REVIEW

Preclinical R&D Department[1]; R&D Department[2]; Analytical Development & Quality Control Department[3]; Clinical Development Department[4], Synthon Biopharmaceuticals B.V., Nijmegen, The Netherlands, Lycalis sprl[5], Bruxelles, Belgium; Regulatory Affairs Department[6], Synthon B.V., Nijmegen, The Netherlands

Comparison of Copaxone® and Synthon's therapeutically equivalent glatiramer acetate

R.J. Arends[1,*], D. Wang[2], M. Buurman[3], J. Luten[3], N.P. Koper[4], C. Wolf[5], M. Scheren[6]

Received April 18, 2019, accepted May 15, 2019

**Corresponding author: Dr. Roel Arends, Preclinical R&D Department, Synthon Biopharmaceuticals B.V., Microweg 22, Nijmegen, The Netherlands*
roel.arends@synthon.com

Pharmazie 74: 449-461 (2019) *doi: 10.1691/ph.2019.9515*

Glatiramer acetate is indicated for the treatment of patients with relapsing forms of multiple sclerosis (RMS). In 2016, an alternative to the originator product was approved in the EU through the hybrid procedure regulatory pathway. This paper reviews the scientifically rigorous and multifaceted program undertaken to demonstrate the equivalence of this glatiramer acetate follow-on product (GTR) and the reference product Copaxone®, which resulted in the EU approval of GTR 20 mg/mL and 40 mg/mL. Establishing therapeutic equivalence for non-biological complex drugs is not trivial and requires a complex and multidisciplinary effort. Ultimately, there is not a single test or study that establishes therapeutic equivalence of two heterogeneous products. Instead, it requires a good understanding of the synthesis process together with a full set of data that includes comparative physicochemical testing, nonclinical *in vitro* and *in vivo* studies, and a comparative clinical study to allow for a valid conclusion that two products are therapeutically equivalent. The detailed understanding of glatiramer's synthesis process and its impact on the characteristics of glatiramer, combined with the results of a scientifically rigorous and multifaceted physicochemical and biological characterization program, and the clinical data from the 794-patient Phase III GATE study, demonstrate that GTR and Copaxone are therapeutically equivalent. The data further demonstrate that Synthon's manufacturing process consistently yields drug substance of the same quality as Copaxone and that switching from Copaxone to GTR is safe and well-tolerated.

1. Introduction

Glatiramer acetate (glatiramer, Copaxone®) 20 mg/mL once daily is indicated for the treatment of patients with relapsing forms of multiple sclerosis (RMS). Copaxone was first approved by the Food and Drug Administration (FDA) in 1996 (Copaxone USPI) and by the European Medicines Agency (EMA) in 2003 (Copaxone SmPC). Copaxone 40 mg/mL three-times weekly was approved in the United States (Teva 2014a) and the European Union (Teva 2014b) in 2014. The first generic versions of the 20 mg/mL and 40 mg/mL strengths received approval in the EU in 2016 (Synthon 2016) and 2017 (Synthon 2017), respectively.

Glatiramer is a synthetic copolymer consisting of a heterogeneous mixture of copolymer chains comprising four amino acids: l-glutamic acid, l-lysine, l-alanine, and l-tyrosine. When applying the manufacturing process as described in literature (US Patents 7,199,098 and 6,048,898; Teitelbaum et al. 1971), each new batch of glatiramer will contain a myriad of copolymer chains and no two batches of glatiramer will ever be identical. Consequently, glatiramer does not have a fixed single sequence or combination of sequences and no exact sequence data can be generated. This batch-to-batch heterogeneity is an inherent characteristic of glatiramer due to its manufacturing process. Bioequivalence against the reference product Copaxone cannot be established using conventional bioequivalence studies, because the active substance is readily absorbed and a large part of the dose is rapidly degraded to smaller fragments already in subcutaneous tissue. Furthermore, no good circulating surrogate biomarkers exist. Therefore, establishing equivalence, both from a quality and clinical perspective, is substantially more complex for glatiramer than for conventional small molecules.

To obtain a marketing authorization in the EU for a generic version of Copaxone, in view of the nature of glatiramer acetate, the regulatory pathway of the hybrid generic as provided for in article 10.3 of Directive EC/2001/83 was successfully followed, in line with advice obtained from the EMA and a number of national medicines agencies. Herein, the scientifically rigorous and multifaceted program undertaken to demonstrate the equivalence of Synthon's glatiramer acetate (GTR) and the reference product Copaxone under the hybrid application procedure (Article 10 (3)) is reviewed. This includes an overview of the data submitted to the EU Reference Member State which resulted in the EU approval of GTR 20 mg/mL and 40 mg/mL.

2. Glatiramer product characteristics

The formulation, dosage form and strengths of GTR and Copaxone are identical. Both are supplied in an aqueous solution for injection in a glass prefilled syringe at strengths of 20 mg/mL and 40 mg/mL. However, demonstrating their equivalence on the level of the drug substance is challenging due to its inherent batch-to-batch heterogeneity.

Glatiramer acetate is a synthetic copolymer consisting of a heterogeneous mixture of copolymer chains comprising four amino acids: l-glutamic acid, l-lysine, l-alanine, and l-tyrosine in a defined molar ratio of 0.129 – 0.153, 0.300 – 0.374, 0.392 – 0.472 and 0.086 – 0.100, respectively (Copaxone SmPC). Glatiramer acetate has a molecular weight of 5,000 – 9,000 Dalton (Copaxone SmPC).

The process used to manufacture GTR is the same as that published in patents (US 7,199,098 and 6,048,898) and in the literature (Teitelbaum et al. 1971) for glatiramer and consists of the following

three steps: (1) Polymerization; (2) Partial depolymerization and deprotection of the glutamic acid residues; (3) Deprotection of the lysine residues and purification.

At the start of the synthesis all amino acid starting materials are dissolved in dioxane. Subsequently, an initiator (diethylamine) is added to the mixture to initiate the polymerization. Under these reaction conditions it is only possible to obtain copolymer chains having random amino acid sequences. It has been calculated that with these four amino acids $>10^{29}$ possible sequence combinations of on average 60 amino acids in length can be generated. Consequently, each new batch of glatiramer will contain a myriad of copolymer chains and no two syringes of glatiramer will ever contain identical copolymers in identical amounts. Based on the certainty of finding this inherent heterogeneity, the best approach to assess drug substance equivalence includes the evaluation of the mixture's overall properties and characterization of its batch-to-batch variability using a broad set of orthogonal bioanalytical methods.

Based on scientific advice from both national health authorities in EU countries and ultimately the EMA, it was determined that demonstrating GTR therapeutic equivalence to the reference product, Copaxone, would require compelling scientific evidence supporting (1) comparability in physicochemical characteristics; (2) comparability in nonclinical *in vitro* and *in vivo* studies and (3) comparability in a clinical study.

3. Methodology

3.1. Physicochemical, nonclinical and clinical comparability

To demonstrate physicochemical, nonclinical and clinical comparability, the following methods were part of the characterization program. Test descriptions are provided in the experimental section.

Primary structure: Amino acid composition, peptide mapping/proteolytic digestion profile. *Higher order structure*: Structure analysis by circular dichroism, Coomassie brilliant blue binding, intrinsic fluorescence spectroscopy. *Other physicochemical properties*: Chromatographic property by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), Ultraviolet (UV) spectroscopy, Molecular Weight Distribution (MWD) by Gel Permeation Chromatography (GPC), Heterogeneity analysis by Capillary IsoElectric Focusing (cIEF). The following *nonclinical in vitro and in vivo studies* were performed to demonstrate nonclinical comparability: Western Blot, Enzyme-linked immunosorbent assay (ELISA, Biological activity by Cell Based Assay (CBA), Micro array study, Experimental Autoimmune Encephalomyelitis (EAE) MOG model in Brown Norway rats, Experimental Autoimmune Encephalomyelitis PLP model in SJL mice and toxicity and safety studies in rats. *Comparability in a clinical study:* EU approval of a non-biological complex drug such as glatiramer also requires evidence of comparability with the reference product in a clinical study. Clinical data used to evaluate the therapeutic equivalence of GTR and Copaxone were generated in a 2-year Phase 3 trial consisting of a 9-month, randomized, multicenter, double-blind, active and placebo-controlled part and a 15-month open-label active treatment extension. The trial included 794 patients with relapsing-remitting multiple sclerosis (RRMS).

3.2. Important methodological considerations

3.2.1. Copaxone batches tested

Since glatiramer is a heterogeneous mixture having inherent batch-to-batch variability, a good understanding of the extent of the variability in the reference product is required. Such data can only be considered representative of the true variability in the reference product if the batches tested cover a sufficiently wide production time period to ensure that different drug substance batches are included in the testing program. The results presented in this article are based on more than 20 different Copaxone batches tested over a period of 10 years.

3.2.2. Discriminatory power

Adequate characterization programs apply physicochemical tests that discriminate between glatiramer batches prepared by using different manufacturing processes. The discriminatory power of the physicochemical tests was established by using a negative control polymer manufactured using a slightly different process and thereby intentionally affecting the primary structure of glatiramer.

In the normal production process, all amino acid starting materials are present in solution when polymerization starts. To produce the negative control polymer, the starting materials were added in 3 blocks at different time intervals, thereby altering the polymerization process. As a result, the final drug substance has the same overall amino acid composition and molecular weight as Copaxone but has different physicochemical properties as a consequence of the changes in its primary structure.

4. Results

4.1. Physicochemical characterization

4.1.1. Primary structure

Amino acid composition: The amino acid composition of Copaxone and GTR were within the defined molar fraction range of glatiramer and showed low variability (Fig. 1).

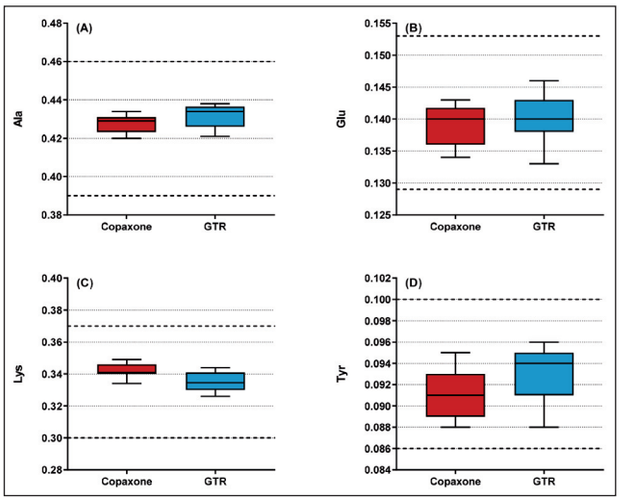

Fig. 1: Amino acid composition of the GTR and Copaxone batches. Y-axis represents molar fraction. (A) Ala = l-alanine, (B) Glu = l-glutamic acid, (C) Lys = l-lysine and (D) Tyr = l-tyrosine. Dashed lines indicate defined molar fraction range of glatiramer. Data are depicted in Box & Whiskers plot (min to max). Number of batches: Copaxone 20, GTR 24.

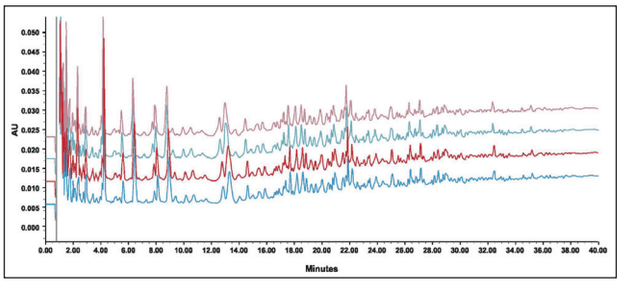

Fig. 2: UPLC-UV (214 nm) chromatograms of trypsin digested Copaxone (2 batches, red) and GTR (2 batches, blue).

Peptide mapping/proteolytic digestion profile: Analysis of digested Copaxone and GTR consistently resulted in comparable digestion fingerprints for all batches containing a comparable number of peaks at comparable retention times and with comparable intensities. Fig. 2 shows representative chromatogram examples of

the trypsin digested GTR and Copaxone batches. In the negative control polymer additional peaks were found e.g. around retention time 3.8 min for Trypsin digestion (Supplementary Fig. 14).
The high comparability of the chromatograms of both products can only be achieved if the same polypeptide fragments have been obtained for digested Copaxone and GTR. As such, the primary structure of the polypeptide chains comprising Copaxone and GTR must be comparable.

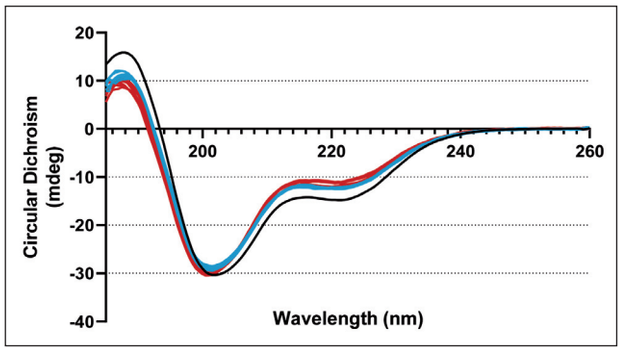

Fig. 3: Circular dichroism spectra of Copaxone (6 batches, red), GTR (6 batches, blue) and negative control polymer (black).

4.1.2. *Higher order structure*

Circular dichroism (CD): All CD spectra of GTR and Copaxone were comparable, indicating that the secondary structures of the polypeptides were comparable in both products. An example of CD spectra of six GTR batches and six Copaxone batches are shown in Fig. 3. The CD spectrum for the negative control polymer did not fall within the variation of the Copaxone batches as shown in Fig. 3. A parallel-shifted spectrum from 200 to 235 nm as well as the highest zero and positive mean residue ellipticity at 187 nm were observed, confirming the discriminatory power of the method.

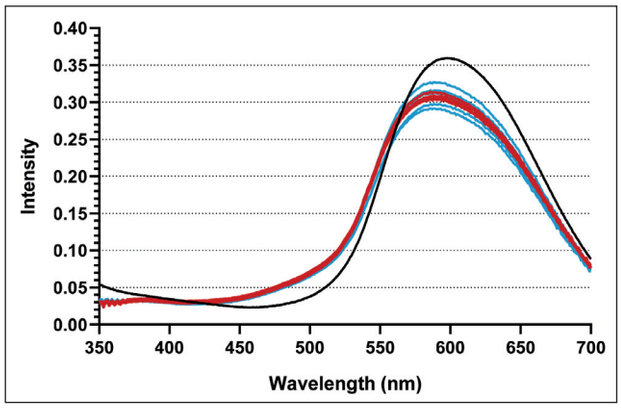

Fig. 4: UV absorbance spectra of the CBB complexes formed with Copaxone (6 batches, red), GTR (6 batches, blue), and negative control polymer (black).

Coomassie Brilliant Blue (CBB) binding: Comparable UV-Vis absorbance spectra of the CBB complexes for Copaxone and GTR indicate that the polypeptides in both drug substances adopt comparable higher order structures (Fig. 4). The UV spectrum found for the negative control polymer was not the same as that found for Copaxone and GTR. The peak maximums were shifted towards a higher wavelength.
Intrinsic fluorescence spectroscopy: The intrinsic fluorescence emission spectra for all Copaxone and GTR batches were similar and the variability in fluorescence intensity of GTR was within the range of variability for Copaxone (Supplementary Fig. 15). The results for the negative control polymer tests for intrinsic fluorescence showed higher fluorescence intensity at the maximum emission wavelength compared to Copaxone and GTR. These results indicate comparable polypeptide structures in both Copaxone and GTR.

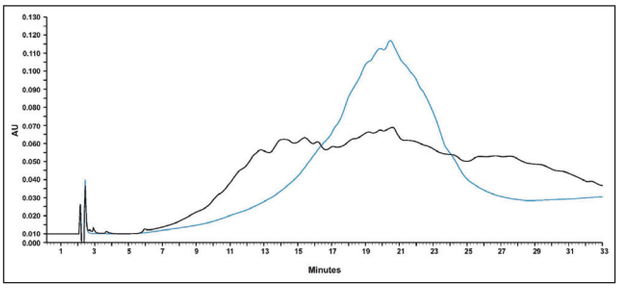

Fig. 5: Reversed Phase-High Performance Liquid Chromatogram of GTR (blue) and negative control polymer (black).

4.1.3. *Other physicochemical properties*

Reversed Phase-High Performance Liquid Chromatography (RP-HPLC): RP-HPLC of GTR batches show comparable profiles, i.e. the same characteristic multi-component peak, compared to Copaxone (Supplementary Table 1). This indicates that the polypeptides in GTR have the same hydrophobic interaction properties, and thus comparable composition of amino acid sequences, compared to Copaxone. A clearly different chromatogram was obtained for the negative control polymer. A much broader distribution was observed (Fig. 5), which confirmed the discriminatory power of reversed phase HPLC.

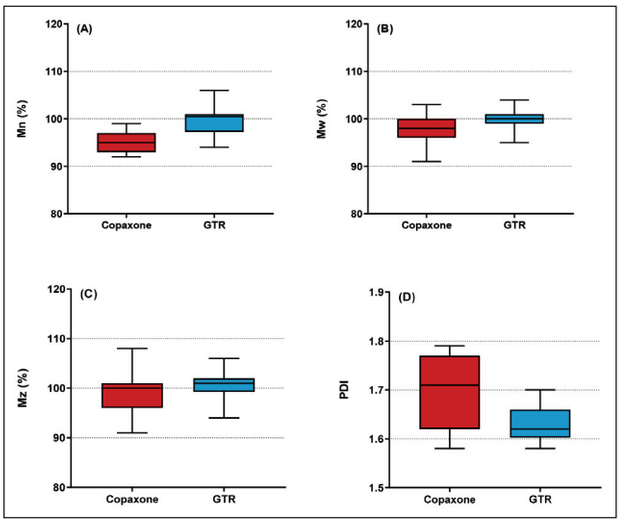

Fig. 6: Molecular weight distribution of Copaxone and GTR batches determined with GPC-UV. Molecular weight expressed relative to reference material; (A) Mn, (B) Mw, (C) Mz and polydispersity index; (D) PDI. Data are depicted in Box & Whiskers plot (min to max). Number of batches: Copaxone 19, GTR 24.

UV spectroscopy: UV spectroscopy clearly showed the three characteristic UV absorbance maxima of glatiramer (absorbance of the peptide bond around 200 nm, the α-helix around 220 nm and the tyrosine moieties around 280 nm) for each GTR and Copaxone batch (Supplementary Fig. 16). Hence, it can be concluded that the polypeptides in GTR contain the same conformational regions as Copaxone. For the negative control polymer, a parallel-shifted spectrum at 220 nm was observed.
Molecular Weight Distribution (MWD): The relative MWD (GPC-UV) and absolute MWD (GPC-MALS), compared to a reference

Table 1: Reverse Phase-High Performance Liquid Chromatography properties

	Retention time window of three highest multi-component peaks (minutes)	Result (specification 17 – 22 minutes)
GTR		
Batch 1	19.9 - 20.8	Comply
Batch 2	19.9 - 20.8	Comply
Batch 3	19.8 - 21.0	Comply
Batch 4	20.0 - 21.2	Comply
Batch 5	19.7 - 21.7	Comply
Batch 6	19.6 - 20.7	Comply
Copaxone		
Batch 1	18.8 - 20.8	Comply
Batch 2	18.4 - 20.1	Comply
Batch 3	19.3 - 20.6	Comply
Batch 4	19.4 - 20.7	Comply
Batch 5	19.6 - 20.7	Comply
Batch 6	19.1 - 20.7	Comply

RP-HPLC retention time window: hydrophobic interactions of Copaxone and GTR were assessed by evaluation of the position of multi-component peaks.

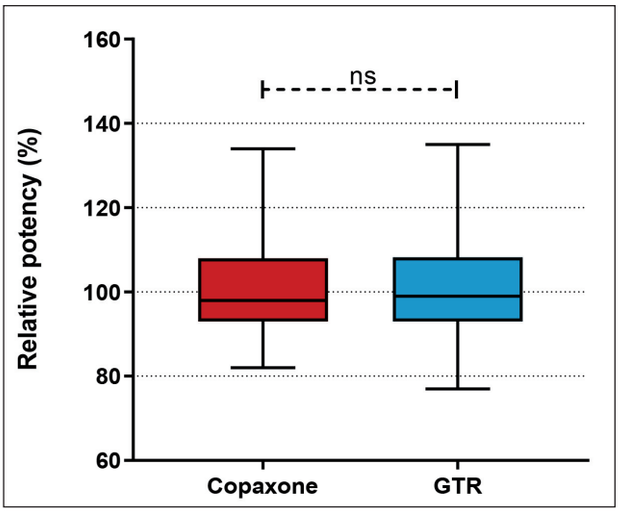

Fig. 7: Relative potency in cell-based assay in THP-1 cells. Data are depicted in Box & Whiskers plot (min to max). Number of batches: Copaxone 33, GTR 40, ns = not significant, unpaired t-test.

standard, of the GTR and Copaxone batches showed that the MWD of GTR is within the range of Copaxone (Fig. 6). This indicates that the MWD of Copaxone and GTR are comparable.

Capillary IsoElectric Focusing (cIEF): The cIEF of GTR and Copaxone were comparable in the number of detected peaks and the distribution of these peaks across the entire cIEF pattern (data not shown). A much broader distribution in polypeptide charge heterogeneity was observed for the negative control polymer (Supplementary Fig. 17). The results indicate the heterogeneity of the polypeptides in both products are comparable.

4.2. Nonclinical evaluation

4.2.1. Western Blot

The Western blot profiles (data not shown) show that in the Copaxone and GTR batches tested, both anti-GTR and anti-Copaxone polyclonal antibodies (pAb) identify a similar variety of epitopes. This

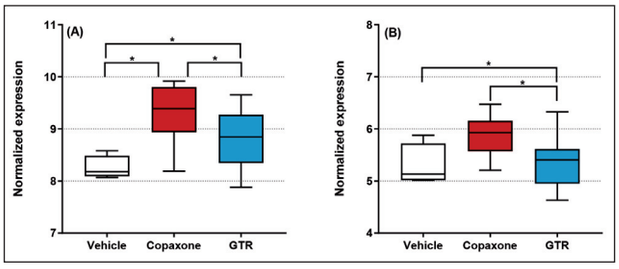

Fig. 8: Normalized expression profiles (log2 scale) for the two probe sets (EGR1: A and SRGN: B) differentially expressed between Copaxone and GTR. Data are depicted in Box & Whiskers plot (min to max). Fold change ≥ 1.4, * adjusted p-value < 0.05.

suggests that both products have similar affinities to the same antibodies, indicating similar recognition moieties and specificities.

4.2.2. ELISA

The results (Supplementary Fig. 18) demonstrated that the relative affinity of anti-GTR pAb to Copaxone and GTR is around 100 %, which indicates that GTR contains the same epitopic polypeptide sequences as Copaxone.

4.2.3. Cell-based assay

The relative potencies of GTR batches and Copaxone showed no statistical differences in relative mean potencies of Copaxone and GTR and no difference in variance between the Copaxone and GTR drug product batches were found (Fig. 7). The results of the THP-1 assay indicated that Copaxone and GTR induce production of sIL-1Ra in human monocytes to a comparable extent and thus exhibit the same mode of action with respect to the anti-inflammatory sIL-1Ra response.

4.2.4. Micro array study

Gene expression was evaluated on Affymetrix 24 HT HG U133+ PM arrays after exposure of THP-1 cells *in vitro* to either Copaxone or GTR. Five batches of GTR were compared with five batches of Copaxone. The experiment included four independent biological replicates of each condition.

In total, 40 probe sets were significantly modulated by both Copaxone and GTR, 20 were significantly modulated by Copaxone only and twelve were significantly modulated by GTR only (Supplementary Tables 2 and 3). The twelve probe sets that were statistically significantly modulated by GTR but not by Copaxone, were also modulated to a comparable extent by Copaxone, but statistical significance was not achieved. Furthermore, the 20 probe sets significantly modulated by Copaxone were also modulated to a comparable extent by GTR, but statistical significance was not achieved. Direct comparison of GTR and Copaxone gene expression profiles revealed that two genes, early growth response protein 1 (EGR1) and serglycin (SRGN), were upregulated more by Copaxone compared to GTR (Fig. 8). EGR1 was also upregulated by GTR compared to vehicle treatment; however, EGR1 was more upregulated by Copaxone (Fig. 8). The function of all modulated genes was evaluated by Ingenuity Pathway Analysis, which demonstrated that the same canonical pathways were modulated by GTR and Copaxone. Genes linked to differentially expressed probe sets are predominantly involved in chemokine/cytokine signaling, cell adhesion and migration, and communication between innate and adaptive immune system (Supplementary Table 4).

The microarray study demonstrated comparable gene modulation and no relevant differences between the Copaxone and GTR in a human monocytic cell line THP-1.

4.2.5. EAE rat test

In a MOG-induced EAE model in rats the effects of GTR and Copaxone on survival of retinal ganglion cells (RGCs), the neurons

Table 2: 52 differentially modulated probe sets GTR compared to vehicle sorted on associated gene symbol (adjusted p-value < 0.05, fold change ≥ 1.4)

Gene Symbol	Probe Set ID	Ave Expr	LogFC	P-Value	Adj P-Val	Gene Title
---	231513_at	7.39	0.62	5.39E-05	1.80E-03	---
---	235094_at	8.04	0.70	1.47E-08	1.64E-06	---
---	238666_at	5.68	0.77	1.63E-07	1.37E-05	---
ABCA1	203504_s_at	5.68	-1.40	4.89E-23	1.49E-19	ATP-binding cassette, sub-family A (ABC1), member 1
ABCA1	203505_at	7.14	-1.16	3.89E-21	5.91E-18	ATP-binding cassette, sub-family A (ABC1), member 1
ANKRD22	238439_at	9.73	0.52	1.17E-21	2.22E-18	ankyrin repeat domain 22
CACNA2D3	219714_s_at	7.86	-0.53	3.34E-12	9.59E-10	calcium channel, voltage-dependent, alpha 2/delta subunit 3
CD274	227458_at	9.32	0.72	4.13E-23	1.49E-19	CD274 molecule
CFH	215388_s_at	9.15	0.53	5.41E-13	1.83E-10	complement factor H
CXCL10	204533_at	11.31	0.84	2.21E-21	3.74E-18	chemokine (C-X-C motif) ligand 10
CXCL11	211122_s_at	10.59	0.99	4.61E-23	1.49E-19	chemokine (C-X-C motif) ligand 11
CXCL9	203915_at	10.97	0.66	1.72E-22	3.75E-19	chemokine (C-X-C motif) ligand 9
DUSP6	208892_s_at	9.19	0.53	4.84E-05	1.66E-03	dual specificity phosphatase 6
EGR1	201694_s_at	8.97	0.93	3.88E-13	1.37E-10	early growth response 1
EGR1	227404_s_at	7.27	1.16	3.01E-11	7.16E-09	early growth response 1
FDFT1	241954_at	5.66	0.71	9.46E-09	1.16E-06	farnesyl-diphosphate farnesyltransferase 1
GCH1	204224_s_at	11.34	0.61	1.08E-15	6.60E-13	GTP cyclohydrolase 1
GIMAP1-GIMAP5	64064_at	6.53	0.61	2.17E-19	2.20E-16	GIMAP1-GIMAP5 read-through
HEG1	212822_at	7.28	0.54	3.99E-11	9.21E-09	heart development protein with EGF-like domains 1
HEG1	213069_at	9.18	0.52	4.71E-19	4.48E-16	heart development protein with EGF-like domains 1
HMGCR	202539_s_at	8.75	0.53	1.90E-14	8.75E-12	3-hydroxy-3-methylglutaryl-CoA reductase
HMGCR	202540_s_at	8.72	0.60	6.24E-17	4.75E-14	3-hydroxy-3-methylglutaryl-CoA reductase
HMGCS1	205822_s_at	6.07	0.72	3.67E-10	6.81E-08	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
IER2	202081_at	9.02	0.54	6.48E-14	2.82E-11	immediate early response 2
IFIT3	229450_at	8.52	0.52	3.47E-15	2.03E-12	interferon-induced protein with tetratricopeptide repeats 3
IL18BP	222868_s_at	6.93	0.62	5.42E-09	7.24E-07	interleukin 18 binding protein
IL1RN	212659_s_at	6.49	0.55	1.18E-08	1.35E-06	interleukin 1 receptor antagonist
INSIG1	201625_s_at	6.60	0.70	1.98E-10	3.86E-08	insulin-induced gene 1
INSIG1	201626_at	8.86	0.97	8.33E-20	9.75E-17	insulin-induced gene 1
INSIG1	201627_s_at	8.05	0.94	3.69E-26	5.62E-22	insulin-induced gene 1
JAG1	209099_x_at	7.77	0.58	2.80E-16	2.03E-13	jagged 1
JAG1	216268_s_at	7.68	0.76	8.38E-10	1.34E-07	jagged 1
KCNJ2	206765_at	8.02	0.70	2.34E-20	2.96E-17	potassium inwardly-rectifying channel, subfamily J, member 2
LDLR	202068_s_at	6.63	1.58	2.07E-19	2.20E-16	low density lipoprotein receptor
LEF1	221558_s_at	7.93	0.51	3.35E-14	1.50E-11	lymphoid enhancer-binding factor 1
LRRK2	229584_at	6.68	0.52	7.77E-07	5.23E-05	leucine-rich repeat kinase 2
MAFB	218559_s_at	8.84	0.57	4.50E-13	1.56E-10	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)
MAFB	222670_s_at	7.52	0.68	5.91E-17	4.74E-14	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)
MAFF	36711_at	8.41	0.57	4.63E-15	2.52E-12	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)
MAML2	235457_at	6.76	0.63	6.00E-11	1.34E-08	mastermind-like 2 (Drosophila)
MSMO1	209146_at	9.09	1.26	1.16E-22	2.94E-19	methylsterol monooxygenase 1
MYLIP	220319_s_at	5.77	-0.60	9.03E-07	5.95E-05	myosin regulatory light chain interacting protein

Table 3: 60 differentially modulated probe sets Copaxone compared to vehicle sorted on mapped gene symbol (adjusted p-value < 0.05, fold change ≥ 1.4)

Gene Symbol	Probe Set ID	Ave Expr	LogFC	P-Value	Adj P-Val	Gene Title
---	231513_at	7.39	0.73	2.86E-06	1.42E-04	---
---	235094_at	8.04	1.06	3.92E-14	1.35E-11	---
---	238666_at	5.68	0.92	1.71E-09	2.32E-07	---
ABCA1	203504_s_at	5.68	-1.17	4.36E-20	5.53E-17	ATP-binding cassette, sub-family A (ABC1), member 1
ABCA1	203505_at	7.14	-0.98	2.44E-18	2.18E-15	ATP-binding cassette, sub-family A (ABC1), member 1
ANKRD22	238439_at	9.73	0.54	9.86E-23	2.50E-19	ankyrin repeat domain 22
BTG2	201236_s_at	6.63	0.71	9.69E-12	2.23E-09	BTG family, member 2
CACNA2D3	219714_s_at	7.86	-0.62	1.73E-14	6.59E-12	calcium channel, voltage-dependent, alpha 2/delta subunit 3
CD274	227458_at	9.32	0.67	6.12E-22	1.33E-18	CD274 molecule
CEP97	235918_x_at	6.34	0.51	1.61E-04	3.67E-03	centrosomal protein 97kDa
CNTLN	239989_at	7.03	0.59	2.75E-16	1.61E-13	centlein, centrosomal protein
CXCL10	204533_at	11.31	0.90	6.18E-23	1.88E-19	chemokine (C-X-C motif) ligand 10
CXCL11	211122_s_at	10.59	1.01	8.45E-24	4.29E-20	chemokine (C-X-C motif) ligand 11
CXCL9	203915_at	10.97	0.62	7.09E-22	1.35E-18	chemokine (C-X-C motif) ligand 9
DACH1	228915_at	7.08	0.53	3.22E-09	4.05E-07	dachshund homolog 1 (Drosophila)
DKK2	219908_at	7.69	0.56	7.20E-16	3.78E-13	dickkopf WNT signaling pathway inhibitor 2
DUSP6	208891_at	9.06	0.60	6.00E-18	4.56E-15	dual specificity phosphatase 6
DUSP6	208892_s_at	9.19	0.61	3.54E-06	1.72E-04	dual specificity phosphatase 6
EGR1	201694_s_at	8.97	1.53	9.06E-22	1.53E-18	early growth response 1
EGR1	227404_s_at	7.27	1.54	1.26E-15	5.99E-13	early growth response 1
FDFT1	241954_at	5.66	0.76	1.05E-09	1.51E-07	farnesyl-diphosphate farnesyltransferase 1
FOS	209189_at	7.76	0.79	1.67E-15	7.68E-13	FBJ murine osteosarcoma viral oncogene homolog
GBP1	202270_at	10.22	0.51	7.10E-10	1.07E-07	Guanylate-binding protein 1, interferon-inducible
GCH1	204224_s_at	11.34	0.59	2.62E-15	1.17E-12	GTP cyclohydrolase 1
GIMAP1-GIMAP5	64064_at	6.53	0.59	4.10E-19	4.46E-16	GIMAP1-GIMAP5 readthrough
HLA-DPB1	201137_s_at	7.29	0.53	4.71E-09	5.64E-07	major histocompatibility complex, class II, DP beta 1
IER2	202081_at	9.02	0.80	8.70E-21	1.20E-17	immediate early response 2
IFI16	208965_s_at	9.34	0.51	4.31E-15	1.87E-12	interferon, gamma-inducible protein 16
IFI44L	204439_at	6.77	0.67	8.28E-13	2.38E-10	interferon-induced protein 44-like
IFIT3	229450_at	8.52	0.60	5.98E-18	4.56E-15	interferon-induced protein with tetratricopeptide repeats 3
IL18BP	222868_s_at	6.93	0.66	5.91E-10	9.08E-08	interleukin 18 binding protein
IL1RN	212659_s_at	6.49	0.61	5.39E-10	8.37E-08	interleukin 1 receptor antagonist
IL1RN	216243_s_at	7.49	0.58	6.42E-11	1.16E-08	interleukin 1 receptor antagonist
IL8	202859_x_at	7.99	0.93	7.06E-14	2.33E-11	interleukin 8
INSIG1	201625_s_at	6.60	0.53	1.56E-07	1.19E-05	insulin-induced gene 1
INSIG1	201626_at	8.86	0.87	3.77E-18	3.18E-15	insulin-induced gene 1
INSIG1	201627_s_at	8.05	0.84	2.87E-24	2.19E-20	insulin-induced gene 1
JAG1	209099_x_at	7.77	0.61	1.85E-17	1.23E-14	jagged 1
JAG1	216268_s_at	7.68	0.73	1.93E-09	2.59E-07	jagged 1
KCNJ2	206765_at	8.02	0.80	4.71E-23	1.79E-19	potassium inwardly-rectifying channel, subfamily J, member 2
LDLR	202068_s_at	6.63	1.53	3.52E-19	4.12E-16	low density lipoprotein receptor
LOC100506965	226587_at	5.57	-0.57	6.16E-04	1.02E-02	uncharacterized LOC100506965
LRRK2	229584_at	6.68	0.59	3.18E-08	2.88E-06	leucine-rich repeat kinase 2

Table 4: Results of the Ingenuity Pathway Analysis conducted in the *in vitro* gene expression study in THP-1 cells

Canonical Pathway	Likelihood		Modulated genes in pathway	Modulated genes in pathway
	GTR/Veh	COP/Veh	GTR/Veh	COP/Veh
LXR/RXR Activation	8.04	5.97	(7) MYLIP, LDLR, IL1RN, TNF, HMGCR, ABCA1, FDFT1	(6) MYLIP, LDLR, IL1RN, TNF, ABCA1, FDFT1
IL-17A Signalling in Gastric Cells	4.61	8.13	(3) CXCL11, CXCL10, TNF	(5) FOS, CXCL11, CXCL10, TNF, CXCL8
Pathogenesis of Multiple Sclerosis	6.03	5.75	(3) CXCL11, CXCL9, CXCL10	(3) CXCL11, CXCL9, CXCL10
Superpathway of Cholesterol Biosynthesis	8.43	2.58	(5) HMGCS1, SQLE, HMGCR, FDFT1, MSMO1	(2) FDFT1, MSMO1
Granulocyte Adhesion and Diapedesis	4.52	5.24	(5) CXCL11, CXCL9, IL1RN, CXCL10, TNF	(6) CXCL11, CXCL9, IL1RN, CXCL10, TNF, CXCL8
Agranulocyte Adhesion and Diapedesis	4.39	5.09	(5) CXCL11, CXCL9, IL1RN, CXCL10, TNF	(6) CXCL11, CXCL9, IL1RN, CXCL10, TNF, CXCL8
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	3.95	5.27	(3) IL1RN, CXCL10, TNF	(4) IL1RN, CXCL10, TNF, CXCL8
Cholesterol Biosynthesis I	5.50	3.22	(3) SQLE, FDFT1, MSMO1	(2) FDFT1, MSMO1
Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)	5.50	3.22	(3) SQLE, FDFT1, MSMO1	(2) FDFT1, MSMO1
Cholesterol Biosynthesis III (via Desmosterol)	5.50	3.22	(3) SQLE, FDFT1, MSMO1	(2) FDFT1, MSMO1
Epoxysqualene Biosynthesis	5.29	2.25	(2) SQLE, FDFT1	(1) FDFT1
Communication between Innate and Adaptive Immune Cells	3.23	4.30	(3) IL1RN, CXCL10, TNF	(4) IL1RN, CXCL10, TNF, CXCL8
Airway Pathology in Chronic Obstructive Pulmonary Disease	1.80	3.79	(1) TNF	(2) TNF, CXCL8
Role of Cytokines in Mediating Communication between Immune Cells	2.20	3.37	(2) IL1RN, TNF	(3) IL1RN, TNF, CXCL8
IL-6 Signalling	1.54	3.49	(2) IL1RN, TNF	(4) FOS, IL1RN, TNF, CXCL8
IL-10 Signalling	1.97	3.03	(2) IL1RN, TNF	(3) FOS, IL1RN, TNF
IL-17 Signalling	1.93	2.95	(2) CXCL11, CXCL10	(3) CXCL11, CXCL10, CXCL8
Toll-like Receptor Signalling	1.93	2.95	(2) IL1RN, TNF	(3) FOS, IL1RN, TNF
Notch Signalling	2.49	2.31	(2) JAG1, MAML2	(2) JAG1, MAML2
Graft-versus-Host Disease Signaling	2.47	2.29	(2) IL1RN, TNF	(2) IL1RN, TNF
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.00	2.65	(3) CXCL9, TNF, TNFSF10	(4) CXCL9, TNF, TNFSF10, CXCL8
LPS/IL-1 Mediated Inhibition of RXR Function	2.93	1.70	(4) HMGCS1, IL1RN, TNF, ABCA1	(3) IL1RN, TNF, ABCA1
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.58	2.93	(3) IL1RN, TNF, LEF1	(5) FOS, IL1RN, TNF, DKK2, CXCL8
PPAR Signalling	1.74	2.68	(2) IL1RN, TNF	(3) FOS, IL1RN, TNF
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	1.90	2.51	(3) IL1RN, TNF, LEF1	(4) FOS, IL1RN, TNF, DKK2
Tetrahydrobiopterin Biosynthesis II	2.16	2.07	(1) GCH1	(1) GCH1
Tetrahydrobiopterin Biosynthesis I	2.16	2.07	(1) GCH1	(1) GCH1
Cholecystokinin/Gastrin-mediated Signalling	1.67	2.56	(2) IL1RN, TNF	(3) FOS, IL1RN, TNF
Airway Inflammation in Asthma	2.04	1.95	(1) TNF	(1) TNF
Role of MAPK Signalling in the Pathogenesis of Influenza	2.04	1.86	(2) CXCL10, TNF	(2) CXCL10, TNF

Functional analysis of genes associated with the differentially expressed probe sets for GTR/vehicle and Copaxone/vehicle (adjusted p-value < 0.05, fold change ≥ 1.4) using Ingenuity Pathway Analysis. Depicted are the top 30 most significant pathways sorted on total significance for GTR/vehicle and Copaxone/vehicle treatment (total $-\log_{10}$ p-value results from the Fisher's Exact test across all the observations). Pathway -log p-value > 1.3 or p-value < 0.05 indicates a statistically significant association. Adjusted p-value is p-value corrected for multiple testing. Grey scale indicates magnitude of adjusted p-value.

that form the axons of the optic nerve were investigated. GTR and Copaxone both increased survival of RGCs to a comparable extent (Fig. 9).

4.2.6. EAE Mouse test

Response to treatment was determined by calculating the percentage inhibition of EAE score compared to vehicle treated animals ((Geomean Max EAE score Vehicle – Geomean Max EAE score Treatment)/Geomean Max EAE score Vehicle*100). There was no statistically significant difference between the response of GTR and Copaxone (Fig. 10).

The data indicate that GTR has biological activity and significantly inhibited the PLP139-151-induced EAE response in SJL mice. The *in vivo* biological activity of GTR is the same as Copaxone.

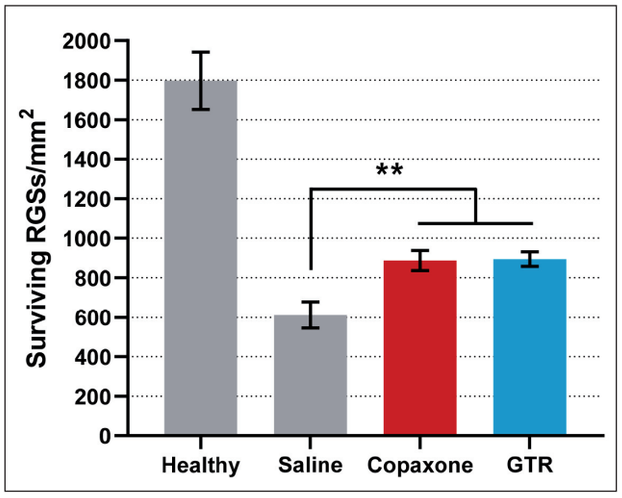

Fig. 9: Survival of retinal ganglion cells in EAE model in Brown Norway rats. Healthy are non-diseased animals, Saline are animals with maximum disease, Copaxone and GTR animals were treated daily with 50 µg per animal. Data are mean +- SEM. ** p<0.01 vs Saline, ANOVA followed by Dunnett's multiple Comparison test.

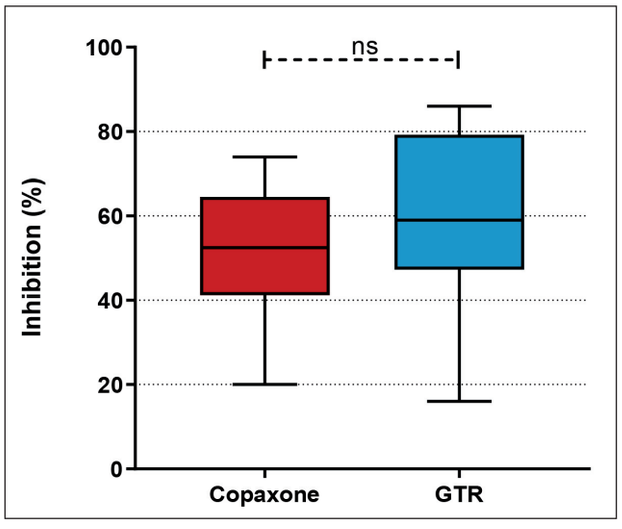

Fig. 10: Percentage inhibition of disease in EAE model in SJL mice. Animals were treated prophylactically by adding per animal 0.5 mg Copaxone or GTR to immunization mixture. Data are depicted in Box & Whiskers plot (min to max). Number of batches: Copaxone 12, GTR 32, ns = not significant, unpaired t-test.

4.2.7. *Nonclinical toxicity and safety*

Long-term toxicity and safety of GTR in comparison to Copaxone was assessed in a 28-day and 90-day repeat dose study in rats. Both studies were performed in male and female rats at dose levels ranging from 10 to 40 mg/kg/day. For GTR 20 mg/mL doses used in the toxicity studies (10 or 40 mg/kg/day) correspond to 31 and 125 times the human therapeutic dose on mg/kg basis (assumed body weight of 62.5 kg). A vehicle arm was included in all studies and all compounds were dosed daily by subcutaneous injection.

Local tolerability data show that GTR induces mild local effects not different from Copaxone. No treatment-related changes were observed for mortality, clinical appearance, body weights, food consumption, during ophthalmoscopic examination or urine and IgG analysis after treatment with Copaxone or GTR. Treatment-related local effects at the injection sites were recorded in control animals and in all treated animals with the highest incidence and severity in the high dose-treated animals. Treatment-related microscopic findings were recorded in the injection sites, stomach (only in 28-day study), kidney (only in 28-day study) and liver. The alterations were not observed at the end of the 28-day treatment-free period. Findings observed during clinical pathology might be related to the observed microscopic findings in liver and kidney. In general, the magnitude of these effects was comparable between the Copaxone and GTR treated animals.

Treatment with 10 or 40 mg/kg/day Copaxone or GTR resulted in an increased incidence and severity of local effects at the injection sites, perilobular fibrosis of liver and systemic perivascular (lympho) plasmacytic infiltrates in kidneys, liver, parotid glands and injection sites. Furthermore, several clinical pathology parameters were affected in animals treated with Copaxone or GTR. The toxic potential of GTR was comparable to that of Copaxone.

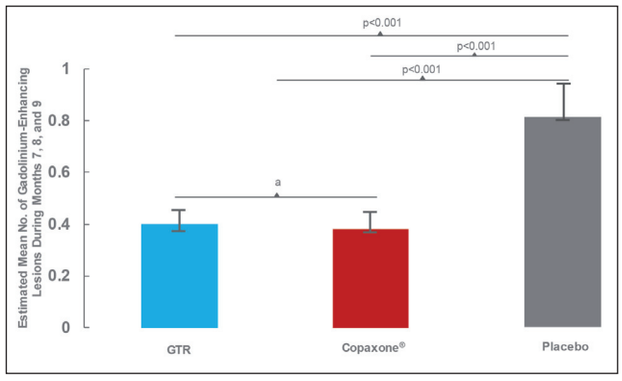

Fig. 11: Number of T1-GdE lesions. [a] The estimated mean number of new and persisting gadolinium-enhancing lesions during months 7 through 9 for the combined GTR (blue) and Copaxone® (red) treatment groups was 0.40. Reproduced with permission from Cohen et al. (2015) Copyright© (2015) American Medical Association. All rights reserved.

4.3. *Clinical evaluation*

4.3.1. *The GATE study*

The therapeutic equivalence of GTR and Copaxone was assessed in a 2-year, multicenter Phase III trial consisting of a 9-month, double-blind, randomized, placebo-controlled part comparing the efficacy, safety and tolerability of GTR (Copaxone (20 mg/mL), and placebo in subjects with RRMS followed by a 15-month open-label GTR treatment part. The design and the results of both part of the GATE study were published elsewhere (Cohen et al. 2015; Selmaj et al. 2017). In brief, patients were 18 to 55 years old with at least 1 relapse in the prior year and 1 to 15 gadolinium-enhancing brain magnetic resonance imaging (MRI) lesions at study entry. Patients were randomized 4.3:4.3:1 to receive GTR, Copaxone, or placebo by daily subcutaneous injection for 9 months. The primary end point was the total number of gadolinium-enhancing lesions during months 7, 8, and 9.

In total, 794 patients were randomized and treated with GTR (n = 353), Copaxone (n = 357), or placebo (n = 84) and more than 90% completed the 9-month double-blind treatment period (Cohen et al. 2015). The results (Fig. 11) showed that the estimated mean numbers of gadolinium-enhancing lesions with GTR and Copaxone were lower than with placebo (ratio, 0.488; 95% CI, 0.365-0.651; $P < 0.001$), confirming study sensitivity. For gadolinium-enhancing lesions, the estimated ratio of GTR to Copaxone was 1.095 (95% CI, 0.883-1.360). Point estimate and the boundaries of the 95% CI were within the predefined equivalence margin of 0.727 to 1.375. Incidence, spectrum, and severity of reported adverse events, including injection site reactions, were similar in the GTR and Copaxone groups. As expected, 80-90% of patients in both the active groups reported injection site reactions. The most common serious adverse events were MS relapse (2 GTR patients and 4 Copaxone patients), bronchitis (2 Copaxone patients), anaphylactoid reaction (1 GTR patient and 1 Copaxone patient), and angioedema (1 GTR patient and 1 (2019) Copaxone patient). All other serious adverse events occurred in

single patients. Overall, the results of the double-blind, placebo-controlled part establish therapeutic equivalence for GTR and Copaxone as treatment for RRMS.

4.3.2. *Open-label extension part*

During the 15-month open-label part, the longer-term efficacy, safety, and tolerability of GTR treatment were assessed; in addition, the efficacy, safety, and tolerability of switching from Copaxone to GTR treatment were also assessed (Selmaj et al. 2017).

More than 90 % of the originally randomized patients entered the open-label part and received GTR 20 mg/mL daily. In general, assessments for safety and efficacy were done every three months. The mean number of gadolinium-enhancing lesions in patients who continued on GTR (GTR/GTR) and for patients switching from Copaxone to GTR (Copaxone/GTR) was similar until the end of the open-label part. The change in other MRI parameters was also similar in the GTR/GTR and Copaxone/GTR groups. The annualized relapse rate (ARR) did not differ between the GTR/GTR and Copaxone/GTR groups, 0.21 and 0.24, respectively. The incidence, spectrum, and severity of reported adverse events did not differ between the GTR/GTR and Copaxone/GTR groups. In addition to comparable efficacy and safety being maintained over two years, switching from Copaxone to GTR was shown to be safe and well-tolerated.

4.3.3. *Glatiramer anti-drug antibodies*

The presence of glatiramer anti-drug antibodies (ADAs) was tested at baseline and months 1, 3, 6, 9, 12, 18, and 24. Glatiramer ADA titers for patients in the both active arms were comparable during the 9-month double blind-part and also when continuing on GTR for the overall 2-year study duration and when switching from Copaxone to GTR for the remaining 15 months of the study (Fig. 12).

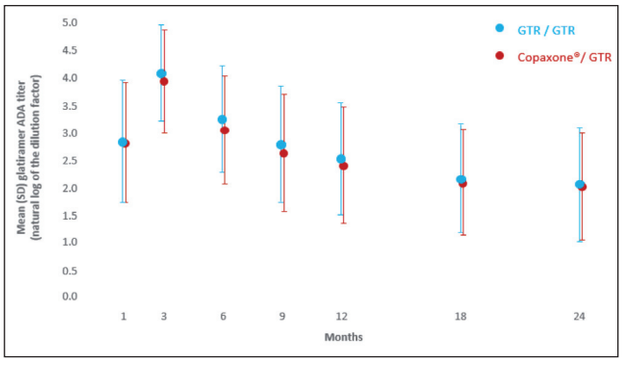

Fig. 12: Mean (SD) glatiramer ADA titer values in patients treated with GTR (blue) over 24 months and patients treated with Copaxone (red) for 9 months then switched to GTR for 15 months. This figure was originally published by SAGE in Selmaj et al. (2017).

4.3.4. *Conclusion on therapeutic equivalence of GTR 20 mg/mL to Copaxone 20 mg/mL*

The characterization program convincingly showed comparability of GTR and Copaxone in physicochemical characteristics. This was further supported by a robust set of nonclinical *in vitro* and *in vivo* studies and an adequately controlled randomized double-blind clinical trial to evaluate clinical efficacy, safety and tolerability. Based on these results, Synthon was granted marketing authorization for GTR 20 mg/mL pre-filled syringe in all 28 member-states of the European Union, and Iceland, Liechtenstein, and Norway.

4.3.5. *Bridging strategy for demonstrating equivalence between Copaxone 40 mg/mL and GTR 40 mg/mL*

When comparing the quality aspects of the 20 and 40 mg/mL products for both GTR and Copaxone it can be concluded that the 20 and 40 mg/mL products contain the identical drug substance and identical excipients in the same amounts. Moreover, the drug product is filled in an identical container closure system and manufactured utilizing an identical, well-controlled, straightforward drug product manufacturing process. Hence, the sole difference is the higher concentration of glatiramer in the 40 mg/mL formulation.

Demonstrating the safety and efficacy of GTR 40 mg/mL three times a week (TIW) was therefore based on a straightforward and comprehensive bridging strategy comprising three "bridges" (Fig. 13). including reference to the pivotal registration study of Copaxone TIW vs. placebo in patients with RRMS (Khan et al. 2013).

Bridge 1 (Physicochemical similarity, *in vitro,* and *in vivo* studies *plus* the clinical GATE study) confirmed the equivalence of the critical and complex drug substance of GTR and Copaxone at the level of the 20 mg/mL product.

Bridge 2 (Physicochemical similarity, *in vitro,* and *in vivo* studies) confirmed the equivalence of GTR 20 mg/mL to GTR 40 mg/mL and that a doubling of the concentration did not affect any of the product's characteristics.

Bridge 3 (Physicochemical similarity, *in vitro,* and *in vivo* studies) confirmed the equivalence of GTR 40 mg/mL and Copaxone 40 mg/mL.

Together, the three bridges show that the drug substances in GTR 20 and 40 mg/mL and Copaxone 20 and 40 mg/mL are the same and that a doubling of the concentration does not affect any of the drug substance's characteristics. An additional head-to-head clinical study was not undertaken because, unlike physicochemical testing, there is no clinical study design methodology that would be sufficiently sensitive to detect minute differences that could have resulted from changes in drug-substance concentration. Efficacy and safety of the 40 mg/mL TIW dosing regimen was supported by the data from the pivotal registration study of Copaxone TIW vs. placebo in patients with RRMS (Khan et al. 2013).

Based on this bridging strategy, Synthon was granted marketing authorization for GTR 40 mg/mL pre-filled syringe in all 28 member-states of the European Union, and Iceland, Liechtenstein, and Norway approximately a year and a half after approval of the 20 mg/mL pre-filled syringe (Synthon 2016, 2017).

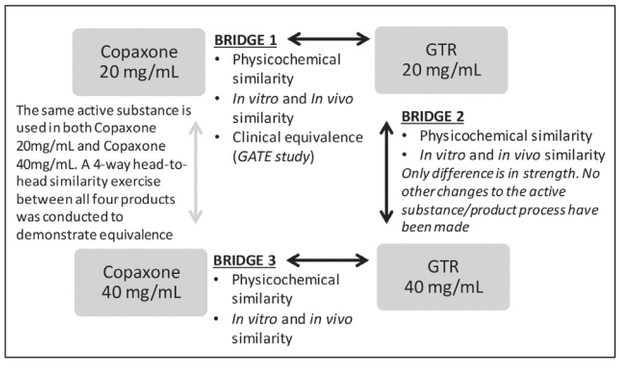

Fig. 13: Bridging strategy

5. Discussion

Glatiramer acetate is a synthetic heterogeneous mixture of copolymer chains (see above) indicated for the treatment of patients with relapsing forms of multiple sclerosis. It is a synthetic compound and for such compounds one of two approval pathways should be followed to obtain marketing authorization in the EU: generic or hybrid. Considering glatiramer's heterogeneity and that demonstrating therapeutic equivalence requires a phase III clinical study, obtaining market authorization in Europe for a therapeutically equivalent alternative to Copaxone could not be obtained through the regulatory pathway for generic drugs. Instead, the hybrid approval pathway had to be followed. This paper summarizes the underlying data supporting the market authorization of this complex follow-on drug.

The identity, quality, and consistency of glatiramer is dependent on the manufacturing process and an extensive characterization program on drug substance and drug product is required to demonstrate adequate process control and control on the batch-to-batch variability and biological activity. Moreover, comparability to the reference product Copaxone had to be established. From scientific advice from both national health authorities in EU countries and ultimately the EMA, it was concluded that demonstrating GTR therapeutic equivalence to the reference product, Copaxone, should be based on scientific evidence supporting comparability in physicochemical characteristics, nonclinical *in vitro* and *in vivo* studies, and an adequately sensitive double-blind clinical trial in RRMS patients. Orthogonal analytical techniques (i.e., different techniques to elucidate various physicochemical properties of the drug) were used for testing primary structure, higher order structure, and other physicochemical properties. All methods were qualified or validated and numerous Copaxone batches have been characterized in the various methods to determine intra-assay, inter-assay and batch-to-batch variability. Some methods are semi-quantitative or descriptive by nature and therefore exact specifications cannot always be set. For such methods relevant ranges were determined based on various negative controls (e.g. negative control polymer) and on intra-assay, inter-assay and batch-to-batch variability. This negative control polymer was manufactured using a slightly different process affecting the primary structure of glatiramer while retaining the same overall amino acid composition and molecular weight as Copaxone. The different physicochemical properties as a consequence of the changes in its primary structure allow its use in the bioanalytical assays. In descriptive methods, the spectroscopic fingerprints of the negative control polymer were different compared to GTR and Copaxone, demonstrating the discriminatory power of the physicochemical tests with regard to microheterogeneities.

Demonstrating comparability of non-biological complex drug is a relevant and highly debated topic in scientific discussions and for regulatory authorities (Hussaarts et al. 2017). A single set of critical attributes that determine essential product characteristics for glatiramer are not yet defined and to obtain market authorization it is up to the manufacturer to propose, justify, and execute a scientifically rigorous strategy to demonstrate therapeutic equivalence.

The methods to demonstrate comparability between Copaxone and GTR in physicochemical characteristics provided information on e.g. amino acid composition, molecular weight distribution as well as structural and spectroscopic fingerprints. Biological assays, *in vitro* and *in vivo*, were conducted to demonstrate biological activity, nonclinical safety and gene expression profiling. Obviously, no single method is able to determine all critical attributes of such a complex drug, but a well-designed set of physicochemical and biological orthogonal methods can very accurately describe the product characteristics leading to a therapeutically equivalent drug. Despite the long list of orthogonal methods that have been used, no set of methods will ever be able to provide an unambiguous and full characterization of heterogeneous compounds such as glatiramer.

Microheterogeneities are an important factor for non-biological complex drugs, but do not necessarily have biological relevance. It should be noted that "comparable" does not equal "identical" as such minor differences also exist between batches of the same product with the definition of "minor difference" remaining arguable. Closely related is the (mis)interpretation that a statistical difference de facto results in a biologically relevant effect. The latter is in particularly relevant when comparing two complex drugs in gene expression studies and biological assays. Gene expression profiling is a useful tool in the analysis of mechanism of action, but it is less suitable to demonstrate comparability of complex drugs. Significant differences in expression of 98 genes were found in T-cells between a generic version of glatiramer and Copaxone (Bakshi et al. 2013). In a study from Towfic et al. (2014), Copaxone induced a significantly higher expression of FoxP3 and Gpr83 compared to a generic version of glatiramer, though both genes were strongly upregulated compared to control medium. The authors link this statistical difference to a potential biological effect and suggest an impact on clinical safety and efficacy. In a gene expression study with a very similar experimental set-up Foxp3 and Grp83 were not changed at all by Copaxone or a generic version of glatiramer compared to a control medium (D'Alessandro et al. 2015).These discrepant results are likely due to differences in experimental set-up and/or methodological differences. Melamed-Gal et al. (2018) reported microheterogeneities between GTR as compared with Copaxone. No data was provided that any of the claimed microheterogeneities have any biological effect or clinical ramification (Cohen et al. 2018).

What ultimately matters are differences that are relevant for clinical efficacy or safety. The 2-year GATE study in 794 RRMS patients established comparable safety, efficacy, tolerability, and immunogenicity (Cohen et al. 2015; Selmaj et al. 2017). Furthermore, switching from Copaxone to GTR was shown to be safe and well-tolerated. In addition, equivalence of GTR 40 mg/mL TIW and Copaxone 40 mg/mL TIW was demonstrated using an appropriate bridging strategy incorporating physicochemical characterization methods as well as available biological and clinical data. These results provide evidence that the observed microheterogeneities in physicochemical and non-clinical assays are clinically irrelevant.

In conclusion, establishing therapeutic equivalence for non-biological complex drugs is not trivial and requires a complex and multidisciplinary effort. Ultimately, there is not a single test or study that establishes therapeutic equivalence of two heterogeneous products. Instead, it requires a good understanding of the synthesis process together with a full set of data that includes comparative physicochemical testing, nonclinical *in vitro* and *in vivo* studies, and a comparative clinical study to allow a founded conclusion that two products are therapeutically equivalent.

The detailed understanding of glatiramer's synthesis process and its impact on the characteristics of glatiramer, combined with the results of a scientifically rigorous and multifaceted physicochemical and biological characterization program, and the clinical data from the GATE study, demonstrate that GTR and Copaxone are therapeutically equivalent. The data further demonstrate that Synthon's manufacturing process consistently yields drug substance of the same quality as Copaxone and that switching from Copaxone to GTR is safe and well-tolerated.

6. Experimental

All *in vivo* studies and protocols were approved by the institutional animal care and use committees according to European regulations for the care and use of laboratory animals.

Central and local ethics committees approved the clinical study. Participants gave written informed consent before any study-related procedures were performed. The study was conducted in accord with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH 1996) guidelines for good clinical practice and principles of the Declaration of Helsinki (ICH 1996; WMA 2013).

6.1. Primary structure

Amino acid composition: Provides information with respect to the molar fraction of amino acids comprising the drug substance. *Peptide mapping/proteolytic digestion profile*: The enzyme trypsin is a serine protease and cleaves, when used for the digestion of glatiramer, at the carboxyl side of the amino acid lysine. The fragments were analyzed by means of liquid chromatography to identify distinguishable and repeatable sequences, a so-called 'fingerprint'.

6.2. Higher order structure

Structure analysis by Circular Dichroism: Circular Dichroism (CD) was used to determine the secondary structures of the polypeptides in glatiramer. This method provides information related to α-helix, β-sheet, and/or random coil conformational structures. *Coomassie Brilliant Blue binding*: The Coomassie Brilliant Blue (CBB) binding test was used to evaluate the interaction of glatiramer with CBB (a dye that exhibits affinity to various proteins and peptides). This interaction, visualized by UV-light, is contingent on glatiramer-specific chemical properties and higher order structure. Thus, differences in the binding behavior of CBB is indicating differences in higher order structure.

Intrinsic Fluorescence Spectroscopy: Fluorescence spectroscopy was used to examine structural differences of the polypeptides in glatiramer. The intrinsic fluorescence of the polypeptides in these batches originates from the aromatic residue tyrosine present in the polypeptide chains of glatiramer.

6 3. Other physicochemical properties

Chromatographic property by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC): RP-HPLC was used to identify the characteristic polypeptides in glatiramer via the assessment of specific hydrophobic interactions. Specifically, a characteristic multi-component peak is expected based on evaluations of glatiramer. The hydrophobic interactions of glatiramer were assessed by evaluation of the position of multi-component peaks. *Ultraviolet (UV) spectroscopy*: UV spectroscopy was used to identify and quantify characteristic polypeptides in glatiramer. Specifically, absorbance maxima at three characteristic transition wavelengths for the polypeptides in glatiramer. *Molecular Weight Distribution (MWD) by Gel Permeation Chromatography (GPC)*: GPC was used to determine the Molecular Weight Distribution (MWD) profile of the polypeptides in glatiramer. It provides information on the number average molecular weight, weight average molecular weight, Z average molecular weight, and polydispersity index. Coupled with a UV detector, GPC was used to measure MWDs relative to characteristic protein molecular weight markers. Coupled with a Multi Angle Light Scattering (MALS detector), GPC was used to determine absolute *MWDs.Heterogeneity analysis by Capillary IsoElectric Focusing (cIEF)*: cIEF is a high-resolution separation technique which, based on differences in isoelectric points, can be used to determine the identity and charge distribution of molecules. This method was used to determine the heterogeneity of the polypeptides in the glatiramer batches.

6.4. Comparability in nonclinical in vitro and in vivo studies

Western Blot: Western blotting technique was used to evaluate the relative binding of glatiramer to anti-GTR and anti-Copaxone polyclonal antibodies.*Enzyme-linked immunosorbent assay (ELISA)*: This plate-based assay technique was used to determine the relative binding of anti-GTR polyclonal antibodies to Copaxone and GTR. *Biological activity by Cell Based Assay (CBA)*: CBA was used to quantify *in vitro* biological activity of GTR or Copaxone based on glatiramer-induced secretion of soluble Interleukin-1 Receptor antagonist (sIL-1Ra) by the human monocytic THP-1 cell line. Soluble IL-1Ra is considered to be a relevant mediator in the pathogenesis of multiple sclerosis (MS). THP-1 cells were incubated with IFN-γ to induce differentiation to a monocytic phenotype. Soluble IL-1Ra was, thereafter, determined using ELISA and biological activity was quantified relative to reference standard and expressed as relative potency. *Micro array study*: Gene expression was evaluated on Affymetrix 24 HT HG U133+ PM arrays after exposure of THP-1 cells *in vitro* to Copaxone or GTR.

Experimental Autoimmune Encephalomyelitis (EAE) MOG model in Brown Norway rats: In a rat model of EAE, the effects of GTR or Copaxone on survival of retinal ganglion cells were investigated (Maier et al. 2006). EAE was induced by intradermal injection of an emulsion containing 100 µg myelin-oligodendrocyte-glycoprotein in saline with complete Freund's adjuvant containing 200 µg heat-inactivated Mycobacterium tuberculosis. Animals were treated daily subcutaneously with 50 µg per animal Copaxone or GTR. Clinical EAE signs and disease severity was assessed and survival of retinal ganglion cells (RGCs), the neurons that form the axons of the optic nerve was quantified.

Experimental Autoimmune Encephalomyelitis $PLP_{139-151}$ model in SJL mice: This is an in vivo model for MS and was used to determine the *in vivo* biological activity of GTR or Copaxone in MS research (Teitelbaum et al. 1996). EAE was induced by subcutaneous injection of 75 µg $PLP_{139-151}$ emulsified in incomplete Freund's adjuvant supplemented with *Mycobacterium tuberculosis*. After 1 and 3 days, mice were injected intravenously with heat killed *Bordetella pertussis* bacteria to increase the permeability of the blood-brain barrier. Animals were treated prophylactically by adding per animal 0.5 mg Copaxone or GTR to the immunization mixture. Clinical EAE signs and disease severity was assessed using a scale from 0 to 5. The response to treatment was determined by calculating the percentage inhibition of EAE score compared to vehicle treated animals ((Maximum EAE score vehicle – maximum EAE score treatment) / maximum EAE score vehicle * 100). *Toxicity and safety studies in rat*: Toxicity and safety was assessed in 28-day and 90-day toxicity studies in male and female rats. Doses used in the toxicity studies (10 or 40 mg/kg/day) correspond to 31 and 125 times the human therapeutic dose on mg/kg basis (assumed body weight of 62.5 kg) and 5 and 20 times the human therapeutic dose based on a mg/m^2 basis. A vehicle arm was included in all studies and all compounds were dosed daily by subcutaneous injection.

Acknowledgements: The authors thank J. van Orsouw for graphic support and J. Salem of Global Research Services LLC for support in preparing the draft manuscript of this publication.

Conflicts of Interest: R.J. Arends, M. Buurman, J. Luten and N.P. Koper are employees of Synthon Biopharmaceuticals B.V. M. Scheren and D. Wang are employees of Synthon B.V. C. Wolf is a partner at Lycalis sprl and reports compensation for his organization for consulting from Novartis, Teva, Celgene, Mylan, Synthon, BBB, ICON and Desitin; and for speaking from Mylan and Synthon.

Authors' Contributions: Conception and design: R.J. Arends, M. Scheren, D. Wang. Development of methodology: R.J. Arends, M. Buurman, N.P. Koper, J. Luten, D. Wang.
Acquisition of data: R.J. Arends, M. Buurman, N.P. Koper, J. Luten, D. Wang. Analysis and interpretation of data: R.J. Arends, M. Buurman, N.P. Koper, J. Luten, M. Scheren, C. Wolf, D. Wang.
Writing, review, and/or revision of the manuscript: R.J. Arends, D. Wang, M. Buurman, J. Luten, N.P. Koper, C. Wolf, M. Scheren.

References

Bakshi S, Chalifa-Caspi V, Plaschkes I, et al. (2013) Gene expression analysis reveals functional pathways of glatiramer acetate activation. Expert Opin Ther Targets 17: 351-362

Cohen J, Belova A, Selmaj K, et al. (2015) Equivalence of generic glatiramer acetate in multiple sclerosis a randomized clinical trial. JAMA Neurol 72: 1433-1441.

Cohen JA, Wolf C, Selmaj KW, Arends R (2018) Follow-on glatiramer acetate. eNeurologicalSci. 13: 51-52

Copaxone SmPC https/www.medicines.org.uk/emc/product/183/smpc#AUTHDAT [accessed 17Apr2019].

Copaxone USPI : https/www.accessdata.fda.gov/drugsatfda_docs/label/2014/020622s089lbl.pdf. [accessed 17Apr2019].

D'Alessandro J.S, Duffner J, Pradines J, et al. (2015) Equivalent gene expression profiles between Glatopa and Copaxone(R). PLoS One 10: e0140299.

Hussaarts L, Muhlebach S, Shah VP, et al. (2017) Equivalence of complex drug products: advances in and challenges for current regulatory frameworks. Ann NY Acad Sci 1407: 39-49.

ICH (1996) International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH harmonized tripartite guideline: guideline for good clinical practice: E6(R1). Current step 4 version dated June 10, 1996. http://www.ich.org/fileadmin/Public_WebSite/ICH_Products/Guidelines/Efficacy/E6/E6_R1_ Guideline.pdf. [accessed 17Apr2019].

Khan O, Rieckmann P, Boyko A, et al. (2013) Three times weekly glatiramer acetate in relapsing- remitting multiple sclerosis. Ann Neurol 73: 705-713.

Maier K, Kuhnert AV, Taheri N, et al. (2006) Effects of glatiramer acetate and interferon-beta on neurodegeneration in a model of multiple sclerosis: a comparative study. Am J Pathol 169: 1353-1364.

Melamed-Gal S, Loupe P, Timan B, et al. (2018) Physicochemical, biological, functional and toxicological characterization of the European follow-on glatiramer acetate product as compared with Copaxone. eNeurologicalSci 12: 19-30.

Selmaj K, Barkhof F, Belova, A, et al. (2017) Switching from branded to generic glatiramer acetate: 15-month GATE trial extension results. Mult Scler 23: 1909-1917.

Synthon(2016): https://www.synthon.com/es/acerca-de-synthon/comunicados-de-prensa/synthon-obtains-approval-glatiramer-acetate-20-mgml-europe [accessed 17Apr2019].

Synthon(2017):https://www.synthon.com/press-releases/regulatory-approval-europe-granted-synthons-glatiramer-acetate-40-mgml-1 [accessed 17Apr2019].

Teitelbaum D, Meshorer A, Hirshfeld T, Arnon R, et al. (1971) Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. Eur J Immunol 1: 242-248.

Teitelbaum D, Fridkis-Hareli M, Arnon R, et al. (1996) Copolymer 1 inhibits chronic relapsing experimental allergic encephalomyelitis induced by proteolipid protein (PLP) peptides in mice and interferes with PLP-specific T cell responses. J Neuroimmunology 64: 209-217.

Teva (2014a) : https://www.tevapharm.com/news/teva_announces_u_s_fda_approval_of_three_times_a_week_copaxone_glatiramer_acetate_injection_40mg_ml_01_14.aspx [accessed 17Apr2019].

Teva (2014b) : https://www.tevapharm.com/news/teva_receives_positive_outcome_in_europe_for_three_times_a_week_copaxone_glatiramer_acetate_40_mg_ml_for_the_treatment_of_relapsing_forms_of_multiple_sclerosis_rms_12_14.aspx [accessed 17Apr2019].

Towfic F, Funt JM, Fowler KD, et al. (2014) Comparing the biological impact of glatiramer acetate with the biological impact of a generic. PLoS One 9: e83757

WMA (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310: 2191-2194.

Supplementary material

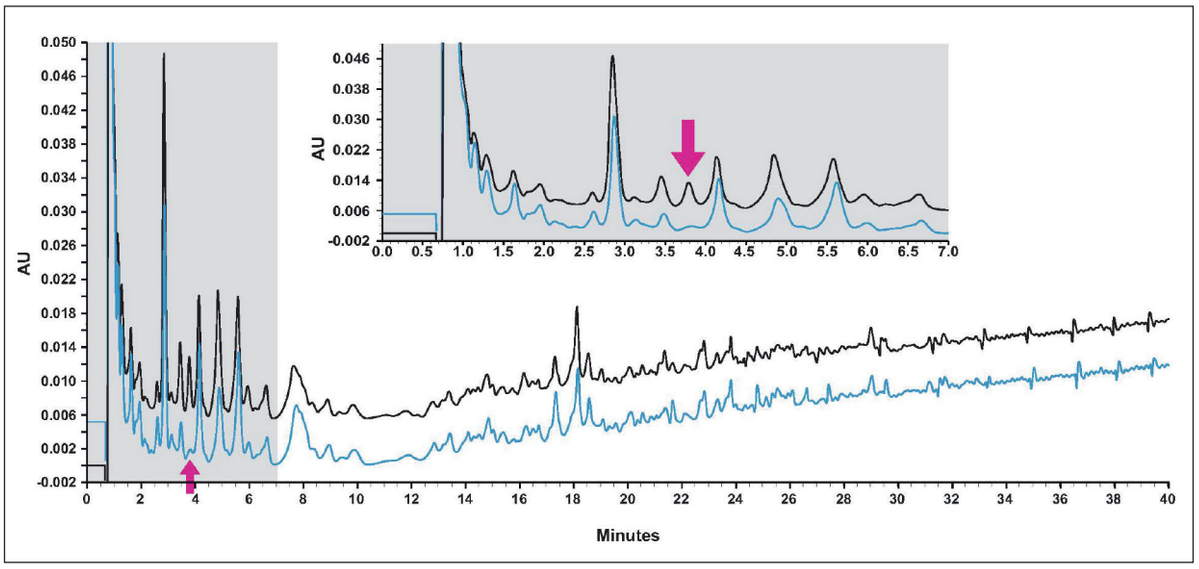

Fig. 14: UPLC-UV (214 nm) chromatograms of trypsin digested GTR (blue) and negative control polymer (black). Zoom 2-7 minutes shows a peak at 3.8 minutes in in negative control polymer but not in GTR.

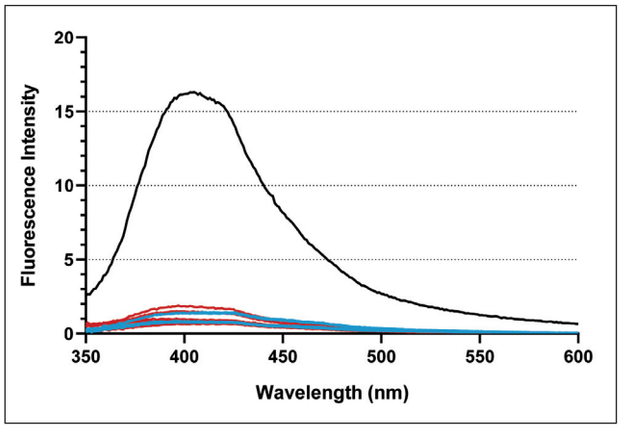

Fig. 15: Fluorescence emission spectra of Copaxone (6 batches, red), GTR (6 batches, blue) and negative control polymer (black).

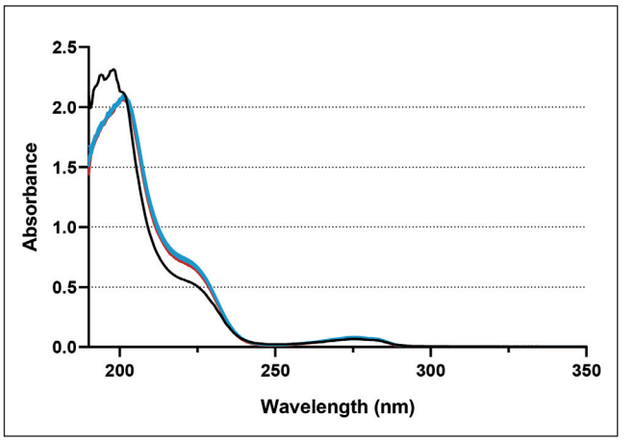

Fig. 16: UV-Vis spectra of Copaxone (6 batches, red), GTR (6 batches, blue) and negative control polymer (black).

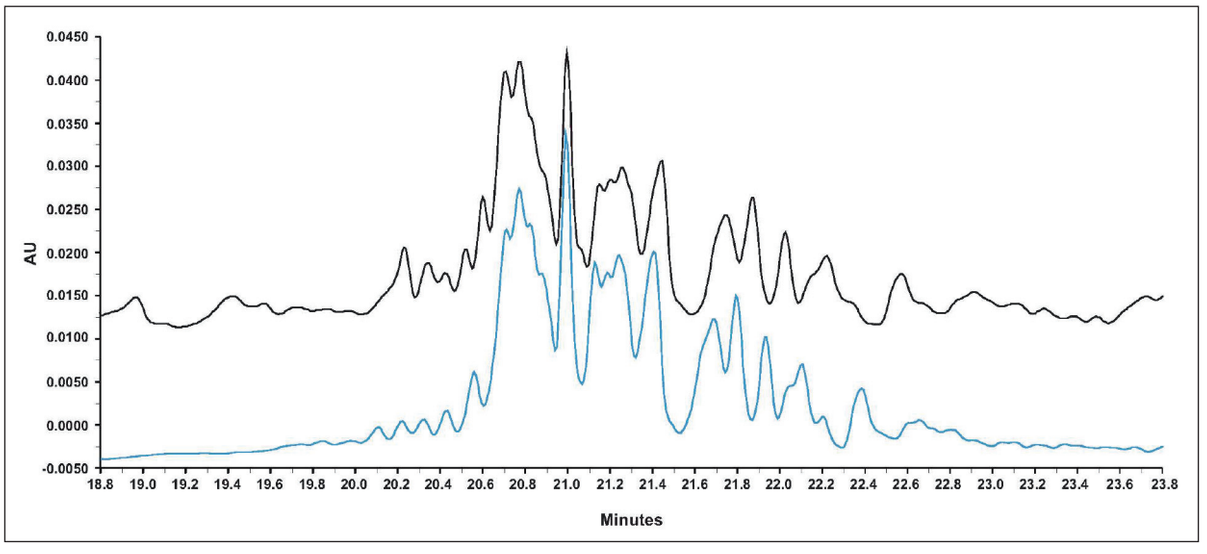

Fig. 17: cIEF results (UV 280nm) of GTR (blue) in overlay with that of negative control polymer (black).

Supplementary material

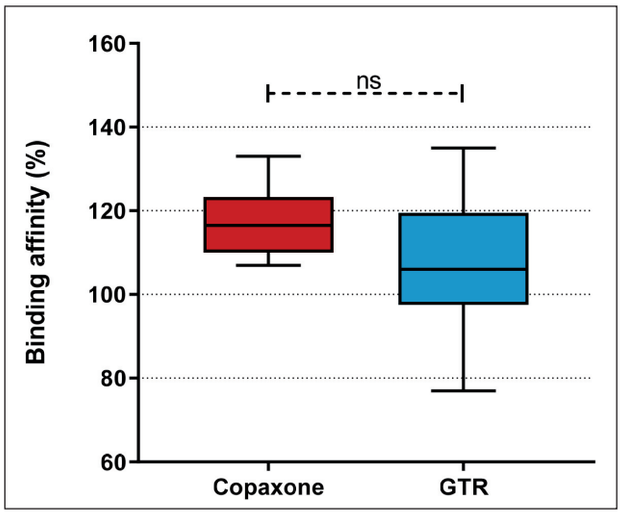

Fig. 18: ELISA analysis of Copaxone and GTR batches using anti-GTR polyclonal antibodies. Data are depicted in Box & Whiskers plot (min to max). Number of batches: Copaxone 6, GTR 13 ns = not significant, unpaired t-test.