenetics to disease risk or course. Since the genetic and epigenetic changes in cell subsets may be masked in the mixed population of peripheral blood mononuclear cells (PBMCs), an immune cell-specific approach is important.

Fifteen MG twin pairs across Europe and America filled a specific questionnaire: 5 concordant and 10 discordant, of which 3 concordant and 4 discordant females pairs were recruited for the epigenetic study. The unique recruitment of these rare MG twin pairs consent is due to worldwide collaboration during the FIGHT-MG (FP7) project. We organized the collect of blood samples for the different pair

of twins and purified the main peripheral blood cell subsets to prepare DNA and RNA for the epigenetic and transcriptome studies, respectively.

Hundreds of deregulated genes (DEGs) and thousands of deregulated methylated regions (DMGs) were found differently expressed between healthy and MG patients, in the difference cell subsets (CD4, CD8, B cells, monocytes). In brief, this study revealed that healthy twin had an epigenetic profile very similar to his MG twin, highlighting his high risk to the development of the disease. However, many genes were found differentially expressed between the healthy and the MG twin. These genes are particularly relevant to explain the onset of the disease in one of the twins, and need further investigations.

Altogether, these results generated very large sets of data, and allowed to identify genes that could explain why a monozygotic twin developed a myasthenia disease while his twin does not. This work not yet published has been disseminated via an interview and video (Youris.com).

3. How does MG develop?

Several defects in the immune system have been described in MG disease. The thymus presents frequent thymic abnormalities, namely thymic follicular hyperplasia in the young patients and thymoma in the older ones. The pathogenic mechanisms explored in FIGHT-MG included several molecules contributing to immune regulations. Among them, we have investigated the role of IL-17, VAV1 and AIRE. We have also addressed the role of miRNAs that play a major role in basic cell biology, such as proliferation, function or death.

3.1 Role of Th17 cells

Treg cells have a major role in the induction of tolerance by reducing the proliferation of effector T cells. In an inflammatory environment, Treg cells could become pathogenic and express a Th17 profile. We showed that Treg cells from MG patients were defective in suppressing the proliferation of control T conventional cells (Tconv), and we demonstrated that MG Tconv cells were resistant to Treg cell suppression (Gradolatto, Nazzari et al. 2014). To investigate the factors that could explain these differences, we analyzed the transcriptomes of purified thymic Treg and Tconv cells from MG patients in comparison to CTRL cells. Many of the pathways revealed by this analysis are involved in other autoimmune diseases, and T cells from MG patients exhibit a Th1 (inflammatory), Th17 (inflammatory) and Tfh (follicular helper) signature. Since Th1 and Th17 cells have strong inflammatory properties, these results might enlighten the chronic activation observed in MG patients that could explain the escape of thymic T cells from regulation in the MG thymus.

3.2 Investigation of Vav-1 in the susceptibility to MG

Vav1 has a key role in T cell antigen receptor signaling in natural Tregs development and susceptibility to T cell mediated diseases (Colacios, Casemayou et al. 2011). We demonstrated that the Vav1^{R63W} mutation: (1) is responsible for high numbers of thymic and peripheral Foxp3+Treg cells; (2) impacts on thymic development of conventional T cells and favors their commitment into Th2

phenotype (3) diminishes the susceptibility to CNS inflammation by reducing the production of encephalitogenic cytokines by autoreactive CD4 T cells; (4) enhances the susceptibility to experimental autoimmune myasthenia gravis by impacting thymic negative selection; (5) reduces Vav1 adaptor functions without affecting its enzymatic activity (GEF). We also set-up original technologies concerning the purification and characterization of Vav1 signalosome in primary CD4 T cells and obtained exciting results concerning the Vav1 signalosome in Tconv cells. Based on these studies, we provided evidence that the interaction between the Vav1^{R63W} and Themis is mandatory for Treg suppressive function (*Pedros et al in preparation*). In the future, this new technology will be used to compare Vav1 interactome between Treg and Tconv and the impact of Vav1^{R63W} on its composition. The knowledge of Vav1 pathways in these studies will allow testing the Vav1 partners in association studies in human MG. Interestingly VAV1 is one of the genes discovered in the genetic study, for its significant association with MG (Avidan, Le Panse et al. 2014).

3.3 Role of AIRE

We showed that the transcription factor AIRE is involved in the susceptibility of MG. More generally, AIRE seems also to be implicated in the susceptibility of women to autoimmune diseases. Aire knockout (KO) mice as well as mouse strains that are susceptible to experimental autoimmune myasthenia gravis (EAMG) have lower thymic expression of acetylcholine receptor (AChR- the main autoantigen in MG), compared to wild type (WT) mice and EAMG-resistant mouse strains, respectively. In addition, Aire KO mice have a significant and reproducible lower frequency of CD4⁺Foxp3⁺ cells and a higher expression of Th17 markers in their thymus, compared to wild type (WT) mice. These findings led us to expect that Aire KO mice would display increased susceptibility to EAMG. Surprisingly, when EAMG was induced in young (2 month-old) mice, EAMG was milder in Aire KO than in WT mice for several weeks until the age of about 5 months. However, when EAMG was induced in relatively aged (6 month-old) mice, Aire KO mice presented higher disease severity than WT controls. This age-related change in susceptibility to EAMG correlated with an elevated proportion of Treg cells in the spleens of young but not old KO, compared to WT mice, suggesting a role for peripheral Treg cells in the course of disease (Aricha, Feferman et al. 2011). Our observations point to a possible link between Aire and Treg cells and suggest an involvement for both in the pathogenesis of myasthenia.

3.4 Role of miRNA

MiRNAs are small RNAs that play a major role in the regulation of genes and proteins. The thymic miRnome of different categories of MG patients versus controls was analyzed by microarrays. This work required several technical challenging steps that were successful. These experiments generated large sets of data. Many miRNA differentially expressed in MG patients were discovered. As an example, for miRNAs differentially expressed in MG patients versus woman controls, 53 small miRNAs were identified as significantly dysregulated with 20 and 33 that were up-regulated and down-regulated in MG, respectively. Several of these dysregulated miRNAs were validated by PCR: miR-486-3p and miR-196b were clearly confirmed up- and down-regulated in MG patients, respectively.

These first analyses revealed already very interesting observations, but many other analyses need to be done. In addition, the potential role of the deregulated MiR in the pathogenesis of MG needs further investigation.

4. What happens in the muscle of MG patients?

Myasthenia Gravis (MG) patients suffer from chronic fatigue of skeletal muscles, even after initiation of proper immunosuppressive medication. Although the antibodies to molecules of the neuromuscular endplate (AChR, MuSK, LRP4) are known to be pathogenic, their mechanism of actions on muscle function, and the molecular mechanisms associated were almost unknown. Several models and approaches were developed to answer these questions.

4.1 Changes in the neuromuscular junction in a mouse model with anti-MuSK antibodies

A mouse model (EAMG) with anti-MuSK antibodies (MuSK+) has been successfully established. These mice have detectable serum antibodies against MuSK and display muscle weakness/fatigue in the facial, bulbar and paraspinal muscles, which resembles the human disease. At the neuromuscular junction of the MuSK-MG model, the number and size of acetylcholine receptors (AChR) were decreased, and in many cases, the AChRs appeared fragmented (Punga, Lin et al. 2011). Muscles with low muscle-intrinsic MuSK levels, such as the masseter, were more vulnerable to the postsynaptic perturbation of MuSK antibodies with subsequent denervation and atrophy, indicating differential response of the different muscles in the disease (Punga, Maj et al. 2011). These findings augment the understanding of the sometimes severe, facio-bulbar phenotype of MuSK+ MG.

4.2 Role of NOS in the anti-AChR model

The chronic fatigue in MG is puzzling since it remains although immunosuppressive medication has been initiated. Since the localization of neuronal nitric oxide synthase (nNOS) at the muscle membrane is important for sustained muscle contraction, we studied the localization of nNOS in muscles from mice with AChR experimental autoimmune MG (EAMG). At EAMG disease grade 3 (severe myasthenic weakness), the triceps, sternomastoid and masseter muscles were collected for analysis. Immunohistological and biochemical analysis showed that nNOS was lost from the muscle membrane and accumulated in the cytosol, which is likely the consequence of blocked neuromuscular transmission. Atrophy of all examined EAMG muscles were supported by up-regulated transcript levels of the atrogenes atrogin-1 and MuRF1, as well as MuRF1 protein, in combination with reduced muscle fiber diameters (Meinen, Lin et al. 2012). We propose that loss of sarcolemmal nNOS provides an additional mechanism for the chronic muscle fatigue and secondary muscle atrophy in EAMG and MG.

4.3 Satellite cells

The regeneration of affected muscle is carried out by local stem cells called satellite cells (SC), however, molecular and cellular mechanisms of myogenesis in MG disease were totally unknown. Muscle biopsies from MG patients and healthy age-matched controls were collected. We evaluated the number of satellite cells and their function. In human MG muscle biopsies, we found a higher number of satellite cells compared to non-MG muscle biopsies. To evaluate their function, satellite cells were isolated from the muscle biopsies and cultured to obtain a sufficient number of cells. The analysis of these cells in the MG muscle revealed new and unexpected findings. Even after several

days of culture, SCs from MG patients had a higher proliferation rate, and a higher differentiation index compared to non-MG muscle biopsies. In addition, sera from MG patients and anti-AChR monoclonal antibodies had also a functional effect (increased differentiation) on non-MG SCs, suggesting that the anti-AChR antibodies have a direct effect on the differentiation of satellite cells (Attia et al., ICNMD, 2014).

These findings demonstrate for the first time the activation of SCs in MG muscle as well as functional differences between SC properties from healthy and MG muscles. The autoimmune attack in MG might lead to important changes in the number and function of SC that could represent a mechanism of compensation to regenerate muscle fibres that have been damaged by the autoantibodies. These results obtained *in vitro* are novel and bring new hypothesis on the mechanism of action of the anti-AChR antibodies.

4.4 Pathogenic factors in the sera

MG is due to autoantibodies directed to proteins of the muscle endplate. However, whether the pathogenic factor is included exclusively in the antibodies was not demonstrated, especially in the MuSK-MG form. Whole sera, purified anti-MuSK antibodies, or fraction without antibodies were injected in mice to identify the pathogenic fraction. It was shown that the pathogenic agent was in the anti-MuSK antibody fraction only. Similar studies were performed in AChR-MG patients, and showed similar results. The removal of the antibodies was sufficient to prevent the onset of symptoms. Furthermore, it was shown that the autoantibodies vary in pathogenicity depending on their target specificity, since the antibodies directed against the α AChR subunit were more pathogenic than antibodies directed against the β subunit, shedding light into the underlying pathophysiology of MG (Kordas et al. submitted).

4.5 MiRNA analysis in the muscle

MicroRNAs (miRNAs) are an abundant class of non-coding RNAs that are important in many biological processes due to their ability to regulate gene expression. miRNA molecules play an important role in cell growth, differentiation, proliferation, apoptosis. It therefore seems likely that miRNA expression levels can be used as novel diagnostic markers.

miRNA distribution was analyzed in muscle samples in both MG patients and experimental autoimmune MG in rats, using miRNA chips including all the miRNA available. These studies included several technical challenging steps and generated large sets of data. In human and rat studies, lists of miRNA deregulated in MG were identified. Interestingly, three miRNAs appear common between human and rat: miR19b, miR92a and miR185. Although the analysis of these specific miRNA was never addressed in the muscle, these results are promising to identify their role in the pathogenic mechanisms occurring in the muscle after the autoimmune attack. Whether these molecules play a role in the pathogenesis or the compensatory mechanisms in MG need further investigation. Validation steps and functional analysis are still ongoing.

5. How to improve the diagnostic and monitoring?

Autoimmune MG is characterized by muscle weakness and is due to autoantibodies against components of the neuromuscular junction. Most autoantibodies are directed against the acetylcholine receptor (AChR) and sometime against the Muscle-specific kinase (MuSK). However, some MG patients are still not diagnosed because the autoantibody target is not known. Moreover MG is a fluctuating disease and no biological markers are available for the follow-up of MG patients as autoantibody titers are not well correlated with the severity of the disease. Several approaches were developed to improve diagnosis and monitoring.

5.1 New sensitive assays

Cell based assays are very sensitive and were developed for the detection of anti-LRP4, anti-AChR and anti-MuSK antibodies. These assays were validated using several hundreds of sera from MG patients, patients with other neuroimmune diseases and healthy controls obtained from partners of FIGHT-MG and collaborating partners. These new very sensitive diagnostic assays have been validated and can detect low titer of antibodies from patients with previously undetectable antibodies (Trakas, Zisimopoulou et al. 2011) (Zisimopoulou, Evangelakou et al. 2013).

5.2 LRP4, a novel antigen in MG

A novel autoantigen, LRP4, has been discovered and efforts were made to develop a sensitive diagnostic assay for this antigen. Samples from MG patients were collected, from seronegative-MG (SNMG), patients in order to perform large scale screenings and epidemiological analysis of LRP4-MG. From a cohort of 635 seronegative MG (SNMG) patients that represent about 10% of MG patients, obtained from 10 countries including six partners of FIGHT-MG, the overall frequency of LRP4-MG was 18.7%. At disease onset, symptoms were mild (81% had MGFA grade I or II), with some identified thymic changes (32% hyperplasia, none with thymoma). Contrary to MuSK-MG patients, 27% of ocular SNMG patients were LRP4 antibody positive. The prevalence was higher in women than in men (female/male ratio 2.5/1), with an average disease onset at ages 33.4 for females and 41.9 for males. This study allowed a better clinical characterization of the LRP4-MG subgroup.

These data will contribute to improving diagnosis and identification of new subgroup of MG patients (Zisimopoulou, Evangelakou et al. 2013).

5.3 New seric biomarkers

5.3.1 Proteomic Studies

We performed a proteomic analysis from the sera in order to define new markers to diagnose patients that are seronegative for AChR and MuSK antibodies, associated with the severity grade in MG patients, and serum proteins targeted by glucocorticoid treatment and that could be involved in the beneficial effect of this treatment.

Representative samples from five groups of patients (SNMG ocular, SNMG generalized, SPMG ocular, SPMG generalized, controls) have been used for a differential proteomic approach towards protein biomarkers distinguishing the various disease conditions. To reduce the background, the seven most

abundant serum proteins were removed by an affinity procedure. Dual radioisotope labeling and subsequent 2D-PAGE and mass spectrometry revealed a set of 10-15 plausible biomarker candidates. Three proteins were selected because they showed interesting differences in the proteomic analyses and were analyzed in larger groups of patients by ELISA: **Alpha 1-antitrypsin (A1AT)**, **Complement C9 (C9)** and **Vitamin D binding protein (VitD BP)**. A1AT is a protease inhibitor. Serum levels of A1AT were significantly increased in all patients with MG. However, A1AT was especially discriminating for SN patients compared to controls. VitD BP binds vitamin D metabolites but could have other functions. Serum levels of VitD BP were significantly lower in patients with MG (especially AChR+ patients) compared to controls. Moreover, the levels were especially lower in ocular forms of the disease. C9 complement is a member of the membrane attack complex. Serum levels of C9 were significantly higher in SN patients compared to AChR+ patients, suggesting a different involvement of the complement cascade in these two MG groups (Schrattenholz et al, Myasthenia 2013, Paris).

Altogether, these data point out biomarker candidates to discriminate the different subgroups of MG patients. Our preliminary analyzes pointed out severity markers that could be helpful to follow the evolution of MG for each patient, since few biomarkers appear differently expressed in ocular versus generalized forms.

5.3.2 Circulating miRs

168 miRNAs were analyzed in serum samples from four AChR+ MG patients and four healthy controls using Exiqon Focus miRNA PCR. Specific accumulation pattern of 13 miRNAs from the discovery set was subsequently investigated in the sera of 16 AChR+ MG patients and 16 healthy controls. All patients were without immunosuppressive treatment. Selected specific miRNAs were further analyzed in the serum of nine MG patients before and after thymectomy to assess the effect of thymus removal on the accumulation of the candidate miRNAs in patient sera. Three miRNAs were specifically dysregulated in AChR+ MG patient sera samples. **Hsa-miR150-5p**, which induces T-cell differentiation, as well as **hsa-miR21-5p**, a regulator of Th1 versus Th2 cell responses, were specifically elevated in MG sera. Additionally, **hsa-miR27a-3p**, involved in natural killer (NK) cell cytotoxicity, was decreased in MG. Hsa-miR150-5p levels had the highest association with MG and were significantly reduced after thymus removal in correlation with disease improvement (Punga, Le Panse et al. 2014 (in press)).

We propose that the validated miRNAs: hsa-miR150-5p, hsa-miR21-5p, and hsa-miR27a-3p can serve as novel serum biomarkers in AChR+ MG. Hsa-miR-150-5p could be a helpful marker to monitor disease severity.

5.4 How to detect the patients non-responders to Azathioprine?

Azathioprine (AZA) is an immunosuppressant drug used to treat autoimmune diseases such as MG. Some MG patients are intolerant to this drug. AZA is mainly catabolized through the thiopurine S-methyltransferase (TPMT) and xanthine oxidase (XO) pathway. The *TPMT* gene SNPs was first analyzed in Italian MG patients and a new haplotype was identified, designated haplotype *TPMT*3E*, which includes known haplotype *TPMT*3A* in association with intronic polymorphism T140+114A. To identify other genes involved in drug metabolism in MG patients, a total of 140 MG patients were genotyped, including 112 patients treated with azathioprine and glucocorticosteroids for a period ranging from 9 days to 14 years and 28 MG controls who had not taken azathioprine, by using DMET

gene chip. This is an Affymetrix GeneChip, which covers a large repertoire of common SNP variants for a wide range of drugs. By applying the multifactorial dimensionality reduction (MDR) tool we selected four multilocus SNPs as statistically significant predictors of responsive patients (rs3869579, CYP2A7 gene; rs1143672, SLC15A2 gene; rs7751481, PPARD gene and rs1045642, ABCB1 gene) (Colleoni, Kapetis et al. 2013).

In conclusion, these results demonstrated that variants in genes involved in metabolite transport, together with those in TPMT gene, are able to predict the response to azathioprine in MG patients, paving the way towards a 'personalized' treatment in MG.

6. How to improve therapies in MG?

Current treatments do not lead to remission and entail considerable side-effects stressing the need for improved therapies. Several therapies targeting different types of molecules were addressed.

6.1 Regulation of immune responses

Since Immune responses are dysregulated in MG, therapies aiming to regulate immune responses, and to stimulate Treg cells were tested. Suppressive regulatory T cells (Treg) and pathogenic T helper 17 (Th17) cells are two lymphocyte subsets with opposing activities in autoimmune diseases, and the balance between these 2 subsets are major to maintain tolerance.

6.1.1 Antibodies to IL-6

The proinflammatory cytokine IL-6 is a potent factor in switching immune responses *in vivo* from the induction of Treg to pathogenic Th17 cells. We studied the Treg and Th17 cell compartments in experimental autoimmune myasthenia gravis (EAMG) and healthy control rats in order to assess whether the equilibrium between Treg and Th17 cells is perturbed in the disease. We found that Th17 cell-related genes are upregulated and Treg-related genes are downregulated in EAMG. The shift in favor of Th17 cells in EAMG could be reversed by antibodies to IL-6. **Administration of anti-IL-6 antibodies to myasthenic rats suppressed EAMG when treatment started at the acute or at the chronic phase of disease.** Suppression of EAMG by anti-IL-6 antibodies was accompanied by a decrease in the overall rat anti-AChR antibody titer and by a reduced number of B cells as compared with control treatment. Administration of anti-IL-6 antibodies led to down-regulation of several Th17 related genes including IL-17, IL-17R, IL-23R and IL-21 but did not affect the number of Treg cells in the lymph nodes. These data identify IL-6 as an important target for modulation of autoimmune responses (Aricha, Mizrachi et al. 2011).

6.1.2 Treg cell therapy

Treg cells transferred from healthy rat donors to myasthenic rats suppress EAMG. However, Treg cells from sick animals do not have the same *in vivo* suppressive activity as those from healthy donors. The frequency of Treg cells within the spleen and PBL was decreased in EAMG rats as compared to naive and CFA-immunized healthy controls. Treg cells from myasthenic rats were less

effective than Treg cells from controls in suppressing the proliferation of CD4(+)T effector cells in response to ConA and of B cells in response to LPS. Moreover, Treg cells from EAMG rats exhibited an elevated extent of apoptosis and expressed upregulated levels of FAS and of Th17-associated cytokines (Gertel-Lapter, Mizrahi et al. 2013). Since EAMG is an induced disease, these quantitative and qualitative alterations in Treg cells do not reflect predisposing impairments and seem to be associated with the specific autoimmune response resulting from AChR immunization. These results indicate that the inflammatory environment linked to the autoimmune response could alter the Treg cells used for therapies. However using a high number of Treg cells appear to be efficient to suppress EAMG. To this end, a high number of Treg cells were obtained by coculture with bone marrow (BM) cells and were used to treat EAMG in rats. The treatment was very efficient: the disease suppression was accompanied by reduced levels of total AChR specific antibodies in the serum and elevated numbers of Treg cells in the spleen.

6.1.3 Mesenchymal Stem Cells (MSCs)

MSC are multipotent progenitor cells that have been shown to possess broad immunoregulatory and anti-inflammatory capabilities, making them a promising tool to treat autoimmune diseases (Ben-Ami, Berrih-Aknin et al. 2011). Among their mechanism of actions, MSC appear to decrease the expression of the CD8 molecule as well as of co-stimulatory molecules such as CD28. In addition, the effects of MSC appear to be mediated by antigen-presenting cells (Hof-Nahor, Leshansky et al. 2012).

We established an animal model that recapitulate all MG features from thymus abnormalities to clinical signs, in a human immune system in order to test mesenchymal cell therapies. As MG thymus provides all the cellular elements required for the autoimmune reaction, including B cells that produce anti-AChR antibodies, thymus fragments from AChR-seropositive patients were subcutaneously grafted into NOD-*scid* IL2Rgamma^{null} (NSG) immunodeficient mice. We observed that ectopic thymus is preserved and human cells can be maintained in mice for several weeks. We could thus conclude that the MG-NSG model is pertinent for the investigation of cellular therapeutic approaches.

Ben Ami et al. demonstrated that MSC inhibit less efficiently the proliferation of T cells from MG patients than T cells from healthy control (Ben-Ami, Miller et al. 2014). It was hypothesized that MSC need to be activated to be fully effective. Thus we compared the immunosuppressive effect of MSC previously activated (conditioned MSC or cMSC) or not (resting MSC or rMSC) *in vitro* in the MG-NSG model.

We observed that **MSC treatment reduced MG clinical signs occurrence and prevented endplate AChR loss**. Whatever the parameter analyzed, the effect of conditioned MSC was superior to that of resting MSC. These data are very promising for the development of new cell therapies for human MG, and more generally for autoimmune diseases.

6.2 Elimination of pathogenic antibodies

We aimed to express recombinant domains of the AChR and MuSK proteins, in order to immobilize them onto a suitable matrix, which will be used for the antigen-specific clearance of MG patients' sera from the pathogenic autoantibodies (immunoabsorption). We have created mutant forms of the extracellular domains of the human AChR and MuSK proteins, which are easier to express for large-

scale applications, such as the antigen-specific therapy proposed herein (Lagoumintzis, Zisimopoulou et al. 2010). We have shown that these mutants are equally efficient or better at autoantibody binding than their wild type counterparts. Furthermore, we addressed safety aspects with respect to adsorbent stability and the aphaeresis procedure using immunized rabbits, and evaluated the efficiency of immunoadsorption *in vivo* using immunized rats. It, therefore, appears that the proposed method is feasible and safe, for use in MG therapy, and the progression towards clinical trials will allow the final assessment of the procedure before it can be applied in the clinic (Lazaridis, Zisimopoulou et al. 2012) (Lagoumintzis, Zisimopoulou et al. 2014).

6.3 Regulation of plasminogen activator System

Tissue plasminogen activator (tPA), a component of the PA/plasmin system, is elevated in inflammatory areas and plays a role in inflammatory neurological disorders (Gur-Wahnon, Mizrahi et al. 2013). We explored the involvement of tPA and the potential immunomodulatory activity of tPA in experimental autoimmune myasthenia gravis (EAMG). Mice deficient in tPA (tPA^{-/-}) present with a markedly more severe disease than wild type EAMG mice. In an attempt to treat EAMG with an 18aa peptide derived from the PA system inhibitor (PAI-1), designed to tether out the endogenous inhibitor, a significant suppression of disease severity was demonstrated. The more severe disease in tPA^{-/-} mice was accompanied by a higher level of anti-AChR antibodies and increased expression of B-cell markers. In view of the essential role of B-cell activating factor (BAFF) in B-cell maturation, the expression of BAFF family components was tested. An increase in BAFF and BAFF receptor was observed in EAMG tPA^{-/-} mice, whereas BCMA expression was reduced, consistent with the increased level of pathogenic antibodies and the more severe disease. Given the importance of T regulatory cells (Tregs) in EAMG, they were evaluated and their number was reduced in tPA^{-/-} mice, in which EAMG was aggravated, whereas following PAI-1dp treatment, Tregs were replenished and the disease was ameliorated (Gur-Wahnon, Mizrahi et al. 2014).

The results show the involvement of tPA in EAMG, implying a **protective role for tPA in EAMG pathogenesis**. The amelioration of EAMG by PAI-1dp treatment suggests that the PA system may be considered a potential site for therapeutic intervention in neuroimmune diseases.

6.4 Targeting epigenetic regulators

Epigenetic research has experienced a major revolution in the past few years. This is largely due to the recognition of the role of epigenetic alterations in a growing number of human diseases. Epigenetics is defined as heritable changes in gene activity and expression that occur without alteration in DNA sequence. It is known these non-genetic alterations are tightly regulated by two major epigenetic modifications: histone modifications and DNA methylation.

Azacytidine (AzaC) is a chemical analogue of cytosine, a nucleoside present in DNA and RNA. It is a hypomethylating agent, after it has been metabolized, it can become a substrate for the DNA replication machinery and it is incorporated into DNA, as a consequence, methylation marks become lost during DNA replication upon treatment with AzaC.

AzaC has been reported to induce Foxp3 expression in CD4⁺CD25⁻ T cells both *in vitro* and *in vivo*. It is known that T regulatory (Treg) cells play a major role in EAMG. We tested the efficacy of AzaC as an epigenetic modulator of EAMG. Lewis rat were treated with AzaC by i.p injections, 6 times per week for a period of 4 weeks. Treatment started at the acute phase of the disease, 2 days after

disease induction. The preliminary data shows that treatment with AzaC at the acute phase of the disease ameliorates the course of EAMG. Moreover, 50% of the AzaC treated rats were totally healthy as compared to the untreated EAMG group where 100% developed significant myasthenic symptoms. These results suggest that targeting epigenetic regulators is also an alternative for new therapy in MG.

7. Ethical issues

Ethical issues have been a priority during the whole work of Fight-MG consortium. A specific ethics presentation has been held at each meeting. All ethical documents required in the project synopsis have been provided.

All ethical requirements according to national and international regulations have been fulfilled in the project. Moreover, at the last meeting, discussion focused on future ethical challenges particularly concerning the common database of the consortium, with the help and advice of EAB Eugenia Lamas.

Anticipation and prevention of possible ethical problems has been a constant objective. The most challenging part of the consortium's work lays in the use of personal data across borders. For each particular study involving patients' samples/data ethical consents of patients have been scrupulously obtained. Great care has been taken in order to avoid non-compliance with any national/European requirement, particularly in studies involving inclusion from different nationalities (ex. the twin study).

The future use of the existing database has also been thoroughly discussed in an ethical perspective. A discussion led by EAB Eugenia Lamas was specifically devoted to this topic during the final meeting. An agreement between the partners was elaborated so that the database may continue to be used until at least 2024.

Expected final results and impacts

Our proposal aimed to shed light on the course of MG, but also on the etiological and pathological mechanisms of MG. The expected impacts are as follows:

1. The **epidemiological studies** improved the knowledge on clinical, psycho-social and environmental factors affecting the course of MG, and revealed that hormonal changes and smoking are associated with the development of MG. A solid EuroMG DB was successfully developed. It includes already 4300 MG patients, and all the partners included in FIGHT-MG agreed for a continuation of the database after the end of FIGHT-MG project.
2. At the level of **etioloical factors**, this research improved significantly our knowledge of the molecular events associated with the origin of MG diseases, including genetic and environmental factors. The role of EBV infection, and of interferon type I was demonstrated. In the same time, the genetic studies revealed that the genetic contribution is important: new genes associated with MG were discovered, and monozygotic twin without MG has an epigenetic signature very similar to his myasthenia twin. This study on monozygotic twins identified a list of genes that are differentially expressed between the healthy and the MG twin, these genes could explain how and why the disease starts in one of the twin and not the other.
3. At the level of the **pathological mechanism** at the neuromuscular junction, this project provided knowledge on the molecular mechanisms occurring at the neuro-muscular junction and on the stability of the endplates after the autoimmune attack. The role of satellite cells and the contribution of small RNA (miRNA) were also investigated for the first time and identified new pathogenic pathways. In the immune system, we could show the contribution of the Th17 cells and Toll like receptors in the physiopathology of human MG and their role in the thymus pathology. The role of two other molecules AIRE and VAV1 were investigated and found to play a role in autoimmunity and regulation of immune responses.
4. At the **diagnostic and monitoring levels**, the antibodies were found to be pathogenic, while protective factors in the serum could not be demonstrated. Resistance to Azathioprine treatment was associated with a mutated genotype of the TPMT (thiopurine S-methyltransferase) gene. This knowledge could be applied in the clinical practice. Finally, the development of new cell-based assays increased sensitivity of the diagnostic tests for AChR and MuSK, as well as the novel anti-LRP4 assay allow to reduce significantly the number of undiagnosed patients.
5. At the level of **innovative therapies**, our project included several novel pharmacological, molecular and cell-based therapies. New pre-clinical models were developed, and several therapies were efficient. Among cell therapies, use of Treg cells or Mesenchymal Stem cells appear to be highly relevant for MG disease. The use of plasminogen mutant protein was promising in the rat model. A new approach using a hypomethylating agent was also innovative. The important ramifications of this network with associations of patients and many neurologists through this European project will facilitate translational research.

Publications

- Aricha, R., T. Feferman, H. S. Scott, M. C. Souroujon, S. Berrih-Aknin and S. Fuchs (2011). "The susceptibility of Aire(-/-) mice to experimental myasthenia gravis involves alterations in regulatory T cells." *J Autoimmun* **36**(1): 16-24.
- Aricha, R., K. Mizrachi, S. Fuchs and M. C. Souroujon (2011). "Blocking of IL-6 suppresses experimental autoimmune myasthenia gravis." *J Autoimmun* **36**(2): 135-141.
- Askmark, H., L. Haggard, I. Nygren and A. R. Punga (2012). "Vitamin D deficiency in patients with myasthenia gravis and improvement of fatigue after supplementation of vitamin D3: a pilot study." *Eur J Neurol* **19**(12): 1554-1560.
- Avidan, N., R. Le Panse, S. Berrih-Aknin and A. Miller (2013). "Genetic basis of myasthenia gravis - A comprehensive review." *J Autoimmun*.
- Avidan, N., R. Le Panse, H. F. Harbo, P. Bernasconi, K. Poulas, E. Ginzburg, P. Cavalcante, L. Colleoni, F. Baggi, C. Antozzi, F. Truffault, S. Horn-Saban, S. Pöschel, Z. Zagoriti, A. Maniaol, B. A. Lie, I. Bernard, A. Saoudi, Z. Illes, C. Casasnovas Pons, A. Melms, S. Tzartos, N. Willcox, A. Kostera-Pruszczyk, C. Tallaksen, R. Mantegazza, S. Berrih-Aknin and A. Miller (2014). "VAV1 and BAFF, via NF κ B pathway, are genetic risk factors for myasthenia gravis." *Annals of Clinical and Translational Neurology*.
- Ben-Ami, E., S. Berrih-Aknin and A. Miller (2011). "Mesenchymal stem cells as an immunomodulatory therapeutic strategy for autoimmune diseases." *Autoimmun Rev* **10**(7): 410-415.
- Ben-Ami, E., A. Miller and S. Berrih-Aknin (2014). "T cells from autoimmune patients display reduced sensitivity to immunoregulation by mesenchymal stem cells: role of IL-2." *Autoimmun Rev* **13**(2): 187-196.
- Berrih-Aknin, S. (2014). "Myasthenia Gravis: Paradox versus paradigm in autoimmunity." *J Autoimmun*.
- Cavalcante, P., P. Cufi, R. Mantegazza, S. Berrih-Aknin, P. Bernasconi and R. Le Panse (2013). "Etiology of myasthenia gravis: innate immunity signature in pathological thymus." *Autoimmun Rev* **12**(9): 863-874.
- Cavalcante, P., R. Le Panse, S. Berrih-Aknin, L. Maggi, C. Antozzi, F. Baggi, P. Bernasconi and R. Mantegazza (2011). "The thymus in myasthenia gravis: Site of "innate autoimmunity"?" *Muscle Nerve* **44**(4): 467-484.
- Cavalcante, P., L. Maggi, L. Colleoni, R. Caldara, T. Motta, C. Giardina, C. Antozzi, S. Berrih-Aknin, P. Bernasconi and R. Mantegazza (2011). "Inflammation and Epstein-Barr virus infection are common features of myasthenia gravis thymus: possible roles in pathogenesis." *Autoimmune Dis* **2011**: 213092.
- Cavalcante, P., B. Serafini, B. Rosicarelli, L. Maggi, M. Barberis, C. Antozzi, S. Berrih-Aknin, P. Bernasconi, F. Aloisi and R. Mantegazza (2010). "Epstein-Barr virus persistence and reactivation in myasthenia gravis thymus." *Annals of Neurology* **67**(6): 726-738.
- Colacios, C., A. Casemayou, A. S. Dejean, F. Gaits-Iacovoni, C. Pedros, I. Bernard, D. Lagrange, M. Deckert, L. Lamouroux, M. Jagodic, T. Olsson, R. S. Liblau, G. J. Fournie and A. Saoudi (2011). "The p.Arg63Trp polymorphism controls Vav1 functions and Foxp3 regulatory T cell development." *J Exp Med* **208**(11): 2183-2191.
- Colleoni, L., D. Kapetis, L. Maggi, G. Camera, E. Canoni, P. Cavalcante, N. Kerlero de Rosbo, F. Baggi, C. Antozzi, P. Confalonieri, R. Mantegazza and P. Bernasconi (2013). "A new thiopurine S-methyltransferase haplotype associated with intolerance to azathioprine." *J Clin Pharmacol* **53**(1): 67-74.
- Cordiglieri, C., R. Marolda, S. Franzini, C. Cappelletti, C. Giardina, T. Motta, F. Baggi, P. Bernasconi, R. Mantegazza and P. Cavalcante (2014). "Innate immunity in myasthenia gravis thymus: Pathogenic effects of Toll-like receptor 4 signaling on autoimmunity." *J Autoimmun*.
- Cufi, P., N. Dragin, N. Ruhlmann, J. M. Weiss, E. Fadel, A. Serraf, S. Berrih-Aknin and R. Le Panse (2014). "Central role of interferon-beta in thymic events leading to myasthenia gravis." *J Autoimmun*.
- Cufi, P., N. Dragin, J. M. Weiss, P. Martinez-Martinez, M. H. De Baets, R. Roussin, E. Fadel, S. Berrih-Aknin and R. Le Panse (2013). "Implication of double-stranded RNA signaling in the etiology of autoimmune myasthenia gravis." *Ann Neurol* **73**(2): 281-293.
- Cufi, P., P. Soussan, F. Truffault, R. Fetouchi, M. Robinet, E. Fadel, S. Berrih-Aknin and R. Le Panse (2014). "Thymoma-associated myasthenia gravis: On the search for a pathogen signature." *J Autoimmun*.
- Fulvio, B. and R. Mantegazza (2014). "European Database for Myasthenia Gravis: A model for an international disease registry." *Neurology* **83**(2): 189-191.
- Gertel-Lapter, S., K. Mizrachi, S. Berrih-Aknin, S. Fuchs and M. C. Souroujon (2013). "Impairment of regulatory T cells in myasthenia gravis: studies in an experimental model." *Autoimmun Rev* **12**(9): 894-903.
- Gradolatto, A., D. Nazzari, F. Truffault, J. Bismuth, E. Fadel, M. Foti and S. Berrih-Aknin (2014). "Both Treg cells and Tconv cells are defective in the Myasthenia gravis thymus: Roles of IL-17 and TNF-alpha." *J Autoimmun*.

Gur-Wahnon, D., T. Mizrahi, F. Y. Maaravi-Pinto, A. Loubopoulos, N. Grigoriadis, A. A. Higazi and T. Brenner (2013). "The plasminogen activator system: involvement in central nervous system inflammation and a potential site for therapeutic intervention." *J Neuroinflammation* **10**: 124.

Gur-Wahnon, D., T. Mizrahi, S. Wald-Altman, A. Al-Roof Higazi and T. Brenner (2014). "Tissue plasminogen activator involvement in experimental autoimmune myasthenia gravis: Aggravation and therapeutic potential." *J Autoimmun.*

Hof-Nahor, I., L. Leshansky, S. Shvitiel, L. Eldor, D. Aberdam, J. Itskovitz-Eldor and S. Berrih-Aknin (2012). "Human mesenchymal stem cells shift CD8+ T cells towards a suppressive phenotype by inducing tolerogenic monocytes." *J Cell Sci* **125**(Pt 19): 4640-4650.

Lagoumintzis, G., P. Zisimopoulou, G. Kordas, K. Lazaridis, K. Poulas and S. J. Tzartos (2010). "Recent approaches to the development of antigen-specific immunotherapies for myasthenia gravis." *Autoimmunity* **43**(5-6): 436-445.

Lagoumintzis, G., P. Zisimopoulou, N. Trakas, E. Grapsa, K. Poulas and S. J. Tzartos (2014). "Scale up and safety parameters of antigen specific immunoadsorption of human anti-acetylcholine receptor antibodies." *J Neuroimmunol* **267**(1-2): 1-6.

Lazaridis, K., P. Zisimopoulou, G. Lagoumintzis, L. Skriapa, N. Trakas, P. Evangelakou, I. Kanelopoulos, E. Grapsa, K. Poulas and S. Tzartos (2012). "Antigen-specific apheresis of autoantibodies in myasthenia gravis." *Ann N Y Acad Sci* **1275**: 7-12.

Maniaol, A. H., M. Boldingh, C. Brunborg, H. F. Harbo and C. M. Tallaksen (2013). "Smoking and socio-economic status may affect myasthenia gravis." *Eur J Neurol* **20**(3): 453-460.

Maniaol, A. H., C. Brunborg and C. M. Tallaksen (2010). "Development and validation of a self-administered questionnaire for myasthenia gravis patients." *Neuroepidemiology* **34**(4): 253-261.

Meinen, S., S. Lin, M. A. Ruegg and A. R. Punga (2012). "Fatigue and muscle atrophy in a mouse model of myasthenia gravis is paralleled by loss of sarcolemmal nNOS." *PLoS One* **7**(8): e44148.

Punga, A. R., S. Lin, F. Oliveri, S. Meinen and M. A. Ruegg (2011). "Muscle-selective synaptic disassembly and reorganization in MuSK antibody positive MG mice." *Exp Neurol* **230**(2): 207-217.

Punga, A. R., M. Maj, S. Lin, S. Meinen and M. A. Ruegg (2011). "MuSK levels differ between adult skeletal muscles and influence postsynaptic plasticity." *Eur J Neurosci* **33**(5): 890-898.

Punga, T., R. Le Panse, M. Andersson, F. Truffault, S. Berrih-Aknin and A. R. Punga (2014 (in press)). "Circulating miRNAs in myasthenia gravis: miR-150-5p as a new potential biomarker." *Annals of Clinical and Translational Neurology*.

Trakas, N., P. Zisimopoulou and S. J. Tzartos (2011). "Development of a highly sensitive diagnostic assay for muscle-specific tyrosine kinase (MuSK) autoantibodies in myasthenia gravis." *J Neuroimmunol* **240-241**: 79-86.

Zisimopoulou, P., P. Evangelakou, J. Tzartos, K. Lazaridis, V. Zouvelou, R. Mantegazza, C. Antozzi, F. Andreetta, A. Evoli, F. Deymeer, G. Saruhan-Direskeneli, H. Durmus, T. Brenner, A. Vaknin, S. Berrih-Aknin, M. Frenkian Cuvelier, T. Stojkovic, M. Debaets, M. Losen, P. Martinez-Martinez, K. A. Kleopa, E. Zamba-Papanicolaou, T. Kyriakides, A. Kostera-Pruszczyk, P. Szczudlik, B. Szyluk, D. Lavrnica, I. Basta, S. Peric, C. Tallaksen, A. Maniaol and S. J. Tzartos (2013). "A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis." *J Autoimmun.*

Main dissemination activities and exploitation of results

- ✚ Project Website: www.fight-mg.eu
- ✚ Altogether 57 publications in peer-reviewed journals were published, and at least 10 other ones are in preparation.
- ✚ Communications via several networks (Commhere, Youris, Horizonhealth.eu) and associations (AFM) led to press releases, interviews, video, articles on Internet network
- ✚ Articles in « VLM » (Vaincre les myopathies)
 - Sonia Berrih-Aknin, sur tous les fronts de la Myasthénie, VLM, 2011
 - Myasthenia 2013 : du nouveau dans la prise en charge des pistes thérapeutiques
 - Posters of AFM presented during meetings between families and clinicians/scientists
- ✚ Press release on the viral etiology of MG:
 - La piste des virus dans les maladies auto-immunes se confirme La Croix
 - L'origine virale de la myasthénie auto-immune se confirme Le quotidien du médecin
 - Maladie auto-immune : la piste virale confirmée (CNRS, INSERM)
 - This press release was disseminated via many websites :
 - ✚ <http://www.science.gouv.fr>
 - ✚ <http://scienceindex.com>
 - ✚ forums.phoenixrising.me
 - ✚ <http://www.mdlinx.com>
 - ✚ <http://medicalxpress.com>
 - ✚ <http://www.techno-science.net>
 - ✚ <http://www.viva.presse.fr>
 - ✚ <http://www.medicalnewstoday.com>
 - ✚ <http://www.medilexicon.com>
 - ✚ Facebook, twitter
- ✚ Interviews
 - MG: from cause to cure (June 2013, Horizonhealth.eu)
 - **How rare models suggest new treatment strategies** (July 2014, Youris) : this interview was disseminated via several websites :
 - ✚ <https://cordis.europa.eu/wire/index.cfm?fuseaction=article.Detail&RCN=45469&rev>
 - ✚ <http://www.topix.com/forum/health/myasthenia-gravis/TMFBI00I4NE357VAU>
 - ✚ <http://medicalxpress.com/news/2014-07-rare-treatment-strategies.html>
- ✚ Videos: **Twins help progress and diagnosis of rare Myasthenia** (July 2014, Youris)
- ✚ **Article: Network of experts join forces to fight rare disease** (July 2014, Youris) disseminated via several websites :
 - ✚ <https://cordis.europa.eu/wire/index.cfm?fuseaction=article.Detail&RCN=45468&rev=0>
 - ✚ <http://www.news-medical.net/news/20140722/2316/Korean.aspx>
 - ✚ <http://pewjournal.com/2014/07/22/network-of-experts-join-forces-to-fight-rare-disease/>
 - ✚ <http://pressgrab.com/FR/paris/?p=212175>
 - ✚ <https://cordis.europa.eu/wire/index.cfm?fuseaction=article.Detail&RCN=45469&rev=0>
 - ✚ <http://www.topix.com/forum/health/myasthenia-gravis/TMFBI00I4NE357VAU>
 - ✚ <http://medicalxpress.com/news/2014-07-rare-treatment-strategies.html>

Project Poster:

Myasthenias: a group of immune mediated neurological diseases: from etiology to therapy

Myasthenia Gravis (MG) is a rare and heterogeneous neuromuscular disease affecting the neuromuscular junction and leading to abnormal fatigability of various skeletal muscles. The molecular events causing and maintaining MG are still unknown and current treatments do not lead to remission and entail considerable side-effects. Thanks to its translational approach linking basic science to clinical research and its close collaboration with MG patient associations, FIGHT-MG will address the etiological and pathophysiological mechanisms of MG that will lead to the development of new and more effective diagnostic assays and therapies for MG.

The FIGHT-MG consortium is coordinated by the French National Institute of Health and Medical Research (INSERM) in Paris, with the support of 11 research teams of international standing from France, Greece, Israel, Switzerland, Germany, Norway and Italy.

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GERMANY	Proteomics	Proteomics	Dario TAMARCOLO

FIGHT-MG is funded by the European Commission's 7th Framework Programme, Grant Agreement Number 342216.

www.fight-mg.eu

Project Leaflet:

PARTNERS

The FIGHT-MG consortium is a translational research project coordinated by the French National Institute of Health and Medical Research (INSERM) in Paris, with the support of 11 research teams of international standing from France, Greece, Israel, Switzerland, Germany, Norway and Italy.

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FIGHT-MG is a 4-year collaborative research project funded by the European Commission's 7th Framework Programme, contract number 342216.

www.fight-mg.eu

CONTACTS

Thanks to its translational approach linking basic science to clinical research and its close collaboration with MG patient associations, FIGHT-MG will address the etiological and pathophysiological mechanisms of MG that will lead to the development of new and more effective diagnostic assays and therapies for MG.

CONTEXT

Myasthenia Gravis (MG) is a rare and heterogeneous neuromuscular disease affecting the neuromuscular junction and leading to abnormal fatigability of various skeletal muscles. The molecular events causing and maintaining MG are still unknown and current treatments do not lead to remission and entail considerable side-effects. Thanks to its translational approach linking basic science to clinical research and its close collaboration with MG patient associations, FIGHT-MG will address the etiological and pathophysiological mechanisms of MG that will lead to the development of new and more effective diagnostic assays and therapies for MG.

OBJECTIVES

The main scientific and technological objectives of FIGHT-MG are:

- To define the etiological and pathophysiological mechanisms of MG.
- To identify new therapeutic targets.
- To develop new diagnostic assays.
- To improve the quality of life of MG patients.

STRATEGY

To achieve these objectives, FIGHT-MG partners will carry out the following steps:

- To perform basic research on the neuromuscular junction, to evaluate the molecular events causing and maintaining MG, and to identify new therapeutic targets.
- To develop new diagnostic assays for the detection of MG, based on the identification of new biomarkers.
- To improve the quality of life of MG patients, through the development of new therapeutic strategies.

EXPECTED OUTCOMES

Identifying and validating new therapeutic targets, developing new diagnostic assays, identifying new biomarkers, and improving the quality of life of MG patients.

Photography legends

- Microscopic view of a neuromuscular junction.
- Microscopic view of a muscle fiber.
- Microscopic view of a nerve terminal.
- Microscopic view of a synapse.
- Microscopic view of a motor unit.
- Microscopic view of a muscle fiber bundle.
- Microscopic view of a nerve terminal.
- Microscopic view of a synapse.
- Microscopic view of a motor unit.
- Microscopic view of a muscle fiber bundle.



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