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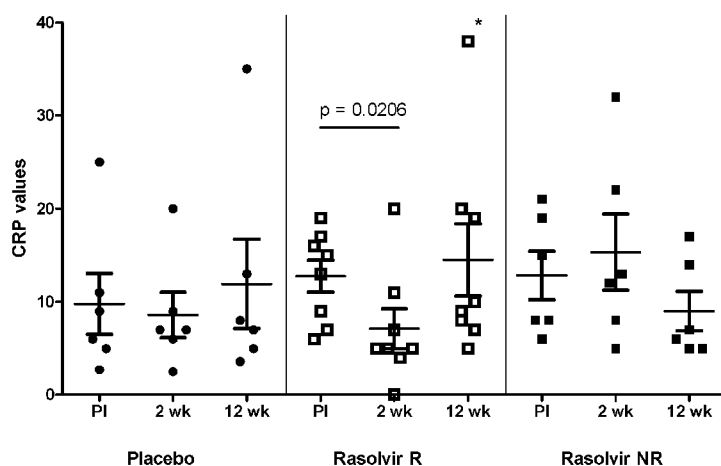
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(54) **Title:** BIOMARKERS FOR RESPONSE TO BIP (RASOLVIR™)

Figure 1



(57) **Abstract:** The invention relates to a method of determining responsiveness to BiP administration in a subject, the method comprising (a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP (b) comparing said concentration(s) determined in (a) to reference concentration(s) for said biomarker(s) wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) indicates that the subject is responsive to Bi P administration. The invention also relates to kits, devices, uses, processes and syringes.

**BIOMARKERS FOR RESPONSE TO BIP (RASOLVIR™)**BACKGROUND

5 The invention is in the field of treatment of rheumatoid arthritis and/or bone loss and/or bone resorption using BiP (binding immunoglobulin protein).

BiP (binding immunoglobulin protein) refers to the 78kD endoplasmic reticulum chaperone protein as disclosed in WO 00/21995.

10

WO 00/21195 discloses treatment of inflammatory disease using BiP.

WO 2006/111720 discloses use of BiP in the manufacture of a medicament for the prevention or treatment of bone loss or bone resorption.

15

The treatment of rheumatoid arthritis (RA) has been transformed by the use of targeted protein biologics [1]. Despite these advances in the management of RA, the great unmet need is the development of therapies that induce “cure”: prolonged periods of drug-free remission [2]. Anti-cytokine biologics are only functional while an effective serum  
20 concentration is maintained i.e. pharmacodynamics (PD) and pharmacokinetics (PK) are concordant requiring frequent repeat dosing [3]. In contrast, Rituximab, an anti-CD20 antibody, by killing CD20+ B cells, can have a prolonged beneficial clinical effect ie PD in excess of PK [4]. However, Rituximab has the disadvantage [5] of being a cellular ablative therapy.

25

There is now overwhelming evidence that immune response homeostasis is maintained by immune regulatory cells of varying descriptions but mainly B-cells [6] and T-cells [7]. The induction of regulatory cells may be the best prospect for developing curative therapies for RA as defined above [8]. Developing cellular therapies is confronted by  
30 the dual problems of consistent methods for expanding regulatory cell populations and then determining optimal doses and frequency of administration [9,10]. Stress proteins have been demonstrated to generate regulatory functions, suggesting they may be a suitable alternative way of generating this response. Our pre-clinical studies have shown that systemically administered Rasolvir™ (BiP, 78kD glucose regulated protein),  
35 an endoplasmic reticulum resident chaperone and stress protein, has potent anti-inflammatory and immunomodulatory properties [11]. More pertinently Rasolvir™, through deactivation of human monocytes and abrogation of dendritic cell maturation,

leads to induction of CTLA-4+ regulatory T cells [12]. A single injection of Rasolvir™ in mice with collagen induced arthritis delivers a prolonged, sustained therapeutic response that can be transferred by Rasolvir™ sensitised spleen and lymph node cells in the absence of additional Rasolvir™ [12]. Furthermore, a single intravenous dose of Rasolvir™ in mice with severe combined immunodeficiency (SCID), bearing subcutaneous transplants of RA synovial membrane, led to suppression of rheumatoid inflammation [13]. This model has been used to test biologics, such as anti-TNFα and anti-IL-6 receptor antibodies [14,15].

US 2008/0155704 discloses methods and compositions for determining *in vivo* activity of BiP. In particular, this document discloses a range of markers which might be determined in a sample, which are mostly focussed on cell determinants (CD antigens). There is no mention of the panel of biomarkers of the present invention in this document.

Kirkham *et al.* (Panels of Rheumatic Diseases 2015, Volume 74 (Supp. 2: 480) discloses Results Of A Single Dose Ascending First In Man Trial Of A Novel Biologic, Human Stress Protein Rasolvir (BiP) In Rheumatoid Arthritis (RA): The Ragulo Trial. This document contains no disclosure of any method of assessing the effectiveness of BiP after it has been administered to a patient.

The BiP Study (“THE BIP STUDY\_FIRST IN HUMAN STUDY TO INVESTIGATE THE EFFECTS OF BIP IN PATIENTS WITH RA”) is an outline of the BiP 001 study published in 2015 (see <http://www.khpcto.com/BIP/about.aspx?lang=1>). There is no mention of the panel of biomarkers of the present invention in this document.

A seminar entitled “Rasolvir™: From bench to bedside and back again” was delivered by inventor Corrigan on 5 May 2015. ). There was no mention of the panel of biomarkers of the present invention in this seminar.

An abstract FRI0159 “Results of a Single Dose Ascending First-in-Man Trial of a Novel Biologic, Human Stress Protein Rasolvir (BIP) in Rheumatoid Arthritis (RA): The Ragulo Trial” was published in 2015 (Ann Rheum Dis 2015 vol. 74 Suppl. 2: 480). There is no mention of the panel of biomarkers of the present invention in this document.

It has been a problem in the field to find markers indicative of a response to therapy. This is a problem in the art.

#### SUMMARY OF THE INVENTION

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It is a problem in rheumatoid arthritis that there are no objective measures of the effectiveness of therapy. Assessing the effectiveness of therapy involves interviewing patients. Patients' responses to the interview questions are typically very subjective and vary widely. Often a strong placebo effect is observed.

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In contrast, the inventors have surprisingly identified robust biomarkers which are indicative of response to treatment of subjects with BiP, such as treatment of rheumatoid arthritis with BiP. In particular, the present invention enables the separation of responders from non-responders by examination of biomarkers. This has utility not only in assessment of patients' progress but also in the selection of treatment regimes and aiding a physician's decision whether or not to treat (or retreat) with BiP.

15

Thus, in one aspect the invention provides a method of determining responsiveness to BiP administration in a subject, the method comprising

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(a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP

(b) comparing said concentration(s) determined in (a) to reference

25

concentration(s) for said biomarker(s)

wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) indicates that the subject is responsive to BiP administration.

In one aspect, the invention relates to a method of determining the likelihood of remission of rheumatoid arthritis following administration of BiP to a subject or treatment of a subject with BiP, the method comprising

30

(a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject

wherein said sample was obtained from said subject at about 2 to 12 weeks after

35

administration of BiP

(b) comparing said concentration(s) determined in (a) to reference

concentration(s) for said biomarker(s)

wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) indicates an increased likelihood of remission of rheumatoid arthritis in said subject.

- 5 In one aspect, the invention relates to a method of selecting a treatment regime for a subject with rheumatoid arthritis, the method comprising:
- (a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained after administration of BiP to a subject or treatment
- 10 of a subject with BiP, wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP
- (b) comparing said concentration(s) determined in (a) to reference concentration(s) for said biomarker(s)
- 15 wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) selects a treatment regime comprising a second or further administration of BiP to said subject.

Suitably the concentration of at least one of VEGF and IL-8 is determined.

20

Suitably the concentrations of at least two of said biomarkers are determined in (a).

Suitably the concentrations of both VEGF and IL-8 are determined.

25 Suitably the concentrations of all of said biomarkers are determined in (a).

Suitably determining the concentration of each biomarker comprises:

- (bi) detection by contacting the sample with an antibody or antigen binding fragment thereof capable of specifically binding the biomarker; and
- 30 (bii) quantification of said binding.

Suitably determining the concentration of each biomarker comprises detection of the mRNA for the biomarker, wherein detection of the mRNA comprises:

- (bi) contacting the sample with specific nucleic acid probe(s) or primer(s) for the
- 35 biomarker; and
- (bii) quantification of said probe(s) or primer(s).

Suitably said sample is a sample of serum or plasma.

In one aspect, the invention relates to a kit comprising reagent(s) for the specific detection of each of the following biomarkers: VEGF, IL-8, and CRP.

5

In one aspect, the invention relates to a kit comprising reagents for the specific detection of mRNA encoding each of the following biomarkers: VEGF, IL-8, and CRP.

10 In one aspect, the invention relates to a device comprising an array of materials which together are capable of specifically binding each of the following biomarkers: VEGF, IL-8, and CRP, each material within the array being capable of specifically binding one of said biomarkers.

15 In one aspect, the invention relates to a device comprising an array of materials which together are capable of detecting mRNA specific for each of the following biomarkers VEGF, IL-8, and CRP, each material within the array being capable of specifically detecting one of said mRNAs.

20 In one aspect, the invention relates to use of BiP for treatment of rheumatoid arthritis in a subject, wherein the method as described above is carried out for said subject, wherein if it is determined that said subject is responsive to BiP administration then BiP is administered to said subject.

25 Suitably a treatment regimen of administering 5 mg to 15 mg BiP to said subject is selected.

Suitably a treatment regimen of administering a second or further 5 mg to 15 mg BiP to said subject is selected.

30 In one aspect, the invention relates to a method of treating a subject comprising carrying out the method as described above, wherein if it is determined that the subject has a decrease in the concentration(s) in the sample compared to the reference concentration(s), BiP is administered to said subject.

35 In one aspect, the invention relates to a process for selecting a treatment regimen, said process comprising

carrying out the method as described above, wherein if it is determined that the subject has a decrease in the concentration(s) in the sample compared to the reference concentration(s), then a treatment regimen of a second or further administration of BiP is selected.

5

In one aspect, the invention relates to a syringe containing a composition comprising an effective amount of BiP for treatment of rheumatoid arthritis in a subject, wherein the method as described above is carried out for said subject, wherein if it is determined that the subject has a decrease in the concentration(s) in the sample compared to the reference concentration(s), then said syringe is used to administer said composition to said subject.

In one aspect, the invention relates to a syringe as described above wherein said effective amount is 5 mg to 15 mg of BiP.

15

In one aspect, the invention relates to use of a combination of materials each of which recognises, specifically binds to or has affinity for one of the following biomarkers: VEGF, IL-8, and CRP, wherein said combination includes at least one such material for each of said biomarkers, for determining responsiveness to BiP administration in a subject. Suitably said material comprises an antibody or antigen binding fragment thereof.

In one aspect, the invention relates to use for determining responsiveness to BiP administration in a subject, of a combination of materials each of which recognises, specifically binds to or has affinity for mRNA of one or more of the following biomarkers: VEGF, IL-8, and CRP. Suitably said material comprises a nucleic acid primer or probe.

In one aspect, the invention relates to an apparatus comprising logic configured to carry out the method as described above.

In one aspect, the invention relates to a computer program product operable, when executed on a computer, to perform the method steps as described above.

In one aspect, the invention relates to a method of treatment of Juvenile Idiopathic Arthritis (JIA), said method comprising administering BiP to said subject, suitably 5 mg to 15 mg of BiP to said subject.

In one aspect, the invention relates to BiP for treatment of Juvenile Idiopathic Arthritis (JIA).

- 5 In one aspect, the invention relates to use of BiP in the treatment of Juvenile Idiopathic Arthritis (JIA).

In one aspect, the invention relates to a method of treatment of spondylitic disease, said method comprising administering BiP to said subject, suitably 5 mg to 15 mg of  
10 BiP to said subject.

In one aspect, the invention relates to BiP for treatment of spondylitic disease.

In one aspect, the invention relates to use of BiP in the treatment of spondylitic disease.  
15

In one aspect, the invention relates to a method of treatment of psoriatic arthritis, said method comprising administering BiP to said subject, suitably 5 mg to 15 mg of BiP to said subject.

- 20 In one aspect, the invention relates to BiP for treatment of psoriatic arthritis.

In one aspect, the invention relates to use of BiP in the treatment of psoriatic arthritis.

#### DETAILED DESCRIPTION OF THE INVENTION

25 The invention has particular value in post-treatment assessment of efficacy. Biomarker data as taught by the invention has special value because most of the current measures used in the art to assess effectiveness of rheumatoid arthritis treatments are based on how a patient “feels”. This leads to confounding of the assessment. This leads to  
30 exacerbation and widespread placebo effects. The present invention advantageously overcomes these problems.

It is known that BiP can expand regulatory T-cells in the body. This is particularly true of CD39+ regulatory T-cells (Tregs), which are the most efficient Tregs in the body. In  
35 particular, the inventors have observed that BiP responders show a rise in these Treg populations which comes up faster and lasts longer than non-responders. In contrast, placebo treated subjects do not show any change in their Tregs. Thus, in one aspect the

invention relates to assessment of CD39+ Tregs in a subject to whom BiP has been administered.

The concentration of C-reactive protein (CRP)[ Milman N, Karsh J, Booth RA. Clin  
 5 Biochem. 2010 Nov;43(16-17):1309-14. Correlation of a multi-cytokine panel with  
 clinical disease activity in patients with rheumatoid arthritis], vascular endothelial  
 growth factor (VEGF)[ Kurosaka D, Hirai K, Nishioka M, Miyamoto Y, Yoshida K, Noda  
 K, Ukichi T, Kurosaka D, Hirai K, Nishioka M, Miyamoto Y, Yoshida K, Noda K, Ukichi  
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 10 Yanagimachi M, Furuya K, Takahashi E, Kingetsu I, Fukuda K, Yamada A. J  
 Rheumatol. 2010 Jun;37(6):1121-8. Clinical significance of serum levels of vascular  
 endothelial growth factor, angiopoietin-1, and angiopoietin-2 in patients with  
 rheumatoid arthritis.] and interleukin-8 (IL-8)[ van den Brink HR, van Wijk HJ,  
 Geerrtzen HJ, Bjilmsa HJ. J Rheumatol 1994, 21 (3), 430-4. Influence of corticosteroid  
 15 pulse therapy on the serum levels of soluble interleukin-2 receptor, interleukin-6 and  
 interleukin-8 in patients with rheumatoid arthritis] are elevated in the serum of  
 patients with rheumatoid arthritis (RA). The concentration fluctuates with disease  
 activity. CRP is produced by the liver and is a measure of systemic or generalised  
 inflammation. VEGF reflects the degree of vascular inflammation and synovitis in the  
 20 inflamed rheumatoid joint. Finally, IL-8 is produced by activated neutrophils and is a  
 measure of rheumatoid synovial inflammation.

Although these biomarkers have been shown to fluctuate with variations in RA disease  
 activity none of them have been shown to predict changes in disease in a randomised  
 25 placebo controlled drug trial. In the present invention patients receiving BiP who show  
 decreases in serum CRP, VEGF and IL-8 concentrations at 2 weeks correspond to  
 patients who are responding at 3 months following the initial single pulse intravenous  
 dose of BiP (see example section especially Figures 1 and 2 for further details).

30 Thus patients showing decreased serum concentrations in these biomarkers at 2 weeks  
 may delay repeat dosing of BiP while patients in whom serum concentrations do not  
 decrease may receive a repeat dose at 2 weeks with the aim of achieving a meaningful  
 clinical response.

35 The invention addresses safety and patient response as indicated by biomarker changes  
 to Rasolvir™ (BiP) in the Phase I/IIa RAGULA clinical trial in rheumatoid arthritis.

Suitably the reference concentration is the patient's own biomarker level(s) (concentration(s)) determined pre-treatment with BiP. Suitably the reference concentration(s) are from a time point within 4 weeks of treatment of BiP treatment (i.e. within 4 weeks before BiP treatment).

- 5 Clearly each biomarker assessed has its own reference concentration(s). Suitably levels/concentrations are compared to those of the same biomarker.

### BONE LOSS

- 10 Disorders associated with or related to bone loss, include, but are not limited to, Paget's Disease, primary and secondary osteoporosis, post menopausal osteoporosis, senile osteoporosis, glucocorticoid-induced osteoporosis, periodontal disease, alveolar bone loss, post-osteotomy and childhood idiopathic bone loss, long term complications of osteoporosis - such as curvature of the spine and loss of height - and prosthetic surgery,  
15 as well as loosening of prosthetic joints such as hips, knees and the like.

Bone loss may be characterised by bone destruction, bone erosion, bone thinning or bone digestion. As will be apparent herein, this may be brought about by a pathological change in the balance between bone deposit and bone resorption.

20

- The invention may be used to assess response when BiP is used in the treatment of condition(s) associated with low bone mass. Such conditions will be apparent where the level of bone mass is below the age specific normal as defined in standards by the World Health Organization Assessment of Fracture Risk and its Application to  
25 Screening for Postmenopausal Osteoporosis (1994). Report of a World Health Organization Study Group. World Health Organization Technical Series 843. The phrase "condition(s) which present with low bone mass" also refers to a vertebrate, e.g., a mammal known to have a significantly higher than average chance of developing diseases – such as osteoporosis (e.g., post-menopausal women, men over the age of  
30 50). Other bone mass augmenting or enhancing uses may include bone restoration, increasing the bone fracture healing rate, replacing bone graft surgery entirely, enhancing the rate of successful bone grafts, bone healing following facial reconstruction or maxillary reconstruction or mandibular reconstruction, prosthetic ingrowth, vertebral synostosis or long bone extension.

35

The invention may be used to assess responses when BiP is used for the prevention or treatment of bone resorption. In the context of the present invention, bone resorption

is prevented or treated by modulating (eg. preventing) bone resorption by the direct or indirect alteration of osteoclast formation or activity. Bone resorption may be modulated by inhibiting the removal of existing bone either from the mineral phase and/or the organic matrix phase, through direct or indirect alteration of osteoclast formation or activity. A variety of disorders in humans and other mammals involve or are associated with abnormal bone resorption. Such disorders include, but are not limited to, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma.

Suitably the bone loss or bone resorption underlies musculoskeletal disease – such as osteoporosis or Paget's disease. Suitably the condition associated with bone loss or bone resorption is osteoporosis.

The presence of bone loss or bone resorption may be determined using various methods that are known in the art – such as by performing a Bone Resorption Assessment. This is a non-invasive evaluation of the biochemical markers of bone loss. It can also be used to measure the rate of bone loss long before problems can be detected using other measures – such as bone density. As the matrix framework of bone undergoes resorption – crosslinks that stabilize collagen molecules – such as deoxypyridinium (D-pyd) and/or pyridinium (Pyd) - are excreted in the urine and their levels relative to normal levels are an indication of how quickly bone is being lost. When these markers are high, it is an indication that bone is being lost at a rate greater than the body is capable of replacing it.

Alternatively, bone loss or resorption may be determined by clinical techniques such as dual energy X-ray absorption (DEXA) scan of the spine and/or neck of the femur.

### APPLICATIONS

It is an advantage of the invention that a physician may be aided in their decision regarding retreatment. This applies equally to both responder and non-responder subjects, since the decision not to retreat can be as important as the decision to retreat. Suitably responders are retreated. Suitably non-responders are not retreated.

Currently, assessment of patients is based on the “DAS28” methodology. It is an advantage of the invention that biomarker assessments taught herein are more robust and more reliable than DAS28 assessments.

- 5 Suitably biomarker MMP3 is assessed. Suitably assessment of MMP3 biomarker is in addition to (as well as) assessment of IL-8 and/or VEGF. Most suitably, VEGF, IL-8 and MMP3 are each assessed for a subject. Most suitably, VEGF, IL-8, MMP3 and CRP are each assessed for a subject.

10 SAMPLE

The sample may be from a subject. The subject is suitably a mammal, most suitably a human.

- 15 Suitably the sample is blood.

Suitably the sample is blood supernatant.

Suitably said sample is a sample of serum or plasma.

20

Most suitably the sample is serum. Serum may be obtained as the fluid collected from a blood sample which has been clotted.

- 25 Serum may be prepared from clotted blood, serum drawn off as cell-free clear straw coloured liquid and aliquots stored at -80 degrees Celsius.

30

Suitably the sample is plasma. Plasma may be obtained as the fluid collected from a blood sample which has been centrifuged to pellet the blood cells present. Alternatively plasma may be obtained by filtration to remove the blood cells present.

Suitably said serum or plasma is essentially cell free.

35

Suitably the methods do not involve actual collection of the sample. Suitably the sample is an *in vitro* sample.

Methods of the invention are suitably performed on an isolated sample from the subject being investigated. Thus, suitably the methods are methods which may be conducted

in a laboratory setting without the need for the subject to be present. Suitably the methods are carried out *in vitro* i.e. suitably the methods are *in vitro* methods. Suitably the methods are extracorporeal methods.

- 5 Suitably the invention may be applied to analysis of nucleic acids. Suitably, nucleic acid is prepared from the sample collected from the subject of interest, e.g. by extraction of nucleic acid from white blood cells in the sample. Suitably, the sample comprises nucleic acid. Suitably, the sample consists of nucleic acid. Suitably, the nucleic acid comprises, or is, mRNA or cDNA, suitably mRNA.

10

Most suitably the invention may be applied to analysis of protein biomarkers.

- Advantages of protein based analysis include avoiding difficulties with nucleic acid based analysis, for example when translation from nucleic acid may or may not occur  
15 and/or where post-translational changes also may affect levels of protein produced. Thus most suitably proteins in the sample are analysed.

Suitably protein biomarkers are assessed by ELISA.

- 20 Suitably the sample is an *in vitro* sample.  
Suitably the sample is an extracorporeal sample.

Suitably the sample is from a subject at about 2 to 12 weeks after administration of BiP; most suitably about 2 weeks after administration of BiP.

25

### BiP

- BiP refers to the "Binding Immunoglobulin Protein" 78kD endoplasmic reticulum chaperone protein as disclosed in WO 00/21995. Suitably the BiP polypeptide has the  
30 amino acid sequence as shown in appendix 2 of WO00/21995 at page 23. Preferably, the BiP protein has the amino acid sequence given in WO 00/21995 as SEQ ID NO. 1 or SEQ ID NO. 2. Preferably the BiP sequences used herein are devoid of tags such as the 6His tags present in the polypeptides referred to above. As used herein, the term  
"amino acid sequence" is synonymous with the term "polypeptide" and/or the term  
35 "protein". In some instances, the term "amino acid sequence" is synonymous with the term "peptide". In some instances, the term "amino acid sequence" is synonymous with the term "protein".

Preferably, the BiP protein is encoded by the nucleotide sequence given in WO 00/21995 as SEQ ID NO. 3. The nucleotide sequence may be DNA or RNA or genomic, synthetic or recombinant origin e.g. cDNA. The nucleotide sequence may be double-  
 5 stranded or single-stranded whether representing the sense or antisense strand or combinations thereof. The nucleotide sequence may be prepared by use of recombinant DNA techniques (*e.g.* recombinant DNA). In particular, methods for the expression of BiP in *E. coli* and purification of the recombinant protein are disclosed in WO 00/21995. The nucleotide sequence may be the same as the naturally occurring  
 10 form, or may be a fragment, homologue, variant or derivative thereof.

As used herein, the term “BiP activity” refers to the level and/or pattern of BiP expression and/or activity.

15 In case any further guidance is needed, suitably the sequence of BiP is as in the publically available NCBI Genbank accession number X87949 (most suitably GenBank: X87949.1 - <http://www.ncbi.nlm.nih.gov/nuccore/X87949>) Nucleic Acids Research, 2013 Jan;41(D1):D36-42; GenBank, National Center for Biotechnology Information, National Library of Medicine, 38A, 8N805, 8600 Rockville Pike, Bethesda, MD 20894,  
 20 USA; NCBI-GenBank Flat File Release 211.0 (15 December 2015), or more suitably UniProt accession number P11021 (<http://www.uniprot.org/uniprot/P11021>; The UniProt Consortium, “UniProt: a hub for protein information”, Nucleic Acids Res. 43: D204-D212 (2015); UniProt release 2015\_12, Published 9 December, 2015;) as shown below:

25

BiP: UniProt accession number P11021 - **SEQ ID NO: 1**

|    |            |            |            |            |            |
|----|------------|------------|------------|------------|------------|
|    | 10         | 20         | 30         | 40         | 50         |
|    | MKLSLVAAML | LLLSAARAE  | EDKKEDVGT  | VGIDLGTTYS | CVGVFKNGRV |
|    | 60         | 70         | 80         | 90         | 100        |
| 30 | EIIANDQGNR | ITPSYVAFTP | EGERLIGDAA | KNQLTSNPEN | TVFDAKRLIG |
|    | 110        | 120        | 130        | 140        | 150        |
|    | RTWNDPSVQQ | DIKFLPFKVV | EKKTKPYIQV | DIGGGQTKTF | APEEISAMVL |
|    | 160        | 170        | 180        | 190        | 200        |
|    | TKMKETAEAY | LGKKVTHAVV | TVPAYFNDAQ | RQATKDAGTI | AGLNVMRIIN |
| 35 | 210        | 220        | 230        | 240        | 250        |
|    | EPTAAAIAYG | LDKREGEKNI | LVFDLGGGTF | DVSLLTIDNG | VFEVVATNGD |
|    | 260        | 270        | 280        | 290        | 300        |

5    THLGGEDFDQ RVMHFIFKLY KKKTGKDVRK DNRAVQKLRR EVEKAKRALS  
      310                320                330                340                350  
      SQHQARIEIE SFYEGEDFSE TLTRAKFEEL NMDLFRSTMK PVQKVLDS  
      360                370                380                390                400  
 10    LKKSDIDEIV LVGGSTRIPK IQQLVKEFFN GKEPSRGINP DEAVAYGAAV  
      410                420                430                440                450  
      QAGVLSGDQD TGDVLVLLDVC PLTLGIETVG GVMTKLIPRN TVVPTKKSQI  
      460                470                480                490                500  
      FSTASDNQPT VTIKVYEGER PLTKDNHLLG TFDLTGIPPA PRGVPQIEVT  
 15    510                520                530                540                550  
      FEIDVNGILR VTAEDKGTGN KKNKITITNDQ NRLTPEEIER MVNDAEKFAE  
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      EDKKLKERID TRNELESYAY SLKNQIGDKE KLGGKLSSD KETMEKAVEE  
      610                620                630                640                650  
 15    KIEWLESHQD ADIEDFKAKK KELEEIVQPI ISKLYGSAGP PPTGEEDTAE  
      654  
      KDEL

### BiP Manufacture

20    BiP protein is provided as a sterile solution of protein at 5.0 mg/mL in phosphate buffered saline pH 7.4 (Ph Eur 4005000). Suitably dose will be a single infusion of BiP over the dose range of 1-15mg per patient.

25    The BiP protein solution is supplied by the manufacturer as sterile 1 mL aliquots in 2 mL clear borosilicate glass injection vials, sealed with rubber stoppers and aluminium caps.

30    The ingredients of the formulation buffer (listed below) are all Ph Eur compliant. All but the WFI are sourced from the EMPROVE range of pharmaceutical grade chemicals (Merck Chemicals). The WFI, made by distillation, is sourced from Baxter.

35    Disodium hydrogen phosphate  
      Potassium dihydrogen phosphate  
      Sodium chloride  
      Orthophosphoric acid  
      Water for Injections (WFI)

The formulation buffer, following preparation by CBC, is tested for bioburden, pH, conductivity and osmolality prior to use. The filter used for processing and filtration into the final bioprocess container is filter integrity tested.

- 5 To ensure that 1.0 ml can be withdrawn by syringe, there is an overfill of 0.3 mL.

Manufacture of BiP is carried out in accordance with the requirements of cGMP by:-  
Clinical Biotechnology Centre (CBC), Bristol Institute for Transfusion Sciences,  
University of Bristol, Langford House, Lower Langford, Bristol BS40 5DU, UK.

10

The National Health Service Blood and Transplant (NHSBT) has been granted a Manufacturer's Authorisation for the manufacture of investigational medicinal products (MIA(IMP) 25224) by the MHRA. The authorisation covers the manufacture of gene therapy products, biotechnology products and recombinant protein products at the CBC (Site Number 89442). Final batch release and certification of the IMP is the responsibility of the Qualified Person (QP) named on the licence, who must be satisfied that the requirements to manufacture IMP to current GMP as described in EU Directive 2003/94/EEC have been met.

15

- 20 The batch size for the clinical batch of BiP protein (Batch No ABO14) was 180 bottles, corresponding to 0.5 x the working volume of the fermenter (the working volume of the fermenter was up to 11 litres).

The product is manufactured according to the current Ph Eur monograph

- 25 "Recombinant DNA Technology, Products of" (01/2008:0784).

#### REFERENCE SEQUENCES

Suitably the reference sequences of the biomarkers of interest are as defined in the following table:

30

| <b>Biomarker</b> | <b>Protein<br/>Accession<br/>Number<br/>(UniProtKB) or<br/>amino acid<br/>sequence</b> | <b>Canonical<br/>sequence</b> | <b>Protein<br/>Isoforms</b> | <b>Gene<br/>name</b> | <b>mRNA<br/>Accession<br/>Number<br/>(RefSeq<br/>Release 73)</b> |
|------------------|----------------------------------------------------------------------------------------|-------------------------------|-----------------------------|----------------------|------------------------------------------------------------------|
| IL-8             | P10145                                                                                 | P10145-1                      | P10145-2                    | CXCL8                | NM_000584.3                                                      |

|      |                 |          |                                                                                                                                                                                                       |                                                                                           |                                                                                                                                                                                                                                                                                                                                                                   |
|------|-----------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| VEGF | P15692          | P15692-1 | P15692-2<br>P15692-3<br>P15692-4<br>P15692-5<br>P15692-6<br>P15692-8<br>P15692-9<br>P15692-10<br>P15692-11<br>P15692-12<br>P15692-13<br>P15692-14<br>P15692-15<br>P15692-16<br>P15692-17<br>P15692-18 | <i>VEGFA</i>                                                                              | NM_001025366.2<br>NM_001025367.2<br>NM_001025368.2<br>NM_001025369.2<br>NM_001025370.2<br>NM_001033756.2<br>NM_001171622.1<br>NM_001171623.1<br>NM_001171624.1<br>NM_001171625.1<br>NM_001171626.1<br>NM_001171627.1<br>NM_001171628.1<br>NM_001171629.1<br>NM_001171630.1<br>NM_001204384.1<br>NM_001204385.1<br>NM_001287044.1<br>NM_001317010.1<br>NM_003376.5 |
| CRP  | <b>P02741-1</b> | P02741-1 | P02741-1<br>P02741-2                                                                                                                                                                                  | <i>CRP/P</i><br><i>TXI</i>                                                                | NM_000567.2<br>NP_000558.2                                                                                                                                                                                                                                                                                                                                        |
| MMP3 | <b>P08254</b>   | P08254-1 |                                                                                                                                                                                                       | <i>SLI/ST</i><br><i>MY/ST</i><br><i>MYI/</i><br><i>STRI/T</i><br><i>RANSI</i><br><i>N</i> | NM_002422.2<br>J03209.1                                                                                                                                                                                                                                                                                                                                           |

The sequences are hereby incorporated herein by reference.

- 5 When referring to 'CRP' or C-reactive protein, suitably C-reactive protein, pentraxin-related [ Homo sapiens (human) ] Uniprot P02741 is assayed/determined. Suitably the CRP precursor protein is not assayed/determined. CRP is a pentamer; the precursor/mature sequences given above are for a single unit.

The skilled worker only has to identify the correct gene/protein being used in the analysis. The guidance provided is not intended to restrict the invention rigidly to the specific single exemplary sequences provided. Gene sequences (and therefore protein sequences) are known to vary between individuals e.g. due to allelic variance or mutations between individuals. The information provided is to assist the operator in working the invention by assaying the correct gene. Ultimately the gene product (such as mRNA or more suitably protein) is actually assayed. Therefore minor or minimal allelic or mutational differences between individuals are not important, what is important is that the correct gene (gene product) is assayed using the guidance provided.

Suitably where the biomarker name is used, this means the corresponding amino acid or nucleic acid sequence from the above table. Suitably for amino acid sequences, the canonical sequence is preferred. Suitably for nucleic acid sequences, the most recent (e.g. highest numbered) nucleic acid sequence is preferred.

It will be understood that the invention may equally make use of detection of fragment(s), variant(s) or mutant(s) of these biomarkers. Suitably any such fragment(s), variant(s) or mutant(s) have at least 80% sequence identity to the reference sequences along the whole length of said fragment(s), variant(s) or mutant(s), suitably 90%, suitably 95%, suitably 98% sequence identity along the whole length of said fragment(s), variant(s) or mutant(s).

Sequence identity may be calculated using any suitable technique known in the art. For example, a suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux et al., 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 *ibid* – Chapter 18), FASTA (Atschul et al., 1990, J. Mol. Biol., 403-410) and the GENWORKS suite of comparison tools. Suitably default settings are used for any gap penalties or other such values required for the calculation.

#### DETECTION

Suitably biomarkers of the invention are detected as proteins. Suitably detection is via use of antibodies or antigen binding fragments thereof which specifically bind to the biomarkers of interest, and assaying binding to determine concentration. Suitably the

antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')<sub>2</sub> fragment, a scFv, a Fv, a rIgG, and a diabody.

Procedures well known in the art may be used for the production of antibodies to  
5 biomarkers of interest. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. Neutralising antibodies, ie., those which may modulate the biological activity BiP, are especially preferred for diagnostics and therapeutics.

10 For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc. may be immunised by injection with one or more of the polypeptides described herein or any portion, variant, homologue, fragment or derivative thereof or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include,  
15 but are not limited to, Freund's, mineral gels - such as aluminium hydroxide - and surface active substances - such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (Bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants which may be employed.

20

Suitably, the antibody is a monoclonal antibody.

Suitably the serum biomarkers are determined/measured using a cytokine multiplex detection system. Suitably that system is commonly available and has been validated  
25 by the manufacturing and supplying company. Such a kit (which can be customised on purchase) can detect several cytokines in the serum simultaneously. Suitably the supplier is Millipore although other companies also supply the beads which are produced by a company called Luminex. All materials and reagents are commonly available and/or available from the MerckMillipore Custom Assay Development Service  
30 Suite 21, Building 6, Croxley Green Business Park, Watford, Hertfordshire, WD18 8YH, United Kingdom. The above service includes customisation according to operator choice.

Suitably the assay is carried out on all individual patient samples (samples from a  
35 single subject) at the same (e.g. pre-BiP-infusion to 12 weeks; samples are suitably frozen in aliquots at -80 until required). This has the advantage of ameliorating or eliminating assay differences which might otherwise affect the data. Each plate has a

standard curve that covers the range of concentrations expected in the serum. If a sample shows very high levels the series of serum samples will be repeated at a higher dilution. The concentration of cytokine detected in the serum is calculated from the standard curve as in all immunoassays.

5

Detection of cell surface antigens are described in the “METHODS AND PROTOCOLS BiP STUDY RESEARCH FLOW CHART” section of the Examples.

CRP may be detected by clinical laboratory using commercially validated kits.

10

Suitably biomarker detection/measurement is accomplished using commercial enzyme linked immunosorbent assay (ELISA) (Becton Dickinson, Pharmingen, Oxford, UK).

15

Suitably biomarker detection/measurement is accomplished using Luminex reagents such as kits with catalogue numbers: L5000GRIRU / L1000GRIRU 6 x Human Cytokine Group I, 10-plex, 1 x 96-well Flat Bottom Plate, x-Plex Assay format. Includes : premixed Coupled Magnetic Beads, Detection Antibodies, Standards, Reagents and Diluents for detecting Human Cytokine IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-17A, IFN-gamma, MCP-1 (MCAF), TNF-alpha and VEGF all in one kit available from Bio-Rad Laboratories Ltd., Bio-Rad House, Maxted Road, Hemel Hempstead, Herts, HP2 7DX, UK. Suitably the manufacturer's instructions are followed. When the sample is blood, any pre-processing is selected by the operator in conjunction with the manufacturer's instructions.

20

## 25 DATABASE RELEASE

Sequences deposited in databases can change over time. Suitably the current version of sequence database(s) are relied upon. Alternatively, the release in force at the date of filing is relied upon.

30

As the skilled person knows, the accession numbers may be version/dated accession numbers. The citeable accession numbers for the current database entry are the same as above, but omitting the decimal point and any subsequent digits e.g. for VEGF a version/dated accession number is P15692-18; the current entry is obtained using P15692 and so on.

35

GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA; Nucleic Acids Research, 2013 Jan;41(D1):D36-42) and accession numbers provided relate to  
5 this unless otherwise apparent. Suitably the GenBank database release referred to is 15 December 2015, NCBI-GenBank Release 211.0.

UniProt (Universal Protein Resource) is a comprehensive catalogue of information on proteins ('UniProt: a hub for protein information' Nucleic Acids Res. 43: D204-D212  
10 (2015)). For the avoidance of doubt, UniProt Release 2015\_11 is relied upon. In more detail, the UniProt consortium European Bioinformatics Institute (EBI), SIB Swiss Institute of Bioinformatics and Protein Information Resource (PIR)'s UniProt Knowledgebase (UniProtKB) Release 2015\_12, (9-Dec-2015) is relied upon.

## 15 ADMINISTRATION

Administration of BiP to subjects such as humans is known in the art.

The BiP may be formulated into a pharmaceutical composition, such as by mixing with  
20 one or more of a suitable carrier, diluent or excipient, by using techniques that are known in the art.

Suitably BiP is administered using any suitable formulation known in the art. Suitably the BiP is recombinantly expressed in *E.coli* and purified. Suitably BiP is formulated as  
25 a complete replica of the endogenous molecule as disclosed in WO 00/21195 and/or WO 2006/111720.

Suitably BiP is administered using any suitable route known in the art. Suitably administration is intravenous (i.v.).

30 Suitably a dose in the range 1mg to 100mg BiP is administered to an adult human. Suitably 1mg, 5mg or 15mg BiP is administered to an adult human, more suitably 5mg or 15mg. Actual doses may be determined by a physician.

35 Retreatment follows the same guidance as treatment regarding dose, formulation, administration and any further details. Retreatment refers to a second or further

administration of BiP. In other words, to be 'retreated' a subject must have previously experienced a first treatment of BiP.

Suitably 'retreatment' is at least 72 hours after the subject's most recent treatment such as first treatment. Suitably retreatment is at a time point in the range 72 hours to 12

5 weeks after the subject's most recent treatment such as first treatment. Suitably retreatment is at a time point in the range 72 hours to 2 weeks after the subject's most recent treatment such as first treatment. Suitably retreatment is at a time point in the range 2 weeks to 12 weeks after the subject's most recent treatment such as first treatment.

10 Suitably retreatment is at least 72 hours after the subject's most recent treatment such as first treatment.

Suitably retreatment is at least 2 weeks after the subject's most recent treatment such as first treatment.

15 Suitably retreatment is at least 12 weeks after the subject's most recent treatment such as first treatment.

Suitably retreatment is at about 72 hours after the subject's most recent treatment such as first treatment.

Suitably retreatment is at about 2 weeks after the subject's most recent treatment such as first treatment.

20 Suitably retreatment is at about 12 weeks after the subject's most recent treatment such as first treatment.

Suitably retreatment is at 72 hours after the subject's most recent treatment such as first treatment.

25 Suitably retreatment is at 2 weeks after the subject's most recent treatment such as first treatment.

Suitably retreatment is at 12 weeks after the subject's most recent treatment such as first treatment.

#### FURTHER APPLICATIONS

30

The invention is useful in showing that intravenous Rasolvir™ (BiP) is safe in this first clinical trial in patients with unresponsive RA.

We demonstrate that Rasolvir™ induces significant falls in the biomarkers CRP, VEGF  
35 and IL-8 despite high background placebo clinical response.

It shows utility that after a single intravenous infusion Rasolvir™ may induce remission lasting up to 3 months.

A method of determining the effectiveness or efficacy in treating rheumatoid arthritis of administration of BiP to a subject, the method comprising

- 5 (a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP
- 10 (b) comparing said concentration(s) determined in (a) to reference concentration(s) for said biomarker(s) wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) indicates an increased effectiveness or efficacy in treating rheumatoid arthritis in said subject.

15

A method of determining a change in pathology of rheumatoid arthritis following administration of BiP to a subject or treatment of a subject with BiP, the method comprising

- (a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP
- 20 (b) comparing said concentration(s) determined in (a) to reference concentration(s) for said biomarker(s) wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) indicates an improvement in pathology of rheumatoid arthritis of rheumatoid arthritis in said subject.

Suitably the reference concentration is the patient's own biomarker levels determined pre-treatment with BiP. Suitably the reference concentrations are from a time point within 4 weeks of treatment of BiP treatment (i.e. within 4 weeks before BiP treatment).

A method of identifying a subject as a responder to BiP administration, the method comprising

- 35 (a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject

wherein said sample was obtained after administration of BiP to a subject or treatment of a subject with BiP,

wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP

- 5 (b) comparing said concentration(s) determined in (a) to reference concentration(s) for said biomarker(s)

wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) subject said subject as a responder to BiP administration.

- 10 Regarding US 2008/0155704, the inventors observe that this document relates only to *in vitro* studies. The data in the present application all come from patients treated with BiP the effect of which could be very difficult to predict.

#### ADDITIONAL APPLICATIONS

15

The invention may also be applied to seropositive (rheumatoid factor, ACPA positive) erosive rheumatoid arthritis, erosive but seronegative arthritis, seronegative pelvospondylitic arthritis, and arthritis with psoriasis, arthritis with inflammatory

- 20 invention may also be applied to autoimmune diseases, reasoning that they are driven by the same mechanisms which drive these immune inflammatory joint diseases.

Without wishing to be bound by theory, all these diseases are driven by the same basic autoimmune mechanism. That is why the inventors assert application to human autoimmune diseases in general. It is observed that anti-TNFs and other biologics were  
25 never trialled in a suitable spondylitic model because one does not exist.

- BiP undoubtedly acts by regulating immune mediated inflammation through the induction of regulatory cells. The inventors have pre-clinical and clinical evidence of the efficacy of BiP, direct preclinical evidence of the regulatory effect and indirect  
30 evidence of the regulatory effect. The inventors therefore assert the following uses of BiP:

- BiP can be used in human immune mediated diseases in which its ability to induce immune cells that can regulate the immune response can suppress the immune-  
35 mediated inflammation. Such conditions include:

- 1 Seropositive rheumatoid arthritis (see above)
- 2 Seronegative arthritis (see above)

- 3 Pelvospondylitic disease
- 4 Psoriatic disease
- 5 Inflammatory bowel disease (ulcerative colitis or Crohn's disease)
- 6 Solid organ, bone marrow, or blood cell transplantation.

5

The inventors assert that there is indirect support for these embodiments from the mode of action of BiP. The inventors point out that science works by extrapolating from a base of known knowledge. The inventors know BiP induces regulatory cells. The inventors point out its subsequent actions in various diseases.

10

Thus the invention relates to a method of treatment of human immune mediated diseases in which its ability to induce immune cells that can regulate the immune response can suppress the immune-mediated inflammation, seropositive rheumatoid arthritis, Seronegative arthritis, Pelvospondylitic disease, Psoriatic disease,

15 Inflammatory bowel disease (ulcerative colitis or Crohn's disease), Solid organ, bone marrow, or blood cell transplantation, or autoimmune diseases, said method comprising administering BiP to said subject, suitably 5 mg to 15 mg of BiP to said subject.

20 Thus the invention relates to BiP for treatment of human immune mediated diseases in which its ability to induce immune cells that can regulate the immune response can suppress the immune-mediated inflammation, seropositive rheumatoid arthritis, Seronegative arthritis, Pelvospondylitic disease, Psoriatic disease, Inflammatory bowel disease (ulcerative colitis or Crohn's disease), Solid organ, bone marrow, or blood cell  
25 transplantation, or autoimmune diseases.

Thus the invention relates to use of BiP in the treatment of human immune mediated diseases in which its ability to induce immune cells that can regulate the immune response can suppress the immune-mediated inflammation, seropositive rheumatoid  
30 arthritis, Seronegative arthritis, Pelvospondylitic disease, Psoriatic disease, Inflammatory bowel disease (ulcerative colitis or Crohn's disease), Solid organ, bone marrow, or blood cell transplantation, or autoimmune diseases.

Further particular and preferred aspects are set out in the accompanying independent  
35 and dependent claims. Features of the dependent claims may be combined with features of the independent claims as appropriate, and in combinations other than those explicitly set out in the claims.

Where an apparatus feature is described as being operable to provide a function, it will be appreciated that this includes an apparatus feature which provides that function or which is adapted or configured to provide that function.

5

#### BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will now be described further, with reference to the accompanying drawings, in which:

- 10 Figure 1 shows serum C-reactive protein levels: CRP was quantified in serum from patients taken pre-infusion, and at 2 and 12 weeks post infusion. Three groups of patients: placebo, Rasolvir™ responders(R) and Rasolvir™ non responders (NR) are shown. \* denotes protocol violator who had ceased concurrent medication.
- 15 Figure 2 shows changes in biomarker levels in Rasolvir™ treated patients. Serum concentrations of vascular endothelial growth factor (VEGF) and interleukin (IL)-8 were measured by Luminex bead technology and the change from pre-infusion serum concentration calculated for each patient at 2 and 12 weeks. A, shows the change in VEGF concentration and B, shows change in concentration of IL-8. Data show placebo
- 20 group (n = 6), responder group (R) (n = 8 (2w) – 6 (12w)) and the total patient group treated with Rasolvir™ (n = 14 (2w) – 12 (12w)) who remained in the study at 12 weeks. Range of concentration (all patients) VEGF, 4-195pg/ml; and for IL-8, 0.7 – 19 pg/ml.

Figure 3 shows a bar chart.

- 25 Figure 4 shows a bar chart.

Figure 5 shows a bar chart.

Figure 6 shows a bar chart.

Figure 7A shows bar charts; Figure 7B shows plots.

#### 30 EXAMPLES

##### **Overview**

Objectives. Rasolvir™ (BiP) is a human endoplasmic reticulum-resident stress protein.

- 35 In pre-clinical studies it has anti-inflammatory properties due to the induction of regulatory cells. This randomised placebo-controlled, dose ascending double blind Phase I/IIA trial of Rasolvir™ in patients with active RA, who had failed accepted

therapies, had the primary objective of safety. Potential efficacy was measured by disease activity score 28-erythrocyte sedimentation rate (DAS28-ESR) and changes in biomarkers.

Methods. 24 patients with active RA who had failed one or more DMARDs were sequentially assigned to three groups each of eight patients randomly allocated to receive placebo (two patients) or Rasolvir™ (six patients), 1mg, 5mg or 15mg. Patients received a single intravenous infusion over one hour and were observed as inpatients overnight. A twelve week follow-up for clinical, rheumatological and laboratory assessments for safety, efficacy (DAS28-ESR) and biomarker analysis was performed.

Results. No infusion reactions or serious adverse drug reactions were noted. Adverse events were evenly distributed between placebo and Rasolvir™ groups with no Rasolvir™-related toxicities. Haematological, renal and metabolic parameters showed no drug-related toxicities. Remission was only achieved by patients in the 5mg and 15mg groups and no patient who received placebo or 1mg Rasolvir™. Good DAS28-ESR responses were achieved in all treatment groups. The Rasolvir™ responding patients showed significantly lower serum concentrations of C-reactive protein, 2 weeks post-infusion compared with pre-infusion levels, and of vascular endothelial growth factor and interleukin-8 from the placebo group.

Conclusion. Rasolvir™ ( $\leq 15$ mg) is safe in patients with active RA. Some patients had clinical and biological improvements in RA activity. Rasolvir™ merits further study.

TRIAL REGISTRATION: Trial registration study numbers ISRCTN2288225 (<http://isrctn.com>) and EudraCT (<https://eudract.emea.europa.eu>) number 2011-005831-19.

An intravenous (IV) infusion of Rasolvir™ should have a prolonged therapeutic effect. The Rheumatoid Arthritis ReGULAtory (RAGULA) is a Phase 1/IIA randomised placebo controlled, dose escalating, first-in-man clinical trial, designed to test, firstly, for safety; secondly, for efficacy in patients with active RA who have failed disease modifying anti-rheumatic drugs (DMARDs) and, in some patients, biologics; and, thirdly, to explore PK and biomarker data as related to clinical and biological endpoints following infusion of Rasolvir™.

The Treg data shows a drop in general CD4+ Tregs but crucially a sustained rise in the CD39+ , CD4, CD25hi CD127lo T regs.

## PATIENTS AND METHODS

### Study Design

This study was a double-blind, randomised, placebo controlled, parallel group, single ascending dose design in 3 treatment cohorts of 8 female or male patients with active RA as stipulated by the Medicines and Healthcare Products Regulatory Agency (MHRA). Within each treatment cohort, 6 patients were randomised to receive Rasolvir™ and 2 to receive placebo (normal saline). Three doses of Rasolvir™, 1.0 mg, 5.0 mg, and 15.0 mg were investigated in ascending order. The study was approved by the MHRA (reference 40945/0001/001-0001) and the London Bridge Research Ethics Committee (12/LO/0012). It was performed in accordance with the principles of the Declaration of Helsinki. Signed informed consent for the study was obtained from each patient before any study related procedures were undertaken. The full study protocol is available from the sponsor. An independent Data Monitoring and Safety Committee based outside our institutions reviewed all clinical and laboratory results for safety after each dosing cohort and gave consent to proceed to the next dosing level.

Following screening, eligible patients were admitted within 1 week to the Quintiles Drug Research Unit. Patients were admitted on Day -1 for final eligibility checks and baseline assessments, and then randomized to receive Rasolvir™ or placebo. The randomisation list was generated by the study statistician using the R statistical package and maintained on the MedSciNet database for authorised access as patients passed final safety tests at the Quintiles facility. A sentinel strategy was employed, where, of the first two patients in each cohort, one was randomised to receive Rasolvir™, and the other placebo. On the morning following admission, patients received a single IV infusion of Rasolvir™ or placebo over one hour. There was an appropriate stagger of at least 24 hours between dosing each patient in each cohort. Patients were monitored for 24 hours following infusion and discharged from the Quintiles Unit on Day 2 after final assessments had been performed. Following discharge, patients were assessed for safety and efficacy at Weeks 1, 2, 3, 4, 8 and 12, at the Guy's Hospital Clinical Research Facility.

#### Patients

The study randomised 24 patients aged from 18 to 75, with RA as defined by the 1987-revised American College of Rheumatology (ACR) diagnostic criteria [16] for at least 6 months with active RA defined by having at least 6 swollen and 6 tender joints, C-Reactive Protein (CRP) >4mg/L, and/or erythrocyte sedimentation rate (ESR) >15mm/hour despite adequate dosage of at least one disease modifying anti-rheumatic drug (DMARD) with a normal chest X-ray within 3 months of randomisation. Major

exclusion criteria were treatment with any biologic drug within 3 months of screening (6 months for Rituximab), functional Class IV by ACR Criteria [17], safety screening pathology results outside pre-defined ranges, hepatitis B or C, HIV positive, any other active systemic infection within 2 weeks before baseline. Patients with a history of malignancy (except basal cell carcinoma or adequately treated carcinoma in situ of the cervix), significant cardiac, renal, neurological, psychiatric, endocrine, metabolic, or hepatic disease were also excluded. Patients could continue the following medications at a stable dose for at least 4 weeks before the baseline visit and during the study: sulphasalazine (up to 3 g/d), methotrexate (up to 25 mg/week), hydroxychloroquine (up to 400mg/d), leflunomide (up to 20mg/d), prednisolone or prednisolone equivalent (up to 10 mg/d), and non-steroidal anti-inflammatory drugs.

#### Doses of Rasolvir™

Rasolvir™ (recombinant human BiP) was produced under good manufacturing practice guidelines by the NHS Blood Transfusion Clinical Biotechnology Centre, University of Bristol, Bristol, UK, stored as frozen liquid (5mg/ml dose vials) at -80°C. As this was the first-in-human dosing of Rasolvir™, the MHRA requested the first dose should be just within the therapeutic range as determined in pre-clinical animal studies. Pre-clinical models showed no toxicity over a wide dose range, so a no observed adverse effect level dose was not achieved to calculate a possible highest dose. Based on pre-clinical modelling three doses were chosen, 1mg/patient (Cohort 1), 5mg/patient (Cohort 2) and 15mg/patient (Cohort 3).

#### Study Endpoints

##### 1) Safety.

The primary endpoint was safety, assessed clinically, and by laboratory and ECG measures. During the inpatient admission at Quintiles, ECG was performed twice prior to infusion, then hourly for 4 hours, then twice again before discharge. Changes in laboratory safety measures were graded using the Common Terminology Criteria for Adverse Events (CTCAE) [18].

##### 30 (2) Efficacy

The main efficacy endpoint was DAS28-ESR response, graded according to the EULAR Response Criteria [19] into good, moderate and non-response with remission defined as a DAS28-ESR < 2.6. and the ACR 20, 50 and 70 responses [20]. Biological efficacy endpoints were changes in ESR and CRP.

5 (3) Exploratory PK and biomarkers.

a) PK

Serum Rasolvir™ concentrations were measured by a sensitive ELISA technique developed in our laboratory at the screening visit and at 24 hours after the intravenous infusion of Rasolvir™. These values also include endogenous BiP as the two molecules  
10 could not be distinguished.

b) Serum vascular endothelial growth factor (VEGF) and interleukin (IL)-8 concentration

Gene array data (unpublished) showed that VEGF and IL-8 production from human peripheral blood monocytes was inhibited by Rasolvir™. Serum VEGF and IL-8  
15 concentration was measured before infusion and at 2 and 12 weeks by Luminex technology (Bio-Rad, Hemel Hempstead, UK). Only patients remaining in the study at 12 weeks were included in this analysis.

Statistical Analysis

a) Safety: Due to the exploratory nature of this study no formal sample size calculations  
20 were performed. The study design was based on the results of the pre-clinical studies. With six subjects per cohort receiving Rasolvir™, the probability of observing at least one patient with an adverse event is  $\geq 90\%$ , for an underlying event rate  $\geq 33\%$ . A cohort of size 8, with no observed events in the 6 active patients, would provide a 95% confidence interval of 0% to 46% for the underlying adverse event rate.

25 b) Efficacy: It was expected that the last two doses would show significant benefit. Descriptive statistics, mean and standard errors were used for continuous secondary outcome, DAS28 score. Plots with these summary statistics as a function of time were performed to show how these measurements change over time. The AUC over time for each participant was used as a summary accumulated effect and described for each drug  
30 group using means and 95% confidence intervals. Differences between placebo and the

three dose groups were tested using two-sample T-tests or Mann-Whitney tests, and non-randomised comparisons of the changes within cohorts involved paired t-tests and Wilcoxon test. For response thresholds, the proportion of patients achieving the corresponding response at each time point for each drug group were described. Effects were considered significant if  $P < 0.05$ .

c) Biomarker data was analysed using non-parametric tests, non-paired, Mann Whitney and paired Spearman Rank test where appropriate.

## RESULTS

42 patients were screened, and 24 were randomised to receive either Rasolvir™ or placebo. The demographics of the patients are shown in Table 1. The four groups of patients, placebo, Cohort 1, Cohort 2 and Cohort 3 were comparable in most demographic data (Table 1), with the exception that no male received placebo, and the groups who received 1mg and 5mg doses had failed more therapies.

Table 1: Demographic details of patients recruited into the RAGULA trial

| Characteristics                | Rasolvir™<br>1mg<br>(n = 6) | Rasolvir™<br>5mg<br>(n = 6) | Rasolvir™<br>15mg<br>(n = 6) | Placebo<br>(n = 6) | Total<br>(n = 24) |
|--------------------------------|-----------------------------|-----------------------------|------------------------------|--------------------|-------------------|
| Female/male<br>n (%)           | 6/0 (100/0)                 | 6/0 (100/0)                 | 3/3 (50/50)                  | 6/0 (100/0)        | 21/3 (87/13)      |
| Mean age<br>years (SD)         | 50.8 (7.6)                  | 53.5 (10.4)                 | 53.5 (11.7)                  | 56 (10)            | 52.5 (9.5)        |
| Race Caucasian<br>n (%)        | 5 (83)                      | 6 (100)                     | 4 (67)                       | 4 (67)             | 19 (79)           |
| Disease duration<br>years (SD) | 11.3 (4.3)                  | 12.3 (9.3)                  | 11.5 (11.6)                  | 8.2                | 10.5 (10)         |
| RF positive<br>n (%)           | 5 (83)                      | 6 (100)                     | 5 (83)                       | 3 (50)             | 19 (79)           |
|                                |                             |                             |                              |                    |                   |

|                                           |        |        |        |        |         |
|-------------------------------------------|--------|--------|--------|--------|---------|
| <b>DMARD<br/>Concurrent use<br/>n (%)</b> | 4 (66) | 5 (83) | 5 (83) | 5 (83) | 19 (79) |
| Methotrexate                              | 1 (17) | 4 (66) | 4 (66) | 5 (83) | 14(58)  |
| Hydroxychloroquine                        | 0      | 1 (17) | 1 (17) | 1 (17) | 3 (13)  |
| Sulphasalazine                            | 2 (33) | 5 (87) | 1 (17) | 1 (17) | 9 (37)  |
| Leflunomide                               | 0      | 0      | 0      | 1 (17) | 1 (4)   |
| Prednisolone                              | 3 (50) | 1 (17) | 1 (17) | 1 (17) | 6(25)   |
| <b>Failed biologics</b>                   | 3 (50) | 1 (17) | 0      | 0      | 4 (17)  |
| Infliximab                                | 1 (17) | 1 (17) | 0      | 0      | 2 (8)   |
| Adalimumab                                | 3 (50) | 0      | 0      | 0      | 3 (13)  |
| Etanercept                                | 2 (33) | 1 (17) | 0      | 0      | 3 (13)  |
| Certolizumab                              | 1 (17) | 0      | 0      | 0      | 1 (4)   |
| Golimumab                                 | 1 (17) | 0      | 0      | 0      | 1 (4)   |
| Rituximab                                 | 2 (33) | 0      | 0      | 0      | 2 (8)   |
| Tocilizumab                               | 2 (33) | 0      | 0      | 0      | 2 (8)   |
| Abatacept                                 | 2 (33) | 0      | 0      | 0      | 2 (8)   |

Most patients had failed multiple DMARD therapies, and remained on a DMARD during the study. Four patients had failed up to eight biologic therapies, three in Cohort 1 and the fourth in Cohort 2. Three patients were not on current DMARD therapy. Most patients completed the study after infusion. In the placebo group, no patient was withdrawn because of worsening disease; in Cohort 1 two patients were withdrawn at week 4 for worsening disease and another patient withdrew at week 8, because she wished to travel overseas while she felt so well. In Cohort 2, one patient was withdrawn at week 4 because of active disease, and in Cohort 3 one patient was withdrawn at week 4 for active disease and one patient at week 8 because of a protocol violation: she discontinued methotrexate therapy as she felt so well.

Primary endpoint: safety

No serious adverse events were observed at the time of the infusion of Rasolvir™, during the 24 hour inpatient observation period or the subsequent 12 week follow up.

No patient was withdrawn because of adverse events, and no pattern of adverse events were noted that could be ascribed to Rasolvir™. Adverse events occurred in 23 subjects, evenly distributed amongst all groups, and none required specific intervention (Table 2).

5

Table 2: Clinical adverse events affected more than 10% of the study population. Each group shows the number of events recorded during the 54 follow-up visits and is independent of the number of patients affected by any adverse event. There was no significant difference between the groups in the prevalence, severity or type of adverse events.

10

| Adverse event                     | Rasolvir™ (n = 6/group) |     |      | Placebo | Total<br>n = 24 |
|-----------------------------------|-------------------------|-----|------|---------|-----------------|
|                                   | 1 mg                    | 5mg | 15mg |         |                 |
| Upper respiratory tract infection | 1                       | 2   | 4    | 2       | 9               |
| Limb pain                         | 1                       | 3   | 3    | 2       | 9               |
| Diarrhoea/Gastroenteritis         | 0                       | 1   | 4    | 3       | 8               |
| Headache                          | 2                       | 2   | 1    | 2       | 7               |
| Backache                          | 1                       | 1   | 1    | 2       | 5               |
| Abdominal pain                    | 0                       | 0   | 3    | 1       | 4               |
| Nausea                            | 1                       | 0   | 1    | 1       | 3               |
| Dizziness                         | 1                       | 0   | 0    | 2       | 3               |

Laboratory safety measures also did not change in a way that suggested any drug-related toxicity, with changes occurring in all groups including those who received placebo. All changes were CTCAE Grade I, with the exception of one subject in the 15mg group with grade II haemoglobin values pre-treatment and visit 4. In “Supplementary Materials” on-line complete laboratory safety monitoring values are given. ECGs had no medically relevant changes throughout the monitoring period.

20 Secondary endpoint: clinical efficacy

In terms of the EULAR Response Criteria (Table 3) at 12 weeks, there was one good, four moderate and one non-responder in the placebo group; one good, one moderate and

four non-responders in Cohort 1; two good, one moderate and three non-responders in Cohort 2; and two good, one moderate and three non-responders in Cohort 3.

Table 3: DAS28-ESR values and remission responses

5

| Patient ID | VISITS:                  |      |      |                    |      |      |                    |                    | EULAR<br>Response at W12 |
|------------|--------------------------|------|------|--------------------|------|------|--------------------|--------------------|--------------------------|
|            | 0                        | 2    | 4    | 5                  | 6    | 7    | 8                  | 9                  |                          |
|            | Screen.                  | W0   | W1   | W2                 | W3   | W4   | W8                 | W12                |                          |
|            | <b>Placebo</b>           |      |      |                    |      |      |                    |                    |                          |
| 1          | 5.00                     | 6.73 | 4.53 | 3.72               | 6.07 | 4.18 | 5.10               | 3.75               | Moderate                 |
| 3          | 4.90                     | 4.96 | 4.62 | 3.66               | 3.68 | 3.99 | 3.93               | 3.48               | Moderate                 |
| 9          | 6.43                     | 6.49 | 5.51 | 5.40               | 5.21 | 5.74 | 6.44               | 6.33               | None                     |
| 14         | 5.44                     | 5.45 | 3.99 | 4.19               | 3.70 | 4.47 | 3.69               | 4.63               | Moderate                 |
| 18         | 6.68                     | 6.92 | 6.10 | 6.17               | 6.08 | 4.94 | 5.87               | 4.84               | Moderate                 |
| 20         | 4.66                     | 5.89 | 1.71 | 3.37               | 4.30 | 3.73 | <b><u>2.67</u></b> | <b><u>2.63</u></b> | <b>Good</b>              |
|            | <b>1.0 mg Rasolvir™</b>  |      |      |                    |      |      |                    |                    |                          |
| 2          | 5.17                     | 5.69 | 5.10 | 5.07               | 5.85 | 4.08 | 5.98               | 6.43               | None                     |
| 4          | 6.35                     | 6.02 | 6.42 | 5.92               | 4.59 | 5.12 | 6.00               | 5.45               | None                     |
| 5          | 8.13                     | 8.28 | 7.72 | 8.24               | 8.37 | 8.56 | 8.56               | 8.56               | None                     |
| 6          | 6.77                     | 6.52 | 6.08 | 6.93               | 7.00 | 7.62 | 7.62               | 7.62               | None                     |
| 7          | 7.49                     | 7.49 | 4.43 | 4.18               | 3.40 | 3.24 | 3.58               | 3.04               | <b>Good</b>              |
| 8          | 6.38                     | 6.43 | 5.72 | 5.99               | 5.47 | 5.14 | 4.95               | 4.95               | Moderate                 |
|            | <b>5.0 mg Rasolvir™</b>  |      |      |                    |      |      |                    |                    |                          |
| 10         | 5.03                     | 5.41 | 4.83 | 5.82               | 5.78 | 6.03 | 5.13               | 4.85               | None                     |
| 11         | 7.35                     | 7.30 | 7.85 | 7.49               | 7.12 | 7.51 | 7.72               | 7.72               | None                     |
| 12         | 4.18                     | 2.95 | 4.04 | <b><u>2.19</u></b> | 2.93 | 3.36 | 3.14               | 2.92               | Good                     |
| 13         | 4.45                     | 4.53 | 4.24 | 2.97               | 2.94 | 4.03 | 3.69               | 3.22               | Moderate                 |
| 15         | 4.28                     | 4.15 | 3.12 | <b><u>2.66</u></b> | 2.77 | 3.46 | 2.77               | <b><u>2.47</u></b> | <b>Good/Remission</b>    |
| 16         | 5.59                     | 6.42 | 5.59 | 5.02               | 5.07 | 4.96 | 5.50               | 6.39               | None                     |
|            | <b>15.0 mg Rasolvir™</b> |      |      |                    |      |      |                    |                    |                          |
| 17         | 7.04                     | 6.34 | 6.81 | 6.57               | 6.9  | 5.87 | 6.48               | 6.16               | None                     |

|    |      |      |      |      |                    |      |                    |                    |                       |
|----|------|------|------|------|--------------------|------|--------------------|--------------------|-----------------------|
| 19 | 4.62 | 4.52 | 3.52 | 3.22 | 3.48               | 3.58 | 4.49               | 4.94               | None                  |
| 21 | 5.78 | 6.07 | 6.63 | 5.87 | 6.40               | 6.66 | 6.66               | 6.66               | None                  |
| 22 | 4.72 | 4.38 | 3.41 | 3.35 | <b><u>2.61</u></b> | 3.06 | 3.40               | <b><u>2.33</u></b> | <b>Good/Remission</b> |
| 23 | 4.37 | 4.56 | 4.26 | 3.60 | 2.72               | 3.06 | <b><u>2.52</u></b> | <b><u>2.52</u></b> | <b>Good/Remission</b> |
| 24 | 6.89 | 6.84 | 7.20 | 7.02 | 5.72               | 5.81 | 5.62               | 5.62               | Moderate              |

Screen: Screening visit. ID Patient identity number

DAS28-ESR shown in bold, underlined are patients in remission at that visit.

5 DAS28 area under the curve analysis over the 12 week period did not contribute any more useful data. At 12 weeks, remission was achieved by one patient (17%) in Cohort 2 and by two patients (33%) in Cohort 3, and no placebo patient or in Cohort 1. In those patients who achieved remission, low DAS28 scores were seen by 3 weeks (2.77, 2.61 and 2.72) (Table 3).

10 Sustained ACR20 responses (defined as ACR20 response in at least half of all follow up visits) were seen in 33% of placebo, Cohort 1 and 2 patients and in 66% of Cohort 3 patients. ACR 50 and 70 were seen in subjects who achieved good EULAR responses.

15 Placebo patients did not show sustained changes in CRP levels in the moderate EULAR responders, with a decrease in the one good responder. In contrast, in patients receiving Rasolvir™, CRP decreased in all patients with good responses, one of three patients with moderate responses, and two patients with no response. Patients responding to Rasolvir™ at 2 weeks showed a significant fall in CRP (  $p = 0.0206$ ) from pre-infusion level (Figure 1), although the levels were not significantly different from the other groups. All CRP values are summarised in the Supplementary Table S3.

20

Exploratory PK and biomarker detection:

a) PK

25 The ELISA for the detection of Rasolvir™, a homologue of the endogenous protein, BiP does not distinguish between the proteins. Individual serum levels of Rasolvir™/BiP in all groups of patients, whether placebo or those receiving the drug, did not change from baseline over the initial 24h period after the infusion.

b) Biomarker detection: Serum VEGF and IL-8 concentrations

Due to the small numbers in each treatment group the biomarker analysis was undertaken across the dosing groups according to whether the patient showed a EULAR response or not (Table 3). The 6 placebo patients were kept as a group regardless of their EULAR clinical response. Both at 2 and 12 weeks post-infusion, when compared with those patients receiving placebo, serum VEGF levels in Rasolvir™ treated patients were significantly reduced. Unlike the Rasolvir™ responder (R) patient group or the total group of Rasolvir™ treated patients placebo patients generally showed increased serum levels (change from pre-infusion at 2 weeks; placebo,  $9.9 \pm 8.5$  pg/ml, range, 0.5 to 22.0 pg/ml *versus* Rasolvir™ responders,  $-7.9 \pm 12.5$  pg/ml, range, -29.9 to 7.5pg/ml,  $p = 0.059$ ; or total treated group,  $-5.06 \pm 14.4$  pg/ml, range, -29 – 19.8 pg/ml,  $p = 0.031$ . 12 weeks post infusion ; placebo,  $8.3 \pm 11.6$  pg/ml, range, -8.4 to 19.9 pg/ml *versus* Rasolvir™ responders,  $-8.9 \pm 12.1$ pg/ml, range, -33.0 to 2.6 pg/ml,  $p = 0.028$ ; or total treated group,  $-5.7 \pm 11.9$ , range, -33 – 12.2 pg/ml,  $p = 0.032$ ) (Figure 2A). Similarly, changes in serum IL-8 were significantly lower at 2 weeks but less so at 12 weeks post-infusion (Figure 2B) when compared with the placebo group (2 weeks, placebo,  $1.2 \pm 1$  pg/ml, range 1-3 pg/ml *versus* Rasolvir™ R,  $-0.6 \pm 1.2$  pg/ml, range -2.7 -1 pg/ml,  $p = 0.014$  or total treated group,  $0.09 \pm 2$  pg/ml, range -2.7 – 5.5 pg/ml,  $p = 0.028$ ; 12 weeks post infusion, placebo 0.8-1.4 pg/ml range -0.5 – 2.6 pg/ml *versus* Rasolvir™ R,  $-0.37 \pm 1.1$ , range -1.9 – 0.9 pg/ml,  $p = \text{ns}$ , or total treated group,  $-0.8 \pm 1.1$  pg/ml, range, -2.4 – 0.9 pg/ml,  $p = 0.035$ ). Within this very small number of patients no correlation between serum VEGF concentrations and CRP was observed (see supplementary data Figure 1)

## DISCUSSION

In this exploratory clinical trial, a single intravenous infusion of Rasolvir™ at increasing doses to 15mg is safe. No Rasolvir™-related serious adverse or adverse events were observed in the clinical, laboratory tests or ECG measures.

Efficacy was the secondary outcome. A very heterogeneous group of patients was entered to this safety study, compared to those entering large efficacy studies. Consequently, this trial was particularly confounded by the high level of clinical response in the placebo group, which makes judgement of efficacy difficult. Clinically, good EULAR responses were more common in those treated with higher doses of Rasolvir™ with sustained DAS28 remission (from 3-12 weeks) observed in three

patients who received Rasolvir<sup>TM</sup>, compared to no patients who received placebo. Remarkably, these findings parallel our pre-clinical observations in the murine CIA model in which the response occurred early and was sustained for several weeks after a single dose of BiP. Analysis of biomarkers proved useful and suggestive of some biological changes. Baseline CRP levels were heterogeneous overall, but limited change occurred in the placebo group, in contrast to the patients who responded to Rasolvir<sup>TM</sup> who showed a significant drop in CRP at 2 weeks. Similarly, quantification of serum VEGF and IL-8, common biomarkers used in biologic clinical trials because they correlate well with measurement of synovitis and monocyte infiltration, respectively, showed they were significantly lowered following Rasolvir<sup>TM</sup> treatment. Furthermore, biomarkers failed to provide support for any clinical improvement observed in placebo patients. Strikingly, despite the clinical response observed in the placebo patients at 12 weeks significantly fewer patients showed reduced serum VEGF and IL-8 (17% and 50% respectively). In the Rasolvir<sup>TM</sup> R group 71% and 83% of patients had reduced VEGF and IL-8 serum levels, respectively and even the Rasolvir<sup>TM</sup> NR group showed serum concentrations reduced in 66% and 83% patients respectively, indicative of a change in the pathology of their disease. Further studies would be useful to further confirm this data and to establish an optimum dose and frequency of administration of the drug.

The RAGULA trial should be seen in the context of a long history of research suggesting that microbial and human stress proteins, heat shock proteins (HSP) and their specific peptides can induce regulatory T cell differentiation and down-regulate inflammatory responses. These consistent pre-clinical findings triggered clinical trials of the proteins or their peptides as novel therapies for type 1 diabetes (HSP60 DiaPep277 [26]), JIA (HSP dnaJ-derived epitope) [27] and for RA [28]. These early phase clinical trials were inconclusive, partly due to sub-optimal study design, for example, the HSP, chaperonin 10, trial in RA [29], had no placebo arm, making interpretation of effects difficult. Thus the present findings with Rasolvir<sup>TM</sup> are exciting and novel. To our knowledge this is the only double-blind, placebo-controlled fully randomised clinical trial of a human stress protein in an autoimmune disease that shows safety and suggests efficacy. The prolonged therapeutic activity suggests induction of regulatory cells, supporting the original hypotheses regarding therapeutic action of

stress proteins. Further biomarker analysis is underway to provide definitive proof of concept. If so, many clinical applications can be envisaged for Rasolvir™.

In summary, a single intravenous infusion of Rasolvir™ ( $\leq 15\text{mg}$ ) is well tolerated with no drug-related adverse events. There are suggestions of clinical efficacy with clinical  
 5 outcomes supported by biological end points. These results were achieved early after therapy and sustained for 12 weeks suggesting that Rasolvir™ has immunomodulatory effects. Larger studies would be advantageous to confirm and expand these data, particularly the optimum dose and frequency of Rasolvir™ administration. Immunological analysis of cellular and humoral biomarkers monitored during the trial  
 10 should reveal whether Rasolvir™ can induce regulatory cell activity. In that event Rasolvir™ may have therapeutic uses in other indications such as JIA, spondylitic diseases and psoriatic arthritis.

## EXAMPLE 2: MMP3

15 Analysis of MMP3 is advantageous, especially in conjunction with the other biomarkers, because there is a distinct difference between the responders and non responders.

The responders show significantly lower levels of MMP3 than the non responders, at  
 20 point 3 72h, 0.08 (approaching significance); 7 days 0.05; 14 days 0.03; 28 days 0.02 and going back up at 84 days 0.07.

The actual statistical analysis of the serum levels of MMP3 is below:

25 MMP3 0.04 at time6 (4 weeks) with otherwise a p between 0.10 to 0.15 from time 3 to time 7 but higher p at time2.

Time 4

Placebo v Responder: 1

30 Placebo v Nonresponder: 0.08

Nonresponder v Responder: 0.02

At time points 3 to 7 (apart from point 6 above):

- for Placebo v Responder p-values are all high
- 35 - for Placebo v Nonresponder p-values are 0.18 at times 3, 4, 7 and 0.37 at time 5

- for Nonresponder v Responder p-values are 0.08, 0.05, 0.03, (point 3 to 5) and 0.07 (point 7)

5       -       **Nonresponders are different from responders (and somewhat from placebo)**

-       **there is extra support from neighbouring time-points so AUC should with graph over time should useful here.**

### 10       **EXAMPLE 3: MMP3**

Here we show data for the change in serum levels of MMP-3 . Different analysis from the statistical data in Example 2 that looked at actual serum levels. The data really give a very good idea of whether the patients will or will not respond to BiP.

15

This means that at 2 weeks the VEGF and IL-8 levels (lower than placebo levels) will show that BiP has had an immunological effect on the patient and as early as 72h but also 2weeks and later the change in level of MMP3 from patients' pre-treatment baseline will give some indication as to whether the patient will be a responder or not.

20

We refer to Figure 3, Figure 4, Figure 5 and Figure 6.

Thus we show very exciting data especially when taken in the context of the high placebo clinical response.

### 25       **EXAMPLE 4: DATA FOR THE REGULATORY T CELLS**

Figure 7A and Figure 7B show upregulation of CD39 on CD3+CD4+ CD25hi CD127lo regulatory T cells. Heparinised whole blood samples taken from patients pre infusion and post treatment at 24hours(h), 72h and 84 day time points, were stained with the required fluorochrome conjugated monoclonal antibodies to CD45, CD3, CD4, CD25, CD127 and CD39 including a live/dead indicator. Cells were analysed within 4 hours by FACSCanto flow cytometer. The change in expression of CD39 on CD3+CD4+ CD25hi CD127lo regulatory T cells was calculated in the placebo, BiP/Rasolvir responder (R) and non-responder (NR) groups and total treated group.

35

## METHODS AND PROTOCOLS

**BiP STUDY RESEARCH FLOW CHART**

- 1) Retrieve cell sample from liquid N<sub>2</sub> (bottom of Liq N<sub>2</sub> Tank 2/Tower 2)  
 5 Thaw sample ready for use as internal control and add 10µl NHS before staining. Resuspend in PBS 200µl and dispense 50µl into each of 4 tubes  
 Keep on ice until needed
- 2) **Immunofluorescent staining**  
 Take 1ml hep blood in eppendorf and put aside  
 10 Put conjugated antibodies into eppendorfs for each of the panels (3 +1 unstained) and the isotope (2) panels

| Panel 1      |         | CD4 CD25hi Treg |              |                      |                      |       |        |
|--------------|---------|-----------------|--------------|----------------------|----------------------|-------|--------|
| Colour       | Isotype | CD Number       | Manu. Number | ul in 100 ul WB      | ul in 50 ul WB       | Laser | Filter |
| Pacific Blue | IgG1k   | *45             | 304021       | 2                    | 1                    | 405   | 450/50 |
| APC Cy7      | igG1k   | *3              | 344817       | 1                    | 0.5                  | 633   | 780/60 |
| FITC         | IgG2bk  | *4              | 317407       | 2                    | 1                    | 488   | 530/30 |
| APC          | IgG1k   | 25              | 302609       | 2                    | 1                    | 633   | 660/20 |
| PE Cy7       | IgG1k   | 39              | 328211       | 2                    | 1                    | 561   | 780/60 |
| PE           | IgG1k   | *127            | 557938       | 1                    | 0.5                  | 488   | 575/26 |
| eFluor 506   | N/A     | Live/Dead       | 65-0866-14   | 1ul of 1in5 dilution | 1ul of 1in5 dilution | 405   | 525/20 |

| Panel 2      |         | CD8 Treg  |              |                      |                      |       |        |
|--------------|---------|-----------|--------------|----------------------|----------------------|-------|--------|
| Colour       | Isotype | CD Number | Manu. Number | ul in 100 ul WB      | ul in 50 ul WB       | Laser | Filter |
| Pacific Blue | IgG1k   | 45        | 304021       | 2                    | 1                    | 405   | 450/50 |
| APC Cy7      | igG1k   | 3         | 344817       | 1                    | 0.5                  | 633   | 780/60 |
| FITC         | IgG1k   | 8         | 344703       | 4                    | 2                    | 488   | 530/30 |
| PE Cy7       | IgG1k   | 28        | 302925       | 4                    | 2                    | 561   | 780/60 |
| APC          | IgG1k   | *56       | 318309       | 1                    | 0.5                  | 633   | 660/20 |
| PE           | IgG1k   | 183       | 353705       | 5                    | 2.5                  | 488   | 575/26 |
| PerCP 5-5    | IgG1k   | *62L      | 304823       | 5                    | 2.5                  | 488   | 695/40 |
| eFluor 506   | N/A     | Live/Dead | 65-0866-14   | 1ul of 1in5 dilution | 1ul of 1in5 dilution | 405   | 525/20 |

| Panel 3      |         | Monocyte  |              |                 |                |       |        |
|--------------|---------|-----------|--------------|-----------------|----------------|-------|--------|
| Colour       | Isotype | CD Number | Manu. Number | ul in 100 ul WB | ul in 50 ul WB | Laser | Filter |
| Pacific Blue | IgG1k   | 45        | 304021       | 2               | 1              | 405   | 450/50 |
| PerCP 5-5    | IgG1k   | 14        | 325621       | 5               | 2.5            | 488   | 695/40 |
| FITC         | IgG2bk  | 20        | 302303       | 5               | 2.5            | 488   | 530/30 |

|               |        |           |            |                         |                         |     |        |
|---------------|--------|-----------|------------|-------------------------|-------------------------|-----|--------|
| APC           | IgG2bk | 86        | 305411     | 1                       | 0.5                     | 633 | 660/20 |
| PE Cy7        | IgG2ak | *HLA-DR   | 307615     | 0.5                     | 0.25                    | 561 | 780/60 |
| PE            | IgG1k  | 80        | 305207     | 5                       | 2.5                     | 488 | 575/26 |
| eFluor<br>506 | N/A    | Live/Dead | 65-0866-14 | 1ul of 1in5<br>dilution | 1ul of 1in5<br>dilution | 405 | 525/20 |

5

**For the first panel master mix**

|  |       | For 100µl | Master mix |
|--|-------|-----------|------------|
|  | CD45  | 2         | 3          |
|  | CD3   | 1         | 1.5        |
|  | CD4   | 2         | 3          |
|  | CD25  | 2         | 3          |
|  | CD39  | 2         | 3          |
|  | CD127 | 1         | 1.5        |

**NB 100µl blood needed for this panel**

∴ sample volume 9.8µl/ tube internal control 4.8µl/tube

**For Second panel master mix**

|  |              | For 100µl | Mastermix |
|--|--------------|-----------|-----------|
|  | <b>CD45</b>  | <b>2</b>  | <b>2</b>  |
|  | <b>CD3</b>   | <b>1</b>  | <b>1</b>  |
|  | <b>CD8</b>   | <b>4</b>  | <b>4</b>  |
|  | <b>CD28</b>  | <b>4</b>  | <b>4</b>  |
|  | <b>CD56</b>  | <b>1</b>  | <b>1</b>  |
|  | <b>CD183</b> | <b>5</b>  | <b>5</b>  |
|  | <b>CD62L</b> | <b>5</b>  | <b>5</b>  |

10

For 50µl blood and internal control 10.5µl/tube

**For Third panel and FMO**

|  |  |  | Mastermix |
|--|--|--|-----------|
|--|--|--|-----------|

|  |        |                  |     |
|--|--------|------------------|-----|
|  | CD45   | 2                | 3   |
|  | CD14   | 5                | 7.5 |
|  | CD20   | 5                | 7.5 |
|  | CD86   | 1                | 1.5 |
|  | HLA_DR | 0.5 (NOT IN FMO) |     |
|  | CD80   | 5                | 7.5 |

For FMO mix and remove 9µl before you **add 0.5µl HLA-DR, mix**

9µl/sample tube and 9µl/internal control tube

5

**NB also keep 50µl of blood for the unstained control**

Isotype control tubes:

Add 2µl / isotype control . Two panels IgG1 and IgG2a

|                   |       |
|-------------------|-------|
| <b>isoPanel 1</b> |       |
| Pac Blue          | IgG1k |
| APC Cy7           | IgG1k |
| PE Cy7            | IgG1k |
| PE                | IgG1k |
| PerCP Cy5.5       | IgG1k |
| APC               | IgG1k |
| FITC              | IgG1k |

|                   |        |
|-------------------|--------|
| <b>isoPanel 2</b> |        |
| APC               | IgG2bk |
| PE Cy7            | IgG2ak |
| FITC              | IgG2bk |

10

Add 14µl to sample tube for isotype panel 1 and 6µl for isotype panel 2

- 3) **When all antibodies have been dispensed add 100µl heparinised blood to panel 1 sample tube and 50µl for all other tubes. There should be 6 tubes for the sample and 3 for the internal control**

15

- 4) **Vortex and leave on ice for 24h**

- 5) Lyse red blood cells with 1x NH<sub>4</sub>Cl solution on ice for 7 mins
- 6) Spin down and re-lyse for 5mins
- 7) wash x1 in PBS

5

**PREPARE LIVE/DEAD STAIN WHILE CELLS ARE STAINING**

- a) Lyse 100ul of blood as described above and wash in PBS
- b) Split into 2 and heat one half at 65°C for 5 mins. Recombine the cells
- 8) Add 1 µl of diluted Live/Dead stain (bottom shelf of -80 dilute 1/5 in PBS) to all cells
- 9) Incubate at 4°C for 30 minutes
- 10) Wash x2 with 1ml of IF buffer at 250 x g
- 11) Centrifuge and fix in IF buffer+ 2%para at RT for 10mins
- 12) Wash in IF buffer x2 and put in fridge for analysis

15

**WHILE THE SAMPLES ARE STAINING**

- 9) Prepare the control beads—
  - a) vortex well and place one drop in a FACs tube
  - b) Resuspend in 800µl and dispense 100µl vols into 7 tubes
  - c) Add 0.5µl of the following: CD45, CD3, CD4, CD127, CD56, CD62L, HLA-DR
  - d) incubate for 30' on ice
  - e) wash x2 with IF buffer and resuspend in 200µl IF buffer

20

PBMC separation: Heparin and EDTA

**Lymphoprep to get maximum number of cells wash x2 count and set up in freezing vials at 10.10<sup>6</sup>/900µl.**

25

**NE freeze 2ml plasma from top of Lymphoprep in 500µl aliquots (freeze at -80, top shelf BiP trial box) LABEL clearly with PLASMA**

**Freeze cells for a minimum 4h in freezing pot in -80 and then transfer to liquid N<sub>2</sub>.**

30

**Liquid Nitrogen Tank 2, Tower2, lower 4 shelves**

**SERUM separation**

**After 1 h separate the serum and aliquot at 500µl/eppendorf.**

35

**Freeze in -80 (top shelf in BiP Trial Box)**

**RNA separation**

- Take PBMC after second wash and remove all supernatant from pellet with pipette. Resuspend in 720µl TriZol and pipette vigorously/ vortex and freeze at -80

40

Although illustrative embodiments of the invention have been disclosed in detail herein, with reference to the accompanying drawings, it is understood that the invention is not limited to the precise embodiments and that various changes and modifications can be effected therein by one skilled in the art without departing from the scope of the invention as defined by the appended claims and their equivalents.

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- 25

CLAIMS

1. A method of determining responsiveness to BiP administration in a subject, the method comprising
- 5 (a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP
- (b) comparing said concentration(s) determined in (a) to reference
- 10 concentration(s) for said biomarker(s) wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) indicates that the subject is responsive to BiP administration.
2. A method of determining the likelihood of remission of rheumatoid arthritis
- 15 following administration of BiP to a subject or treatment of a subject with BiP, the method comprising
- (a) determining the concentration of one or more biomarkers selected from the group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained from said subject at about 2 to 12 weeks after
- 20 administration of BiP
- (b) comparing said concentration(s) determined in (a) to reference concentration(s) for said biomarker(s) wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) indicates an increased likelihood of remission of rheumatoid arthritis
- 25 in said subject.
3. A method of selecting a treatment regime for a subject with rheumatoid arthritis, the method comprising:
- (a) determining the concentration of one or more biomarkers selected from the
- 30 group consisting of VEGF, IL-8, and CRP in a sample from said subject wherein said sample was obtained after administration of BiP to a subject or treatment of a subject with BiP, wherein said sample was obtained from said subject at about 2 to 12 weeks after administration of BiP
- (b) comparing said concentration(s) determined in (a) to reference
- 35 concentration(s) for said biomarker(s)

wherein a decrease in the concentration(s) in the sample compared to the reference concentration(s) selects a treatment regime comprising a second or further administration of BiP to said subject.

5     4.     A method according to any preceding claim wherein the concentration of at least one of VEGF and IL-8 is determined.

5.     A method according to any preceding claim wherein the concentrations of at least two of said biomarkers are determined in (a).

10

6.     A method according to any preceding claim wherein the concentrations of both VEGF and IL-8 are determined.

7.     A method according to any preceding claim wherein the concentrations of all  
15 three of said biomarkers are determined in (a).

8.     A method according to any preceding claim wherein determining the concentration of each biomarker comprises:

- (bi)    detection by contacting the sample with an antibody or antigen binding  
20 fragment thereof capable of specifically binding the biomarker; and  
(bii)   quantification of said binding.

9.     A method according to any of claims 1 to 7 wherein determining the concentration of each biomarker comprises detection of the mRNA for the biomarker,  
25 wherein detection of the mRNA comprises:

- (bi)    contacting the sample with specific nucleic acid probe(s) or primer(s) for the biomarker; and  
(bii)   quantification of said probe(s) or primer(s).

30     10.    A method according to any preceding claim wherein said sample is a sample of serum or plasma.

11.    A kit comprising reagent(s) for the specific detection of each of the following biomarkers: VEGF, IL-8, and CRP.

35

12.    A kit comprising reagents for the specific detection of mRNA encoding each of the following biomarkers: VEGF, IL-8, and CRP.

13. A device comprising an array of materials which together are capable of specifically binding each of the following biomarkers: VEGF, IL-8, and CRP, each material within the array being capable of specifically binding one of said biomarkers.

5

14. A device comprising an array of materials which together are capable of detecting mRNA specific for each of the following biomarkers VEGF, IL-8, and CRP, each material within the array being capable of specifically detecting one of said mRNAs.

10

15. Use of BiP for treatment of rheumatoid arthritis in a subject, wherein the method according to any of claims 1 to 10 is carried out for said subject, wherein if it is determined that said subject is responsive to BiP administration then BiP is administered to said subject.

15

16. Use according to claim 15 wherein a treatment regimen of administering 5 mg to 15 mg BiP to said subject is selected.

17. Use according to claim 15 or claim 16 wherein a treatment regimen of administering a second or further 5 mg to 15 mg BiP to said subject is selected.

20

18. A method of treating a subject comprising carrying out the method according to any of claims 1 to 10, wherein if it is determined that the subject has a decrease in the concentration(s) in the sample compared to the reference concentration(s), BiP is administered to said subject.

25

19. A process for selecting a treatment regimen, said process comprising carrying out the method according to any of claims 1 to 10, wherein if it is determined that the subject has a decrease in the concentration(s) in the sample compared to the reference concentration(s), then a treatment regimen of a second or further administration of BiP is selected.

30

20. A syringe containing a composition comprising an effective amount of BiP for treatment of rheumatoid arthritis in a subject, wherein the method according to any of claims 1 to 10 is carried out for said subject, wherein if it is determined that the subject has a decrease in the concentration(s) in the sample compared to the reference

35

concentration(s), then said syringe is used to administer said composition to said subject.

21. A syringe according to claim 20 wherein said effective amount is 5 mg to 15 mg  
5 of BiP.

22. Use of a combination of materials each of which recognises, specifically binds to  
or has affinity for one of the following biomarkers: VEGF, IL-8, and CRP, wherein said  
combination includes at least one such material for each of said biomarkers, for  
10 determining responsiveness to BiP administration in a subject.

23. Use according to claim 22 wherein said material comprises an antibody or  
antigen binding fragment thereof.

15 24. Use for determining responsiveness to BiP administration in a subject, of a  
combination of materials each of which recognises, specifically binds to or has affinity  
for mRNA of one or more of the following biomarkers: VEGF, IL-8, and CRP.

25. Use according to claim 24 wherein said material comprises a nucleic acid  
20 primer or probe.

26. An apparatus comprising logic configured to carry out the method of any one of  
claims 1 to 10.

25 27. A computer program product operable, when executed on a computer, to  
perform the method steps of any one of claims 1 to 10.

28. A method of treatment of Juvenile Idiopathic Arthritis (JIA), said method  
comprising administering BiP to said subject, suitably 5 mg to 15 mg of BiP to said  
30 subject.

29. BiP for treatment of Juvenile Idiopathic Arthritis (JIA).

30. Use of BiP in the treatment of Juvenile Idiopathic Arthritis (JIA).  
35

31. A method of treatment of spondylitic disease, said method comprising  
administering BiP to said subject, suitably 5 mg to 15 mg of BiP to said subject.

32. BiP for treatment of spondylitic disease.
33. Use of BiP in the treatment of spondylitic disease.
- 5 34. A method of treatment of psoriatic arthritis, said method comprising administering BiP to said subject, suitably 5 mg to 15 mg of BiP to said subject.
35. BiP for treatment of psoriatic arthritis.
- 10 36. Use of BiP in the treatment of psoriatic arthritis.
37. A method of treatment of human immune mediated diseases in which its ability to induce immune cells that can regulate the immune response can suppress the
- 15 immune-mediated inflammation, seropositive rheumatoid arthritis, Seronegative arthritis, Pelvospondylitic disease, Psoriatic disease, Inflammatory bowel disease (ulcerative colitis or Crohn's disease), Solid organ, bone marrow, or blood cell transplantation, or autoimmune diseases, said method comprising administering BiP to said subject, suitably 5 mg to 15 mg of BiP to said subject.
- 20 38. BiP for treatment of human immune mediated diseases in which its ability to induce immune cells that can regulate the immune response can suppress the immune-mediated inflammation, seropositive rheumatoid arthritis, Seronegative arthritis, Pelvospondylitic disease, Psoriatic disease, Inflammatory bowel disease (ulcerative
- 25 colitis or Crohn's disease), Solid organ, bone marrow, or blood cell transplantation, or autoimmune diseases.
39. Use of BiP in the treatment of human immune mediated diseases in which its ability to induce immune cells that can regulate the immune response can suppress the
- 30 immune-mediated inflammation, seropositive rheumatoid arthritis, Seronegative arthritis, Pelvospondylitic disease, Psoriatic disease, Inflammatory bowel disease (ulcerative colitis or Crohn's disease), Solid organ, bone marrow, or blood cell transplantation, or autoimmune diseases.

35

Figure 1

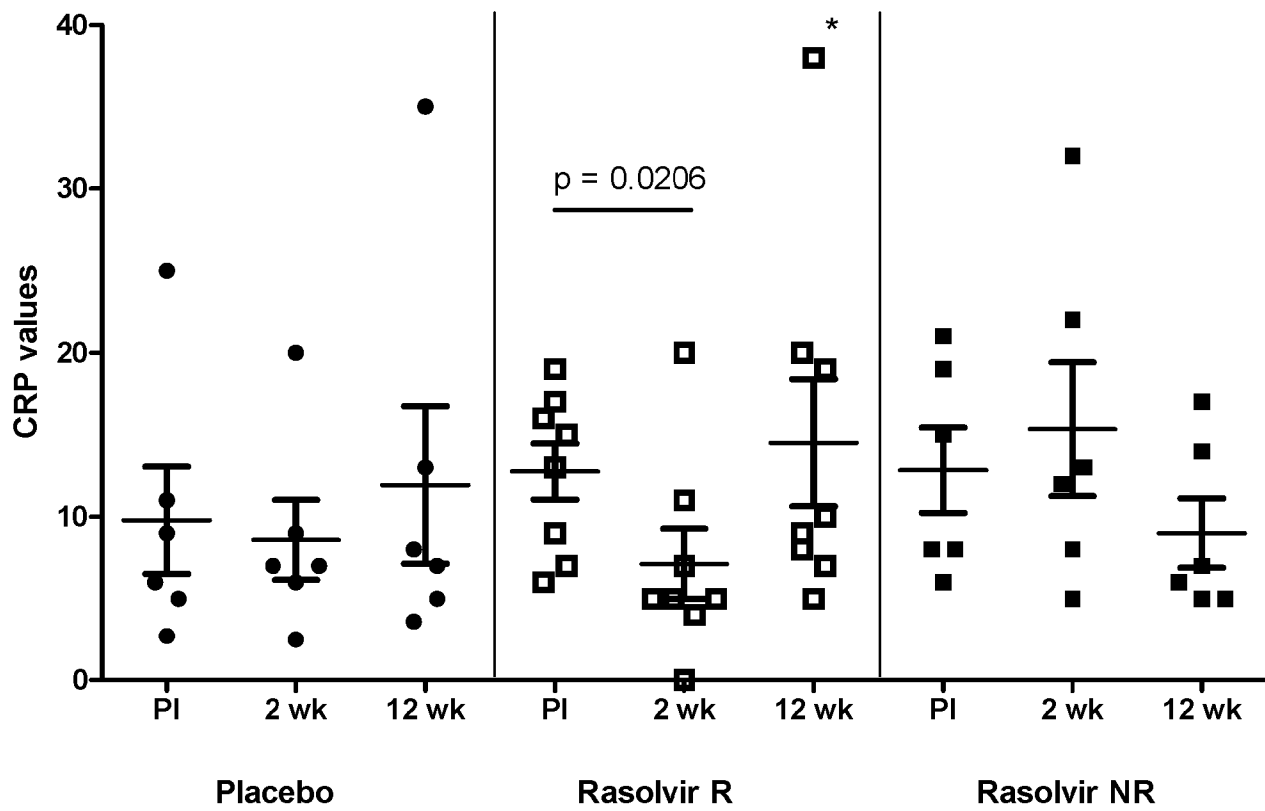


Figure 2

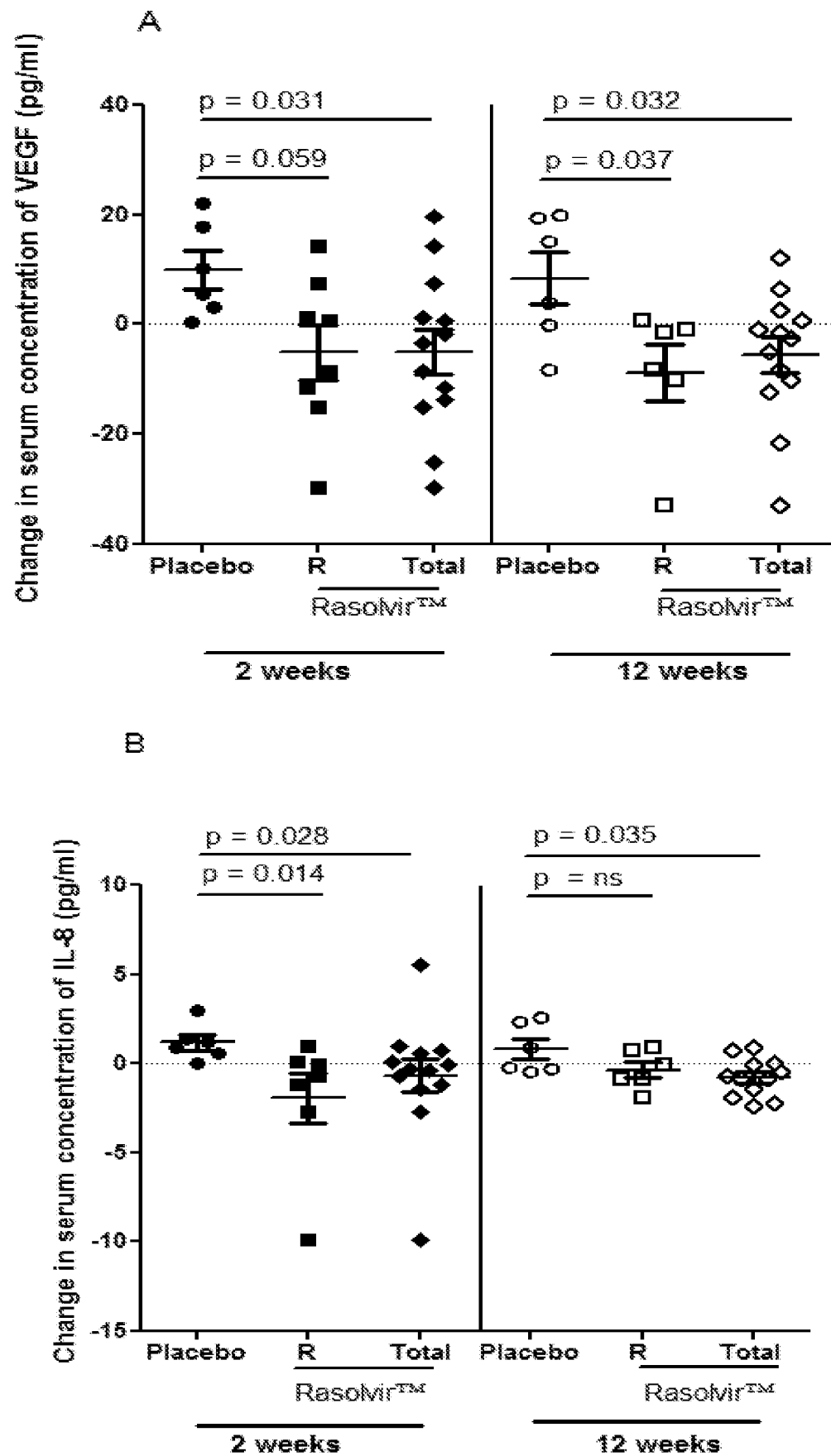


Figure 3

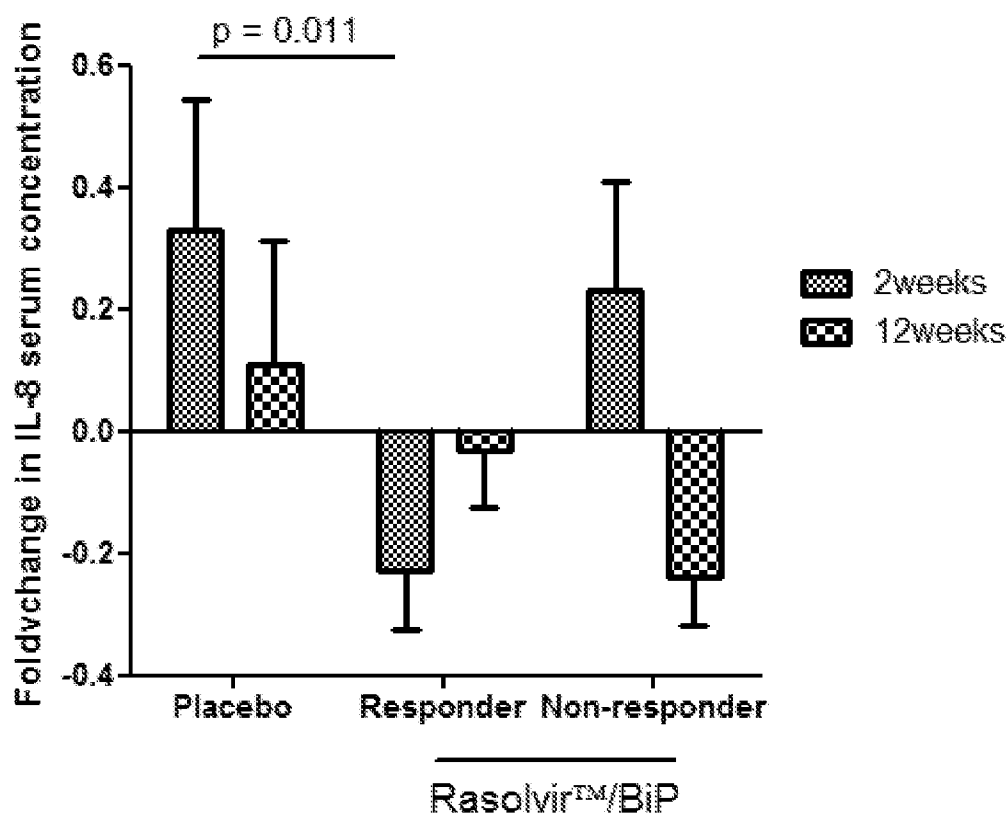


Figure 4

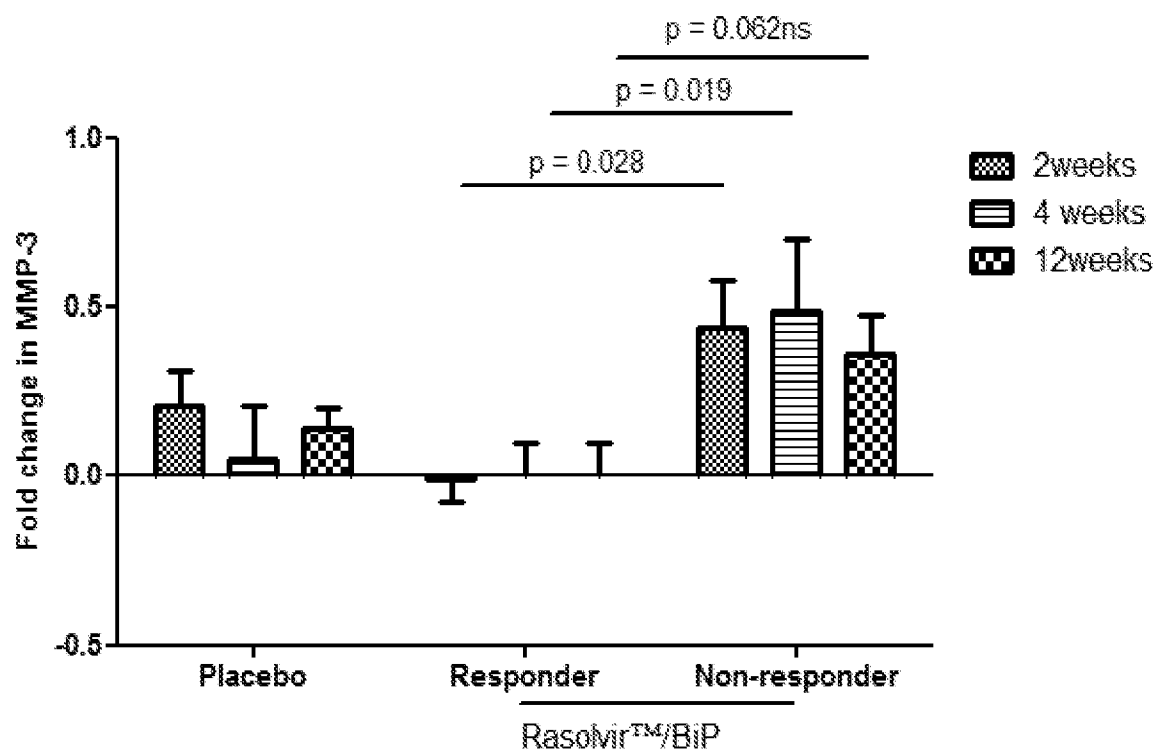


Figure 5

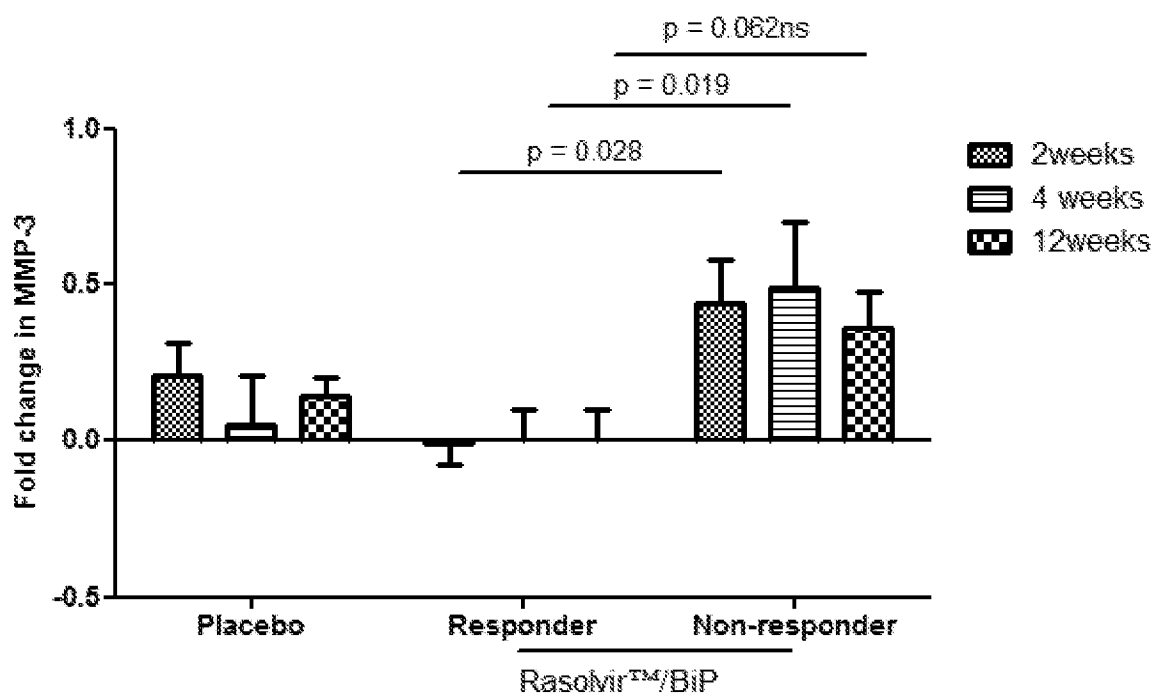


Figure 6

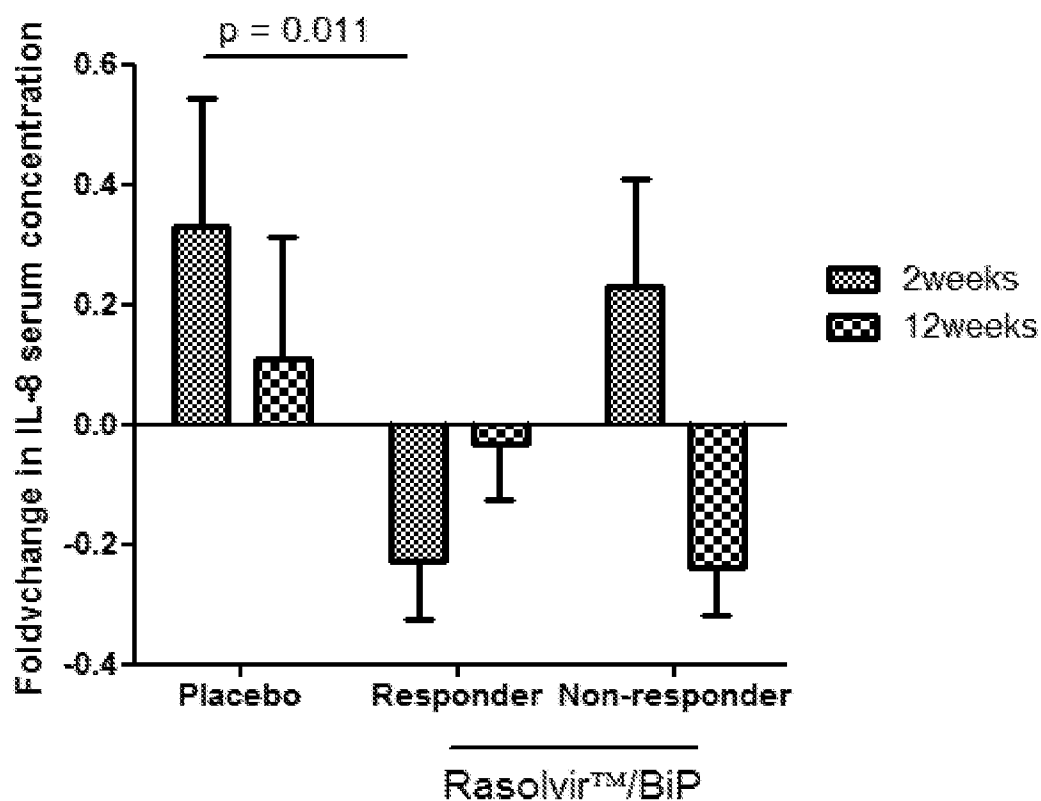


Figure 7A

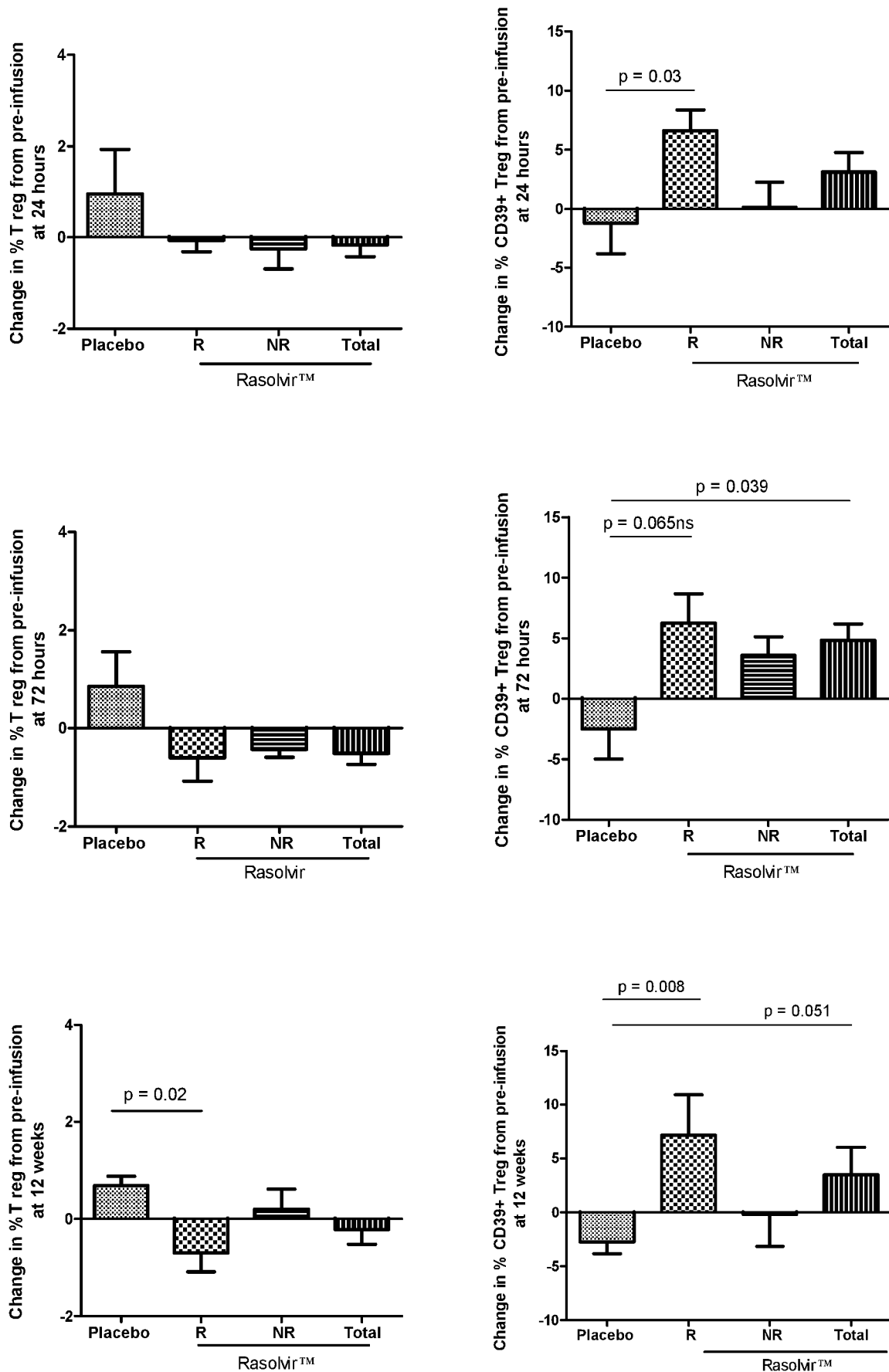
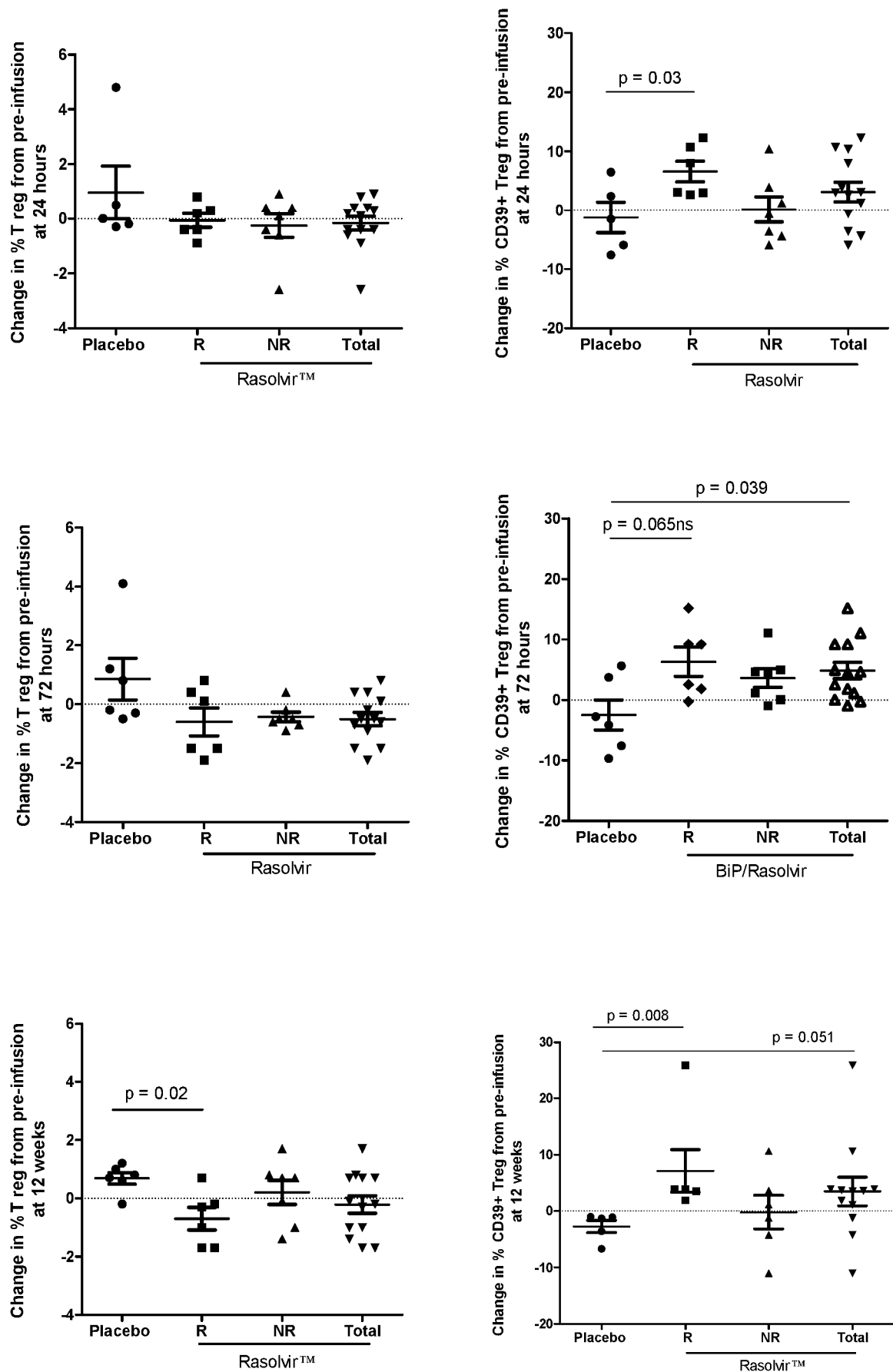


Figure 7B



# INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2017/050486

## A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/564 A61K38/17  
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, BIOSIS, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                       | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y         | <p>ALLY M.M.T.M. ET AL.: "Circulating anti-citrullinated peptide antibodies, cytokines and genotype as biomarkers of response to disease-modifying antirheumatic drug therapy in early rheumatoid arthritis", BMC MUSCULOSKELETAL DISORDERS, vol. 16, no. 1, 130, 29 May 2015 (2015-05-29), pages 1-9, XP021222792, abstract page 3 tables 2,4</p> <p>-----<br/>-/--</p> | 1-27                  |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 April 2017

Date of mailing of the international search report

19/06/2017

Name and mailing address of the ISA/

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Authorized officer

Giry, Murielle

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2017/050486

### Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a. ☒ forming part of the international application as filed:
    - ☒ in the form of an Annex C/ST.25 text file.
    - ☐ on paper or in the form of an image file.
  - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
    - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
    - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB2017/050486

### Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-27

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-27

Methods and products for determining responsiveness to BIP administration (in particular to treat rheumatoid arthritis) and use thereof.

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2. claims: 28-30

Method of treating juvenile idiopathic arthritis (JIA).

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3. claims: 31-33

Method of treating spondylitic disease.

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4. claims: 34-36

Method of treating psoriatic arthritis.

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5. claims: 37-39

Method of treating any human immune mediated disease.

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# INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2017/050486

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                       | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y         | TALAAT R.M. ET AL.: "Therapeutic effect of dimethyl dimethoxy biphenyl dicarboxylate on collagen-induced arthritis in rats", CHIN. J. INTEGR. MED., vol. 21, no. 11, November 2015 (2015-11), pages 846-854, XP035579926, abstract                                                       | 1-27                  |
| Y         | -----<br>US 2008/155704 A1 (PANAYI G.S. [GB] ET AL.) 26 June 2008 (2008-06-26) cited in the application abstract paragraphs [0003] - [0009] figures 6-9 examples 7-8                                                                                                                     | 1-27                  |
| A         | -----<br>ALLY M.M.T.M. ET AL.: "Serum matrix metalloproteinase-3 in comparison with acute phase proteins as a marker of disease activity and radiographic damage in early rheumatoid arthritis", MEDIATORS OF INFLAMMATION., vol. 2013, 2013, pages 1-6, XP055364035, abstract           | 1-27                  |
| A         | -----<br>YOO S.-A. ET AL.: "A novel pathogenic role of the ER chaperone GRP78/BiP in rheumatoid arthritis", J. EXP. MED., vol. 209, no. 4, 19 March 2012 (2012-03-19), pages 871-886, XP055363898, the whole document                                                                    | 1-27                  |
| A         | -----<br>SHIELDS A.M. ET AL.: "Systemic gene transfer of binding immunoglobulin protein (BiP) prevents disease progression in murine collagen-induced arthritis", CLIN. EXP. IMMUNOL., vol. 179, no. 2, 2 January 2015 (2015-01-02), pages 210-219, XP055364023, the whole document      | 1-27                  |
| A         | -----<br>PANAYI G.S. ET AL.: "Immunoglobulin heavy-chain-binding protein (BiP): a stress protein that has the potential to be a novel therapy for rheumatoid arthritis", BIOCHEM. SOC. TRANS., vol. 42, no. 6, December 2014 (2014-12), pages 1752-1755, XP055364020, the whole document | 1-27                  |
|           | -----<br>-/--                                                                                                                                                                                                                                                                            |                       |

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2017/050486

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                         | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X,P       | <p>KIRKHAM B. ET AL.: "Safety and patient response as indicated by biomarker changes to binding immunoglobulin protein in the phase I/IIA RAGULA clinical trial in rheumatoid arthritis",<br/>RHEUMATOLOGY,<br/>vol. 55, no. 11,<br/>7 August 2016 (2016-08-07), pages<br/>1993-2000, XP009194099,<br/>the whole document</p> <p>-----</p> | 1-27                  |

## INTERNATIONAL SEARCH REPORT

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