Supplementary Information

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Supplementary Information Methods 1

Intervention and study rational. We are using high dosage of 1:1 omega-3:omega-6 polyunsaturated (PUFA) aiming to overpass and normalize global diet regional traditions and habits in relation to abnormal PUFA/saturated fatty acids (SFA)/monounsaturated fatty acids (MUFA) daily ratio consumption irrespective of the quantities consumed; and enough to equilibrate patients' diet in line with the recommended more physiologic omega-3:omega-6 ratio of about 1:1-4 wt/wt as reported by Simopoulos, 2002. In addition to correct existing deficiencies, cell membrane abnormalities, specifically of the immunopathological system and blood mononuclear peripheral cells, and high enough for availability and immediate ongoing modulation of the involved pathogenic mechanisms and network of events in MS. The high dosage is also required to overpass the quantity limitations, previously discussed, of diet-consumed PUFAs for cellular incorporation, especially in the central nervous system (CNS) of adults. Additionally, fatty acids (FAs) must first cross the intestinal epithelium before reaching the different tissues, where digestion and absorption constitute further problems in their availability (Carlier, H, 1991). Omega-3 PUFA are used in re-esterified form to eliminate unwanted disturbances, at the sides of action, by other fatty acids and molecules present in crude fish oils but also to increase the bioavailability of the FA since triglycerides have been shown to be associated with much higher bioavailability (Dyerberg et al, 2010). Linoleic (LA) and gamma linolenic acid (GLA) are essential structure molecules and important for any physiological (re)generation of cell membrane. GLA quantity is doubled to LA to ensure high direct production of dihomo-gamma-linolenic acid (DGLA), from GLA when LA cannot be metabolized, due to desaturase deficiency or malfunction. Such a reduced capacity to convert LA to GLA has been associated with aging, diabetes, alcoholism, atopic dermatitis, premenstrual syndrome, rheumatoid arthritis, cancer and cardiovascular diseases (Bolton-Smith et al, 1997; Horrobin, 1990; Leventhal et al, 1993). This is going to result in the increase of DGLA relative to arachidonic acid (AA) with DGLA promoting production of prostaglandin (PG)E1 but also inhibition of phospholipase (PL)A2: two major reasons and rational for their use. If other metabolic problems are involved within the omega-6 series and the normal metabolites are not produced then the eicosapentaenoic acid EPA available in PLP10 will substitute the function of DGLA, as a competitive inhibitor of AA for PLA2. In both cases the pro-inflamatory leucotrienes, prostaglandines of the 2-series (PG2) and thromboxanes of the 2 series including the platelet-activator factor (PAF) will be attenuated. The synthesis of AA from DGLA by Δ5 desaturase promoted by LA/GLA supplementation is very limited in humans as a result of limited activity of the enzyme (Yang-Yi & Robert, 1998). AA in the body is mostly available through diet. EPA and docosahexaenoic acid (DHA) are both physiologically important and crucial structured molecules able to substitute excess AA and SFA within the cell membranes. EPA will contribute to the inhibition (competitive to AA) of PLA2, joining the co-supplied omega-6 PUFA but will also participate in the production of anti-inflammatory leukotrienes, prostaglandins of the 3-series (PG3) and thromboxane (TX3) along with DHA, both found in the PLP10 intervention. Moreover EPA will replace AA of the membrane phospholipids and both omega-3 PUFA will contribute replacing abnormal quantities of SFA and excess AA. DHA is used in 3:1 ratio to EPA to cover any possible inabilities of EPA to be metabolized, high enough to strongly promote high production of the aforementioned anti-inflammatory

eicosanoids and cytokines and to be incorporated into CNS cell membranes where DHA should be the major PUFA present, replacing other FA, probably saturated and excess of AA. EPA, DHA, LA and GLA along with the rest of the other ingredients used ("other" omega-3 PUFA, SFA and MUFA that are usually found in the cell membranes of healthy people in limited quantities) in the intervention regimen are for their availability as minor structural constituents of physiological cellular membranes integrity, fluidity and overall function as building blocks for myelin repair and/or myelination. Furthermore the PUFA used within the cocktail intervention aimed to manipulate all other pathophysiological pathways that are reported to be able to: as previously discussed including gene transcription for neuroprotection and remyelination. Furthermore, PUFA are used to ensure the integrity of blood brain barrier (BBB) and to modulate the gelatinases responsible for the T cell migration within the CNS. Three different antioxidant vitamins (vitamin E, mostly as alpha-tocopherol, gamma (γ)-tocopherol (vitamin E isoform) and vitamin A) are used in the regimen preparation to support the cellular antioxidant defenses but also to protect peroxidation of the supplied increased amounts of PUFA. Alpha-tocopherol low-molecular-weight antioxidants will contribute to radical scavenging, interfering with gene transcription, protein expression, enzyme activity and metal chelation (van Meeteren et al, 2005). Vitamin E (alpha tocopherol) and vitamin A are used as antioxidants for the protection of the excess supplemented PUFA, with alpha-tocopherol been demonstrated to protect against peroxynitrite-induced oxidative damage, as well as able to efficiently detoxify hydroxyl, perhydroxyl and superoxide free radicals (the elevated reactive oxygen species (ROS)); each one with different mechanism of action, increasing the effect capability (Vatassery et al, 1998b; van Meeteren, 2005). Gamma-tocopherol is used in high dosage since its half life is very short compared to alpha-tocopherol and has been demonstrated to specifically protect against nitro-radicals. Tocopherols can also exert non-antioxidant properties, including modulation of cell signaling and immune function, regulation of transcription, and induction of apoptosis as previously discussed (van Meeteren et al, 2005).

PLP10 is the first preparation ever developed for MS therapy that is composed by the use of all different previously discussed PUFA, MUFA, SFA in a cocktail preparation mixed with the specific aforementioned antioxidant vitamins that have never been all together used before within a specific formulation. The ingredients ratio, quality, structural form and mostly the high dosage has never been before tested. Furthermore, the knowledge and chronotherapy as well as other unique limitations associated with the individual molecules used, have never been accounted, discussed, proposed or reported for any previous therapeutic regimen.

Through systems medicine therapeutic philosophy, by the use of PLP10, potentially MS patients have the opportunity to be treated holistically, by natural source isolated molecules, demonstrated as able of affecting and modulating all known pathophysiological, immunopathological, habitual, gene related factors; thus the dynamic interconnected complex network of events simultaneously. Possibly synergistic effects between PLP10 ingredients are also feasible. Moreover we can speculate that treatment efficacy of PLP10, when used as

adjunct to existing pharmaceuticals produced by reductionism, can be proven therapeutically superior to any available treatment for MS.

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Supplementary Information Methods 2

Interventions specifications. The specific omega-3 (re-esterified glycerides) and omega-6 (glycerides) raw materials were purchased according to the required interventions' PUFA-fraction specification (molecular structure, quantity/ratio and quality) with vitamin E (alpha-tocopherol) used as antioxidant stabilizer by the supplier. The vitamins and masking aroma were purchased separately. The mixing of fractions to the final required intervention-composition specification was always performed by the same team of scientists under the supervision of the involved medical biochemist and lipidology specialist, under appropriate conditions every six months. Interventions were stored refrigerated in dark until use.

The ratios of the different ingredients used were as follows: omega-3, EPA to DHA (about 1 to 3 wt/wt), omega-6, LA to GLA (about 2 to 1 wt/wt), omega-3 (EPA + DHA) to omega-6 (LA + GLA) (about 1 to 1 wt/wt). The total omega-3 (EPA + DHA + "other" omega-3 PUFA) used as re-esterified triglycerol (minimum value 60%), diglyceride (about 33%), monoglyceride (about 2%) structural form mixture and about 2% ethyl ester structural form, with no less than 80% re-esterified triglycerol content to be DHA and EPA as a result of PUFA triglycerides re-esterification of fish body oils. The "other" group of omega-3 on the re-esterified glycerols included the 18:3 (alpha-linolenic acid), 18:4 (stearidonic acid), 20:4 (eicosatetraenoic acid) and 22:5 (docosapentaenoic acid) PUFA. The fraction of omega-6 (LA + GLA) used as triglycerides with no less than 50-65% triglycerol content to be LA and GLA in a ratio of 2 to 1 with 18:1 (oleic acid) 14-20% and 20:1 (eicosenoic acid), 22:1 (docosenoic acid), 24:1 (tetracosenic acid) as additional monounsaturated fatty acids and minor quantities of 16:0 (palmitic acid) 4-16%, 18:0 (stearic acid) 2-5% saturated fatty acids from Borage oil source. The vitamins used were vitamin A as beta-carotene, vitamin E (alpha-tocopherol) and pure gamma-tocopherol (vitamine E isoform). Citrus extract was used as masking aroma and pure virgin olive oil as delivery vehicle.

The daily intervention formula agent dosages were:

Intervention formula A daily dosage: EPA (1650mg) / DHA (4650mg) / GLA (2000mg) / LA (3850mg) / total other omega-3 (600mg) / total monounsaturated fatty acids (MUFA) (18:1 1300mg, 20:1 250mg, 22:1 82mg, 24:1 82mg) + total saturated fatty acids (SFA) (18:0 160mg, 16:0 650mg) / vitamin A (0.6mg) / vitamin E (22mg).

Intervention formula B (PLP10) daily dosage: EPA (1650mg) / DHA (4650mg) / GLA (2000mg) / LA (3850mg) / total other omega-3 (600mg) / total MUFA (18:1 1300mg, 20:1 250mg, 22:1 82mg, 24:1 82mg) + total SFA (18:0 160mg, 16:0 650mg) / vitamin A (0.6mg) / vitamin E (22mg) / gamma- tocopherol (γ-tocopherol) (760 mg).

Intervention formula C daily dosage: γ-tocopherol (760 mg) (in 16137 mg pure virgin olive oil as a vehicle).

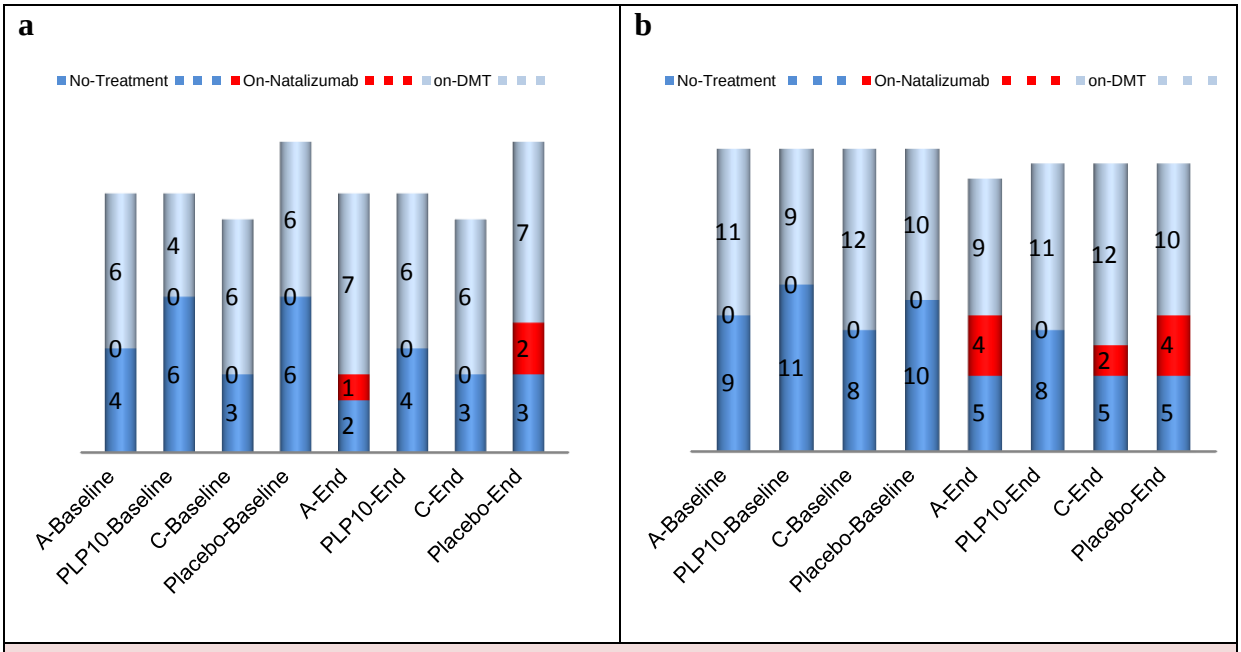
Intervention formula D daily dosage**:** pure virgin olive oil (16930mg).

Citrus aroma was added in each intervention formula to make up a total dosage of 19.5ml of solution per day.

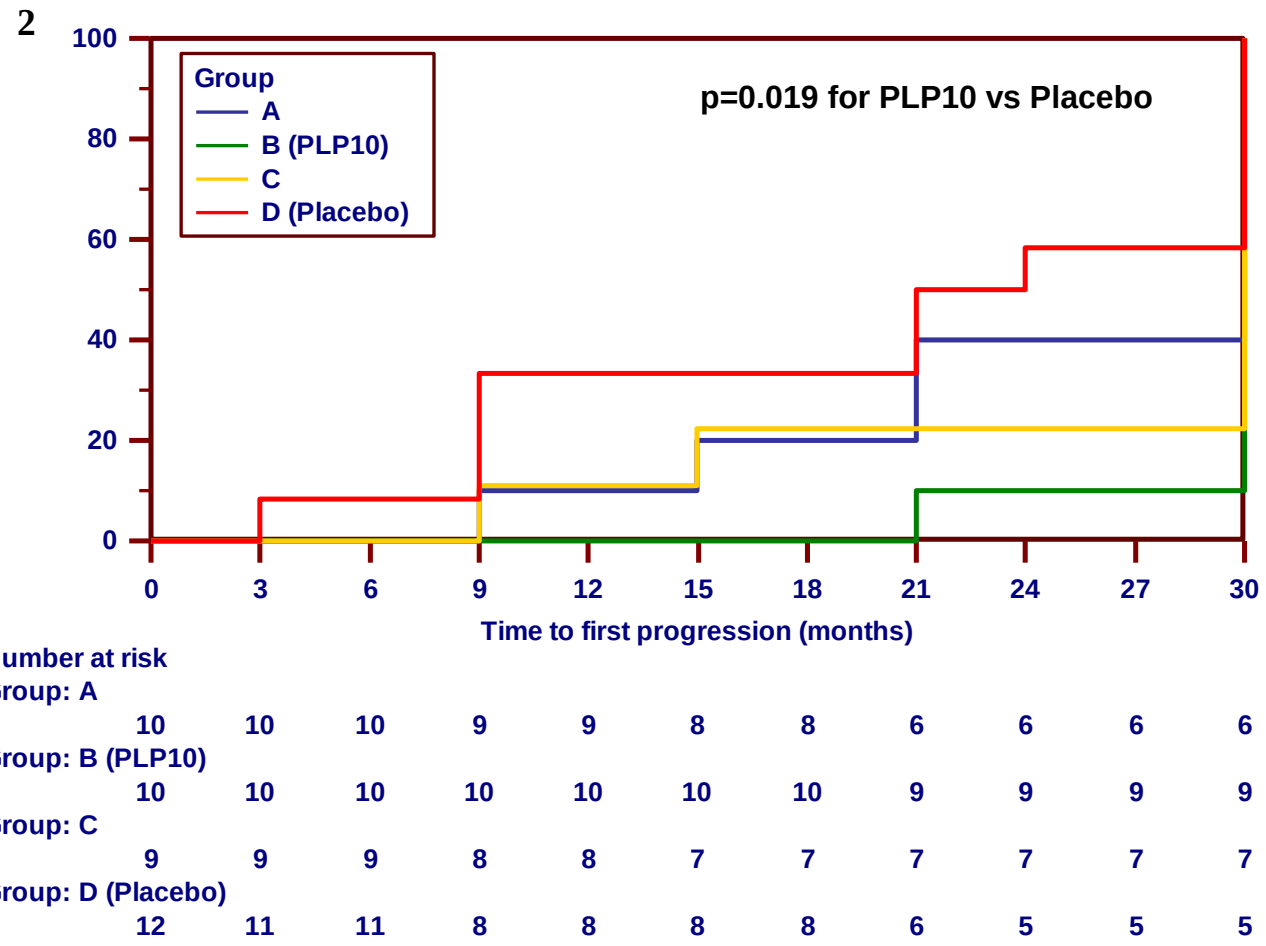
The specific omega-3 related fraction, according to specifications required for the interventions was prepared and purchased from EPAX AS, Aalesund, Norway; as re-esterified glycerides from fish body oils as a source. The specific omega-6 PUFA, MUFA and SFA related fraction, according to required specifications, was prepared and purchased from Goerlich Pharma International GmbH, Edling, Germany, as triglycerides from Borage seed oil (organic, cold pressed) *"Borago officinalis"* as a source. Both omega-3 and omega-6 fractions were delivered stabilized by the producer (vitamine E (alpha-tocopherol) ~ 4.5 mg/g was used as antioxidant).

Vitamins: vitamin A as beta-carotene (HealthAid Ltd., Middlesex, United Kingdom) and pure gamma-tocopherol (Tama Biochemical Co. Ltd., Shinjuku-ku Tokyo, Japan).

Citrus aroma (Givaudan Schwaiz AG, Dubendorf, Switzerland).



Supplementary Information Figure 1 | Population on DMT and/or natalizumab. (a) Demonstrates the all-time on-study population per treatment arm that was receiving or not receiving DMT at entry baseline and the same population at the end of the trial (including patients on natalizumab). No statistical significant differences were calculated. The all-time on-study patients per treatment-arm that were receiving DMT at entry baseline were six patients out of ten (60%) within group A, four out of ten (40%) within PLP10 group, six out of nine (66%) within group C and six out of 12 (50%) within placebo. When the study completed, 80% of the patients in group A, 60% in PLP10 group, 66% in group C and 75% of the patients in placebo ended up on treatment. Within group A one out of eight and within placebo two out of nine patients on DMT transferred on natalizumab. (b) Demonstrates the total randomized population per treatment arm that was receiving or not receiving DMT at entry baseline and the same population at the end of the trial without lost to follow (including patients on natalizumab). A total of 61% of group A patients were on DMT at entry baseline and became 72% at the end; for PLP10 group 41% and became 53%; for group C 73% and became 74% and for placebo 53% and became 74% at study completion. At the end: for group A, four out of 13 patients on DMT transferred on natalizumab; for PLP10 group no patient was on natalizumab; for Group C two out of 14 patients on DMT transferred on natalizumab; and for placebo group four out of the 14 patients on DMT transferred on natalizumab. No significant differences measured at entry baseline between the groups.



Supplementary Information Figure 2 | Kaplan–Meier estimates for the time to disability progression. Kaplan–Meier plot of the time to sustained progression of disability among all-time on-study patients, including patients on natalizumab, receiving intervention A, PLP10 and C *vs.* placebo. Intervention PLP10 reduced the risk of sustained progression of disability by 83% over two years (p=0.019). The cumulative probability of progression was 10% in the intervention B group and 58% in the placebo group. Intervention formula A reduced the risk of sustained progression of disability by 32% (p=0.301) and intervention formula C by 62% (p=0.109).

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